Chapter-1

Introduction
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1.1 Introduction of self-emulsifying drug delivery systems

Due to the prevalence of high-throughput screening and combinatorial chemistry in drug development programs, nearly 40-70% of new drug compounds are found to be poorly water soluble. This issue demands concern of formulation scientist as it creates jeopardy in dissolution and absorption in the gastrointestinal tract and thereby leading to poor and variable bioavailability reduced dose proportionality, substantial food effect, gastric irritancy and slow onset of action (Robinson 11-13). There are several proven techniques, which are being used for dissolution enhancement purpose for years, which include micronization, salt formation, complexation including use of cyclodextrin, nanoparticles, and solid dispersions. However, these techniques have their own drawbacks (Serajuddin 1058-66). Use of lipid based drug delivery systems has evolved as a useful alternative to above mentioned techniques. The successful examples include simple oily solution, emulsion, microemulsion, nanoemulsion, micellar solution and more recently self-emulsifying drug delivery systems (Gursoy and Benita 173-82).

Fundamentally, a self-emulsifying drug delivery system (SEDDS) or self-emulsifying oil formulation (SEOF) is mixture of natural/synthetic oil(s), solid/semisolid surfactant(s) ideally isotropic sometimes containing co-solvent(s) which upon introduction into aqueous phase, readily emulsifies to produce fine oil in water emulsion or microemulsion (SMEDDS). This whole emulsification procedure requires very little agitation, same as the peristaltic motion prevailing in the gut. Self-microemulsifying drug delivery systems (SMEDDS) produce droplets having a size range of 100-300 nm while self-nanoemulsifying drug delivery systems (SNEDDS) produce droplets of less than 100 nm size. In comparison to traditional emulsion formulations which are thermodynamically unstable dosage forms and requires high energy input, S(M)EDDS are kinetically stable and spontaneous in emulsion formation. This facet of self-emulsifying drug delivery systems makes them stand alone in the category of oral lipid based formulations.

The SEDDS is advantageous over conventional emulsion in terms of easy manufacturing & scale up and good physical stability. Furthermore they allow formulation of final drug delivery in the form of liquid which can even be added to food such as apple sauce or juice and more recently as soft gelatin capsule or even tablet (Gao and Morozowich 273-302; Mahmoud, Bendas and Mohamed 183-92).
1.1.1. Theory of self-emulsification

There is no proved theory of self-emulsification but there many empirical hypotheses. Generally it is believed that self-emulsification takes place when erosion of fine cloud of small droplets occurs from the surface of large droplet rather than a progressive decrease in the droplet size. This can be understood due to the presence of a lot of surfactants in S(M)EDDS (Pouton 47-58). While another theory suggested that self-emulsification takes place when the entropy change favouring dispersion is greater than the energy required to increase the surface area of the dispersion (Reiss 61-70).

Moreover the theory has also been presented in a different way that the free energy in a conventional emulsion formulation is a direct function of the energy required to create a new surface between the oil and water phases. In order to reduce the interfacial area and thus the free energy of the systems, the two phases of the emulsion gradually tend to separate. The conventional surfactants form a layer around the emulsion droplets and hence reduce the interfacial energy, as well as provide a mechanical barrier to coalescence. When SEDDS is introduced in aqueous media, it forms fine oil–water emulsions with only little agitation. Since the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous (Craig et al. 147–55).

In addition, one of the phenomena believed to be responsible for spontaneous emulsification is that initially surfactant or surfactant mixture in oil phase favors the formation of oil continuous microemulsion. After surfactant(s) come in contact with water i.e. GI fluids, water diffusion and/or chemical reaction make the surfactant more hydrophilic and lead to increased capability of microemulsion to solubilize water and decreased capability to solubilize oil. Eventually, microemulsion becomes unable to solubilize all the oil present and oil droplets nucleate. Moreover, the microemulsion itself inverts to become water continuous and miscible with water, so that final state is oil droplets dispersed in an aqueous phase (Sjoblom).

The ease of emulsification was proposed to be related to the ease of water penetration into the various liquid crystal or gel phases formed on the surface of the droplet (Wakerly et al. 242-55). When binary mixture (oil/non-ionic surfactant) is added to water, the interface between the oil and aqueous continuous phases is formed (Wakerly et al. 242-55). Water then gets solubilized within the oil phase as a result of aqueous penetration through the interface. This will continue until the solubilisation limit approaches close to the interface. Further aqueous penetration will lead to the
formation of the dispersed liquid crystal phase. Eventually, everything that is in close vicinity to the interface will be liquid crystal, the actual amount of which depends on the surfactant concentration in the binary mixture. Thus, following gentle agitation of the self-emulsifying system, water will rapidly penetrate into the aqueous cores and lead to interface disruption and droplet formation. As a consequence of the liquid crystal interface formation surrounding the oil droplets, SEDDS become very stable to coalescence.

In short, we can say that self-emulsification is a complex procedure, which is strongly influenced by the interplay of different types and proportions of oils, surfactants and co-surfactants. The formulator should have clear understanding of identifying the formation of emulsion and micro/nano emulsion and constructing the phase diagrams which gives idea about the final composition of optimization formulation.

1.1.2. Components of SEDDS

Self-emulsification is specific to: the nature of the oil/surfactant pair, the surfactant concentration, and oil/surfactant ratio; and the temperature at which self-emulsification occurs (Gursoy and Benita 173-82). So it can be inferred that only a certain blend of pharmaceutical Excipients can result in efficient self-emulsifying systems. Hence acquiring a basic understanding about excipients is appropriate at starting point of research.

1.1.2.1. Natural oily excipient

A number of natural oils, obtained primarily from plant sources and treated to remove impurities or to separate various fractions of the original product, are available and suitable for use in encapsulated oral formulation products. Natural oils and fats comprises of mixtures of triglycerides which contain fatty acids of different chain lengths and degrees of unsaturation. The melting point of any oil increases in proportion to the fatty acid chain lengths and decreases with increasing degree of unsaturation, which in turn predisposes them to oxidation. Triglycerides are classified into short (<5 carbons), medium (6–12 carbons), or long-chain (>12 carbons) and may be synthetically hydrogenated to decrease the degree of unsaturation, thereby conferring resistance to oxidative degradation (Hauss 667-76).
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The oil is one of the most important and major component in the SEDDS formulation not only because it can solubilize marked amounts of the lipophilic drug or facilitate self-emulsification but also and mainly because it can increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride. Usually vegetable oil and their derivatives are more preferred for SEDDS. Both long and medium chain triglyceride oils with different degrees of saturation have been used for the design of self-emulsifying formulations (Hasan 580-90; Bandyopadhyay, Katare and Singh 50-61). Furthermore, edible oils which could represent the logical and preferred lipid excipient choice for the development of SEDDS are not frequently selected due to their poor ability to dissolve large amounts of less lipophilic drugs (Gursoy and Benita 173-82). The common examples of natural oils used in SEDDS include, but not limited to, corn oil, olive oil, peanut oil, rapeseed oil, sesame oil, soybean oil, hydrogenated vegetable oils, hydrogenated soybean oil (Gao and Morozowich 273-302).

1.1.2.2. Semisynthetic oily excipient

In recent times polyglycolyzed glycerides with varying fatty acids and polyethylene glycol chain lengths having different HLBs along with vegetable oils are being used to solubilize poorly water-soluble drugs and to improve their bioavailability. They are derived from food grade vegetable oils and treated with pharmaceutical grade PEGs so that they are well tolerated by the body (Constantinides 1561-72). Generally, these semisynthetic excipients exist in both liquid and thermo-softening state, which is suitable to be filled with hard and soft gelatin and HPMC capsules. These excipients find application as drug-solubilizing vehicles, surfactants, and wetting agents and as emulsifiers and co-emulsifiers in SEDDS and self-micro emulsifying drug delivery systems (SMEDDS) (Hauss 667-76).

Medium chain glycerides obtained from coconut oil are preferred for formulating oral emulsions because, a) they are food grade products which are well recognized by FDA, b) They can be used at ambient temperature with a variety of compositions and c) they improve intestinal absorption of co-formulated drug (Constantinides 1561-72). Commonly used excipients used in this category include medium-chain triglycerides of coconut oil and palm seed oil and organic liquids/semi-solids such as DL-α-tocopherol, medium chain mono- and diglycerides, propylene glycol esters of fatty acids.
1.1.2.3. **Synthetic oily excipient**

A variety of fully-synthetic oily excipients are used as solvents for formulating poorly water-soluble drugs. They exist as monomeric and polymeric liquid and semi-solid excipients and reported as fairly non-toxic (Jannin et al. 385-92). These excipients, when used alone or in combination with other lipid excipients have the potential to improve the overall solubilizing capacity of the formulation. However, their marked water miscibility can reduce formulation performance due to uncontrolled precipitation of the drug after dilution in the aqueous contents of the GIT. This generally leads to dose-dependent bioavailability enhancement. The classic examples in this category are the polyethylene glycols (PEGs), Propylene glycol, and the poloxamers, which are co-polymers of poly-oxy-ethylene and poly-oxy-propylene (Hauss 667-76).

1.1.2.4. **Surfactants**

Surfactants are amphiphilic in nature and they can dissolve or solubilize relatively high amounts of hydrophobic drug compounds. Safety is the important criteria for selection of surfactants, because large amount may cause gastric irritation. Therefore natural surfactants are preferred because of comparative better safety profile than the synthetic surfactants (Hauss et al. 164-69). However, the natural excipients have a limited self-emulsification ability. Non-ionic surfactants are less toxic than ionic surfactants but they may lead to reversible changes in the permeability of the intestinal lumen (Wakerly et al. 242-55). Usually, large amount of surfactant are used to prepare efficient SEDDS mostly in the concentration ranges of 30 and 60% w/w. The surfactant used for formulation of SEDDS should have a relatively high HLB and hydrophilicity to facilitate immediate formation of oil globules in water continuous phase. An efficient surfactant should be able to prevent precipitation of the candidate drug upon dilution in ever changing gastric conditions and also keep drug in solubilized form until absorbed (N.H. Shah et al. 15–23).

There is a strong influence of surfactant concentration on the globule size. It is widely reported that, increasing the surfactant concentration could lead to droplets with smaller mean globule size (L. Wei, P. Sun, et al. 785-94; Hong et al. 332-38). This phenomenon can be explained by the stabilization of the oil globules due to localization of the surfactant molecules at the oil−water interface (Levy and Benita 29–37). Further it could also be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to the ejection of oil droplets into the aqueous phase (Pouton 47-58). On
the other hand, in some cases the mean droplet size may increase with increasing surfactant concentrations (Kommuru et al. 233-46). Various non-ionic surfactants such as the polysorbates (e.g., Tween® 80) and polyoxyls (e.g., Cremophor® EL), which cover the HLB range from 2 to 18, are frequently used in combination with lipid excipients to promote self-emulsification or micro-emulsification (Gao and Morozowich 273-302). Surfactants with low HLB values are generally used as co-surfactants. They provide stability to formed oil globules by providing flexibility to surfactant film at the interface. They help to prevent creaming, coalescence and phase separation.

1.1.2.5. Co-solvents
In the formulation of an efficient SEDDS, higher amount (generally more than 30%) of surfactants is required. This big amount of surfactants needs an external solvent to get solubilized in the lipid base. For this purpose, various solvents such as ethanol, propylene glycol (PG), and polyethylene glycol (PEG) are suitable for oral delivery. These solvents can even act as co-surfactants in microemulsion systems. On the other hand, alcohols and other volatile co-solvents have the disadvantage of evaporating into the shells of the soft gelatin, or hard, sealed gelatin capsules in conventional SEDDS leading to drug precipitation (Gursoy and Benita 173-82). Thus, alcohol-free formulations have been designed (Constantinides 1561-72), but their lipophilic drug dissolution ability may be limited.

1.1.3. Alternatives for the design of SEDDS
Self-emulsifying drug delivery systems have no longer been a novel concept now. The researchers have exploited this fantastic phenomenon to the fullest to come up with better and better formulations every time. The methods discussed below in this section tale bear the fact that in recent years the concept of self-emulsification has gained sizable recognition in pharmaceutical research.

1.1.3.1. Liquid self-emulsifying drug delivery systems
The traditional method of SEDDS requires the dose of the drug to be incorporated in the oil, surfactant, co-surfactant and/or co-solvent, generally in an amount to be incorporated in the appropriate hard or soft gelatin or HPMC capsule. The extent and efficiency of drug loading in SEDDS depends on physicochemical compatibility between drug and the system. Drugs are
reported to interfere with self-emulsification process so as to change optimal oil/surfactant ratio. The efficiency of a SEDDS can be changed either by halting charge movement through the system by direct complexation of the drug with some of the components in the mixture through its interaction with the liquid crystalline phase, or by penetration into the surfactant interfacial monolayer (Craig et al. 147–55). Further it is reported that the interference of the drug compound with the self-emulsification process may lead to change in droplet size distribution that changes with drug concentration (S. A. Charman et al. 87-93). It should also be considered that emulsions with smaller oil globules in more complex formulations are more susceptible to changes caused by the addition of the drug compound (Constantinides 1561-72). Therefore this approach seems very simple but it requires careful consideration for following points:

- A systematic quantitative solubility study should be carried out for the drug in various oil, surfactants, co-surfactants and co-solvents or their combinations.
- Based on the solubility study, excipients should be selected and phase diagram should be drawn to identify the blend and concentration of excipients that would result in fine emulsion spontaneously.
- The SEDDS should be prepared by dissolving drug into the excipients homogenously.
- Stability and precipitation of drug in the system should be carefully observed upon storage.

1.1.3.2. Supersaturatable self-emulsifying drug delivery systems

The surfactants that are commonly employed in SEDDS formulations can increase the commonness of GI side-effects and, therefore, a controlled amount of surfactant should be used in the formulations (Wignot et al. 420–27; Sherman and Fish 908–14; FGJ Poelma et al. 317–24; FGJ. Poelma et al. 392 –97). The low level of surfactant would lead to precipitation of the drug upon dilution with water in the gastrointestinal tract. This problem necessitates the exploration of strategy to maintain the drug in the solution i.e. something related to supersaturation. The concept of supersaturation for increasing the bioavailability of poorly soluble drugs has been recognized by Higuchi for more than four decades (Higuchi 85–97).

The problem of precipitation of the drug in GI tract can be overcome by maintaining the supersaturation in GI tract. For this reason, one need to formulate the dosage form that can yield supersaturated state only after administration In-vivo. Such dosage forms are known as supersaturatable dosage forms. Some common ingredients that can generate supersaturation are
polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), hydroxypropyl methylcellulose phthalate, and sodium carboxymethylcellulose (NaCMC). The cellulosic polymers are excellent crystal growth inhibitors and they are effective in maintaining the supersaturated state of the drugs (Gao and Morozowich 273-302). In addition, supersaturation can also be achieved by applying sonication followed by heating at moderately high temperature for short time and cooling at room temperature (Thomas, Holm, Garmer, et al. 219-27). The literature are available showing the development of supersaturatable S-SEDDS formulations using some poorly soluble drugs, like, docetaxel and silybin (Chen et al. 278-86; Y. Wei et al. 22-28).

1.1.3. Solid self-emulsifying drug delivery systems
Since years the solid dosage forms have been the favorite dosage form for manufacturers and patients as well. Anything that comes as the solid thing is well accepted in terms of performance and stability. The liquid SEDDS pre-concentrate present a problem of leakage of drug from the capsule and it may also lead to dehydration of capsule cell. Another issue with liquid SEDDS is that solubilization of a complete dose of drugs in single capsule volume suitable for oral administration is sometimes not possible.

The liquid pre-concentrate can be mixed along with some solid and/or semisolid excipients to prepare solid dispersion SEDDS (Laddha, Suthar and Butani 91-100). Solid dispersion formulations provide alternative to liquid SEDDS in cases where incorporating the entire dose in the system is not possible because, they may not require full solubilization of the drug in the excipient matrix (Gao and Morozowich 273-302). Some amount of the drug contained in a solid dispersion dissolves immediately upon contact with the GI fluid, resulting in a saturated or supersaturated solution for rapid absorption, and the excess drug precipitates in the GI fluid, forming amorphous or crystalline particles in the sub-micron size range with high surface area and hence lead to high dissolution and absorption rate. These characteristics often result in substantially improved drug absorption from a solid dispersion as compared to a conventional tablet or capsule formulation.

The hypothesis made by researchers suggest that structurally SEDDS solid dispersion can exist as eutectic solid dispersion, amorphous solid dispersion and/or solid solution. A eutectic mixture is a two-phase system with specific composition in which the drug and the carrier(s) exhibit complete
miscibility in the molten state, but are immiscible in the solid state and form an intimate mixture of the finely divided crystalline drug and carrier. Amorphous solid dispersions result when the drug and carrier are miscible in the molten state, but upon cooling, the drug loses miscibility in the carrier and solidifies in its amorphous state. Solid solutions are homogeneous, single-phase systems in which the components are completely miscible with one another, on a molecular scale, in the solid state. These three modalities governs the release from the solid dispersion. Drug release from a SEDDS solid dispersion formulation is believed to proceed following sequence: (1) dissolution of water-soluble excipient components, (2) exposure of lipid and/or drug to aqueous media, (3) lipid micellization or emulsification, (4) equilibrium partitioning of drug from the oily phase to the aqueous phase, and (5) in some cases, digestion of lipid. Commonly used ingredients to prepare SEDDS solid dispersion are Gelucires®, block copolymers (Fernandez-Tarrio et al. 471-79) (Pluronic F68), d-\(\alpha\)-tocopheryl polyethylene glycol 1000 succinate, lipid-polysorbate mixtures, Polyethylene Glycol-Polysorbate 80 Mixtures and colloidal silica (Gao and Morozowich 273-302; Agarwal et al. 44–52).

While preparing the solid dispersions two points need a special emphasis i.e. solubility of the drug in carrier and stability of the drug in the carrier. SEDDS solid dispersions are prepared by three methods. The first method is direct filling in hard gelatin capsules which include filling up of the capsule with molten excipients and allowing for solidification of the material inside. The second method is hot melt extrusion in which a mixture of drug substance and one or more excipients is continuously fed into a heated extruder barrel containing rotating horizontal screw(s) and extruded through an orifice at the opposite end of the barrel. Thirdly, melt pelletization is employed in which a meltable carrier or surfactant such as Gelucire® are mixed with drug substance and other excipients of SEDDS under high shear in a granulating bowl to produce pellets or granules of desired particle size.

Several SEDDS pellets are reported in literature, for instance nimesulide pellets were prepared by wet granulation with microcrystalline cellulose, lactose, mono-, and diglycerides and polysorbate 80 (Franceschinis et al. 87-97). In another study, a self-emulsifying pellet formulation of progesterone was prepared by extrusion and spheronization. A 50:50 mixture of oil (mono- and diglycerides) and polysorbate 80 was prepared by melting the glycerides at 50°C, adding the surfactant, and cooling the mixture to room temperature, yielding a liquid in which the progesterone
was dissolved. The solution was combined with microcrystalline cellulose and small amounts of water and ethanol, resulting in solidified mass that was extruded, spheronized and filled into hard gelatin capsules. (Abdalla, Klein and Mäder 457-64). In recent study the researchers developed and evaluated the solid self-emulsifying (SE) pellets of poorly soluble nitrendipine (NTD). These pellets were prepared via extrusion/spheronization technique, using liquid SEDDS (NTD, Miglyol® 812, Cremophor® RH 40, Tween 80, and Transcutol® P), adsorbents (silicon dioxide and crospovidone), microcrystalline cellulose and lactose (Wang et al. 1–6). It is important to mention here that in all the examples cited above, the performance of liquid SEDDS was unchanged upon conversion into solid SEDDS both in-vitro and in-vivo (Tuleu C, Newton M and Rose J 1495-502).

Other than solid dispersion, there are several other techniques to convert liquid or semisolid SEDDS into solid, which include but not limited to physical adsorption onto solid carriers, spray-drying, freeze drying and rotary evaporation, all of which convert liquid SEDDS into free flowing powders (Tan, Rao and Prestidge 2993-3017). Solid carriers used in above techniques work either by adsorbing liquid on to their surface or by encapsulating the dispersed oil globules prior to drying. Commonly used solid carriers include the water-insoluble, highly porous silica-based adsorbents, the water-soluble polysaccharide-based, polymeric and protein-based carriers etc. among others.

Silica based adsorbents highly popular because of their very high specific surface area, porosity and oil adsorption capacity. They are mainly used for adsorption of liquid SEDDS to prepare free flowing dosage form. Examples of such agents include fumed silica (Aerosil® series), micronised porous silica gels (Syloid® and Sylysia®), precipitated silica (Neosyl®, magnesium aluminometasilicate (Neusilin®) as well as calcium silicates (Florite® and Hubersorb®). Out of these agents, larger sized and highly porous inorganic carriers (e.g. Neusilin US2) are better in producing powders with better flowability and compressibility than the smaller sized Aerosil200 and other classes of non-porous, polymeric and organic carriers (Tan, Rao and Prestidge 2993-3017). Thus in the literature several SEDDS tablets are reported using silica based carriers such as Neusilin US2 for model drugs like Probucol, Cyclosporin, and Carvedilol (Gumaste, Dalrymple and Serajuddin 3186-99; Sander and Holm; L. Wei et al.)
Polysaccharide based carriers, which are mainly used for spray drying, freeze drying and rotary evaporation technique, include lower molecular weight mannitol, sorbitol, sucrose, lactose, trehalose, and the higher molecular weight maltodextrins, cyclodextrins, dextrins, gum acacia and starch sodiumoctenyl succinate. However, it is worthwhile to note that some of these carriers exist in crystalline form which after process are converted to high energy amorphous forms (Christensen, Pedersen and Kristensen 187-94). This phenomenon may be useful in promoting solubilization of encapsulated drugs.

Polymeric carriers, being amphiphilic, possess solubility in aqueous media and great solubilizing power for lipophilic compounds, and hence are being employed as emulsifiers and solid carriers in lipid-based formulation design. This class of carriers include but not limited to Poloxamers (or Pluronics/Kolliphor), hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC) sodium and polyvinylpyrrolidone (PVP). These carriers have benefit that they behave as precipitation inhibitor and supersaturation promoter (Chen et al. 278-86). All the agents listed in this class are hygroscopic and absorb moisture at elevated temperature and relative humidity except poloxamers which are less hygroscopic than others listed above and hence they are widely used for improving the stability of moisture sensitive drugs (Tan, Rao and Prestidge 2993-3017).

Protein based carriers such as gelatin and glycine find less applicability in solidification of emulsions because of their poor compressibility. However, they are widely used to entrap oil and oil soluble vitamins in fine size beads. Glycine is used reported to have superior ability to act as lyophilization aid for emulsions. Flurbiprofen is reported to be formulated as solid SMEDDS using gelatin as carrier (D. W. Kim et al. 323-30).

1.1.4. Evaluation of SEDDS

1.1.4.1. Physical evaluation

The primary method of assessing self-emulsification is visual evaluation (Craig et al. 147–55; Gursoy et al. 2420–27; Gershnik and Benita 147–57). The efficiency of self emulsification could be estimated by determining the time required for self-emulsification and droplet size distribution. Turbidity measurements can be carried out to determine the rapid equilibrium reached by the dispersion and to check reproducibility of this process (Gursoy et al. 2420–7).
1.1.4.2. Particle size of undissolved drug

This test is applicable in the case of SEDDS solid dispersion. *In-vitro* performance of such formulation can be assessed not only by estimating the dissolution rate, but also by determining the particle size of the released, but undissolved, drug in the dissolution medium. For instance, if the undissolved drug particles are in the sub-micron range then the chances of complete dissolution of the dose *in-vivo*, during GI transit, increase, because of the relatively high drug particle surface area.

1.1.4.3. Globule Size

The globule size of the emulsion is a decisive factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption (Tarr and Yalkowsky 40–3). Photon correlation spectroscopy (PCS), or more specifically dynamic light scattering (DLS) is a useful method for determination of emulsion globule size (Gershanik and Benita 147–57) especially when the emulsion properties do not change upon infinite aqueous dilution, a necessary step in this method. Various laser particle size analyzers are also used which have facility of auto dilution. Dispersed globules undergo Brownian movement. The larger the globule, the slower the Brownian motion will be. DLS monitors the brownian movement with light scattering. DLS measures the speed at which the globules are diffusing due to Brownian motion by recording the rate at which the intensity of the scattered light fluctuates. Smaller globules cause the intensity to fluctuate more rapidly than large globules. Light intensity fluctuations are monitored and correlated to derive hydrodynamic radius as output (NSF-NNIN- Bulletin). The polydispersibility index mentioned in the DLS report gives important information regarding homogeneity of globule size distribution. Value near to zero indicates homogenous distribution of globules throughout the continuous phase.

1.1.4.4. Pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams are used for assessing efficiency of self-emulsification. Generally the oil, surfactant and co-surfactant or co-solvent ratios are changed in order to identify self emulsifying regions and/or other types of intermediate phases. % isotropic region can be estimated and any composition within that region should be able to produce the emulsion efficiently. Moreover these diagrams give idea about the extent of dilution, that any given composition of oil and surfactant mixture can withstand without change in self-emulsification efficiency. Finally,
appropriate excipient concentrations are established by means of ternary diagram studies allowing formulation of the required SEDDS and/or SMEDDS.

1.1.4.5. Droplet polarity

Emulsion droplet polarity is also a very important factor in characterizing emulsification efficiency (Shah et al. 15–23). The HLB, chain length and degree of unsaturation of the fatty acid, molecular weight of the hydrophilic portion and concentration of the emulsifier have an impact on the polarity of the oil droplets. Polarity represents the affinity of the drug compound for oil and/or water and the type of forces formed. Rapid release of the drug into the aqueous phase is promoted by polarity.

1.1.4.6. Zeta potential

The charge of the oil globules of SEDDS is another property that should be assessed (Gershanik and Benita 147–57). Globules with zeta potentials more positive than +30 mV or more negative than -30 mV are generally considered stable (Malvern 1-6). The charge of the oil droplets in conventional SEDDS is negative due to the presence of free fatty acids; however, incorporation of a cationic lipid, such as oleylamine at a concentration range of 1.0−3%, will yield cationic SEDDS. Thus, such systems have a positive $\zeta$-potential value of about 35−45 mV (Gershanik, Benzeno and Benita 863–9; Gershanik et al. 29–36). The positively charged oil droplets formed by SEDDS could produce strong interaction with the mucosal surface, improve the adhesion of the positively charged droplets to the intestinal mucosa, and increase drug uptake from the mucosa, further improving the oral bioavailability (Gu et al. 485-91).

1.1.4.7. In-vitro release study

All the SEDDS formulations are required to be tested for In-vitro release in suitable dissolution or more precisely bio-relevant media. The liquid formulations are generally kept in semipermeable dialysis membrane and is put in USP type I (basket) or type II (paddle) apparatus. Use of USP type III (Reciprocating cylinder) and type IV (flow through cell) are being used now a days as they allow biorelevant volumes to be employed in the study and hence more reliable dissolution data can be achieved (Sunesen, Pedersen, et al.). Amount of drug released in the medium is checked periodically using various analytical technique.
1.1.4.8. *In-vitro* dynamic lipolysis study

Some studies have revealed that small intestinal fluid contains various surfactants, including bile salts (BS) and phospholipids (PL), which in combination with dietary or endogenous fats, form mixed micelles with high solubilizing capacity for many BCS class II compounds. The relation between the small intestinal lipid digestion and drug absorption has been well established now. The dynamic lipolysis model simulates small intestinal lipid digestion and holds potential for predicting formulation performance in humans in both the fed and fasted states.

Dynamic lipolysis experiments are conducted in a jacketed thermostatically controlled reaction vessel maintained at 37°C and agitated continuously with a magnetic stirring device. The reaction medium consists of a mixture of Bile Salt, Phospholipid, buffer, and lipid substrate (e.g., dietary lipid or lipid-based formulation incorporating the drug substance). Lipolysis is initiated by addition of the lipase solution and the pH and free calcium concentration of the reaction mixture is maintained by the computer-controlled addition of sodium hydroxide and calcium chloride solutions, respectively. Samples of the reaction medium are withdrawn immediately following addition of the lipase solution and at sequential time points subsequent to the initiation of lipolysis. The lipolysis reaction is inhibited by addition of the lipase inhibitor, 4-bromobenzene boronic acid or by freezing the samples immediately after withdrawal, and the samples are subsequently ultra-centrifuged, resulting in the formation of three distinct phases:

- A pellet comprised largely of insoluble calcium soaps of fatty acids,
- An intermediate aqueous layer, consisting of bile salt mixed micelles and various lipid vesicles, and
- An uppermost, oily layer comprised of diglycerides, and unhydrolyzed triglycerides.

The aqueous phase is of greatest interest in this study of the GI absorption of hydrophobic drugs. As it contains the drug in dissolved form within micelles, it gives indirect idea about amount available for absorption post-digestion.

1.1.4.9. *In-vivo* bioavailability study

For any dosage form, *in-vitro* release study is not enough to predict the behavior of formulation in the body. Reason being the unpredictability of the gastrointestinal tract environment in different individuals and difficulty in mimicking the body conditions outside, it has now been the need of
an hour to carry out *in-vivo* bioavailability study for successful formulation of the drug delivery system. Suitable animal model can be used to study the effect of formulation on the body.

### 1.1.5. Mechanisms underlying bioavailability enhancement by SEDDS

#### 1.1.5.1. Effect of surfactants

The surfactants used in these formulations are known to improve the bioavailability by various mechanisms including: (a) improved drug dissolution; (b) increased intestinal epithelial permeability; (c) increased tight junction permeability; and (d) decreased: inhibited p-glycoprotein drug efflux (Kommuru et al. 233-46). Surfactants improve the permeability of the single layer of the epithelial cell membrane by interfering with the lipid bilayer. The lipid bilayer along with the unstirred aqueous layer, forms the rate limiting barrier to drug absorption/diffusion. Hence, passive transcellular route is the major mode of absorption for most drugs. Moreover the surfactants partition into the cell membrane and disorder the structural organization of the lipid bilayer leading to permeation enhancement (Nakarani 5-58; H.-J. Kim et al. 523-29). Positively charged oil droplets interact with the negatively charged surface components of the GI lumen. Another mechanism for dissolution enhancement is believed to be that surfactants cause droplet size to decrease. Such a decrease in droplet size may be the result of more surfactant being available to stabilize the oil–water interface. Furthermore, the decrease in the droplet size behavior reflects the formation of a better close-packed film of the surfactant at the oil–water interface, thereby stabilizing the oil droplets. The smaller oil droplets provide a large interfacial area for pancreatic lipase to hydrolyze triglycerides and thereby promote the rapid release of the drug and/or formation of mixed micelles of the bile salts containing the drug (Kommuru et al. 233-46). Micellar solubilization of lipophilic drugs with high proportions of surfactants in the formulation affect the amount of free drug and extent of absorption. The intestinal absorption of griseofulvin in rats was reported to decrease in the presence of high bile salt concentration (20 mM taurocholate) as a result of micellar solubilization (Gursoy and Benita 173-82).

#### 1.1.5.2. Effect of lipids

Lipids are established to improve the bioavailability of lipophilic drugs via several mechanisms altering the biopharmaceutical attributes of the drug. These mechanisms include improved dissolution rate and hence intrinsic solubility in gastrointestinal fluids, protection of enclosed drug from chemical and enzymatic degradation, inducing the digestion through pancreatic and gastric
lipase leading to formation of bile salt-phospholipid micelles to increase solubilization, promoting
the lymphatic transport and avoiding first pass effect. The solubilization, absorption and
distribution of the drug formulated as lipid based drug delivery system is highly influenced by the
fatty acid chain length present in the lipid employed for formulation, its degree of saturation and
the total lipid load incorporated as the dose. The transport of drug enclosed in lipids requires the
presence of lipoprotein which is further dependent on the capacity of the lipid to stimulate the
production of bile salts and lipoproteins. Short and medium chain fatty acids (with a carbon chain
length shorter than 12 carbon atoms) are transported to the systemic circulation by the portal blood
and are not incorporated to a great extent in chylomicrons. In contrast, long chain fatty acids and
monoglycerides are re-esterified to triglycerides within the intestinal cell, incorporated into
chylomicrons and secreted from the intestinal cell by exocytosis into the lymph vessels (Nakarani
5-58).

In addition to the stimulation of the lymphatic transport, lipid based formulation enhance the
concentration of drug in blood, as compared to non-lipid formulation, by presentation as a
solubilized formulation (thereby avoiding solid-state limitations) and by induced changes to the
atmosphere of the gastrointestinal tract (Porter, Trevaskis and Charman 231-48). As reported in
literature, the most important criteria for improving the dissolution of the lipophilic drug is to keep
them solubilized by preventing the precipitation. When a lipophilic drug is administered with
dietary lipids or formulated as lipid based drug delivery, the drug gets distributed between the
colloidal species. This process prevents drug precipitation and leads to an increase in effective
aqueous solubility of the co-administered drug (Cherniakov, Domb and Hoffman 1121-33).
Indigenously present bile salts, monoglycerides, cholesterol, lecithin and lysolecithin further
emulsify the large fat droplets upon their entry to the intestine, and smaller droplets of 0.5−1 μm
mean diameter are formed. Pancreatic lipase then act on the surface of the droplets and catalyze
the digestion of lipid droplets, which results into formation of mixed micelles and bile salts.
Following their penetration through the aqueous layer and mucin, mixed micelles and
microemulsions are absorbed either by pinocytosis, diffusion or endocytosis. The drug compound
then reaches the systemic circulation via the portal vein or lymphatic system (Gao and Morozowich
273-302).
1.1.5.3. Effect of P-glycoprotein (P-gp) inhibition

The reason behind increased uptake of lipophilic drugs by permeation can be modification in the fluidity of gastrointestinal membrane by either alteration in the conformation of membrane-bound transporters or promotion of the inhibition of membrane-bound efflux transporters. Certain non-ionic surfactants such as Tweens, Spans, Cremophors (EL and RH 40 grades), Vitamin E TPGS and block co-polymers are reported to inhibit the P-gp efflux action (Constantinides and Wasan 235-48). Though poor permeability is not a barrier for absorption of BCS class II compounds, effect of efflux transport by P-gp can significantly impede their oral bioavailability. Therefore increased permeation of such drugs are more attributed to inhibition of P-gp efflux than passive diffusion. In-contrast to above mechanism, some studies also show that surfactants like Cremophors can inhibit uptake transporters such as organic anion transporting polypeptide (OATP) which is expressed in the apical membrane of enterocytes. This in turn can reduce the permeation of some drugs (Cherniakov, Domb and Hoffman 1121-33). However, more detailed research is not found reported.

1.1.5.4. Influence of self-emulsifying lipid-based formulations on effect of food

Improved absorption of lipophilic drug when taken with or after food is well documented phenomenon. It has been hypothesized that maximum extent of absorption possible when the drug is formulated as lipidic formulation resembles the degree of the absorption enhancement exhibited due to food (Porter et al. 1405–12). The effect of food on the bioavailability of lipophilic drugs is governed by multiple factors, including the physicochemical properties of the drug, the dose, the nature of the formulation and the amount and composition of the ingested food (W. N. Charman et al. 269–82). Postprandial changes in the GIT that can increase drug absorption, relative to the fasted state, include: (i) increased drug solubilization by bile salt mixed micelles, and (ii) increased intestinal membrane permeability secondary to the presence of bile and lipid digestion products.

Although very few, but studies showing the efficacy of self-emulsifying lipid-based formulations for mitigating food effect have been described in the literature. Grove et al. (Grove et al. 8-15) studied the influence of food on the bioavailability of seocalcitol in minipigs following administration as either a solution in MCT, a MC-SMEDDS, or a solution in propylene glycol (PG). The fasted state bioavailability of seocalcitol was 15%, 21%, and 28% for the PG, MCT, and MC-SMEDDS formulations, respectively. In the postprandial state, the seocalcitol bioavailability
from the PG solution nearly doubled to 29%, but was unchanged, relative to the fasted state, for both the MCT and MC-SMEDDS formulations. These results show magnitude by which food and lipid-based formulations improve the absorption of poorly soluble drugs. Other poorly soluble drugs for which reports suggest that lipid-based formulations have reduced the effect of food on drug absorption include danazol (W. Charman et al. 381–86) L-683,453 (Matuszewska et al. 147–54) and cyclosporine (Mueller et al. 151–55).

1.1.6. Rewards of SEDDS over simple oral dosage forms (Sarpal, Pawar and Bansal 42-49)

✓ Improvement in oral bioavailability: The ability of lipid based formulations to present the drug to GIT in solubilized and micro emulsified form (globule size between 1-100 nm) and subsequent increase in specific surface area, enables more efficient drug transport through the intestinal aqueous boundary layer and through the absorptive brush border membrane, leading to improved bioavailability.

✓ Ease of manufacture and scale-up: Ease of manufacture and scale-up is one of the most important advantage that makes lipid based formulations unique when compared to other bioavailability enhancement techniques like solid dispersions, liposomes and nanoparticles. SEDDS require very simple and economical manufacturing facilities for large-scale production.

✓ Reduction in inter-subject and intra-subject variability and food effects.

✓ Prevention of enzymatic hydrolysis in GIT: One unique property that makes lipid based formulations superior as compared to the other drug delivery systems is their ability to deliver macromolecules like peptides, hormones, enzyme substrates and inhibitors enclosed in oil globules and to offer protection from enzymatic hydrolysis.

✓ Increased drug loading capacity: SEDDS provide the advantage of increased drug loading capacity because solubility of BCS class 2 drugs with intermediate partition coefficient are typically low in natural lipids and much greater in amphiphilic surfactants, co-surfactants and co-solvents.

1.1.7. Conclusion

SEDDS promise to enhance the dissolution of poorly water soluble drugs and ultimately the oral bioavailability. It improves physicochemical properties of the hydrophobic drug and eases the
formulation problems. The ability of the system differs from drug to drug and therefore SEDDS formulation should be chosen very carefully. SEDDS employs high amount of surfactants so, toxicity of surfactants should be considered seriously. A sensible compromise should be made between toxicity and self emulsifying ability of surfactant to be used. The size and charge of droplets of resultant emulsion are two deciding factors for efficiency of GI absorption. There are many successful examples of marketed SEDDS and still 40% of newly discovered drugs are found to be hydrophobic. Therefore SEDDS are expected to be explored and employed more and more for harnessing their unique advantages to improve the performance of poorly water soluble, lipophilic drugs.

1.2. Introduction to biorelevant dissolution media

In recent years, there has been a strong push to identify bioavailability (BA) problems of a drug formulation based on the results of appropriately designed dissolution experiments. To save time and costs associated with the need for pharmacokinetic and clinical studies, dissolution test systems capable of predicting BA by means of an in-vitro–in-vivo correlation would represent a valuable tool. Ideally, the methodology should be as simple as possible, reliable and reproducible, and make it possible to discriminate appropriately between different degrees of product performance. However, to achieve adequate predictability of the in-vivo release behaviour of a dosage form by use of in-vitro dissolution data, physicochemical properties of the drug and its formulation as well as the relevant physiological conditions have to be considered in equal measure (Klein 397-406).

It is well documented that dissolution of poorly water soluble drugs in gastrointestinal tract depends on several factors such as pH, buffer capacity, ionic strength, volume available for dissolution and presence of surfactants etc. And the dissolution medium used for in-vitro study should closely represent these conditions in order to provide meaningful results that are capable of giving some insight of in-vivo behaviour (Galia et al. 698-705).

In the last two decades, knowledge of these gastrointestinal (GI) luminal conditions (including the lower gut) has improved vividly. As a result, various in-vitro performance tests which mimic the intragastric performance of orally administered formulations, i.e. biorelevant tests, have been proposed. Biorelevant dissolution/release testing systems are useful for the evaluation of formulation and food effects on plasma drug concentrations after administration of oral dosage forms. Luminal disintegration times of immediate release (IR) dosage forms and the bile acid
sequestering activity of resins in the lumen can also be successfully predicted with biorelevant *in-vitro* performance testing (Reppas et al. 1867-76). To mimic the conditions of the GI tract, use of biorelevant media for the *in-vitro* dissolution study had been proposed and updated time to time (Dressman et al. 591-602; Jantratid et al. 1663-76).

### 1.2.1. Factors to be considered for designing biorelevant media

There are some physicochemical and physiological factors which have been identified over the time, to influence the dissolution of almost all drugs. While designing biorelevant media, it is essential to gain thorough understanding about them. Hence some of the very important factors are discussed below.

#### 1.2.1.1. pH (Hörter and Dressman 75-87)

The solubility of weak acids and bases is dependent on their ionization constants, $K_a$ and the pH of the dissolution medium. The intrinsic solubility of weak acids increases linearly at high pH values exceeding $pH = pK_a + 1$, while for weak bases the solubility increases with decrease in the pH. Therefore the pH of the gastrointestinal fluids is one of the most important parameter while assessing the dissolution of ionisable drugs. The pH varies widely with location in the gastrointestinal tract. pH of the stomach, duodenum, jejunum and ileum in fasted state is reported to be 1.3, 6.5, 6.6, 7.4 respectively and in fed state the values are changed to 4.9, 5.4, 5.2 - 6.0 and 7.5 respectively. There are complex variations in pH between the fed and fasted state. Upon ingestion of a meal, the gastric pH at first increases because of buffering effects of food components. In response to food ingestion, however, gastric acid is secreted, and by 3–4 h after the meal intake, the fasted state pH has usually been re-established.

Weakly basic drugs like itraconazole, ketoconazole and dipyridamole will suffer poor dissolution after ingestion of food because gastric pH is less acidic. However, this issue will be offset by increased gastric emptying time providing sufficient time for dissolution of the drugs. It should also be considered that pH of the luminal fluids is dependent on some other factors also, such as, age, pathophysiological conditions like achlorhydria and AIDS and simultaneous drug treatment of H$_2$ receptor antagonists and proton pump inhibitors. For weakly basic drugs elevated gastric pH due to any of the conditions outlined above, leads to poor dissolution and absorption. The pH in the small intestine after exposure to food due to release of acidic chime form the stomach but after
sometime it is re-established due to release of pancreatic bicarbonates. This transient change in pH cerates problem for poorly water soluble weakly acidic drug, which have none or very less dissolution in gastric pH and undergo dissolution in small intestine for the first time. This phenomenon leads to poor dissolution and absorption of weakly acidic drugs. Hence the pH is an unavoidable factor to be considered while designing biorelevant media.

1.2.1.2. Viscosity
The effect of viscosity on the dissolution of the drug is inevitable. Dissolution rate is inversely proportional to the viscosity (Braun and Parrott 175-8). One of the study reported that intrinsic dissolution rate of the candidate drug decreased at high viscosity induced by addition of hydroxypropyl methylcellulose in the dissolution medium (Nielsen et al.). Despite of these findings available in the literature, viscosity is the least considered parameter in the field of biorelevant dissolution media.

1.2.1.3. Surface tension
The surface tension of human gastric juice is nearly independent of pH and secretion rate, lying normally in the range from 35–45 21mNm. The surfactants responsible for the relatively low surface tension were not identified. Finholt and his co-worker have reported that the rate of dissolution of hydrophobic drugs increases with decreasing particle size when the dissolution medium has a low surface tension, decreases when the surface tension is high (Finholt and Solvang 1322-26). This finding was further supported by some other study, which noted linear relationship between the surface tension of the dissolution medium and the dissolution time in the case of phenacetin. Further experiments showed no improvement in the solubility of phenacetin with increasing surfactant concentration, it was concluded that the main effect of the surfactants on the dissolution rate was the decrease in the interfacial tension (Hörter and Dressman 75-87).

1.2.1.4. Volume of media
One of the breakthrough study reported increment in absolute bioavailability of lipophilic drug, Danazol by 55% when administered with 1000 ml water as compared to 200 ml (Sunesen, Vedelsdal, et al. 297-303). The volume available for the drug to dissolve depends on the volume of co-administered fluids as well as secretions from the organs surrounding the gastrointestinal tract into the lumen. These secretions usually occur at quite different rates, depend on whether they
occur at basal conditions or in response to food or co-administered drug. Therefore it is justified to use the volume of the medium according to the site in the gastrointestinal tract being studied and the conditions of administration (Dressman and Reppas S73-S80). Dressman et al. further proposed biorelevant volumes to be used with standard USP paddle apparatus as: fasted state stomach, 300–500 ml; fed state stomach, 900 ml; fasted state small intestine, 500 ml and fed state small intestine, 900–1000 ml. Where, 300 ml would represent the volume of secretions in the stomach under baseline conditions in addition to 250 ml co-administered fluid and 500 ml is also the lowest volume that can be used with the paddle still immersed in the medium. However, the volumes in the upper small intestine in postprandial condition approaches to as much as 1.5 l, the maximum volume that can be incorporated in the conventional dissolution apparatus is 1 l. In spite of the capacity of the apparatus, the volumes suggested by them are still in the physiologically relevant (Dressman and Reppas S73-S80).

1.2.1.5. Bile salt concentration

Bile salts increases the dissolution of lipophilic drugs by (1) promoting wetting of the drugs with high contact angles and (2) improving the solubilization due to formation of micelles (Bates, Gibaldi and Kanig 191-9). However, they primarily increase the dissolution of less lipophilic drugs (Log P 1-2) by improving wetting of the drugs with high contact angle. While in case of highly lipophilic drugs (Log P* 6), bile salts act by facilitating solubilization through micelle formation. Usually the levels of bile salt in the fasting condition ranges between 3–6 mM, which can decrease the contact angle remarkably (Miyazaki et al. 2468-72). For instance, as reported in one of the study, in the case of phenylbutazone, which has a contact angle of 90° with water, addition of bile salts to the medium resulted in a large increase in the dissolution rate. On the other hand, bile salts had little influence on the dissolution rate of indomethacin, which has a contact angle with water of 28° (Hörter and Dressman 75-87). The decrease in the contact angle increases effective surface area of the drug available for dissolution. Therefore the most important parameters in Noyes-Whitney equation, effective surface area (A) and the saturation solubility of the compound (C_s), are improvised by bile salts and ultimately the rate of dissolution increases. The Noyes-Whitney equation is denoted below:

\[ DR = \frac{dc}{dt} = \frac{A C_s D}{V h} \]

Where, \( DR \) dissolution rate depends on, \( A \) effective surface area, \( C_s \) saturation solubility, \( D \) diffusion co-efficient, \( V \) volume for dissolution medium and \( h \) the boundary layer thickness.
1.2.1.6. Presence of food

Role of food in enhanced bioavailability of poorly water soluble drug can be summarized as increased absorption due to (1) a delay in the gastric emptying allowing prolonged release into the small intestine, (2) improved solubilization of the drug due to secretion of bile salts and lipase from pancreas in response to food, and (3) an hence increase in the dissolution rate of the drug (W. Charman et al. 381–86). One of the ways in which food intake influences the dissolution rate is through the increase in volume of the GI contents. Fluids ingested with the meal can increase the available gastric volume by as much as 1.5 l. Not only do the ingested food and fluids directly influence the volume in the upper GI tract, they also stimulate secretion of gastric acid, bile and pancreatic juice as mentioned earlier. Furthermore, ingestion of hypertonic substances can stimulate net water efflux across the intestinal wall into the GI lumen (Hörter and Dressman 75-87). Food induced digestion produce bile salt-phospholipid mixed micelles which carry the drug to membranes promoting their absorption in portal vein as well as into lymphs. Lipophilic drugs show increased plasma concentrations even sometimes unexpected toxicity if taken with or after food, (W. N. Charman et al. 269–82), and therefore clinical trial guidelines routinely require studies comparing drug exposure in fed and fasted subjects.

Looking to the various important aspects of human gut environment as presented above, an attempt was made in the present thesis to address them judiciously, while preparing biorelevant media for the study

1.2.2. *In-vitro* lipid digestion study

1.2.2.1. Significance of lipid digestion in dissolution enhancement

Lipid based drug delivery systems (LBDDS) i.e. SEDDS in the present context, improve drug solubilization due to surfactants and lipids present in the formulation and their digestion products that result subsequently to their exposure to gastrointestinal bile salts, phospholipids and cholesterol. The physicochemical nature of LBDDS changes dramatically after oral ingestion due to interaction between formulation components and biliary and pancreatic secretions in the small intestine. This process is very much identical to the digestion of food derived lipids which is facilitated primarily by pancreatic lipases and esterases that are secreted in response to exposure of exogenous lipid in the upper small intestine and secondarily, by acid-stable lipases in the stomach. The products generated by lipid digestion are further solubilized by bile salt–phospholipid–
cholesterol-mixed micelles secreted in bile. This process leads to formation of different colloidal structures like unilamellar and bilamellar vesicles and micelles. These structures have the capacity to carry the drugs along with digestion products to the intestinal wall for absorption. (Williams et al. 3360-80)

The determining factors of in-vitro and in-vivo performance of SEDDS are: (1) the capacity of the formulation to maintain solubilization capacity on dilution and digestion, (2) the rate of release of drug during digestion of the formulation and production of digestion products (3) the solubilization capacity of the localized gastrointestinal content in the presence of products of the digested formulation. These factors affect the fate of all the formulations that contain digestible lipid &/or surfactant. Digestion of poorly dispersed LBDDS containing lipids with very less or no surfactants leads to the generation of more amphiphilic lipid digestion products that are more readily incorporated into bile salt–phospholipid-mixed micelles. Thus lipid solutions are likely produce colloidal structures with high drug solubilization capacities. Hence, it can also be said that digestion is the primary and the only mechanism by which lipid solution gets dispersed and promote the solubilization of poorly water soluble drugs. In contrast, SEDDS which contain high proportions of surfactant and co-solvent, do not require digestion to reduce particle size because initial dispersion of the formulation normally leads to the production of nano meter sized colloidal droplets. It can be inferred from this theory that digestion of SEDDS is inevitable and detrimental to absorption in some cases where digestion can lead to drug precipitation. This is especially concerned with presence of digestible surfactant in the formulation. Figure 1.1 indicates mechanism of lipid digestion which leads to formation of different colloidal structures.

According to the figure 1.1, as described in the report of porter et al., after ingestion, of the dietary lipid or formulation containing lipid, digestion is usually begun by gastric lipase. The mechanical mixing in stomach as propulsion, grinding and retropulsion, process the exogenous lipid which when combined with diglycerides and fatty acids produced by initial lipid digestion further form crude emulsion. Generally in case of diet, lipid digestion starts in mouth with lingual lipase but in case of dosage forms which is generally swallowed, this step is bypassed. Usually it is observed that lipids containing medium-chain triglycerides are digested faster as compared to long-chain triglycerides, both reactions producing diglycerides, monoglycerides and free fatty acids. In the small intestine, pancreatic lipase with its cofactor co-lipase completes the hydrolysis of triglyceride
to diglyceride, monoglyceride and fatty acid. The pancreatic phospholipase A2 digest phospholipids derived from either food or formulation and yield lyso-phosphatidylcholine and fatty acid (Thomas, Holm, Rades, et al. 860-71). Additionally, the exogenous lipid stimulates the secretion of endogenous bile salts, phospholipids and cholesterol from gall bladder, and this high concentration of bile salt helps in accommodating the digestion products into different colloidal structures such as multilamellar and unilamellar vesicles, mixed micelles and micelles. Ultimately these colloidal species set the capacity of small intestine to solubilize the lipolysis products and administered drugs to the supreme level (Porter, Trevaskis and Charman 231-48).

Figure 1.1. Lipid digestion mechanism as reported by Porter and his co-workers (Porter, Trevaskis and Charman 231-48). The oil droplet in the intestine is represented in different colours to indicate undigested triglyceride in the core (orange) and digested products such as fatty acid (blue) and monoglyceride (green) on the surface of the droplet.
1.2.2.2. Method employed

To understand the performance of a wide range of LBDDS effectively, *in-vitro* lipid digestion models have been proposed as a possible mechanism by which the complex series of *in-vivo* interactions may be simulated and predicted *in-vitro* (Dahan and Hoffman 2165-74). In these models, the lipid formulation containing drug is dispersed in a digestion medium that represents upper small intestine in fasting condition, and digestion of the formulation is initiated by addition of a porcine-derived pancreatic extract containing pancreatic lipase and other pancreatic enzymes. The digestion of the formulation liberates fatty acid from either glyceride lipids or surfactant fatty acid esters, and hence a drop in the pH of the digestion medium is observed. The pH is monitored and maintained at set point by addition of equimolar sodium hydroxide using pH-stat titrator. The rate and extent of lipid digestion can be estimated by measuring the rate of addition of base to neutralize free fatty acids liberated during hydrolysis (Sek, Porter and Charman 651-61).

![Figure 1.2. Schematic set-up of in-vitro lipolysis model as presented by Fatouros and his co-author (Fatouros and Müllertz 257-71)](image-url)
1.2.2.3. Expected outcomes

Samples from the digestion medium may be removed at intervals during the digestion test and the lipolysis is inhibited by adding lipolysis inhibitor before the separation by high-speed ultracentrifugation. Ultra-centrifugation results in the separation of three discrete phases: a pellet phase containing precipitated drug; an aqueous colloid phase containing solubilized drug; and an oily phase containing a mixture of incompletely digested lipid, any phase-separated digestion products, and incorporated drug.

1.2.2.4. Scope in the present thesis

A prerequisite for absorption is that the drugs are dissolved in the aqueous phase of the intestinal content, which contains mixed BS micelles. Therefore, the concentration of a drug in the aqueous phase is of the greatest interest when studying drug behaviour during hydrolysis in in-vitro lipid digestion models (Christensen et al. 287-96; Larsen, Sassene and Müllertz 245-55). In the present thesis, aqueous phase was investigated primarily to estimate the amount of drug available ready for absorption.

1.3. In-silico methods for prediction of in-vivo fate of an optimized formulation

In formulation development process of any drug delivery system, predictions of the drug behaviour in the human gastro-intestinal lumen when given through different dosage forms will significantly simplify the development process and might enable faster entry into clinical trials (Reppas et al. 1867-76). Several different models can be used to predict the performance of dosage forms after oral administration, e.g. in vivo and ex vivo nonclinical models, in vitro and in silico models. Animal models can, however, discriminate between the species better as compared to man, they are expensive and the capacity is limited (Kataoka et al. 1674–80). Furthermore, the pharmaceutical industries are now committed to reduce the use of preclinical animal models wherever possible; thus, making in-vitro or in-silico models the preferred choice whenever applicable (Berthelsen et al. 356-65). Previous studies using either in-vitro and/or in-silico models to predict the bioavailability of BCS class II compounds have shown good in vitro–in vivo correlations (IVIVCs) (Galía et al. 698-705; Sunesen, Pedersen, et al. 305-13), greatly supporting their use in formulation development.
The *in-silico* method estimates parameters with the aid of computational technology and has proven useful in many studies in the field of pharmaceutical sciences for predicting bioavailability during the process of developing formulations (Yu et al. 921-25; Tubic-Grozdanis, Bolger and Langguth 213-26; Pelkonen, Turpeinen and Raunio 483–91).

1.3.1. Convolution

The convolution method provides simple and practical approach to develop IVIVC and product evaluation. It is prospective *in-vitro-in-vivo* relationship model, where, no pilot in-vivo plasma concentration data are available and the pharmacokinetic profile is used as reported in the literature (Chakraborty, Pandya and Aggarwal 1-7). The convolution approach provides direct correlation of dissolution data and pharmacokinetic parameters ($C_{\text{max}}$, $T_{\text{max}}$). Predicting plasma concentration-time profile in a single step using convolution method is relatively simple when compared to other computational methods. It uses *in-vitro* dissolution data to derive blood drug levels using pharmacokinetic parameters of a test product. The required pharmacokinetic parameters can be obtained from literature or from a standard text book of pharmacology (Qureshi 38-47). One of the study reported the successful prediction of $C_{\text{max}}$ and AUC of aprepitant and donepezil using convolution method and found less than 15% prediction error with respect to reported pharmacokinetic data (Chakraborty, Pandya and Aggarwal 1-7). In the same study drug release in biorelevant media were used for calculation of plasma concentration with convolution method. Detailed procedure is mentioned in the chapter 7 of this thesis.

1.3.2. Computer simulation using software

Computer simulations have been used in the assessment of waiving bioequivalence studies and the determination of IVIVC for class II, III and IV drugs, reducing both research time and the cost of developing new generic medicines. An example of such an *in-silico* method is GastroPlus™,a software which has applications based on BCS theory and the advanced compartmental transit and absorption model (ACAT model), which simulates gastrointestinal absorption and the different pharmacokinetic parameters of medications (Grbic et al. 165-71). The form of ACAT model implemented in GastroPlus™ is demonstrated by a system of coupled linear and nonlinear rate equations used to simulate the effect of physiological conditions on drug absorption as it transits through successive GI compartments.
The equations include the consideration of six states (unreleased, undissolved, dissolved, degraded, metabolized, and absorbed), 18 compartments (stomach, seven compartments for the small intestine, colon, and nine enterocyte compartments), three states of excreted material (unreleased, undissolved, and dissolved), and the amount of drug in up to three PK compartments (when PK parameters are available). The total amount of absorbed material is summed over the integrated amounts being absorbed/exsorbed from each absorption/transit compartment. Besides physiological parameters, this model requires certain input parameters regarding drug physicochemical and pharmacokinetic data along with some dosage form properties. It should be kept in mind that such parameters should adequately reflect drug biopharmaceutical properties.

GastroPlus™ can also aid in the development of new formulations and the selection of biorelevant dissolution conditions with IVIVC. This software has been used for predicting pharmacokinetic behavior and establishing the IVIVC for various class II drugs (Arthur. Okumu, Marie. DiMaso and Raimar. Löbenberg 2778-85; Arthur. Okumu, Marie. DiMaso and Raimar. Löbenberg 91-98).

To summarize, a validated in-silico model, and accordingly, identification of biorelevant dissolution method can help in reducing the number of human in-vivo studies during the development of generic formulations, their approval by the regulatory agencies, and certain post-approval changes (Grbic et al. 165-71).
1.4. References


Chapter 1


