FORMULATION AND EVALUATION OF SOLID SELF MICROEMULSIFYING DRUG DELIVERY SYSTEM

A Thesis submitted to Gujarat Technological University

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In

Pharmacy [Pharmaceutics]

By

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GUJARAT TECHNOLOGICAL UNIVERSITY, AHMEDABAD

[January – 2017]

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ABSTRACT

Candesartan cilexetil is an orally administered ACE inhibitor for the treatment of hypertension and cardiac failure, but its solubility, stability and oral bioavailability are poor. The objective of our investigation was to formulate a self microemulsifying drug delivery system (SMEDDS) of candesartan cilexetil using minimum surfactant concentration that could improve its solubility, stability and oral bioavailability. The composition of optimized formulation [C7IIB] consist of Capryol 90 as oil, Labrasol as surfactant and Captex 500 as cosurfactant , containing 32 mg of candesartan cilexetil showing drug release for liquid SMEDDS formulation (99.91%), droplet size (9.15 nm), Zeta potential (-23.2), viscosity (0. 8824 cP) and infinite dilution capability. *In-vitro* drug release of the C7IIB was highly significant ($p < 0.05$) as compared to marketed conventional tablet (M). The C7IIB was further used for the preparation of various Solid SMEDDS(S-SMEDDS) formulations (Tablet). These tablets were prepared via adsorption to solid carrier technique, using optimized liquid SMEDDS formulation [C7IIB] whereas Aeropearl 300 pharma as optimized adsorbents .The resulting S-SMEDDS tablet exhibited particle size (78.3 nm) whereas the liquid SMEDDS showed (9.15 nm). The *in vitro* release was almost similar for the S-SMEDDS as well liquid i.e. 78.32% and 84.6% respectively within 5 min. Also, one of the main objective to enhance the oral bioavailability of drug (15%) which was enhanced to 1.78 folds. In conclusion, our studies illustrated that adsorption to solid carrier technique could be a useful method to prepare the solid SMEDDS tablets from liquid SMEDDS, which can improve oral absorption of candesartan cilexetil, nearly equivalent to the liquid SMEDDS, but better in the formulation stability, drugs leakage and precipitation, etc.

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At times our own light goes out and is rekindled by a spark from another person. Each of us has cause to think with deep gratitude of those who have lighted the flame within us.

-- Albert Schweitzer

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Jill Shukla

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LIST OF ABBREVIATION

ACE	Angiotensin Converting Enzyme
ANOVA	Analysis Of Variance
ARBs	Angiotensin Receptor Blockers
AT_1	Angiotensin II Type 1
AUC	Area Under Curve
BCS	Biopharmaceutics Classification System
BS	Bile Salts
CH	Cholesterol
Cmax	Maximum Concentration
DG	Di- Glycerides
DOCA	Deoxycorticosterone Acetate
DSC	Differential Scanning Calorimetry
GI	Gastrointestinal
GPCR	G-Protein Coupled Receptor
GRAS	Generally Recognized As Safe
HLB	Hydrophilic-Lipophilic Balance
LCT	Long Chain Triglyceride
LFCS	Lipid Formulation Classification System
MCC	Microcrystalline Cellulose
MCT	Medium Chain Triglyceride
o/w	Oil-In-Water
PDI	Polydispersibility Index
PEG	Polyethylene Glycol
PG	Propylene Glycol
PL	Phospholipids
PSD	Particle Size Distribution
SEDDS	Self-Emulsifying Drug Delivery Systems
SEM	Scanning Electron Microscopy
SEOF	Self-Emulsifying Oil Formulations
SMEDDS	Selfmicroemulsifying Drug Delivery System

Smix	Surfactant And Cosurfactants Mixture
S-SMEDDS	Solid Selfmicroemulsifying Drug Delivery System
UGT1A3	Uridine Diphosphate Glucuronosyltransferase 1A3
UNX	Uninephrectomized
VA	Visual Assessment

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1. INTRODUCTION

Approximately 40% of new drug candidates have poor water solubility and the oral delivery of such drugs is frequently associated with low bioavailability, high intra- and inter-subject variability, and a lack of dose proportionality [1]. To overcome these problems, various formulation strategies are exploited including the use of surfactants, lipids, permeation enhancers, micronisation, salt formation, cyclodextrins, nanoparticles and solid dispersions [1]. Recently, much attention has been paid to lipid-based formulations with particular emphasis on self-emulsifying drug delivery systems (SEDDS) to improve the oral bioavailability of lipophilic drugs [2, 3]. SEDDS or self-emulsifying oil formulations (SEOF) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants or alternatively, one or more hydrophilic solvents and co-solvents/ surfactants. [4] Upon mild agitation followed by dilution in aqueous media, such as gastrointestinal (GI) fluids, these systems can form fine oil-in-water (o/w) emulsions or microemulsions or Selfmicroemulsifying drug delivery system (SMEDDS). Fine oil droplets would pass rapidly from the stomach and promote wide distribution of the drug throughout the GI tract, thereby minimizing the irritation frequently encountered during extended contact between bulk drug substances and the gut wall. When compared with emulsions, which are sensitive and metastable dispersed forms, SMEDDS are physically stable formulations that are easy to manufacture. An additional advantage of SMEDDS over simple oily solutions is that they provide a large interfacial area for partitioning of the drug between oil and water.

Different formulation approaches like micronization, solid dispersion, and complexation with cyclodextrins have come up for the poorly water soluble drugs. [5] Indeed, in some selected cases, these approaches have been successful but they offer many other disadvantages. The main problem with micronization is chemical / thermal stability. Many drugs may degrade and lose bioactivity when they are micronized by conventional method. For solid dispersion the amount of carriers used is often large, and thus if the dose of active ingredient is high, the tablets or capsules formed will be large in volume and difficult to swallow. Moreover, since the carriers used are usually expensive and freeze-drying or spray-drying method requires particular facilities and processes, leading to high production cost. Though, traditional solvent method can be adopted instead, it is difficult to deal with co-

precipitates with high viscosity. Complexation with cyclodextrins techniques is not applicable for drug substances which are not soluble in both aqueous and organic solvents. Realization that the oral bioavailability of poor water soluble drugs may be enhanced when co-administered with meal rich in fat has led to increasing recent interest in the formulation of poorly water soluble drugs in lipids. Lipid suspension, solutions and emulsions have all been used to enhance the oral bioavailability but, more recently there have been much focus on the utility of self-microemulsifying drug delivery systems (SMEDDS).[6]

1.1 LIPID FORMULATION CLASSIFICATION SYSTEM

The different lipid drug delivery systems available include lipid solution, lipid emulsion, microemulsion, dry emulsion. To get a clear picture of all these different systems and due to large number of possible excipient combinations that may be used to assemble these lipid-based formulations, self emulsifying systems in particular a classification system have been established called as lipid formulation classification system (LFCS). This classification helps to better understand the fate of different lipid formulation *in vivo*, it also helps to use a systematic & rational formulation approach avoid "trial-and-error" iterations and provide framework to guide regulatory agencies. LFCS was established by Pouton in 2000 and recently updated .[7] The LFCS briefly classifies lipid-based formulations into four types according to their composition and the possible effect of dilution and digestion on their ability to prevent drug precipitation, as shown in Table 1.1.

TABLE 1.1: Compositions of lipid-based formulation [7]

Composition	Type I	Type II	Type III		Type IV
	Oil	SEDDS	III A SEDDS	III B SMEDDS	Oil-Free
Glycerides (TG, DG, MG)	100%	40-80%	40-80%	< 20%	-
Surfactants (HLB < 12)	-	20-60%	-	-	0-20%
(HLB > 12)	-	-	20-40%	20-50%	20-80%
Hydrophilic co-solvents	-	-	0-40%	20-50%	0-80%
Particle size of dispersion(nm)	Coarse	100-250	100-250	50-100	< 50

Type I systems consist of formulations which comprise drug in solution in triglycerides and/or mixed glycerides or in oil-in-water emulsion stabilized by low concentrations of emulsifiers such as 1% (w/v) polysorbate 60 and 1.2% (w/v) lecithin. Generally, these systems exhibit poor initial aqueous dispersion and require digestion by pancreatic lipase/ co-lipase in the GIT to generate more amphiphilic lipid digestion products and promote drug transfer into the colloidal aqueous phase. Type I lipid formulations therefore represent a relatively simple formulation option for potent drugs or highly lipophilic compounds where drug solubility in oil is sufficient to allow incorporation of the required payload (dose).

TABLE 1.2: Typical properties of Type I, II, III and IV lipid formulations [7]

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

Type II lipid formulations constitute SEDDS. Self-emulsification is generally obtained at surfactant contents above 25% (w/w). However at higher surfactant contents (greater than 50–60% (w/w) depending on the materials) the progress of emulsification may be compromised by the formation of viscous liquid crystalline gels at the oil/water interface. Type II lipid-based formulations provide the advantage of overcoming the slow dissolution step typically observed with solid dosage forms and as described above generate large interfacial areas which in turn allows efficient partitioning of drug between the oil droplets and the aqueous phase from where absorption occurs. [8, 9]

Type III lipid-based formulations, commonly referred to as self-microemulsifying drug delivery systems (SMEDDS), are defined by the inclusion of hydrophilic surfactants (HLB>12) and co-solvents such as ethanol, propylene glycol and polyethylene glycol. Type III formulations can be further segregated (somewhat arbitrarily) into Type IIIA and Type IIIB formulations in order to identify more hydrophilic systems (Type IIIB) where the content -of hydrophilic surfactants and co-solvents increases and the lipid content reduces. Type IIIB formulations typically achieve greater dispersion rates when compared with Type IIIA although the risk of drug precipitation on dispersion of the formulation is higher given the lower lipid content. [10]

Type IV: In order to capture the recent trend towards formulations which contain predominantly hydrophilic surfactants and co-solvents, this category was recently added. Type IV formulations do not contain natural lipids and represent the most hydrophilic formulations. These formulations commonly offer increased drug payloads when compared to formulations containing simple glyceride lipids and also produce very fine dispersions when introduced in aqueous media. Little is known however, as to the solubilisation capacity of these systems *In vivo* and in particular whether they are equally capable of maintaining poorly water soluble drug in solution during passage along the GIT when compared with formulations comprising natural oils (Type II and Type III). An example of a Type IV formulation is the current capsule formulation of the HIV protease inhibitor amprenavir (Agenerase) which contains TPGS as a surfactant and PEG 400 and propylene glycol as co-solvents. [11]

1.1.1 Biopharmaceutical Classification System (BCS):

Biopharmaceutics Classification System (BCS) was introduced in 1995 as a basis for predicting the likelihood of *In vitro-In vivo* correlations for immediate release dosage forms, based on the recognition that drug solubility/dissolution properties and gastrointestinal permeability are the fundamental parameters controlling the rate and extent of drug absorption. According to BCS, drug substances are classified as, shown in Table 1.3;

TABLE 1.3: BCS classification [10]

Class I	**High solubility High permeability**
Class II	**Low solubility High permeability**
Class III	**High solubility Low permeability**
Class IV	**Low solubility Low permeability**

The FDA has set specifications regarding the solubility and permeability class boundaries used for this BCS classification. **[10]**

Solubility:

A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over a pH range of 1 to 7.5 (equilibrium solubility at 37°C). **[10]**

Permeability:

In the absence of evidence suggesting instability in the gastrointestinal tract, a drug substance is considered highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on mass balance determination or in comparison to an intravenous reference dose (absolute bioavailability study). **[11]**

1.2 SELF MICRO EMULSIFYING DRUG DELIVERY SYSTEMS

SMEDDS are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants that have a unique ability of forming fine oil-in-water (o/w) microemulsions upon mild agitation followed by dilution in aqueous media, such as GI fluids. [11] SMEDDS spread readily in the GI tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification. The basic difference between self emulsifying drug delivery systems (SEDDS) also called as self emulsifying oil formulation (SEOF) and SMEDDS is SEDDS typically produce opaque emulsions with a droplet size between 100 and 300 nm while SMEDDS form transparent micro emulsions with a droplet size of less than 100 nm also the concentration of oil in SMEDDS is less than 20 % as compared to 40-80% in SEDDS. When compared with emulsions, which are sensitive and metastable dispersed forms, SMEDDS are physically stable formulations that are easy to manufacture. Thus, for lipophilic drug compounds which exhibit dissolution rate-limited absorption, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood-time profiles. The key step is to find a suitable oil surfactant mixture that can dissolve the drug within the required therapeutic concentration. The SMEDDS mixture can be filled in either soft or hard gelatin capsules. A typical SMEDDS formulation contains oils, surfactants and if required an antioxidants. Often co-surfactants and co-solvents are added to improve the formulation characteristics.

1.2.1 Advantages of SMEDDS:

- **Improvement in oral bioavailability:**

Dissolution rate dependant absorption is a major factor that limits the bioavailability of numerous poorly water soluble drugs. The ability of SMEDDS to present the drug to GIT in solubilised and micro emulsified form (globule size between 1-100 nm) and subsequent increase in specific surface area enable more efficient drug transport through the intestinal aqueous boundary layer and through the absorptive membrane leading to improved bioavailability. E.g. In case of halofantrine approximately 6-8 fold increase in bioavailability of drug was reported in comparison to tablet formulation. [12]

- **Ease of manufacture and scale-up:**

Ease of manufacture and scale- up is one of the most important advantage that makes SMEDDS unique when compared to other drug delivery systems like solid dispersions, liposomes, nanoparticles, etc., dealing with improvement of bio-availability. SMEDDS require very simple and economical manufacturing facilities like simple mixer with agitator and volumetric liquid filling equipment for large-scale manufacturing. This explains the interest of industry in the SMEDDS. [12]

- **Reduction in inter-subject and intra-subject variability and food effects:**

There are several drugs which show large inter-subject and intra-subject variation in absorption leading to decreased performance of drug and patient non-compliance. Food is a major factor affecting the therapeutic performance of the drug in the body. SMEDDS are a boon for such drugs. Several research papers specifying that, the performance of SMEDDS is independent of food and SMEDDS offer reproducibility of plasma profile are available. [13]

- **Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT:**

One unique property that makes SMEDDS superior as compared to the other drug delivery systems is their ability to deliver macromolecules like peptides, hormones, enzyme substrates and inhibitors and their ability to offer protection from enzymatic hydrolysis. The intestinal hydrolysis of prodrug by cholinesterase can be protected if polysorbate 20 is emulsifier in micro emulsion formulation. [14] These systems are formed spontaneously without aid of energy or heating thus suitable for thermo labile drugs such as peptides. [15]

- **No influence of lipid digestion process:**

Unlike the other lipid-based drug delivery systems, the performance of SMEDDS is not influenced by the lipolysis, emulsification by the bile salts, action of pancreatic lipases and mixed micelle formation. SMEDDS are not necessarily digested before the drug is absorbed as they present the drug in micro-emulsified form which can easily penetrate the mucin and water unstirred layer. [15]

- **Increased drug loading capacity:**

SMEDDS also provide the advantage of increased drug loading capacity when compared with conventional lipid solution as the solubility of poorly water soluble drugs with intermediate partition coefficient (2<log P>4) are typically low in natural lipids and much greater in amphilic surfactants, co surfactants and co-solvents. [15]

1.2.2 Advantages of SMEDDS over Emulsion:

- SMEDDS not only offers the same advantages of emulsions of facilitating the solubility of hydrophobic drugs, but also overcomes the drawback of the creaming of emulsions after long time. SMEDDS can be easily stored since it belongs to a thermodynamically stable system.[15]
- Microemulsions formed by the SMEDDS exhibit good thermodynamics stability and optical transparency. The major difference between the above microemulsions and common emulsions lies in the particle size of droplets. The size of the droplets of common emulsion ranges between 0.2 and 10 µm, and that of the droplets of microemulsion formed by the SMEDDS generally ranges between 2 and 100 nm (such droplets are called droplets of nano particles).Since the particle size is small, the total surface area for absorption and dispersion is significantly larger than that of solid dosage form and it can easily penetrate the gastrointestinal tract and be absorbed. The bioavailability of the drug is therefore improved.
- SMEDDS offer numerous delivery options like filled hard gelatin capsules or soft gelatin capsules or can be formulated in to tablets whereas emulsions can only be given as an oral solutions.[15]

1.2.3 Excipients Used In SMEDDS:

Pharmaceutical acceptability of excipients and the toxicity issues of the components used makes the selection of excipients really critical. There is a great restriction as which excipients to be used. Early studies revealed that the self-microemulsification process is specific to the nature of the oil/surfactant pair, the surfactant concentration and oil/surfactant ratio, the concentration and nature of co-surfactant and surfactant/co-surfactant ratio and the temperature at which self-microemulsification occurs. These important discoveries were further supported by the fact that only very specific combinations of pharmaceutical excipients led to efficient self- microemulsifying systems. [16]

- **OILS:**

The oil represents one of the most important excipients in the SMEDDS formulation not only because it can solubilize the required dose of the lipophilic drug or facilitate self emulsification mainly because it can increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride. Both long and medium chain triglyceride (LCT and MCT) oils with different degrees of saturation have been used for the design of self-emulsifying formulations. Furthermore, edible oils which could represent the logical and preferred lipid excipient choice for the development of SMEDDS are not frequently selected due to their poor ability to dissolve large amounts of lipophilic drugs. Modified or hydrolyzed vegetable oils have been widely used since these excipients form good emulsification systems with a large number of surfactants approved for oral administration and exhibit better drug solubility properties. They offer formulative and physiological advantages and their degradation products resemble the natural end products of intestinal digestion. Novel semisynthetic medium chain derivatives, which can be defined as amphiphilic compounds with surfactant properties, are progressively and effectively replacing the regular medium chain triglyceride oils in the SMEDDS. This is in accordance with findings of Deckelbaum showing that MCT is more soluble and have a higher mobility in the lipid/water interfaces than LCT associated with a more rapid hydrolysis of MCT. In general, when using LCT, a higher concentration of cremophor RH40 was required to form microemulsions compared with MCT. [16]

- **SURFACTANTS:**

Several compounds exhibiting surfactant properties may be employed for the design of self-emulsifying systems, but the choice is limited as very few surfactants are orally acceptable. The most widely recommended ones being the non-ionic surfactants with a relatively high hydrophilic-lipophilic balance (HLB). The commonly used emulsifiers are various solid or liquid ethoxylated polyglycolyzed glycerides and polyoxyethylene 20 oleate (Tween 80). Safety is a major determining factor in choosing a surfactant. Emulsifiers of natural origin are preferred since they are considered to be safer than the synthetic surfactants. However, these surfactants have a limited self-emulsification capacity. Non-ionic surfactants are less toxic than ionic surfactants but they may lead to reversible changes in the permeability of the intestinal lumen. Usually the surfactant concentration ranges between 30 and 60% w/w in order to form stable SMEDDS. It is very important to determine the surfactant concentration properly as large amounts of surfactants may cause GI irritation. Surfactants are amphiphilic in nature and they can dissolve or solubilize relatively high amounts of hydrophobic drug compounds. The lipid mixtures with higher surfactant and co-surfactant/oil ratios lead to the formation of SMEDDS. **[16]**

There is a relationship between the droplet size and the concentration of the surfactant being used. In some cases, increasing the surfactant concentration could lead to droplets with smaller mean droplet size, this could be explained by the stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil-water interface on the other hand, in some cases the mean droplet size may increase with increasing surfactant concentrations. This phenomenon could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase. The surfactants used in these formulations are known to improve the bioavailability by various mechanisms including: improved drug dissolution, increased intestinal epithelial permeability, increased tight junction permeability and decreased/inhibited p-glycoprotein drug efflux. However, the large quantity of surfactant may cause moderate reversible changes in intestinal wall permeability or may irritate the GI tract. Formulation effect and surfactant concentration on gastrointestinal mucosa should ideally be investigated in each case. **[16]**

- **CO-SOLVENTS:**

The production of an optimum SEDDS requires relatively high concentrations (generally more than 30% w/w) of surfactants, thus the concentration of surfactant can be reduced by incorporation of co surfactant. Role of the co-surfactant together with the surfactant is to lower the interfacial tension to a very small even transient negative value. At this value the interface would expand to form fine dispersed droplets, and subsequently adsorb more surfactant and surfactant / co-surfactant until their bulk condition is depleted enough to make interfacial tension positive again. This process known as 'spontaneous emulsification' forms the microemulsion. However, the use of co-surfactant in self emulsifying systems is not mandatory for many non-ionic surfactants. The selection of surfactant and co-surfactant is crucial not only to the formation of SMEDDS, but also to solubilization of the drug in the SMEDDS. Organic solvents, suitable for oral administration (ethanol, propylene glycol (PG), polyethylene glycol (PEG), etc) may help to dissolve large amounts of either the hydrophilic surfactant or the drug in the lipid base and can act as co-surfactant in the self emulsifying drug delivery systems, although alcohol- free self-emulsifying microemulsions have also been described in the literature. Indeed, such systems may exhibit some advantages over the previous formulations when incorporated in capsule dosage forms, since alcohol and other volatile co-solvents in the conventional self-emulsifying formulations are known to migrate into the shells of soft gelatin or hard sealed gelatin capsules resulting in the precipitation of the lipophilic drug. On the other hand, the lipophilic drug dissolution ability of the alcohol free formulation may be limited. Hence, proper choice has to be made during selection of components. **[16]**

1.2.4 The Self Emulsification Process:

Self-emulsification is a phenomenon which has been widely exploited commercially in formulations of emulsifiable concentrates of herbicides and pesticides. Concentrates of crop-sprays are to be diluted by the user, such as farmers or house-hold gardeners, allowing very hydrophobic compounds to be transported efficiently. In contrast, SMEDDS, using excipients acceptable for oral administration to humans, have not been widely exploited and knowledge about their physicochemical principles is therefore limited.

(a)Mechanism of Self Emulsification:

In emulsification process the free energy (ΔG) associated is given by the equation: [31]

$$\Delta G = \sum N_i \pi r_i$$

In which 'N' is Number of droplets with radius 'r' and 'σ' is interfacial energy

It is apparent from equation that the spontaneous formation of the interface between the oil and water phases is energetically not favored. The system commonly classified as SEDDS have not yet been shown to emulsify spontaneously in the thermodynamic sense. The process of self-emulsification was observed using light microscopy. Groves and Mustafa developed a method of quantitatively assessing the ease of emulsification by monitoring the turbidity of the oil-surfactant in a water stream using phosphated nonylphenoloxylate (PNE) and phosphated fatty alcohol ethoxlate (PFE) in n-hexane. Pouton has argued that the emulsification properties of the surfactant may be related to phase inversion behavior of the system. For example, on increase the temperature of oil in water system stabilized using nonionic surfactant; the cloud point of the surfactant will be reached followed by phase inversion. The surfactant is highly mobile at the phase inversion temperature; hence the o/w interfacial energy is minimized leading to a reduction in energy required to cause emulsification. The specificity of surfactant combination required to allow spontaneous emulsification may be associated with a minimization of the phase inversion temperature, thereby increasing the ease of emulsion. Phase studies are also necessary for liquid crystal formation in self-emulsification. These indicate that good formulations are usually operating

close to a phase inversion region and in a region of enhanced close to a phase inversion region and in a region of enhanced aqueous solubilization. In the phase diagram of the system (30 % w/w tween and 85/70 % w/w MCT oil) for dilution in water over a range of temperature shows that the phase inversion region is at approximately 40° C and the system works well at ambient temperature up to 60°C above which water in oil emulsion tend to form.[17]

The emulsification process may be associated with the ease with which water penetrates the oil-water interface with the formation of liquid crystalline phases resulting in swelling at the interface thereby resulting in greater ease of emulsification. However, for system containing co- surfactant, significant partitioning of components between the oil and aqueous phases may take place leading to a mechanism described as "diffusion and stranding", where by the oil is solubilized, leading to migration in to the aqueous phase. [17]

b) Dilution phases

Upon dilution of a SMEDDS formulation, the spontaneous curvature of the surfactant layer changes via a number of possible liquid crystalline phases. The droplet structure can pass from a reversed spherical droplet to a reversed rod-shaped droplet, hexagonal phase, lamellar phase, cubic phase and various other structures until, after appropriate dilution, a spherical droplet will be formed again (Fig. 1.1). [17]

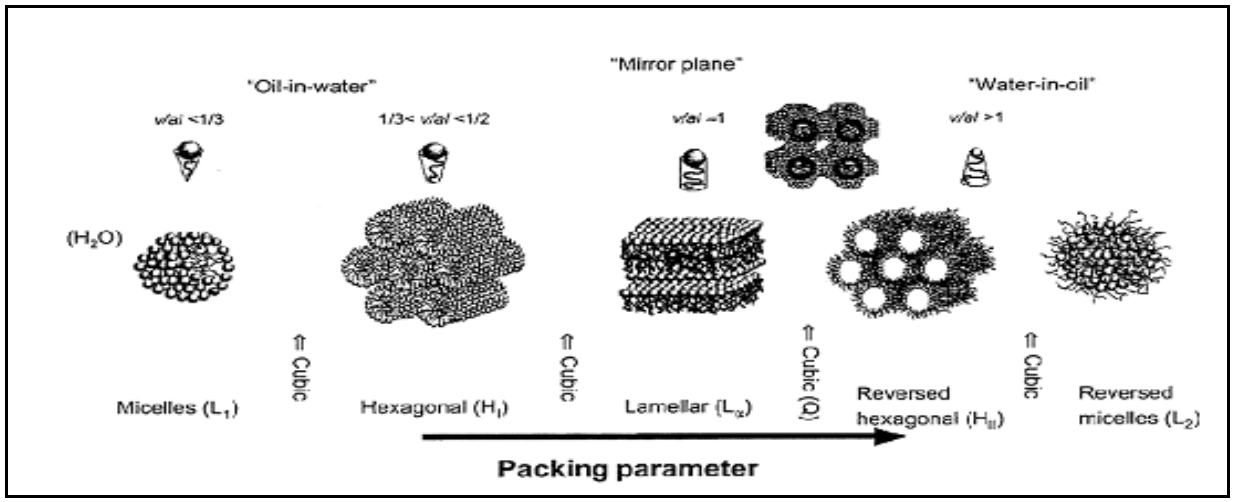


Figure 1.1: Representation of the most commonly encountered phases upon addition of water to an oil-surfactant combination

Many roles have been described to the occurrence of liquid crystalline phases upon aqueous dilution of a lipid formulation. Early work of Groves and Mustafa related the emulsification behaviour to the phase behaviour of the surfactant-oil mixtures with systems forming liquid crystals showing shorter emulsification times [18]. The authors suggested that the ease of emulsification could be associated with the passage of water into the droplet, more precisely the ease with which the solvent may penetrate into the liquid crystalline phases formed on the surface of the droplet. The structures formed upon dilution have been ascribed an important role in the stability of the diluted microemulsion and the rate of drug release . This can be explained by the fact that a layer of liquid crystalline material surrounds the oil droplets, affecting drug dissolution and formulation digestion. Some examples are shown in Table 1.4;

TABLE 1.4:Examples of SEDDS for Oral Delivery of Lipophilic Drugs [18]

Type of delivery system	Oil	Surfactant(s)	%w/w	Solvent(s)	Drug compound	Drug content
SEDDS	A mixture of mono-and di-glycerides of oleic acid	Solid, polyglycolyzed mono-di and triglycerides, Tween 80	80 or 20	-	Ontazolast	7.5
SEDDS (Sandimmune)	Olive oil	Polyglycolyzed glycerides	30	Ethanol	CsA	10
SEDDS (positively charged)	Ethyl oleate	Tween 80	25	Ethanol	CsA	10
SEDDS (positively charged)	Ethyl oleate	Tween 80	25	Ethanol	Progesterone	2.5
SEDDS	Myvacet 9-45 or captex 200	Labrasol or Labrafac CM10	5-30 0-25	-	CoQ10	5.66
SEDDS(Norvir)	Oleic acid	Polyoxyl 35 castor oil	NA	Ethanol	Ritonavir	8
SEDDS (Fortovase)	dl-alpha tocopherol	Medium chain mono-and diglycerides	NA	-	Saquinqvir	16

1.2.5 Factors Affecting SMEDDS:

- **Nature and dose of the drug:**

Drugs which are administered at very high dose are not suitable for SMEDDS unless they exhibit extremely good solubility in at least one of the components of SMEDDS, preferably lipophilic phase. The drugs which exhibit limited solubility in water and lipids (typically with log P values of approximately 2) are most difficult to deliver by SMEDDS. The ability of SMEDDS to maintain the drug in solubilised form is greatly influenced by the solubility of the drug in oil phase. As mentioned above if surfactant or co-surfactant is contributing to the greater extent in drug solubilization then there could be a risk of precipitation, as dilution of SMEDDS will lead to lowering of solvent capacity of the surfactant or co-surfactant. Equilibrium solubility measurements can be carried out to anticipate potential cases of precipitation in the gut. However, crystallization could be slow in the solubilising and colloidal stabilizing environment of the gut. Pouton's study reveal that such formulations can take up to five days to reach equilibrium and that the drug can remain in a super-saturated state for up to 24 hours after the initial emulsification event. It could thus be argued that such products are not likely to cause precipitation of the drug in the gut before the drug is absorbed, and indeed that super-saturation could actually enhance absorption by increasing the thermodynamic activity of the drug. There is a clear need for practical methods to predict the fate of drugs after the dispersion of lipid systems in the gastro-intestinal tract. [19]

- **Polarity of the lipophilic phase:**

The polarity of the lipid phase is one of the factors that govern the drug release from the microemulsions. The polarity of the droplet is governed by the HLB, the chain length and degree of unsaturation of the fatty acid, the molecular weight of micronized for their propensity to inhibit crystallization and, thereby, generate and maintain the supersaturated state for prolonged time periods. [19] A supersaturable self-microemulsifying drug delivery system (S-SMEDDS) of paclitaxel was developed employing HPMC as a precipitation inhibitor with a conventional SMEDDS formulation. *In vitro* dilution of the S-SMEDDS formulation resulted in formation of a microemulsion, followed by slow crystallization of paclitaxel on standing. This result indicated that the system was supersaturated with respect to

crystalline paclitaxel, and the supersaturated state was prolonged by HPMC in the formulation. In the absence of HPMC, the SMEDDS formulation underwent rapid precipitation, yielding a low paclitaxel solution concentration. A pharmacokinetic study showed that the paclitaxel S-SMEDDS formulation produced approximately a 10-fold higher maximum concentration (Cmax) and a 5-fold higher oral bioavailability (F ~ 9.5%) compared with that of the orally administered Taxol formulation (F~2.0%) and the SMEDDS formulation without HPMC (F ~ 1%). [19]

1.2.6 Biopharmaceutical Aspects:

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

a) Alterations (reduction) in gastric transit, thereby slowing delivery to the absorption site and increasing the time available for dissolution. [20]
b) Increases in effective luminal drug solubility. The presence of lipids in the GI tract stimulates an increase in the secretion of bile salts (BS) and endogenous biliary lipids including phospholipids (PL) and cholesterol (CH), leading to the formation of BS/PL/CH intestinal mixed micelles and an increase in the solubilization capacity of the GI tract. However, intercalation of administered (exogenous) lipids into these BS structures either directly (if sufficiently polar), or secondary to digestion, leads to swelling of the micellar structures and a further increase in solubilization capacity. [20]
c) Stimulation of intestinal lymphatic transport. For highly lipophilic drugs, lipids may enhance the extent of lymphatic transport and increase bioavailability directly or indirectly via a reduction in first-pass metabolism. A hydrophilic drug is less likely to be absorbed through the lymphatic (chylomicron) and instead may diffuse directly in to the portal supply. Hence in this case, increased dissolution from the large surface area afforded by emulsion may be a contributing factor to enhanced absorption of drugs.[20]
d) Changes in the biochemical barrier function of the GI tract. It is clear that certain lipids and surfactants may attenuate the activity of intestinal efflux transporters, as indicated by the p glycoprotein efflux pump, and thus reduce the extent of enterocyte-based metabolism.

e) Changes in the physical barrier function of the GI tract. Various combinations of lipids, lipid digestion products and surfactants have been shown to have permeability enhancing properties. For the most part, however, passive intestinal permeability is not thought to be a major barrier to the bioavailability of the majority of poorly water-soluble, and in particular, lipophilic drugs.[20]

1.2.7 Susceptibility to Digestion:

The well known positive effect of food on the bioavailability of many poorly water soluble drugs is often ascribed to the ingested lipid and points to the beneficial role of lipids on drug absorption. Although the form, content and volume of dietary lipids is markedly different to oil phases included in a pharmaceutical formulation, possible food effects on drug bioavailability can be a starting point for the design of lipid self-emulsifying formulations for such drugs. The presence of lipids in the GI tract increases drug solubilization and thus drug dissolution via a number of potential mechanisms.

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

The co-administration of drugs with lipids can also have an effect on their absorption path.

1.2.8 *In-Vitro* Characterization of SEDDS/SMEDDS:

Pouton classified lipid-based formulations into three categories based on the polarity of the excipient blends (Table 1.5). Due to their relative simplicity Type I formulations, which are simple solutions of the drug in triglycerides and/or mixed glycerides, are a reasonable starting point in the search for a lipid-based formulation. Type II formulations that add a lipophilic surfactant (HLB 12), are employed when SEDDS and greater drug solubilizing capacity is desired in a formulation. Type III formulations include the further addition of hydrophilic surfactants (HLB -12) and co solvents to further improve the self-emulsification process in the

GIT, thereby yielding a SMEDDS formulation. Type III formulations are further subdivided into Types IIIA and IIIB, where Type IIIB contains a greater ratio of hydrophilic to lipophilic components than the former. While Type IIIB formulations are associated with more facile self-emulsification and smaller lipid droplet size than Type IIIA, they carry a greater risk of drug precipitation as the hydrophilic components may separate from the oil phase during dispersion in the GIT leading to a loss of drug-solubilizing capacity. [23]

TABLE 1.5: Classification Of Lipid Based Formulations

INCREASING HYDROPHILIC CONTENT				
Composition (%)	**Type I**	**Type II**	**Type III**	**Type IV**
Triglycerides or mixed glycerides	100	40–80	40–80	<20
Surfactants	—	20–60 (HLB <12)	20–40 (HLB <11)	20–50 (HLB <11)
Hydrophilic Cosolvents	—	—	0–40	20–50
In vivo performance Particle size of dispersion (nm)	Coarse	100–250	100–250	50–100
Significance of aqueous dilution	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity	Significant phase changes and potential loss of solvent capacity
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial but likely to inhibited	Not required and not likely occur

Excipient combinations yielding SEDDS/SMEDDS formulations are identified by construction of ternary phase diagrams. Each point in the phase diagram represents a given combination of oil, surfactant, and co surfactant. In instances where combinations of more than three excipients must be tested, a fixed ratio between two of the excipients (e.g., the surfactant and co surfactant) is selected and treated as a single component. As a practical example, mixtures consisting of different amounts of the selected excipients are evaluated for

their self-emulsifying properties by the addition of pharmaceutically-relevant amounts of the formulation to 250mL of water or a biorelevant, simulated physiological fluid. The resulting dispersion is examined by direct visualization and by dynamic light scattering to accurately determine the lipid droplet size, thereby allowing classification of the formulation as a SEDDS or SMEDDS. The number of combinations of drug and excipients resulting in a microemulsion, which is typically small, defines the microemulsion existence field on the ternary phase diagram: the area enclosed in the broken line in Figure 1.3 represents the microemulsion existence field for various combinations of medium chain triglycerides (MCT) or LCT, Cremophor RH40 and Akoline MCM or Peceol. [23]

1.2.9 Influence of Emulsion Droplet Size on Drug Absorption:

Although improved drug absorption is generally assumed to be associated with smaller lipid droplet size, many examples exist in which drug absorption is not influenced by droplet size. Khoo et al evaluated the bioavailability of the poorly soluble antimalarial drug, halofantrine, in dogs following administration of either MC-SEDDS (mean lipid droplet size of 119nm) or MC-SMEDDS (mean lipid droplet size of 52nm) formulations; both yielded comparable bioavailability. [24]

Studies conducted in humans comparing the Sandimmune® formulation of cyclosporine, which forms a crude emulsion in the GIT, to that of the self-microemulsifying Neoral® formulation demonstrated improved performance of the latter with regard to the rate , extent, uniformity and linearity of cyclosporine exposure as a function of dose. In addition, absorption of cyclosporine from the Neoral formulation was relatively unaffected by food as compared to the Sandimmune formulation. [24]

From the foregoing discussion, it is difficult to determine the impact of lipid droplet size on drug absorption. It should be noted, however, that the cited studies utilized different lipid and surfactant systems, which can also influence drug absorption and confound the experimental results, thus making it difficult to draw conclusions. However, these findings collectively suggest that lipid droplet size may be less likely to impact formulation performance unless the normal lipid digestion process, which inherently produces a fine emulsion from ingested lipid, is compromised. [24]

1.2.10 *In-Vivo* Studies with SEDDS/SMEDDS:

Several published studies describing modest to substantial increases in drug bioavailability from SEDDS and SMEDDS formulations, relative to conventional solid dosage forms, water-miscible glycol solutions [e.g., PEG and propylene glycol (PG)] or simple oil solutions are summarized in Table 1.1. Relative to conventional solid dosage forms, increases in drug bioavailability from self-emulsifying formulations ranged from 1.5-fold for simvastatin to approximately seven-fold for L-365,260 (cholecystokinin antagonist). The results of these studies suggest that the physicochemical properties of the drug substance, as well as the excipients selected for the formulation, appear to determine the bioavailability enhancing potential of a particular formulation for a given drug substance. [24]

1.2.11 Effect of Dispersion on Bioavailability:

Compared to simple oil solutions of the drug, only modest improvements in drug bioavailability were generally observed from self-emulsifying formulations. However, it is important to note that these studies were conducted in different species, with different formulations and with different lipid and surfactant doses, which sometimes differed within an individual study. It should also be noted that only healthy test subjects, with fully functioning GI lipid handling pathways, were studied. Self-emulsifying formulations appear to provide better absorption enhancement, when the normal physiological processes enabling lipid digestion and dispersion are compromised.

Studies conducted by Porter et al. demonstrated a significant increase in the bioavailability of danazol, administered as either a LCT solution or a LC-SMEDDS formulation, relative to either a conventional solid dosage form or a MC-SEDDS formulation. The presence of a high concentration of surfactant in the SMEDDS containing long chain triglycerides (LC-SMEDDS) formulation did not improve danazol absorption over that seen from the simple LCT solution, which supported the findings of who demonstrated similar bioavailability of seocalcitol, when administered to rats as simple MCT or LCT solutions or following addition of high concentrations of surfactant to yield MC-SMEDDS or LC-SMEDDS formulations, respectively. It should be noted that the SMEDDS formulations of

danazol were not controlled for the ratio amounts of oil, surfactant or cosurfactant, which makes it difficult to accurately assess the impact of dispersion on drug absorption. [25]

- **Some examples of marketed Pharmaceutical SEDDS formulations are as shown below:** [32]

Table 1.6: Examples Of Marketed SEDDS Formulations

Drug Name	Compound	Dosage form	Company	Indication
Neoral	Cyclosporine A/I	Soft gelatin capsule	Novartis	Immune suppressant
Norvir	Ritonavir	Soft gelatin capsule	Abbott Laboratories	HIV antiviral
Convulex	Valproic acid	Soft gelatin capsule	Pharmacia	Antiepileptic
Lipirex	Fenofibrate	Hard gelatin Capsule	Genus	Antihyper-lipoproteinemic
Sandimmune	Cyclosporine A/II	Soft gelatin capsule	Novartis	Immuno Suppressant

1.3 Solid Self-Microemulsifying Drug Delivery System (S-SMEDDS):

SMEDDS can exist in either liquid or solid states. SMEDDS are usually, limited to liquid dosage forms, because many excipients used in SMEDDS are not solids at room temperature. Given the advantages of solid dosage forms, S-SMEDDS have been extensively exploited in recent years, as they frequently represent more effective alternatives to conventional liquid SMEDDS.

From the perspective of dosage forms, S-SMEDDS mean solid dosage forms with self-emulsification properties. S-SMEDDS focus on the incorporation of liquid/semisolid SE ingredients into powders/ nanoparticles by different solidification techniques (e.g. adsorptions to solid carriers, spray drying, melt extrusion, nanoparticle technology, and so on). Such powders/nanoparticles, which refer to SE nanoparticles/dry emulsions/solid dispersions are usually further processed into other solid SE dosage forms, or, alternatively, filled into capsules (i.e. SE capsules). SE capsules also include those capsules into which liquid/semisolid SEDDS are directly filled without any solidifying excipient. [26]

In the 1990s, S-SEDDS were usually in the form of SE capsules, SE solid dispersions and dry emulsions, but other solid SE dosage forms have emerged in recent years, such as SE pellets/tablets, SE microspheres/nanoparticles and SE suppositories/implants.

1.3.1 Solidification Techniques for Transforming Liquid/Semisolid SMEDDS to S-SMEDDS:

Various solidification techniques are as listed below;

- **Capsule filling with liquid and semisolid self-emulsifying formulations:**

Capsule filling is the simplest and the most common technology for the encapsulation of liquid or semisolid SE formulations for the oral route.

For semisolid formulations, it is a four-step process:

(i) Heating of the semisolid excipient to at least 20˚C above its melting point.

(ii) Incorporation of the active substances (with stirring).

(iii) Capsule filling with the molten mixture.

(iv) Cooling to room temperature.

For liquid formulations, it involves a two-step process: filling of the formulation into the capsules followed by sealing of the body and cap of the capsule, either by banding or by microspray sealing. [27]

The advantages of capsule filling are simplicity of manufacturing; suitability for low- dose highly potent drugs and high drug loading potential [up to 50% (w/w)].

- **Spray drying:**

Essentially, this technique involves the preparation of a formulation by mixing lipids, surfactants, drug, solid carriers, and solubilization of the mixture before spray drying. The solubilized liquid formulation is then atomized into a spray of droplets. The droplets are introduced into a drying chamber, where the volatile phase (e.g. the water contained in an emulsion) evaporates, forming dry particles under controlled temperature and airflow conditions.

Such particles can be further prepared into tablets or capsules. The atomizer, the temperature, the most suitable airflow pattern and the drying chamber design are selected according to the drying characteristics of the product and powder specification. [27]

- **Adsorption to solid carriers:**

Free flowing powders may be obtained from liquid SE formulations by adsorption to solid carriers. The adsorption process is simple and just involves addition of the liquid formulation onto carriers by mixing in a blender. The resulting powder may then be filled directly into capsules or, alternatively, mixed with suitable excipients before compression into tablets. A significant benefit of the adsorption technique is good content uniformity. SEDDS/SMEDDS can be adsorbed at high levels [up to 70% (w/w)] onto suitable carriers. [28]

- **Melt granulation:**

Melt granulation is a process in which powder agglomeration is obtained through the addition of a binder that melts or softens at relatively low temperatures. As a 'one-step' operation, melt granulation offers several advantages compared with conventional wet granulation, since the liquid addition and the subsequent drying phase are omitted. Moreover, it is also a good alternative to the use of solvent. [27]

- **Melt extrusion/extrusion spheronization:**

Melt extrusion is a solvent-free process that allows high drug loading (60%), as well as content uniformity. Extrusion is a procedure of converting a raw material with plastic properties into a product of uniform shape and density, by forcing it through a die under controlled temperature, product flow, and pressure conditions. [29]

1.3.2 DOSAGE FORM DEVELOPMENT OF S-SMEDDS:

Various dosage forms of S-SMEDDS are as listed below; [30]

- Dry emulsions
- Self-emulsifying capsules
- Self-emulsifying sustained/controlled-release tablets
- Self-emulsifying sustained/controlled-release pellets
- Self-emulsifying solid dispersions
- Self-emulsifying beads
- Self-emulsifying sustained-release microspheres
- Self-emulsifying nanoparticles
- Self-emulsifying suppositories
- Self-emulsifying implants

2. AIM OF THE PRESENT WORK

SMEDDS spread readily in the GI tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification. When compared with emulsions which are sensitive and metastable dispersed forms, SMEDDS are physically stable formulations that are easy to manufacture. Thus, for lipophilic drug compounds which exhibit dissolution rate-limited absorption, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood-time profiles.

Candesartan cilexetil is an esterified prodrug of candesartan, a nonpeptide angiotensin II type 1(AT_1) receptor antagonist used in the treatment of hypertension. Based on its solubility across physiological relevant pH conditions and absorption characteristic, candesartan cilexetil is classified in the Biopharmaceutical classification system as a class II drug. Low solubility of candesartan cilexetil across the physiological pH range is reported to result in incomplete absorption from the GI tract and hence is reported to have an oral bioavailability of about 15%.candesartan cilexetil is a highly lipophilic compound and has good solubility in tri and diglyceride oils. These factors, may contribute toward absorption via the lymphatic route.

The main objective of this work is to prepare S-SMEDDS for oral solubility and bioavailability enhancement of poorly water soluble drug.

- To formulate a stable liquid SMEDDS formulation using suitable excipients.
- To enhance the solubility, dissolution rate and bioavailability of drugs using suitable vehicles and excipients.
- To compare the dissolution rate of optimized liquid SMEDDS and S-SMEDDS with marketed formulation.
- To perform the stability study of optimized SMEDDS formulation as well as marketed formulation as per ICH guidelines and to find out shelf life of the developed S-SMEDDS.
- To perform the bioavailability assessment of optimized S-SMEDDS formulation.

3. LITERATURE REVIEW

Linjie Liu et al. [33] has formulated self-microemulsifying drug delivery systems (SMEDDS) in order to enhance the solubility, release rate, and oral absorption of the poorly soluble drug, silymarine. *In vitro* release was investigated using a bulk-equilibrium reverse dialysis bag method. Differences in the release medium significantly influenced the drug release from SMEDDS and the release profiles of silymarine from SMEDDS was higher than that for commercial capsules (Legalon, Germany), and significantly higher than that for commercial tablets (Yiganling China). The optimal formulation of SMEDDS is an alternative oral dosage form for improving the oral absorption of silymarine.

Ashok R Patel et al. [34] formulated a SMEDDS (self-microemulsifying drug delivery system) of fenofibrate and evaluated it's *in vitro* and *in vivo* potential. SMEDDS formulations were tested for microemulsifying properties, and the resultant microemulsions were evaluated for clarity, precipitation, and particle size distribution. The optimized SMEDDS formulation showed complete release in 15 minutes as compared with the plain drug, which showed a limited dissolution rate. Comparative pharmacodynamic evaluation was investigated in terms of lipid-lowering efficacy, using a Triton-induced hypercholesterolemia model in rats. The SMEDDS formulation significantly reduced serum lipid levels in phases I and II of the Triton test, as compared with plain fenofibrate.

Sagar D. Mandawgade et al. [35] has invested self-microemulsifying drug delivery systems (SMEDDS) using a novel, indigenous natural lipophile (N-LCT) as an oily phase. SMEDDS based on N-LCT and commercially available modified oil (Capryol90) was formulated. BAM-loaded SMEDDS were characterized with respect to mean globule size and *in vitro* drug release profile in comparison to the marketed formulation (Larither®). Comparative *in vivo* anti-malarial performance of the developed SMEDDS was evaluated against the (Larither®) in Swiss male mice infected with lethal ANKA strain of Plasmodium berghei. Both the BAM–SMEDDS showed excellent self-microemulsification efficiency and released>98% of the drug in just 15 min whereas (Larither ®) showed only 46% drug release at the end of 1h. The mean globule size for optimized BAM–SMEDDS was <100nm.The anti-malarial studies revealed that BAM–SMEDDS resulted in significant improvement in the anti-

malarial activity (P <0.05) as compared to that of (Larither®) and BAM solubilized in the oily phases and surfactant.

Annette Mullertz et al. [36] has formulated Self microemulsifying delivery system (SMEDDS) to improve the lymphatic transport and the portal absorption of a poorly water-soluble drug, halofantrine. Two different structured triglycerides were incorporated in SMEDDS; (MLM) and (LML). Apreviously optimized SMEDDS formulation for halofantrine, comprising of triglyceride, Cremophor EL, Maisine 35-1 and ethanol was selected for bioavailability assessment. The extent of lymphatic transport via the thoracic duct was 17.9% of the dose for the animals dosed with the MLM SMEDDS and 27.4% for LML. Also the plasma availability was affected by the triglyceride incorporated into the multi-component delivery system and availabilities of 56.9% (MLM) and 37.2% (LML) were found. These data indicate that the structure of the lipid can affect the relative contribution of the two absorption pathways. The MLM formulation produced a total bioavailability of 74.9%, which is higher than that of total absorption previously observed after post-prandial administration.

Jing Yao et al. [37] has prepared nobiletin self-microemulsifying drug delivery systems (SMEDDS) and investigate its intestinal transport behavior using the single-pass intestinal perfusion (SPIP) method in rat. SPIP was performed in each isolated region of the small intestine over three concentrations of nobiletin (15, 30 and 60 μg/mL) and the effective permeability coefficients (Peff) in rats were calculated. The intestinal permeability of nobiletin in SMEDDS, sub-microemulsions and micelles was compared. The Peff in jejunum at 15 μg/mL was significantly higher than that at 60 μg/mL ($p< 0.01$). There was no statistical difference in Peff at each same concentration in jejunum, duodenum and ileum. The estimated human absorption of nobiletin for the SMEDDS dilutions was higher than that for sub-microemulsions ($p<0.01$) and similar to that of the micelles ($p>0.05$).

Abhijit A. Date et al. [38] has investigated and evaluated the potential of the microemulsions to improve the parenteral delivery of propofol. The propofol microemulsions were evaluated for globule size, Physical and chemical stability, osmolarity, in vitro hemolytic, pain caused by injection using rat paw-lick test and in vivo anesthetic activity. The microemulsions exhibited globule size less than 25 nm and demonstrated good physical and chemical stability.

Propofol microemulsions were slightly hypertonic and resulted in less than 1% hemolysis after 2h of storage with human blood at 37°C. Rat paw-lick test indicated that propofol microemulsions were significantly less painful as compared to the marketed propofol formulation.

Nianping Feng et al. [39] developed self-microemulsifying drug delivery system (SMEDDS) of ordonin to enhance its oral bioavailability. The influence of the oil, surfactant and co-surfactant types on the drug solubility and their ratios on forming efficient and stable SMEDDS were investigated in detail. The SMEDDS were characterized by morphological observation, droplet size and zeta-potential determination, cloud point measurement and in vitro release study. The optimum formulation consisted of 30% mixture of Maisine 35-1 and Labrafac CC (1:1), 46.7% Cremopher EL, and 23.3% Transcutol P. In vitro release test showed a complete release of Oridonin from SMEDDS in an approximately 12h. The absorption of Oridonin from SMEDDS showed a 2.2-fold increase in relative bioavailability compared with that of the suspension.

Lanlan Wei et al. [40] developed a new self-emulsifying drug delivery system (SEDDS) and self-microemulsifying drug delivery system (SMEDDS) of carvedilol to increase the solubility, dissolution rate, and ultimately, oral bioavailability. The minimum self-emulsification time was found at a Tween 80 content of 40.Benzoic acid had a dual function; it improved the self-emulsification performance of SEDDS and SMEDDS in 0.1 N HCl and lead to a positively charged emulsion. The in vitro dissolution rate of carvedilol from SEDDS and SMEDDS was more than two-fold faster compared with that from tablets. The developed SEDDS formulations significantly improved the oral bioavailability of carvedilol significantly, and the relative oral bioavailability of SEDDS compared with commercially available tablets was 413%.

Saroj Kumar Ghosal et al. [41] to improve the solubility and bioavailability and to get faster onset of action of celecoxib developed the self-microemulsifying drug delivery system (SMEDDS). Composition of SMEDDS was optimized using simplex lattice mixture design. Dissolution efficiency, t85%, absorbance of diluted SMEDDS formulation and solubility of celecoxib in diluted formulation were chosen as response variables. The SMEDDS formulation optimized via mixture design consisted of 49.5% PEG-8 caprylic/capric

glycerides, 40.5% mixture of Tween20 and Propylene glycol monocaprylic ester (3:1) and 10% celecoxib, which showed significantly higher rate and extent of absorption than conventional capsule. The relative bioavailability of the SMEDDS formulation to the conventional capsule was 132%.

K. C. Ofokansi et al. [42] used Peanut oil and Tween 80 blends devoid of any cosurfactant and employed in the formulation of different batches of liquid self-microemulsifying drug delivery systems (LSMEDDS) and their suitability as vehicles for the delivery of a typical lipophilic drug griseofulvin was investigated. The release profile of griseofulvin from the optimized LSMEDDS was evaluated in citrate/phosphate buffer solutions of pH 2.0, pH 6.5, and pH 7.4. The results obtained indicated that there was significantly higher ($a \leq 0.05$) percentage cumulative amounts of griseofulvin released from the LSMEDDS in comparison with that released from peanut oil alone. The release of griseofulvin from the LSMEDDS into aqueous media of pH 6.5 and pH 7.4 showed enhanced and controlled dissolution of the drug from the formulation. Incorporation of griseofulvin into this proposed formulation is suggested as a strategy to overcome the irregular dissolution and absorption behaviors often associated with conventional griseofulvin tablets.

PATENTS

Sr. no.	Approaches	Patent Number	Claim
1.	Self-microemulsifying drug delivery system composition containing coenzyme Q 10 and method for preparing the same.[43]	KR2008/004373 Assignee: Daewoong pharmaceuticals CO., Ltd.	Improvement in solubility and bioavalability using polyglucerine fatty acid ester as surfactants and polyethylene-sorbitan fatty acid ester as cosurfactant.
2.	Self emulsifying and self microemulsifying formulations for oral administration of taxoids.[44]	EP1648517 B1 Assignee: Aventis pharma S.A	Development od self microemulsifying formulation for oral administration of taxoids using cremophor EL as surfactant, and at least one oil and cosurfactant.
3.	Self-microemulsifying drug delivery systems of a HIV protease inhibitor. [45]	US20070104740 Assignee:Voorspoels, Jody Firmin Marceline	The present invention relates to pharmaceutical formulations of (3R,3aS,6aR)-hexahydrofuro [2,3-b]furan-3-yl(1S,2R)-3-[[(4-aminophenyl)sulfonyl](isobutyl) amino]-1-benzyl-2-hydroxypropylcarbamate forms thereof, which are self-microemulsifying drug delivery systems and comprise as carrier a lipophilic phase, one or more surfactants, a hydrophilic solvent and a nucleation inhibitor.

4.	Self microemulsions As Solid Dosage Forms For Oral Administration. [46]	US6280770 B1 Assignee: Cima Labs Inc, USA	To form a free flowing and compressible powder comprising an admixture of drug containing SMEDDS and solid particle adsorbents.
5.	Self-Microemulsifying Drug Delivery Systems. [47]	EP1961412 A1 Assignee: Lek Pharmaceuticals D.D	To enhance the solubility of pharmaceutical ingredients comprising a polyoxyethylene sorbitan fatty acid ester emulsifier; a fatty acid ester co-emulsifier and an oil.
6.	Self-Microemulsifying Drug Delivery Systems. [48]	US7736666 Assignee: Nicox S.A.; (Sophia Antipolis, FR)	To formulate a emulsion pre-concentrate comprising of a compound , surfactants, oil or semi-solid fat and one or more short chain alcohols used in the treatment of pain and inflammation.
7.	Novel capsule SMEDDS formulations of etoposide for oral use. [49]	US20050220866 Assignee:Dr. Reddy's laboratories, inc.	The present invention relates to self microemulsifying pharmaceutical compositions comprising Etoposide that are encapsulated comprising a drug phase comprising Etoposide, and a solvent; a co-solvent and an emulsifying base comprising a lipid, a surfactant and a stabilizer.
8.	Self-microemulsifying dosage forms of low solubility active	US20060275358 Assignee: Cardinal Health - Dublin, OH.	The present invention includes a SMEDDS comprising a combination of a pair of

	ingredients such as co-enzyme Q10. [50]		hydrophilic and lipophilic surfactant. It also contains a lipophilic solvent. The formulations exhibited excellent dissolution properties and storage stability.
9.	Method and formulation for increasing the bioavailability of poorly water-soluble drugs. [51]	US5993858 Assignee: Port Systems L.L.C. (Ann Arbor, MI)	A self-microemulsifying excipient formulation for increasing the bioavailability of a drug which includes an emulsion including oil or other lipid material, a surfactant, and a hydrophilic co-surfactant.
10.	Butylphthalide Self-microemulsifying Drug Delivery System, Its Preparation Method and Application.[52]	US8728518 B2 Assignee: Fish & Richardson P.C.	The drug delivery system comprises as essential ingredients 1% to 65% of butylphthalide and 10% to 65% of a emulsifying agent, together with various excipients as required depending on the desired dosage forms. The present invention significantly increases the contact area between butylphthalide and the mucous membrane of the gastrointestinal tract, and therefore improves the absorptivity of the drug.

4. DRUG PROFILE

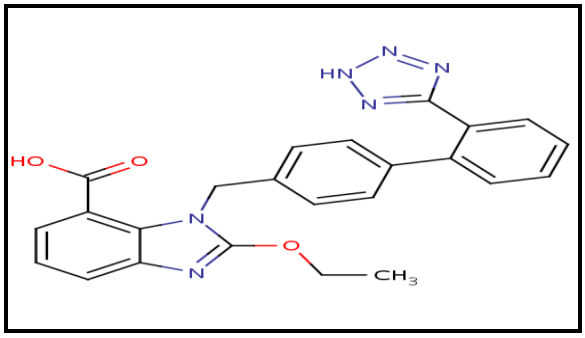
Name: Candesartan Cilexetil

4.1 Introduction:

Candesartan is an angiotensin II receptor antagonist used mainly for the treatment of hypertension. The prodrug candesartan cilexetil is marketed by Astrazeneca and takeda Pharmaceuticals, commonly under the trade names Blopress, Atacand, Amias and Ratacand. [53, 54]

4.2 Physicochemical properties: [54,55]

- **Pharmacopoeial specification:** Official in European Pharmacopoeia 2012 and USP-NF 35-30
- **Description:** White or almost white crystalline powder.
- **Structure:**



- **Chemical name:** 2-ethoxy-1-({4-[2-(2H-1,2,3,4-tetrazol-5yl) phenyl] phenyl} methyl) -1H-1, 3-benzodiazole-7-carboxylic acid
- **Molecular formula:** $C_{24}H_{20}N_6O_3$
- **State:** Solid
- **Molecular weight:** 440.45g/mol
- **Solubility:**
  - Insoluble in water (7.71e-03 g/l)
  - Soluble in methanol
- **pKa:** 7.4
- **log P:** 4.0

4.3 Mechanism of Action: [56]

Candesartan selectively blocks the binding of angiotensin II to AT1 in many tissues including vascular smooth muscle and the adrenal glands. This inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in an overall decrease in blood pressure. Candesartan is greater than 10,000 times more selective for AT1 than AT2. Inhibition of aldosterone secretion may increase sodium and water excretion while decreasing potassium excretion.

4.4 Pharmacodynamics: [56]

Candesartan cilexetil is an ARB prodrug that is rapidly converted to candesartan, its active metabolite, during absorption from the gastrointestinal tract. Candesartan confers blood pressure lowering effects by antagonizing the hypertensive effects of angiotensin II via the RAAS. RAAS is a homeostatic mechanism for regulating hemodynamics, water and electrolyte balance. During sympathetic stimulation or when renal blood pressure or blood flow is reduced, renin is released from granular cells of the juxtaglomerular apparatus in the kidneys. Renin cleaves circulating angiotensinogen to angiotensin I, which is cleaved by angiotensin converting enzyme (ACE) to Angiotensin II. Angiotensin II increases blood pressure by increasing total peripheral resistance, increasing sodium and water reabsorption in the kidneys via aldosterone secretion, and altering cardiovascular structure. Angiotensin II binds to two receptors: type-1 angiotensin II receptor (AT1) and type-2 angiotensin II receptor (AT2). AT1 is a G-protein coupled receptor (GPCR) that mediates the vasoconstrictive and aldosterone-secreting effects of angiotensin II. Studies performed in recent years suggest that AT2 antagonizes AT1-mediated effects and directly affects long-term blood pressure control by inducing vasorelaxation and increasing urinary sodium excretion. Angiotensin receptor blockers (ARBs) are non-peptide competitive inhibitors of AT1. ARBs block the ability of angiotensin II to stimulate pressor and cell proliferative effects. Unlike ACE inhibitors, ARBs do not affect bradykinin-induced vasodilation. The overall effect of ARBs is a decrease in blood pressure.

4.5 Pharmacokinetics: [56]

- **Absorption:**

Following administration of the candesartan cilexetil prodrug, the absolute bioavailability of candesartan was estimated to be 15%. Food with a high fat content has no affect on the bioavailability of candesartan from candesartan cilexetil.

- **Metabolism:** [56]

The prodrug candesartan cilexetil undergoes rapid and complete ester hydrolysis in the intestinal wall to form the active drug, candesartan. Elimination of candesartan is primarily as unchanged drug in the urine and, by the biliary route, in the feces. Minor hepatic metabolism of candesartan (<20%) occurs by O-deethylation via cytochrome P450 2C9 to form an inactive metabolite. Candesartan undergoes N-glucuronidation in the tetrazole ring by uridine diphosphate glucuronosyltransferase 1A3 (UGT1A3). O-glucuronidation may also occur. 75% of candesartan is excreted as unchanged drug in urine and feces.

- **Route of elimination:** [56]

When candesartan is administered orally, about 26% of the dose is excreted unchanged in urine. Candesartan is mainly excreted unchanged in urine and feces (via bile).

- **Half life:** Approximately 9 hours.
- **Clearance:** 0.37 mL/min/kg

4.6 Indications: [56]

Indications for its \use include:

- May be used as a first line agent to treat uncomplicated hypertension, isolated systolic hypertension and left ventricular hypertrophy.
- May be used as a first line agent to delay progression of diabetic nephropathy.
- Candesartan may be also used as a second line agent in the treatment of congestive heart failure, systolic dysfunction, myocardial infarction and coronary artery disease in those intolerant of ACE inhibitors.

4.7 Cautions: [56]

- Pregnancy
- If renal artery stenosis or impairment
- If hepatic impairment
- If volume depletion
- If hyponatremia

4.8 Side-effects: [56]

- Back pain
- Dizziness
- Upper respiratory tract infection
- Pharyngitis
- Rhinitis

4.9 Dose: [56]

- 2 to 32 mg per day.
- The dosage is based on the desired antihypertensive effect and on how the individual patient tolerates the medicine.
- **Recommended initial dose:** Usual recommended starting dose is 16 mg once daily when used as monotherapy.
- **Maximum permitted daily dose:** 32 mg.
- It can be administered once or twice daily with total daily doses ranging from 8 to 32 mg.

4.10 Interactions for Candesartan Cilexetil:

Not substantially metabolized by CYP isoenzymes; has no effect on CYP isoenzymes at therapeutic concentrations. [57]

Table 4.7: Interactions for Candesartan Cilexetil

Drug	Interaction	Comment
Cardiac drugs (e.g., digoxin, enalapril, hydrochlorothiazide, nifedipine)	Pharmacologic interactions unlikely[57,58,59]	-
Contraceptives, oral	Pharmacokinetic interaction unlikely[57,58,59]	-
Glyburide	Pharmacologic interaction unlikely[57,58]	-
Lithium	Increased serum lithium concentrations; possible toxicity[57]	Closely monitor serum lithium concentrations
Warfarin	Pharmacologic interaction unlikely[57,58,59]	-

5. EXCIPIENT PROFILE

5.1 Polysorbate 80:[68]

- **Nonproprietary Name**

  BP: Polysorbate80

  JP: Polysorbate80

  Ph Eur: Polysorbate80

  USP-NF: Polysorbate80

- **Synonym:** Capmul POE-O; CremophorPS80; Crillet4; polyoxyethylene 20 oleate ; polysorbatum 80 and Tween80.

- **Empirical Formula:** $C_{64}H_{124}O_{26}$

- **Molecular weight:**1310 g/mol

- **Chemical names and CAS Registry Number:**

  Polyoxyethylene 20 Sorbitan mono oleate, [9005-65-6]

- **Functional Category:** Dispersing agent ; emulsifying agent ; nonionic surfactant ; solubilizing agent ; suspending agent ; wetting agent

- **Odor:** Characteristic odor

- **Taste :** Bitter taste

- **Colour and Physical form at 25 ˚ C:** Yellow oily liquid

- **Solubilities of Polysorbate 80 in various solvents:**

Ingredient	**Solvents**			
Tween 80	**Ethanol**	**Mineral oil**	**Vegetable oil**	**Water**
	Soluble	Insoluble	Insoluble	Soluble

- **Typical Properties:**

HLB value	**Acid value (%)**	**Hydroxyl Value**	**Moisture content**	**Specific Gravity at 25 ˚ C**	**Viscosity (mPa s)**
15.0	2.0	65-80	3.0	1.08	425

- **Incompatibilities:**

  Discoloration and / or precipitation occur with various substances, especially phenols, tannins, tars, and tar like materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates.

- **Storage and stability:**

  Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides. Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

5.2 Polyethylene Glycol 400:[68]

- **Nonproprietary Names**

  BP: Macrogols

  JP: Macrogol400

  PhEur: Macrogols

  USP-NF: Polyethylene Glycol

- **Synonyms:** Carbowax; Carbowax Sentry; Lipoxol; Lutrol E; macrogola; PEG; PluriolE and polyoxyethylene glycol.

- **Empirical formula:** $HOCH_2(CH_2OCH_2)_{8.7}CH_2OH$

- **Molecular weight:** 380-420 g/mol

- **Chemical Name and CAS Registry Number:**

  α-Hydro-ω-hydroxypoly (oxy-1,2-ethanediyl) , [25322-68-3]

- **Functional Category:** Polyethylene glycols (PEGs) are widely used in a variety of pharmaceutical formulations, emulsion stabilizers, parenteral, topical, ophthalmic, oral, and rectal preparations.

- **Odor:** characteristic

- **Taste :** Bitter

- **Colour and Physical form at 25 ˚ C:** Colorless liquid

- **Solubilities of PEG 400 in various solvents:**

Ingredient	Solvents					
PEG 400	**Water**	**Acetone**	**Alcohols**	**Benzene**	**Glycerin**	**Glycols**
	Soluble					

- **Typical Properties:**

HLB value	Hydroxyl Value	Freezing point	Density	pH (5% w/w solution)	Viscosity (mPa s)
16	264-300	4-8˚c	1.120	4.0-7.0	105-130

- **Incompatibilities:**

The chemical reactivity of polyethylene glycols is mainly confined to the two terminal hydroxyl groups, which can be either esterified or etherified. However, all grades can exhibit some oxidizing activity owing to the presence of peroxide impurities and secondary products formed by autoxidation. Liquid and solid polyethylene glycol grades may be incompatible with some coloring agents.

- **Stability and storage:**

Polyethylene glycols are chemically stable in air and in solution, although grades with a molecular weight less than 2000 are hygroscopic. Polyethylene glycols do not support microbial growth, and they do not become rancid. Polyethylene glycols and aqueous polyethylene glycol solutions can be sterilized by autoclaving, filtration, or gamma irradiation.

5.3 Isopropyl Myristate:[68]

- **Nonproprietary Name**

  BP: Isopropyl Myristate

  PhEur: Isopropyl Myristate

  USP-NF: Isopropyl Myristate

- **Synonym:** EstolIPM; HallStar IPM-NF; isopropyl ester of myristic acid; Isopropyl myristat; isopropylis myristas; Kessco IPM95 and Lexol IPM-NF.
- **Empirical Formula:** $C_{17}H_{34}O_2$
- **Molecular weight:**270.5 g/mol
- **Chemical names and CAS Registry Number:**

  1-Methyl ethyl tetradecanoate, [110-27-0]
- **Functional Category:** Emollient; oleaginous vehicle; skin penetrant; solvent.
- **Odor:** Practically Odorless
- **Taste :** Bitter taste
- **Colour and Physical form at 25 ˚ C:** Clear colorless liquid
- **Solubilities of Isopropyl myristate in various solvents:**

Soluble in acetone, chloroform, ethanol (95%), ethyl acetate, fats, fatty alcohols, fixed oils, liquid hydrocarbons, toluene, and waxes. Dissolves many waxes, cholesterol, or lanolin. Practically insoluble in glycerin, glycols and water.

- **Typical Properties:**

HLB value	Boiling Point	Flash point	Freezing point	Viscosity (mPa s)
11.5	140.2˚c at 266 pa	153.5˚c	≈ 5˚c	425

- **Incompatibilities:**

When isopropyl myristate comes into contact with rubber, there is a drop in viscosity with concomitant swelling and partial dissolution of the rubber. Isopropyl myristate is incompatible with hard paraffin, producing a granular mixture. It is also incompatible with strong oxidizing agents.

- **Stability and Storage Conditions:**

Isopropyl myristate is resistant to oxidation and hydrolysis and does not become rancid. It should be stored in a well-closed container in a cool, dry place and protected from light.

5.4 Colloidal silicon dioxide:[67,68]

- **Nonproprietary Name**

  BP: Colloidal Anhydrous Silica
  JP: Light Anhydrous Silicic Acid
  PhEur: Silica, Colloidal Anhydrous
  USP-NF: Colloidal Silicon Dioxide

- **Synonym:** Aerosil; Aeropearl 300 pharma; Cab-O-Sil; Cab-O-SilM-5P;colloidal silica; fumed silica; fumed silicon dioxide and hoch disperses silicum dioxid.
- **Empirical Formula:** SiO_2
- **Molecular weight:** 60.08 g/mol
- **Chemical names and CAS Registry Number:** Silica , [7631-86-9]
- **Functional Category:** Adsorbent; anti caking agent; emulsion stabilizer; glidant; suspending agent; tablet disintegrant; thermal stabilizer; viscosity-increasing agent; Carrier for liquid and pasty (active) ingredients, Converts liquid and pasty substances into free-flowing powders.
- **Odor:** Odorless
- **Taste :** Tasteless

- **Colour and Physical form at 25 ˚ C:** Bluish-white amorphous powder

- **Solubilities of Colloidal silicon dioxide in various solvents:**
  Practically insoluble in organic solvents, water and acids, except hydrofluoric acid; soluble in hot solutions of alkali hydroxide. Forms a colloidal dispersion with water. For Aerosil, solubility in water is 150mg/L at 258˚C (pH 7).

- **Typical Properties:**

PROPERTY	INFERENCE
Bulk density	0.029–0.042g/cm^3
Tapped density	0.05 g/cm^3
Melting point	1600˚C
Particle size distribution	7–16 nm
Refractive index	1.46
Surface gravity	2.2
pH	3.5-5.5
Specific surface area	100–400m^2/g depending on grade

- **Incompatibilities:**

Incompatible with diethylstilbestrol preparations.

- **Stability and Storage Conditions:**

Colloidal silicon dioxide is hygroscopic but adsorbs large quantities of water without liquefying. When used in aqueous systems at a pH 0–7.5, colloidal silicon dioxide is effective in increasing the viscosity of a system. However, at a pH greater than 7.5 the viscosity-increasing properties of colloidal silicon dioxide are reduced and at a pH greater than 10.7 this ability is lost entirely since the silicon dioxide dissolves to form silicates.

Benefits:

- Excellent absorbent for liquid API's.
- Moisture scavenger for improved storage stability of tablets.
- Improves the bioavailability of BCS class II drugs.
- Low dust, high density granulate.

5.5 Plurol Oleique CC 497: [65]

- **Empirical Formula:** $C_{36}H_{70}O_{14}$
- **Molecular weight:** 726.93 g/mol
- **Chemical names :**

  Polyglyceryl-3 Dioleate
- **Functional Category:** Bioavailability enhancer, solubilizer
- **Odor:** Characteristic
- **Color and Physical form at 25 ˚ C:** yellowish viscous liquid.
- **Typical Properties:**

HLB value	pH(at 10% in water)	Hydroxyl value	Water content	Refractive index at 20 ˚C
6	3.5 – 7.5	196-244 mg KOH/g	<0.50%	1.465-1.485

5.6 Labrafil M 1944CS: [65]

- **Empirical Formula:** $C_{43}H_{88}O_{10}$
- **Molecular weight:** 765.15g/mol
- **Chemical names :**

  EP: Oleoyl Macrogol-6 glycerides

  NF: Oleoyl polyoxyl-6 glycerides
- **Functional Category:** Bioavailability enhancer, solubilizer, penetration enhancer
- **Odor:** Faint
- **Colour and Physical form at 25 ˚ C:** Colorless liquid.

- **Typical Properties:**

HLB value	Viscosity at 20˚C	Hydroxyl value	Water content	Refractive index at 20 ˚C
4	75-95 mPa.s	45-65 mg KOH/g	<0.50%	1.465-1.475

5.7 Transcutol P: [65]

- **Empirical Formula:** $C_6H_{14}O_3$
- **Molecular weight:**134.17g/mol
- **Chemical names :**

  EP and USP NF: Purified diethylene glycol monoethyl ether
- **Functional Category:** solubilizer, as oily vehicle
- **Odor:** Faint
- **Colour and Physical form at 25 ˚ C:** Colorless limpid liquid.
- **Typical Properties:**

Water content	Refractive index at 20 ˚C
<0.10%	1.426-1.428

5.8 Capryol 90: [65]

- **Chemical names :**
  Propylene glycol monocaprylate 90%
- **Functional Category:** Bioavailability enhancer, solubilizer, penetration enhancer, co-surfactant in microemulsion
- **Odor:** Faint
- **Colour and Physical form at 25 ˚ C:** Colorless oily liquid.

- **Typical Properties:**

HLB value	Water content	Saponification value
6	1.00%	270-290 mgKOH/g

5.9 Peceol: [65]

- **Empirical Formula:** $C_{21}H_{40}O_4$
- **Molecular weight:** 356.54g/mol
- **Chemical names :**

  EP: Glyceryl monooleate 40
- **Functional Category:** solubilizer , co-surfactant
- **Odor:** Faint
- **Colour and Physical form at 25 ˚ C:** Practically crystallized liquid.
- **Typical Properties:**

HLB value	Water content	Saponification value
3	1.00%	150-175 mgKOH/g

5.10 Labrasol: [65]

- **Chemical names :**

  EP: Caprylocaproyl macrogol-8 glycerides

  NF: Caprylocaproyl polyoxyl-8 glycerides
- **Functional Category:** solubility and bioavailability enhancer, surfactant
- **Odor:** Faint
- **Colour and Physical form at 25 ˚ C:** Colorless oily liquid.

- **Typical Properties:**

Viscosity at 20˚C	Refractive index at 20˚C	HLB value	Water content	Saponification value
80-110 mpa.s	1.450-1.470	14	1.00%	85-105 Mg KOH/g

5.11 Capmul MCM (C8):[66]

- **Empirical Formula:** $C_{11}H_{22}O_4$
- **Molecular weight:**218.29g/mol
- **Chemical names and CAS Registry Number**
  Monoglyceride of capylic acid, [26402-26-2]
- **Functional Category:** solubility ,surfactant
- **Odor:** Faint
- **Colour and Physical form at 25 ˚ C:** Colorless liquid/semi solid.
- **Typical Properties:**

Hydroxyl value	Water content	Saponification value	Solubility
324-396	1.00%	252-308 mgKOH/g	Slightly water soluble

- **Storage:**
  Keep away from heat and flame. Store in a dry area.

5.12 Captex 200: [66]

- **Chemical names and CAS Registry Number**
  Mixed of caprylic/capric acids diesters of propylene glycol, [68583-51-7]
- **Functional Category:** oily vehicle
- **Odor:** Neutral
- **Colour and Physical form at 25 ˚ C:** Crystal clear liquid.
- **Typical Properties:**

Viscosity at 77 ˚F	Hydroxyl value	Water content	Saponification value	Solubility in water
9.0 centistoke	0.2	0.01%	324 mgKOH/g	Insoluble

- **Incompatibility:** Incompatible with oxidizers
- **Storage:**
  Keep away from heat and flame. Store in a dry area.

5.13 Captex 355: [66]

- **Chemical names and CAS Registry Number**
  Glycerol Caprylate Caprate, Octanoic / Decanoic Acid Triglyceride, [65381-09-1]
- **Functional Category:** Oily vehicle
- **Odor:** Neutral
- **Colour and Physical form at 25 ˚ C:** Light Yellow/Clear Liquid
- **Typical Properties:**

Viscosity at 20 ˚C	Hydroxyl value	Water content	Saponification value
25 – 35 cP	10	0.1%	325-340 mgKOH/g

- **Storage:**
  Store in a dry location at ambient temperature.

6. METHODOLOGY

6.1 List of materials:

TABLE 6.8: List of materials required for Research Work

Sr. no.	Name	Category	Supplier of material
1.	Candesartan cilexetil	API	Alembic Pharmaceuticals
2.	Capryol 90	Oil	Gattefosse,France
3.	Tween 80	Surfactant	S.D fine Chem
4.	Polyethylene Glycol 400 (PEG 400)	Co-surfactant	Suvidhinath Chemicals
5.	Labrasol	Surfactant	Gattefosse,France
6.	Peceol	Co-surfactant	Gattefosse,France
7.	Transcutol P	Surfactant	Gattefosse,France
8.	Captex 200	Oil	Abitec Corporation
9.	Captex 200 P	Oil	Abitec Corporation
10.	Plurol Oleique CC497	Surfactant	Gattefosse,France
11.	Labrafil 1944 CS	Oil	Gattefosse,France
12.	Capmul MCM	Surfactant	Abitec Corporation
13.	Isopropyl myristate	Oil	S.D fine Chem
14.	Aerosil 200 pharma	Adsorbent	Evonik Deggussa
15.	Aeropearl 300 pharma	Adsorbent	Evonik Deggussa
16.	Fujicalin SG	Adsorbent	Gangwalchem
17.	Microcystalline cellulose 102	Diluent	Remedy Labs
18.	Lactose monohydrate (SuperTab 11 SD)	Diluent	DMV Fonterra excipients
19.	Mannitol (Pearlitol 200 SD)	Diluent	Signet chemical corporation pvt ltd
20.	Pregelatinized starch	Disintegrant	DMC/Roquette

6.2 List of Equipments:

TABLE 6.9: List of equipments required for research work

Sr. No.	Instruments	Manufactures/ Suppliers
1.	Electronic Balance	Mettler Toledo
2.	Digital pH meter	Labindia
3.	Magnetic Stirrer	Remi equipment Ltd.
4.	Hardness Tester	Electro lab
5.	Friability test apparatus	Electro Lab
6.	Compression Machine	Cadmach
7.	U.V Spectrophotometer	Shimadzu
8.	Tablet Dissolution Tester	Electro Lab
9.	Tap Density Tester	Electro Lab
10.	Tablet Disintegration test apparatus	Electro Lab
11.	H.P.L.C	Shimadzu

6.3 Analytical method:

6.3.1 HPLC Analysis of Candesartan cilexetil: [71]

Candesartan cilexetil was analyzed by SHIMADZU Prominence LC 20 AD series with UV detection. The chromatographic conditions are;

Column: Inertsil ODS-3, C-18 250×4.6 mm, 5µm stainless steel column (Agilent Technologies, USA)

Column temperature: 25^0C

Flow rate: 2ml/min

Mobile phase : Mixture of buffer (0.02 M monobasic potassium phosphate), acetonitrile, and triethylamine in the ratio of 40:60:0.2, pH - 6.0 using phosphoric acid.

Run time: 15 min

UV wavelength: 254 nm

TABLE 6.10: Calibration curve of Candesartan cilexetil by HPLC

Peak area	Concentration (mg/mL)			Average Concentration
	I	II	III	
0	0	0	0	0
0.08	1806	1804	1807	1806
0.12	2398	2398	2395	2397
0.16	3347	3349	3347	3348
0.2	4069	4071	4071	4070
0.24	4921	4923	4925	4923

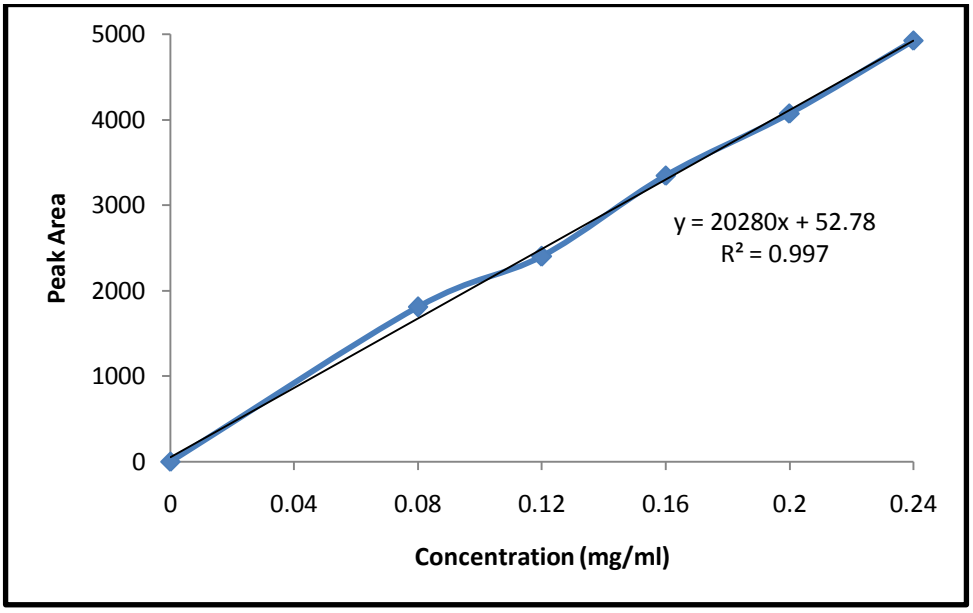


FIGURE.6.2: Calibration curve of drug by HPLC

6.3.2 UV Spectrophotometry Analysis of Candesartan cilexetil: [72]

6.3.2.1 Calibration curve in methanol:

Preparation of standard stock solution

Standard drug solution of drug was prepared by dissolving 10 mg of standard drug in 20 ml methanol in 100ml volumetric flask. It was sonicated for 5 minutes for the complete solubility of drug. After dissolving the drug the final volume was made up to 100ml by adding methanol to obtain a 100 µg/ml concentration.

Preparation of Calibration curve

Calibration curve of drug was prepared in methanol and absorbance was taken at λmax 254 nm using Shimadzu UV-1601 spectrophotometer. From the stock solution of 100µg/ml serial dilution of 10, 20, 30, 40, 50 µg/ml were prepared by using same solvent. The absorbance was taken at 254nm corresponding to each concentration and was recorded. Calibration curve was plotted taking absorbance at Y-axis and concentration at X-axis. The experiment was done in triplicate and reading expressed as ± SD.

TABLE 6.11: Calibration curve of Candesartan cilexetil in methanol

Concentration (µg/ml)	Absorbance			Average absorbance
	I	II	III	
0	0	0	0	0
10	0.238	0.237	0.237	0.237
20	0.375	0.377	0.377	0.376
30	0.579	0.577	0.578	0.578
40	0.770	0.771	0.773	0.771
50	0.896	0.899	0.898	0.897

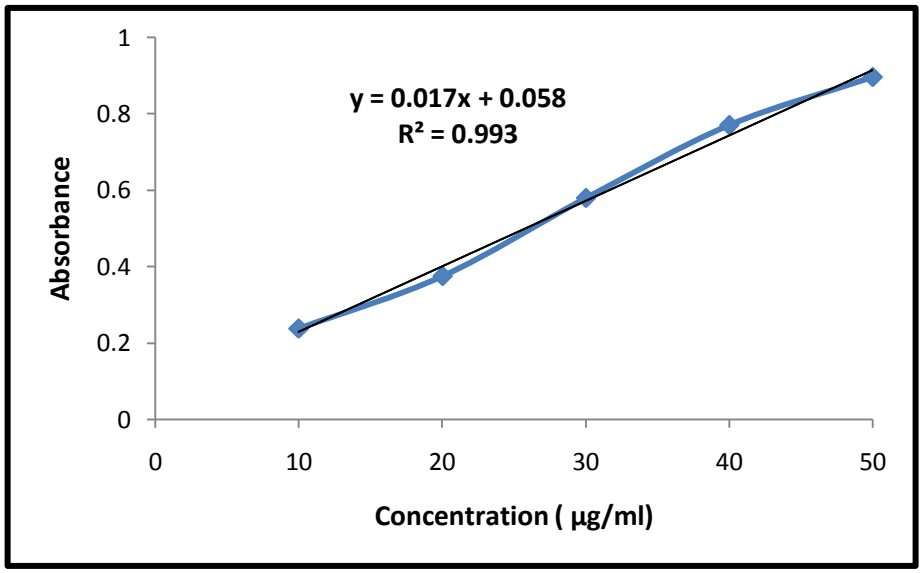


FIGURE.6.3: Calibration curve of Candesartan cilexetil in methanol

6.3.2.2 Calibration curve in 0.1 N HCL: [72]

Preparation of standard stock solution

Standard drug solution of drug was prepared by dissolving 10 mg of standard drug in 20 ml 0.1 N HCL in 100ml volumetric flask. It was sonicated for 5 minutes for the complete solubility of drug. After dissolving the drug the final volume was made up to 100ml by adding 0.1 N HCL to obtain a 100 µg/ml concentration.

Preparation of Calibration curve

Calibration curve of CC was prepared in 0.1 N HCL and absorbance was taken at λmax 254 nm using Shimadzu UV-1601 spectrophotometer. From the stock solution of 100µg/ml serial dilution of 10, 20, 30, 40, 50 µg/ml were prepared by using same solvent. The absorbance was taken at 254nm corresponding to each concentration and was recorded. Calibration curve was plotted taking absorbance at Y-axis and concentration at X-axis. The experiment was done in triplicate and reading expressed as ± SD.

TABLE 6.12: Calibration curve of Candesartan cilexetil in 0.1 N HCL

Concentration (µg/ml)	Absorbance			Average absorbance
	I	II	III	
0	0	0	0	0
10	0.097	0.098	0.097	0.097
20	0.175	0.176	0.175	0.175
30	0.264	0.265	0.267	0.265
40	0.37	0.371	0.371	0.371
50	0.45	0.453	0.451	0.451

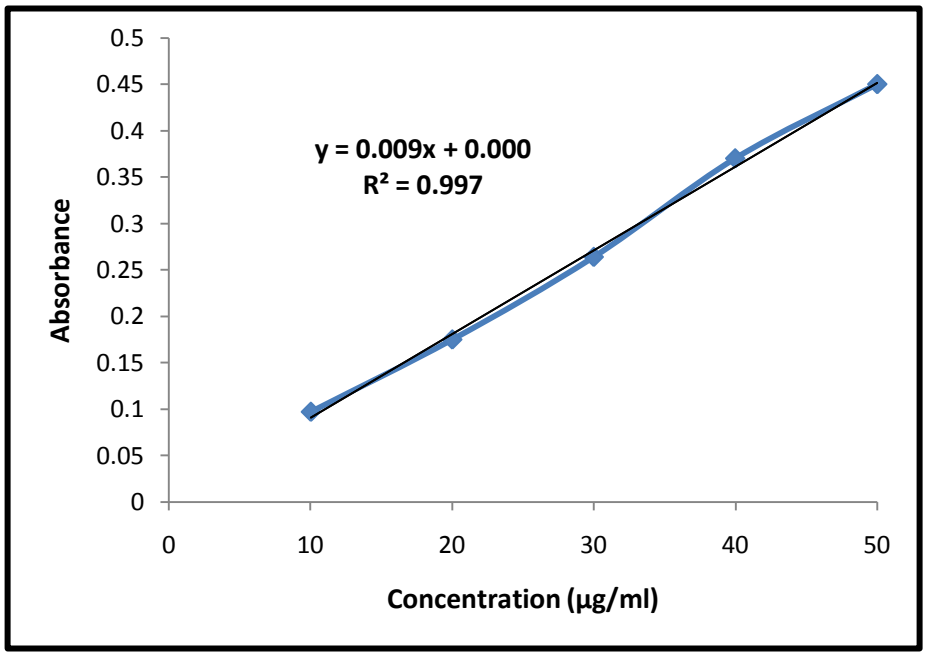


FIGURE.6.4: Calibration curve of Candesartan cilexetil in 0.1 N HCL

6.4 METHODOLOGY FOR LIQUID SMEDDS

6.4.1 Screening of components:

6.4.1.1 Solubility study

The most important criteria for the screening of components for SMEDDS is the solubility of poorly soluble drug in oils, surfactants and co surfactants. Since the aim of this study is to develop an oral formulation, therefore, solubility of drug in oils is more important as the ability of SMEDDS to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in oil phase. The solubility of candesartan cilexetil in various oils and distilled water was determined by adding an excess amount of drug in 2mL of selected oils (capryol 90, isopropyl myristate, Labrafil 1944 CS, captex 200, captex 200 P, captex 355) and distilled water separately in 5mL capacity stopper vials, and mixed using a vortex mixer. The mixture vials were then kept at 25±1.0 ^{o} C in an isothermal shaker for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of candesartan cilexetil was determined in oils and water using UV spectrophotometer at 254 nm. [72]

6.4.1.2 Preparation of candesartan cilexetil SMEDDS:

A series of SMEDDS formulations were prepared using various oil, Surfactant and Co-surfactant as shown in Table 6.13. In all the formulations, the level of candesartan cilexetil was kept constant (i.e. 32 mg). The amount of SMEDDS should be such that it should solubilize the drug (single dose) completely. The candesartan cilexetil (32 mg) was added in the mixture. Then the components were mixed by gentle stirring and vortex mixing, then heated at 40˚C.The mixture was stored at room temperature until further used.

TABLE 6.13: Formulations of Candesartan Cilexetil SMEDDS

Ingredients	I				II				III			
	A	B	C	D	A	B	C	D	A	B	C	D
Candesartan cilexetil	**32 mg**											
C 1												
Ca 90	5	5	5	5	10	10	10	10	15	15	15	15
PO	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Peceol	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15
C 2												
Ca 90	5	5	5	5	10	10	10	10	15	15	15	15
Tr-P	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Lauroglycol	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15
C 3												
Ca 90	5	5	5	5	10	10	10	10	15	15	15	15
Lauroglycol	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Tr-P	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15
C 4												
Ca 90	5	5	5	5	10	10	10	10	15	15	15	15
Capmul MCM(C8)	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Labrasol	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15
C 5												
Ca 90	5	5	5	5	10	10	10	10	15	15	15	15
Cap MCM(EP)	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Labrasol	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15
C6												
Ca-90	5	5	5	5	10	10	10	10	15	15	15	15
Acconon CC-6	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Tween 80	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15
C7												
Ca-90	5	5	5	5	10	10	10	10	15	15	15	15
Captex 500	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Labrasol	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15
C8												
Ca-90	5	5	5	5	10	10	10	10	15	15	15	15
Acconon CC-6	47.5	60	35	70	45	60	30	70	42.5	56.6	28.4	70
Labrasol	47.5	35	60	25	45	30	60	20	42.5	28.4	56.6	15

Where ;various ratios of S/CoS are A-1:1; B-2:1; C-1:2 and D-3:1; I-5% oil conc.; II – 10% oil conc. and III-15% oil conc.

6.4.1.3 Drug and surfactant compatibility study:

Physical compatibility of the water-insoluble drug with surfactants should be used in surfactant selection procedure. Physical compatibility may include precipitation/crystallization, phase separation and color change in the drug –surfactant solution during course study. Chemical compatibility is primarily regarded as the chemical stability of the drug in a surfactant solution. A surfactant was considered for further development only if it was physically and chemically compatible with drug. [74]

6.4.1.4 Pseudoternary phase diagram:

The existence of microemulsions regions were determined by using pseudo-ternary phase diagrams. SMEDDS were diluted under agitation conditions using water titration method: The mixture of oil and surfactant/cosurfactant at certain weight ratios were diluted with water in a dropwise manner. Distill water was used as an aqueous phase for the construction of phase diagrams. Oil, surfactants and co surfactants were grouped in four different combinations for phase studies. Surfactant and cosurfactant (Smix) in each group were mixed in different weight ratios (1:1, 2:1, 1:2, 2:1, 3:1). These Smix ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams for formulation of SMEDDS (Fig.8.6). For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different weight ratios from 1:1 to 3:1 in different glass vials. Twelve different combinations of oil and Smix were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo-ternary phase diagrams were developed using aqueous titration method. [75] The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived from the weight measurements. These values were then used to determine the boundaries of the microemulsion domain corresponding to the chosen value of oils, as well as the S/CoS mixing ratio. [76]On the basis of the solubility studies of drug, Capryol 90 was selected as the oil phase. The physical state of the SMEDDS was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (Smix ratio).

6.5 CHARACTERIZATION OF SMEDDS OF CANDESARTAN CILEXETIL[80]

6.5.1 Viscosity and pH:

The viscosities were measured to determine rheological properties of formulations. Brookfield LVDV 111+ CP viscometer at 30˚C with a CPE 42 spindle at 5 rpm was used to serve this purpose. The pH of the formulations was measured using pH meter.

6.5.2 Thermodynamic stability:

a) **Heating cooling cycle:** Six cycles between refrigerator temperature 4˚C and 45˚C with storage at each temperature of not less than 48h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

b) **Centrifugation:** Passed formulations were centrifuged at 3500 rpm for 30min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

c) **Freeze thaw cycle:** Three freeze thaw cycles between 4˚C and +25 ˚C with storage at each temperature for not less than 48h was done for the formulations. Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility test for assessing the efficiency of self-emulsification. The formulations were observed visually for any phase separation or color change.

6.5.3 Dispersibility test:

The efficiency of self-emulsification of oral SMEDDS was assessed using a USP dissolution apparatus 2. [73] One milliliter of each formulation was added to 500 ml of water at 37±0.5 ˚C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The *in-vitro* performance of the formulations was visually assessed using the following grading system:

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

Grade B: Rapidly forming, slightly less clear microemulsion, having a bluish white appearance.

Grade C: Fine milky microemulsion that formed within 2 min.

FIGURE 6.5: Visual assessment of liquid SMEDDS formulations

6.5.4 Particle size distribution (PSD) and ζ-potential analysis:

SMEDDS formulation was diluted 100 times with distilled water and 0.1 mol/l HCl, at 37 ± 0.5˚C. The resultant emulsions were prepared by gentle agitation for 10 min using a magnetic stirrer. PSD and ζ-potential of the final microemulsion were determined using, Malvern zetasizer.

6.5.5 % Transmittance Measurement:

The percent transmittance of various formulations was measured at 254 nm using UV spectrophotometer keeping water as a blank.

6.5.6 Polydispersibility Index:

The procedure is same as in 6.5.4 for particle size distribution.

6.5.7 *In-vitro* diffusion study:

In-vitro drug diffusion study was carried out by using dialysis bag method. Dialysis bag was soaked overnight in 0.1 N HCl for saturation purpose and then it was further used for experimental procedure.1 ml of candesartan cilexetil SMEDDS diluted with aqueous phase was instilled in dialysis bag and one end was tied with thread and was placed in 900 ml of 0.02% Tween 20 in 0.1 N HCl as dissolution medium at 37±0.5˚C temperature. The

revolution speed of paddle was maintained at a rate of 50 rpm. [19] An aliquot of 5mL was withdrawn at regular time intervals of 0, 5, 10, 20, 30, 45 and 60 min. The SMEDDS formulation was compared with the conventional marketed tablet formulation M (Atacand 32 mg tablet) and the suspension of pure drug (S). The samples were analyzed for the drug content using HPLC method at 254nm.

6.5.8 Comparison of *In-vitro* dissolution of SMEDDS formulation with Marketed formulation

Two criteria for comparison of dissolution:

- If both test and reference product show > 85 % of dissolution within 15 mins the profile considered to be similar. If not then,
- Calculate f_2 value.

The *in-vitro* drug release profile of prepared batches with Market product's release profile was compared using similarity factor (f_2).

$$f_2 = 50 \times \log \{[1+(1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$$

Where, R_t, T_t are the percentage release of the reference and test profile, respectively, at the t time point. n is total number of sample times.

TABLE 6.14: Specification of Similarity factor value and its significance

SIMILARITY FACTOR (F2)	SIGNIFICANCE
< 50	Test and reference profiles are dissimilar
50 – 100	Test and reference release profiles are similar
100	Test and reference release profiles are identical
> 100	The equation yields a negative value

A value of 100% for the similarity factor suggests that the test and reference profiles are identical. Values between 50 and 100 indicate that the dissolution profiles are similar whilst smaller values imply an increase in dissimilarity between release profiles.

6.5.9 Stability of candesartan cilexetil SMEDDS: [81]

Candesartan cilexetil SMEDDS samples were filled in glass vials with rubber stopper and then placed in Stability chambers at 25 ±0.5˚C / 60 ± 5 % RH and 40±0.5˚C / 75 ± 5 % RH for 3 months. Duplicate samples were withdrawn at 0, 15, 30, 60, and 90 days to evaluate their physical and chemical stabilities. The physical stability was evaluated by visual inspection for physical changes (such as phase separation and drug precipitation), and a particle size analyzer was used to determine the mean particle size after dilution with water.

7. PREPARATION OF SOLID SELF MICROEMULSIFYING DELIVERY SYSTEM (S-SMEDDS) OF CANDESARTAN CILEXETIL:

7.1 ADSORPTION TO SOLID CARRIERS:

The optimized liquid SMEDDS formulation was converted into free flowing powders by adsorption onto solid carriers. The solid carriers used for adsorption comprised of materials that provided a high surface area with good disintegration characteristics. The solid carriers used includes Aerosil 200 pharma (A1), Aeropearl 300 Pharma (A2) and Fujicalin SG(F1).The carrier chosen can absorb at the levels up to 70% (w/w). The conversion process involved addition of liquid formulation onto carriers under continous mixing in a blender. The powder was dried and was further evaluated for various parameters before comprising it as a tablet formulation. The combination of adsorbent and Liquid SMEDDS which showed the best result was used for developing final tablet formulation.

7.1.1 Adsorbent Selection For Optimized Liquid SMEDDS Formulation (C7IIB):

The optimized liquid SMEDDS formulation (C7IIB) was converted into free flowing powder by adsorption of liquid onto solid carriers. The solid carriers used for adsorption materials that provided a high surface area with good disintegration characteristic. The soild carriers used include Aerosil 200 pharma (A1), Aeropearl 300 Pharma (A2) and Fujicalin SG (F1).The carriers chosen can adsorb upto 70% (w/w).The conversion process involved addition of liquid formulation on solid carriers under continuous mixing. 0.2 ml optimized liquid SMEDDS i.e. C7IIB (containing drug 32 mg) was used to convert into solid SMEDDS. The amount of adsorbents required to achieve a free flowing powder is as shown below in table 37; [95]

- **Bulk density:** Bulk density is determined from bulk volume and the weight of dry powder in a graduated cylinder. [96]

$$\text{Bulk density}(\rho 0) = \frac{\text{weight of powder}}{\text{bulk volume}}$$

- **Tapped density:** It is obtained by mechanically tapping the measuring cylinder containing powder. [96]

$$\text{Tapped density}(\rho t) = \frac{\text{weight of powder}}{\text{Tapped volume}}$$

- **Carr's index:** %compressibility of powder is a direct measure of the potential powder bridge strength or arch is calculated according equation, [96]

$$\text{Carr}'\text{iindex} \quad = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}}$$

Decreasing the voids causes decrease in the tapped density (w/v) results in to decrease in the Carr's index, so achieving good flow properties. The various limits of Carr's index are as follows:

TABLE 7.15: Interpretation of Carr's index for powder flow

Carr's Index	Type of flow
5-15	Excellent
12-16	Good
18-21	Fair to passable
23-35	Poor
33-38	Very poor
>40	Extremely poor

- **Hausner's ratio:** it is related to interparticle friction and could be used to predict powder flow property. [96]

$$\text{Hausner}'\text{s ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

The various limits of hausner's ratio are as follows:

Value < 1.25 indicate good flow (=20% Carr)

While > 1.50 indicate poor flow (=35% Carr)

- ***In-vitro* dissolution:**

Dissolution test was carried out by using USP type II apparatus. The paddle was rotated at 50 rpm. 0.02% Tween 20 in 0.1 N HCl was used as dissolution medium (900 ml) and was maintained at 37±0.5˚C. Samples of 5 ml were withdrawn at predetermined intervals (0, 5, 10, 15, 30, 45 and 60 min) filtered and replaced with 5 ml of fresh dissolution medium. The collected samples were suitably diluted with dissolution fluid, where ever necessary and were analyzed for the drug at 254 nm by using HPLC. Each dissolution study was performed for three times and mean values were taken. The results are as shown in Table 8.39; [99]

- **Scanning electron microscopy: [100]**

Some amount of optimized formulations was mounted on the stub. This specimen was then sputter coated with gold particles and observed with a SEM (JSM-5610, JEOL, Japan) at an accelerating voltage of 10 kV. Surfaces of powder were photographed.

- **Differential scanning calorimetry [DSC] [100]**

Thermal properties of drug, placebo, and solid SMEDDS formulations were investigated using a Perkin-Elmer DSC-7 differential scanning calorimeter/TAC-7 thermal analysis controller with an intracooler-2 cooling system (Perkin-Elmer Instruments, USA). About 3 to 5 mg of product was placed in perforated aluminum sealed 50-µl pans, and the heat runs for each sample was set from 40°C to 200°C at 5°C/min, under an inert environment using nitrogen. The apparatus was calibrated using pure metals like indium with known melting points and heat of fusion (ΔH_{fusion}).

7.2 CHARACTERIZATION OF MARKETED SAMPLE

TABLE 7.16: Marketed sample characterization

Brand Name	Atacand 32 mg
Mfg. By	Astrazeneca
Label claim	Each tablet contains Candesartan cilexetil 32 mg
Pack size	30 or 90 tablets
Storage Condition	store at 25˚C Keep container tightly closed

Market sample was characterized for following physicochemical parameters:

- Weight
- Thickness
- Hardness
- Disintegration time
- Dissolution test
- Initial assay
- Assay at accelerated condition (40 $\pm$ 2 ˚C / 75 $\pm$ 5 %RH)

7.3 FORMULATION DEVELOPMENT OF SOLID SELF MICRO EMULSIFYING DRUG DELIVERY SYSTEM (S-SMEDDS):

The 3^2 Full factorial design was applied for the tablet SMEDDS formulation of candesartan cilexetil. The composition is as shown below:

TABLE 7.17: 3^2 Full factorial design for S-SMEDDS

Ingredients(mg)	T1	T2	T3	T4	T5	T6	T7	T8	T9
S-SMEDDS	120	120	120	120	120	120	120	120	120
Lactose monohydrate	74			69			64		
Mannitol		74			69			64	
Microcrystalline cellulose-102			74			69			64
Pre-gelatinized starch	5	5	5	10	10	10	15	15	15
Magnesium stearate	1	1	1	1	1	1	1	1	1
Total(mg)	200	200	200	200	200	200	200	200	200

7.3.1 TABLET EVALUATION:

Prepared tablets were evaluated by following tests;

- **Weight variation:**

Every individual tablet in a batch should be in uniform weight and weight variation within permissible limits. Weight control is based on a sample of 20 tablets. Twenty tablets were randomly selected and accurately weighed using an electronic balance. The results are expressed as mean values of 20 determinations.

- **Thickness:** [101]

The thickness of ten randomly selected tablets was determined using a digital vernier caliper. The results are expressed as mean values of 10 determinations.

- **Hardness:** [101]

The hardness of the tablets was determined using an Electolab hardness tester.

- **Friability:** [101]

The friability of the tablets was measured in a Roche friabilator. Tablets of a known weight (W_0) or a sample of 10 tablets are dedusted in a drum for 100 revolutions at a speed of 25 RPM and weighed (W) again. Percentage friability was calculated from the loss in weight as given in below equation. The weight loss should not be more than 1 % w/w with no breakage of any tablet.

$$\boldsymbol{Friability = \frac{W0 - W}{Wo} \times 100}$$

- ***In -Vitro* Dissolution Testing:**

Method is same as shown in 7.1.1.

- **Assay of Candesartan cilexetil Tablet**

10 tablet of Candesartan were crushed in mortar and pestle. 193.1 mg powder equivalent to 10 mg drug was taken and transferred in 100 ml volumetric flask. 20 ml methanol was added and the solution was then sonicated for 5 minutes. Volume of the solution in the flask was made up to the 100 ml by adding methanol. It was then filtered through Whatman filter paper. The filtrate was then diluted to 10µg/ml by adding methanol. Absorbance was measured against blank and assay of the tablet was done by using standard calibration curve. The validated method was also applied to determine the assay of the Candesartan cilexetil marketed tablet. (Candesartan cilexetil contains not less than 98.7% and not more than 101.0% of $C_{33}H_{34}N_6O_6$, calculated on anhydrous basis).[72]

- **Comparison of *In-vitro* dissolution of prepared tablet with marketed formulation**

Here *in-vitro* dissolution of prepared tablet is compared with the marketed tablet formulation (Atacand Tablet).

- **Stability Study:**

The stability study was carried out for selected formulation as per ICH guidelines. An accelerated stability study was performed at $40^0C \pm 2^0C$ and 75% ± 5% RH and real time stability study was performed at 25 ±0.5˚C / 60 ± 5 % RH for a period of three months. The tablets of the best formulation were blister packed and placed in a stability chamber. The samples were analyzed for physical appearance, particle size and zeta potential at regular interval.

7.4 *IN VIVO* STUDIES:

7.4.1 Measurement of systolic blood pressure:

The studies were performed for optimized batch of solid SMEDDS formulation i.e. T4. A pharmacodynamic method was applied to determine enhancement in bioavailability to S-SMEDDS (T4) of drug as compared to plain drug suspension. Candesartan cilexetil inhibits the pressor effects of Angiotensin II infusion in a dose-dependent manner. [102,103] Hence, decrease in pressor effect can be directly correlated with the amount of drug that reaches the systemic Circulation, higher the bioavailability of the administered formulation. The pharmacodynamic study was thus based on this hypothesis.

DOCA [deoxycorticosterone acetate] salt model was applied to induce hypertension in rats.[104] After induction of hypertension, treatment was started with plain drug suspension and S-SMEDDS and blood pressure was measured by tail-cuff method using LE 5002 Storage Pressure Meter [Letica Scientific Instruments]. The animal experiments are conducted in full compliance with Institutional Animal Ethical Committee [IAEC] regulations, as per CPCSEA guidelines. The registration number of our institute is 282/14/a/CPCSEA.

- **DOCA salt hypertensive rats**

Female Wistar rats [weight approximately 200–250 g] obtained from Nishka labs, Hyderabad, India were used for the study. These animals were divided into six groups, each containing four rats. All rats were uninephrectomised under anesthesia with intraperitoneal ketamine [100 mg/kg]. Kidneys were visualized by a right lateral abdominal incision. The right kidney was removed after ligation of adjoining renal vasculature and ureter with sutures i.e. uninephrectomy. After one week recovery period, uninephrectomized rats were given either no further treatment [UNX rats] or 1% NaCl in drinking water with subcutaneous injections of deoxycorticosterone acetate [DOCA; 25mg in corn oil every fourth day] [DOCA-salt rats] . DOCA-salt rats were further sub-grouped into five according to treatment given to them: DC1 & DC2-low dose S-SMEDDS and plain drug suspension, respectively [0.5mg/kg/day], DC3 & DC4-high dose S-SMEDDS and plain drug suspension, respectively [5mg/kg/day], DC0-no treatment [DOCA control]. To get bulk drug suspension, plain drug with equivalent quantity

of S-SMEDDS was suspended into distilled water before administration, while S-SMEDDS was administered as such after suitable dilution. After 14 days, all subgroups of DOCA-salt rats except DC0 subgroup were orally administered daily for further 7 days.

- **Measurement of systolic blood pressure**

Systolic blood pressure was measured once a week before drug administration for first two weeks [using tail-cuff method]. During treatment, systolic blood pressure was measured daily for all subgroups of DOCA salt rats except DC0, 2–3 hours after administration. In UNX rats and DC0 rats, blood pressure was measured once a week throughout the experiment.

- **Statistical analysis**

ANOVA was applied followed by t-test to determine differences in decrease in blood pressure between groups; $p < 0.05$ was considered significant.

7.4.2 Bioavailability Assessment Of S-SMEDDS Of Candesartan Cilexetil Tablet:

Bioavailability of candesartan cilexetil S-SMEDDS formulation (T4) was compared with suspension of marketed (M) Atacand tablet 32 mg (Astrazeneca). Candesartan cilexetil S-SMEDDS suspension was prepared as mentioned above and diluted to a definite volume using the same vehicle afterwards. Six rats (200–250 g) were allocated at random to two treatment groups and administered S-SMEDDS and Atacand suspension in a crossover design. The washout period between the two treatments was 7 days. Female rats (weighing approximately (200–250 g) were fasted for 12 h prior to the experiment and water was available *ad lib*. After oral administration of drug dose (5.6 mg/1.5 kg body weight), about 2 mL of blood sample was collected through retro-orbital plexus into heparinized tubes at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h. Blood samples were centrifuged at 5,000 rpm for 10 min using a high-speed centrifuging machine, and plasma samples were withdrawn and stored at −18°C.

8. RESULT AND DISCUSSION

8.1 RESULTS FOR LIQUID SMEDDS OF CANDESARATAN CILEXETIL:

8.1.1 Screening of components:

The important criterion for selection of the materials was that all the components are pharmaceutically acceptable for oral administration and fall under GRAS (Generally recognized as safe) category. The maximum solubility of the drug in the oil phase is important for the SMEDDS to maintain the drug in solubilized form. If the surfactant or cosurfactant is contributing to drug solubilization, there could be a risk of precipitation, as dilution of SMEDDS in GIT will lead to lowering of solvent capacity of surfactant or cosurfactant. [76] The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC under the prevailing conditions of temperature, pH and ionic strength. [76] Thus, for the current study, one oil from various categories of triglyceride as well as synthetic monoglyceride oils was selected, so that highest solubility of candesartan cilexetil could be achieved.

Safety is a major determining factor in choosing a surfactant as large amounts of surfactants may cause GI irritation. Nonionic surfactants are less toxic than ionic surfactants as typically nonionic surfactants have lower CMCs than their ionic counter parts. SMEDDS dosage forms for oral or parenteral use based on nonionic surfactants are likely to offer *in-vivo* stability. [81] An important criterion for selection of the surfactants is that their required HLB value to form SMEDDS greater than 10. The right blend of low and high HLB surfactants leads to the formation of a stable SMEDDS upon dilution with water. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of single surfactant, usually needs the addition of a cosurfactant. The presence of cosurfactants decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition. Thus, various cosurfactants were selected for the study that again are nonionic surfactants.

The solubility of candesartan cilexetil in different oils and water was determined (Table 8.18). The solubility of candesartan cilexetil was found to be highest in oil Capryol 90 (80.12 mg/mL) as compared to other oils while in water it was 0.09±0.01mg/mL. This may be

attributed to the polarity of the poorly water soluble drugs that favor their solubilization in small / medium molecular volume oils such as medium chain triglycerides or mono- or diglycerides. Thus, Capryol 90 was selected as the oil phase for the development of the formulation.

TABLE 8.18: Solubility study of Candesartan cilexetil in various vehicles

Solvent	Solubility(mg/mL)
Transcutol P	253.1±0.27
Plurol oleique	169.21±2.19
Labrasol	159.7±3.53
Capryol 90	80.12±4.04
Labrafil 1944 CS	49.76±1.13
Captex 200	5.67±0.68
Captex 200 P	7.29±0.94
Captex 355	10.31±1.02
Capmul MCM	35.02±1.32
Tween 80	261.09±2.85
PEG 400	108.13±3.22
IPM	22.54±0.29
Lauroglycol FCC	177.05±1.54
Capmul MCM (C8)	198.70±2.13
Acconon CC-6	181±1.76
Captex 500	191.35±2.78
Capmul MCM EP	173.64±1.19
Distill water	0.09±0.01

***Mean±SD, n=3**

8.1.2 Drug and surfactant compatibility study:

Physical and chemical compatibility of the water-insoluble drug candesartan cilexetil with various surfactants and co-surfactants was carried out to check the physical as well as chemical compatibility. As shown in Table 8.19, all the formulations passed the physical as well as chemical compatibility tests. The formulations did not show any changes during the compatibility studies and were found to be stable. Further studies were carried out using this formulation.

TABLE 8.19: Drug surfactant compatibility study

Formulation	Precipitation	Crystallization	Phase separation	Color change
C1	√	√	√	√
C 2	√	√	√	√
C 3	√	√	√	√
C 4	√	√	√	√
C 5	√	√	√	√
C6	√	√	√	√
C7	√	√	√	√
C8	√	√	√	√

Where, √-Passed and ×-Failed

8.1.3 Pseudoternary phase diagram:

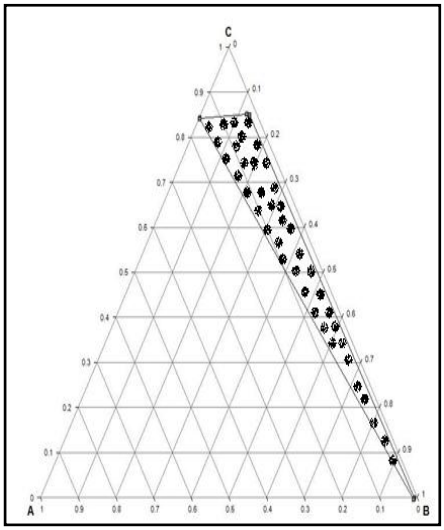
Self-microemulsifying systems form fine oil-water emulsions with only gentle agitation, upon their introduction into aqueous media. Surfactant and co surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the microemulsion formulation. Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the formation of the microemulsion.

Constructing phase diagrams is time consuming, particularly when the aim is to accurately delineate a phase boundary. [77] Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form a microemulsion is very low, the formation is thermodynamically spontaneous. The relationship between the phase behavior of a mixture and its composition can be found with the aid of a phase diagram. Pseudo-ternary phase diagrams were constructed separately for each group (Fig.8.6), so that SMEDDS regions could be identified.

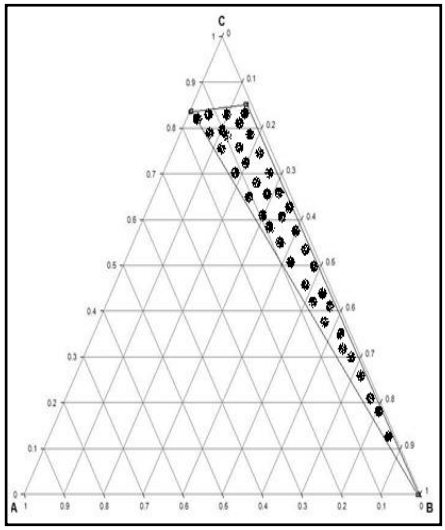
In Fig.8.6 the formulation C2 is shown. It can be observed that when Transcutol-P was used along with lauroglycol as S/CoS mixture, amount of oil (10-15%w/w) could be solubilized at a high concentration (70%w/w) of surfactant. It was observed that increase in the

concentration of surfactant increased the microemulsion region in this formulation. In Fig.8.6 formulation C4 is shown. Labrasol and Capmul MCM(C8) was used as S/CoS mixture. The amount of oil solubilized was 15%w/w by 70%w/w of surfactant. The lower concentrations of surfactant give a smaller microemulsion region. In Fig. 8.6 the formulation C8 is shown in which 5-15% of oil can be solubilized by using 35-70% of surfactant. With the decrease in concentration of surfactant, increase in microemulsion region can also be observed. In Fig 8.6 formulations C7 was observed which gave the appropriate microemulsion region in all the concentrations. The results of visual assessment showing the amount of water required for dilution are as shown in Table 8.20.

In the present study Capryol 90 was tested for phase behavior studies with Labrasol and Captex 500 as the S/CoS mixture. As seen from the ternary plot C7IIB gave a wider microemulsion region at all S/CoS ratios. The microemulsion area increased as the S/Cos ratios increased. However, it was observed that increasing the surfactant ratio resulted in a loss of flowability. Thus, an S/CoS ratio 10% 2:1 was selected for the formulation study.



C2IIID



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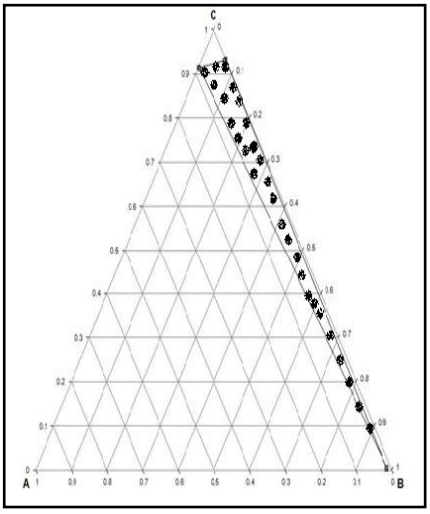
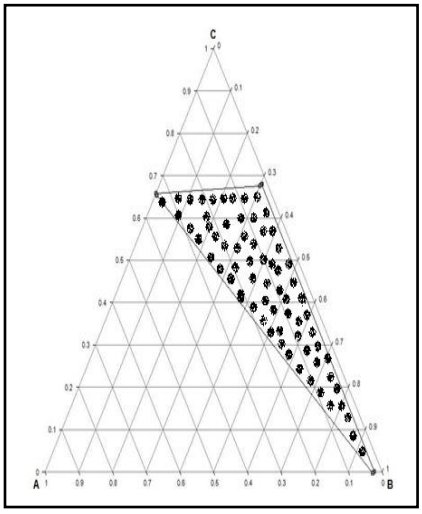


C7IC



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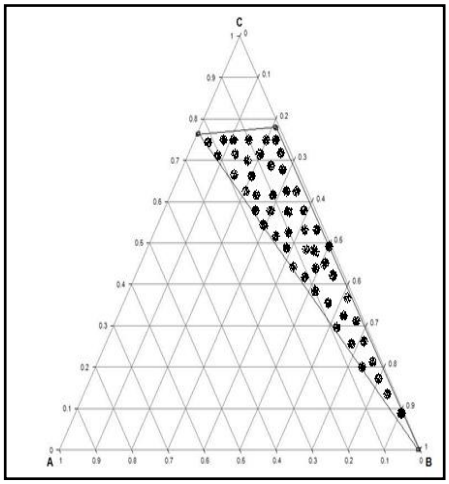
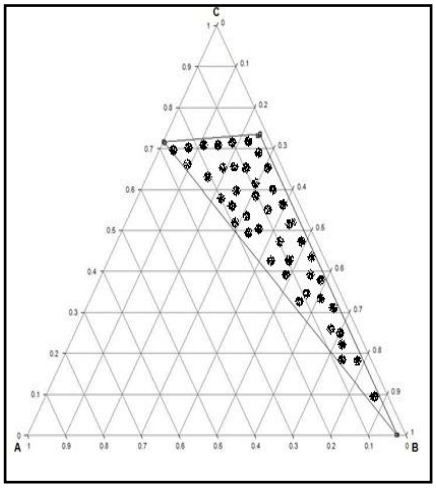
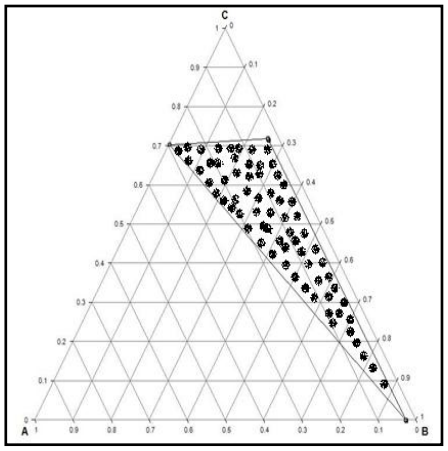


C7IIA



C7IIB



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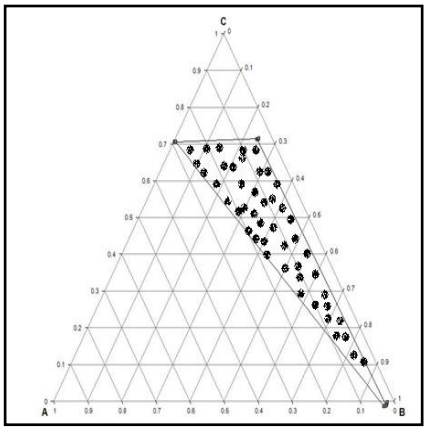
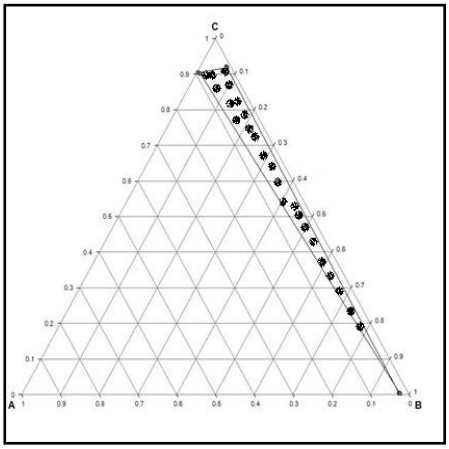
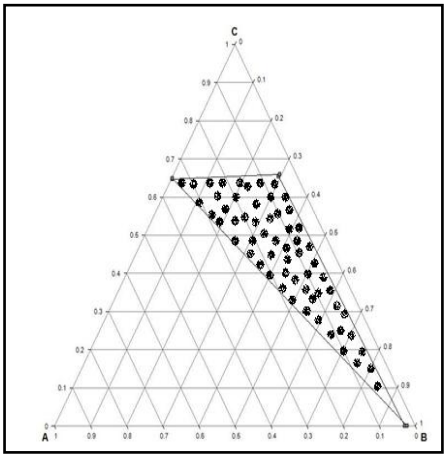


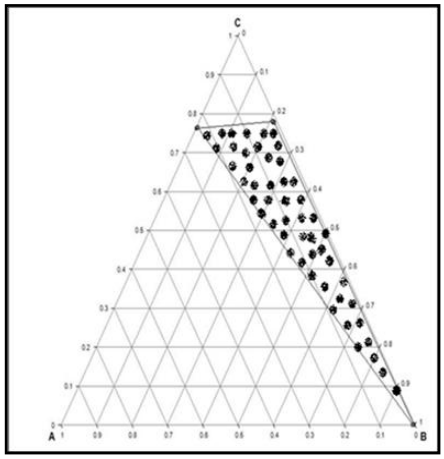
C7IIIA



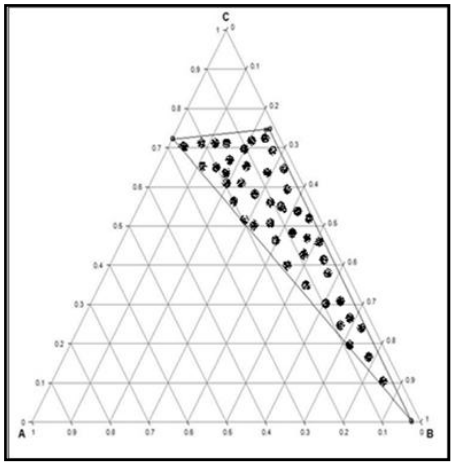
C7IIIC



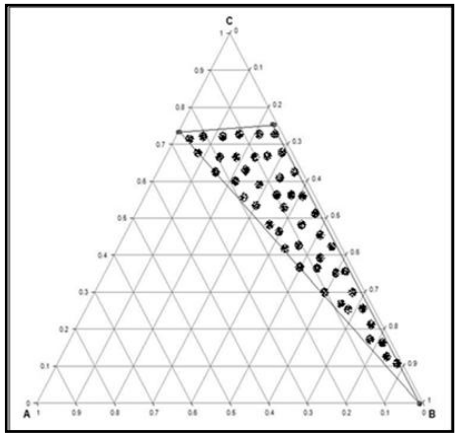
C7IIID



C7IIIB



C4IIB



C4IIIB

FIGURE 8.6: Phase diagrams for various liquid SMEDDS formulations

TABLE 8.20: Visual assessment of candesartan cilexetil SMEDDS formulations showing amount of water needed for dilution

I								
Batch	**A**	**VA**	**B**	**VA**	**C**	**VA**	**D**	**VA**
C2	58.5	**b**	50.4	**b**	60.5	**b**	33.5	**c**
C4	17.8	**a**	16.9	**a**	72.5	**b**	13.9	**a**
C7	17.6	**a**	15.3	**a**	67.8	**b**	10.6	**a**
C8	18.6	**a**	17.4	**a**	69.3	**b**	14.4	**a**
II								
Batch	**A**	**VA**	**B**	**VA**	**C**	**VA**	**D**	**VA**
C2	56.3	**b**	52.6	**b**	56.8	**b**	27.5	**a**
C4	16.2	**a**	14.8	**a**	65.8	**b**	13.2	**a**
C7	14.7	**a**	13.6	**a**	67.5	**a**	12.5	**a**
C8	18.2	**b**	16.4	**a**	63.8	**b**	15.4	**a**
III								
Batch	**A**	**VA**	**B**	**VA**	**C**	**VA**	**D**	**VA**
C2	54.7	**b**	47.2	**b**	55.5	**b**	27.5	**a**
C4	14.5	**a**	13.2	**a**	63.5	**a**	12.8	**a**
C7	12.5	**a**	10.8	**a**	57.8	**a**	9.5	**a**
C8	15.7	**a**	12.2	**a**	62.5	**a**	13.8	**a**

Where, VA- Visual assessment, a-transparent, and b- Whitish. Values in table indicate Amount of water in ml required to form microemulsion.

It was found that formulations C4 and C7 are clear whereas C2 and C8 become cloudy on dilution. Higher concentration of co-surfactant(C) as compared to concentration of surfactant (A, B and D) showed poor microemulsion. From the results of pseudoternary phase diagram it was revealed that formulation C7II covers the maximum microemulsion region as compared to other formulations whereas other formulations makes microemulsion which are unstable on dilution and have poor microemulsion region.

8.1.4 Viscosity and pH:

The viscosity of microemulsion systems can be monitored by standard rheological techniques. All the formulation has viscosity which is highly similar to that of water i.e.1.0. Thus, it shows that SMEDDS forms o/w microemulsion and water remains as external phase. The results of viscosity are as shown in Table 8.21.

The excipients used in the formulation decide the pH of the final preparation. The change in the pH may affect the zeta potential of the formulation which in turn can affect the stability of preparation. All the formulations showed similar pH values in the range of 5.1 to 6.0. Thus pH is not affecting stability. Therefore it can be assumed that drug is not diffusing in the external phase and remains in the oil phase. Since, water is the external phase entire system showed pH of water. Candesartan cilexetil is unstable in alkaline pH. Here the formulations show acidic to neutral pH which is suitable for stability of Candesartan cilexetil.

TABLE 8.21: Viscosity and pH of various SMEDDS formulations

Formulation code	Viscosity (cp)	pH
C 4III D	0.8865	5.12
C7III D	0.8887	5.57
C4 II B	0.8812	5.88
C7 II B	0.8824	5.14

8.1.5 Dispersibility test:

When microemulsion formulation is infinitely diluted , there is every possibility of it to phase separate leading to precipitation of a poorly soluble drug as microemulsion are formed at a particular concentration of oil, surfactant and water. For oral microemulsions the process of dilution by the GI fluids will result in the gradual desorption of surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC. [88] In the present study, we used distilled water as a dispersion medium because it is well reported that there is no significant difference in the microemulsions prepared using nonionic surfactants, dispersed in either water or simulated gastric or intestinal fluid. Formulations that passed Dispersibility test in Grade A and B were taken for further study, as Grade A and B formulations will remain as microemulsions when dispersed in GIT. All the formulation that were falling in Grade C, D and E of Dispersibility tests were discarded for further study. Keeping the criteria of increasing oil concentration and minimum amount of surfactant used for its solubilization, one formulation for each percent of oil (5%, 10% and 15%) was selected irrespective of the Smix ratio used for that percent of oil. Optimized formulations were taken for *in-vitro* release study, globule size and viscosity determination**.** The results for the Dispersibility test are as shown in Table 8.22.

8.1.6 Thermodynamic stability:

SMEDDS are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano-or microemulsion from emulsions that have kinetic stability and will eventually phase separate.[92] Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which survived thermodynamic stability tests, were taken for Dispersibility test. It was observed that formulation C1, C3, C5 and C6 did not pass the thermodynamic stress tests and thus were dropped for further study .The results are as shown in Table 8.22.

TABLE 8.22: Thermodynamic stability and dispersibility test of different formulations

(A) Formulations I with 5% oil concentration

Formulation code	Oil:S/CoS ratio	H/C	Cent.	Freeze Thaw.	Disperse. Grade	Inference
C2	A	**X**	**X**	**X**	Xx	Failed
	B	**X**	**X**	**X**	Xx	Failed
	C	**X**	**X**	**X**	Xx	Failed
	D	**X**	**X**	**X**	Xx	Failed
C4	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	**X**	**X**	**X**	Xx	Failed
	D	√	√	√	+	Passed
C7	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	√	√	√	+	Passed
	D	√	√	√	+	Passed
C8	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	√	√	√	+	Passed
	D	√	√	√	+	Passed

(B) Formulations II with 10% oil concentration

Formulation code	Oil:S/CoS ratio	H/C	Cent.	Freeze Thaw.	Disperse. Grade	Inference
C2	A	**X**	**X**	**X**	Xx	Failed
	B	**X**	**X**	**X**	Xx	Failed
	C	**X**	**X**	**X**	Xx	Failed
	D	√	√	√	+	Passed
C4	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	√	√	√	+	Passed
	D	√	√	√	+	Passed
C7	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	√	√	√	+	Passed
	D	√	√	√	+	Passed
C8	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	**X**	**X**	**X**	Xx	Failed
	D	√	√	√	+	Passed

(C) Formulations III with 15% oil concentration

Formulation code	Oil:S/CoS ratio	H/C	Cent.	Freeze Thaw.	Disperse. Grade	Inference
C2	A	**X**	**X**	**X**	Xx	Failed
	B	**X**	**X**	**X**	Xx	Failed
	C	**X**	**X**	**X**	Xx	Failed
	D	√	√	√	+	Passed
C4	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	√	√	√	+	Passed
	D	√	√	√	+	Passed
C7	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	√	√	√	+	Passed
	D	√	√	√	+	Passed
C8	A	√	√	√	+	Passed
	B	√	√	√	+	Passed
	C	√	√	√	+	Passed
	D	√	√	√	+	Passed

Where; √- passed and X- Failed. Whereas, +- clear, xx- Slightly whitish and Xx- whitish. Heating cooling cycle (H/C), centrifugation (Cent.), freeze-thaw cycle (Freez. Tha.), Dispersibility test (Disperse.)

8.1.7 Particle size distribution (PSD) and ζ-potential analysis:

From the results of pseudoternary phase diagram, formulations C4 and C7 were further characterized for measurement of particle size and zeta potential.

The droplet size of the emulsion is a crucial factor in self-emulsification process because it determines the rate and extent of drug release as well as drug absorption. Also, it has been reported that the smaller particle size of the emulsion dro1plets may lead to more rapid absorption as well as enhance the bioavailability of the formulation. [93] Fig.8.7 (a) and 7(b) shows the particle size distribution of candesartan cilexetil SMEDDS diluted with water and 0.1mol/l HCl, respectively. The average particle size of candesartan cilexetil SMEDDS is as shown in Table 8.23-8.26. The optimal batch was C7IIB with mean particle size 9.15 nm in water.

The resulting microemulsion produced was with a small mean size and a narrow particle size distribution regardless of the dispersion medium. The charge of SEDDS is another important property that should be assessed. [94] The effect of drug on the ζ-potential is shown in Table 8.26. All formulations were diluted with purified water to avoid error caused by the dispersion medium and the ζ-potential of the resulting emulsions was measured using a Coulter counter. The blank SMEDDS formulation exhibited almost no charged emulsion whereas a negatively charged emulsion was obtained with drug-loaded SMEDDS. This may be because the emulsifier used in the formulation was a nonionic-surfactant. The optimal batch C7IIB had the least zeta potential i.e. -23.2 mV with highest zeta potential towards negative side. The zeta potential governs the stability of microemulsion, it is important to measure its value for stability samples. The high value of zeta potential indicates electrostatic repulsion between two droplets. DLVO theory states that electric double layer repulsion will stabilize microemulsion where electrolyte concentration in the continuous phase is less than a certain value.

TABLE 8.23: Particle size of the various SMEDDS formulations

Formulation code	Avg. Particle size	
	Distilled Water	**0.1 NHCL**
C7 II D	56.4	105.7
C7II B	**9.15**	**24.5**
C4 II D	226	421
C4II B	157	275
C7 I D	50.5	55.8
C7 I B	32.35	137.9
C4I D	224	258
C4 I B	102.1	93.6

TABLE 8.24: Particle size distribution of C7IIB in 0.1 N HCl

Parameter	**Size (nm)**
Di (90)	**24.5**
Di (50)	**11.3**
Di (10)	**6.7**

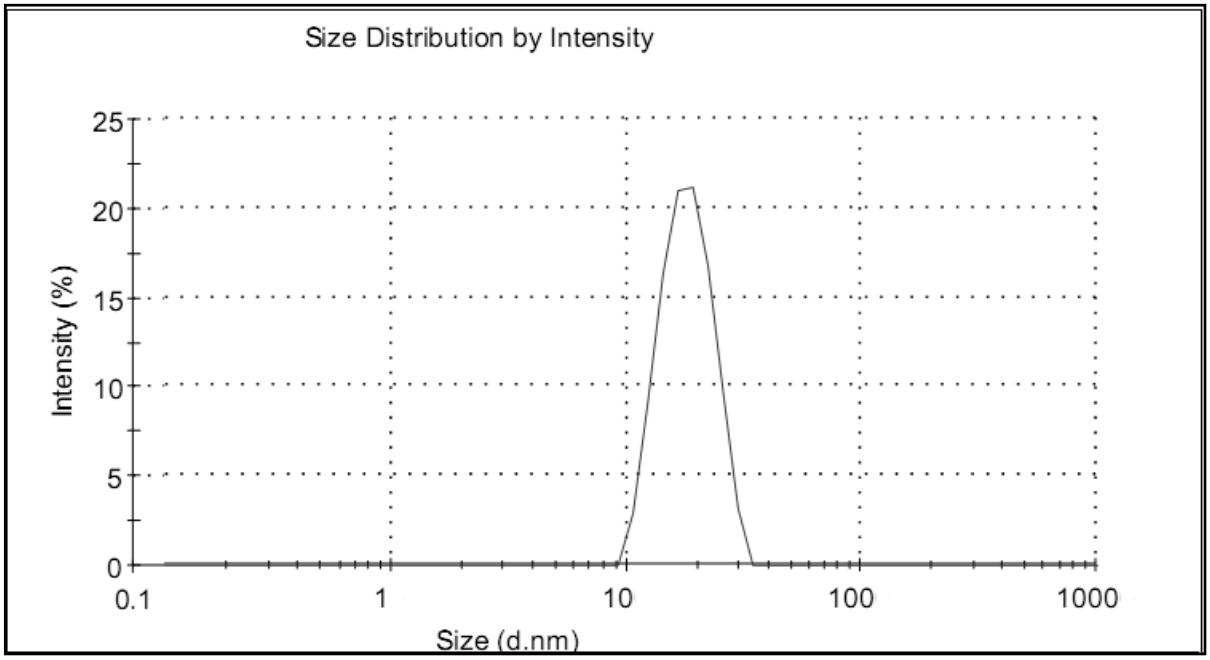


FIGURE 8.7 (a): Particle size distribution of formulation C7IIB in 0.1 N HCL

TABLE 8.25: Particle size distribution of C7IIB in water

Parameter	Size (nm)
Di (90)	**9.15**
Di (50)	**7.58**
Di (10)	**5.11**

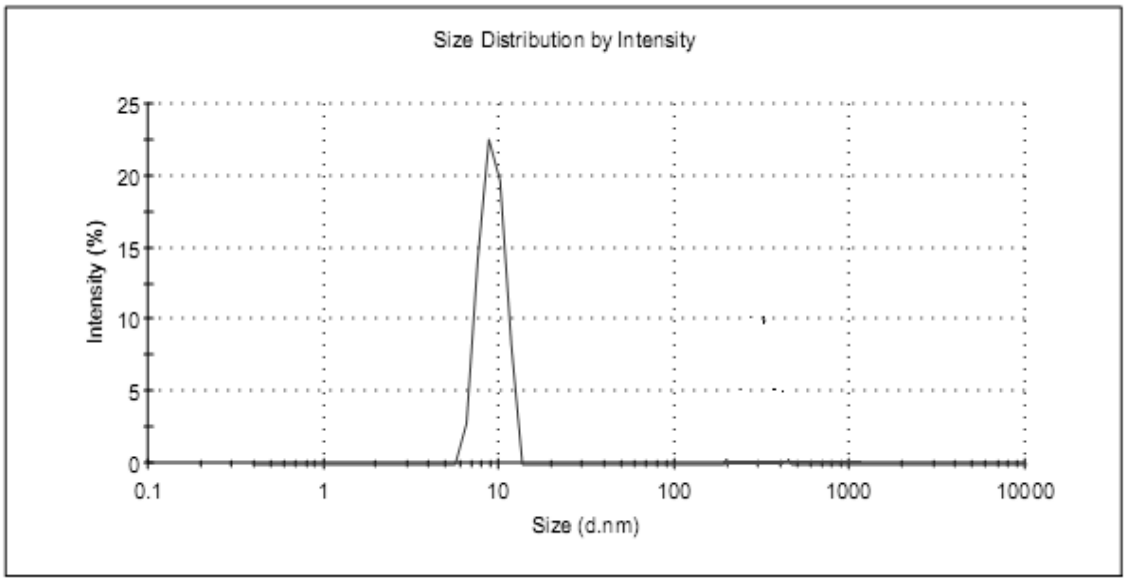


FIGURE 8.7 (b): Particle size distribution of formulation C7IIB in Water

The zeta potential for various candesaratn cilexetil SMEDDS formulations are as shown in table as follows;

TABLE 8.26: Zeta potential of the various SMEDDS formulations

Formulation code	Zeta potential
C7II D	-16.9
C7II B	**-23.2**
C4II D	-14.4
C4II B	-12.3
C7I D	-19.1
C7I B	-20.2
C4I D	-12.6
C4I B	-6.54

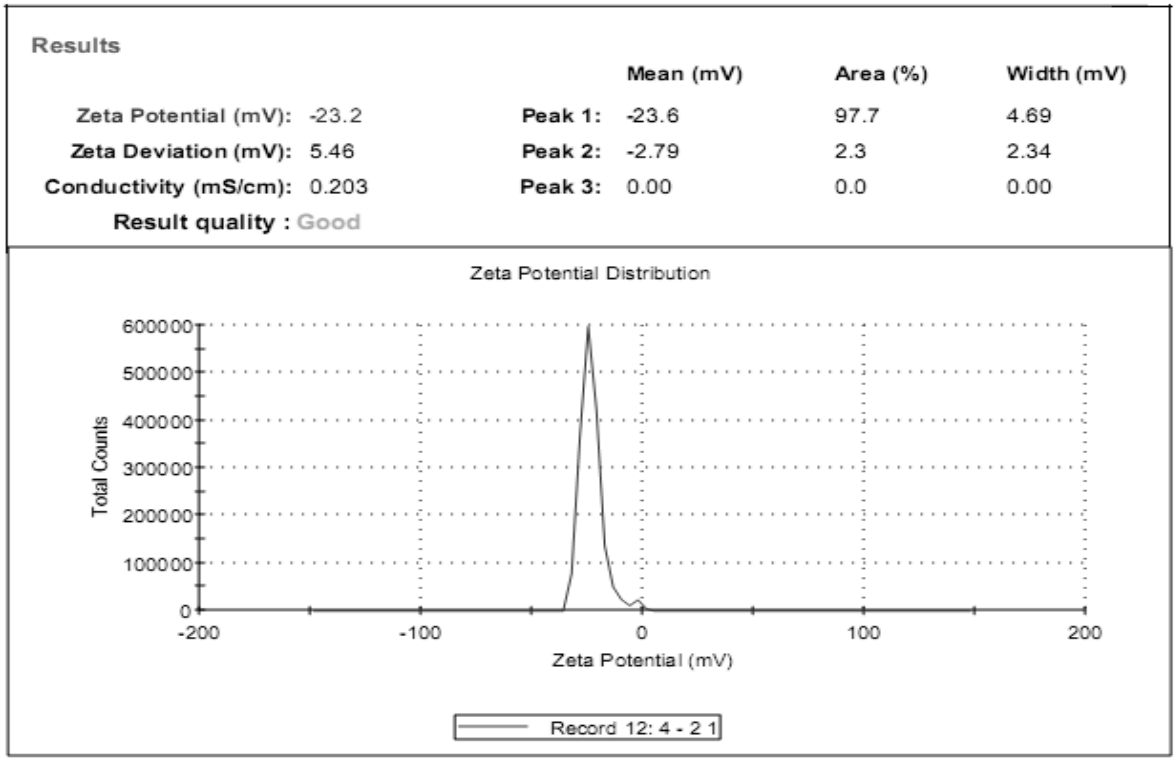


FIGURE 8.8: Zeta potential for formulation C7IIB

8.1.8 % Transmittance:

The clarity of microemulsions was checked by transparency, measured in terms of transmittance (%T). SMEDDS forms o/w microemulsion since water is external phase. Formulation C7 has % transmittance value greater than 99%.These results indicate the high clarity of microemulsion. The results of %T are as shown in Table 8.27.

TABLE 8.27: % Transmittance for C7IIB formulation

Period (months)	%T	
	25˚C	40˚C
0	99.7±0.2	99.6±0.3
1	99.6±0.5	99.4±0.2
2	99.4±0.3	99.2±0.5
3	99.3±0.6	95.4±0.7
6	99.1±0.2	80.3±0.6

***Mean; n=3**

8.1.9 Polydispersibility Index (PDI):

Polydispersibility which determines size range of particles in the system is measured by;

$$\frac{\textbf{No.of particles having size greater than 100 nm}}{\textbf{No. of particles having size less than 100 nm}}$$

It is expressed in terms of polydispersibility index (PDI). An ideal SMEDDS should be widely distributed with particles less than 100 nm and so PDI should be less than 0.3 or in other words particles having size more than 100 nm should be maximum up to 23%.The data are as shown in table 8.28. The results show that formulations C3ID and C3IB does not pass the test as they have PDI more than 0.3 whereas remaining all formulations pass the test as they have PDI less than 0.3.

TABLE 8.28: Polydispersibility index of Candesartan cilexetil SMEDDS formulations

Formulation Code	PDI
C4 II D	0.136
C4II B	0.096
C7 II D	0.246
C7II B	0.221

8.1.10 *In- vitro* diffusion study:

Sink conditions are often violated when using conventional release methods for dispersed systems. So, methods must be developed for SMEDDS to separate the dissolved drugs from micro emulsion-associated drugs before their determination. It has been reported that a dialysis method and an ultra filtration method have been applied to candesartan cilexetil SMEDDS, and a relatively high release rate was obtained using the latter. In this study, a bulk-equilibrium reverse dialysis bag method was developed to allow an increase in the membrane surface area available for transport from the donor to the receiver phases and,

hence, to maintain sink conditions in the donor phase by infinite dilution of the emulsion in the outer vessel.

In the dissolution media, 0.02% of tween 20 was added since it provided better discrimation between the formulations. The faster dissolution from SMEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and upon exposure to dissolution medium results in small droplets that can dissolve rapidly in the dissolution medium.

The dissolution profile for formulations C2IIB, C4IIB, C7IIB and C8IIB is as shown in the Fig. 8.9. The formulation C7IIB showed highest release rate among all the liquid SMEDDS formulations i.e. 92.01% in 10 min which is highest among all batches. In this case, the drug was present in form of micro globules of microemulsion and water was aqueous phase. Due to low size of microemulsion particles, they easily diffuse through the dialysis membrane. The results indicate that candesartan cilexetil SMEDDS can be diluted previously with aqueous phase before performing the *in-vitro* release test in dialysis bag. Thus, *in-vitro* study concludes that release of candesartan cilexetil was greatly enhanced by SMEDDS formulation. The batch C7IIB was thus taken for further studies and comparison.

TABLE 8.29: Comparison of *In-vitro* drug release of various liquid SMEDDS formulations

Time (min)	C2IIB	C4IIB	C7IIB	C8IIB
0	0	0	**0**	0
5	40.3	65.2	**84.6**	51.40
10	56.55	76.63	**92.01**	67.74
20	72.14	91.24	**96.36**	75.90
30	80.68	96.54	**98.76**	84.23
45	93.20	98.50	**99.48**	95.18
60	96.80	99.41	**99.91**	98.73

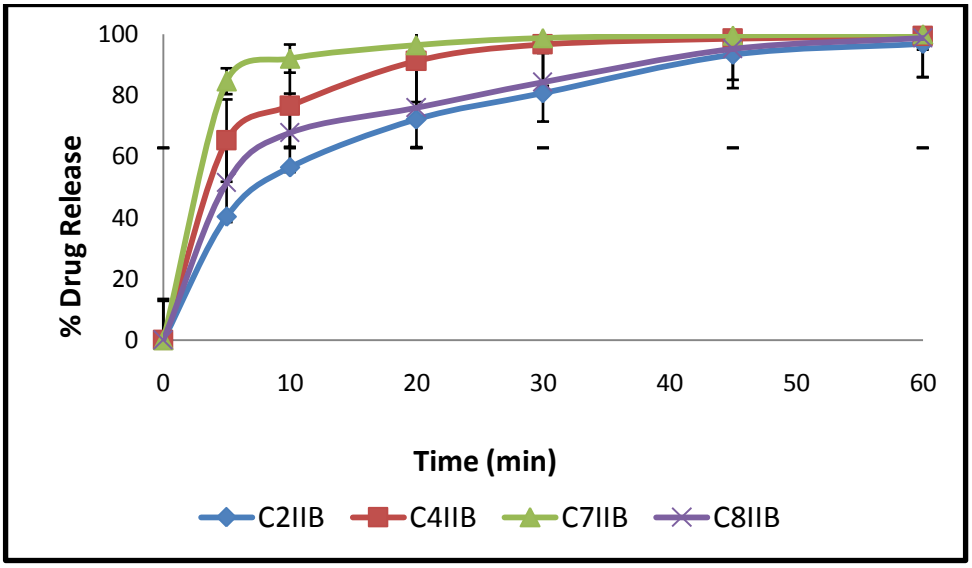


FIGURE 8.9:*In-vitro* diffusion study of various SMEDDS formulation

The results obtained from the comparison of release rate of various liquid SMEDDS formulations were subjected to Two way ANOVA. The results are as shown in Table 8.30.

TABLE 8.30: ANOVA for comparison of *In-vitro* drug release of various liquid SMEDDS formulations

Source	Degree of freedom	Sum of sq. (S.S)	Mean of S.S	F-Ratio	P-Value
Between rows (RSS) (Time)	5	24716	4943.2	27.22	<0.0001
Between columns (CSS) (Ratio of S/Cos)	4	14159.4	3539.85	19.49	<0.0001
Error	20	3631.42	181.57		
Total	29				

Calculated F (20,4) 19.49 > 2.87 (Table value $F_{20,4,\ 0.05}$)

Hence, it can be concluded that there is significant difference because the calculated value is more than the table value. It can also be said that the change in the time have a significant effect on the release rate of the formulation. The compositions of various formulations i.e. combination of oil and S/CoS also affect the release rate of drug from formulation.

The comparison of *in-vitro* release of C7IIB, M and pure drug (S) are as shown in Fig.8.10.

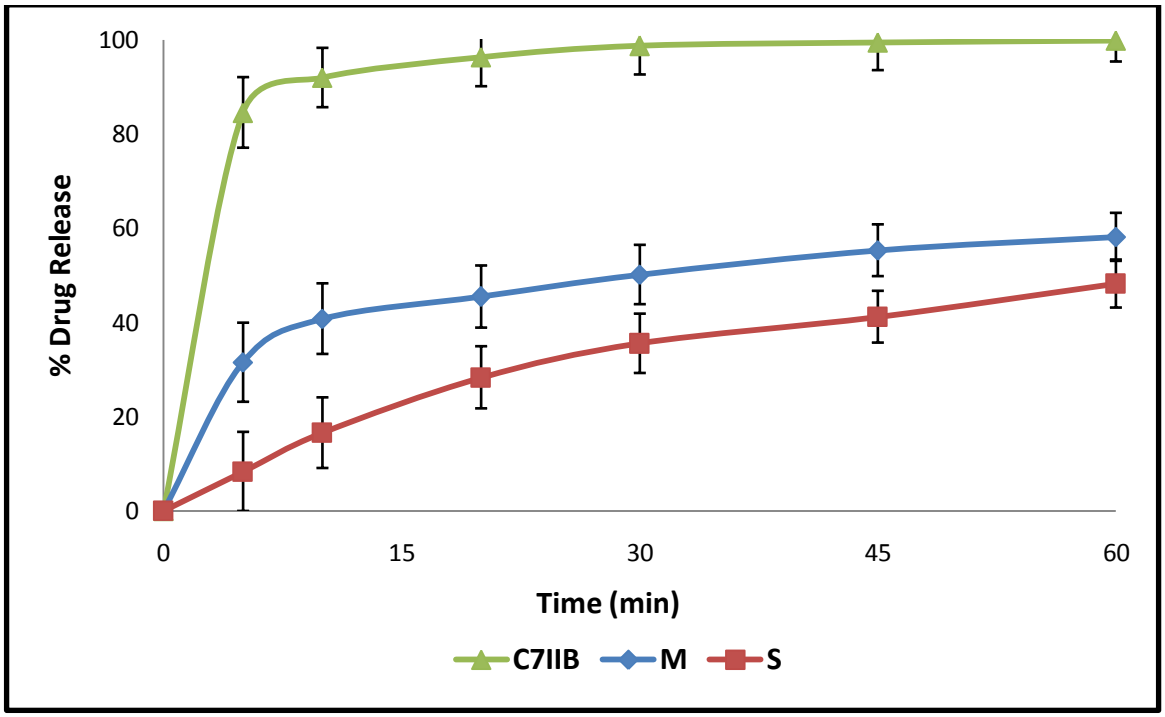


FIGURE 8.10: *In-vitro* diffusion study of C7IIB , M and S

The results obtained from the comparison of *In vitro* dissolution study of the optimized liquid formulations C7IIB with marketed tablet (M) and pure drug (S) were also subjected to two way ANOVA. The results are as shown in Table 8.31.

TABLE 8.31: ANOVA for comparison of *In-vitro* drug release of various formulations

Source	Degree of freedom	Sum of sq. (S.S)	Mean of S.S	F-Ratio	P-Value
Between rows (RSS) (Time)	5	13096.3	2619.26	9.069	0.00039
Between columns (CSS) (Type of dosage form)	3	13779.4	4593.29	15.903	<0.0001
Error	15	4232.22	288.81		
Total	23				

Calculated $F_{(15, 3)}$ 15.903 > 3.29 (Table value $F_{15, 3, 0.05}$)

As per the hypothesis it can be said that there is significant difference, as calculated value is more than table value. The formulation C7IIB has the maximum release rate at all the time as compared to the Atacand Tablet (M) and pure drug (S).From the results of all the characterizations, it was found that formulation C7IIB was the optimized liquid SMEDDS formulation and thus it was carried for stability studies and for further solidification study. The various results of C7IIB are as shown in Table 8.32;

TABLE 8.32: Various result of C7IIB

PARAMETERS	RESULTS
Particle size distribution (PSD)	9.15 nm
Zeta potential	-23.2 mV
Polydispersibility Index(PDI)	0.221
Viscosity	0.8824
pH	5.14
In-vitro drug release (10 min)	92.01%

8.1.11 Stability Studies:

On the basis of results of all characterization points it was found that formulation C7IIB was the optimized formulation therefore its stability study was performed.

The stability study was performed as per ICH guidelines, conditions can be decided based on that particular zone. An accelerated stability (40±2˚C / 75 ± 5 %) and real time stability study (25±2˚C / 60 ± 5 %) was performed on the formulation C7IIB for a period of three months.

Formulation C7IIB shows decreased in the assay content at the accelerated condition which indicates instability of formulation at this condition. Results were shown in Table 8.33.

TABLE 8.33: Results of characterization tests initially and after accelerated stability studies

Parameters	Initial data	Data after Accelerated stability studies		
		1M	2M	3M
Assay (%)	100	93.58	87.23	81.76
Particle size (nm)	9.15 nm	9.30	10.68	11.16
Zeta potential (mV)	-23.2	-23.2	-22.9	-22.2

TABLE 8.34: Results of characterization tests initially and after Real time stability studies

Parameters	Initial Data	Data after Real time stability Studies		
		1M	2M	3M
Assay (%)	100	99.56	98.94	98.26
Particle size (nm)	9.15 nm	9.20	10.24	10.87
Polydispersibility Index	0.221	0.209	0.192	0.186
Zeta potential (mV)	-23.2	-23.0	-22.8	-22.2
% Transmittance	99.1	98.92	98.80	98.65

TABLE 8.35: Particle size distribution of C7IIB after 3 month Real time stability study

Parameter	Size (nm)
Di (90)	10.87
Di (50)	9.58
Di (10)	6.11

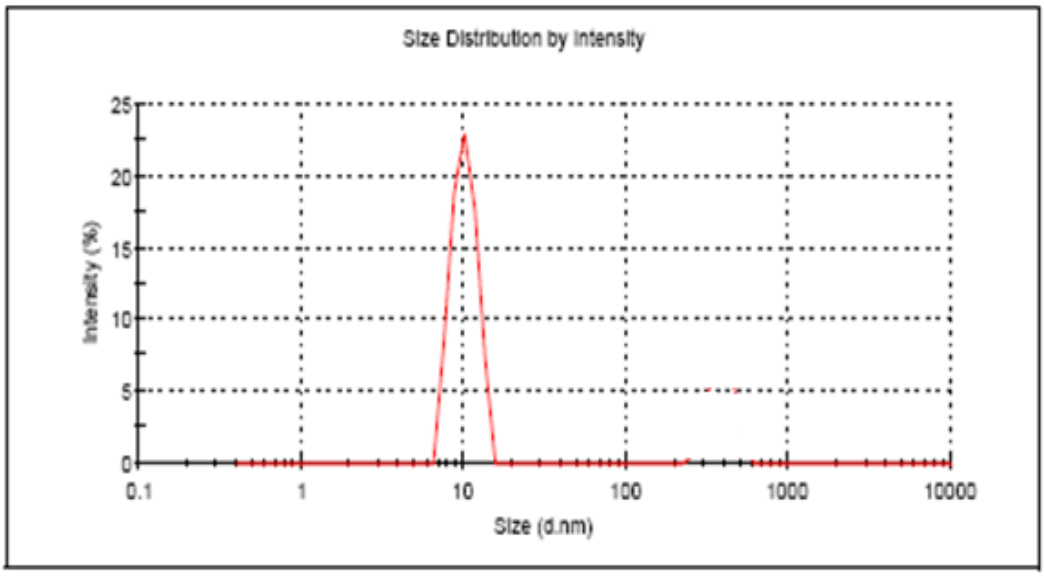


FIGURE.8.11 :Particle size distribution of C7IIB in Water after 3 month Real time stabiity study

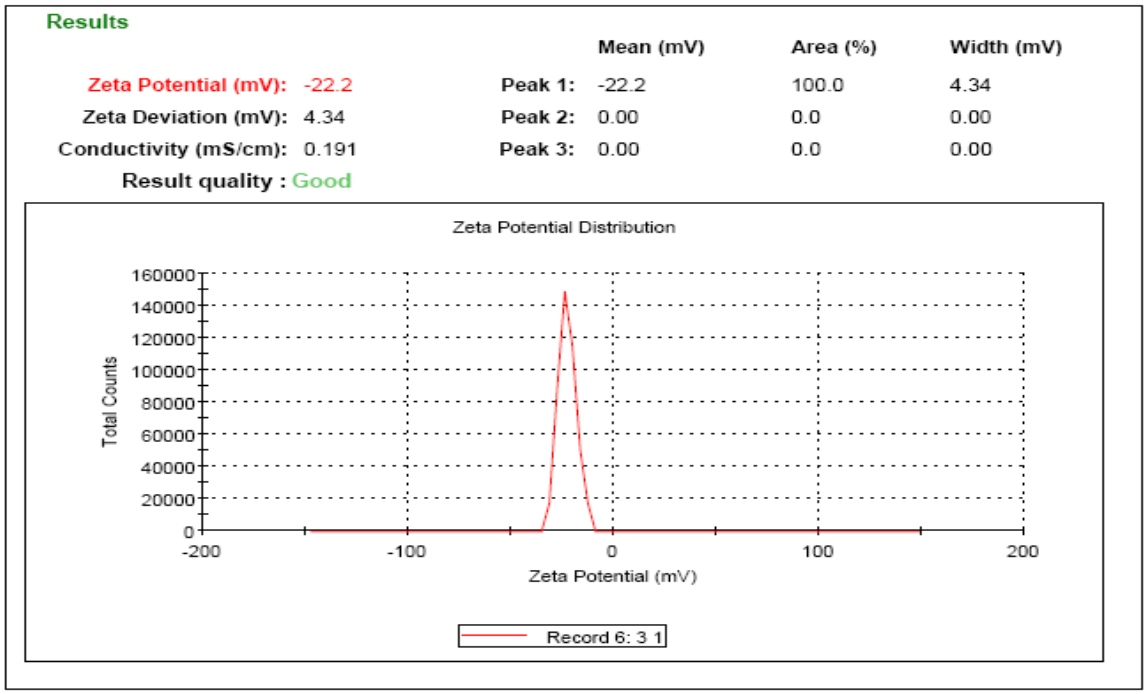


FIGURE 8.12: Zeta potential of C7IIB after 3 month Real time stability study

TABLE 8.36: *In-vitro* dissolution of C7IIB after real time study

Time (min)	% Drug released			
	Initial	1M	2M	3M
0	0	0	0	0
5	84.60	83.23	82.93	81.54
10	92.01	91.10	91.04	90.61
20	96.36	96.22	96.02	96.00
30	98.76	98.55	98.05	97.98
45	99.48	99.2	99.03	98.84
60	99.91	99.57	99.35	99.10

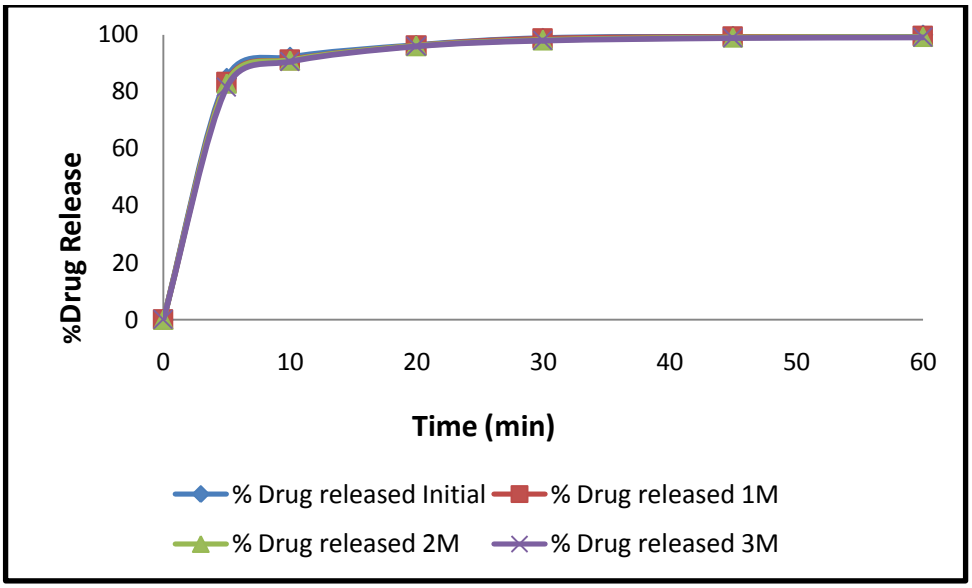


FIGURE. 8.13: *In-vitro* dissolution of C7IIB after real time study

The values clearly prove that after the stability study, formulation C7IIB doesn't show significant difference for particle size, zeta potential and PDI. After 3 months stability study the particle size of C7IIB was found to be 10.87 nm in water and the initial particle size was 9.15 nm, so no significant difference was found. The PDI was found to be 0.221 initially and 0.186 after stability study. The zeta potential was initially found to be -23.2 mV and after stability study it was found to be -22.2 mV. Results for the *in-vitro* dissolution were found to be satisfactory and it was shown in Table 8.36.

This result indicates that all the excipients used are compatible and hence form stable microemulsion with almost same particle size. Since, zeta potential governs the stability of microemulsion, it is important to measure its value for stability samples. The high value of zeta potential indicates electrostatic repulsion between two droplets. DLVO theory states that electric double layer repulsion will stabilize microemulsion where electrolyte concentration in the continuous phase is less than a certain value. A negative force means a negative potential between the droplets.

8.2 RESULTS OF SOLID SELF MICRO EMULSIFYING SYSTEM OF CANDESARTAN CILEXETIL:

8.2.1 Adsorbent selection:

- Three different adsorbent (Aerosil 200 pharma, Aeroperl 300 pharma and Fujicalin SG) were used to convert liquid SMEDDS into free flow powder. Among this adsorbents Aeroperl 300 pharma require only 60 mg to convert liquid SMEDDS (0.2 ml containing 32 mg drug) into free flow powder whereas Aerosil 200 pharma require 180 mg and Fujicalin SG require 110 mg. Final weight of adsorbent after addition of liquid SMEDDS was measured by using electronic balance. All results are shown in Table 8.37.
- Results of powder characteristic were shown in Table 8.38.From the results it was found that powder of all adsorbent have good flow property.
- Free flow powder of all three different adsorbent containing 32 mg of drug was filled into capsule and *in-vitro* dissolution was performed. Aeroperl 300 pharma based free flow powder gives 63 % drug release within 5 min and 90% drug release within 15 min which was faster drug release as compare to two other adsorbents based on free flow granules of liquid SMEDDS. Results were shown in Table 8.39.
- From the above results it was concluded that Aeroperl 300 pharma was better adsorbent as compare to other adsorbent used in the study so solid state characterization was performed for Aeroperl 300 pharma based free flow powder by SEM.
- SEM of Aeroperl 300 pharma and solid SMEDDS was shown in Fig. 8.15. Surface of Aeroperl 300 pharma was rough before and after adsorption of liquid SMEDDS a smooth surface was observed which indicate that liquid SMEDDS was adsorbed on the surface of Aeroperl 300 pharma.

TABLE 8.37: Adsorbent selection

Adsorbent	Amount of Liquid SMEDDS (ml)	Amount of adsorbent required to get free flow powder (mg)
A1	0.2	180
A2	0.2	60
F1	0.2	110

TABLE 8.38: Powder characteristics of all adsorbent after adsorption of liquid SMEDDS

Adsorbent	Parameters				Inference
	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's index %	Hausner's ratio	
A1	0.397	0.543	14.6	1.36	Passable
A2	0.594	0.744	15.0	1.25	Excellent
F1	0.471	0.601	13.2	1.27	Passable

TABLE 8.39: *In vitro* release of candesartan cilexetil from free flow powder of different adsorbents

Time (min)	% Drug released from free flow powder		
	A1	A2	F1
0	0	0	0
5	35.18± 2.3	63.23± 1.5	51.84± 3.6
10	50.84± 3.0	76.40± 2.0	63.71± 4.7
15	68.27± 2.5	90.72± 3.6	80.69± 2.1
30	80.34± 1.8	98.37± 1.4	91.78± 3.3
45	92.16± 2.0	101.14± 2.2	97.12± 2.5
60	92.67±3.2	100.32± 1.7	98.54± 2.8

***Mean of n=3**

The graphical representation of *in-vitro* study release of candesartan cilexetil from free flow powder of different adsorbents with standard errors is as shown below in Fig 8.14;

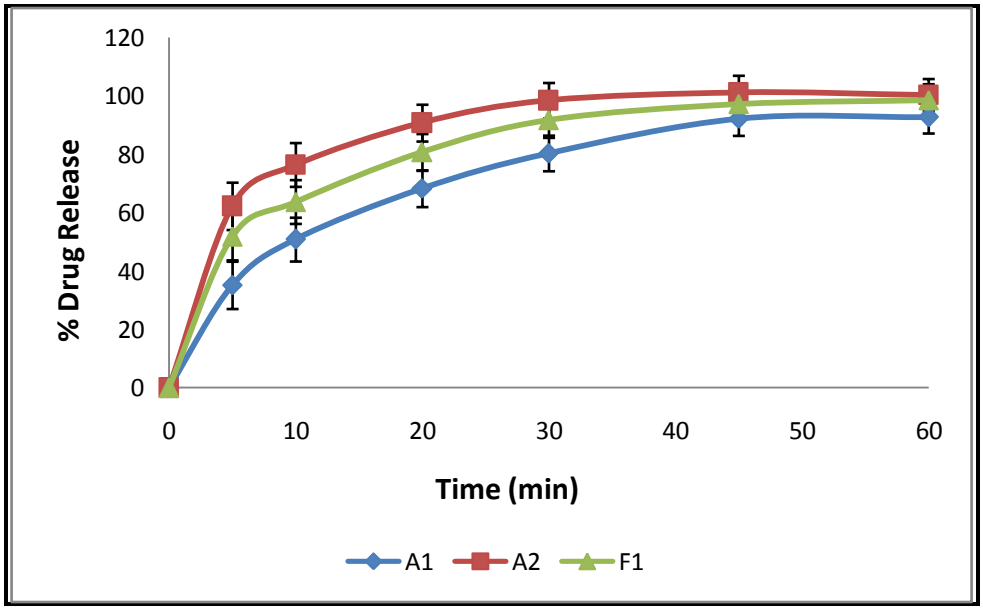


FIGURE.8.14: *In-vitro* dissolution study of drug from various adsorbent

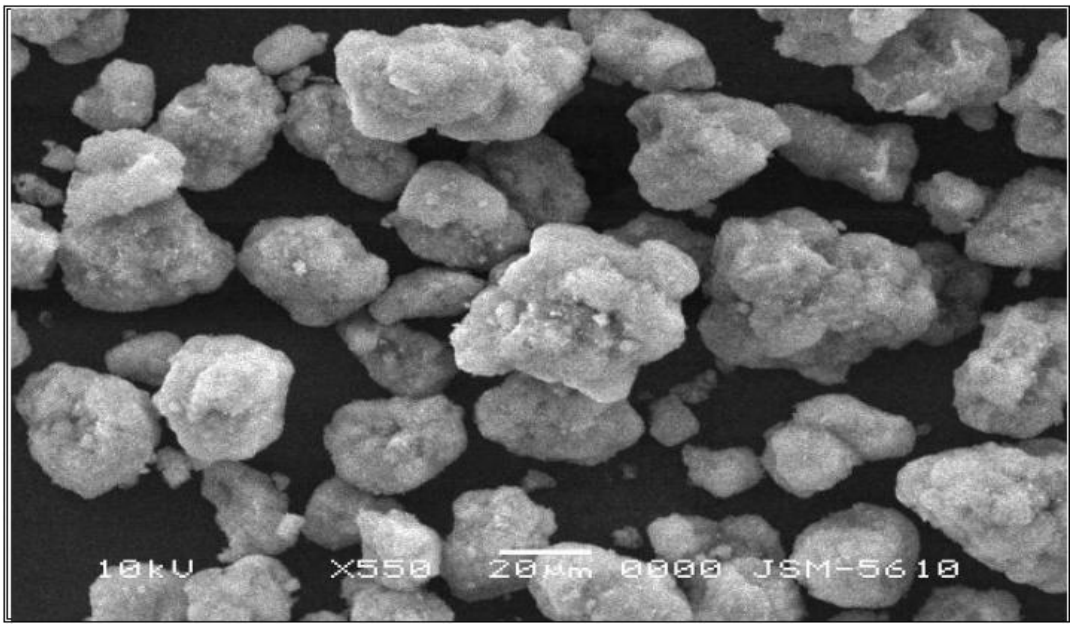


FIGURE.8.15 (a): SEM OF AEROPERL 300 pharma

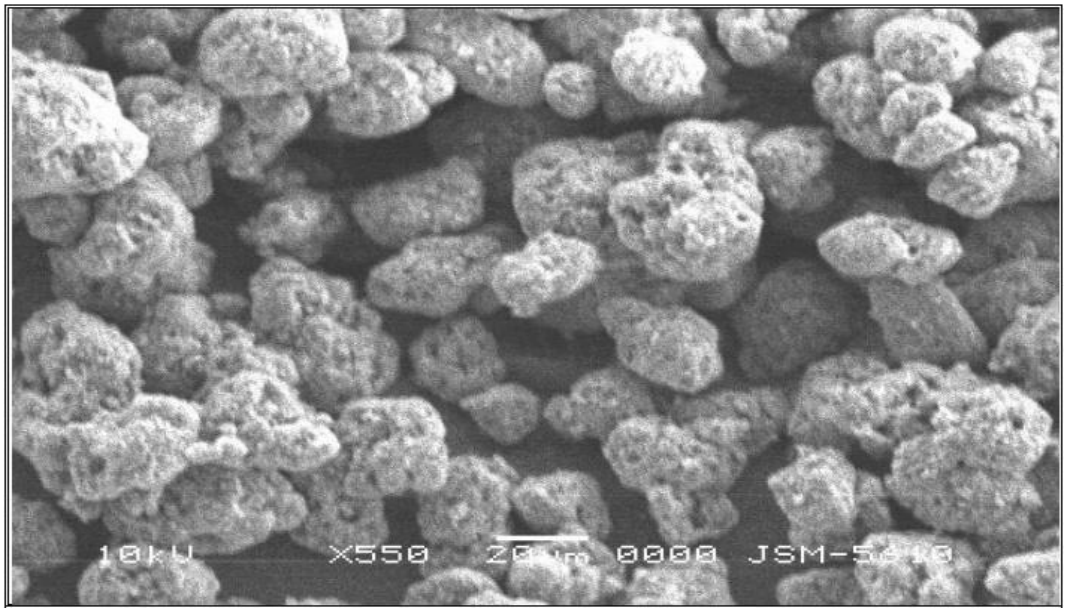


FIGURE. 8.15(b): SEM OF AEROPERL 300 Pharma AFTER ADSORPTION

8.2.2 CHARACTERIZATION OF MARKETED SAMPLE

Marketed sample Atacand 32 mg tablet was characterized for different parameters and the results were shown in Table 8.40.

TABLE 8.40. Characterization of marketed sample

Sr No	Parameters	Results	
1.	Weight	288.27 mg	
2.	Thickness	10 mm	
3.	Hardness	3.1	
4.	Shape	ROUND (circular, biconvex-shaped)	
5.	Dissolution test	**Time (min)**	**% Drug released**
		5	32.47
		10	43.81
		15	47.59
		30	51.87
		45	56.32
		60	59.26

8.2.3 RESULTS FOR CANDESARTAN CILEXETIL S-SMEDDS TABLET FORMULATION:

8.2.3.1 EVALUATION OF TABLETS

- Tablet evaluation parameters of batch T1 to T9 were shown in Table 8.42. All parameters were found to be satisfactory and within the specification for candesartan cilexetil tablet.
- *In-vitro* drug release was performed for the batch T1 to T9 and the results were shown in Table 8.43.All batches shows approximately 70% drug released within 5 minutes but among this batch T4 shows 78.32 % drug released in 5 minutes which was faster as compare to other batches. Only batch T4 and T7 prepared form lactose monohydrate shows almost 90 % drug released within 10 min. In batch T7 amount of disintegrant pregelatinized starch was higher (7.5%) as compare to batch T4 (5%) so batch T4 was considered as an optimized batch and was used for further study.
- The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as drug absorption. Particle size of the batch T4 in water was found to be 78.3 nm which was higher as compare to optimized liquid SMEDDS formulation C7IIB which has particle size 9.15 nm. Results were shown in Table 8.41.
- Zeta potential of the formulation was found to be -17.4 mV for the batch T4 which was higher as compare to optimized liquid SMEDDS formulation C7IIB which has zeta potential value -23.2 mV.
- The DSC thermogram of pure drug Candesartan cilexetil and Solid SMEDDS are as shown in Fig.8.19. Pure drug substance shows a sharp endothermic peak at 169^0Cwhich shows the highly crystalline behavior of drug. Whereas, no peak was observed in S-SMEDDS formulation which shows the change in melting behavior of drug and inhibition of crystallization following granulation using lipid surfactants.

TABLE 8.41: Particle size distribution of T4 in water

Parameter	Size (nm)
Di (90)	78.3
Di (50)	45.7
Di (10)	20.1

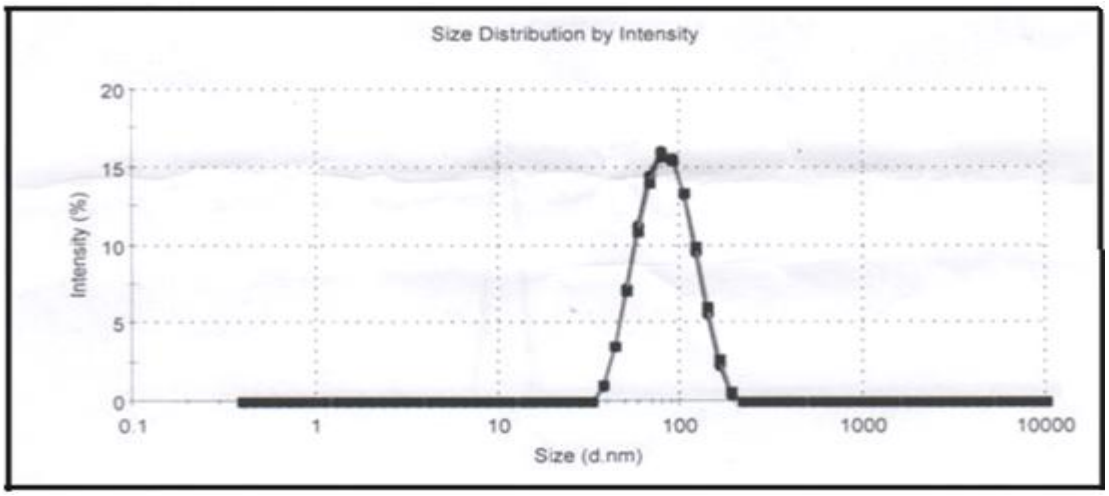


FIGURE.8.16: Particle size distribution of T4 in water

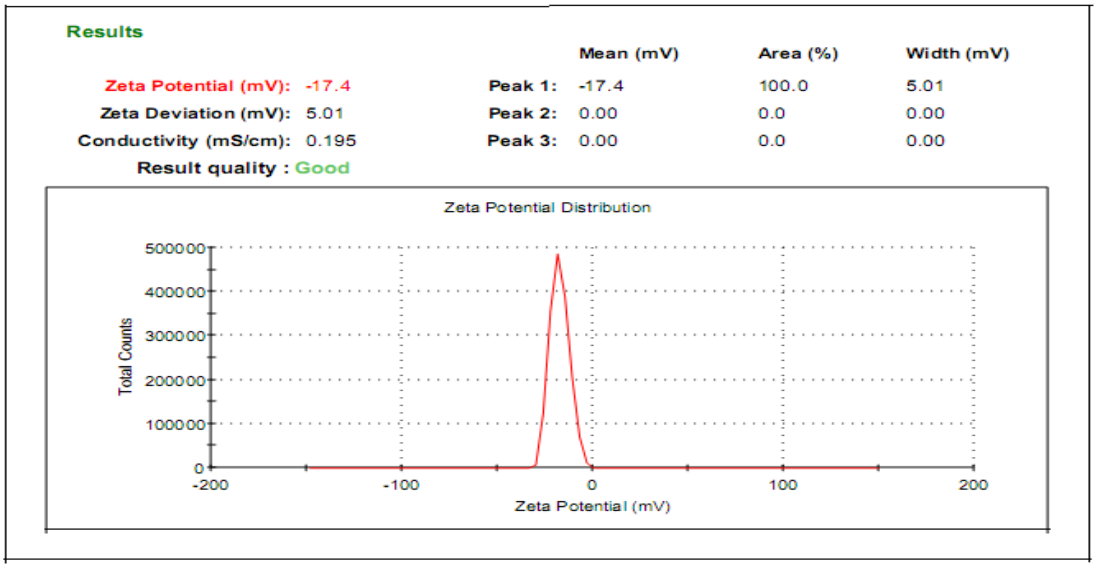


FIGURE.8.17: Zeta potential of T4 in water

TABLE 8.42: Evaluation parameters for S-SMEDDS tablet of Candesartan Cilexetil

Batch	Evaluation Parameters				
	Weight variation (mg)	**Hardness (kg/cm^2)**	**Thickness (mm)**	**Friability (%)**	**Disintegration time (sec)**
T1	205±2.1	2.3	3.12 – 3.26	0.26	50
T2	195±1.5	2.8	3.10 – 3.21	0.28	70
T3	207±1.9	3.8	3.16 – 3.32	0.14	78
T4	201±1.3	3.2	3.15 – 3.24	0.15	30
T5	198±2.5	4.1	3.11 – 3.22	0.30	60
T6	200±2.3	4.2	3.14 – 3.28	0.17	66
T7	199±2.7	2.5	3.18 – 3.30	0.10	42
T8	200±1.7	2.7	3.20 – 3.31	0.24	56
T9	197±1.9	4.2	3.17 – 3.22	0.21	58

TABLE 8.43*: In vitro* dissolution study of S-SMEDDS formulations

Time (min)	%Drug released								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
0	0	0	0	0	0	0	0	0	0
5	72.54	70.56	71.52	78.32	72.12	73.16	74.31	71.86	72.16
10	88.23	82.88	86.12	90.38	80.72	88.32	90.68	86.18	84.21
15	93.36	88.12	94.51	97.66	86.26	95.50	95.40	90.14	92.44
30	99.12	93.24	98.44	99.36	92.31	99.13	99.67	95.36	96.63
45	100.45	96.62	101.26	101.40	95.48	99.82	101.20	99.84	102.10
60	100.08	97.72	102.22	101.45	96.01	99.80	100.45	99.54	101.79

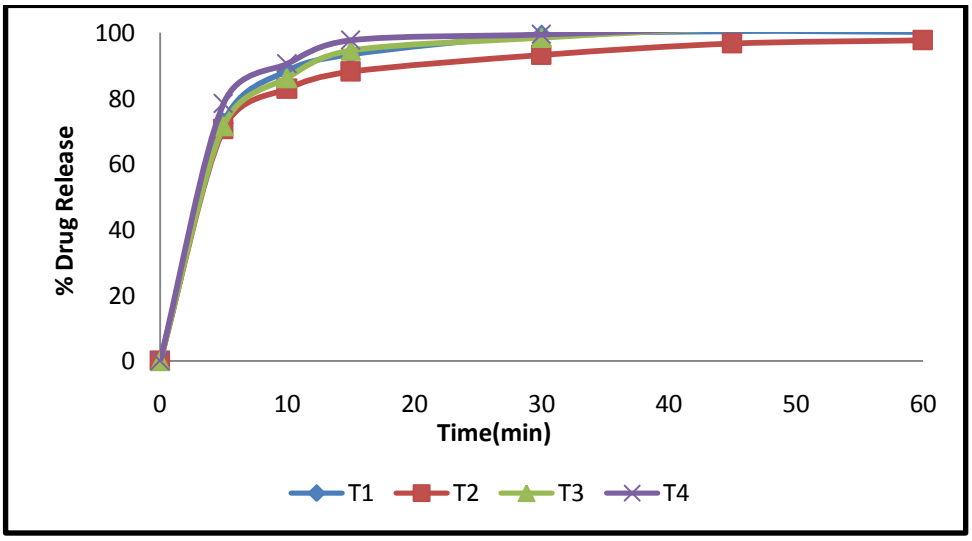


FIGURE.8.18 (a): *In vitro* dissolution of batch T1 to T4

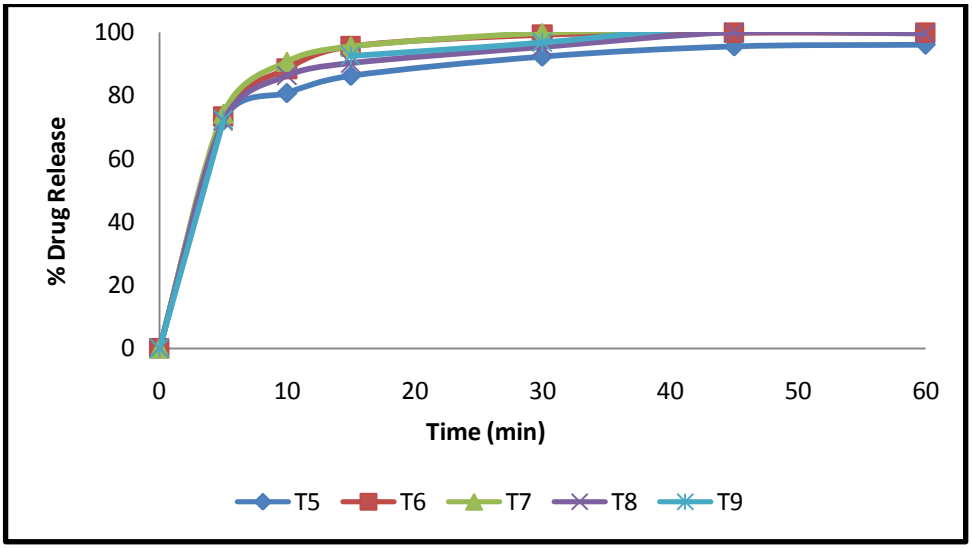


FIGURE.8.18(b): *In vitro* dissolution of batch T5 to T9

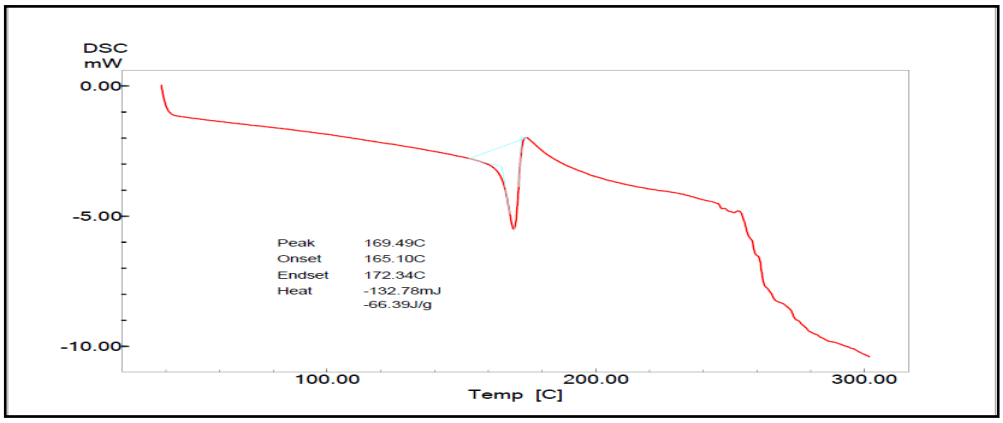


FIGURE.8. 19 (a): DSC thermogram of Pure candesartan cilexetil

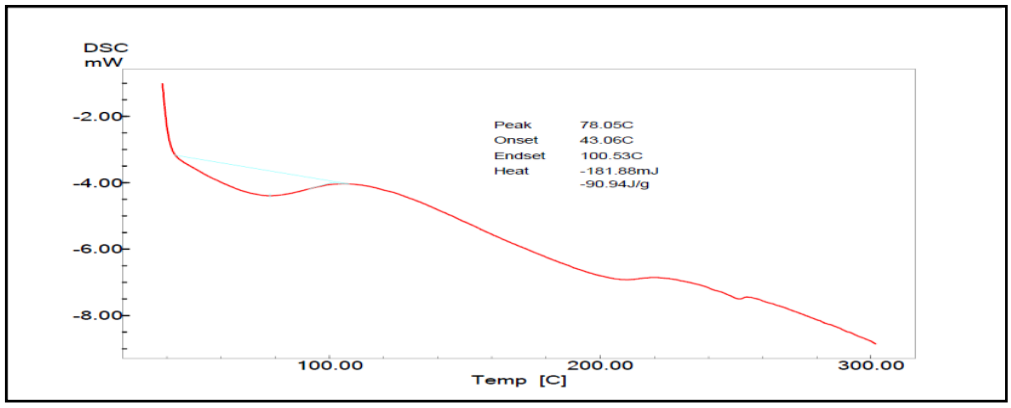


FIGURE.8.19(b): DSC thermogram of S-SMEDDS of candesartan cilexetil (T4)

8.3 3^2 FULL FACTORIAL DESIGN

- Lactose monohydrate, Mannitol and Microcrystalline cellulose (MCC) 102 were used as diluents. Tablets were found to be satisfactory when evaluated for weight variation, thickness, hardness, friability, and *in-vitro* release.
- The effect of various diluents (Lactose monohydrate, Mannitol and MCC) and the concentration of pre gelatinized starch were kept as independent variables X1 and X2 respectively.
- A statistical model, $Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1X_1 + b_{22}X_2X_2$, incorporating interactive and polynomial terms was used to evaluate the responses; where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1X_1 and X_2X_2) are included to investigate nonlinearity. The data clearly indicate that the DT, T70 and T90 values are strongly dependent on the selected independent variables. The fitted equations (full and reduced) relating the responses DT (sec), T70 (min) and T90(min) to the transformed factors are shown in Table 8.44-8.46. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., positive or negative).

TABLE 8.44: Various data for full factorial design

3^2 FULL FACTORIAL DESIGN					
Batch no.	**Variables levels in coded form**		**DT (sec)**	**T_{70} (min)**	**T_{90} (min)**
	X_1	**X_2**			
T1	**-1**	**-1**	**50**	**5**	**15**
T2	**-1**	**0**	**70**	**10**	**30**
T3	**-1**	**+1**	**78**	**10**	**15**
T4	**0**	**-1**	**30**	**5**	**10**
T5	**0**	**0**	**60**	**10**	**30**
T6	**0**	**+1**	**66**	**10**	**30**
T7	**+1**	**-1**	**42**	**5**	**10**
T8	**+1**	**0**	**56**	**10**	**15**
T9	**+1**	**+1**	**58**	**10**	**15**

TRANSLATION OF CODED LEVELS IN ACTUAL UNITS

Variables level	**Low (-1)**	**Medium (0)**	**High (+1)**
Pre-gelatinized starch conc. (X_1)	**5**	**10**	**15**
Effect of Diluents (X2)	**Lactose monohydrate**	**Mannitol**	**Microcrystalline cellulose 102**

TABLE 8.45: Data for the full model

Data	Correlation coefficient						
	B0	**B1**	**B2**	**B12**	**B11**	**B22**	**Multiple R**
DT (sec)	57.33	-7	13.33	-3	7	-8	0.97
T70 (min)	6.66	-1.66	1.66	-1.25	3.93E-17	-3.1E-16	0.88
T90 (min)	25	-4.16	1.66	1.25	-2.5	-10	0.82

The above table can be explained as follows;

Disintegration time:

Y=57.33-7x1+13.33x2-3X12+X11-8x22

The use of various diluents affects the disintegration time of the formulations.

T_{70}(min):

Y=6.66-1.66x1+1.66x2-1.25X12+3.93E-17X11-3.1E-16x22

The use of various diluents has more effect on this factor.

T_{90}(min):

Y=25-4.16x1+1.66x2+1.25X12-2.5X11-10x22

The use of various diluents has more effect on this factor.

TABLE 8.46: Data for the reduced model

Data	Correlation coefficient						
	B0	**B1**	**B2**	**B12**	**B11**	**B22**	**Multiple R**
DT (sec)	57.33	-	13.33	-	7	-	0.97
T70 (min)	6.66	-	1.66	-	3.93E-17	-	0.88
T90 (min)	25	-	1.66	1.25	-	-	0.82

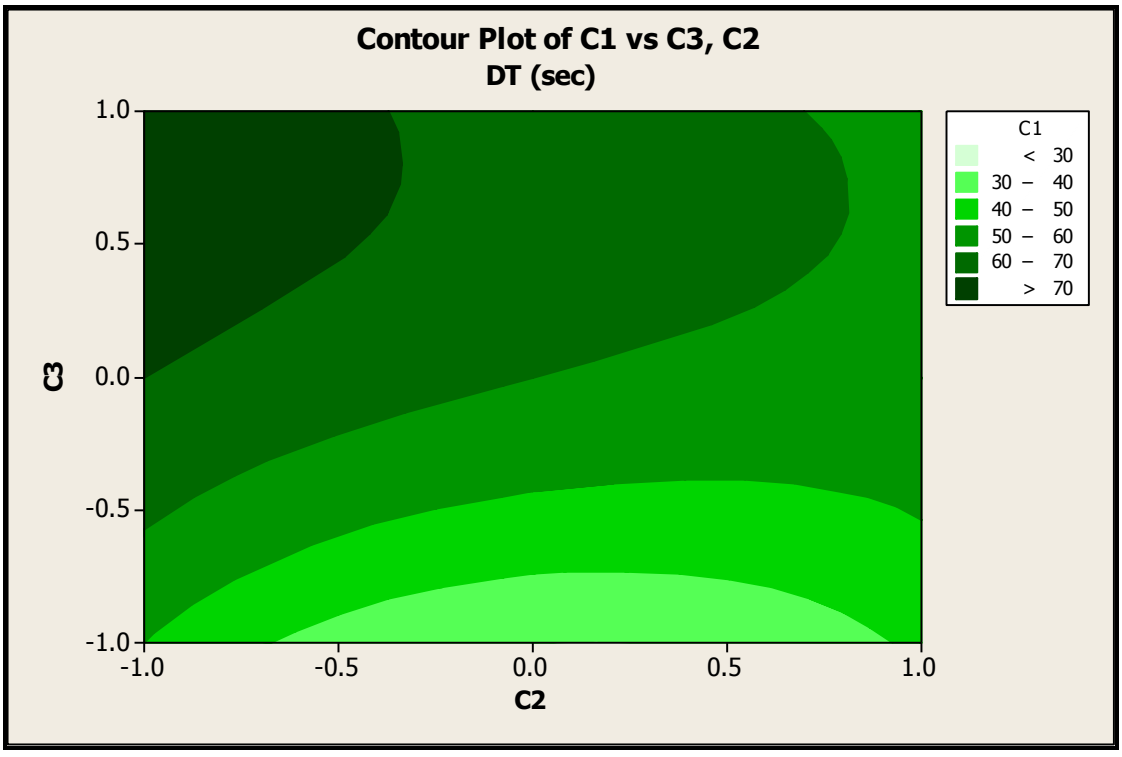


FIGURE.8.20 (a): Contour plot for DT (sec)

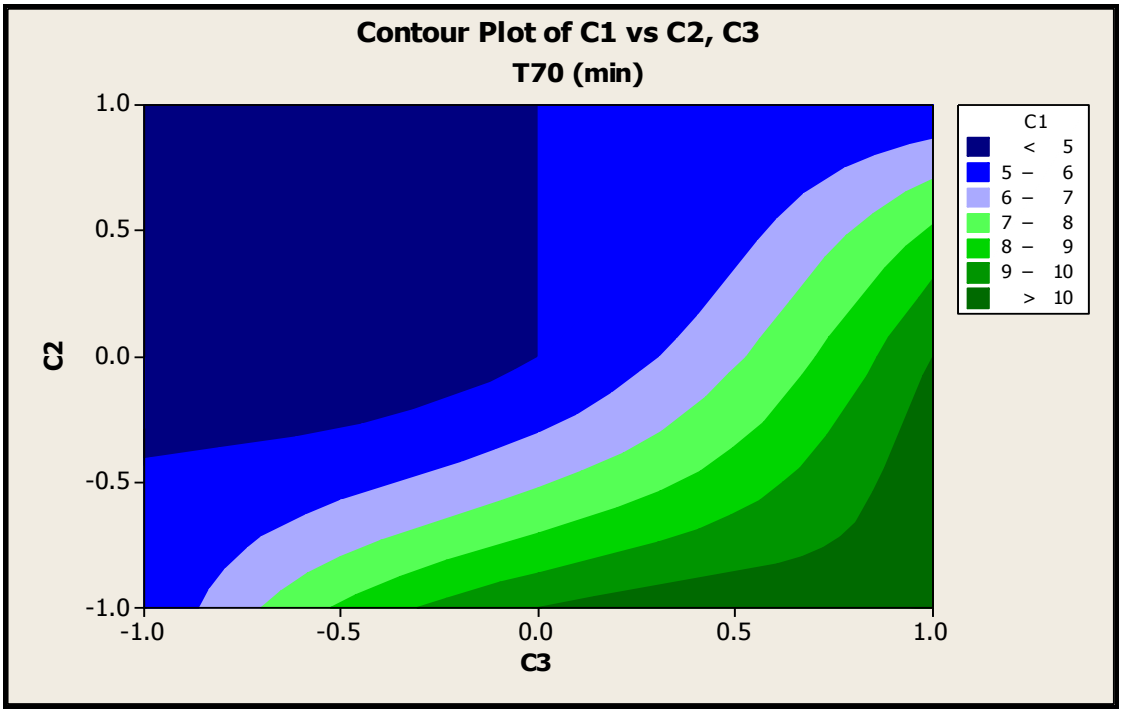


FIGURE.8.20 (b):Contour plot for t_{70} (min)

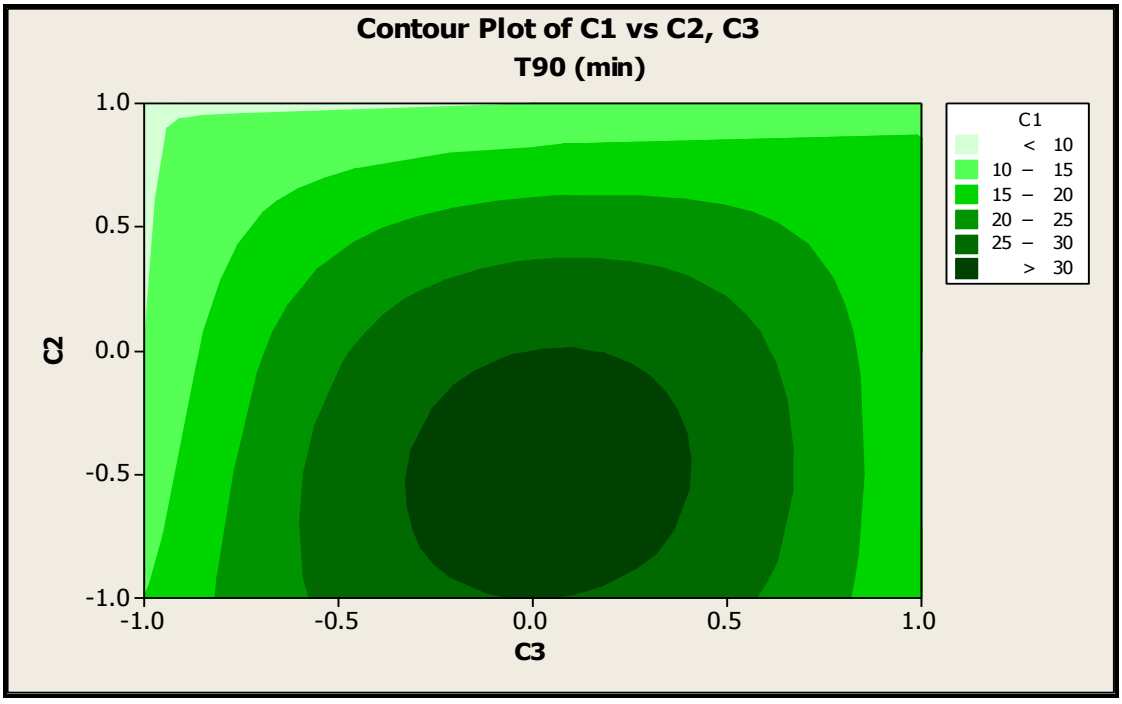


FIGURE.8.20 (C):Contour plot for t_{90} (min)

8.4 COMPARISON OF *IN-VITRO* DISSOLUTION OF BATCH T4 WITH MARKETED FORMULATION (ATACAND TABLET)

- *In-vitro* drug release of batch T4 was compare with the marketed tablet formulation i.e. Atacand 32 mg tablet (M). Marketed formulation shows just 32.47% drug release in 5 min whereas tablets of batch T4 shows 78.32 % drug release in same time. Batch T4 shows 90.38 % drug release in 10 min whereas marketed formulation shows 59.26 % drug release in 60 min. This data clearly indicate that by formulating S-SMEDDS formulation of candesartan cilexetil, solubility and thus dissolution profile of candesartan cilexetil was increased. Similarity factor (F2) value was found to be 14.89 and dissimilarity factor (F1) value was found to be 96.26 when both formulations were compared. So data shows that both formulations were dissimilar with respect to *in-vitro* drug released.
- *In-vitro* drug release of batch T4 was also compared with the optimized liquid SMEDDS formulation C7IIB. Batch T4 shows almost same dissolution profile as that of batch C7IIB. Initial drug release was found to me slightly slower in T4 as compared to C7IIB.
- Similarity factor (F2) value was found to be 65.84 and dissimilarity factor (F1) value was found to be 3.79 when *in-vitro* dissolution of solid SMEDDS (batch T4) was compare with the liquid SMEDDS (C7IIB).

TABLE 8.47: *In-vitro* dissolution comparison of M, T4 and C7IIB

Time (min)	% Drug released		
	M	T4	C7IIB
0	0	0	0
5	32.47	78.32	84.6
10	43.81	90.38	92.01
15	47.59	97.66	96.36
30	51.87	99.36	98.76
45	56.32	101.4	99.48
60	59.26	101.45	99.91

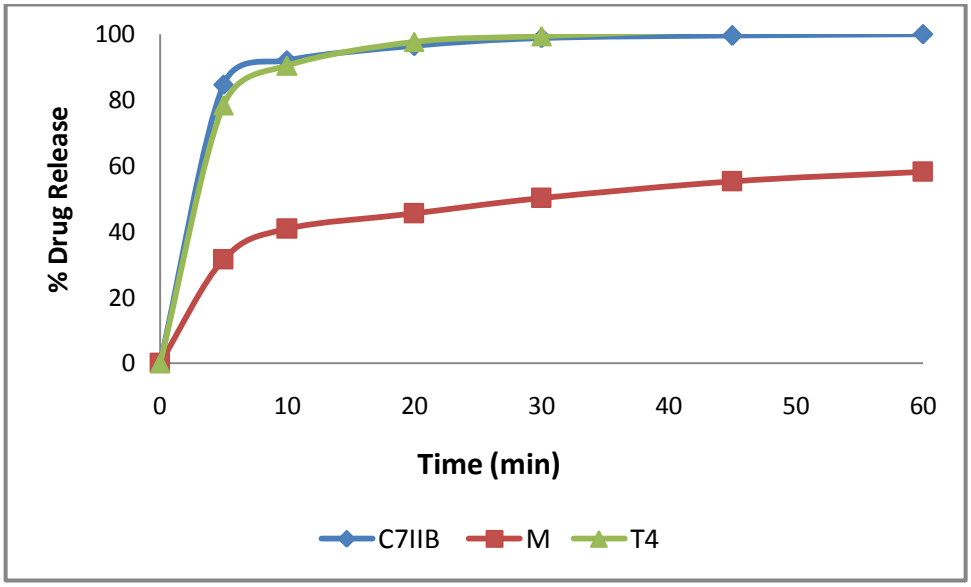


FIGURE.8.21: *In-vitro* dissolution comparison of M, T4 and C7IIB

8.5 STABILITY STUDIES:

- Accelerated stability study ($40^0C \pm 2^0C$ /75% ± 5% RH) and real time stability study ($25^0C \pm 2^0C$ /60% ± 5% RH) was performed on batch T4 for a period of three months. No significant changes were observed in appearance, average weight, hardness, thickness and friability of the tablets for both the condition. Results were shown in Table 8.48.
- Assay was decreased to 84.76 % in the sample stored at accelerated condition after three months which indicate that formulation were not stable at higher temperature. So other evaluation parameters like particle size, zeta potential and *in-vitro* dissolution was not performed on the sample store at accelerated condition. After three months storage of batch T4 at real time condition assay value was fond to be 98.83% which indicate formulation were stable at this condition.
- Initial value for particle size of the batch T4 was 78.3 nm and after stability it was found to be 79.2 nm. Zeta potential value after stability was found to be -17.1 mV and the initial value was -17.4 mV so data indicate that formulation was stable.

TABLE 8.48: Results of Accelerated stability study of Batch T4

Parameters	Initial	1M	2M	3M
Description	White colored, round shaped tablets plain on both side.			
Weight (mg)	205± 1.2%	200± 0.9%	200 ± 1.7%	198 ± 1.3%
Hardness (kg/cm2)	2.0 -3.0	3.0 -4.0	3.0 -4.0	2.5 -3.0
Thickness (mm)	3.15 – 3.24	3.10-3.22	3.18-3.24	3.14-3.25
Friability (%)	0.15	0.15	0.18	0.28
Assay (%)	100.4	94.80	91.25	84.76

TABLE 8.49: Results of Real time stability study of Batch T4

Parameters	Initial	1M	2M	3M
Description	White colored, round shaped tablets plain on both side.			
Weight (mg)	201 ± 5.2%	198 ± 1.7%	200±0.5%	200 ± 1.8%
Hardness (kg/cm2)	2.0 -3.0	2.5 -3.5	3.0 -4.0	3.0 -4.0
Thickness (mm)	3.17 – 3.23	3.12-3.20	3.14-3.24	3.10-3.23
Friability (%)	0.12	0.17	0.20	0.23
Assay (%)	100.4	99.75	99.18	98.83
Particle size distribution	78.3	78.6	79.0	79.2
Zeta potential	-17.4	-17.2	-17.0	-17.1

TABLE 8.50: Log % drug remained at different temperature

Time (days)	Temperature (°C)	For S-SMEDDS (T4)	
		%Drug remained	Log %Drug remained
0	30±0.5	100	2
15	30±0.5	99.3	1.99695
30	30±0.5	98.8	1.99476
45	30±0.5	98.2	1.99211
60	30±0.5	97.6	1.98945
0	40±0.5	100	2
15	40±0.5	97.32	1.9882
30	40±0.5	94.4	1.97497
45	40±0.5	92.34	1.96539
60	40±0.5	88.5	1.94694
0	50±0.5	100	2
15	50±0.5	70.21	1.8464
30	50±0.5	50.03	1.69923
45	50±0.5	39.81	1.59999
60	50±0.5	32.1	1.50651

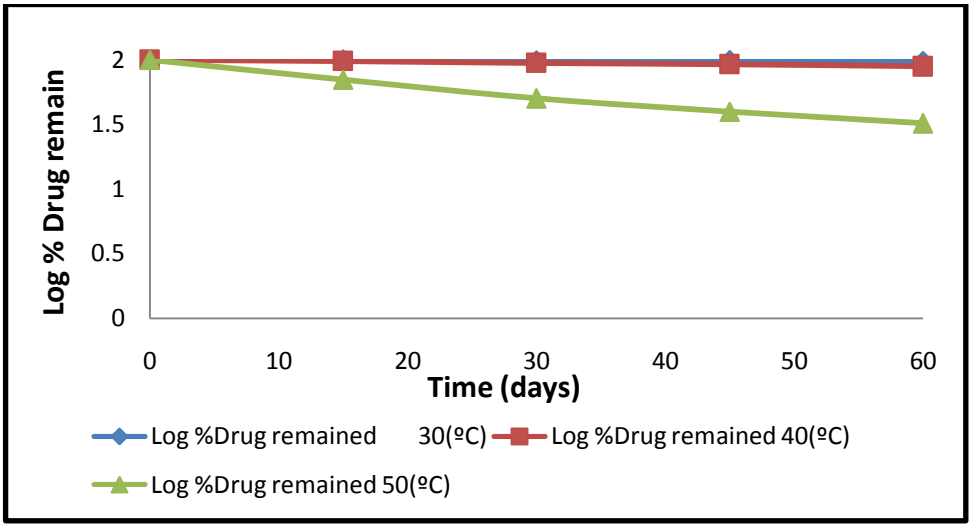


FIGURE.8.22: Log percent concentration of drug remaining versus time plot T4

TABLE 8.51: Various data required for Arrhenius Plot

Temp˚C	Temp ˚K	1/T*1000 k^{-1}	For T4		
			Slope	k*10^{-4}	log K
30	303	3.30033	-0.00018	4.0495	-3.39259
40	313	3.194888	-0.00088	20.3649	-2.69111
50	323	3.095975	-0.008	184.24	-1.73462
25	298	3.355705	-	1.28	-3.89114

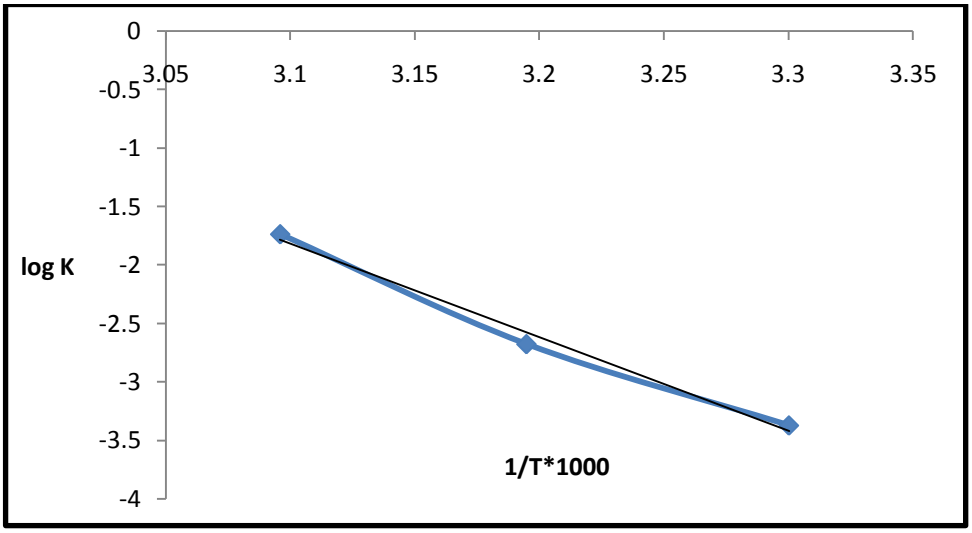


FIGURE.8.23: Arrhenius plot for T4

8.6 *In vivo* studies

8.6.1 Measurement of systolic Blood Pressure:

As shown in Table 8.52, systolic blood pressure of UNX rats after 4 weeks was 132.0±7.5 mmHg compared to DOCA-salt rats 176.0±2.1 mmHg, i.e. only uninephrectomy was not sufficient to develop significant rise in systolic blood pressure. All DOCA-salt rats showed mild but significant hypertension in two weeks. Table 8.52 shows % decrease in systolic blood pressure of rats after one week of treatment.

TABLE 8.52: % Decrease in systolic blood pressure

Group		Systolic blood pressure(mmHg)					% Decrease in systolic blood pressure after treatment**
		0 d	7 d	14 d	17 d	21 d	
UNX rats		104±1.5	117±2.6	122±3.1	–	132±7.5	–
DOCA rats	DC0	110±4.6	127±1.9	154±4.3	–	176±2.1	–
	DC1	107±3.2	120±2.6	154±3.1	137±2.2	127±3.2	17.94±0.357[a]
	DC2	108±3.3	125±3.6	152±4.8	144±3.2	137±3.3	13.69±0.415
	DC3	116±5.4	128±5.2	158±4.7	126±6.2	117±3.1	26.75±0.336[a]
	DC4	116±3.5	129±3.6	152±4.2	135±5.2	124±3.7	18.0±0.358

Where, ** %decrease in systolic blood pressure after one week of treatment in comparison to that of after 14 days (Actual measurements were done daily in all treatment groups but here for comparison one week time point was used); Each value represents the mean (n=4); a is $P < 0.05$, compared with rats receiving plain drug suspension; DC0: DOCA control (no treatment); DC1: rats receiving low dose S-SMEDDS suspension; DC2: rats receiving low dose plain drug suspension; DC3: rats receiving high dose S-SMEDDS suspension; DC4: rats receiving low dose plain drug suspension.

After one week treatment, DC1 rats [receiving low dose S-SMEDDS suspension] showed 17.94±0.357% and DC2 rats [receiving low dose plain drug suspension] showed 13.69±0.415% decrease in systolic blood pressure. Similarly, DC3 rats [receiving high dose S-SMEDDS suspension] showed 26.75±0.336% and DC4 rats [receiving high dose plain drug suspension] showed 18.0±0.358% decrease in systolic blood pressure. This significant enhancement in antihypertensive activity was clearly observed in Fig. 8.24 and attributed to micro sizing of candesartan cilexetil.

Thus, it was confirmed that Candesartan cilexetil decreases blood pressure in a dose-dependent manner and hence decrease in pressor effect can be directly correlated with the amount of drug that reaches systemic circulation i.e. bioavailability of drug. In other words, higher the inhibition of pressor effect, more the bioavailability of drug from administered formulation. The data also clearly demonstrated that inhibition of pressor effect was greater in rats receiving S-SMEDDS suspension in comparison to rats receiving plain drug suspension at both doses. Based on this pharmacodynamic study, it could be concluded that bioavailability of drug was higher from S-SMEDDS suspension in comparison to plain drug suspension.

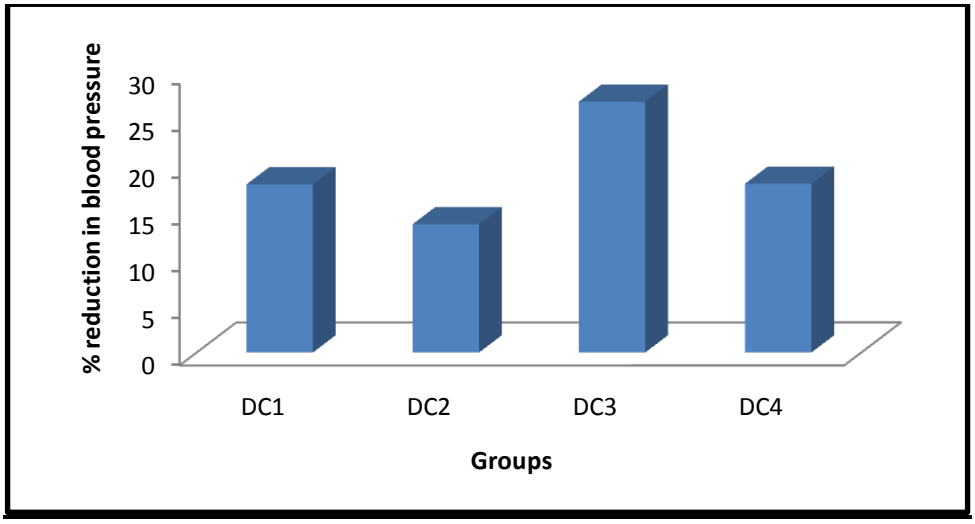


FIGURE.8. 24: % Decrease in systolic blood pressure after treatment in different groups.

8.6.2 Bioavailability Study:

In vivo pharmacokinetic behaviors of candesartan cilexetil with SMEDDS (T4) and marketed formulation (Atacand) were studied in rat. Mean plasma concentration was plotted as a function of time as shown in Fig. 8.25. The noncompartment model is used to evaluate pharmacokinetic parameters of candesartan cilexetil absorption which are summarized in Table 8.53. The linear trapezoidal rule is used to calculate the area under curve ($AUC_{0\rightarrow t}$)

Relative bioavailability was calculated using following formulae:

$$\text{Relative BA } (\%) = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{reference}}} \times \frac{\text{Dose}_{\text{reference}}}{\text{Dose}_{\text{test}}}$$

Plasma concentration C_{max} and $AUC_{0\rightarrow t}$ are significantly increased for S-SMEDDS than those for the Atacand suspension. T_{max} is decreased for S-SMEDDS(T4) and it was 1 h for S-SMEDDS(T4) and 1.36 h for Atacand formulation. Relative bioavailability is increased 1.78-fold. The results of $AUC_{0\rightarrow\infty}$ were compared using *t* test, and it was found that it is highly significant ($p < 0.01$) when S-SMEDDS and Atacand formulation were compared.

The non compartment model was used to evaluate pharmacokinetic paremeters of candesratan cilexetil absorption which is summarized in Table 8.53;

TABLE 8.53: Pharmacokinetic Parameters for T4 and M

Parameters	T4	M
t_{max} (h)	1 ± 0.37	1.36 ± 0.42
C_{max} (ng/mL)	115.51 ± 9.11	69.54 ± 3.87
$AUC_{0\rightarrow t}$ (ng h/mL)	606.93 ± 45.25	446.36 ± 72.32
$AUC_{0\rightarrow\infty}$ (ng h/mL)	1123.37 ± 79.66	893.72 ± 116.56
$AUMC_{0\rightarrow t}$ (ng h/mL)	4751.96 ± 103.70	3746.13 ± 265.20
$AUMC_{0\rightarrow\infty}$ (ng h/mL)	37936.75 ± 1702.08	33794.48 ± 1861.19
$MRT_{0\rightarrow\infty}$ (h)	34.73 ± 1.13	38.72 ± 1.40
Relative bioavailability (%)	178.75	–

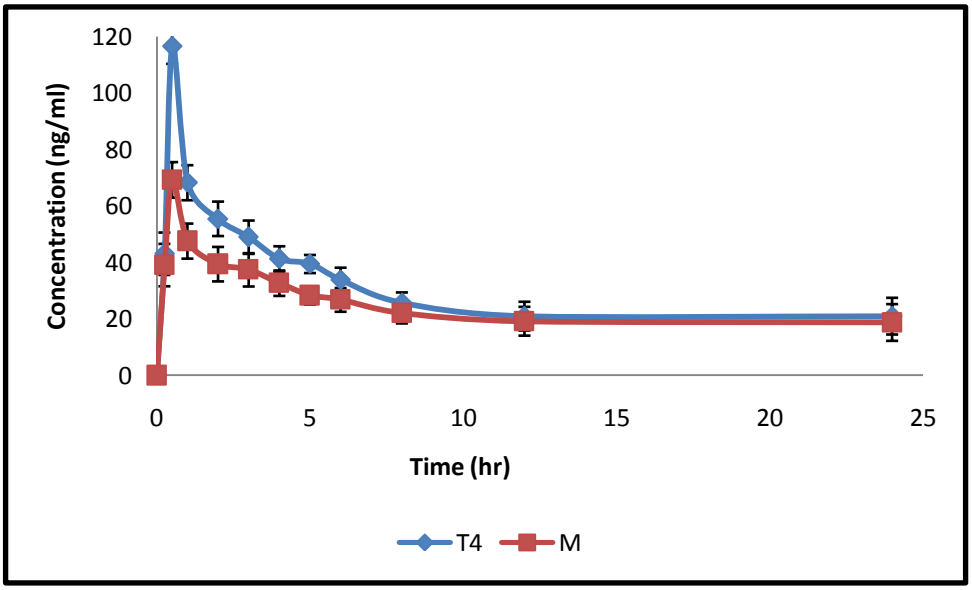


FIGURE.8.25: Plasma concentration v/s time curve

The consistency in the intrinsic properties of drug may be contributing factor. Increased bioavailability of S-SMEDDS (T4) may due to its lymphatic transport through transcellular pathway. It is also reported that the long-chain oils promote lipoprotein synthesis and

subsequent lymphatic absorption. The main rate-limiting barrier for drug absorption/diffusion is the single layer of intestinal epithelial cell. High content of surfactants in S-SMEDDS (T4) could increase the permeability by disturbing the cell membrane. It should be noted that the surfactant with best enhancement ability requires both hydrophilic and lipophilic domains reaching a balance with intermediate values of HLB. Its structural characteristics impart both lipophilic and hydrophilic properties to the surfactant, allowing it to partition between lipid and protein domains. Surfactant also demonstrated a reversible effect on the opening of tight junction; it may interact with the polar head groups of the lipid bilayers, modifying hydrogen bonding and ionic forces between these groups. It may also insert itself between the lipophilic tails of the bilayers, resulting in a disruption of the lipid-packing arrangement. On the basis of *in vitro* and *in vivo* correlation, it can be assumed that increase in release profile of candesartan cilexetil from S-SMEDDS (T4) can lead to increase of bioavailability of candesartan cilexetil.

9. CONCLUSION

Candesartan cilexetil is an orally administered ACE inhibitor for the treatment of hypertension and cardiac failure, but its solubility, stability and oral bioavailability are poor. The objective of our investigation was to formulate a self microemulsifying drug delivery system (SMEDDS) of candesartan cilexetil using minimum surfactant concentration that could improve its solubility, stability and oral bioavailability. The composition of optimized formulation [C7IIB] consist of Capryol 90 as oil, Labrasol as surfactant and Captex 500 as cosurfactant , containing 32 mg of candesartan cilexetil showing drug release for liquid SMEDDS formulation (99.91%), droplet size (9.15 nm), Zeta potential (-23.2), viscosity (0. 8824 cP) and infinite dilution capability. *In-vitro* drug release of the C7IIB was highly significant ($p < 0.05$) as compared to marketed conventional tablet (M). The C7IIB was further used for the preparation of various Solid SMEDDS(S-SMEDDS) formulations (Tablet). These tablets were prepared via adsorption to solid carrier technique, using optimized liquid SMEDDS formulation [C7IIB] whereas Aeropearl 300 pharma as optimized adsorbents .The resulting S-SMEDDS tablet exhibited particle size (78.3 nm) whereas the liquid SMEDDS showed (9.15 nm). The *in vitro* release was almost similar for the S-SMEDDS as well liquid i.e. 78.32% and 84.6% respectively within 5 min. Also, one of the main objective to enhance the oral bioavailability of drug (15%) which was enhanced to 1.78 folds. In conclusion, our studies illustrated that adsorption to solid carrier technique could be a useful method to prepare the solid SMEDDS tablets from liquid SMEDDS, which can improve oral absorption of candesartan cilexetil, nearly equivalent to the liquid SMEDDS, but better in the formulation stability, drugs leakage and precipitation, etc.

Conclusion

- The solubility of candesartan cilexetil was found to be highest in Capryol 90 (80.12±4.04mg/mL) as compared to other oils while in water it was (0.09±0.01mg/mL). Thus, Capryol 90 was selected as the oil phase for the development of the formulation.
- From the prepared liquid SMEDDS formulations, C7 and C4 are clear whereas remaining became cloudy on dilution.
- Addition of higher concentration of co-surfactant (C) as compared to concentration of surfactant (A, B and D) showed poor microemulsion.
- From the results of pseudoternary phase diagram it was revealed that formulation C7IIB covers the maximum microemulsion region as compared to other formulations whereas other formulations makes microemulsion which are unstable on dilution and have poor microemulsion region.
- Higher concentration of oil in SMEDDS may provide greater opportunity for the solubilization and incorporation of higher concentration of candesartan cilexetil.
- It was observed that the viscosity of all the formulations is less than 1 cp which shows that all SMEDDS forms o/w microemulsion.
- All the formulations showed similar pH values in the range of 5.1 to 6.0;thus pH is not affecting stability. Therefore it can be assumed that drug is not diffusing in the external phase and remains in the oil phase. Since, water is the external phase entire system showed pH of water. Candesartan cilexetil is unstable in alkaline pH. Here the formulations show acidic to neutral pH which is suitable for stability of Candesartan cilexetil.
- It was observed that formulation C1, C3, C5 and C6 did not pass the thermodynamic stress tests and thus were dropped for further study.
- Formulation C7IIB was found out to have minimum average particle size 9.15 nm in water.

Conclusion

- The optimal batch C7IIB had the least zeta potential i.e. -23.2 mV with highest zeta potential towards negative side. The zeta potential governs the stability of microemulsion, it is important to measure its value for stability samples. The high value of zeta potential indicates electrostatic repulsion between two droplets. DLVO theory states that electric double layer repulsion will stabilize microemulsion where electrolyte concentration in the continuous phase is less than a certain value.

- Formulation C7 has % transmittance value greater than 99% which indicates the high clarity of microemulsion.

- The results show that formulations C3ID and C3IB does not pass the test as they have PDI more than 0.3 whereas remaining all formulations pass the test as they have PDI less than 0.3.

- The formulation C7IIB showed highest release rate among all the liquid SMEDDS formulations i.e. 92.01% in 10 min which is highest among all batches.The *in-vitro* study concludes that release of candesartan cilexetil was greatly enhanced by SMEDDS formulation. The batch C7IIB was thus taken for further studies and comparison.

- The formulation C7IIB has the maximum release rate at all the time as compared to the Atacand Tablet (M) and pure drug (S).

- From Two way ANOVA it can also be said that the change in the time and compositions of various formulations i.e. combination of oil and S/CoS have significant effect on the release rate of the formulation.

- From the stability studies it was revealed that formulation C7IIB is more stable as compared to marketed tablet Atacand.

- The values clearly prove that after the stability study, formulation C7IIB doesn't show significant difference .After 3 months stability study the particle size of C7IIB was found to be 10.87 nm in water and the initial particle size was 9.15 nm, so no significant difference was found. The PDI was found to be 0.221 initially and 0.186 after stability

study. The zeta potential was initially found to be -23.2 mV and after stability study it was found to be -22.2 mV.

- This result indicates that all the excipients used are compatible and hence form stable microemulsion with almost same particle size. From, all th results batch C7IIB was selected as optimized formulation and further used for solidification and convertin it into a solid dosage form (Tablet).

- Adsorption to solid carrier technique was used and adsorbent Aeroperl 300 pharma (A2) was selected as optimized adsorbent as it require only 60 mg to convert liquid SMEDDS (0.2 ml containing 32 mg drug) into free flow powder. Also, A2 showed better results for other tests like powder flow properties as well as *in-vitro* dissolution.

- Thus, solid state characterization was performed for Aeroperl 300 pharma based free flow powder by SEM. Surface of Aeroperl 300 pharma was rough before and after adsorption of liquid SMEDDS a smooth surface was observed which indicate that liquid smedds was adsorbed on the surface of Aeroperl 300 pharma.
- Further solid SMEDDS formulation i.e. tablets was prepared using the mixture of optimized liquid SMEDDS (C7IIB) and adsorbent Aeroperl 300 pharma (A2).
- Various tablet evaluation parameters of batch T1 to T9 were found to be satisfactory and within the specification for candesartan cilexetil tablet.
- All batches shows approximately 70% drug released within 5 minutes but among this batch T4 shows 78.32 % drug released in 5 minutes which was faster as compare to other batches.

- The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as drug absorption. Particle size of the batch T4 in water was found to be 78.3 nm which was higher as compare to optimized liquid SMEDDS formulation C7IIB which has particle size 9.15 nm.

Conclusion

- Zeta potential of the formulation was found to be -17.4 mV for the batch T4 which was higher as compare to optimized liquid SMEDDS formulation C7IIB which has zeta potential value -23.2 mV.
- The DSC thermogram was taken for pure drug Candesartan cilexetil and optimized Solid SMEDDS formulation (T4). Pure drug substance shows a sharp endothermic peak at 169^0Cwhich shows the highly crystalline behavior of drug. Whereas, no peak was observed in S-SMEDDS formulation which shows the change in melting behavior of drug and inhibition of crystallization following granulation using lipid surfactants.
- 3^2 full factorial design was applied for the tablet formulations. The effect of various diluents (Lactose monohydrate, Mannitol and MCC) and the concentration of pre gelatinized starch were kept as independent variables X1 and X2 respectively. The dependent variables were DT, T70 and T90. The data shows that values are strongly dependent on the selected independent variables.
- *In-vitro* drug release of batch T4 was compare with the marketed tablet formulation i.e. Atacand 32 mg tablet (M). Marketed formulation shows just 32.47% drug release in 5 min whereas tablets of batch T4 shows 78.32 % drug release in same time. This data clearly indicate that by formulating S-SMEDDS formulation of candesartan cilexetil, solubility and thus dissolution profile of candesartan cilexetil was increased.

- *In-vitro* drug release of batch T4 was also compared with the optimized liquid SMEDDS formulation C7IIB and marketed formulation M. Batch T4 shows almost same dissolution profile as that of batch C7IIB. Initial drug release was found to me slightly slower in T4 as compared to C7IIB.
- Accelerated stability study (40^0C ± 2^0C /75% ± 5% RH) and real time stability study (25^0C ± 2^0C /60% ± 5% RH) was performed on batch T4 for a period of three months. No significant changes were observed in appearance, average weight, hardness, thickness and friability of the tablets for both the condition.
- Assay was decreased to 84.76 % in the sample stored at accelerated condition after three months which indicate that formulation were not stable at higher temperature. So other evaluations were not performed on the sample store at accelerated condition. After three

months storage of batch T4 at real time condition assay value was fond to be 98.83% which indicate formulation were stable at this condition.

- Initial value for particle size of the batch T4 was 78.3 nm and after stability it was found to be 79.2 nm. Zeta potential value after stability was found to be -17.1 mv and the initial value was -17.4 mv so data indicate that formulation was stable.
- *In-vivo* studies were performed using optimized S-SMEDDS (T4) and marketed sample Atacand (M) .The decrease in systolic blood pressure was observed in DC3 rats [receiving high dose S-SMEDDS suspension] which showed 26.75±0.336% and DC4 rats [receiving high dose plain drug suspension] showed 18.0±0.358% decrease in systolic blood pressure. Thus, significant enhancement in antihypertensive activity was clearly observed attributed to microsizing of candesartan cilexetil.
- Thus, it was confirmed that Candesartan cilexetil decreases blood pressure in a dose-dependent manner and hence decrease in pressor effect can be directly correlated with the amount of drug that reaches systemic circulation i.e. bioavailability of drug. Based on this pharmacodynamic study, it could be concluded that bioavailability of drug was higher from S-SMEDDS suspension in comparison to plain drug suspension.
- *In vivo* pharmacokinetic behaviors of candesartan cilexetil with SMEDDS (T4) and marketed formulation (Atacand) were studied in rat. Plasma concentration C_{max} and $AUC_{0\rightarrow t}$ are significantly increased for S-SMEDDS than those for the Atacand suspension. T_{max} is decreased for S-SMEDDS(T4) and it was 1 h for S-SMEDDS(T4) and 1.36 h for Atacand formulation. Relative bioavailability is increased 1.78-fold.

- Increased bioavailability of S-SMEDDS (T4) may due to its lymphatic transport through transcellular pathway. It is also reported that the long-chain oils promote lipoprotein synthesis and subsequent lymphatic absorption. On the basis of *in vitro* and *in vivo* correlation, it can be assumed that increase in release profile of candesartan cilexetil from S-SMEDDS (T4) can lead to increase of bioavailability of candesartan cilexetil.

From all above results it can be concluded that the proposed objective of the present research work of enhancing bioavailability of candesartan cilexetil, a low solubility antihypertensive drug, by improving solubility of drug was achieved successfully.

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List of Publications

1. Jill B. Shukla and Dr. Girish K Jani. Formulation And Evaluation Of Oral Self Microemulsifying Drug Delivery System Of Candesartan Cilexetil. International Journal of Pharmacy and Pharmaceutical Sciences. ISSN- 0975 – 1491. Vol 8; Issue 5, 2016.

2. Jill B. Shukla and Dr. Girish K Jani. Formulation And Evaluation Of Solid Self Microemulsifying Drug Delivery System Of Candesartan Cilexetil. International Journal of Pharmacy and Pharmaceutical Sciences. ISSN- 0975 – 1491. Vol 8; Issue 5, 2016.

3. Jill B. Shukla and Dr. Girish K Jani. Preparation and Evaluation Of Solid Self Microemulsifying Drug Delivery System Containing Candesartan Cilexetil. Asian Journal of Pharmaceutical Sciences (AJPS). ISSN- 1818-0876.