CHAPTER 2:

REVIEW OF LITERATURE
2.0 REVIEW OF LITERATURE

2.1 Background: Oral delivery of anticancer drugs

In the past years an increasing interest can be seen towards oral administration of cytotoxic agents with several new oral analogues or oral formulations of commonly used cytotoxic drugs [Demario and Ratain 1998]. Examples are etoposide and analogues, topotecan and related compounds, cyclophosphamide and trophosphamide, idarubicin, vinorelbine, miltefosine and several prodrugs of 5-fluorouracil (5-FU) [Terwogt et al. 1999]. Oral chemotherapy is to be preferred in the first place for its convenience for patients and its potential to improve patients’ quality of life [Liu et al. 1997]. Oral drug treatment is convenient to patients as oral drugs can be taken at home eliminating the need for hospital admission. In addition, oral treatment avoids the discomfort of an injection and the risks of infection and extravasation that are associated with intravenous access lines. Rightly, patients’ quality of life is increasingly becoming a central consideration in cytotoxic drug treatment especially in palliative treatment regimens. A further argument for oral treatment of cytotoxic drugs is that the oral route facilitates the use of more chronic treatment regimens. This is especially important for cell cycle specific agents and agents with a predominately cytostatic effect such as angiogenesis inhibitors and signal transduction inhibitors.

For these agents prolonged exposure to the drug may have pharmacodynamic advantages over intermittent intravenous administration [Kakolyris et al. 1998]. Finally, in view of increasing costs of anticancer therapy oral treatment of cytotoxic agents is attractive, as oral administration eliminates the need for hospitalization, physician and nursing assistance and infusion equipment.

2.2. Considerations in oral drug administration

An obvious prerequisite for oral drug treatment with cytotoxic agents is sufficient bioavailability after oral administration. Bioavailability concerns the rate and extent to which a drug is absorbed into the systemic circulation. Important factors which limit the oral bioavailability of drugs are structural instability in the gastro-intestinal fluids, limited aqueous solubility and dissolution, and/or affinity for intestinal and liver cytochrome P450
metabolic enzymes and the multidrug efflux pump P-glycoprotein, which serve to protect the body from xenotoxins [Bardelmeijer et al. 2000]. Another limitation associated with poor bioavailability is the substantial interpatient variability in oral pharmacokinetics. This is important as cytotoxic drugs have in general a narrow therapeutic window. It is evident that caution is warranted with oral cytotoxic treatment since either toxic or sub-therapeutic dosing may easily occur. Another major impetus in oral treatment of cytotoxic drugs is patient non-compliance. Inability of patients to comply with adequate oral drug intake is thought to be a major source of therapy failure for many diseases. However, patient non-compliance may be less of an issue for oral cancer therapy, because the seriousness of the disease may provide adequate motivation for adherence to the prescribed regimen. Another factor complicating oral treatment with cytotoxic agents is the risk of local irritation of the gastrointestinal tract by the cytotoxic drug and/or its formulation, which can result in side effects such as nausea, vomiting and diarrhea. Finally, the medical condition of the patient may preclude oral drug therapy, such as in obstructive disorders of the gastro-intestinal tract and motility disorders.

2.2.1. Oral Bioavailability
The therapeutic effect of an orally administered drug depends on the rate and extent of drug absorbed from the gastrointestinal (GI) tract. Optimization of the dose fraction reaching the systemic circulation in chemically unchanged form is a central factor during drug development. Bioavailability is the rate and extent to which the active drug or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action [Amidon et al., 1995]. As a consequence of modern drug discovery techniques, there has been a consistent increase in the number of new pharmacologically active lipophilic compounds that are poorly water-soluble. A great challenge facing pharmaceutical scientists is rendering these molecules into orally administered medications with sufficient bioavailability. Oral delivery is the preferred route for drug administration due to several advantages over the other routes (more natural, non-invasive, self administered, less expensive, etc.) and it is by far the easiest and most convenient method for drug delivery during chronic therapy. Several drugs have to be administered parenterally due to their low water solubility [Aungst, 1993]. Nearly 40% of new drug candidates exhibit low water solubility and hence high intra- and inter-subject variability and lack of dose proportionality
[Robinson, 1996; Lipinski et al., 1997; Lipinski, 2000; Gursoy and Benitan, 2004]. Although the relationship between a dose and the corresponding systemic exposure as determined by the total area under time concentration (AUC) curve is highly complex, a host of variables has been identified that limit and affect oral bioavailability. These include:

- Poor dissolution or low aqueous solubility
- Degradation of the drug in gastric or intestinal fluids
- Poor intestinal membrane permeation
- Pre-systemic intestinal or hepatic elimination

The absorption of most drugs depends on two processes:
- Dissolution of the drug in the physiological fluids and
- Absorption process itself.

The large majority of drugs is absorbed by passive diffusion, i.e. spontaneous migration of drug molecules from a region of high concentration to that of low concentration. A smaller percentage is absorbed by facilitated or active transport, which involves expenditure of energy by the body. In either event, the dissolution of the drug is the first step in the absorption process unless the drug is administered as a solution. Drug dissolution is the process in which a drug is released, made available in solution and ready to be absorbed. Physicochemical properties of a drug, such as solubility, and the gastrointestinal environment are the most important parameters affecting drug dissolution [Wadke et al., 1989].

2.3. Taxanes: Overview

Taxanes are the most common anticancer drugs used against a broad range of human malignancies, including ovarian cancer [Green et al., 2009], breast cancer [Overmoyer, 2008], non-small cell lung carcinoma [Schittenhelm et al. 2009] and melanoma [Rowinsky and Donehower 1995]. Paclitaxel and docetaxel are approved for clinical use by the Food and Drug Administration (FDA) board for the treatment of breast cancer, ovarian cancer, non-small-cell lung cancer (NSCLC) and prostate cancer. Taxol® and Taxotere® are commercially available formulations of paclitaxel and docetaxel, respectively, which are administered intravenously at different dosages and infusion schedules [Singla et al., 2002]. The taxanes are a unique class of hydrophobic antineoplastic agents that exhibit cytotoxic activity by binding to tubulin and promoting inappropriately stable, non-functional microtubule formation [Schiff et al. 1979]. Interference with microtubule function leads to
disrupted mitosis and cell death. The toxicity profiles for these agents are somewhat different; paclitaxel has been most widely associated with peripheral neuropathies and myalgias/athralgias, whereas the use of docetaxel most commonly results in cumulative fluid retention that may in some cases be dose-limiting.

Paclitaxel was first discovered in the early 1960s as part of a National Cancer Institute (NCI) screening study to identify natural compounds with antineoplastic activity. Paclitaxel was identified as the crude extract from the bark of the North American pacific yew tree, *Taxus brevifolia*, in the early 1970s and found to exert significant cytotoxic effects in preclinical studies against many tumors [Wani et al. 1971]. However, clinical development was slowed until the early 1980s owing to the scarce supply of the pacific yew tree bark and its poor solubility.

Docetaxel is a semisynthetic compound produced from 10-deacetylbaaccatin-III, which is found in the needles of the European yew tree, *Taxus baccata*. Although slightly more water-soluble than paclitaxel, docetaxel also requires a complex solvent system for its commercial formulation. The semisynthetic production process of docetaxel circumvented the availability problems that first plagued the development of paclitaxel. A semisynthetic process has now been developed for paclitaxel production, as well [Michaud et al. 2000].

Despite overcoming the initial difficulties surrounding a limited drug supply, problems related to solubility of both agents remain challenging. After much investigation, Cremophor EL® (CrEL), a polyoxyethylated castor oil vehicle, and dehydrated ethanol USP (1:1, v/v) was identified as the most viable option for the solvent system employed in the commercial formulation of paclitaxel. CrEL has been widely used as the vehicle for a number of hydrophobic pharmacologic agents, including propofol, diazepam and cyclosporine.

Notably, the concentration of CrEL in the therapeutic dose of parenteral paclitaxel is relatively high compared with other agents using this solvent. In contrast, docetaxel is solubilized in another polyoxyethylated surfactant, polysorbate 80 (Tween 80), for clinical use. Both solvents are both biologically and pharmacologically active. A number of biologic effects related to both of these drug formulation vehicles have been described, including acute hypersensitivity reactions and peripheral neuropathies. In addition, several reports have linked these solvents to alterations in the pharmacokinetic profiles of both paclitaxel and docetaxel.
2.3.1. Drawbacks of current taxane formulations

2.3.1.1. Toxicities of vehicles

In the early development of paclitaxel, a high incidence of acute hypersensitivity reactions characterized by respiratory distress, hypotension, angioedema, generalized urticaria and rash were observed [Weiss et al. 1990]. It is generally felt that CrEL contributes significantly to the hypersensitivity reactions, as the vehicle has induced similar release reactions in dogs. These reactions appear to be associated with an increased rate of infusion. Researchers initially investigated alternative excipients such as polyethylene glycol for paclitaxel solubilization; however, this compound appeared to decrease the antitumor activity of paclitaxel in murine models. Thus, Cr EL has remained the standard solvent. Despite premedications with corticosteroids and histamine antagonists, minor reactions (e.g., flushing and rash) still occur in approximately 40% of all patients, and nearly 3% of patients experience potentially life-threatening reactions. Prolonging the infusion does not eliminate the risk of hypersensitivity reactions [Weiss et al. 1990]. In addition, the cumulative toxicities of dexamethasone used as a premedication may contribute to treatment-related morbidity.

Docetaxel has been known to cause infusion-related reactions in the absence of premedication; however, these reactions have occurred at a decreased frequency when compared with paclitaxel and can be effectively managed by premedication with corticosteroids and histamine receptor antagonists.

Agents formulated in CrEL have been known to cause peripheral neurotoxicity. Whether CrEL is the sole causative agent remains unknown. Electrophysiologic studies in patients who developed neurotoxicity after paclitaxel treatment have shown evidence of both axonal degeneration and demyelination [Onetto et al. 1993]. Administration of intravenous cyclosporine, which contains CrEL in its formulation, results in development of peripheral neuropathies in 25% of patients. The oral formulation, on the other hand, never induces this adverse effect, which is consistent with the observations that CrEL is not absorbed through the gastrointestinal tract. Furthermore, CrEL plasma concentrations achieved after administration of therapeutic doses of both paclitaxel and intravenous cyclosporine have been noted to cause axonal swelling, vesicular degeneration and demyelination in rat dorsal root ganglion neurons exposed to the formulation vehicle. Recent evidence suggests that the
ethoxylated derivatives of castor oil account for much of the neuronal damage observed [Brat et al. 1992].

Polysorbate 80 is also capable of producing vesicular degeneration. Although sensory neuropathies have been associated with docetaxel administration, the incidence is much lower than that seen with paclitaxel administration. However, the polysorbate 80-containing epipodophyllotoxin etoposide is not a known neurotoxin, suggesting that the mechanism of taxane-induced neuropathy may be multifactorial, at least in part contributed by the vehicle formulation.

2.3.1.2. Influence of vehicles on the pharmacokinetics of taxanes
CrEL and polysorbate 80 have also been demonstrated to alter the disposition of intravenously administered paclitaxel and docetaxel. The pseudo-non-linear plasma pharmacokinetics of paclitaxel in patients has long been established; however, the cause of this phenomenon is less well understood. Pharmacokinetic studies conducted in mouse models first described that the non-linear pharmacokinetics of paclitaxel result exclusively from CrEL [Sparreboom et al. 1996]. At the higher doses administered most often on a thrice weekly schedule and shorter infusion rates (3 h versus 24 h), the plasma concentration of paclitaxel appears to exceed the metabolic capacity of its elimination pathways. The overall resulting effect is a substantial increase in systemic exposure to paclitaxel with a concomitantly reduced systemic clearance, leading to altered pharmacodynamic characteristics of the solubilized drug. This is a result of the micellar entrapment of paclitaxel by CrEL in plasma, and these micelles subsequently act as the principal carriers of paclitaxel in systemic circulation. It has been shown that the percentage of total paclitaxel trapped in micelles increases disproportionately with the administration of higher doses of CrEL, thereby making it less available for tumor tissue distribution, metabolism and biliary excretion [Sparreboom et al. 1999]. Diminished clearance and prolonged exposure to high concentrations of the chemotherapeutic agent place patients at risk for severe systemic toxicities. Winer and colleagues demonstrated that dose escalation of standard formulation paclitaxel resulted in increased toxicity, but with no improvement in efficacy [Winer et al. 2004]. Moreover, weekly paclitaxel administration has improved response rates in patients with metastatic breast cancer and rates of pathologic complete remission in those receiving
treatment in the neo-adjuvant setting [Green et al. 2009]. Notably, CrEL micelles may entrap other hydrophobic drugs (e.g., doxorubicin) or inhibit drug uptake in the plasma (e.g., cisplatin), and place patients at risk for increased adverse effects and diminished efficacy when these agents are used in combination with paclitaxel. An additional problem linked to the CrEL solvent is the leaching of plasticizers from polyvinylchloride (PVC) bags and infusion sets used routinely in clinical practice. Consequently, paclitaxel must be prepared and administered in either glass bottles or non-PVC infusion systems with in-line filtration.

It has long been thought that polysorbate 80 is rapidly degraded in plasma and does not interfere with kinetics of docetaxel, but recent evidence suggests that this vehicle may indeed influence binding of docetaxel in plasma in a concentration-dependent manner [Loos et al. 2003]. Furthermore, Baker and colleagues reported a similar pharmacokinetic profile when docetaxel was administered weekly compared with 3-weekly [Baker et al. 2004]. In this study, the weekly regimen was administered over 30 min while the 3-weekly regimen was given over 1 h, resulting in similar polysorbate 80 concentrations at the end of the docetaxel infusion in either arm. This practice was deemed consistent with current modes of docetaxel administration.

2.3.1.3. The impact of vehicles on efficacy

Some in vitro models have demonstrated that CrEL and polysorbate 80 may enhance cytotoxic activity by modulating P-glycoprotein and inhibiting multidrug resistance gene expression [Woodcock et al. 1990]. However, in vivo studies have failed to replicate this finding with either surfactant. This lack of efficacy in vivo is likely due to the low volume of distribution of CrEL and rapid degradation of polysorbate 80 in plasma. Historically, overexpression of multidrug transporter P-glycoprotein by intestinal enterocytes has limited the oral absorption of the taxanes. Oral administration of both paclitaxel and docetaxel has been attempted with P-glycoprotein inhibitors, but there are limited human clinical data supporting this alternative for administration [Asperen et al. 1997; Richel et al. 1999].

The intrinsic cytotoxic activity of CrEL is thought to result from free radical formation by polyunsaturated fatty acids in its formulation. Conversely, some investigators have demonstrated that CrEL may antagonize the cytotoxicity of paclitaxel through cell cycle arrest when administered at therapeutic concentrations [Liebmann et al. 1994]. The exact
contribution of polysorbate 80 to antitumor effects has not been clarified; however, several reports suggest that it may be linked to the release of oleic acid, a fatty acid known to interfere with malignant cell proliferation, and inhibition of angiogenesis [Kimura 2002]. Owing to the complexity of the effects of CrEL and Polysorbate 80 on taxane administration and antitumor activity, changing the solvent system and replicating clinical data previously published is a daunting task. Still, there is good evidence that these formulation vehicles are responsible for a number of severe adverse effects and clinically relevant drug–drug interactions. The drawbacks of the taxane formulation vehicles have spurred interest in the development of surfactant-free taxane formulations, while continuing to maximize the proven activity of the taxanes against many solid tumors. Several strategies are in progress to develop alternative formulations of paclitaxel and docetaxel, including the use of albumin nanoparticles, prodrugs, polyglutamates, analogs, emulsions and liposomes, to avoid vehicle-related adverse effects, overcome resistance attributed to P-glycoprotein and the multi-drug resistance (MDR) gene, and increase response rates beyond those achieved with standard taxanes [Nuijen et al. 2001].

2.3.2. Oral Delivery of Taxanes

Taxane delivery by the oral route would be very valuable with respect to improving patient compliance and ease of administration, as well as the development of chronic treatment schedules, which would in addition decrease the costs of therapy [Peltier et al., 2006; Malingre et al. 2001a]. However, oral treatment with taxanes is not possible until now because of their low oral bioavailability owing to several factors. Taxanes show limited aqueous solubility and dissolution, affinity to the membrane-bound drug efflux pump P-glycoprotein (P-gp) [Malingré et al., 2001b; Sparreboom et al. 1997a], and metabolism by cytochrome P450 3A4 (CYP3A4) [Walle, 1996]. Numerous strategies have been developed so far to enhance the oral bioavailability of taxanes. Nano-sized carriers such as polymeric micelles [Tsallas et al., 2009; Bromberg, 2008], self micro emulsifying drug delivery systems (SMEDDS) [Yang et al., 2004], solid self-emulsifying drug delivery systems (S-SEDDS) [Gao et al., 2003], microemulsions [Nornoo et al., 2009; Yin et al., 2009], lipid nanocapsules [Roger et al., 2010; Roger et al.,
2009; Peltier et al., 2006], and nanoparticles [Saremi et al., 2011; Agüeros et al., 2009; Feng et al., 2009; Chavanpatil et al., 2006; Zhang and Feng, 2006] have been prepared to enhance oral bioavailability of taxanes.

2.3.2.1. Preclinical studies on oral delivery of taxanes

Taxanes paclitaxel and docetaxel are potent anticancer drugs with proven activity against a broad range of human malignancies, including ovarian and breast cancer and non-small cell lung carcinoma [Rowinsky and Donehower 1995; Huizing et al. 1995]. The drugs are currently administered intravenously at different dosages and infusion schedules. Oral treatment has not appeared feasible because of the low oral bioavailability of paclitaxel and docetaxel (less than 1% and 8%, respectively) [Fujita et al. 1994; Eiseman et al. 1994]. Recent studies using wild-type and mdr1a P-glycoprotein knock-out mice have shed new light on this issue [Schinkel et al. 1994; 1997]. P-glycoprotein is an energy-dependent multidrug efflux pump, which was initially discovered by its ability to confer multidrug resistance (MDR) [Juliano and Ling 1976]. This MDR phenotype is based on a drug accumulation defect in tumor cells caused by P-glycoprotein, which functions as an outward directed drug efflux pump for a broad array of drugs, including many anticancer agents such as anthracyclines, vincaalkaloids, epipodophyllotoxins and taxanes [Endicott and Ling 1989; Gottesman and Pastan 1993].

Later, high expression of P-glycoprotein was also discovered in normal tissues having an excretory function such as liver and kidney and in tissues that fulfill an important barrier function such as endothelial cells in the brain, the testis and the placenta and in the intestinal epithelium [Thiebaut et al. 1987; Fojo et al. 1987; Van Asperen et al. 1997; Van Asperen et al. 1998]. The normal physiological function of these P-glycoproteins is still a matter of conjecture, but the idea is that they serve to protect the organism against toxins. Human P-glycoprotein is encoded by the MDR1 gene. In mice, two P-glycoproteins, encoded by mdr1a and mdr1b, perform the same function as the single human protein [Tang-Wai et al. 1995; Borst et al. 1993; Borst and Schinkel 1996]. The mdr1a knock-out mice are particularly useful for studying the role of P-glycoprotein in the intestine, because the mdr1a gene is the only murine P-glycoprotein expressed in this tissue. Because paclitaxel is a very good substrate of P-glycoprotein, the hypothesis was raised that the low oral bioavailability of
paclitaxel results from P-glycoprotein activity in the gut. This was investigated in wild-type and mdr1a P-glycoprotein knock-out mice receiving orally administered paclitaxel and intravenous paclitaxel [Sparreboom et al. 1997b]. After oral drug administration, the plasma area under the concentration-time curve (AUC) of paclitaxel was 6-fold higher in mdr1a P-glycoprotein knock-out than in wild-type mice. After intravenous administration of paclitaxel the AUC was 2-fold increased in P-glycoprotein knock-out mice compared to wild type mice.

It was also investigated whether the pattern of drug excretion had been altered in mdr1a knock-out mice compared to wild-type mice. After intravenous administration of paclitaxel, the fecal excretion of unaltered drug was 40% of the delivered dose in wild-type mice and was markedly reduced to only 1.5% of the administered dose in mdr1a knock-out mice. Also after oral administration of paclitaxel large differences in fecal excretion were observed. In mdr1a P-glycoprotein knock-out mice, only 2% of the orally delivered dose was recovered in the feces as unchanged drug, whereas in wild-type mice almost 90% was excreted unchanged. Biliary secretion was not significantly different in wild-type mice and mdr1a P-glycoprotein knock-out mice. It was then concluded that P-glycoprotein in the epithelium of the gut limits the bioavailability of orally administered paclitaxel. Intestinal P-glycoprotein also contributes to the elimination of parenterally administered paclitaxel by a direct secretion of drug into the intestinal lumen. These findings provided a rationale for attempts to improve the low and variable oral bioavailability of paclitaxel by concomitant administration of P-glycoprotein inhibitors. This rationale was tested in wild-type mice receiving either oral paclitaxel as a single agent or oral paclitaxel in combination with the experimental P-glycoprotein inhibitor SDZ PSC 833 [Van Asperen et al. 1997]. Combined treatment with SDZ PSC 833 resulted in an approximately 10-fold increase in the AUC of paclitaxel.

An estimation of the oral bioavailability was made using the data of a previously performed study of intravenous administered paclitaxel [Sparreboom et al. 1996]. AUCs obtained after intravenous administration of paclitaxel in Cremophor EL-free formulations were used as Cremophor EL causes nonlinear pharmacokinetic behavior of paclitaxel. Although the oral formulation used in this study contained Cremophor EL, the systemic uptake of this compound from the gastro-intestinal tract was very low (plasma levels were undetectable). Treatment with SDZ PSC 833 increased the bioavailability from 20% to 210%, suggesting that, apart from the effect of SDZ PSC 833 on intestinal paclitaxel uptake by P-glycoprotein...
inhibition, the increased systemic exposure also results from the interaction of this agent with drug elimination pathways. Various mechanisms may contribute to this decreased clearance, e.g. both paclitaxel and cyclosporins are substrates for the cytochromeP450 isozymes [Shet et al. 1993; Harris et al. 1994], which may cause a metabolic interaction after simultaneous administration.

To further study on the feasibility of a clinically effective oral formulation of paclitaxel it was investigated whether co-treatment with a commonly applied and commercially available P-glycoprotein blocker, e.g. cyclosporin A, had a similar effect [Van Asperen et al. 1998]. The effect of cyclosporin A on the pharmacokinetics of orally and intravenously administered paclitaxel was investigated in wild-type mice. Calculated relative to the AUC of intravenously administered paclitaxel (with Cremophor EL) in mice treated without cyclosporine A, the oral bioavailability of paclitaxel increased from 9% up to 67% with co-administration of cyclosporine A. The effect of cyclosporin A on the systemic exposure after orally administered paclitaxel was the result of both a significantly increased uptake and decreased clearance. Histological examination revealed that the enhanced absorption was not caused by gastrointestinal toxicity. It was concluded that cyclosporin A is very effective in increasing the systemic exposure to orally administered paclitaxel. Importantly, these data enabled the development of a clinically useful oral formulation of paclitaxel in combination with oral cyclosporin A.

2.3.2.2. Clinical studies on oral delivery of taxanes

Based on the extensive preclinical research, a ‘proof of concept’ study of orally administered paclitaxel in cancer patients was initiated [Terwogt et al. 1999]. Patients received either one course of oral paclitaxel of 60 mg/m² as a single agent or oral paclitaxel 60 mg/m² in combination with 15 mg/kg oral cyclosporin A. In all subsequent courses patients received 3-weekly intravenous paclitaxel 175 mg/m² administered as a 3-hr infusion. The low oral dose of 60 mg/m² was selected for safety reasons because the results in mice indicated increased systemic exposure to paclitaxel after oral administration in combination with the P-glycoprotein inhibitor SDZ PSC 833 as compared with intravenous administration of paclitaxel. On all occasions patients were pre-medicated with standard paclitaxel pretreatment. Co-administration of cyclosporin A resulted in an approximately 7-fold increase in the plasma AUC of paclitaxel. The oral bioavailability of paclitaxel, calculated as
the ratio of the AUC after oral drug administration divided by the AUC after intravenous administration with a correction for the difference in dose, was 4% for oral paclitaxel administered as a single agent and 28% when oral paclitaxel was combined with cyclosporin A. These oral bioavailabilities, however, are significant underestimations of the true oral bioavailability of paclitaxel, which is due to the non-linear pharmacokinetics of intravenously administered paclitaxel [Gianni et al. 1995]. Recalculation of the oral bioavailability of paclitaxel using the AUC value of intravenous paclitaxel at a lower dose [Huizing et al. 1997], which is more realistic for comparison purposes, resulted in an apparent bioavailability of 6% without cyclosporin A and 47% of oral paclitaxel with cyclosporin A. The increase in oral bioavailability is most likely caused by inhibition of intestinal P-glycoprotein by cyclosporin A. In addition, inhibition of paclitaxel metabolism by cyclosporin A may also have contributed as both paclitaxel and cyclosporin A are substrates for the cytochrome P450 3A4 metabolic system [Shet et al. 1993]. Co-administration of cyclosporin A resulted in a significant reduction of the formation of the paclitaxel metabolite 30 p-hydroxypaclitaxel which is suggestive for cytochrome P450 3A4 inhibition.

The oral combination of paclitaxel and cyclosporine A was very well tolerated and did not induce gastro-intestinal toxicities such as nausea, vomiting and diarrhea. Co-administration of a P-glycoprotein inhibitor might cause toxicities, which are related to the physiological protective function of P-glycoprotein. P-glycoprotein inhibition could cause an increase of the paclitaxel levels in P-glycoprotein protected brain and cardiac tissue and may therefore enhance the risk of central neurotoxicity or cardiotoxicity [Van Asperen et al. 1999]. In clinical and animal studies no signs of central neurotoxicity or cardiotoxicity were observed. The single oral dose of 15 mg/kg cyclosporin A resulted in peak and trough values which were in the therapeutic range for immunosuppression and may be associated with toxicity, in particular renal toxicity. Toxicity nor any other side effects clearly related to the single administered cyclosporin A dose were observed. This has demonstrated the ‘proof of concept’ of efficient oral uptake of paclitaxel in cancer patients induced by concomitant administration of the P-glycoprotein blocker cyclosporin A. Subsequently, it was investigated whether an increase in the cyclosporin A dose or fractionated cyclosporine A administration would result in an increase in paclitaxel AUC values. Dose-increment of cyclosporine A to 30 mg/kg and changing the schedule to two administrations of 15 mg/kg separated by 2 hr.
did not result in a further increase in the AUC of paclitaxel. Apparently, P-gp inhibition was maximal at a single dose of cyclosporin A of 15 mg/kg. It remained, however, unclear whether cyclosporine A inhibited P-glycoprotein completely. In addition, incomplete distribution of cyclosporin A over the mucosal wall may also have contributed to the possible incomplete P-glycoprotein inhibition by cyclosporine A.

In an attempt to further increase the systemic exposure of orally administered paclitaxel and to determine the dose limiting toxicity and maximum tolerated dose, dose-escalation of oral paclitaxel was investigated [Malingré et al. 2001a]. Dose limiting toxicity was reached at the dose level of 360 mg/m$^2$ and consisted of acute nausea and vomiting. The maximum tolerated dose was then defined at 300 mg/m$^2$. Pharmacokinetic analysis of oral paclitaxel revealed that dose-escalation of oral paclitaxel from 60 to 300 mg/m$^2$ resulted in significant increases in the AUC of paclitaxel, however, these increases were moderate and not proportional with increases in dose. It was hypothesized that this non-linear absorption pharmacokinetic behavior of oral paclitaxel was due to the poor aqueous solubility of paclitaxel and consecutive limited dissolution in the gastro-intestinal tract. A similar non-linear pharmacokinetic absorption pattern due to poor aqueous solubility was observed for the oral anticancer drugs etoposide and the platinum complex JM216 [Hande et al. 1993]. At all investigated oral paclitaxel dose levels, plasma levels of the co-solvent Cremophor EL were undetectable. Apparently, Cremophor EL is not absorbed following oral administration of the paclitaxel intravenous formulation. This is important because systemic exposure to Cremophor EL can induce severe hypersensitivity reactions requiring extensive premedication [Weiss et al. 1990]. Absence of systemic exposure to Cremophor EL after oral drug administration justifies paclitaxel treatment without premedication.

In another study of orally administered paclitaxel in combination with cyclosporin A [Duchin et al. 1998], oral paclitaxel was administered without premedication and no hypersensitivity reactions were observed. Furthermore, Cremophor EL is responsible for the non-linear pharmacokinetic behavior of intravenous paclitaxel [Sparreboom et al. 1999; Malingré et al. 2001b]. It entraps paclitaxel in the plasma compartment, which results in a more than proportional increase in plasma paclitaxel levels with increasing doses. However, these higher total drug levels in plasma do not result in higher drug levels in tissue. This pseudo-non-linearity of intravenous paclitaxel has two important implications for the pharmacology
of oral paclitaxel. Firstly, it will result in a significant underestimation of the true bioavailability of oral paclitaxel. This has been discussed above. Secondly, the pseudo-non-linearity of intravenous paclitaxel implies that after oral administration, when Cremophor EL is not systemically present, plasma levels of paclitaxel represent a higher fraction of free drug, which will result in enhancement of the availability of paclitaxel for the (tumor) tissues. Therefore, interpretation of differences between paclitaxel plasma levels after oral and intravenous administration, without and with Cremophor EL in the systemic circulation, respectively, should be done with great caution. At the maximum tolerated oral paclitaxel dose of 300 mg/m\(^2\) a mass balance study was performed. Excretion of the drug after intravenous administration was also investigated. After intravenous administration of paclitaxel, the major excretory route of paclitaxel and metabolites was feces, viz. 56% of the administered dose. The major compounds detected in feces were the metabolites, viz. 47% of the administered dose, of which the metabolite 6a-hydroxypaclitaxel accounted for 37%. Following oral paclitaxel administration in combination with cyclosporin A, the major excretion route of paclitaxel and metabolites was also with feces, viz. 76% of the administered dose. The major compound recovered in feces after oral drug administration was paclitaxel, accounting for 61% of the administered dose. In the preclinical studies of oral paclitaxel in wild-type mice and mdr1a P-glycoprotein knock-out mice fecal excretion of paclitaxel was significantly decreased from 87% in wild-type mice to 2% in the mdr1a knock-out mice. This large decrease in fecal excretion of paclitaxel suggested almost complete (re)uptake of the drug from the gastro-intestinal tract in P-glycoprotein knock-out mice. Thus, according to the preclinical studies, only a small fraction of the paclitaxel dose excreted in the feces instead of the observed 61% was expected. The most plausible explanation for this large amount of paclitaxel recovered in feces is excretion of unabsorbed drug, which is supported by the significant lower plasma AUC value of orally administered paclitaxel (300 mg/m\(^2\)) compared to intravenous administered paclitaxel (175 mg/m\(^2\)). Because of the non-linear oral pharmacokinetics of paclitaxel with only moderate further increases of the AUC with doses up to 300 mg/m\(^2\) and the large amount of original drug recovered in feces after oral paclitaxel administration at a dose of 300 mg/m\(^2\), an oral paclitaxel dose of 180 mg/m\(^2\) is considered most appropriate for further investigation. The safety of the oral combination at this dose-level is very good.
Based on the non-linear drug absorption a split dose regimen was investigated to achieve a greater overall daily systemic exposure. Oral paclitaxel was administered in two doses 7 hr apart at dose levels of 2 X 60, 2 X 90 and 2 X 120 mg/m². In this study with oral paclitaxel, besides the AUC value, the pharmacokinetic parameter time above the threshold concentration of 0.1 µmol (T>0.1 µmol) was considered. Previous clinical work has suggested that time above this threshold concentration is related to the activity of the drug [Huizing et al. 1993]. The pharmacokinetic data revealed that bi-daily dosing of oral paclitaxel also shows non-linear absorption pharmacokinetics as was observed after single dose administration of the drug. Comparison with the pharmacokinetic data after single dose administration revealed that fractionated administration of the drug resulted in higher AUC and T>0.1 µmol values of paclitaxel. Therefore, a multiple dosing regime may be a realistic option to further increase the systemic exposure after oral administration of paclitaxel.

For docetaxel, a similar clinical ‘proof of concept’ study was initiated as had been done for oral paclitaxel [Richel et al. 1999]. Patients received either one course of oral docetaxel 75 mg/m² as a single agent or oral docetaxel in combination with cyclosporin A. Patients continued on a 3-weekly schedule of 100 mg/m² intravenous docetaxel administered as a 1-hr infusion. Standard docetaxel pretreatment was given in all courses. Pharmacokinetic data showed that co-administration of oral cyclosporin A strongly enhanced the systemic exposure of orally administered docetaxel. Docetaxel administered as a single agent exhibited poor oral bioavailability of only 8%, whereas oral docetaxel in combination with cyclosporin A exhibited a bioavailability of 90%. Furthermore, the variance in the systemic exposure after oral drug administration was of the same order as after intravenous administration.

Hence oral administration did not result in a notable increase in the inter-patient difference in systemic exposure. The oral combination of docetaxel and cyclosporin A was very well tolerated. Thus, oral docetaxel may become a realistic alternative to the current intravenous treatment of docetaxel. In addition, as recent clinical studies have shown that administration of intravenous docetaxel on a weekly schedule decreases the hematological toxicity profile of the drug while therapeutic activity is maintained [Hainsworth et al. 1998; Löffler et al. 1998; Climent et al. 1999], the feasibility of oral drug administration may stimulate and facilitate the use of weekly treatment schedules of docetaxel. The concept of modulation of
bioavailability by a P-gp inhibitor may well be applied for other (cytotoxic) drugs that show affinity for the multidrug efflux pump and are associated with poor or moderate oral bioavailability.

2.4. Multidrug Resistance (MDR)

Multidrug resistance (MDR) in tumor cells is a significant obstacle to the success of chemotherapy in many cancers. Multidrug resistance is a phenomenon whereby tumor cells in vitro that have been exposed to one cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds. The drug resistance that develops in cancer cells often results from elevated expression of particular proteins, such as cell-membrane transporters, which can result in an increased efflux of the cytotoxic drugs from the cancer cells, thus lowering their intracellular concentrations [Gottesman et al. 2002]. In addition, MDR occurs intrinsically in some cancers without previous exposure to chemotherapy agents [Fardel et al. 1996]. The cytotoxic drugs that are most frequently associated with MDR are hydrophobic, amphipathic natural products, such as the taxanes (paclitaxel, docetaxel), vinca alkaloids (vinorelbine, vincristine, vinblastine), anthracyclines (doxorubicin, daunorubicin, epirubicin), epipodophyllotoxins (etoposide, teniposide), topotecan, dactinomycin, and mitomycin C [Krishna and, Mayer 2000].

A number of different mechanisms can mediate the development of MDR, including increased drug efflux from the cell by adenosine triphosphate (ATP)-dependent transporters, decreased drug uptake into the cell, activation of detoxifying enzymes, and defective apoptotic pathways [Gottesman et al. 2002]. The etiology of MDR may be multifactorial, but the classic resistance to the cytotoxic drugs mentioned above has most often been linked to the overexpression of P-glycoprotein (P-gp), a 170-kd ATP dependent membrane transporter that acts as a drug efflux pump [Gottesman and Pastan 1993]. In addition to cytotoxic drugs, P-gp also transports several other exogenous compounds, including digoxin, opiates, polycyclic aromatic hydrocarbons, technetium (99mTc) sestamibi, and rhodamine 123. The last two compounds have been used in imaging and in surrogate marker assays of P-gp function in normal and malignant human cells [Witherspoon et al. 1996]. The surrogate marker assay as an indicator of in vivo modulator drug activity relies on examination of the CD56+ subset of peripheral blood lymphocytes that express functional P-gp. Hence, the
changes seen in rhodamine 123 (a substrate for P-gp) uptake by CD56+ lymphocytes from modulator-treated and untreated whole blood are used as the basis for these types of studies.

2.4.1. P-glycoprotein (P-gp)
P-gp belongs to the ATP-binding cassette (ABC) family of transporters, currently numbering 48 members that share sequence and structural homology [Dean et al. 2001]. It is believed that, while this class of transporters has a large number of members, only 10 or so are reported to confer the drug-resistant phenotype. These transporters use the energy that is released when ATP is hydrolyzed to drive the transport of various molecules across the cell membrane. In addition to their physiologic expression in normal tissues, many are expressed and, importantly, over-expressed, in human tumors.

In cancerous tissue, the expression of P-gp is usually highest in tumors that are derived from tissues that normally express P-gp, such as epithelial cells of the colon, kidney, adrenal, pancreas, and liver, resulting in the potential for resistance to some cytotoxic agents before chemotherapy is initiated. In other tumors, the expression of P-gp may be low at the time of diagnosis but increases after exposure to chemotherapy agents, thereby resulting in the development of MDR in those cells [Fardel et al. 1996].

There is a growing body of literature that links the failure of certain chemotherapeutic agents to the expression of P-gp. Indeed, the induction of MDR1 RNA can be rapid following exposure of tumor cells to chemotherapy. Inhibiting P-gp as a way of reversing MDR has been extensively studied for more than 2 decades. Many agents that modulate the function of P-gp have been identified, including calcium channel blockers, calmodulin antagonists, steroidal agents, protein kinase C inhibitors, immunosuppressive drugs, antibiotics, and surfactants [Ferry et al. 1996]. It has also been reported that the expression of P-gp was a significant prognostic marker in certain childhood malignancies [Chan et al. 1990].

Cyclosporin A was investigated in combination with chemotherapy in retinoblastoma patients and found to cause high cure rate (91% of previously untreated patients remained relapse-free, with salvage therapy combining cyclosporin and chemotherapy prolonging survival in those previously untreated with cyclosporin) [Chan et al. 1996]. Although these trials were limited in size, they raised substantial interest in the cancer research community. However, it is now widely acknowledged that the major limitation of many of the early
agents is that they typically reverse MDR at concentrations that result in unacceptable toxicity [Krishna and Mayer 2000]. This, together with unfavorable pharmacokinetic interactions, prompted the development of a number of new molecules that are more potent and selective for the P-gp transporter.

2.4.2. P-gp Modulators

2.4.2.1. First-Generation P-gp Modulators

Many agents that modulate the P-gp transporter, including verapamil, cyclosporin (cyclosporin A), tamoxifen, and several calmodulin antagonists, were identified in the 1980s [Krishna and Mayer 2000]. These agents often produced disappointing results in vivo because their low binding affinities necessitated the use of high doses, resulting in unacceptable toxicity [Ferry et al. 1996]. Many of the first chemosensitizers identified were themselves substrates for P-gp and thus worked by competing with the cytotoxic compounds for efflux by the P-gp pump; therefore, high serum concentrations of the chemosensitizers were necessary to produce adequate intracellular concentrations of the cytotoxic drug. In addition, many of these chemosensitizers are substrates for other transporters and enzyme systems, resulting in unpredictable pharmacokinetic interactions in the presence of chemotherapy agents. To overcome these limitations, several novel analogs of these early chemosensitizers were tested and developed, with the aim of finding P-gp modulators with less toxicity and greater potency [Krishna and Mayer 2000].

2.4.2.2. Second-Generation P-gp Modulators

The second-generation P-gp modulators included verapamil, dextriguldipine, valspodar (PSC 833), and biricodar (VX-710). These agents are more potent than their predecessors and also less toxic. The best characterized and most studied of these agents is valspodar, a derivative of cyclosporin D that inhibits P-gp with 10- to 20-fold greater activity than cyclosporin A. Valspodar has been studied in numerous clinical trials in combination with cytotoxic agents [Advani et al. 2001; Baekelandt et al. 2001; Fracasso et al. 2001]. A study by Coley et al. that used fresh tumor material from patients with soft-tissue sarcomas indicated that valspodar at 1 nM had a modest effect (20% increase) on anthracycline accumulation in P-gp positive samples [Coley et al. 2000]. Moreover, in another study examining MDR in epithelial
ovarian cancer, the effect was of a similar magnitude in similar experiments and may go some way toward explaining the disappointing results in clinical trials [Coley et al. 2002]. The pipercolinate derivative biricodar citrate (VX-710) has also undergone extensive clinical development. This molecule interferes with drug efflux by directly binding with high affinity to the P-gp pump and also by inhibiting the ABC transporter MRP1 [Germann et al. 1997a,b]. The co-administration of second-generation P-gp modulators and chemotherapy agents in clinical trials has resulted in the reversal of MDR and some limited success in treating refractory cancers [Chico et al. 2001].

2.4.2.2.1. Limitations of Second-Generation P-gp Modulators

Second-generation P-gp modulators have a better pharmacologic profile than the first-generation compounds, but they also retain some characteristics that limit their clinical usefulness. In particular, these compounds significantly inhibit the metabolism and excretion of cytotoxmic agents, thus leading to unacceptable toxicity that has necessitated chemotherapy dose reductions in clinical trials. In response to cytotoxic agents, cytochrome P450 enzymes are often induced along with members of the ABC transporter family, and it is thought that the genes of these families share overlapping regulatory elements [Lum et al. 1995]. In fact, many of the cytotoxic agents that are substrates for P-gp are also substrates for the cytochrome P450 isoenzyme 3A4. It is not surprising then that the agents affected by the development of MDR are also metabolized by cytochrome P450 3A4. Several of the second-generation P-gp modulators, including valspodar and biricodar, are substrates for this enzyme. The competition between cytotoxic agents and these P-gp modulators for cytochrome P450 3A4 activity has resulted in unpredictable pharmacokinetic interactions. For example, valspodar inhibits the cytochrome P450 3A4-mediated metabolism of paclitaxel and vinblastine [Wandel et al. 1999], resulting in increased serum concentrations of the cytotoxic agents and potentially putting patients at risk of cytotoxic drug overexposure [Fischer et al. 1998]. Similarly, in a pharmacokinetic study in patients with solid tumors, biricodar administered in a 24-hour intravenous infusion decreased the clearance of paclitaxel in a dose-dependent manner [Rowinsky et al. 1998]. It has been suggested that this interaction may be due in part to the inhibition of cytochrome P450 3A4 by biricodar, thereby interfering with the metabolism of paclitaxel.
The most common response of clinical researchers to this drug interaction has been to reduce the dose of the cytotoxic agent. However, it should be noted that since the pharmacokinetic interactions between chemosensitizers and cytotoxic agents are unpredictable and cannot be determined in advance, reducing the dose of a cytotoxic agent by a set percentage may result in under- or over-dosing in a significant number of patients [Gottesman et al. 2002]. The unpredictability of the effects of second-generation P-gp modulators on cytochrome P450 3A4-mediated drug metabolism has made it difficult to establish a safe but effective dose of the co-administered chemotherapy agent and thus limits the use of these second-generation modulators in the treatment of multidrug-resistant cancers.

In addition to inhibiting P-gp, many second-generation modulators also function as substrates for other transporters, particularly those of the ABC transporter family, inhibition of which could lessen the ability of normal cells and tissues to protect themselves from cytotoxic agents. Many of these transporters have well defined physiologic roles, often involving the elimination of xenobiotics (in the case of those transporters in the liver, kidney, and gastrointestinal tract) [Krishna and Mayer 2000]. In addition, ABC transporters are involved in regulating the permeability of the central nervous system (blood-brain barrier), the testes, and the placenta, thus systems from being exposed to cytotoxic agents circulating in the blood.

Many of the early-generation P-gp modulators inhibited several other ABC transporters as well as the P-gp transporter. For instance, valspodar and biricodar are not specific solely to P-gp; both of these agents affect MRP1 [Rowinsky et al. 1998]. It is possible that this inhibition of non-target transporters may lead to greater adverse effects of anticancer drugs, including neutropenia and other myelotoxic effects. For example, the ABC transporter BCRP is a functional regulator of hematopoietic stem cells [Bunting 2002] and its inhibition may contribute to these effects.

2.4.2.3. Third-Generation P-gp Inhibitors

Third-generation molecules that specifically and potently inhibit P-gp function have been developed by using structure-activity relationships and combinatorial chemistry to overcome the limitations of the second generation P-gp modulators. These agents do not affect cytochrome P450 3A4 at relevant concentrations [Dantzig et al. 1999], thus explaining, at
least in part, why they do not alter the plasma pharmacokinetics of paclitaxel in rats [Mistry et al. 2001; Roe et al. 1999]. Similarly, third-generation agents typically do not inhibit other ABC transporters (albeit they have not been tested against all of the ABC transporters). This specificity for the P-gp pump minimizes the possibility that the blockade of more than one pump might result in altered bioavailability or excretion of the chemotherapy agents. These preclinical data have translated to clinical trials, in which none of the third-generation agents have caused clinically relevant alterations in the pharmacokinetics of the co-administered cytotoxic agents (see below). Consequently, chemotherapy dose reductions have been unnecessary.

The third generation P-gp inhibitors currently in clinical development include the anthranilamide derivative tariquidar (XR9576) [Roe et al. 1999], the cyclopropyl dibenzosuberane zosuquidar (LY335979) [Starling et al. 1997], laniquidar (R101933) [van Zuylen et al. 2000], and the substituted diarylimidazole ONT-093 [Newman et al. 2000]. Despite having diverse chemical structures and origins, these agents have in common a high potency and specificity for the P-gp transporter. The modulator elacridar (GF120918/GG918) has been shown to inhibit breast cancer resistance protein BCRP [Maliepaard et al. 2001]. One of the most promising third-generation P-gp inhibitors is tariquidar, which binds with high affinity to the P-gp transporter and potently inhibits its activity [Martin et al. 1999]. Second-generation P-gp modulators compete as a substrate with the cytotoxic agent for transport by the pump. In contrast, tariquidar specifically and noncompetitively binds to the pump with an affinity that greatly exceeds that of the transported substrates [van Zuylen et al. 2000]. It is not clear whether XR9576 binds to ATP binding site on P-gp, but like other modulators such as GF120918, it inhibits the ATPase activity of P-gp [Martin et al. 1999].

The inhibitory effects of tariquidar on the P-gp transporter pump greatly exceed those of first- and second-generation P-gp modulators with respect to potency and duration of action. In an in vitro study, P-gp pump transport remained blocked for more than 22 hours after tariquidar had been removed from the culture medium; in the same assay, the clearance time for cyclosporin was 60 minutes. Pharmacokinetic studies in healthy subjects showed that single doses of tariquidar up to 2 mg/kg intravenously or 750 mg orally are well tolerated and provide complete P-gp inhibition for at least 24 hours, as shown by rhodamine 123 accumulation in CD56+ lymphocytes [Stewart et al. 2000]. A single intravenous dose of
tariquidar in patients with cancer inhibited the efflux of rhodamine from CD56+ cells for up to 48 hours. Tariquidar showed no effect on the pharmacokinetics of paclitaxel, vinorelbine, or doxorubicin when it was administered to patients with solid tumors [Abraham et al. 2001; Ferry et al. 2001]. This allowed the use of standard doses of these chemotherapeutic agents without the need for dose reduction. Tariquidar is currently in phase III trials in patients with non–small-cell lung cancer. The cyclopropyl dibenzosuberane modulator LY335979 was shown to competitively inhibit the binding of vinblastine to P-gp [Dantzig et al. 1999]. In clinical studies in both solid and hematologic malignancies, LY335979 showed no significant pharmacokinetic interactions with doxorubicin, etoposide, daunorubicin, vincristine, or paclitaxel [Cripe et al. 2001]. R101933 and ONT-093 are two other third-generation P-gp inhibitors that have been shown to be effective in inhibiting P-gp with no effect on the pharmacokinetics of docetaxel [van Zuylen et al. 2000] and paclitaxel [Newman et al. 2000]. Because of their specificity for P-gp transporters and lack of interaction with cytochrome P450 3A4, third-generation P-gp inhibitors offer significant advantages over the second-generation agents. The results of clinical trials to date show that third-generation P-gp inhibitors such as elacridar, tariquidar, LY335979, R101933, and ONT-093 can be given with full therapeutic doses of cytotoxic agents and with minimal interference with the pharmacokinetics of the cytotoxic agents.

2.4.2.3.1. GF120918 (Elacridar)

GF120918 is an acridonecarboxamide derivative and has been shown to be a potent blocker of P-glycoprotein in tumor cells in vitro and in vivo [Hyafil et al. 1993]. Elacridar (GF120918) is a selective inhibitor of the drug pumps P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP; A3CG2). This compound was discovered in the search for potent and selective P-gp inhibitors. Many MDR reversing agents appeared to be weak competitive P-gp inhibitors, or potent inhibitors of cytochrome P4503A4 (CYP3A4), causing undesirable pharmacokinetic interactions [Sparreboom et al. 1997b]. Elacridar, on the other hand, is about 100-fold more potent than the widely used P-gp inhibitor cyclosporin A and has very low affinity for CYP3A4 [Hyafil et al. 1993; Sparreboom et al. 1999]. In comparison to the early generation of chemosensitizers (e.g., cyclosporin-A, verapamil), which are not optimized for MDR reversal [Teodori et al. 2002; Belpomme et al. 2000],
GG918 has a higher potency for P-gp and a lower cellular toxicity. It also has a very low affinity for other enzyme systems such as CYP3A4, and is therefore less likely to cause undesirable pharmacokinetic interactions [Wallstab et al. 1999; Sparreboom et al. 1999].

2.5. Lipid based drug delivery system

The main purpose of the lipid formulation classification system (LFCS) is to enable in-vivo studies to be interpreted more readily, and subsequently to facilitate the identification of the most appropriate formulation for specific drugs. Table 2.1 describes the lipid formulation classification system (LFCS). Type I systems consist of formulations, which comprise drug in solution in triglycerides and/or mixed glycerides or in an oil-in-water emulsion stabilized by the low concentration of emulsifiers. Generally, these system exhibited poor initial aqueous dispersion and require digestion by pancreatic lipase/co-lipase in the GIT to generate more amphiphilic lipid digestion products and promote drug transfer into colloidal aqueous phase.

Type I lipid formulations therefore represent a relatively simple formulation option for potent drugs or highly lipophilic compounds where drug solubility in oil is sufficient to allow incorporation of the required dose.

<table>
<thead>
<tr>
<th>Excipients in formulation</th>
<th>Content of formulation (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
</tr>
<tr>
<td>Oils: triglycerides or mixed mono- and diglycerides</td>
<td>100</td>
</tr>
<tr>
<td>Water-insoluble surfactant (HLB&lt;12)</td>
<td>-</td>
</tr>
<tr>
<td>Water-soluble surfactant (HLB&gt;12)</td>
<td>-</td>
</tr>
<tr>
<td>Hydrophilic co-solvents (e.g., PEG, Propylene glycol, Transcutol)</td>
<td>-</td>
</tr>
</tbody>
</table>
Type II formulations are water-insoluble self-emulsifying drug delivery systems (SEDDS), which are isotropic mixtures of lipids and lipophilic surfactants (HLB < 12) that self-emulsify to form fine oil-in-water emulsions when introduced in aqueous media. Self-emulsification is generally obtained at surfactant contents above 25% (w/w). However, at higher surfactant contents (greater than 50–60% (w/w) depending on the materials) the progress of emulsification may be compromised by the formation of viscous liquid crystalline gels at the oil/water interface. Poorly water-soluble drugs can be dissolved in SEDDS and encapsulated in hard or soft gelatin capsules to produce convenient single unit dosage forms. Type II lipid-based formulations provide the advantage of overcoming the slow dissolution step typically observed with solid dosage forms and as described above generate large interfacial areas which in turn allows efficient partitioning of drug between the oil droplets and the aqueous phase from where absorption occurs.

Type III lipid-based formulations, commonly referred to as self-microemulsifying drug delivery system (SMEDDS), are defined by the inclusion of hydrophilic surfactants (HLB > 12) and co-solvents such as ethanol, propylene glycol and polyethylene glycol. Type III formulations can be further segregated (somewhat arbitrarily) into Type IIIA and Type IIIB formulations in order to identify more hydrophilic systems (Type IIIB) where the content of hydrophilic surfactants and co-solvents increases and the lipid content reduces. Type IIIB formulations typically achieve greater dispersion rates when compared with Type IIIA although the risk of drug precipitation on dispersion of the formulation is higher given the lower lipid content. The distinction between SEDDS (Type II) and SMEDDS (Type III) formulations is also commonly made on the particle size and optical clarity of the resultant dispersion. Thus SEDDS formulation typically provide opaque dispersions with particle sizes > 100 nm whereas SMEDDS formulations (which contain higher concentrations of hydrophilic surfactants and co-solvents) disperse to give smaller droplets with particle sizes < 100 nm, and provide optically clear or slightly opalescent dispersions, more consistent with the presence of a microemulsion.

Table 2.1 also includes an additional category (Type IV) to represent the recent trend towards formulations, which contain predominantly hydrophilic surfactants and co-solvents. Type IV formulations contain no oils and represent extremely hydrophilic formulations. The advantage of blending a surfactant with a co-solvent to give a Type IV formulation is that the
surfactant offers much greater good solvent capacity on dilution (as a micellar solution) than the co-solvent alone. The co-solvent is useful to facilitate dispersion of the surfactant, which is likely to reduce variability and irritancy caused by high local concentrations of surfactant [Pouton, 2006]. A Type IV formulation is useful for drugs, which are hydrophobic but not lipophilic, this type of formulations (Type IV) may not be well tolerated if the drug is to be used on a chronic basis (e.g., marketed formulation amprenavir, an HIV protease inhibitor).

The general characteristics, advantages and disadvantages of each type of lipid formulation are shown in table 2.2. The performance of lipid formulations, and the fate of the drug in the gastrointestinal tract, depends on the physical changes that occur on dispersion and dilution of the formulation, and the influence of digestion on drug solubilization. The main advantage of lipid formulation is that the drug remains in solution throughout its passage through the gastrointestinal tract. If precipitation occurs at any stage the advantage of a lipid formulation is lost. Precipitation of drug is more common from lipid systems, which contain more hydrophilic excipients.

2.5.1. Mechanism of absorption from lipid drug delivery system

The ability of lipid vehicles to enhance the absorption of lipophilic drugs has not been fully understood. The current understanding that lipids may enhance bioavailability via a number of potential mechanisms, namely [Porter and Charman, 2001].

<table>
<thead>
<tr>
<th>LFCS Type</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Non-dispersing; requires digestion</td>
<td>GRAS status; simple; excellent capsule Compatibility</td>
<td>Formulation has poor solvent capacity unless drug is highly lipophilic</td>
</tr>
<tr>
<td>Type II</td>
<td>SEDDS without water soluble components</td>
<td>Unlikely to lose solvent capacity on Dispersion</td>
<td>Turbid o/w dispersion (particle size 0.25–2µm)</td>
</tr>
</tbody>
</table>
Type III A  
SEDDS/SMEDDS with water soluble components  
Clear or almost clear dispersion; drug absorption without digestion  
Possible loss of solvent capacity on dispersion; less easily digested

Type III B  
SMEDDS without water soluble components and low oil content  
Clear dispersion; drug absorption without digestion  
Likely loss of solvent capacity on dispersion

Type IV  
Oil free formulation based on surfactants and cosolvents  
Good solvent capacity for many drugs; disperses to micellar solution  
Loss of solvent capacity on dispersion; may not be digestible

2.5.1.1. Enhanced dissolution/solubilization

The presence of lipids in the GI tract stimulates gall bladder contractions and biliary and pancreatic secretions, including bile salts (BS), phospholipids (PL) and cholesterol [Fleisher et al., 1999]. These products, along with the gastric shear movement, form a crude emulsion, which promotes the solubilization of the co-administered lipophilic drug. Recently, it has been shown that even small amounts of lipid may stimulate gall bladder contractions. In addition to the increase in biliary secretions, the exogenous lipid component of the delivery system is subjected to enzymatic digestion. Esters are rapidly hydrolyzed in the presence of pancreatic lipase, and the lipolytic products, upon interaction with BS/PL, form different micellar species that prevent the co-administered lipophilic drug precipitation. Exogenous surface-active agents incorporated into the delivery system may further stimulate the solubilization of the lipophilic compound [O'Driscoll, 2002].

2.5.1.2. Prolongation of gastric residence time

Lipids in the GI tract provoke delay in gastric emptying, i.e. gastric transit time is increased. As a result, the residence time of the co-administered lipophilic drug in the small intestine increases. This enables better dissolution of the drug at the absorptive site, and thereby improves absorption [Charman et al., 1992; Gursoy and Benitan, 2004].
2.5.1.3. Stimulation of lymphatic transport
Bioavailability of lipophilic drugs may be enhanced also by the stimulation of the intestinal lymphatic transport pathway. This issue will be further detailed separately [Charman et al., 1992; Gursoy and Benitan, 2004].

2.5.1.4. Affecting intestinal permeability
A variety of lipids have been shown to change the physical barrier function of the gut wall, and thus, to enhance permeability [Constantinides and Wasan, 2007]. For BCS class II compounds, permeability through the GI wall is not a limiting step towards absorption, and hence, this mechanism is not thought to be a major contributor for the absorption enhancement of lipophilic drugs [Charman et al., 1992; Gursoy and Benitan, 2004].

2.5.1.5. Reduced metabolism and efflux activity
Recently certain lipids and surfactants have been shown to reduce the activity of efflux transporters in GI wall, and hence, to increase the fraction of drug absorbed. Because of the interplay between P-gp and CYP3A4 activity this mechanism may reduce intra-enterocyte metabolism as well. Possible mechanisms of intestinal drug absorption, using lipid-based formulations are summarized in figure 2.1. These mechanisms include, an increase in membrane fluidity facilitating transcellular absorption, opening of the tight junctions to allow paracellular transport, mainly relevant for ionized drugs or hydrophilic macromolecules, inhibition of P-gp and/or CYP450 to increase intracellular concentration and resistance time, and stimulation of lipoprotein/chylomicron production. The last two mechanisms are potentially the most promising methods for intestinal lymphatic drug targeting [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. Generally, compounds processed by the intestinal lymph are transported to the systemic circulation in association with the lipid core of lipoproteins [Pocock and Vost, 1974], and as such require co-administered lipid 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 largely in
chylomicrons [Kiyasu et al., 1952]. 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. Bile salts, monoglycerides, cholesterol, lecithin and lyssolecithin further emulsify the large fat droplets upon their entry into the intestine, and smaller droplets of 0.5-1 µm mean diameter are formed. Pancreatic lipase then catalyzes the metabolism of these droplets [Charman et al., 1992], which later on form mixed micelles with bile salts [Constantinides, 1995]. Following their penetration through the aqueous layer and mucin, mixed micelles and microemulsions are absorbed by pinocytosis, diffusion or endocytosis [Georgakopoulos et al., 1992].
Figure 2.1. Intestinal drug transport via lipid based formulation (Adapted from O’Driscoll 2001).

Schematic diagram of the mechanisms of intestinal drug transport from lipid-based formulations via the portal and mesenteric lymphatic routes. The main effect shown includes:
(A) Increased membrane fluidity facilitating transcellular absorption
(B) Opening of tight junction to allow paracellular transport
(C) Inhibition of P-gp and or CYP450 to increase intracellular concentration and residence time and
(D) Stimulation of lipoprotein/chylomicron production.
2.5.2. Self-micro emulsifying drug delivery systems

In recent years much attention has been focused on lipid based formulations [Humberstone and Charman, 1997] with particular emphasis on self-emulsifying drug delivery systems to improve oral bioavailability of lipophilic drugs [Cui et al., 2005; Constantinides, 1995; Pouton, 1997; Singh et al., 2008]. The term “self –emulsifying” denotes a transparent or translucent homogenous product can be formed spontaneously without precipitation or phase separation when oil phase, a surfactant, a co-surfactant and aqueous phase are mixed together with little or no input of energy. The process may be spontaneous or may require low levels of shear unlike the conventional emulsification system, which requires high shear.

Self-micro emulsifying formulation comprises isotropic mixture of natural or synthetic oils, surfactant, and co-surfactant, which upon dilution with aqueous media spontaneously form fine O/W microemulsion with less than 50nm in droplet size [Nazzal et al., 2002]. The digestive motility of the stomach and intestine provide the agitation necessary for self-emulsification in vivo. Factors controlling the in vivo performance of SMEDDS include their ability to form small droplets of oil (< 100 nm) and the polarity of the oil droplets to promote faster drug release into aqueous phase [Shah et al., 1994]. The smaller oil droplets provide a large interfacial area for pancreatic lipase to hydrolyze triglycerides and thereby promote the rapid release of the drug and/or formation of mixed micelles of the bile salts containing the drug [Tarr and Yalkowsky, 1989]. The surfactants used in these formulations are known to improve the bioavailability by various mechanisms, including: (a) improved drug dissolution [Constantinides, 1995]; (b) increased intestinal epithelial permeability [Swenson et al., 1992]; (c) increased tight junction permeability; and (d) decreased: inhibited p-glycoprotein drug efflux [Nerurkar et al., 1996].

A prerequisite for the use of SEDDS/SMEDDS for oral administration is that the doses of the compound should be soluble in the preconcentrate and stay solubilized in the vehicle after dispersion. Solubility in the preconcentrate is the limiting factor for a number of poorly water-soluble compounds, which are also poorly soluble in lipids.

For selecting a suitable self-emulsifying vehicle, it is important to assess:

1. The drug solubility in various components
2. The area of self-emulsifying region in the phase diagram
3. Droplet size distribution following self-emulsification [Kang et al., 2004].
2.5.2.1. Factor affecting absorption of drugs via SMEDDS

The efficiency of SMEDDS depends on two main factors:

1. Uniform fine particle size of oil droplets on exposure to aqueous media; and
2. The polarity of the resulting oil droplets.

Both properties control the rate of release of the drug from the oil to the aqueous phase. Once exposed to the aqueous phase, SMEDDS form oil-in-water (o/w) microemulsions. The resulting o/w emulsions are produced spontaneously because SMEDDS are thermodynamically stable, as opposed to the regular emulsions, which are thermodynamically unstable. There are two factors that favor emulsion stability in the case of SMEDDS:

1. Relatively small volume of the dispersed oil phase; and
2. Narrow range of droplet size distribution.

For a given combination of components, emulsions with small, uniform droplet size will take longer to break. Larger droplets are less stable than smaller droplets due to their larger area to volume ratio, and so will tend to grow at the expense of the smaller droplets. The smaller droplets will have a larger interfacial surface area per unit volume. The diffusion path for a drug will decrease with the reduction of the radius of the droplets.

Another important factor for the performance of SMEDDS is the polarity of the oil droplets. The polarity of the oil droplets is governed by the hydrophilic-lipophilic balance (HLB), the chain length and degree of unsaturation of the fatty acid, the molecular weight of the hydrophilic portion and the concentration of the emulsifier. The combination of small droplets together with the appropriate polarity of the drug will permit an acceptable rate of release of the drug.

2.5.2.2. Various modes of enhanced absorption from the SMEDDS

- Drugs may be absorbed through the lymphatic system via chylomicron formation of the fatty components of the digestible oil phase of emulsion. A lipophilic drug, which preferably remains in the oil droplets, may in fact be absorbed via bile salt micelles along with metabolite of the lipid carrier.
- Inhibition of gastric motility caused by the presence of the lipid phase of emulsion might allow more time for dissolution and absorption of drug from lipid phase [Bates and Sequeria, 1975].
- Increase in mucosal permeability via incorporation of lipid from mixed micelles and enhanced mesenteric lymph flow may be responsible for enhanced drug absorption.
- Increase dissolution from the large surface area afforded by emulsion may be a contributing factor to enhanced absorption of drugs.
- A relatively less focused consideration is the presence of surfactant in formulation, which may also play a role in increasing the absorption of the drugs.

2.5.2.3. Stability of self-dispersing systems

The essential distinction between emulsion and microemulsion are presented in the table 2.3. Emulsions are considered ‘kinetically stable’ whereas the latter is ‘thermodynamically stable’. The stability of the microemulsion can be influenced by addition of salt, other additives, temperature or pressure. Normal emulsions age by coalescence of droplets and Ostwald ripening (transfer of material from small droplets to larger ones), since these processes lead to a decrease in the free energy of dispersion (the system is inherently thermodynamically unstable).

2.5.2.4. Thermodynamics of microemulsion formation and phase behavior

Thermodynamic stability of the microemulsion has been proposed by Reckentein who considered that the free energy of formation comprises interfacial free energy, interaction energy between droplets and entropy of dispersion have proposed thermodynamic stability of the microemulsions. The interaction energy between droplets has been shown to be negligible and the free energy of formation can be zero or even negative if the interfacial tension is of the order of 10–2–10–3 mN/m. 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].
<table>
<thead>
<tr>
<th>Emulsions</th>
<th>Microemulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermodynamically unstable</td>
<td>Thermodynamically stable</td>
</tr>
<tr>
<td>Inefficient molecular packing</td>
<td>Efficient molecular packing</td>
</tr>
<tr>
<td>Direct oil/water contact at the interface</td>
<td>No direct oil/water contact at the interface</td>
</tr>
<tr>
<td>Interfacial surface tension 20-50m Nm$^{-1}$</td>
<td>Interfacial surface tension $10^{-4}$ -20m Nm$^{-1}$</td>
</tr>
<tr>
<td>Multiple phase only</td>
<td>May be single or multiple phase</td>
</tr>
<tr>
<td>Micelle diameter 20nm</td>
<td>Micelle diameter 3-20nm</td>
</tr>
<tr>
<td>Cloudy colloidal systems</td>
<td>Optically transparent (Isotropic)</td>
</tr>
</tbody>
</table>

The free energy of a conventional emulsion formulation is a direct function of the energy required to create a new surface area between the oil and water phases and can be described by the following equation:

$$\Delta G = \sum N_i \pi r^2 \alpha$$

Where $\Delta G$ is the free energy associated with the process (ignoring the free energy of mixing), $N$ is the number of droplets of radius $r$ and $\alpha$ represents the interfacial energy.

With time, the two phases of the emulsion will tend to separate, in order to reduce the interfacial area and subsequently, the free energy of the systems. Therefore, the emulsions resulting from the aqueous dilution are stabilized by conventional emulsifying agent, which form a monolayer around the emulsion droplets, and hence, reduce the interfacial energy, as well as providing a barrier to coalescence. In the case of self-emulsifying systems, the free energy required to form the emulsion is either very low and positive, or negative (then, the emulsification process occurs spontaneously).

The free energy of microemulsion formation can be considered to depend on the extent to which surfactant lowers the surface tension of the oil-water interface and the change in the entropy of the system such that,
$$\Delta G_f = \gamma \Delta A - T \Delta S$$

Where $\Delta G_f$ is the free energy of formation, $\gamma$ is the surface tension of the oil-water interface, $\Delta A$ is the change in the interfacial area on microemulsification, $\Delta S$ is the change in entropy of the system which is effectively the dispersion entropy, and $T$ is the temperature. When microemulsion is formed the change in $\Delta A$ is very large due to the large number of very small droplets formed (figure 2.2). Microemulsions form spontaneously only when IFT (interfacial tension) is small (in the order of $10^{-3}$ mN/m).

![Figure 2.2. Thermodynamics of microemulsion formation](image)

**Figure 2.2. Thermodynamics of microemulsion formation**

$\Delta G_m =$ free energy change for microemulsion formation

$\Delta G_1 =$ free energy change due to increase in total surface area

$\Delta G_2 =$ free energy change due to interaction between droplets

$\Delta G_3 =$ free energy change due to adsorption of surfactant at the oil/water interface

$\Delta S =$ increase in entropy due to dispersion of oil as droplets

### 2.5.2.5. Factors affecting SMEDDS

#### 2.5.2.5.1. Nature and dose of the drug

Drugs, which are administered at very high doses are not suitable for SMEDDS unless they exhibit extremely good solubility in at least one of the components of SMEDDS, preferably the lipophilic phase. Drugs exhibiting limited solubility in both water and lipids (typically at
log P values of approximately 2) are most difficult to deliver by SMEDDS. The ability of SMEDDS to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase. If the surfactant or co-surfactant is contributing to the greater extent in drug solubilisation then there could be a risk of precipitation, as dilution of SMEDDS will lead to lowering of solvent capacity of the surfactant or co-surfactant. Equilibrium solubility measurements can be carried out to anticipate potential cases of precipitation in the gut. However, crystallisation could be slow in the solubilising and colloidal stabilising environment of the gut. Pouton’s study reveal that such formulations can take up to five days to reach equilibrium and that the drug can remain in a super-saturated state for up to 24 hours after the initial emulsification event [Pouton et al. 2008]. It could thus be argued that such products are not likely to cause precipitation of the drug in the gut before the drug is absorbed, and indeed that super-saturation could actually enhance absorption by increasing the thermodynamic activity of the drug. There is a clear need for practical methods to predict the fate of drugs after the dispersion of lipid systems in the gastro-intestinal tract.

2.5.2.5.2. Polarity of the lipophilic phase:
The polarity of the lipid phase is one of the factors that govern the drug release from microemulsions. The polarity of the droplet is governed by the HLB, the chain length and degree of unsaturation of the fatty acid, the molecular weight of the hydrophilic portion and the concentration of the emulsifier. In fact, the polarity reflects the affinity of the drug for oil and/or water, and the type of forces formed. The high polarity will promote a rapid rate of release of the drug into the aqueous phase. The highest release is obtained with the formulation that had oil phase with highest polarity.

2.5.2.6. Advantages/Application of self emulsifying formulation

2.5.2.6.1. Improvement in oral bioavailability
Dissolution rate-dependent absorption is a major factor that limits the bioavailability of poor water-soluble drugs. Microemulsion is a novel approach to improve the water solubility and ultimately, bioavailability of lipophilic drugs. Microemulsion presents the drug to the gastrointestinal tract in solubilized and micro-emulsified form; subsequent increase in
specific surface area enables more efficient drug transport through intestinal aqueous boundary layer and through the absorptive brush border membrane leading to improved bioavailability.

2.5.2.6.2. **Reduction in inter-subject and intra-subject variability**

There are several drugs which show large inter-subject and intra-subject variation in absorption, leading to decrease performance of drug and patient non-compliance. Microemulsion drug delivery system is a proven approach to overcome inter- and intra-subject variation [Ghosh and Murthy, 2006].

2.5.2.6.3. **Reduction of food effects**

The poor bioavailability of water insoluble, highly lipophilic drugs can often result in a large food effect, i.e. much higher exposure in the fed than fasted state, which can lead to the greater sensitivity of the pharmacokinetic profile to the fat content of meals and timing of food administration. A widely used approach for overcoming poor fasted state bioavailability of lipophilic drugs is to utilize solution in lipid vehicles containing surfactants that constitute a self-microemulsifying drug delivery system (SMEDDS), to effect spontaneous emulsification upon contact of the oil with the fluid in the GI tract [Shah et al., 1994; Constantinides, 1995; Perlman et al., 2008; Ghosh and Murthy, 2006].

2.5.2.6.4. **Ease of manufacturing and scale-up**

This is one of the most important advantages that makes microemulsion a unique delivery system when compared to solid dispersions, liposomes, nanoparticles, etc. dealing with improvement of bioavailability. Microemulsion requires very simple and economical manufacturing facilities like simple mixers with agitator and volumetric liquid filling equipment for large-scale manufacturing. This explains the interest of the industry for microemulsion based drug delivery systems.

2.5.2.6.5. **Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT**
One unique property of microemulsion is the ability to deliver macromolecules like peptides, hormones, enzyme substrate and inhibitors and the ability to offer protection from enzymatic hydrolysis.

2.5.2.6.6. No influence of lipid digestion process
Unlike other lipid-based drug delivery systems, the performance of microemulsion is not influenced by the lipolysis, emulsification by the bile salts, action of pancreatic lipases and mixed micellar formation. Microemulsions are not necessarily digested before the drug is absorbed, as they present the drug in micro emulsified form, which can easily penetrate the mucin, and unstirred water layer covering the epithelial surface.

2.5.2.6.7. As solid dosage form for oral administration
Microemulsions can be converted into various solid dosage forms by adsorbing onto solid surfaces [Ghosh and Myrthy, 2006].

2.5.2.7. Formulation considerations for self-emulsifying formulation
Pharmaceutical acceptability of excipients and their toxicity issues of the components used, make the formulation of microemulsion really critical. There is great restriction in selection of the components that are orally acceptable. Self-emulsification has been shown to be specific to the nature of the oil/surfactant pair, the surfactant concentration and oil/surfactant ratio, and the temperature at which self-emulsification occurs. It has also been demonstrated that only very specific pharmaceutical excipients lead to efficient self-emulsifying systems. Microemulsion formulation usually involves a combination of three to five components: an oil phase, an aqueous phase, a primary surfactant and in many cases, a secondary surfactant (co-surfactant) and sometimes an electrolyte. The tendency towards a w/o or an o/w microemulsion is dependent on the properties of both the oil and surfactant [Wakerly et al., 1987; Gurosy and Benita, 2004; Constantinides, 1995]. Table 2.4 lists some of the factors that affect the choice of excipients.
Table 2.4. Factors affecting the choice of excipients for lipid-based formulations

<table>
<thead>
<tr>
<th>Regulatory issues—irritancy, toxicity, knowledge and experience</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent capacity</td>
</tr>
<tr>
<td>Miscibility</td>
</tr>
<tr>
<td>Morphology at room temperature (i.e. melting point)</td>
</tr>
<tr>
<td>Self-dispersibility and role in promoting self-dispersion of the formulation</td>
</tr>
<tr>
<td>Digestibility and fate of digested products</td>
</tr>
<tr>
<td>Capsule compatibility</td>
</tr>
<tr>
<td>Purity, chemical stability</td>
</tr>
<tr>
<td>Cost of goods</td>
</tr>
</tbody>
</table>

2.5.2.7.1. Oils

The oil represents important excipient in the SMEDDS formulation not only because it can solubilize marked amounts of the lipophilic drug but also increase the fraction of lipophilic drug transport via the intestinal lymphatic system. Both long- and medium-chain triglycerides (MCT) oils with different degrees of saturation have been used for the design of self-emulsifying formulations. Triglyceride vegetable oils have many advantages as the foundation of lipid-based delivery systems. They are commonly ingested in food, fully digested and absorbed, and therefore do not present safety issues. Vegetable oils are glyceride esters of mixed unsaturated long-chain fatty acids, commonly known as long-chain triglycerides (LCT). Coconut oil is distilled to produce ‘medium-chain triglycerides’ (MCT) (also known as glyceryl tricaprylate/caprate) which is comprises glyceryl esters with predominantly saturated C8 (50–80%) and C10 (20–45%) fatty acids. Triglycerides are highly lipophilic and their solvent capacity for drugs is commonly a function of the effective concentration of the ester groups, thus on a weight basis MCT generally has higher solvent capacity than LCT [Anderson, 1999]. In addition MCT is not subject to oxidation, so MCT is a popular choice for use in lipid-based products.
2.5.2.7.2. Surfactants

Surfactants increase the 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.

The surfactant chosen must be able to:

(a) Lower interfacial tension to a very small value to aid dispersion process during the preparation of the microemulsion.

(b) Provide a flexible film that can readily form round droplets.

(c) Be of the appropriate lipophilic character to provide the correct curvature at the interfacial region for the desired microemulsion type i.e., for o/w, w/o or bicontinuous.

The process of dilution of SMEDDS with aqueous media and formation of droplets in the nanometer range is a thermodynamically driven process. However, the process of dilution will result in the gradual desorption of surfactant located at the droplet interface. The process is thermodynamically driven by the requirements of surfactant to maintain an aqueous phase concentration equivalent to its critical micelle concentration (CMC) under the prevailing conditions of temperature, pH and ionic strength. Because non-ionic surfactants typically have lower CMCs than their ionic surfactants counterparts, o/w microemulsion dosage forms based on non-ionic surfactants offer superior in-vivo and in-vitro stability.

Toxicity is an independent issue, and is important with regard to the choice of surfactants. Water-insoluble surfactants penetrate and fluidize biological membranes and water-soluble surfactants have the potential to solubilize membrane components. All surfactants are potentially irritant or poorly tolerated as a result of these non-specific effects. In general terms cationic surfactants are more toxic than anionic surfactants, which in turn are more toxic than non-ionic surfactants.

Self-emulsifying formulations usually only include non-ionic surfactants so it is pertinent to compare the toxicity of non-ionic surfactants. In general bulky surfactants such as polysorbates or polyethoxylated vegetable oils are less toxic than single-chain surfactants, and esters are less toxic than ethers (which are non-digestible). Non-ionic surfactants are generally considered to be acceptable for oral ingestion, and the emergence of several successful marketed products [Strickly, 2004; Strickly, 2007] has given the industry confidence in lipid-based products. The oral and intravenous LD50 values for most non-ionic
surfactants are in excess of 50 g/Kg and 5 g/Kg respectively, so 1 g surfactant in a formulation is well-tolerated for uses in oral drug administration [Atwood and Florence, 1983].

The marketed HIV protease inhibitors products, such as Agenerase, Kaletra and Norvir, contain a considerable mass of surfactants in each capsule and several capsules are administered 2–4 times daily, so that patients are ingesting 2–3 g Cremophor or TPGS daily. Most non-ionic surfactants have similar LD50 values. IIG limits for the commonly used non-ionic surfactants (Cremophore RH40 & Cremophore ELP) are presented in table 2.5 [www.fda.gov/cder].

Another practical consideration relates to the chemical complexity of excipients. The use of vegetable oils from different plants is an immediate source of diversity, and subsequent chemical derivation by hydrolysis and esterification introduces more diversity. Further processing is required to produce nonionic surfactants, usually esters of polyoxyethylene or polyglycerol, or products of reaction with ethylene oxide. The polyoxyethylene or polyglycerol chains are polymeric in nature, which means that a typical surfactant product based on mixed glycerides is comprised of dozens of separate chemical entities at different proportions. For practical purposes these products are usually given a simple chemical name which represents their ‘average’ composition but which hides their complexity. The formulator of lipid-based products has to accept that there will be differences between excipients products, which appear to have the same chemical name, and there will also be a finite level of diversity between batches of the same product.

**Table 2.5. “Inactive ingredients database” of Cremophore RH40 & Cremophore ELP**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Excipients</th>
<th>Route dosage form</th>
<th>Maximum potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Cremophore ELP (Polyoxyl 35 Hydrogenated Castor Oil)</td>
<td>IV (infusion); Injection</td>
<td>65%</td>
</tr>
<tr>
<td>02</td>
<td>Cremophore ELP (Polyoxyl 35 Hydrogenated Castor Oil)</td>
<td>Intravenous Injection</td>
<td>50%</td>
</tr>
<tr>
<td>03</td>
<td>Cremophore ELP (Polyoxyl 35 Hydrogenated Castor Oil)</td>
<td>IV (infusion; solution) Injection</td>
<td>52.75%</td>
</tr>
</tbody>
</table>
2.5.2.7.3. Co-surfactants

Role of the co-surfactant, usually a short chain alcohol is to increase the interfacial fluidity by penetrating the surfactant film and consequently creating a disordered film due to the void space among surfactant molecules. Organic solvents suitable for oral administration (ethanol, polypropylene glycol, polyethylene glycol, etc.) may help to dissolve large amounts of either the hydrophilic surfactant or the drug in the lipid base. Alcohols and other volatile solvents have the disadvantage of diffusing into the shell of the soft gelatin or hard gelatin capsules leading to the drug precipitation.

Table 2.6 provides the regulatory status of some of the commonly used excipients for the preparation of SMEDDS.
Table 2.6. Regulatory status of commonly used excipients for SMEDDS formulation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Excipients</th>
<th>Regulatory Status</th>
<th>Chemical description</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Capryol\textsuperscript{TM} PGMC</td>
<td>FCC/USFA/JPED/JSFA</td>
<td>Monoester of propylene glycol with caprylic acid (HLB 5)</td>
</tr>
<tr>
<td>02</td>
<td>Capryol\textsuperscript{TM} 90</td>
<td>FCC/USFA/JPED/JSFA</td>
<td>Propylene glycol monopropylate (HLB 6)</td>
</tr>
<tr>
<td>03</td>
<td>Lauroglycol\textsuperscript{TM} FCC</td>
<td>FCC/USFA/JPED/JSFA</td>
<td>Propylene glycol laurate (HLB 5)</td>
</tr>
<tr>
<td>04</td>
<td>Lauroglycol\textsuperscript{TM} 90 EP/JPED/USFA/E477/JSFA</td>
<td>Propylene glycol monolaurate (HLB 5)</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>Plurol\textsuperscript{®} Oleique</td>
<td>JPED/USFA/E475/JSFA</td>
<td>Polyglyceryl oleate (HLB 10)</td>
</tr>
<tr>
<td>06</td>
<td>Transcutol\textsuperscript{®} HP</td>
<td>EP/USP-NF</td>
<td>Diethylene glycol monoethyl ether</td>
</tr>
<tr>
<td>07</td>
<td>Labrasol\textsuperscript{TM}</td>
<td>EP/USP-NF</td>
<td>Capryolocaproyl macrogolglycerides (HLB 14)</td>
</tr>
<tr>
<td>08</td>
<td>Cremophore RH 40</td>
<td>EP/USP-NF</td>
<td>Polyoxy 40 Hydrogenated Castor Oil (HLB 14)</td>
</tr>
<tr>
<td>09</td>
<td>Cremophore ELP</td>
<td>EP/USP-NF</td>
<td>Polyoxy 35 Hydrogenated Castor Oil (HLB 12.5)</td>
</tr>
<tr>
<td>10</td>
<td>Tween 80</td>
<td>EP/USP-NF</td>
<td>Polyoxyethylene (20) sorbitan monooleate (HLB 15)</td>
</tr>
</tbody>
</table>

All the excipients had GRAS (generally regarded as safe) status for oral use.

2.5.2.8. Clinical overview of self-emulsifying formulation

A self-microemulsifying formulation of cyclosporine A (Neoral\textsuperscript{®}) was shown to enhance fasting state bioavailability, decrease the food effect, increase dose linearity and reduce the variability in exposure. Decreasing the emulsion droplet size by homogenization increased the rate of absorption [Tarr and Yalkowsky, 1989] as a result of the increased surface area that facilitated digestion of the glycerides by intestinal lipases to form micelle thus promoted absorption. In recent years, self-emulsifying drug delivery systems have been increasingly employed to enhance the oral bioavailability of poorly water-soluble drugs. Examples of
increased drug bioavailability following oral administration of SEDDS and self-microemulsifying drug delivery systems (SMEDDS) are listed in table 2.7 and a comprehensive list of commercially available formulations is shown in table 2.8.

**Table 2.7. Studies describing the bioavailability enhancement of lipophilic drugs after administration of SEDDS and SMEDDS formulations**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formulation(s)</th>
<th>Study design</th>
<th>Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Win 54954</td>
<td>SEDDS (35% drug, 40% Neobee M5 (MCT) and 25% Tagat TO) or PEG 600 solution</td>
<td>Relative BA in dogs</td>
<td>No difference in BA but improved reproducibility, increased $C_{max}$</td>
<td>[Charman et al., 1992]</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>Sandimmum (SEDDS: corn oil and ethanol) or Neoral (SMEDDS: Corn oil glycerides, Cremophor RH40, PG, dl-α-tocopherol and ethanol)</td>
<td>Relative BA in humans</td>
<td>Increased BA and $C_{max}$ and reduced $T_{max}$ from SMEDDS</td>
<td>[Trull et al., 1994]</td>
</tr>
<tr>
<td>Sandimmum (SEDDS) or Neoral (SMEDDS)</td>
<td>Relative BA in humans</td>
<td>Increased $C_{max}$, AUC and dose linearity and reduced food effect from SMEDDS</td>
<td>[Mueller et al., 1994]</td>
<td></td>
</tr>
<tr>
<td>Sandimmum (SEDDS) or Neoral (SMEDDS)</td>
<td>Relative BA in humans</td>
<td>Reduced intra- and inter-subject variability from SMEDDS</td>
<td>[Kovarik et al., 1994]</td>
<td></td>
</tr>
<tr>
<td><strong>Compound</strong></td>
<td><strong>Formulation(s)</strong></td>
<td><strong>Study design</strong></td>
<td><strong>Observations</strong></td>
<td><strong>Reference</strong></td>
</tr>
<tr>
<td>----------------</td>
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<td>-----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Halofantrine</td>
<td>5% drug in MCT SEDDS (47% Captex 355, 23% Capmul MCM, 15% Cremophor EL and ethanol), MCT SMEDDS (33% Captex 355, 17% Capmul MCM, 35% Cremophor EL and ethanol) or LCT SMEDDS (29% Soybean oil, 29% Maisine 35-1, 30% Cremophor EL and 7% ethanol)</td>
<td>Relative BA in dogs</td>
<td>Trend to higher BA from LCT SMEDDS</td>
<td>[Khoo et al., 1998]</td>
</tr>
<tr>
<td>Ontazolast</td>
<td>Soybean oil emulsion, drug solution in Peceol, drug suspension or two semi-solid SEDDS comprising Gelucrie 44/14 and Peceol in the ratios 50:50 and 80:20</td>
<td>Absolute BA in rats</td>
<td>BA increases of at least 10-fold from all lipid-based formulations</td>
<td>[Hauss et al., 1998]</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>SEDDS (Tween 80:Span 80:palm oil (LCT) in a 4:2:4 ratio) or soybean oil (LCT) solution</td>
<td>Relative BA in humans</td>
<td>BA 3-fold higher from SEDDS</td>
<td>[Julianto et al., 2000]</td>
</tr>
<tr>
<td>Coenzyme Q&lt;sub&gt;10&lt;/sub&gt;</td>
<td>SMEDDS (40% Myvacet 9-45, 50% Labrasol and 10% lauroglycol) or powder formulation</td>
<td>Relative BA in dogs</td>
<td>BA 2-fold higher from SEDDS</td>
<td>[Kommuru et al., 2001]</td>
</tr>
<tr>
<td>Ro-15-0778</td>
<td>SEDDS (polyglycolysed glycerides and peanut oil), PEG 400 solution, wet-milled spray-dried powder or tablet</td>
<td>Relative BA in dogs</td>
<td>BA 3-fold higher from SEDDS when compared with other</td>
<td>[Shah et al., 1994]</td>
</tr>
<tr>
<td>Compound</td>
<td>Formulation(s)</td>
<td>Study design</td>
<td>Observations</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------------------------------------------------------</td>
<td>--------------------</td>
<td>---------------------------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>SMEDDS (37% Capryol 90, 28% Cremophor EL, 28% Carbitol) or tablet</td>
<td>Relative BA in dogs</td>
<td>BA 1.5-fold higher from SMEDDS</td>
<td>[Kang et al., 2004]</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>SEDDS (70% ethyl oleate and 30% Tween 85) or powder formulation</td>
<td>Relative BA in rats</td>
<td>BA significantly increased from SEDDS</td>
<td>[Kim and Ku, 2000]</td>
</tr>
<tr>
<td>Progesterone</td>
<td>SEDDS (mono-di-glycerides:polysorbate 80, 50/50 w/w) or aq suspension</td>
<td>Relative BA in dogs</td>
<td>BA 9-fold higher from SEDDS</td>
<td>[Tuleu et al., 2004]</td>
</tr>
<tr>
<td>Danazol</td>
<td>LC-SMEDDS (long chain lipids, cremophor EL and ethanol), MC-SMEDDS (medium chain lipids, cremophor EL and ethanol) or LCT solution</td>
<td>Relative BA in dogs</td>
<td>BA from LCT solution and LC-SMEDDS 7-fold and 6-fold higher than that from MC-SMEDDS</td>
<td>[Porter et al., 2004]</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>SEDDS (labrafil M1944CS, tween 80 and transcutol) and tablet</td>
<td>Relative BA in dogs</td>
<td>BA 4-fold higher from SEDDS</td>
<td>[Wei et al., 2005]</td>
</tr>
<tr>
<td>Solvent green 3</td>
<td>Semi-solid SMEDDS (Gelucire 44/14) or soybean oil emulsion</td>
<td>Relative BA in rats</td>
<td>BA 1.7-fold higher from SMEDDS</td>
<td>[Iwanaga et al., 2006]</td>
</tr>
<tr>
<td>Silymarin</td>
<td>SMEDDS (Tween 80, ethyl alcohol and ethyl linoleate), PEG 400 solution or PEG 400 suspension</td>
<td>Relative BA in rabbits</td>
<td>BA approximately 2- and 50-fold higher from SMEDDS than that of PEG 400</td>
<td>[Wu et al., 2006]</td>
</tr>
<tr>
<td>Compound</td>
<td>Formulation(s)</td>
<td>Study design</td>
<td>Observations</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------------------------------------</td>
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<td>-----------------------------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>Three SMEDDS (Cremophor RH40, propylene glycol and labrafil, estol or labrafac) or tablet</td>
<td>Relative BA in dogs</td>
<td>BA significantly increased from all SMEDDS formulations</td>
<td>[Shen and Zhong, 2006]</td>
</tr>
<tr>
<td>Itroconazole</td>
<td>SEDDS (Transcutol, pluronic L64 and tocopherol acetate) or conventional capsule</td>
<td>Relative BA in rats</td>
<td>Increased BA and reduced food effect from SEDDS</td>
<td>[Hong et al., 2006]</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>Two SMEDDS (long chain lipids, ethanol and cremophor EL or Pluronic L121) or aq susp</td>
<td>Relative BA in dogs</td>
<td>BA 3-fold higher from SMEDDS</td>
<td>[Sek et al., 2006]</td>
</tr>
<tr>
<td>Seocalcitol</td>
<td>LC-SMEDDS (sesame oil, Peceol, Cremophor RH40) versus MC-SMEDDS (Vicscoleo (MCT), Akoline MCM (medium chain mono and di-glyceride) and Cremophor RH40)</td>
<td>Absolute BA in rats</td>
<td>BA LC-SMEDDS = MC-SMEDDS</td>
<td>[Grove et al., 2006]</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>SEDDS formulation comprising Transcutol, Pluronic L64 and tocopherol acetate versus commercial Sporanox formulation</td>
<td>Relative BA under differing dietary conditions in rats</td>
<td>More consistent (and in some cases enhanced) BA from the SEDDS formulation across the differing dietary</td>
<td>[Hong et al., 2006]</td>
</tr>
</tbody>
</table>
Table 2.8. Commercially available self-emulsifying formulation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formulation(s)</th>
<th>Study design</th>
<th>Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neoral ®</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(Cyclosporine)</td>
<td>25 mg and 100 mg</td>
<td>Upto 1 g/day</td>
<td>Mono-di-triglycerides, polyoxyl 40 castor oil, DL-α-tocopherol and propylene glycol</td>
<td>20 – 50% compared to Sandimmune®</td>
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<tr>
<td><em>Novartis</em></td>
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<tr>
<td><strong>Norbir®</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(Ritonavir)</td>
<td>100 mg</td>
<td>600 mg bid (12 caps/day)</td>
<td>Caprylic/capric triglycerides, polyoxyl 35 castor oil, citric acid, ethanol, polyglycolized glycerides, polysorbate 80 and propylene glycol</td>
<td>Similar to an oral solution</td>
</tr>
<tr>
<td><em>Abott</em></td>
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<tr>
<td><strong>Fortovase®</strong></td>
<td>200 mg</td>
<td>1200 mg tid (18 caps/day)</td>
<td>Medium chain mono and diglycerides, povidone and DL α-tocopherol</td>
<td>AUC increase 3.3 fold compared to Invirase®</td>
</tr>
<tr>
<td>(Saquinavir)</td>
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</tr>
<tr>
<td><em>Abott</em></td>
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</tr>
<tr>
<td><strong>Agenerase®</strong></td>
<td>150 mg</td>
<td>1200 mg bid (18 caps/day)</td>
<td>Tocopheryl polyethylene glycol 1000 succinate, polyethylene glycol 400 and propylene glycol</td>
<td>Conventional oral formulation gave no detectable blood level</td>
</tr>
<tr>
<td>(Amprenavir)</td>
<td></td>
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<tr>
<td><em>GSK</em></td>
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