Development and characterization of oil-in-water nanoemulsions from self-microemulsifying mixtures

Ishan Shah

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A Thesis

entitled

Development and Characterization of Oil-in-Water Nanoemulsions from Self-Microemulsifying Mixtures

by

Ishan Shah

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Pharmaceutical Sciences with Industrial Pharmacy option

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May 2011
An Abstract of

Development and Characterization of Oil-in-Water Nanoemulsions from Self-Microemulsifying Mixtures

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Self-Microemulsifying Drug Delivery Systems (SMEDDS) are isotropic mixtures of oil, surfactant and/or cosurfactants, and a drug that spontaneously forms an oil-in-water nanoemulsion upon gentle agitation with water. When dispersed in the gastrointestinal (GI) tract, the motility of stomach provides necessary agitation for emulsification. SMEDDS incorporated with a poorly water soluble drug demonstrates improved drug absorption since it maintains the drug in a solubilized state in the GIT tract. The purpose of this research was to screen lipid excipients for their self-microemulsification efficiency and to develop SMEDDS based formulation in liquid and solid forms using ibuprofen as a model drug. Excipients evaluated for SMEDDS were Tween 80 and Cremophor RH 40 as surfactants, Transcutol P, Capyrol 90 and PEG 400 as cosurfactants and Labrafac Lipophile WL 1349 (a medium chain triglyceride) as the oil. Self Microemulsifying (SME) mixtures containing various proportions of these components were tested for their self-microemulsification ability and were characterized by ternary phase diagrams. Based on these results, a particular mixture containing Tween 80-PEG 400-LL WL 1349 was selected and optimized for drug delivery purpose. Liquid SMEDDS was formulated by
dissolving ibuprofen in the SME mixture. Solid SMEDDS was formulated by adsorbing Liquid SMEDDS onto an inert carrier Neusilin US2 by physical mixing. SMEDDS were analyzed for their droplet size and zeta potential. Solid state characterization of Solid SMEDDS was performed using scanning electron microscopy, differential scanning calorimetry and powder x-ray diffractometry. Finally, in vitro drug release studies were performed on Liquid and Solid SMEDDS and the results compared to plain ibuprofen dissolution. Ibuprofen was found to be physically and chemically stable in the SMEDDS and did not precipitate upon aqueous dilution. Solid and Liquid SMEDDS showed a droplet size of less than 50 nm and possessed a neutral zeta potential. Solid state characterization of Solid SMEDDS confirmed the presence of ibuprofen in a molecularly dissolved state in the formulation. In vitro drug release studies showed that 75 % of drug was released from Solid and Liquid SMEDDS within first five minutes of the dissolution time. A SMEDDS based dosage form was successfully developed and shows potential for application in the delivery of poorly water soluble drugs.
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Chapter 1


Approximately one third of the drugs emerging from drug discovery programs are poorly water soluble, presenting the pharmaceutical scientist with several problems when developing formulations for such active pharmaceutical ingredients (API). Conventional oral dosage forms for poorly water soluble drugs present the drug in a solid form to the gastrointestinal tract which means the drug has to dissolve in the GI fluids before it can be absorbed. Thus, their rate and extent of absorption is largely dependent on the rate of dissolution. The formulation technique plays an important role in overcoming this shortcoming of poorly water soluble drugs. According to the Biopharmaceutical Classification System (BCS) classification, two classes of drugs show poor aqueous solubility namely BCS II and BCS IV. BCS II drugs possess poor aqueous solubility but have good permeation properties. BCS class IV drugs are poorly water soluble and poorly permeable. Developing a formulation for a class IV drug is nearly impossible
unless the dose necessary is very small. Most of the times, such drugs are withdrawn at its lead optimization stage of drug discovery and reworked to improve its physico-chemical properties. Developing a formulation for a drug belonging to BCS II is often challenging as it requires improved dissolution characteristics. Popular formulation techniques used for delivering a poorly water soluble drug include: (a) micronized crystalline solids (b) amorphous formulation or solid solutions and (c) lipid based formulations. When particles of drug are milled to smaller particle sizes, there is an increase in surface area resulting in an increased dissolution of the drug. Micronization using an air jet mill will yield particles in the size range of 2-5 µm. Using a new technique NanoCrystal®, which employs high-speed attrition process, particles can be reduced in nanometer ranges. Such powders can be processed into tablets and capsules [1]. Solid dispersions can be defined as a “dispersion of one or more active ingredient in an inert excipient or matrix” wherein the active ingredient exists in a finely crystalline, solubilized or amorphous state [2]. When solid dispersions are exposed to aqueous media, the matrix dissolves and releases the drug as very fine colloidal particles. This results in increased dissolution of the drug. This research project involves development of lipid based formulations and hence I will elaborate more on this topic below.

1.1 Lipid based drug delivery

Lipid based drug delivery consists of delivering a drug dissolved in a mixture of one or more excipients which may be a mono, di and tri-glyceride, lipophilic and hydrophilic surfactants and a cosurfactant. When a drug is delivered through lipid formulations, it remains in the dissolved state throughout its transit in the GI tract. The absorption of the
drug when presented in a solubilized form within a colloidal dispersion is enhanced since the drug dissolution step is partially evaded.

In the year 2000, Pouton classified lipid based formulations into three categories based on their composition and properties. In 2006, this classification was updated by adding one more class (Type IV) as shown in Table 1.1 [3].

Table 1.1: Lipid formulation classification system (LFCS) showing typical proportions of lipid formulations

<table>
<thead>
<tr>
<th>Excipient in formulation</th>
<th>Content of formulation (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
</tr>
<tr>
<td>Oils: triglyceride or mixed mono and diglyceride</td>
<td>100</td>
</tr>
<tr>
<td>Water insoluble surfactants (HLB*&lt;12)</td>
<td>-</td>
</tr>
<tr>
<td>Water soluble surfactants (HLB&gt;*12)</td>
<td>-</td>
</tr>
<tr>
<td>Hydrophilic cosolvents (eg. PEG, propylene glycol, transcutol)</td>
<td>-</td>
</tr>
</tbody>
</table>

*HLB: Hydrophilic Lipophilic Balance

Type I formulations are simply oil based, type II systems are water-insoluble self-emulsifying drug delivery systems (SEDDS), type III systems are SEDDS or self-microemulsifying drug delivery systems (SMEDDS) and type IV systems are oil-free formulations. One common requirement for all lipid formulation types is that they should be able to keep the drug in the solubilized form. If by any chance the drug precipitates, the advantage of lipid formulations is nullified. Each lipid formulation type has specific features as described in Table 1.2 by Pouton [3].
Table 1.2: Characteristics, advantages and disadvantages of lipid formulations

<table>
<thead>
<tr>
<th>LCFS type</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Non-dispersing , require digestion</td>
<td>GRAS status, simple, excellent capsule</td>
<td>Poor solvent capacity unless the drug is highly lipophilic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>compatibility</td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>SEDDS without water soluble components</td>
<td>Unlikely to lose solvent capacity on dispersion</td>
<td>Turbid o/w emulsion (0.25-2 µm)</td>
</tr>
<tr>
<td>Type IIIA</td>
<td>SEDDS/SMEDDS with water soluble components</td>
<td>Clear or almost clear dispersion, drug absorption without digestion</td>
<td>Possible loss of solvent capacity on dispersion, less easily digested</td>
</tr>
<tr>
<td>Type IIIB</td>
<td>SMEDDS with water-soluble components and low oil content</td>
<td>Clear dispersion, drug absorption without digestion</td>
<td>Likely loss of solvent capacity on dispersion</td>
</tr>
<tr>
<td>Type IV</td>
<td>Oil-free formulation based on surfactant and cosolvents</td>
<td>Good solvent capacity for many drugs, disperses to micellar solution</td>
<td>Loss of solvent capacity on dispersion, may not be digestible</td>
</tr>
</tbody>
</table>

**Formulation of Type I systems**

Type I systems are comprised of only one excipient which is a triglyceride or a mixture of a triglyceride with a mono or diglyceride. They have no or limited solubility in water and used only if the drug is sufficiently soluble in the glyceride mixture. Highly lipophilic drugs with logP >5 can be delivered by this formulation. To improve solvent capacity of the drug, triglycerides may be blended with mono or diglycerides. These formulations are very safe. These can be easily administered via oral route resulting in complete intestinal absorption of the drug molecules. They are poorly dispersible since they do not have any surfactant. Upon digestion in intestine the digested products are
solubilized in mixed micelles forming a colloidal dispersion of drug from which the drug partitions out [4]. Examples of commercially available type I formulations (in soft gelatin capsules) are Progesterone dissolved in peanut oil (Prometrium®), testosterone undecanoate dissolved in oleic acid (Restandol®) and valproic acid dissolved in corn oil (Depakene®).

**Formulation of Type II formulations**

These formulations contain a lipophilic surfactant (HLB<12) in addition to the triglycerides. Surfactants aid in the emulsification of these systems as well as provide solvent capacity for the drug. If the surfactant is not sufficiently hydrophilic, it exists as a dispersed phase, either within or separated from the oily components. Such formulations do retain solvent capacity after dispersion in aqueous media. They require at least 25% of surfactant to self-disperse. If surfactant concentration exceeds 65% w/w, emulsification progress is affected by formation of a liquid crystalline phase at the oil-water interface [5]. A SEDDS comprising of a medium chain triglyceride and a polyoxyethylene-(25)-glyceryl trioleate (Tagat TO) is an example of type II system [6].

**Formulation of Type III systems**

Addition of a hydrophilic surfactant (HLB>12) and /or water soluble cosolvent to the triglyceride makes the formulation self-emulsifying. Addition of cosolvent has a double effect: (a) along with the surfactant it is able to form a very fine dispersion with droplet size of less than 100 nm and (b) it increases the solvent capacity of the formulation since it can dissolve large quantities of a drug. Only disadvantage of using cosolvents is loss of
solvent capacity upon dispersion. The cosolvent upon dispersion will separate from oily components and dissolve in the aqueous phase causing partial drug precipitation. Thus, it is essential to consider this factor while formulating such systems. Type III formulations are further classified into two categories based on the amount of hydrophilic components. Type III B systems, also referred to as SMEDDS have large quantities of cosolvents but contain lesser amount of oil. Such formulations possess highest risk of precipitation.

Most of the marketed lipid formulations belong to class III. Table 1.3 shows few examples of such formulations

Table 1.3: Examples of Type III lipid based formulations [2]

<table>
<thead>
<tr>
<th>Drug/Trade name/Manufacturer</th>
<th>Glyceride</th>
<th>Surfactant</th>
<th>Cosolvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine/Neoral®/Novartis</td>
<td>Partial glycerides</td>
<td>Cremophor RH 40</td>
<td>Ethanol, propylene glycol</td>
</tr>
<tr>
<td>Lopinavir and Ritonavir/Kaletra®/Abbott</td>
<td>Oleic acid</td>
<td>Cremophor EL</td>
<td>Propylene glycol</td>
</tr>
</tbody>
</table>

**Formulation of Type IV system**

These systems are comprised of just surfactants or a mixture of surfactant and a cosolvent. If the drug is formulated in a pure solvent, there are chances of drug being precipitated as amorphous or fine crystalline particles. If the drug is formulated in pure surfactants, there will be less chance of precipitation but owing to formation of a liquid crystalline state at the oil-water interface, surfactants will take more time to disperse in water. Moreover, use of surfactants in high concentrations may cause gastric irritation and local damage [4]. Commercially examples of such a system is Amprenavir
(Agenerase®, GSK) which is formulated in a blend of tocopheryl polyethylene glycol 1000 succinate (TPGS), PEG 400, and propylene glycol.

1.2 Self-Emulsifying / Microemulsifying drug delivery systems

Self-Emulsifying / Microemulsifying drug delivery systems (S(M)EDDS) are isotropic mixtures of oil, hydrophilic surfactant and/or a cosurfactant, and a solubilized drug. They can be encapsulated in hard or soft gelatin capsules or can be converted to solid state (Solid SEDDS/SMEDDS). These formulations spontaneously form a fine oil-in-water emulsion in case of SEDDS and a nanoemulsion in the case of SMEDDS upon dilution with water. In the GI tract, they are readily dispersed, where the motility of the stomach and small intestine provides the gentle agitation necessary for emulsification. SEDDS produces coarse emulsions while SMEDDS produces droplets of size less than 100 nm. This property of S(M)EDDS makes them a natural choice for delivery of hydrophobic drugs that have adequate solubility in oil-surfactant blends. S(M)EDDS improves the rate and extent of absorption of hydrophobic drugs, whose absorption is considered to be dissolution rate-limited. Upon aqueous dilution the drug remains in the oil droplets or as a micellar solution since the surfactant concentration is very high in such formulations [4]. The drug in the oil droplet may partition out in the intestinal fluid as shown in figure 1-1
1.3 Excipient selection for lipid based formulations

Chemically, lipids are considered as one of the most versatile excipient classes available today. There are various subcategories of lipids available and there is a constant influx of new lipid based excipients in the market. This provides flexibility to the formulator in terms of selecting a suitable excipient, but at the same time the formulator should be cautious while selecting a particular excipient. Pouton et al. described few factors that should be considered while selecting a lipid excipient. They are: (a) regulatory issues- irritancy, toxicity (b) solvent capacity (c) miscibility (d) morphology at room temperature (e) self-dispersibility (f) digestibility and fate of digested products (g) capsule compatibility (h) purity, chemical stability and (i) cost [4]. Apart from these factors, lipids have been shown to increase the bioavailability of drug by other means, as described in the section 1.5.

The following description on lipid based excipients is in relation to the S(M)EDDS.
1.3.1 Oils

Oils play a critical role in S(M)EDDS because it is responsible for solubilization of the hydrophobic drug, aiding in self-emulsification and moreover contributes to the intestinal lymphatic transport of the drug. The emulsification property of the oil is said to be dependent on the molecular structure of the oil [7]. Oils used in self-dispersing systems can be classified into three categories.

**Triglyceride vegetable oils:** They are easily ingested, digested and absorbed presenting no safety issues. Depending on the vegetable source, they can have different proportions of long chain triglycerides (LCT) and medium chain triglycerides (MCT). Generally vegetable oils are rich in unsaturated LCT with the exception of coconut oil and palm kernel oil which are rich in saturated MCT. They are highly lipophilic and their effective concentration of ester group determines its solvent capacity. MCT’s are preferred over LCT’s in lipid based drug delivery owing to its good solvent capacity and resistance to oxidation [4]. Vegetable oils are not widely used in SEDDS because of their poor solubility for the hydrophobic drug and due to poor self dispersing property.

**Vegetable oils derivatives:** Popular vegetable oil derivatives are hydrogenated vegetable oil, mixed glycerides, polyoxylglycerides, ethoxylated glycerides and esters of fatty acids with various alcohols. Hydrogenated vegetable oils are produced by hydrogenation of the unsaturated bonds present in the oil. Usually vegetable oils are hydrogenated before they are transformed into their derivatives since hydrogenation increases chemical stability. Examples of such oils are hydrogenated cottonseed oil
(Lubritab), hydrogenated palm oil (Dynasan), hydrogenated castor oil (Cutina HR) and hydrogenated soybean oil (Lipo) [4].

**Mixed Partial Glycerides**: They are formed by partial hydrolysis of triglycerides present in the vegetable oil resulting in a mixture of mono-, di- and tri-glycerides. The physical state, melt characteristics, and the HLB of the partial glycerides depend on the nature of the fatty acid present and the degree of esterification. Glycerides with medium chain or unsaturated fatty acids are used for improving bioavailability, while ones with saturated long chain fatty acids are used for sustained-release purposes [8]. Examples of glycerides with medium chain fatty acids are glyceryl monocaprylocaprate (Capmul MCM) and ones with long chain fatty acids are glyceryl monoleate (Peceol) and glyceryl monolinoleate (Maisine 35-1).

**Polyoxylglycerides / Macrogolglycerides**: They are formed by polyglycolysis of vegetable oil (hydrogenated or not hydrogenated) with polyethylene glycols (PEG) of a particular molecular weight. It has a fixed composition of a mixture of mono-, di- and triglycerides and mono and diesters of PEG. They are readily dispersible in water making them a good choice for SEDDS. Like glycerides, they may be composed of unsaturated long chain fatty acids such as oleyl polyoxylglycerides (Labrafil 1944CS) and linoleyl polyoxylglycerides (Labrafil M 2125CS) or medium chain fatty acids such as caprylocaproyl polyoxylglycerides (Labrasol) and lauroyl polyoxylglycerides (Gelucire 44/14).

**Ethoxylated glycerides**: They are formed from ethoxylation (etherification) of ricinoleic acid (present in glyceride) of castor oil. This reaction makes the oil hydrophilic.
Examples of such glycerides are ethoxylated castor oil (Cremophor EL) and ethoxylated hydrogenated castor oil (Cremophor RH40 and Cremophor RH 60). Because of its amphiphilic nature, Cremophor’s are widely used as surfactants in the formulation of SEDDS. Moreover, they can dissolve large quantities of drugs, have good self-emulsification property, and their degradation products are similar to those obtained from intestinal digestion [9, 10].

**Polyalcohol esters of fatty acids:** These are newer oil derivatives that possess surfactant properties because of its amphiphilic nature and are effective in replacing conventionally used oils [10]. Their composition is based on nature of alcohol used. They can be polyglycerol (Plurol Oleique CC 497), and propylene glycol (Capryol), and polyoxyethylene glycol (Mirj).

### 1.3.2 Surfactants

Surfactants are surface active molecules which concentrate at the oil-water interface and stabilize the internal phase in an emulsion. Surfactants are critical components of S(M)EDDS systems since they are responsible for forming a stable emulsion upon aqueous dilution. Nonionic surfactants are commonly used in this type of formulation. Proper selection of the surfactant is based on its Hydrophilic Lipophilic Balance (HLB) value and safety considerations. Nonionic surfactants with high hydrophilicity are required for SEDDS. A surfactant with an HLB value of more than 12 is necessary in SMEDDS to spontaneously form a fine oil-in-water nanoemulsion when dispersed in the GI tract fluids. Surfactants used in lipid based drug delivery are usually polyethoxylated lipid derivatives [11]. These lipids can be fatty acids, alcohols or glycerides which are
linked to a certain number of repeating polyethylene oxide units through ester linkage (fatty acids and glycerides) and ether linkage (alcohols). The polyethylene groups provide hydrophilic characteristics to the surfactant. Examples of such surfactants are polyethoxylated fatty acid ester (Myrj and Solutol HS 15), polyethoxylated alkyl ethers(Brij), polyethoxylated sorbitan esters(Tweens), and polyethoxylated glycerides (Cremphors, Labrasol) [11]. The most commonly used surfactants in SMEDDS are Tweens, Cremophors, and Labrasols. Block copolymers such as Pluronics have also been used in SEDDS [12]. Emulsifiers of natural origin are preferred due to safety considerations but are not widely used because of their poor self emulsification property [10]. Nonionic surfactants are less toxic and possess good emulsion stability over wider range of ionic strength and pH than ionic surfactants [13], but may cause changes in intestinal lumen permeability [14]. The surfactant concentration necessary to form a stable S(M)EDDS ranges from 30%w/w to 60%w/w [15]. The least possible surfactant concentration should be used so as to prevent gastric irritation. Extremely small droplet size produced in case of SMEDDS promotes rapid gastric emptying and low local concentration of surfactant, thereby reducing the gastric irritation [16]. The surfactant concentration is shown to have varied effects on emulsion droplet size. Increase in surfactant concentration causes a decrease in droplet size associated with stabilization of surfactant molecules at the oil-water interface [17], while the reverse is possible due to enhanced water penetration into oil droplets leading to breakdown of oil droplets [18]. The surfactants being amphiphilic can dissolve large quantities of the hydrophobic drug. They can contribute to the total solubility of the drug in S(M)EDDS, thus preventing
drug precipitation upon aqueous dilution and keep the drug in solubilized state in GI tract for further absorption [17].

1.3.3 Cosolvents

Water soluble cosolvents are widely used in lipid based dosage forms. Ethanol, polyethylene glycol (PEG), propylene glycol, and glycerol are examples of cosolvents used. Their role is: (a) to increase the solvent capacity of the drugs which are freely soluble in them. But this is associated with the risk of drug precipitation when S(M)EDDS are dispersed in water, (b) to dissolve large quantities of the hydrophilic surfactant in the oil. S(M)EDDS requires use of high concentration of surfactants to ensure proper dispersion of the formulation, (c) to increase the stability of nanoemulsion by wedging themselves between surfactant molecules [19]. There are several key issues that have to be considered before using a particular cosolvent. The cosolvents are miscible with the oil only up to a certain limit. There are some incompatibilities of using alcohol since it may penetrate into soft and hard gelatin shell causing precipitation of the drug.

1.4 Role of SEDDS/SMEDDS in improvement of oral absorption

S(M)EDDS partially avoids the additional drug dissolution step prior to absorption in the GI tract. They increase the amount of solubilized drug in the intestinal fluids resulting in good drug absorption. Apart from this, absorption of the drug may also be enhanced by using lipid based excipients in the formulation. There are several mechanisms through
which increased absorption can be achieved; the following schematic diagram describes these mechanisms.

![Figure 1-2: Pathways for drug absorption from lipid based formulations][20]

**Retardation of gastric emptying time:** Surfactants are believed to play a role in retardation of gastric transit time, thereby increasing the time available for the drug to dissolve and get absorbed. Surfactants may slow down gastric emptying for a period of time by formation of viscous mass in the gastric and intestinal lumen. Labrasol (a caprylocaproyl macrogolglyceride) was shown to improve bioavailability of an investigational compound by retarding gastric emptying time [21].

**Increase in effective drug solubility in lumen:** When exogenous lipid excipients are encountered in the gastric environment, they are digested by gastric lipases. Triglycerides
are digested to di-glycerides and fatty acids. The duodenum secretes bile salts (BS), phosphatidylcholine (PL) and cholesterol (Ch) from the gall bladder and pancreatic lipases from pancreas. These agents in combination with lipid digestion products get adsorbed to the surface of emulsion droplet and transform into small, stable droplets. They also produce a series of colloidal particles such as micelles, mixed micelles, and vesicles as shown in figure 1.2. The drug contained in the oil droplet partitions into these micellar structures making them a drug reservoir at the absorption site. This results in an increased solubilization capacity of the drug in the GI tract. This capacity is dependent on the type (medium chain or long chain triglycerides) and quantity of the lipids, presence of additional lipid excipients such as surfactants and cosurfactants, and the level of endogenous BS and PL present [22]. The micelles and nanoemulsions can be absorbed through following mechanisms: pinocytosis, diffusion, or endocytosis [9]. The partition of the drug from the oil droplets depends on their size and polarity. Nano sized droplets will result in faster partitioning since the drug can diffuse faster from smaller droplets [23]. In case of SMEDDS, it has been shown that digestion of the resultant nanoemulsion acts independently of bile salts [24] and the polarity of the oil droplets is not significant because the drug reaches the capillaries within the oil droplets [19].

**Lymphatic transport of the drug:** Most of the drugs delivered using S(M)EDDS are absorbed systematically via portal vein except for certain type of drugs. Lymphatic transport of the drug occurs when the drug is highly lipophilic (logP >5) and shows high solubility in triglycerides (>50mg/ml) [8]. Such drugs are absorbed via lymph vessels in the intestine which are responsible for absorption of lipids. Since the drug is cleared by the lymph vessels, they bypass the liver metabolism. This results in an increased
bioavailability of these drugs. The bioavailability of Ontazolast, an extensively first-pass metabolized drug was improved when delivered in a lipid based formulation. The drug was absorbed via lymphatic pathway and thus bypassed first-pass metabolism [25].

**Enterocyte based drug transport:** Few endogenous lipid transporters have been identified which are responsible for intestinal passage of lipophilic drugs. At low lipid concentrations drugs are actively transported, while at high lipid concentrations drugs are passively permeated. P-glycoprotein (P-gp) is an efflux transporter present in enterocytes that acts as a substrate for many lipophilic drugs. Surfactants are reported to inhibit these P-gp efflux transporters resulting in an increase in permeability of poorly permeated drugs [20]. Labrasol was identified as the most effective surfactant in inhibiting the P-gp.

**Increasing membrane permeability:** Lipids are responsible for causing fluidization of intestinal cell membrane and opening of tight junctions resulting in increased membrane permeability. Labrasol has a dual property of increasing membrane permeability by both the mechanisms, while Cremphor EL and Tween 80 act by opening the tight junction barrier [8]. Surfactants also penetrate into the intestinal cell membrane and disrupt the structural organization of the membrane leading to an increased permeability [17].
1.5 Formulation of SEDDS/SMEDDS

Formulation Composition

S(M)EDDS are composed of oil, hydrophilic surfactant, and a cosolvent. The process of self-emulsification is only specific to certain combinations of pharmaceutical excipients. It depends on the type of oil and surfactant pair, their ratios, the surfactant concentration and the temperature at which self-emulsification occurs. The primary step during formulation of a S(M)EDDS is the identification of these specific combinations of excipients and construct a phase diagram which shows various concentrations of excipients that possess self-emulsification. Mutual miscibility of these excipients is also important for producing a stable liquid formulation. Long chain triglycerides (LCT) are usually immiscible with hydrophilic surfactants and cosolvents. Polar oils such as mixed glycerides show an affinity towards hydrophilic surfactants and thus are miscible with the surfactant and also aids in self-dispersion of the formulation. The diversity of chemical nature of lipids used may lead to immiscibility on long-term storage, so it is essential to perform physical stability tests on the formulation. If a waxy excipient is used, they should be melted before weighing and then mixed with other liquid excipients [4].

Drug incorporation

Poorly water soluble drugs are often a choice for S(M)EDDS based dosage form. It is essential that the therapeutic dose of the drug be soluble in an acceptable volume of self-emulsifying mixture. The use of newer synthetic oils that are amphiphilic in nature can
dissolve large quantities of the drug when compared to conventionally used pure
vegetable oils or its derivatives. Surfactants also provide good solvency for the drug.
Although, the cosolvent is capable of dissolving a large quantity of the drug, they may
cause drug precipitation on aqueous dilution due to loss of solvent capacity. This
necessitates 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 Liquid Crystalline (LC) phase of
some of the mixture components causing blockage of charge movement through the
system [26] or may penetrate the surfactant monolayer [27]. The incorporated drug may
increase or decrease the self-emulsifying efficiency or may not affect it at all [28, 29].
Hence S(M)EDDS 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 [30]. Because of these reasons, pre-formulation solubility and
phase diagrams should be thoroughly evaluated when choosing the optimized
formulation.

**Capsule compatibility**

Liquid S(M)EDDS filled in hard and soft gelatin capsules are more acceptable as dosage
forms. Presence of hygroscopic material in the liquid formulation may cause dehydration
of capsule shell or polar molecules such as polyethylene glycol or alcohol may penetrate
into the capsule shell. Thus it is necessary to investigate such effects at an early stage of
development [10]. Solid S(M)EDDS possess an advantage in this regard due to lack of
contact of liquid material with the capsule shell.
1.6 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 expansion is created 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 \[31\]. In case of S(M)EDDS, 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 formation. This LC phase is considered to be responsible for the high stability of the resulting nanoemulsion against coalescence \[32, 33\].

1.7 Conversion of Liquid SEDDS to Solid SEDDS

Liquid SEDDS can be filled in soft or hard gelatin capsule. Recently, there have been efforts by research groups working on SEDDS to convert liquid SEDDS to solid state SEDDS. These Solid SEDDS can be made into tablets or be encapsulated. The primary reason to formulate SEDDS in a solid form is to consolidate the advantages of Liquid SEDDS with convenience of solid oral dosage forms. Oral solid dosage forms have the
following advantages [34]: (a) low production cost (b) convenience of process control (c) high stability and reproducibility and (d) better patient compliance. Generally, the formulated S(M)EDDS are liquid in state, but sometimes it could be in a semisolid state depending on the physical state of excipients used. Researchers have adopted various techniques to obtain this conversion. Solid SMEDDS also offers added versatility in terms of possible dosage forms. The following description elaborates various Liquid to Solid SMEDDS conversion techniques.

**Spray drying:** Spray drying is the most widely used technique to convert Liquid SEDDS into solid state. In this method the Liquid SEDDS is mixed with a solid carrier in a suitable solvent. The solvent is then atomized into a spray of fine droplets. These droplets are introduced into a drying chamber, where the solvent gets evaporated forming dry particles under a controlled temperature and airflow conditions [34]. The process parameters required to be controlled are inlet and outlet temperature, feed rate of solvent, and aspiration and drying air flow rate. The dry particles can then be either filled into capsules or made into tablets after addition of suitable excipients. Various solid carriers that have been used for this purpose are: Aerosil 200 suspended in ethanol [35] and aqueous solution of Dextran 40 [36].

**Adsorption to solid carriers:** The Liquid SEDDS can be made to adsorb onto free flowing powders that possess very large surface area and are capable of adsorbing high quantities of oil material. The adsorption can be done either by mixing Liquid SEDDS and the adsorbent in a blender or by simple physical mixing. The resulting powders can be either filled into capsules or can be made into tablets after addition of appropriate
excipients. The adsorbents are capable of adsorbing Liquid SEDDS up to 70 %w/w of its own weight. Solid carriers used for this purpose can be microporous inorganic substances, high surface area colloidal inorganic substances or cross-linked polymers [34]. Categories of solid adsorbents used are: silicates, magnesium trisilicate, talcum, crospovidone, cross-linked sodium carboxymethyl cellulose and cross-linked polymethyl methacrylate [37]. Oral solid heparin and gentamicin SMEDDS were prepared using three kinds of adsorbents: microporous calcium silicate (Florite RE), magnesium aluminometa silicate (Neusilin US₂) and silicon dioxide (Sylysia 320) [38, 39].

**Encapsulation of Liquid and Semisolid SEDDS:** It is one of the simplest techniques for conversion of Liquid SEDDS to solid oral dosage form. Liquid SEDDS can be simply filled in capsules, sealed using a microspray or a banding process. For a semisolid SEDDS, it is a four step process: (1) heating the semisolid excipients to at least 20°C above its melting point; (2) adding the drug in the molten mixture while stirring; (3) filling the drug loaded molten mixture into the capsule shell and (4) cooling the product to room temperature. The compatibility of the excipients used with the capsule shell should be well investigated. Lipid excipients compatible with the capsule shell are described in the work by Cole et al [40]. Capsule filling of SEDDS is suitable for low dose highly potent drugs and allows high drug incorporation [34].

**Extrusion Spheronization:** This is a solvent free technique that converts Liquid SEDDS into pellets using extrusion and spheronization processes. In this method the Liquid SEDDS is first mixed with a binder, followed by addition of water until the mass is suitable for extrusion. The extruded mass is then spheronized to form uniform sized
pellets. The pellets are then dried and size separated. The relative quantity of water and Liquid SEDDS used in the process has an effect on size distribution, extrusion force, surface roughness of pellets, and disintegration time [15]. High drug incorporation can be achieved by using this technique. Abdalla et al. used microcrystalline cellulose (MCC) as a binder in preparation of progesterone self-emulsifying pellets [41]. A mixture of silicon dioxide, glycercyl behenate, pregelatinized starch, sodium croscarmellose, and MCC were used by Setthacheewakul et al. in the preparation curcumin loaded SMEDDS pellets [42].

**Melt Granulation:** Melt Granulation is another solvent free technique for converting Liquid SEDDS. In this method, Liquid SEDDS is mixed with a binder that melts or softens at relatively low temperature. This melted mixture can be granulated. This technique is advantageous since it does not require addition of a liquid binder and subsequent drying unlike conventional wet granulation. The variables to be controlled in this process are impeller speed, mixing time, binder particle size, and the viscosity of the binder [34]. A mixture of mono-, di- and triglycerides and esters of polyethylene glycol (PEG) called as Gelucire are used as binders to prepare immediate release pellets by melt granulation and as a self-emulsifying drug delivery system by capsule moulding or as powder obtained by cryogenic grinding [43].

### 1.8 Dosage form development of Solid SEDDS

**Dry Emulsions:** Dry emulsions are powdered solid dosage forms which spontaneously emulsify with the addition of water. Dry emulsions could be obtained by emulsifiable glass system, freeze drying, and spray drying. Lipid based surfactant free emulsifiable glass system was developed by Myers et al [44]. In this method a poorly water soluble
drug dissolved in a vegetable oil is mixed with aqueous solution of sucrose. The mixture is then evaporated under vacuum producing dry foam. This dry foam produces an emulsion when added to water. Attempts have been made to deliver cyclosporin A by via method [45]. Freeze drying of oil-in-water emulsions using amorphous cryoprotectants was described by Bamba et al [46]. Vyas et al. prepared dry emulsion of griseofulvin using mannitol as the cryoprotectant [47]. Corveleyn et al. studied parameters affecting the preparation of lyophilized dry emulsion tablets [48]. Dry emulsion of Amlodipine was produced by spray drying of an emulsion using dextrin as a carrier [49]. An enteric coated dry emulsion for the delivery of peptides and proteins was developed by Toorisaka et al [50].

**Capsules:** Solid S(M)EDDS prepared by various techniques mentioned above can be filled into capsule shells. This prevents physical incompatibility of Liquid S(M)EDDS with the capsule shell. If semi-solid excipients are used in the formulation, they are first melted and then filled into capsules. Contents of the capsule then solidify at room temperature.

**Tablets:** Nazzal et al. [51] formulated eutectic based self-emulsifying tablets in which irreversible precipitation of the drug within the formulation was inhibited. A eutectic forming combination of a drug and suitable semi-solid oil was used in the formulation. Using the melting point depression method the oil phase containing the drug melts at body temperature producing emulsion droplets in the nanometer size range. During preparation of such tablets maltodextrin, modified povidone, and microcrystalline
cellulose (MCC) were used as additional excipients. The drug release from such tablets can be sustained by modulating the particle size of MCC.

**Pellets:** Pellets are convenient multiple unit dosage forms, which are made by extrusion/spheronization technique mentioned previously.

**Solid dispersions:** Availability of self-dispersing waxy semi-solid excipients have reduced the manufacturing and stability problems associated with solid dispersions. Excipients such as Gelucire 44/14 and Gelucire 50/02 are used for this purpose. These are semisolid excipients which can be directly filled into capsules in a molten state. Gelucire’s have high surface activity which enhances dissolution of poorly water soluble drugs. Absorption of drug is also improved when Gelucire is used as a carrier in solid dispersions [2]. The bioavailability of an investigational compound was reported to have enhanced using Gelucire 44/14 relative to its conventional PEG based formulation [52].

**Beads:** Patil et al. used porous polystyrene beads for delivering self-emulsifying formulations. The formulation is incorporated into microchannels of the bead through capillary action. The beads were prepared by copolymerizing styrene and divinyl benzene [53].

**Microspheres:** Sustained release microspheres of Zedoary turmeric oil (traditional Chinese medicine) were prepared by a quasi-emulsion-solvent-diffusion method. The microspheres reported in this research work were made using hydroxypropyl methylcellulose acetate succinate and Aerosil 200 [54].
**Nanoparticles:** Self-emulsifying nanoparticles can be formulated using solvent injection technique, wherein the excipients and drug are melted together and injected into a non-solvent solution. Resulting nanoparticles can be separated by centrifugation and lyophilization. Self-emulsifying nanoparticles of drugs were prepared using goat fat and Tween 65 using this method [55]. Glyceryl monooleate (GMO) which has self-emulsifying property was used along with chitosan for preparation of paclitaxel nanoparticles. Chitosan was responsible for bioadhesion of nanoparticles, while 100% drug incorporation was achieved because of self-emulsifying property of GMO [56].

**Implants:** Self-emulsified 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine, BCNU) was incorporated into PLGA wafer and used as an implant. The SEDDS formulation retarded the exposure of BCNU from the aqueous media and thus improved its stability and shelf-life. The formulation comprised of tributyrin, cremophor RH 40, Labrafil 1944 and BCNU [57].

**Suppositories:** Glycerrhizin self-emulsifying suppositories were formulated using C$_6$-C$_{18}$ fatty acid glycerol ester and C$_6$-C$_8$ fatty acid macrogol ester. The formulation demonstrated good drug absorption as indicated by high plasma drug levels when delivered via rectal/vaginal route [58].
Chapter 2

Instrumentation

2.1 Dynamic Light Scattering

Dynamic Light Scattering (DLS) technique is the most frequently used technique for determining the size of submicron particles. DLS is also called as Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering. It can determine size of colloidal suspensions and solutions (microemulsions, micelles).

**Principle [59]:** Brownian motion of particles is utilized by the instrument to measure the particle size. Brownian motion is the random thermal, translational, and rotational motion (diffusion) of the particles in the solution. When these particles come in the path of laser light, they scatter the light. Because of the constant motion of the particles, there is a temporal variation in the intensity of the scattered light. These variations are recorded and detected by the detectors in the form of intensity versus time profile. The autocorrelator of the instrument creates a correlation function, which is an exponentially decaying time function. The decay constant of this function is related to the diffusion coefficient in the Stokes-Einstein equation (Equation 2.1).
\[ D = \frac{kT}{3\pi\eta r} \]

where \( D \) is the diffusion coefficient, \( r \) is the hydrodynamic diameter of the particle, \( k \) is the Boltzmann constant, \( T \) is the temperature in Kelvin scale and \( \eta \) is the viscosity of the medium. DLS can provide three types of particle size information: intensity based (\( Z \) average diameter), volume based, and number based.

**Instrumentation:** The primary components of a DLS instrument are a laser source, laser delivering optics, sample holder, scattered light collecting optics, detector, and an autocorrelator (Figure 2-1).

Figure 2-1: Schematics of Dynamic Light Scattering instrument [60]
A monochromatic, vertically polarized, and coherent laser light emitted from a He-Ne laser is used as a light source. Argon ion laser and diode lasers are the other less commonly used laser light sources. Delivering optics consists of apertures and collimators that focus the light in a small area of the sample. The sample is placed in a glass cuvette which is placed in the sample holder. The scattered light collecting optics consists of lenses and apertures which collect the light at a specific angle. Generally these are placed at 90° to the source, but multi angle collecting optics is also available. Commonly used detectors are photomultiplier tubes (PMT) and photodiodes (PD).

**Sample Preparation:** Various parameters such as solvent viscosity, refractive index, and sample temperature are to be considered before measurements [61]. The instrument software uses this information when calculating the particle size. Samples should be dilute to minimize inter-particle interactions. Samples should also be free from contaminants and air bubbles. Dry solid particulate samples should be suspended in a liquid medium before measurements.

**Applications:** DLS is the most popular technique used for measuring particle size and distribution of colloidal dispersions, emulsions, microemulsions, polymers, micelles, and proteins. Rapid measurements, easy sample preparation, and low sample volume requirement are few advantages associated with DLS [62].
2.2 Electrophoretic Light Scattering

Electrophoretic Light Scattering (ELS) is used to characterize the zeta potential or surface charge of colloids in solution. Zeta potential is useful in evaluating the charge stability of the colloidal dispersions. A particle in solution can acquire charge either by adsorption of ions present in solution, by ionization of its surface groups or due to difference in dielectric constant between particle and dispersing medium. Depending on the surface charge, there will be two layers: a stern layer comprising of tightly bound ions surrounding the charged particles and a diffuse layer containing less firmly associated ions. These two layers form an electric double layer and the potential difference between the double layer and the electro-neutral region of solution is called the zeta potential. The double layer acts as the true surface of the moving particle dictating its stability.

**Principle:** ELS measures the electrophoretic mobility of particles moving in a liquid medium under the effect of electric field. Charged particles move towards anode or cathode with a mobility proportional to their zeta potential. When these particles in motion are illuminated by laser light, they cause light scattering. The mobility of particles can be measured from the shift in frequency of the incident laser beam caused by moving particles [59]. Zeta potential can be calculated from the particle mobility using Smoluchowski equation (Equation 2.2)

\[ \mu = \frac{\zeta \varepsilon}{\eta} \]  

(Equation 2.2)

where \( \mu \) is the electrophoretic mobility of particles, \( \zeta \) is the zeta potential, \( \varepsilon \) is the electric permittivity and \( \eta \) is the viscosity of the solution.
**Instrumentation:** The instrumentation for ELS is similar to that of DLS since they operate on same principles and detect similar signals. Main components of ELS are the laser source, delivering optics (beam splitter, focusing lens), sample holder, collecting optics, detectors (Photomultiplier tube), and an auto-correlator (Figure 2-2). A monochromatic coherent laser is divided into two beams of equal intensity. These two beams cross each other at a point called as measurement volume. The beam are scattered by moving particles at the measurement volume and the scattered light reaches the detector [63].

![Figure 2-2: Schematics of Electrophoretic Light Scattering](image)

**Sample Preparation:** Sample requirements and preparation is similar to that of DLS.

**Applications:** Zeta Potential is an indicator of the magnitude of repulsive forces existing between particles and hence provides information about colloid stability in solution [65].
2.3 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is widely used to visualize the surface topography and chemical composition of material of various types.

**Principle:** SEM uses high energy electrons for creating an image of the sample. A focused beam of electrons falls on a sample, the interactions between the electrons and the sample atoms leads to generation of various signals. The interactions can be classified into elastic and non-elastic interactions. Both types of interactions occur when incident electrons interact with atomic nucleus or electrons of the sample. This leads to generation of back scattered electrons (BSE) when incident electrons are elastically scattered at an angle of more than 90°. High energy BSE’s provides deep seated information about the sample. Inelastic interactions involve transfer of energy from incident electrons to the sample atom leading to generations of secondary electrons (SE). The signal produced from SE’s provides information about surface texture and roughness with high resolution. Since they have low energy, they are only emitted from the surface of the sample. In addition to these, Auger electrons, x rays and cathodeluminescence (visible/UV/IR) signals are also produced. The X-ray signals are particularly useful to probe the chemical composition of a sample [66].

**Instrumentation:** Principal components of an SEM include an electron gun, lenses, sample stage, detectors, data output device, and a vacuum system (Figure 2-3). The electron gun produces a stable electron beam which rasters across the specimen surface. Electromagnetic lenses control the size of the beam and focus it on the sample. Scanning coils around the electron beam correct the astigmatism and contribute to improvement in...
the resolution of the acquired image. The incident beam of electrons after interacting with the sample generates signals which are detected and amplified. The amplified signals are processed and displayed in the output device. The entire optical system is maintained in a vacuum in order to prevent electron-air molecule interactions [67].

![Diagram of SEM](image)

Figure 2-3: Schematics of Scanning Electron Microscopy [68]

**Sample preparation:** Sample preparation depends on the nature of the sample and the type of image desired. If the sample is conductive, then it can be directly mounted on a carbon or copper tape for imaging. For non conductive material (organics, polymers and biological samples), a thin layer of conductive material (Gold, Palladium, or Platinum) should be coated on the sample surface to make the surface conductive. The coating further prevents excessive charging of the sample surface. Non conductive and non
coated materials can be visualized by Environmental SEM (ESEM). This method allows the sample to remain in a moist state unlike conventional SEM where samples are required to be completely dry [69].

**Applications:** SEM has been used to analyze shape and surface topography of pharmaceutical solids. It is widely used for characterization of excipients and powder mixtures, granulations, pellets, tablets, coatings, and spray and freeze dried products [65, 70]. Nanometer size colloids are visualized in three dimensions using SEM. However its applicability is limited to solid samples that are stable in vacuum.

### 2.4 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a thermo analytical technique used for analyzing thermal transitions involving thermal energy with a great sensitivity.

**Principle:** DSC measures the heat energy necessary to maintain a zero temperature difference between a sample and an inert reference material when both the sample and reference are exposed to identical temperatures. In other words, DSC measures the difference in the heat flow rate between the sample and the reference, when both are subjected to identical controlled temperature program [71]. It is essential to understand the response of drugs and their formulations to thermal stress in order to develop a stable product. DSC provides details about thermodynamic properties of the sample. Whenever a material undergoes any physical or chemical change it is associated with an exothermic
or an endothermic event. DSC provides accurate information about such events occurring in the material [72].

**Instrumentation:** A typical thermogram obtained from a DSC experiment is shown in the figure 2-4. When there is no thermal event occurring in the sample a baseline thermogram is recorded. With the occurrence of an enthalpic event, a peak is displayed in the thermogram at the temperature corresponding to the temperature at which event occurred. The peak area related to this enthalpic event is proportional to the change in heat capacity.

![Figure 2-4: A typical thermogram obtained from DSC [73]](image)

There are two common types of DSC systems commercially available: (1) Power compensated DSC and (2) Heat flux DSC

**Power compensated DSC** (Figure 2-5): In a power compensated DSC, the sample and the reference pan are kept in isolated furnaces (that is both of them are heated by separate
heat sources). The power required to maintain a zero temperature differential between the sample and reference is measured. At the onset of any enthalpic event (exothermic/endothermic), the sample temperature would change relative to the reference; hence in order to maintain isothermal conditions energy is supplied to one or the other pan depending on the nature of the enthalpic event. During an exothermic reaction, heat is supplied to the reference, while heat is supplied to the sample during an endothermic reaction [72]. A change in power signal results in a peak in the thermogram.

Figure 2-5: Power compensated DSC [74]

**Heat flux DSC** (Figure 2-6): In heat flux DSC, both the sample and reference pans are enclosed in a single furnace and are heated by a thermoelectric disk. A thermocouple placed below the pans is used to measure the temperature differential which is
transformed into heat flow using mathematical equations.

![Figure 2-6: Heat flux DSC [74]](image)

**Sample preparation:** The samples are placed in metal pans that are inserted into the sample holders of the instrument. Material that possesses high thermal conductivity and compatibility with sample material are used to fabricate sample pans. The pans may be open, sealed, covered, or pin-holed depending on the nature of the sample used. A reference pan of identical to the sample pan is placed in reference sample holder. It may be filled with an inert material or remain empty. The sample size is ideally 3-5 mg. Low density powders should be lightly pressed into the bottom of the pan to ensure good thermal contact. For calculation of heat capacity or heat of fusion, the samples should be accurately weighed and sample weight should be entered into instrument software.

**Applications:** DSC is used in pharmaceutical industry for characterization of active pharmaceutical ingredients and excipients. Any material that exhibits a transition in its physical state or reacts chemically with associated heat exchange can be evaluated using
DSC. DSC may be used for single material characterization or multi-components investigations. Some pharmaceutical applications of DSC include studies involving:

- Melting point, crystallization, sublimation, dehydration, desolvation, and glass transition [75].
- Measurement of heat of reaction and heat capacities [76]
- Purity of materials
- Degree of crystallinity
- Polymorphism [77]
- Decomposition, degradation and stability kinetics [78]
- Protein unfolding [79]
- Freeze drying [80]
- Drug-excipient compatibility studies [81-83]
- Drug-Polymer interactions in polymeric drug delivery [84]
- Solid dispersions [85-87]

2.5 Powder X-ray Diffraction

X-rays are electromagnetic radiations having a wavelength of about 1 °A, which is approximately the size of an atom (7). It is used for analysis of crystalline solids at an atomic level.

**Principle [88, 89]:** The atoms in a crystalline solid are arranged in discrete parallel planes separated by a distance d. Such planes exist in a number of different orientations, each with its own specific d. When a monochromatic X ray beam of wavelength of λ is incident on the parallel planes at an angle of θ, diffraction only occurs if the distance travelled by reflected rays from successive planes differs by a complete n number of wavelengths (Figure 2-7). When this occurs, the reflected rays constructively interfere according to the Bragg’s law. Bragg’s law (Equation 2.3) states that if the wavelengths of
reflected X rays differ by an integer multiple $n$ of wavelength $\lambda$, then they constructively interfere and the angle of diffraction $\theta$ will be equal to the angle of incidence $\theta$.

$$n \cdot \lambda = 2d \sin \theta \quad \text{..................................................(Equation 2.3)}$$

When the angle $\theta$ is changed, Bragg law’s conditions are satisfied at various $d$ spacing’s of crystalline material resulting in diffracted X rays. A plot of intensities of the diffracted X ray peak’s versus $2\theta$ (angular positions) gives a pattern that is characteristic of crystalline material, also called as “fingerprint” of the material.

**Figure 2-7: Diffraction of X ray by a crystalline material [90]**

**Instrumentation** [88, 89]: The PXRD instrument (Figure 2-8) essentially consists of an X-ray tube, sample holder, and an X-ray detector. X rays of suitable wavelength and intensity are produced by the x ray tube.
Figure 2-8: Schematics X ray diffractometer [91]

The X-ray tube consists of an anode and a cathode. A tungsten filament cathode is heated to produce electrons. These electrons are bombarded on a metal anode (target) which results in the generation of X-ray spectra. A commonly used target is copper, although molybdenum, chromium, silver, and rhodium can also be used. The X-ray spectra consist of Kα and Kβ components with a certain wavelength characteristic to the type of the target used. Copper produces CuKα rays with a wavelength of 1.5418 Å. Monochromatic X-rays are produced by filtering the X-ray spectra using foils or a crystal monochromator. These monochromatic X-rays are collimated on the sample at an angle of θ. The goniometer in the instrument simultaneously moves the sample holder and the detector in such a way that incident X-ray beam hits the sample and the reflected X-ray is detected at an angle of 2θ. At a certain angle of θ, where Bragg’s law is obeyed constructive interference is detected by the detector. The detector processes the signal and converts it
into a count rate which is displayed as a function of intensity verses 2θ (diffracting angle).

**Sample Preparation**: The sample quantity analyzed depends on the capacity of sample holder used. Coarse samples should be grounded to about 200 mesh. A flat compact bed of sample powder should be made on the sample holder using a glass slide or a razor blade. The upper surface of the sample should be flat to ensure random distribution of lattice orientation.

**Applications**: PXRD is a characterization technique used by material scientists working in pharmaceuticals, geologicals, engineering, and environmental sciences areas. The advantages associated with this technique are: non-destructive technique, rapid output of results, minimal sample preparation, and easy spectra interpretation [92]. PXRD is used to assess solid state properties in general. In pharmaceuticals, PXRD finds its use in the following ways:

- Identification of drugs in pharmaceutical dosage forms [93]
- Studying solid-solid interactions and properties of different polymorphs, solvate forms and their phase transformations [94, 95]
- Drug-excipient compatibility [96]
- Alterations in crystallinity of materials [97]
2.6 UV/Visible Spectroscopy

UV/Visible spectroscopy uses UV (200-400 nm) and visible (400-800 nm) radiations to analyze samples. It is one of the simplest and most widely used spectroscopic techniques in quantitative determination of organic, inorganic and biological samples.

**Principle:** With the absorption of UV/Visible light, valence electrons of the molecule are excited and undergo an electronic transition from their highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). In UV/Visible spectroscopy (Figure-2.9), (n to π*) and (π to π*) transitions are observed because the energy required for this transitions fall in the UV/Visible range. Such transitions are possible in alkenes, aromatics, and conjugated dienes and trienes because of the presence of unsaturated double bonds. Molecules that have these functional groups are capable of absorbing UV/Visible light are called as chromophores. The vibrational and rotational spectra also gets superimposed on the electronic spectra of the chromophore (or the molecule), which makes the spectra to look like a broad band. The Beer’s-Lambert law (Equation 2.4) states that the absorbance of a solution is directly proportional to the concentration of the absorbing species present in the sample. This law can be used to measure concentration of absorbing species in the solution.

\[
A = -\log \left( \frac{I}{I_o} \right) = \varepsilon \cdot c \cdot L \quad \text{..........................(Equation 2.4)}
\]

where A is the measured absorbance, I₀ is the intensity of incident light, I is the intensity of transmitted light, L is the pathlength of light through the sample, c is the concentration of the absorbing species and ε is the molar absorptivity or extinction coefficient of the
species. $\varepsilon$ is characteristic of a species under specific conditions of solvent, wavelength, and temperature.

![Energy diagram showing various energy transitions](image)

**Figure 2-9**: Energy diagram showing various energy transitions [98]

**Instrumentation**: A typical UV/Visible spectrophotometer consists of a light source (deuterium and tungsten lamps), wavelength selector (prism, gratings monochromator, interferometer), sample holder and sample cells (cuvette, 96 well plate), detector (photomultiplier tube, photodiode array, CCD camera), and data acquisition system (computer). There are two kinds of spectrophotometers: (1) Single beam and (2) Double beam spectrophotometer. In a single beam spectrophotometer (Figure 2-10), the transmittance of the sample and reference (blank) is to be measured separately (first blank and then sample), while a double beam spectrophotometer (Figure 2-11) measures both the transmittances simultaneously by splitting the light beam into two halves [73, 99].
Sample Preparation: Samples for UV-Visible spectroscopy are generally solutions. Samples are placed in a cuvette which is made of UV transparent material such as fused silica, quartz glass, or plastic. Commonly used solvents with their wavelength cutoff for UV/Visible measurements are acetonitrile (190 nm), water (191 nm), cyclohexane (195 nm), methanol (201 nm), ethanol (204 nm), ether (215 nm), and methylene chloride (220 nm).
**Applications:** Qualitative applications of UV/Vis spectroscopy is limited to the extent of identification of functional groups in the absorbing species based on the absorption peaks observed in the spectra. Results obtained from UV are often used in conjunction with other techniques such as Nuclear Magnetic Resonance (NMR), Infra red (IR), and Mass Spectroscopy (MS) for complete chemical structure elucidation. Quantitative estimation of organic and inorganic species can be made with good accuracy and a detection limit of $10^{-4}$ to $10^{-5}$ M is possible. Derivative spectroscopy is used to measure concentration of an analyte in a mixture of two or more interfering analytes with ease. Derivative spectroscopy yields a plot of derivative of absorbance with respect to wavelength as a function of wavelength rather than a conventional absorbance verses wavelength plot [73, 101]. Difference spectroscopy is used for detecting two compounds having a very close absorption maxima are present in a sample or where there is a matrix (solvent) interference in the sample [65].
Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Tween 80

Source: Spectrum Chemicals, Lot # C136790, CAS 156-27-1

Chemical name: Polyoxyethylene 20 sorbitan monoleate

Molecular Formula: $C_{64}H_{124}O_{26}$

Molecular weight: 1310 gram/mole

Specific gravity: 1.08

Physical state: Yellow oily liquid

Soluble in alcohol, methanol, toluene, insoluble in mineral oil
Figure 3-1: Chemical structure of Tween 80

Tween 80 is the partial fatty acid ester of sorbitol and it forms anhydrides as a result of copolymerization of approximately 20 moles of ethylene oxide for each mole of sorbitol. They are hydrophilic nonionic surfactants (HLB-15) and are used as emulsifiers in the preparation of oil-in-water emulsions, oral and injectable suspensions and solutions [102], and as solubilizing agents for oil soluble vitamins [103].

Tween 80 has been reported to protect proteins from surface induced denaturation during freeze drying [104]. It is one of the most widely used surfactant in the preparation of self dispersing type of formulations [9]. It is approved by the FDA for oral use. Peroxide impurities present in Tween 80 causes protein denaturation when such products are stored for extended periods of time.

3.1.2 Cremophor RH 40

Source: BASF Chemicals, Lot #72809647G0, CAS #61788-85-0

Chemical name: Polyethoxylated castor oil

Other names: PEG 40 Hydrogenated Castor oil, polyoxyethylene 40 castor oil
Soluble in water, ethanol, chloroform

Physical state: white, semisolid paste at 20°C

Figure 3-2: Chemical structure of Hydrogenated castor oil

It is a polyoxyethylene of derivative castor oil containing 70% of components which are hydrophobic in nature with an HLB of 14-16. Cremophor RH 40 contains fatty acid esters of glycerol polyethylene glycol and fatty acid esters of polyethylene glycol. It aids in improving aqueous solubility of propellant in water based aerosol vehicles [103].

It is used as a solubilizing agent for various hydrophobic Active Pharmaceutical Ingredient’s (API), fat soluble vitamins, and essential oils; and as an emulsifier in the preparation of pharmaceutical emulsions and SEDDS. It aids in solubilization of Lopinavir and Ritonavir in Kaletra® oral solution and Cyclosporine in Neoral® oral microemulsions [105].
3.1.3 Labrafac Lipophile WL 1349

Source: Gattefosse, Batch # 123717

Chemical name: Caprylic /Capric Triglyceride

Physical state: Colorless viscous liquid

Boiling point: >150°C

Specific gravity: 0.93-0.96

Soluble in ethanol, chloroform, methylene chloride, vegetable oils, insoluble in water.

Labrafac Lipophile WL 1349 is a medium chain triglyceride of fractionated vegetable C8 and C10 fatty acids (mainly fractionated coconut oil or palm kernel oil) with an HLB of 1. It is a non rancidable fluid used as a vehicle in oral and topical preparations, emulsions, self-emulsifying drug delivery systems, suspensions, ointments, suppositories, and creams. It can be used as filler in capsules and as an anti adherent in tablets. In combination with long chain triglycerides, it serves as a total parenteral nutrition (TPN)
component. They possess excellent spreadability, skin penetration, and solvent properties when compared to long-chain triglycerides.

### 3.1.4 Polyethylene Glycol 400

**Source:** Spectrum Chemicals, Lot # YW0048, CAS 25322-68-3

**Chemical name:** α-Hydroxy-ω-hydroxy-poly(oxy-1,2-ethanediyl)

**Chemical formula:** $C_{2n}H_{4n+2}O_{n+1}$

**Molecular weight:** 380-420 (average)

![Chemical structure of PEG 400](image)

**Figure 3-4:** Chemical structure of PEG 400

**Physical state:** Colorless viscous liquid

**Specific gravity:** 1.1254

Soluble in water, acetone, alcohol, glycerin, benzene

Polyethylene glycols (PEG) have a wide range of applications including topical, oral, parenteral, ophthalmic and rectal delivery. Liquid grade PEG’s are used as a water miscible co-solvents which possess good solvent properties for poorly water soluble
drugs. Due to this property, they are widely used in lipid based drug delivery systems such as solid dispersions and self-emulsifying mixtures. When used in soft gelatin capsules, they are known to cause hardening of capsule shells by absorption of moisture from gelatin in the shell [106].

3.1.5 Capyrol 90

![Chemical structure of Capyrol 90](image)

Fig 3-5: Chemical structure of Capyrol 90

Source: Gattefosse, Batch # 118148, CAS # 85883-73-4

Chemical name: Propylene glycol monocaprylate

Chemical formula: $\text{C}_{11}\text{H}_{22}\text{O}_3$

Molecular weight: 202.29

Physical state: Colorless oily liquid

Specific gravity: 0.935-0.955

Soluble in ethanol, chloroform, methylene chloride, and vegetable oils, insoluble in water

Capryol 90 contains more than 90% monoester of $\text{C}_8$ fatty acid (caprylic acid). It is used as an emulsifier in oil-in-water emulsions and self-emulsifying drug delivery systems. It
is reported to possess bioavailability enhancing properties due to its inhibitory action on CYP3A4 enzyme [106].

3.1.6 Transcutol P

![Chemical structure of Transcutol P](#)

**Figure 3-6: Chemical structure of Transcutol P**

Source: Gattefosse, Batch # 450844010, CAS # 111-90-0

Chemical name: Diethylene glycol monoethyl ether

Chemical formula: C_{6}H_{14}O_{3}

Molecular weight: 134.17

Physical state: Colorless limpid liquid

Specific gravity: 0.985-0.991

Soluble in water, and ethanol, insoluble in mineral oils

Transcutol P has good solvent properties for poorly water soluble drugs. It enhances drug penetration, permeation, and produces a drug depot effect [103]. It is used as a co-solvent in the formulation of SEDDS.
3.1.7 Ibuprofen

![Ibuprofen chemical structure]

Figure 3-7: Chemical structure of Ibuprofen

Source: PCCA, Lot # C136790, CAS # 15687-27-1

Chemical name: 2-(4-isobutylphenyl) propionic acid

Chemical formula: C\textsubscript{13}H\textsubscript{18}O\textsubscript{2}

Molecular weight: 206.29

Physical state: White color powder

Slightly soluble in water, soluble in alcohol and other organic solvents

Ibuprofen is a non steroidal anti inflammatory drug (NSAID), used for management of pain and fever. It belongs to the class of propionic acid derivatives possessing anti-inflammatory activity [107]. Ibuprofen is a nonselective cyclooxygenase inhibitor (COX-I, II). These COX enzymes are responsible for producing prostaglandins that are considered to be pain and inflammatory mediators in the body. The analgesic, anti-inflammatory, and antipyretic activity of ibuprofen is manifested by action of COX-I
enzyme, while action on COX-II causes unwanted actions like GI irritation and platelet aggregation [108]. Recently, it has been shown that ibuprofen inhibits up-regulation of pro-inflammatory genes like tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β) in the nucleus of cell [109]. Ibuprofen is used in conditions such as rheumatoid arthritis, osteoarthritis, body pain, dysmenorrhea and dental pain. It is absorbed rapidly, binds extensively to plasma proteins, undergoes hepatic metabolism, and is excreted through urine [107].

Commercial dosage forms and dose

Marketed products: Motrin®, Advil®, Nuprin®, Rufen®, Medipren®

Dosage forms: Tablets, Caplets, Sofgels, Suspensions, Capsules, Gelcaps

Dose: 200-400 mg every 4-6 hours for analgesic purpose, 300-400 mg every 6-8 hours for rheumatoid arthritis and osteoarthritis.

3.1.8 Neusilin US₂

Source: Fuji Chemicals, Lot # 901001, CAS 12511-31-8

Chemical Name: Magnesium Aluminometasilicate

Chemical Formula: Al₂O₃.MgO.1.7SiO₂.xH₂O

Physical State: White powder

Specific gravity: 2.0-2.2

Insoluble in water and ethanol
Neusilin US₂ is a very fine powder of amorphous magnesium aluminosilicate. It possesses very large surface area enabling it to adsorb oils up to three times of its weight. It has good flowability and compressibility and can be directly compressed into tablets. It has been used as an adsorbent for oil-emulsifier mixtures [39] in SEDDS and for melt granulation in solid dispersion technology [110]. Upon co-grinding with a crystalline drug, it converts the drug into an amorphous form [111].

3. 2 Methods

3.2.1 Screening of lipid excipients for SMEDDS

A SMEDDS is an isotropic mixture of oil, surfactant, and cosurfactant. The property of self-microemulsification is only exhibited by certain combination of these components. In this study, excipients shown in Table 3.1 were evaluated for potential self-emulsification property.

Table 3.1: Lipid excipients used in SMEDDS

<table>
<thead>
<tr>
<th>Oil</th>
<th>Surfactant</th>
<th>Cosurfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labrafac Lipophile WL 1349</td>
<td>Tween 80</td>
<td>Capyrol 90</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>Transcutol P</td>
<td>PEG 400</td>
</tr>
</tbody>
</table>

A total of six self emulsifying mixtures comprising of components shown in Table 3.1 were evaluated.

1. Tween 80-PEG 400-LL WL 1349
2. Tween 80-Capyrol 90-LL WL 1349
3. Tween 80-Transcutol P-LL WL 1349
4. Cremophor RH 40-PEG 400-LL WL 1349
5. Cremophor RH 40-Capyrol 90-LL WL 1349
6. Cremophor RH 40-Transcutol P-LL WL 1349

All six combinations were prepared with the ratios of Oil:(Surfactant/Cosurfactant) as 1:1, 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7. The Surfactant/Cosurfactant ratio’s (k_m) of 1:1, 2:1 and 3:1 were evaluated. Surfactant/cosurfactant mixtures possessing various k_m ratio’s were prepared by weighing appropriate amount of surfactant and cosurfactant and were vortexed for 30 min to produce a homogenous mixture. Mixtures with 12-50% of the oil, 25-66% of the surfactant and 12-44% of cosurfactant were evaluated for their self-emulsifying properties. These mixtures were then mixed with the oil phase to form an isotropic self microemulsifying (SME) mixture. Thus, each combination had a total of 21 samples with different proportions of oil, surfactants and cosurfactants.

3.2.2 Test for Self Emulsification

A test for self emulsification was performed on all the six SME combinations described in section 3.2.1 according to the method suggested by Craig et al [26].

0.6 ml of each of the 21 SME samples was placed in 400 ml of water and contents were agitated via a magnetic stirrer. The spontaneity of emulsification, clarity of dispersion, and apparent stability were evaluated. The emulsion so formed was either a visually clear nanoemulsion, or a slightly turbid emulsion, or a milky emulsion that immediately phase
separated. The formulations that were emulsified into a clear, transparent nanoemulsion and showed no signs of instability for 24 hours were delineated in ternary phase diagrams using Sigmaplot® software.

3.2.3 Drug Solubility

Excess amount of Ibuprofen was added to 2ml of each excipient placed in microtubes and the mixture was vortexed, heated to 40°C in a water bath to facilitate drug solubilization. The mixture was finally kept at ambient room temperature (25°C) under continuous shaking for 48 hours to attain equilibrium. The mixtures were then centrifuged at 3000 rpm for 15 min. Aliquots of supernatant were diluted with methanol, and the drug content was quantified using a UV spectroscopic method. The solubility of drug was determined from a calibration curve of Ibuprofen in methanol.

3.2.4 Preparation of Liquid SMEDDS

After careful evaluation of phase diagrams, Tween 80-Labrafac Lipophile WL 1349-PEG 400 were selected as a SME mixture for drug delivery. Liquid SMEDDS formulation was prepared by dissolving 200 mg of Ibuprofen in the optimized SME mixture consisting of Tween 80(50%w/w), Labrafac Lipophile WL 1349(25%) and PEG 400(25%). Drug containing SME mixture was vortexed until a clear solution was obtained. These mixtures were observed for any signs of turbidity or phase separation for a period of 48 hours.
3.2.5 Preparation of Solid SMEDDS

The Liquid SMEDDS described in section 3.2.4 was adsorbed onto Neusilin US2 by physical mixing in a small mortar and pestle. The resulting Solid SMEDDS was a free flowing powder that was subsequently subjected to solid state characterization and dissolution studies. The formula of the optimized Solid SMEDDS is shown in table 3.2.

Table 3.2: Composition of an optimized Solid SMEDDS

<table>
<thead>
<tr>
<th>Formula components</th>
<th>Proportions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>200 mg</td>
</tr>
<tr>
<td>Tween 80</td>
<td>750 mg</td>
</tr>
<tr>
<td>Labrafac Lipophile WL 1349</td>
<td>375 mg</td>
</tr>
<tr>
<td>PEG 400</td>
<td>375 mg</td>
</tr>
<tr>
<td>Neusilin US2</td>
<td>500 mg</td>
</tr>
</tbody>
</table>

3.2.6 Droplet size and Zeta potential of nanoemulsions

Liquid SMEDDS and Solid SMEDDS were dispersed in 400 ml of water to obtain to a nanoemulsion. The droplet size and zeta potential of the resultant nanoemulsion was measured using Dynamic Light Scattering (Nicomp 380 ZLS, Particle Sizing Systems, CA). The nanoemulsion samples were taken in disposable Durex borosilicate glass culture tubes (VWR Scientific products) and volume weighted diameter was determined by placing the sample in the path of a Helium Neon laser of wavelength 658 nm at a scattering angle of 90°C and a temperature of 23°C.
3.2.7 Morphological Analysis of Solid SMEDDS

The surface morphology of Solid SMEDDS, Ibuprofen, and Neusilin US₂ was analyzed in an FEI Quanta 3D FEG Dual Beam Electron Microscope. The samples were fixed on an aluminum stub using a double sided carbon adhesive tape and were made electrically conductive by coating with palladium under vacuum. An accelerating voltage of 5 kV was used to visualize the samples.

3.2.8 Differential Scanning Calorimetry of Solid SMEDDS

The physical state of Ibuprofen in Solid SMEDDS was characterized by differential scanning calorimetry (Diamond DSC, PerkinElmer) equipped with an Intercooler 1P. Samples of Ibuprofen powder, Solid SMEDDS, Neusilin US₂ and physical mixture (Ibuprofen+Neusilin US₂) were run on DSC. The samples (about 3-6 mg) were placed in standard 20μl aluminum pans using nitrogen as effluent gas at 20 ml/min. Samples were scanned at a temperature ramp speed of 10°C/min from 0 to 140°C. The acquired data was analyzed using Pyris Manager (v 1.3) software.

3.2.9 Powder X-ray Diffraction of Solid SMEDDS

The physical state of ibuprofen in Solid SMEDDS was characterized by X ray powder scattering (PXRD) measurements using X ray diffractometer (X:Pert PRO with X-Pert Data Collecter, PAN analytical). The measurements were performed at room temperature using monochromatic CuKα-radiation at 40mA and at 40kV over a 2Θ range of 7 to 80° with a continuous scanning speed of 4°/min. The analyzed samples were compactly packed in the cavity of an aluminum sample holder using a glass slide. PXRD was
performed on samples of Ibuprofen powder, Solid SMEDDS, Neusilin US<sub>2</sub>, and a physical mixture of Ibuprofen and Neusilin US<sub>2</sub>.

3.2.10 In vitro drug release studies

*In vitro* drug release studies from Solid SMEDDS were performed using USP Type II dissolution apparatus (Vanderkamp® 600 six spindle dissolution tester) with number of paddle rotations set to 50 rpm. The dissolution medium consisted of 900 ml of phosphate buffer pH 7.2 maintained at 37 ±0.5°C. Solid SMEDDS containing 200 mg of Ibuprofen was introduced into the dissolution medium. At predetermined time intervals 5ml of aliquot was withdrawn, filtered using 0.45µm syringe filter and an equivalent volume of fresh dissolution medium was immediately added. The amount of drug released was estimated by measuring absorbance at 264 nm using a single beam spectrophotometer (Agilent UV spectrophotometer 8453). Dissolution of Liquid SMEDDS and plain ibuprofen powder was also determined in identical manner. The calibration curve of ibuprofen was made in phosphate buffer pH 7.2 and at 264 nm. Release data was statistically analyzed using Sigma Plot software employing a one way ANOVA followed by a Tukey’s test to determine group differences. Statistical significance was set at p<0.001.
Chapter 4

Results and Discussion

4.1 Screening of lipid excipients

Successful formulation of lipid based drug delivery systems is dependent on proper selection of lipid excipients. With a variety of lipid based excipients currently available commercially, a formulator has more options for selecting an excipient than was possible in the past. It is essential that the selected excipients should possess properties required for the desired type of lipid based drug delivery. The choice of selecting excipients for developing Self Micro-Emulsifying Drug Delivery System (SMEDDS) is based on following criteria

1. The self micro-emulsifying (SME) mixture should instantly self-microemulsify forming a fine oil-in-water nanoemulsion upon aqueous dilution in the GIT.

2. The SME mixture should be capable of solubilizing the entire drug dose in a volume that is acceptable for unit oral administration.

3. The drug should remain physically and chemically stable in the SME mixture.

4. The excipients should not produce any systemic toxicity.
Six SME mixtures listed below were evaluated for self micro-emulsifying performance and one of them was selected for drug delivery based on its self-microemulsifying efficiency, drug solubility, and drug stability studies. All the excipients selected for the study were FDA ‘Generally regarded as safe’ (GRAS) material.

1. Tween 80-PEG 400-LL WL 1349
2. Tween 80-Capyrol 90-LL WL 1349
3. Tween 80-Transcutol P-LL WL 1349
4. Cremophor RH 40-PEG 400-LL WL 1349
5. Cremophor RH 40-Capyrol 90-LL WL 1349
6. Cremophor RH 40-Transcutol P-LL WL 1349

4.2 Ternary Phase diagrams

Self micro-emulsifying performance of SME mixture was assessed from their ternary phase diagrams and time taken to produce a fine nanoemulsion. Only certain combinations of oil, surfactant and a cosurfactant in a certain composition range will produce a fine nanoemulsion upon aqueous dilution. To check emulsification efficiency of SME mixtures, test for emulsification was performed on all six combinations and the resultant dispersions were visually assessed. Resulting dispersions either formed a clear nanoemulsion, a slightly turbid emulsion or a milky emulsion which immediately phase separated as shown in Figure 4-1. Table 4.1 shows the time taken by SME combinations to form a clear nanoemulsion. It also shows corresponding concentrations (%w/w) of mixture components based on the ratio of surfactant to cosurfactant (k_m) under test.

Ternary phase diagrams provide a lucid representation of the concentration range of the
three SME components (Figures 4-2~4-7). The area bound by the points in the phase
diagram displays the concentration range of SME mixture components that resulted in a
clear nanoemulsion out of all the trial concentrations. All the combinations under test
formed a nanoemulsion in certain concentrations, but the combination with wider SME
region is considered to be a better combination in terms of self-microemulsification
efficiency. Figures 4-2~4-7, clearly indicate that Tween 80-PEG 400-LL WL 1349,
Cremphor RH 40-PEG 400-LL WL 1349, and Cremophor RH 40-Transcutol P-LL WL
1349 have the largest SME region in the ternary phase diagram. The imaginary line on
which all the points lie represents the $k_m$ ratio chosen. The other factor taken into
consideration for selection of best SME mixture is the time taken for complete
emulsification. All the time readings were taken in triplicates. No substantial
disimmilarity was observed between the time readings. Results from table 4.1 shows that
SME mixtures containing Cremophor RH 40 took more time to emulsify than the ones
with Tween 80. This may be attributed to the semi-solid nature of Cremophor RH 40. At
a fixed $k_m$ ratio, an increase in surfactant concentration resulted in faster emulsification
of the SME mixture. This is possibly due to spontaneous stabilization of oil droplets by
surfactant monolayers owing to their high concentration. The Tween 80-PEG 400-LL
WL 1349 mixture possessed the largest SME region in the phase diagrams and took the
least time to micro-emulsify. They were completely emulsified within a minute and the
dispersion was clear and transparent. It has been reported that drug incorporation into
SMEDDS can affect the SME performance [10]. This can be due to the drug interacting
with the liquid crystalline phase of the SME mixture causing blockage of drug charge
movement in the system [26] or due to drug penetration into the surfactant monolayer
producing perturbations at the interface [10, 27]. To verify this, ibuprofen loaded SME mixtures (10% w/w) were subjected to the test for emulsification. No differences in self-emulsification region in the phase diagram and time taken for complete emulsification were observed between the drug loaded and blank SME mixtures. This suggests that the presence of ibuprofen does not affect self micro-emulsifying property of the SME mixture.

Figure 4-1: Results from test of emulsification. (a) milky emulsion (b) slightly turbid emulsion (c) clear nanoemulsion.
Table 4.1: Time taken various SME mixtures*(I-VI) to completely self-microemulsify

<table>
<thead>
<tr>
<th>$K_m$ ratio</th>
<th>Oil (%w/w)</th>
<th>K. surfactant (%w/w)</th>
<th>Cosurfactant (%w/w)</th>
<th>Time (sec)</th>
<th>Time (sec)</th>
<th>Time (sec)</th>
<th>Time (sec)</th>
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<tbody>
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<td>25</td>
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<td>No ME</td>
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<td>+300</td>
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<td></td>
<td>33.3</td>
<td>44</td>
<td>22</td>
<td>35</td>
<td>No ME</td>
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<td>48</td>
<td>+300</td>
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<td>25</td>
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<td>25</td>
<td>24</td>
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<td>26.6</td>
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<td>28</td>
<td>60</td>
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* I. Tween 80-PEG 400-oil

II. Tween-Capyrol 90-oil

III. Tween 80-Transcutol P-oil

IV. Cremphor RH 40-PEG 400-oil

V. Cremophor RH 40-Capyrol 90-oil

VI. Cremophor RH 40-Transcutol P-oil
Figure 4-2: Ternary phase diagrams of SME mixture with Tween 80, Capyrol 90, and LL WL 1349

Figure 4-3: Ternary phase diagrams of SME mixture with Cremophor RH 40, Capyrol 90 and LL WL 1349
Figure 4-4: Ternary phase diagrams of SME mixture with Cremophor RH 40, Transcutol P, and LL WL 1349

Figure 4-5: Ternary phase diagrams of SME mixture with Tween 80, Transcutol P, and LL WL 1349
Figure 4-6: Ternary phase diagrams of SME mixture with Cremophor RH 40, PEG 400, and LL WL 1349

Figure 4-7: Ternary phase diagrams of SME mixture with Tween 80, PEG 400 and LL WL 1349
4.3 Drug Solubility studies

Table 4.2: Solubility of Ibuprofen in various excipients

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<th>Excipient</th>
<th>Solubility (mg/ml)</th>
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<tr>
<td>Tween 80</td>
<td>182.56 ± 23.39</td>
</tr>
<tr>
<td>LL WL 1349</td>
<td>77.39 ± 6.82</td>
</tr>
<tr>
<td>Capyrol 90</td>
<td>208.11 ± 8.95</td>
</tr>
<tr>
<td>PEG 400</td>
<td>207.20 ± 19.69</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>145.35 ± 3.29</td>
</tr>
<tr>
<td>Transcutol P</td>
<td>319.02 ± 23.21</td>
</tr>
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For a successful delivery of drugs via SMEDDS, the entire dose of the drug should be soluble in an acceptable volume of SME mixture. If the drug solubility is inadequate there are chances of drug precipitation upon aqueous dilution. Equilibrium solubility measurements of the drug in SME mixture excipients should be carried out to negate the risk of drug precipitation. Thus the solubility of the drug in the excipients is an important criterion for selection apart from the excipient’s self-microemulsifying tendency.

Equilibrium solubility measurements of Ibuprofen were carried out in all lipid excipients tested. Ibuprofen, a poorly water soluble drug showed good solubility in all the excipients (Table 4.2). Ibuprofen showed highest solubility in Tween 80 and Transcutol P among surfactants and cosurfactants respectively. There are more chances of drug precipitation when cosurfactants contribute greatly to the overall drug solubility. Cosurfactants such as polyethylene glycols, propylene glycol, and alcohols help to solubilize large quantities of a drug in the oil. Upon aqueous dilution, the cosurfactants will separate from oil components forming a micellar dispersion producing a reduction in solvent capacity for
the drug [5]. Thus it necessary for the drug to have good solubility in the oil as well as the surfactant. Ibuprofen showed good solubility in Labrafac Lipophile WL 1349. Ibuprofen is reported to have good solubility in medium chain triglycerides and polysorbates (Tweens) [112]. All the evaluated excipients demonstrated good solubility for Ibuprofen suggesting that their use in drug delivery would be appropriate. Based on the results of ternary phase diagrams and drug solubility studies, Tween 80(50%w/w)-PEG 400(25%w/w)-LL WL 1349(25%w/w) was selected as an optimized SME mixture or blank formulation for drug delivery. The concentrations of excipients selected are within the usual range as recommended by the classification of lipid based formulations reported by Pouton [5] and used by other research groups [2].

4.4 Liquid and Solid SMEDDS

Ibuprofen is available as an over the counter product in a 200 mg strength dosage form. Hence, 200 mg of ibuprofen was incorporated in Liquid and Solid SMEDDS. It is necessary that the dose of Ibuprofen(200mg) should be soluble in an acceptable volume of Tween 80(50%w/w)-PEG 400(25%w/w)-LL WL 1349(25%w/w) so as to limit the excess use of surfactant and cosurfactant in the formulation. To evaluate this criterion 200 mg of Ibuprofen was dissolved in various quantities of blank formulation and observed for any signs of phase separation or precipitation of the drug. The minimum amount of Tween 80-PEG 400-LL WL 1349 SME mixture required was found to be 1.5 gm. The drug was soluble in this mixture and exhibited no signs of phase separation, discoloration, and precipitation for a period of 48 hours. This suggests that the drug is physically stable in the formulation. Test for emulsification was performed on the
formulated liquid SME mixture. It showed a progression of emulsification similar to that of a blank formulation and formed a clear nanoemulsion. The nanoemulsion showed no signs of drug precipitation over a period of 24 h which suggests that the drug was present in the oil droplets and the surfactant portion of the nanoemulsion. Also, Ibuprofen’s appreciable solubility in the cosurfactant did not cause loss of solvent capacity upon aqueous dilution. To convert Ibuprofen loaded Liquid SMEDDS to Solid SMEDDS, it was adsorbed onto 500 mg Neusilin US2 using a glass mortar pestle. On physical mixing, a free flowing powder with adsorbed liquid SME mixture was obtained. It was suitable for further solid state characterization studies. Neusilin US2 has high oil adsorbing capacity (about 3.2 mg/ml) because of its porous amorphous structure [113]. Few researchers have reported conversion of Liquid SEDDS to solid intermediates using Neusilin US2 [38, 39]. Solid SMEDDS developed in this project were also tested for emulsification test and the results were similar to that obtained from Liquid SMEDDS. Liquid SMEDDS took about 30 seconds to completely emulsify, whereas Solid SMEEDS took about 38 seconds. These observations confirmed the presence of Ibuprofen in a solubilized form in the solid formulation. The physical state of ibuprofen in the Solid SMEDDS was further verified using DSC and PXRD.
4.5 Characterization of Liquid and Solid SMEDDS

4.5.1 Droplet size analysis.

The droplet size of the nanoemulsion is important since it determines the rate and extent of drug release and absorption. The drug can diffuse faster from smaller droplets into the aqueous phase, thereby increasing the drug dissolution [23]. Smaller droplet size presents large surface area for drug absorption. Reduction in droplet size improved bioavailability of cyclosporin emulsion when compared to a coarse emulsion [114]. Increase in surfactant concentration decreases the droplet size up to a certain size but thereafter anymore increase in surfactant concentration results in an increase in droplet size [115]. The reduction in droplet size can be attributed to the stabilization of oil droplets due to localization of surfactant monolayers at the oil-water interface [17]. Increase in surfactant concentration causes enhanced water penetration into oil droplets leading to breakdown of oil droplets and resultant bigger droplets [18]. The droplet size of the nanoemulsion was measured using dynamic light scattering. The Ibuprofen loaded SMEDDS displayed a Gaussian distribution of droplet sizes. The mean droplet size (volume weighted) obtained from liquid SME mixture was 18.70 ± 0.82 nm (Figure 4-8), while the mean droplet size obtained from Solid SMEDDS was 19.17 ± 2.29 nm (Figure 4-9). The Solid SMEDDS showed a bimodal (Nicomp®) distribution due to the presence of Neusilin US2 particles in the sample. For Solid SMEDDS samples, the large Neusilin US2 particles were allowed to sediment and then the sample of nanoemulsion was tested for droplet size. In their bi-modal distribution, more than 90% of the particles by volume
constitute the nanoemulsion oil droplet, while the rest shows the size of the Neusilin US$_2$ particles.

Figure 4-8: Droplet size distribution of the nanoemulsion obtained from Liquid SMEDDS

Figure 4-9: Droplet size distribution of nanoemulsion obtained from Solid SMEDDS
4.5.2 Zeta potential measurements

The surface charge (zeta potential) of the nanoemulsion formed from SMEDDS is believed to play a role in its bioavailability. Because of the presence of fatty acids in the structure of the excipients used, generally the surface charge of the droplet is negative. The zeta potential of the nanoemulsion formulated from dispersion of Liquid SMEDDS in water was found to be neutral. Surface charge of the oil droplets present in the nanoemulsion may affect its interaction with the luminal intestinal mucosal cells. These cells are negatively charged with respect to mucosal solution in lumen [116]. If the surface charge of the droplet is positive, then there may be an electrostatic interaction between the mucosal cell surface and the droplets. This leads to an increased absorption of the administered drug and hence its bioavailability. It was observed that if a cationic lipid like oleyl amine (upto 3% concentration) is used as one of the components of the SME mixture, positively charged droplets were obtained. Oleyl amine localizes at the oil-water interface and renders a positive charge to the oil droplets [117]. It was reported that such positively charged emulsions get electrostatically attracted to the mucosal cell surface resulting in an improved bioavailability of the drug. Such observations were only found true in case of the coarse emulsions, whereas finer emulsions or nanoemulsions showed a higher bioavailability than positively charged coarse emulsions irrespective of its surface charge [118, 119].
4.5.3 Morphology of SMEDDS.

For converting a Liquid SMEDDS into the solid state, a highly porous powder with good oil adsorbing capacity is required. Such powders can adsorb oil components of the Liquid SMEDDS and convert them into a free flowing powder. Neusilin US\textsubscript{2} has a highly porous structure which is capable of adsorbing up to three times its weight of oil [113, 120]. Scanning electron microscopy reveals the morphology of solid SMEDDS. From the figure 4-11(c), ibuprofen appeared to be made of smooth rectangular crystalline structures. Similar observations about ibuprofen micrographs were made by other researchers [121]. Neusilin US\textsubscript{2} appears to be spherical porous particles of size of...
approximately 100 µm. Micrographs of Solid SMEDDS shows Liquid SMEDDS adsorbed onto the surface of Neusilin US₂ particles. Since the formulation process involved facilitating adsorption through physical mixing, partially covered Neusilin US₂ are also visible in the field of vision. Crystalline structures characteristic of solid ibuprofen are not seen in Solid SMEDDS micrographs suggesting that the drug is present in a completely dissolved state in the Solid SMEDDS.
Figure 4-11: Scanning Electron Microscopy images of: (a) Neusilin US₂ (b) Solid SMEDDS (c) Ibuprofen
4.5.4 Differential Scanning Calorimetry

The physical state of the drug present in solid SMEDDS was confirmed from DSC studies. The physical state of a drug in the dosage form affects the *in vitro* and *in vivo* release characteristics of the drug [35]. Ibuprofen shows a sharp endothermic peak at 69.92°C with heat of enthalpy of 93.89 joule/gm. Neusilin US₂ did not show any peak in the temperature range studied which conforms to data reported by Vadher et al [122]. The physical mixture comprising equal amounts of Neusilin US₂ and Ibuprofen showed a less intense melting point peak at 67.89°C due to presence of Ibuprofen. The Solid SMEDDS did not show any peaks under the studied temperature range indicating that Ibuprofen in Solid SMEDDS was present either in an amorphous form or in a disordered crystalline form of a molecular dispersion [36].

![Differential Scanning Calorimetry (DSC) of Ibuprofen, Physical mixture (Ibuprofen and Neusilin US₂), Neusilin US₂ and Solid SMEDDS.](image)

Figure 4-12: Differential Scanning Calorimetry (DSC) of Ibuprofen, Physical mixture (Ibuprofen and Neusilin US₂), Neusilin US₂ and Solid SMEDDS.
4.5.5 Powder X ray Diffraction (PXRD)

PXRD further verified the physical state of the drug in the Solid SMEDDS. In figure 4-13 the presence of sharp peaks is indicative of the presence of Ibuprofen in a highly crystalline form. Neusilin US₂ is in amorphous state as demonstrated by the absence of sharp diffraction patterns. The physical mixture (1:1) of Ibuprofen and Neusilin US₂ showed some crystalline peaks due to the presence of Ibuprofen in the mixture. In contrast to the physical mixture of Ibuprofen and Neusilin US₂, the Solid SMEDDS did not show significant crystalline peaks, which further confirms the molecularly dispersed state of Ibuprofen in the formulation.

Figure 4-13: Powder X-ray Diffraction (PXRD) of Ibuprofen, Physical mixture (Ibuprofen and Neusilin US₂), Neusilin US₂ and Solid SMEDDS.
4.6.6 Drug release studies

The *in vitro* dissolution of the Liquid and Solid SMEDDS was performed in phosphate buffer (PBS) of pH 7.2 using a USP Type II dissolution apparatus with a paddle speed of 50 RPM. Liquid SMEDDS, Solid SMEDDS, and plain ibuprofen were placed in the dissolution medium and were sampled at time intervals of 5, 10, 15, 20, 30, 40, 50 and 60 minutes. The amount of drug released was calculated from the calibration curve of ibuprofen (figure 4-14) dissolved in PBS of pH 7.2.

**Calibration curve of Ibuprofen in PBS 7.2**

![Calibration curve of Ibuprofen in PBS 7.2](image)

Figure 4-14: Calibration of Ibuprofen in phosphate buffer (PBS) of pH 7.2 (n=3)
For both the formulations, it was observed that 80% of the drug was released within 30 minutes dissolution time with about 75% of the drug released within first five minutes of the dissolution (Table 4.3).

Table 4.3: Cumulative percentage drug release (expressed as Mean±S.D.) from Liquid SMEDDS, Solid SMEDDS and plain Ibuprofen (n=3)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
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<th>Ibuprofen (%)</th>
<th>Solid SMEDDS (%)</th>
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<td>0</td>
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<td>5</td>
<td>75.60±3.48</td>
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<td>10</td>
<td>76.15±2.46</td>
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<td>79.34±0.69</td>
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<tr>
<td>15</td>
<td>76.90±2.45</td>
<td>86.40±3.01</td>
<td>80.96±2.06</td>
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<td>20</td>
<td>78.95±1.52</td>
<td>93.96±4.25</td>
<td>82.17±1.81</td>
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<td>30</td>
<td>80.45±1.39</td>
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<td>83.26±1.52</td>
<td>95.59±3.95</td>
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<td>83.10±6.36</td>
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<td>60</td>
<td>87.65±0.67</td>
<td>99.06±1.40</td>
<td>84.83±1.66</td>
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However, the total amount of drug released from the Liquid SMEDDS was 88%, while that from Solid SMEDDS was about 85% (Table 4.3). The dissolution rate was compared with that of plain Ibuprofen. Ibuprofen shows a pH dependent solubility with highest solubility shown at pH 7.2 [123]. The initial release of the drug from the formulations was faster as compared to the plain drug dissolution (Figure 4-15).
Figure 4-15: Cumulative drug release profile of Liquid SMEDDS, Plain Ibuprofen and Solid SMEDDS (n=3)

One way ANOVA followed by Tukey’s multiple comparison test showed that the drug release from Liquid SMEDDS and Solid SMEDDS significantly differed from that of plain drug (p<0.001). There was no significant difference observed between Solid and Liquid SMEDDS (p=0.086). The rapid release of the drug from the oil droplets suggests
that the polarity of the oil and the logP value of Ibuprofen was appropriate, thus enabling
the drug to partition out from the oil droplet. This establishes that SMEDDS can
effectively increase the drug dissolution rate of poorly water soluble drugs and can be
formulated as an immediate release dosage form for poorly water soluble drugs.
Nanoemulsion drug delivery has been successfully utilized for the delivery of anti-cancer
agents, anti-retroviral drugs, nutraceuticals, and cholesterol reducing statins. There is a
concomitant increase in bioavailability of these drugs when delivered through via
nanoemulsions because of a large increase in surface to volume ratio of these
nanoemulsions [124-126].

Results of one way ANOVA followed by Tukey’s test as obtained from Sigma Plot®

One Way Analysis of Variance

Data source: Data 1 in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.330)

Equal Variance Test: Passed (P = 0.453)

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Source of Variation

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82
Between Groups 2 320.560 160.280 72.547 <0.001
Residual 6 13.256 2.209
Total 8 333.816

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor:

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<td>3</td>
<td>12.532</td>
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Conclusions

In this study, self-microemulsifying (SME) mixtures containing surfactant, cosurfactant and a medium chain triglyceride were prepared and their tendency to efficiently emulsify was evaluated. Upon aqueous dilution, such mixtures spontaneously emulsified forming an oil-in-water nanoemulsion. This property was dependent on the composition of the excipients as well as their individual concentration in the mixture. Excipients evaluated for self-microemulsification were Tween 80 and Cremophor RH 40 as surfactants, Transcutol P, Capyrol 90 and PEG 400 as cosurfactants and Labrafac Lipophile (LL) WL 1349 (a medium chain triglyceride) as an oil. All the excipients showed a tendency to form a nanoemulsion with varying degrees of efficiency. A particular SME mixture comprising of Tween 80-PEG 400-LL WL 1349 was selected and optimized for the purpose for delivering a model drug, ibuprofen. Self-microemulsifying drug delivery system (SMEDDS) is known to improve dissolution characteristics of a poorly water soluble drug since they maintain the drug in a solubilized state in the GI tract. Using the optimized SME mixture, ibuprofen loaded Liquid and Solid SMEDDS were prepared, evaluated for their self-microemulsification tendency and characterized. The resulting nanoemulsions from the trial formulations showed a droplet size of approximately 20 nm and a neutral zeta potential. Differential Scanning Calorimetry and Powder X-Ray Diffractometry confirmed the presence of ibuprofen in a molecularly dissolved state in
the Solid SMEDDS. *In vitro* drug release studies showed a 70% drug release within first five minutes of dissolution time from the trial formulations. Our studies indicated that SMEDDS can be potentially used for delivering a poorly water soluble drug. Ongoing studies related to this project include *in vitro* drug permeability testing using Caco-2 cell cultures.
References


