1. INTRODUCTION

1.1 ORAL DRUG DELIVERY

Oral route is the major route of drug delivery for the chronic treatment of many diseases. However, oral delivery of many drugs is hampered because of the high lipophilicity of the drug itself. Nearly 40% of new drug candidates exhibit low solubility in water, which leads to poor oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality. Add to this, oral bioavailability is significantly affected by absorption mechanisms in the gastro-intestinal (GI) tract, first pass metabolism in the gut and liver and efflux transporter systems (Dressman et al. 2010). Figure 1 depicts the aforesaid problems of reduced drug bioavailability through conventional oral dosage forms. Thus, for such compounds, the absorption rate from the GI lumen is controlled by dissolution (Amidon et al. 1995a).

Figure 1: Pathways depicting the reduction in drug bioavailability of conventional oral dosage forms [Figure adapted from Singh et al. 2009]

Oral delivery of drug compounds gets thwarted owing to their low and variable bioavailability. Besides poor aqueous solubility, other ostensible cause(s) attributing the inadequate rate and/or extent of bioavailability of such drugs like hypolipidemic, antihypertensives, etc. encompass extensive first-pass effect, P-glycoprotein (P-gp) efflux, suboptimal gastrointestinal permeability, intestinal metabolism by cytochrome P450 isoforms, acid lability, etc. To attain desired
plasma levels, these drugs have to be administered in doses higher than actually required, leading invariably to increased toxicity problems. Suitable formulation approaches, therefore, are expedient to improve drug bioavailability.

Modification of the physicochemical properties, such as salt formation and particle size reduction of the compound may be one approach to improve the dissolution rate of the drug (Serajuddin 2007). However, these methods have their own limitations. For instance, salt formation of neutral compounds is not feasible and the synthesis of weak acid and weak base salts may not always be practical. Moreover, the salts that are formed may convert back to their original acid or base forms and lead to aggregation in the GI tract. Particle size reduction may not be desirable in situations where handling difficulties and poor wettability are experienced for very fine powders (Qiu et al. 2009).

To overcome these drawbacks, various other formulation strategies have been adopted which include the use of cyclodextrins, nanoparticles, solid dispersions and permeation enhancers. Indeed, in some selected cases, these approaches have been successful too. In recent years, much attention is focused on lipid-based formulations to improve the oral bioavailability of poorly water soluble drug compounds. In fact, the most popular approach is the incorporation of the drug compound into inert lipid vehicles such as oils, emulgent dispersions, self-emulsifying formulations, emulsions and liposomes with particular emphasis on self-emulsifying drug delivery systems (SEDDS) (Pouton 1985).

In an attempt to attain its ultimate therapeutic objective(s), a drug delivery product has to encounter various phases in its in vitro and in vivo milieu. Albeit, the objectives of any drug delivery systems (DDS) are the clinical ones, the means to accomplish the same are invariably pharmaceutical in nature. Accordingly, the interaction of a medicament with the desired receptor site can effectively be modulated by monitoring its pharmacokinetic availability in blood stream. This pharmacokinetic availability of a therapeutic agent, in turn, is a distinct function of its pharmaceutical characteristics. The sojourn of a drug molecule in the light of these three steps viz. pharmaceutical, pharmacokinetic and pharmacodynamic, has been depicted as Figure 2.
1. INTRODUCTION

SITE OF ACTION

EFFECT
Pharmaceutical Phase Pharmacokinetic Phase Pharmacodynamic Phase

Figure 2: Various phases of drug sojourn following oral administration of its delivery system(s)

1.2 BIOAVAILABILITY

Therapeutic effectiveness of a drug depends upon the ability of the dosage form to deliver the medicament to its site of action at a rate and amount sufficient to elicit the desired pharmacodynamic response. Accordingly, the term, "bioavailability" construes measurement of both true rate and total amount of drug that reaches the general circulation from an administered dosage form by absorption. The concept of bioavailability, known hitherto as physiological availability, was first introduced by (Oser et al. 1945) when they compared vitamin oral solutions with vitamin tablets. Ideally, it reflects the concentration of active moiety at the site of action, i.e., receptor site.

The United States Food and Drug Administration (2008) defined bioavailability as "the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action". However, it is quite impractical to determine drug concentration at the receptor site; hence, the International Consensus Statement is more widely accepted and used in industrial practice. It states bioavailability as "the rate at which and the extent to which a drug reaches systemic circulation" (Ritschel 1992). Verily, all the federal definitions encompass both the rate and extent of absorption from the GI tract.

The influence of route of administration on drug bioavailability is generally in the order of: parenteral > oral > rectal > topical, barring only a few exceptions. Within the parenteral route, intravenous injection of drug results in 100% bioavailability as the absorption process is bypassed (Shargel et al. 2004).
Making reliable estimates of oral bioavailability in humans for the selection of the best developmental candidates is a considerable challenge to the pharmaceutical industry. In recent years, several promising in vitro models became available for the study of the absorption potential of new compounds (Tukker 2000). The dose available to the patient in case of oral administration is called as the bioavailable dose, which is often less than the administered dose. The amount of drug that reaches the systemic circulation (i.e., extent of absorption) is called as the systemic availability. The term, bioavailable fraction (F), refers to the fraction of administered dose that enters the systemic circulation (Gibaldi and Perrier 1982).

The rate at which a drug reaches the systemic circulation is relatively more important consideration for the drugs used to treat acute condition, as pain or insomnia, which can be ameliorated even with a single dose. A drug that is absorbed slowly may not concentrate at the site of action to show the desired effect or intensity of effect, even if the entire dose is absorbed. Figure 3 (A & B) illustrates the case of three oral drug formulations having same extent of absorption (AUC) but grossly differing in their rates of absorption. The Figure clearly explains that if the rate of absorption is very slow (case B), the levels may not at all be pharmacodynamically productive, as minimum effective level is not reached. Further, extremely fast absorption (case C) also at times is unwelcome, as the levels are likely to cross minimum toxic level and exhibit signs of drug overdosage.

Figure 3: Three formulations having (A) the same extent but different rates of absorption (B) the same rate but different extents of absorption

Hence, a plasma concentration time profile should behave in an ideal manner, with moderate fast absorption rate (case A). Herein in case A, the levels tend to remain...
1. INTRODUCTION

in the therapeutic window for a sizable duration, thus attaining optimal therapeutic effects with minimal toxicity (Ritschel 1992). The extent of absorption, on the other hand, is usually more important factor for the drugs that are administered repetitively for the treatment of only chronic conditions as asthma, arthritis, diabetes, hypertension or epilepsy.

1.2.1 Factors Affecting Oral Bioavailability of Drugs

By and large, the bioavailability of a drug is controlled by three principal factors. These variants are namely,

- Rate and extent of release of the drug from the dosage form
- Subsequent absorption from the solution state, and
- Biotransformation during the process of absorption

Figure 4 portrays a variety of factors that can play influential role in determining the rate and extent of drug absorption following oral administration. The variants include, slow release of the drug from the dosage form, instability of the drug in the GI tract, poor permeability of the drug through GI mucosa and first-pass metabolism of the drug in the gut wall or liver (Dressman 2000).

![Figure 4: Pictorial depiction of various factors limiting oral bioavailability of drugs](image-url)

Oral drug absorption can simplistically be considered as a consecutive process of dissolution and permeation. For a chemically stable drug, poor absorption can be thought to be caused by an inadequate rate and extent of drug dissolution and/or low permeation. Low permeation could be caused by poor membrane permeability
1. INTRODUCTION

and/or by a low drug concentration (solubility) in the intestinal lumen. This implies that oral drug absorption or bioavailability can be limited by drug dissolution rate, membrane permeability or solubility (Wyatt 1999; Shargel et al. 2004). Dissolution rate and solubility are usually linearly related properties. Poorly soluble compounds, therefore, are only expected to exhibit dissolution-limited absorption.

To get an insight into the likely mechanisms of absorption enhancement, Wyatt proposed the use of simple parameters like dose volume, dissolution time and absorbable dose. A brief account of these parameters is as under:

Dose volume \( (V_{\text{Dose}}) \) is the minimum amount of water required to dissolve a dose of drug. It can be calculated using Eqn 1.

\[
V_{\text{Dose}} = \frac{M_0}{C_S} \quad \ldots (1)
\]

Where, \( M_0 \) is the dose and \( C_S \) is the solubility. If the calculated volume of water required to dissolve the dose is more than 250 ml, then the absorption is likely to be solubility limited.

Dissolution time \( (T_{\text{Diss}}) \) is the theoretical time that would take a drug particle to dissolve. It can be calculated from various physicochemical parameters, as in Eqn 2.

\[
T_{\text{Diss}} = \frac{\rho h r_0}{3D C_S} \quad \ldots (2)
\]

where, \( \rho \) is the particle density, \( h \) is the thickness of the diffusion layer around the drug particle, \( r_0 \) is the radius of the drug particle, \( D \) is the diffusion coefficient and \( C_S \) is the solubility. If \( T_{\text{Diss}} \) is significantly longer than the residence time of the drug at the site of absorption, then drug absorption will be dissolution-limited. Since the rate of dissolution can be enhanced by particle size reduction, this calculation can be used to deduce the possible effect that micronization of substance might have on absorption.

Absorbable Dose \( (D_{\text{abs}}) \) is the maximum amount of drug that can be absorbed from the GI tract. It can be calculated from a few assumptions about the residence time of dissolved drug in the small intestine and a measure of permeability, as in Eqn 3.
1. INTRODUCTION

\[ D_{\text{abs}} = P_{\text{eff}} C_s A < T_{\text{si}} > \]  \hspace{1cm} \ldots (3)  

Where, \( P_{\text{eff}} \) is the effective permeability, \( C_s \) is the solubility, \( A \) is the surface area of the small intestine (around 630 cm\(^2\)), and \( < T_{\text{si}} > \) is the mean small intestinal transit time (around 199 minutes). Ideally, the permeability data would be derived from human studies, but it can be a surrogate data obtained from in vitro ‘cells on sheets’ or from animal studies. Table 1 episonizes the diverse factors influencing GI drug absorption in the light of the magnitude of these Wyatt parameters (Wyatt 1999).

Table 1: Summary of the factors limiting oral drug absorption

<table>
<thead>
<tr>
<th>Factor</th>
<th>Physiochemical constraints</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility limited</td>
<td>( T_{\text{diss}} &lt; 2 \text{ minutes} ) ( P_{\text{eff}} &gt; 2 \times 10^{-4} \text{ cm/s} )</td>
<td>Dissolution is relatively fast. Permeability is fast. Solubility limited absorption often occurs because the gut is saturated by a high dose. Absorption does not increase with increased dose.</td>
</tr>
<tr>
<td>Dissolution limited</td>
<td>( T_{\text{diss}} &gt; ) residence in small intestine ( P_{\text{eff}} &gt; 2 \times 10^{-4} \text{ cm/s} )</td>
<td>Dissolution is slow and absorption limiting, even though permeability is fast. Dissolution rate can be enhanced by particle size reduction. Absorption increases with increasing dose.</td>
</tr>
<tr>
<td>Permeability limited</td>
<td>( T_{\text{diss}} &lt; 50 \text{ minutes} ) ( P_{\text{eff}} &lt; 2 \times 10^{-4} \text{ cm/s} )</td>
<td>Permeability is low regardless of solubility of the drug substance. The absolute amount of drug absorbed increases with increasing dose.</td>
</tr>
</tbody>
</table>

1.3 ORAL DRUG ABSORPTION

Drug absorption is determined by physicochemical properties of drugs, their formulations and routes of administration. For oral administration, the most common route, absorption refers to the transport of drugs across membranes of the epithelial cells in the GI tract. Absorption after oral administration is confounded by differences in luminal pH along the GI tract, surface area per luminal volume, blood perfusion, the presence of bile and mucus and the nature of epithelial membranes (Wikman et al. 1993; Pade and Stavchansky 1998). Acids are absorbed faster in the intestine than in the stomach, apparently contradicting the hypothesis that unionized drug crosses the membranes more readily. However, the apparent contradiction is explained by the larger surface area and greater
permeability of the membranes in the small intestine.

1.3.1 GI tract and different sites of drug absorption

**Oral mucosa:** The oral mucosa has a thin epithelium and a rich vascularity that favors absorption, but contact is usually too brief, even for drugs in solution, for appreciable absorption to occur. A drug placed between the gums and cheek (buccal administration) or under the tongue (sublingual administration) is retained longer so that absorption is more complete.

**Stomach:** The stomach has a relatively large epithelial surface, but because it has a thick mucous layer and the time that the drug remains there is usually relatively short, absorption is limited. Absorption of virtually all drugs is faster from the small intestine than from the stomach. Therefore, gastric emptying is the rate-limiting step. Food, especially fatty foods, slows gastric emptying (and the rate of drug absorption), explaining why some drugs should be taken on an empty stomach when a rapid onset of action is desired. Food may enhance the extent of absorption for poorly soluble drugs (e.g., griseofulvin), reduce it for drugs degraded in the stomach (e.g., penicillin G), or have little or no effect (Christensen et al. 2004). Drugs that affect gastric emptying (e.g., parasympatholytic drugs) affect the absorption rate of other drugs. Figure 6 represents different sites of drug absorption in GI tract.

**Small intestine:** The intestinal mucosa is characterized by the presence of villi that constitute the anatomical and functional unit for nutrient and drug absorption (Pacha 2000). The small intestine has the largest surface area for drug absorption in the GI tract. The intraluminal pH is 4 to 5 in the duodenum but becomes progressively more alkaline, approaching 8 in the lower ileum. Certain drugs are inactivated by GI micro flora, reducing their absorption. Decreased blood flow (e.g., in shock) may lower the concentration gradient across the intestinal mucosa and decrease absorption by passive diffusion (decreased peripheral blood flow also alters drug distribution and metabolism).

**Intestinal transit time:** can influence drug absorption, particularly for drugs which are absorbed by active transport (e.g., vitamin B complex), that dissolve slowly (e.g., griseofulvin), or that are too polar (i.e., poorly lipid-soluble) to cross
membranes readily (e.g., many antibiotics). For such drugs, transit may be too rapid for absorption to be complete (Yu and Amidon 1999; Sunesen et al. 2005). For controlled-release dosage forms, absorption may occur primarily in the large intestine, particularly when drug release continues for > 6 h, the time for transit to the large intestine.

1.3.2. Factors altering the rate of drug absorption

(a) Physiochemical Characteristics of Drug

- Number of Nitrogens and Oxygens
  - < 5 -NH’s –OH groups enhances absorption
  - > 10 –O, -N, Hydrogen bond acceptors, reduced absorption
- Molecular Weight (< 500)
- Log P (< 5)

(b) Formulation Factors and Physiochemical Properties of Formulations

- Drug solubility, Particle size, Salt formation
- Disintegrant(s), Diluent(s), Inert filler(s), Granulating Agent(s), Coating Ingredient(s), Lubricant(s), Emulgent(s), Coloring Agent(s), etc.

(c) Physiological Factors

- GI pH and pKa of drug
- Gastric emptying process
- Intestinal motility
- First pass extraction
- Food
- Disease states

1.3.3 Absorption from various dosage forms

Absorption from solution: A drug given orally in solution is subjected to numerous GI secretions and, to be absorbed, must survive encounters with low pH and potentially degrading enzymes. Usually, even if a drug is stable in the enteral environment, little of it remains to pass into the large intestine. Drugs with low lipophilicity (i.e., low membrane permeability), such as aminoglycosides, are
absorbed slowly from solution in the stomach and small intestine; for such drugs, absorption in the large intestine is expected to be even slower because the surface area is smaller. Consequently, these drugs are not candidates for controlled release (Evrard et al. 2002).

**Absorption from solid forms:** Most drugs are given orally as tablets or capsules primarily for convenience, economy, stability and patient acceptance. These products must disintegrate and dissolve before absorption can occur as shown in Figure 5. Disintegration greatly increases the drug's surface area in contact with GI fluids, thereby promoting drug dissolution and absorption. Disintegrants and other excipients (e.g., diluents, lubricants, emulgents, binders, and dispersants) are often added during manufacture to facilitate these processes (Banker and Anderson 1991). Emulgents increase the dissolution rate by increasing the wetability, solubility and dispersibility of the drug. Disintegration of solid forms may be retarded by excessive pressure applied during the tableting procedure or by special coatings applied to protect the tablet from the digestive processes of the gut. Hydrophobic lubricants (e.g., magnesium stearate) may bind to the active drug and reduce its bioavailability (Banker and Anderson 1991).

![Diagram of drug absorption process](image)

**Figure 5:** Rate limiting steps in the absorption of drugs from orally administered formulations

### 1.4 BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS)

The BCS is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability. When combined with the *in vitro* dissolution characteristics of the drug product, the BCS takes into account three major factors: solubility, intestinal permeability, and dissolution rate, all of which govern the rate and extent of oral drug absorption from IR solid oral-dosage forms.
Table 2 provides a classification of drugs based on the above-mentioned criteria as per the BCS.

Thus, in order to be absorbed into systemic circulation, the drug(s) must be hydrophilic enough to be dissolved (i.e., soluble), yet lipophilic enough to get across the GI membrane (i.e., permeable). The drug in the dosage form is released and dissolves in the surrounding GI fluid to form a solution. Once the drug is in the solution form, it passes across the membranes of the cells lining the GI tract. Then onwards, the drug is absorbed into systemic circulation.

For a chemically stable drug, poor absorption can be thought to be caused by an inadequate rate and extent of drug dissolution and/or low permeation. Low permeation could be caused by poor membrane permeability and/or by a low drug concentration (solubility) in the intestinal lumen.

Table 2: Classification of drugs as per BCS

<table>
<thead>
<tr>
<th>Class I drugs</th>
<th>Class II drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>High solubility</td>
<td>Low solubility</td>
</tr>
<tr>
<td>High permeability</td>
<td>High permeability</td>
</tr>
<tr>
<td>(Examples: Diltiazem, Captopril, Propranolol, Phenylalanine, Caffeine, etc.)</td>
<td>(Examples: Flurbiprofen, Diclofenac, Naproxen, Piroxicam, Ketoprofen, etc.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class III drugs</th>
<th>Class IV drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>High solubility</td>
<td>Low solubility</td>
</tr>
<tr>
<td>Low permeability</td>
<td>Low permeability</td>
</tr>
<tr>
<td>(Example: Famotidine, Nadolol, Atenolol, Cimetidine, Ranitidine, etc.)</td>
<td>(Examples: Furosemide, Cyclosporin, Terfenedine, etc.)</td>
</tr>
</tbody>
</table>

This implies that oral drug absorption or bioavailability can be limited by drug dissolution rate, membrane permeability or solubility, as depicted in Figure 6.

1.4.1 Solubility

The solubility of a substance is the amount of substance that has passed into solution when equilibrium is attained between the solution and excess (i.e., undissolved) substance, at a given temperature and pressure. A drug substance is considered "highly soluble" when the highest dose strength is soluble in ≤ 250 ml
water over a pH range of 1 to 7.5.

Figure 6: Three vital criteria of BCS

The volume estimate of 250 ml is derived from the typical volume of water consumed during the oral administration of dosage form, which is about a glassful, or 8 ounces of water. This boundary value is a reflection of the minimum fluid volume anticipated in stomach the time of drug administration. The pH-solubility profile of the drug substance is determined at $37 \pm 1 \, ^\circ C$ in aqueous medium with pH in the range of 1-7.5. A sufficient number of pH conditions should be evaluated to accurately define the pH-solubility profile. The number of pH conditions for a solubility determination depends upon ionization characteristics of the test drug substance. A minimum of three replicate determinations of solubility in each pH condition should be carried out. Standard buffer solutions described in pharmacopoeias are considered appropriate for use in solubility studies. If these are not suitable for physical or chemical reasons, other buffer solutions can also be used provided the pH of these solutions is verified. Methods other than shake flask method are also used with justification to support the ability of such methods to predict equilibrium solubility of test drug substance e.g., acid or base titration methods. The concentration of drug substance in selected buffers or pH conditions should be determined using a validated solubility-indicating assay that can distinguish between the drug substances from its degradation products. If degradation of drug is observed as a function of buffer composition and/or pH, it should also be taken into consideration.
1.4.2 Permeability

A drug substance is considered "highly permeable" when the extent of absorption in humans is determined to be ≥ 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose. Permeability is the ability of drug molecule to permeate through the biological membrane into the systemic circulation. A compound may traverse the biological barrier by a variety of routes, i.e., passive transcellular transport which is dependent on the ability of a compound to partition into the lipid bilayer of the cell membrane and traverse the barrier; paracellular route which is the pathway through the tight junction and the lateral intercellular spaces; carrier mediated trans-membrane transport which involves a carrier in the absence (passive) or presence of ATP (active). The methods that are routinely used for determination of permeability include in vivo or in situ intestinal perfusion in a suitable animal model, in vitro permeability methods using excised intestinal tissues, monolayer of suitable epithelial cells e.g. Caco-2 cells or TC-7 cells. Intestinal perfusion models and in vitro methods are recommended for passively transported drugs. The observed low permeability of some drug substances in humans could be attributed to the efflux of drug by various membrane transporters like P-gp. This leads to misinterpretation of the permeability of drug substance. An alternative to intestinal tissue models is the use of well-established in vitro systems based on the human adenocarcinoma cell line, i.e., Caco-2 cell lines. These cell lines serve as a model of small intestinal tissue. The differentiated cells exhibit the microvilli typical of the small intestinal mucosa and the integral membrane proteins of the brush-border enzymes. In addition, they also form the fluid-filled domes typical of a permeable epithelium.

Accordingly, for a drug to have adequate permeability across the lipoproteinaceous biological membrane in GI tract, it is ought to possess one of the following two prerequisites:

- High lipophilicity for moderately sized drug molecules by transcellular transport

- High polarity for small sized drug molecules through intracellular transport.

Figure 13 shows the general structure of biological membrane
1.4.3 Dissolution

A drug product is considered to be “rapidly dissolving” when ≥ 85% of the labeled amount of drug substance dissolves within 30 minutes using USP 30 apparatus II in a volume of ≤ 900 ml buffer solutions. Drug dissolution is the process by virtue of which drug is released, dissolved and becomes ready for absorption. It is directly proportional to the solubility of the drug and this can be explained on the basis of a mathematical relationship, proposed by Noyes and Whitney in 1897 (Eqn 3).

\[
\frac{dC}{dT} = K(C_s - C_b)
\]  \hspace{1cm} \text{... (3)}

where, \(dC/dT\) is the rate of dissolution, \(K\) is the first-order dissolution rate constant, \(C_s\) is the concentration of drug in the stagnant water layer (also called as the equilibrium solubility, saturation solubility, or maximum solubility) and \(C_b\) is the concentration of drug in the bulk of the solution as a function of time, \(t\).

Brunner (1904) modified the Noyes Whitney equation by incorporating Fick’s first law of diffusion and proposed another relationship, as described in Eqn 4.

\[
\frac{dC}{dT} = \frac{DAK_{w/o}}{Vh} (C_s - C_b)
\]  \hspace{1cm} \text{... (4)}

where, \(D\) is the diffusion coefficient (diffusivity) of the drug, \(A\) is the surface area of the dissolving solid, \(K (w/o)\) is the water/oil partition coefficient of the drug (since the rapidity with which a drug dissolves depends on the \(K (w/o)\), also known as intrinsic dissolution rate constant), \(V\) is the volume of dissolution medium, \(h\) is the thickness of the stagnant layer, and \((C_s - C_b)\) is the concentration gradient for diffusion of drug. The influence of the parameters on drug dissolution rate is summarized in Table 3.

Pragmatically, the dissolution rate of a drug can be enhanced by altering its surface area and/or solubility. The diffusion and partition behavior of a drug remaining invariant, all other parameters cannot be significantly influenced looking into the simulation of GI conditions \textit{in vivo}. Whereas, the diffusion coefficient is inversely related to medium viscosity, the degree of agitation can diminish the thickness of the diffusion layer and hasten the process of dissolution. Further, it is impossible to modify the film thickness or diffusion coefficient in an \textit{in vivo} environment. Figure
7 is a representation of various strategies to enhance the solubility and permeability of a drug.

**Table 3: Significance of various Fick’s parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Influence on drug dissolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion coefficient of drug</td>
<td>D</td>
<td>Greater the value, faster the dissolution</td>
</tr>
<tr>
<td>Surface area of solid drug</td>
<td>A</td>
<td>Greater the surface area, faster the dissolution</td>
</tr>
<tr>
<td>Water/oil partition coeff of drug</td>
<td>K</td>
<td>Higher the value, more the hydrophilicity and faster the dissolution in aqueous fluids</td>
</tr>
<tr>
<td>Concentration gradient of drug</td>
<td>(C_s-C_b)</td>
<td>Greater the concentration gradient, faster the diffusion and drug dissolution</td>
</tr>
<tr>
<td>Thickness of unistirred water layer</td>
<td>H</td>
<td>More the thickness, lesser the diffusion and drug-drug dissolution</td>
</tr>
</tbody>
</table>

1.4.4 BCS: Key parameters

The interaction of the physiological environment (e.g., intestinal pH, transit time, GI motility, luminal metabolism, endogenous substances such as bile salts, exogenous substances such as nutrients, etc.) with physicochemical characteristics of the drug (e.g., pKa, solubility in the gut lumen, dissolution rate, aqueous diffusivity, partition coefficient, chemical and enzymatic stability in the intestine, etc.) can have profound influence on the availability of the drug at the absorption site.

![Figure 7: Possible formulation strategies for the solubility-dissolution characteristics of BCS Class II, III and IV drug nature to BCS Class I](Figure adapted from Singh et al. 2009)
The BCS is a fundamental guideline that tends to integrate the physicochemical properties of drug compounds with that of the physiological factors to predict the fraction of dose absorbed during the GI transit.

The BCS is associated with drug dissolution and absorption model, which identifies the key parameters controlling drug absorption as a set of dimensionless numbers, namely the absorption number, dissolution time, the dissolution number, dose number, absorbable dose and dose volume. The absorption number ($A_n$) is the ratio of the mean residence time ($T_{res}$) to the mean absorption time ($T_{abs}$), and is calculated as in Eqn 5:

$$A_n = \frac{T_{res}}{T_{abs}} = \frac{\pi R^2 L}{Q} \frac{R}{P_{eff}}$$  \hspace{1cm} \text{(5)}

Dissolution time ($T_{diss}$) is the theoretical time taken by a drug particle to dissolve (Eqn 6).

$$T_{diss} = \frac{\rho r_0}{3DC_s}$$  \hspace{1cm} \text{(6)}

The dissolution number ($D_n$) is a ratio of mean residence time ($T_{res}$) to mean dissolution time ($T_{diss}$) and could be estimated as Eqn 7:

$$D_n = \frac{T_{res}}{T_{diss}} = \frac{\pi R^2 l/}{Q} \frac{\rho r_0}{3DC_{sm}}$$  \hspace{1cm} \text{(7)}

The dose number ($D_0$) is the dose divided by an uptake volume of 250 ml and the drug's solubility, expressed as Eqn 8:

$$D_0 = \frac{\text{Dose}}{V_0 \times C_{sm}}$$  \hspace{1cm} \text{(8)}

Absorbable dose ($D_{abs}$) is the maximum amount of drug that can be absorbed (Eqn 9).

$$D_{abs} = P_{eff} C_s A(T_{si})$$  \hspace{1cm} \text{(9)}

where, $L$ = tube length, $R$ = tube radius, $Q$ = fluid flow rate, $r_0$ = initial particle radius, $V_0$ = gastric volume (250 ml), $\rho$ = particle density, $h$ = thickness of the diffusion layer, $P_{eff}$ = effective permeability, $T_{si}$ = mean small intestine transit time and $C_{sm}$ = minimum aqueous solubility in the physiological pH range 1 – 8.
mean residence time ($T_{res}$) is the average of residence time in the stomach, small intestine and the colon.

The dose volume ($V_{dose}$) is the ratio of the amount of dose ($M_0$) and the drug solubility ($C_s$), Eqn. 10

$$V_{dose} = \frac{M_0}{C_s}$$  ... (10)

1.4.5 BCS and dissolution specifications

The absorption of drug into systemic circulation depends upon the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the GI tract, as shown in Figure 8.

The term “$k_d$” in Figure 8 represents the dissolution rate and is a function of solubility of drug and nature of the formulation; “$k_{dd}$” represents disintegration rate and is dependent upon the formulation characteristics; “$k_{id}$” represents solubilization rate and is dependent on the solubility of API; “$k_p$” represents permeability rate which depends upon the molecular structure and nature of API and excipients.

![Diagram of various factors affecting absorption of orally administered drug](image)

**Figure 8: Various factors affecting absorption of orally administered drug**

In case of Class I drugs, both $k_d$ and $k_p$ are high, whereas for Class II and IV drugs, $k_d \gg k_p$. On the other hand, if $k_d << k_p$, the absorption of drug becomes dissolution controlled. The dissolution controlled absorption can be intrinsic dissolution controlled if $k_{dd} \gg k_{id}$, and disintegration controlled if $k_{dd} << k_{id}$.

An orally administered drug product has to pass through a variety of physiological environments of GI tract before it gets absorbed. As shown in Figure 9, BCS in
conjunction with the numerous of compendial and physiological media available, can be employed as a fundamental guidance for designing appropriate biorelevant dissolution conditions, leading to a more meaningful prediction of in vivo performance.

Figure 9: Various pH conditions in the gastrointestinal tract

For class I drugs, simple and mild aqueous dissolution media, such as simulated gastric fluid (SGF) without pepsin is suitable, while milk as dissolution medium might be appropriate for specific food/formulation interaction. For neutral class II drugs, simulated intestinal fluid (SIF) in fasted state, i.e., FaSSIF, reflects the dissolution in the upper GI tract under fasted conditions. If a class II drug is a weak base, SGF could be used to assess the dissolution of the drug in the stomach under fasted state conditions. To verify the possibility of drug precipitation under intestinal conditions, performing dissolution in FaSSIF may be appropriate. Comparison of dissolution results obtained under pre-prandial (FaSSIF) to that of post-prandial (fed state SIF, FeSSIF) could be a good indicative of whether the formulation should be administered before or after meals. In case of class II weak acids, dissolution could be performed in FaSSIF as a suitable representative of intestinal fasted state conditions. Milk with its composition of lipids and proteins or FeSSIF containing high bile salt/lecithin levels can be employed to simulate the fed state conditions.
1.5 BIOAVAILABILITY ENHANCEMENT OF BCS CLASS II DRUGS

Various approaches can be adopted to augment oral bioavailability of poorly soluble BCS class II drugs, described as under:

1. **Micronization:** As particle size decreases, the surface area per unit volume of solute increases and subsequently more drug is exposed to the solvent (Serajuddin *et al.* 1990). Furthermore, as particle size decreases, the free energy of the surface molecules increases as well as dissolution rate of the drug (Kaneniwa and Watari 1978). Decrease in particle size generally increases the rate of absorption and bioavailability, but the finely reduced particles tend to form agglomerates due to stronger van-der Waal’s attraction, resulting eventually in decreased bioavailability. Examples of drugs whose bioavailability have been increased by micronization include griseofulvin and several steroidal and sulfa drugs.

2. **Use of Cosolvents:** The addition of a water-miscible or partially miscible organic solvent (i.e., cosolvent to water) is a common and an effective way to increase the solubility of a nonpolar drug. In order for an organic compound to be appreciably miscible with water, it must have some degree of hydrogen bond donating and/or hydrogen bond accepting ability. Cosolvents with hydrocarbon regions do not interact as strongly. Thus the addition of a cosolvent to water decreases the water-water interactions and the ability to ‘squeeze out’ a nonpolar organic solute in the cosolvent systems. Aqueous solubilization via cosolvency is dependent on both the solute and cosolvent physical properties (Williams and Amidon 1984).

3. **Micellar Solubilization:** Emulgents are the compounds that have two distinct regions, a polar region and a nonpolar region. The polar portion of the emulgent associates with the more polar phase and the nonpolar portion remain in the nonpolar region. In water, as the concentration of emulgent increases, the emulgent accumulates at the interface until it (interface) gets saturated (Elworthy *et al.* 1968). The surface-active agents enhance dissolution rate primarily by promoting wetting and penetration of dissolution fluid into the solid drug particles. They are generally used in concentration below their critical micelle concentration (CMC) values since above CMC, the drug entrapped in the micelle structure fails to
I. INTRODUCTION

partition in the dissolution fluid. A nonpolar drug which is squeezed from the water can locate within the micelle core. A semipolar drug can locate between or partially within the core and the mantle. Since the micelles are soluble in water, any drug incorporated into the micelle is also soluble in water (Christian and Scamehorn 1995). Nonionic emulgents like polysorbates are widely used. Examples of drugs whose bioavailability have been increased by use of emulgents in the formulation include steroids like spironolactone. Figure 10 shows the mechanism of incorporation of poorly soluble drug in micelle.

4. Use of Adsorbents: A highly active adsorbent such as the inorganic clays like bentonite can enhance the dissolution rate of poorly water soluble drugs such as griseofulvin, indomethacin and prednisolone by maintaining the concentration gradient at its maximum. The two reasons suggested for the rapid release of drugs from the surface of clays are the weak physical bonding between the adsorbate and the adsorbent, and the hydration and swelling of the clay in the aqueous media (Monkhouse and Lach 1972).

![Figure 10: Micelle formation from amphiphilic monomers and drug incorporation into the micelle core by both covalent attachment to the hydrophobic fragment of the monomer and by non-covalent incorporation into the hydrophobic corope micelle](image)

5. Use of Salt Forms: Salts have improved solubility and dissolution characteristics in comparison to the original drug. Alkali metal salts of acidic drugs like penicillins and strong acid salts of basic drugs like atropine are more water-soluble than the parent drug. However, all of these techniques are associated with one or more practical limitations.
6. Metastable Polymorphs: When a substance exists in more than one crystalline form, the different forms are designated as polymorphs and the phenomenon as polymorphism. Depending on their relative stability, one of the several polymorphic forms will be physically more stable than the others. The remaining polymorphs are called as metastable forms that represent the higher energy state, having lower melting points, and higher aqueous solubility. Since the metastable forms have greater apparent solubility, they show better bioavailability and are therefore preferred in formulations (Urakami 2005; Blagden *et al.* 2007).

However because of their poor thermodynamic stability, aging of dosage forms containing such metastable forms usually result in formation of less soluble, stable polymorph. Such a transformation of metastable to stable form can be inhibited by dehydrating the molecule environment or by adding viscosity building agents such as PVP, CMC, pectin or gelatin that prevent such a conversion by adsorbing onto the surface of the crystals of the metastable forms of the drug (Govindaraj and Suryanarayanan 2006). A metastable polymorph is more soluble than the stable polymorph of a drug that exhibits polymorphism. For instance, the B form of chloramphenicol palmitate is more water soluble than the A and the C forms.

7. Solute-Solvent Complexation: Solvates of drugs with organic solvents (also called as pseudopolymorphs) generally have higher aqueous solubility than their respective hydrates or the original drug (Lu and Rohani 2009). Much higher solubility can be attained by freeze drying such a solute in solution with an organic solvent with which it is known to form a solvate. Such a process results in a powder of particles of submicron size, e.g., 1:2 griseofulvin benzene solvate. However, one should take care that the solvent is nontoxic.

8. Solvent Deposition: In this method, the poorly aqueous soluble drug such as nifedipine is dissolved in an organic solvent like alcohol and deposited on an inert, hydrophilic, solid matrix such as starch or microcrystalline cellulose by evaporation of solvent.

9. Solid Solutions: The three means by which the particle size of a drug can be reduced to submicron level are

- Use of solid solutions
1. INTRODUCTION

- Use of eutectic mixtures, and
- Use of solid dispersions

In all these cases, the solute is frequently a poorly water soluble drug acting as the guest and the solvent is a highly water-soluble compound or polymer acting as a host or carrier. A solid solution is a binary system comprising of a solid solute molecularly dispersed in a solid solvent. Since the two components crystallize together in a homogeneous one phase system, solid solutions are also called as molecular dispersions or mixed crystals. Because of reduction in particle size to the molecular level, solid solutions show greater aqueous solubility and faster dissolution than eutectics and solid dispersions (Gresensa 1981).

6. Eutectic Mixtures: These systems are also prepared by fusion method. Eutectic melts differ from solid solutions in that the fused melt of solute-solvent show complete miscibility but negligible solid-solid solubility i.e. such systems are basically intimately blended physical mixture of two crystalline components.

Table 4: Current approaches for enhancing the delivery of PWSDs

<table>
<thead>
<tr>
<th>S. No</th>
<th>Advanced approaches</th>
<th>Concept</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Solid Dispersions</td>
<td>Intimate mixture of drug substance and diluents, such as PEG and PVP. The modified drug is often in an amorphous, more soluble state. There is a need for scale up manufacturing aspect for large scale production.</td>
<td>(Sethia and Squillante 2003; Karolewicz et al. 2012)</td>
</tr>
<tr>
<td>2</td>
<td>Microemulsions</td>
<td>Micellar dispersion of oil/solvent-dissolved drug as nanometer size droplets in water. The drug can be directly absorbed from the droplets administered as liquids. There are some concerns about toxicity of high emulgents and co-solvent levels and the possibility of precipitation.</td>
<td>(Bagwe et al. 2001; He et al. 2010; Hathout and Woodman 2012)</td>
</tr>
<tr>
<td>3</td>
<td>Self-emulsifying Systems</td>
<td>Mixture of drug, oil, emulgents, and co-solvents that form an emulsion upon administration. Can be administered as a solid dosage form in capsules. To prevent precipitation of drug water soluble cellulosic polymers can be used.</td>
<td>(Pouton 2000; Gao and Morozowich 2006; Singh et al. 2009a; Wadhwa et al. 2012)</td>
</tr>
<tr>
<td>4</td>
<td>Complexation</td>
<td>Cyclodextrins are the most common complexing agents used to enhance drug absorption.</td>
<td>(Arima et al. 2012)</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

<table>
<thead>
<tr>
<th>S. No</th>
<th>Advanced approaches</th>
<th>Concept</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Liposomes</td>
<td>Encapsulation of a drug in uni-or multilayered vesicles of phospholipids. Specific sites can be targeted and certain drugs can be protected from inactivation.</td>
<td>(Levchenko et al. 2012)</td>
</tr>
<tr>
<td>6</td>
<td>Particle size reduction</td>
<td>Increased particle surface area enhances rate of solubilization.</td>
<td>(Liversidge and Cundy 1995)</td>
</tr>
<tr>
<td>7</td>
<td>Wet Milling</td>
<td>Particle size reduction to nanosized particles through attrition in the presence of stabilizing agents.</td>
<td>(Peltonen and Hirvonen 2010)</td>
</tr>
<tr>
<td>8</td>
<td>Homogenization</td>
<td>Particle size reduction by high-shear processing of aqueous slurry of drug and stabilizing agents.</td>
<td>(Hecq et al. 2006; Sun et al. 2011)</td>
</tr>
<tr>
<td>9</td>
<td>Controlled Particle Formation</td>
<td>Growth of drug particles with controlled morphology.</td>
<td>(Palakodaty and York 1999; Elder et al. 2007)</td>
</tr>
<tr>
<td>10</td>
<td>Supercritical Fluid-Based Approach</td>
<td>Engineered particle growth using Supercritical fluid as solvent.</td>
<td>(Yasuji et al. 2008; Elizondo et al. 2012)</td>
</tr>
</tbody>
</table>

When the eutectic mixture is exposed to water, the soluble carrier dissolves leaving the drug in a microcrystalline state which solubilizes rapidly. Examples of eutectics include paracetamol-urea, griseofulvin-urea, Chloramphenicol-urea, lidocaine-tetracaine, etc. (Goldberg et al. 1966; Ohzeki et al. 2008). Table 4 shows the advanced approaches used to deliver poorly soluble drugs.

1.6 SELF-EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS)

Self-emulsifying drug delivery systems (SEDDS) or self-emulsifying oil formulations are isotropic mixtures of drug, lipids (natural or synthetic oils), emulsifiers (solid or liquid), usually with one or more of hydrophilic co-solvents/co-emulsifiers (Craig 1993; Shah et al. 1994; Craig et al. 1995). This novel drug delivery system falls under the category of micelle solubilization method for enhancing the bioavailability of poorly water soluble lipophilic drug molecules. The principal characteristic of this type of system is its ability to form fine oil-in water (o/w) emulsions upon capsule disintegration and subsequent dilution in gastric fluids, with gastric motility supplying the necessary agitation. Such emulsions can be coarse emulsions, microemulsions or nanoemulsions, depending upon the size of oil droplets. The distinction between SNEDDS and SMEDDS formulations is commonly made on the particle size and optical clarity of the resultant dispersion. Upon mild agitation followed by dilution with aqueous media, these systems can
form fine oil-in-water emulsions instantaneously, SNEDDS formulation typically provide opaque dispersions with particle sizes ≤200 nm whereas SMEDDS formulations (which contain higher concentrations of hydrophilic emulgents and co-solvents) disperse to give smaller droplets with globules sizes ranging between >200 nm, and provide optically clear or slightly opalescent dispersions, more consistent with the presence of a microemulsion (Figure 11) (Constantinides 1995; Anton and Vandamme 2011).

These self emulsifying formulations are more physically stable and easier to manufacture than conventional emulsions globules size ranging between 0.1 μm to 100 μm. Due to digestive motility of the stomach and agitation provided by intestine the SEDDS spread readily in the GI tract (Shah et al. 1994).

1.6.1 Formulation aspects of SEDDS

The SEDDS formulation should form a clear dispersion instantaneously that should remain stable on dilution. The solubilization of drug in the GI tract and its bioavailability depends predominantly on the intraluminal processing to which lipids are subjected prior to absorption.

When the lipid formulation comes in contact with gastric media oil globules breakdown into an emulsion of high surface area. The emulsified lipids are digested by intestinal enzymes leading to quantitative production of one molecule
of 2-monoglyceride (MG) and two fatty acid (FA) molecules for each triglyceride (TG) molecule. Bile salts present in intestine react with these FAs and MGs to form mixed micelles. It is in this step that the drug released from the formulation due to either precipitation or dissolution in the gastric media is resolubilised as micelles or mixed micelles by emulsification, which can affect the in vivo performance of the formulation significantly.

For lipophilic drug compounds that exhibit dissolution rate-limited absorption, the SEDDS may offer an improvement in the rate and extent of absorption resulting in more reproducible blood-time profiles. Thus, SEDDS may be a promising alternative to orally administered emulsions because of their relatively better physical stability, manufacture and higher convenience of delivery using hard and soft gelatin capsules. Besides, these systems offer several advantageous features, as enumerated in Box 1.

1.6.1.1 Excipient characteristics

Historically, excipients were considered inert substances that would be used mainly as diluents, fillers, binders, lubricants, coatings, solvents, and dyes, in the manufacture of drug products. Over the years, however, advances in pharmaceutical science and technology have facilitated the availability of a wide range of novel excipients. In some cases, known and/or unknown interactions can occur between an excipient and active ingredient, other inactive ingredient(s), biological surroundings, or even container closure system (Hauss 2007a).

In the United States, the Food and Drug Administration (FDA) has published listings in the Code of Federal Regulations (CFR) for GRAS substances that are generally recognized as safe (U.S. Food and Drug Administration). Over the years, FDA also maintains a list entitled Inactive Ingredient Guide (IIG) for excipients that have been approved and incorporated in the marketed products (http://www.fda.gov/Drugs/InformationOnDrugs/ucm080123.htm). This guide is helpful in that it provides the database of allowed excipients with the maximum dosage level by route of administration or dosage form for each excipient. Both GRAS listings and IIG information can be used by industry as an aid in developing drug products. For new drug development purposes, once an inactive ingredient
has appeared in an approved drug product for a particular route of administration, the inactive ingredient is not considered new and may require a less extensive review the next time it is included in a new drug product.

**Box 1: Advantages of SEDDS**

- Enhanced oral bioavailability enabling reduction in dose.
- More consistent temporal profiles of drug absorption.
- Selective targeting of drug(s) toward specific absorption window in GIT.
- Protection of drug(s) from the hostile environment in gut.
- Control of delivery profiles.
- Reduced variability including food effects.
- Protective of sensitive drug substances.
- High drug payloads.
- Improved understanding of the manner in which lipids enhance oral bioavailability and reduce plasma profile variability.
- Better characterization of lipoidic excipients, formulation versatility and the choice of different DDS.
- Reduction in particle size enables more efficient drug transport through intestinal aqueous boundary layer and absorptive brush border membrane.
- Smaller particle size makes the drug formulation less dependant on lipolysis thus enabling a more rapid onset and extended duration of therapeutic action.
- Robustness of composition to dilution keeping the drug in solubilized state in the time frame relevant for absorption, hence resulting in faster dissolution.
- Thermodynamic stability for a time period relevant for absorption, thereby reducing any variability in bioavailability.
- Less susceptibility to gastric emptying delays, resulting eventually in faster absorption and reduction in unwanted retention.
- Surface activity provided by permeation enhancers yield more efficient and faster drug transport.

For a drug or biological product subject to premarketing approval, their excipients are reviewed and approved as 'components' of the drug or biological product in the application. From a scientific standpoint, this regulatory process is appropriate since excipients play an integral part to the formulation and cannot be reviewed separately from the drug product. This is particularly true for lipid excipients in view of their distinct physicochemical properties and potential complex interactions with other ingredients or physiological environment, which may occur in vivo.
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1.6.1.1 Lipidic excipients

Since SEDDS is a lipid based drug delivery system, lipid is an indispensable constituent of the formulations. These formulations typically contain long- or medium-chain triglyceride lipids, long- or medium-chain mixed mono-and diglycerides, individual or mixed emulgents, and various hydrophilic emulgents (Charman 2000). Lipid excipients solubilize marked amounts of lipophilic drugs within the dosage form matrix and can augment the fraction of lipophilic drugs transported via intestinal lymphatic system, thereby increasing its absorption from the GI tract (Gershanik and Benita 2000).

Natural edible oils, in this regard, are not frequently preferred owing to their poor ability to dissolve large amounts of lipophilic drugs. Modified long and medium chain triglyceride oils, with varying degrees of saturation or hydrolysis, have widely been used for the design of SEDDS. These offer distinct formulation and physiological advantages with their degradation products resembling the natural end products of intestinal digestion, these amphiphilic excipients are progressively and effectively replacing the conventional medium chain triglyceride oils in the SEDDS systems (Constantinides 1995). These semi-synthetic derivatives form good emulsification systems when used in conjunction with a large number of solubility enhancing emulgents approved for oral administration (Kimura et al. 1994; Constantinides and Scalart 1997; Hauss 2007b). Amongst the vast list of these modified triglycerides, Table 5 give an accounts for nomenclature of various fatty acids used in lipid excipients and Table 6 enlist some of vegetable oils along with their fatty acid composition infrequently employed for the formulation of SEDDS.

1.6.1.1.2 Emulgents

An emulsifier is obligatory to provide the essential emulsifying characteristics to SEDDS. Emulgents, being amphiphilic in nature, invariably dissolve (or solubilize) relatively high amounts of hydrophobic drug compounds (Aungst 2000). The twin issues that govern the selection of a emulgent are its HLB and safety. The HLB of a emulgent provides vital information on its potential utility in the formation of SEDDS.
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Table 5: Nomenclature of various fatty acids used in lipid based excipients.

<table>
<thead>
<tr>
<th>Fatty acid chain length (number of carbons)</th>
<th>Number and position (Δ) of unsaturated bonds</th>
<th>Common Name</th>
<th>Melting temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>08</td>
<td>0</td>
<td>Caprylic acid</td>
<td>16.5</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>Capric acid</td>
<td>31.6</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>Lauric acid</td>
<td>44.8</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>Myristic acid</td>
<td>54.4</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>Palmitic acid</td>
<td>62.9</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>Stearic acid</td>
<td>70.1</td>
</tr>
<tr>
<td>18</td>
<td>1 Δ 9</td>
<td>Oleic acid</td>
<td>16.0</td>
</tr>
<tr>
<td>18</td>
<td>2 Δ 9, 12</td>
<td>Linoleic acid</td>
<td>-5.0</td>
</tr>
<tr>
<td>18</td>
<td>3 Δ 6, 9, 12</td>
<td>γ-Linoleic acid</td>
<td>-11.0</td>
</tr>
<tr>
<td>18</td>
<td>1 Δ 9 (-OH:12)</td>
<td>Ricinoleic acid</td>
<td>6.0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>Arachidic acid</td>
<td>76.1</td>
</tr>
<tr>
<td>22</td>
<td>0</td>
<td>Behenic acid</td>
<td>80.0</td>
</tr>
</tbody>
</table>

For imparting high self-emulsifying properties to the SEDDS formulation the emulsifier should have a relatively high HLB (i.e., high hydrophilicity) for immediate formation of o/w droplets and/or rapid spreading of the formulation in the aqueous media. It would keep the drug for a relatively prolonged period of time at the site of absorption for effective absorption as the precipitation of drug compound within the GI lumen can be prevented (Serajuddin et al. 1988; Shah et al. 1994).

Lipid-based delivery systems usually only include non-ionic emulgents such as polysorbates or polyethoxylated vegetable oils, as these are less toxic than single-chain emulgents. The most widely recommended emulsifiers with high HLB values are solid or liquid ethoxylated polyglycolyzed glycerides and polyoxyethylene-20 oleate (e.g., Tween 80).

From safety perspectives, the emulsifiers from the natural origin are regarded as safer than the synthetic ones. However, as these possess only limited self-emulsification capacities, they are infrequently employed for the formulation of SEDDS. Non-ionic emulgents 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.
Table 6: Vital hydrogenated vegetable oils and Semisynthetic triglycerides and their Fatty acid composition of fat

<table>
<thead>
<tr>
<th>Oil</th>
<th>Fatty acid</th>
<th>Caprylic</th>
<th>Capric</th>
<th>Lauric</th>
<th>Myristic</th>
<th>Palmitic</th>
<th>Myristoleic</th>
<th>Palmitoleic</th>
<th>Margaric</th>
<th>Margaroleic</th>
<th>Stearic</th>
<th>Oleic</th>
<th>Linoleic</th>
<th>Linolenic</th>
<th>Eicosanoic</th>
<th>Eicosadienoic</th>
<th>Docosanoic</th>
<th>Robenic</th>
<th>Erucic</th>
<th>Tetracosenoic</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Carbons</td>
<td></td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>16</td>
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<td>18</td>
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<td>20</td>
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<td>22</td>
<td>22</td>
<td>24</td>
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<tr>
<td>No. of double bonds</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunflower oil</td>
<td></td>
<td>0.1</td>
<td>7</td>
<td>0.1</td>
<td>0.1</td>
<td>5</td>
<td>19</td>
<td></td>
<td></td>
<td>68</td>
<td>0.8</td>
<td>0.4</td>
<td>0.1</td>
<td>0.7</td>
<td>-18</td>
<td>-12</td>
<td></td>
<td>-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castor oil</td>
<td></td>
<td>0.1</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>87</td>
<td>3</td>
<td></td>
<td></td>
<td>60</td>
<td>1</td>
<td>0.4</td>
<td>0.1</td>
<td>-10</td>
<td>-6</td>
<td>-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td>0.1</td>
<td>11</td>
<td>0.2</td>
<td>0.1</td>
<td>2</td>
<td>25</td>
<td></td>
<td></td>
<td>60</td>
<td>1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.4</td>
<td>-10</td>
<td>-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olive oil (virgin)</td>
<td></td>
<td>0.1</td>
<td>9</td>
<td>0.6</td>
<td>3</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0.7</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut oil</td>
<td></td>
<td>0.1</td>
<td>11</td>
<td>0.2</td>
<td>0.1</td>
<td>1</td>
<td>47</td>
<td></td>
<td></td>
<td>32</td>
<td>1</td>
<td>2</td>
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<td>1</td>
<td>-5</td>
<td>-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td></td>
<td>0.1</td>
<td>0.7</td>
<td>22</td>
<td>0.6</td>
<td>0.1</td>
<td>1</td>
<td>3</td>
<td>19</td>
<td>54</td>
<td>0.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>17-22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canola oil</td>
<td></td>
<td>0.1</td>
<td>0.4</td>
<td>0.1</td>
<td>2</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>9</td>
<td>0.7</td>
<td>1</td>
<td>0.3</td>
<td>0.7</td>
<td>0.2</td>
<td>17-22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td></td>
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Albeit, the nonionic emulgents are considered as safer than ionic emulgents, they may cause reversible change(s) in the permeability of intestinal lumen (Wakerly et al. 1986; Swenson et al. 1994). The oral and intravenous LD50 values suggested by IIG for most non-ionic emulgents are in excess of 50 g/Kg and 5 g/Kg respectively, so 1 g emulgent in a formulation is well-tolerated for uses in acute oral drug administration. The marketed HIV protease inhibitors products, such as Agenerase®, Kaletra® and Norvir®, contain a considerable mass of emulgents in each capsule, and several capsules are administered 2–4 times daily, so that patients are ingesting 2–3 g Cremophor or TPGS daily. Following the selection of safe and effective emulgent, it becomes vital to explore their pragmatic concentration ranges. Usually for forming stable SEDDS, the emulgent concentration usually ranges between 30 and 60% w/w, as higher concentrations of emulgents may be irritating to the GI mucosa. Inverse relationship between the droplet size and the concentration of the emulgent is usually observed. This could be attributed by the stabilization of the oil droplets as a result of the localization of the emulgent molecules at the oil-water interface. However in some cases, the droplet size tends to augment after attaining a critical concentration. This phenomenon could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets, mediated by the increased emulgent concentration, leading ultimately to the ejection of oil droplets into the aqueous phase (Wakerly et al. 1986; Craig et al. 1995; Kommuru et al. 2001).

1.6.1.1.3 Co-emulgents/Cosolvents
As discussed above, the formulation of an effective SEDDS requires quite high concentrations of emulgent. Accordingly, cosolvents like ethanol, propylene glycol and polyethylene glycol are required to enable the dissolution of large quantity of hydrophilic emulgent. The lipid mixtures with higher emulgent and co-emulgent/oil ratios lead to the formation of SNEDDS and SMEDDS (Vonderscher and Meinzer 1994; Meinzer et al. 1995). Alcohol and other volatile co-solvents have a serious limitation of getting evaporated from the shells of sealed gelatin capsules, leading eventually to the precipitation of drug inside the shell fully ascertained. Newer cosolvents like Transcutol® HP and Glycofural™ have several stellar

30
advantages over the traditional ones.

1.6.2 Mechanism of Self-emulsification

Till date the mechanism by which self-emulsification occurs has not yet been thoroughly reported. Nevertheless, it has been suggested that self-emulsification takes place when the entropy change favoring dispersion is greater than the energy required to increase the surface area of the dispersion (Reiss 1975). The thermodynamic relationship for net free energy change is described by Equation 12. The free energy of a conventional emulsion formulation is a direct function of the energy required to create a new surface between the oil and water phases.

\[ \Delta G = \sum N_i 4\pi r_i^2 \sigma \]

\( \Delta G \) = Free energy associated with the process
\( r_i \) = radius of droplets
\( N_i \) = number of droplets
\( \sigma \) = interfacial energy

The two phases of the emulsion tend to separate with time to reduce the interfacial area and thus the free energy of the system(s). The conventional emulsifying agents stabilize emulsions resulting from aqueous dilution by forming a monolayer around the emulsion droplets, reducing the interfacial energy and forming a barrier to coalescence. On the other hand, emulsification occurs spontaneously with SEDDS, as the free energy required to form the emulsion is low, whether positive or negative (Constantinides 1995). For emulsification to take place, it is vital for the interfacial structure to offer no resistance against surface shearing (Dabros et al. 1999). The ease of emulsification has been suggested to be related to the ease of water penetration into the various liquid crystals or gel phases formed on the surface of the droplet (Groves et al. 1974; Rang and Miller 1999). The interface between the oil and aqueous continuous phases is formed upon addition of a binary mixture (oil/non-ionic emulgent) to water. This is followed by solubilization within the oil phase, as a result of aqueous penetration through the interface. Invariably, this occurs until the solubilization limit is attained close to the interphase. Further, aqueous penetration will lead to the formation of the dispersed liquid crystal phase. Ultimately, everything that is in close proximity...
with the interface will be liquid crystal, the actual amount of which depends on the
emulgent concentration in the binary mixture. Thus, following gentle agitation of
the self-emulsifying system, water rapidly penetrates into the aqueous cores
leading to interface disruption and droplet formation. As a consequence of the
liquid crystal interface formation surrounding the oil droplets, SEDDS become
quite stable to coalescence. Moreover, the presence of the drug compound may
alter the emulsion characteristics, plausibly by interacting with the liquid crystal
phase. Nevertheless, the correlation between the liquid crystal formation and
spontaneous emulsification has still not been properly established (Craig et al.
1993; Gursoy et al. 2003).

1.6.3 Lipid Digestion and Solubilization

Whilst drug solubility in the formulation (i.e. potential drug load) and the ease of
dispersion remain important design criteria for SEDDS, assessment of the impact of
type of lipid and lipid digestion of a formulation is also required to accurately
explain in vivo performance. The solubilization of drug in the GI tract and its
bioavailability depend predominantly on the intraluminal processing to which
lipids are subjected prior to absorption. Figure 12 gives a diagrammatic
presentation of the same. Knowledge of the sojourn of lipids from the GI lumen to
the systemic circulation is of great significance for interpretation of the
biopharmaceutical properties of oral lipid-based formulations and successful
product development (Ljusberg-Wahren et al. 2005). After oral administration of a
lipid-based drug formulation it undergoes three main phases:

1. Digestive phase
2. Absorption phase
3. Circulatory uptake

Digestive phase involves three sequential steps

i. Dispersion of oil globule into an emulsion of high surface area
ii. Enzymatic hydrolysis of the fatty acid glyceryl esters at oil/water interface
iii. Dispersion of the digestion products into an absorbable form

Digestive phase starts with the physical breakdown of lipid formulation into a
coarse emulsion of high surface area due to shear produced by antral contraction,
1. INTRODUCTION

retropulsion and gastric emptying.

Figure 12: A diagrammatic representation of lipid digestion in human body

The reduction in particle size provides an increase in the surface area available for binding of the pancreatic lipase/co-lipase complex, an interfacial enzyme system that preferentially acts at the surface of the emulsified triglyceride droplets, leading to quantitative production of one molecule of 2 MG and 2 FA molecules for each TG.

Dispersion of TG lipid → lipolysis → 2 FA + 2 MG BS solubilization

The dispersed lipid digestion products along with the undigested lipids then empty into the duodenum (Carey et al. 1983). As the acidic gastric content reaches the duodenum, the low gastric pH causes the release of secretin from the duodenal mucosa. This stimulates the pancreas to produce and secrete bicarbonate (along with lipase and co-lipase) into the duodenum to create a pH-neutral environment, which in turn maximizes the activity of pancreatic lipase and co-lipase. In the presence of FAs, cholecystokinin is released into the portal circulation which additionally stimulates the pancreas to release TG lipase and co-lipase required to facilitate the TGs digestion within emulsified particles. Being partially ionized, FAs and MGs are also potent emulsifiers which promote binding of the co-lipase–lipase complex to the emulsion surface (Borgstrom 1980; Bernback et al. 1989). Thus, the
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Lipolysis is an autocatalytic process capable of enhancing the emulsification when lipolytic products are produced. Both enzymes being water soluble act at the water/lipid interface and hydrolyze TGs to MGs and FAs.

The digestion phase ends with the formation of mixed micelles by the interaction of FAs and MGs with bile salts, while a part of the TGs and FAs may form vesicles after digestion in this preabsorptive phase. It is at this phase that the drug released from the formulation due to either precipitation or dissolution into the gastric media is resolubilized as micelles or mixed micelles by emulsifications, which can play a significant role on the performance of the formulation (Hernell et al. 1990).

The overall in vivo solubilization capacity depends on both the lipophilicity and chemical structure of the drug and the nature of the endogenous and exogenous lipids involved in the formation of colloidal species (Kossena et al. 2007). The enzymatic action being an interfacial process, the rate of lipolysis is enhanced in formulations with good dispersibility like self-nano/microemulsifying drug delivery systems. These types of formulations maximize the rate of drug partitioning into the aqueous intestinal fluids and provide consistent bioavailability.

In the Absorption phase the colloidal species produced, in the form of micelles, mixed micelles, vesicles and free FAs as a result of lipid digestion, are taken up by passive diffusion, facilitated diffusion and active transport through the enterocyte membrane. In the cytosol, a fatty acid-binding protein transports them from the apical membrane to the smooth endoplasmic reticulum (ER). Thereby, a concentration gradient facilitates the uptake of FAs into the cell by a carrier-mediated process (Stremmel 1988). In the smooth ER, FAs and MGs are resynthesized into TGs and phospholipids, respectively, which are transferred to the golgi apparatus and stored into secretory vesicles to be released by exocytosis into the extracellular space via basolateral membrane. Another critical step is the association of the absorbed free drug with the intestinal lipoproteins (chylomicrons) within the enterocyte. These chylomicrons are relatively large (<1 μm in diameter) and colloidal in nature which eventually lead to selective intestinal lymphatic transport of the lipophilic compound. (Charman and Porter 1996)

Circulatory uptake The majority of orally administered drugs gain access to the
systemic circulation by absorption into the portal blood. However, some extremely 
lipophilic drugs gain access to the systemic circulation via lymphatic route, which 
avoids hepatic first-pass metabolism. Therefore, highly metabolized lipophilic 
drugs may be potential candidates for lipid based drug delivery. Compounds 
showing increased bioavailability in the presence of lipids (dietary or lipid-based 
formulation) are absorbed via the intestinal lymph as they are generally 
transported in association with the long-chain TGs lipid core of intestinal 
lipoproteins formed in the enterocyte after re-esterification of free FAs and MGs. 
Short-chain and medium-chained TGs are primarily absorbed directly in the portal 
blood (Caliph et al. 2000). Direct uptake of long-chained TGs and phospholipids 
into the bloodstream is not possible, though the portal blood is approximately 500-
fold higher than that of the intestinal lymph. This is because their large molecular 
size restricts them to pass through capillary fenestration spaces.

The walls of lymphatic capillaries consist of a single layer of squamous epithelial 
cells, and this thin wall makes it possible for tissue fluid (interstitial fluid) from the 
interstitial space to enter the lymphatic capillary. Moreover, the endothelial 
arquitecture of the lymphatic vessels facilitates the size-selective transport of high 
molecular weight substances like chylomicrons for which facile access across the 
blood capillary endothelium is restricted (Leak 1976). Studies have shown that the 
free FA chain length and the composition and size of the lymph lipid precursor pool 
in the enterocyte play major role in lymphatic drug transport. In general, free fatty 
acids (FFA) of chain length 6-12 carbons are absorbed primarily by means of the 
portal blood, whereas FFA with chain lengths >12 carbons are re-esterified and 
transported via intestinal lymph (Trevaskis et al. 2008). Additionally, increase in 
the degree of unsaturation produces larger size lymph lipoproteins and selectively 
enhances lymphatic uptake (Ockner et al. 1972; Green and Glickman 1981). The 
lymph fluid is then emptied (average 3 litres per day) via thoracic duct into the 
subclavian vein, thus protecting the drug from hepatic first-pass metabolism. The 
drug being transported in the circulatory system, in the form of either micelles or 
mixed micelles, may then be available in its free form, since upon dilution with a 
large volume of the lymph/blood, emulgent concentration may reduce below its 
cmc value and micelle may dissociate into monomers (Porter et al. 2007). The drug
transported as lipid vesicles may remain intact for extended periods and, thereby, can result in prolonged release of the encapsulated drug. Figure 13 is a diagrammatic presentation of the various mechanisms by which lipids enhance the bioavailability of drug.

1.6.4 Mechanism of drug transport from SEDDS

The small droplets formed in contact with the gastrointestinal fluid are also responsible for transporting the drug substance through the unstirred water layer to the gastrointestinal membrane for absorption (de Smidt et al. 2004).

Figure 13: Various mechanisms of enhancement of drug bioavailability in the presence of lipids: (a) solubilization of drug in the intestinal fluid by formation of colloidal species viz., vesicles, mixed micelles and micelles; (b) interference with enterocyte-based transport, thereby potentially changing drug uptake, efflux, disposition and the formation of metabolites (M) within the enterocyte; (c) by selective lymphatic uptake which reduces first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation [Figure adapted from Porter et al. 2008]

Route and mechanism of drug transport depends upon the type of lipids used. In the literature, medium chain triglycerides (MCT) have been preferred in SEDDS due to
the higher fluidity, better solubility properties and self-emulsification ability compared with long chain triglycerides (LCT), as well as a better chemical stability of drug substance in MCT due to the purity of the lipid and the lack of double bonds, that can catalyse oxidation (Charman et al. 1992; Shah et al. 1994).

The two lipids are differently transported in the body: MCT is directly transported by the portal blood to the systemic circulation, whereas LCT is transported in the intestinal lymphatics (Porter and Charman 1997). Lipid-based drug delivery systems containing LCT are likely to enhance the lymphatic transport of a lipophilic drug substance and as the lymphatic transport circumvents the liver, the first-pass metabolism of a drug substance may be reduced (Palin et al. 1986; Porter and Charman 1997; Caliph et al. 2000). SEDDS containing either MCT or LCT have been studied with halofantrine and danazol and in both cases LCT-SEDDS were found to give the maximum bioavailability owing to avoidance of first-pass metabolism of the drug (Khoo et al. 1998; Cuine et al. 2008). Studies comparing the bioavailability of drug substance from SEDDS and other lipid-based drug delivery systems e.g. pure lipid solutions are still limited in number and until now most studies have been comparing self-emulsifying systems with solid dosage forms.

The main mechanism of intestinal lymphatic drug absorption is via intracellular association of the drug with the lipid core of the chylomicron, a lipoprotein that is synthesized in situ inside the enterocyte (Gershkovich and Hoffman 2005; Dahan and Hoffman 2006). Following this association, the chylomicrons are transported to interacellular spaces via basolateral membrane of enterocytes. In intracellular spaces chylomicrons are absorbed into the absorbed into the porous mesenteric lymph vessels and travels, with the lipophilic molecule in it, along the lymphatics until it drains into the systemic blood circulation.

At cellular level, three pathways to potentially target the drugs into the intestinal lymphatics have been investigated.

1. Paracellular route with the aid of absorption enhancers
2. M cells and gut associated lymphoid tissues (GALT)
3. Transcellular route

The choice of pathway depends on physicochemical properties of drug candidate
and design of drug delivery systems. Figure 14 represents the cellular mechanisms of drug access from the intestinal lumen to the mesenteric lymphatic system. Figure 15 represents various absorption pathways of fats and drug entrapped SEEDS across the gastro-intestinal tract.

1.6.4.1 Paracellular Pathway

Emulgents, one of the components of SEDDS, may open up the paracellular route, resulting in increased permeability of hydrophilic molecule or macromolecule conjugates. Due to porous structure of the lymphatic capillaries, macromolecular targeting may be possible.

![Diagram showing the transport of lipids and drug](image)

**Figure 14:** The pictures portray the transport of (a) lipids and (b) drug [Figure adapted from Porter and Charman 2001]

Yoshikawa *et al.* (1983) formed a non-covalent complex between bleomycin and dextran sulphate, and on co-administration with mixed micelles reported preferential uptake by lymphatics. This route has an additional advantage for the delivery of protein and peptide drugs due to lower enzymatic activity. However,
there are certain limitations too. The transport capacity of this route is limited due to relatively low surface area and safety issues particularly due to chronic use of absorption enhancers such as toxicity, selectivity and reversibility of the membrane effects.

1.6.4.2 Transcellular mechanism

The third route is transcellular via intestinal lipid transport systems. The precise mechanism of drug transport via this route is not fully understood. At the cellular level, stimulation of chylomicrons production is a key factor in enhancing lymphatic transport of lipophilic compounds.

Figure 15: Absorption pathways of fats and drug entrapped SEDDS across the GI tract

This can be achieved through formulation design and the incorporation of an excipient, such as long chain unsaturated fatty acids, to drive chylomicrons production. The loading of the drug is influenced by the physicochemical properties of drug candidate. Drug should have ideally log P of 5 and triglyceride solubility of 50 mg/ml (Charman and Stella 1986). Pre-absorptive events in the gut lumen have also been shown to influence the degree of lymphatic transport, i.e., physiological processes of lipid digestion and absorption play a role in drug uptake
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and lymphatic transport (Humberstone and Charman 1997).

1.6.4.3 Effect of P-glycoprotein (P-gp) inhibition

There may be other possible reasons for enhanced uptake of hydrophobic and/or lipophilic drugs formulated as SEDDS from the GI tract, like a decrease in the P-gp drug efflux (Lo et al. 1998; Yu et al. 1999). In addition to a multidrug efflux pump, phase-I metabolism by the intestinal cytochrome P450’s is now becoming recognized as a significant factor in oral drug bioavailability (Woo et al. 2003). In some cases, as shown recently, excipients incorporated in SEDDS can inhibit both presystemic drug metabolism and intestinal efflux mediated by P-gp resulting in an increased oral absorption of cytotoxic drugs (Chervinsky et al. 1993; Dintaman and Silverman 1999). Figure 16 depicts a typical P-gp pump on transcellular membrane.

![P-gp Efflux Pump](Figure adapted from Pauwels et al. 2007)

1.6.5 Effect of constituents

Lipidic constituents of SEDDS formulations tend to have significant impact on the oral bioavailability of the drug compound. They exert their effects possibly through several complex mechanisms that can lead to alteration in the biopharmaceutical properties of the drug, such as increased dissolution rate of the drug and solubility in the intestinal fluid, protection of the drug from chemical as well as enzymatic degradation in the oil droplets and the formation of lipoproteins promoting the lymphatic transport of highly lipophilic drugs (Borgstrom 1980; Green and Glickman 1981; Charman 2000; O’Driscoll 2002).

The absorption profile and the blood/lymph distribution of the drug compound are
affected by the acid chain length of the triglyceride, saturation degree, and volume of the lipid administered (Deckelbaum et al. 1990). Generally, compounds processed by the intestinal lymph are transported to the systemic circulation in association with the lipid core of lipoproteins, and as such require coadministered lipid(s) to stimulate lipoprotein formation. Short and medium chain fatty acids (with a carbon chain length shorter than 12 carbon atoms) are transported to the systemic circulation by the portal blood and are not incorporated to a great extent in chylomicrons. In contrast, long chain fatty acids and monoglycerides are re-esterified to triglycerides within the intestinal cell, incorporated into chylomicrons and secreted from the intestinal cell by exocytosis into the lymph vessels. In addition to the stimulation of the lymphatic transport, administration of lipophilic drugs with lipids may enhance drug absorption into the portal blood when compared to non-lipid formulations (MacGregor et al. 1997; Sek et al. 2002; Gershkovich and Hoffman 2005).

Emulgents increase drug permeability by interfering with the lipid bilayer of the single layer of the epithelial cell membrane, which with the unstirred aqueous layer forms the rate-limiting barrier to drug absorption/diffusion. Therefore, most drugs are absorbed via the passive transcellular route. Emulgents partition into the cell membrane and disrupt the structural organization of the lipid bilayer leading to permeation enhancement. These also exert their absorption enhancing effects by increasing the dissolution rate of the drug (Christian and Scamehorn 1995; Li et al. 2005; Mullertz et al. 2010).

Extensive studies have been carried out to improve the oral bioavailability of CsA, which eventually led to the formulation of Sandimmune® and later on to even a better performing microemulsion formulation of CsA, Sandimmun Neoral® (Vonderscher and Meinzer 1994). The administration of cyclosporin in positively charged SEDDS led to higher blood levels as compared to the corresponding negatively charged formulation. Positively charged oil droplets interact with the negatively charged surface components of the GI lumen. It is interesting to note that larger droplets in sizes of a few microns are less neutralized by mucin than smaller droplets in submicron sizes formed by the same formulation. It appears that there is a more complex correlation between droplet size and bioavailability in
positively charged formulations. CoQ₁₀ is available on the market as oil-based and powder-filled capsule formulations, which exhibit high variations in oral bioavailability. Thus, a new SEDDS approach was evaluated for improved oral bioavailability of CoQ₁₀ leading to improvement in bioavailability (Kommuru et al. 2001).

Many other extensive studies like above were carried out in animals for assessment of oral bioavailability of hydrophobic drugs. Owing to their immense utility in industrial pharmacy, numerous SEDDS formulations have been patented too (Table 7). The success story has paved the way for the commercial development of effective therapeutic systems which could be marketed in the form of SEDDS as is evident from Table 8.

Table 7: Patents on SEDDS formulations

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<tr>
<td>Self-emulsifying drug delivery Labrasol®, Cremophor ELP® and Gelucire 44/14®</td>
<td>US20100310661</td>
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<td>Butylphthalide self-</td>
<td>HK1111299 (A2)</td>
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<td>emulsifying drug delivery system, its preparation method and application (2008)</td>
<td>LIYING YANG</td>
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<td>Soft Gelatin Capsule And Injection Of Ibuprofen Using SMEDDS As Solubilization Method</td>
<td>KR20020071037 (A)</td>
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<td>Self-nanoemulsifying oily formulation for the administration of poorly water-soluble drugs</td>
<td>US/2006/0292186</td>
<td>JEAN-SEBASTIEN GARRIGUE, GREGORY LAMBERT, ALAIN RAZAFINDRATSITA</td>
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<td>Butylbenzene phthalein self emulsifying drug delivery system, its preparation method and application (2006, 2007)</td>
<td>CA 2578130 MX2007002335 (A)</td>
<td>Zhentao Liu, Liying Yang, Hanyu Yang, Yuqing Gao, Dongmin Shen, Wenmin Guo, Xiaolong Feng and Jia Zheng</td>
<td>Shijiazhuang Pharma, China</td>
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<td>Self-emulsifying formulations of fenofibrate and/or fenofibrate derivatives with improved oral bioavailability and/or reduced food effect (2006)</td>
<td>US7022378B2</td>
<td>Likan Liang, Amir H. Shojaei, Scott A. Ibrahim and Beth A. Burnside</td>
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<td>Pharmaceutical composition for hyperlipidemia treatment of self emulsifying drug delivery system to increase bioabsorption and improve stability of active ingredient (2005)</td>
<td>KR20050011323 (A)</td>
<td>Cho Sun Hang and Jeong Sang Young</td>
<td>Korea Research Institute of Chemical Technology</td>
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<td>Self-emulsifying and self-microemulsifying formulations for the oral administration of taxoids</td>
<td>US 2005/0025792 A1</td>
<td>Peracchia Maria-Teresa, Cote Sophie and Gaudel Gilbert</td>
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<td>Self emulsifying drug</td>
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### 1. INTRODUCTION

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<td>Self emulsifying drug delivery system, wherein the fatty agent is optional (2004)</td>
<td>SI1267832 (T1)</td>
<td>Holmberg Christina and Siekmann Britta</td>
<td>Astra Zeneca</td>
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<tr>
<td>Self-emulsifying ibuprofen solution and soft gelatin capsule for use therewith this invention relates to a self-emulsifying solution of ibuprofen suitable for encapsulation into a soft gelatin capsule (2001)</td>
<td>US 6221391</td>
<td>Mark T. Rouffer</td>
<td>Accucaps Industries Limited, Cannada</td>
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<tr>
<td>Self-emulsifiable formulation and oil-in-</td>
<td>US 5965160</td>
<td>Simon Benita, Jackie Kleinstern</td>
<td>Yissum Research Development</td>
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</table>
## 1. INTRODUCTION

<table>
<thead>
<tr>
<th>Title/Year</th>
<th>Patent Number</th>
<th>Inventors</th>
<th>Company</th>
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<tr>
<td>water emulsion (1999) and Tatyana Gershanik</td>
<td></td>
<td></td>
<td>Company of the Hebrew University of Jerusalem</td>
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<tr>
<td>Polymeric micromulsions (novel self-emulsifying diblock copolymer)</td>
<td>US20060034797</td>
<td>and Tatyana Gershanik</td>
<td>Johnson &amp; Johnson</td>
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<td>Delivery systems for hydrophobic drugs</td>
<td>US6,096,338</td>
<td>Lacy, Jonathan E. Embleton, Jonathan K. Perry and Elizabeth A.</td>
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<td>Supersaturated cationic self-emulsified drug delivery system and its preparation method (2007)</td>
<td>CN101057829 (A)</td>
<td>Gong Mingtao Chen</td>
<td>Shanghai Inst Pharm Industry, China</td>
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<td>Hemlock parsley oil self-emulsifiable oral medicine delivery system and preparing method thereof (2008)</td>
<td>CN101229205 (A)</td>
<td>Qin Cai and Long Liang</td>
<td>Sichuan Pearl Pharmaceutical, China</td>
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<td>Self-emulsifying active substance formulation and use of this</td>
<td>US 2004/0013697 A1</td>
<td>Gunther Berndl, Jorg Breitenbach, Robert Heger,</td>
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<td>formulation</td>
<td></td>
<td>Michael Stadler, Peter Wilke and Jorg Rosenberg</td>
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<td>Self-emulsifying pigments this invention relates to a surface-modified pigment having at least two surface-active agents chemically immobilized onto the surface of the pigment</td>
<td>US7276113</td>
<td>Mark G. Le Page, William Zavadoski, Shigeru Kishida and Yoshiaki Kawasaki</td>
<td>U.S. Cosmetics Corporation, US</td>
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<td>New self emulsifying drug delivery system</td>
<td>CA200124018</td>
<td>Christina Holmberg and Britta Siekmann</td>
<td>Astrazeneca, Sweden</td>
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<td>Siekmann Britta and Holmberg Christina</td>
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<td></td>
<td>US 2003/0077303</td>
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<td>AU2001237875</td>
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<td>Self-Emulsifying Drug Delivery Systems For Extremely Water-Insoluble, Lipophilic Drugs</td>
<td>AU2001277099 (B2)</td>
<td>Ping Gao and Narmada Shenoy</td>
<td>Pharmacia and Upjohn Company</td>
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<td></td>
<td>US 2003/0147927</td>
<td>Mansoor A. Khan and Sami Nazzal</td>
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<td>A1</td>
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<td>Formulation For Self Emulsifying Matrix Type Mucosal And Transdermal Absorbent</td>
<td>KR20010093728 (A)</td>
<td>Hong Cheong Il and Ki Min Hyo</td>
<td>Chong Kun Dang Pharm Corp, Korea</td>
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<td>Vinpocetine oral self-micro-emulsification medicine-releasing system and preparation</td>
<td>CN101103962 (A)</td>
<td>Gao Li and Ying Chen</td>
<td>Tongji Medical College of Huaz, China</td>
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### 1. INTRODUCTION

<table>
<thead>
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<th>Title/Year</th>
<th>Patent Number</th>
<th>Inventors</th>
<th>Company</th>
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<td>method thereof</td>
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<td>Self-emulsifying matrix type transdermal preparation</td>
<td>US 2003/0129219 A1</td>
<td>Chung Il Hong, Hee Jong Shin, Min Hyo Ki, Seok Kyu Lee and Don Sun Kweon</td>
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</table>

#### Table 8: Various drugs marketed as SEDDS formulations

<table>
<thead>
<tr>
<th>Trade Name (Company)</th>
<th>Drug Molecule</th>
<th>Type of Formulation</th>
<th>Excipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accutane® (Roche)</td>
<td>Isotretinoin</td>
<td>Soft gelatin capsule (10, 20, 40 mg)</td>
<td>Beeswax, hydrogenated soybean oil, hydrogenated vegetable oil, soybean oil</td>
</tr>
<tr>
<td>Agenerase® (GaxoSmithKline)</td>
<td>Amprenavir</td>
<td>Soft gelatin capsule (50 mg)</td>
<td>TPGS, PEG 400, Propylene glycol, Sodium chloride</td>
</tr>
<tr>
<td>Aptivus® (Boehringer Ingelheim)</td>
<td>Tipranavir</td>
<td>Soft gelatin capsule (250 mg)</td>
<td>Cremophor EL, medium-chain mono- and diglycerides, dl-α-tocopherol</td>
</tr>
<tr>
<td>Cyclosporine Capsules® (Sidmak)</td>
<td>Cyclosporine A</td>
<td>Soft gelatin capsule (100 mg)</td>
<td>Caprylic/capric triglycerides (Labrafac), dl-α-tocopherol, glyceryl caprylate, PEG-8 caprylyl/capric glycerides (Labrasol), PEG-35 castor oil (Cremophore EL)</td>
</tr>
<tr>
<td>Fenogal® (Genus)</td>
<td>Fenofibrate</td>
<td>Hard gelatin capsule (200 mg)</td>
<td>Lauryl macrogol-glycerides (Gelucre 44/14)</td>
</tr>
<tr>
<td>Fortovase® (Roche)</td>
<td>Saquinavir</td>
<td>Soft gelatin capsule (200 mg)</td>
<td>Medium-chain mono- and diglycerides, dl-α-tocopherol</td>
</tr>
<tr>
<td>Gengraf® (Abbott)</td>
<td>Cyclosporine A</td>
<td>Hard gelatin capsules (25, 100 mg)</td>
<td>Polyethylene glycol, cremophor EL, polysorbate 80, sorbitan monooleate</td>
</tr>
<tr>
<td>Infree® (Eisai Co.)</td>
<td>Indomethacin</td>
<td>Soft gelatin capsule (200 mg)</td>
<td>Polyoxoy 60 hydrogenated castor oil (Cremophor RH 60), hydrogenated oil, glyceryl monooleate</td>
</tr>
<tr>
<td>Kaletra® (Abbott)</td>
<td>Lopinavir &amp; Ritonavir</td>
<td>Soft gelatin capsule lopinavir (133.3 mg) &amp; ritonavir (33.3 mg)</td>
<td>Oleic acid, polyoxyl 35 castor oil (Cremophor EL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral solution</td>
<td>Polyoxoy hydrogenated castor oil (Cremophor RH)</td>
</tr>
<tr>
<td>Trade Name (Company)</td>
<td>Drug Molecule</td>
<td>Type of Formulation</td>
<td>Excipients</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
<td>---------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Lipirex® (Sanofi-Aventis)</td>
<td>Lopinavir (80 mg/ml) &amp; ritonavir (20 mg/ml)</td>
<td>Hard gelatin capsule</td>
<td>Lauryl macrogol-glycerides (Gelucire 44/14), PEG 20000, peppermint oil</td>
</tr>
<tr>
<td>L-OROS® SOFTCAP™ (ALZA Corp)</td>
<td>Guaiphenesin</td>
<td>Osmotic pump</td>
<td>Soft gelatin capsule containing oily self-emulsifying liquid</td>
</tr>
<tr>
<td>Neoral® (Novartis)</td>
<td>Cyclosporine A</td>
<td>Soft gelatin capsule (10, 25, 50, 100 mg)</td>
<td>dl-a-tocopherol, corn oil-mono-di-triglycerides, Propylene glycol, cremophore RH 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral solution (100 mg/ml)</td>
<td>dl-a-tocopherol, corn oil-mono-di-triglycerides, polyoxyl 40, Cremophore RH 40</td>
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<tr>
<td>Norvir® (Abbott)</td>
<td>Ritonavir</td>
<td>Soft gelatin capsule (100 mg)</td>
<td>Oleic acid, polyoxyl 35 castor oil (Cremophor EL)</td>
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<tr>
<td>Rapamune® (Wyeth)</td>
<td>Sirolimus</td>
<td>Oral solution</td>
<td>Phosal 50PG, Propylene glycol, Phosphatidylcholine</td>
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<tr>
<td>Rocaltrol® (Roche)</td>
<td>Calcitriol</td>
<td>Soft gelatin capsule (0.25, 0.5 μg)</td>
<td>Medium chain triglycerides, BHT</td>
</tr>
<tr>
<td>Sandimmune® (Sandoz)</td>
<td>Cyclosporine A</td>
<td>Soft gelatin capsule (25, 50, 100 mg)</td>
<td>Corn oil, Polyhydroxy castor oil, Labrafil M 2125 CS, Ethanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral solution (100 mg/ml)</td>
<td>Olive oil, Labrafil M 1944 CS</td>
</tr>
<tr>
<td>Solufen® (Sanofi-Aventis)</td>
<td>Ibuprofen</td>
<td>Hard gelatin capsule</td>
<td>Oral solution containing oily excipients like soya phospholipids, Gelucire 44/14</td>
</tr>
<tr>
<td>Targretin® (Ligand)</td>
<td>Bexarotene</td>
<td>Soft gelatin capsule (75 mg)</td>
<td>Macrogol, Polysorbate, Povidone, BHT</td>
</tr>
<tr>
<td>Vesanoïd® (Roche)</td>
<td>Tretinoin</td>
<td>Soft gelatin capsule (10 mg)</td>
<td>Beeswax, BHA, EDTA, hydrogenated soybean oil flakes, hydrogenated vegetable oils, soybean oil</td>
</tr>
</tbody>
</table>

1.6.6 Characterization of SEDDS

A number of techniques have been employed to identify the physical nature of
SEDDS and to ascertain the feasibility of process. It is a Herculean task to assume the *in vivo* state in the light of complexity of diverse structures and components involved in these systems, and limitations associated with each technique. A brief account of the methods is described as under.

1.6.6.1 Equilibrium phase behavior

Although self-emulsification is a dynamic non-equilibrium process involving interfacial phenomena, yet information can be obtained about self-emulsification using equilibrium phase behavior. There appears to be a correlation between emulsification efficiency and (i) region of enhanced water solubilization (i.e., a typical property of nonionic emulgent system) and phase inversion region, and (ii) formation of lamellar liquid crystalline dispersion phase on further incorporation of water. It allows comparison of different emulgents and its synergy with the co-solvent/emulgent. The boundaries of one phase region can be assessed easily by visual observation of the samples. Phase behavior of three-component system can be represented pictorially by a ternary phase diagram.

1.6.6.2 Particle size measurement

1.6.6.2.1 Droplet size

Droplet size can be measured by small-angle X-ray scattering (SAXS), small angle neutron scattering (SANS), and static as well as dynamic light scattering (DLS) techniques. During DLS, the sample is illuminated with a laser beam and the intensity of the resulting scattered light produced by the particles fluctuates at a rate that is dependant on the size of particles (Goddeeris *et al.* 2006).

1.6.6.2.2 Cryo-TEM studies

The samples for the Cryo-TEM studies are prepared in a controlled environment vitrification system (CEVS). A small amount of the sample (5 μl) is put on carbon film supported by a copper grid and blotted with filter paper to obtain a thin liquid film on the grid. The grid is quenched in liquid ethane at –180°C and transferred to liquid nitrogen (–196°C). The samples are characterized with a TEM microscope (Fatouros *et al.* 2007).
1.6.6.3 Zeta potential

The charge on the oil droplets of SEDDS is another property that should be assessed. The charge in conventional SEDDS is negative due to the presence of free fatty acids. However, incorporation of a cationic lipid, like oleylamine in the concentration range of 1-3%, will yield cationic SEDDS. Thus, such systems have a positive potential value of about 35-45 mV. This positive z-potential value is preserved following the incorporation of the drug compounds (Gershanik et al. 1998; Gershanik and Benita 2000).

1.6.6.4 Emulsification time

This test is designed to estimate time required by SEDDS for emulsification in conditions similar to agitation provided in GI tract. A quantity of 1.0 g of each of the formulations was added to 0.5% w/v SLS solution (500 mL) under continuous stirring (50 rpm) using a USP 31 Apparatus 2 (Labindia, DS 8000, Mumbai, India) at 37 ± 0.5 °C. Time required to disperse the system completely and uniformly was determined, and was recorded as the emulsification time (Singh et al. 2011b).

1.6.6.5 Pseudo-ternary phase diagram study

The pseudo-ternary phase diagrams of oil, emulgent: coemulgent and water are developed using water titration method: the mixtures of oil and emulgent/coemulgent at certain weight ratios are diluted with water in a dropwise manner. For each phase diagrams at a specific ratio of emulgent/coemulgent, transparent and homogenous mixture of oil and drug get formed under the mixing by magnetic stirring (Cirri et al. 2007). Then, each mixture is titrated with water and visually observed for phase clarity and flow ability. After identification of microemulsion region in the phase diagrams, the microemulsion formulations are selected at desired component ratios (Shafiq et al. 2007). Figure 17 shows a pseudoternary phase diagram showing the formation of microemulsion region.

1.6.6.6 Robustness to dilution

Microemulsions resulting from dilution with dissolution media must be robust to all dilutions and should not show any separation even after 24 h of storage.
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Figure 17: Microemulsion diagram depicting the formation of microemulsion region

1.6.6.7 Rheological studies

As a prelude to dissolution and permeation across GI lumen, drug molecules form the SEDDS formulation have to undergo dilution in the GI milieu to form nano/microemulsion. Accordingly, estimation of their rheological behavior during this transition is quite vital, next only to the assessment of solubility and permeability across GI tract (Groves and de Galindez 1976). On dilution, the oil and emulsifier(s) present in SEDDS form an interface with GI fluids, termed as intermediate liquid crystalline phase. The rheological studies, consequently, focus on exploration of the viscoelastic properties of this intermediate liquid crystalline phase and to evaluate its effect on self-emulsification performance (Biradar et al. 2009). Additionally, assessment of rheological properties of SEDDS is indispensable to have better understanding of their phase behavior during extreme conditions of temperature, humidity, transportation, etc.

Rheological behavior of micro/nanoemulsions formed after dilution has been determined using digital instruments coupled with either cup and bob or a co-axial measuring device. A rotational viscometer has also been used for viscosity measurements on fresh microemulsions and those stored for long periods (Cirri et al. 2007). A typical rheometer test program for rheological characterization (i.e.,
flow, thixotropy, static yield, creep value) presents a data analysis for flow curves, quality control min/max limits, mathematical models, data averaging and many more analytical functions (Newton et al. 2005; Biradar et al. 2009). Generally, viscosity measurements indicate that on dilution with distilled water (e.g. 10 and 100 times), viscosity of a formulation decreases, thus construing that the drug absorption is likely to be faster from stomach (Patel and Sawant 2007). Besides, the effect of concentration of excipients on the rheological profile of SEDDS can also be examined.

1.6.7 Permeation Studies

The intestinal epithelium is a gatekeeper, i.e. it controls the entry of nutrients and xenobiotics e.g., medicines). Knowledge of the absorption and metabolism of these substances at the intestinal mucosal level is of particular importance, since the oral bioavailability of a drug is defined as the fraction of an oral dose that reaches the systemic circulation.

1.6.7.1 In vitro / ex vivo models

**Brush border membrane vesicles:** In this approach, cell homogenates or intestinal scrapings are treated by CaCl₂ precipitation method using centrifugation (Osiecka et al. 1985). The final pellet contains the luminal wall-bound proteins and phospholipids, which contain most of the brush border enzymatic and carrier activity. Resuspension of the pellet in buffer results in the formation of vesicles. These vesicles are mixed with the permeant in buffer and filtered after a fixed time, the amount of permeant taken up by the vesicles is then determined. Despite drawbacks like the need for a radiolabelled compound and day-to-day variation in precipitation, this method is useful for mechanistic studies of the drug absorption process.

**Organotypic models:** All intestinal cell types (e.g., enterocytes, calciform cells and lymphocytes) are present in organotypic models, which are used to study formulation effects (with the possible use of parenteral lipid emulsions, such as intralipid, or bovine serum albumin for drugs having low solubility), intestinal metabolism/stability, and regional differences in permeability. Some studies have shown that permeability to various marker molecules varies along the intestinal
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canal. In general, permeability decreases in the order: jejunum > ileum > colon (Davis et al. 1982). The half-lives of these models are short (i.e., 1-3 h).

**In situ single pass perfusion technique:** In this technique perfusion solution is passed through the intestinal segment by cannulating it at both the ends and effective permeability is calculated from amount of drug unabsorbed from the intestine. This technique provides experimental conditions closer to that occurring in vivo and are able to predict exact mechanism of absorption i.e., passive absorption, carrier mediated absorption or active transport (Varma and Panchagnula 2005). Figure 18 describes the schematic representation of SPIP technique.

*Figure 18: Schematic representation of single pass intestinal perfusion technique*

**Non everted gut sac technique:** In this method, a 2 to 4 cm section of the intestine is tied off at one end and everted using a glass rod or a thread. This method can be used to determine kinetic parameters with high reliability and reproducibility (Barthe et al. 1998). Oxygenated tissue culture media and specific preparation techniques ensure tissue viability for up to 2 h. The technique can be used to study drug transport across the intestine and into the epithelial cells, provided that sensitive detection methods are employed. Radiolabelled compounds are most
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appropriate. It is used mainly to quantify the paracellular transport of hydrophilic molecules, and to estimate the effects of potent enhancers on their absorption (Leppert and Fix 1994). Molecules that cross the epithelial barrier by a transcellular route have a much higher permeability, which can also be accurately quantified by using the everted sac system.

This kind of model is suitable for measuring absorption at different sites in the small intestine (Chowhan and Amaro 1977), and for performing preliminary experiments on the colon (Levet-Trafit et al. 1996; Barthe et al. 1998; Barthe et al. 1999). A potential disadvantage of this approach is the presence of the muscularis mucosa, which is not usually removed from everted sac preparations. Therefore, this model does not reflect the actual intestinal barrier, because compounds under investigation pass from the lumen into the lamina propria (where blood and lymph vessels are found) and across the muscularis mucosa. Thus, the transport of compounds with a propensity to bind to muscle cells might be underestimated.

**Isolated and perfused intestinal segments:** During the last decade, a wide range of isolated organ systems have been developed for biomedical and pharmaceutical research. The availability of sophisticated equipment, increased manual skills, and the routine use and standardization of models and protocols, have led to the increased reproducibility and validity of experimental results under circumstances that are virtually "true-to-life". These methods contribute to the reduction of animal experimentation. Figure 19 shows the place of isolated perfused organs in biomedical research, compared with cell cultures of either human or animal origin.

The results are predictive of the in vivo situation including absorption at the organ level (Levet-Trafit et al. 1996; Barthe et al. 1999). Isolated perfused organs have the advantage that the scientist works with an intact organ, where physiological cell-to-cell contacts and normal intracellular matrixes are preserved. The major limitation is the short duration of the experiments, since changes occur rapidly.

**Diffusion cells using tissue(s):** In this method, diffusion across small section of intestine representing mucosal environment etc. is studied into a system with specific fluid pH, temperature etc. representing secrosal environment. The buffer solution at both side of the membrane is gassed continuously with carbogen.
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(Barthe et al. 1999). The same method can be used for tissue other than intestinal tissue (e.g., buccal, esophageal, gastric, rectal, nasal, lung, and skin tissue).

Figure 19: The place of isolated perfused organs in biomedical research

The usefulness of these cells for intestinal transport studies has long been recognized, and they have also been used to study the intestinal metabolism of xenobiotics. In this system, the drug can be exposed at either the mucosal level or the serosal level. The simplicity of these cells makes them an attractive in vitro model system for studying drug transport. This type of study may add additional information on the pharmacological behavior of the test compound (Roediger and Truelove 1979; Soderholm et al. 1998).

1.6.7.2 Cell models

**Epithelial cell models:** The study of absorption mechanisms is best performed in a model that contains only absorptive cells, without the confounding contributions of mucus, the lamina propria and/or the muscularis mucosa. Therefore, much attention is currently paid to the use of epithelial cell cultures for studies of drug transport mechanisms.

**Isolated intestinal cells:** These cells from the intestine of animal or human origin can be used as uptake systems in the assessment of oral bioavailability. However, the use of isolated intestinal epithelial cells has been slow to gain popularity, because they are difficult to culture and have limited viability (Roediger and Truelove 1979; Ishii et al. 1994).
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The development of human cell culture systems has been limited by the loss of important in vivo anatomical and biochemical features. Attention has therefore turned to the use of human adenocarcinoma cell lines, such as HT-29 and Caco-2 that reproducibly display a number of properties characteristic of differentiated intestinal cells (Kedinger et al. 1987). The limitations of cell models must not be overlooked, but they offer the advantage of relative simplicity, and are suitable for automated procedures and HTS. These cell lines originated from tumors, and are out of the in vivo physiological environment; therefore, extrapolation of the data to the in vivo situation may be difficult (as is true of most in vitro systems).

**Non-intestinal cell systems:** Madin Darby canine kidney (MDCK) cells were isolated from a dog kidney by Madin & Darby. They are currently used to study the regulation of cell growth, drug metabolism, toxicity and transport at the distal renal tubule epithelial level. MDCK cells have been shown to differentiate into columnar epithelial cells, and to form tight junctions when cultured on semi-permeable membranes. The use of these cells as a cellular barrier model for assessing intestinal epithelial drug transport was discussed by (Cho et al. 1989).

**Caco-2 cells:** These are the most popular cellular model in studies on passage and transport. These were derived from a human colorectal adenocarcinoma. In culture, they differentiate spontaneously into polarised intestinal cells possessing an apical brush border and tight junctions between adjacent cells, and they express hydrolases and typical microvillar transporters. Caco-2 cells, despite their colonic origin, express in culture the majority of the morphological and functional characteristics of small intestinal absorptive cells, including phase I and phase II enzymes, detected either by measurement of their activities toward specific substrates, or by immunological techniques (Bailey et al. 1996; Sermkaew et al. 2013).

1.6.8 Types of SEDDS

There are many problems associated with the conventional liquid SEDDS formulations like possibility of leakage of contents from capsular cell, GIT irritation due to high concentrations of surfactants, decreased transcellular and/or paracellular drug transportation, etc. Hence, the advanced types of SEDDS viz,
solid, supersaturable and cationic SEDDS were able to surmount the above-mentioned problems. Supersaturable systems are able to generate and maintain a metastable supersaturated state *in vivo* by preventing or minimizing the precipitation of the drug through the use of a suitable polymeric precipitation inhibitor. Owing to their cationic charge, cationic SEDDS facilitates increased adhesion of the droplets to the cell surface due to electrostatic attraction. This mechanism is mainly responsible for the preferential uptake of the drugs.

### 1.6.8.1 Cationic SEDDS

Many physiological studies have proved that the apical potential of absorptive cells, as well as that of all other cells in the body, is negatively charged with respect to the mucosal solution in the lumen (Barry and Eggenton 1972; Corbo *et al.* 1990; Rojanasakul *et al.* 1992; Gershanik *et al.* 1998). The drug exposure of the cationic SEDDS has been found to be higher vis-à-vis the conventional formulations especially for bioavailability enhancement. More recently, it has been shown that the enhanced electrostatic interactions of cationic droplets with the mucosal surface of the everted rat intestine are mainly responsible for the preferential uptake of the drugs (Gershanik *et al.* 1998). Table 9 enumerates such literature studies on cationic SEDDS formulations and their compositions.

**Table 9: Literature Updates on Cationic SEDDS**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cationic Inducer</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Carvedilol</td>
<td>Oleylamine</td>
<td>(Singh <em>et al.</em> 2009b)</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>Oleylamine</td>
<td>(Gershanik <em>et al.</em> 1998)</td>
</tr>
<tr>
<td>Fluorescent dye</td>
<td>Oleylamine</td>
<td>(Gershanik <em>et al.</em> 2000)</td>
</tr>
<tr>
<td>DilC_{18}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lovastatin</td>
<td>Oleylamine</td>
<td>(Singh <em>et al.</em> 2010c)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Oleylamine</td>
<td>(Gershanik and Benita 1996)</td>
</tr>
<tr>
<td>Vinpocetine</td>
<td>Oleylamine</td>
<td>(Chen <em>et al.</em> 2008)</td>
</tr>
</tbody>
</table>

The binding of the cationic SEDDS has been found to be much higher compared with the anionically charged formulation, suggesting increased adhesion of the
droplets to the cell surface due to electrostatic attraction (Humberstone and Charman 1997; Gershanik et al. 2000). In almost all the cationic SEDDS formulations, ethyl oleate has been utilized as the lipidic carrier, while oleylamine has remained as the charge-inducer of the choice. Hence, studies on the successful formulation of some cationic SEDDS have been undertaken.

1.6.8.2 Supersaturable SEDDS (S-SEDDS)

High levels of surfactant typically present in SEDDS formulations can invariably lead to severe GI side-effects. Hence, a new class of SEDDS formulations, i.e., supersaturable SEDDS (S-SEDDS) has been designed to reduce the amount of surfactant by incorporating a water soluble polymeric precipitation inhibitor (PPI) (Gao and Morozowich 2006). Such formulations have been developed specifically to reduce the surfactant side-effects and achieve rapid absorption of poorly soluble drugs (Poelma et al. 1991; Gao et al. 2003; Gao et al. 2004). The system is intended to generate and maintain a metastable supersaturated state in vivo by preventing or minimizing the precipitation of the drug through the use of a suitable PPI. Supersaturation is intended to increase the thermodynamic activity to the drug beyond its solubility limit, and therefore, to result in an increased driving force for transit into and across the biological barrier (Dahan and Hoffman 2008). The S-SEDDS formulations have been demonstrated to improve both the rate and extent of the oral absorption of poorly water-soluble drugs quite effectively (Gao et al. 2006; Gao and Morozowich 2006; Morozowich et al. 2006). The inclusion of cellulosic polymers in the S-SEDDS formulation tends to effectively suppress the precipitation of drugs (Gao et al. 2003).

Various viscosity grades of hydroxypropyl methylcellulose (HPMC) are well-recognized for their ability to inhibit crystallization and, thereby, generate and maintain their supersaturated state for extended time periods (Pellett et al. 1994; Pellett et al. 1997b; Raghavan et al. 2000). The comparative in vitro studies (Gao et al. 2009) have indicated that the presence of a small amount HPMC in the formulation is critical to achieve a stabilized supersaturated state of drug upon mixing with water. Applying the supersaturable SEDDS approach, a reduced amount of surfactant is deliberately used with HPMC in order to produce a temporarily supersaturated state with reduced solubilization (Gao and
I. INTRODUCTION

Morozowich 2006). This is to obtain a high free drug concentration through generating and maintaining a supersaturated state in vivo and to increase the driving force for absorption (Gao et al. 2004).

The mechanism underlying the inhibited crystal growth and stabilized supersaturation using these polymers is poorly understood, even although several studies have been carried out to investigate the same (Hasegawa et al. 1988; Pellett et al. 1997a; Raghavan et al. 2001). Table 10 enlists various studies reported in literature on the supersaturated SEDDS formulations and their respective compositions. Methyl cellulose derivatives like HPMC have invariably been used as PPI’s in such formulations. Besides in some studies, polymers like polyvinylpyrrolidone (PVP) have also employed as PPI’s.

Table 10: A Literature Update on Various Reports on Supersaturable SEDDS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymeric Precipitation Inhibitor (PPI)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMG 517</td>
<td>PVP 12PF and K30 &amp; HPMC E4M, E5LV, E50LV, K100 and K3</td>
<td>(Gao et al. 2009)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>PVP K30, K60 and K90, Methylcellulose, Sodium carboxymethylcellulose, HPMC</td>
<td>(Zhang et al. 2011)</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>PVP K30 and VA64; Pluronic F68 and F127; Soluplus</td>
<td>(Song et al. 2013)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>PVP K30 and K90; HPMC.</td>
<td>(Gosangari and Dyakonov 2013)</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>PVPK30; HPMC K100, K15M and K100M</td>
<td>(Chen et al. 2011)</td>
</tr>
<tr>
<td>Indirubin</td>
<td>PVP K17; HPMC E6</td>
<td>(Chen et al. 2012)</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>HPMC 2910 E5LV</td>
<td>(Gao et al. 2003)</td>
</tr>
<tr>
<td>PNU 91325</td>
<td>HPMC 2910 E5LV and E50LV</td>
<td>(Gao et al. 2004)</td>
</tr>
<tr>
<td>Silybin</td>
<td>HPMC E50LV</td>
<td>(Wei et al. 2012)</td>
</tr>
</tbody>
</table>

HPMC: Hydroxypropyl methylcellulose, PVP: Povidone, PEG: Polyethylene glycol

1.6.8.3 Solid SEDDS

Usually, the SEDDS are prepared as liquid dosage forms. These have to be,
therefore, administered in the soft gelatin capsules incurring higher production costs and resulting in lower stability, lower portability and lower drug loading (Prajapati and Patel 2007). This calls for the development of the solid SEDDS which tends to combine the advantages of the conventional SEDDS (i.e., enhanced solubility and bioavailability) with those of solid dosage forms (e.g., low production cost, convenience of process control, high stability and reproducibility, and better patient compliance (Singh et al. 2009a). The solid SEDDS focus on the incorporation of liquid/semisolid ingredients into powders employing diverse solidification techniques like spray drying (Abdalla et al. 2008; Yi et al. 2008b), melt granulation (Gupta et al. 2001; Gupta et al. 2002), moulding (Attama et al. 2003), melt extrusion (Newton et al. 2007; Iosio et al. 2008) and nanoparticle technology (Attama and Nkemnele 2005).

The powders can then be formulated as solid dosage forms (Jannin et al. 2008; Tang et al. 2008) like self-emulsifying tablets (Nazzal et al. 2002; Attama et al. 2003; Nazzal and Khan 2006) and self-emulsifying pellets (Franceschinis et al. 2005; Abdalla and Mader 2007; Iosio et al. 2008). Alternative approaches for the development of solid SEDDS comprise adsorption by solid carriers like microcrystalline cellulose (Abdalla and Mader 2007; Abdalla et al. 2008), colloidal silica (Dixit and Nagarsenker 2008) and use of high melting point solid excipients like Lutrol® and Gelucire® (Chambin and Jannin 2005). The idea of blending the potential of the SEDDS with that of the pellets through the inclusion of a self-emulsifying mixture into microcrystalline cellulose, and the production of pellets using extrusion-spheronization was first introduced by Newton (Newton et al. 2001). Table 11 enlists various literature studies on solid SEDDS formulations and their respective compositions.

Table 11: Literature Reports on Various Solid SEDDS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipients for Solidification</th>
<th>Technique for Solidification</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>MCC</td>
<td>Extrusion/Spheronization</td>
<td>(Matsaridou et al. 2012)</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>Silicon dioxide and sodium starch</td>
<td>Extrusion/Spheronization</td>
<td>(Mukherjee and Plakogiannis 2012)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipients for Solidification</th>
<th>Technique for Solidification</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifendate</td>
<td>MCC, lactose, and mannitol</td>
<td>Extrusion/Spheronization</td>
<td>(Yanyu et al. 2012)</td>
</tr>
<tr>
<td>Candesartan cilexetil</td>
<td>Colloidal silicon dioxide, MCC</td>
<td>Adsorption on solid carriers</td>
<td>(Nekkanti et al. 2010)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Neusilin UFL2 and FL2, Sylsia 320 and 350</td>
<td>Adsorption on solid carriers</td>
<td>(Milovic et al. 2012)</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>Lutrol F 127</td>
<td>-</td>
<td>(Singh et al. 2013)</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>Hydroxypropyl cellulose</td>
<td>Spray drying</td>
<td>(Onoue et al. 2012)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Aerosil 200</td>
<td>Spray drying</td>
<td>(Yan et al. 2011)</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Magnesium aluminometasilicate</td>
<td>Direct compression</td>
<td>(Sander and Holm 2009)</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>PVP K30</td>
<td>Spray drying</td>
<td>(Lei et al. 2012)</td>
</tr>
<tr>
<td>Danazol</td>
<td>Neusilin US2</td>
<td>Adsorption on solid carrier</td>
<td>(Van Speybroeck et al. 2012)</td>
</tr>
<tr>
<td>Dexamprofen</td>
<td>Aerosil 200</td>
<td>Adsorption on solid carrier</td>
<td>(Balakrishnan et al. 2009)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>MCC</td>
<td>Extrusion/Spheronization</td>
<td>(Abdalla and Mader 2007)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Goat fat</td>
<td>Moulding</td>
<td>(Attama et al. 2003)</td>
</tr>
<tr>
<td>Doxetaxel</td>
<td>Colloidal silica</td>
<td>Spray drying</td>
<td>(Quan et al. 2012)</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>Aerosil 200</td>
<td>Mixing</td>
<td>(Dixit and Nagarsenker 2008)</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>Dextran</td>
<td>Spray drying</td>
<td>(Kim et al. 2012b)</td>
</tr>
<tr>
<td>Fenofibrate and probucol</td>
<td>Poloxamer 188</td>
<td>Dispersion</td>
<td>(Shah and Serajuddin 2012)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Dextran</td>
<td>Spray drying</td>
<td>(Marasini et al. 2013)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Gelatin</td>
<td>Spray drying</td>
<td>(Kim et al. 2012a)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Colloidal silica and dextran</td>
<td>Spray drying</td>
<td>(Oh et al. 2011)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Silicon dioxide, Magnesium stearate</td>
<td>Spray drying</td>
<td>(Kang et al. 2012)</td>
</tr>
<tr>
<td>Glyburide</td>
<td>Neusilin US2</td>
<td>Adsorption on solid carriers</td>
<td>(Mura et al. 2012)</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>Magnesium aluminum silicate</td>
<td>Adsorption on solid carriers</td>
<td>(Agarwal et al. 2009)</td>
</tr>
<tr>
<td>Drug</td>
<td>Excipients for Solidification</td>
<td>Technique for Solidification</td>
<td>References</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------</td>
<td>------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Fujicalin (R)</td>
<td>Adsorption on solid carriers</td>
<td>(Kang et al. 2011)</td>
</tr>
<tr>
<td>Loratadine</td>
<td>Porous polystyrene</td>
<td>Beads formation by evaporation</td>
<td>(Khan et al. 2004)</td>
</tr>
<tr>
<td>Methyl Paraben &amp; Propyl Paraben</td>
<td>Avicel PH101</td>
<td>Extrusion/Spheronization</td>
<td>(Serratoni et al. 2007)</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>Microcel 101</td>
<td>Spray drying</td>
<td>(Franceschinis et al. 2005)</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>Dextran 40</td>
<td>Spray drying</td>
<td>(Yi et al. 2008b)</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>Dextran</td>
<td>Spray drying</td>
<td>(Yi et al. 2008b)</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>HPMC</td>
<td>Spray drying</td>
<td>(Yi et al. 2008a)</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>Syloid 244 FP Kollidon CL-SF Flowlac 100 Avicel PH 101</td>
<td>Extrusion/Spheronization</td>
<td>(Wang et al. 2010)</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>Sylysia (350, 550, and 730) and Neusilin US2</td>
<td>Adsorption on solid carrier</td>
<td>(Beg et al. 2013)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>MCC</td>
<td>Extrusion/Spheronization</td>
<td>(Abdalla et al. 2008)</td>
</tr>
<tr>
<td>Puerarin</td>
<td>—</td>
<td>Extrusion/Spheronization</td>
<td>(Zhang et al. 2012)</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>Neusilin US2</td>
<td>Adsorption on solid carrier</td>
<td>(Deshmukh and Kulkarni 2013)</td>
</tr>
<tr>
<td>Scutellarin</td>
<td>Lactose, HPMC and MCC</td>
<td>Adsorption on solid carriers &amp; spray drying</td>
<td>(Li et al. 2013)</td>
</tr>
<tr>
<td>Silymarin</td>
<td>MCC &amp; Lactose monohydrate</td>
<td>Extrusion/Spheronization</td>
<td>(Iosio et al. 2011)</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>MCC, Lactose and Na CMC</td>
<td>Extrusion/Spheronization</td>
<td>(Hu et al. 2012)</td>
</tr>
<tr>
<td>Tetrahydro curcumin</td>
<td>Silicon dioxide, Glycerol behenate, Pregelatinized starch, SSG &amp; MCC</td>
<td>Extrusion/Spheronization</td>
<td>(Setthacheewakul et al. 2011)</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>Maltodextrin and MCC</td>
<td>Adsorption on solid carriers</td>
<td>(Nazzal et al. 2002)</td>
</tr>
</tbody>
</table>
Development of an oral DDS invariably involves handling a plethora of drugs, polymers, excipients and processes. Optimizing the formulation composition and the manufacturing process of such a drug delivery product to furnish the desired quality traits is, therefore, a Herculean task. The traditional approach of optimizing a formulation or process essentially involves studying the influence of one variable at time (OVAT), while keeping others as constant. Using this OVAT approach, the solution of a specific challenging property can be achieved somehow, but attainment of the true optimum composition or process is never guaranteed (Singh et al. 2005a). This may ostensibly be ascribed to the presence of interactions, i.e., the influence of one or more variables on others. The final product may be satisfactory, but mostly sub-optimal, as a better formulation might still prevail for the studied conditions.

Design of experiments (DoE), on the other hand, is an optimization technique meant for products and/or processes, developed to evaluate all the potential factors simultaneously, systematically and rapidly. Its implementation invariably encompasses the use of statistical experimental designs, generation of mathematical equations and graphic outcomes, portraying a complete picture of
variation of the response(s) as a function of the factor(s), which can never be obtained employing the traditional OVAT approach.

Lately, a holistic DoE-based philosophy of Quality by Design (QbD) has been slowly permeating into the mindset and practice in the industrial environs (Skrdla et al. 2009; Huang et al. 2011). This popularity of QbD in pharma circles is largely attributable to the recent impetus provided by the ICH, FDA and EMEA through their respective federal guidance's. Since DoE has much wider domain of application, recently we have proposed, on the heels of QbD, a terser jargon, viz. "Formulation by Design (FbD)", applicable specifically to the use of DoE in drug formulation development (Singh et al. 2011a). Table 12 succinctly enumerates the merits of FbD over the OVAT methodology. Usually, specific terminology, both technical and otherwise, is employed during FbD practice. To facilitate better clarity of precepts of FbD of DDS, important terms have been compiled in Box 2.

**Table 12: Comparison of OVAT and FbD methodology**

<table>
<thead>
<tr>
<th>Attribute</th>
<th>OVAT</th>
<th>FbD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choice of optimum formulation</td>
<td>May result only in sub-optimal solutions</td>
<td>Yields the best possible formulation</td>
</tr>
<tr>
<td>Interaction among the ingredients</td>
<td>Inept to reveal possible interactions</td>
<td>Estimates any synergistic or antagonistic interaction among constituents</td>
</tr>
<tr>
<td>Scale-up and post approval changes</td>
<td>Very difficult to design a formulation slightly differing from the desired formulation</td>
<td>Changes in the optimized formulation can easily be incorporated, as all response variables are quantitatively governed by a set of input variables</td>
</tr>
<tr>
<td>Resource-economics</td>
<td>Highly resource-intensive, as it leads to unnecessary runs and batches</td>
<td>Economical, as it furnishes information on product/process performance using minimal trials</td>
</tr>
<tr>
<td>Time-economics</td>
<td>Highly time-consuming, as each product is individually evaluated for its performance</td>
<td>Can simulate the product or process behavior using model equations</td>
</tr>
</tbody>
</table>

Based on prior multi-disciplinary knowledge impacting various product attributes and/or process parameters, the foremost task ahead for a researcher is to identify the "knowledge space" i.e., entire space worth exploring from the possible vast
1. INTRODUCTION

ocean of scientific information. A "knowledge space", thereby, encompasses all those product and process variables that may even minutely affect the overall product quality. A "design space" has to be demarcated involving “selected few” most influential variables from the "knowledge space". “Control space”, a further subset construct of this "design space", is the experimental domain earmarked for detailed studies within the refined ranges of input variables.

Box 2: Vital terminology employed during FbD of drug delivery

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimize</td>
<td>Make as perfect, effective, or functional as possible</td>
</tr>
<tr>
<td>Optimized</td>
<td>Improved product to accomplish the objectives of a development scientist using DoE and computers</td>
</tr>
<tr>
<td>Optimization</td>
<td>Implementation of systematic approaches to achieve “the best” combination of product and/or process characteristics under a given set of conditions</td>
</tr>
<tr>
<td>Independent Variables</td>
<td>Input variables, which are directly under the control of the product development scientist</td>
</tr>
<tr>
<td>Quantitative Variables</td>
<td>Variables that can take numeric values</td>
</tr>
<tr>
<td>Categorical Variables</td>
<td>Qualitative variables which cannot be quantified</td>
</tr>
<tr>
<td>Runs or Trials</td>
<td>Experiments conducted according to the selected experimental design</td>
</tr>
<tr>
<td>Factors</td>
<td>Independent variables, which influence the product/process characteristics or output of the process</td>
</tr>
<tr>
<td>Design Matrix</td>
<td>Layout of experimental runs in matrix form, as per experimental design</td>
</tr>
<tr>
<td>Knowledge Space</td>
<td>Scientific elements to be considered and explored on the basis of previous knowledge as product attributes and process parameters</td>
</tr>
<tr>
<td>Design Space</td>
<td>Multidimensional combination and interaction of input variables and process parameters, demonstrated to provide quality assurance</td>
</tr>
<tr>
<td>Control Space</td>
<td>Domain of design space selected for the detailed study</td>
</tr>
<tr>
<td>Levels</td>
<td>Values assigned to the factor</td>
</tr>
<tr>
<td>Constraints</td>
<td>Restrictions imposed on the factor levels</td>
</tr>
<tr>
<td>Response Variables</td>
<td>Characteristics of the finished drug product or the in-process material</td>
</tr>
<tr>
<td>Critical Attributes</td>
<td>Parameters ranging within appropriate limits, which ensure the desired product quality</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Critical Process Parameters</strong></td>
<td>Independent process parameters most likely to affect the quality attributes of a product or intermediates</td>
</tr>
<tr>
<td><strong>Critical Formulation Attributes</strong></td>
<td>Formulation parameters affecting critical quality attributes</td>
</tr>
<tr>
<td><strong>Interaction</strong></td>
<td>Lack of additivity of factor effects</td>
</tr>
<tr>
<td><strong>Antagonism</strong></td>
<td>Undesired negative change due to interaction among factors</td>
</tr>
<tr>
<td><strong>Synergism</strong></td>
<td>Desired positive change due to interaction between factors</td>
</tr>
<tr>
<td><strong>Effects Plot</strong></td>
<td>Plot between the magnitude of various coefficients for the effects and/or interactions against the response variable</td>
</tr>
<tr>
<td><strong>Main effect</strong></td>
<td>The effect of a factor averaged over all the levels of other factors</td>
</tr>
<tr>
<td><strong>Nuisance Factor</strong></td>
<td>Uncontrollable factors which complicate the estimation of main effect or interactions</td>
</tr>
<tr>
<td><strong>Orthogonality</strong></td>
<td>If the estimated effects are due to the main factor of interest and independent of interactions</td>
</tr>
<tr>
<td><strong>Confounding</strong></td>
<td>Lack of orthogonality</td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>The measure of the degree of confounding</td>
</tr>
<tr>
<td><strong>Coding (or normalization)</strong></td>
<td>Process of transforming a natural variable into a non-dimensional coded variable</td>
</tr>
<tr>
<td><strong>Factor Space</strong></td>
<td>Dimensional space defined by the coded variables</td>
</tr>
<tr>
<td><strong>Experimental Domain</strong></td>
<td>Part of the factor space, investigated experimentally for optimization</td>
</tr>
<tr>
<td><strong>Blocks</strong></td>
<td>A set of relatively homogenous experimental conditions, wherein every level of the primary factor occurs the same number of times with each level of nuisance factor.</td>
</tr>
<tr>
<td><strong>Response Surface</strong></td>
<td>Graphical depiction of the mathematical relationship</td>
</tr>
<tr>
<td><strong>Empirical Model</strong></td>
<td>Mathematical model describing factor-response relation using polynomial equations</td>
</tr>
<tr>
<td><strong>Response Surface Plot</strong></td>
<td>3-D graphical representation of a response plotted between two independent variables and one response variable</td>
</tr>
<tr>
<td><strong>Contour Plot</strong></td>
<td>Geometric illustration of a response obtained by plotting one independent variable against another, while holding the magnitude of response and other variables as constant</td>
</tr>
</tbody>
</table>

"Design space" uses systematic approach coupled with archival data to convert the "knowledge space" to "control space". Figure 20 portrays the hierarchy of knowledge, design and control space.
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Verily, FbD hits the bull’s eye using five key strengths viz. apt choice of experimental designs, accurate computer-aided optimization, meticulous drug product development, precise definition of design and control space, and identification of critical quality attributes (CQAs), critical formulation attributes (CFAs) and critical process parameters (CPPs). Figure 21 pictorially illustrates the concept.

The theme of FbD optimization methodology provides thought-through and thorough information on diverse FbD aspects organized in a five-step sequence illustrated in Figure 22.
I F/VTR O DIICTIO/V
• The FbD study begins with Step I, where an endeavor is made to explicitly ascertain the drug delivery objective(s). Various CQAs or response variables, which pragmatically epitomize the objective(s), are earmarked for the purpose. All independent product/process variables are also listed.

• In Step II, the response variables which directly represent the product quality (e.g., particle size for nanoparticles, emulsification time for self-emulsifying systems) are selected. Also, selection of “prominent few” influential factors among the “possible many” input variables is conducted using experimental designs, the process popularly termed as “screening” (Murphy 2003). The formulator, at times, can even bypass the rigors of screening process to choose these factors, viz. CFAs and/or CPPs by virtue of his experience, wisdom and previous knowledge. Factor influence studies are usually conducted later to quantify the effect of factors and to determine the interactions, if any. Experimental studies are also undertaken to define the broad range of factor levels.

• During Step III, a suitable experimental design is worked out, accordingly, to map the responses on the basis of the study objective(s), responses being explored, number and the type of factors, and factor levels, viz. high, medium or low. The niceties of using important experimental designs along with their pros and cons are discussed in subsequent sections. A design matrix is subsequently generated to guide the drug delivery scientist. The drug delivery formulations are experimentally prepared according to the chosen experimental design, and the chosen response variables are evaluated meticulously.

• In Step IV, a suitable numeric model is proposed on the basis of experimental data thus generated, and its statistical significance discerned. Response surface methodology (RSM) is employed to relate a response variable to the levels of input variables. Optimum formulation compositions are searched within the experimental domain, employing graphical or numerical techniques.

• Step V is the ultimate phase of the FbD exercise, involving validation of response predictive ability of the proposed design model. Drug delivery performance of some studies, taken as the confirmatory runs, is assessed vis-à-
vis that predicted using RSM, and the results are critically compared. The optimum formulation is scaled-up and set forth ultimately for the production cycle.

1.7.1 FbD experimental designs

An experimental design constitutes the pith of entire DoE exercise (Singh et al. 2008). Before the selection of an experimental design, it is essential to demarcate the experimental domain (i.e., the area to be investigated) within the factor space (i.e., the broad range of factor studies).

![Figure 22: Five step strategy of formulating DDS using FbD [Figure adapted from Singh et al. 2011]](image_url)

To accomplish this task, first a pragmatic range of experimental domain is embarked upon and the levels and their number are selected so that the optimum lies within its realm. While selecting the levels, one must see that the increments between them should be realistic. Too wide increments may miss finding the useful information between the levels, while a too narrow range may not yield accurate results (Doornbos and Haan 1995a).

There are numerous types of experimental designs to choose from. Various
commonly employed experimental designs for RSM, screening and factor-influence studies during pharmaceutical product/ process development include:

1. **Factorial Designs (FD)**
2. **Fractional Factorial Designs (FFD)**
3. **Plackett-Burman Designs (PBD)**
4. **Star Designs**
5. **Central Composite Designs (CCD)**
6. **Box-Behnken Designs (BBD)**
7. **Center of Gravity Designs**
8. **Equiradial Designs**
9. **Mixture Designs (SMD)**
10. **Taguchi Designs**
11. **Optimal Designs**
12. **Rechtschaffner Design**

### 1.7.1.1 Choosing experimental designs

The choice of a design depends upon proposed model, shape of the domain and objective of the study. The selection is usually a compromise between the information required and the number of experimental studies to be conducted (Tye 2004). It depends largely upon the objectives of the study and number of the factors to be investigated. If the primary purpose of the experiment is to screen few important main effects from the many less important ones, low resolution screening designs are used. These are usually FDs, PBDs or Taguchi designs. Screening designs support only the linear responses. Thus, if a nonlinear response is detected or a more accurate picture of response surface is needed, a more complex design-type is necessary.

For interaction models, high resolution designs are usually preferred. Designs such as BBD or CCD, which support nonlinear responses, are commonly used for RSM optimization applications. When the formulator has several factors that are proportions of a mixture formulation, mixture designs are specifically favored (Cornell 1990; Lewis 2002). On the whole, the first-order experimental designs must enable estimation of the first-order effects, preferably free from interference by the interactions among factors and other variables (Montgomery 2012). These designs also allow testing for “goodness of fit” of the proposed model. Computer-aided designs like D-optimal are more suited to cases wherein a large number of qualitative factors are incorporated in the design and/or when the resultant experimental domain is irregular in shape (Lewis 2002; Myers 2010).
**1.7.1.2 Central composite designs (CCD)**

For nonlinear responses requiring second-order models, the CCDs are most frequently employed designs (Schwartz and Connor 1996a; Bolton 1997; Myers 2010). Also known as Box-Wilson design, the "composite design" contains an embedded \( (2^k) \) FD or \( (2^{k-1}) \) FFD, augmented with a group of star points \( (2k) \) and a "central" point (Box and Wilson 1951). The FDs involve studying the effect of all the factors \( (k) \) at various levels \( (x) \), including the interactions amongst them, with total number of experiments as \( x^k \) (Li 2010). FDs can be investigated at either 2 levels (2\(^k\) FD) or more than 2 levels. The star points allow estimation of curvature and establish new extremes for the low and high settings for all the factors (Doornbos and Haan 1995a; Araujo and Brereton 1996). Hence, CCDs are the second-order designs that effectively combine the advantages of FDs and star design. Total number of factor combinations in a CCD is \( 2^k + 2k + 1 \).

If the distance from center of the design space to a factorial point is ±1 unit for each factor, the distance from the center of the design space to a star point is ± \( \alpha \) with \( |\alpha| > 1 \). The axial points for 2-factor problem include, \((± \alpha, 0)\) and \((0, ± \alpha)\). A 2-factor CCD is identical to a \( 3^2 \) FD with rectangular experimental domain at \( \alpha = ±1 \), as shown in Figure 23(a). On the other hand, the experimental domain is spherical in shape for \( \alpha = \sqrt{2} \ (≈1.414) \), as shown in Figure 23(b). The CCD is quite popular in response surface optimization during pharmaceutical product development (Box and Wilson 1951; Singh et al. 2005b).

![Figure 23: Representation of central composite design (a) rectangular domain with \( \alpha=1 \); (b) spherical domain with \( \alpha = 1.414 \).](image-url)

**1.7.2 Modelization**

Following choice and implementation of an apt experimental design, apt models
1. INTRODUCTION

need to be generated. Generally, the polynomial mathematical equations are obtained, their statistical significance determined and the choice of the apt model made taking the help of model diagnostic plots (Box and Draper 1987; Montgomery 2012).

1.7.2.1 Calculation of the coefficients of polynomial equations

Regression is most widely used method for quantitative factors. It cannot be used for qualitative factors, as interpolation between categorical factor values is meaningless. In ordinary least-squares regression (OLS), a linear model, expressed as Eqn 11, is fitted to the experimental data, for estimating the values of $\beta$ such that the sum of squared differences between predicted and observed responses is minimized.

$$
Y = \beta_0 + \beta_1 X_1 \text{ or } y = \beta_0 + \beta_1 X_1 + \beta_1 X_1^2 
$$

... (11)

Multiple linear regression analysis (MLRA) can be performed for more factors, $X_i$, interactions, $X_1X_2$, and higher order terms, as depicted in Eqn 12.

$$
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_1\beta_2 X_1X_2 + ... 
$$

... (12)

In certain situations, wherein the factor-response relationship is nonlinear, multiple nonlinear regression analysis (MNLRA) may also be performed. Regression analysis can only be performed on the coded data or the original values after one or several models have been postulated, the choice being based on some expectation of the response surface. In multivariate studies where there are large number of variables, the method of partial least squares or principal component analysis can also be employed for regression (Kettaneh-Wold 1991; Myers 2000; Liu and Schisterman 2010).

1.7.2.2 Model diagnostic plots

The goodness of fit of a model can be investigated using one or more of the plots explained as under:

**Actual vs. predicted:** A graph is plotted between the actual and the predicted response values. This helps in detecting a value or a group of values that are not easily predicted by the model.
Residuals vs. predicted: Residuals (or error) is the quantitative difference between the observed and the predicted response(s). Studentized residuals are the residuals converted to their standard deviation units.

Residuals vs run: This is a plot of the residuals versus order of the experimental run (Cochran and Cox 1992). It checks for any trends indicating a time-related "lurking variables" that may have influenced the response during the experiment.

Residuals vs factor: This is a plot of the residuals versus any selected factor. It checks whether the variance, not accounted for by the model, is different for different levels of a factor.

Normal probability plot: It investigates the normal probability distribution of residuals, as judged from the linear trend of the points, when plotted on a probit scale (Montgomery 2012).

Outlier T: This is a measure of by how many standard deviations the actual value deviates from the value predicted after deleting the point in question.

Cook’s distance: This provides measures of the influence, potential or actual, of the individual runs (Cook 1977).

Leverage: This is a measure of degree of influence of each point on the model fit. (Montgomery 2012).

Box-Cox plot for power transforms: The Box-Cox plot is a tool to help in determining the most appropriate power transformation for application to response data (Box and Cox 1964).

1.7.2.3 Search for an optimum

From the models thus selected, optimization of one response or the simultaneous optimization of multiple responses needs to be accomplished graphically and/or numerically (Takayama and Nagai 1989; Podczeck 1996; Schwartz and Connor 1996a).

1.7.2.3.1 Graphical Optimization

Known popularly as response surface analysis, graphical optimization displays the area of feasible response values in the factor space (Schwartz and Connor 1996a;
Bolton 1997). The experimenter has to make a choice, "trading off" one objective for other(s), according to the relative importance of the objectives considered. The success in locating an optimum lies in the sagacious interpretation and/or comparison of the resulting plots, leading to attainment of the best compromise (Doornbos and Haan 1995a). One or more of the following techniques may be employed for this purpose.

1.7.2.3.1.1 Location of the stationary point

After completing the experimental work, often the goal of the formulation scientist is to locate the optimum. The nature of the response surface is interpreted graphically, and a stationary point is located, which may be maximum, minimum, or a target value. At this point, the partial derivatives of the response with respect to the design variable are all zeroes. The case in which the stationary point is neither a maximum nor a minimum is known as the saddle point.

1.7.2.3.1.2 Search methods

Search methods are employed for choosing the upper and lower limits of the responses of interest (Doornbos and Haan 1995a; Schwartz and Connor 1996a; Bolton 1997). The response surfaces in these search methods, as defined by the appropriate equations, are searched to find the combination of independent variables yielding the optimum. Two major steps, viz. feasibility search and grid search are used. Together, these techniques are also referred to as the brute force method. The feasibility search method is used to locate a set of response constraints that are just at the limit of possibility. One selects several values for the responses of interest, and a search of the response surface is made to determine whether a solution is feasible. Subsequently, the exhaustive grid search is applied, wherein the feasible experimental range is divided into a grid of smaller specific sizes and searched methodically.

1.7.2.3.1.3 Overlay plots

The response surfaces or contour plots are superimposed over each other to visually search for the best compromise (Bolhuis et al. 1995; Westerhuis et al. 1996). Minimum and maximum boundaries are set for acceptable objective values. The region is highlighted wherein all the responses are acceptable. This is termed
as an overlay plot or a combined contour plot. Within this area, an optimum is located, trading off different responses. Figure 24 illustrates the overlay plot generated during formulation optimization of SEEDS of carvedilol (Singh et al. 2013).

![Overlay Plot]

Figure 24: An overlay plot showing the location of the optimized formulation

1.7.2.3.2 Mathematical optimization

Graphical analysis is usually preferred in the case of single response (Lewis et al. 1999). However, in case of multiple responses, it is usually advisable to conduct numerical optimization first to uncover a feasible region.

1.7.2.3.2.1 Desirability functions

Desirability function is a way of overcoming the difficulty of multiple, sometimes opposing responses. In this method, each response is associated with its own partial desirability function. The optimum is the point with the highest value for desirability. The experimenter should study the contour plot of desirability surface around the optimum and combine this with contour plots of the most important responses. A large area or volume of high desirability will indicate a robust formulation or set of processing conditions. Although the method requires appropriate computer software, yet it is a highly useful and pragmatic method of
optimization (Derringer and Suich 1980; Anderson and Whitcomb 2005).

Besides the techniques of “objective function” and “sequential unconstrained minimization technique (SUMT)” have also been utilized to optimize drug delivery systems numerically.

1.7.2.3.2.2 Sequential search methods

Despite the numerous merits of simultaneous approaches, there are situations where there is hardly any a priori knowledge about the effects of variables (Doornbos and Haan 1995b; Schwartz and Connor 1996b). Such situations arise when choosing a very extensive experimental domain is difficult or the possible experimental domain is not known at the beginning of the study, thus calling for the application of the sequential optimization methods. In sequential approach, optimization is attempted in a step-wise fashion. Experimentation is started at an arbitrary point in the experimental domain and responses are evaluated (Takayama and Nagai 1989; Lipp and Heimann 1996; Schwartz and Connor 1996a).

The inherent advantages of these methods are:

- No need to plan all the experiments simultaneously
- A priori knowledge of the response surface is not essential
- Interactive

However, various disadvantages encompass:

- Number of experiments to reach an optimum can not be predicted
- Optimum found may not be the global optimum
- Robustness is not known
- Unsuitable for multiple objective problems
- Attainment of optimum is judged only by the expert developmental scientist(s)
- Mathematical model and complete response surface is not generated
- Yields unreliable results when multiple optima exist
- Applicable only when response surface is continuous

Steepest ascent (or descent) methods are direct optimization methods for first-order designs esp. when the optimum is outside the domain and is to be arrived at
rapidly (Lewis 2002).

1.7.2.4 Selection of optimum search methodology

In case of single response, graphical analysis is often opted for (Lewis et al. 1999). However in case of multiple response variables, certain responses can oppose one another. Accordingly, changes in a factor that improve one response may have a negative effect on another. Since it is not usually possible to obtain the best values for all the responses, optimization principally embarks upon finding experimental conditions where different responses are most satisfactory, over all. Nevertheless, there is a certain degree of subjectivity in weighing up their relative importance.

1.7.3 Optimization of self-emulsifying drug delivery systems

Table 13 illustartes a comprehensive account on literature search of systematic optimization studies on various types of SEDDS. While optimizing them, various formulation components have been investigated as independent factors, with drug release characteristics as the major response variables. The independent variables have been the quantity of oil, emulgent and co-emulgent, however response varies.

1.8 PHARMACOKINETIC ANALYSIS AND MODELING

Pharmacokinetics plays key role in the development of new drugs and drug delivery systems. Notwithstanding the in vitro design considerations of an immediate oral DDS, its end objective is always pharmacokinetic, i.e., sustenance of the in vivo plasma level time profile of the drug in its therapeutic window for extended periods of time. This is usually accomplished by modulating drug release kinetics such that the desired plasma levels of drug are are maintained for the desired period (Liu et al. 1995; Homero de Souza Filho et al. 2010; Khandave et al. 2010).

Pharmacokinetics, during the last five decades, has emerged from a purely mathematical description of time course of drug concentration in the body to a discipline that is fully integrated with pharmaceutics, mathematical modeling and clinical therapeutics. Usually for such pharmacokinetic studies, the data on the drug levels in biological fluids, at periodic intervals, is processed by a series of equations involving mathematical and statistical precepts.
Table 13: Literature Reports on Various Self-Emulsified Formulations Optimized using "Design of Experiments"

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type of Design</th>
<th>Factors Employed</th>
<th>Response Variables</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astilbin</td>
<td>CCD (2 factors, 3 levels)</td>
<td>Capryol PGMC, Tween 20 and Transcutol HP</td>
<td>Droplet size, equilibrium solubility, and cumulative percentage of drug released in 15 min</td>
<td>(Marasini et al. 2012)</td>
</tr>
<tr>
<td>Albendazole</td>
<td>FFD (2 factors, 3 levels)</td>
<td>Spray rate, inlet air temperature, inlet air flow, and atomization air pressure</td>
<td>Particle size distribution, morphology, density, and flow</td>
<td>(Mezghrani et al. 2011)</td>
</tr>
<tr>
<td>All-trans-retinol acetate</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Amount of added oil, surfactant, and cosurfactant</td>
<td>Particle size, turbidity, and cumulative amount of the active ingredient emulsified after 10 and 30 min</td>
<td>(Mukherjee and Plakogiannis 2012)</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>CCD (2 factors, 3 levels)</td>
<td>Capmul MCM, Nikkol HCO 50 &amp; Transcutol</td>
<td>Droplet size, emulsification time, liquefaction time intestinal absorption rate</td>
<td>(Singh et al. 2008b)</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>CCD (2 factors, 3 levels)</td>
<td>Capmul MCM, Nikkol HCO 50 &amp; Transcutol</td>
<td>Droplet size, emulsification time, liquefaction time intestinal absorption rate</td>
<td>(Singh et al. 2013)</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>Face-Centered Cubic design (3 factors, 3 levels)</td>
<td>Lemon oil, Cremophor EL &amp; Capmul MCM C8</td>
<td>Amount of silicone dioxide, magnesium stearate, compression force, hardness, Carr's flow index, friability.</td>
<td>(Nazzal and Khan 2006)</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>BBD (3 factors, 3 levels)</td>
<td>R-(+)-limonene, Cremophor EL &amp; Capmul GMO 550</td>
<td>cumulative percentage of drug released after 5 minutes, turbidity, particle size, and zeta potential</td>
<td>(Palamakula et al. 2004)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>SLD (3 factors, 4 levels)</td>
<td>Ethyl oleate, Emulsifier OP, Cremophor EL &amp; PEG 400</td>
<td>Solubility of drug in SMEDDS, the mean particle size of micro-emulsification</td>
<td>(Cui et al. 2009)</td>
</tr>
</tbody>
</table>
### Table: Response Variables

<table>
<thead>
<tr>
<th>Drug (Delivery System)</th>
<th>Type of Design</th>
<th>Factors Employed</th>
<th>Response Variables</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>CCD (2 factors, 3 levels)</td>
<td>Oil, surfactant and co-surfactant percentages</td>
<td>Solubility, droplet size, polydispersity index and emulsifying time</td>
<td>(Wang et al. 2011)</td>
</tr>
<tr>
<td>Cyclosporine A (SNEDDS)</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Orange oil, Emulphor EL 620 &amp; Capmul MCM C8</td>
<td>Particle size, turbidity, cumulative percent of drug released after 5 min, emulsification rate (10 min), lag time</td>
<td>(Zidan et al. 2007)</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>CCD (2 factors, 3 levels)</td>
<td>Lipids (Maisine 35-1 and Capryol 90) and emulgents (Labrasol and Tween 80)</td>
<td>% drug release in 15 min, globule size and amount permeated (45 min)</td>
<td>(Bandyopadhyay et al. 2012)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Inlet temperature, feed rate and carrier concentration</td>
<td>Moisture content, yield, drug content and droplet size</td>
<td>(Marasini et al. 2013)</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Cremophor EL, Capmul MCM C8 and lemon essential oil</td>
<td>Emulsification efficacy, turbidity, droplet size, polydispersity index and drug release</td>
<td>(Villar et al. 2012)</td>
</tr>
<tr>
<td>Genistein</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Maisine 35-1 and Labrafac Lipophile WL 1349, Cremophor EL and Labrasol, and Transcutol P</td>
<td>Droplet size, turbidity, dissolution percentage</td>
<td>(Zhu et al. 2009 (In press))</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>FD (2 factors, 3 levels)</td>
<td>Capmul MCM and Silicon dioxide</td>
<td>Droplet size, in vitro drug diffusion</td>
<td>(Patil et al. 2004)</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>CCD (2 factors, 3 levels)</td>
<td>Capmul MCM, Nikkol HCO 50 &amp; Lutrol F 127</td>
<td>Amount permeated (45 min), % dissolution efficiency in 15 min, MDT, liquefaction time emulsification time</td>
<td>(Singh et al. 2008a)</td>
</tr>
<tr>
<td>Lu 28-179, crystalline free base (SNEDDS &amp; SMEDDS)</td>
<td>D-OD</td>
<td>Akolene MCM, medium-chain triglyceride, Cremophor EL, Tween 80 &amp; PEG 200</td>
<td>Droplet size, solubility, weight change at the two storage conditions below 3%</td>
<td>(Holm et al. 2006)</td>
</tr>
<tr>
<td>Model drug A</td>
<td>MD (3 factors, 2 levels)</td>
<td>PEG 400, Cremophor EL, and a mixture of glycerol dioleate, and glycerol monooleate</td>
<td>Dispersion performance, droplet density</td>
<td>(Gao et al. 2004)</td>
</tr>
<tr>
<td>Drug (Delivery System)</td>
<td>Type of Design</td>
<td>Factors Employed</td>
<td>Response Variables</td>
<td>References</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Nimesulide (SEDDS)</td>
<td>MD (5 factors, 2 levels)</td>
<td>Mono- and di-glycerides, Polysorbate 80</td>
<td>Median diameter of pellets, percentage in modal fraction</td>
<td>(Franceschinis et al. 2005)</td>
</tr>
<tr>
<td>Oridonin</td>
<td>CCD (2 factors, 5 levels)</td>
<td>Maisine 35-1 &amp; Labrafac CC, Cremophor EL &amp; Transcutol P</td>
<td>Droplet size, polydispersity, equilibrium solubility, intestinal absorption rate</td>
<td>(Liu et al. 2009)</td>
</tr>
<tr>
<td>QH917</td>
<td>CCD (2 factors, 3 levels)</td>
<td>Oil content (%) and the weight ratio of surfactant and cosurfactant (Km)</td>
<td>self-microemulsifying time, mean particle size and polydispersity index</td>
<td>(Zhang et al. 2007)</td>
</tr>
<tr>
<td>Raloxifene hydrochloride</td>
<td>D-OD (2 factors, 3 levels)</td>
<td>Capmul MCM, tween 80, transcutol HP propylene glycol &amp; ethan</td>
<td>percent release in 10 min, percent dissolution efficiency in 15 min, mean dissolution time, permeation in 30 min</td>
<td>(Ahuja et al. 2009)</td>
</tr>
<tr>
<td>Self emulsifying pellets</td>
<td>CDD (2 factors, 5 levels)</td>
<td>Mono and diglycerides, Polysorbate 80</td>
<td>Ratio of lactose to MCC, ratio of MP to water, extrusion force, size spread, disintegration time, tensile strength.</td>
<td>(Franceschinis et al. 2005)</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>MD (3 factors, 2 levels)</td>
<td>Labrafac CC &amp; Cremophor EL</td>
<td>Amount of simulated gastrointestinal fluid, oil and surfactant</td>
<td>(Meng and Zheng 2007)</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Amounts of copolyvidone, maltodextrin microcrystalline cellulose</td>
<td>Cumulative percent of Ubiquinone emulsified in 45 min with constraints on weight, flowability index, tensile strength, friability and disintegration time</td>
<td>(Nazzal et al. 2002)</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Polyoxyl 35 castor oil, medium-chain mono-and diglyceride and lemon oil</td>
<td>Cumulative percentage of ubiquinone emulsified in 10 minutes</td>
<td>(Nazzal and Khan 2002)</td>
</tr>
<tr>
<td>Valsartan</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Capmul MCM, Labrasol and Tween 20</td>
<td>Viscosity, refractive index, globule size and zeta potential</td>
<td>(Beg et al. 2012)</td>
</tr>
<tr>
<td>Valsartan</td>
<td>BBD (3 factors, 3 levels)</td>
<td>Labrafil M2125 CS, Tween 20 and Capryol 90</td>
<td>Particle size, polydispersity index (PDI), dissolution after 15 min and equilibrium solubility</td>
<td>(Poudel et al. 2012)</td>
</tr>
</tbody>
</table>
Consequently, the collection, analysis and presentation of pharmacokinetic data have become an essential part of developing not only the new drugs but diverse DDS too. Use and scope of the pharmacokinetic approaches have expanded enormously by encompassing pharmacodynamic vistas, physiologically based modes, nonlinear kinetics, population methods and system theory methods.

Box 3 outlines some vital definitions associated with pharmacokinetics and allied sciences. Today, several approaches and computational techniques have been postulated and put into use for estimating pharmacokinetic parameters and simulating the pharmacokinetic performance of a drug from its delivery device. Besides the traditional compartmental modeling approach, these include, the relatively more recent noncompartmental stochastic and system deconvolution approaches (Almeida et al. 2010; Tassaneeyakul et al. 2010; Ullah et al. 2010; Jackson et al. 2011; Onyamboko et al. 2011). Eventually, for drugs exhibiting dissolution-limited absorption, in vitro-in vivo relationships/correlations (IVIVR/IVIVC) need to be explored using model-independent approach of statistical moments or system theory. Consequently, this leads to successful simulation of the in vivo performance of any oral immediate DDS from its in vitro drug release profile.

Box 3: Terminology of important terms related to classical pharmacokinetics

<table>
<thead>
<tr>
<th>Terms and their Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biodisposition</strong>: Cumulative process of distribution and elimination of drugs from the body</td>
</tr>
<tr>
<td><strong>Biotransformation</strong>: Chemical alteration of a drug that occurs by virtue of its sojourn in a biological system. Also “drug metabolism”</td>
</tr>
<tr>
<td><strong>Disposition</strong>: The study of time course of drug distribution to various body tissues, and elimination from the body primarily through renal excretion and biotransformation</td>
</tr>
<tr>
<td><strong>Model</strong>: A hypothetical structure that can be used to characterize, with reproducibility, the behavior and fate of a drug in a biological system, when given through a certain route of administration and in a specific dosage form</td>
</tr>
<tr>
<td><strong>Empirical Model</strong>: Mathematical model describing the pharmacokinetic behavior of a drug using differential or polynomial equations</td>
</tr>
<tr>
<td><strong>Explanative Model</strong>: Schematic flow chart depicting pharmacokinetic behavior of a drug</td>
</tr>
<tr>
<td><strong>Compartment</strong>: An entity described by certain volume and concentration, with maximal intra-compartmental homogeneity and maximum inter-compartmental heterogeneity. Usually, it is a tissue or a group of tissues with similar blood flow rate</td>
</tr>
</tbody>
</table>
### Terms and their Explanation

- **Multi-Compartment Model**: A hypothesis envisioning finite number of well-connected pools

- **Lag Time**: Latent period of time elapsed between drug intake and perceptible drug concentration

- **Steady state levels, Cpss**: Relatively invariant plasma drug levels during the equilibrium plateau phase obtained following multiple administration of drug

- **$C_{max}$**: An acronym for “peak” drug concentration in plasma/serum, after its administration prior to administration of subsequent dose during single or multi-dose kinetics, usually in $\mu$g/ml.

- **$C_{min}$**: Minimum or “trough” concentration of a drug observed after its administration just prior to the administration of a subsequent dose during multi-dose pharmacokinetics

- **Swing**: Proportional rise in plasma drug conc., $\frac{(C_{max} - C_{min})}{C_{min}}$, during multi-dose kinetics

- **Therapeutic Window**: Difference between minimum effective and toxic levels of a drug

- **Clearance, Cl**: The rate at which a drug is cleared from a body fluid, e.g., plasma or blood (usually in L/h or ml/min). Clearance may be from total body (Cl$_b$) or kidneys (Cl$_k$) or liver (Cl$_l$)

- **Biological Half-Life, $t_{1/2}$**: Time (usually in h) for drug levels in the terminal linear phase of plasma conc.-time curve to be halved. Mathematically obtained as $0.693/\lambda$.

- **Urinary Availability, $f_r$**: Fraction of drug excreted (%) through renal pathway

- **Volume of Distribution, $V_d$**: It is volume where a drug appears to distribute, given as the ratio of dose to drug conc. (L or L/Kg). It may be $V_{d_{app}}, V_{d_{ss}}, V_{d_{area}}, V_{d_{p}}, V_{d_{c}}, V_{d_{t}},$ etc.

### 1.8.1 One Compartmental Body Model

Most drugs make the body "behave" as if it consists of a single pharmacokinetic compartment. Tissue and plasma levels of drug rapidly and simultaneously reach equilibrium in all the tissues to which it is distributed. A plot of plasma level ($C_p$) versus time after intravenous administration can be rectified into only a single straight line of negative slope, which can intersect the ordinate at only one point; only one volume of distribution can be calculated. Empirically, a one compartment open body model (1-CBM) is represented as Figure 25.

### 1.8.2 Noncompartmental pharmacokinetics

Despite the global popularity of compartmental modeling, controversy and confusion have invariably existed for quite sometime regarding its validity for
Figure 25: Diagrammatic representation of one compartment body model

pharmacokinetic data treatment. There have been a number of questions and misgivings about the use of such models. Several disadvantages of compartmental analysis, given below in Box 4, call for the alternative approaches to the compartmental modeling.

Box 4: Potential shortcomings of classical compartmental analysis

- The basic assumption of compartmental modeling, i.e., the body is consisting of relatively few, kinetically homogeneous compartments, seems to be quite unrealistic and difficult to justify from physiological reality.
- Unambiguous identification of correct model is often not possible because more than one model of comparable complexity can be consistent with available data.
- Model identification and parameter estimation are confounded by vanishing exponentials.
- Microscopic rate constants do not conform to reality.
- Likelihood of flip-flop situation particularly for drugs with short biological half-life following non-instantaneous administration.
- Differing number of exponential terms are frequently employed in the empirical mathematical expression of pharmacokinetic models.
- Higher models are intricate in expression and comprehension.
- Differing model selection for the same drug in the same or different animals.
- Quantitative magnitude of classical pharmacokinetic parameters is a function of the selected model.
- There is no parameter to depict the performance of controlled release drug products.
- Pharmacokinetic comparison amongst congeneric or non-congeneric drug series can not be pragmatically plausible.
- Computation for drugs exhibiting nonlinear pharmacokinetic behavior is
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Incongruity exists for in silico prediction of ADME parameters using Quantitative Structure Pharmacokinetic Relationship (QSPkR) Studies. Elaborate computer software is almost an imperative requirement. Only use of effective experimental designs shows optimal usage of data.

Noncompartmental approaches have developed and evolved as both alternatives and adjuncts to compartmental models. Albeit these methods are hardly new, their usage for pharmacokinetic data analysis has been novel and significant. Many relationships used in the former practice have been derived originally in the compartmental context for supplemental use in these models.

Motivating factors for the development of noncompartmental methods include criticisms of the compartmental approach as discussed above. Another impetus for noncompartmental method has been the desire to develop methods of estimating pharmacokinetic parameters that do not require tedious and somewhat subjective method of nonlinear regression. The approach has also some times been known as model-independent or model-free approach. Box 5 outlines various advantageous features. Amongst the popular noncompartmental methods propounded for pharmacokinetic data analysis are the statistical moment approach, linear system analysis and the recirculatory models.

The information on the myriad perspectives of the subject lie scattered as over a scores of sparse reviews and book chapters. Linear system analysis tends to describe the entire time course of plasma drug concentration in body in a relatively more comprehensive manner. This approach may be exploited for prediction of systemic drug concentration by convolution, and estimation of time course of drug absorption by deconvolution, and has even been used for establishing Level A in vitro-in vivo correlations.

The recirculatory models tend to describe drug disposition in terms of repeated cycles through the body circulatory system (Moriwaki et al. 2004; Avram et al. 2007; Naidoo et al. 2009; Masui et al. 2010). It can be argued that such models reflect better than even the so called physiologically based compartmental models.
1.8.2.1 Statistical moment approach

The technique is doubtlessly the most accepted and indisputable noncompartmental approach for pharmacokinetic data analysis. It has gained a significant degree of use and acceptance in pharmacokinetic literature in the last two decades. Although the statistical moments have been used extensively as a practical technique in various sciences since the fifties, the first report on their use in describing cholesterol kinetics appeared in 1969 (Perl and Samuel 1969). The application of statistical moment approach to pharmacokinetics was virtually simultaneously reviewed by Yamaoka et al. (1978) and Cutler (1978). Chanter (1985) questioned the validity of using statistical moments for estimating MRT. In the same year, Landaw & Katz (1985) indicated errors in the Chanter postulate and Benet (1985) suggested some alternative measures to compute MRT for 2-CBM kinetics. Gillespie and Veng-Pedersen (1985) ultimately ended the controversy in the same year by re-establishing the application of moment theory for all the linear pharmacokinetic systems. Assumptions made while hypothesizing such a non-compartmental model are fewer, less restrictive, easily verifiable and realistic ones. Another impetus for these "model-independent" methods has been the desire to develop methods of estimating pharmacokinetic parameters that do not require tedious and somewhat subjective method of nonlinear regression.

1.8.2.1.1 Mean time concept

Based on integration of data, the statistical moment technique banks upon parameters like mean residence time (MRT), applicable to virtually any linear body model. After the drug is administered, the drug molecules distribute themselves throughout the body and stay in the body for differing time periods. Some
molecules may stay longer in the body while other will leave immediately after absorption. Thus, the term, MRT describes the average time for which the drug molecules reside in the body. The residence time for the drug molecules in the dose may be sorted according to their residence time into groups. The total residence time is the summation of the number of molecules in each group multiplied by their residence times. Thus, MRT is the ratio of sum of the residence times of all the drug molecules divided by total number of such molecules. To accomplish this, the drug dose may be converted to number of moles by dividing the dose in grams by molecular weight. Multiplying the number of moles by the Avogadro's number \((6.023 \times 10^{23})\) yields the number of molecules which turns out to tune of \(10^{20}\). Hence for estimation of pharmacokinetic parameters, one needs to determine residence times of each of such \(10^{20}\) molecules and eventually calculate their mean and variance as MRT and variance of residence time (VRT) respectively.

Residence times of such large number of molecules can be rationally considered to follow a normal distribution curve, as per the Gaussian Eqn 13.

\[
P(X) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(x - \mu)^2}{2\sigma^2}} \quad \cdots (13)
\]

Accordingly, as per the Gaussian relationship, the residence times of drug molecules in body following a single dose, when drawn versus their number, can be regarded as a statistical normal frequency distribution curve.

For such Gaussian normal distribution, the curve tends to be symmetric about the axis with the area lying under particular range as ascertainable. Hence the value corresponding to the zenith of this frequency curve or peak of this unimodal distribution is MRT. As it is known that nearly 68.23% of area under normal distribution curve is encompassed between mean \((\mu) \pm 1\) standard deviation \((\sigma)\), the value of \(\sigma\) and eventually, its square, variance \((i.e., \sigma^2)\) can easily be monitored. Verily, using conventional means of averaging the residence times of zillions of the drug molecules is a daunting rather impossible task. Statistical moments, on the other hand, make the task enormously simplified.

Pharmacokinetically, the movement of individual drug molecules through a body compartment is governed by probability. Some drug molecules may hit the target
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(stocha) as all may not get absorbed (or metabolized or distributed or excreted) and rest may form a random scatter around a general cluster. The analysis of statistical moments is, in fact, the analysis of the probability distribution resulting as a consequence of this stochastic process.

1.9 IN VITRO/IN VIVO CORRELATIONS (IVIVC)

The term IVIVC first appeared in pharmaceutical literature as a result of awareness of concept of in vivo bioavailability and of in vitro dissolution rate determination. An endeavor to relate in vitro dissolution and in vivo pharmacokinetic results is referred to as IVIVC analysis (Amidon et al. 1995b; Uppoor 2001; Emami 2006). Thus, IVIVC can verily be defined as “a quantitative rational relationship between a biological property (e.g., Cmax, AUC or tmax), or a parameter derived from a biological property produced by a dosage form, with a physicochemical property or characteristic of the same dosage form”.

The United States Pharmacopeia (USP) defines IVIVC as “the establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a dosage form and a physicochemical property or characteristic of the same dosage form”.

However, the US Food and Drug Administration (FDA) describes IVIVC as “a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant in vivo response.” Generally, the in vitro property is the rate or extent of drug dissolution or release, while the in vivo response parameter is the plasma drug concentration or amount of drug absorbed.

In the last a few years, the concept of IVIVC has extensively been discussed by pharmaceutical scientists, particularly for extended release (ER) drug products. The ability to predict the expected bioavailability characteristics accurately and precisely through dissolution profile characteristics is a long sought-after goal of the pharmaceutical scientists.

Dissolution testing was considered useful only for process control, stability, minor formulation changes, and manufacturing site changes.

1.9.1 Significance of IVIVC

The concept of IVIVC has gained tremendous attention in pharmaceutical industry, academia, and regulatory sector. It acts as a tool to reliably correlate in vitro drug dissolution data and in vivo drug absorbed. Such a tool shortens the drug development period, economizes the resources and leads to improved product quality (Byron et al. 2010; Yang 2010).

The main objective of establishing IVIVC is to facilitate in vitro dissolution studies to serve as surrogate for in vivo bioequivalence testing, thus supporting bio waiver, especially during scale-up and post approval changes (SUPAC). Understanding and controlling the relationship between in vitro release and in vivo response in a compound plays a critical role in development of modified release formulations, generics, fixed dose combination products, and drug delivery systems. During the manufacturing and marketing of any therapeutic agent, development and optimization of formulation are integral parts which are indeed time consuming and costly procedures. Optimization process may require alteration in formulation composition, manufacturing process, equipment and batch sizes. If these types of changes are applied to a formulation, studies in healthy human volunteers may be required to prove that the new formulation is bioequivalent with the old one. The implementation of these requirements not only halts the marketing of the new formulation but also increases the cost of the optimization processes. A validated IVIVC, here, can be used to predict in vivo behavior of the formulation to assess likelihood of success before entering it in a biostudy, thus, eliminating many biostudies that are unnecessary.

With increasing introduction of modified release and novel drug delivery systems, it is obligatory to understand the concept of IVIVC in greater depth. Conducting dissolution analysis with IVIVC is a fast and inexpensive method for obtaining optimal formulations as opposed to slow and expensive bioavailability or bioequivalence studies that provide "hit or miss" results. Earlier, the IVIVC is implemented in the drug development process, easier and more cost-effective is its
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Implementation for all the future changes in the formulation. Box 6, in a nutshell, summarizes the key advantages of IVIVC in a typical pharmaceutical R & D set-up.

1.9.2 IVIVC model development

In the process of IVIVC development, the *in vitro* drug release parameters which resemble *in vivo* drug performance are identified. The appropriate design of *in vitro* dissolution tests, capable of discriminating between the formulations with different bioavailabilities, plays a major role in the ability of the IVIVC predictability.

**Box 6: Key advantages of IVIVC**

- Bypasses the hassles of biostudy by serving as a surrogate for human bioequivalence studies
- Helps to reduce costs by obviating the need to perform expensive bioavailability/bioequivalence human trials
- Speeds up the product development process
- Demonstrates bioequivalence when certain pre-approval changes are made in formulation, equipment, manufacturing process or in manufacturing site.
- Improves product quality using more meaningful dissolution specifications
- Reduces "regulatory burden" due to biostudies

Therefore, it is essential that *in vitro* dissolution tests closely reflect *in vivo* situations, when they are used to establish an IVIVC. Thus for all categories, it is anticipated that well-designed dissolution test is a key prognostic tool in the assessment of both, the drug's potential for oral absorption and the bioequivalence of its formulations.

1.9.3 Levels of IVIVC

Five correlation levels have been defined in the FDA guidance namely level A, level B, level C, level D, and multiple level C. The concept of correlation level is based upon the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of given dosage form.
1.9.3.1 Level A IVIVC

This is the most common type of correlation observed in new drug applications (NDAs) submitted to the FDA. From regulatory perspectives, it is considered to be the most useful. It is the highest category of correlation, where a point to point correlation exists between the entire in vitro dissolution (or drug release) time course and the entire in vivo response time course, e.g., the time course of plasma drug concentration or amount of drug absorbed, or in vivo dissolution of the drug from the dosage form (Donato et al. 2008; Ghosh et al. 2008; Ochoa et al. 2010; Scheubel et al. 2010). This estimation of in vivo absorption profile from the concentration-time data can be achieved through the two-stage deconvolution methods. Wagner-Nelson (WN, Eqn 14) is specifically suited for cases obeying one-compartmental (1-CBM) open body model.

\[
\frac{(X_a)_T}{(X_a)_\infty} = \frac{C_T + K \int_0^T C dT}{K \int_0^T C dT} \quad \ldots (14)
\]

Analogously, for cases obeying two-compartmental (2-CBM) open body model, Loo-Riegelman (LR, Eqn 15) method is more suited.

\[
\frac{(X_a)_T}{(X_a)_\infty} = \frac{C_T + K_{10} \int_0^T C dT + \left( \frac{1}{V_c} \right) (X_p)_T}{K_{10} \int_0^T C dT} \quad \ldots (15)
\]

where, \((X_a)_T\) = amount of drug absorbed into systemic circulation at time ‘t’; \((X_a)_\infty\) = amount of drug absorbed into systemic circulation at infinite time; \(C_T\) = plasma concentration of drug at time ‘t’; \(K\) = first-order elimination rate constant; \(K_{10}\) = apparent first order elimination rate constant of drug form the central compartment; \(X_p\) = amount of drug in the central compartment; \(V_c\) = apparent volume of the central compartment

Subsequent to the estimation of the in vivo absorption profile, the relationship with in vitro dissolution is evaluated. An example of establishing "level A" IVIVC in case of slow, medium and fast dissolution is depicted in Figure 26 (a,b,c).
Figure 26: "Level A" IVIVC in case of (a) slow dissolution, (b) medium dissolution, (c) fast dissolution

In "Level A" IVIVC, generally a linear correlation model is observed which can be expressed as a simple equation (Eqn 16) between the *in vivo* drug absorption and *in vitro* drug dissolved (released).

\[ Y_{\text{in vivo absorbed}} = mX_{\text{in vitro dissolved}} + C \]  \hspace{1cm} \ldots (16)

In this equation, \( m \) is the slope of the relationship, and \( C \) is the intercept. Ideally, \( m = 1 \) and \( C = 0 \), indicating a linear relationship. However, depending on the nature of the modified-release system, some data are better fitted using nonlinear models, such as Sigmoid, Weibull, Higuchi, or Hixson-Crowell.

Eqn 15 may be applied to most formulations with comparable *in vitro* and *in vivo* duration of release. However, for dosage forms with complicated mechanisms of release, which are of longer duration, *in vitro* release may not be in the same time scale as the *in vivo* release. Thus, in order to model such data, it is necessary to incorporate time-shifting and time-scaling parameters within the model. This is the kind of data that is routinely encountered in the development of sustained-release dosage forms.

*In vivo* release rate \( (X'_{\text{vivo}}) \) can also be expressed as a function of *in vitro* release rate \( (X'_{\text{rel,vitro}}) \) with parameters \( (a, b) \), which may be empirically selected and refined using appropriate mathematical processes as shown in Eqn 17. An iterative process may be used to compute the time-scaling and time-shifting parameters.

\[ X'_{\text{vivo}} (t) = X'_{\text{rel,vitro}} (a + bt) \]  \hspace{1cm} \ldots (17)

Nonlinear IVIVC are observed when *in vitro* dissolution profile runs ahead or
behind the *in vivo* input profile as seen in Figure 27a. The resulting non-linear IVIVC can be made linear by log transformation (Figure 27b) using an exponential equation for correlation.

### 1.9.3.2 Level B IVIVC

If the entire profile can not be used for correlation, data reduction that leads to comparable and preferably analogous parameters on the *in vivo* and the *in vitro* side is required. The statistical moment theory, a model-independent method, uses empirical distribution functions for the purpose.

![Figure 27: (a) Nonlinear IVIVC (b) Log transformation of nonlinear IVIVC](image)

Level B correlation, as depicted in Figure 28, is established by comparing the mean *in vitro* dissolution time (MDT\textsubscript{vitr}) of the product to either mean *in vivo* residence time (MRT) or the mean *in vivo* dissolution time (MDT\textsubscript{vivo}). Level B correlation, like Level A, utilizes all of the *in vitro* and *in vivo* data but is not considered to be a point-to-point correlation because it does not uniquely reflect the actual *in vivo* plasma level curve, since there are a number of different *in vivo* curves that will produce similar mean residence time values.

Besides MRT, Mean Transit time (MTT) and Mean Absorption Time (MAT) are other parameters used in oral pharmacokinetics. While MTT, quite similar to MRT, is mean time a molecule needs to leave a kinetic space, MAT can be estimated as per Eqn 18.

\[
MAT = MRT_{oral} - MRT_{iv} \quad \cdots (18)
\]

In shear contrast to the traditional compartmental pharmacokinetics, one of the remarkable features of the stochastic approach is that even drug formulation...
performance can be determined using moment parameters. Some instances of such moment parameters include Mean Dissolution Time (MDT, Eqn 19) and Mean Disintegration Time (MDIT).

\[ MDT = MAT_{solution} - MAT_{solid} \quad \ldots (19) \]

The most vital advantage of mean time parameters is their rational additivity across the whole gamut of processes involved in the drug fate of solid oral dosage forms, both \textit{in vitro} and \textit{in vivo}, encompassing disintegration, dissolution, absorption and disposition. The continuum of parameters is pictorially represented in Figure 29.

\textbf{1.9.3.3 Level C IVIVC}

It establishes a single point relationship between \textit{in vitro} dissolution parameter (e.g., \(t_{50\%}\) or percent dissolved in 4 h) and a pharmacokinetic parameter (AUC, \(t_{\text{max}}\) or \(C_{\text{max}}\)). One dissolution time point (like \(t_{50\%}, t_{90\%}\) etc.) is usually compared to one pharmacokinetic parameter (like AUC, \(t_{\text{max}}, C_{\text{max}}\) etc.).

Therefore, it represents a single point correlation and does not reflect the complete shape of the plasma profile, which is a crucial factor that indicates the performance of modified-release products.

\[ \text{Figure 28: "Level B" correlation} \]
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Figure 29: Continuum of mean time parameters in correlation of in vitro and in vivo fate of drugs

Level C is the weakest level of correlation, as the partial relationship between absorption and dissolution is only established. Because of its obvious limitations, a level C correlation has limited usefulness in predicting in vivo drug performance and is subject to the same caveats as a Level B correlation in its ability to support product and site changes as well as justification of quality control standard extremes. Level C correlations can be useful in the early stages of formulation development when pilot formulations are being selected. While the information may be useful in formulation development, waiver of an in vivo bioequivalence study (bio waivers) is generally not possible.

1.9.3.4 Multiple Level C correlation

A multiple point level C relationship is almost equivalent to level A. It relates one or more pharmacokinetic parameters of interest (like C_{max}, AUC, or any other suitable metrics) to the amount of drug dissolved at various time points. It can be employed to justify biowaiver(s) provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. A relationship should be demonstrated at each time point at the same parameter such that the effect on the in vivo performance of any change...
in dissolution can be assessed, at least three dissolution time points covering the early, middle, and late stages of the dissolution profile are required. If such a multiple level C correlation is achievable, then the development of a "level A" correlation is also likely.

1.9.3.5 Level D correlation

It is a nonparametric rank order correlation between the *in vitro* dissolution parameter and an *in vivo* pharmacokinetic parameter. It is usually based on ordinal (but not quantitative) data, thus considered to be a weaker correlation.

1.9.4 IVIVC in the Light of BCS

Class I compounds like metoprolol, propranolol, labetolol, diltiazem, verapamil, enalapril, phenylalanine and caffeine possess high permeability and solubility. They are expected to be well absorbed owing to their high $A_n$ and high $D_n$, unless they are unstable, form insoluble complexes, are secreted directly from gut wall, or undergo first pass metabolism. When a class I drug is formulated as an ER product in which the release profile controls the rate of absorption, and the solubility and permeability of the drug is site independent, a level A correlation is most likely. However, once the permeability is site dependent a level C correlation is expected. The major challenge in development of drug delivery system for class I drugs is to achieve a target release profile associated with a particular pharmacokinetic and/or pharmacodynamic profile. Formulation approaches include both control of release rate and certain physicochemical properties of drugs like pH-solubility profile of drug (Singh *et al.* 2010b)

Class II compounds like phenytoin, danazol, ketoconazole, mefenamic acid, nifedipine, flurbiprofen, diclofenac, naproxen, piroxicam and ketoprofen, possess high permeability and low solubility. They have a high $A_n$ but low $D_n$, which means absorption is faster than dissolution rate i.e., drug is absorbed quickly after dissolution, thus dissolution is the rate limiting step. For class II drugs, therefore, a strong correlation between dissolution rate and the *in vivo* performance can be established. When a class II drug is formulated as an ER product, and the solubility and permeability of the drug is site independent, a level A correlation is expected. However, once the permeability is site dependent little or no IVIVC is expected. The
systems that are developed for class II drugs are based on their solubility enhancement. The key techniques include, micronization, lyophilization, addition of emulgents, emulsion formulations, microemulsion systems, and use of complexing agents like CD.

Class III drugs, like cimetidine, acyclovir, neomycin, famotidine, nadolol, atenolol and ranitidine, possess low permeability and high solubility. They are rapidly dissolving and permeability is the rate-controlling step in drug absorption. Furthermore, Class III drugs exhibit a high variability in rate and extent of absorption, but if dissolution is fast such that 85% of drug dissolves in 15 minutes, the variation could be attributed to GI transit, luminal contents, and membrane permeation rather than dosage form factors. As drug permeation is rate controlling, limited or no IVIVC is expected. Class III drugs require the technologies that address to fundamental limitations of absolute or regional permeability. Peptides and proteins constitute the part of class III and the technologies handling such materials are on rise now days.

Class IV like taxol, furosemide, cyclosporine and terfenedine possess low permeability and solubility. This class of drugs exhibit significant problems for effective oral delivery, no IVIVC is expected. Class IV drugs present a major challenge for development of drug delivery system and the route of choice for administering such drugs is parenteral with the formulation containing solubility enhancers.

1.9.5 Applications of IVIVC

1.9.5.1 Biowaivers for changes in the manufacturing of a drug product

A biowaiver, using an IVIVC developed with two formulations/release rates, for a non-narrow therapeutic index drug will likely be granted for an ER drug product for Level 3 manufacturing and non-release controlling excipient changes and as defined in SUPAC-MR (Singh et al. 2010a; Singh and Beg 2012).

1.9.5.1.1 Biowaivers for lower strengths

If an IVIVC is developed with the highest strength, waivers for changes made on the highest strength and any lower strength may be granted if these strengths are compositionally proportional or qualitatively the same, the \textit{in vitro} dissolution
profiles of all the strengths are similar, and all strengths have the same release mechanism.

1.9.5.1.2 Approval of new strengths

This biowaiver is applicable to strengths lower than the highest strength, within the dosing range that has been established to be safe and effective, if the new strengths are compositionally proportional or qualitatively the same; have the same release mechanism; have similar *in vitro* dissolution profiles; and are manufactured using the same type of equipment and the same process at the same site as other strengths that have bioavailability data available. For generic products to qualify for this biowaiver, one of the situations listed in Box 7 should exist.

**Box 7: Conditions for generic products to qualify for biowaiver**

- Bioequivalence has been established for all strengths of the reference product
- Dose proportionality has been established for the reference product, and all reference product strengths are compositionally proportional or qualitatively the same
- Bioequivalence is established between the generic product and the reference product at the highest and lowest strengths and, for the reference product, all strengths are compositionally proportional or qualitatively the same
- Obtaining "category 2d" biowaivers: The difference in predicted means of $C_{\text{max}}$ and AUC should be no more than 10%, based on dissolution profiles of the highest strength and the lower strength product

1.9.5.2 Product development

The pharmaceutical applications of IVIVC are not limited merely to obtaining biowaivers for new formulations. The concepts of IVIVC can be well-utilized at various stages of product development resulting in the formulation of more valuable and robust formulations. The long standing dream of product development scientists of predicting *in vivo* behavior of formulations by examining their *in vitro* release profiles can only be realized by IVIVC. A newer approach of *in vitro/ in vivo* matching (IVIVM) comes to the rescue of the scientists in this regard. The technique principally involves simulation of *in vivo* profiles from *in vitro* data using model-independent deconvolution techniques, as shown in Figure 30.
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Figure 30: In vitro/ in vivo matching (IVIVM) using rational computer simulations

1.9.5.2.1 Setting-up of dissolution specifications

In vitro dissolution specifications should generally be based on the performance of the clinical/bioavailability lots. These specifications may sometimes be widened so that scale-up lots, as well as stability lots, meet the specifications associated with the clinical/bioavailability lots. This approach is based on the use of the in vitro dissolution test as a quality control test without any in vivo significance, even though in certain cases (e.g., ER formulations), the rate limiting step in the absorption of the drug is the dissolution of the drug from the formulation. An IVIVC adds in vivo relevance to in vitro dissolution specifications, beyond batch-to-batch quality control. In this approach, the in vitro dissolution test becomes a meaningful predictor of in vivo performance of the formulation, and dissolution specifications may be used to minimize the possibility of releasing lots that would be different in in vivo performance.

It is relatively easier to establish a multipoint dissolution specification for modified-release dosage forms. The FDA guidance describes the procedures of setting dissolution specifications in cases of level A, multiple level C and level C correlation and where there is no IVIVC. Once an IVIVC developed, it should be used to set specifications in such a way that the fastest and lowest release rates allowed by the upper and lower dissolution specifications result in a maximum difference of 20% in the predicted $C_{\text{max}}$ and AUC. Predicted plasma concentration, and the consequent AUC and $C_{\text{max}}$, can be calculated using convolution or any other
appropriate modeling techniques, described earlier.

1.10 CARDIOVASCULAR DISEASES

Cardiovascular diseases (CVD's) are caused by disorders of the heart and blood vessels. The major CVD's include, coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. CVD's are the number one cause of death globally (Gaziano et al. 2006). About 82% of CVD's mortalities take place in low- and middle-income countries, almost equally among men and women. It is estimated that by 2030, almost 23.6 million people will die from CVDs, mainly from heart disease and stroke. The Indian subcontinent, which is home to about 20 per cent of the world's population, is one of the regions with the highest burden of CVD's across the globe (http://www.who.int/topics/cardiovascular_diseases/en/).

The major medical risk factors for CVD's are raised blood pressure, raised cholesterol, overweight, obesity and diabetes. The behavioural risk factors for CVD's comprises smoking, poor diet, physical inactivity and alcohol consumption (Scarborough et al. 2010). Various drugs are available in the market for controlling and monitoring the above problems. Such drugs can be introduced into the vascular system for systemic effects or targeted to an organ via the regional blood supply.

1.10.1 Hyperlipidemia

The most common cause underlying the CVD's is the build-up of fatty deposits, i.e., lipids, on the inner walls of the blood vessels that supply the heart. Lipid is the scientific term for fats circulating in the blood. At proper levels, lipids tend to perform vital functions in our body. But these lipids can cause health problems, if they are present in excess in systemic circulation, i.e., hyperlipidemia. When the excess of lipids in systemic circulation are in association with proteins, the condition is termed as hyperlipoproteinemia (Bandyopadhyay et al. 2011). Lipid and lipoprotein abnormalities are extremely common in the general population, and are regarded as a highly modifiable risk factor for CVD's, especially atherosclerosis (Nicholls 2009). Figure 31 portrays the progression of
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atherosclerosis.

1.10.1.1 Types of Lipoproteins in Systemic Circulation

Lipids circulating in the blood mainly comprises of cholesterol and triglycerides. They are carried in combination with phospholipids and proteins, usually called as lipoproteins. There are five major families of blood (plasma) lipoproteins;

- Chylomicrons
- Very low-density lipoproteins (VLDL)
- Intermediate-density lipoproteins (IDL)
- Low-density lipoproteins (LDL)
- High-density lipoproteins (HDL)

**Figure 31: Progressive build-up of plaques ultimately leading to blockade of arteries [adopted from (Bandyopadhyay et al. 2011)]**

**Chylomicrons** are the least dense of all the lipoproteins constituting of 90 percent triglycerides (i.e., fat) in combination with phospholipids, cholesterol, and miniscule amount of protein. The **VLDL** comprises of a high amount of fat, some phospholipids and cholesterol. The **IDL**, on the other hand, is basically a lipid-protein complex with a density between those of VLDL and LDL.(Grundy et al. 2004).

With a relatively short half-life, the IDL is normally present in blood in very low concentrations. The **LDL** is very high in cholesterol concentration (approximately 101...
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45%). The **HDL** is a "spherical blob" comprising mostly of protein, phospholipids, and very little cholesterol content. The densest of all the lipoproteins, HDL, is the healthiest one.

Clinically important lipoproteins are the LDL and HDL. Excess LDL contributes to the blockage of arteries, which eventually leads to heart attack. Studies have clearly shown that higher the level of LDL, greater the risk of heart disease. (Grundy et al. 2004) Hence, LDL has been labeled as the "bad" cholesterol. In contrast, higher levels of HDL greatly diminish the risk of coronary heart disease. (Nicholls 2009) As a result, HDL is commonly referred to as the "good" cholesterol. Either way, the balance between the "good cholesterol" and the "bad cholesterol" tends to be disturbed. Figure 32 illustrates the basic mechanism of both the lipoproteins.

![Figure 32: Mechanistic outline of the effects of HDL and LDL](Figure adopted from (Bandyopadhyay et al. 2011))

1.10.1.2 Atherosclerosis

High lipidic levels *viz.* LDL, triglycerides, etc., can enhance the process of atherosclerosis or hardening of the arteries. Normally, arteries are smooth and unobstructed on the inside, but a sticky substance called plaque forms with age in the walls of the arteries. This plaque is made of lipids and other materials circulating in the blood. As more plaque builds up, arteries narrow-up and get
stiffened. Eventually, enough plaque may build up to reduce and/or occlude the blood flow through the arteries (Greene et al. 1998). Figure 33 represents the accumulation of high levels of cholesterol in the arteries, consequently resulting in plaque formation. The effects of chronic hyperlipidemia are complex, as it not only results in the deposition of lipids in the atheromatous lesions, but it may produce the primary endothelial injury that initiates the process of atherosclerosis as well.

Figure 33: Various stages of plaque formation [Figures adopted from (Bandyopadhyay et al. 2011)]

1.10.1.3 Diagnosis of Hyperlipidemia

Hyperlipidemia usually doesn’t cause any perceptible symptoms and tends to be diagnosed during routine examination or evaluation for atherosclerotic CVD. Hence, besides medical history and physical examination, it requires a blood test for proper diagnosis. The blood test will show the levels of different lipids in our blood. The range of different lipids helps in diagnosing the hyperlipidemia and ultimately the type of treatment.
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The treatment may be a modification in lifestyle, medical treatment, or both. Based on all these facts, a physician will determine whether a person has atherosclerosis or other risk factors for heart disease and may further prescribe dietary changes, weight reduction and exercise too. If lifestyle modifications cannot bring about the optimal control of lipid levels, then medications may be necessary. Most blood tests measure levels of LDL, HDL, total cholesterol (LDL plus HDL), and triglycerides.

Albeit the desired threshold of lipidic levels varies from one country region to another, yet the most agreeable levels have been compiled as Box 8. To have a low risk of heart disease, the desirable lipid levels must be within the above mentioned limits.

**Box 8: Recommended limits of lipidic levels in India**

- LDL cholesterol levels of ≤ than 100 mg/dl
- Optimal HDL levels of ≥ 40 mg/dl (men) or 50 mg/dl (women)
- Total cholesterol levels of ≤ 200 mg/dl, and
- Triglyceride levels of ≤ 150 mg/dl.

1.10.1.4 Treatment of Hyperlipidemia

1.10.1.4.1 Anti-hyperlipidemic Drugs

There are several classes of hypolipidemic drugs available for the treatment of hyperlipidemia ([http://wwwvascularweb.org/patients/NorthPoint/Hyperlipidemia.html](http://wwwvascularweb.org/patients/NorthPoint/Hyperlipidemia.html); Kelly 2010) The hypolipidemic drugs may differ in their impact on the cholesterol profile. For example, some may lower the "bad cholesterol", i.e., LDL, while others may preferentially increase the "good cholesterol", i.e., HDL. Clinically, the choice of an agent is evaluated on the basis of patient’s cholesterol profile, cardiovascular risk, and the liver and kidney functions of the patient. Medications most commonly used to treat various high lipid levels include, statins, fibrates, bile acid sequestrants, phytosterols, ezetimibe, niacin and orlistat.

**Statins** are particularly prescribed for lowering LDL, the cholesterol with the strongest links to cardiovascular diseases (Sadowitz et al. 2010). In studies using standard doses, statins have been found to lower LDL by 18% to 55%, depending
on the specific statin being used. However, a severe risk of muscle damage (i.e., myopathy and rhabdomyolysis) has been reported with statins. Hence, they are mostly administered as combination therapy.

**Fibrates** are another promising class of antihyperlipidemic drugs indicated for hypertriglyceridemia. Fibrates typically lower triglycerides by 20% to 50% and increase the levels of HDL. Fibrates may decrease LDL, though generally to a lesser degree than statins (Abourbih et al. 2009). Similar to statins, there are reports of severe muscle damage (myopathy & rhabdomyolysis) with fibrates too and like statins mostly administered as combination therapy.

**Bile Acid Sequestrants (Resins)** are particularly effective for lowering LDL by sequestering the cholesterol-containing bile acids released into the intestine and preventing their reabsorption from the intestine. They decrease LDL by 15-30% and raise HDL by 3-5%. Though they have little effect on triglycerides, yet can cause a slight increase. Bile acid sequestrants may cause gastrointestinal problems, and may also reduce the absorption of other drugs and vitamins from the gut.

**Niacin**, like fibrates, is also well-suited for lowering triglycerides by 20-50%. It may also lower LDL by 5-25% and increase HDL by 15-35%. Niacin may cause hyperglycemia, and may also cause liver damage.

**Ezetimibe** is a selective inhibitor of dietary cholesterol absorption. It may be used alone when other cholesterol-lowering medications are not tolerated, or together with statins when cholesterol levels are unable to be controlled on statins alone.

**Phytosterols** are the phytoconstituents found naturally in plants. Similar to ezetimibe, phytosterols reduce the absorption of cholesterol from the gut. Hence, they are most effective when consumed with meals.

**Orlistat** is particularly designed to control obesity. Its primary function is to prevent the absorption of about 30% of fats from the human diet; thereby reducing caloric intake by inhibiting pancreatic lipase, an enzyme that breaks down triglycerides in the intestine.

**1.10.1.4.2 Newer Investigational Drugs**

**CETP** (cholesteryl ester transfer protein) **inhibitors** are still under
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development. It is expected that these drugs will mainly increase HDL while also lowering LDL. (Kolovou et al. 2009) The synthetic development of one of these drugs, torcetrapib, was halted in 2006 when phase III studies showed excessive mortality. Another one, anacetrapib, is currently in Phase III trial and is being studied by Merck Co., USA.

Lapaquistat, a squalene synthase inhibitor, is currently under investigation for lowering cholesterol levels in the prevention of cardiovascular disease. Lapaquistat metabolites inhibit squalene synthase, during the final stages in the synthesis of cholesterol.

ApoA-1 Milano is a naturally occurring mutated variant of the apolipoprotein A1 protein found in human HDL, the lipoprotein particle that carries cholesterol from tissues to the liver and is associated with protection against cardiovascular disease. It significantly reduces cardiovascular disease, even though it causes a reduction in HDL levels and an increase in the triglyceride levels.

1.10.1.4.3 Newer Promising drugs for hyperlipidemia

Elevated LDL concentrations are associated with increased risk of coronary atherosclerosis, and subsequent morbidity and mortality from coronary heart disease. The conventional/mainstream basis of lipid lowering therapy today is only statins. But the residual risk of cardiovascular events amongst individuals treated with statins remains a major healthcare concern. Newer vistas for lipid lowering therapy target pathways that regulate lipoprotein assembly include, lipoprotein clearance and pro-atherogenic lipoprotein modification (Paras et al. 2010).

These emerging drugs have novel mechanisms including,

- Inhibition of lipoprotein assembly (antisense mRNA inhibitors of apolipoprotein B and microsomal transfer protein inhibitors)
- Enhanced lipoprotein clearance (proprotein convertase subtilisin kexin type 9, thyroid hormone analogues)
- Inhibition of pro-atherogenic lipoprotein remodeling (cholesterol ester transfer protein inhibitors viz dalcetrapib, anacetrapib)
- Activation of peroxisome proliferator (GFT-505, aleglitazar)
• Inhibition of lipoprotein modification (heme oxygenase-1 inhibitor viz succinobucol)
• Inhibition of Phospholipase A(2) (varespladib, darapladib)

1.10.2 Hypertension

Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a cardiac chronic medical condition in which the arterial blood pressure is elevated. It is classified as either primary (essential) hypertension or secondary hypertension. About 90–95% of cases are categorized as "primary hypertension", which means high blood pressure with no obvious medical cause (Carretero and Oparil 2000). The remaining 5–10% of cases (i.e., secondary hypertension) are caused by other conditions that affect the kidneys, arteries, heart or endocrine system. Hypertension is a major risk factor for stroke, myocardial infarction (heart attacks), heart failure, aneurysms of the arteries (e.g. aortic aneurysm), peripheral arterial disease and is a cause of chronic kidney disease.

1.10.2.1 Classification

In case of people aged 18 years or older, hypertension is defined as a systolic and/or a diastolic blood pressure measurement consistently higher than an accepted normal value (i.e., 139 mmHg systolic, 89 mmHg diastolic). However, lower thresholds are used (i.e., 135 mmHg systolic or 85 mmHg diastolic) if measurements are derived from 24-hour ambulatory or home monitoring (http://www.nice.org.uk/nicemedia/live/13561/56007/56007.pdf).

As per the latest international hypertension guidelines, categories have also been created below the hypertensive range which indicates a continuous risk towards higher blood pressures. Hypertension is also sub-classified into hypertension stage I, hypertension stage II and isolated systolic hypertension. Isolated systolic hypertension refers to elevated systolic pressure with normal diastolic pressure and is common in the elderly (Chobanian et al. 2003; Williams et al. 2004; Mancia et al. 2007). Table 14 illustrates the various classifications along with the ranges of both systolic and diastolic pressure.
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1.10.2.2 Signs and symptoms

Hypertension is rarely accompanied by any symptoms, and its identification is usually through screening, or when seeking healthcare for an unrelated problem. A proportion of people with high blood pressure reports headaches (particularly at the back of the head and in the morning), as well as lightheadedness, vertigo, tinnitus (buzzing or hissing in the ears), altered vision or fainting episodes (Fisher and Williams 2005).

Table 14: Various stages of hypertension

<table>
<thead>
<tr>
<th>Classification</th>
<th>Systolic pressure</th>
<th>Diastolic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmHg</td>
<td>kPa</td>
</tr>
<tr>
<td>Normal</td>
<td>90–119</td>
<td>12–15.9</td>
</tr>
<tr>
<td>Prehypertension</td>
<td>120–139</td>
<td>16.0–18.5</td>
</tr>
<tr>
<td>Stage 1</td>
<td>140–159</td>
<td>18.7–21.2</td>
</tr>
<tr>
<td>Stage 2</td>
<td>≥160</td>
<td>≥21.3</td>
</tr>
<tr>
<td>Isolated systolic hypertension</td>
<td>≥140</td>
<td>≥18.7</td>
</tr>
</tbody>
</table>

1.10.2.3 Complications

Hypertension is the most important preventable risk factor for premature death worldwide. It increases the risk of ischemic heart disease strokes, peripheral vascular disease, and other cardiovascular diseases, including heart failure, aortic aneurysms, diffuse atherosclerosis, and pulmonary embolism. Hypertension is also a risk factor for cognitive impairment and dementia, and chronic kidney disease. Other complications include hypertensive retinopathy and hypertensive nephropathy (Lewington et al. 2002; Chobanian et al. 2003; Eoin et al. 2007; Singer and Kite 2008). Figure 34 illustrates the various complications associated with hypertension.

1.10.2.4 Pathophysiology

In hypertension, the heart is required to work harder than normal to circulate blood through the blood vessels. Blood pressure is summarised by two
measurements, systolic and diastolic, which depend on whether the heart muscle is contracting (systole) or relaxed between beats (diastole).

![Diagram showing complications of persistent high blood pressure]

Figure 34: Main complications of persistent high blood pressure

Normal blood pressure at rest is within the range of 100-140 mmHg systolic and 60-90 mmHg diastolic. High blood pressure is said to be present if it is persistently at or above 140/90 mmHg.

In most people with established essential (primary) hypertension, increased resistance to blood flow (total peripheral resistance) accounting for the high pressure while cardiac output remains normal (Conway 1984). There is evidence that some younger people with prehypertension or 'borderline hypertension' have high cardiac output, an elevated heart rate and normal peripheral resistance, termed hyperkinetic borderline hypertension. These individuals develop the typical features of established essential hypertension in later life as their cardiac output falls and peripheral resistance rises with age (Palatini and Julius 2009).

Pulse pressure (the difference between systolic and diastolic blood pressure) is frequently increased in older people with hypertension. This can mean that systolic pressure is abnormally high, but diastolic pressure may be normal or low, a condition termed isolated systolic hypertension (Chobanian 2007). The high pulse pressure in elderly people with hypertension or isolated systolic hypertension is...
explained by increased arterial stiffness, which typically accompanies aging and may be exacerbated by high blood pressure (Zieman et al. 2005).

Many mechanisms have been proposed to account for the rise in peripheral resistance in hypertension. Most evidence implicates either disturbances in renal salt and water handling (particularly abnormalities in the intrarenal renin-angiotensin system) and/or abnormalities of the sympathetic nervous system (Esler et al. 2010; Navar 2010). These mechanisms are not mutually exclusive and it is likely that both contribute to some extent in most cases of essential hypertension. It has also been suggested that endothelial dysfunction and vascular inflammation may also contribute to increased peripheral resistance and vascular damage in hypertension (Marchesi et al. 2008; Versari et al. 2009).

1.10.2.5 Diagnosis

Hypertension is diagnosed on the basis of a persistently high blood pressure. Initial assessment of the hypertensive people should include a complete history and physical examination. Once the diagnosis of hypertension has been made, physicians will attempt to identify the underlying cause based on risk factors and other symptoms, if present. Secondary hypertension is more common in preadolescent children, with most cases caused by renal disease. Primary or essential hypertension is more common in adolescents and has multiple risk factors, including obesity and a family history of hypertension (Luma and Spiotta 2006).

Laboratory tests can also be performed to identify possible causes of secondary hypertension, and to determine whether hypertension has caused damage to the heart, eyes, and kidneys. Additional tests for diabetes and high cholesterol levels are usually performed because these conditions are additional risk factors for the development of heart disease and may require treatment (Carretero and Oparil 2000). Serum creatinine is measured to assess for the presence of kidney disease, which can be either the cause or the result of hypertension. Estimation of glomerular filtration rate (eGFR) can also provides a baseline measurement of kidney function that can be used to monitor for side effects of certain antihypertensive drugs on kidney function (Chobanian et al. 2003). Additionally,
testing of urine samples for protein is used as a secondary indicator of kidney disease.

Electrocardiogram (EKG/ECG) testing is done to check for evidence that the heart is under strain from high blood pressure. It may also show whether there is thickening of the heart muscle (left ventricular hypertrophy) or whether the heart has experienced a prior minor disturbance such as a silent heart attack. A chest X-ray or an echocardiogram may also be performed to look for signs of heart enlargement or damage to the heart (Eoin et al. 2007).

1.10.2.6 Prevention and therapy

Apart from various preventive measures like, reduced dietary sodium intake, regular aerobic physical activity, limit alcohol consumption, consumption of a diet rich in fruit and vegetables, etc. the patients needs to be administered medications in order to achieve the goal of a normal levels of blood pressure.

Several classes of medications, collectively referred to as antihypertensive drugs, are currently available for treating hypertension. Prescription should take into account the person’s cardiovascular risk (including risk of myocardial infarction and stroke) as well as blood pressure readings, in order to gain a more accurate picture of the person’s cardiovascular profile (http://www.australianprescriber.com/magazine/33/4/108/12). A reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease (http://www.hta.ac.uk/fullmono/mon731.pdf). The aim of treatment should be to reduce blood pressure to <140/90 mmHg for most individuals, and lower for those with diabetes or kidney disease (http://www.webmd.com/hypertension-high-blood-pressure/guide/new-low-for-high-blood-pressure).

The majority of patients require more than one drug to control their hypertension. In most of the cases, the preferred combinations are based on renin–angiotensin system inhibitors either with calcium channel blockers or diuretics (Sever and Messerli 2011). Commonly used prescription drugs include, ACE inhibitors (e.g. captopril), α-blockers (e.g. prazosin), angiotensin II receptor blockers, β-blockers
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(e.g. propranolol), calcium channel blockers (e.g. verapimil), diuretics (e.g. hydrochlorothiazide) and direct renin inhibitors (e.g. aliskiren).

1.10.2.7 Angiotensin II receptor blockers (ARBs)

Angiotensin II receptor antagonists, also known as angiotensin receptor blockers (ARBs), AT1-receptor antagonists or sartans, represent a newer class of antihypertensive agents whose mechanism of action differs from that of the angiotensin-converting enzyme (ACE) inhibitors, which also affect the renin-angiotensin system. The ARBs were developed to overcome several of the deficiencies of ACE inhibitors (Burnier and Brunner 2000);

- Competitive inhibition of ACE results in a reactive increase in renin and angiotensin I levels, which may overcome the blockade effect
- ACE is a relatively nonspecific enzyme that has substrates in addition to angiotensin I, including bradykinin and other tachykinins, and thus, inhibition of ACE may result in accumulation of these substrates
- Production of angiotensin II can occur through non-ACE pathways as well as through the primary ACE pathway, and these alternative pathways are unaffected by ACE inhibition
- Specific adverse effects are associated with ACE inhibitor effects on the enzyme
- ARBs offer more complete angiotensin II inhibition by interacting selectively with the receptor site

All 7 drugs in this class (i.e., losartan, valsartan, irbesartan, candesartan, telmisartan, eprosartan and olmesartan) are approved by the USFDA for the treatment of hypertension, either alone or in combination with other drugs.

1.10.2.7.1 Pharmacology

The renin-angiotensin system, specifically angiotensin II, is implicated in the pathogenesis of essential hypertension, renovascular hypertension, congestive heart failure, and renal diseases associated with albuminuria (Burnier and Brunner 2000; Burnier 2001). Angiotensin II is a very potent chemical that causes muscles surrounding blood vessels to contract, thereby narrowing blood vessels. This narrowing increases the pressure within the vessels and can cause high blood
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Pressure (hypertension). The ARBs are medications that selectively block the action of angiotensin II by competitive antagonism of the angiotensin II receptors thereby preventing angiotensin II from binding to angiotensin II receptors on blood vessels. This mechanism leads to reversible of angiotensin II-induced vasoconstriction, aldosterone release, catecholamine release, arginine vasopressin release, water intake, and hypertrophic response (Burnier 2001). As a result, blood vessels enlarge (dilate) and blood pressure is reduced. Reduced blood pressure makes it easier for the heart to pump blood and can improve heart failure. In addition, the progression of kidney disease due to high blood pressure or diabetes is slowed. ARBs have effects that are similar to angiotensin converting enzyme (ACE) inhibitors, but ACE inhibitors act by preventing the formation of angiotensin II rather than by blocking the binding of angiotensin II to muscles on blood vessels. Figure 35 depicts the pathway via which it inhibits angiotensin II receptors leading to lowered blood pressure.

![Mechanism of action of Angiotensin II Receptor Blockers (ARBs)](image)

**Figure 35: Mechanism of action of Angiotensin II Receptor Blockers (ARBs)**
1.11 DRUG MONOGRAPHS

1.11.1 Ezetimibe: Drug Profile

1.11.1.1 Physicochemical properties

Chemically, ezetimibe is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone with an empirical formula of C$_{24}$H$_{21}$F$_{2}$NO$_{3}$ (Figure 36) and a molecular weight of 409.4. It is white, crystalline powder that is freely to very soluble in ethanol, methanol, and acetone and practically insoluble in water. It is highly permeable drug with log P of 4.59.

1.11.1.2 Absorption and distribution

After oral administration, ezetimibe is rapidly absorbed across intestinal mucosa and extensively conjugated to a pharmacologically active phenolic glucuronide (ezetimibe-glucuronide). After a single 10 mg dose to fasted adults, mean ezetimibe peak plasma concentrations ($C_{\text{max}}$) of 3.4 to 5.5 ng.mL$^{-1}$ were attained within 4 to 12 hours ($T_{\text{max}}$). There was, however, no substantial deviation from dose proportionality between 5 and 20 mg indicating linear pharmacokinetics (Araujo et al. 2005).

![Figure 36: Chemical structure of ezetimibe](image_url)

The absolute bioavailability of ezetimibe cannot be determined, as the compound is virtually insoluble in aqueous media suitable for injection. Ezetimibe has variable bioavailability; the coefficient of variation, based on inter-subject variability, was 35 to 60% for AUC values. Relative bioavailability of the drug for
the tablet formulation is about 30% vis-à-vis its suspension drug formulation. Concomitant food administration (high fat or non-fat meals) had no effect on the extent of absorption of ezetimibe when administered as 10 mg tablets. However, the \( C_{\text{max}} \) value of ezetimibe was increased by 38% with consumption of high fat meals. It can be administered with or without food (Araujo et al. 2005). Ezetimibe and ezetimibe-glucuronide are highly bound (>90%) to human plasma proteins.

1.11.1.3 Metabolism and Excretion

Ezetimibe is primarily metabolized in the small intestine and liver via glucuronide conjugation (a phase II reaction) with subsequent biliary and renal excretion (Yamamoto et al. 2007). Minimal oxidative metabolism (a phase I reaction) has been observed in all species evaluated. In humans, ezetimibe is rapidly metabolized to ezetimibe-glucuronide. Ezetimibe and ezetimibe-glucuronide are the major compounds detected in plasma, constituting approximately 10 to 20% and 80 to 90% of the total drug in plasma, respectively. Both ezetimibe and ezetimibe-glucuronide are slowly eliminated from plasma with a half-life of approximately 22 hours for both ezetimibe and ezetimibe-glucuronide. Plasma concentration-time profiles exhibit multiple peaks, suggesting enterohepatic recycling (Yamamoto et al. 2007). Ezetimibe was the major component in feces and accounted for 69% of the administered dose, while ezetimibe-glucuronide was the major component in urine and accounted for 9% of the administered dose.

1.11.1.4 Effects of age, gender, race, hepatic and renal impairment

The effect of ezetimibe was observed in populations in a multiple dose study. In a dose of 10 mg once daily for 10 days with ezetimibe, plasma concentrations for total ezetimibe were about 2-fold higher in older (≥65 years) healthy subjects compared to younger subjects. Further, the absorption and metabolism of ezetimibe were similar in adolescents (10 to 18 years) and adults. Based on total ezetimibe, there are no pharmacokinetic differences between adolescents and adults. Pharmacokinetic data in the pediatric population <10 years of age are not available. The plasma concentrations for total ezetimibe were slightly higher (<20%) in women than in men. Based on a meta-analysis of multiple-dose pharmacokinetic studies, there were no pharmacokinetic differences between
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Blacks and Caucasians. There were too few patients in other racial or ethnic groups to permit further pharmacokinetic comparisons.

In the same study, the mean area under the curve (AUC) for total ezetimibe was increased approximately 1.7-fold in patients with mild hepatic insufficiency, compared to healthy subjects. The mean AUC values for total ezetimibe and ezetimibe were increased approximately 3-4 fold and 5-6 fold, respectively, in patients with moderate or severe hepatic impairment. In a 14-day, multiple-dose study (10 mg daily) in patients with moderate hepatic insufficiency, the mean AUC values for total ezetimibe and ezetimibe were increased approximately 4-fold on Day 1 and Day 14 compared to healthy subjects. Due to the unknown effects of the increased exposure to ezetimibe in patients with moderate or severe hepatic insufficiency, ezetimibe is not recommended in these patients. After a single 10-mg dose of ezetimibe in patients with severe renal disease (n=8; mean CrCl <30 mL/min/1.73m²), the mean AUC values for total ezetimibe, ezetimibe-glucuronide, and ezetimibe were increased approximately 1.5-fold, compared to healthy subjects (n=9).

1.11.1.5 Mechanism of action

Ezetimibe potently and selectively prevents the absorption of cholesterol from dietary and biliary sources by inhibiting the mechanism by which cholesterol is transported through the intestinal wall. It does so without interfering with the absorption of triglycerides, fat-soluble vitamins, fatty acids, or bile acids (Nutescu and Shapiro 2003). Specifically, it appears to bind to a critical mediator of cholesterol absorption, the Niemann-Pick C1-Like 1 (NPC1L1) protein on the g.i. tract epithelial cells as well as in hepatocytes (Yamamoto et al. 2007).

1.11.2 Valsartan: Drug Profile

1.11.2.1 Physicochemical properties

Chemically valsartan is N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl) [1,1′-biphenyl]-4-yl][methyl]-L-valine with an empirical formula of C_{24}H_{29}N_{5}O_{3} (Figure 37) and a molecular weight of 435.5. It is a white to practically white fine powder melting at 105-110 °C with decomposition. It is soluble in ethanol and methanol, and slightly soluble in water. It is also a highly permeable drug with log P of 5.8.

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1.11.2.2 Absorption and distribution

In the rat, marmoset and human, valsartan was absorbed to a moderate extent. Its peak plasma concentration is reached 2 to 4 hours after dosing. Absolute bioavailability for the capsule formulation is about 25% (range 10%-35%) (Flesch et al. 1997; Martin and Krum 2002).

![Chemical structure of valsartan](image)

Food decreases the exposure (as measured by AUC) to valsartan by about 40% and peak plasma concentration ($C_{\text{max}}$) by about 50%. It is highly bound to proteins of plasma or serum (95%), mainly serum albumin (Csajka et al. 1997). Valsartan does not accumulate appreciably in plasma following repeated administration. The steady state volume of distribution of valsartan after intravenous administration is small (17 L), indicating that valsartan does not distribute into tissues extensively (Mitsuyama 2007).

1.11.2.3 Metabolism and Excretion

Valsartan was poorly metabolized in animals and humans. The enzyme(s) responsible for valsartan metabolism have not been identified but do not seem to be CYP 450 isoforms. It shows biexponential decay kinetics following intravenous administration, with an average elimination half-life of about 6 hours (Kimura et al. 2002). When administered as an oral solution, it is primarily recovered in feces (about 83% of dose) and urine (about 13% of dose). The recovery is mainly as unchanged drug and the enzyme(s) responsible for its metabolism have been identified to be CYP2C9 (Nakashima et al. 2005). Following intravenous
1. INTRODUCTION

administration, plasma clearance of valsartan is about 2 Lh\(^{-1}\) and its renal clearance is 0.62 Lh\(^{-1}\) (i.e., about 30% of total clearance) (Brookman et al. 1997).

1.11.2.4 Effects of age, gender, race, hepatic and renal impairment

Exposure (measured by AUC) to valsartan is higher by 70% and the half-life is longer by 35% in the elderly than in the young. The pharmacokinetics of valsartan have not been investigated in patients < 18 years of age. Pharmacokinetics of valsartan does not differ significantly between males and females.

On average, patients with mild-to-moderate chronic liver disease have twice the exposure (measured by AUC values) to valsartan of healthy volunteers (matched by age, sex and weight). In general, no dosage adjustment is needed in patients with mild-to-moderate liver disease. Although, care should be exercised in patients with liver disease. There is no apparent correlation between renal function (measured by creatinine clearance) and exposure (measured by AUC) to valsartan in patients with different degrees of renal impairment. Consequently, dose adjustment is not required in patients with mild-to-moderate renal dysfunction. No studies have been performed in patients with severe impairment of renal function (creatinine clearance < 10 mL/min). Valsartan is not removed from the plasma by hemodialysis. In the case of severe renal disease, exercise care with dosing of valsartan

1.11.2.5 Mechanism of action

Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin-converting enzyme (ACE, kininase II). Angiotensin II is the principal pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. Valsartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and angiotensin II circulating levels do not overcome the effect of valsartan on blood pressure.
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1.12 EXCIPIENT MONOGRAPHS

1.12.1 Long Chain Triglycerides (LCT’s)

**Ethyl oleate:** Chemically it is Ethyl (Z)-octadec-9-enoate with a molecular formula of \( C_{20}H_{38}O_2 \) and molar mass of 310.51 g.mol\(^{-1}\). It is a fatty acid ester formed by the condensation of oleic acid and ethanol. It is light yellow colored liquid having a boiling point of 205 to 208°C with HLB of 11.3. It is miscible with chloroform, ethanol (95%), ether, fixed oils, liquid paraffin, and most other organic solvents but practically insoluble in water. It is used as a solvent for pharmaceutical drug preparations involving lipophilic substances and vehicle in certain parenteral preparations intended for intramuscular administration (Rowe et al. 2009).

**Groundnut oil:** It is also known as peanut oil or arachis oil which is a mild tasting vegetable oil derived from peanuts. Its major component fatty acids are oleic acid (46.8% as olein), linoleic acid (33.4% as linolein) and palmitic acid (10.0% as palmitin). The oil also contains some stearic acid, arachidic acid, arachidonic acid, behenic acid, lignoceric acid and other fatty acids. It is light yellow colored liquid having a high boiling point of 475°C. It is very slightly soluble in ethanol (95%), soluble in benzene, carbon tetrachloride, and oils and miscible with carbon disulfide, chloroform, ether, and hexane but which is insoluble in cold water. It is used as an excipient in pharmaceutical formulations primarily as a solvent for sustained-release intramuscular injections. Therapeutically, emulsions containing peanut oil have been used in nutrition regimens and also widely used as edible oil (Rowe et al. 2009).

**Lauroglycol™ FCC:** Chemically it is Propylene glycol monolaurate (type I) with a molecular formula of \( C_{15}H_{30}O_3 \) and molar mass of 258.39 g.mol\(^{-1}\). It is transparent liquid with HLB of 4.0. It is water insoluble and forms a coarse dispersion i.e. emulsion (SEDDS) or a fine dispersion i.e. microemulsion (SMEDDS). It also acts as bioavailability enhancer by inhibiting the enterocytic drug metabolizing enzyme CYP3A4. It can be easily filled in hard gelatin and soft gelatin capsules.

**Lauroglycol™ 90:** Chemically it is Propylene glycol monolaurate (type II) with a molecular formula of \( C_{15}H_{30}O_3 \) and molar mass of 258.39 g.mol\(^{-1}\). It is transparent liquid with HLB of 5.0. It is water insoluble and forms a coarse dispersion i.e.,
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emulsion (SEDDS) or a fine dispersion i.e. microemulsion (SMEDDS). It also acts as bioavailability enhancer by inhibiting the enterocytic drug metabolizing enzyme CYP3A4. It can be easily filled in hard gelatin and soft gelatin capsules.

**Maisine™ 35-1:** Chemically it is Glyceryl monolinoleate with a molecular formula of \( C_{21}H_{38}O_4 \) and molar mass of 354.52 g.mol\(^{-1} \). It is yellow colored viscous liquid with HLB of 4.0. It is oily vehicle for use in self-emulsifying lipid formulations to obtain a coarse dispersion i.e., emulsion (SEDDS) or a fine dispersion i.e. microemulsion (SMEDDS). It also acts as bioavailability enhancer by facilitating lymphatic transport of drugs and thus reduces the hepatic first-pass metabolism. It is a good solvent for lipophilic API and can be easily filled in hard gelatin and soft gelatin capsules.

**Olive oil:** it is a fat obtained from the fruits of olive tree (*Olea europaea*, family Oleaceae), a traditional tree vrop of the Mediterranean Basin. Its major components are saturated fats (palmitic acid: 7.5–20.0%, stearic acid: 0.5–5.0%, arachidic acid: <0.6%, behenic acid: <0.3%, myristic acid: <0.05%, lignoceric acid: <0.2%), unsaturated fats, monounsaturated fats (oleic acid: 55.0–83.0%, palmitoleic acid: 0.3–3.5%) and polyunsaturated fats (linoleic acid: 3.5–21.0 %, \( \alpha \)-linolenic acid: <1.0%). It is light yellow colored liquid having a high boiling point of 300°C with HLB of 7.0. It is slightly soluble in ethanol (95%); miscible with ether, chloroform, light petroleum (50–70°C), and carbon disulfide. It is used as a vehicle for oily injections including targeted delivery systems and has also been used in oral capsules and solutions. It is used widely in the food industry as cooking oil and for preparing salad dressings (Rowe et al. 2009).

**Sesame oil:** It is also known as gingelly oil or til oil which is an edible vegetable oil derived from sesame seeds. Its major component fatty acids are palmitic acid (7-12%), palmitoleic acid (0.5%), stearic acid (3.5-6%), oleic acid (35-50%), linoleic acid (1%) and eicosenoic acid (1%). It is light yellow colored liquid having a high boiling point of 440°C with HLB of 7.0. It is insoluble in water, practically insoluble in ethanol (95%) and miscible with carbon disulfide, chloroform, ether, hexane, and light petroleum. The major use of sesame oil in pharmaceutical formulations is as a solvent in the preparation of sustained-release intramuscular injections. It is used as a solvent in the preparation of emulsions, dry emulsions and...
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Soybean oil: It is a vegetable oil extracted from the seeds of the soybean (*Glycine max*). The major unsaturated fatty acids in soybean oil triglycerides are 7-10% α-linolenic acid, 51% linoleic acid and 23% oleic acid. It also contains the saturated fatty acids, 4% stearic acid and 10% palmitic acid. It is light yellow to yellow colored liquid having a high boiling point of 510°C. It is practically insoluble in ethanol (95%) and water, miscible with carbon disulfide, chloroform, ether, and light petroleum. In pharmaceutical preparations, soybean oil emulsions are primarily used as a fat source in total parenteral nutrition (TPN) regimens. Emulsions containing soybean oil have also been used as vehicles for the oral and intravenous administration of drugs. It is also consumed as edible oil (Rowe *et al*. 2009).

1.12.2 Medium Chain Triglycerides (MCT’s)

**Capmul® MCM:** Chemically it is mono-diglyceride of medium chain fatty acids (mainly caprylic and capric). It is light yellow colored liquid with HLB of 4.0. It is an excellent solvent for many organic compounds including steroids. It is also a useful emulsifier for water-oil systems. This can be used as carrier (vehicle), solubilizer, emulsifier/co-emulsifier and bioavailability enhancer.

**Capmul® MCM L8:** Chemically it is Mono/diglycerides of caprylic acid composed of mono and diglycerides of medium chain fatty acids (mainly caprylic). It is an excellent solvent for many organic compounds, including steroids. It is also a useful emulsifier for water-oil systems. It is also used as carrier (vehicle), solubilizer, emulsifier/co-emulsifier and bioavailability enhancer.

**Capmul® PG8:** Chemically it is Propylene glycol monocaprylate and transparent liquid with HLB of 6.0. It is a derivative of propylene glycol monoester of caprylic acid. It is an excellent solvent and a good bioavailability enhancer for many complex, poorly soluble drug compounds. It is primarily used as a carrier in soft and hard gelatin capsules.

**Capryol™ 90:** Chemically it is Propylene glycol monocaprylate (type II) and transparent liquid with HLB of 6.0. It is oily vehicle for use in self-emulsifying lipid formulations to obtain a coarse dispersion i.e., emulsion (SEDDS) or a fine
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dispersion ie. microemulsion (SMEDDS). It also acts as bioavailability enhancer by inhibiting the enterocytic drug metabolizing enzyme CYP3A4. It can be easily filled in hard gelatin and soft gelatin capsules.

Captex® 200 P: Chemically it is propylene glycol diesters of saturated fatty acids, mainly of caprylic and capric acid of vegetable origin. The low viscosity, excellent lubricity and relatively non-oily character makes it ideally suited for a variety of pharmaceutical and nutritional applications.

Captex® 300: Chemically it is mono-diglyceride of medium chain fatty acids (mainly caprylic and capric). The low viscosity excellent lubricity and relatively non-ionic character make it ideally suited for variety of pharmaceutical, nutritional, personal care, and cosmetic application.

Captex® 355: Chemically it is manufactured by the esterification of glycerin and fatty acids (mainly caprylic and capric) which originate from coconut and/or palm kernel vegetable sources. It is used as carrier (vehicle), solubilizer, emulsifier/ co-emulsifier and bioavailability enhancer.

Labrafac Lipophile™ WL 1349: Chemically it is Medium-chain triglycerides and transparent liquid with HLB of 1.0. It is oily vehicle for use in self-emulsifying lipid formulations to obtain a coarse dispersion i.e., emulsion (SEDDS) or a fine dispersion ie. microemulsion (SMEDDS). It can be easily filled in hard gelatin and soft gelatin capsules.

1.12.3 Emulgents

Cremophor® EL: Chemically it is Polyoxyl 35 castor oil which is is a nonionic solubilizer and emulsifier made by reacting castor oil with ethylene oxide in a molar ratio of 1:35. The main component is polyethylene-glycolricinoleate. It is a pale yellow viscous oily liquid that is clear above 26°C. It has a faint but characteristic odour. The HLB lies between 12 and 14. It forms clear solutions in water and also soluble in many organic solvents, e.g. ethyl alcohol, n-propyl alcohol, isopropyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, trichloroethylene, toluene and xylene. In contrast to anionic emulsifying agents, Cremophor EL becomes less soluble in water at higher temperatures. Thus, aqueous solutions become turbid at a certain temperature.
Labrafac® PG: Chemically it is Propylene glycol dicaprylocaprate and yellow colored liquid with HLB of 2.0. It is oily vehicle for use in self-emulsifying lipid formulations to obtain a coarse dispersion i.e., emulsion (SEDDS) or a fine dispersion i.e. microemulsion (SMEDDS). It can be easily filled in hard gelatin and soft gelatin capsules.

Labrasol®: Chemically it is Caprylocaproyl polyoxy-8 glycerides and transparent liquid with HLB of 14.0. It is a non-ionic water dispersible surfactant composed of well-characterised polyethylene glycol (PEG) esters, a small glyceride fraction and free PEG. It is able to self-emulsify on contact with aqueous media forming a fine dispersion i.e., microemulsion (SMEDDS). It is used as a solubilizer and wetting agent for improving the solubility and wettability of active pharmaceutical ingredients, in vitro and in vivo. It also acts as bioavailability enhancer by inhibiting the enterocytic efflux transporter (known as P-gp inhibition). It can be easily filled in hard gelatin and soft gelatin capsules.

Tween®: These are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides. Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions. They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for P-glycoprotein (Nerurkar et al. 1996) and are also widely used in cosmetics and food products (Rowe et al. 2009).

Tween® 80: Chemically it is Polyoxyethylene (20) sorbitan monooleate with a molecular formula of C_{64}H_{124}O_{26} and molar mass of 1309.68 gmol⁻¹. It is a nonionic emulgent derived from polyethoxylated sorbitan and oleic acid. It is amber colored viscous liquid (300–500 mPas) with HLB of 15. It is soluble in water and other solvents like, ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene.

Tween® 60: Chemically it is Polyoxyethylene (20) sorbitan monostearate with a
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molecular formula of $C_{64}H_{126}O_{26}$ and molar mass of 1311.90 g mol$^{-1}$. It is a nonionic emulgent ethoxylated (20) sorbitan ester based on a natural fatty acid (stearic acid). It is dark yellow colored viscous liquid (600 mPas) with HLB of 14.9. It is soluble in water and ethanol, and insoluble in vegetable oils.

**Tween® 40:** Chemically it is Polyoxyethylene (20) sorbitan monopalmitate with a molecular formula of $C_{62}H_{122}O_{26}$ and molar mass of 1283.54 g mol$^{-1}$. It is a nonionic emulgent ethoxylated (20) sorbitan ester based on a natural fatty acid (palmitic acid). It is yellow colored viscous liquid (500 mPas) with HLB of 15.6. It is soluble in water and ethanol, and insoluble in vegetable oils.

**Tween® 20:** Chemically it is Polyoxyethylene (20) sorbitan monolaurate with a molecular formula of $C_{58}H_{114}O_{26}$ and molar mass of 1127.72 g mol$^{-1}$. It is a nonionic emulgent ethoxylated (20) sorbitan ester based on a natural fatty acid (lauric acid). It is yellow to yellow-green colored viscous liquid (400 mPas) with HLB of 16.7. It is soluble in water and ethanol, and insoluble in vegetable oils.

1.12.4 Polymer precipitation inhibitors (PPI’s)

**Methocel™ (Hydroxyethylmethylcellulose, HPMC):** Chemically it is Cellulose, 2-hydroxyethyl methyl ester which is partly O-methylated and O-(2-hydroxypropylated) cellulose with molar mass of 10,000–150,000 g mol$^{-1}$. It is a white, yellowish-white or grayish-white powder or granules, hygroscopic after drying. It is practically insoluble in hot water (above 60°C), acetone, ethanol (95%), ether, and toluene. It dissolves in cold water to form a colloidal solution (Rowe et al. 2009).

It has similar properties to methylcellulose, but the hydroxyethyl groups make it more readily soluble in water and solutions are more tolerant of salts and have a higher coagulation temperature. As a protective colloid, it can prevent droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments. It is used as an excipient in a wide range of pharmaceutical products, including oral tablets and suspensions, and topical gel preparations.

Various grades may be distinguished by appending a number (for e.g. hypromellose 1828) indicative of the apparent viscosity, in mPa s, of a 2% w/w aqueous solution at 20°C. The first two digits refer to the approximate percentage
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content of the methoxy group (OCH$_3$). The second two digits refer to the approximate percentage content of the hydroxypropoxy group (OCH$_2$CH(OH)CH$_3$), calculated on a dried basis. E type possesses about 29% methyl group substitution and hydroxypropoxy group substitution is almost 8-10%. Also, the E type HPMC is more hydrophobic and have different viscosity grades (corresponding to different average molecular weight) available. From the nomenclature of HPMC polymers, the viscosity grade and the average MW of these HPMC polymers follows the same rank order: HPMC E4M (4000 mPas) > HPMC E50LV (50 mPas) > HPMC E15LV (15 mPas) > HPMC E5LV (5 mPas) (Rowe et al. 2009).

**Kollidon® (Polyvinylpyrrolidone, PVP):** Chemically it is poly[1-(2-oxo-1-pyrrolidinyl)ethylene] with a molecular formula of (C$_6$H$_{11}$NO)$_n$ and molar mass of 2,500–3,00,0000 gmol$^{-1}$. It is a synthetic polymer consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, the differing degree of polymerization of which results in polymers of various molecular weights. It is a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder. It is freely soluble in acids, chloroform, ethanol (95%), ketones, methanol, and water; practically insoluble in ether, hydrocarbons, and mineral oil (Rowe et al. 2009).

Although povidone is used in a variety of pharmaceutical formulations, it is primarily used in solid-dosage forms. It is used as a solubilizer in oral and parenteral formulations, and has been shown to enhance dissolution of poorly soluble drugs from solid-dosage forms. The solubility of a number of poorly soluble active drugs may be increased by mixing with povidone. Both PVP 17PF and PVP 25 are water soluble free-flowing white powders with with molar mass of 10,000 gmol$^{-1}$ and 30,000 gmol$^{-1}$, respectively. The viscosities of the polymers are in the range of 1.5 – 3.5 mPas and 3.5 – 5.5 mPas.

1.12.5 Cationic charge inducers

**Oleylamine:** Chemically it is (Z)-9-Octadecen-l-amine with a molecular formula of C$_{68}$H$_{137}$N and molar mass of 267.50 g.mol$^{-1}$. It is clear to slight yellow liquid with boling point between 348 and 350°C. It can function as a cationic surfactant, solvent for the reaction mixture and as a coordinating agent to stabilize the surface of the particles.
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**Oleylamine:** Chemically it is 1-Aminooctadecane with a molecular formula of $C_{18}H_{39}N$ and molar mass of 269.52 g mol$^{-1}$. It is white to off-white solid with boiling point between 47 and 53°C. It is practically insoluble in water. It is used as a cationic surfactant and facilitate stabilization of dispersion of solid materials stabilizer.

**Chitosan:** It is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acytlated unit). On average, the molecular weight of commercially produced chitosan is between 3800 and 20,000 Daltons. It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide. It is sparingly soluble in water, practically insoluble in ethanol (95%), other organic solvents and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric and sulfuric acids). It occurs as odorless, white or creamy-white powder or flakes. Chitosan is a cationic polyamine with a high charge density at pH < 6.5, and so adheres to negatively charged surfaces and chelates metal ions. Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable (Rowe et al. 2009).