Literature Review

Chapter 2

2.1. Oral Delivery

Poorly water-soluble drug candidates are more prevalent nowadays [1] and became a noteworthy and frequently encountered problem for pharmaceutical scientists [2]. Poor solubility of drugs not only influence oral bioavailability but also hinder the development of suitable delivery systems [3]. It is estimated that between 40% to 70% of all new chemical entities identified in drug discovery programs are insufficiently soluble in aqueous media [4, 5]. Poorly soluble drugs pose a challenge for the formulation scientists to develop suitable dosage form which can enhance their bioavailability. Poor bioavailability can be attributed to poor solubility, degradation in GI lumen, presystemic elimination, Permeability glycoprotein (P-gp) based efflux and poor membrane permeation [6]. Various strategies have been widely investigated to enhance the bioavailability of poorly absorbed drugs in order to increase their clinical efficacy upon oral administration [4]. Recently, much attention has been focused on lipid based formulations to improve the oral bioavailability of poorly water soluble drugs. The primary mechanism by which these systems enhance bioavailability is through solubilization of the drug. Other mechanisms for absorption enhancement are also reported and include reduction of P-glycoprotein mediated efflux, mitigation of hepatic first pass metabolism through enhanced lymphatic transport, prolongation of gastrointestinal transit time or protection from degradation in GI tract [7]. The absorption of drug from lipid based formulation depends on numerous factors, including particle size, degree of emulsification, rate of dispersion and precipitation of drug upon dispersion [8]. Lipid based formulations consists of surfactant dispersions, microemulsions, nanoemulsions, self-emulsifying formulations, self-micro/nanoemulsifying formulations, emulsions, liposomes etc. Out of these promising drug delivery technologies, nanoemulsion and self micro emulsifying drug delivery system have been applied in the present project to enhance the oral bioavailability of selected poorly water soluble drugs.

2.2. Lipid Based Drug Delivery Systems (LBDDS)

Lipid-based drug delivery is a broad field covering the use of lipids and lipid-based technologies in pharmaceutical formulations intended for oral, transdermal, pulmonary, parenteral, rectal and ocular administration [9].However, oral route is the most preferred route because of the properties like non invasiveness, ease of manufacturing, patient convenience and compliance [8]. Within these applications, lipid-based drug delivery

systems (LBDDS) are used for a number of reasons: bioavailability enhancement by increased solubility, targeting lymphatic transport, modulation of enterocytes-based drug transport; to produce physically and chemically stable formulations that offer safe and effective means to deliver drugs to the intended site of absorption/action; and to overcome patient compliance obstacles by improving taste, overall palatability, dosing frequency and tolerability [9]. Lipid based formulations offer a potential platform for improving oral bioavailability of drugs especially those belonging to Biopharmaceutical Classification System (BCS) class II and class IV. A primary indication of the potential utility of lipid based formulation can be obtained by assessing the drug lipophilicity (Log P) and its solubility in pharmaceutically-acceptable lipid excipients, which should be sufficient to allow the entire dose of the drug to be administered in a single dosage unit [10]. In addition to improving solubility and bioavailability, lipid drug delivery also offers several advantages over conventional formulations for many poorly soluble compounds, including:

- Lowering of therapeutic dose due to improved drug absorption
- Keeping drug molecule in pre-dissolved state in the lipid component which aids in avoiding rate limiting dissolution step in GIT, thereby achieving increased and consistent bioavailability
- Reduction or elimination of intra- and inter- subject variability due to reduced effects of gastrointestinal variability on solubilization
- Reduction or elimination of food effects on bioavailability of a compound thus improving dosing flexibility
- Reduction in hepatic metabolism due to potential transport of compounds through lymphatic system
- Improved dose uniformity [9].

2.2.1. The Lipid Formulation Classification System (LFCS)

The Lipid Formulation Classification System (LFCS) was introduced as a working model in 2000 by Pouton [11] and an extra 'type' of formulation was added in 2006 by Pouton and Porter [12] to identify the factors affecting the *in vivo* behaviour of formulations. One of the main objectives of this classification system was to identify the most suitable formulation system for specific drugs based on their physicochemical properties and the same for the excipients [13]. Table 2.2.1 describes the composition, characteristic features, advantages and disadvantages of the four essential types of lipid

formulations in LFCS [8, 11, 13].The most straightforward lipid-based formulation is a lipid solution, classified as a Type I formulation. The apparent advantage of this formulation is its virtual simplicity. However, these formulations are highly dependent on the digestion process and suffer from low solvent capacity. Unless the drug is sufficiently lipophilic (log P>4), formulation as an oil solution is limited to highly potent compounds. Solvent capacity can be increased by adding surface active agents as is the case in type II and III formulations. In addition, the most polar formulations, comprising hydrophilic surfactants and represented by class III, often exhibit self emulsifying properties. Type IV systems are essentially pure surfactants or mixtures of surfactants and cosolvents, do not contain natural lipids, and represent the most hydrophilic formulations [13].

Table 2.2.1: Lipid formulation classification system (LFCS)

Formulation Type	Composition		Characteristics	Advantages	Disadvantages
Type I	Oils	100%	Non-dispersing, poor solvent capacity unless drug is highly lipophilic, requires digestion	Generally recognized as safe; simple and excellent capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type II	Oils Low-HLB surfactants (water insoluble surfactants)	40-80% 20-60%	SEDDS without water-soluble components, turbid O/W dispersion	Unlikely to lose solvent capacity on dispersion	Turbid o/w dispersion (particle size 0.25–2 μm)
Type III	Oils High-HLB surfactants (water soluble and insoluble surfactants) Cosolvents	<20-80% 20-50% 0-50%	SEDDS / SMEDDS with water-soluble components, clear or bluish dispersion, possible loss of solvent capacity on dispersion, less easily digested	Clear or almost clear dispersion, drug absorption without digestion	Possible loss of solvent capacity on dispersion, less easily digested
Type IV	Low-HLB surfactants High-HLB surfactants Cosolvents	0-20% 30-80% 0-50%	Micellar solutions, loss of solvent capacity on dispersion, may not be digested	Formulation has good solvent capacity for many drugs	Likely loss of solvent capacity on dispersion, may not be digestible

2.2.2. Formulation components for LBDDS

Lipid-based drug delivery systems are of increasing interest to the pharmaceutical scientist because of their potential to solubilize drug molecules that may be otherwise difficult to develop. Several potential excipients for the lipid-based formulations are available in the market. Oral lipid-based drug delivery systems include a broad range of oils, surfactants and cosolvents. These components are selected according to their objectives: (a) to achieve maximal drug loading; (b) to achieve minimal self-emulsification time and droplet size in the gastric environment for maximal absorption; (c) to reduce variation in the emulsion droplet size as a function of pH and electrolyte content of the aqueous medium; and/or (d) to prevent/minimize drug degradation/metabolism in physiological environment [13].

Various excipients/components used in the formulations are:

2.2.2.1. Oils/Lipids

Oil represent one of the most important excipients in the LBDDS not only because it can solubilize the required dose of the lipophilic drug but also can increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the gastro intestinal tract (GIT) depending on the molecular nature of the triglyceride[14]. Both long and medium chain triglyceride (LCT & MCT) oils with different degrees of saturation have been used for the design of lipid based formulations. Unmodified edible oils which represents the natural and preferred basis for lipid vehicles are not selected because of their poor ability to dissolve large amounts of hydrophobic drugs and their relative difficulty in efficient self-emulsification. In contrast, modified or hydrolyzed vegetable oils or their derivatives have contributed widely to the success of the above systems since they exhibit formulation and physiological advantages[13, 14]. Triglycerides are classified as short (SCT) (<5 carbons), medium (MCT) (6-12 carbons), long chain (LCT) (12-22 carbons) and very long chain (VLCT) (>22 Carbons). Triglycerides are highly lipophilic and their solvent capacity for drugs is commonly a function of the effective concentration of the ester groups. Thus on a weight basis, MCT generally has higher solvent capacity than LCT. In addition, as MCT is not subject to oxidation, it is a popular choice for use in lipid-based products [15, 16]. Table 2.2.2 shows the examples of different triglycerides [17].

Table 2.2.2: Examples of Different Triglycerides

Number of carbon	Common name	Appearance
Short Chain Triglyceride (SCT)		
C4	Butyric Acid	Colorless liquid
Medium Chain Triglyceride (MCT)		
C6	Caproic Acid	Oily Liquid
C8	Caprylic Acid	Oily Liquid
C10	Capric Acid	White Crystals
C12	Lauric Acid	White powder
Long Chain Triglyceride (LCT)		
C14	Myristic Acid	Crystalline solid
C16	Palmitic Acid	White Crystals
C18	Stearic Acid	White Solid
C20	Arachidic Acid	White Crystalline Solid
C22	Behenic Acid	White to yellowish crystals or powder
Very Long Chain Triglyceride (VLCT)		
C24	Lignoceric Acid	Crystalline Solid
C26	Cerotic Acid	Crystalline Solid

2.2.2.2. Surfactants

Next to oil, the other most vital component for LBDDS is surfactant. The surfactants or surface-active agents are amphiphilic by nature, and therefore usually able to dissolve and even solubilize relatively high quantities of the hydrophobic drug which further prevents precipitation within the GI lumen and allows prolonged existence of the drug molecules in soluble form, which is vital for effective absorption [18]. The two issues that govern the selection of a surfactant are its hydrophilic–lipophilic balance (HLB) and

safety [19]. Non-ionic surfactants are known to be less toxic compared to ionic surface-active agents, but they may cause moderate reversible changes in intestinal wall permeability. The usual surfactant concentration in self-emulsifying formulations required to form and maintain an emulsion state in the GI tract ranged from 30 to 60% w/w of the formulation. A large quantity of surfactant may irritate the GIT [14]. Thus, the safety aspect of the surfactant vehicle should be carefully considered. Non-ionic surfactants with a relatively high HLB are recommended for the design of self-dispersing systems. The high HLB and subsequent hydrophilicity of surfactants is necessary for the immediate formation of o/w droplets and/or rapid spreading of the formulation in the aqueous environment, providing a good dispersing/self emulsifying performance. Based on HLB value, surfactants can be water insoluble or water soluble [13] (Table 2.2.3).

Apart from this, there is a relationship between the droplet size and the concentration of the surfactant being used. Generally, increasing the surfactant concentration 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 [20]. On the other hand, in some cases the mean droplet size may increase with increasing surfactant concentrations [21]. This phenomenon could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration, leading to ejection of oil droplets into the aqueous phase [22]. The surfactants used in these formulations are known to improve the bioavailability by various mechanisms like: improved drug dissolution, increased intestinal epithelial permeability, increased tight junction permeability and decreased/inhibited P-gp drug efflux.

2.2.2.3. Cosurfactants

Generally, co-surfactant of HLB value 10-14 is used with surfactant to decrease 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 until their bulk condition is depleted enough to make interfacial tension positive again [14]. Organic solvents suitable for oral administration like ethanol, propylene glycol, polyethylene glycol may help to dissolve large amounts of either the hydrophilic surfactant or the drug in the lipid base and can act as cosurfactant in the microemulsion systems.

2.2.2.4. Cosolvents

Cosolvents include short chain alcohols such as ethanol, glycerin, polyethylene glycol and propylene glycol [23]. Cosolvents increase the solvent capacity of the formulation for drugs and aid in the dispersion of systems which contain a high proportion of water soluble surfactants, thus enhancing the solubilization process [24]. However, there are certain limitations to the use of cosolvents viz precipitation of the solubilized drug from the solvent due to loss of the solvent capacity following dilution, immiscibility of some cosolvents with oils and incompatibilities of low molecular weight solvents with capsule shells.

Table 2.2.3.: Examples of Various Surfactants and Cosurfactants/ Cosolvents

Types/Class	Examples
Low HLB (<10) surfactants	
Phosphatidyl choline and Phosphatidyl choline/ solvent mixture	Phosphatidyl choline; Phosphatidyl choline in propylene glycol, Phosphatidyl choline in medium chain triglycerides, Phosphatidyl choline in safflower oil/ethanol
Unsaturated poly glycolized glycerides	Oleoyl Macrogol glyceride, Linoleoy lMacrogol glyceride
Sorbitan esters	Sorbitan monooleate, sorbitan mono stearate, Sorbitan mono laurate, Sorbitan mono palmitate
High HLB (>10) surfactants	
Polyoxyethylene Sorbitan Esters	Polysorbate 20, Polysorbate 40, Polysorbate 60, Polysorbate 80
Polyoxyl castor oil derivatives	Polyoxyl 35 castor oil, Polyoxyl 40 Hydrogenated castor oil
Polyoxyethylene Polyoxypropylene block copolymer	Poloxamer 188, Poloxamer 407
Saturated Polyglycolized glycerides	Lauroyl Macrogol glyceride, Stearoyl Macrogol glyceride
PEG-8 caprylic/capric glycerides	Caprylo caproyl Macrogol glyceride
Vitamin E Derivative	Tocopherol PEG Succinate
Cosurfactants/ Cosolvents	
Alcohols and Polyols	Ethanol, Isopropranol, Butanol, Benzyl alcohol, Ethylene glycol, Propylene glycol, Butanediols
Esters of propylene glycols	Tetrahydrofuryl alcohol, PEG ether (glycofural) or Methoxy PEG

2.2.3. Applications of LBDDS in Oral Delivery

Various modes of enhanced drug absorption from LBDDS can be hypothesized as follows:

Stimulation of body secretions that help in digestion of lipids: Administration of lipid can stimulate the biliary and pancreatic secretions which are helpful for the digestion of lipids. The enzymes present in the secretions are water soluble and act at water/lipid interface. Fatty acids liberated from the lipid digestion process interact with the bile salts and result in the formation of mixed micelles and micelles in which the drug gets solubilized.

Prolongation of GI residence time: Administration of lipid along with the drug allows the drug to be present for prolonged duration of period in the GIT which facilitates the absorption of the drug.

Stimulation of lymphatic transport: Highly lipophilic drugs (log*P*> 5) which has high solubility in triglycerides (>50mg/mL) can undergo lymphatic transport when co-administered with esters of unsaturated long chain fatty acids; thereby improving bioavailability. This restricted lymphatic transport is mainly due to low lymph-to-blood flow ratio. This enhanced lymph delivery of the drug can bypass the first pass extraction whereby the bioavailability of drugs that undergo extensive first pass effect can be improved.

Increased intestinal wall permeability: Opening of tight junctions in the intestine caused by lipids contributes to the increased permeability of poorly permeable drugs. Although this mechanism is not essential in case of BCS Class II drugs, it leads to marked improvement in absorption of Class IV drugs which have both dissolution and permeability rate limited absorption.

Reduced efflux of the drug in the GIT: Lipids such as anionic phospholipids (cardiolipin and phosphatidyl serine) may inhibit P-gp by interaction with membrane lipids. So, the drugs which have propensity to be effluxed from the GIT can be formulated as LBDDS for the improvement of bioavailability. The inhibitory effect is due to competition for binding with the transporter and due to membrane perturbation caused by the excipients, mainly surfactants. The residence time of the drug can be prolonged by this inhibition of efflux [13].

2.3. Drug Delivery Systems Selected for Present Study

2.3.1. Self-Micro Emulsifying Drug Delivery System (SMEDDS)

A self-emulsifying system is a mixture of oil, surfactant and sometimes cosurfactant or cosolvent that emulsifies in water under conditions of gentle agitation, *in vivo* provided by gastrointestinal motility. Such mixtures may be spontaneously emulsifying if the entropy change favoring dispersion is larger than the energy required to increase the surface area of the dispersion [13]. SMEDDS are defined as isotropic mixtures of natural or synthetic oils, surfactants and/or one or more hydrophilic cosolvents. SMEDDS formulations easily spread in the GI tract and the gentle agitation required for self-emulsification of formulation is provided by motility of the stomach and the intestine [25]. Depending on the excipient selection and relative composition of the formulation, aqueous dilution will result in spontaneous formation of droplets in size from approximately 100 nm (SMEDDS) to less than 20 nm [26].

Furthermore, these formulations are known to reduce inter- and intra-individual variations in bioavailability, which is believed to be caused by a decreased sensitivity of formulation performance to pre-absorptive solubilisation and dietary status. SMEDDS can enhance drug absorption by a number of ancillary mechanisms, including reduction of gastric motility and alteration of the physical and biochemical barrier function of the gastro-intestinal mucosa [27].

2.3.1.1. Advantages of SMEDDS

- **Oral bioavailability improvement:** Owing to its small globule size (between 1 to 100 nm) SMEDDS maintain the drug in solubilised form in the GIT. This leads to subsequent increase in surface area and drug transport through the intestinal aqueous boundary layer and through the absorptive brush border membrane of intestine, leading to improved bioavailability.
- **Ease of manufacture and scale-up:** It is one of the most important advantage that make SMEDDS unique when compared to other drug delivery systems like solid dispersion, liposomes, nanoparticles etc.
- **Reduction in inter-subject and intra-subject variability:** Drugs having poor solubility show large inter-subject and intra-subject variation in their absorption, leading to decreased performance of drug and patient noncompliance. Formulation of such drugs into SMEDDS can overcome above limitations.

- **Prevention of enzymatic hydrolysis in GIT**: SMEDDS can protect drugs against enzymatic hydrolysis in the GI tract and can reduce presystemic clearance in the GI mucosa and hepatic first-pass metabolism.
- **Increased drug loading capacity:** SMEDDS can increase drug loading capacity of drugs by dissolving in oil phase as compared to conventional lipid solutions. The lipophilic drugs having partition coefficient ($2<\log P<4$) whose solubility is less in natural lipids can be improved by amphiphilic surfactant and/or cosolvents [13].

2.3.1.2. Limitations of SMEDDS

In spite of these aforementioned advantages, SMEDDS also have the following limitations [14]:

- SMEDDS lack good predictive *in vitro* models for assessment of the formulations.
- Drugs unstable in oil phase are difficult to formulate.
- High surfactant concentrations in formulations (approximately 30%–60%) may irritate the GI tract.
- Volatile cosolvents in the conventional self microemulsifying formulations are known to migrate into the shells of soft or hard gelatin capsules, resulting in the precipitation of the lipophilic drugs.
- The precipitation tendency of the drug on dilution may be higher due to the dilution effect of the hydrophilic solvent.

2.3.1.4. Role of SMEDDS in Improvement of Oral Absorption

SMEDDS partially avoid the additional drug dissolution step prior to absorption in the GIT thereby resulting in improved drug absorption. There are several mechanisms through which increased absorption can be achieved such as: retardation of gastric emptying time; increase in effective drug solubility in lumen; lymphatic transport of the drug; enterocyte based drug transport; increasing membrane permeability etc [28]. In case of SMEDDS, it has been shown that the oil/water partition coefficient of the drug and droplet size can modulate drug release. The droplet size upon dilution with aqueous media is primarily controlled by the nature and concentration of the emulsifier. Phase diagrams of the oil/ surfactant/ drug can be constructed to identify regions where maximum self-microemulsification occurs. The higher the concentration of emulsifier, the smaller the droplet sizes of the resulting emulsion and the faster is the drug release. The combination of small droplets along with a low oil/water partition coefficient will

allow for an optimum drug release from SMEDDS. Similarly, drug release from microemulsion (o/w and w/o), depends on a number of process parameters, such as oil/aqueous phase ratio, droplet size, distribution of drug in the phases of microemulsion system and its diffusion rate in both phases. Though direct determination of drug distribution between the aqueous and oil phases of microemulsion is difficult, water/oil partitioning studies using the aqueous and oil phases of the corresponding microemulsion should be conducted and correlated to the observed oral bioavailability and/or *in vitro* permeability [29].

2.3.1.5. Mechanism of Self Emulsification

Conventional emulsions are formed by mixing two immiscible liquids namely water and oil stabilized by an emulsifying agent. When an emulsion is formed, surface area is expanded between the two phases. The emulsion is stabilized by the surfactant molecules that form a film around the internal phase droplet. In conventional emulsion formation, the excess surface free energy is dependent on the droplet size and the interfacial tension. If the emulsion is not stabilized using surfactants, the two phases will separate reducing the interfacial tension and the free energy [30]. It was reported by Reiss[31]that self-emulsification process occurs when entropy of the dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of the conventional emulsion is a direct function of the energy required to create a new surface between the oil and water phases [32] and can be described by the following equation:

$$\Delta G = \Sigma_i (N_i 4\pi r_i^2 \sigma) \qquad \ldots\ldots Eqn\ 2.3.1$$

where ΔG is the free energy associated with the process, N is the number of droplets of radius r and σ is the interfacial energy.

In case of SMEDDS, the free energy of formation is very low and positive or even negative which results in thermodynamic spontaneous emulsification. It has been suggested that self-emulsification occurs due to penetration of water into the Liquid Crystalline (LC) phase that is formed at the oil/surfactant-water interface into which water can penetrate assisted by gentle agitation during self-emulsification. After water penetrates to a certain extent, there is disruption of the interface and a droplet forms. This LC phase is considered to be responsible for the high stability of the resulting nanoemulsion against coalescence [13, 33].

2.3.1.6. Fabrication of SMEDDS

SMEDDS are physically stable and can be easily manufactured for oral delivery in softand hard gelatin capsules. The primary step during formulation of a SMEDDS is the identification of specific combinations of excipients and constructing a phase diagram which shows various concentrations of excipients necessary for self-emulsification. Selection of excipients for SMEDDS lies in identifying excipients combination which will solubilise the entire dose of drug in volume acceptable for unit oral administration. Mutual miscibility of these excipients is also important for producing a stable liquid formulation. Excipients combinations yielding SMEDDS formulations are identified by construction of pseudo-ternary phase diagram. Pseudo-ternary phase diagram can be represented in a triangular format (triangle) which has three coordinates. Each coordinate represents one component of microemulsion system. Various components for the fabrication of SMEDDS are as explained in section 2.2.2-'Formulation components for LBDDS'.

The uses of newer synthetic oils that are amphiphilic in nature and can dissolve large quantities of the drug when compared to conventionally used pure vegetable oils or its derivatives has increased tremendously. Surfactants also provide good solvency for the drug. Although the cosolvents are capable of dissolving a large quantity of the drug, they may cause drug precipitation on aqueous dilution due to loss of solvent capacity. This demands for performing equilibrium solubility measurements of the drug in the excipients under use. The drug may affect the self-emulsification efficiency by changing optimal oil/surfactant ratio. It may interact with the LC phase of some of the mixture components causing blockage of charge movement through the system or may penetrate the surfactant monolayer [34]. The incorporated drug may increase or decrease the self-emulsifying efficiency or may not affect it at all [35]. Hence, SMEDDS should also be evaluated for its self-emulsification efficiency in the presence of the drug. SMEDDS are known to be more sensitive towards any changes in the ratio of excipients. Because of these reasons, pre-formulation solubility and phase diagrams should be thoroughly evaluated when choosing the optimized formulation.

2.3.1.7. Characterization of SMEDDS

Different characterization and evaluation parameters for SMEDDS include percent transmittance, cloud point measurement, globule size, polydispersity index and zeta potential, robustness to dilution, self emulsification time, drug content estimation, thermodynamic stability, surface morphology by Transmission Electron Microscopy,

viscosity determination, *in vitro* release studies, *ex vivo* studies, cell line studies and *in vivo* studies.

2.3.1.8. Review of Work Done

Table 2.3.1: Review of work done on SMEDDS

Author (Reference no.)	**Drug**	**Excipients used**	**Conclusion**
V. Pandey, et al.2017[36]	Pioglitazone	Lipid- Capryol 90, surfactant- Cremophor ELP and co-surfactant Transcutol HP	SMEDDS formulations showed remarkable enhancement in percentage drug release in contrast to conventional marketed preparation (tablet) and pure drug
Suhua Li et al. 2017 [37]	Danazol, Indomethacin and Haloperidol	Lipid- Capmul MCM and caprylic acid, Surfactant- Cremophor RH40 Cosurfactant- PEG 400	Weaklyacidic/ basic drugs but not neutral drugs reduced the droplet size of the microemulsions, indicating that ionization of drug also had significant impact on droplet size formation of the SMEDDS due to various intermolecular forces between the drugs and SMEDDS components
Yang Xu, et al.2016 [38]	[6]-Gingerol	Oils- ethyl oleate, surfactant- Cremophor EL35 and co-surfactant- 1,2-propanediol	Mean droplet size (73.06 ± 0.49 nm), zeta potential (−2.45 ± 0.41) and encapsulation efficiency (89.40 ± 1.11%) was achieved. The *in vitro* release profile of [6]- Gingerol indicated that the solubility was significantly enhanced and also exhibited prolonged plasma circulation which led to 6.58-fold

			increase in oral bioavailability compared with the free drug
Li Q. et al 2015[39]	Curcumin-piperine (CUR-PIP)	Oil- Capryol 90, Surfactant- Cremophor RH40 Cosurfactant- Transcutol HP	The mean size of microemulsion droplet formed from CUR–PIP-SMEDDS was 15.87 ± 0.76 nm, and the drug encapsulation efficiency of SMEDDS for CUR and PIP were (94.34 ± 2.18)% and (90.78 ± 2.56)%, respectively.CUR–PIP-SMEDDS exhibited definite anti-colitis activity by directing CUR–PIP-SMEDDS to inflammatory colon tissue through retention enema administration
Nipun T.S. et al. 2014 [40]	Gliclazide	Oil- Capryol 90, Surfactant-Tween 80 Cosurfactant-Transcutol HP	Droplet size was found to be 50.959 µm. The test formulation showed significant reduction in plasma glucose level, after oral administration.
Negi L.M. et al 2013 [41]	Irinotecan	Oil- Capmul MCM-C8, Surfactant- Cremophor EL Cosurfactant- Pluronic L-121	Nano-scale oil droplets (130 ± 2.13 nm) were produced on spontaneous emulsification. A much deeper penetration to the intestine was observed with SMEDDS by using confocal laser scanning microscopy. Flow-cytometric studies also revealed the greater uptake of fluorescent probe in Caco-2 cell-lines with the use of SMEDDS. The $AUC_{0\rightarrow t}$ of Irinotecan from

			the optimized SMEDDS formulation was found to be 4 folds higher than that from Irinotecan suspension on oral administration
Sha X. et al 2012 [42]	Probucol	Oil- Olive oil, Lauroglycol FCC Surfactant- Cremophor EL, Tween 80 Cosurfactant- PEG-400	Relative bioavailability of probucol SMEDDS was dramatically enhanced in an average of 2.15 and 10.22 fold than that of oil solution and suspension respectively
Lili Zhao et al 2012 [43]	Apigenin	Oil- Capryol 90 Surfactant- Cremophor EL, Cosurfactant- Transcutol HP	The average particle size was 17.1 nm and zeta potential −5.18 mV. In vitro dissolution studies showed about 95% of apigenin was released within 10 min. All of the results showed that SMEDDS could enhance the solubility and dissolution of apigenin, and would be a potential carrier to improve the oral absorption of apigenin, a poorly water soluble drug.
Dixit A. R. et al. 2010 [44]	Valsartan	Oil- Capmul MCM Surfactant- Tween 80 Cosurfactant- polyethylene glycol 400	The particle size distribution, zeta potential and polydispersity index were determined and were found to be 12.3 nm, -0.746, and 0.138, respectively. The AUC and T_{max} showed significant improvement as the values obtained were 607 ng h/mL and 1 h for SMEDDS in comparison

			to 445.36 and 1.36 h for marketed formulation suggesting significant increase ($p < 0.01$) in oral bioavailability of valsartan SMEDDS
Zhang P et al. 2008 [45]	Oridonin	Oil- Maisine 35-1 and Labrafac CC (1:1) Surfactant- Cremophor EL Cosurfactant- Transcutol P	*In vitro* release study showed complete release of oridonin from SMEDDS in approximately 12 h. The relative bioavailability of oridonin from SMEDDS was increased 2.2-fold as compared to that of the suspension
X. Sha et al.2005[46]	Mannitol as paracellular marker	Negatively charged SMEDDS- Oil- Maisine 35-1 Surfactants mixture Cremophor EL and Labrasol Cosurfactant- Transcutol P For positively charged SMEDDS, Oleylamine was additionally added into the above solution	Negatively charged SMEDDS with different dilutions had no effect on the TEER, but significantly increased the permeability of mannitol. In contrast, the positively charged formulation showed a dilution-dependent reduction in TEER. A corresponding increase in mannitol permeability of up to 29.4-fold to 64.7-fold greater than the control was also observed across the monolayer. Labrasol with the concentration of 0.1 and 1% was shown to increase the permeability of mannitol by 4.6-fold and 33.8-foldrespectively.The mechanism involved in drug release was opening of tight junctions

2.3.2. Nanoemulsion

The use of nanotechnology in pharmaceuticals and medicine has progressed to large extent in last few years. The pharmaceuticals developed on the basis of nanotechnolgy are termed as Nanopharmaceuticals. The various nanopharmaceuticals currently being used or in the process of development are Nanoemulsions, nanosuspensions, nano spheres, nanotubes, nanoshells, nanocapsules, etc [47]. All these utilize nanosizing technology in one or the other way. Nanosizing technology (nanonization) is one of the most promising approaches to improve the solubility of drugs by an increase in surface area via reduction of the particle size below 1 μm, typically a few hundred nanometers [48].One of the nanoscience approaches that has increasingly received considerable attention within the pharmaceutical sciences is the formulation as nanoemulsions.

Nanoemulsions (NEs) are group of dispersed particles used in pharmaceutical and biomedical aids or as vehicles that prove enormous promise for the opportunity of cosmetics, diagnostics, drug therapies and biotechnology for future [47].

NEs (mini-emulsions/ultrafine emulsions) are transparent (or translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than 100 nm[13].Nanoemulsion provides ultra low interfacial tensions and large o/w interfacial areas[49].The nanosized droplets leading to enormous interfacial areas associated with nanoemulsions would influence the transport properties of the drug, an important factor in sustained and targeted drug delivery[50]. The attraction of formulating o/w nanoemulsion systems lies in their ability to incorporate hydrophobic drugs into the oil phase thereby enhancing their solubility[51].The uniqueness of these systems is often characterized by their increased drug solubility, rapid dissolution velocity, and enhanced bioavailability after oral administration [52]. There have been numerous investigations on nanoemulsions as an alternative drug delivery strategy to increase bioavailability of water insoluble drugs[49]. The prevailing mechanisms responsible for the increase in the bioavailability are due to improvement of drug solubilization, protection against enzymatic hydrolysis and increased specific surface area of droplets that lead to wide distribution of the drug in the GIT as well as surfactant-induced permeability changes [53]. Hence, nanoemulsion would be an efficient, convenient, flexible and more patient compliant approach in comparison to solid oral dosage forms like tablets and capsules [54].

The very small droplet size provides nanoemulsion stability against sedimentation and creaming, along with a transparent or slightly turbid appearance suitable for food

applications [55]. These emulsions are easily produced by mixing water immiscible oil phase into an aqueous phase with a high stress. Nanoemulsions may possess a relatively high kinetic stability, even for several years [52]. The long-term physical stability of nanoemulsions makes them unique and they are sometimes referred to as 'Approaching Thermodynamic Stability'[55]. Nanoemulsions due to their characteristic size appear transparent or translucent to the naked eye (Figure. 2.3.1)

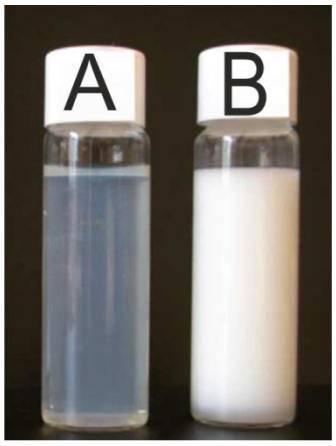

Figure 2.3.1: (A) Nanoemulsion and (B) Macro-emulsion with droplet diameters of <100 nm and >1 µm, respectively [55]

2.3.2.1. Advantages of Nanoemulsions

- NEs have much smaller size, hence high surface area and free energy than macroemulsions, therefore makes it an effective transport system.
- NEs do not show the problems of inherent creaming, flocculation, sedimentation and coalescence: The very small droplet size causes a large reduction in the gravity force, Thus, no creaming or sedimentation occurs during storage. The small droplet size also prevents any flocculation of the droplets. Weak flocculation is prevented, which enables the system to remain dispersed with no separation. The small droplets also prevent their coalescence. Because these droplets are elastic, surface fluctuations are prevented [56].
- The transparent nature of the system, their fluidity (at reasonable oil concentrations),as well as the absence of any thickeners may give them a pleasant aesthetic character [55].

- NEs can be formulated into different formulations such as foams, creams, liquids and sprays and are non toxic and non irritant , hence can be applied on skin and mucous membrane [57].
- Unlike microemulsions (which require a high surfactant concentration, usually in the region of 20% and higher), nanoemulsions can be prepared using a reasonable surfactant concentration. For a 20% o/w nanoemulsion, a surfactant concentration in the region of 5%–10% may be sufficient [55].
- The use of nanoemulsions as delivery systems can improve the efficacy of certain drugs, allowing the total dose to be reduced and thus minimizing side effects [56].

2.3.2.2. Limitations of Nanoemulsions

- The preparation of NEs requires, in many cases, special application techniques such as the use of high-pressure homogenizers as well as ultrasonics [55].
- There is a perception in the personal care and cosmetic industry that nanoemulsions are expensive to produce because they require expensive equipment [57].
- There is a lack of understanding of the mechanism of production of submicron droplets and the role of surfactants and cosurfactants.
- There is a lack of demonstration of the benefits that can be obtained from using nanoemulsions compared with classical macroemulsion systems [56].

2.3.2.3.Formulation Aspects of Nanoemulsions

There are two basic types of nanoemulsion based on the relative spatial organization of the oil and aqueous phases: oil-in-water (O/W) and water-in-oil (W/O). The O/W nanoemulsion consists of oil droplets dispersed in a water phase and the W/O nanoemulsion consists of water droplets dispersed in an oil phase [58]. The components used to prepare nanoemulsions are described in section 2.2.2- Formulation component for LBDDS. Formation of Nanoemulsions is based on thermodynamic theory.

2.3.2.3.1. Thermodynamics Theory

External energy is needed to prepare nanoemulsion using oil, surfactant, cosurfactant/cosolvent and water. This can be understood from a consideration of the energy required to expand the interface, $\Delta A\gamma$ (where ΔA is the increase in interfacial area when the bulk oil with area $A1$ produces a large number of droplets with area $A2$; $A2 >> A1$, γ is the interfacial tension). Since γ is positive, the energy to expand the

interface is large and positive. This energy term cannot be compensated by the small entropy of dispersion $T\Delta S$ (which is also positive) and the total free energy of formation of an emulsion, ΔG is positive.

$$\Delta G = \Delta A\gamma - T\Delta S \quad \text{..... Eqn 2.3.2}$$

Thus, emulsion formation is non-spontaneous and energy is required to produce the droplets. The formation of large droplets (few micrometers) as is the case for macroemulsions is fairly easy and hence high speed stirrers such as the Ultraturrax or probe sonicator are sufficient to produce the emulsion. In contrast, the formation of small drops is difficult and this requires a large amount of surfactant and energy.

The high energy required for formation of nanoemulsions can be understood from a consideration of the Laplace pressure (the difference in pressure between inside and outside the droplet). To break up a drop into smaller ones, it must be strongly deformed and this deformation increases Laplace pressure. Consequently, the stress needed to deform the drop is higher for a smaller drop. Since the stress is generally transmitted by the surrounding liquid via agitation, higher stresses need more vigorous agitation, hence more energy is needed to produce smaller drops. Surfactants play major role in the formation of nanoemulsions: By lowering the interfacial tension, p is reduced and hence the stress needed to break up a drop is reduced. Surfactants prevent coalescence of newly formed drops.

Various processes occur during emulsification namely; break up of droplets, adsorption of surfactants and droplet collision (which may or may not lead to coalescence to occur). Each of these processes occurs numerous times during emulsification and the time scale of each process is very short, typically a microsecond. This shows that the emulsification process is a dynamic process and events that occur in a microsecond range could be very important[13, 55]

2.3.2.4. Fabrication of Nanoemulsion

The fabrication of nanoemulsions can be roughly categorized as either high energy or low-energy approaches, depending on the underlying principle. The high energy approaches disrupt the oil and aqueous phases into tiny droplets using mechanical devices such as high-pressure homogenizers, microfluidizers, and sonicators [59]. In low-energy approaches, nanoemulsions are formed as a result of phase transitions that

occur during the emulsification process when the environmental conditions (either temperature or composition) are altered, e.g., phase inversion temperature (PIT) and spontaneous emulsification methods [60]. The performance of the method of preparation is generally assessed by the size of the nanoemulsion droplet and stability of the system. The high energy methods have control over particle size distribution and ability to produce fine emulsion from large variety of materials. Low-energy emulsification methods are also reported for preparation of nanoemulsions which involve transitional inversion induced by changing factors that affects HLB of the system or by catastrophic inversion induced by increasing dispersed phase volume fraction [61].

2.3.2.4.1. High Energy Approaches

Intense disruptive force is required for the sample to be emulsified in high-energy approaches. Two opposing processes that is, droplet disruption and droplet coalescence, take place inside the system [62]. The attainment of a balance between these two processes leads to the production of smaller droplets.When the applied shear is greater than the Laplace pressure of the emulsion, droplet break-up occurs. The surfactant plays a critical role in both droplet break-up and coalescence. The surfactant aids droplet break-up by lowering the interfacial tension and prevents the immediate re-coalescence of newly formed droplets by rapid adsorption to, and stabilization of, the newly formed interface. The efficiency of droplet break-up is controlled by the nature and intensity of the shear [59]. This can be achieved using ultrasonic devices, high pressure homogenizer and microfludizers.

2.3.2.4.1.1. Ultrasonication

Different types of ultrasonic devices have been developed for nanoemulsion preparation. Premix emulsion is agitated at ultrasonic frequency causing the droplet to break into nano droplets. The emulsion is then circulated through region of high shear to produce uniform droplet size distribution [63]. Cavitation is the main phenomenon of ultrasonically induced effects, which is the formation and collapse of vapor cavities in a flowing liquid [64]. Two mechanisms are proposed for ultrasonic emulsification. First, the application of an acoustic field produces interfacial waves resulting in the dispersion of the oil phase in the continuous phase in the form of droplets. Secondly, the application of ultrasound causes acoustic cavitation causing the formation and subsequent collapse of microbubbles by the pressure fluctuations of a simple sound wave, which creates extreme levels of highly localized turbulence. Therefore, the turbulent micro-implosions

break up primary droplets into sub-micron size [49]. Since the emitted sound field is typically inhomogeneous in most ultrasonic devices, it is necessary to recirculate the emulsions through the region of high power so that all droplets experience the highest shear rate [61, 64].

2.3.2.4.1.2. High Pressure Homogenization (HPH)

This technique makes use of high pressure homogenizer/piston homogenizer to produce nanoemulsions of extremely low particle size (up to 1nm) [65]. This method is executed by applying a high pressure over the coarse emulsion having oil phase, aqueous phase and surfactant or co-surfactant formed using high speed mixer (ultra turrax). This coarse emulsion is then forced to pass through a small inlet orifice at very high pressure (500 to 5000 psi), which subjects the product to intense turbulence and hydraulic shear resulting in extremely fine particles of emulsion [66]. As per figure 2.3.2, homogenization is completed in the area between the valve and the seat, where the emulsion experiences a combination of intense disruptive forces that cause the larger droplets to be broken down to smaller ones [67].

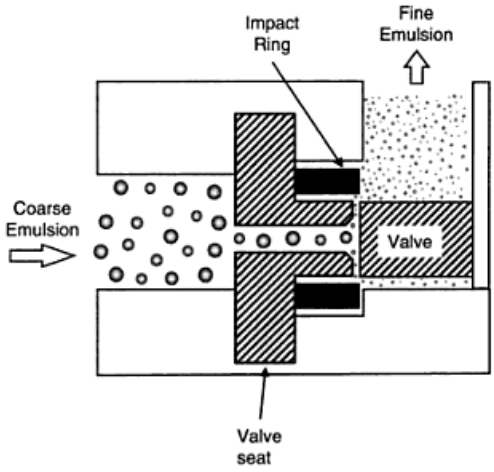

Figure 2.3.2. Schematic representation of high pressure valve homogenizer. Reprinted from Ref [67].

2.3.2.4.1.3. Microfluidization

Microfluidization involves the use of patented mixing technology and makes use of high pressure on a coarse emulsion for the fabrication of nanoemulsions with the help of a device called microfiudizer [47]. It produces smaller droplets and narrow size distribution of droplet compared with traditional emulsification techniques. A microfluidizer is similar to a high-pressure homogenizer; however the design of the

channels for the flow of emulsion is different. The emulsion initially flowing through a channel is further divided into two streams and each stream is passed through a separate fine channel. At the interaction chamber, the two fast-moving streams are directed at each other, creating intense disruptive forces that lead to highly efficient droplet disruption [58]. These extreme shear rates and impact effects generated under high pressure creates exceptionally fine nanoemulsion.

2.3.2.4.2. Low Energy Approaches

Low-energy methods use the internal chemical energy of the system for emulsion production [55] and are more cost-effective than high-energy methods. Commonly used low-energy emulsification methods are self-emulsification and phase inversion methods.

2.3.2.4.2.1. Self-Emulsification Method

This method involves the spontaneous formation of emulsion through the rapid diffusion of surfactant and/or solvent molecules from the dispersed phase to the continuous phase with the help of the chemical energy released due to the dilution process [68]. This method can be used to produce nanoemulsions by dilution of microemulsions at stabilized mixing conditions. Dilutions can be brought about by addition of an organic phase containing hydrophobic oil, a hydrophilic surfactant, and a water-miscible organic solvent to water[69], or by addition of water to an organic phase containing hydrophobic oil, water-miscible organic solvent, and surfactant [70]. In this way, when surfactant, oil, and water contents are optimized, nanoemulsions can be produced [58].

2.3.2.4.2.2. Phase Inversion Methods

Phase inversion methods utilize the chemical energy resulting from phase transitions occur through emulsification method to form fine dispersions. Nanoemulsions have been formed by adequate phase transitions from w/o or o/w or vice versa either by changing the composition at constant temperature or by changing the temperature at constant composition [65]. The phase inversion temperature (PIT) method was introduced based on the changes made in the physiochemical properties of nonionic surfactants, co-surfactants and excipients used in the formulation, leading to the generation of emulsion droplets in the nanometric range with change in temperature[69]. The Phase Inversion Composition (PIC) method involves the progressive addition of one of the components either water or oil to a mixture of the other two components, either oil/ surfactant or water /surfactant respectively [71]. Emulsion Inversion Point (EIP) method involves catastrophic phase inversion generated by altering the ratio of oil to water phases, either

by increasing or decreasing the volume of dispersed phase in an emulsion to certain extent [72].

2.3.2.5.Characterization of Nanoemulsions

Different characterization and evaluation parameters for nanoemulsions include NE droplet size and polydispersity, zeta potential, pH, drug content, surface morphology (by transmission electron microscopy), viscosity determination, *in vitro* release studies, cell line studies, *ex vivo* studies, *in vivo* studies and stability studies.

2.3.2.7. Review of Work Done

Table 2.3.2: Review of work done on Nanoemulsions

Author (Reference no.)	**Drug**	**Technique**	**Oil/ surfactant, cosurfactant used**	**Conclusion**
Xiaoyu Li, et al. 2017 [73]	Hohenbuehelia serotina polysaccharides	High speed homogenization	Oil Phase- Polycaprolactone in DCM Emulsifier- polyvinyl alcohol (PVA) in aqueous solution	Encapsulation efficiency, particle size and zeta potential were 75.42±0.69% 410.1±2.3 nm and -52.34±5.62 mV respectively. Moreover, NE showed sustained-release characteristics in simulated gastric fluid
T. Mehmood et al 2017 [74]	Alpha tocopherol	High speed homogenization followed by ultrasonic homogenization	Oil- Olive oil Surfactant- Tween 80	Co-surfactant free, olive-oil based alpha tocopherol NE was prepared. The optimum

				emulsifying conditions were determined. Particle size obtained was 151.68 nm
A. Nagi et al. 2017 [75]	Silymarin	high pressure homogenization (HPH) technique	Oil- Capryol 90, surfactant- Solutol HS 15 and co-surfactant Transcutol HP	Antioxidant activity of silymarin increased by formulating NE. Improved *in vitro* drug release, improvement in pharmacokinetics and thus enhanced bioavailability was observed
A. Khunt et al. 2015 [76]	Itraconazole	High-speed stirring, followed by probe sonication	Oil- Capmul MCM C8 Surfactant- Cremophor EL Cosurfactant- Pluronic F68	Nanoemulsion of ITR with particle size of 100.9 nm and zeta potential of 35.9 ± 1.2 mV was reported
S.Y. Tang et al. 2012 [77]	Aspirin	Ultrasonic cavitation method	Oil- Lauroglycol 90 Surfactant- Cremophor EL Cosurfactant- Transcutol	Nanoemulsion demonstrated enhanced anti-inflammatory and analgesic effect compared to reference suspension with mean droplet diameter of 215.6 nm and 0.289 PDI.

N. Belhaj et al. 2012 [78]	Coenzyme Q10 PUFA's	Sonication followed by HPH	Oil-Salmon oil Surfactant-Salmon Lecithin	Mean droplet size was found to be 167.08 ± 1.07 with PDI of 0.12 ± 0.02. NE formulation showed twice the area under curve value of CoQ10
Syafinaz Zainol et al. 2012 [79]	Levodopa	Sonication followed by HPH	Oil- Palm and MCT oil Surfactant-Lecithin and Cosurfactant-Cremophor EL	Response Surface Methodology was used as a beneficial tool for carrying out the optimization study of levodopa nanoemulsion formulations
Shailaja et al. 2012 [80]	Olanzapine	Ultrasonication	Oil-Seasame oil Surfactant- Soy Lecithin Cosurfactant-Tween 80	Optimized NE showed an average size of 65.1nm to 74.21nm and surface charge of -18.9 mv to -25.23mv. TEM revealed spherical morphology, Box Behnken statistical design was applied to optimize the selected formulation
Chang Wei Hseih et al. 2012 [81]	Glabridin	HPH	Oil-Capric tryglyceride Emulsifier-	RSM Box Behnken design was used to predict the response

			Tween 80 and Span 80.	of the droplet size of Glabridin NE. The optimal conditions for preparing glabridin NE were predicted.
S.Y. Tang et al. 2012 [81]	Aspirin	Ultrasonic cavitation	Oil- Lauroglyycol 90 Emulsifier- Cremophor EL Cosurfactant- Transcutol HP	Central composite design (CCD) was successfully employed to develop empirically a second order polynomial regression model for predicting the variation of the average droplet size and PDI of aspirin NE formulations
K.K. Singh, S.K. Vingkar. 2008[82]	Primaquine	High speed stirring (ultra turrax) followed by HPH	Oil- Miglylol 812, Surfactant- Lutrol F 68 Cosurfactant- glycerol	Lipid nanoemulsion of primaquine exhibited improved oral bioavailability and was taken up preferentially by the liver with 45% higher drug concentration as compared to the plain drug.
Y. Yuan et al. 2008 [83]	β-carotene	HPH	Oil- MCT	The z-average of the dispersed particles containing

				β-carotene ranged from 132 to 184 nm and the size distribution was unimodal.
S. Shafiq et al. 2007 [49]	Ramipril	Spontaneous emulsification (Titration method)	Oil- Sefsol 218 Surfactant- Tween 80 Cosurfactant- Carbitol	Thermodynamically stable and dilutable nanoemulsion were prepared having particle size 80.9 nm. The relative bioavailability of ramipril NE to that of conventional capsule form was found to be 229.62% whereas to that of drug suspension it was 539.49%.

2.4. Experimental Design by Quality by Design Approach

Quality by Design (QbD) is a systematic approach that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management[84]. It involves statistical design of experiment (DoE) for optimization to minimize expenditure in terms of time, money and efforts when compared to conventional OFAT (one factor at a time) approach [85]. QbD based product development has gained significant attention as it is recommended by most regulatory bodies all over the world [86]. An experimental design is a strategy for laying out a detailed experimental plan in advance to the conduct of the experimental studies. Before the selection of experimental design, it is essential to isolate the experimental domain within the factor space [87]. There are numerous types of experimental designs. Various commonly employed experimental designs for response surface methodology

(RSM), screening, and factor influence studies in pharmaceutical product development are: factorial design, fractional factorial design, Plackett–Burman design, star design, central composite design, Box–Behnken design, center of gravity design, equiradial design, mixture design, Taguchi design, optimal design, Rechtschaffner design, Cotter design etc.

RSM is an empirical modelling tool consisting of a group of mathematical and statistical techniques that can be used to develop, improve and optimize processes in which the response is influenced by several variables. It defines the effect of the independent variables, alone or in combination regarding the studied processes [88]. RSM allows researchers to conduct optimization study with ease as it helps to reduce the number of experimental trials required to as minimum as possible [89].

The purpose of optimization of any pharmaceutical process is to determine and evaluate independent variables that affect formulation response. Design of experiments (DoE), on the other hand, is an optimization technique meant for products and/or processes, developed to evaluate all the potential factors simultaneously, systematically and speedily. Its implementation invariably encompasses the use of statistical experimental designs, generation of mathematical equations and graphic outcomes, portraying a complete picture of variation of the response(s) as a function of the factor(s) [90].

DoE optimization methodology encompass planning the study objectives, screening of influential variables, experimental designs, postulation of mathematical models for various chosen response characteristics, fitting experimental data into these model(s), mapping and generating graphic outcomes, and design validation using model-based response surface methodology [85].DoE is an efficient procedure for planning experiments in such a way that the data obtained can be analyzed to yield valid and unbiased conclusions [91].

In the present study, Statistical D-Optimal mixture design was used to optimize the Dabigatran etexilate SMEDDS, three level two factor full factorial design was used to optimize Dabigatran etexilate nanoemulsion, Nisoldipine SMEDDS and Nisoldipine nanoemulsion formulations. These experimental designs were used as a statistical tool to quantify relationship between critical formulation variables and responses measured [92].

The D-optimal mixture design is one of the most popular response surface methodologies for optimizing formulation of a SMEDDS. The D-optimal mixture design

diminishes the variation associated with evaluation of coefficients in a model and generates the best possible subset by considering the criteria for maximizing information matrix determinants. The D-optimal mixture design considers the total system of SMEDDS as 100% unlike other designs. The regression equation of this design is somewhat different from traditional polynomial equation and is restricted to $X_1+X_2+\ldots+X_n= 1$, termed as canonical polynomial [93]. Factorial designs are used to study the effect of independent variables on the dependent variables of any formulation. Based on the principle of design of experiments, factorial design is employed to evaluate the effect of two independent factors. The regression equation for the response is calculated using the equation:$Y=b_0+b_1X_1+b_2b_2+b_3X_{12}+b_4X_{22}+b_5X_1X_2$. In this mathematical approach, each experimental response (Y) can be represented by a quadratic equation of the response surface. Y is the measured response and b is the estimated coefficient for the factor X. The coefficients corresponding to linear effects (X_1 and X_2), interaction (X_1X_2) and the quadratic effects (X_{12} and X_{22}) [94].

2.5. Drug Profiles

2.5.1. Dabigatran Etexilate

Dabigatran (DAB) is a potent, synthetic, non-peptide, competitive, rapidly acting oral direct thrombin inhibitor belonging to BCS class II. It is poorly absorbed following oral dosing; hence it is administered in the form of pro-drug: Dabigatran Etexilate (DE) which does not possess anticoagulant activity. After oral administration, DE is rapidly absorbed and converted to DAB by esterase-catalysed hydrolysis in plasma and in the liver. DE is used in its salt form Dabigatran Etexilate Mesylate (DEM) which specifically and reversibly inhibits thrombin, the final enzyme in the coagulation cascade (Figure 2.5.1).By inhibiting thrombin, dabigatran prevents the conversion of fibrinogen into fibrin, positive feedback amplification of coagulation activation, cross-linking of fibrin monomers, platelet activation, and inhibition of fibrinolysis [95]. Since thrombin enables the conversion of fibrinogen into fibrin during the coagulation cascade, its inhibition prevents the development of thrombus. DAB also inhibits free thrombin, fibrin-bound thrombin and thrombin induced platelet aggregation. The granted indication is primary prevention of venous thrombo embolism in patients undergoing elective major orthopedic surgery (total knee replacement or total hip replacement surgery) [96].

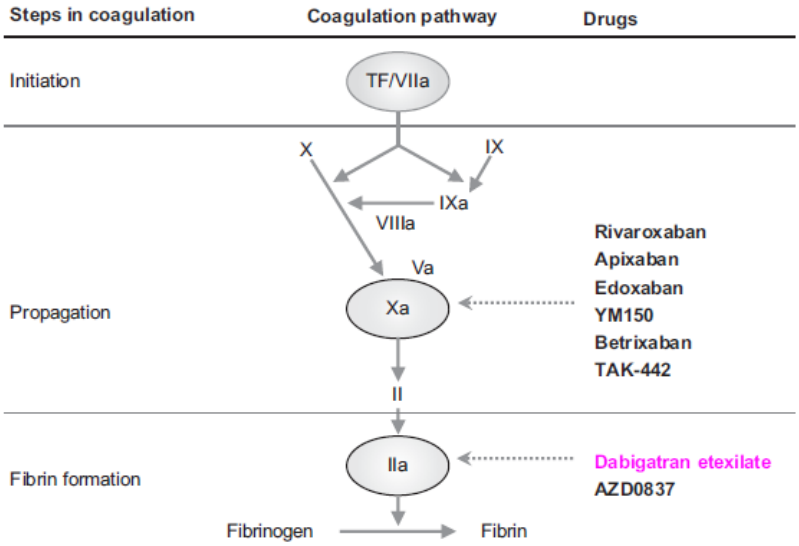

Figure 2.5.1. Site of action of new anticoagulants in the coagulation cascade[95]

The chemical (IUPAC) name of dabigatran etexilate is (ethyl-3-[[2-[[[4-(N0-hexoxycarbonylcarbamimidoyl)phenyl]amino]methyl]-1-methylbenzimidazole-5-carbonyl]-pyridin-2-ylamino]propanoate)corresponding to the molecular formula $C_{34}H_{41}N_7O_5$. The molecular mass is 627.75g/mol. DE is a white to off white crystalline powder. The structural formula is:

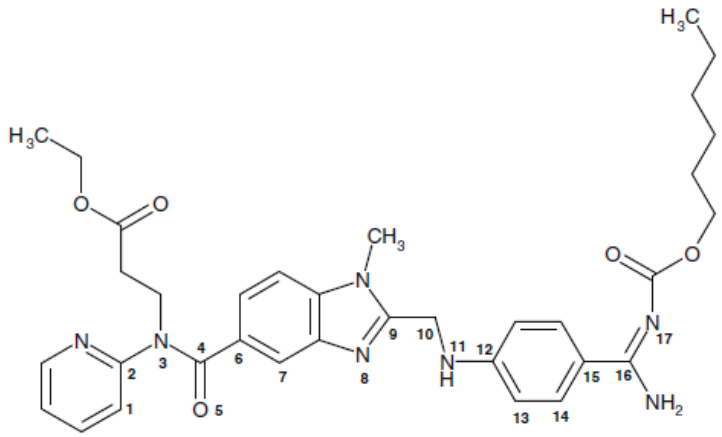

Figure 2.5.2: Structural formula of Dabigatran Etexilate

Solubility is strongly pH dependent with increased solubility at acidic pH. DEM is freely soluble in methanol, soluble in ethanol, sparingly soluble in iso-propanol, very slightly soluble in acetone and practically insoluble in ethyl acetate. DEM has affinity for the efflux transporter P-glycoprotein.

The absolute bioavailability of dabigatran following oral administration is approximately 6.5%. This low bioavailability is attributed to the low solubility, Pgp efflux and acid hydrolytic degradation. C_{max} is attained within 0.5 to 2.0 h post administration. Food does not affect the bioavailability of dabigatran etexilate but delays the time to peak plasma concentrations by 2 h. The biological half-life is 12-17h [97, 98]. Formulation of DE into nano system would increase the solubility. Also, incorporation of Pgp inhibitor (surfactant and co-surfactant) could reduce the drug efflux thereby increasing the bioavailability.

2.5.1.1. Physical and Biopharmaceutical Properties of DE

Some of the physical and biopharmaceutical properties of DE [97] are given in Table 2.5.1.

Table 2.5.1.: Physicochemical and biopharmaceutical properties of DE

Parameter	Value/Remarks
Melting point	130°-133°C
Volume of Distribution	50-70L
$t_{½}$	12-17h
pKa	$pKa_1 = 4.0 \pm 0.1$ $pKa_2 = 6.7 \pm 0.1$
Log P (Neutral form/free base)	3.8
Plasma protein Binding	35%
First pass metabolism	No
PgP Substrate	Yes
Absolute Bioavailability	3-7%

2.5.1.2. Mechanism of action

Dabigatran and its acyl glucuronides are competitive, direct thrombin inhibitors. Because thrombin (serine protease) enables the conversion of fibrinogen into fibrin during the coagulation cascade, its inhibition prevents the development of a thrombus. Both free and clot bound thrombin and thrombin induced platelet aggregation are inhibited by the active moieties [98].

2.5.1.3. Pharmacokinetics

2.5.1.3.1. Absorption

The absolute bioavailability of dabigatran following oral administration of dabigatran etexilate is approximately 3 to 7%. Dabigatran etexilate is a substrate of the efflux transporter P-gp. After oral administration of dabigatran etexilate in healthy volunteers, Cmax occurs at 1 hour post-administration in the fasted state but delayed by approximately 2 hours when coadministred with high-fat meal [99].

2.5.1.3.2. Distribution

Dabigatran is approximately 35% bound to human plasma proteins. The red blood cell to plasma partitioning of dabigatran measured as total radioactivity is less than 0.3. The volume of distribution of dabigatran is 50 to 70 L. Pharmacokinetics of dabigatran are dose proportional after single doses of 10 to 400 mg.

2.5.1.3.3.Metabolism

After oral administration, dabigatran etexilate is converted to dabigatran. The cleavage of the dabigatran etexilate by esterase-catalyzed hydrolysis to the active principal dabigatran is the predominant metabolic reaction. Dabigatran is not a substrate, inhibitor, or inducer of CYP450 enzymes. Dabigatran is subject to conjugation forming pharmacologically active acyl glucuronides.

2.5.1.3.4.Elimination

Dabigatran is eliminated primarily in the urine. Renal clearance of dabigatran is 80% of total clearance after intravenous administration. After oral administration of radio labelled dabigatran, 7% of radioactivity was recovered in urine and 86% in faeces. The half-life of dabigatran in healthy subjects is 12 to 17 hours [98].

2.5.1.4. Adverse effects

Most common adverse reactions are bleeding and gastritis-like symptoms.

2.5.1.5. Analytical methods

The reported analytical methods were slightly modified for estimation of Dabigatran Etexilate.

2.5.1.5.1. UV-spectrophotometric method

A simple accurate and sensitive UV-spectrophotometric method for estimation of DE was developed in 0.01N hydrochloric acid at 325nm [100].

Since DE is having high solubility in methanol, UV-spectrophotometric method was developed in methanol at 315nm [101].

In order to carry out drug release study in biorelevant media, the UV-spectrophotometric method was also developed in phosphate buffer pH 6.8 at 316nm [101].

2.5.1.5.2. HPLC method

A simple HPLC method was reported for DE analysis in plasma by Bernardi et al. with slight modification in mobile phase [102].

2.5.1.6. Formulations available

Till date, no dosage form is available for DE in the market but only capsule of Dabigatran Etexilate Mesylate (DEM) are available in strengths of 75 mg and 150 mg with the brand name Pradaxa® by Boehringer Ingelheim Pharmaceuticals, Inc. USA.

2.5.1.7. Dosage and administration

Recommended dose: For patients with creatinine clearance (CrCl) >30 mL/min, the recommended dose of Pradaxa is 150 mg taken orally, twice daily, with or without food.

For patients with severe renal impairment (CrCl 15-30 mL/min), the recommended dose of Pradaxa is 75 mg twice daily [98].

Table 2.5.2. : Review of work done on DE

Author (Reference no.)	Formulation	Technique/ Method	Polymer/ Excipient used	Conclusion
Chai, Sun & Ding et al. 2016 [101]	Solid self-nano emulsifying system	DE SNEDDS dispersible tablets by the direct compression method	Oils- (Maisine 35–1: MCT = 1:1, w/w) Surfactants - (Cremophor RH40 Gelucire 44/14, OP-10) Co-surfactants- (1,2-propanediol, Transcutol P) Diluent (MCC 102), disintegrating	SNEDDS was developed for improving oral absorption and bioavailability. Optimized SNEDDS had increased relative bioavailability compared with Pradaxa®

			agent (PVPP), lubricants (colloidal silicon dioxide and magnesium stearate), absorbent (MCC KG 802)	capsules.
Mei Hu.et al. 2016 [103]	binary mixed micelles system	Thin film hydration method.	Soluplus® and D-alpha tocopheryl polyethylene glycol 1000 succinate.	Dabigatran etexilate loaded micelles displayed an average size distribution of around 83.13 nm. The cellular uptake in Caco-2 cell monolayer was significantly enhanced by 2 to 2.6 fold over time as compared with drug suspension. The oral bioavailability in rats was 3.37 fold higher than that of drug suspension.

PATENTS

Inventor(s)	**Title (Patent No.)**	**Description**
Ali Turkyilmaz, Ali Hasan Turp, Mehtap Saydam [104]	Oral pharmaceutical formulations comprising dabigatran (WO 2014060561 A1)	The present invention relates to a novel pharmaceutical enteric coated pellet formulations comprising dabigatran etexilate free base as an active agent

C.S. Kandi, N.P.V. bhotla Sreekanth Manikonda, A.K Reddy, S.K. Meenakshi sunderam, A.R. Panchada [105]	Stabilized pharmaceutical compositions of dabigatran and process for preparation thereof (US 20150157618 A1)	The present invention relates to stabilized pharmaceutical compositions comprising dabigatran etexilate, or pharmaceutically acceptable salts, esters, hydrates and solvates thereof, process of preparation and method of using the same

2.5.2. Nisoldipine

Nisoldipine, a calcium channel blocker of the dihydropyridine class, is used in the treatment of hypertension. It acts primarily on vascular smooth muscle cells by stabilizing voltage gated L-type calcium channels in their inactive conformation. It is a BCS class II drug. The chemical name (IUPAC) of Nisoldipine is 3, 5-pyridinedicarboxylic acid, 1, 4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methyl-propyl ester corresponding to molecular formula of $C_{20}H_{24}N_2O_6$. The structural formula[106] is:

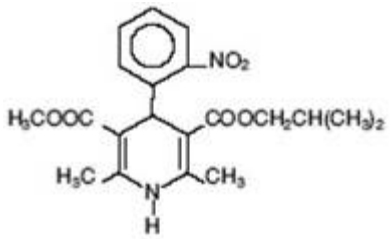

Figure 2.5.3.: Structural formula of Nisoldipine

Nisoldipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. It has a molecular weight of 388.42 g/mol. Nisoldipine is relatively well absorbed into the systemic circulation with 87% of the radiolabeled drug recovered in urine and faeces. The absolute bioavailability of nisoldipine is about 5% while the half life is 7-12 h. The low bioavailability is due to pre-systemic metabolism in the gut wall

[107]. A pronounced food-effect is observed when the product is administered with a high-fat meal resulting in an increased peak concentration (C_{max}) of up to 245%. It has also been reported that incorporation of lipids can enhance the dissolution property of nisoldipine [108].

Formulation of nisoldipine into nano system could increase the solubility and avoid first pass metabolism by lymphatic uptake. Since it is reported that high fat meal increases the absorption of nisoldipine, lipid based system could enhance the systemic bioavailability of nisoldipine.

2.5.2.1.Physical and Biopharmaceutical Properties of Nisoldipine

Some of the physical and biopharmaceutical properties of Nisoldipine [109] are given in Table 2.5.3.

Table 2.5.3.: Physicochemical and biopharmaceutical properties of Nisoldipine

Parameter	Value/Remarks
Melting point	155-160°C
$t_{½}$	7-12h
pKa	5.32
Log P	3.26
First pass metabolism	Yes
Systemic Bioavailability	5%
Light sensitive	Yes

2.5.2.2. Mechanism of Action

Nisoldipine is a member of the dihydropyridine class of calcium channel antagonists (calcium ion antagonists or slow channel blockers) that inhibit the trans membrane influx of calcium into vascular smooth muscle and cardiac muscle. It reversibly competes with other dihydropyridines for binding to the calcium channel. Because the contractile process of vascular smooth muscle is dependent upon the movement of extracellular calcium into the muscle through specific ion channels, inhibition of the calcium channel results in dilation of the arterioles. *In vitro* studies show that the effects of nisoldipine on contractile processes are selective, with greater potency on vascular smooth muscle than on cardiac muscle. Although, like other dihydropyridine calcium channel blockers, nisoldipine has negative inotropic effects. *In vitro* studies conducted in intact

anesthetized animals have shown that the vasodilating effect occurs at doses lower than those that affect cardiac contractility. The effect of nisoldipine on blood pressure is principally a consequence of a dose related decrease of peripheral vascular resistance. While nisoldipine, like other dihydropyridines, exhibits a mild diuretic effect, most of the antihypertensive activity is attributed to its effect on peripheral vascular resistance [108].

2.5.2.3. Pharmacokinetics

Nisoldipine pharmacokinetics are independent of the dose across the clinical dosage range of 17 mg to 51 mg, with plasma concentrations proportional to dose.

2.5.2.3.1. Absorption

Nisoldipine is relatively well absorbed into the systemic circulation with 87% of the radiolabeled drug recovered in urine and faeces. The absolute bioavailability of nisoldipine is about 5%. The low bioavailability is due to pre-systemic metabolism in the gut wall. A pronounced food-effect is observed when nisoldipine extended-release is administered with a high-fat meal resulting in an increased peak concentration (C_{max}) up to 245%. Total exposure (AUC) is decreased by 25%. As a result, nisoldipine should be taken on an empty stomach (1h before or 2 h after a meal).

2.5.2.3.2. Distribution

Maximal plasma concentrations of nisoldipine are reached 9.2 ± 5.1 h after dosing. The terminal elimination half-life (reflecting post absorption clearance of nisoldipine) ranges from 13.7 ± 4.3 h. The plasma protein binding of nisoldipine is very high, with less than 1% unbound over the plasma concentration range of 100 ng/mL to 10 mcg/mL.

2.5.2.3.3. Metabolism and elimination

Nisoldipine is highly metabolized; five major urinary metabolites have been identified. Although 60% to 80% of an oral dose undergoes urinary excretion, only traces of unchanged nisoldipine are found in urine. The major biotransformation pathway appears to be the hydroxylation of the isobutyl ester. A hydroxylated derivative of the side chain, present in plasma at concentrations approximately equal to the parent compound, appears to be the only active metabolite, and has about 10% of the activity of the parent compound. Cytochrome P enzymes are believed to play a major role in the metabolism of nisoldipine. The particular isoenzyme system responsible for its metabolism has not been identified, but other dihydropyridines are metabolized by cytochrome P IIIA4. Nisoldipine should not be administered with grapefruit juice as this has been shown, in a study of 12 subjects, to interfere with nisoldipine metabolism, resulting in a mean

increase in C of about 3-fold (ranging up to about 7-fold) and AUC of almost 2-fold (ranging up to about 5-fold). A similar phenomenon has been seen with several other dihydropyridine calcium channel blockers [107, 108].

2.5.2.4. Adverse effects

The more common side effects that can occur with nisoldipine include headache, dizziness, nausea, or swelling of the ankles, feet, or hands. They also include chest pain, sore throat, nasal congestion, or rash [108].

2.5.2.5. Analytical methods

The reported analytical methods were slightly modified for estimation of Nisoldipine.

2.5.2.5.1. UV-spectrophotometric method

A simple accurate and sensitive UV-spectrophotometric method for estimation of Nisoldipine in 0.1N Hydrochloric acid with 0.5% SLS at 238nm [110].

Since Nisoldipine has high solubility in methanol, UV-spectrophotometric method was developed in methanol at 236nm [111].

In order to carry out drug release study in biorelevant media the UV-spectrophotometric method was also developed in phosphate buffer pH 6.8 with 0.5% SLS at 236nm [112].

2.5.2.5.2. HPLC method

A reported HPLC method by Marinkovic et al.2003,with slight modification in mobile phase was used for NISO analysis in plasma [113].

2.5.2.6. Formulations available

Nisoldipine extended release tablets having strengths 8.5mg, 17 mg, 25.5 mg and 34 mg are commercially available [108].

2.5.2.7. Dosage and administration

The dosage of nisoldipine extended-release tablets must be adjusted to each patient's needs. Therapy usually should be initiated with 17 mg orally once daily, then increased by 8.5 mg per week or longer intervals, to attain adequate control of blood pressure. Usual maintenance dosage is 17 mg to 34 mg once daily. Doses beyond 34 mg once daily are not recommended. Nisoldipine extended-release tablets should be administered orally once daily. Nisoldipine extended release tablets should be taken on an empty

stomach (one h before or 2 h after a meal). Grapefruit products should be avoided before and after dosing. Nisoldipine extended-release tablets are an extended release dosage form and tablets should be swallowed whole, not bitten, divided or crushed [108].

Table 2.5.4.: Review of work done on Nisoldipine

Author (Reference no.)	**Formulation**	**Technique**	**Polymer/ Excipients used**	**Conclusion**
S. Chopra et al.2013 (122)	Lipid bearing pellets	Extrusion–spheronization	Microcrystalline cellulose, soy phosphatidyl choline (SPC), granulating fluid, lactose	Pellets were found to be spherical, 600–850 µm size with <0.01% friability and had >70% yield. SEM studies showed a smooth external surface and a porous internal matrix
Yan Xiao et al. 2012 [114]	Microemulsion-based hydrogel	Simple mixing	Isopropyl myristate (IPM, 3%, w/w), Cremophor EL (15%, w/w), Transcutol® (22%, w/w) and water (60%, w/w), Carbomer as hydrogel matrix	The size of the NS-MEs was 23.1± 2.4 nm and the spherical droplets existed in both NS-MEs and NS-MEs-H. The *in vitro* skin permeation and *in vivo* pharmacokinetic studies showed that NSMEs-H could maintain a steady skin permeation rate

Nepolean. R et al 2012[115]	Nanoparticles	Nano precipitation method	Eudragit RLPO, Tween 80, Water	The mean particle size of the NP's was found to 400-600 nm with narrow size distribution with zeta potential of about -25mV. The *in vitro* release from Nisoldipine loaded Eudragit RLPO nanoparticles showed 11% release at pH 1.2, 55%at pH 5.0 and 90%at pH 6.8 within 24 h
Gupta et al 2009 [116]	Extended release buccal tablets	Progressive hydration technology	Carbopol 972P (CP), Hypromellose K15M (HPMC) and Polycarbophil (PC)	High polymer contribution was able to control the release and retain sufficient buccoadhesive strength
Fakharul Hassnain, et al 2012 [117]	Solid Dispersion	Solvent evaporation method	Polyvinyl pyrrolidone (PVP) k-25 and polyethylene glycol (PEG) 4000	The particle size was found in range of 43.52 to 45.12 µm. Dissolution studies indicated better release for solid dispersions and solubility was also increased 15 folds than pure drug
Mohan et al 2012 [118]	Solid dispersion	Solvent evaporation and Melt Method	PEG 6000 and mannitol	The Drug: Carrier ratio of 1:5 was found to be optimum for

				improving the dissolution rate of Nisoldipine as compared to other formulations. Solvent method showed faster dissolution rate when compared with that of the pure drug and other complexes.
Ali et al.2014 [112]	Fast dissolving tablet	Direct compression	Sodium starch Glycolate, Cross Povidone, Aspartame, Magnesium stearate and Mannitol	Nisoldipine FDTs using 6%w/w of combined superdisintegrants showed faster disintegration, dissolution profile, increased onset of action and improved bioavailability

PATENTS

Inventor(s)	**Title (Patent No.)**	**Description**
Pascal Grenier, Guy Vergnault, Alain Nhamias, [119]	Controlled Release Nisoldipine Compositions (US 2008/ 0221174 A1)	Controlled release oral dosage formulations containing calcium channel blockers, and methods of use thereof, are provided for the once-a-day treatment of cardiovascular disorders, such as hypertension, angina, and cardiac arrhythmia. The active agent is preferably a dihydropyridine calcium channel blocker, such as nisoldipine. The formulation provides an increase in the

		bioavailability of the calcium channel blocker as compared to the bioavailability of the calcium channel blocker in other drug delivery formulations known in the art. In one embodiment, the formulation provides an increase in the bioavailability of the calcium channel blocker, nisoldipine, as compared to the same dose of nisoldipine in the coat-core version of the drug (Sular®). The formulation can be in the form of a trilayer tablet containing a core or central layer and one or more barrier layers
Kuhl Alexander, Brendel Erich, Brocker Frank, Funke Adrian, Ohm Andreas, Kvesic Dennis, Volkmer Thomas [120]	Pharmaceutical dosage form comprising nifedipine or nisoldipine and an angiotensin-II antagonist and/or a diuretic (EP 2370065 B1)	Pharmaceutical dosage form comprising an active compound combination of nifedipine or nisoldipine and at least one angiotensin II antagonist and/or at least one diuretic, characterized in that the core is an osmotic release system

2.6. Excipients Profile

Detailed profiles of excipients are given in Appendix I.

2.7. Conclusions

Literature review for Dabigatran Etexilate and Nisoldipine revealed that these poorly soluble drugs belonging to BCS class II exhibited low bioavailability (3-7% and 5% respectively). There exists a strong need to improve the bioavailability of these drugs. Considerable research has been done on lipid based drug delivery systems and it is a scalable technique with some commercial products already reaching the market.

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