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Lipid-Based Nano-Delivery for Oral Administration of Poorly Water Soluble Drugs (PWSDs): Design, Optimization and *in vitro* Assessment

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<http://dx.doi.org/10.5772/65710>

Abstract

Currently, more than 90% of compounds identified are water insoluble and or poorly water soluble, which is a bottle neck in the development of many new drug candidates. These poorly soluble drug molecules are difficult to formulate using conventional approaches and are associated with numerous formulation-related performance issues. Formulating these compounds using lipid-based systems is one of the rapidly growing interests and suitable drug delivery strategies. Lipid formulations such as self-emulsifying/microemulsifying/nanoemulsifying drug delivery systems (SEDDS/SMEDDS/SNEDDS) have been attempted in many researches to improve the bioavailability and dissolution rate for their better dispersion properties. One of the greatest advantages of incorporating the poorly soluble drug into such formulation products is their spontaneous emulsion and or microemulsion/nanoemulsion formation in aqueous media. The performance and ongoing advances in manufacturing technologies have rapidly introduced lipid-based drug formulations as commercial products into the marketplace with several others in clinical development. The current chapter aims to present the characteristics feature, development and utilization of oral lipid-based nanoformulations within the drug delivery regime. The content of the chapter also provides an insight into the *in vitro* evaluation of lipid-based nanosystems and their limitations.

Keywords: lipid-based formulation, self-nanoemulsifying drug delivery systems (SNEDDS), poorly water soluble drugs (PWSDs), lipid formulation classification systems (LFCS), solubility enhancement

1. Introduction

Due to the continuous rise in the number of low solubility drug molecules and lack of more targeted drug therapies, the drug development has become more complex and challenging job within the industry. In fact, up to 90% of today's drug candidates are suffering from low aqueous solubility, which is commonly associated with low bioavailability, high intra- and inter-subject variability and lack of dose suitability [1, 2]. In keeping these challenges in mind, drug formulators must seek new techniques and innovative formulation approaches to overcome such hurdles and ensure effective treatments for the patients in need.

It is more than a decade, when lipid-based formulations have been considered as a well-established strategy for improving oral bioavailability and minimizing variable food effect of poorly soluble compounds. Lipids have been used as carriers in various delivery systems for drug administration, including solutions, suspensions, emulsions, and more attractively self-emulsifying/microemulsifying/nanoemulsifying drug delivery systems (SEDDES/SMEDDES/SNEDDES) that are designed to increase solubility and bioavailability of drugs belonging to the BCS Class II–IV [3]. Among several approaches, which are currently available to incorporate active pharmaceutical drugs into lipid vehicles in a variety of dosage forms, SEDDES, SMEDDES and or SNEDDES have proved to be the most successful approaches in improving the bioavailability [4]. The initial key achievement of these formulation systems (SEDDES/SMEDDES/SNEDDES) is to increase the solubilization of the poorly water soluble drugs (PWSDs) by the formation of emulsions and or micellar systems (colloidal solutions).

These systems advantageously present the drug in solubilized form, and their relatively smaller droplet sizes provide a large interfacial area enhancing the activity of pancreatic lipase to hydrolyze triglycerides and thereby promoting faster drug release containing mixed micelles of bile salts. The development of Neoral® (cyclosporin A) as a commercial product exhibits an excellent example of the utilization of these systems [5].

Nanotechnology has become a buzzword for scientific experts, and efforts are ongoing to extend its applications in various medical and pharmaceutical aspects. The nanoscale technologies can be generally categorized into: lipid-based nanocarriers, polymeric nanocarriers, inorganic nanocarriers, and drug nanoparticles or nanosuspensions [6]. Within the lipid-based nanocarriers category, there has been a resurgence of interest in nanoemulsions since low energy emulsification methods, such as spontaneous or self-nanoemulsification, have been developed. SNEDDES are anhydrous homogenous liquid mixtures, composing oil, surfactant, drug and/or cosolvents, which spontaneously form transparent nanoemulsion (20–250 nm droplet size) upon aqueous dilution with mild agitation [6, 7].

Being nanosized, SNEDDES offer a strong alternative to the more conventional oral formulations of lipophilic compounds. SNEDDES introduce the drug in solution within nanosized oil droplets. These fine droplets are emptied rapidly from the stomach resulting in faster drug release all over the gastrointestinal (GI) tract. An additional advantage of SNEDDES over simple oily solutions is granting much larger interfacial area for partitioning of the drug between oil and water leading to ease of dispersibility [8]. In contrast to oily solutions, SNEDDES does not

depend on the action of bile salts, enzymes and/or other effects related to the (fed/fasted) state of the stomach [9]. Thus, SNEDDS can reduce the variability in rate and extent of absorption and grant more reproducible plasma concentration levels [10].

Compared with conventional nanoemulsions, SNEDDS can offer the advantages of improved physical and/or chemical stability of the formulation and ability to fill them into unit dosage forms, such as soft/hard capsules, which improve their commercial viability, patient compliance/tolerability and reduce palatability-related concerns [6]. A key feature of a successful SNEDDS formulation is its capability to hold the drug in solution, throughout the GIT, for sufficient time to allow for absorption [11]. Many PWSDs have high solubility in SNEDDS formulations but could make a risk of precipitation after aqueous dispersion of the formulation or during its digestion in the intestine [12].

The current chapter will provide all the information to probe factors which influence the selection of successful lipid nanoformulations and affect the fate of the PWSDs after oral administration. The investigational research presented in this chapter will also provide additional information regarding current practice of lipid formulations with a particular emphasis on self-nanoemulsifying drug delivery systems (SNEDDS), the trends and perspectives and the fate of PWSDs formulated in SNEDDS.

2. Lipid nanoformulations: design approach

Lipid excipients are comprised of a large group of physically and chemically diverse glycerides, which may be used in simple (single oil solutions of the drug substance) or in more complex nanocarriers (SMEDDS/SNEDDS, drug dissolved in the mixture of glyceride, surfactant and or cosolvent), with considerable flexibility in formulation design [12].

Simple oil formulations are generally composed of mono-, di-, or triglycerides or their derivatives and differ on the content of medium- (C_6 - C_{10} in chain length) or long-chain (C_{12} - C_{24} in chain length) fatty acids. Glyceride esters are water immiscible, and their solvent capacities for drug substances vary according to the fatty acid chain length. Many lipid excipients (oils, surfactants), which are regarded as acceptable food grade materials, expected to be well tolerated by the body [13], even as parenteral emulsion dosage form [14]. These excipients have a history of use in a wide variety of pharmaceuticals.

In simple terms, lipid nanoformulations can be distinguished according to their dispersion and digestion in the aqueous content of the gut [15, 16]. Emulsion droplet size has been considered to be an important part in the performance of self-nanoemulsifying systems since particle size can determine the rate and extent of drug release *in vitro* [17]. However, the relative digestion rate would be expected to vary if the formulation is modified, and the critical factor is the fate of the drug after digestion of the formulation, in particular whether or not the drug remains in a solubilized state.

Function	Composition and description	Commercial name	Supplier
Oil	Medium-chain triglycerides (C₈-C₁₀): Fractionated coconut oil and palm seed oil, triglycerides of caprylic/capric acid	Miglyol 812, 810, Capmul MCM, Captex 355, etc.	Gattefosse corporation, France; Abitec Corp., Janesville, USA;
	Long-chain triglycerides (C₁₄-C₂₂): Vegetable oils are glyceride esters of mixed unsaturated long-chain fatty acids, commonly known as long-chain triglycerides	Soybean oil, sesame oil, corn oil, olive oil, peanut oil, cottonseed oil, rapeseed oil, etc.	Sasol GmbH, Witten, Germany; Nikko Chemicals Co., Tokyo, Japan;
	Mixed mono-, di- and triglycerides: Novel semisynthetic medium- and long-chain derivatives. Esters of propylene glycol and mixture of mono- and diglycerides of caprylic/capric acid	Imwitor 988, Imwitor 308, Maisene 35-1, etc.	Cremer Oleo GmbH & Co. KG , Hamburg, Germany; Lipoid, Germany; BASF Co., Germany
	Polar oil: Some excipients which are traditionally thought of as hydrophobic surfactants, such as sorbitan fatty acid esters	Span 80, 85, etc.	
Nonionic surfactant	Water insoluble: Oleate esters, such as polyoxyethylene (20) sorbitan trioleate, PEG-6-sorbitan oleate and polyoxyethylene (25) glyceryl trioleate are commonly used in the pharmaceutical industries	Polysorbate 85 (Tween 85), TO-106 V, Tagat TO, etc.	
	Water soluble: The popular castor oil derivatives with saturated alkyl chains resulting from hydrogenation of materials derived from a vegetable oil. Other derivatives include polysorbate 80 which are predominantly ether ethoxylates and phospholipids	Cremophor RH40, Cremophor EL, HCO30, Tween 20, 80, poloxamer 407, various Labrasols, Labrafac Labrafils, Gelucires, Soy phosphatidylcholine, etc.	
Cosolvent	The most popular water soluble cosolvents are propylene glycol, polyethylene glycol, ethanol and glycerol. Others are diethylene glycol monoethyl ether, propylene carbonate, tetrahydrofurfuryl alcohol, polyethylene glycol ether	PG, PEG 300, PEG 400, 600, transcutol, glycofurol, etc.	
Other excipient	Many oil-soluble antioxidants	α -Tocopherol, β -carotene, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, ascorbyl palmitate, etc.	

Table 1. Common excipients for designing self-nanoemulsifying formulations and the list of their suppliers.

2.1. Excipients used to design lipid-based nanoformulations

Excipients play a key role in designing successful nanoformulations with a sound control strategy and influence business-critical and clinically significant drug product performance outcomes such as stability, bioavailability and manufacturability. The design of lipid-based nanoformulations, particularly SNEDDS, is comparatively simple as the drug need to be incorporated into a suitable oil-surfactant mixture, which could be filled in a soft or hard gelatin capsules. Various choices of lipid excipients are available in the market. Numerous lipids are amphiphilic in nature, which contain both hydrophilic and lipophilic portions (fatty acid) [18]. The morphology of the lipids should be assessed as melting point increases when the length of fatty acid chain increases, but it decreases when unsaturation of the fatty acid increases [19]. Choice of excipients for successfully designed lipid-based nanocarriers is determined based on factors, such as miscibility; solvent capacity; self-dispersibility; digestibility; irritancy; toxicity; purity; chemical stability; compatibility with capsule; melting point; and cost. Since these excipients can affect the drugs bioavailability, it is necessary to identify the characteristics of these excipients. Details of the lipids (oils, nonionic surfactants, cosolvents), their compositions and list of suppliers are given in **Table 1**.

3. Lipid nanocarriers and recent advancements in oral drug delivery

Lipid-based nanoformulations as drug delivery vehicles signify a promising strategy that incorporates or encapsulates the drug molecules and are biodegradable or biocompatible. They are containing nanosized droplets typically ranging from 0 to 250 nm [20]. The entrapped drug molecules can be taken intact and protected against degradation by gastrointestinal (GI) fluids, while drug absorption through the GI epithelium or lymphatic transport can be enhanced. Possible mechanisms of transport of these nanocarriers across GI mucosa are introduced later in the chapter. These focus on effects of size and surface properties of the nanocarriers on the nonspecific or targeted uptake by enterocytes and/or M cells. Applications of various oral nanocarrier formulations, such as lipid nanoparticles and SMEDDS/SNEDDS, are reviewed in several recent publications [4, 21, 22]. **Figure 1** shows an encapsulated SNEDDS designed for oral administration, which are the most efficient formulations for improving the apparent aqueous solubility of PWSDs.

Within the scope of the current chapter, the most advanced SNEDDS and/or SMEDDS systems have been explored as potential nanocarriers, which are much more stable kinetically and thermodynamically and showed great potential for improving the bioavailability of orally administered drugs. In a pure drug nanoparticle formulation, submicron size particles of drugs are stabilized in aqueous medium with generally regarded as safe (GRAS) listed excipients blend. Such formulation can be used for drugs with poor solubility in water and oil, high melting point, high log *P* and high dose.

When saquinavir (HIV protease inhibitor) in 1995 was marketed for the first time as mesylate salt formulation in a hard gelatin capsule (Invirase®), its bioavailability was only 4% and highly variable [23]. Later on, after 2 years, a self-nanoemulsifying formulation of saquinavir (Forto-

vase®) containing medium-chain mono- and diglycerides, povidone and α -tocopherol was able to increase bioavailability threefold higher than Invirase® in humans [23, 24]. Several other published [10, 25, 26] and unpublished case studies are also available that established the significance of rational approach in designing SMEDDS/SNEDDS which can improve the *in vivo* absorption of the PWSDs. The commercial product such as amprenavir (agenerase), ciprofloxacin (cipro), fenofibrate (fenogal), liponavir/ritonavir (kaletra, norvir), etc., have been formulated using suitable SMEDDS/SNEDDS [24, 27].

Figure 1. The encapsulated SNEDDS designed for oral administration of PWSDs. *Adapted with permission from Ref. [21].

4. Concept of nanoemulsions within lipid-based formulation

The potentiality of nanoemulsions within lipid-based drug delivery systems was explored almost four decades ago. In simple term, nanoemulsions are the emulsions comprising nanosized droplets and they are well dispersed, transparent and kinetically stable for several months. Their physical stability can be improved by careful selection of surfactants and the ratio of oil/water/surfactant and also the efficiency of equipment used to reduce droplet sizes.

Not only for oral delivery, nanoemulsions are used as greater transmucosal and transdermal drug delivery vehicles due to their remarkable wetting, spreading and penetration abilities.

4.1. SMEDDS vs SNEDDS

SMEDDS and SNEDDS are almost similar lipid dosage form which can be prepared from same materials comprising a simple mixture of oils, surfactants and possibly cosolvents. SMEDDS have the ability to form fine oil in water (O/W) microemulsion, and SNEDDS produce nanoemulsion upon mild agitation in the presence of an aqueous (preferably intestinal) media [28]. The structure provides both SMEDDS and SNEDDS as good candidates for oral delivery of PWSDs with adequate solubility in oil only or oil/surfactant blends and establishes the desired reproducible pharmacokinetic profile. Upon dilution, SMEDDS form transparent microemulsions, with a droplet size of <50 nm [11], while SNEDDS produce transparent dispersions of oil and water stabilized by surfactants, with droplet sizes between 20 and 250 nm and kinetically but not thermodynamically stable systems [29]. These two systems are the most famous colloidal dispersions within lipid-based systems but physicochemically different. Structures and properties of nanoemulsion can be changed on long-term storage but not for microemulsions at same temperature, pressure and composition. The formation of SMEDDS is spontaneous, and SNEDDS need high-energy methods for their fabrication, but both systems need some external energy to overcome kinetic energy barriers and support mass transport. In comparison, SNEDDS need lesser surfactant-to-oil ratio than SMEDDS. The preparation of SNEDDS involve specific mixing order in which surfactant must be mixed first with oil phase, whereas SMEDDS do not need any specific mixing order for their preparation. Ternary phase diagrams are required to have a suitable selection of both systems which should be coherent with different phases involved in preparation.

An important best-known example is Sandimmune Neoral® which was introduced in 1994 became the turning point for development of SMEDDS in oral lipid-based formulations of PWSD [30]. This formulation contains Cremophor RH40 (polyoxyl hydrogenated castor oil), corn oil glycerides, propylene glycol and ethanol, which emulsifies spontaneously into a microemulsion with a particle size smaller than 100 nm. This new formulation (Sandimmune Neoral®) resulted in a twofold increase in the bioavailability compared to the earlier product Sandimmune® [31]. Recent years, SMEDDS and SNEDDS have gained lots of interest as potential drug delivery vehicles largely due to their clarity, simplicity of preparation, thermodynamic stability and their abilities to be filtered and to incorporate a wide range of drugs of varying lipophilicity.

4.1.1. SMEDDS/SNEDDS within lipid formulation classification systems

By considering several factors in mind, Pouton [20, 32] introduced a lipid formulation classification system (LFCS) into four Types (I–IV) which differentiate lipid-based formulations from one to another that is being used as a framework to categorize nanoformulations. These four Types of formulations were classified on the basis of formulation compositions, their aqueous dispersibility and the potential effects of lipid digestion and possible drug precipitation from lipids. Among the LFCS, Type III systems are the most attractive formula-

tions as they produce microemulsions/nanoemulsions (SMEDDS and SNEDDS) of lipid-surfactant mixtures with particle sizes in the range of 0–250 nm upon dispersion. The microemulsions can be used for many other drug delivery/application systems, such as topical, intra venous, trans-dermal, etc. There are several marketed products available which were developed as Type III formulations since the drugs may be absorbed from the microemulsions and or nanoemulsions without the digestion of lipids and/or surfactants present. Type III systems further divided into subtype IIIA and IIIB according to the hydrophilic content of the SMEDDS and SNEDDS. Type IV systems are efficient formulations as they also produce SMEDDS and/or SNEDDS and have high drug loading ability but may loss solvent capacity upon dilution with aqueous media.

4.2. Solidification of SMEDDS/SNEDDS

The excipients commonly used in designing SNEDDS are liquid at room temperature, and their compatibility with semi-solid and solid dosage forms allows encapsulating into soft/hard gelatin capsules for oral delivery. This could be a great challenge as the interaction between liquid formulation and capsule shell may result in either brittleness or softness of the shell [33]. In addition, the stability of liquid formulations could be another major issue (e.g., leaching and rancidity) since some drugs might suffer significant chemical instability in both aqueous and oily formulations. Apart from that, manufacturing liquid-filled soft gelatin capsules is a slow process and requires specialized equipment, having risk of formulation components migrating into capsule shell [23].

Therefore, to address this limitation, incorporation of liquid lipid formulations into a solid dosage form is convincing and desirable. Liquid lipid formulations could be transformed into acceptable free flowing fine powder by loading the formulation on a suitable solid carrier as solid SNEDDS [34, 35]. Only few studies have attempted to investigate the conversion of such formulations into free flowing powders by adsorption using various inorganic high surface area materials (i.e., neusilin, syloid, aeroperal and aerosol) that are amenable to encapsulation or tableting [36, 37]. On the other hand, the final powder preparation should have acceptable flow properties to achieve the best content uniformity and weight variation. The current interest in solidification technique by both the industry and academia is raised enormously due to the attractive properties including independence of gastric transit, flexibility in dose dividing, decrease in intra- and inter-subject variability, highest safety profile and physical/chemical stability improvement.

5. Equilibrium solubility of diluted nanoformulations

For lipid nanoformulations, drug solubility determines the maximum drug loading capacity (single unit dose) and is increased when the drug is highly lipid soluble or when the formulation contains high proportions of surfactant or cosolvent. The solubilization capacity of the nanoformulations (SNEDDS) is likely to decrease when excipients are dispersed and digested

in the GI tract. As a result, the drug concentrations in the GI fluids are elevated from the equilibrium solubility and could cause extreme precipitation.

To predict the likely fate of the drug on dispersion effectively, one should investigate its solubility in the formulations during aqueous dilution. The solubility of PWSDs within the diluted nanoformulations can be determined using a shake-flask method to observe how the drug solubility is changed as water is incorporated into the system. The samples are prepared by adding an excess amount of drug to the formulation, which is then shaken and thoroughly mixed with a vortex mixer. The samples are incubated in a dry heat incubator at 37°C for 7 days and centrifuged to separate excess solid drug from the dissolved drug. An aliquot of the supernatant is weighed and diluted in an appropriate solvent. The dissolved drug concentration can be analyzed by UV-vis spectrophotometer.

Figure 2. Effect of aqueous dilution on solubility of fenofibrate in nanoformulations representing LFCS Types IIIB and IV systems. Data are presented as mean $\pm$ SD ($n = 3$).

Figure 2 shows the fenofibrate solubility in nanoformulations (SMEDDS/SNEDDS) of LFCS Type IIIB and IV systems which was studied over 10–100 dilution with water. The results suggest how fenofibrate solubility decreased markedly, with several Type IIIB and IV nanoformulations, as the formulation was diluted with water [12]. After adding only 10% w/w water to the anhydrous formulation (drug dissolved at 80% of its equilibrium solubility), the one-third drug solubility had dropped down from the initial solubility of the formulation. The data predict that if fenofibrate was dissolved at its equilibrium solubility in the anhydrous formulations, its solubility would be exceeded in all cases when the formulation is diluted 1 in 10 or 1 in 100.

6. Drug release and the justification of dispersion test for nanoformulations

In vitro release studies assess the ability of lipid-based nanoformulations to disperse into various types of media and to evaluate whether the drug partitions from the vehicle into the aqueous medium. It can estimate how much drug will be in solution before absorption thus predicts the fate of the drug *in vivo*. A range of biorelevant dissolution test media and experimental methodologies has been developed by Dressman's group that have established application in drug release studies from lipid-based oral formulations [38, 39].

Technically, it is difficult to characterize drug release from emulsions *in vitro*, particularly under sink condition. Since solubility of the drug in sink phase may be poor, large volumes of aqueous content may be needed to maintain the sink conditions. It is hard to separate the oil droplets due to their smaller size from the dissolved or released drug in the sink solution levy. In a previous study, our group has conducted an *in vitro* dissolution of anti-histaminic drug, cinnarizine (CN, week base) from various SNEDDS systems and commercial product Stuger-on[®] tablet [4]. Dissolution was carried out in simulated gastric fluid (SGF, pH 1.2) for first 2 h and subsequently shifted into simulated intestinal fluid (SIF, pH 6.8) for another 2 h.

Figure 3. Dissolution profiles of cinnarizine SNEDDS 1 [MCT/MCDM/T85 (25/25/50)], SNEDDS 2 [MCT/MCM/T85 (25/25/50)] and Stuger-on[®] tablets. Data are expressed as mean $\pm$ S.E, $n = 3$. **Abbreviations: MCT—medium-chain triglycerides (M810); MCDM—mixture of medium-chain di- and monoglycerides (I988); MCM—medium-chain monoglycerides (I308); T85—Tween 85.

In SGF, all the SNEDDS showed superior dissolution profiles with respect to Stuger-on[®] tablet (**Figure 3**). At 15 min, Stuger-on[®] tablet managed to release only 66.5% drug in solution where the optimal formulations were able to release 84–95% drug in solution. This indicates the ability of these formulations to provide more efficient and rapid release of CN with respect to the marketed tablet. Upon shifting from SGF to SIF, Stuger-on[®] showed significant precipitation (87–92% precipitated), while the SNEDDS were able to hold high amount of CN (78–93%) in solution (**Figure 3**). This finding suggests the immense need for developing a SNEDDS that could enhance the drug dissolution profile and resist the sharp pH-dependent changes particularly for week bases.

A standard USP dissolution apparatus is suitable for the establishment of a dispersion test, but emphasis should be on precipitation rather than dissolution [4]. This is why, dynamic dispersion test is highly considered for the prediction of whether precipitation is likely to occur prior to digestion. In the dispersion study, samples are removed from the dispersion vessel at various intervals for at least 24 h and analyzed to determine the likelihood of precipitation during GI transit. Dispersion testing is vital for LFCS Types III and IV formulations (produce SNEDDS), which may lose solvent capacity on dispersion due to migration of water soluble components into the bulk aqueous phase. Care is needed in the design of lipid-based nanoformulations to ensure that the precipitation of the drug is minimized.

7. Mechanism of drug supersaturation: role of SMEDDS/SNEDDS

When the lipid nanoformulations approach to the high volume of gastric fluid, it is dispersed rapidly and reduces solubilization capacity of the drug due to the high content of surfactant/water soluble cosolvent, thus potentially generates supersaturation. Even though supersaturation in the stomach is not desirable as most of the drugs are absorbed in the small intestine, it poses threat for drug precipitation before the drug enters to small intestine. Therefore, SMEDDS/SNEDDS should be designed to minimize supersaturation in the stomach or at least to maintain a period sufficient to allow gastric emptying prior to drug precipitation.

Correlations between the investigations of the equilibrium solubility of the drug in the aqueous diluted formulation (10–99% diluted) and corresponding dynamic dispersion tests could help to predict whether precipitation is likely to take place, and whether it would affect bioavailability [12]. The imbalance between high initial solubilized drug concentrations and lower equilibrium drug solubilities during lipid dispersion and digestion *in vivo* does not immediately result in precipitation but stimulates drug supersaturation. This supersaturation is more likely to occur in the formulations that contain high proportions of water soluble surfactants or cosolvent. In some cases, during the process of lipid (or surfactant) digestion where hydrolysis occurs to form more polar post digestion products also stimulates changes to colloidal structure, thus lead to changes in drug solubility and may facilitate drug precipitation.

This is why, SNEDDS must contain drugs less than equilibrium solubility (approximately 50–90% of the equilibrium solubility) to avoid any precipitation. In recent studies, precipitation inhibitors have been introduced in supersaturated SNEDDS to overcome the risk of precipitations [40]. Supersaturated SNEDDS inhibit and minimize the nucleation process and subsequent drug precipitation in GIT by achieving and then sustaining the metastable supersaturated state. The commonly used water soluble precipitation inhibitors are PVP, HPMC, NaCMC and MC polymers [41].

7.1. The risk of drug precipitation from nanoformulations

Triglycerides alone (LFCS Type I) are poor solvents for most of the hydrophobic drugs but suitable for highly lipophilic compounds. If lipid-based formulations contain mixed glycer-

ides, polar oils, surfactants and/or cosolvents (LFCS Type II and III), it is likely to improve the solvent capacity of the formulation. Therefore, formulators are always preferred to add water soluble surfactants and cosolvents against pure oils, ultimately sometimes resulting in the complete exclusion of oily excipients to produce oil-free formulations (LFCS Type IV). However, the formulator must keep well balance between oils and surfactants/cosolvents in the formulation to avoid risk of drug precipitation on aqueous dispersion. Several studies showed that small changes in formulation compositions are not expected to cause large changes in drug solubility, but there could be a dramatic drop in solvent capacity upon aqueous dilution [4, 12, 42]. Dilution of a cosolvent implies a substantial loss of solvent capacity, while the loss of solvent capacity may not be suffered with the use of surfactant. This could be possible due to the linearity between solubilized drug to the number of micelles present and therefore to the surfactant concentration. Drugs which are more soluble in surfactant or cosolvent than pure oil are at high precipitation risk because solvent capacity of surfactant and cosolvent decreases upon dilution but not pure oil. Hence, increasing the solubility of a drug by including a cosolvent is generally a poor strategy than using a nonionic surfactant [11, 43]. **Figure 4** shows the extreme precipitation of an anticancer drug, paclitaxel using LFCS Types III and IV formulations.

Figure 4. Percentage of the original dose of paclitaxel remaining in solution after 1:100 dilutions in the dispersion medium (paclitaxel was originally dissolved at 80% of the equilibrium solubility in the anhydrous mixture). One gram formulation was dispersed in 100 ml water, and then, the samples were withdrawn periodically over 24 h to examine the drug precipitations. Data are presented as mean $\pm$ SD ($n = 3$).

It is quite difficult to predict the fate of the PWSDs on dispersion of a typical LFCS Type IIIA lipid formulation. The hydrophilic surfactant used in Type IIIA systems is substantially separated from the oily components, forming a micellar solution in the continuous phase.

Hence, one might question: does this system lower the overall solvent capacity for the drug or not? However, this may depend on the $\log P$ of the drug, and to what extent the surfactant was contributing to its solubilization within the formulation. At present, there are no established techniques available to help formulators assessing the risk of precipitation. It is worth mentioning that in some cases, Type III formulations can take several days to reach equilibrium and the drug remains in a supersaturated state for up to 24 h time [12]. It could be argued that such formulations are not likely to cause precipitation in the gut before the drug is absorbed, and possibly the supersaturation acts as an absorption enhancer by increasing the thermodynamic stability of the drug [44].

8. Lipid digestion and drug absorption: mechanism

8.1. Lipid metabolism

Following ingestion of a lipid-based dosage form (capsule/tablet), the formulation is initially dispersed in the stomach where the digestion of exogenous dietary lipid is started by the action of gastric lipase on the lipid-water interface. Gastric lipase releases about 15% of free fatty acids from lipids [45]. Within the small intestine, pancreatic lipase together with its co-lipase completes the breakdown of dietary glycerides to diglyceride, monoglyceride and fatty acid. The presence of exogenous lipids in the small intestine also stimulates secretion of endogenous biliary lipids including bile salt, phospholipid and cholesterol from the gallbladder [45]. In the presence of elevated bile salts concentrations, lipid digestion products are subsequently incorporated into a series of colloidal structures including multilamellar/unilamellar vesicles, bile salt phospholipid mixed micelles and micelles [46]. Together these species significantly expand the solubilization capacity of the small intestine for both lipid digestion products and drugs, and this can be studied relatively easily as a preformulation exercise.

8.2. Drug absorption

Sufficient aqueous solubility along with good intestinal permeability is crucial for adequate drug absorption, ultimately leading to sufficient bioavailability. On the other hand, PWSDs are associated with poor and variable absorption and often affected by the various food intakes. Several studies have already documented lipid-based nanoformulations, particularly SNEDDS, as an absorption enhancer for PWSDs when administered orally [4, 47]. Possible mechanisms for improving drug absorption include: (i) an increase in the membrane fluidity facilitating transcellular absorption, (ii) larger surface area provided by the fine emulsion droplets, hydrolysis and formation of mixed micelles, (iii) paracellular transport by opening tight junction mainly for ionized drugs or hydrophilic macromolecules, (iv) inhibition of P-gp and/or CYP450 to increase intracellular concentration and residence time, and (v) stimulation of lipoprotein/chylomicron production. The natural process of digestion offers the possibility that very lipophilic drugs could be taken up into the lymphatic system by partitioning into chylomicrons in the mesentery. This is expected to be a mechanism of absorption for drugs

with log *P* values >6.0 and has been demonstrated to be crucial in a past study for the absorption of the antimalarial compound halofantrine [48, 49].

The mixed micelles substantially transport digestion products across the unstirred water layer and reach the vicinity of the aqueous-microvillus interface to allow for lipid absorption through the mucosal cells. However, it is possible that digestion of a lipid formulation could reduce the solubility of the drug in the gut lumen, which would result in the precipitation of the drug and a decrease in the absorption rate. Therefore, more investigation on *in vitro* lipolysis is needed to clearly understand drug precipitation during digestion for better absorption.

9. *In vitro* digestion (lipolysis): significance

The fate of the lipid carrier in the GI tract is essentially important for the absorption of the incorporated drug and therefore has to be closely analyzed. It is evident that the solvent capacity of the formulation can be lost on digestion, leading to drug precipitation [26, 50]. However, the investigation of the lipolysis by *in vivo* experiments is complex, costly and time-consuming. Thus, the *in vitro* model simulating the enzymatic degradation of lipid-based formulations is highly significant as an alternative method of monitoring the digestion process in the simulated gastrointestinal media under fed and fasted conditions.

Lipolysis can be carried out as an *in vitro* test using a pH-stat titration unit to maintain pH and using the lipase/co-lipase content of porcine pancreatin to serve as model for human pancreatic juice. Bile salt lecithin-mixed micelles are used in the reaction mixture to provide a sink for solubilization of degradation products. Composition of mixture that used in the *in vitro* lipolysis studies is provided in **Table 2**.

Substance of the mixture for 10 ml aqueous media		
Lipid 250 mg		
Pancreatic lipase 1 ml (800 TBU/ml)		
Lipolysis buffer 9 ml		
Composition of the lipolysis buffer	Concentration (fed state)	Concentration (fasted state)
Bile salt (BS, mM)	20	5
Phospholipid (PL, mM)	5	1.25
Trizma maleate (mM)	0.5	0.5
Ca ⁺ (mM)	0.05	0.05
Na ⁺ (mM)	1.5	1.5

Table 2. Composition of mixture for *in vitro* lipolysis experiments. *Adapted with permission from Ref. [51].

Lipolysis is allowed to proceed for a fixed time (30–60 min), the reaction is then subjected to high-speed ultracentrifugation, and further drug analysis in the various phases allows predicting whether the drug will remain solubilized in the intestinal lumen after digestion of the formulation. However, if the drug is partially precipitated, then drug will be found in the pellet, which may be still in solution. The rate and extent of lipolysis can be quantified by the data generated from the pH-stat. This technique was recently applied in LFCS Types I–IV formulations to predict the effect of formulation on the fate of a number of drug compounds and assumed that surfactants are subjected to digestion, probably for SMEDDS and SNEDDS, where water soluble surfactants are used predominantly. Lipolysis experiments may play a vital role in the near future for establishing strong methods for *in vitro in vivo* correlations (IVIVCs).

10. *In vitro in vivo* correlation (IVIVC) for lipid nanoformulations

The IVIVCs play a major role in drug development, particularly on the optimization of suitable formulations which is time-consuming and a highly expensive process. Formulation optimization requires modifications in composition, equipment, manufacturing process and batch sizes. If such changes applied to the formulation, the *in vivo* bioequivalence studies in human are necessary to be conducted to confirm the similarity of the new formulation. This process will increase the load of carrying out a number of bioequivalence studies and therefore will increase the cost of process optimization and marketing of the new formulation.

To overcome these issues, it is necessary to develop *in vitro* tests that can imitate the bioavailability data. The IVIVC can be used in the development of new pharmaceuticals to decrease the number of human trials during the formulation development and to support biowaivers.

In the beginning of 1980s, the IVIVC theory was established based on many published research studies, which can be used as a prediction tool for correlating *in vitro* and *in vivo* data. The IVIVC is usually used in the development stages of pharmaceuticals to enhance the formulation and dosage optimization with fewer trials in human [51–56] or additional bioavailability studies. The FDA defines IVIVC as “a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response (e.g., plasma drug concentration or amount of drug absorbed).” For drugs that are administered orally, dissolution and intestinal permeation are considered as the rate-limiting steps for the absorption. Therefore, if an excellent correlation exists between *in vitro* dissolution test and a bioavailability parameter, then controlling the dissolution profile will permit the evaluation of bioavailability [57–59].

There are several tools which can be used to establish IVIVC. The *in vitro* drug release studies of the formulations can be performed using dissolution, dynamic dispersion and digestion tests, whereas the *in vivo* pharmacokinetic studies can be performed on various animal models. However, there are only a limited number of IVIVC studies so far have been conducted using lipid formulations. To obtain more robust *in vitro* and *in vivo* relationship, a large number of

model compounds should be explored along with more human clinical data sets and complete characterizations of *in vitro* and *in vivo* solubilization of PWSDs formulated in lipid vehicles.

11. Conclusion

For many drugs with poor aqueous solubility, the technique of developing SMEDDS/SNEDDS provides a powerful and effective solution to improve their solubility in the aqueous contents of the GI tract that is the main obstacle for such drugs. The most critical step in designing the nanoformulations of lipid-based systems for PWSDs is the selection of the most suitable oil, surfactant and/or cosolvent for a particular drug with certain physicochemical properties. So, the formulators must keep a balance and make compatibility between the factors of different formulations such as self-emulsification efficiency, drug loading capacity, droplet size distribution, *in vitro* dispersion/release profile in acidic and basic media and *in vitro* digestion by using fed and fasted state. In summary, SMEDDS/SNEDDS provide a robust formulation approach to enhance GI solubilization and to promote drug absorption after oral administration. If there is a successful *IVIVC* made for lipid nanoformulations, confidence in the development of the pharmaceutical product and its quality are likely to improve, and the drug development time may be shortened.

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References

- [1] Lei, Y., et al., Solid self-nanoemulsifying cyclosporin A pellets prepared by fluid-bed coating: preparation, characterization and *in vitro* redispersibility. *Int. J. Nanomed.*, 2011. 6: p. 795–805.
- [2] Kommuru, T.R., et al., Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. *Int. J. Pharm.*, 2001. 212(2): p. 233–246.

- [3] Mohsin, K., A.A. Shahba, and F.K. Alanazi, Lipid based self emulsifying formulations for poorly water soluble drugs-an excellent opportunity. *Indian J. Pharm. Educ. Res.*, 2012. 46(2): p. 88–96.
- [4] Shahba, A.A., K. Mohsin, and F.K. Alanazi, Novel self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of cinnarizine: design, optimization, and *in-vitro* assessment. *AAPS PharmSciTech*, 2012. 13(3): p. 967–977.
- [5] Mueller, E.A., et al., Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. *Pharm. Res.*, 1994. 11(2): p. 301–304.
- [6] Date, A.A., et al., Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances. *Nanomedicine (Lond)*, 2010. 5(10): p. 1595–1616.
- [7] Date, A.A. and M.S. Nagarsenker, Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. *Int. J. Pharm.*, 2007. 329(1–2): p. 166–172.
- [8] Charman, S.A., et al., Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. *Pharm. Res.*, 1992. 9(1): p. 87–93.
- [9] Charman, W.N., et al., Effect of food and a monoglyceride emulsion formulation on danazol bioavailability. *J. Clin. Pharmacol.*, 1993. 33(4): p. 381–386.
- [10] Gursoy, R.N. and S. Benita, Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother.*, 2004. 58(3): p. 173–182.
- [11] Pouton, C.W., Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. *Eur. J. Pharm. Sci.*, 2006. 29(3–4): p. 278–287.
- [12] Mohsin, K., M.A. Long, and C.W. Pouton, Design of lipid-based formulations for oral administration of poorly water-soluble drugs: precipitation of drug after dispersion of formulations in aqueous solution. *J. Pharm. Sci.*, 2009. 98(10): p. 3582–3595.
- [13] Duro, R., et al., Interfacial adsorption of polymers and surfactants: implications for the properties of disperse systems of pharmaceutical interest. *Drug Dev. Ind. Pharm.*, 1999. 25(7): p. 817–829.
- [14] Floyd, A.G., Top ten considerations in the development of parenteral emulsions. *Pharm. Sci. Technol. Today*, 1999. 4(2): p. 134–143.
- [15] Pouton, C.W., Effects of the inclusion of a model drug on the performance of self emulsifying formulations. *J. Pharm. Pharmacol.*, 1984. 36: p. 51.
- [16] Pouton, C.W., Self emulsifying drug delivery systems: assessment of the efficiency of emulsification. *Int. J. Pharm.*, 1985. 27: p. 335–348.

- [17] Tarr, B.D. and S.H. Yalkowsky, Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size. *Pharm. Res.*, 1989. 6(1): p. 40–43.
- [18] Humberstone, A.J. and W.N. Charman, Lipid based vehicles for the oral delivery of poorly water soluble drugs. *Adv. Drug Deliv. Rev.*, 1997. 25(1): p. 103–128.
- [19] Jannin, V., J. Musakhanian, and D. Marchaud, Approaches for the development of solid and semi-solid lipid-based formulations. *Adv. Drug Deliv. Rev.*, 2008. 60(6): p. 734–746.
- [20] Pouton, C. and C. Porter, Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. *Adv. Drug Deliv. Rev.*, 2008. 60(6): p. 625–637.
- [21] Mohsin, K., et al., Development of self-nanoemulsifying drug delivery systems for the enhancement of solubility and oral bioavailability of fenofibrate, a poorly water-soluble drug. *Int. J. Nanomed.*, 2016. 11: p. 2829–2838.
- [22] Cerpnjak, K., et al., Lipid-based systems as a promising approach for enhancing the bioavailability of poorly water-soluble drugs. *Acta Pharm.*, 2013. 63(4): p. 427–445.
- [23] Perry, C.M. and S. Noble, Saquinavir soft-gel capsule formulation. A review of its use in patients with HIV infection. *Drugs*, 1998. 55(3): p. 461–486.
- [24] Hoffmann-La-Roche, *Fortovase (saquinavir) Soft Gelatin Capsules. Product Information*. (CAS registry number: 127779-20-8), 2003; <http://www.medicines.org.au/files/ropforto.pdf>
- [25] Martin-Facklam, M., et al., Dose-dependent increase of saquinavir bioavailability by the pharmaceutical aid cremophor EL. *Br. J. Clin. Pharmacol.*, 2002. 53(6): p. 576–581.
- [26] Porter, C.J. and W.N. Charman, *In vitro* assessment of oral lipid based formulations. *Adv. Drug Deliv. Rev.*, 2001. 50(Suppl 1): p. S127–S147.
- [27] Talegaonkar, S., et al., Microemulsions: a novel approach to enhanced drug delivery. *Recent Pat. Drug Deliv. Formul.*, 2008. 2(3): p. 238–257.
- [28] Singh, B., et al., Self-emulsifying drug delivery systems (SEDDS): formulation development, characterization, and applications. *Crit. Rev. Ther. Drug Carrier Syst.*, 2009. 26(5): p. 427–521.
- [29] Cherniakov, I., A.J. Domb, and A. Hoffman, Self-nano-emulsifying drug delivery systems: an update of the biopharmaceutical aspects. *Expert Opin. Drug Deliv.*, 2015. 12(7): p. 1121–1133.
- [30] Klyashchitsky, B.A. and A.J. Owen, Drug delivery systems for cyclosporine: achievements and complications. *J. Drug Target*, 1998. 5(6): p. 443–458.
- [31] Mueller, E.A., et al., Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation. *Pharm. Res.*, 1994. 11(1): p. 151–155.

- [32] Pouton, C.W., Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur. J. Pharm. Sci.*, 2000. 11(Suppl 2): p. S93–S98.
- [33] Nazzal, S. and M. Khan, Controlled release of a self-emulsifying formulation from a tablet dosage form: stability assessment and optimization of some processing parameters. *Int. J. Pharm.*, 2006. 315(1–2): p. 110–121.
- [34] Tang, B., G. Cheng, J-C. Gu, and C-H. Xu, Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. *Drug Discov. Today*, 2008. 13: p. 606–612.
- [35] Katteboina, S., P. Chandrasekhar, and S. Balaji, Approaches for the development of solid self-emulsifying drug delivery systems and dosage forms. *Asian J. Pharm. Sci.*, 2009. 4: p. 240–253.
- [36] Agarwal, V., A. Siddiqui, H. Ali, and S. Nazzal, Dissolution and powder flow characterization of solid self-emulsified drug delivery system (SEDDS). *Int. J. Pharm.*, 2009. 366: p. 44–52.
- [37] Shazly, G. and K. Mohsin, Dissolution improvement of solid self-emulsifying drug delivery systems of fenofibrate using an inorganic high surface adsorption material. *Acta Pharm.*, 2015. 65(1): p. 29–42.
- [38] Dressman, J.B. and Klein, S., eds. Development of dissolution tests on the basis of gastrointestinal physiology. *Pharmaceutical Dissolution Testing*, eds. Dressman, J., and Krämer, J., 2005, Taylor and Francis: NY, USA. pp. 193–227.
- [39] Dressman, J.B., Alsenz, J., Schamp, K., and Beltz, K., eds. Characterizing release from lipid-based formulations. *Oral Lipid-based Formulations: Enhancing the Bioavailability of Poorly Water-soluble Drugs*, eds. Hauss, DJ., 2007, Informa Healthcare, Inc.: NY, USA. pp. 242–245.
- [40] Thomas, N., K. Richter, T.B. Pedersen, R. Holm, A. Müllertz, and T. Rades, *In vitro* lipolysis data does not adequately predict the *in vivo* performance of lipid-based drug delivery systems containing fenofibrate. *AAPS J.*, 2014. 16(3): p. 539–549.
- [41] Gao, P., et al., Enhanced oral bioavailability of a poorly water soluble drug PNU-91325 by supersaturatable formulations. *Drug Dev. Ind. Pharm.*, 2004. 30(2): p. 221–229.
- [42] Mohsin, K. and F. Alanazi, The fate of paclitaxel during *in vitro* dispersion testing of different lipid-based formulations. *J. Drug Deliv. Sci. Technol.*, 2012. 22(2): p. 197–204.
- [43] Devraj, R., et al., *In vitro* assessment of drug-free and fenofibrate-containing lipid formulations using dispersion and digestion testing gives detailed insights into the likely fate of formulations in the intestine. *Eur. J. Pharm. Sci.*, 2013. 49(4): p. 748–760.
- [44] Gao, P., B.D. Rush, W.P. Pfund, T. Huang, J.M. Bauer, W. Morozowich, M.S. Kuo, and M.J. Hageman, Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved oral bioavailability. *J. Pharm. Sci.*, 2003. 92: p. 2386–2398.

- [45] Porter, C.J., N.L. Trevaskis, and W.N. Charman, Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov.*, 2007. 6(3): p. 231–248.
- [46] Kossena, G.A., et al., Influence of the intermediate digestion phases of common formulation lipids on the absorption of a poorly water-soluble drug. *J. Pharm. Sci.*, 2005. 94(3): p. 481–492.
- [47] Stegemann, S., et al., Self-emulsifying drug delivery systems facing the bioavailability challenge in drug delivery. *Pharm. Ind.*, 2009. 71(8): p. 1409–1416.
- [48] Humberstone, A.J., C.J. Porter, and W.N. Charman, A physicochemical basis for the effect of food on the absolute oral bioavailability of halofantrine. *J. Pharm. Sci.*, 1996. 85(5): p. 525–529.
- [49] Boyd, M., et al., A stepwise surgical procedure to investigate the lymphatic transport of lipid-based oral drug formulations: cannulation of the mesenteric and thoracic lymph ducts within the rat. *J. Pharmacol. Toxicol Methods*, 2004. 49(2): p. 115–120.
- [50] Pouton, C.W. and C.J. Porter, Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. *Adv. Drug Deliv. Rev.*, 2008. 60(6): p. 625–637.
- [51] Uppoor, V.R., Regulatory perspectives on *in vitro* (dissolution)/*in vivo* (bioavailability) correlations. *J. Control. Release*, 2001. 72(1–3): p. 127–132.
- [52] Ghosh, P., M. Milewski, and K. Paudel, *In vitro/in vivo* correlations in transdermal product development. *Ther. Deliv.*, 2015. 6(9): p. 1117–1124.
- [53] Anand, O., et al., Dissolution testing for generic drugs: an FDA perspective. *AAPS J.*, 2011. 13(3): p. 328–335.
- [54] FDA, Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Food and Drug Administration, Rockville, 1997.
- [55] Committee, I.C.o.H.S. Pharmaceutical Development Q8; Draft Consensus Guideline, Released for Consultation at Step 2 of the ICH Process; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use in International Conference on Harmonisation. 2004.
- [56] European Agency for the Evaluation of Medicinal Products, H.M.E.U., Committee for Proprietary Medicinal Products, CPMP/QWP/155/96, *Note for Guidance on Development Pharmaceuticals*, H.M.E.U. European Agency for the Evaluation of Medicinal Products, Committee for Proprietary Medicinal Products, CPMP/QWP/155/96, Editor 1998: London, UK.
- [57] Hwang, S.S., W. Bayne, and F. Theeuwes, *In vivo* evaluation of controlled-release products. *J. Pharm. Sci.*, 1993. 82(11): p. 1145–1150.

- [58] Abd-Elbary, A., M.I. Tadros, and A.A. Alaa-Eldin, Development and *in vitro/in vivo* evaluation of etodolac controlled porosity osmotic pump tablets. AAPS PharmSciTech, 2011. 12(2): p. 485–495.
- [59] Benet, L.Z., Pharmacokinetics of sustained-release etodolac. Rheumatol. Int., 1993. 13(Suppl 2): p. S3–S5.

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