Mechanistic Studies
and
Modeling
of
Self-Emulsifying Drug Delivery Systems
for the
Oral Delivery
of Hydrophobic Compounds

A Dissertation Presented

By

Fulden Buyukozturk

to

The Department of Chemical Engineering

In partial fulfillment of the requirements
For the degree of

Doctor of Philosophy

In the field of

Chemical Engineering

Northeastern University
Boston, Massachusetts

August 22nd, 2012
ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my advisor Dr. Rebecca Carrier for giving me the opportunity to conduct this research, for her efforts in creating a happy working environment, for her friendship, guidance, support and for giving me the freedom to grow as an independent researcher.

I would like to acknowledge my committee members Dr. Shashi Murthy, Dr. Mansoor Amiji, Dr. David Budil and Dr. David Julian McClements for finding the time out of their busy schedule to provide valuable insight for my research. I would like to acknowledge collaborators on this project; Dr. James Benneyan for very useful insight and direction on statistical design and statistical modeling, Dr. David Budil for sharing his expertise on EPR, Dr. Sow-Hsin Chen for making SANS analysis possible, Dr. David Cohen for letting use his laboratory space and for giving me the opportunity to learn HPTLC, and Dr. Robert Campbell and Dr. Heather Clark for letting use their dynamic light scattering equipment. I would like to acknowledge Rob Eagan for all the technical support he offered.

I would like to thank Lin Wang for not only being an amazing senior labmate but for also being an amazing friend. Thank you for being the cheerful person you are, you made 132 Mugar a perfect place to work. I would like to acknowledge Selena Di Maio, with whom I had the chance to work towards similar research goals, for useful discussions and collaboration. I would like to acknowledge all my labmates; Brian McMahon, Courtney Pfluger, Mukul Ashtikar, Andrew Michealson, Hasan Yildiz, Sean Kevlehan, and Ece Gamsiz for contributing to the pleasant working environment and friendship over the years. I would like to acknowledge Bing Sun for much needed
distractions from work and for her company. I would like to acknowledge Yasin Celik for challenging me with his curious questions about this project, for providing graphic design help, for being the visionary person he is and for endless support over the years. I would like to acknowledge my family for being such an academic inspiration in my life, especially my uncle Oral Buyukozturk and my aunt Hulya Buyukozturk for giving me endless guidance, support and love throughout my graduate school endeavor.

Lastly, I would like to acknowledge Department of Chemical Engineering and Northeastern University for providing the environment to advance as a researcher.
ABSTRACT

The oral route for drug delivery is not possible for approximately 50% of currently marketed drug compounds due to low solubility in water. Lipid based drug delivery systems, and in particular self-emulsifying drug delivery systems (SEDDS), show great potential for enhancing oral bioavailability, as well as offering the advantage of minimal processing and inherent stability, but have not been broadly applied, largely due to lack of general formulation guidance and lack of knowledge of how these systems function to enhance bioavailability. It is hypothesized that a systems-based model incorporating key processes involved in oral absorption will enable prediction of the fate of compounds co-administered with self-emulsifying drug delivery systems.

In this investigation, in order to understand how formulation design influences physicochemical emulsion properties and associated function in the gastrointestinal environment, a broad range of SEDDS formulations was studied and used to develop a quantitative predictive model. Twenty-seven representative formulations were designed using $3^3$ factorial design. Key functions of emulsion-based drug delivery systems, permeability enhancement, drug release, digestion kinetics, and lymphatic transport were studied separately in vitro and statistically related to three formulation properties — oil structure, surfactant hydrophilic lipophilic balance (HLB) values, and surfactant-to-oil ratio. Three surfactants with HLB values ranging from 10 to 15 and three structurally different oils (long chain triglyceride, medium chain triglyceride, and propylene glycol dicaprylate/ dicaprate) were combined at three different weight ratios (1:1, 5:1, 9:1). Strong influences of certain formulation parameters and interactions on emulsion function were observed. It was shown that regression modeling could be used to estimate
key parameters reflective of performance of specific formulations such as digestion kinetics with high degrees of predictability ($R^2=0.897$).

A system-based model was constructed for the first time to enable quantitative prediction of overall absorption enhancement achievable with SEDDS based on drug and formulation properties. The model includes gastrointestinal mass transport processes of a drug orally administered with SEDDS using process kinetic constants and differential equations obtained from mechanistic studies. The following kinetic processes were considered building the model; formulation digestion, drug release from formulation and drug absorption. Simulation results for simultaneous formulation digestion and drug release were compared with combined in vitro digestion and release Electron Paramagnetic Resonance (EPR) experimental results. Close comparison between simulation results and experimental findings indicated the validity of assumptions made.

The model was combined with a one compartment pharmacokinetic model to predict absorbed drug plasma concentration profiles. There was an inverse correlation between the rate of digestion and the amount of drug absorbed whereas surprisingly, there was no effect of variations in drug release constant on the amount of drug absorbed. Simulation results were further used to compare predictions of the extent of drug absorption in cases upon oral dosing in solid form versus within SEDDS, to assess enhancement (or not) in oral bioavailability due to SEDDS. Results demonstrate a strong predicted dependence of improvement in oral absorption on initial formulation loading; improvement in oral absorption was observed with SEDDS with 400 mg load but not a 200 mg drug load. The model presented here would be of direct benefit to formulation scientists trying to achieve oral formulations of low solubility compounds with acceptable bioavailability.
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1.0 INTRODUCTION

The oral route is the most preferred method of administration of drugs. Unfortunately, this route is not possible for 50% of currently marketed drug compounds due to their low solubility in water and therefore low oral bioavailability. Lipid-based drug delivery systems are commonly studied for the enhancement of oral bioavailability of hydrophobic drugs. Recent approaches have included the administration of drug components with lipid vehicles such as oils, liposomes and self-emulsifying formulations [1, 2]. However, these systems are not being commonly utilized commercially, partially due to the lack of guidance for formulation and lack of knowledge of how these systems function to enhance bioavailability. Self-emulsifying drug delivery systems (SEDDS), in particular, have shown great promise for enhancing oral bioavailability of low-solubility compounds as well as offering the advantage of minimal processing and inherent stability.

SEDDS spontaneously emulsify in the gently mixed aqueous gastrointestinal (GI) environment [1] and typically have droplet sizes ranging from a few nanometers to hundreds of nanometers and consist minimally of oil, surfactant, and the drug to be delivered. By example, the bioavailability of Tipranavir (TPV), a nonpeptidic protease inhibitor anti-HIV drug, was doubled when dosed to rats in a SEDDS formulation versus delivered as solid powder in a hard filled capsule [3]. Kommuru et al [4] developed a self-emulsifying drug delivery system for Coenzyme Q10 using polyglycolyzed glycerides as emulsifiers with a resultant 150% increase in bioavailability. Numerous other examples exist in the literature demonstrating enhanced bioavailability with self-emulsifying drug delivery systems [5-9], but most focus on a single formulation or small
set of formulations with little explanation of how this formulation was developed. Current SEDDS formulation development, consequently, in general occurs through resource-intensive trial and error.

Some mechanistic studies have been conducted exploring a range of SEDDS formulations and a specific aspect of their function, most commonly ability to emulsify [7, 9]. Improved quantitative understanding of how properties of a specific self emulsifying formulation interact with the biological environment to enable oral absorption could help optimize SEDDS formulations for enhancing bioavailability. Although a significant amount of literature exists from other fields concerning the function of emulsions [10-13], pharmaceutical formulations have not been broadly studied with respect to fundamental aspects of emulsion function, with few studies statistically developing predictive models that relate emulsion properties with formulation parameters [14-17]. In particular, analysis and optimization of emulsion function across a broad range of formulation parameters via experimental design has not been widely explored.

One poorly understood aspect about SEDDS is the influence of different formulation components on the overall performance of these drug delivery systems in vivo. Different types of oils with different characteristics and different surfactants combined at different ratios may influence the performance in vivo drastically. There is no guidance currently available for formulating a drug with specific properties with self emulsifying drug delivery formulations. It is therefore necessary to investigate the influence of formulation components with a quantitative and statistically designed and analyzed manner.
Another knowledge in which there is a lack of understanding is the mechanism of how self-emulsifying drug delivery systems work to enhance the overall bioavailability of a hydrophobic drug orally. Suggested mechanisms responsible of functioning of SEDDS in the GI tract environment includes the increased drug solubilization in the aqueous lumen phase due to alterations in the composition and character of colloidal environment in the GI tract fluid and increased drug absorption due to enhanced permeability permeability (e.g. widening of tight junctions, changes to cellular processing) and lymphatic transport [18]. Important mechanisms that would influence drug solubilization in the lumen is the rate and extent of digestion of lipidic formulation components. Another one is the rate at which the drug is released from oil droplets especially during the process of “degradation” of the emulsified drug carriers by the digestive enzymes in vivo. It is essential to investigate the rate of digestion of self-emulsifying formulation lipids, rate of drug release, as well as the rate and extent of drug transport across intestinal monolayer incorporated with SEDDS in order to understand and predict formulation functioning in GI tract. Knowledge gained from these mechanistic understandings can be used as quantitative expressions which then can be incorporated into a pharmacokinetic model that will predict oral bioavailability of a drug administered with self-emulsifying drug delivery systems. To define and understand challenges involved with oral delivery of hydrophobic drug compounds it is necessary to present an overview of current technologies.

1.1 **Oral delivery of hydrophobic compounds**

40% percent of prospective drugs coming out of discovery pipelines are lipophilic compounds, which leads to difficulty in achieving acceptable bioavailability with oral
formulations [5]. There are several factors responsible for low oral absorption of hydrophobic drugs and one very particular one is poor absorption due to slow and/or incomplete drug dissolution and precipitation in the gastro-intestinal lumen or other aqueous media [19]. Therefore in order to be delivered orally and to achieve acceptable bioavailability, lipophilic drugs require a co-administered drug delivery system.

1.2 Oral drug delivery systems for hydrophobic drugs

Compounds that have low aqueous solubility are class II (low solubility, extensive metabolism) and class IV (low solubility, poor metabolism) drugs classified by BDDCS. These drugs get eliminated by the biological environment either as metabolites or unchanged forms [20]. Figure 1 shows the schematic representation of critical steps in oral drug absorption.

![Schematic of critical steps in drug absorption](image-url)

**Figure 1: Schematic of critical steps in drug absorption**
In order to enable oral bioavailability of a water insoluble drug, one common approach is the use of a carrier that can enhance the amount and the time of dispersed drug in the gastrointestinal fluid. In order to increase the amount of drug administered orally, excipients that solubilize high amounts of hydrophobic drug are being used. By selecting the optimum liquid vehicle composition, it is possible to eliminate or at least minimize drug precipitation [19]. Drug delivery systems that alter the drug solubilization in the biological environment will be discussed in the following sections.

1.2.1 Overview of technologies

Excipients that are being used for solubilizing drugs as oral dosage forms include pH modifiers, water-soluble organic solvents, surfactants, water insoluble organic solvents, medium chain triglycerides, long chain triglycerides, cyclodextrins and phospholipids. The majority of the commercially available oral formulations are solid dosage forms either as tablets or capsules. But also there are solubilized oral formulations as syrups, or solutions filled into soft or hard capsules[21].

Water insoluble drugs can be dissolved in water soluble organic or organic and aqueous mixture solvents. Commercially available water soluble organic solvents are PEG 400, ethanol, propylene glycol, glycerin, and non-ionic surfactants. Ritonavir, an HIV protease inhibitor, has an inherent solubility of 1 µg/ml and it also has two weak basic groups with low pKa’s of 1.8 and 2.6 which limits the use of a pH modifier solvent. A co-solvent mixture of 42% ethanol, water, glycerin, surfactant Cremophor RH 40, and peppermint oil was previously used to solubilize 20 mg/ml Ritonavir in oral dosage forms. Another example is Sirolimus, a non-ionizable and water-insoluble immunosuppressant which was solubilized using the surfactant polysorbate 80 (Tween
80), and a proprietary solution Phosal 50 PG, that is composed of phosphatidylcholine, propylene glycol, mono- and diglycerides, 1.5-2.5 % ethanol, soy fatty acids, and ascorbyl palmitate. The resultant drug’s oral bioavailability was 14% [22, 23].

Another approach to formulate lipophilic drugs is the use of water insoluble solvents for the solubility enhancement. Such solvents include; long-chain triglycerides, peanut oil, hydrogenated vegetable oils, hydrogenated soybean oil, beeswax, vitamin E, oleic acid and the medium-chain triglycerides derived from coconut oil and palm seed. Drugs solubilized into these solvents are usually encapsulated in gelatin capsules [21]. Liposomes for instance, are amphilic phospholipid molecules that are arranged in a closed spherical bilayer which are utilized to increase the bioavailability of lipophilic compounds.

A drug with a basic functional group can be solubilized in acidic solutions with pKₐs lower than the drug’s and pH can be controlled adjusting the salt forms used, hydrochloric acid, tartaric acid, benzoic acid, or citric acid. An example of the pH modified drugs is Loratadine, a drug for allergic treatments. The drug is soluble in citric acid, water, glycerin and propylene glycol mixture at 1 mg/ml whereas it is not soluble in neutral pH water [21].

Another approach to increase oral bioavailability of water insoluble drugs is to use cyclodextrin complexation. Cyclodextrins are cyclic (α-1,4) linked oligosaccharides of a α-D-glucopyranose containing a hydrophilic outer surface and a relatively hydrophobic central cavity [24]. In the past years, there have been a tremendous amount of research done on cyclodextrins and currently there are more than 21 commercial formulations available. Most commonly used solid form of cyclodextrins is β-
cyclodextrin. Even though its solubility is limited to 18 mg/ml in water it is possible to alter this value with covalent modifications which will also result with a biologically safer formulation.

Surfactants are also utilized to solubilize hydrophilic drug compounds. However due to biological toxicity issues they have been avoided most of the time. Surfactants are structures that have both a hydrophobic head and a hydrophilic tail. These structures self-assemble to form micellar structures at above critical micellar concentration that varies with the type of the surfactant. Thus, drugs can be solubilized in monomers, in micellar structures, or in both. Micelles, once formed, are known to have a capacity of capturing higher amounts of compounds compared to monomers. A significant bioavailability enhancement is enabled by 20% d-α-tocopherol polyethylene glycol 1000 succinate (TPGS) containing formulation of drug Amprenavir. The bioavailability of Amprenavir in conventional capsule or tablet formulations is nearly zero. However when formulated with TPGS, the bioavailability was increased up to 70% in beagle dogs. The reason to this significant increase in bioavailability was showed to be due to the TPGS potent inhibitor structure of an active flux which was tested across Caco-2 cell monolayers [25]. This effect indicates that surfactants not only enhance bioavailability by solubilizing high amounts of drug but also play an important role in efflux inhibition and overall permeability enhancement. Surfactants can also be utilized as components of self-emulsifying drug delivery systems, other than being a direct co-solvent for the drug. In self-emulsifying drug delivery system formulations surfactants are incorporated with an oily component to form low surface energy oil droplets as drug carriers [21]. This carrier system will be further discussed in the following section. There are similar approaches to
those explained above for injectable formulations for the bioavailability enhancement of water insoluble drugs. However these formulations are not the focus of the current work and will not be covered.

1.2.2. Microemulsions or Self-Emulsifying Drug Delivery Systems (SEDDS)

Emulsions in general are thermodynamically unstable systems. The droplets of the dispersed phase are large. Microemulsions on the other hand are emulsion systems that have a droplet size of a few to hundreds of nanometers and are typical complex fluids that consist of three essential components: two immiscible fluids and a surfactant. Typically these are water-in-oil or oil-in-water microemulsions where the rheological properties of these two liquids and microstructure of the surfactant strongly affect the resulting microemulsion [1].

Microemulsions and micellar solutions are distinguished from emulsions by the fact that the average drop size does not grow with time, which is a manifestation of thermodynamic instability. Micellar solutions and microemulsions on the other hand are assumed to be thermodynamically stable [26].

Reasons why there is tremendous attention on SEDDS include industrial trend towards the discovery and development on hydrophobic drugs and the resolution of technology transfer, stability and regulatory issues by SEDDS and the fact that they have proven pharmaceutical benefit with commercially available compounds of up to 5 fold increase in bioavailability (cyclosporine, lipid soluble vitamins, HIV protease inhibitors etc.) [27].
1.3 Modeling oral drug absorption

It is very important to identify compounds for their pharmacokinetic properties and pharmacokinetic problems that may be present at the early stages of drug discovery in order to assess absorption limitations in humans and animals. Therefore computational models are very useful tools in the drug discovery processes. Computer based simulation models are also called as “in silico” models. Several examples of commercially available (e.g., GastroPlus™, iDEA™, Intellipharm®PK, and PK-Sim®) simulation software packages have shown to predict human absorption properties of fairly accurately [28].

There are two main approaches to modeling of drug bioavailability; a) qualitative models, and b) quantitative models [29]. Qualitative models are relatively simple models that aim to correlate physicochemical and physiological properties to the oral absorption of drugs. These models, however don’t work in all cases. Quantitative models on the other hand, predict the extent of absorption based on the assessment of underlying mechanisms. These quantitative models include mass balance models, mixing tank models, and compartmental absorption and transit models. Due to a series interrelated differential equations nature of these models, numerical integration techniques are generally used to constitute numerical solutions.

Mixing tank model describes the intestine as one or more well mixed tanks with uniformity. If the intestine is considered as different tanks in between solid drug and dissolved drug, then these two tanks are considered to be two tanks in series with first order kinetics [29, 30]. Compartmental absorption and transit models are based on the transit flow of drugs in the gastrointestinal tract. Yu et al. [31], for example, in a study, described the intestinal transit process in seven different compartments.
In silico absorption models can be very useful tools especially for solubilized drug delivery approaches of the water insoluble drugs in the formulation development phase. Availability of these models might alter the desire to use these formulations as well as the potential to reduce the use of animal models [28].
2.0 CRITICAL LITERATURE REVIEW

As described above, self-emulsifying drug delivery systems show great promise for enhancing and enabling oral bioavailability of low aqueous drug molecules, which unless otherwise may not be delivered orally. However, how these drug delivery systems influence biological environment and how they get influenced to enhance bioavailability is poorly understood. Several mechanisms that are believed to play major role is digestion of emulsion droplets in the intestine, drug solubilization into drug the digestion process, and alterations in intestinal permeability and lymphatic transport.

2.1 Self-emulsifying drug delivery systems

Self-emulsifying drug delivery systems by definition are mixtures of an oil, one or more surfactants and optionally a co-solvent or co-surfactant which when introduced into an aqueous media, under gentle agitation, forms fine oil-in-water emulsions. These systems when incorporated with a drug compound, drug is distributed in the aqueous solution entrapped inside oil droplets [32].Figure 2 shows a schematic of a SEDDS. SEDDS enable distribution of hydrophobic drug component in the aqueous media and creates a drug solubilization in the gastrointestinal environment. Distribution of drug inside oil droplets prevents drug from being an undisolved substance, precipitating and being excreted from body. However their mechanisms of action in the body are not limited to solubilization enhancement and also include other processes such as intestinal permeability and lymphatic transport enhancement.
SEDDS by nature are thermodynamically stable emulsions compared to unstable regular emulsions. Their stability is thought to be dependent on their relatively small dispersed oil droplet size and narrow range of droplet distribution [33]. SEDDS are typically composed of emulsion droplets having a diameter of 50 nanometers to 500 nm whereas systems having droplet size less than 50 nm are called self nano emulsifying drug delivery systems (SNEDDS) [5].

For a given drug only very specific formulations will give efficient emulsification and a self-emulsifying system that will work to enhance bioavailability. Efficiency of SEDDS therefore, as explained in detail by Gursoy et al., is governed by surfactant concentration, oil/surfactant ratio, polarity of the emulsion, droplet size and charge of the droplets [5]. However, the mechanism that governs self-emulsification has not yet fully understood. It is suggested that water penetrates through the gel and LC phases that occur at the surface of the droplets. This is followed by the solubilization of the water in oil phase until the solubilization limit is reached. After the limit is reached, formation of

**Figure 2: Schematic of an emulsion system in which an oil droplet is stabilized by a surfactant which also forms micelles in the free solution.**

![Schematic of an emulsion system in which an oil droplet is stabilized by a surfactant which also forms micelles in the free solution.](image)
dispersion of LC phase is formed and this depends on the surfactant concentration. With this formation, SEDDS become resistant to coalescence [34].

Emulsion stability is governed by a variety of factors such as physical nature of the interfacial film, presence of electrostatic or steric barriers on the droplet, viscosity of the continuous phase, droplet size distribution, oil to water ratio, temperature and the amount of surfactant that is absorbed on the surface of the oil droplet. The more surfactant is absorbed on the surface, the more decreased the interfacial tension between oil and water which consequently yields delayed coalescence of droplets by electrostatic and steric repulsion [35, 36]. Although with the addition of high amounts of drug, which is common case for potential oral dosage forms, it is harder to have stabilized emulsions. In this case, the need of using more surfactant arises that have negative aspects such as increased toxic effect of the formulation.

The total interfacial area that is generated is proportional to the work done to the system, as described with equation below [37];

\[ W = \gamma(A \Delta A) \]

where \( W \) is the amount of work put into the system, \( \gamma \) is the interfacial tension and \( A \) is the change in the interfacial area. Therefore systems with smaller droplets have a decreased interfacial tension.

2.1.1 Characterization of SEDDS

Emulsion science in general is linked to various aspects of the following disciplines: phase transitions, surface forces and wetting, hydrodynamic instabilities, mechanical properties and flow and important factors for their stability are, in addition to the surface free energy used in the mixed film theory, are a) the Van der Waals attraction
potential between the dispersed droplets, b) the repulsive potential from the compression of the diffuse electric double layer, and c) the entropic contribution to the free energy from the space position combinations of the dispersed droplets. Among physical measurements that are useful for identifying microemulsion systems are light scattering, optical birefringence, sedimentation, centrifugation, rheology, conductivity and nuclear magnetic resonance (NMR) [38]. For emulsions and microemulsions used as drug delivery systems, on the other hand, characterization techniques and methods used have been relatively narrow and simpler.

In order to assess SEDDS efficiency, early attempts have been quantifying the time required for emulsification. It was found that, formulations with surfactant concentration of 20-50% emulsified more rapidly [39]. The second method for assessment that is still valid is particle size measurement. Surfactant ratios at which SEDDS have the lowest mean droplet diameter were assessed to be efficient drug delivery systems. Wakerly et al. [40] showed that low particle size is maintained at 30-65% surfactant concentration with a study where Targat TO and medium chain triglycerides were used. However, self-emulsifying systems are too complex to assess with only surfactant concentration. Hence, recently more methods for characterization of SEDDS, especially to predict their effectiveness are employed. Most popular approach to determine optimum SEDDS component ratios has been to plot ternary or pseudo-ternary phase diagrams in order to map the efficient emulsification areas for single drug compounds in the pharmaceutical research [4, 9, 14, 41-43] where points of the ternary diagram were oil concentration, surfactant, water and optionally a co-surfactant or a co-solvent. Diagrams were plotted by keeping one or two of the component concentrations
constant and changing the others gradually. This approach was and has been used to find the optimum formulation parameters for given formulation components.

Another characterization is measuring turbidity. Turbidity is measured in order to determine the rate at which the equilibrium is reached and repeatability of the system [44]. Polarity also rises as an important parameter for characterization of SEDDS since it is known to be correlated with the rate of drug release from emulsion droplets. Charge of the droplets is another important characterization parameter. Charge of the droplets should be at least ±25mV in order to SEDDS to be classified as stable and therefore efficient. Charge of the droplets for conventional SEDDS is negative due to free fatty acids composition in the formulation unless a cation is added to the formulation [5].

2.1.2 Formulations of SEDDS

Excipients used to formulate SEDDS have great importance on the performance since self-emulsification is known to be directly related to the physicochemical properties of oil-surfactant pair [5]. So far the general approach to formulate a single drug with SEDDS has been to test several oil and surfactant combinations at different ratios and to plot pseudo-ternary phase diagrams and eventually try to find the optimum formulation parameters. There are tens of oils, surfactants, cosurfactants and cosolvents in the literature which are used to formulate efficient SEDDS formulations by researchers. Table 1 summarizes the self-emulsifying system components used in the literature. Optimum formulation is selected considering the parameters of smaller droplet size, spontaneous emulsification and sometimes other factors explained above in the section 2.1.1 which all are indications of “efficient” emulsification however may not necessarily be indications of an efficient drug delivery system. In order to find the optimum
formulation for the specific drug, the common approach is trial and error and there is no established guidance for rational design of SEDDS formulations.

When designing self-emulsifying formulations the general desire is to use minimum amount of excipients with the ultimate efficiency in terms of good emulsification and bioavailability. Studies in the literature support that it is possible to formulate SEDDS using one of the triglycerides and preferably a non-ionic surfactant in order to obtain efficient emulsification. Sometimes a third formulation component, such as an alcohol based cosolvent is required in order to solubilize more amount of hydrophobic drug. Also, in some instances it is preferable to use a second emulsifier or cosolvent in order to avoid using high concentrations of potentially toxic nonionic surfactant [45]. However this usually is not a guarantee for increase in drug bioavailability in vivo since the exact mechanism of SEDDS functioning in GI tract environment is not fully understood.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Oil</th>
<th>Surfactant</th>
<th>Other</th>
<th>Methods of Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>[46]</td>
<td>Imwitor 742</td>
<td>Tween 80</td>
<td>NA</td>
<td>Visual assessment, Particle size, Dielectric measurements</td>
</tr>
<tr>
<td>[47]</td>
<td>Captex 200, Myglyol 812, Myvace, Soybean</td>
<td>Ariacel 80, 186, Capmul MCM, Centrophase 31, Cremophor EL, Labrafac CM10, Labrafil M 1944 CSD, Myverol 18-92, Tagat TO, Tween 80</td>
<td>NA</td>
<td>Conductance, Interfacial tension, Density, Refractive index, Viscosity, Particle size</td>
</tr>
<tr>
<td>[48]</td>
<td>Labrafil M2125 CS</td>
<td>Tween 80</td>
<td>Drug: L-365, 260</td>
<td>Particle size, Dielectric measurements, Surface tension</td>
</tr>
<tr>
<td>[43]</td>
<td>Peanut oil, Neobee M5</td>
<td>Tween 80, PEG 25, Polyglycolyzed glycerides, Glyceryl monocaprylate/caprate</td>
<td>Drug: Aratinoid drug</td>
<td>Droplet size</td>
</tr>
<tr>
<td>[50]</td>
<td>Peanut oil, Soybean oil, Safflower oil</td>
<td>Sorbitan fatty acid esters, Polyoxyethylene fatty acid esters, Glycerol esters</td>
<td>Aratinoid drug</td>
<td>Conductivity, Photomicrographic study, Drop point determination</td>
</tr>
<tr>
<td>[51]</td>
<td>Corn oil</td>
<td>PEG 400</td>
<td>Polyoxyethylene 20, Polyoxyl 60</td>
<td>Compressibility, Uniformity, Stability</td>
</tr>
<tr>
<td>[52]</td>
<td>Lemon oil</td>
<td>Cremophor Capmul</td>
<td>Drug: Coenzyme Q10</td>
<td>Compressibility, Uniformity, Stability</td>
</tr>
</tbody>
</table>
Table 1 cont. SEDDS formulation components and formulation methods of characterization.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Oil</th>
<th>Surfactant</th>
<th>Other</th>
<th>Methods of Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>[53]</td>
<td>Captex 200</td>
<td>Tween 80, Capmul</td>
<td>Gelling agent: Aerosil 200, Drug: Ketoprofen</td>
<td>Viscosity, Droplet size</td>
</tr>
<tr>
<td>[54]</td>
<td>Goat fat</td>
<td>Tween 65</td>
<td>Drug: Diclofenac</td>
<td>Uniformity, Liquefaction time</td>
</tr>
<tr>
<td>[55]</td>
<td>Soybean oil</td>
<td>Tween 80</td>
<td>Drug: Solvent green 3, Gelucire 44/14</td>
<td>Stability</td>
</tr>
<tr>
<td>[56]</td>
<td>Propylene glycol</td>
<td>Cremophor RH40, TPGS</td>
<td>Drug: Paclitaxel, Vitamin E, Deoxycholic acid sodium salt</td>
<td>Droplet size (PCS), Zeta potential</td>
</tr>
<tr>
<td>[53]</td>
<td>Captex 200</td>
<td>Tween 80</td>
<td>CoSurf: Capmul MCM, Drug: Ketoprofen, Gelling Agent: Aerosil</td>
<td>Droplet size (PCS), Polydispersity, Turbidimetry, Emulsification time, Viscosity</td>
</tr>
<tr>
<td>[57]</td>
<td>Oleylamine, Ethyl oleate</td>
<td>Tween 80</td>
<td>Solvent: Ethanol, DiLC18</td>
<td>Visual observations, Particle size, Zeta potential</td>
</tr>
<tr>
<td>[6]</td>
<td>Paceol</td>
<td>Gelucire 44/14</td>
<td>Drug: Ontazolast</td>
<td>Particle size</td>
</tr>
<tr>
<td>[32]</td>
<td>Medium chain triglyceride saturated fatty acids</td>
<td>PEG 25</td>
<td>WIN 54954</td>
<td>Particle size</td>
</tr>
</tbody>
</table>
2.1.2.1 **Oils**

Lipids, a major component of SEDDS, play a crucial role in the function of all living organisms. Therefore they have been widely studied in nutrition and food related research. Lipid based drug delivery systems are well investigated both in physical and chemical aspects however physiological impacts that affects their functioning and performance is not well valued [19].

Lipids used in self-emulsifying drug delivery systems are most commonly triglycerides from homolipids class. The most common type of glycerides used in oral formulations is long or medium chain triacylglycerols. Triacylglycerols are long (C14-C24) or medium chain (C6-C12) fatty acids linked to a glycerol molecule and can be also referred to as triglycerides [19]. Medium chain triglycerides are preferable drug carriers because they are thought to have no pharmacological action [58]. They are subject to intraluminal hydrolysis and are mainly absorbed as free fatty acids. There are distinct differences among the processes of intraluminal digestion, uptake by the mucosal cells and transport to the systemic circulation between long chain triglycerides and medium chain triglycerides [19]. Edible oils on the other hand are not commonly utilized because of their poor ability to dissolve lipophilic compounds [5]. Structured lipids are also used in oral formulations. They are triacylglycerols that contain mixtures of either medium chain or short chain fatty acids or both, and a long chain fatty acid most preferably on the same glycerol molecule. Modified or hydrolyzed vegetable oils are widely used not only because of their ability to dissolve high amounts of lipophilic compounds but also because their degradation products are natural end products of intestinal digestion [5, 19].
The importance and effect of digestion of these oils on oral drug absorption is discussed in detail in the following sections.

The non-glyceride fraction of vegetable and animal oil minor components have specific functional important properties for topical formulations but very little is known about their impact on oral formulations. These components include sterols, squalene, triterpenic alcohols and vitamins [19].

Phospholipids, a class of heterolipids, are also used to formulate oral formulations however not widely due to their limited resources. Phospholipids source is limited with vegetable oil seeds, the egg yolk and milk [19].

Oils used in the SEDDS formulations are crucial as a formulation component for many reasons which include solubilizing the hydrophobic drug compound, in some instances promoting lymphatic transport of the lipophilic drug and alteration of postprandial drug absorption and bioavailability [27].

2.1.2.2 Surfactants

Surfactants are amphiphilic molecules that contain both a hydrophilic head and a hydrophobic tail. In emulsions the tails attach to the oil droplet whereas the head gets surrounded by water molecules. Therefore the oil is stabilized in the aqueous media. Once the critical micelle concentration is reached, free surfactant in the solution self-assemble to form micellar structures [21]. Mixed micellar structures exhibit characteristic properties that are remarkably different from those individual components. Mixed micelles are also important in biological consideration. Bile salts that are naturally present in the gallbladder and intestinal fluid are types of micellar structures that exhibit surfactant like properties [59, 60].
In self-emulsifying formulations, surfactants are characterized by their hydrophilic lipophilic balance values (HLB), which is the ratio of the molecular weight of the hydrophilic end to the molecular weight of the lipophilic end, resulting with an arbitrary scale of 0 to 20 [61]. HLB approach used in formulation of SEDDS requires the usage of surfactants with a HLB value of 10 and higher in order to form fine emulsions. Even though it is the most common and practical method in drug delivery research, the determination of HLB value is qualitative and the rules that can be derived from HLB is crude to a certain extent in physics [62]. Liu et al.[63] used a MD (molecular dynamic) simulations and surfactant chain model to investigate the emulsification process and the effect of conformation interaction parameters on emulsification. It is shown that the surfactant with stronger tail-oil attraction promotes the emulsification and therefore yields more stable oil droplets.

Safety is the key parameter when formulating SEDDS. Natural surfactants are preferred over synthetic surfactants since they are considered to be safer. However these excipients exhibit poor emulsification properties. Also non-ionic surfactants were found to be less toxic than ionic surfactans and therefore have been the most commonly used class [47, 64].

Surfactants are known to affect the resulting self-emulsifying system droplet size directly. In some cases, increasing surfactant concentration results with decrease in droplet size. This can be explained by better stabilization of oil droplets with adequate surfactant amounts. Whereas in some cases, mean droplet size was reported to increase with increasing surfactant concentration [4]. James-Smith et. al [65] showed that increased surfactant concentration in an emulsion system is linearly proportional to the
amount of surfactant at the oil-water interface and this trend might be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets promoted by the increased surfactant concentration. The impact of oil to surfactant ratio on emulsion performance was recently studied by Cuinè et al. [66]. Authors utilized Soybean oil: Maisine 35-1 (1:1 w/w) as the oil phase, Cremophor EL as the surfactant and ethanol as the co-surfactant and formulated excipients at the following oil to surfactant ratios 2:1, 1.6:1, 1.5:1, 1.2:1, 1.1:1, 0.7:1, 0.3:1. A notable effect of oil to surfactant ratio on emulsion particle size and drug release rate was not observed with this relatively narrow range of ratios; however, drug solubilization into the aqueous phase during in vitro digestion was highly affected by oil to surfactant ratio, which was also shown to affect overall oral absorption. Therefore the amount of surfactant that is used in formulations should be carefully optimized.

2.2 Enhancement of Oral Bioavailability of Hydrophobic Drugs with SEDDS

SEDDS show up to 5 fold increase in oral bioavailability for certain formulated hydrophobic drugs. The reason to limited number of commercially available SEDDS formulations is partially the lack of guidance on the prediction of “when” and “how” these systems work and partially the toxicity issues correlated arising with the need of using high concentrations of surfactant in order to stabilize emulsion systems. In order to overcome issues that limit the use of SEDDS, it is important to fully understand the mechanistic performance of these systems in the biological environment.
2.2.1. Examples of SEDDS formulations and bioavailability enhancement

There are only a few commercially available orally delivered SEDDS formulations in the market; Sandimmune®, Snadimmun Neoral® (Cyclosporin A), Norvir® (Ritonavir), and Fortovase® (saquinavir). Each of these products’ formulation components are designed for each specific drug [5]. There is also several SEDDS formulation designs formulated for some specific drugs in the literature. Studies so far has been established to either optimize or create new formulations with better efficiency for already formulated drugs or use model drugs to design optimum SEDDS formulations. Gursoy et al. summarized in detail a range of formulation designs from late eighties to late nineties [5]. In the recent years Kommuru and coworkers developed a self-emulsifying drug delivery system for Coenzyme Q10 using polyglycolyzed glycerides as emulsifiers [4]. They combined Coenzyme Q10, a lipid soluble compound, with Myvacet 9-45 as the oil, Labrasol as the surfactant and lauroglycol as the co-surfactant. Bioavailability tests conducted with Coonhound dogs showed that SEDDS formulation resulted with a two fold increase in bioavailability when compared with powder formulation. Kang et al. developed a self-emulsifying formulation for Simvastin and studied the bioavailability enhancement of the formulation in beagle dogs [9]. Developed formulation consisted of Carpyol 90 (37%), Cremophor EL (28%) and Carbitol (28%) considering optimum case for particle size, dissolution and bioavailability. It was shown that release rate of drug from SEDDS is significantly higher than conventional tablet Zocor® and bioavailability increased by 1.5 fold compared to conventional tablet. On the other hand, bioavailability of Tipranavir (TPV), a nonpeptidic protease inhibitor anti-HIV
drug, was doubled compared to drug delivered as solid powder in a hard filled capsule when dosed to rats in a SEDDS formulation [3].

There are also studies in the literature where polar drugs instead of lipophilic compounds were incorporated with self-emulsifying formulations. Patil et al studied the bioavailability of Ketoprofen incorporated in gelled self-emulsifying formulations [8]. Orally administered hard gelatin capsules of SEDDS formulation neither significantly altered nor retarded the bioavailability of Ketoprofen when compared with its immediate release solid dosage form. Authors have previously investigated Ketoprofen release from the gelled SEDDS formulation and have showed that release is retarded with incorporation of gelled self-emulsifying formulation [53].

### 2.2.2 Mechanisms responsible for enhancement of bioavailability

Enhancement of oral bioavailability of hydrophobic compounds incorporated with SEDDS formulations has been reported several times in the literature. However there are instances where unpredictable cases occur and enhancement of oral bioavailability is not achieved. Unpredictable functioning of SEDDS in body is primarily due to the lack of knowledge on how SEDDS functioning is affected by the biological environment and how they influence the GI tract environment. The latter one is better studied in the literature whereas the effect of the intestinal environment on self-emulsifying systems performance is less considered.

Formulation component physicochemical properties have a direct influence on emulsification process but they also affect intestinal physiological characteristics at a certain extent that it governs the overall absorption of a drug. These properties include the chemical structure of the formulation components, formulation component ratio,
particle size and emulsion stability. Therefore, depending on the formulation component physicochemical characteristics, intestinal responses to administered formulations vary. These changes include; changes in the permeability of the intestinal monolayer, P-gp and cytochrome P450 inhibition, promoted lymphatic transport of the lipophilic drug, and postprandial solubilization due to oil component digestion [6, 67, 68]. For instance, commonly used surfactants polyoxyethylene sorbitan fatty acid esters (e.g Tween 80) and polyethoxylated castor oil (e.g. Cremophor EL) inhibited P-gp-mediated drug efflux in cell culture systems below critical micellar concentration.

All of these factors above combine together to determine the overall efficiency and therefore oral bioavailability of the co-administered drug. Some of the most significant mechanisms affecting enhancement of oral bioavailability with SEDDS are discussed in the following sections of this paper.
2.3 Permeability Enhancement of Intestinal Monolayer

Surfactants are believed to be capable of modifying biological membranes. Permeability enhancement, as well as inhibition of absorption and pharmacological activity of drugs has been all shown in the presence of surfactants [67]. A work
performed by Levy et al. showed that the nonionic surfactant Polysorbate 80 (Tween 80) with a concentration below CMC (Critical micellar concentration) increased the absorption of barbiturates across goldfish membrane while it decreased absorption at concentrations above the CMC. The increase in absorption was interpreted as being due to the enhanced permeability of the biological membrane [69]. The decrease at higher concentrations, conversely, might have been due to the increased solubilizing effect of the micellar surfactant structures slowing the rate of absorption or to the layering of free surfactant monomers on the cell culture monolayer and consequent blocking of drug transport. Surfactants, in some cases, are also capable of enhancing drug permeability with an desired effect, toxicity. In a recent study by Sha et al. [70] performed on Caco-2, it was shown that Labrasol alone induced cytotoxicity dramatically at dilutions higher than 1:100 (w/v). However, an increase in paracellular drug transport with no toxicity was reported with a formulation consisting of 30% Maisine 35-1, 38% Cremophor EL, 10% Labrasol, 23% Transcutol P; enhancement in paracellular permeability was suggested to be due to Labrasol at 1:1000 overall dilution.

Other than surfactants, lipids are also suggested to change the characteristics of the intestinal biological membrane. Monoglycerides, fatty acids and bile salts are known to have inherent permeability enhancement abilities. Monoglycerides, fatty acids and bile salts get directly absorbed into intestinal enterocytes while carrying the dissolved drug with them. Therefore lipid digestion products together with bile salts may change intestinal permeability to drug compound [3].

One of the challenges in testing the effect of oils used in formulation of SEDDS is the fact that they are insoluble in aqueous cell culture mediums. Since oils are not
miscible with the cell culture medium such as Dulbecco’s Modified Eagle Medium (DMEM), low density oils float on the surface of the aqueous medium and do not contact with the cell layer they are getting tested on [10]. Therefore the most convenient way to test these compounds on cell layer is to introduce them as emulsified forms. Pamakula and coworkers investigated the effect of different sample preparation techniques in order to facilitate contact with the oil phase and cells [10]. Systems such as suspensions, dispersions, homogenized microemulsions, and nanoemulsions with low surfactant concentrations were used. There was at least one order difference between the homogenized oil (thousand nm range) and nanoemulsion (hundred nm range) particle size. However neither the particle size nor the preparation technique of oily samples (homogenized vs nanoemulsion) did have an effect on the toxicity of the compounds. On the other hand suspensions of Captex-200, Captex-355, and Neobee M-5 and Corn oil showed decreased cell viability compared to the cytotoxicity measurements where other sample preparation methods were used. This was explained as the settling down of the higher density oils on the cell culture surface and causing more toxicity due to the increased exposed surface area [10].

Caco-2 cell model is a commonly used model for testing the effect of formulation components or formulation as a whole on the permeability enhancement of the intestinal monolayer. It has been previously shown that Caco-2 cell culture grown on 96 well plate or polycarbonate membranes is adequate for conducting cytotoxicity or transport tests [71]. To test the influence of drug delivery systems on the tight junction integrity, transport studies across model cell culture system with paracellular markers are commonly being used. Radiolabeled Mannitol is a very common pracellular marker [10]
and has shown to reflect paracellular track more precisely then other drugs of paracellular route [72]. Yet, other compounds are also worth testing for paracellular drug transport experiments if radioactive material usage is being avoided.

2.4 Drug Release from Emulsions

When a drug is introduced into body, it is thought to follow the following sequence: dissolution of drug from solid form into the solution, gastrointestinal fluid in this case, and then the absorption of the drug to the systemic circulation. The rate limiting process in this sequence is the slowest step which in many cases is dissolution [67]. Dissolution rate of a tablet dosage form is known to be determined by two parameters: solubility of the drug in the dissolution medium and the surface area of the drug exposed to the medium [73]. In the case of self-emulsified drug delivery systems, solubilization of drug component is enabled by dispersed oil droplets and coexisting micelles that are capable of dissolving water insoluble drug.

![Figure 4: Schematic of possible pathways involved in the release of a drug from an emulsion droplet.](image)

Available mechanisms for transport of a solute from an emulsion droplet into an
aqueous micellar solution were discussed by Dungan et al [74]. As shown in Figure 4, solubilization of the drug, entrapped in the oil droplets, can follow one of the either pathways. Pathway 1 suggests that solute moves as an individual molecule and transports through the aqueous medium once releases from the oil droplet. Whereas in pathway 2, solute is incorporated with micellar structures while leaving the oil layer and transports in the aqueous medium inside micelles and eventually releases to water. In both of the pathways there is interfacial transport, bulk diffusive/convective transport and reactive transport of the solute molecule [74]. Drug ideally chooses the most convenient way of transport mechanism available among these two. Without considering any of these mechanisms a simple population balance model [75] was applied to SEDDS systems by Dungan et al. [74]:

\[
\frac{dm}{dt} = k_{\text{eff}} A (C_{D,aq} - C_{D,aq}^{eq})
\]

Equation 2

where the concentrations \(C_{D,aq}\) and \(C_{D,aq}^{eq}\) are the total solute concentrations of free drug and drug in micelles at time \(t\) and saturation concentration at equilibrium respectively. \(A\) is the total interfacial area of droplets and \(m\) represents the lost of solute compound from emulsion droplets. Authors developed a series of equations for each of the scenarios described above and compared model predicted rate expressions of alkane solubilization in oil in water emulsions with measured \(k_{\text{eff}}\) values (Eq. 2). Firstly, authors assessed the micelle mediated mechanism (Pathway 1). Measured rate coefficients were 10 to 100 times smaller than that predicted from bulk micellar transport mechanism (pathway 2) and the measured values did not show dependence on aqueous viscosity and micellar diffusion was not found to be contributing to the observed rates. Therefore it was suggested that a micelle mediated mechanism can only be valid for the observed kinetics
if the rate limiting step in that pathway is interfacial transport. Together with micelle-mediated mechanism, influence of molecular mechanism was also investigated, again by comparing the molecular mechanism derivation predicted of rate coefficients with measured $k_{\text{eff}}$ values. It was observed that the molecular mechanism predicted coefficients were in accordance with the measured kinetics. Besides these observations, in both of the cases an interfacially-dominant transport mechanism was suggested to be consistent with experimental conditions.

In accordance with findings of Dungan et al. assuming the interfacial transport step (among interfacial, diffusive and reactive transport steps) as the rate limiting step, in 1970, Bikhazi et al. [76] described compound transport process in and out of an emulsion droplet as:

$$
\frac{dC_{D,\text{aq}}}{dt} = -\frac{A_{\text{em}} P_{\text{rel}}}{V_{\text{aq}}} (C_{D,\text{aq}} - C'_{D,\text{aq}}) 
$$

Equation 3

Here $C_{D,\text{aq}}$ is the drug concentration in the aqueous media (buffer and micelles) and outside of oil droplets, $V_{\text{aq}}$ is the volume of the aqueous media, $P_{\text{rel}}$ is the permeability constant for the oil water interface, $A_{\text{em}}$ is the total interfacial surface area of the oil droplets, and $C'_{D,\text{aq}}$ is the hypothetical aqueous concentration of the solute in equilibrium with compound concentration inside emulsion droplet, $C_{D,\text{em}}$. $C'_{D,\text{aq}}$ is defined by the oil-aqueous phase partition coefficient as:
\[ K = \frac{C_{D,em}}{C_{D,aq}} \]  \hspace{1cm} \text{Equation 4}

Expression developed by Bikhazi et al. is similar to the one used by Dungan et al. (Equation 2) with the major difference being the definition of equilibrium compound solubility expression.

With the assumption that compound releases from inside the emulsion droplet to the outside aqueous solution under sink conditions, scientist [11, 77] have used a Fick’s second law derivation by Guy et al. [78] to calculate the rate of release expressing the interfacial transport from spherical particles;

\[ \ln\left(1 - \frac{M_t}{M_0}\right) = -\frac{3kt}{r^2} \]  \hspace{1cm} \text{Equation 5}

where \(M_t/M_0\) is the fraction of released drug from oil droplets at time \(t\) and \(r\) is the droplet radius. Even though it was suggested that interfacial transport is independent of the surfactant compound delivery by diffusion to the oil droplet surface and bulk aqueous viscosity, parameters that would influence the interfacial transport mechanism are still yet to be identified [79]. On the other hand, in the case of amphiphilic solute solubilization (for instance fatty acids) as opposed to hydrophobic solutes, a faster interfacial transfer may make the micelle-mediated pathway kinetically available to acid molecule, allowing to it to avoid molecular transport through water, therefore resulting in a relatively fast [80] (compared to hydrophobic solutes) solubilization in the micellar media.

Measuring the rate of release of a drug from microemulsions in situ is not easy since it is a relatively fast occurring process where several different phases are involved and it is not likely to separate each phase without disrupting the dispersed phase equilibrium [77]. In order to overcome the problem of separation of phases, Washington
et al. [81] introduced an in situ model applicable to weak acidic or basic properties. It was shown that it is possible to monitor the rapid release of solutes from submicron emulsions with the change in pH. Another method that was used by scientists [11, 53] is the dialysis technique where emulsions containing drug is entrapped inside a cellulose membrane which is immersed into a receiver fluid and the release rates are monitored as a function of drug concentration in the receiver fluid outside the membrane.

Shah and coworker defined important parameters influencing drug release as; uniform oil droplet distribution, polarity of the droplets, and viscosity [43]. The factors governing the polarity were HLB value, the chain length, and degree of unsaturation of the fatty acid [33]. A lipid of long chain fatty acid structure creates a more lipophilic environment whereas a medium chain length fatty acid enables a less lipophilic environment for the drug [43]. Shah et al. also claimed that the efficiency of SEDDS depend on the fast release of drug [43] however, “too fast” release of the drug compound from the droplets may result with drug precipitation. Formulation physicochemical properties, overall, have been reported to show a big impact on drug release profiles from microemulsions. Montenegro et al. [11] studied the influence of oil phase lipophicity on in vitro drug release rates. Three different lipids: isopropyl mysristate (IPM), isopropyl palmitate (IPP), and isopropyl stearate (IPS), with a lipophilicity order of IPM < IPP < IPS were used. Microemulsions were formed with combining these lipids with low amount (7%) of surfactant mixture isoceteth-20/glyceryl oleate (5:2) in order to avoid the formation of micellar structures. Release tests were conducted in vitro using cellulose membranes with Franz-type diffusion cells and rate of release from each microemulsion system was calculated. Statistical anaylsis of data with one-way ANOVA coupled with
Bonferroni’s t-test yielded that release profiles were dependent on oil phase lipophilicity and drug partition coefficients. It was also shown that cumulative amount of drug released at 22 hour is inversely related to the drug lipophilicity which favors the fact that higher the drug oil-water partition coefficient the lower the amount of drug released when equilibrium reached. Influence of drug logP was associated with the composition of the dissolution medium it releases into [12]. Attama et al. in a study of drug release from microemulsions, used three different drug compounds of different lipophilicity and combined these with individual SEDDS formulations containing Tween 65 and Homolipid at different ratios [12]. The drug release from three formulations was evaluated in three different dissolution mediums; SGF, SIG and distilled water. Release rate profiles in all the receiver mediums led to the conclusion that the most lipophilic drug showed the slowest release, which was also the system with the smallest particle size. Tendency of small particle size emulsions to favor the rate of drug release was shown in another study where the dissolution rate of a lipophilic drug from SEDDS formulations with polyglycolyzed glycerides (PGG) varying in fatty acid chain lengths, and another SEDDS formulations formulated with medium chain monoglyceride (Capmul MCM90) and PEG (25) trioleate (Tagat TO) were compared [43]. Authors showed that the formulations with PPGs increased the rate of release of the drug when compared to other SEDDS formulations and powder forms. This is explained by “energetically more favorable situation’ enabled with PGG, which in other words refer to small particle size of that system. The influence of formulation viscosity on the rate of drug release, another important formulation parameter, was highlighted by Patil et al. [53]. Authors formulated Keteprofen using $3^2$ factorial design with Captex 200 as oil,
Tween 80 as the surfactant, and Capmul MCM as the cosurfactant and capsulated contents in a gelled form with the addition of gelling agent Aerosil. Release of ketoprofen from gelled self-emulsifying systems was evaluated with an in vitro dialysis technique. Viscosities of the systems were evaluated with a programmable rheometer. Gelling agent, Aerosil, was found to increase LC phase viscosity and consequently retard the rate at which the drug releases. It should be noted that when it comes to microemulsions, changing one parameter in the system will result with a change in a majority of other parameters as well. For example changing oil lipophilicity in a formulation will most probably result with a change in the mean droplet size of the microemulsion system also. Therefore, when addressing the influence of one parameter on drug release performance as discussed above, it is of great importance to use a statistically designed experimental approach and to consider all the important variables simultaneously.

In in vivo conditions once a self emulsifying formulation disperses in the gastrointestinal tract in the presence of digestive enzymes oil droplets start digesting which results with formation of different phases on the outer surface of the droplet and at the same time results with formation of solubilizing species (micelles and vesicles) in the aqueous environment. Therefore it is important to address the influence of phase transformations and presence of micelles and vesicles on the rate and extent of drug release. Influence of phase transformation on the drug release from microemulsions was recently studied by Trotta et al. [77]. The effect of different phases, namely, emulsions, liquid crystals, microemulsions on the rate of drug release was studied by monitoring the change in pH. 9 different colloidal systems were formed combining Isopropyl myristate,
lecithin, lysolecithin, water and alcohol (ethanol, 1-propanol, 1-butanol, 1-pentanol or benzyl alcohol) at different ratios. A direct correlation between release rates and the microemulsion mean diameter was reported. However it should be noted that the equation authors used to calculate the release rates includes the mean emulsion diameter expression, therefore the correlation between the droplet size and release rate is not an independent correlation. It was also noted that systems with liquid crystals showed slower and sustained release rates followed by coarse fine emulsions and faster release was obtained from microemulsions. It is worth mentioning that there was no indication of the statistical significance of the trends observed.

Possible supporting or hampering effect of micelles in favor of drug absorption was proposed by Charman et al. Micelles may support absorption by facilitating the diffusion of the drug compound by solubilizing it in micelles and with another hypothesis, conversely solubilization of drug in micelles would decrease the intermicellar free drug amount and consequently decrease the total absorption [3]. The effect of nonionic surfactant concentrations and micellar surfactant structures on the release of model drugs from emulsions was studied previously [82]. CMC values of each emulsion system were calculated by measuring surface tension measurements and plotting a graph of surface tension versus surfactant concentration. It was shown that transport rates of model drugs PPA and benzocaine from emulsions increased with increased micellar phase concentration. However, increasing micellar concentrations more than 1% w/v resulted with a decrease in the transport rate. This was explained to be due to the possible structural changes in the micellar structures and consequently increased solubilizing properties of these structures [82]. The decrease in the rate of transport with exceeding a
certain micelle concentration might be also due to clogging of the membrane. The change of micellar structures from spherical to ellipsoidal might be clogging the pores of the dialysis membrane taking into account that the decrease is more significant with the same experiment conducted with 1KD and less significant and almost negligible with 50 KD molecular cutoff membranes.

Drug release profiles from microemulsions was recently investigated during in vitro formulation digestion. Fatouros et al. reported that the rate and extent of drug release from oil formulations (Sesame oil: Maisine 35-1 mixture incorporated with cosolvent ethanol) was significantly lower compared to self micro and nano emulsifying drug delivery systems (both composed of Sesame oil: Maisine 35-1: Cremophor RH 40 at different ratios). In vitro digestion was performed using pH-stat unit. 20 ml samples were taken at 0, 15, 30, 45 and 60 minute time points. 4-bromobenzeneboronic acid was added to each sample followed by centrifugation at 40,000 rpm for 135 minutes and drug, probucol, concentration was determined by HPLC [83]. Release profiles from three drug carriers were reported as percentage drug released and all formulations seems to be leveled off after 15 minute time point. As mentioned earlier, the possible effect of the long centrifugation process on the release profiles should also be considered when analyzing these findings. An in situ and real time method for analysis of amount of compound in each phase (water, micelles, and formulation) during in vitro digestion was recently employed by Rube et al. [84] using Electron Paramagnetic Resonance (EPR) Spectroscopy. EPR is a non-invasive method, which requires the presence of paramagnetic molecules. Because most drug delivery systems are EPR silent, the analysis required the presence of a spin probe, Tempo Benzoate, instead of real drug molecule.
EPR has been previously proposed [85] as a powerful tool to monitor drug delivery processes and is an advantageous alternative to conventional techniques for measuring drug release from SEDDS and partitioning into colloidal phases as it is non-invasive, and online method unlike above discussed methods.

2.5 Lymphatic Transport

Most of the orally administered drugs associate with portal vein in order to get into systematic circulation however some very lipophilic compounds, especially in the presence of absorbed digested lipid products in intestinal cells, are transported via the lymphatic way avoiding first-pass metabolism [3]. Usually drugs which are included in the systematic circulation through lymphatic transport, associates with the triglyceride lipid core of the lipoproteins. Fatty acids with a carbon chain length shorter than 12 (short and medium chain) are not transported to the systematic circulation via lymphatic pathway, they incorporate with blood vessels [86]. On the other hand long chain fatty acids and monoglycerides, digestion products of SEDDS formulations, are re-esterified to triglycerides inside the intestinal cell and by exocytosis they are secreted into lymph vessels [5]. Hauss et al. showed that the bulk amount of drug compound Onzalast formulated with triglyceride absorbed via lymphatic pathway [6]. In vitro tests on rats showed that the drug transported into systemic circulation is directly proportional with the amount of triglyceride transported via lymph. Emulsion systems containing soybean oil, a long chain triglyceride, showed the highest transport via lymphatic pathway. However, there are reports in literature where medium chain fatty acids are transported via intestinal lymph and long chain fatty acids absorbed by portal blood [87]. Lipid digestion products after getting absorbed into enterocyte, either directly diffuse through
the cell and enter the portal vein or involve with Endoplasmic reticulum inside the cell and are re-synthesized to triglycerides and further constitute the lipid component of the intestinal lipoproteins. Due to large colloidal particle nature of the lipoproteins, following exocytosis from the enterocyte, these particles preferentially passes through the highly permeable lymphatic endothelium rather than portal vein [18].

Another factor that determines the lymphatic drug transport is the nature of the drug. Most of the drugs with low molecular mass are known to be absorbed with portal route. The reason to this is based on the fact that the blood flow rate is almost 500 times higher than of that lymph flow [18]. Similarly, even though the exact mechanism is unknown (it is believed that lipophilic drug association with lipoproteins during transport through the enterocyte) studies suggest that a drug is required to have a logP value of more than 5 and triglyceride solubility of minimum 50 mg/ml for lymphatic transport to be a major contributor to oral bioavailability. On the other hand sometimes formulation parameters might limit the lymphatic transportation of the lipophilic drug. Seeballuck et al., using Caco-2 cell culture model, showed that Cremophor EL at a concentration higher than 0.025% w/v inhibits P-glycoprotein efflux as well as chylomicron secretion [68]. Cremophor EL’s inhibition of chylomicron secretion reduces the potential lymphatic transport of co-administered drug compound and might consequently affect the overall oral absorption.

The most common approach for the assessment of lymphatic transport of drugs is the rat model. However there are quite a few variations in reported literature possibly due to the differences in the invasive techniques, animal treatment before and after surgery, and the conscious state of the animal [18]. An alternative approach to animal model is the
use of cultured intestinal epithelial cells, Caco-2 model. Caco-2 systems were used to assess the influence of lipids and lipid based system excipients on drug incorporation into lipoprotein [68, 88, 89]. Recently, Seeballuck et al. [88] compared the in vitro Caco-2 intestinal lipoprotein processing with the rat model and it was shown that even though Caco-2 model mirrored the rat model qualitatively, there was no quantitative correlation between two models.

2.6 Digestion of Emulsion Droplets in the Intestine

Many of the hydrophobic drugs can be absorbed orally when administered after meals or when administered with the food. Even though it is not yet understood which of the mechanisms contributes to enhancement in bioavailability, possibilities include one or more of the following:

(a) delayed gastric emptying, allowing more drug to dissolve before reaching the small intestine, therefore producing a longer residence time at specific absorption sites in the small intestine,

(b) direct interaction and solubilization of drug by food (high fat meals)

(c) food-related increase in hepatic blood flow causing a decrease in first-pass metabolism

(d) increased gastrointestinal secretions (e.g. bile) improving drug solubility[90].

Most of the knowledge we have on triglycerides originates from nutritional literature and this knowledge provides reasonable information on postprandial food effect bioavailability with regard to lipid induced responses as mentioned above such as enhanced wetting, solubilization, transit changes, increased intestinal secretions, changed local blood flow. Although these concepts provide information on the semi-empirical
design of the lipid based formulations they do not address the distinctions between a meal and a formulation dosage form or the additional drug and formulation specific luminal and extraluminal processing [27].

Upon entry to intestine, large fat droplets are emulsified by bile salts, monoglycerides, cholesterol, lecithin and lysolecithin and typically form droplets of 0.5-1 µm. Metabolism products of these droplets are then catalyzed with pancreatic lipase (PL) and mixed micelles are formed [3, 47]. Lipids undergo several steps of processes when interact with digestion enzyme lipase. They first break down into fatty acid (FA) and diglyceride (DG). Further hydrolysis produces a second fatty acid (FA) and 2-monoglyceride (2-MG). Even though it is believed to be limited in vivo, in some cases 2-MG might undergo isomerization to 1-monoglyceride (1-MG). 1-MG further gets hydrolyzed to a third fatty acid and glycerol [91]. Digestion products of long chain triglycerides get solubilized in micelles and involve in the trafficking of lipid digestion products to the surface of the enterocyte. Triglyceride digestion is therefore a complex process with the sequential lipolysis to diglyceride, monoglyceride, and fatty acids, and transportation of the lipolytic products to enterocyte surface via micelles [27]. Digestion products and mixed micelles after penetrating through the aqueous gastrointestinal fluid and mucin, get absorbed by one of the following ways: pinocytosis, diffusion or endocytosis [82, 92]. Above mentioned processes apply for microemulsion formulations as well.

Substances used in the simulation of fat digestion in the body have direct influences on pancreatic lipase activity. Bile salts at high concentrations inhibit pancreatic lipase activity and at higher concentration they prevent enzyme from binding
the substrate. Addition of colipase, however, may restore lipolyiss [93]. On the other hand solubilization of digestion products fatty acids during oil droplet digestion may directly influence the rate of lipolysis. Long chain fatty acids released from triolein for example have poor water solubility and will remain in the substrate interface. This accumulation in the interface may influence lipolysis unless adequate amount of fatty acid “acceptors” such as bile salts or albumin are present in the media [93]. Therefore, one of the challenges, when studying digestion of fats and lipid based systems is simulating intestinal fluid in vitro. Parameters of the intestinal fluid in vivo such as, osmolality, pH, molar concentrations of bile salts and phospholipids, total volume, surface tension and so forth are very much dependent from person to person. Therefore developing one good simulated model to represent the in vivo case is not intuitive. Kalantzi et al. [94] studied the upper gastrointestinal luminal composition with the ultimate goal of designing in vitro studies to explain/predict food effect on dosage form performance. Authors used twenty healthy human subjects who received 250 ml water or 500 ml Ensure plus® (a complete nutrition drink) and collected aspirated samples from gastric antrum or duodenum for a period of to 3.5 hours. It was reported that for the fasted duodenum, the median pH was 6.2 (ranged 2.5-7.5), which is acclaimed to be in accordance with previously published data of 5.95 to 6.72. Median osmolality was reported to be 178 mOsm/kg (ranged 100-300 mOsm/kg) and median bile salt concentration was reported to be 2.6 mM (ranged 1-4 mM) which is lower than previously reported values of 4.3 to 6.4 [95]. Even though fed state pH in the duodenum depends on the energy content of the intake meal, with the energy content of 458 kcal after 30 minutes median pH was found to be 6.6 (ranged 6.2-7.2) and dropped down to
5.2 in 210 minutes. Fed state osmolality values were shown to be hyperosmotic with high variability however shown to reach isoosmolatility, 287 mOsm (ranged 200-400 mOsm) at 210 minutes. Bile salt concentration in fed state was also reported to show higher variability compared to fasted state and median value at 30 minutes was 11.2mM (ranged 2.5-23). Previous research has shown a mean of 14.5mM at 30 minute after administration of the meal [96]. Other reported median values for the fasted and fed state conditions respectively are as follows; buffer capacity: 5.6 mmol/LΔpH, 28 mmol/ LΔpH, surface tension: 32.3 mN/m, 28.1 mN/m, total protein content: 3.1 mg/ml, 12 mg/ml [94]. Schersten et al. [97] reported that body secretes bile salts and phospholipids at the ratio of 4 to 1. However at fed state conditions, phospholipids are introduced into small intestine in this case, at some extent with the food intake. Therefore, while simulating intestinal fluid it might be meaningful to consider this ratio to be slightly higher.

Emulsion components, such as oils and surfactants are highly complex structures consisting of too many compounds. Fliszar et. al. by using a reverse-phase HPLC, previously profiled three representative commercially available medium chain triglyceride oils: Imwitor 742, Miglyol 812 and Capmul MCM [98]. However, while studying the possible influence of different lipid excipients (complex derivatized oils) on extent and rate of digestion instead of complex oils (for instance derivatized oils) usually representative simple triglycerides has been used in order to understand digestion properties of lipid formulations. Therefore complex oil and oil digestion products’ characterization in order to monitor lipid digestion is avoided. For example, long chain triglycerides are commonly represented by triolein [91], a triglyceride consisting of three oleic acid molecules attached to glycerol compound. Lipase breaks down double ester
bonds between oleic acid molecules and glycerol which results with a mixture of diolein, monoolein and oleic acid as digestion products of triolein. A common simple triglyceride to represent medium chain triglycerides is tricaprylin [91]. It is also formed of a glycerol molecule linked to caprylic acids by double ester bonds. Tricaprylin’s digestion products are dicaprylin, monocaprylin and caprylic acid. One approach to characterize structures of digestion products during in vitro digestion is HPTLC analysis [91]. With this technique it is possible to distinguish among triglycerides, diglycerides and monoglycerides.

2.6.1 Lipase kinetics

The classical Michaelis-Menten kinetic model assumes that the enzymatic reaction must take place in an isotropic medium, in which both the enzyme and the substrate must be present in the same phase [99]. Therefore classical Michaelis-Menten model can not be applied to emulsions. Verger et al. [100] described a modified Michealis-Menten model for hydrolysis of emulsions and other lipid particles where all the enzyme is assumed to be bound to the particles:

\[
v_p = \frac{dP}{dt} = \frac{k_{cat}}{K_M + S} E_0 S = Q_p E_0 S \tag{6}
\]

where \(v_p\) is the rate of hydrolysis of the particles, \(Q_p\) is overall kinetic constant, \(S\) is substrate concentration, \(E_0\) is the total enzyme concentration, \(k_{cat}\) is catalytic rate constant, \(K_M^*\) is interfacial Michealis-Menten constant, \(P\) is product concentration and \(t\) is time.
Recently, a mathematical model was developed in order to explain SEDDS formulation digestion kinetics [101]. This expression relates the rate of fatty acid production to the droplet surface area available to the enzyme:

$$\frac{dm_{FA,aq}}{dt} = k_{dig} S_{em} V_{aq}$$

Equation 7

where $m_{FA,aq}$ is the mass of digestible fatty acid (FA) in the aqueous phase, $k_{dig}$ is the digestion rate constant, $S_{em}$ is the total oil droplet surface area per total volume and $V_{aq}$ is the aqueous solution volume. This model of digestion kinetics assumes that the total number of droplets in the system remain constant but their diameter decreases as they are digested and as the digested lipids leave the droplet. Therefore it was also assumed that all the free fatty acids that are digested do not stay on the droplet but leave the oil droplets.

2.6.2 Phase changes during lipid based formulation digestion

Rate of hydrolysis with pancreatic lipase varies with the substrate and its physical state. For instance monomers of a triglyceride are much poorer substrates than of aggregates of the same triglyceride [102] and the physical state of the substrate may change during hydrolysis. Overall performance of SEDDS in increasing bioavailability is suggested to be highly dependent on the morphologies of the intermediate phases produced during lipid digestion [103]. Therefore there is a need for a correlation between formulation characteristics and the phases occurring during lipid digestion in order to have an understanding on how self emulsifying systems work to enhance bioavailability.

It is known that digestion of lipids result with complex phase behavior in aqueous fluids [104, 105]. It is suggested that during oil droplet digestion, liquid crystalline phases starts
forming at the oil-water interface. Based on the structure of the triglyceride, diglyceride, or monoglyceride (long chain vs. medium chain) these liquid crystalline phases show lamellar (Lα), or cubic (C) architectures [18]. Endogenous species lysophospholipids and bile salts on the other hand are structurally partly soluble in water and form unstable monomolecular films and above CMC they form micellar solutions [99]. Simulated lipid digestion studies suggested that systems with long chain lipids exhibited cubic and coexisting colloidal liquid (L1) structures whereas in the presence of medium chain lipids, lamellar phases were observed [106]. In addition to these phases occurring at the oil-water interface during in vitro digestion, as a result of the solubilization of lamellar and cubic phases inside micelles in the aqueous phase, L1 phase formation occurs. The colloidal liquid phase consists of micelles, multilamellar and/or unilamellar vesicles. The effect of these colloidal structures on drug solubilization is considered to be very significant. Compared to endogenous bile salts, phospholipids and cholesterol, drug solubilization capacity of these colloidal species was reported to be 50 fold higher [106]. Influence of formation of different phases during digestion on solubilization capacity of these phases together with the influence of the surfactants, present in SEDDS, on the formation of different phases present an important factor in order to understand drug solubilization patterns of SEDDS in vivo [107].
Kossena et al. [103] studied the effects of intermediate digestion phases on oral absorption of model drug Cinnarizine in a rat model. Representative colloidal (L$_\alpha$), Lamellar (L$_m$), and cubic liquid crystalline (C) digestion phases were formed by mixing fatty acids and monoglycerides of C8, C12, C18:1 lipids. It was observed that administration of drug with L$_m$ phases of C8 and C12 lipids (medium chain length) increased systemic drug circulation significantly compared to control. On the other hand, C phases of C18:1 lipids (long chain length) caused a decrease and also a sustainability of Cinnarizine over a 8 hour period. This was suggested to being possibly due to several reasons including high drug affinity for C phases, viscosity-associated mucoadhesion, or due to C phases sustaining drug release in a matrix like manner. Sek et al. [108] investigated in vitro digestion profiles of long and medium chain glycerides and the
phase behavior of digestion products. Common formulation lipids were used to study in vitro digestion: Miglyol 812 (medium chain triglyceride), Capmul MCM (C$_8$/C$_{10}$ monoglyceride/ diglyceride mixture), soybean oil (long chain triglyceride, LCT) and Maisine 35-1 (C$_{18}$ monoglyceride/diglyceride mixture). Digestion was monitored with pH-stat titration and HPTLC analysis. It was suggested that digestion products of long chain triglycerides gets solubilized into the aqueous phase proportional to bile salt concentration whereas medium chain lipolytic products show a bile salt independent manner. It was also reported that digestion of diglyceride/monoglyceride mixtures of both long and medium chain lipid mixtures were more rapid compared to those of triglycerides.

In order to establish an understanding of the rate and extent of self emulsifying drug delivery formulation digestion it is important to monitor phases occurring in vitro. In a recent study by Fatouros et. al [107] the structural development of colloidal phases during lipid digestion utilizing small-angle x-ray scattering was investigated. For the SEDDS formulation, a mixture of long chain triglycerides (30% Sesema oil, 30% Maisine 35-1) and 30% surfactant, Cremophor RH 40, and 10 % cosolvent ethanol were used. Samples were taken at 0, 5, 15, 30, 60 and 90 minutes and analyzed by small angle x-ray scattering. X-ray analysis indicated that at 5 min there was a formation of lamellar phases followed by a lamellar and hexagonal phases at 60 minute till 90 minute time point.
Small-angle neutron scattering offers several advantages compared to small-angle x-ray scattering such as; such as probing the internal structure of the materials in the nanometer and micrometer scale [109]. Hjelm et al. investigated particle size and morphology in monoolein-bile salt mixtures [110], bile salt-phosphatidylcholine [111], and bile salt-fatty acid-monoglyceride mixed colloids using small-angle neutron scattering (SANS). They also studied the effects of dilution on particle morphology in samples of glycocholate and lecithin [112] and of lecithin and bile salt [113]. Samples were prepared in D₂O buffer (pH 6.7) at different concentrations and analyzed by 30 M SANS instrument. These extensive studies showed that the size, shape, and composition of colloidal particles were highly dependent on type of compounds, concentration and molar ratio of samples. However, these lipid particles were investigated in equilibrated model systems and in absence of enzymatic lipolysis processes. Equilibrated systems might not reflect physiological dynamic processes occurring in the human GI tract. Adding the action of lipases will be a more realistic representation of these processes. It is therefore necessary to analyze and compare the nature and dynamic evolution of colloidal structures generated during the proposed in vitro lipid digestion simulation, which mimics more closely non-equilibrium in vivo conditions.

2.7 Predicting Bioavailability of Drugs Administered with SEDDS

Currently, there are no mathematical models available that is based on the kinetic and physical processes of self emulsifying systems in the biological environment which predicts the oral bioavailability of a drug that is administered with SEDDS orally.

The most common approach to rationalize formulation design with lipid based drug delivery systems has been the use of in vitro lipolysis models in the recent few years
There is a few amount of work done in order to correlate drug dissolution with in vivo bioavailability data using in vitro lipolysis models that gives good correlation with the in vivo solubility despite the differences in the methods of lipolysis models among different labs. These models mimic the pre-enterocyte phases of the drug therefore, correlates the impact of drug solubilization followed by lipid digestion with the overall drug bioavailability of a drug administered with lipidic formulations. In a recent study with progesterone, good correlation was observed between in vitro lipolysis model and in vivo AUC values [115]. Progesterone was incorporated in 3 lipid formulation components; a long chain triglyceride, a medium chain triglyceride and a short chain triglyceride in the absence of a surfactant component. Authors showed a lipid performance rank order based on the percent progesterone (logP 3.87) solubilized in the aqueous phase following the in vitro lipolysis in which the end product of MCT lipid showed 4 fold greater solubilization capacity with respect to LCT and 11 fold greater than the SCT. This trend was also observed with the in vivo studies as well as the good correlation with the percent dose solubilized ($R^2$>0.99). Same lab further investigated the distribution and solubilization pattern of two different drugs, dexamethasone (2.55) and griseofulvin (logP 2.2) across different phases of the in vitro lipolysis resulting again from different lipids of LCT, MCT and SCT class [116]. Trends observed regarding percent griseofulvin dose that was solubilized in the aqueous phase in the in vitro lipolysis model were in good correlation with observed AUC values in the in vivo.

In the context of in vivo-in vitro correlation modeling, Fatouros et al. [83] used Adaptive Neuro-Fuzzy Modeller (AFM) approach that combines artificial neural networks (ANN) with fuzzy logic (FL). In vitro study was performed using pH-stat unit
and 20 ml samples were taken at 0, 15, 30, 45 and 60 minute time points. 4-
bromobenzeneboronic acid was added to each sample followed by centrifugation at
40,000 rpm for 135 minutes and drug, probucol, concentration was determined by HPLC
[83]. However, drug release from colloidal structures is a relatively quick process and
centrifugation for 135 minutes cannot provide a real time drug measurement. Authors
conducted in vivo bioavailability studies in mini-pigs fed with a high fat meal prior to
treatment. Representative drug delivery vehicles were formulated with Sesame oil:
Maisine 35-1: Cremophor RH 40 at varying ratios to prepare following systems; a self
emulsifying formulation (26.7:26.7:26.7:20), a self nano emulsifying formulation
(30:30:30:10) and an oil mixture (Sesame oil: Maisine 35-1: Ethanol, 45:45:10).
However more complex prediction formations may be needed for accurate prediction of
bioavailability where different types of drugs and different formulation parameters such
as different oil, surfactant and ratio are involved.

Above mentioned results show good correlation between the drug solubilization
followed by in vitro lipidic formulation lipolysis with in vivo bioavailability data
(Correlation with AUC) for certain drug compounds tested but it is hard to generalize this
predictability across different compounds and different formulation parameters such as
different oil, surfactant and ratio. In vitro-in vivo correlation models described above
does not provide a time based bioavailability information. Besides, these models most
likely to lack prediction in the cases where drug absorption is affected by other
mechanisms of actions of lipid based drug delivery systems such as lymphatic transport,
or enterocyte based bioavailability enhancement since these models describe only pre-
enterocyte phases. A quantitative model which would predict the lipophilic drug
absorption in a time dependent manner considering all the possible kinetic processes occurring simultaneously based on the underlying mechanisms of lipid based drug delivery systems could significantly streamline the SEDDS formulation process.
3.0 EXPERIMENTAL

One barrier to commercial usage of self-emulsifying drug delivery systems is the lack of information on how these systems “work” to enhance oral bioavailability of drugs which leads to resource intensive obstacles in the drug formulation stage. An improved mechanistic understanding of their performance in vivo together with a modeling approach in order to predict bioavailability may provide guidance for self-emulsifying drug delivery system formulation.

3.1 Formulation and SEDDS Preparation

To study emulsion mechanics and formulation effects on intestinal environment, representative formulations were prepared inside plastic centrifuge tubes. Formulations were left overnight on a shaker, equilibrated for 24 hours and were kept at room temperature until used. Self-emulsifying systems were prepared by warming 10 ml of deionized (DI) water to 37° C in a 15 ml centrifuge tube. 1:100 dilution and 1:1000 dilution emulsions were prepared by introducing 10 or 100 µl of formulation to the aqueous phase and vortexing for 30 seconds. All formulations were prepared three times and assessed initially visually for spontaneous emulsification and uniform fine emulsion formation.

3.1.1 Statistical design of a broad range of formulations

In order to study a wide variety of formulation components and SEDDS characteristics, a $3^3$ factorial experimental design was used to combine oils of three different structural classes and surfactants with three HLB values (ranging from 10 to 15) at three different oil-to-surfactant weight ratios: 9:1, 5:1, 1:1 (Table 2). Naproxen was
incorporated into formulations as the model drug at 3 mg/ml for characterization studies, and at 7 mg/ml for drug release studies.

**Table 2: 3^3 factorial design of SEDDS formulations**

<table>
<thead>
<tr>
<th>SEDDS formulation no.</th>
<th>Oil type</th>
<th>Surfactant HLB</th>
<th>Oil to surfactant ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>long chain triglyceride</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>long chain triglyceride</td>
<td>15</td>
<td>5</td>
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<td>3</td>
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<td>13</td>
<td>5</td>
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<tr>
<td>6</td>
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* Excipients that correspond to above characteristics are as follows; long chain triglyceride: Soybean oil, medium chain propylene glycol diester: Captex 200, Medium chain triglyceride: Neobee M5, surfactant HLB 15: Tween 80, HLB 13: Cremophor EL, HLB 10: 56% Labrasol 44% Capmul

Formulations without drug were prepared for characterization, permeability, and cytotoxicity studies. Each formulation was prepared in polycarbonate 2 ml centrifuge
tubes, vortexed, further mixed using a rotating shaker for three hours at 37\(^{\circ}\) C, and was equilibrated at room temperature for 24 hours before use.

3.1.2 Characterization of emulsions

Droplet size and zeta potential of freshly prepared emulsions was measured using laser light scattering (Brookhaven 90 Plus). Droplet size and zeta potential was measured on three independently prepared formulations, with each measurement averaged from three readings per formulation. All SEDDS formed were observed visually with light microscope for large particles which are not detectable with particle sizing equipment.

3.1.3 Model Drug Compounds

Model drug compounds used in experiments were Naproxen, Lucifer Yellow, Danazol and Tempo Benzoate. Naproxen was used in the initial characterization, permeability and drug release under experiments under sink conditions. Lucifer Yellow was used as a model paracellular drug compound in permeability experiments in order to assess the influence of SEDDS on cell monolayer tight junctions. Danazol was used as a more hydrophobic model drug compound for permeability in the presence of biorelevant media. Lastly, Tempo Benzoate was used due to its spin probe properties in EPR analyses in order to perform combined digestion and release experiments and drug release under non-sink conditions.

3.2 Estimating Kinetic Parameters of Functional SEDDS mechanisms

Key functional mechanisms of SEDDS were proposed to be effect on intestinal drug transport, oil phase digestion, drug releases into aqueous phase, and lymphatic transport. Therefore kinetics of these mechanisms were investigated.
3.2.1 Kinetics of Digestion of Formulations

Kinetics of digestion of formulations was assessed with simulated intestinal digestion conditions in vitro. Maleate buffer containing 100mM tris-maleate, 65 mM NaCl, 5mM CaCl-2H2O at pH 6.5 was prepared as for the digestion buffer. 3mM Sodium azide was added to digestion buffer to prevent bacteria growth. In order to mimic fasted intestinal conditions 5mM NaTDC/1.25mM PC content was included in digestion buffer. Briefly, BS/PL solution was prepared dissolving all of the materials in digestion buffer [91]. The solution was stirred and equilibrated for 12 hours until forming a clear solution. Freshly prepared pancreatin was used to perform formulation digestion. Pancreatin extract was prepared by adding 2 g of porcine pancreatin powder (containing pancreatic lipase and colipase) to 10 ml of digestion buffer and stirring for 15 minutes [91]. Solution was centrifuged at 1600g for 5°C for 15 minutes, supernatant was collected and stored on ice.

18ml of digestion buffer was dispensed into a beaker with a stir bar and 180 µl of formulation was introduced to the solution at 37°C on a heated stir plate and was stirred for 10 minutes at 50 rpm. Digestion of emulsions was initiated with the addition of 2 ml pancreatin extract. Change in pH was monitored with a digital pH stat titration unit. Fatty acids produced by digestion of long chain triglycerides were titrated with 0.2M NaOH whereas fatty acids produced due to digestion of medium chain triglycerides was titrated with 0.6M NaOH. Samples of 200 µl was collected at specific time intervals (0, 1, 2, 3, 4, 5, 10, 20, and 30 min) in polypropylene tubes and digestion was stopped with the addition of enzyme inhibitor, 4-BPB. Enzyme inhibitor was prepared at 0.5M in methanol and was directly added to samples at 9 µl/ml of solution [117].
3.2.1.1 Evaluation of digestion rate constant with a mathematical model of digestion kinetics

Fatty acid production was assessed by measuring total NaOH added to stabilize pH at 6.5 and digestion rate constants were calculated using an equation relating rate of fatty acid production to the droplet surface area available to the enzyme [101]:

\[
\frac{dm}{dt} = k_{dig} A_d V_E
\]

Equation 8

where \( dm/dt \) is the rate of free fatty acids leaving the oil droplets, \( k_{dig} \) is the rate constant, \( A_d \) is the droplet surface area per unit emulsion volume, and \( V_E \) is total aqueous volume (oil emulsions and aqueous phase). It was assumed that the droplet size of oil in water emulsions decreases as digestion proceeds, as fatty acids, products of digestion of oil and surfactant, leave oil droplets. Setting \( m \), total moles of “digestible” fatty acids in the droplet, to \( m=m_0 \) at \( t=0 \), Equation 8 was integrated to give:

\[
\frac{m}{m_0} = \left(1 + \frac{3k_{dig} M t}{n_0 d_0 \rho}\right)^{-2}
\]

Equation 9

where \( m \) is the moles of digestible fatty acids remaining in the droplet, \( m_0 \) is the initial amount of digestible fatty acids in the droplet, \( M \) is the averaged molecular weight of the oil and surfactant, \( \rho \) is the averaged density of the oil, \( n_0 \) is the averaged number of digestible fatty acids per oil and surfactant molecule, and \( d_0 \) is the initial emulsion droplet size. \( \frac{dm}{dt} \) was determined experimentally by rate of fatty acid formation from neutralization reaction and \( k_{dig} \), rate of digestion constant was calculated for each formulation seperately.
3.2.1.2 Quantifying rate of fatty acid formation based on acid neutralization

Digestion products of hydrolysis of emulsion droplets are fatty acids of carboxylic acid group. These acids react with the titrant NaOH at 1 to 1 molar ratio. Therefore fatty acid formed for each time interval was determined with the following equation.

\[ M = C_i V \]  \hspace{1cm} \text{Equation 10}

where \( M \) is released fatty acid amount, \( C_i \) is the titrant concentration, \( V \) is the total titrant volume added, and \( t \) is titration time. Based on the assumption of hydrolysis of one molecule triglyceride yields to 2 molecules of fatty acid and 1 molecule of monoglyceride (which then forms 2-monoglyceride) \([18]\), hydrolysis of 1 mol triglyceride correspond to formation of 2 moles of fatty acids and as a result consumption of 2 moles NaOH.

3.2.1.3 Monitoring the phases during digestion

Rate of digestion was correlated with substrate’s and products’ physical state \([102]\) and formulation derived digestion products are believed to play a significant role in the solubilization behavior of the administered drug \([108]\). Therefore phases present before, was assessed using (1) dynamic light scattering and (2) small angle neutron scattering.

3.2.1.4 Droplet size and zeta potential analysis

Samples taken at specific time intervals were analyzed for change in the mean droplet diameter using dynamic light scattering. (Brookhaven 90 Plus). 200 µl samples were collected and mixed with enzyme inhibitor inside plastic cuvettes. Mean droplet size was measured with each measurement averaged from three readings per sample.
3.2.1.5 Small angle neutron scattering analysis

Fasted state biorelevant media and self-emulsifying drug delivery systems in biorelevant media were investigated using Small-Angle Neutron Scattering in order to gain more insight into size, structure and shape of colloidal species that exist in these complex fluids. Information regarding size and shape was extracted from scattering data. Fasted state biorelevant media was prepared in D$_2$O instead of H$_2$O, and pure Triolein was used as the oil component of formulations instead of Soybean oil in order to eliminate impurities that might originate from Soybean oil. Measurements were performed over a Q range of 0.0018 to 0.045 Å$^{-1}$. Experiments were performed in the High Flux Isotope Reactor (HFIR) facility at Oak Ridge National Laboratory, Oak Ridge, TN.

3.2.2 Kinetics of Drug Release from Formulations

Drug release from emulsions was assessed considering two conditions; 1) In the absence of digestive enzymes and 2) in the presence of digestive enzymes.

3.2.2.1 Drug release in the absence of digestive enzymes

In the absence of digestive enzymes drug release experiments were conducted separately under sink conditions and non-sink conditions which more closely mimics physiological conditions. Under sink conditions, a regenerated cellulose dialysis membrane (Fisherbrand, MWCO 3500) with 3 ml volume was used to carry release experiments. Emulsion formulations containing model drug compound Naproxen were introduced inside the tubular membrane at a formulation to PBS ratio of 1:100. The dialysis membrane then was immersed in 35 ml PBS solution in a beaker that served as the receiver fluid maintaining sink conditions. A stir bar was used at a mixing speed of
350 rpm to enable mixing inside the beaker, and a hot plate was used to maintain the temperature inside the beaker at 37°C. Samples were taken every 15 minutes for three hours from the receiver compartment and replaced with fresh PBS. Samples were analyzed using UV spectrophotometry drug compound content.

The rate of drug release from emulsions, characterized by the release coefficient \( k_{\text{eff}} \), was calculated using the mass transfer equations

\[
\ln \left(1 - \frac{M_t}{M_0}\right) = 3 * k_{\text{eff}} * t / r^2 \quad \text{Equation 11}
\]

and

\[
\frac{dM_R}{dt} = \rho * (C_w - C_{w,\text{OUT}}) \quad \text{Equation 12}
\]

where \( M_t/M_0 \) is the fraction of the released drug at time \( t \), \( M_R \) is the drug amount inside the receiver fluid outside the dialysis membrane, and \( C_{w,\text{OUT}} \) is the concentration of drug in the receiver fluid measured throughout the release experiment as described above. The parameter \( r \) in Equation 11 the oil droplet radius, calculated from the average particle size of the emulsions formed at 1:100 ratio in PBS, assuming spherical shape. In order to calculate the concentration of free drug in solution inside the membrane \( (C_w) \), the permeability of the membrane to free drug, was be measured using a drug release experiment (with no emulsion) together with Equation 12. Briefly, 3 ml of 0.14 mg/ml drug solution in PBS was placed inside a dialysis membrane immersed in 35 ml PBS, as described above, and samples were taken from the receiver compartment every 15 minutes for 3 hours.

In order to calculate the fraction of drug released from oil droplets at time \( t \), drug concentration in the aqueous phase inside the dialysis membrane, meaning both micelles
and water phase, was calculated using $C_w$ and an experimentally determined partition coefficient $K_m$ [118],

$$K_m = \frac{C_m}{C_w}$$  \hspace{1cm} \text{Equation 13}

where $C_m$ is the drug concentration in the micellar phase and $C_w$ is the drug concentration in water. Briefly, 7 mg/ml drug was dissolved in surfactant and 60 ul of surfactant containing drug will was mixed with 3 ml PBS inside of a dialysis membrane. The membrane then was immersed in a PBS receiver compartment as described above for release experiments. A sample was taken after 24 hours from the receiver compartment and was used to calculate $C_w$. $C_m$ then was calculated by mass balance and used to determine the partition coefficient. Drug partitioning into micelles was assumed to be instantaneous.

Micelle volume in each emulsion system, required to calculate the partition coefficient, was measured by a previously established method [65]. Emulsions with Tween 80 was centrifuged for 20 minutes through nanoporous regenerated cellulose centrifuge filters (Amicon Ultra, Millipore Corp, Billerica, MA) of molecular weight cut off 100,000. For emulsions of Cremophor EL, an ultracentrifugation unit (Amicon 8400, Millipore Corporation) and a membrane of 500,000 molecular weight cut off was used. Micelle molecular weight, calculated from the aggregation number and the monomer molecular weight, was used to select the membrane molecular weight cut off that allows micelles but not oil droplets to pass through the membranes [119]. The filtrates from both centrifugation and ultracentrifugation processes containing the micelles and surfactant monomers were analyzed using a methylene blue complexation method [120].
percentage of surfactant introduced that formed micelles and monomers (as opposed to that associated with emulsion droplets) were calculated.

Under non-sink conditions, drug release tests were performed in a beaker on a hot stirring plate, with stirring speed of 180 rpm. 400 µl of formulation including 50 mg/ml Tempo Benzoate was added into 40 ml fasted state biorelevant media. Over time, 2 ml samples were taken from the beaker, filtered through 0.1 µm Ptf filters in order to separate aqueous solution containing micelles from formulation emulsion droplets. Filtered solution was analyzed using HPLC for Tempo Benzoate content. Error was calculated using two independent measurements. Drug release rate was calculated based on expression by Higuchi et al. [76] considering interfacial resistance at the oil-water interface as a limiting barrier to compound transport:

\[ \frac{dC_{D,aq}}{dt} = -\frac{A_{em} P_{rel}}{V_{aq}} (C_{D,aq} - C'_{D,aq}) \]  \hspace{1cm} \text{Equation 14}

Here \( C_{D,aq} \) is the drug concentration in the aqueous media (buffer and micelles) and outside of oil droplets, \( V_{aq} \) is the volume of the aqueous media, \( P_{rel} \) is the permeability constant for the oil water interface, \( A_{em} \) is the total interfacial surface area of the oil droplets, and \( C'_{D,aq} \) is the hypothetical aqueous concentration of the solute in equilibrium with compound concentration inside emulsion droplet, \( C_{D,em} \). \( C'_{D,aq} \) is defined by the formulation-aqueous phase partition coefficient, \( K \), as;
$$K = \frac{C_{D_{em}}}{C_{D_{aq}}}$$  
\[Equation 15\]

### 3.2.2.2 Drug release during in vitro formulation digestion

Experiments were performed for combined drug release and digestion experiments in order to assess drug partitioning into aqueous phase in the presence of digestive enzymes. In order to measure drug concentration in aqueous phase, $C_{aq}$, in vitro digestion experiments were performed using formulations containing Tempo Benzoate as model drug compound. Samples collected at different time points throughout digestion were analyzed immediately by Electron Paramagnetic Resonance Spectroscopy (EPR) [84] in order to quantify model drug compound, spin probe, in the aqueous phase (micelles and vesicles excluding oil droplets), $C_{aq}$ over time. This technique enables an online study on the distribution of the drug between water, oil and colloidal species in aqueous phase. In an effort to perform real time quantitative non-invasive tracking of drug partitioning and drug release into colloidal phases during in vitro digestion, electron paramagnetic resonance (EPR) (9.1-9.9 GHz, X-Band; Bruker EMX) was used to track the model drug (spin probe: Tempo Benzoate). In order to simulate the digestion process in vitro, lipolysis experiments were conducted in biorelevant simulated intestinal fluid containing 100 mM tris-maleate, 65 mM NaCl, 5mM CaCl-$2H_2O$ at pH 6.5; with the addition of 5 mM NaTDC/1.25 mM PC solution (digestion buffer) mimicking fasted intestinal conditions. Formulations including spin probe Tempo Benzoate were added to 18 ml digestion buffer at 1:100 ratio and digestion was started with the addition of 2 ml pancreatin extract. Change in pH was monitored with a pH meter. Fatty acids produced due to digestion of long chain triglycerides were titrated with NaOH. Samples were
collected at specific time intervals (0, 5, 20, 50 minutes), the digestion process was terminated by the addition of enzyme inhibitor, 4-BPB (4-bromophenacyl bromide) and samples were analyzed by EPR.

3.2.2.3 Solubility measurements

Solubilities of Tempo Benzoate (TB) and Danazol were measured separately by adding excess amount of compound into each phase, formulation and maleate buffer, and fasted state biorelevant media. Samples were stirred at 37°C for 72 hours on a hot plate. After 72 hours, 2 ml samples of aqueous solutions were centrifuged at 1700 g and 37°C for 30 minutes, and formulation was centrifuged at 16100 g for 30 minutes using Eppendorf Microcentrifuge 5415R (Hauppauge, NY). The aliquots were filtered through 0.45 µm nylon filters, and was analyzed by HPLC for compound content. Solubility of Danazol in mixed BS/PL, Tween 80 micelles was measured by adding excess amount of compound in fasted media with emulsified formulation at 1:100 ratio. Samples were taken upon 24-hour equilibration, filtered through 0.1 µm (Pttf Polytetrafluoroethylene) filters in order to separate aqueous solution containing micelles from formulation emulsion droplets. Drug content was analyzed using HPLC.

3.2.2.4 Estimation of Tempo Benzoate Diffusion Coefficient

In order to simulate solid dosage form oral drug dissolution, Noyes-Whitney equation [121] was utilized:

$$\frac{dC_{D,aq}}{dt} = \frac{A_p}{h} D_D \left(C_{sol} - C_{D,aq}\right)$$

Equation 16

where $C_{D,aq}$ is the concentration of drug dissolved in bulk aqueous phase, $A_p$ is the surface area of dissolving drug particle, $h$ is the stationary diffusion layer around
dissolving particle, $D_D$ is the drug diffusion coefficient in water, and $C^s_{D_{aq}}$ is the drug solubility in water. Drug diffusion coefficient of Tempo benzoate, $D_D$, was estimated using Wilke-Chang equation [122].

$$D_{AB} = 7.4 \times 10^{-8} \sqrt[0.6]{\frac{\Psi_B MW_B}{\eta_B V_A}} T \quad \text{Equation 17}$$

where $D_{AB}$ is the diffusivity of compound A in solvent B (cm$^2$/s), $\Psi_B$ is the constant which accounts for solvent/solvent interactions (2.6 for water), $T$ is temperature (K), $\eta_B$ is the viscosity of solvent B (cP), $MW_B$ is molecular weight of solvent B (g/mol), and $V_A$ is the molar volume of compound A.

### 3.2.3 Intestinal Absorption with SEDDS Formulations

Possible intestinal absorption enhancement of drugs incorporated with SEDDS was assessed with (1) Cytotoxicity measurements, (2) Drug transport studies and, (3) Transepithelial Electric Resistance (TEER) Measurement Studies.

#### 3.2.3.1 Cytotoxicity Measurements

Toxicity of formulations on Caco-2 cell monolayers was tested with the MTT toxicity assay. Cells was seeded on 96 well cell culture plates at a seeding density of $2.5 \times 10^5$ cells/ml in 200 µl medium 7 days prior to the cytotoxicity assay. Medium was changed every 3 days. On the eighth day, the cell culture medium was removed, and cells were rinsed with HBSS twice and incubated with each of the 27 emulsion formulations for three hours at 37$^0$ C. Three wells were used to test each formulation. Emulsion systems were prepared at a 1:100 formulation-to-buffer ratio without the model drug (Naproxen) in HBSS. After three hours, wells were rinsed twice with HBSS and then incubated with 3-4,5-Dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT
substrate) solution at 10µl/100µl HBSS for three hours. MTT solubilization solution then
was added to solubilize any formazan crystals formed. After 12 hour incubation, UV
absorbance of wells was measured at 570 nm. Wells incubated with HBSS only was used
as negative control and was assumed to result with 100% cell viability. 0.1% Sodium
dodecyl sulfate (SDS) solution was used as a positive control. Percent viabilities were
determined relative to the negative control.

Cytotoxicity of biorelevant media contents and formulations on Caco-2 viability
due to 20 hour incubation required for lymphatic transport assessment was investigated
similar to as described above with MTT toxicity assay. On the fifth day, the cell culture
medium was removed, and cells were rinsed with HBSS twice and incubated with each of
the 18 emulsion formulations prepared at 1:1000 dilutions in DMEM or biorelevant
media for 20 hours at 37 °C. Three wells were used to test each formulation. After 20
hours, wells were rinsed twice with HBSS and then incubated with MTT substrate
solution at 10 µl/100 µl HBSS for three hours. MTT solubilization solution was then
added to solubilize any formazan crystals formed. Following incubation, UV absorbance
of wells was measured at 570 nm. Wells incubated with HBSS only were used as
negative control. 0.1% Sodium dodecyl sulfate (SDS) solution was used as positive
control. Percent viabilities were determined relative to the negative and positive controls.

3.2.3.2 Drug transport measurements across Caco-2

Transport across intestinal epithelial cells was tested using model drug
compounds Naproxen and Lucifer Yellow. Cells was seeded at 2 x 10^6 cells/ml and
cultured on 24 well plate Transwell® permeable supports (0.4 um pore size) for 27 days.
Cell culture medium was changed every other day. On day 27, cell culture medium was
removed from both the apical and basolateral compartments, and cells were rinsed once with HBSS. Apical compartment solution was replaced with emulsion in HBSS (with no drug), and basolateral compartment solution was replaced with HBSS. Emulsion systems were prepared at 1:100 dilution with the model drug in HBSS. Blank HBSS and 0.1% SDS served as the negative and positive controls, respectively. Cells were exposed to emulsions for 3 hours inside the incubator. After three hours, samples were taken from each compartment and analyzed for drug content using a UV spectrophotometer.

Influence of the formulations on the paracellular drug transport was tested following the same procedure with flourescence drug Lucifer Yellow and drug content was measured using a fluorescence plate reader at 485 nm excitation and 528 nm emission wavelengths.

Similar to above described protocol, transport studies across cell culture membrane was carried out separately dosed in the biorelevant media and including model drug Danazol. Cells ranging in passage number 44-46 were seeded at 6x10^4 cells per cm^2 and cultured on 12 well plate Transwell® permeable supports (0.4 um pore size) for 27 days. Cell culture medium was changed every other day. On day 27, cell culture medium was removed from both the apical and basolateral compartments, and cells were rinsed twice with HBSS. Apical compartment solution was replaced with biorelevant medium containing drug compound Danazol in DMEM with 1% antibiotics, and basolateral compartment solution was replaced with 4% Bovine serum albumin in HBSS. Biorelevant medium was 2.5mM Sodium taurodeoxycholate (NaTDC) and 1.25mM L-α-Phosphatidylcholine (PC) in 1% antibiotics in DMEM. Danazol in 1% antibiotics in DMEM served as control. Cells were exposed to drug solutions for 2 hours inside the
incubator. After 2 hours, samples were taken from each compartment and analyzed for Danazol content using HPLC (Shimadzu, Japan) with a diode array detector at 287 nm wavelength. The column used was Agilent Zorbax RX-C18 4.6 × 75 mm, 3.5 µm. The column temperature was maintained at 40°C, and the flow rate was 1 mL/min. The mobile phase contained distilled water with 0.15% Trifluoroacetic acid (TFA) and methanol, and the gradient method was 90:10 to 10:90 (TFA:methanol) over 12 min.

Transport studies were combined with Trans Epithelial Electrical Resistance (TEER) measurements before and after exposure to formulations (EVOM, World Precision Instruments, Sarasota, FL) in order to assess effect of formulations on cell monolayer integrity. Apparent permeability coefficient, Papp, of drug for each formulation was calculated according to the following equation [123]:

$$P_{app} = \frac{(dQ/dt)(1/AC_0)}{dQ/dt}$$

Equation 18

where $dQ/dt$ is the steady-state flux, $A$ is the surface area of the membrane, and $C_0$ is the initial drug concentration in the donor compartment.

3.2.4 Evaluation of Lymphatic Transport

In vitro lymphatic transport studies were performed in order to gain qualitative understanding of formulation influence on lymphatic drug transport. Potency of self-emulsifying drug delivery systems to alter lymphatic transport was assessed by quantification level of Apolipoprotein B secreted by cells upon incubation with formulations. Lipophilic drug associates with the triglyceride lipid core of the lipoproteins, and it has been suggested that amount of drug transported through the lymphatic pathway is proportional to the amount of lipoprotein secreted by intestinal cells [88]. ApoB incorporates into triglyceride rich lipoproteins (TRL) in a 1:1 ratio, and hence
presents a suitable means of quantifying the number of secreted lipoproteins and the triglyceride.

Cells ranging in passage number 48-50 were cultured on 12 well polycarbonate 0.4 µm pore size membranes, Corning® permeable Transwell® inserts, to form differentiated monolayers. Cells were seeded at 2.6x10⁵ cells/cm². Cell culture medium was changed every 3 days [124]. Sensitivity of differentiated Caco-2 monolayers to digesting oils was compared with 2 h in vitro digestion with pancreatin in the simulated intestinal media previously [88]. It was shown that overall fatty acid production via in vitro 2 h digestion of lipid based formulation components with pancreatin was comparable to fatty acid production at the end of 20-hour incubation of formulations on Caco-2 monolayers [88]. On day 27 of culture, monolayers were incubated with 1:1000 dilution formulations in DMEM containing 1% antibiotic for 20 hours. Samples taken from basolateral compartment were analyzed for Apolipoprotein B (ApoB) content using an ELISA kit (Bioo Scientific, Austin, TX). 250 µl samples were collected from the basolateral compartment at the end of 20-hour incubation with emulsions and centrifuged at 3,000 x g for 10 minutes at 4°C [88, 89]. 200 µl samples were taken from the upper layer of the supernatant, and were applied (100 µl per well) to an antibody coated 96-well plate in duplicate. 0.1% Pluronic L 81, a surfactant which was shown to inhibit ApoB secretion [68], biorelevant media, and DMEM were used as controls.

3.3 Incorporation of kinetic parameters into a model predicting oral bioavailability

A model incorporating fundamental processes in the GI tract namely; emulsion formation, emulsion digestion, drug release from emulsions, permeation enhancement
and lymphatic transport was developed to predict the influence of emulsions on drug absorption.

The model simulates the processes occurring when an oil/surfactant/drug mixture is dosed to the GI tract. Kinetic parameters that pertain to process differential equations were calculated based on the mechanisms described above. A pharmacokinetic model was incorporated to enable bioavailability prediction.

Process differential equations, and mass balances, were incorporated into MATLAB® code and was solved using a built in ordinary differential equation solver, ODE45, in Matlab® that uses Runge-Kutta numerical solution technique. The program input was physical and chemical properties of the drug and emulsion system as well as assumed properties of the biological environment. Program output was the concentration of absorbed drug as a function of time as well as drug in the intestinal lumen, either emulsion associated or free drug.

3.4 Comparison of experimental values with model predictions

It is important to test combined digestion and absorption experiments since combination and interaction of these processes may have an interaction with each other. In vitro experiments were run to test model predictions of emulsion system influence on bioavailability. Model predictions for drug release in the presence of digestion were compared with combined release and digestion experiments analyzed by EPR as described in Section 3.2.2.2. Experimental aqueous drug concentration, $C_{D,aq}$, values obtained using EPR during digestion were compared with simulation results for $C_{D,aq}$ over time upon 7 mg/ml drug load in formulation.
4.0 RESULTS AND DISCUSSION

4.1 Preparation and Characterization of Emulsions

In order to study a wide variety of formulation components and SEDDS characteristics, a $3^3$ factorial experimental design was used to combine oils of three different structural classes and surfactants with three HLB values (ranging from 10 to 15) at three different oil-to-surfactant weight ratios: 9:1, 5:1, 1:1 (Table 3). Naproxen was incorporated into formulations as a model drug at 3 mg/ml for characterization studies, and at 7 mg/ml for drug release studies. Formulations without drug were prepared for characterization, permeability, and cytotoxicity studies. Each formulation was prepared in polycarbonate 2 ml centrifuge tubes, vortexted, further mixed using a rotating shaker for three hours at $37^\circ$C, and equilibrated at room temperature for 24 hours before use.

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<tr>
<td>Neobee M5</td>
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</table>

For testing of ability to emulsify and emulsion characterization, 10 ml of deionized (DI) water was warmed to $37^\circ$C in a 15 ml centrifuge tube. 1:100 dilution and 1:1000 dilution emulsions were prepared by introducing 10 or 100 µl of formulation to the aqueous phase and vortexing for 30 seconds. All formulations were prepared three times and assessed visually for spontaneous emulsification and uniform fine emulsion
formation. Droplet size and zeta potential of freshly prepared emulsions were measured using laser light scattering (Brookhaven 90 Plus). Droplet size was measured on three independently prepared formulations, with each measurement averaged from three readings per formulation, whereas zeta potential was analyzed on two independent formulations, with each measurement averaged from four readings per formulation.

The visual observation upon gentle mixing of formulations in aqueous media indicated that the HLB value of surfactant is highly important for formation of a fine, uniform emulsion. Formulations containing surfactant with the lowest HLB value tested (10) did not result in uniform emulsifications. Surfactants that have been used for formulating SEDDS generally range in HLB value from 4 to 15, but it has been previously suggested that surfactants should have a relatively high HLB for spontaneous oil in water emulsion formation [5]. In a study utilizing HLB 10 surfactant, Labrafac CM 10, good emulsification was possible only with a surfactant ratio higher than 50% (1:1) [43]. Our findings imply that for a reasonable oil-to-surfactant ratio (≥ 1:1), a surfactant with HLB value greater than 10 would be necessary for fine and uniform self emulsification. It should be noted that head group structure, in addition to HLB, may play a role in the emulsification process [5]. The formulations that did not spontaneously form emulsions upon gentle mixing were not further analyzed in the permeability enhancement and drug release studies. All studied systems that did form visibly uniform emulsions, including different formulations and different dilutions in aqueous media, contained droplet sizes in the 100 to 500 nm range (Table 4). Formulations that did not form uniform fine emulsions had larger droplet sizes, with measured averages in the range of hundreds of nanometers to several microns. However, data pertaining to these
systems are not reported, since samples taken from these solutions are not representative of the entire system due to lack of uniformity, and the light scattering instrument used accurately measures particle sizes only up to 6 microns.

All three formulation design parameters (oil type, HLB value, oil-to-surfactant ratio) had strong direct or interaction influences on emulsion mean droplet size. Regression analysis yielded good predictive models (Table 5) for mean droplet size for both formulations with drug \( p < 0.0005 \), adjusted \( R^2 = 0.444 \) and without drug \( p < 0.0005 \), adjusted \( R^2 = 0.709 \). For 1:1000 dilution emulsions in DI water with drug, an analysis of variance (Table 6) indicated that the primary factors affecting mean droplet size were oil-to-surfactant ratio \( p = 0.007 \), oil type \( p = 0.005 \), and surfactant-ratio interaction \( p < 0.001 \), but interestingly not surfactant alone \( p = 0.251 \). A higher oil-to-surfactant ratio resulted in a larger mean droplet size, with post-hoc pairwise comparisons indicating that mean droplet sizes from the 9:1 oil-to-surfactant ratio systems are significantly larger than those from the 1:1 ratio systems \( p = 0.005 \). This relationship agrees with previous findings where a medium chain triglyceride oil and Tagat TO, an HLB 11.5 surfactant, were combined at 9 different surfactant concentrations between 30% and 70% [40]. However, an increase in mean droplet diameter once the surfactant concentration exceeded 60% was reported. The effect of surfactant concentration on droplet size has been attributed to viscous gel formation above a certain surfactant level and a consequent retarding of self emulsification [39].
<table>
<thead>
<tr>
<th>Oil</th>
<th>Surfactant</th>
<th>Weight ratio</th>
<th>1:1000 dilution with 3 mg/ml drug in DI water</th>
<th>Mean</th>
<th>S.D.</th>
<th>1:1000 dilution without drug in DI water</th>
<th>Mean</th>
<th>S.D.</th>
<th>1:100 dilution without drug in HBSS</th>
<th>Mean</th>
<th>S.D.</th>
<th>1:50 dilution with 7 mg/ml drug in PBS</th>
<th>Mean</th>
<th>S.D.</th>
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<td></td>
<td>443.6</td>
<td>141.1</td>
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</table>

a Each measurement was repeated with three independent samples. M.D. indicates mean diameter and S.D. indicates standard deviation. Droplet size measurements of formulations with HLB 10 surfactant, which did not spontaneously emulsify, are not reported here.
The influence of oil appears to be related to oil lipophilicity, which is related to the fatty acid carbon chain length of the oil (Figure 6). The oil studied with the longest carbon chain length, Soybean oil (C16-C22), had a significantly larger mean particle diameter ($p = 0.012$) than Captex 200 and Neobee M5 as indicated by Tukey’s multiple comparison post-hoc tests. Conversely, medium chain triglyceride, Neobee M5, and Captex 200, which has a carbon chain length similar to that of Neobee M5, both showed similar effects on droplet size. This relationship agrees with the results of James-Smith et al [65], who correlated carbon chain length with mean emulsion diameter. Their studies, conducted with SDS as the surfactant and alkane oils ranging in carbon chain length from 8 to 16, also showed that droplet size is largest for systems containing oil with chain length closest to that of the surfactant. Surfactants utilized in our formulations, Tween 80 and Cremophor EL, have alkyl carbon chain lengths of 17 and 18, respectively, which are in the range of Soybean oil chain lengths [125]. The phenomenon occurring might be low levels of partitioning of the surfactant at the oil water interface as a result of the low driving force, which would be the difference in carbon chain lengths between surfactant and oil. It has been proposed that oil with carbon chain length similar to that of surfactant penetrates more into the interfacial film, decreasing surfactant partitioning and droplet stabilization [126].
Figure 6: Droplet size of 50% surfactant concentration formulations with drug in DI water at 1:1000 dilution. For all oils, particle size is larger for formulations with Tween 80 (HLB 15) and smaller for formulations with Cremophor EL (HLB 13). Droplet size of the emulsion system with the most lipophilic triglyceride (soybean oil) was the largest for both surfactants ($p = 0.012$, Tukey post-hoc test). Error bars indicate standard deviation of three independent measurements. '*' shows significance compared to other values (without **).

Our results also graphically suggest that for each oil type, particle size tends to be smaller for formulations with HLB 13 surfactant (Cremophor EL) compared to HLB 15 surfactant (Tween 80) (Figure 6). It was somewhat surprising, however, that there was not significant dependence of particle size on surfactant HLB over the 13 to 15 range ($p = 0.251$), given the inability of low HLB surfactant to spontaneously form emulsions, although this may be due to the narrow remaining test range (13 to 15 HLB) or the low statistical power of 3 measurements.

For systems without drug formed with 1:1000 dilution in water, factors demonstrating statistically significant influences (ANOVA) on droplet size were oil-to-
surfactant ratio (\( p = 0.034 \)), oil type (\( p < 0.001 \)), surfactant HLB (\( p < 0.001 \)), oil-
surfactant interaction (\( p = 0.018 \)), and oil-ratio interaction (\( p = 0.037 \)) (Table 5). Tukey’s
post-hoc tests again indicated that mean diameters of 1:1 oil to surfactant ratio systems
were significantly smaller compared to 9:1 ratio emulsions (\( p = 0.032 \)), agreeing with the
above results for 1:1000 dilution emulsions in DI water with drug. Mean droplet size of
formulations containing Captex 200 was smaller than that of systems containing other
oils (\( p < 0.0005 \)), again indicating that a shorter oil carbon chain length relative to
surfactant may contribute to a smaller droplet size.

Overall, formulations with drug had slightly smaller mean droplet size compared
to those without drug at 1:1000 dilutions in DI water. This can be attributed to the drug
partitioning at the oil water interface. Naproxen has a logP of 3.29 and is thus relatively
hydrophobic, but may still partition between the oil and aqueous layer along with the
surfactant due to limited solubility in oil. Therefore a decrease in interfacial tension
might occur that leads to an overall decrease in droplet size. Another potential reason for
the change in droplet size may be the change in surfactant critical micelle concentration
(CMC) due to addition of drug, which can increase surfactant aggregation number and
decrease CMC [127]. At the same surfactant concentrations, systems with lower CMC
values tend to have smaller droplet size [128]. The opposite effect of drug, however, has
been observed for some systems. When Simvastin concentration was increased from
2.4% to 18.3% in formulations containing Capryol 90, Lauroglycol 90, and Cremophor
EL, an increase in droplet size from 33 nm to 150 nm was observed [9]. These
phenomena are likely drug dependent, and drugs with a wider range of properties need to
be studied to better understand the influence of drug on emulsion characteristics.
Particle sizes of systems prepared with 1:100 dilution in HBSS without drug and 1:50 in PBS with 7 mg/ml Naproxen are also summarized in Table 4. The 1:100 dilution emulsions in HBSS without drug were used to test the influence of emulsions on intestinal monolayer permeability, and 1:50 dilution formulations in PBS with 7 mg/ml Naproxen were used to test drug release kinetics. Droplet sizes of 1:1000 dilution systems with no drug in DI water appear similar to those of 1:100 dilution systems with no drug in HBSS, indicating a lack of impact on particle size of a range of dilution factors typically observed in the literature. The 1:50 formulations have larger mean emulsion particle size than emulsions prepared at greater dilution levels, possibly related to the increase in drug concentration or the decrease in dilution factor.

Zeta potential measurements of emulsions confirmed the stability of the systems and ranged from approximately -18 mV to -41 mV for all systems except for 1:50 dilution formulations in PBS with 7 mg/ml drug concentration (Table 7). For these systems, zeta potential ranged from -6 to -15 mV and was generally of smaller magnitude compared to those of other systems. This decrease in zeta potential magnitude can be correlated with the comparatively larger droplet sizes as well as the increased drug concentration of these same systems. Surface charge of the droplets was not statistically related to any of the formulation parameters ($\rho = 0.424$); the regression model therefore is not shown in Table 5.
Table 5: Linear regression analysis of formulation design variables and emulsion characteristics

<table>
<thead>
<tr>
<th>Response</th>
<th>$Y_i$</th>
<th>Model</th>
<th>$p$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet size</td>
<td>Droplet size of 1:1000 dilution emulsions in DI water with drug in nm</td>
<td>$Y_1 = 797.2 - 138.5*A_1 + 43.9*A_2 - 279.6*B$ - $245.5*C + 62.2*A_1*C + 62.9*A_1*B - 24.8*A_2*B - 3*A_2*C + 121*C*B$</td>
<td>&lt;0.0005</td>
<td>0.444</td>
</tr>
<tr>
<td></td>
<td>Droplet size of 1:1000 dilution emulsions in DI water without drug in nm</td>
<td>$Y_2 = 677.3 - 59.9*A_1 - 137*A_2 - 229.2*B - 35.4*C - 39.2*A_1*C + 88.4*A_1*B - 76.3*A_2*C + 121.4*A_2*B + 30.9*C*B$</td>
<td>&lt;0.0005</td>
<td>0.709</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>Percentage cell viability</td>
<td>$Y_3 = 80.7 - 11.8*A_1 + 1.7*A_2 + 17.5*B + 30.6*C - 0.4*A_1*B + 4.3*A_1*C + 9.8*A_2*B - 7.6*A_2*C - 21.2*B*C$</td>
<td>&lt;0.0005</td>
<td>0.466</td>
</tr>
<tr>
<td>Permeability</td>
<td>Percentage Naproxen permeation across cell monolayer</td>
<td>$Y_4 = 35.8 + 3.7*A_1 - 0.9*A_2 - 4.6*B - 3.4*C - 1.6*A_1*B + 3*A_1*C + 3.5*A_2*B - 0.9*A_2*C + 2.2*C*B$</td>
<td>0.028</td>
<td>0.289</td>
</tr>
<tr>
<td>Release Coefficient</td>
<td>Release constant of Naproxen in nm$^2$/min</td>
<td>$Y_5 = 2300.1 - 1793.6*A_1 - 799.5*A_2 + 542.7*B - 28.6*C + 1267.3*A_1*B + 52.9*A_1*C - 691.*A_2*B + 498.3*A_2*C - 193.8*C*B$</td>
<td>&lt;0.0005</td>
<td>0.594</td>
</tr>
</tbody>
</table>

$^b$ Independent variables are: $A_1$: Soybean Oil, $A_2$: Captex 200; $B$: Surfactant HLB; $C$: Oil to surfactant ratio. Oil type is coded as (0,1) indicator variables, with Soybean oil included by setting $A_1=1$ and $A_2=0$, Captex 200 included by setting $A_1=0$ and $A_2=1$, and Neobee M5 included by setting $A_1=0$ and $A_2=0$ (note only one oil type can be included in any given model). Zeta potential and integrity were statistically insignificant and are not included in the table.
Table 6: Analysis of variance of formulation design parameters affecting emulsion performance.

<table>
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<tr>
<th>Response</th>
<th>Main effects</th>
<th>Interactions</th>
</tr>
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<tr>
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<td>$P$</td>
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<td>Droplet size without drug</td>
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</tr>
<tr>
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Table 7: Surface charges of emulsion droplets

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<th>1:1000 with 3 mg/ml drug in DI water</th>
<th>1:1000 without drug in DI water</th>
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<th>1:50 with 7 mg/ml drug in PBS</th>
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<td>Mean</td>
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<td>7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:1</td>
<td>-26</td>
<td>2.9</td>
<td>-33.6</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Tween 80</td>
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<td>17.4</td>
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<tr>
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<td>13.6</td>
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<tr>
<td></td>
<td></td>
<td>5:1</td>
<td>-20.3</td>
<td>5.7</td>
<td>-31</td>
<td>16.5</td>
</tr>
</tbody>
</table>

c Each measurement is repeated with two independently prepared samples. M.Z. indicates mean zeta potential value and S.D. indicates standard deviation. Zeta potential measurements of formulations with HLB 10 surfactant, which did not spontaneously emulsify, are not reported here.
4.2 Effect of emulsions on Caco-2 cell monolayer cytotoxicity and permeability

Since intestinal permeability enhancement sometimes can be attributed to damage to the intestinal epithelial barrier, Caco-2 cell monolayers were used to test the cytotoxicity of the formulations. Toxicity of formulations on Caco-2 cell monolayers was tested with the MTT toxicity assay. Cells were seeded on 96 well cell culture plates at a seeding density of $2.5 \times 10^5$ cells/ml in 200 µl medium 7 days prior to the cytotoxicity assay. Medium was changed every 3 days. On the eighth day, the cell culture medium was removed, and cells were rinsed with HBSS twice and incubated with each of the 27 emulsion formulations for three hours at 37°C. Three wells were used to test each formulation. Emulsion systems were prepared at a 1:100 formulation-to-buffer ratio without the model drug (Naproxen) in HBSS. After three hours, wells were rinsed twice with HBSS and then incubated with 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT substrate) solution at 10µl/100µl HBSS for three hours. MTT solubilization solution then was added to solubilize any formazan crystals formed. After a 12 hour incubation, UV absorbance of wells was measured at 570 nm. Wells incubated only with HBSS were used as a negative control, assumed to have 100% cell viability. 0.1% Sodium dodecyl sulfate (SDS) solution was used as a positive control. Percent viabilities were determined relative to the negative control.

The surfactant seems to be the major factor in cytotoxicity either as a main or interaction effect, with cytotoxicity in the MTT assays affected by surfactant type ($p < 0.001$), oil-to-surfactant ratio ($p < 0.001$), and surfactant-ratio interaction ($p < 0.001$). Cells exposed to formulations composed of 1:1 ratio mixture surfactant (56% Labrasol, 44% Capmul MCM), regardless of the oil type, showed significant cytotoxicity ($p <$
0.001) after three hours of exposure compared to the negative control (Figure 7). The significant toxicity of the unstable formulations (HLB 10 surfactant at 1:1 oil-to-surfactant ratio (but not the higher ratios)) implies that emulsion stability might be an important factor in maintaining cell viability. The cytotoxicity also might be due, however, to the toxic nature of the specific surfactant mixture, as suggested by the statistically significant surfactant-ratio interaction above. This was investigated by exposing Caco-2 monolayers to single surfactant solutions in buffer (data not shown). Surfactant mixtures of Labrasol and Capmul MCM showed higher cytotoxicity levels compared to other surfactants; however, even though the concentrations of surfactant were higher in solution in these experiments than in tests of emulsion cytotoxicity, surfactant alone did not decrease cell viability as much as emulsion formulations with surfactant at 1:1 ratio. Testing all formulation components individually on Caco2 was not possible since oils without surfactant do not form a uniform mixture in the aqueous cell culture medium.
Changes in intestinal permeability were assessed by measuring drug transport across epithelial cell layers, and epithelial tight junction integrity was assessed by measuring transepithelial electrical resistance (TEER). Two model drugs were used in permeability assessment: Naproxen and Lucifer Yellow. Lucifer Yellow is known to be transported by the paracellular route while Naproxen is transported mostly by the transcellular route. Cells were seeded at 2 x 10^6 cells/ml and cultured on 24 well plate Transwell® permeable supports (0.4 um pore size) for 27 days. Cell culture medium was changed every other day. On day 27, cell culture medium was removed from both the apical and basolateral compartments, and cells were rinsed once with HBSS. Apical compartment solution was replaced with emulsion in HBSS (with no drug), and basolateral compartment solution was replaced with HBSS. Emulsion systems were

Figure 7: Effect of emulsion formulations on intestinal cell monolayer viability. Formulations of low HLB surfactant (56% Labrasol, 44% Capmul MCM), at 1:1 surfactant ratios, showed toxic effect on intestinal cells (p < 0.001). Error bars indicate standard deviation of three independent measurements. ‘*’ shows significance with respect to negative control HBSS.
prepared at 1:100 dilution ratio without the model drug, Naproxen, in HBSS. Blank HBSS and 0.1% SDS served as the negative and positive controls, respectively. Cells were exposed to emulsions for 3 hours inside the incubator. To test transport, a solution containing 0.3 mg/ml Naproxen was prepared in PBS. Apical compartment solution was removed and replaced with drug solution after rinsing cells once with HBSS, basolateral compartment solution was replaced with blank PBS, and cells were placed in the incubator. After three hours, samples were taken from each compartment and analyzed for Naproxen content using a UV spectrophotometer at 330 nm wavelength. Paracellular transport was tested following the same procedure except instead of Naproxen, 1 mg/ml Lucifer Yellow was used, and drug content was measured using a fluorescence plate reader at 485 nm excitation and 528 nm emission wavelengths. Transport studies were combined with Trans Epithelial ElectricalResistance (TEER) measurements before and after exposure to formulations (EVOM, World Precision Instruments, Sarasota, FL) in order to assess effect of formulations on cell monolayer integrity. Apparent permeability coefficient, $P_{app}$, is calculated as [123]

$$P_{app} = \left(\frac{dQ}{dt}\right)\frac{1}{AC_0}$$

Equation 19

where $dQ/dt$ is the steady-state flux, $A$ is the surface area of the membrane, and $C_0$ is the initial drug concentration in the donor compartment.
Figure 8: Effect of formulations on transport of Naproxen across cell monolayers. There is no observed influence of formulations on Naproxen permeability. Permeability values were in the range of 1.8 to 2.3 \times 10^{-3} \text{ cm/min. Error bars indicate standard deviation of two independent measurements.}

Formulations tested did not appear to influence the transcellular transport route. No increase in permeability to Naproxen was observed with exposure to emulsion systems (Figure 8); permeability values were in the range of 2.9 to 3.5 \times 10^{-5} \text{ cm/s. In contrast, the integrity of tight junctions as assessed by TEER values appeared to be influenced by the emulsion formulations. TEER values were measured before and after exposure to emulsion systems during transport studies. Cell monolayer resistance was between 503 and 630 ohms for all wells before exposure to emulsion systems. 4 shows the decrease in cell monolayer integrity due to exposure to SEDDS. Formulations with Tween 80 at 1:1 oil-to-surfactant ratio resulted in the most notable decrease in cell monolayer integrity; although only decreases after exposure to formulations with Soybean oil (at a 1:1 ratio) were found to be statistically significant ($p = 0.01$) compared to negative control HBSS. Since the formulations that decreased monolayer integrity do
not result in transcellular permeability enhancement or a toxic affect on cells as indicated by the MTT assay results, the decrease in cell monolayer integrity can be attributed to the loosenings of tight junctions due to Tween 80. Decrease in cell-cell junction integrity due to the surfactant Tween 80 was also indicated in a study by Levy et al [69]. They studied transport of barbituates across goldfish membrane and showed that the nonionic surfactant Polysorbate 80 (Tween 80) with a concentration below the CMC increased drug absorption, while it decreased absorption at concentrations above the CMC [69]. The increase in absorption was interpreted as being due to the enhanced permeability of the biological membrane. The decrease at higher concentrations, conversely, might have been due to the increased solubilizing effect of the micellar surfactant structures slowing the rate of absorption or to the layering of free surfactant monomers on the cell culture monolayer and related blocking of drug transport.

Figure 9: Effect of formulations on cell monolayer integrity. Exposure to SEDDS formulations with high levels of high HLB surfactant (Tween 80) resulted in decreased cell layer integrity potentially indicating loosenings of tight junctions. Soybean oil-Tween 80 formulation at 1:1 oil to surfactant weight ratio decreased cell integrity significantly ($p = 0.01$). Error bars indicate standard deviation of three independent measurements. ‘*’ shows significance with respect to negative control HBSS.
Lucifer Yellow transport, another indicator of paracellular transport and thus tight junction integrity, was influenced by exposure to different emulsion systems (Figure 10). Due to large variances in experimental measurements, it was not possible to distinguish any significant enhancement in permeability due to any specific formulation, although additional experiments might increase statistical power. To test the specific influence of Tween 80, the effect of surfactants alone on Lucifer Yellow transport also was studied. Exposure to Tween 80 solution for three hours tended to improve paracellular drug transport (Figure 10).

Figure 10: Effect of emulsion systems and surfactants on Lucifer Yellow transport across Caco-2 cell monolayers. Tween 80 solution in HBSS at 1:100 dilution tends to improve Lucifer Yellow permeability. Error bars indicate standard deviation of two independent measurements.
4.3 Kinetics of Drug Release from Emulsions

Compound release from inside of SEDDS formulation emulsion droplets to the outside aqueous media was investigated separately in the absence and presence of digestive enzymes. In the absence of digestive enzymes a dialysis membrane was used in order to separate formulation components from aqueous solution and experiments were carried out in sink conditions. Naproxen was used as a model drug compound. In the presence of digestive enzymes experiments were run in non-sink conditions and EPR was utilized for the analysis of model drug compound, spin probe, Tempo Benzoate in different phases over time; aqueous (micelles and water) and formulation emulsion.

4.3.1 In the absence of digestive enzyme

Drug release experiments were conducted using a regenerated cellulose dialysis membrane (Fisherbrand, MWCO 3500) with 3 ml volume. Emulsion formulations containing 7 mg/ml Naproxen were introduced inside the tubular membrane at a formulation to PBS ratio of 1:50 and gently shaken to enable mixing. The dialysis membrane then was immersed in 35 ml PBS solution in a beaker that served as the receiver fluid maintaining sink conditions. A stir bar was used at a mixing speed of 330 rpm to enable mixing inside the beaker, and a hot plate was used to maintain the temperature inside the beaker at 37°C. Samples (200 ml) were taken every 15 minutes for three hours from the receiver compartment and replaced with fresh PBS. A sample was also taken at 24 hours to assess the amount of drug released at equilibrium. Samples were analyzed using UV spectrophotometry at 271 nm.

The rate of drug release from emulsions, characterized by the release coefficient \( k_{eff} \), was calculated using the mass transfer equations
\[ \ln\left(1 - \frac{M_t}{M_0}\right) = 3 \cdot k_{\text{eff}} \cdot \frac{t}{r^2} \]  

Equation 20

and

\[ \frac{dM_g}{dt} = \rho \left( C_w - C_w^{\text{OUT}} \right) \]  

Equation 21

where \( M_t/M_0 \) is the fraction of the released drug at time \( t \), \( M_R \) is the drug amount inside the receiver fluid outside the dialysis membrane, and \( C_w^{\text{OUT}} \) is the concentration of drug in the receiver fluid measured throughout the release experiment as described above. It was assumed that initially drug was only incorporated inside oil droplets. In order to calculate the concentration of free drug in solution inside the membrane (\( C_w \)), the permeability of the membrane to free drug, \( r \), was measured using a drug release experiment (with no emulsion) together with Equation [3]. Briefly, 3 ml of 0.14 mg/ml Naproxen solution in PBS was placed inside a dialysis membrane immersed in 35 ml PBS, as described above, and samples were taken from the receiver compartment every 15 minutes for 3 hours.

In order to calculate the fraction of drug released from oil droplets at time \( t \), drug concentration in the aqueous phase inside the dialysis membrane, meaning both micelles and water phase, was calculated using \( C_w \) and an experimentally determined partition coefficient \( K_m \) [118],

\[ K_m = \frac{C_m}{C_w} \]  

Equation 22

where \( C_m \) is the drug concentration in the micellar phase and \( C_w \) is the drug concentration in water. Briefly, 7 mg/ml drug was dissolved in surfactant and 60 ul of surfactant containing drug was mixed with 3 ml PBS inside of a dialysis membrane. This membrane then was immersed in a PBS receiver compartment as described above for
release experiments. A sample was taken after 24 hours from the receiver compartment and used to calculate $C_w$. $C_m$ then was calculated by mass balance and used to determine the partition coefficient. Drug partitioning into micelles was assumed to be instantaneous.

Micelle volume in each emulsion system, required to calculate the partition coefficient, was measured by a previously established method [65]. Emulsions with Tween 80 were centrifuged for 20 minutes through nanoporous regenerated cellulose centrifuge filters (Amicon Ultra, Millipore Corp, Billerica, MA) of molecular weight cut off 100,000. For emulsions of Cremophor EL, an ultracentrifugation unit (Amicon 8400, Millipore Corporation) and a membrane of 500,000 molecular weight cut off were used. Micelle molecular weight, calculated from the aggregation number and the monomer molecular weight, was used to select the membrane molecular weight cut off that allows micelles but not oil droplets to pass through the membranes[119]. The filtrates from both centrifugation and ultracentrifugation processes containing the micelles and surfactant monomers were analyzed using a methylene blue complexation method [120]. The percentage of surfactant introduced that formed micelles and monomers (as opposed to that associated with emulsion droplets) was calculated.

The parameter $r$ in Equation 20 is the oil droplet radius, calculated from the average particle size of the emulsions formed at 1:50 ratio in PBS, assuming spherical shape.

Drug release from emulsions involves interfacial transport across the layer of surfactant coating the emulsion droplet followed by diffusive and convective transport through the surrounding aqueous medium. The appropriate kinetic expression depends upon (1) whether the drug enters the aqueous phase as a free molecule which then
“reacts” with micelles or enters the aqueous phase by partitioning directly into a micelle and (2) the rate-limiting step in transport: interfacial transport, bulk transport, or reaction with micelles. In order to explore drug release kinetics and dependence on emulsion formulation characteristics, we used a mass transfer model that assumes interfacial transport limited kinetics (Equation 20) [78] to calculate the release coefficient from emulsions. The magnitude of this coefficient can be compared to that expected if different mechanisms were dominating drug release for insight into drug release mechanism [129].

Table 8: Release coefficients of Naproxen from emulsion oil droplets. Values are the average and standard deviation of three independent measurements.

<table>
<thead>
<tr>
<th>Oil</th>
<th>Surfactant</th>
<th>Ratio</th>
<th>$k_{\text{eff}}$ (nm$^2$/min)</th>
<th>% drug released at 15 min</th>
<th>% drug released at 24 hr</th>
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<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Std Dev</td>
<td></td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>Cremophor EL</td>
<td>9:1</td>
<td>904.2</td>
<td>90.4</td>
<td>69.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5:1</td>
<td>941.9</td>
<td>94.2</td>
<td>58.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:1</td>
<td>1085.9</td>
<td>260.6</td>
<td>75.1</td>
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<tr>
<td>Tween 80</td>
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<td>4138.3</td>
<td>289.7</td>
<td>94.3</td>
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<td></td>
<td></td>
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<td>2170.4</td>
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<tr>
<td></td>
<td></td>
<td>1:1</td>
<td>890.6</td>
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</tr>
<tr>
<td>Captex 200</td>
<td>Cremophor EL</td>
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<td>851.5</td>
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<td>69.8</td>
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<td></td>
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<td></td>
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<td>1:1</td>
<td>245.6</td>
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<td>2830.6</td>
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<tr>
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<td></td>
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<td>1:1</td>
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<td>265.4</td>
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For most of the formulations, release of Naproxen from oil droplets was mostly completed in the first fifteen minutes of the experiment. However, it was also observed
that for some formulations release was not complete even after 24 hours. Release coefficients reported as $k_{eff}$ represent effective release coefficients for formulations calculated for the first fifteen minutes of the experiment. Based on the analysis performed with release rate constants ($k_{eff}$) all of the formulation parameters (oil type $p < 0.0005$, surfactant HLB $p < 0.0005$, oil-to-surfactant ratio $p < 0.0005$) had significant influence on rate of release constant. It was statistically observed that release rate constants were higher for formulations with the least surfactant ratio (oil to surfactant ratio 9:1) compared to other ratios ($p < 0.0005$, Tukey’s post-hoc test) which might be due to the low level of partitioning of the surfactant at the interface resulting in a decrease in the interfacial barrier. It was previously suggested that decreasing the total surfactant amount in a system may cause a decrease in the amount of surfactant located at the interface [65]. Release coefficients were also overall highest for formulations containing Soybean oil and lowest for those containing Captex 200 ($p < 0.0005$, post-hoc tests) (Table 8). Soybean oil has a carbon chain length closest to that of the surfactants used. As proposed above, penetration of soybean oil into the interfacial film [126] might decrease the interfacial barrier at the oil water interface, which is proposed to be the rate-limiting factor for release from emulsion systems [77].

4.3.2 In the presence of digestive enzyme

Upon inception of digestion, digestion products are released from the surface of SEDDS into aqueous media forming complex colloidal structures. Meanwhile, drug compounds, originally located inside oil emulsion droplets, are released from SEDDS into intestinal fluid, and a portion of released drug further partitions into colloidal structures. In an effort to perform real time quantitative non-invasive tracking of drug
partitioning and drug release into colloidal phases during in vitro digestion, electron paramagnetic resonance (EPR) (9.1-9.9 GHz, X-Band; Bruker EMX) was used to track a model drug (spin probe: Tempol Benzoate). Tempo Benzoate was first analyzed in static solutions in order to fit and obtain parameters and tensors that represent the spectral characteristics of TB in each microenvironment (Figure 11).

![EPR spectra of TB in static solutions](image)

**Figure 11**: EPR spectra of TB in static solutions. Y axis indicates signal intensity and x axis indicates Magnetic field (G). A) TB in Fasted state buffer: less micelles, B) TB in Fed state buffer: more micelles, c)TB in formulation dispersed in fasted state media: oil droplets together with micelles, show how the spectra changes based on TB microenvironment. The dotted line on to the left donates a less hydrophilic microenvironments whereas the line to the right denotes a more hydrophilic environment.
Next, EPR analysis was performed during in vitro digestion experiments. Differences in peak to peak distances ($a_N$) and peak widths among EPR spectra, especially evident on the third peak (Figure 12), give information on the probe distribution among different phases (oil vs. vesicles and micelles vs. water). For the specific formulation studied (Figure 12), Soybean oil with Tween 80 at 1:1 ratio, results indicate an increased partitioning of the probe into the micellar and buffer phase over time, which is indicated by the increased height of the “x” peak compared to the “y” peak on the spectra. Multi-component analysis of spectra allowed quantitative determination of the ratio of the spin probe in each phase over time (Figure 13). Before the inception of digestion, model drug distribution in oil, micelle and water phase was: 60%, 35% and 5% respectively. Over the course of digestion, there was a decrease in the relative amount of drug inside oil and an increase in buffer and micelle phase; at 90 minutes the distribution was 26%, 52% and 22%.
Figure 12: EPR spectra of 1:100 dilution SEDDS formulation (Soybean oil-Tween 80 at 1:1 ratio) including probe Tempol Benzoate during in vitro lipolysis. A:0, B:5, C:25, D:50, E:90 minute into digestion. “x” indicates the peak location of oil, “y” indicates the aqueous phase. Y axis indicates signal intensity and x axis indicates Magnetic field (G).
4.4 Kinetics of SEDDS Digestion

A major process central to emulsion function is the enzymatic degradation of SEDDS oil in water emulsions in the intestinal lumen. Upon entry into the intestine, self-emulsifying drug delivery systems are known to spontaneously emulsify in the aqueous gastrointestinal fluid to form oil droplets tens to hundreds of nanometers in size. These droplets are then catalyzed with pancreatic lipase (PL), due to their lipid nature, resulting in changes in colloidal phases, drug solubility, and drug release kinetics [3, 47]. These changes to SEDDS and GI tract due to digestion are believed to play an important role in determining the degree of oral absorption and bioavailability of hydrophobic compound, and are believed to be highly dependent on the specific SEDDS formulation. Effects of formulation digestion on SEDDS performance have been broadly studied [108, 117, 130-132]; however, a statistical or predictive mechanistic modeling approach that relates

**Figure 13:** Distribution of model drug compound, Tempo Benzoate, in different phases present over the course of in vitro formulation digestion.
digestion to formulation design parameters has yet to be applied but could facilitate rational design of broad ranges of SEDDS drug delivery vehicles.

In order to gain a greater understanding of the dependence of SEDDS emulsion digestion in the GI tract on formulation design, kinetics of formulation digestion in vitro across a broad range of statistically designed SEDDS formulations was studied, relating kinetics to formulation design parameters using predictive regression modeling and analysis of variance tests.

4.4.1 Characterization of Biorelevant Media and SEDDS in Media

In order to characterize SEDDS emulsion formation and calculate digestion rates, statistically designed formulations were analyzed using dynamic light scattering (Brookhaven 90Plus) upon dispersion in fasted state simulated intestinal fluid. Mean droplet diameter of self-emulsified oil droplets ranged from 96 to 700 nm (Figure 14). Overall, systems with high surfactant ratio (1:1 oil:surfactant) had smaller droplet sizes. Factors demonstrating statistically significant influences (ANOVA) on droplet size were oil type (p<0.0005) and oil to surfactant ratio (p<0.0005). On the other hand, influences of surfactant type and pair interactions of the factors on droplet size were not significant. Tukey's post-hoc tests indicated that mean diameters of 1:1 oil to surfactant ratio systems were significantly smaller compared to 9:1 and 5:1 ratio emulsions (p <0.0005). Mean droplet size of formulations containing Soybean oil was larger than that of systems containing other oils (p < 0.0005). Micelle diameter of fasted state biorelevant medium was 4.2 ±1.1 nm.
Fasted state biorelevant media and self-emulsifying drug delivery systems were investigated using Small-Angle Neutron Scattering in order to gain more insight into size, structure and shape of colloidal species that exist in these complex fluids. Information regarding size and shape was extracted from scattering data. Fasted state biorelevant media was prepared in D$_2$O instead of H$_2$O, and pure Triolein was used as the oil component of formulations instead of Soybean oil in order to eliminate impurities that might originate from Soybean oil. Measurements were performed over a Q range of 0.0018 to 0.045 Å$^{-1}$.

Low Q measurements enable detection of larger particles in the range of 10s to 100s of nm, which in our case are oil-in-water emulsions that originate from formulation,
whereas high Q measurements enable detection of smaller particles of a few nanometers size, which in this case are bile salt/phospholipid micelles. Using SANS, it was possible to detect oil droplets together with co-existing micelles, which wasn’t achieved with other techniques such as dynamic light scattering. High Q scattering lines on Figure 15 correspond to scattered intensities from bile salt/phospholipid micelles, which is evident with a curved line, and scattering intensity lines on low Q region corresponds to emulsion droplets. Figure 15 shows change in size and possibly shape of oil-in-water emulsions and bile salt/phospholipid micelles with varying concentration of formulations (Soybean oil and Tween 80 at 1:1 or 9:1 weight ratio) or just Soybean oil. It is interesting that there is a change in bile salt/phospholipid micelles with the addition of 9.2 mM formulation (1:100 volume ratio in aqueous phase) including Soybean oil and Tween 80 at 1:1 ratio into the micellar solution, while changes to micelles are not evident with other formulations with other ratios and concentrations.

**Figure 15:** Scattering intensity profiles of self-emulsifying formulations of varying concentration or just soybean oil in fasted state biorelevant medium including bile salt/phospholipid micelles.
Fasted state biorelevant media prepared in D$_2$O was investigated separately for micellar structures. A theoretical model for compact particles with ellipsoid rotation was fit to the scattered intensity in order to extract information on size and shape of bile salt/phospholipid micelles (Figure 16). Micelle particle radius, $R_g$, was calculated as 1.6 nm, which is in close agreement with values previously obtained from dynamic light scattering, 1.9 nm.

**Figure 16:** Guiner analysis of fasted state biorelevant media micelles for compact particles with ellipsoid rotation.
4.4.2 Calculation of Digestion Kinetic Constants

Oil droplets undergo multiple chemical processes when interacting with digestive enzyme lipase. During lipolysis, triglyceride molecules first break down into fatty acid (FA) and diglyceride (DG). Further hydrolysis produces a second fatty acid (FA) and 2-monoglyceride (2-MG). Enzymatic hydrolysis of 1 molecule triglyceride thus produces 2 molecules FA and 1 molecule 2-MG. Although it is believed to be limited, 2-MG might undergo isomerization to 1-monoglyceride (1-MG), in which case 1-MG is further hydrolyzed to a third fatty acid and glycerol [3, 47, 91]. Products of digested lipids then dissolve into endogenous micelles and vesicles forming mixed micelles and complex vesicles [106].

In order to compare rates of digestion of emulsions of statistically designed formulations and in order to determine influences of formulation design parameters such as oil type, oil to surfactant ratio and surfactant HLB on kinetics of self-emulsifying system digestion, kinetic constants of digestion were calculated based on Equation 9. (Figure 17) shows a comparison of experimental fatty acid production profiles and model fits over time of two formulations; medium chain triglyceride with Cremophor EL and long chain triglyceride with Tween 80, both at 1:1 oil to surfactant ratio. Model fits were in close agreement with experimental profiles for all formulations studied. Digestion rate constants ranged between 0.0022-0.2753 mol/m²*s (Table 9).
Figure 17: A comparison of measured digestion rate profiles (diamond and triangle dots) of two different formulations and model fits (continuous lines), expressed as percent fatty acid released. Error bars correspond to calculated standard errors based on 2 measurements.

Table 9: Digestion rate constants of self-emulsifying drug delivery systems

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Oil*</th>
<th>Surfactant</th>
<th>Rate constant mol/m2*s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>1A</td>
<td>LCT</td>
<td>Cremophor EL</td>
<td>0.0062</td>
</tr>
<tr>
<td>1B</td>
<td>LCT</td>
<td>Cremophor EL</td>
<td>0.0022</td>
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<td>2B</td>
<td>MCD</td>
<td>Cremophor EL</td>
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<tr>
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</tr>
<tr>
<td>6C</td>
<td>MCT</td>
<td>Cremophor EL</td>
<td>0.1003</td>
</tr>
</tbody>
</table>

* LCT: long chain triglyceride, MCPGD: medium chain propylene glycol diester, MCT: medium chain triglyceride
4.4.3 Linear regression analysis of digestion rates

Linear regression and Anova tests were performed using SPSS software to map out significant formulation design parameters based on digestion rates calculated. Digestion rate constants were expressed as a function of formulation design parameters; oil type, oil to surfactant ratio, and surfactant type (Table 10). Analysis of variance test showed that all of the formulation design parameters tested, namely oil type, surfactant HLB, and oil to surfactant ratio, affected digestion rate constant significantly. Linear regression analysis indicated that among all the design parameters studied, oil to surfactant ratio had the least effect on digestion rate constant.

Table 10: Linear regression analysis of formulation design parameters and emulsion digestion kinetics.

<table>
<thead>
<tr>
<th>Response, Y (mmol/m²s)</th>
<th>Model</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestion rate constant</td>
<td>( Y = -1354.9 + 19.3A_1 + 56.2A_2 + 115.8B - 5.246C - 136.5A_1B + 41.9A_1C - 130.8A_2B + 30.4A_2C - 42.3BC )</td>
<td>&lt;0.0005</td>
<td>0.897</td>
</tr>
</tbody>
</table>

Independent variables are: \( A_1 \): soybean oil, \( A_2 \): Captex 200; \( B \): surfactant HLB; \( C \): oil to surfactant ratio. Oil type is coded as (0,1) indicator variables, with soybean oil included by setting \( A_1 = 1 \) and \( A_2 = 0 \), Captex 200 included by setting \( A_1 = 0 \) and \( A_2 = 1 \), and Neobee M5 included by setting \( A_1 = 0 \) and \( A_2 = 0 \) (note only one oil type can be included in any given model).

Effect of oil lipophilicity on rate of formulation digestion was highlighted with the fact that digestion rates were higher for formulations with medium chain oils compared to long chain oils (\( p<0.05 \)). As shown in Figure 18, regardless of the surfactant ratio or type, formulations of long chain triglyceride, Soybean oil, have lower digestion
rates than those of medium chain propylene glycol diester and triglyceride. However, there was no clear trend observed related to medium chain propylene glycol diester. The dependence of digestion kinetics on triglyceride chain length is in agreement with earlier studies where higher rates of digestion with medium chain oils than long chain oils were demonstrated. [101, 108] Sek et al.[108] investigated in vitro digestion profiles of medium (Miglyol 812) and long chain triglycerides (Soybean oil) crudely emulsified in fasted and fed state biorelevant media using titration and HPTLC techniques. Similarly, they reported a higher digestion rate with medium chain triglyceride oil than long chain triglyceride. This difference in rate of digestion due to chain length was attributed to higher aqueous solubility of medium chain triglycerides and its lipolytic products compared to long chain triglycerides and its digestion products due to corresponding polarities. Accumulation of the digestion products at the digesting oil droplet interface could retard digestion. It was suggested that digestion products of long chain triglycerides get solubilized into the aqueous phase proportional to bile salt concentration whereas medium chain lipolytic products show bile salt independent aqueous solubilization. Formation of different phases during digestion of different chain length oils may also play a role in aqueous and micellar solubilization. Fatouros et al. [107] recently studied structural change of micro-emulsions during in vitro digestion using small angle x-ray scattering (SAXS). The formulation studied consisted of a mixture of long chain triglycerides (LCT) as the oil component: Sesame oil (30% w/w), containing mono-, di- and triacylglycerides (primarily oleic and linoleic), Maisine 35-1 (30% w/w) mainly mono-, and diacylglycerides (primarily containing oleic and linoleic fatty acids), the surfactant Cremophor RH 40 (30% w/w) and ethanol (10% w/w), which acts as a co-
solvent. The authors demonstrated that before the start of lipolysis, samples did not contain any visible liquid crystallinity, however during the lipolysis process, first a lamellar and then a hexagonal phase appeared. In another study [106], where crosspolarizing light microscopy was used to investigate simulated lipid digestion phases, results suggested that systems with long chain lipid digestion products exhibited cubic crystalline structures, whereas in the presence of medium chain lipids, lamellar phases were observed. Structured interfacial layers tend to provide better stability to emulsions [133]. The presence of lamellar phases at the oil water interface may result in higher steric hindrance for enzyme, therefore inhibiting lipolysis, compared to cubic phases as a result differences in structure. On the other hand, slower transformation of cubic phases of LCT digestion products at the digesting oil water interface into colloidal vesicles compared to lamellar phases of MCT/D digestion products may cause retardation of the digestion process.
Overall, formulations of Tween 80 had higher digestion rates compared to formulations of Cremophor EL (p<0.05) (Figure 18). Tween 80 and Cremophor EL are both nonionic surfactants; therefore, we do not expect any electrostatic effects to play a role in the interfacial characteristics of these emulsions. Li et al. [134] studied the effect of a range of non-ionic, anionic and cationic, low-molecular weight surfactants on the digestibility of lipids in corn oil-in-water emulsions using an in vitro digestion model. Among non-ionic surfactants studied, they reported the ability of the surfactants to inhibit lipid digestion studied in the following order: Tween 80 > Tween 20 > Brij35, with a corresponding HLB value order of 15>16>17. This is in agreement with the correlation we see with HLB effect and digestion rate; Tween 80 (HLB 15) has a higher HLB value than Cremophor EL (HLB 13). A higher HLB value refers to more hydrophilic nature of...
the molecule, which would mean higher affinity for lower HLB surfactant Cremophor EL for oil phase than Tween 80. Therefore, high HLB surfactant may be more prone to be dislocated by enzyme or enzyme-bile salt complex than low HLB surfactant during competitive adsorption, providing increased surface area for enzyme. It was suggested that there may be competitive adsorption between the enzyme complex, surface active agents (bile salts, phospholipids, surfactants initially adsorbed to the lipid droplet surfaces), and digestion products, which could interfere with the binding of the lipase to the droplet surfaces [135]. On the other hand, in vitro digestion experiments in fasted state simulated media of formulations of a blend of long chain triglycerides with a range of surfactants including Cremophor EL and Pluronic 121, 72, 43, 61 and 68 were conducted previously [136]. The rate of digestion of formulations in fasted simulated intestinal fluid did not follow any order that correlated to surfactant HLB. However, it is important to note that only formulations of Cremophor EL and Pluronic 68 were reported to form dispersions that gave mean droplet sizes of 196 nm and 460 nm, respectively, and the rest of the formulations studied were not dispersed enough to enable droplet size measurements. Therefore, dispersion of the oil droplets may be a dominating factor over digestion rate in this system, unlike ours.
Influence of SEDDS on Lymphatic Transport

Most orally administered drugs enter the systemic circulation via the hepatic portal vein; however, some very lipophilic compounds are transported via the lymphatic pathway, avoiding first-pass metabolism [3]. Lipid digestion products, upon absorption into enterocytes, either directly diffuse through the cell and enter the portal vein or interact with endoplasmic reticulum and undergo re-synthesis into triglycerides and further constitute the lipid component of intestinal lipoproteins. Due to the large colloidal particle nature of lipoproteins, following exocytosis from the enterocyte, these particles preferentially pass through the highly permeable lymphatic endothelium rather than the portal vein [2, 5]. Drugs that are included in the systemic circulation via lymphatic transport generally associate with the triglyceride lipid core of lipoproteins. Hauss et al.

Figure 19: Influence of surfactant ratio and surfactant type on rate of formulation digestion for formulations of A) Soybean oil, B) Captex 200, and C) Neobee M5. The error bars represent standard error from two independent measurements.

4.5 Influence of SEDDS on Lymphatic Transport

Most orally administered drugs enter the systemic circulation via the hepatic portal vein; however, some very lipophilic compounds are transported via the lymphatic pathway, avoiding first-pass metabolism [3]. Lipid digestion products, upon absorption into enterocytes, either directly diffuse through the cell and enter the portal vein or interact with endoplasmic reticulum and undergo re-synthesis into triglycerides and further constitute the lipid component of intestinal lipoproteins. Due to the large colloidal particle nature of lipoproteins, following exocytosis from the enterocyte, these particles preferentially pass through the highly permeable lymphatic endothelium rather than the portal vein [2, 5]. Drugs that are included in the systemic circulation via lymphatic transport generally associate with the triglyceride lipid core of lipoproteins. Hauss et al.
demonstrated that the bulk of drug compound Onzalast formulated with triglyceride is absorbed via the lymphatic pathway. Drug transported into systemic circulation was directly proportional to the amount of triglyceride transported via lymph in in vivo tests on rats. Formulation parameters might also limit the lymphatic transport of the lipophilic drug. Seeballuck et al. [68], using Caco-2 cell culture model, showed that Cremophor EL at a concentration higher than 0.025% w/v inhibits P-glycoprotein efflux as well as chylomicron secretion.

Digestion products of SEDDS are believed to interact with intestinal absorption pathways and stimulate lymphatic transport as an alternative route to absorption.

Potential effects of each formulation on lymphatic transport were also studied qualitatively using a Caco-2 model.

We assessed the influence of formulation composition on lymphatic drug transport in vitro by quantifying secretion of apolipoprotein B (ApoB), a lipoprotein secretion marker, which is secreted proportional to the increase of lymphatic drug transport, in intestinal epithelial Caco-2 cells. Lipophilic drug associates with the triglyceride lipid core of the lipoproteins, and it has been suggested that amount of drug transported through the lymphatic pathway is proportional to the amount of lipoprotein secreted by intestinal cells [88]. ApoB incorporates into triglyceride rich lipoproteins (TRL) in a 1:1 ratio, and hence presents a suitable means of quantifying the number of secreted lipoproteins and the triglyceride. However, it was shown that Caco-2 model via in vitro ApoB determination does not quantitatively correlate well with conventional in vivo cannulated rat lymphatic model transport results, therefore in this study ApoB quantification was used as means of a qualitative determination of likelihood of
formulation contribution to lymphatic transport and not as a quantitative assessment [2, 88].

4.5.1 Effect of self-emulsifying drug delivery systems and conventional biorelevant media on Caco-2 viability

In vitro tests for ApoB secretion require 20-hour incubation of formulations with Caco-2 cells. Therefore, along with the ApoB test, MTT toxicity tests were carried out to assess the possible cytotoxicity of formulations due to the long incubation period. Bile salt/phospholipid concentrations that would not be toxic to cells after 20 hours of incubation were sought. Conventional fasted (5mM NaTDC/1.25mM PL) and fed state (12mM NaTDC/4mM PL) concentrations together with another bile salt/phospholipid solution with lower bile salt concentration were tested. The conventional fasted and fed state concentrations were found to be toxic to cells with 20 hours of incubation (Figure 20). The concentration that is referred to as “biorelevant media” in Figure 20 and Figure 21 had the following concentration: 2.5mM NaTDC/1.25mM PL, and did not cause toxicity at the end of 20 hours. It was thus used as representative of the biorelevant conditions for ApoB tests. The reason of toxicity due to physiologically relevant phospholipid/bile salt concentrations on Caco-2 cells may be the absence of a mucus layer on Caco-2 cell cultures unlike in vivo conditions. Mucus layer has been shown to protect intestinal cells from injury [137].
Formulations were also tested for potential cytotoxicity at 1:1000 dilution in both DMEM and in biorelevant media upon 20 hours incubation with cells. After 20-hour incubation with microemulsions formed in only DMEM, cell viability ranged 87-100 %, and those formulations were not considered toxic. On the other hand, incubation with microemulsions formed in biorelevant media decreased cell viability down to 5%, especially with high surfactant ratio formulations (50%) (data not shown). Therefore formulations emulsified in DMEM at 1:1000 ratio were selected for incubation for ApoB analysis.

4.5.2 Apolipoprotein B analysis

ApoB is incorporated into triglyceride rich lipoproteins (TRL) in a 1:1 ratio, and hence presents a suitable means of quantifying the number of secreted lipoproteins, and the triglyceride: ApoB ratio can be used to indicate the triglyceride loading of secreted lipoproteins [88]. Quantification of ApoB secretion suggested the formulations tested
would have minimal to no impact on lymphatic transport. Some formulations slightly inhibited ApoB secretion compared to controls DMEM and biorelevant media (Figure 21) Formulations overall induced similar levels of ApoB secretion, with no significant increase in ApoB secretion when compared to controls DMEM and biorelevant media. Caco-2 cultures exposed to formulation of Tween 80 and Neobee M5 at 1:1 weight ratio showed higher levels of ApoB secretion compared to control biorelevant media, although not significantly. In vitro digestion experiments showed that this same formulation also has the fastest digestion rate and liberated highest amount of free fatty acid upon digestion. Higher amounts of free fatty acids may induce ApoB production. Seebullack et al. [88] studied the effects of Tween 80 and 60 on intestinal lipoprotein processing using Caco-2 monolayers in vitro, and cannulated rat model in vivo. Both in vitro and in vivo model findings showed that Tween 80 (0.25 w/v %) stimulated lipoprotein production, and this effect was attributed to digestion of Tween 80 by Caco-2 cells that liberated oleic acids, which stimulated lipoprotein secretion.
4.6 Development of a systems-based mathematical model describing oral drug absorption with SEDDS

A systems based model was developed explaining oral drug absorption of a compound co-administered with SEDDS. Developed model was solved using Runge Kutta numerical solution technique in Matlab®. Model simulations were compared with experimental findings.

4.6.1 Model Development

Kinetic expressions describing oral drug absorption administered with self-emulsifying drug delivery systems were developed considering three major kinetic processes that occur in vivo: formulation digestion, drug release from formulation droplets, and drug absorption across intestinal membrane. Formulation digestion in stomach was ignored and formulation was assumed to enter directly to small intestine upon oral administration.
4.6.1.1 Digestion kinetics

Digestion of the formulation oil droplets interacting with the enzyme lipase can be described with the following Equation:

\[
\frac{dm_{FA,aq}}{dt} = k_{dig}S_{em}V_{aq}
\]  
Equation 23

where \( m_{FA,aq} \) is the mass of digestible fatty acid (FA) in the aqueous phase, \( k_{dig} \) is the digestion rate constant, \( S_{em} \) is the total oil droplet surface area per total volume and \( V_{aq} \) is the aqueous solution volume. Equation 23 can be expressed in terms of FA concentration, \( C_{FA,aq} \) in aqueous solution as follows:

\[
\frac{dC_{FA,aq}}{dt} = k_{dig} S_{em}
\]  
Equation 24

It is assumed that during digestion, oil droplets will shrink in size over time due to enzymatic reaction and this change in particle diameter, \( D(t) \) can be related to the fraction of fatty acids released from the droplets due to digestion. The ratio of the droplet volume during digestion to the initial droplet volume is defined by

\[
\frac{V_{em}(t)}{V_{em,0}} = \frac{1}{6} \pi \left( \frac{D(t)}{3} \right)^3 = \frac{m_{FA,em}}{m_{FA,0}}
\]  
Equation 25

Hence, time dependent emulsion particle diameter \( D(t) \) and emulsion surface area are

\[
D(t) = D_0 \left( \frac{m_{FA,em}}{m_{FA,0}} \right)^{\frac{1}{3}}
\]  
Equation 26

\[
A_{em}(t) = N \pi \left( D_0 \left( \frac{m_{FA,em}}{m_{FA,0}} \right)^{\frac{1}{3}} \right)^2
\]  
Equation 27
where \( N \) is the number of oil droplets present, \( m_{FA,em} \) is the moles of digestible fatty acids remaining in the emulsion droplet, \( m_{FA,0} \) is the initial moles of digestible fatty acids in the droplet, \( D_0 \) is the initial droplet diameter of emulsion droplets, and \( A_{em} \) is the total surface area of the emulsion droplets, related with \( S_{em} \) as:

\[
S_{em} = \frac{A_{em}}{V_{aq}} \quad \text{Equation 28}
\]

Using mass balance, we can relate the mass of digestible FA remaining in the emulsion droplet with the mass of FA released and digested in the aqueous phase:

\[
m_{FA,em} = m_{FA,0} - m_{FA,aq} = m_{FA,0} - C_{FA,aq} V_{aq} \quad \text{Equation 29}
\]

Inserting this expression into Equation 27, we get:

\[
A_{em}(t) = N\pi \left( D_0^3 \sqrt{\frac{m_{FA,0} - C_{FA,aq} V_{aq}}{m_{FA,0}}} \right)^2 \quad \text{Equation 30}
\]

Equation 24 can thus be expressed as:

\[
\frac{dC_{FA,aq}}{dt} = k_{dig} N\pi \left( D_0^3 \sqrt{\frac{m_{FA,0} - C_{FA,aq} V_{aq}}{m_{FA,0}}} \right)^2 \quad \text{Equation 31}
\]

### 4.6.1.2 Drug release kinetics

Another process that occurs in parallel to formulation digestion is drug release from inside of oil droplets to the surrounding aqueous media. Drug release was expressed using a kinetic expression developed by Higuchi et al. [76] considering interfacial resistance at the oil-water interface as a limiting barrier to compound transport:

\[
\frac{dC_{D,aq}}{dt} = -\frac{A_{em} P_{rel}}{V_{aq}} (C_{D,aq} - C_{D,aq}') \quad \text{Equation 32}
\]
Here $C_{D,aq}$ is the drug concentration in the aqueous media (buffer and micelles) and outside of oil droplets, $V_{aq}$ is the volume of the aqueous media, $P_{rel}$ is the permeability constant for the oil water interface, $A_{em}$ is the total interfacial surface area of the oil droplets, and $C'_{D,aq}$ is the hypothetical aqueous concentration of the solute in equilibrium with compound concentration inside emulsion droplet, $C_{D,em}$.

Along with the concentration gradient driven transport of compounds from inside the oil droplets into the aqueous media, there will be a compound transport from oil droplets into the aqueous media due to detachment of oil digestion products from the surface of the droplets. In order to account for compound transport due to digestion, Equation 32 was modified to give

$$\frac{dC_{D,aq}}{dt} = -\frac{A_{em}P_{rel}}{V_{aq}}(C_{D,aq} - \frac{C_{D,em}}{K}) + k_{dig}S_{em} \frac{1}{n} V_{molar,em} C_{D,em}$$

Equation 33

where $n$ is the moles of fatty acid produced per mole of formulation and $V_{molar,em}$ is averaged molar volume of the formulation content. $C'_{D,aq}$, is defined by the formulation-aqueous phase partition coefficient, $K$, as;

$$K = \frac{C'_{D,aq}}{C_{D,aq}} = \frac{\frac{C_{D,em}}{C_{D,water}K_2 + C_{D,water}^{eq}}}{C_{D,em}} = \frac{C_{D,em}}{C_{D,aq}}$$

Equation 34

where $C_{D,em}^{eq}$ the equilibrium solubility concentration of drug in oil, $C_{D,aq}^{eq}$ is the equilibrium solubility concentration of drug in the aqueous phase and $K_2$ is water-micelle partition coefficient defined by;

$$K_2 = \frac{C_{D,micelle}^{eq}}{C_{D,water}^{eq}} = \frac{C_{D,aq}^{eq} - C_{D,water}^{eq}}{C_{D,water}^{eq}}$$

Equation 35
where $C_{D,\text{micelle}}^{eq}$ is the equilibrium solubility concentration of drug in micelles and $C_{D,\text{water}}^{eq}$ is the equilibrium solubility concentration of drug in the water phase. During digestion, we assume that digestion products that leave oil droplets incorporate into micelles, and as a result, the solubilization capacity of aqueous phase, $C_{D,aq}^{eq}$ increases. Therefore, a constant term, molar solubilization capacity of micelles, was introduced in order to express $K_2$ as a function of amount of surfactant that incorporates into micelles. Molar solubilization capacity of micelles, is defined [138]:

$$
\chi = \frac{C_{D,aq}^{eq} - C_{D,\text{water}}^{eq}}{C_{\text{surf}} - \text{cmc}}
$$

Equation 36

where $C_{\text{surf}}$ is the molar concentration of total surfactant at a given point in time and cmc is the critical micelle concentration. $C_{\text{surf}}$ is described as:

$$
C_{\text{surf}} = C_{BS} + C_{PL} + C_{FA,aq}
$$

Equation 37

where $C_{BS}$ is bile salt concentration, and $C_{PL}$ is phospholipid concentration. Combining Equations 35 and 36 yields the following equation where the partition coefficient is expressed as a function of the amount of surfactant that is associated with micelles and $\chi$:

$$
K_2 = \frac{C_{D,\text{micelle}}^{eq}}{C_{D,\text{water}}^{eq}} = \frac{C_{D,aq}^{eq} - C_{D,\text{water}}^{eq}}{C_{D,\text{water}}^{eq}} = \chi \left( \frac{C_{\text{surf}} - \text{cmc}}{C_{D,\text{water}}^{eq}} \right)
$$

Equation 38

From the definition for $C_{D,aq}^{eq}$, Equation 34, Equation 32 can be rewritten to give:
Therefore change in aqueous drug concentration is expressed as a function of $C_{D,em}$ and $K$, a parameter that will change over time due to digestion. $K$ is related to $K_2$ (Equation 38), which will also change over time and is a function of the amount of surfactant associated in micelles, $C_{surf}$ and micelle molar solubilization capacity. Micelle molar solubilization capacity is a constant and can be experimentally determined. $C_{surf}$ is defined by Equation 37 and is a function of $C_{FA,aq}$. On the other hand, using mass balance, $C_{D,em}$ can be expressed as:

$$\frac{dC_{D,em}}{dt} = \frac{V_{aq}}{V_{em}} \left( \frac{A_{em} P_{rel}(C_{D,aq} - C_{D,em})}{V_{aq}} - \frac{C_{D,em}}{K} \right) - k_{dig} \cdot \frac{1}{n} V_{molar,em} C_{D,em}$$

Equation 40

since $V_{em}$ will be changing due to digestion, it can be expressed as:

$$V_{em} = \frac{m_{FA,em}}{m_{FA,0}} V_{molar,em} = \frac{m_{FA,0} - C_{FA,aq}}{n} V_{aq} V_{molar,em}$$

Equation 41

and $C_{D,em}$ can be rearranged to give:

$$\frac{dC_{D,em}}{dt} = \frac{V_{aq}}{n} \left( \frac{A_{em} P_{rel}(C_{D,aq} - C_{D,em})}{V_{aq}} - k_{dig} \cdot \frac{1}{n} V_{molar,em} C_{D,em} \right)$$

Equation 42

### 4.6.1.3 Absorption and clearance kinetics

Kinetic expression describing free drug absorption across the intestinal membrane was defined by a first order Equation:

$$\frac{dm_{D,abs}}{dt} = P_{int} A_{int} C_{D,water}$$

Equation 43

where $P_{int}$ is the permeability of intestinal membrane, $A_{int}$ surface area of intestine,
$C_{D,\text{water}}$ is concentration of drug in water. Equation 43 can be written in terms of absorbed drug concentration, $C_{D,\text{abs}}$, as:

$$\frac{dC_{D,\text{abs}}}{dt} = \frac{P_{\text{int}} A_{\text{int}} C_{D,\text{water}}}{V_{\text{plasma}}}$$  \hspace{1cm} \text{Equation 44}

Using water-micelle partition Equation 38, can be expressed as:

$$C_{D,\text{water}}^{eq} K_2 = C_{D,\text{micelle}}^{eq}$$  \hspace{1cm} \text{Equation 45}

Since $C_{D,\text{aq}}^{eq}$ is the sum of $C_{D,\text{water}}^{eq}$ and $C_{D,\text{micelle}}^{eq}$, it can be written as:

$$C_{D,\text{water}}^{eq} K_2 + C_{D,\text{water}}^{eq} = C_{D,\text{aq}}^{eq}$$  \hspace{1cm} \text{Equations 46}

Therefore Equation 44 can be modified to give:

$$\frac{dC_{D,\text{abs}}}{dt} = \frac{P_{\text{int}} A_{\text{int}} C_{D,\text{aq}}}{V_{\text{plasma}} 1 + K_2}$$  \hspace{1cm} \text{Equation 47}

Lastly, we used the following expression for the rate of drug elimination from plasma:

$$\frac{dm_{D,\text{el}}}{dt} = V_{\text{plasma}} \frac{0.693}{t_{1/2}} C_{D,\text{abs}}$$  \hspace{1cm} \text{Equation 48}

where $t_{1/2}$ is drug elimination half life. Therefore the rate of change of drug concentration in blood is given by:

$$\frac{dC_{D,\text{abs}}}{dt} = \frac{P_{\text{int}} A_{\text{int}} C_{D,\text{aq}}}{V_{\text{plasma}} 1 + K_2} - \frac{0.693}{t_{1/2}} C_{D,\text{abs}}$$  \hspace{1cm} \text{Equation 49}

Combining the three kinetic processes, the main differential equations used to express drug and fatty acid concentration profiles are:
4.6.2 Calculation of Kinetic Parameters

Kinetic rate constant parameters for each of the following processes; digestion, drug release and drug absorption were determined in vitro. Other than formulation related parameters, diffusion coefficient of model drug compound Tempo Benzoate was also determined theoretically.

4.6.2.1 Digestion Kinetics

Formulation digestion kinetic constant pertaining to a formulation that consists of Soybean oil and Tween 80 at 1:1 ratio was calculated as explained in Section 4.4.2. The rate constant was calculated to be 1.92 mol/cm$^2$s.

Using the calculated digestion rate constant, model fits of digestion product, free fatty acid, concentration in aqueous solution, $C_{FA,aq}$ were compared with experimental findings from in vitro digestion studies. Model fit was in close comparison with experimental findings for fatty acid concentration over time (Figure 22).

\[
\frac{dC_{FA,aq}}{dt} = k_{dig} \frac{A_{em}}{V_{aq}} \quad \text{Equation 50}
\]

\[
\frac{dC_{D,aq}}{dt} = - \frac{A_{em} P_{rel}}{V_{aq}} (C_{D,aq} - C_{D,eq}) + k_{dig} S_{em} \frac{1}{n} V_{molar,em} C_{D,em} - \frac{P_{int} A_{int}}{V_{int}} \frac{C_{D,aq}}{1 + K_2} \quad \text{Equation 51}
\]

\[
\frac{dC_{D,em}}{dt} = \frac{V_{aq}}{n} \left( \frac{m_{FA,D} - C_{FA,aq} V_{aq}}{V_{molar,em}} \left( \frac{A_{em} P_{rel}}{V_{aq}} (C_{D,aq} - C_{D,eq}) - k_{dig} S_{em} \frac{1}{n} V_{molar,em} C_{D,em} \right) \right) \quad \text{Equation 52}
\]

\[
\frac{dC_{D,abs}}{dt} = \frac{P_{int} A_{int}}{V_{plasma}} \frac{C_{D,eq}}{1 + K_2} - \frac{0.693}{t_{1/2}} C_{D,abs} \quad \text{Equation 53}
\]
4.6.2.2 Drug release kinetics

Some of the variables pertaining to drug release expressions, $C_{D_{aqeq}}$, as well as partition coefficients $K_1$ and $K_2$ are not constants and will be altered as a result of formulation digestion. In other words, they are only constant for a given micellar concentration, but we assume that aqueous micellar concentration and consequently drug solubilization capacity of the aqueous media will be changed due to incorporation of digested formulation products, fatty acids, into bile/salt phospholipid micelles. It was observed with in vitro drug release studies that there is a certain drug concentration in the aqueous phase at time 0. We believe this is due to the fact that once SEDDS formulation is emulsified in the fasted state biorelevant media, certain amount of the surfactant, Tween 80, associates with the micelles with a certain drug concentration that is initially dissolved in the surfactant component of the formulation. This association is, in fact, in agreement with SANS findings (Section 4.4.1) where it was shown that there was an alteration in BS/PL micelles with the addition of formulation into the aqueous system.
This effect is shown in Table 11 by increased drug solubilization at equilibrium in $C_{D,aq}^{eq}$ with Tween 80 compared to $C_{D,aq}^{eq}$, where $C_{D,aq}^{eq}$ is the drug solubility in aqueous phase before formulation is dispersed and $C_{D,aq}^{eq}$ with Tween 80 is the drug solubility in aqueous phase after formulation is dispersed. In order to estimate the amount of Tween 80 that associates into micelles, experimental values for $C_{aq}$ at time 0 in the presence formulation at 1:100 dilution in aqueous phase (and therefore Tween 80 associated in micelles) and initial drug loading in Tween 80 was used. Amount of Tween 80 that associates in micelles, $C_{Tween80}$, when 1:100 dilution formulation in aqueous phase is dispersed, was calculated to be 0.008148 mmols/ml. The initial total aqueous surfactant concentration was calculated using:

$$C_{surf} = C_{BS} + C_{PL} + C_{Tween80}$$  \hspace{1cm} \text{Equation 54}$$

and was used to calculate molar solubilization constant, $\chi$, using Equation 36 as 0.2115.

Other constant variables $C_{D,\text{water}}^{eq}$ and $C_{D,\text{em}}^{eq}$ were calculated experimentally by measuring model drug, Tempo Benzoate, equilibrium solubilities in each phase; formulation and maleate buffer. Table 11 summarizes equilibrium solubility values that were measured in each phase.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Equilibrium solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maleate buffer</td>
<td>0.013</td>
</tr>
<tr>
<td>Fasted state biorelevant media</td>
<td>0.096</td>
</tr>
<tr>
<td>Fasted state biorelevant media with Tween 80</td>
<td>0.3958</td>
</tr>
<tr>
<td>Self-emulsifying formulation</td>
<td>161.1</td>
</tr>
</tbody>
</table>
Tempo benzoate release kinetics was studied by in vitro release experiments in the absence of digestive enzyme. There was an initial “burst” release of the drug in the aqueous media upon dispersion of the oil/surfactant/drug mixture, as discussed above. Over 18 hours, 14% of drug was released (Figure 23) Oil-water interphase permeability constant, \( P_{rel} \), was calculated by in vitro experimental release studies using Equation 32 as \( 5.55 \times 10^{-9} \text{ cm/s} \).

![Graph of drug release over time](image)

**Figure 23:** In vitro drug release from SEDDS in the absence of digestion. Error bars represent variation of two independent measurements.
4.6.2.3 Drug absorption kinetics

Tempo benzoate is, of course, not an actual drug but rather a spin probe that is useful for EPR analysis, and a hydrophobic compound that is assigned as our “model hydrophobic drug compound”. Therefore, in order to perform in vitro absorption experiments, a hydrophobic compound, Danazol, similar to TB in molecular weight and logP, was used. Permeability constant $P_{int}$ was calculated experimentally using Caco-2 cultures.

Figure 24: Absorption kinetics of Danazol across Caco-2 monolayers administered in biorelevant media. Error bars were calculated based on three independent measurements. 25% of drug compound administered in biorelevant solution was absorbed over two hours (Figure 24). In order to calculate free drug concentration, $C_{water}$, in the apical medium from total aqueous concentration over time, drug micelle-water partition coefficient was utilized. Partition coefficient, $K_2$, was calculated as 5.2 according to Equation 35 by measuring Danazol equilibrium solubility in water and micellar aqueous solution respectively.
Danazol permeability across Caco-2 monolayers were calculated using free drug concentration and the following equation:

\[
K_2 = \frac{C_{eq, micelle}^{D}}{C_{D,water}^{eq}} = \frac{C_{eq, D,ag}^{D} - C_{eq, D,water}^{D}}{C_{D,water}^{eq}}
\]

Equation 55

where \( dm_{D,abs} / dt \) is the mass of drug leaving apical compartment per time, \( P_{int} \) is the permeability, \( A \) is the cell culture insert membrane area, \( C_{apic,water}^{D} \) is free drug concentration in the apical compartment, and \( C_{baso,water}^{D} \) is free drug concentration in basolateral compartment. Permeability of Danazol across Caco-2 monolayers was calculated to be 0.0192 cm/min.

### 4.6.2.4 Tempo Benzoate dissolution rate in the absence of SEDDS

Utilizing Wilke-Chang equation, diffusion coefficient of Tempo Benzoate was calculated 6.7x10^{-6} cm²/s. Other input parameters used to compute dissolution rate are given in Table 12.

### 4.6.3 Model Predictions

Kinetic constants calculated separately for each of the processes; formulation digestion, drug release from formulation droplets in the absence of digestive enzyme and free drug absorption across intestinal membrane were used to solve developed mathematical model simultaneously for all the processes occurring over the same time course in order to predict the synergetic effect of all kinetic processes on oral drug absorption to plasma. Developed mathematical models were solved numerically using Runge-Kutta method in Matlab in order to predict oral absorption of model drug...
administered with SEDDS. Input parameters to the developed model are given in Table 12.

**Table 12: Input parameters to the developed model**

<table>
<thead>
<tr>
<th>Input parameters to the model</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physiological parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Volume of intestinal lumen [139]</td>
<td>250 ml</td>
</tr>
<tr>
<td>Volume of plasma [140]</td>
<td>2800 ml</td>
</tr>
<tr>
<td>Radius of intestinal lumen [141]</td>
<td>1.75 cm</td>
</tr>
<tr>
<td>Bile salt concentration</td>
<td>$5 \times 10^{-3} \text{ mmol/ml}$</td>
</tr>
<tr>
<td>Phospholipid concentration</td>
<td>$1.25 \times 10^{-3} \text{ mmol/ml}$</td>
</tr>
<tr>
<td>Critical micelle concentration</td>
<td>$1.6 \times 10^{-3} \text{ mmol/ml}$</td>
</tr>
<tr>
<td><strong>Formulation and drug parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Droplet size of emulsions</td>
<td>403 nm</td>
</tr>
<tr>
<td>Molecular weight of TB</td>
<td>276.35 mg/mmols</td>
</tr>
<tr>
<td>Number of digestible FAs per mole emulsion</td>
<td>2.168</td>
</tr>
<tr>
<td>Initial concentration of TB in formulation</td>
<td>7, 200, 400 mg/ml</td>
</tr>
<tr>
<td>Initial volume ratio of formulation in intestinal lumen</td>
<td>1:100</td>
</tr>
<tr>
<td>Concentration of Tween 80 associated in BS/PL micelles</td>
<td>8.14 mmol/ml</td>
</tr>
<tr>
<td>Molar solubilization of TB in BS/PL micelles</td>
<td>0.2115 mmols/ml</td>
</tr>
<tr>
<td>Solubility of TB in water</td>
<td>$4.7 \times 10^{-5} \text{ mmol/ml}$</td>
</tr>
<tr>
<td>Solubility of TB in formulation</td>
<td>$5.79 \times 10^{-1} \text{ mmol/ml}$</td>
</tr>
<tr>
<td>Stationary diffusion layer</td>
<td>30 µm</td>
</tr>
<tr>
<td>Particle size of TB</td>
<td>30 µm</td>
</tr>
<tr>
<td><strong>Kinetic parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Diffusion coefficient of TB</td>
<td>$6.7 \times 10^{-6} \text{ cm}^2/\text{s}$</td>
</tr>
<tr>
<td>Intestinal permeability of Danazol</td>
<td>$1.628 \times 10^{-4} \text{ mmol/ml}$</td>
</tr>
<tr>
<td>Formulation digestion kinetic constant</td>
<td>$1.92 \times 10^{-9} \text{ mmol/cm}^2 \cdot \text{s}$</td>
</tr>
<tr>
<td>Formulation release constant of TB</td>
<td>$5.55 \times 10^{-9} \text{ cm/s}$</td>
</tr>
<tr>
<td>Elimination constant of Danazol [142]</td>
<td>$4.28 \times 10^{-5} \text{ 1/s}$</td>
</tr>
</tbody>
</table>

4.6.3.1 *Comparison of model predictions with in vitro experimental results*

Model predictions for drug release in the presence of digestion was compared with combined release and digestion experiments analyzed by EPR. Experimental aqueous drug concentration, $C_{D,aq}$, values obtained using EPR during digestion were compared with simulation results for $C_{D,aq}$ over time upon 7 mg/ml drug load in
formulation. Close comparison of simulation results with EPR suggests the validity of the assumptions made constructing the model such as increased aqueous solubilization being proportional to free fatty acid concentration, and shrinkage of emulsion droplet size proportional to the amount of fatty acid leaving droplets.

Figure 25: Comparison of model simulation results (continuous line) with experimental results (data points) pertaining to drug release from inside of emulsion droplets to the outside aqueous media during formulation digestion.
4.6.3.2 Simulation results for absorbed drug: sensitivity to parameter variations

Simulations were run to predict absorbed plasma drug concentration, $C_{D_{abs}}$, distribution over time upon oral administration of a SEDDS formulation with 7 mg/ml formulation drug load. We assumed that only the free drug in intestinal lumen is absorbed across intestinal wall. Another assumption made was regarding the small intestinal emptying time. Simulation results indicated that concentration of drug in the GI tract peaks at around 3 hours and then gradually decrease over 50 hours. Physiologically, 50% of the contents of small intestine empty in 2.5-3 hours [143]. Therefore we assumed that after 6 hours all content in intestinal lumen was emptied.

Sensitivity of absorbed drug plasma profiles to variation in kinetic constant parameters of formulation digestion, drug release across oil water interphase, and transport across intestinal lumen were investigated. Figure 26 demonstrates the effect of digestion kinetic constant, $k_{dig}$, variation on drug absorption profile compared to the original SEDDS formulation value, $k_{dig}=1.92e-9s^{-1}$. A 20-fold increase in digestion kinetic constant resulted with a decreased AUC (area under the concentration curve) whereas a 20-fold decrease resulted with an increased value therefore pointing out an inverse correlation between formulation digestion rate and amount of drug absorbed into plasma. Susceptibility to decreased bioavailability with a high digestion rate SEDDS formulation was previously reported with an in vivo test in beagle dogs[117]. It was shown that Danazol dosed orally at ~5mg/ml concentration in SMEDDS formulation (~3ml) had an increased bioavailability with a long chain triglyceride based formulation compared to solid dosage form and no enhancement with a medium chain SEDDS
formulation. This contrast effect on drug absorption was attributed to differences in formulation digestion rates; a higher digestion rate with medium chain lipid based formulation compared to long chain triglyceride based SEDDS formulation. This effect was contributed to faster digesting SEDDS formulation resulting with drug precipitation. In contrast, what we observe with current simulation is a higher aqueous concentration over time with a faster digestion rate however a lower corresponding free drug concentration due to most probably increased drug partitioning into more lipophilic mixed micellar phase. Free drug concentration is what impacts the overall compound absorption. On the other hand, surprisingly, AUC values showed no sensitivity to drug release parameter, permeability across oil water interphase.

Figure 26: Simulation results for drug absorption with SEDDS demonstrating the effect of variation in digestion kinetic constant on plasma drug concentration profiles. k=1.92e-9 is the original value for SEDDS formulation studied. Units of kinetic constants are 1/s.
Secondly, the effect of varying intestinal drug permeability constant on plasma drug profiles was investigated (Figure 27). As expected, an increase in permeability constant resulted with an increase in AUC and vice versa.

![Graph showing plasma drug concentration profiles](image)

**Figure 27**: Simulation results for drug absorption with SEDDS demonstrating the effect of variation in intestinal drug permeability on plasma drug concentration profiles. \( P=3.24\times10^{-4} \) is the original permeability value for SEDDS formulation studied. Units of permeability are cm/s.

4.6.3.3 *Simulation results for absorbed compound: solid dosage form versus SEDDS*

The effect of SEDDS formulation on enhancing oral drug absorption of model drug was assessed by comparing simulation results of solid drug absorption with simulation of drug absorption with SEDDS. Simulation of solid drug absorption consisted of the dissolution of Tempo benzoate in the intestinal lumen in the absence of SEDDS and then absorption across intestinal cell wall into plasma. We observed that there was a strong dependence of initial drug load on the overall influence of SEDDS on drug absorption (Figure 28). SEDDS formulation resulted with an enhancement in oral absorption compared to solid dosage form, when the drug loading was 400 mg. On the
other hand, when the loading was 200 mg, solid dosage form resulted with a higher degree of absorption compared to SEDDS formulation. In our model, we assume that only the free drug in the intestinal lumen (not associated with mixed micelles) is absorbed to plasma. Therefore the factor that determines the extent of absorption is the free drug concentration in the intestinal lumen over time. At lower drug loading concentrations (i.e. 200 mg), in the presence of SEDDS formulation, even though the total aqueous (in micelles and free drug) drug concentration was higher over time, corresponding free drug concentration (which correlates with the aqueous concentration with partition coefficient $K_2$) was lower compared to the solid dosage form. In the case of a higher initial loading concentration (i.e. 400 mg), there is an increased overall aqueous drug concentration over time in the presence of SEDDS, and consequently the corresponding free drug concentration. On the other hand, dissolution of the solid dosage form in intestinal lumen is governed by drug’s solubility in water based intestinal solution and is not proportional to the initial drug loading beyond certain concentration resulting with almost no change in the dissolution profile.
Figure 28: Simulation results for drug absorption with SEDDS versus solid dosage form for A) 400 mg, B) 200 mg drug load.
5.0 CONCLUSIONS

5.1 Statistical design of SEDDS formulations and investigation of kinetic processes

This study was performed in an effort to develop guidance for emulsion-based formulation design based on experimental mechanistic understandings of how formulation parameters relate to function. Representative formulations of emulsion based drug delivery systems based on different oil structural classes, surfactant HLB, and oil-to-surfactant ratios were prepared and tested for their ability to spontaneously emulsify and several emulsion characteristics. The impact of these formulation parameters on mean droplet size, cytotoxicity, intestinal permeability, release kinetics, and droplet surface charge, formulation digestion and lymphatic transport were studied via analysis of variance and regression models.

Results identify the strong influences of certain formulation parameters and interactions on emulsion characteristics and function. The effect of surfactant HLB on emulsion formation was underscored by formulations with HLB 10 not spontaneously emulsifying. Exposure to high concentrations of high HLB surfactant (Tween 80), however, resulted in decreased cell monolayer integrity, possibly due to loosening of tight junctions. Release rates were related to oil-surfactant pair carbon chain length and surfactant concentration. Digestion rates were higher for formulations with medium chain oils compared to long chain oils, and among all the design parameters studied, oil to surfactant ratio had the least affect on digestion rate constant. Formulations overall induced similar levels of ApoB secretion, in other words likelihood to stimulate lymphatic transport, but Tween 80 and Neobee M5 at 1:1 weight ratio, even though not significantly, showed higher levels of ApoB secretion compared to controls. It was also
shown that regression modeling could be used to estimate key parameters reflective of performance of specific formulations such as digestion kinetics with high degrees of predictability ($R^2=897$).

### 5.2 Development of a system-based model predicting oral compound absorption co-administered with SEDDS

A system-based model was constructed in order to explain gastrointestinal mass transport properties of a drug administered with a self-emulsifying drug delivery system orally. The following kinetic processes of SEDDS were considered building the model; formulation digestion, drug release from formulation and drug absorption. Simulation results for simultaneous formulation digestion and drug release out of formulation emulsion droplets were compared with combined in vitro digestion and release EPR experimental results. There were close comparison between simulation results and experimental findings indicating the validity of assumptions made. The model was further employed into a one compartment pharmacokinetic model in order to predict absorbed drug plasma concentration profiles. Sensitivity of extent of absorbed drug to variations in kinetic constant parameters was assessed. There was an inverse correlation between the rate of digestion and the amount of drug absorbed whereas surprisingly, there was no effect of variations in drug release constant on the amount of drug absorbed. Simulation results were further used to compare the extent of drug absorption in cases where the compound is taken orally as a solid form versus within SEDDS, in order to assess the level enhancement (or not) in oral bioavailability of a drug administered with SEDDS. Results demonstrate a strong dependence of level of enhancement of oral
absorption on initial formulation loading. We believe that the significance of such predictive guidance on drug formulation process would be extremely high.
6.0 RECOMMENDATIONS

The recommendations for future research, based on the presented results, are firstly in regard to the analysis of fundamental mechanisms across broader range of drugs compounds. Studying drugs with a range of different hydrophobicity will help further generalize these results and will strengthen the overall mechanistic model to optimize drug absorption using emulsion-based drug delivery.

Regression models presented here that constitute the first part of this investigation, eventually could be incorporated into the overall systems-based model presented here predicting drug absorption upon dosing with SEDDS. Mathematical simulation model inputs, kinetic constant parameters, that pertain to mass transfer expressions for drug release, digestion kinetics and drug permeation across the intestinal membrane would contain parameters predicted by regression models. In parallel, experimental design methods could be used to determine optimal formulations that balance multiple desirable properties (e.g. multivariate optimization, desirability and loss functions, goal programming, stochastic optimization, et cetera). This may be especially helpful for optimizing inherent tradeoffs between different combinations of formulation values that separately optimize mean particle size, toxicity, permeability, and release. Another set of important results is the significant amount of experimental variability (standard deviation) in specific characteristics within some formulations, and differences between formulations in the size of these within-formulation variabilities, which typically would not be known via physical models. These results have implications on formulation design optimization, as it may be desirable to identify formulations that both give desirable mean results but also high consistency (i.e. low variability) in performance. Future potential
useful analyses from the experimental design field therefore might include variance minimizing and robust designs that perform desirably well with maximum consistency across uncontrollable (e.g. within and between patient) in vivo environments.

Lastly, in vivo testing of specific SEDDS formulation studied for oral drug bioavailability is recommended for validation of the presented model simulations. Simulations presented here were constructed based on assumptions such as the specific formulation studied did not alter lymphatic transport as an alternative route to hepatic transport, and only the free drug in the intestinal lumen is transported across the intestinal membrane in order to associate with blood stream. It is recommended that these assumptions be validated with in vivo experimental tests comparing the actual bioavailability achieved of a drug co-administered with oral SEDDS formulation with model simulations.

As stated above, results demonstrated here were established with the assumption that only free drug in the intestinal lumen (not associated with micelles or with lipids) is transported into the blood stream. However there is literature suggesting otherwise may also be possible. Further investigation of compound absorption associated with lipid digestion products/mixed micelles and of intracellular lipid trafficking, might shed light into contribution of the absorption mechanisms (both intestinal absorption and alteration of lymphatic transport) on overall impact of SEDDS on oral drug bioavailability.
## 7.0 NOMENCLATURE

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{em}$</td>
<td>total emulsion surface area, cm$^2$</td>
</tr>
<tr>
<td>$A_{int}$</td>
<td>surface area of intestine, cm$^2$</td>
</tr>
<tr>
<td>$C_{FA,aq}$</td>
<td>concentration of fatty acids (FA) in aqueous media, mols/cm$^3$</td>
</tr>
<tr>
<td>$C_{D,oil}$</td>
<td>drug concentration in oil, mols/volume oil</td>
</tr>
<tr>
<td>$C_{D,aq}$</td>
<td>drug concentration in the aqueous phase (water and micelles), mols/cm$^3$.</td>
</tr>
<tr>
<td>$C_{D,micelle}$</td>
<td>drug concentration in micelles per aqueous medium, mols/cm$^3$.</td>
</tr>
<tr>
<td>$C_{D,aq}^{eq}$</td>
<td>hypothetical aqueous concentration of solute in the aqueous phase, mols/cm$^3$.</td>
</tr>
<tr>
<td>$C_{D,water}$</td>
<td>concentration of drug in water, mols/cm$^3$</td>
</tr>
<tr>
<td>$C_{D,oil}^{eq}$</td>
<td>equilibrium solubility of drug in oil, mols/cm$^3$ oil</td>
</tr>
<tr>
<td>$C_{D,aq}^{eq}$</td>
<td>equilibrium solubility of drug in the aqueous phase, mols/cm$^3$ aqueous volume</td>
</tr>
<tr>
<td>$C_{D,micelle}^{eq}$</td>
<td>equilibrium solubility of drug in micelles, mols/cm$^3$ aqueous volume</td>
</tr>
<tr>
<td>$C_{D,water}^{eq}$</td>
<td>equilibrium solubility of drug in the water phase, mols/cm$^3$</td>
</tr>
<tr>
<td>D</td>
<td>diameter of oil droplet over time, cm</td>
</tr>
<tr>
<td>$D_0$</td>
<td>initial diameter of an oil droplet, cm</td>
</tr>
<tr>
<td>$k_{dig}$</td>
<td>digestion rate constant, mols/s*cm$^2$</td>
</tr>
<tr>
<td>K</td>
<td>partition coefficient of drug between oil and aqueous phase</td>
</tr>
<tr>
<td>$K_2$</td>
<td>partition coefficient of drug between water and micelles</td>
</tr>
<tr>
<td>$m_{FA,aq}$</td>
<td>amount of free FAs in aqueous solution, mols</td>
</tr>
<tr>
<td>$m_{drug,abs}$</td>
<td>amount of drug absorbed across intestinal membrane, mols</td>
</tr>
<tr>
<td>$m_{FA,em}$</td>
<td>remaining amount digestible FA’s in an oil emulsion droplet, mols</td>
</tr>
<tr>
<td>$m_{FA,0}$</td>
<td>initial amount of digestible FA’s in oil emulsion droplet, mols</td>
</tr>
</tbody>
</table>
n  number of moles FA produced per mole of oil, unitless
P_{rel}  permeability of drug across oil water interphase, cm/s
P_{int}  permeability of intestinal membrane, cm/s
S_{em}  total droplet surface area per total aqueous volume, cm$^2$/cm$^3$
V_{aq}  aqueous volume, cm$^3$
V_{em}  volume of oil emulsions over time, cm$^3$
V_{em,0}  initial volume of oil emulsions, cm$^3$
V_{molar,oil}  molar volume of oil
\chi  molar solubilization capacity of micelles at certain concentration
8.0 REFERENCES


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