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2. Introduction

2.1 Background of poorly water soluble drugs:

An increasing number of recently discovered drug substances exhibit poor water solubility and hence low absorption after oral administration. Technology Catalysts International reported in 2002 that approximately 35-40% of all new chemical compounds suffer from poor aqueous solubility \(^1, 2\). The issue arose in particular when drug discovery moved from wet chemistry to combinatorial chemistry and high throughput screening in the mid 1990's. The properties of new chemical entities (NCE) shifted towards higher molecular weight and increasing lipophilicity, resulting in decreased aqueous solubility. Due to poor aqueous solubility, many drug candidates become unsuccessful to reach the market in spite of exhibiting potential pharmacodynamic activity. Also, poorly water soluble drugs currently on the market are administered at much higher individual doses than actually desired to achieve necessary plasma levels. The introduction of the Biopharmaceutical Classification System (BCS) has provided a basis to categorize drugs based on the two major parameters affecting absorption - solubility and permeability. Therefore, strategies to improve the aqueous solubility and the release rate of drugs are employed and are under constant investigation.

Several strategies to improve the solubility and dissolution of poorly water soluble drugs have been developed and described in literature, which were at start primarily based on modifying the drug's physicochemical properties. Particle size reduction and salt formation became frequently taken paths in a quest for dissolution improvement, but both methods revealed limitations\(^3, 4\). As a result, altering drug solubility or dissolution through formulation approaches has become more and more popular. Methods to improve drug bioavailability may involve the alteration of various key factors that determine drug dissolution, as described by the Noyes-Whitney equation \(^5\):\n
\[
\frac{dM}{dt} = \frac{DA(Cs - Ct)}{h}
\]

In equation, \(dM/dt\) represents the dissolution rate, \(A\) the specific surface area of the drug particle, \(D\) the diffusion coefficient, \(h\) the diffusion layer thickness, \(Cs\) the
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saturation solubility and C1 the drug concentration at time t. That is, dissolution rate can be increased by increasing the surface area from where dissolution can take place, by decreasing the diffusional layer thickness and by altering the solubility of the drug. Lipid formulations and in particular Self Micro Emulsifying Drug Delivery Systems (SMEDDS) can induce a considerable increase in dissolution rate as these strategies can simultaneously alter various of these factors\textsuperscript{[6-9]}. SMEDDS is a pre-mixture of drug, oil, surfactants and co-surfactants and is able to form microemulsion under gentle shaking or stirring spontaneously. Microemulsion is a very clear, isotropic, transparent and thermodynamically stable system with a very small particle size (below 100nm)\textsuperscript{[10]}.

2.2 Introduction to lipid-based formulations:

The different lipid drug delivery systems available include lipid solution, lipid emulsion, microemulsion, dry emulsion. To get a clear picture of all these different systems and due to large number of possible excipient combinations that may be used to assemble these lipid-based formulations, self emulsifying systems in particular a classification system have been established called as lipid formulation classification system (LFCS). This classification helps to better understand the fate of different lipid formulation in vivo, it also helps to use a systematic & rational formulation approach avoid “trial-and-error” iterations and provide framework to guide regulatory agencies. LFCS was established by Pouton in 2000 and recently updated. \textsuperscript{[7]} The LFCS briefly classifies lipid-based formulations into four types according to their composition and the possible effect of dilution and digestion on their ability to prevent drug precipitation, as shown in Table 1\textsuperscript{[11]}.

**Type I** systems consist of formulations which comprise drug in solution in triglycerides and/or mixed glycerides or in oil-in-water emulsion stabilized by low concentrations of emulsifiers such as 1% (w/v) polysorbate 60 and 1.2% (w/v) lecithin. Generally, these systems exhibit poor initial aqueous dispersion and require digestion by pancreatic lipase/ co-lipase in the GIT to generate more amphiphilic lipid digestion products and promote drug transfer into the colloidal aqueous phase. Type I lipid formulations therefore represent a relatively simple formulation option for potent drugs or highly lipophilic compounds where drug solubility in oil is sufficient to allow incorporation of the required payload (dose)\textsuperscript{[12]}.
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Table 1: Compositions of lipid-based formulation\textsuperscript{[11]}

<table>
<thead>
<tr>
<th>Composition</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oil</td>
<td>SEDDS</td>
<td>III A</td>
<td>III B</td>
</tr>
<tr>
<td>Glycerides (TG, DG, MG)</td>
<td>100%</td>
<td>40-80%</td>
<td>40-80%</td>
<td>&lt; 20%</td>
</tr>
<tr>
<td>Surfactants (HLB &lt; 12)</td>
<td>-</td>
<td>20-60%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(HLB &gt; 12)</td>
<td>-</td>
<td>-</td>
<td>20-40%</td>
<td>20-50%</td>
</tr>
<tr>
<td>Hydrophilic co-solvents</td>
<td>-</td>
<td>-</td>
<td>0-40%</td>
<td>20-50%</td>
</tr>
<tr>
<td>Particle size of dispersion (nm)</td>
<td>Coarse</td>
<td>100-250</td>
<td>100-250</td>
<td>50-100</td>
</tr>
</tbody>
</table>

**Type II** lipid formulations constitute SEDDS. Self-emulsification is generally obtained at surfactant contents above 25% (w/w). However at higher surfactant contents (greater than 50–60% (w/w) depending on the materials) the progress of emulsification may be compromised by the formation of viscous liquid crystalline gels at the oil/water interface. Type II lipid-based formulations provide the advantage of overcoming the slow dissolution step typically observed with solid dosage forms and as described above generate large interfacial areas which in turn allows efficient partitioning of drug between the oil droplets and the aqueous phase from where absorption occurs\textsuperscript{[12, 13]}.

**Type III** lipid-based formulations, commonly referred to as self-microemulsifying drug delivery systems (SMEDDS), are defined by the inclusion of hydrophilic surfactants (HLB>12) and co-solvents such as ethanol, propylene glycol and polyethylene glycol. Type III formulations can be further segregated (somewhat arbitrarily) into Type IIIA and Type IIIB formulations in order to identify more hydrophilic systems (Type IIIB) where the content of hydrophilic surfactants and co-solvents increases and the lipid content reduces. Type IIIB formulations typically
achieve greater dispersion rates when compared with Type IIIA although the risk of drug precipitation on dispersion of the formulation is higher given the lower lipid content\textsuperscript{[11]}. 

**Type IV**: In order to capture the recent trend towards formulations which contain predominantly hydrophilic surfactants and co-solvents, this category was recently added. Type IV formulations do not contain natural lipids and represent the most hydrophilic formulations. These formulations commonly offer increased drug payloads when compared to formulations containing simple glyceride lipids and also produce very fine dispersions when introduced in aqueous media. Little is known however, as to the solubilisation capacity of these systems \textit{in-vivo} and in particular whether they are equally capable of maintaining poorly water soluble drug in solution during passage along the GIT when compared with formulations comprising natural oils (Type II and Type III). An example of a Type IV formulation is the current capsule formulation of the HIV protease inhibitor amprenavir (Agenerase) which contains TPGS as a surfactant and PEG 400 and propylene glycol as co-solvents\textsuperscript{[14]}.

### 2.3 Introduction to microemulsion and self-microemulsifying drug delivery system (SMEDDS)

Microemulsions are isotropic, thermodynamically stable, transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a co-surfactant with a droplet size usually in the range of 10-100 nm. These homogeneous systems, which can be prepared over a wide range of surfactant concentration and oil to water ratio, are all fluids of low viscosity\textsuperscript{[15,16]}. 

Microemulsion as drug delivery tool show favorable properties like thermodynamic stability (long shelf-life), easy formation (zero interfacial tension and almost spontaneous formation), optical isotropy, ability to be sterilized by filtration, high surface area (high solubilization capacity) and very small droplet size. The small droplets also provide better adherence to membranes and transport drug molecules in a controlled fashion\textsuperscript{[15,16]}.
2.3.1 Structure of Microemulsion

Microemulsions are dynamic systems in which the interface is continuously and spontaneously fluctuating. Structurally, they are divided into oil-in-water (o/w), water in oil (w/o) and bicontinuous microemulsion. In w/o microemulsion, water droplets are dispersed in the continuous oil phase while o/w microemulsion is formed when oil droplets are dispersed in the continuous aqueous phase as shown in Figure 1. In systems where the amounts of water and oil are similar, a bicontinuous microemulsion may result, i.e. Figure 2.

In all three types of microemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants. The mixture of oil, water and surfactants is able to form a wide variety of structures and phases depending upon the proportions of the components. The flexibility of the surfactant film is an important factor in this regard. A flexible surfactant film will enable the existence of several different structures like droplet like shapes, aggregates and bicontinuous structures, and therefore broaden the range of microemulsion existence. A very rigid surfactant film will not enable existence of bicontinuous structures which will impede the range of existence\[15\].

Besides microemulsion, structural examinations can reveal the existence of regular emulsions, anisotropic crystalline hexagonal or cubic phases, and lamellar structures depending on the ratio of the components. The internal structure of a microemulsion vehicle is very important for the diffusivity of the phases, and thereby also for the diffusion of a drug in the respective phases. Researchers have been trying zealously to understand the complicated phase behavior and the various microstructures encountered in the microemulsion systems. The difference between coarse emulsion and microemulsion is explained in Figure 3 and Table 2.
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![Figure 1: Structure of Microemulsion\textsuperscript{17}](image1)

Figure 1: Structure of Microemulsion\textsuperscript{17}

![Figure 2: Schematic representation of the three most commonly encountered microemulsion microstructures\textsuperscript{17}](image2)

Figure 2: Schematic representation of the three most commonly encountered microemulsion microstructures\textsuperscript{17}

![Figure 3: Emulsions and Microemulsions\textsuperscript{17}](image3)

Figure 3: Emulsions and Microemulsions\textsuperscript{17}
### Table 2: Differences between Emulsions and Microemulsions\[^{15,17}\]

<table>
<thead>
<tr>
<th>Properties</th>
<th>Emulsions</th>
<th>Microemulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Cloudy</td>
<td>Transparent (or translucent)</td>
</tr>
<tr>
<td>Phases</td>
<td>Biphasic</td>
<td>Monophasic</td>
</tr>
<tr>
<td>Optical isotropy</td>
<td>Anisotropic</td>
<td>Isotropic</td>
</tr>
<tr>
<td>Proportion of dispersed phase</td>
<td>30-60%</td>
<td>23-40% without corresponding increase in viscosity</td>
</tr>
<tr>
<td>Interfacial tension</td>
<td>High</td>
<td>Ultra low</td>
</tr>
<tr>
<td>Microstructure</td>
<td>Static</td>
<td>Dynamic (interface is continuously and spontaneously fluctuating)</td>
</tr>
<tr>
<td>Droplet size</td>
<td>&gt; 500 nm</td>
<td>20-200 nm</td>
</tr>
<tr>
<td>Energy requirement</td>
<td>Requires large energy input at the time of preparation</td>
<td>Forms spontaneously, so no energy requirement</td>
</tr>
<tr>
<td>Stability</td>
<td>Thermodynamically unstable (kinetically stable), will eventually phase separate</td>
<td>Thermodynamically stable, long shelf life</td>
</tr>
<tr>
<td>Surfactant concentration</td>
<td>2-3% by weight</td>
<td>&gt;6% by weight</td>
</tr>
<tr>
<td>Nature</td>
<td>They are lyophobic.</td>
<td>They are on the borderline between lyophobic and lyophilic colloids.</td>
</tr>
<tr>
<td>Preparation</td>
<td>Require a large input of energy, higher cost</td>
<td>Facile preparation, relatively lower cost for commercial production</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Higher viscosity</td>
<td>Low viscosity with Newtonian behavior</td>
</tr>
</tbody>
</table>
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2.3.2 Self Microemulsifying Drug Delivery System (SMEDDS)

SMEDDS are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants that have a unique ability of forming fine oil-in-water (o/w) microemulsion upon mild agitation followed by dilution in aqueous media, such as GI fluids. SMEDDS spread readily in the GI tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification. The basic difference between self emulsifying drug delivery systems (SEDDS) also called as self emulsifying oil formulation (SEOF) and SMEDDS is SEDDS typically produce opaque emulsions with a droplet size between 100 and 300 nm while SMEDDS form transparent micro emulsions with a droplet size of less than 100 nm also the concentration of oil in SMEDDS is less than 20 % as compared to 40-80% in SEDDS. When compared with emulsions, which are sensitive and metastable dispersed forms, SMEDDS are physically stable formulations that are easy to manufacture. Thus, for lipophilic drug compounds which exhibit dissolution rate-limited absorption, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood-time profiles. The key step is to find a suitable oil surfactant mixture that can dissolve the drug within the required therapeutic concentration. The SMEDDS mixture can be filled in either soft or hard gelatin capsules. A typical SMEDDS formulation contains oils, surfactants and if required an antioxidants. Often co-surfactants and co-solvents are added to improve the formulation characteristics[14].

Advantages of SMEDDS over Emulsion

- SMEDDS not only offer the same advantages of emulsions of facilitating the solubility of hydrophobic drugs, but also overcomes the drawback of the layering of emulsions after sitting for a long time. SMEDDS can be easily stored since it belongs to a thermodynamically stable system.
- Microemulsions formed by the SMEDDS exhibit good thermodynamics stability and optical transparency. The major difference between the above microemulsions and common emulsions lies in the particle size of droplets. The size of the droplets of common emulsion ranges between 0.2 and 10 μm, and that of the droplets of microemulsion formed by the SMEDDS
2. Introduction

generally ranges between 2 and 100 nm (such droplets are called droplets of nano particles). Since the particle size is small, the total surface area for absorption and dispersion is significantly larger than that of solid dosage form and it can easily penetrate the gastrointestinal tract and be absorbed. The bioavailability of the drug is therefore improved.

- SMEDDS offer numerous delivery options like filled hard gelatin capsules or soft gelatin capsules or can be formulated in to tablets whereas emulsions can only be given as an oral solutions\(^{[18]}\).

**Advantages of Microemulsion/SMEDDS as oral drug vehicle\(^{[16,17]}\):**

- Increases the rate of absorption.
- Eliminates inter-subject and intra-subject variability in absorption.
- Helps to solubilize lipophilic drug.
- Provides an aqueous dosage form for water insoluble drugs.
- Thermodynamically stable system, so for long time they can remain stable without any type of aggregation or creaming.
- Releases drug in controlled fashion.
- Minimizes first pass metabolism.
- Increases bioavailability.
- Helpful in taste masking.
- Provides protection from hydrolysis and oxidation as drug in oil phase in O/W microemulsion is not exposed to attack by water and air.
- Ease of preparation due to spontaneous formation.
- Scale up process is also easy.

**Disadvantages of Microemulsion/SMEDDS as oral drug vehicle\(^{[16,17]}\):**

- Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nano-droplets.
- Limited solubilizing capacity for high-melting substances.
- The surfactant must be nontoxic for using pharmaceutical applications.
- Microemulsion stability is influenced by environmental parameters such as temperature and pH.
- For unique dosage preparation in gelatin capsules, it may produce softening or hardening effect on capsule shell, so for long term storage it is undesirable.
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2.3.3 Biopharmaceutical Aspects

The ability of lipids to enhance the bioavailability of poorly water-soluble drugs has been comprehensively reviewed and though incompletely understood. The currently accepted view is that lipids may enhance bioavailability via a number of potential mechanisms, including\(^\text{19}\):

a) Alterations (reduction) in gastric transit, thereby slowing delivery to the absorption site and increasing the time available for dissolution.

b) Increases in effective luminal drug solubility: The presence of lipids in the GI tract stimulates an increase in the secretion of bile salts (BS) and endogenous biliary lipids including phospholipids (PL) and cholesterol (CH), leading to the formation of BS/PL/CH intestinal mixed micelles and an increase in the solubilization capacity of the GI tract. However, intercalation of administered (exogenous) lipids into these BS structures either directly (if sufficiently polar), or secondary to digestion, leads to swelling of the micellar structures and a further increase in solubilization capacity.

c) Stimulation of intestinal lymphatic transport: For highly lipophilic drugs, lipids may enhance the extent of lymphatic transport and increase bioavailability directly or indirectly via a reduction in first-pass metabolism, Figure 4.

d) Changes in the biochemical barrier function of the GI tract: It is clear that certain lipids and surfactants may attenuate the activity of intestinal efflux transporters, as indicated by the p-glycoprotein efflux pump, and may also reduce the extent of enterocyte based metabolism.

e) Changes in the physical barrier function of the GI tract: Various combinations of lipids, lipid digestion products and surfactants have been shown to have permeability enhancing properties.

Results of study for intestinal lymphatic transport of halofantrine shows the significant effect that small amounts of lipid present within a single lipid-based dose form can have on the transport of a lymphatically transported drug administered in the fasted state. The reported data have implications with regard to (a) the recruitment of the lymphatics as an absorption pathway after fasted administration of a lipid-based formulation, (b) altered drug delivery profiles as lymphatically transported drugs access the mesenteric lymphatics and associated lymph nodes and then empty into the systemic circulation at the junction of the left subclavian vein and the jugular vein, (c) possible changes in the pharmacokinetics and systemic clearance of lipophilic drugs,
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(d) the potential stimulation of lymphatic transport when a lipophilic drug is ingested in a partial-prandial state (e.g., as a consequence of the presence of a small amount of dietary lipid from a snack or previously consumed meal), and (e) safety assessment of lipophilic new drug candidates, which are often administered in conjunction with lipids and/or lipidic excipients to enhance drug exposure\(^\text{[20]}\).

Figure 4: Potential mechanisms for absorption enhancement for lipid based formulation\(^\text{[21]}\)

2.3.4 Enhanced Drug Absorption by Lymphatic Delivery:

Drug candidates for lymphatic transport should have a log P >5 and, in addition, a triglyceride solubility >50 mg/ml. The importance of lipid solubility was illustrated by a comparing the lymphatic transport of DDT (log P -6.19) with hexachlorobenzene (HCB, log P -6.53). While both compounds have similar log P values, the difference in lymphatic transport on administration in oleic acid, 33.5% of the dose in the case of DDT and 2.3% with HCB, was attributed to the 13- fold difference in triglyceride
solubility. However, combination of a high log P and high triglyceride solubility does not always guarantee significant lymphatic transport. Penclomedine, an experimental cytotoxic agent with a log P of 5.48 and a triglyceride solubility of 175mg/ml, was poorly transported in the intestinal lymph, ~3% of the dose. Although enhanced lymphatic transport has been suggested as a potential mechanism of enhanced bioavailability few studies have investigated the lymphotropic potential of SMEDDS. However, one such study investigated the effects of a range of lipid-based formulations on the bioavailability and lymphatic transport of ontazolast, following oral administration to conscious rats. This drug undergoes extensive hepatic first-pass metabolism and it has solubility in soybean oil of 55 mg/ml, and a log P of 4. The formulations of ontazolast investigated included a suspension (lipid-free control), a 20% soybean o/w emulsion, and two SMEDDS containing Gelucire44/14 and Peceol in the ratios 50:50 and 80:20, respectively, and a solution of the drug in Peceol alone. All the lipid formulations increased the bioavailability of ontazolast relative to the control suspension, while the SMEDDS promoted more rapid absorption. Maximum lymphatic transport occurred with the emulsion and the Peceol solution. The emulsion prolonged lymphatic transport and this may be related to the need for preabsorptive lipolysis of the triglyceride vehicle and an associated slower gastric emptying time. The SMEDDS formulations resulted in the highest concentration of ontazolast in the chylomicron triglyceride. The authors suggest that SMEDDS, which promote more rapid absorption of ontazolast, could produce higher concentrations of the drug in the enterocytes during absorption and hence improve lymphatic drug transport by a concentration-partitioning phenomenon\[22\].

**Susceptibility to Digestion:**

The well known positive effect of food on the bioavailability of many poorly water soluble drugs is often ascribed to the ingested lipid and indicate the beneficial role of lipids on drug absorption. Although the form, content and volume of dietary lipids is markedly different to oil phases included in a pharmaceutical formulation, possible food effects on drug bioavailability can be a starting point for the design of lipid self-emulsifying formulations for such drugs. The presence of lipids in the GI tract increases drug solubilization and thus drug dissolution via a number of potential mechanisms.
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- An increased secretion of bile salts and endogenous biliary lipids
- An intercalation of administered lipids into bile salt structures, directly or after digestion
- A reduced gastric transit time, resulting in an increased dissolution time
- Changes of the physical and biochemical barrier function of the intestinal tract.

Various lipid digestion products and surfactants show permeability enhancing properties and/or alternate the activity of intestinal efflux transporters.

The co-administration of drugs with lipids can also have an effect on their absorption path. Although most orally administered compounds gain access to the systemic circulation via the portal vein, some highly lipophilic drugs (e.g. halofantrine) are transported to the systemic circulation via the intestinal lymphatics in association with the lipid core of lipoproteins. Although the absorption capacity is marginal as compared to absorption via the portal vein, lymphatic transport may markedly increase overall drug absorption of drug suffering from significant first-pass metabolism. The extent of drug absorption via the lymphatic system is highly influenced by the characteristics of the co-administered lipid. Medium chain lipids don’t affect lymphatic transport in contrast to the stimulating impact of long chain lipids, of which the digestion products are re-esterified in the intestinal cell, incorporated into chylomicrons and secreted by exocytosis into the lymph vessel[23].

In addition to the effect of the formulation on the GI tract, the intestinal lumen in turn has an impact on the performance of lipid-based formulations. The presence of lipid in the duodenum stimulates the secretion of salts, biliary lipids and pancreatic juice. Pancreatic lipase/co lipase can attach to oil/water interfaces and hydrolyze lipids prior to absorption. The digestion products, which are more water soluble than the parent lipids, are solubilised within bile salt mixed micelles before delivery to the absorptive cells of the gastrointestinal tract. These lipolytic compounds can enhance drug solubilization as they can affect the dissolution medium, self-associate and interact with endogenous biliary derived colloidal components (such as bile salts, phosphatidylcholine, and cholesterol). Digestion products can thus be incorporated into o/w interfaces, resulting in a decrease of the critical micellar concentration (CMC) and swelling of bile salts micelles already present.
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The lipid chain length has an important influence on the solubilization and distribution of poorly water-soluble drugs in the digestion phases. In the case of SMEDDS based on medium chain lipids, digestion phases consist of a colloidal aqueous phase and an undissolved pellet phase. The colloidal aqueous phase formed upon digestion of long chain lipids exhibits an improved solubilization capacity. However, digestion of SMEDDS containing long chain lipids may produce a residual oil phase in addition to the aqueous and pellet phase. The latter can retain highly lipophilic drugs, thereby interfering with the dissolution of highly lipophilic drugs into the aqueous phase. Hence, the solubilization capacity of the aqueous phase upon lipid digestion and the hydrolysis rate will be critical for the absorption of highly lipophilic drugs. The oral administration of less lipophilic drugs may be limited by the drug dose since solubility in the SMEDDS formulation can be low. This can however be encountered by increasing the surfactant concentration. In addition to the lipophilicity of the drug (which dictates the dose and partitioning behavior) and the nature of colloidal structures produced on digestion of the different formulation lipids, solubilization behavior of drugs on SMEDDS digestion is a function of the kinetics of drug transfer between the digesting formulation and the colloidal phases produced. The kinetics of drug solubilization, which occurs together with formulation digestion and dispersion and which leads to temporary supersaturation conditions, may explain irregularities between in-vitro and in-vivo data\[^{23}\].

2.3.5 Theories and Thermodynamics of Microemulsion Formulations

Historically, three different approaches have been proposed to explain microemulsion formation and the stability aspects.

(i) Interfacial or mixed film theories,
(ii) Solubilization theories and
(iii) Thermodynamic theories

The important features of the microemulsion are thermodynamic stability, optical transparency, large overall interfacial area (about 100 m\(^2\)/ml), variety of structures, low interfacial tension and increased solubilization of oil/water dispersed phase. Microemulsion requires more surfactant than emulsion to stabilize a large overall interfacial area.
The interfacial tension between the oil and water can be lowered by the addition and adsorption of surfactant. When the surfactant concentration is increased further, it lowers the interfacial tension till CMC (Critical Micelle Concentration). The micellar formation commences beyond this concentration of surfactant. This negative interfacial tension leads to a simultaneous and spontaneous increase in the area of the interface. The large interfacial area formed may divide itself into a large number of closed shells around small droplets of either oil in water or water in oil and further decrease the free energy of the system. In many cases, the interfacial tension is not yet ultra low when the CMC is reached. It has been studied and observed by Schulman and workers that the addition of a co-surfactant (medium sized alcohol or amine) to the system results in virtually zero interfacial tension. The further addition of a surfactant (where, interfacial tension (\(\gamma\)) is zero) leads to negative interfacial tension.

**Interfacial/Mixed Film Theories:**

The relatively large entropy of mixing of droplets and continuous medium explains the spontaneous formation of microemulsion. Schulman emphasized the importance of the interfacial film. They considered that the spontaneous formation of microemulsion droplets was due to the formation of a complex film at the oil-water interface by the surfactant and co-surfactant. This caused a reduction in oil-water interfacial tension to very low values (from close to zero to negative) which is represented by following equation\[^{[17,18]}\].

\[
\gamma_i = \gamma_{o/w} - \pi_i
\]

Where, \(\gamma_{o/w}\) = Oil-water interfacial tension without the film present

\(\pi_i\) = Spreading pressure

\(\gamma_i\) = Interfacial tension

**Mechanism of curvature of a duplex film:**

The interfacial film should be curved to form small droplets to explain both the stability of the system and bending of the interface. A flat duplex film would be under stress because of the difference in tension and spreading of pressure on either side of it. Reduction of this tension gradient by equalizing the two surface tensions is the driving force for the film curvature. Both sides of the interface expand spontaneously...
with penetration of oil and co surfactant until the pressures become equal. The side with higher tension would be concave and would envelop the liquid on that side, making it an internal phase. It is generally easier to expand the oil side of an interface than the waterside and hence W/O microemulsion can be formed easily than O/W microemulsion.

**Solubilization Theories:**

Shinoda et al. considered microemulsion to be thermodynamically stable monophasic solution of water-swollen (W/O) or oil swollen (O/W) spherical micelles. Rance and Friberg illustrated the relationship between reverse micelles and W/O microemulsion with the help of phase diagrams. The inverse micelle region of ternary system i.e. water, pentanol and sodium dodecyl sulphate (SDS) is composed of water solubilized reverse micelles of SDS in pentanol. Addition of O-xylene up to 50% gives rise to transparent W/O region containing a maximum of 28% water with 5 % pentanol and 6% surfactant (i.e. microemulsions). The quaternary phase diagram constructed on adding p-xylene shows relationship of these areas to the isotropic inverse micellar phase. These four component systems could be prepared by adding hydrocarbon directly to the inverse micellar phase by titration. Thus the system mainly consists of swollen inverse micelle rather than small emulsion droplets.

**Thermodynamic Theories**

This theory explains the formation of microemulsion even in the absence of co surfactant. The free energy of microemulsion formation can be considered to depend on the extent to which surfactant lowers the surface tension of the oil–water interface and the change in entropy of the system such that,

\[ \Delta G_m = \Delta G_1 + \Delta G_2 + \Delta G_3 - T \Delta S \]

\( \Delta G_m \) = free energy change for microemulsion formation  
\( \Delta G_1 \) = free energy change due to increase in total surface area  
\( \Delta G_2 \) = free energy change due to interaction between droplets  
\( \Delta G_3 \) = free energy change due to adsorption of surfactant at the oil/water interface from bulk oil or water  
\( \Delta S \) = increase in entropy due to dispersion of oil as droplets
In other way, we can write it as,

$$\Delta G_f = \gamma \Delta A - T \Delta S$$

Where, $\Delta G_f =$ Free energy of formulation

$\gamma =$ Surface tension of the oil-water interface

$\Delta A =$ Change in surface area on microemulsification

$\Delta S =$ Change in entropy of the system

$T =$ Temperature

Thermodynamic theory takes into account entropy of droplets and thermal fluctuations at the interface as important parameters leading to interfacial bending instability. Originally workers proposed that in order for a microemulsion to be formed a negative value of $\gamma$ was required, it is now recognized that while value of $\gamma$ is positive at all times, it is very small, and is offset by the entropic component. The dominant favorable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. However, there are also expected to be favorable entropic contributions arising from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achievable when large reductions in surface tension are accompanied by significant favorable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable.

Later it was shown that accumulation of the surfactant and co-surfactant at the interface results in a decrease in chemical potential generating an additional negative free energy change called as dilution effect. This theory explained the role of co-surfactant and salt in a microemulsion formed with ionic surfactants. The co-surfactant produces an additional dilution effect and decreases interfacial tension further. The addition of salts to system containing ionic surfactants causes similar effects by shielding the electric field produced by the adsorbed ionic surfactant the adsorption of large amount of surfactant\textsuperscript{[17, 18]}. 
Theory of Self Emulsification Process:

Self-emulsification is a phenomenon which has been widely exploited commercially in formulations of emulsifiable concentrates of herbicides and pesticides. Concentrates of crop-sprays are to be diluted by the user, such as farmers or household gardeners, allowing very hydrophobic compounds to be transported efficiently. In contrast, SMEDDS, using excipients acceptable for oral administration to humans, have not been widely exploited and knowledge about their physicochemical principles is therefore limited \[^{24}\].

(a) Mechanism of Self Emulsification:

In emulsification process the free energy ($\Delta G$) associated is given by the equation:

$$\Delta G = \sum N_i \pi r_i$$

In which ‘N’ is Number of droplets with radius ‘r’ and ‘\(\pi\)’ is interfacial energy

It is apparent from equation that the spontaneous formation of the interface between the oil and water phases is energetically not favored. The system commonly classified as SEDDS have not yet been shown to emulsify spontaneously in the thermodynamic sense. The process of self-emulsification was observed using light microscopy. Groves and Mustafa developed a method of quantitatively assessing the ease of emulsification by monitoring the turbidity of the oil-surfactant in a water stream using phosphated nonylphenoloxylate (PNE) and phosphated fatty alcohol ethoxlate (PFE) in n-hexane. Pouton has argued that the emulsification properties of the surfactant may be related to phase inversion behavior of the system. For example, on increase the temperature of oil in water system stabilized using nonionic surfactant; the cloud point of the surfactant will be reached followed by phase inversion. The surfactant is highly mobile at the phase inversion temperature; hence the o/w interfacial energy is minimized leading to a reduction in energy required to cause emulsification. The specificity of surfactant combination required to allow spontaneous emulsification may be associated with a minimization of the phase inversion temperature, thereby increasing the ease of emulsion. Phase studies are also necessary for liquid crystal formation in self-emulsification. These indicate that good formulations are usually operating close to a phase inversion region and in a region of enhanced close to a phase inversion region and in a region of enhanced aqueous solubilization. In the
phase diagram of the system (30 % w/w tween and 85/70 % w/w MCT oil) for dilution in water over a range of temperature shows that the phase inversion region is at approximately 40°C and the system works well at ambient temperature up to 60°C above which water in oil emulsion tend to form\textsuperscript{[24]}.

The emulsification process may be associated with the ease with which water penetrates the oil-water interface with the formation of liquid crystalline phases resulting in swelling at the interface thereby resulting in greater ease of emulsification\textsuperscript{[25]}.

**b) Dilution phases**

Upon dilution of a SMEDDS formulation, the spontaneous curvature of the surfactant layer changes via a number of possible liquid crystalline phases. The droplet structure can pass from a reversed spherical droplet to a reversed rod-shaped droplet, hexagonal phase, lamellar phase, cubic phase and various other structures until, after appropriate dilution, a spherical droplet will be formed again (Figure 5)\textsuperscript{[25]}.

\[\text{Figure 5: Representation of the most commonly encountered phases upon addition of water to an oil-surfactant combination}\textsuperscript{[25]}\]

Many roles have been described to the occurrence of liquid crystalline phases upon aqueous dilution of a lipid formulation. Early work of Groves and Mustafa related the emulsification behaviour to the phase behaviour of the surfactant-oil mixtures with systems forming liquid crystals showing shorter emulsification times\textsuperscript{[25]}. The authors
suggested that the ease of emulsification could be associated with the passage of water into the droplet, more precisely the ease with which the solvent may penetrate into the liquid crystalline phases formed on the surface of the droplet. The structures formed upon dilution have been ascribed an important role in the stability of the diluted microemulsion and the rate of drug release. This can be explained by the fact that a layer of liquid crystalline material surrounds the oil droplets, affecting drug dissolution and formulation digestion. Some examples are shown in Table 5\textsuperscript{[26]}

### 2.3.6 Factors Affecting Formation and Phase Behavior of Microemulsions/SMEDDS

**Factor affecting formation of Microemulsion system\textsuperscript{[16,27]}**

The formation of oil or water swollen microemulsion depends on the packing ratio, property of surfactant, oil phase, temperature, the chain length, type and nature of co-surfactant.

- **Packing ratio:**

  The HLB (Hydrophilic Lipophilic Balance) of surfactant determines the type of microemulsion through its influence on molecular packing and film curvature. The analysis of film curvature for surfactant associations leading to microemulsion formation has been explained by Israelachvili et al (1976) and Mitchell and Ninham (1977) in terms of packing ratio, also called as critical packing parameter.

  Critical Packing Parameter (CPP) = \(\frac{v}{a} \times l\)

  Where, \(v\) is the partial molar volume of the hydrophobic portion of the surfactant,
  
  \(a\) is the optimal head group area and
  
  \(l\) is the length of the surfactant tail.

  If CPP has value between 0 and 1 interface curves towards water (positive curvature) and o/w systems are favoured but when CPP is greater than 1, interface curves spontaneously towards oil (negative curvature) so w/o microemulsions are favoured. At zero curvature, when the HLB is balanced (\(p\) is equivalent to 1), then either bi continuous or lamellar structures may form according to the rigidity of the film (zero curvature).

- **Property of surfactant, oil phase and temperature:**
The type of microemulsion depends on the nature of surfactant. Surfactant contains hydrophilic head group and lipophilic tail group. The areas of these groups, which are a measure of the differential tendency of water to swell head group and oil to swell the tail area, are important for specific formulation when estimating the surfactant HLB in a particular system. When a high concentration of the surfactant is used or when the surfactant is in presence of salt, degree of dissociation of polar groups becomes lesser and resulting system may be w/o type. Diluting with water may increase dissociation and leads to an o/w system. Ionic surfactants are strongly influenced by temperature. It mainly causes increased surfactant counter-ion dissociation. The oil component also influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chains oils penetrate the lipophilic group region to a great extent and results in increased negative curvature. Temperature is extremely important in determining the effective head group size of nonionic surfactants. At low temperature, they are hydrophilic and form normal o/w system. At higher temperature, they are lipophilic and form w/o systems. At an intermediate temperature, microemulsion coexists with excess water and oil phases and forms bicontinuous structure.

- **The chain length, type and nature of co-surfactant:**
  Alcohols are widely used as a co-surfactant in microemulsions. Addition of shorter chain co-surfactant gives positive curvature effect as alcohol swells the head region more than tail region so, it becomes more hydrophilic and o/w type is favoured, while longer chain co-surfactant favours w/o type w/o type by alcohol swelling more in chain region than head region.

**Factor affecting phase behavior**[^16, ^18]

- **Salinity:**
  At low salinity, the droplet size of o/w microemulsion increases. This corresponds to increase in the solubilization of oil. As salinity further increases, the system becomes bi-continuous over an intermediate salinity range. Increase in salinity leads to formation of continuous microemulsion with reduction in globule size. Further increase in salinity ultimately results in complete phase transition.
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- **Alcohol concentration:**
  Increasing the concentration of low molecular weight alcohol as a co-surfactant leads to the phase transition from w/o to bi-continuous and ultimately to o/w type microemulsion. Exactly opposite phase transition is noticed in case of high molecular weight alcohol.

- **Surfactant hydrophobic chain length:**
  The increase in length of hydrophobic chain length of the surfactant shows the change of o/w microemulsion to w/o via bi-continuous phase.

- **pH:**
  Change in pH influences the microemulsions containing pH sensitive surfactants. This effect is more pronounced in case of acidic or alkaline surfactants. Carboxylic acids and amines change the phase behaviour from w/o to o/w by increasing the pH.

- **Nature of oil:**
  Increase in the aromaticity of oil leads to phase transition from o/w to w/o and is opposite to that of increase in the oil alkane carbon number.

- **Ionic strength:**
  As the ionic strength increases the system passes from o/w microemulsion in equilibrium with excess oil to the middle phase and finally to w/o microemulsion in equilibrium with excess water.

### 2.3.7 Pharmaceutical Applications of Microemulsions/SMEDDS

**Oral Delivery**

Microemulsion formulations offer the several benefits over conventional oral formulation for oral administration including increased absorption, improved clinical potency and decreased drug toxicity. Therefore, microemulsion have been reported to be ideal delivery of drugs such as steroids, hormones, diuretic and antibiotics. The microemulsion droplets dispersed in the gastrointestinal tract provide large surface area and promote a rapid release of dissolved form of the drug substance and/or mixed micelles containing drug substance, and they may be also responsible for transporting the drug through the unstirred water layer to the gastrointestinal membrane for
absorption. In addition to the enhanced dissolution of drugs, another factor contributing to the increasing bioavailability is that lymphatic transport is responsible for a portion of the entire drug uptake as well. The lipid composition of system may be related to facilitate the extent of lymphatic drug transport by stimulating lipoprotein formation and intestinal lymphatic liquid flux.

Pharmaceutical drugs of peptides and proteins are highly potent and specific in their physiological functions. However, most are difficult to administer orally. With on oral bioavailability in conventional (i.e. non-microemulsion based) formulation of less than 10%, they are usually not therapeutically active by oral administration. Because of their low oral bioavailability, most protein drugs are only available as parenteral formulations. However, peptide drugs have an extremely short biological half life when administered parenterally, so require multiple dosing\(^{28}\). A microemulsion formulation of cyclosporine, named Neoral\(^{®}\) has been introduced to replace Sandimmune\(^{®}\), a crude oil-in-water emulsion of cyclosporine formulation. Neoral\(^{®}\) is formulated with a finer dispersion, giving it a more rapid and predictable absorption and less inter and intra patient variability\(^{29}\).

**Parenteral Delivery**

Microemulsion formulations have distinct advantages over macroemulsion systems when delivered parenterally because of the fine particle microemulsion is cleared more slowly than the coarse particle emulsion and, therefore, have a longer residence time in the body. Both O/W and W/O microemulsion can be used for parenteral delivery. The literature contains the details of the many microemulsion which cannot be used for the parenteral delivery because the toxicity of the surfactant and parenteral use. The alternative approach was taken by Von Corsevant and Thoren in which C3-C4 alcohols were replaced with parenterally acceptable co-surfactants, polyethylene glycol (400) / polyethylene glycol (660) 12-hydroxystearate / ethanol\(^{30}\).

**Topical delivery**

Topical administration of drugs can have advantages over other methods for several reasons, one of which is the avoidance of hepatic first pass metabolism of the drug and related toxicity effects. Another is the direct delivery and targetability of the drug to affected area of the skin or eyes. Both O/W and W/O microemulsions have been
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evaluated in a hairless mouse model for the delivery of prostaglandin E1\textsuperscript{[31]}. The microemulsions were based on oleic acid or Gelucire 44/14 as the oil phase and were stabilized by a mixture of Labrasol (C\textsubscript{8} and C\textsubscript{10} polyglycolysed glycerides) and Pluronic Oleique CC 497 as surfactant. The use of lecithin/IPP/water microemulsion for the transdermal transport of indomethacin and diclofenac has also been reported \textsuperscript{[32]}.

Ocular and Pulmonary Delivery

For the treatment of eye diseases, drugs are essentially delivered topically. O/W microemulsions have been investigated for ocular administration, to dissolve poorly soluble drugs, to increase absorption and to attain prolong release profile. The microemulsions containing pilocarpine were formulated using lecithin, propylene glycol and PEG 200 as co-surfactant and IPM as the oil phase. The formulations were of low viscosity with a refractive index lending to ophthalmologic applications. The formation of a water-in-HFA propellent microemulsion stabilized by fluorocarbon non-ionic surfactant and intended for pulmonary delivery has been described \textsuperscript{[33]}.

Microemulsions in Biotechnology

Biphasic media are also used for enzymatic and biocatalytic types of reactions. Enzymes in low water content display and have increased solubility in non-polar reactants and improvement of thermal stability enabling reactions to be carried out at higher temperatures. Enzymatic catalysis in microemulsions has been used for a variety of reactions, such as synthesis of esters, peptides and sugar acetals transesterification, various hydrolysis reactions and steroid transformation. The most widely used class of enzymes in microemulsion-based reactions is of lipases \textsuperscript{[34]}.

Intranasal Drug Delivery

Intranasal administration of microemulsion based systems help in overcoming the first pass hepatic metabolism of labile drugs, thereby assist in improving the therapeutic levels of drug at the site of action and enhance bio availability of drugs. Bhanushali and Bajaj, (2007) reported improved brain targeting and higher brain uptake of Sumatriptan following intranasal administration of mucoadhesive microemulsion formulations of the drug \textsuperscript{[35]}.
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2.3.8 Solid Self-Microemulsifying Drug Delivery System

SMEDDS can exist in either liquid or solid states. SMEDDS are usually, limited to liquid dosage forms, because many excipients used in SMEDDS are not solids at room temperature. Given the advantages of solid dosage forms, solid self Microemulsifying drug delivery (S-SMEDDS) have been extensively exploited in recent years, as they frequently represent more effective alternatives to conventional liquid SMEDDS.

From the perspective of dosage forms, S-SMEDDS mean solid dosage forms with self-emulsification properties. S-SMEDDS focus on the incorporation of liquid/semisolid SE ingredients into powders/nanoparticles by different solidification techniques (e.g. adsorptions to solid carriers, spray drying, melt extrusion, nanoparticle technology, and so on). Such powders/nanoparticles, which refer to SE nanoparticles/dry emulsions/solid dispersions are usually further processed into other solid SE dosage forms, or, alternatively, filled into capsules (i.e. SE capsules). SE capsules also include those capsules into which liquid/semisolid SEDDS are directly filled without any solidifying excipient\cite{36}.

To some extent, S-SMEDDS are combinations of SMEDDS and solid dosage forms, so many properties of S-SMEDDS (e.g. excipients selection, specificity, and characterization) are the sum of the corresponding properties of both SMEDDS and solid dosage forms. For instance, the characterizations of SE pellets contain not only the assessment of self-emulsification, but also friability, surface roughness, and so on.

Solidification techniques for transforming liquid/semisolid SEDDS

- Adsorption to solid carriers

Free flowing powders may be obtained from liquid SE formulations by adsorption to solid carriers. The adsorption process is simple and just involves addition of the liquid formulation onto carriers by mixing in a blender. The resulting powder may then be filled directly into capsules or, alternatively, mixed with suitable excipients before compression into tablets. A significant benefit of the adsorption technique is good content uniformity. SEDDS can be adsorbed at high levels (up to 70% (w/w)) onto suitable carriers\cite{37}. 
Solid carriers can be microporous inorganic substances, high surface-area colloidal inorganic adsorbent substances, cross-linked polymers or nanoparticle adsorbents, for example, silica, silicates, magnesium trisilicate, magnesium hydroxide, talcum, crospovidone, cross-linked sodium carboxymethyl cellulose and crosslinked polymethyl methacrylate \[38\]. Cross-linked polymers create a favorable environment to sustain drug dissolution and also assist in slowing down drug reprecipitation \[39\]. Nanoparticle adsorbents comprise porous silicon dioxide (Sylysia 550), carbon nanotubes, carbon nailohorns, fullerene, charcoal and bamboo charcoal \[40\].

- **Other techniques**

Capsule filling is the simplest and the most common technology for the encapsulation of liquid or semisolid SE formulations for the oral route \[41\]. In parallel with the advances in capsule technology proceeding, liquid-Oros technology (ALZACorporation) has been designed for controlled delivery of insoluble drug substances or peptides \[42, 43\]. The liquid/semisolid lipophilic vehicles compatible with hard capsules were listed by Cole et al. \[44\]. The technique of spray drying involves the preparation of a formulation by mixing lipids, surfactants, drug, solid carriers, and solubilization of the mixture before spray drying. The solubilized liquid formulation is then atomized into a spray of droplets.

Melt granulation is a process in which powder agglomeration is obtained through the addition of a binder that melts or softens at relatively low temperatures \[45\]. The melt granulation process was usually used for adsorbing self emulsifying mass onto solid neutral carriers \[46, 47\]. Melt extrusion is a solvent-free process that allows high drug loading (60%) \[41\], as well as content uniformity \[48\]. Studies suggested that the maximum quantity of this Self emulsifying system that can be solidified by extrusion spheronization occupies 42% of the dry pellet weight \[49\]. It has been shown that Self emulsifying system with a wide range of rheological characteristics can be processed \[50\]. Applying extrusion-spherization, SE pellets of diazepam and progesterone and bi-layered cohesive SE pellets have been prepared \[51-53\].

In the 1990s, S-SEDDS were usually in the form of SE capsules, SE solid dispersions and dry emulsions, but other solid SE dosage forms have emerged in recent years, such as SE pellets/tablets, SE microspheres/nanoparticles and SE
suppositories/implants\textsuperscript{[41]}. Various dosage forms of S-SMEDDS are as listed below:\textsuperscript{[54]}

- Dry emulsions
- Self-emulsifying capsules
- Self-emulsifying sustained/controlled-release tablets
- Self-emulsifying sustained/controlled-release pellets
- Self-emulsifying solid dispersions
- Self-emulsifying beads
- Self-emulsifying sustained-release microspheres
- Self-emulsifying nanoparticles
- Self-emulsifying suppositories
- Self-emulsifying implants
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2.4 Introduction to drugs used in study

The information for detailed profile of drugs has been taken from the official website of USFDA http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm from the label of product.

Cardiovascular disorders are the world’s most prevalent diseases. With the general aging of the world’s population and rapid socio-economic changes in the developing world, cardiovascular diseases are expected to increase further in the future. Hence, there is a great need for adequate pharmacotherapy to provide symptomatic prompt treatment and long-term protection. Among the most beneficial medications currently available are those that interfere with the actions of angiotensin II and others are calcium channel blockers. Angiotensin II has a well-defined tropic effect on vascular and cardiac cells and the extracellular matrix. Over the last decade, several clinical trials have demonstrated the benefits of blocking angiotensin II. In particular, the angiotensin-receptor blockers (ARBs), originally indicated for hypertension, have shown themselves to have cardiovascular benefits beyond lowering blood pressure. An increased understanding of the renin-angiotensin system and the development of angiotensin-converting enzyme (ACE) inhibitors and angiotensin-II receptor antagonists (such as valsartan) has formed a major part in these accomplishments. Renin is released from the juxtaglomerular cells of the kidney and then cleaves its substrate, angiotensinogen, to form angiotensin-I. This is converted, by the angiotensin converting enzyme (ACE), to angiotensin-II. In the cardiovascular system the products of this pathway are important in both blood pressure regulation and sodium and volume homeostasis; angiotensin-II has a number of important and complex physiological actions, notable among which is vasoconstriction which results in blood pressure elevation. ACE inhibitors were first introduced for the treatment of hypertension but subsequent studies have shown that they are also able to reduce mortality and morbidity in congestive cardiac failure, decrease morbidity and mortality after myocardial infarction, prevent re-infarction, influence atherosclerosis and slow diabetic complications, including nephropathy. Nevertheless, there are disadvantages and, in particular, ACE inhibitors degrade bradykinin, resulting in a prolongation if its normally short half-life; this and related effects seem to be responsible for the cough and angioedema that are prominent adverse effects with the
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ACE inhibitors and can usually be avoided by the use of an angiotensin-II receptor antagonist, such as valsartan, olmesartan, irbesartan, losartan\textsuperscript{[55]}. 

Calcium channel blockers work by blocking voltage-gated calcium channels (VGCCs) in cardiac muscle and blood vessels. This decreases intracellular calcium leading to a reduction in muscle contraction. In the heart, a decrease in calcium available for each beat results in a decrease in cardiac contractility. In blood vessels, a decrease in calcium results in less contraction of the vascular smooth muscle and therefore an increase in arterial diameter (CCBs do not work on venous smooth muscle), a phenomenon called vasodilation. Vasodilation decreases total peripheral resistance, while a decrease in cardiac contractility decreases cardiac output. Since blood pressure is determined by cardiac output and peripheral resistance, blood pressure drops. Calcium channel blockers are especially effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients\textsuperscript{[56]}. 

With a relatively low blood pressure, the afterload on the heart decreases; this decreases how hard the heart must work to eject blood into the aorta, and so the amount of oxygen required by the heart decreases accordingly. This can help ameliorate symptoms of ischaemic heart disease such as angina pectoris.

Unlike beta blockers, calcium channel blockers do not decrease the responsiveness of the heart to input from the sympathetic nervous system. Since moment-to-moment blood pressure regulation is carried out by the sympathetic nervous system (via the baroreceptor reflex), calcium channel blockers allow blood pressure to be maintained more effectively than do beta blockers\textsuperscript{[2,56]}. 


2.4.1 Felodipine

Felodipine is a dihydropyridine calcium-channel blocker, is used alone or with an angiotensin-converting enzyme inhibitor, to treat hypertension, chronic stable angina pectoris, and Prinzmetal’s variant angina. Felodipine is official in IP (2010), BP (2010) and USP 32 NF 27.

Therapeutic category: calcium channel blocker and antihypertensive agent.

Structure:

Molecular formula: C_{18}H_{19}Cl_{2}NO_{4}
Molecular weight: 384.3
Chemical name: Ethyl methyl (4RS)-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4 dihydropyridine-3,5-dicarboxylate
Appearance: White or light yellow, crystalline powder.
Usual strength: 2.5/5/10/20mg once a day
Solubility: Practically insoluble in water, freely soluble in acetone; methanol; methylene chloride.
Bioavailability: 15% (oral)
Protein binding: 99%
Log P: 3.8
Half life: 14.1 hours
Melting point: 145 °C
Preparation: Prolong released tablets
BCS class: II
Storage: protect from light
Clinical Pharmacology

**Mechanism of action:** Felodipine is a calcium channel blocker. It reversibly competes with nitrendipine and/or other calcium channel blockers for dihydropyridine binding sites, blocks voltage-dependent calcium currents in vascular smooth muscle and cultured rabbit atrial cells, and blocks potassium-induced contracture of the rat portal vein. By blocking the calcium channels, felodipine inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes and results in a decrease of peripheral vascular resistance.

**Absorption:** Is completely absorbed from the gastrointestinal tract; however, extensive first-pass metabolism through the portal circulation results in a low systemic availability of 15%. Bioavailability is unaffected by food.

**Volume of Distribution:** 10 L/kg

**Protein binding:** 99%, primarily to the albumin fraction.

**Metabolism:** Hepatic metabolism primarily via cytochrome P450 3A4. Six metabolites with no appreciable vasodilatory effects have been identified.

**Route of elimination:** Although higher concentrations of the metabolites are present in the plasma due to decreased urinary excretion, these are inactive. Animal studies have demonstrated that felodipine crosses the blood-brain barrier and the placenta.

**Clearance:** 0.8 L/min [Young healthy subjects]

**Toxicity:** Symptoms of overdose include excessive peripheral vasodilation with marked hypotension and possibly bradycardia. Oral rat LD$_{50}$ is 1050 mg/kg$^{[57-60]}$. 
2.4.2 Valsartan

Valsartan is a nonpeptide, orally active and specific angiotensin II antagonist acting on the AT1 receptor subtype. It is categorized in angiotensin receptor blocker. The Valsartan Heart Failure Trial demonstrated that the use of Valsartan was associated with reduced rates of heart failure related hospitalizations and mortality, as well as shorter duration of hospitalization. Valsartan is official in IP (2010).

**Therapeutic category:** Antihypertensive Agents, Angiotensin II Receptor Antagonists

**Structure:**

![Chemical Structure of Valsartan](image)

**Molecular formula:** C\textsubscript{24}H\textsubscript{29}N\textsubscript{5}O\textsubscript{3}

**Molecular weight:** 435.5188 gm/mol

**Chemical name:** (2S)-3-methyl-2-[N-\{4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl] phenyl\}methyl] pentanamido]butanoic acid.

**Appearance:** White or almost white crystalline powder

**Usual strength:** 80-160 mg/day

**Solubility:** Freely soluble in alcohol, slightly soluble in water

**Oral Bioavailability:** 25%

**Protein binding:** 95%

**Log P:** 5.68

**Half life:** 6 hours

**Melting point:** 116-117°C

**Preparation:** Tablet and capsule

**BCS class:** II

**Storage:** Store protected from light and moisture, at a temperature not exceeding 30°C.
Clinical Pharmacology

Mechanism of action: Valsartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosteronesecreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Valsartan is selective for AT1 and has virtually no affinity for AT2. Inhibition of aldosterone secretion may inhibit sodium and water reabsorption in the kidneys while decreasing potassium excretion. The primary metabolite of valsartan, valeryl 4-hydroxy valsartan, has no pharmacological activity.

Absorption: Valsartan peak plasma concentration is reached 2 to 4 hours after dosing. Valsartan shows bi-exponential decay kinetics following intravenous administration.

Volume of Distribution: The steady state volume of distribution of valsartan after intravenous administration is small (17 L), indicating that valsartan does not distribute into tissues extensively.

Protein binding: Valsartan is highly bound to serum proteins (95%), mainly serum albumin.

Metabolism: Valsartan is minimally metabolized in humans. The primary circulating metabolite, 4-OH-valsartan, is pharmacologically inactive and produced CYP2C9. 4-OH-valsartan accounts for approximately 9% of the circulating dose of valsartan. Although valsartan is metabolized by CYP2C9, CYP-mediated drug-drug interactions between valsartan and other drugs are unlikely.

Route of elimination: 83% of absorbed valsartan is excreted in feces and 13% is excreted in urine, primarily as unchanged drug.

Toxicity:
Cardiovascular: Palpitations
Dermatologic: Pruritus and rash
Digestive: Constipation, dry mouth, dyspepsia, and flatulence
Neurologic and Psychiatric: Anxiety, insomnia, paresthesia, and somnolence[2, 61-64].
2.5 Introduction to excipients used in the study

Pharmaceutical acceptability of excipients and the toxicity issues of the components used makes the selection of excipients really critical. There is a great restriction as which excipients to be used. Early studies revealed that the microemulsion formulation is specific to the nature of the oil/surfactant pair, the surfactant concentration and oil/surfactant ratio, the concentration and nature of co-surfactant and surfactant/co-surfactant ratio and the temperature. These important discoveries were further supported by the fact that only very specific combinations of pharmaceutical excipients led to efficient systems\[^{65, 66}\].

The main components of microemulsion system are:

1) Oil phase
2) Primary surfactant
3) Secondary surfactant (co-surfactant)
4) Co-Solvent

1) Oil phase

The oil represents one of the most important excipients in the formulation not only because it can solubilize the required dose of the lipophilic drug, it can increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride\[^{67}\].

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils penetrate the tail group region to a greater extent than long chain alkanes, and hence swell this region to a greater extent, resulting in increased negative curvature (and reduced effective HLB). Saturated (for example, lauric, myristic and capric acid) and unsaturated fatty acids (for example, oleic acid, linoleic acid and linolenic acid) have penetration enhancing property of their own and they have been studied since a long time. Fatty acid esters such as ethyl or methyl esters of lauric, myristic and oleic acid have also been employed as the oil phase. Lipophilic drugs are preferably solubilized in o/w microemulsions. The main criterion for selecting the oil phase is that the drug should have high solubility in it. This will minimize the volume of the formulation to deliver
the therapeutic dose of the drug in an encapsulated form\textsuperscript{[15]}. Examples of the commonly used oils are given in Table 5.

2) Surfactants

The surfactant chosen must be able to lower the interfacial tension to a very small value which Table 5 facilitates dispersion process during the preparation of the microemulsion and provide a flexible film that can readily deform around the droplets and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region\textsuperscript{[14]}.

Surfactants used to stabilize microemulsion system may be: (i) non-ionic, (ii) zwitterionic, (iii) cationic, or (iv) anionic surfactants. The surfactant used in microemulsion formation could be ionic or nonionic, which determines the stabilizing interactions of the hydrophilic end of the surfactant with the aqueous phase. Thus, while a nonionic surfactant is stabilized by dipole and hydrogen bond interactions with the hydration layer of water on its hydrophilic surface, an ionic surfactant is additionally stabilized by the electrical double layer. Thus, the effect of salt concentration on the stability of an emulsion or a microemulsion is more profound in the case of ionic surfactant than nonionic surfactants. However for pharmaceutical applications, ionic surfactants are not preferred due to toxicological concerns\textsuperscript{[14]}.

Non-ionic surfactants are generally considered to be acceptable for oral ingestion, and the emergence of several successful marketed products has given the industry confidence in lipid-based products. The oral and intravenous LD50 values for most non-ionic surfactants are in excess of 50 g/Kg and 5 g/Kg respectively, so 1 g surfactant in a formulation is well-tolerated for uses in acute oral drug administration. Non-ionic surfactants in commercially available solubilized oral formulations include polyoxyl 35 castor oil (Cremophor EL), polyoxyl 40 hydrogenated castor oil (Cremophor RH 40), polysorbate 20 (Tween 20), polysorbate 80 (Tween 80), \(d\)-\(\alpha\)-tocopherol polyethylene glycol 1000 succinate (TPGS), Solutol HS-15, sorbitan monooleate (Span 80), polyoxyl 40 stearate, and various polyglycolyzed glycerides including Labrafil M-1944CS, Labrafil M-2125CS, Labrasol, Gellucire 44/14, etc\textsuperscript{[68]}.

It is generally accepted that low HLB (3-6) surfactants are favoured for the formulation of w/o microemulsion, whereas surfactants with high HLB (8-18) are
preferred for the formation of o/w microemulsion. Surfactants having HLB greater than 20 often require the presence of co-surfactants to reduce their effective HLB to a value within the range required for microemulsion formation. Examples are given in Table 5.

3) Co-surfactants

In most cases, single-chain surfactants alone are unable to reduce the o/w interfacial tension sufficiently to enable a microemulsion to form. The presence of co-surfactants allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition. If a single surfactant film is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidizing groups (e.g. unsaturated bonds). Short to medium chain length alcohols (C3-C8) are commonly added as co-surfactants which further reduce the interfacial tension and increase the fluidity of the interface\textsuperscript{15}. Examples are given in Table 5.

Typical co-surfactants are short chain alcohols (ethanol to butanol), glycols such as propylene glycol, medium chain alcohols, amines or acids. The role of co-surfactant is to destroy liquid crystalline or gel structures that form in place of a microemulsion phase and co-surfactant free microemulsion in most system cannot be made except at high temperature\textsuperscript{69}.

The role of a co-surfactant is as following\textsuperscript{69}:
1) Increase the fluidity of the interface.
2) Destroy liquid crystalline or gel structure which would prevent the formation of microemulsion.
3) Adjust HLB value and spontaneous curvature of the interface by changing surfactant partitioning characteristic.

4) Co-solvents

The production of an optimum microemulsion requires relatively high concentrations (generally more than 30% w/w) of surfactants. Organic solvents such as, ethanol, propylene glycol (PG), and polyethylene glycol (PEG) are suitable for oral delivery, and they enable the dissolution of large quantities of either the hydrophilic surfactant or the drug in the lipid base. These solvents can even act as co-surfactants in microemulsion systems\textsuperscript{65}.
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### Table 3: Some commonly used components for Microemulsions

<table>
<thead>
<tr>
<th>Components</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oils</strong></td>
<td></td>
</tr>
<tr>
<td>LCDs</td>
<td>Corn oil, soyabean oil, safflower oil, olive oil, etc.</td>
</tr>
<tr>
<td>MCTs</td>
<td>Glyceryl tricaprylate/caprate: Captex 355, Miglyol 810, Neobee M-5, etc.</td>
</tr>
<tr>
<td>Mono/di glycerides</td>
<td>Glyceryl caprylate/caprate (Capmul MCM), Glycerol monooleate (Capmul GMO), etc</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Oleic acid</td>
</tr>
<tr>
<td>Propylene glycol esters</td>
<td>Capmul PG-8, Propylene glycol monolaurate (Lauroglycol)</td>
</tr>
<tr>
<td><strong>Surfactants</strong></td>
<td></td>
</tr>
<tr>
<td>HLB&lt;10</td>
<td>Phosphatidylcholine (Phospholipon), Unsaturated polyglycolized glycerides (Labrafil M 2125), Sorbitan monopalmitate (Span 40), Sorbitan monooleate (Span 80), etc.</td>
</tr>
<tr>
<td>HLB&gt;10</td>
<td>Polyoxyethylene 20 sorbitan monolaurate (Tween 20), Polyoxyethylene 20 sorbitan monooleate (Tween 80), Polyoxyl 35 castor oil (Cremophore EL), PEG-8 caprylic/capric glycerides (Labrasol), polyoxyl 40 hydrogenated castor oil (Cremophore RH 40), etc.</td>
</tr>
<tr>
<td><strong>Co-solvents</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Propylene glycol, Polyethylene glycol, Ethanol, etc.</td>
</tr>
</tbody>
</table>
2.5.1 Capmul MCM

Capmul products are mono-, di- and triglyceride oils prepared through the glycerolysis of select fats and oils. They can be prepared by esterification of glycerin with specific fatty acids. They are lipophilic, insoluble in water and soluble in oils at elevated temperatures. They are used to produce stable emulsions and to modify viscosity.

Capmul MCM function as very effective carrier and solubilizer of active compounds. Mono-diglyceride medium chain esters are particularly recommended for the dissolution of difficult compounds such as sterols and have also exhibited bacteriostatic activity\(^{[70]}\).

**Generic name:** Mono/diglycerides of caprylic acid

**Physiochemical property:**

**Physical State:** Soft solid or liquid.

**Odor:** Fatty odor.

**Appearance:** Soft solid or liquid.

**Vapor Pressure:** < 1 mmHg at 25°C

**Vapor Density:** > 1 (Air = 1)

**Solubility in Water:** Partially soluble.

**Stability and Reactivity:** stable at room temperature

**Hazardous polymerization:** no

**Conditions to avoid:** keep away from temperatures near the flash point. Store away from heat or direct sunlight.

**Polymerization:** product will not undergo polymerization.

**Hazardous decomposition products:** carbon monoxide and carbon dioxide.

**Incompatible materials:** oxidizers.
2.5.2 Polysorbate emulsifiers

2.5.2.1 Tween 20[^71-74]

Generic name: Polyoxyethylene sorbitan monolaurate, polyethylene glycol sorbitan Monolaurate, polysorbate 20.

Functional Category: Dispersing agent, emulsifying agent, nonionic surfactant, solubilizing agent, suspending agent, wetting agent

Physiochemical property

Physical state and appearance: Viscous liquid
Color: yellow to yellow green
Boiling/condensation point: > 100 °C
Odor: almost odorless
Melting/freezing point: no data available
pH of 1% aqueous solution: 5-7
Refractive index: 1.4685
HLB value: 16.7
CMC value: 60 mg/l
Flash point: 110°C - closed cup
Flammability limits: no data available
Water solubility: soluble in water
Density (at 25°C): 1.1 g/cm³

Toxicity Data (Acute)

LD50

Oral (rat): 36700 mg/kg
Intraperitoneal (rat): 3850 mg/kg
Intravenous (rat): 770 mg/kg
Oral (mouse): >33 g/kg
Intraperitoneal (mouse): 2640 mg/kg
Intravenous (mouse): 1420 mg/kg Oral (hamster): 18 ml/kg
2.5.2.2  Tween 80\textsuperscript{[75]}:

**Nonproprietary Name:**

- **BP:** Polysorbate80
- **JP:** Polysorbate80
- **Ph Eur:** Polysorbate80
- **USP-NF:** Polysorbate80

**Synonym:** Capmul POE-O; CremophorPS80; Crillett4; polyoxyethylene 20 oleate: polysorbatum 80 and Tween80.

**Empirical Formula:** $\text{C}_{64}\text{H}_{124}\text{O}_{26}$

**Physiochemical property**

- **Molecular weight:** 1310 g/mol
- **Functional Category:** Dispersing agent, emulsifying agent, nonionic surfactant, solubilizing agent, suspending agent, wetting agent

- **Odor:** Characteristic odor
- **Taste:** Bitter taste
- **Colour and Physical form at 25 °C:** Yellow oily liquid
- **Solubility of Polysorbate 80 in various solvents:** soluble in water and ethanol

**Typical Properties:**

<table>
<thead>
<tr>
<th>HLB value</th>
<th>Acid value (%</th>
<th>Hydroxyl Value</th>
<th>Moisture Content</th>
<th>Specific Gravity at 25 °C</th>
<th>Viscosity (mPs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0</td>
<td>2.0</td>
<td>65-80</td>
<td>3.0</td>
<td>1.08</td>
<td>425</td>
</tr>
</tbody>
</table>

**Incompatibilities:**

Discoloration and / or precipitation occur with various substances, especially phenols, tannins, tars, and tar like materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates.
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**Storage and stability:**

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides. Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

### 2.5.3 PEG 400

**Generic name:** Macrogols, Polyethylene glycol

**Chemical name:** $\alpha$-Hydro-$\omega$-hydroxypoly(oxy-1,2-ethanediyl)

**Empirical formula:** $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)m\text{CH}_2\text{OH}$ where $m$ represents the average number of oxyethylene groups. ($m=8.7$)

**Average Molecular weight:** 380-420

**Chemical formula:** $\text{H(OCH}_2\text{CH}_2)_n\text{OH}$

**Functional category:** Ointment base; plasticizer; solvent; suppository base; tablet and capsule lubricant.

**Physicochemical property**

**Physical state:** Liquid

**Colour:** Clear

**Odour:** Odourless

**Solubility:** Miscible with water and most organic solvents. Immiscible with aliphatic hydrocarbons

**Specific gravity:** 1.1254 (water=1)

**Density:** 1.11–1.14 g/cm$^3$ at 25°C

**Flash point:** 238 °C

**Freezing point:** 4–8 °C

**pH of 5% solution:** 4.0–7.0

**Viscosity (dynamic):** 105-130 cP and 7.4 cSt at 99 °C

**Refractive index:** 1.465

**Stability and Reactivity:** Stable under ordinary conditions of use and storage.
Hazardous Decomposition Products: Carbon dioxide and carbon monoxide may form when heated to decomposition.

Hazardous Polymerization: not occur.

Incompatibilities: Incompatible with polymerization catalysts (peroxides, persulfates) and accelerators, strong oxidizers, strong bases and strong acids.

Conditions to Avoid: Incompatibles[71, 76, 77].

Toxicity Data

LD50
Oral (guinea pig): 15.7 g/kg
Intraperitoneal (mouse): 10.0 g/kg
Intravenous (mouse): 8.6 g/kg
Oral (mouse): 28.9 g/kg
Oral (rabbit): 26.8 g/kg
Intraperitoneal (rat): 9.7 g/kg
Intravenous (rat): 7.3 g/kg[71, 76, 77]
2.6 References


23. Holm, R., et al., Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured
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57. Indian Pharmacopoeia; Government of India; Ministry of Health & Family Welfare; Published by The Indian Pharmacopoeia Commission, Ghaziabad, 2010, 6(I): p. 1333-1336.
59. United States Pharmacopeia National Formulary (USP32NF27); The Official Compendia of Standards; Published by The United States Pharmacopeial Convention; Rockville, 2009, 2: p. 2347-2350.
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