Chapter-3

Review of literature
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3.1. Review of literature on LCH

LCH (LCH) is chemically, 2-[(3,3diphenylpropyl) methyl-amine]-1, 1-dimethylethylmethyl 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5 pyridine dicarboxylic ester. It is a new third generation drug which belongs to the well-known pharmacological active compound series classified as 1, 4-dihydropyridine calcium channel blockers used in treatment of hypertension (Parmar et al. 327-38). The oral bio-availability of LCH is approximately 10% and shows erratic absorption from gastrointestinal tract which is attributed to extensive first pass metabolism and low solubility (Barchielli et al.). A useful parameter for identifying ‘poorly soluble’ drugs is the dose: solubility ratio (D/S) of the drug. The dose: solubility ratio can be defined as the volume of gastrointestinal fluids necessary to dissolve the administered dose. When this volume exceeds the volume of fluids available, incomplete bioavailability from solid oral dosage forms is anticipated. Lercanidipine has D/S ratio of 2439 ml (using solubility value determined in pH 1.6) which clearly indicates that the conditions in GI tract are less than optimal for dissolution, since the sink conditions are less likely to prevail. It should be noted that for drugs having a D/S higher than 1,000 ml, the solubility issues cannot be overcome by bile components. Detailed drug profile is described in table 3.1.

Table 3.1. Profile of Lercanidipine HCl as reported on the web source
(http://www.medicines.org.au/)

<table>
<thead>
<tr>
<th>Properties</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physicochemical properties</strong></td>
<td></td>
</tr>
<tr>
<td>Chemical Name</td>
<td>3,5-pyridinedicarboxylic acid, 1,4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-2-[(3,3diphenylpropyl)methylamino]-1,1-dimethylethyl methyl ester hydrochloride.</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>
**Mechanism of Action**
Lercanidipine is a calcium antagonist of the dihydropyridine group and selectively inhibits the transmembrane influx of calcium into cardiac and vascular smooth muscle, with a greater effect on vascular smooth muscle than on cardiac smooth muscle. The antihypertensive action is due to a direct relaxant effect on vascular smooth muscle which lowers total peripheral resistance and hence blood pressure. LCH has a prolonged antihypertensive activity because of its high membrane partition coefficient. It is devoid of negative inotropic effects and its vascular selectivity is due to its voltage-dependent calcium antagonist activity. Since the vasodilatation induced by LCH is gradual in onset, acute hypotension with reflex tachycardia has rarely been observed in hypertensive patients.

**Adverse Effects**
The common adverse events include flushing, palpitation, tachycardia, dizziness, vertigo, headache, nausea, dyspepsia, abdominal pain, diarrhoea, somnolence and asthenia (including fatigue and muscle weakness). The rare adverse effects may include Hypotension, orthostatic hypotension, periorbital oedema, anginal pain, myocardial infarction, cardiac failure, dyspnoea, migraine, paraesthesia, cramps legs, taste alteration, vomiting, GI disorder NOS, increased GGT, polyuria, urinary frequency, impotence, myalgia, rash, pruritus, allergic dermatitis, hives,
sweating increased, anxiety, insomnia, hypercholesterolemia, chest pain, malaise.

**Drug Interaction**

Since the main metabolic pathway of lercanidipine involves the enzyme CYP3A4, drugs that inhibit or induce this enzyme have the potential to alter the plasma concentration of the compound. Therefore, inhibitors of CYP3A4 (such as ketoconazole, itraconazole, erythromycin, ritonavir and fluoxetine) may increase the plasma concentration of lercanidipine, and such combinations should be used with caution.

When co-administered with CYP3A4 inducers, such as anticonvulsants (e.g. phenytoin, carbamazepine) and rifampicin, the antihypertensive effect of lercanidipine may be reduced and, therefore, blood pressure should be monitored when the co-administration is foreseen. Also, co-administration of LCH with cyclosporine, metoprolol, β-blockers, cardiac glycosides, cimetidine and simvastatin requires close monitoring.

The metabolism of dihydropyridines can be inhibited by grapefruit juice, leading to increased plasma concentration and hypotensive effect. Alcohol should be avoided while taking lercanidipine since it may potentiate the effect of vasodilating antihypertensive drugs.

**Indication and Usage**

Lercanidipine is indicated for the treatment of hypertension. The recommended dose is 10 mg once daily, at least 15 minutes before a meal. The dose may be increased to 20 mg once daily depending on the individual response. Dose titration should be gradual, as it may take about 2 weeks for the maximal antihypertensive effect to be apparent.

**Pharmacokinetic**

**Absorption**

Lercanidipine is completely absorbed after oral administration. Peak plasma levels of 3.30 ng/mL ± 2.09 and 7.66 ng/mL ± 5.90 occur 1.5-3 hours after dosing with 10 mg and 20 mg, respectively. The absolute bioavailability of LCH is about 10%, because of high first pass metabolism. The bioavailability increases 4-fold when LCH is ingested up to 2 hours after a high fat meal, and about 2-fold when taken immediately after a carbohydrate-rich meal. Consequently, LCH should be taken at least 15 minutes before a meal. With oral administration, LCH
Review of literature

exhibits non-linear kinetics. After 10, 20 or 40 mg, peak plasma concentrations observed were in the ratio 1:3:8 and areas under plasma concentration-time curves in the ratio 1:4:18, showing a progressive saturation of first pass metabolism. Accordingly, bioavailability increases as dosage increases. The two enantiomers of LCH have a similar time to peak plasma concentration. The peak plasma concentration and AUC are, on average, 1.2-fold higher for the (S)-enantiomer. No in-vivo interconversion of enantiomers is observed.

| Distribution | Distribution of LCH from plasma to tissues and organs is rapid and extensive. Serum protein binding exceeds 98%. The free fraction of LCH may be increased in patients with renal or hepatic impairment as plasma protein levels are decreased in these disease states. |
| Metabolism | As for other dihydropyridine derivatives, LCH is extensively metabolised by CYP3A4. It is predominantly converted to inactive metabolites; no parent drug is found in the urine or faeces. About 50% of the dose is excreted in the urine. |
| Excretion | The mean terminal elimination half-life of S- and R- LCH enantiomers is 5.8 ± 2.5 and 7.7 ± 3.8 hours, respectively. No accumulation was seen upon repeated administration. The therapeutic activity of LCH lasts for 24 hours, due to its high binding to lipid membranes. |

Considering the physicochemical properties and bioavailability related problem several studies have been reported presenting LCH in different formulation strategies.

Parmar et al. developed and characterized self-nanoemulsifying drug delivery system (SNEDDS) to improve the oral bioavailability of poorly soluble Lercanidipine (LER). They estimated solubility of the LER in various oils, co-surfactants and surfactants to construct pseudo-ternary phase diagrams. Using the phase diagrams, they explained the effect of co-surfactants on the nanoemulsifying area and the effect of number and length of hydrophobic alkyl chains of co-surfactant on its emulsification capacity. The optimized formulation containing Cremophor EL (45% wt/wt), (13.5% wt/wt) Caproyl 90 with (1.5% wt/wt) Transcutol®HP and Maisine oil (10% wt/wt) was characterized for percentage transmittance, emulsification time, viscosity and droplet
size. The mean droplet size of selected formulation was 20.01nm. In their study, the \textit{in-vitro} dissolution profile of LER SNEDDS was found significant in comparison to the marketed LER (Zanidip) tablet and pure drug in pH 1.2, 4.5 and 6.8 buffers. Optimized formulation filled into hard gelatin capsules, when stored in stability conditions of 30°C/65% RH, 40°C/65% RH and 50°C/75% in glass bottles, showed no significant degradation (p>0.05) in 3 months. The study concluded that SNEDDS of LER, owing to its nano-size, has potential to enhance the absorption of drug (Parmar et al. 327-38).

\textbf{Kallakunta et al.} investigated self emulsifying powder (SEP) to improve the solubility of poorly soluble LCH. They formulated liquid SEDDS of LCH with Capmul MCM L8 as oil, Tween (R) 80 as surfactant and PEG 400 as co surfactant after screening various vehicles. They then evaluated prepared formulations for self emulsifying ability, globule size, effect of pH and robustness to dilution, cloud point, thermodynamic stability, surface morphology and drug release. They showed that system was robust to different pH media and dilution volumes and possessed a mean globule size of 169±06 nm and cloud point of 76 °C. They prepared self emulsifying powder by adsorbing the liquid SEDDS on to neusilin as carrier. The SEP formulated was free flowing with similar emulsification characteristics as that of liquid SEDDS. They proved transformation of crystalline structure of LCH because of its molecularly dissolved state in the liquid SEDDS with the help of X-ray diffraction, Differential Scanning Calorimetric studies and scanning electron microscopy of SEP. They concluded increase in dissolution characteristics of LCH due to high dissolution efficiency of SEP compared with pure drug (Kallakunta et al. 375-82).

\textbf{Ranpise et al.} designed LCH-loaded nanostructured lipid carriers to investigate whether the bioavailability of the same can be improved by oral delivery. They prepared LCH nanostructured lipid carriers by the method of solvent evaporation at a high temperature and solidification by freeze drying and evaluated the same for particle size analysis, zeta potential, entrapment efficiency, \textit{in-vitro} drug diffusion, ex vivo permeation studies and pharmacodynamics study. They found that nanostructured lipid carriers had a mean size of 214.97nm and a zeta potential of $-31.6\pm1.5$mV and more than 70% LCH was entrapped in the NLCs. They demonstrated 19.36% release in acidic buffer pH1.2 during \textit{in-vitro} release studies indicating that the drug entrapped in the nanostructured lipid carriers remains entrapped at acidic pH. The proved that the drug release was enhanced from 10% to 60.54% at blood pH in 24h using ex-vivo studies. They showed that NLCs released LCH
in a controlled manner for a prolonged period of time as compared to plain drug during *in-vivo* pharmacodynamic study. Their results clearly indicated that nanostructured lipid carriers are a potential controlled release formulation for LCH and may be a capable drug delivery system for the treatment of hypertension (Ranpise, Korabu and Ghodake 81-87).

**Thenge et al.** prepared LCH patches using different concentration of Eudragit RS100, hydroxypropyl methyl cellulose and ethyl cellulose using solvent casting techniques on a mercury substrate. They prepared formulations using 20 mg (LCH), 10% w/w of propylene glycol and 10% w/w of dibutyl phthalate in ethanol. The formulations showed uniform thickness, weight and drug content. They evaluated the effect of polymer on the various physicochemical characteristics by performing *in-vitro* drug release studies and ex vivo skin permeation studies. They suggested that maximum drug release in 24 h from the formulations were dependent on the hydrophobicity of the polymer. In their study, formulation containing (Eudragit RS100 and hydroxypropyl methyl cellulose) showed sustained and extended drug release over a period of 24 hrs. Further they also showed that patches of ERS 100: HPMC were better as compared to ERS 100: EC patches. Their study concluded that LCH could be administered transdermally over a period of 24 h. through the matrix type transdermal drug delivery systems for effective control of hypertension (Thenge et al. 253-58).

### 3.2. Review of literature on Self-emulsifying drug delivery systems

For poorly water soluble drugs, distinct formulations are essential to be designed. The options can include the micronization of the drug, chemical modifications like salt formation, and some other formulation techniques like solid dispersions, cyclodextrin complexes, or lipid formulations (Klein 397-406). The ability of lipid vehicles (either in the pharmaceutical formulation or in food) to enhance the absorption of lipophilic drugs has been well known for many years. Porter et al. in their prospective review highlighted many of the important fundamental guiding the formulation of poorly water soluble drugs using lipids. They mentioned that formulations containing natural and/or synthetic lipids present a viable means for enhancing the oral bioavailability of some poorly water-soluble, highly lipophilic drugs. They emphasized the capacity of lipids to enhance drug solubilization in the intestinal milieu, recruit intestinal lymphatic drug transport (and thereby reduce first-pass drug metabolism) and alter enterocyte-based drug transport and disposition (Porter, Trevaskis and Charman 231-48).
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Lipid formulations involve incorporation of drug compound into inert lipid vehicles such as oils, surfactant dispersions, emulsions and self-emulsifying formulations. Lipid systems may include triglycerides, mono and diglycerides, lipophilic surfactants, hydrophilic surfactants and co-solvents; excipients with a wide variety of physicochemical properties (Pouton S93-S98). The other mechanisms behind augmented bioavailability include enhanced dissolution and solubilisation of the co-administered lipophilic drug by stimulation of biliary and pancreatic secretions, prolongation of gastrointestinal tract (GIT) residence time, increased intestinal wall permeability and reduced metabolism and efflux activity (Tatyana Gershanik and Simon Benita 179-88). Some of the breakthrough research reports are reported in following subsections.

3.2.1. Liquid self-emulsifying drug delivery systems

Pouton et al. reported in their study that self-emulsifying formulations have potential uses as vehicles for the administration of lipophilic drugs by the oral route. They compared the rates of emulsification by monitoring the relative intensity of light scattered by the dispersion continuously during the process. They also compared particle sizes of resultant emulsions by light microscopy and a Coulter Nano-Sizer. They produced efficient self-emulsifying formulations using the oils Miglyol 812 or Miglyol 840 in combination with the surfactant Tween 85. They concluded that the finest dispersions were produced rapidly and in reproducible time by a mixture of 30% w/w Tween 85 and 70% w/w Miglyol 812 (Colin W 335-48).

Charman et al. formulated a lipophilic compound, WIN 54954 in medium chain triglyceride oil/non-ionic surfactant mixture. They found that an optimized formulation consisting of 25% w/w surfactant, 40% w/w oil and 35% w/w WIN 54954 showed rapid emulsification under gentle agitation in 0.1 N HCL (37°C) and produced dispersions with mean droplet diameters of less than 3 μm. They compared self emulsifying preparation with polyethylene glycol (PEG 600) solution formulation by administering each as prefilled soft gelatin capsules to fasted beagle dogs in a parallel crossover study. The investigators determined pharmacokinetic parameters and calculated absolute bioavailability by comparison to an i.v. injection. They confirmed that SEDDS improved reproducibility of the plasma profile in terms of the maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (t_{max}) (Charman et al. 87-93).
Mohsin et al. investigated changes in phase behavior during dispersion of anhydrous lipid formulations, and also investigated the effect of lipid/surfactant ratios, and the presence of co-solvent on the performance of formulations. They further studied the influence of chain length and combination of mono-, di- and tri-glycerides on the phase behavior. They carried out droplet size studies to assess the influence of lipid and surfactant on the resultant droplet size upon aqueous dilution. They reported that mixture of mono-, di- and tri-glycerides with equal amount of surfactant can promote great absorption of water and produce efficient self-emulsification systems (particularly, self-emulsifying/microemulsifying drug delivery systems). In their study they also stated that increasing the mono glyceride concentration within the oil component enhanced water solubilisation significantly. They found that, among the phase diagram studies, Imwitor 308/Tween 80 systems produced a large optically transparent nano dispersing region. They determined that the selection of glycerides seems to be the most vital oil component in designing optimal self-emulsifying lipid formulations (Mohsin and Pouton 531-40).

Mohsin et al. investigated the precipitation of a lipophilic drug following dispersion of lipid formulations in water. They formulated model drug fenofibrate using medium chain glycerides, polysorbates, and propylene glycol as excipients. They found that aqueous dispersion of water-insoluble self-emulsifying lipid formulations resulted in turbid emulsions, followed subsequently by very slow precipitation of 3–7% of the dose of fenofibrate. They also mentioned that the self-emulsifying formulations that included water-soluble surfactants, dissolved a lower mass of drug in solution at equilibrium, but typically maintained drugs in a metastable state, following dilution with water, for several hours or even days. They found that extensive precipitation of fenofibrate from oil-free formulations, comprising of only surfactants and co-solvents, took place within 30 min. They indicated in their results that the extent of precipitation varied significantly between formulations and was influenced by the extent of supersaturation after dilution. They concluded that the use of hydrophilic formulations for delivery of lipophilic drugs may result in a greater extent of drug precipitation in the stomach (Mohsin, Long and Pouton 3582-95).

Benita et al. developed and characterized a self-emulsifying oil formulation (SEOF) comprised of Tween 80, benzyl alcohol (BA), ethyl oleate (EO), and oleylamine (OA), able to produce positively charged submicron emulsions upon aqueous or buffer dilution. They found that positive charge of the formulation was due to the localization of the cationic lipid, OA, at the oil/water interface of
the diluted SEOFs. In the results of binary phase diagram analysis, they observed that the SEOF elicited progressive inverse phase behavior under continuous aqueous phase dilution. They also found that self-emulsification process was not markedly affected by the variation in pH over the entire physiological range. They concluded that only the positively charged SEOF could be considered a potential effective dosage form for oral administration of progesterone since it elicited the highest and most satisfactory absorption profile when a comparative oral bioavailability studies were conducted in young female rats using several different liquid dosage forms of progesterone (T. Gershanik and S. Benita 147–57).

However, there are only a few SEDDS available in the market, the research fraternity has extensively explored this technology for a wide range of drugs. Some of the self emulsifying drug delivery systems for different drugs reported in the literature over last decade are shown in Table 3.2.
Table 3.2. Self emulsifying drug delivery systems reported in the literature over last decade

<table>
<thead>
<tr>
<th>Drug-Formulation</th>
<th>Oil/s</th>
<th>Surfactant/s:Co-surfactant/s</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin-SEDDS</td>
<td>Fractionated oat oil and medium chain monoglycerides</td>
<td>Galactolipid fraction from oats</td>
<td>Fractionated oat oil promoted absorption of cyclosporin with similar characteristics as to commercial formulation, Sandimmune Neoral®</td>
<td>(Odeberg et al. 375-82)</td>
</tr>
<tr>
<td>Simvastatin-SMEDDS</td>
<td>Capryol 90</td>
<td>Cremophor EL: Carbitol</td>
<td>Absorption of simvastatin from SMEDDS resulted into 1.5 fold increase in bioavailability compared with conventional tablet Zocor®</td>
<td>(Kang et al. 65-73)</td>
</tr>
<tr>
<td>Oleanolic acid-SNEDDS</td>
<td>Sefsol 218 (Propylene glycol monocaprylic ester)</td>
<td>Cremophor EL/Labrasol Transcutol P</td>
<td>Relative bioavailability of SNEDDS showed 2.4 fold increase compared with that of the tablet.</td>
<td>(Xi et al. 172-82)</td>
</tr>
<tr>
<td>Phenytoin-SEDDS</td>
<td>Lauroglycol® FCC Labrafac® CC</td>
<td>Labrasol®/Plurol® Oleique : Transcutol®</td>
<td>The concentration after 30 min of SEDDS administration was 4.9 times higher than that after commercial suspension Dilantin® administration.</td>
<td>(Atef and Belmonte 257-63)</td>
</tr>
<tr>
<td>Drug</td>
<td>Base</td>
<td>Surfactants</td>
<td>Comparative pharmacodynamics</td>
<td></td>
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<td>--------------</td>
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<tr>
<td>Fenofibrate</td>
<td>Labrafac CM10</td>
<td>Tween 80 : polyethylene glycol 400</td>
<td>Evaluation was investigated in terms of lipid-lowering efficacy, using a Triton-induced hypercholesterolemia model in rats. The SMEDDS formulation significantly reduced serum lipid levels in phases I and II of the Triton test, as compared with plain fenofibrate. (Patel and Vavia E344-E52)</td>
<td></td>
</tr>
<tr>
<td>Carvedilol</td>
<td>Labrafil M 1944CS</td>
<td>Tween 80 : Transcutol P</td>
<td>The developed SEDDS formulations significantly improved the oral bioavailability of carvedilol significantly, and the relative oral bioavailability of SEDDS compared with commercially available tablets was 413%. (L. Wei et al. 785-94)</td>
<td></td>
</tr>
<tr>
<td>Valsartan</td>
<td>Capmul MCM</td>
<td>Tween 80 : Polyethylene glycol 400</td>
<td>Diffusion of valsartan SMEDDS showed maximum drug release when compared to pure drug solution and marketed formulation. The area under curve and time showed significant improvement as the values obtained were 607 ng h/mL and 1 h for SMEDDS in comparison to 445.36 and 1.36 h for market formulation suggesting significant increase (p&lt;0.01) in (Dixit, Rajput and Patel 314-21)</td>
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<thead>
<tr>
<th>Compound</th>
<th>Formulations</th>
<th>Literature Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persimmon leaf extract - SNEDDS</td>
<td>Labrafil M1944 CS Cremophor EL : Transcutol P Com-pared with the commercial tablets, the AUC of both quercetin and kaempferol, which are representative active flavonoids of PLE, was increased by 1.5-fold and 1.6-fold respectively following oral administra-tion of PLE-loaded SNEDDS in fasting beagle dogs. (Li et al. 161-71)</td>
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<tr>
<td>Silybin – Supersaturatable SEDDS</td>
<td>Labrafac CC Cremophor RH40 : Labrasol, and 5% HPMC The in-vivo study indicated that the area under the concentration–time curve (AUC0→12h) of the silybin-S-SEDDS increased by nearly 3-fold more than those of the conventional SEDDS without the presence of HPMC at a drug dose of 533 mg/kg. (Y. Wei et al. 22-28)</td>
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<tr>
<td>Amiodarone- Talinolol - SNEDDS</td>
<td>Trilaurin / tricaprin Polyoxyl 40-hydroxy castor oil : Tween 20 : Span 80 Oral administration of amiodarone-SNEDDS and talinolol-SNEDDS resulted in higher and less variable AUC and Cmax (Elgart et al. 3029-44)</td>
<td></td>
</tr>
<tr>
<td>Resveratrol- Self-emulsifying systems</td>
<td>Medium-chain triglycerides (Miglyol 812) Polysorbate 80 (Montanox 80 VG PHA) : ethanol 96% v/v formulation of resveratrol as a SEDDS significantly improved its cellular uptake and potentiated its antioxidant properties on bovine aortic endothelial cells (Amri et al. 418-26)</td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>SNEDDS Component</td>
<td>Cremophor RH 40 : Transcutol P</td>
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<td>--------------------------------</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Glycerol triacetate</td>
<td>propylene glycol mono caprylic ester (1:1)</td>
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<tr>
<td>SNEDDS</td>
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<tr>
<td>Daptomycin</td>
<td>Dermofeel MCT</td>
<td>Cremophor RH 40</td>
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<tr>
<td></td>
<td>and Capmul MCM EP</td>
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3.2.2. Solid self-emulsifying drug delivery systems

Lipid-based formulations for poorly water-soluble drugs are generally formulated as liquids because most of the lipids and surfactants suitable for dissolving drugs exist as liquid at room temperature. But, because the solid dosage forms are used more commonly due to their superior stability, lower cost of manufacture and better patient acceptability than liquid, various attempts for the development of solid lipid-based formulations have been reported in the literature. Usually these approaches include adsorption of liquids onto silicates and other porous carriers and spray-drying of liquid formulations mixed with carriers having high surface area such as sucrose, maltodextrin, dextran and different polymeric materials (Shah and Serajuddin). These methods are, however, accompanied with various formulation related issues such as low drug loading, incomplete drug release, poor flow properties of powders, poor compactibility into tablets, and so forth. Despite of these problems, solid self-emulsifying drug delivery systems are still favorite amongst researchers and some of the good examples are reviewed below.

Abdalla et al. developed a new pellet based self-emulsifying (SE) drug delivery system for the oral delivery of progesterone. Furthermore, they investigated the influence of physiological dilution media and enzymatic digestion on the solubilization capacity of the formulation for the model drug Progesterone. They developed and optimized lipid mixtures composed of Solutol® HS 15 and medium chain glycerides with respect to their self-emulsifying properties. They mixed liquid SE lipid with micro-crystalline cellulose and transformed it into pellets by extrusion/spheronization. They found that the droplet diameter of the dispersed SE mixtures was largely affected by changing the oil to Solutol® HS 15 ratio and digestion of SE mixtures changed the solubilisation capacity for Progesterone. Pellets with good properties (size, shape and friability) have been produced through the incorporation of a selected SE mixture into MCC. In conclusion, extrusion/spheronization is a suitable process to produce solid self-emulsifying pellets with up to 40% load of a liquid SE mixture. Digestion induces a change in lipid composition which affects the solubilization capacity of the lipid phase (Abdalla, Klein and Mäder 457-64).

Dixit et al. formulated self-nanoemulsifying granules (SNGs) of ezetimibe to enhance its solubility. Various modified oils, surfactant and co-surfactant mixtures were used and composition of self-nanoemulsifying system (SNS) was optimized. SNS diluted and resultant emulsion was characterized for mean globule size and stability. The self-nanoemulsifying systems were
formulated into free flowing self-nanoemulsifying granules using varying proportions of hydrophilic colloidal silicon dioxide as an adsorbing agent. Self-nanoemulsifying granules were characterized by X-ray diffraction pattern, differential scanning calorimetry, dissolution profile and for in-vivo performance in rats. X-ray diffraction studies indicated loss of crystallinity and/or solubilisation of ezetimibe in the self-nanoemulsifying granules. It was supported by SEM studies, which did not show evidence of precipitation of the drug on the surface of the carrier. Dissolution studies revealed remarkable increase in dissolution of the drug as compared to plain drug. In-vivo evaluation in rats showed significant decrease in the total cholesterol levels as compared to positive control. The SNGs filled into hard gelatin capsules showed two to threefold increase in the dissolution rate as compared to plain drug filled capsules signifying its potential in improved delivery of lipophilic drugs (Dixit and Nagarsenker 183-92).

Agarwal et al. investigated the dynamics of powder flow upon griseofulvin-self-emulsified drug delivery system (SEDDS) addition to silica and silicates and the effect of these adsorbents on drug release. They prepared the mixtures at SEDDS/adsorbent ratios from 0.25:1 to 3:1 on magnesium aluminum silicate [5 and 80m], calcium silicate [25m], and silicon dioxide [3.6, 20, and 300m]. They evaluated powder flow was using the powder rheometer and compared to angle of repose. They explained that the effect of SEDDS on the flow behavior of the adsorbents could be correlated to stepwise or continuous growing behavior as observed in wet granulation process. They further elaborated that due to the porous nature, adsorbents exhibited an initial lag phase during which no change in flow was observed. They also noted that dissolution of drug from adsorbed-SEDDS was dependent on pore length and nucleation at the lipid/adsorbent interface. They observed increase in dissolution rate with an increase in surface area which was independent of the chemical nature of the adsorbents. They suggested that particle size, specific surface area, type and amount of adsorbent are important parameters to consider for manufacturing free flowing powder containing liquid SEDDS (Agarwal et al. 44–52).

Shanmugam et al. prepared solid self-nanoemulsifying drug delivery system (S-SNEDDS) containing phosphatidylcholine (PC), as oil phase for the delivery of bioactive carotenoid lutein, by spray drying the SNEDDS (liquid system) containing PC using colloidal silica as the inert solid carrier, and also established the enhanced bioavailability (BA) of lutein from S-SNEDDS. They proved absence of crystalline lutein in the S-SNEDDS with the help of SEM, DSC, and XRPD.
They reported 21-folds and 8-folds enhancement of Cmax and 2.74-folds or 11.79-folds increment in relative BA of S-SNEDDS as compared with lutein powder (LP) and commercial product (CP), respectively. Thus, they concluded that S-SNEDDS containing PC as oil phase could be a useful lipid drug delivery system for enhancing the BA of lutein in-vivo (Shanmugam et al. 250-57).

Shah et al. developed solid self-emulsifying drug delivery systems (SEDDS) for lipids using poloxamer 188 as both solidifying and emulsifying agents. They prepared mixtures of various lipids with poloxamer 188 and PEG 8000 at ~75°C followed by cooling to room temperature. They observed that when added to water, the solid systems containing poloxamer 188 started to disperse in water forming oil globules of 200–600 nm. They did not observe any emulsification of lipids from solids containing PEG 8000, indicating that the surfactant property of poloxamer 188 was responsible for emulsification. They established that poloxamer 188 and PEG 8000 maintained their crystallinity in solid systems, while the lipids were interspersed in between crystalline regions, from Powder XRD, DSC and microscopic examination. At the same time they showed that the drug remained solubilized in the lipid phase. They developed a novel solid SEDDS in which the drug can be solubilized in liquid lipids and can be converted to solid mass by dispersing into the microstructure of poloxamer 188 (Shah and Serajuddin 2817-32).

Inugala et al. investigated the potential of solid self-nanoemulsifying drug delivery system (S-SNEDDS) composed of capmul MCM C8 (oil), tween 80 (surfactant) and transcutol P (co-surfactant) in improving the dissolution and oral bioavailability of darunavir. They developed and evaluated liquid self-nanoemulsifying drug delivery systems (L-SNEDDS). They reported formation of nanoemulsion (144±2.3nm) from the optimized L-SNEDDS which showed in-vitro drug release of about 13.3±1.4% within 30 min from L-SNEDDS followed by slow continuous release of entrapped drug and reached a maximum of 62.6±3.5% release at the end of 24h. They physically adsorbed L-SNEDDS onto neusilin US2. They further found faster dissolution of darunavir from the developed S-SNEDDS with 3 times greater mean dissolution rate (MDR) compared to pure darunavir. They confirmed the presence of drug in non-crystalline amorphous state without any significant interaction of drug with the components of S-SNEDDS from the results of solid state studies. Furthermore, in-vivo pharmacokinetic studies in Wistar rats they observed enhanced values of peak drug concentration (Cmax) for L-SNEDDS ((2.98±0.19 µg/mL)}
and S-SNEDDS (3.7±0.28 µg/mL) compared to pure darunavir (1.57±0.17 µg/mL) (Inugala et al. 1-10).

3.3. Review of literature on biorelevant media

Dissolution of the drug from the SEDDS is a complex process, which is not only affected by physicochemical properties of the drug but the physiological factors such as changing pH, motility, presence of food, digestion by pancreatic enzymes and presence of bile salts and phospholipids also play a decisive role. When the SEDDS are gaining the popularity in the academia and industries, it has become extremely important to focus on designing more realistic in-vitro tests that could mimic the gut and be able to predict the behaviour of the dosage form in-vivo. This belief led to discovery of bio-relevant media. The dissolution tests in bio-relevant media not only saves time but unnecessary animal testing is also bypassed. The research in this field has provided some very good examples of such studies over the past few years and some of them are reviewed below.

**Grove et al.** developed simulated intestinal media (SIM) containing bile salt (BS) and phospholipids (PL) with and without medium chain lipolytic products (MC-LP) or long chain lipolytic products (LC-LP), to study the solubility of seocalcitol. They studied both MC-LP and LC-LP in order to investigate the influence of fatty acid chain length on the in-vitro solubility of seocalcitol and they found the same solubility of seocalcitol in both the media containing either MC-LP or LC-LP. They further investigated the oral bioavailability of seocalcitol dissolved in medium chain triglyceride (MCT), long chain triglyceride (LCT), and a reference formulation containing propylene glycol (PG) in-vivo in rats. They discovered that the lipid formulations showed a twofold increase in bioavailability compared with the reference formulation, indicating positive effects of lipids on the bioavailability reflecting a better solubility in the intestine and protection against precipitation of seocalcitol in the gastrointestinal tract. They also confirmed that there was no difference in the in-vivo bioavailability of seocalcitol between the MCT and the LCT solutions, which was correlated with the identical in-vitro solubility of seocalcitol in the SIM containing MC-LP or LC-LP (Grove et al. 1830-38).

**Mullertz et al.** studied the usefulness of selected biorelevant dissolution media (BDM) to predict in-vivo drug absorption. They compared the dissolution profiles of solid formulations of a poorly soluble model compound in BDM simulating fasted and two levels of fed state. They also
investigated a non-physiologically relevant medium containing the cationic surfactant, cetrimide, and found that all the media studied were capable of differentiating between the formulations employed. They carried out an *in-vivo* dog study and tried to obtain a level A correlation between the plasma absorption curves and *in-vitro* dissolution curves, using non-linear regression software. From the results of correlation, they suggested that fed state media containing high levels of both bile salts (BS) and lipolysis products (LP) were best able to predict *in-vivo* pharmacokinetic parameters (Cmax and AUC) with prediction errors lower than 10%. They demonstrated the potential of physiologically relevant media containing both BS and LP for use in formulation and early drug development. They finally concluded that the design and use of appropriate media for *in-vitro* dissolution is extremely important. (Lue et al. 648-57).

Jantratid et al. developed and compared biorelevant dissolution test methods for lipid formulations of RZ-50, an experimental compound, with standard compendial methods in terms of their *in-vivo* predictability. They studied the release of RZ-50, a poorly soluble weakly acidic drug, from lipid suspensions filled in soft gelatin capsules in compendial and biorelevant media using the USP Apparatus 2 (paddle method) and the USP Apparatus 3 (Bio-Dis method) and obtained the pharmacokinetic data in dogs upon oral administration in postprandial state. They evaluated the *in-vitro* methods in terms of their ability to fit the *in-vivo* plasma profiles using level A IVIVC analysis and curve comparison of fraction drug dissolved vs. absorbed using the Weibull distribution. They found very low drug release with the paddle method owing to poor dispersibility of the lipids in the dissolution media, whereas the Bio-Dis method hydrodynamics facilitated release of the drug by emulsifying the formulation in the medium. They obtained the best IVIVC using a dissolution medium representing fed gastric conditions in combination with the Bio-Dis method and discovered that curve comparisons of the fraction drug absorbed and the fraction drug dissolved profiles based on Weibull distribution fits, yielded similar results. They concluded the Bio-Dis/biorelevant *in-vitro* method to be suitable for lipid formulation (Jantratid et al. 776-85).

Dressman et al. evaluated the ability of *in-vitro* biorelevant dissolution tests to predict the *in-vivo* performance of nanosized fenofibrate (Lipidil 145 ONE®) and microsized fenofibrate (Lipidil – Ter®). They carried out *in-vitro* dissolution using USP apparatus 2 (paddle method) with updated biorelevant media to simulate the pre- and postprandial states. They employed membrane filters with different pore sizes and found that filters with pore sizes of 0.1lm and 0.02lm were able to
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separate molecularly dissolved drug from colloidal and undissolved particles. They used STELLA® software to generate simulated plasma profiles from in-vitro results in combination with two different models: (a) under the assumption of no permeability restrictions to absorption and (b) under the assumption of a permeability restriction. They compared the simulated plasma profiles to in-vivo data for the nanosized and the microsized formulation in the fasted and fed states. They noticed that the first model approach resulted in good correlation for the microsized fenofibrate formulation, but the plasma profile of the formulation containing nano-sized fenofibrate was overpredicted in the fasted state and the second model successfully correlated with in-vivo data for both formulations, regardless of prandial state. They conferred that in the fasted state, absorption of fenofibrate from the nanosized formulation is at least partly permeability-limited, while for the microsized formulation the dissolution of fenofibrate appears to be rate-determining (Juenemann et al. 257-64).

Andreas et al. investigated and forecasted the effect of co-administration of a meal on drug release for delayed and/or extended release mesalamine formulations as well as designed in-vitro tests to distinguish among formulations in a biorelevant way. They investigated five different mesalamine formulations (Asacol® 400 mg, Mezavant® 1200 mg, Pentasa® 500 mg and Salofalk® in the 250 mg and 500 mg strengths) with biorelevant dissolution methods using the USP apparatus III and USP apparatus IV (open loop mode) under both fasted and fed state conditions, as well as with the dissolution methods described in pharmacopeia for delayed and extended release mesalamine products. They observed that changes in release of Asacol®, Mezavant®, Pentasa® and Salofalk® in the proximal gut due to meal intake are forecast to be minimal; while they expected the release for Salofalk® 250 mg to occur much earlier under fed state conditions. They noticed that USP apparatus III was inclined to result in faster dissolution rates and forecast more pronounced food effects for Salofalk® 250 mg than the USP apparatus IV. They suggested the biorelevant dissolution gradients to be able to reflect the in-vivo behavior of the formulations. They determined that in-vitro biorelevant models can be useful in the comparison of the drug release patterns from different delayed and extended release mesalamine formulations as well as estimating effects of concomitant meal intake on drug release (Andreas et al. 39-50).

Breitkreutz et al. developed a biorelevant dissolution setup, in view of the mechanical force of the tongue, the saliva flow, the small fluid volume and the saliva composition. They observed that
in the initial phase, dissolution profiles of KTP (ketoprofen) oro-dispersible films by the biorelevant showed a slower drug release than those obtained with conventional dissolution methods. The researchers observed strong influence of the simulated in-vivo conditions on the dissolution profile. They found that by simulating either saliva flow or mechanical force, the KTP release after 100 s was two to three times higher (18.78% and 14.18%) compared to the profiles, measured without considering one of the parameters (6.76%). However, they showed improvement with in-vitro data, confirmation with in-vivo study was essential in the future plan (Krampe et al. 20-25).

3.3.1. Review of literature for in-vitro lipid digestion study

As the invention of lipid based drug delivery systems, more specifically, SEDDS progressed over the time, it was observed in several studies that digestion of lipidic excipients by pancreatic lipase enzyme in the gut is unavoidable consequence after oral administration of these dosage forms. Formation of lipolytic products and their influence on dissolution and ultimately on the absorption of the candidate drug is the paramount parameter to be considered while designing the characterization strategy of SEDDS. Thus several attempts are made in past few years to explore the effect of lipid digestion on the drug release and few of them are reviewed in this chapter.

Firstly, Macgregor et al., in their review reported that bioavailability of hydrophobic drugs from the gastro-intestinal (GI) tract can be enhanced by formulation in digestible oils. They suggested that in many circumstances digestion will be beneficial in that the drug may be solubilized within mixed micelles of bile components and the products of triglyceride lipolysis and the solubility of hydrophobic drugs in the presence of such micelles is greatly enhanced. They further reported that these lipolysis products can disperse the drug extremely finely and allows rapid partitioning of the drug into the aqueous continuum for absorption. However, they also mentioned that formulation of oils with surfactants may inhibit lipolysis, and therefore SEDDS formulation should include an in-vitro assessment of the lipolysis in the characterization agenda (MacGregor et al. 33-46).

Sek et al. in their research described evaluation of the in-vitro digestion profile and phase behaviour of the common formulation lipids Miglyol 812 (medium chain triglyceride, MCT), Capmul MCM (medium chain monoglyceride/diglyceride mixture), soybean oil (long chain...
triglyceride, LCT) and Maisine 35-1 (long chain monoglyceride/diglyceride mixture). They found that the rate and extent of digestion of the medium chain lipids was greater than the corresponding long chain lipids, and independent of bile salt concentration, with complete conversion to monoglyceride and fatty acid occurring after 30 min digestion. They noticed that the long chain lipid digests separated into an oily phase (containing undigested triglyceride and diglyceride), an aqueous phase (containing bile salt, fatty acid and monoglyceride) and a pellet phase (containing approximately 5 mm of fatty acid, presumably as an insoluble soap) after ultracentrifugation. They observed that higher proportions of long chain fatty acid and monoglyceride were dispersed into the aqueous phase with increasing bile salt concentrations. They also mentioned that in contrast to long chain, medium chain lipolytic products separated only into an aqueous phase and a pellet fraction in a bile-salt-independent manner. They concluded with the finding that the digestion of both the medium and long chain monoglyceride/diglyceride lipid mixtures was more rapid than the corresponding triglyceride, especially at early time points (Sek et al. 29-41).

Kossena et al. prepared colloidal mixtures containing bile salts (BS), phosphatidylcholine (PC), and medium and long-chain monoglycerides and fatty acids to represent typical intestinal contents after digestion of lipid excipients under both low (5 mM BS/1.25 mM PC) and high (20 mM BS/5 mM PC) BS and PC conditions. They observed presence of vesicles, mixed micelles, and simple micelles after size-exclusion chromatography of the colloidal species that formed in the medium-chain digests, whereas the long-chain digests were found to contain only vesicles and mixed micelles. They discussed that in the long-chain digests the mixed micellar phase was the primary drug solubilizing species for griseofulvin, danazol, and halofantrine and at the same time also mentioned that for highly lipophilic drugs, the vesicular phase contributed more towards the solubilization capacity. They finally found that the solubilization capacity of the vesicular phase was main in the medium-chain digests, and no clear patterns were evident in the relationship between drug lipophilicity and proportional solubilisation (Kossena et al. 634-48).

Porter et al. assessed the relative oral bioavailability (BA) of halofantrine base (Hf) in male beagle dogs after administration of a medium chain triglyceride (MCT), a long chain triglyceride (LCT), and a blended LCT/MCT lipid solution formulation of Hf and after administration of suspensions of Hf base and Hf HCl in LCT. The researchers performed a series of in-vitro lipid digestion experiments to explain the data obtained. They found that in-vitro drug solubilization profiles were
significantly dependent on the mass of lipid used in lipid digestion experiments. They observed that at high lipid loads (25 mg triglyceride/mL), MCT formulations gave maximal advantage, whereas at low lipid loads (5 mg triglyceride/mL), LCT formulations delivered improved solubilization capacity. They mentioned that these data were consistent with the in-vivo data where the BA of Hf after oral administration was observed in the rank order of the LCT solution>LCT/MCT blend>MCT solution. In the second BA study they noticed variable exposure after oral administration of a suspension of Hf base or Hf·HCl in LCT and this finding was broadly consistent with in-vitro results too (C. J. H. Porter et al. 1110-21).

Porter et al. investigated the effect of lipid formulation type on in-vitro dispersion and digestion properties and the relationship to oral bioavailability, using danazol as a model lipophilic poorly water-soluble drug. They administered three lipid-based danazol formulations [a long-chain triglyceride solution (LCT-solution) and self-microemulsifying drug delivery systems (SMEDDS) based on long-chain (C18) lipids (LC-SMEDDS) and medium-chain (C8–C10) lipids (MC-SMEDDS)] to fasted beagle dogs and compared the results with a micronized danazol formulation administered postprandially and in the fasted state. They reported that the LCT-solution and LC-SMEDDS formulations significantly improved the oral bioavailability of danazol when compared to administration of the powder formulation in fasted condition. In contrast, though MC-SMEDDS displayed excellent dispersion properties, they exhibited little enhancement in danazol bioavailability. In support of the in-vivo findings, researchers reported that in-vitro digestion of the medium-chain formulation resulted in substantial drug precipitation when compared with the long-chain lipid formulations (C. J. Porter et al. 1405–12).

Dahan and Hoffman examined the correlation between the in-vitro solubilization process of lipophilic compounds from different lipid solutions and the corresponding in-vivo oral bioavailability data with a view to assess the influence of intra-enterocyte processes (metabolism and lymphatic absorption) on this correlation. They tested dissolution of progesterone and vitamin D3 in long (LCT), medium (MCT) and short (SCT) chain triglyceride solutions using a dynamic in-vitro lipolysis model. They derived a rank order of MCT > LCT > SCT for both progesterone and vitamin D3 from the results of the dynamic in-vitro lipolysis experiments. They noticed that the bioavailability of progesterone correlated with the in-vitro data, despite its significant pre-systemic metabolism. They obtained, an in-vivo performance rank order of LCT > MCT > SCT for
vitamin D₃ and when the lymphatic transport was blocked the bioavailability of vitamin D₃ correlated with \textit{in-vitro} data. They concluded the study by reporting that the \textit{in-vitro} lipolysis model is useful for optimization of oral lipid formulations even in the case of pre-systemic metabolism in the gut. However, when lymphatic transport is a major route of absorption, the \textit{in-vitro} lipolysis data may not be prognostic for actual \textit{in-vivo} absorption (Dahan and Hoffman 2165-74).

\textbf{Fatouros et al.} visualized the \textit{in-vitro} digestion of a self nano-emulsifying drug delivery system (SNEDDS) by cryogenic transmission electron microscopy (Cryo-TEM) using the dynamic lipolysis model. From the results, they observed that micelles are present throughout lipolysis process. As the lipolysis progressed they observed oil droplets from the self nano-emulsifying drug delivery system transforming to spherical or elongated unilamellar vesicles and low numbers of bilamellar and open vesicles. They further observed a decrease in the number of unilamellar vesicles and oil droplets after 50\% hydrolysis. They noted an increase to the potential of the hydrolyzing SNEDDS droplets in absolute values inferring incorporation of the micelles to the surface. From the data they tried to emphasize that Cryo-TEM combined with the \textit{in-vitro} dynamic lipolysis model can not only offer useful information on the formation of the various colloid phases during \textit{in-vitro} digestion of lipid-based formulations but can provide a better understanding of the \textit{in-vivo} behavior of these systems, the solubilization of lipophilic drug compounds, designing and optimizing oral lipid-based formulations and possibly predicting their \textit{in-vivo} behavior also. They concluded this methodology to be a useful tool for the strategic development of lipid-based formulations (Fatouros, Bergenstahl and Mullertz 85-94).

\textbf{Christiansen et al.} tested the effects of polysorbate 80 (PS 80), \textit{D--}tocopheryl polyethylene glycol (1000) succinate (TPGS), Surfho\textregistered{} sugar ester D-1216 (sucrose laurate), Cremophor EL (Cr EL) and Cremophor RH 40 (CrRH40) on triglyceride digestion by pancreatic lipases, \textit{in-vitro} using olive oil as model substrate. They could rank the extent of inhibitory action in downward order as Cremophor RH 40>TPGS>polysorbate 80>Cremophor EL>sucrose laurate. The effects already occur below the critical micelle concentrations (CMC) of the detergents. They found that at low concentrations of polysorbate 80 the inhibition shows a competitive mechanism. They detected Polysorbate 80, Cremophor EL and Cremophor RH 40 to be susceptible to digestion by pancreatic enzymes. They concluded fatty acid esters of polysorbate80 to hydrolyze with an extent of 14\% (±1.0\%) and Cremophor EL at 14.4\% (±3.3\%), while Cremophor RH 40 had been diedgested to a
lower extent of 6.1% (±2.8%). They also reported TPGS and sucrose laurate to be stable in presence of pancreatic enzymes (Christiansen, Backensfeld and Weitschies 376-82).

Mohsin et al. investigated the lipid formulation digestibility of fenofibrate formulations (Type II, IIIA, IIIB and IV self-emulsifying/microemulsifying lipid delivery systems (SEDDS and SMEDDDS designed for oral administration) in the simulated gastro intestinal media. Formulations were prepared using various medium-chain glyceride components, non-ionic surfactants and cosolvents as excipients. Soybean oil was used only as an example of long-chain triglycerides to compare the effects of formulation with their counter-parts. They observed that digestion rate was faster and almost completed in Type II and IIIA systems. Most of the surfactants used in the studies were digestible. However, the researchers also found that high concentration of surfactant and/or cosolvent used in Type IIIB or IV systems lowered the rate of digestion. They noticed the digestion of medium-chain triglycerides to be faster than long-chain triglycerides, but it kept comparatively less drug in the post digestion products. They conferred the study with suggestion that medium-chain mixed glycerides are good solvents for fenofibrate because they are rapidly digested, but to improve fenofibrate concentration in post digestion products, the use of long-chain mixed glycerides should be considered for further investigations (Mohsin 637-46).

Buyukozturk et al. studied simultaneous lipid digestion, dissolution/release, and drug partitioning experimentally and modeled for two dosing scenarios: solid drug with a food-associated lipid (soybean oil) and drug solubilized in a model SEDDS (soybean oil and Tween 80 at 1:1 ratio). The researchers measured rate constants for digestion, permeability of emulsion droplets, and partition coefficients in micellar and oil phases to numerically solve the developed model. The developed model help them to predict strong influence of lipid digestion on drug release from SEDDS and solid drug dissolution into food-associated lipid emulsion. They recorded 9% and 70% drug release in the absence and presence of digestion, respectively after ninety minutes of introduction of SEDDS into the medium. However, they noted that complete drug dissolution in the presence of food-associated lipids followed over a longer period than without digestion (Buyukozturk et al. 3131-44).

Ibrahim et al. investigated the intraluminal processing of novel oral lipid-based formulations of amphotericin B by means of an in-vitro lipolysis model. Three lipid-based formulations of
Amphotericin B (AmB) were formulated consisting of different lipid components: iCo-009, iCo-010 and iCo-011 and various lipid loads (0.25, 0.5, 1 and 2 g) were digested using the lipolysis model to assess AmB distribution among the lipolysis phases. They reported that the duration of lipolysis was analogous among the three formulations except for 2 g load of iCo-009 which had a significantly longer lipolysis than iCo-010 and iCo-011. They further elaborated that lipid components of iCo-009 experienced lower extent of lipolysis as compared to other formulations. They could measure highest concentration of Amphotericin B in the aqueous phases with iCo-010 which also had the lowest sediment recovery. They showed that Amphotericin B levels in the undigested lipid layers were comparable between iCo-009 and iCo-010 and were higher than with iCo-011. After the observation that iCo-010 had the highest aqueous micellar solubilization and the lowest sediment recovery of AmB among the tested formulations, they expected these results to be used to interpret and predict the in-vivo performance of AmB- SEDDS formulations in future studies (Ibrahim et al. 323-28).

Williams et al. explored the impact of bile salt (sodium taurodeoxy-cholate, NaTDC) concentration and drug loading on the ability of prepared LBFs containing Danazol to generate and sustain drug solubilization and supersaturation during in-vitro digestion testing and identified common driver of the potential for drug precipitation. Upon increasing NaTDC concentrations, they reported, increased digestion of the most lipophilic LBFs and promotion of lipid (and drug) trafficking from poorly dispersed oil phases to the aqueous colloidal phase (APDIGEST). They reported high NaTDC concentrations to be able to reduce drug precipitation, although, at NaTDC concentrations ≥3 mM, NaTDC effects on either digestion or drug solubilization were modest. In contrast, they figure out that increasing drug load had a marked impact on drug solubilization. For LBFs containing long-chain lipids, they observed that drug precipitation was limited even at drug loads leading to saturation in the formulation and concentrations of solubilized drug in APDIGEST increased with increased drug load. On the contrary they mentioned for LBFs containing medium-chain lipids, significant precipitation was evident, especially at higher drug loads. After complete analysis of the results they concluded that the chance of precipitation was almost entirely dependent on the maximum supersaturation ratio (SRM) attained on initiation of digestion which defines the supersaturation “pressure” in the system and is calculated from the maximum attainable concentration in the APDIGEST (assuming zero precipitation), divided by the solubility of the drug in the colloidal phases formed post digestion. For formulations where phase separation of oil phases
did not occur, a threshold value for SRM was obvious, irrespective of formulation composition and drug solubilization reduced noticeably above SRM > 2.5. They proved the threshold SRM to be an effective tool in discriminating between LBFs based on performance (Williams et al. 3286-300).

**Cuine et al.** investigated the effect of changing the proportions of lipid, surfactant and co-solvent on the solubilisation capacity of self-emulsifying formulations of danazol during *in-vitro* dispersion and digestion studies and correlation with *in-vivo* bioavailability in beagle dogs. However they found all formulations to form microemulsions in the presence of water without drug precipitation on dispersion, drug solubilisation was markedly affected by lipase-mediated digestion and a reduction in lipid (and increase in surfactant) content resulted in increased drug precipitation. Consistent to these results, they noted the bioavailability of danazol to decrease significantly with decreasing the lipid content in the formulations. They also observed A rank-order correlation between the patterns of solubilisation achieved during *in-vitro* digestion and the *in-vivo* performance of self-emulsifying formulations of danazol. They concluded that a decrease in the lipid content and an increase in the proportions of surfactant and co-solvent caused reduced danazol bioavailability (Cuine et al. 748-57).

**Fernandez et al.** investigated Labrasol® lipolysis using either individual enzymes (gastric lipase, pancreatic lipase-related protein 2, pancreatic carboxyl ester hydrolase) or a combination of enzymes under *in-vitro* conditions representing the gastric and duodenal phase of lipolysis. They determined which compounds in Labrasol® were preferentially hydrolyzed using specific methods for quantifying lipolysis products. They observed that gastric lipase had a preference for di- and triacylglycerols and the main acylglycerols remaining after gastric lipolysis were monoacylglycerols. Also PEG-8 diesters were hydrolyzed to a large extent by gastric lipase. They found most of the compounds initially present in Labrasol®, totally hydrolyzed after the duodenal phase of lipolysis. They noticed the rate of Labrasol® hydrolysis by individual lipases to be significantly dependent on dilution of the excipient in water and the resulting colloidal structures (translucent dispersion; opaque emulsion; transparent microemulsion) and each lipase showing a different pattern depending on the particle size. The lipases with different substrate specificities used in this study were found to be sensitive probes of phase transitions occurring after Labrasol® dilution. The researchers concluded these enzymes to be helpful tool for not only developing *in-*
*vitro* digestion models, but for the characterization of self-emulsifying lipid-based formulations (Fernandez et al. 3077-87).

Christophersen et al. evaluated the ability of a gastro-intestinal *in-vitro* lipolysis model to predict the performance of two lipid formulations and a conventional tablet containing a poorly soluble drug, cinnarizine, both in the fasted and fed state. In the fasted state *in-vitro* lipolysis model, they recorded the amount of solubilized cinnarizine decreasing in the order SNEDDS-Capsule >SNEDDS-Tablet >Conventional cinnarizine tablet, which correlated well with the results of *in-vivo* bioavailability study in dog. They further found that in the fed state *in-vitro* lipolysis model, cinnarizine was solubilized to the same amount for all formulations except for conventional tablet for which the performance was improved as compared to fasted state, indicating food effect. This correlated with the *in-vivo* study, where the tablet was the only formulation with a significant food effect. They derived that the fasted state model correlated well with the *in-vivo* results, while the fed state model did not accurately predict the fed state *in-vivo* results, but it could predict which formulation would exhibit a food effect (Christophersen et al. 232-39).

### 3.4. Review of literature on *in-silico* methods for prediction of *in-vivo* fate of formulation

The ultimate goal of any successful of formulation development is to be able to predict &/or expect the similar or better performance of the prepared dosage form *in-vivo*, as estimated in *in-vitro* tests. Since now industries on global platform are focusing on reducing unnecessary pre-clinical testing, *in-vitro-in-vivo* correlation (IVIVC) techniques are gaining popularity.

Qureshi in his report mentioned that evaluating an IVIVC is a essential feature for any drug dissolution test to establish relevance and confidence in assessing the quality and safety of oral dosage products. However, success in this field has been limited. One of the causes for this lack of success may be that the approaches described in the literature to achieve IVIVC appear to be instinctive anticipations rather than a realistic end-point based on scientific basis. For example, rather than predicting an *in-vivo* response based on *in-vitro* results, which is the objective of IVIVC, attempts are generally made to match *in-vitro* results with *in-vivo* results by modifying
experimental conditions for in-vitro testing. (Qureshi 38-47). However recently the trend of using computer based simulation software is observed which not only help predict the pharmacokinetic parameters form in-vitro dissolution data but can provide the opportunity to optimize the dosage form by performing parameter sensitivity analysis. Some of the successful attempts reported in the literature are reviewed below.

Amidon et al. developed a microscopic mass balance approach to predict the fraction dose absorbed of suspensions of poorly soluble compounds. Their mathematical model included four fundamental dimensionless parameters to calculate the fraction dose absorbed: initial saturation ($I_s$), absorption number ($A_n$), dose number ($D_o$), and dissolution number ($D_n$). In their report they mentioned that the fraction dose absorbed ($F$) increases with increasing $I_s$, $A_n$, and $D_n$ and with decreasing $D_o$. At higher $D_n$ and lower $D_o$, the fraction dose absorbed approaches the maximum $F$, which depends only on $A_n$. The dissolution number limit on $F$ can appear at both lower $D_o$ and lower $D_n$. Similarly, at higher $D_o$ and $D_n$, the fraction dose absorbed touches a $D_o$ limit. Initial saturation makes a significant variance in $F$ at lower $D_o$ and $D_n$. They showed that the extent of drug absorption is anticipated to be highly variable when $D_n$ and $D_o$ are approximately one. They further suggested that, by calculating these dimensionless parameters for any given compound, a formulation scientist can estimate not only the extent of drug absorption but also the effect, if any, of particle size reduction on the extent of drug absorption (Oh, Curl and Amidon 264-70).

Chakraborty et al. tried to establish prospective IVIVC method for generic products using example of two different drug formulations (aprepitant capsules, immediate release and donepezil tablets, sustained release). They examined in-vitro dissolution of these formulations using USP-II apparatus and different range of dissolution media. They matched the dissolution profile with the deconvoluted profile of drugs obtained from literature data to select biorelevant dissolution media. They established an IVIVC by using the mean fraction dissolved (FD) and mean fraction absorbed (FA) and tried to simulate the plasma profile of these formulations by convolution from optimized dissolution media. They further compared predicted pharmacokinetic parameters with observed parameters in their in-vivo study. They noticed a positive correlation between the FD and FA for both formulations with $r^2 = 0.989$ for aprepitant and $r^2 = 0.995$ for donepezil. They reported the percent prediction error for both Cmax and AUC<sub>1</sub> less than or equal to 15% while predicting the plasma concentration-time profile for human bioequivalence studies for these formulations. From
the results, researchers could support use of prospective method in establishing IVIVC while predicting *in-vivo* pharmacokinetic profile for bioequivalence studies for generic product development (Chakraborty, Pandya and Aggarwal 1-7).

**Wei et al.** predicted the oral absorption of glyburide using biorelevant dissolution methods, combined with permeability measurements and computational simulations to establish *in-vitro/in-vivo* correlations (IVIVCs). They measured solubility of the glyburide powder and dissolution behavior of two commercial tablet formulations in different media. They used two chemical grades of sodium taurocholate: low quality (LQ) = crude and high quality (HQ) = 97% purity, and egg-lecithin: LQ=60% and HQ=99.1% purity to prepare fasted state small intestinal fluid (FaSSIF) and used simulated intestinal fluid (SIF) and blank FaSSIF without lecithin and taurocholate (BL-FaSSIF) as controls. They predicted the fraction dose absorbed using GastroPlus™ using *in-vitro* data as the input function and compared the results of the simulations with actual clinical data taken from a bioequivalence study. They reported highest solubility of glyburide and significantly different dissolution behaviors of two tablet formulations in LQ-FaSSIF. They achieved best prediction of the average AUC and Cmax of the clinically observed values from the dynamic LQ-FaSSIF dissolution data. Their study showed that BCS based parameters combined with software simulations can be used to establish an IVIVC for glyburide and thus *in-vitro/in-silico* tools can potentially be used as surrogate for bioequivalence studies (Wei and Löbenberg 45-52).

**Shono et al.** used *in-vitro* dissolution testing in biorelevant media coupled with *in-silico* physiologically based pharmacokinetic (PBPK) modeling to the prediction of food effects on the absorption of celecoxib, from 200 mg capsules. The researchers developed PBPK model based on STELLA® software using dissolution kinetics, solubility, standard GI parameters and post-absorptive disposition parameters. They observed an approximately 7-fold difference in the maximum percentage dissolved between *in-vitro* dissolution tests designed to represent the fed and fasted states. While in contrast to above finding they saw that, the food effect estimated by simulating the plasma profiles with the PBPK model predicted only a slight delay in the peak plasma level (1 h), and modest increases in the Cmax and AUC of 1.9-fold and 1.3-fold in the fed state, respectively. They confirmed with their results that the PBPK approach, combining *in-silico* simulation coupled with biorelevant dissolution test results, thus resembles much better to the food effect observed for celecoxib *in-vivo*. Additionally, point estimates of AUC and Cmax as well as
differential index calculations confirmed clear advantages of using results in biorelevant rather than compendial media in the PBPK model (Shono et al. 107-14).

**Okumu et al.** compared the dissolution behaviour of etoricoxib in different dissolution media and established *in-vitro/in-vivo* correlation (IVIVC) using computer simulations. They studied the dissolution behaviour of etoricoxib in the USP Apparatus 2 using different dissolution media. They also adopted dissolution transfer model to investigate whether drug stays in solution upon changing pH of the medium. Drug permeability calculation was done using the caco-2 cell culture technique. The *in-vitro* data were employed as input functions in GastroPlus™ to simulate the *in-vivo* profiles of the drug. The authors observed highest solubility of etoricoxib at low pH, and there was no significant difference in the solubility observed between blank buffers and biorelevant media of similar pH. The drug remained solubilised when transferred into simulated intestinal fluids. Using the *in-vitro* data as input function in GastroPlus™, an IVIVC was established. Further simulations confirmed that the drug absorption occurs similar to the absorption of an oral solution. From the results of the solubility behaviour within the physiological pH gradient of the gastrointestinal tract, etoricoxib was classified as an intermediate class 1/2 drug rather than BCS class 2. Authors concluded that *in-vitro* results combined with *in-silico* simulations using GastroPlus™ can help justify scientifically that a biowaiver for immediate release etoricoxib solid oral dosage forms is appropriate (Okumu, DiMaso and Löbenberg 91-98).

**Grbic et al.** developed a drug-specific absorption model for gliclazide (GLK) using mechanistic gastrointestinal simulation technology (GIST) implemented in GastroPlus™ software package. They employed a range of experimentally determined, *in-silico* predicted or literature data as input parameters. They compared the results of the simulations with actual clinical data and discovered that the GIST-model gave exact prediction of gliclazide oral absorption. Their generated absorption model provided the objective *in-vivo* dissolution profile for *in-vitro–in-vivo* correlation and identification of biorelevant dissolution requirements for GLK immediate-release (IR) tablets. Moreover they used a set of virtual *in-vitro* data for correlation purposes. They suggested that dissolution specification of more than 85% GLK dissolved in 60 min may be considered as “biorelevant” dissolution acceptance criteria for GLK IR tablets (Grbic et al. 165-71).
Wagner et al. predicted the plasma profiles of an immediate release (IR) formulation of Compound A in the fasted and fed state. For this purpose, authors conducted in-vitro biorelevant dissolution tests and transfer model experiments. They then combined dissolution and precipitation kinetics with in-vivo post-absorptive disposition parameters using STELLA software. They also revised developed STELLA model to accommodate for less than optimal permeability characteristics as well as precipitation of the drug in the fasted state small intestine because Compound A not only exhibited poor solubility but also poor permeability. They introduced permeability restrictions into the model using an absorption rate constant estimated from the Caco-2 permeability value of Compound A, the effective intestinal surface area and suitable intestinal fluid volumes. They found that the biorelevant dissolution tests are a helpful tool to predict food effects of Compound A qualitatively. However, they also mentioned that the plasma profiles of Compound A could only be predicted quantitatively when the results of biorelevant dissolution test were combined with the newly developed PBPK model (Wagner et al. 127-38).

Berthelsen et al. developed a sensitive and discriminative in-vitro–-in-silico model able to simulate the in-vivo performance of three fenofibrate immediate release formulations containing different surfactants. In addition, they investigated the effect of dissolution volume while predicting the oral bioavailability of the formulations. The authors carried out in-vitro dissolution studies using the USP apparatus II or a mini paddle assembly, containing 1000 mL or 100 mL fasted state biorelevant medium, respectively. They further performed in-silico simulations of small intestinal absorption using the GI-Sim absorption model twice adopting either a total small intestinal volume of 533 mL or 105 mL, in order to examine the implication of free luminal water volumes for the in-silico predictions. They observed that for the tested formulations, the use of a small biorelevant dissolution volume was critical for in-vitro–in-silico prediction of drug absorption. They obtained very good predictions, demonstrating rank order in-vivo–in-vitro–in-silico correlations for C_{max}, with in-silico predictions using a 105 mL estimate for the human intestinal water content combined with solubility and dissolution data performed in a mini paddle apparatus with 100 mL fasted state simulated media (Berthelsen et al. 356-65).
3.5. References


