Chapter 3

Objectives and Plan of Work
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Objectives and Plan of Work
3.1 OBJECTIVES OF THE PRESENT STUDY

Cancer treatment is a multidisciplinary challenge, which needs more and closer collaboration among clinicians, medical and biological scientists and biomedical engineers to eventually find a satisfactory solution. Compared to other drug classes, anticancer drugs particularly the cytotoxic agents, are more reactive, unstable and toxic, and are more diverse in terms of molecular structure and physicochemical properties (Ewesuedo and Ratain, 2003). Administration by the oral route remains the most popular method of drug delivery. Given the tremendous flexibility, SLN-based systems allow (Battaglia et al., 2007), this relatively new class of drug carriers were adopted for the oral delivery of paclitaxel. Lipid nanoparticle drug delivery technology presents significant opportunities for improving medical therapeutics, but the technology’s potential remains unrealized and several technology challenges like appropriate control of particle size and size distribution, lipid crystallinity, drug loading profile, drug release kinetics, and greater control of biodistribution, once administered, remain unsolved.

This research effort was focused on lipid nanoparticle synthesis, improving the formulators control over particle size, size distribution, and drug loading profile through processing and material formulation variables. Also SLN loaded with drug were perfected and utilized to further improve the oral anticancer treatment and side effect profiles of paclitaxel. This research work was primarily divided into the following objectives:

1. To develop an easily available, straightforward and reproducible SLN production method without using technically sophisticated equipment, using bioacceptable and biodegradable lipid materials.
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2. To optimize the formulation variables such as lipid concentration, amount of injected solvent, emulsifier concentration, etc. for the SLN production methods and characterization of produced SLN in terms of their morphology, size, and size distribution to meet this objective.

3. To develop and characterize the paclitaxel loaded SLN using the selected SLN production method. Paclitaxel loaded SLN were characterized in terms of their morphology, size, size distribution, surface charge and entrapment efficiency.

4. To develop an efficient, reproducible and validated high performance liquid chromatography (HPLC) analytical method for determining the encapsulated paclitaxel in SLN formulations.

5. To evaluate and determine the in vitro release behaviour of paclitaxel from the developed paclitaxel loaded SLN formulations.

6. To determine the stability of the optimized paclitaxel loaded SLN formulations.

7. To carry out a comparative in vivo study using mice as animal model to determine the various pharmacokinetic parameters after oral administration of the paclitaxel loaded SLN formulations and standard paclitaxel injection.

3.2 RATIONALE OF USING SLN FOR PACLITAXEL ORAL DELIVERY

Like other type of drug carriers used for cytotoxic drug delivery, such as polymeric systems and liposomes, SLN have the advantages of physical stability, protection of labile drugs from degradation, controlled release, and easy preparation (Wissing et al., 2004). At the same time, SLN avoid some issues encountered by these drug delivery systems. SLN do not carry the relatively high cost required for large-scale production of liposomal formulations. They have fewer storage and drug leakage problems compared to
systems such as liposomes (Heath, 1988; Muller et al., 2000, 2000a). The significant toxicity and acidity associated with a number of biodegradable polymeric materials are also not observed in SLN (Smith and Hunneyball, 1986; Muller et al., 1996, 1997; Fu et al., 2000).

A carrier material, such as polymers, that can bind one cytotoxic drug may not be compatible with another one. The versatility of SLN is therefore extremely valuable for the encapsulation of cytotoxic compounds. Many biocompatible and biodegradable lipids (e.g. triglycerides such as tristearin, tripalmitin, trilaurin; hard fats such as Witepsol series, glyceryl behenate, cetypalmitate, lipid acids such as stearic acid, palmitic acid) are available for the preparation of SLN (Muller et al., 2000). In addition, SLN formulations are compatible with most emulsifiers (e.g. poloxamer 188, polysorbate 80, lecithin, sodium glycocholate) approved by drug regulatory agencies (Smith and Hunneyball, 1986; Muller et al., 2000). The long-circulating SLN may further expand the role of this versatile drug carrier in cancer treatment. Following are the reasons summarized for studying SLN as drug delivery system for anticancer agents:

1. They are made up of natural, nontoxic, and non immunogenic lipid molecules.
2. They are sustained release delivery systems with a possibility of controlled drug release and drug targeting.
3. Coupling of different ligands on the surface of the SLN may be used to obtain specific targeting.
4. They can carry both the hydrophobic and hydrophilic drugs.
5. As the SLN are prepared from natural lipids, they exhibit well-defined biocompatibility, biodegradability and specific interactions with biologic membranes.

6. There are various ways how SLN carry drug molecules e.g. bound to the membrane i.e. lipid core, drug enriched shell; solid solution i.e. homogeneous drug-lipid matrix; or entrapped in the interior core i.e. lipid shell drug enriched core.

7. SLN encapsulation alters the drugs pharmacokinetics and improves the delivery of drugs in the body. It protects the degradation of the drugs in the body, thus increasing drug stability.

8. There are no problems with respect to large scale production and sterilization of SLN.

Cytotoxic anticancer drugs are known for their heterogeneity. They constitute a class of compounds with highly diverse molecular structure and physicochemical properties (Ewesuedo and Ratain, 2003). Several obstacles frequently encountered with anticancer drugs, such as normal tissue toxicity, poor specificity and stability, are at least partially overcome by delivering them using SLN. Moreover, with the advances in surface-engineering technology, the biodistribution of SLN can be further manipulated by modifying the surface physicochemical properties of SLN to target them to the tissue of interest (Mehnert and Mader, 2001). This maximizes the amount of drug that can reach the targeted tumor sites and minimizes systemic drug toxicity.

This versatility of SLN as a drug carrier made SLN a potential generic platform for the oral delivery of paclitaxel. It is anticipated that, in the near future, SLN will be
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Further improved to deliver anticancer drugs in a more efficient, specific and safer manner. Beyond the scope of this doctoral thesis, the long-term goal of this research is the development of a commercially viable novel solid lipid nanoparticle production process that provides robust particle size control, stability, high lipophile drug loading, superior targeted and controlled release capabilities, and delivery of sensitive biomolecules. Future application interests include antiviral formulations, antifungal formulations, nutraceutical formulations, vaccine therapy, and gene delivery.

3.3 PACLITAXEL: A DRUG PROFILE

In 1963, a crude extract from the bark of the Pacific yew tree (Taxus Brevifolia) was found to have significant preclinical activity against a number of tumors. The active ingredient in that crude extract was subsequently identified as paclitaxel (Wani et al., 1971). This compound was the first identified member of a new class of anticancer agents known as the taxanes. Paclitaxel was discovered as part of a National Cancer Institute program in which extracts of thousands of plants were screened for anticancer activity. Paclitaxel was obtained in a pure form in 1969 and its structure was published in 1971, after many complexities due to its low concentration and structure complexities. Although it had a novel chemical structure (Fig. 3-1) and broad preclinical activity, development was slowed because it did not appear to be more effective against experimental tumors than other agents under development at that time. In addition, it was expected that the procurement and preparation of this potentially scarce natural product in sufficient quantities for large-scale development would be arduous. Interest was revived in 1979 when paclitaxel's unique mechanism of action as antitumor drug was identified, and was further stimulated, when impressive activity was demonstrated in the National Institute of Health anti-tumor screen. It was established that paclitaxel was a microtubule stabilizer that prevented separation of mitotic chromosomes, leading to cell death through apoptosis. The fact that it was effective in cell lines resistant to other chemotherapeutic agents opened the door to the development of protocols for standard therapy of various solid tumors and lymphomas. In 1974, paclitaxel was selected as the national cancer institute number one drug candidate for development, with the purpose of finding effective and safe therapeutic regimens. This effort led to the development of paclitaxel in combination with cis-platin with its approval for breast cancer and ovarian cancer in 1992, followed by more indications including lung cancer in 1995, and melanoma and non-small cell lung cancer in 1996. Paclitaxel remains an active area of research with its novel mechanism of action and potential for combination with other agents to increase efficacy.
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Cancer Institute tumor screening and initial phase I trials were begun in 1983 (Schiff et al., 1979; Rowinsky and Donehower, 1995). Paclitaxel was found to have activity against a number of solid tumors in both the refractory and de novo setting leading to its subsequent approval by The Food and Drug Administration in 1992 for the palliative treatment of refractory ovarian and breast cancers. Since 1993, phase III trials have shown that paclitaxel improves survival in several solid tumor disorders, including ovarian, non-small cell lung cancer (NSCLC) and breast cancer (McGuire et al., 1996; Bonomi et al., 1997).

Table 3.1. Stages in the development of paclitaxel as an anticancer drug

<table>
<thead>
<tr>
<th>Year</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>1962-68</td>
<td>NCI screening of cytotoxic agents from natural products</td>
</tr>
<tr>
<td>1967</td>
<td>Antitumor activity detected</td>
</tr>
<tr>
<td>1969</td>
<td>Pure paclitaxel isolated</td>
</tr>
<tr>
<td>1971</td>
<td>Structure elucidated</td>
</tr>
<tr>
<td>1983</td>
<td>Phase I studies initiated</td>
</tr>
<tr>
<td>1986</td>
<td>Hypersensitive reactions observed</td>
</tr>
<tr>
<td>1988</td>
<td>NCI suggests premedication regimen</td>
</tr>
<tr>
<td>1989</td>
<td>Proved effective against ovarian cancer</td>
</tr>
<tr>
<td>1991</td>
<td>Proved effective against breast cancer</td>
</tr>
<tr>
<td>1992</td>
<td>Proved effective against non-small cell lung cancer</td>
</tr>
<tr>
<td>1992</td>
<td>Approved by US FDA for ovarian cancer</td>
</tr>
<tr>
<td>1994</td>
<td>Approved by US FDA for breast cancer</td>
</tr>
<tr>
<td></td>
<td>Total synthesis by Nicolaou and Holton, Independently</td>
</tr>
<tr>
<td>1994</td>
<td>Approved in India for ovarian cancer</td>
</tr>
<tr>
<td>1995</td>
<td>Approved in India for breast cancer</td>
</tr>
</tbody>
</table>
3.3.1 Physico-chemical properties of Paclitaxel

Paclitaxel is a diterpenoid pseudoalkaloid (Fig. 3-1). The chemical name of paclitaxel is 5b,20-epoxy-1,2a,4,7b,10b,13a-hexahydroxytax-11-en-9-one-4,10-diacetate-2-benzoate-13-ester with (2R, 3S)-N-benzoyl-3-phenylisoserine (molecular formula C_{47}H_{51}NO_{14}; molecular weight 853.9 Da). Paclitaxel differs structurally from other currently available antineoplastic agents (Kingston, 1991; Gregory and DeLisa, 1993).

It is a white to off-white crystalline powder. It is highly lipophilic and practically insoluble in water and melts at around 216-217°C. Although paclitaxel analogs appear to be another approach to overcome the problem of availability, the limiting factor is the construction of the taxane framework with its four-membered oxetane ring and a homochiral ester side-chain at C13 which becomes a challenge for synthetic chemists.

Extensive studies have indicated that an intact taxane ring and an ester side-chain are essential for cytotoxic activity (Kingston, 1994). In addition, it was shown that the
presence of an accessible hydroxyl group at position 2' of the ester side-chain enhances the cytotoxic activity of the drug (Guenard et al., 1993). Modification of the side-chain has resulted in a more potent analog, docetaxel (Fabre et al., 1995). These structure-activity relationships are very important in the development of paclitaxel analogs (Fig. 3-2). Paclitaxel undergoes epimerization in culture media and forms 7-epitaxol, which is as effective as paclitaxel (Ringel and Horwitz, 1987).

Figure 3-2. Structure-activity relationships of paclitaxel

3.3.2 Mechanism of action of Paclitaxel

Unlike other antimicrotubule drugs, such as vinca alkaloids, which induce the disassembly of microtubules, paclitaxel promotes the polymerization of tubulin (Schiff et
At subnanomolar concentrations, paclitaxel inhibits the disassembly of microtubules, whereas it increases their mass and numbers at higher, albeit clinically achievable, concentrations (Jordan et al., 1993). The microtubules formed in the presence of paclitaxel are extraordinarily stable and dysfunctional, thereby causing the death of the cell by disrupting the normal microtubule dynamics required for cell division and vital interphase processes. Paclitaxel also induces the expression of the gene for the tumor necrosis factor α, but structure-activity studies indicate that these activities are not related to paclitaxel’s affects on microtubule assembly, raising the issue of what part this cytokines play in the antitumor activity of paclitaxel (Burkhart et al., 1994). Paclitaxel binds to the N-terminal 31 amino acids of the beta-tubulin subunit in the microtubule, rather than to tubulin dimmers (Rao et al., 1994). In intact cells, paclitaxel induces the bundling of microtubules, which may be useful clinical correlate of a lethal drug effect, and the formation of the large number of asters of mitotic spindles (Schiff and Horwitz, 1980). It also enhance the cytotoxic effects of ionizing radiation in vitro, possibly by inducing arrest in the premitotic G2 and mitotic phases of the cell cycle, which are the most radiosensitive phases (Fig. 3-3) (Tishler et al., 1992, 1992a). The feasibility of using paclitaxel in combination with radiation to treat patients with locally advanced lung, head and neck, and esophageal cancers, which are responsive to both kinds of treatment, is currently being evaluated (Choy et al., 1994).

3.3.3 Limitations

The prototypic taxane paclitaxel, which disrupts tubulin dynamics, has demonstrated impressive clinical activity against many common types of solid malignancies, including ovarian, breast, and non-small cell lung carcinomas, and its use in the management of
both early and advanced malignant diseases is increasing (Rowinsky et al., 1990; Lopes et al., 1993; Panchagnula, 1998). There are two limitations for clinical application of paclitaxel.

Because of the complex and unusual chemistry of paclitaxel, it is mainly extracted from the bark of a slow growing Western (Pacific) yew, and yields are about 0.01% of the dry weight of bark (Whiterup et al., 1990). Newer methods of extraction of paclitaxel in large-scale application using chloroform from a single reverse-phase column has increased yields to 0.04% (Rao et al., 1995). Approximately 3000 trees must be sacrificed in order to obtain 1 kg of paclitaxel and is sufficient to treat about 500 patients. Present-day protocol prescribes about 2 g of the drug for the chemotherapy of one patient. This is not affordable from the environmental point of view (Oliver, 1993). An alternative method allowing preparation of the drug in larger yields (i.e., a semisynthetic method using a precursor extracted from needles and twigs of a more...
prevailing yew) has been developed (Nicolaou et al., 1994). Docetaxel, a semisynthetic taxoid, is produced by attaching a semisynthetic side-chain to 10-deacetylbaccatin III, which is readily available, in yields of about 1 kg per 3000 kg of needles, from *Taxus baccata* (Colin et al., 1990). Although total synthesis of paclitaxel was achieved, the total chemical synthesis of paclitaxel on an industrial scale is very difficult and may not be feasible commercially (Holton et al., 1994; Nicolaou et al., 1994a; Jenkins, 1996).

### 3.3.3.2 Solubility

Another limitation of paclitaxel is its difficulty in clinical administration due to its poor solubility in aqueous medium. Paclitaxel is highly hydrophobic with a water solubility ≤ 0.5 mg/l. Paclitaxel lacks functional groups that are ionisable in a pharmaceutically useful range and therefore manipulation in pH does not enhance its solubility. Furthermore, common approaches to improve solubility like addition of charged complexing agents or by producing alternate salts of the drug are not feasible in the case of paclitaxel (Straubinger, 1995). Prodrug synthesis has also been extensively studied to increase the aqueous solubility of paclitaxel (Burt et al., 1995). The preferred position for the preparation of prodrug of paclitaxel is 2'-position since many 2'-acyl-paclitaxel derivatives hydrolyze fairly rapidly back to paclitaxel in blood compartments (Mellado et al., 1984). Since the configuration of C-7 hydroxyl group does not seem to be a factor in determining cytotoxicity, C-7 prodrug ester has also been synthesized (Deutsch et al., 1989). *In vitro*, these prodrugs have been shown to possess cytotoxic activity against tumor cell lines comparable to those of paclitaxel. In addition, human plasma catalyzes the release of active paclitaxel. A prodrug strategy employing polyethylene glycol (PEG)
as a solubilising agent has been successfully demonstrated in case of paclitaxel (Greenwald et al., 1996).

The only dosage form available for the current administration of paclitaxel uses an adjuvant consisting of Cremophor EL (polyoxyethylated castor oil) and dehydrated alcohol. Many of the currently used excipients are not inert vehicles, but can exert a range of intrinsic adverse effects and have the potential to cause clinically significant drug interactions. One of the best-studied excipients is polyoxyethylated castor oil (Cremophor EL; Basf, Ludwigshafen, Germany), which is being used as a vehicle for the solubilization of a wide variety of hydrophobic drugs, including anesthetics, photosensitizers, sedatives, immunosuppressive agents, and anticancer drugs, such as teniposide and paclitaxel (Ten Tijc et al., 2003). The amount of Cremophor administered with such drugs averages 5 mL (range, 1.5-10 mL), although paclitaxel is an exception, as the amount is much higher per administration, about 25mL at the recommended dose of 175 mg/m² once every three weeks. For this reason, there has been a surge of interest within both industry and academia in Cremophor’s toxicological and pharmacologic profile in the context of chemotherapeutic treatment with paclitaxel. Cremophor presents a number of serious concerns when administered intravenously, including various intrinsic toxic side effects that limit the amount of paclitaxel that can be safely administered. The best known among these is an acute hypersensitivity reaction characterized by dyspnea, flushing, rash, and generalized urticaria, and the effects coincide with the use of intravenous paclitaxel formulated in Cremophor. Mostly, the hypersensitivity reaction occurs within the first two courses of paclitaxel and it can be prevented by reducing the infusion rate and by the use of steroids and histamine.
antagonists. Despite extensive premedication, the overall frequency of minor reactions is still estimated as high as 44%, with major reactions, necessitating discontinuation of paclitaxel therapy, occurring in approximately 1.5% to 3% of patients. Various studies have also shown that Cremophor alters the pharmacokinetic profile of paclitaxel by increasing the systemic exposure to the drug and reducing its systemic clearance. Depending on the dose and intravenous infusion rate, this phenomenon contributes to a distinct nonlinear pharmacokinetics profile of paclitaxel, which is most noticeable with 3-hour infusion regimens in the clinically relevant dose range of 100 mg/m$^2$ to 225 mg/m$^2$ (Gianni et al., 1995; Henningsson et al., 2001). Since paclitaxel is one of the most commercially successful anticancer agents with a world-wide sale of US$1.5 billion in 1999, there has been much effort in developing a better dosage form to avoid the usage of the toxic Cremophor EL, and this may have enormous clinical and commercial value.

3.3.4 Dose, dosing and problems

Paclitaxel has a low therapeutic index, and the therapeutic response is always associated with toxic side-effects (Weiss et al., 1990; Nightingale, 1992; Anon, 1993). It should be only used when the potential benefits of paclitaxel therapy outweigh the possible risks. Paclitaxel is generally given at a dose of 135 or 175 mg/m$^2$ as a 3- or 24-h infusion, every 3 weeks (Kramer and Heuser, 1995). Larger dosages and longer infusion periods have also been used (Fields et al., 1996). A report indicates that gut P-glycoprotein is involved in low peroral bioavailability of paclitaxel (Sparreboom et al., 1997). Paclitaxel is administered by intravenous infusion after diluting the paclitaxel concentrate for injection with 0.9% sodium chloride injection or 5% dextrose injection or 5% dextrose and 0.9% sodium chloride injection, or 5% dextrose in Ringer’s injection to a final paclitaxel
concentration of 0.3–1.2 mg/ml (Mead Johnson Oncology Products, Bristol Myers Squibb, 1997). The stability of 0.1 and 1 mg/ml of paclitaxel in 5% dextrose injection and 0.9% sodium chloride injection is for about 3 days (Xu et al., 1994). Contact of undiluted paclitaxel concentrate for injection with plasticized polyvinyl chloride (PVC) equipment or devices used to prepare solutions for infusion is not recommended (Waugh et al., 1991). Cremophor® EL causes leaching of diethylhexylphthalate (DEHP) from PVC containers. To minimize exposure of patients to leached DEHP, diluted paclitaxel solutions should preferably be stored in glass or polypropylene bottles or in plastic (polypropylene or polyolefin) bags, and administered through polyethylene-lined administration sets (Allwood and Martin, 1996; Pfeifer and Hale, 1993).

Paclitaxel concentrate is a clear, colorless to slightly yellow viscous liquid (Dabur Pharmaceuticals, 1994). After dilution in an infusion solution, the drug may exhibit haziness due to the surfactant content of the formulation vehicle rather than precipitation of paclitaxel. Paclitaxel in aqueous solutions is chemically stable for 27 h or longer. A hydrophilic, microporous inline filter of a pore size not more than 0.22 mm is necessary during paclitaxel infusion (Waugh et al., 1991; Dabur Pharmaceuticals, 1994; Mead Johnson Oncology Products, 1997).
### Table 3-2. Summary of therapeutic efficacy and toxicities encountered with paclitaxel therapy

<table>
<thead>
<tr>
<th>1. Tumors responding to paclitaxel</th>
<th>Ovarian cancer, breast cancer, head and neck cancer, small cell lung cancer, colon cancer, multiple myeloma, melanoma, Kaposi’s sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Dose-limiting toxic effects</td>
<td>Neutropenia, mucositis, neurotoxicity, hypersensitivity</td>
</tr>
<tr>
<td>3. Different systems</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Asymptomatic bradycardia, atrioventricular conduction blocks, atrial arrhythmias, ventricular tachycardia, ischemia</td>
</tr>
<tr>
<td>Hematological</td>
<td>Neutropenia, thrombocytopenia</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>Dyspnea with bronchospasm, urticaria, hypotension</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>Peripheral neuropathy, transient myalgia, scintillating scotamata</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Mucositis, nausea, vomiting, diarrhea</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>Elevation of liver function tests</td>
</tr>
<tr>
<td>Others</td>
<td>Alopecia, myopathy, fatigue, pulmonary lipid embolism</td>
</tr>
</tbody>
</table>

### 3.3.5 Clinical Pharmacology

Paclitaxel belongs to the class of medications known as antimicrotubule agents. It promotes microtubule assembly from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of normal dynamic...
reorganization of the microtubule network that is essential for progression through the cell cycle. In addition, paclitaxel induces abnormal arrays of "bundles" of microtubules throughout the cell cycle along with multiple asters of microtubules during mitosis (Schiff and Horwitz, 1980). Evidence suggests that paclitaxel may also induce cell death by triggering apoptosis. Paclitaxel also enhances the cytotoxic effects of ionizing radiation (AHFS Drug Information, 2005).

The pharmacokinetic data for paclitaxel is shown in Table 3-3. Paclitaxel is widely distributed into body fluids and tissues after IV administration. Paclitaxel has a large volume of distribution, suggesting binding to cellular proteins, possibly tubulin that appears to be affected by dose and duration of infusion. Renal clearance accounts for a small proportion (1 to 8 percent) of total clearance, and therefore dose modifications do not appear to be necessary in patients with renal dysfunction (Wiernik et al., 1987; Longnecker et al., 1987; Brown et al., 1991; Rowinsky and Donchower, 1993; Schilder et al., 1994). Hepatic metabolism, biliary excretion, fecal elimination, or extensive tissue binding appears to be responsible for most of the systemic clearance (Monsarrat et al., 1990, 1993). The biliary concentrations of both paclitaxel and its hydroxylated metabolites, formed by cytochrome P450 enzyme systems, are high (Monsarrat et al., 1990). Paclitaxel does not appear to readily penetrate the central nervous system, but paclitaxel has been detected in ascitic fluid following IV infusion. Paclitaxel is in FDA Pregnancy Category D. At plasma concentrations ranging from 0.1 mcg/ml to 50 mcg/ml, 88% to 98% of paclitaxel is bound to plasma proteins. Following IV infusion of paclitaxel over periods ranging from 6 to 24 hours in adults with malignancy, plasma concentrations of paclitaxel appear to decline in a biphasic manner in some studies, with
an average distribution half-life of 0.34 hours and an average elimination half-life of 5 hours. However, additional studies, particularly those in which paclitaxel is administered over a shorter period of infusion, show that the drug exhibits nonlinear pharmacokinetic behavior. In patients receiving paclitaxel 175 mg/m² administered by 3-hour IV infusion the distribution half-life averages 0.27 hours and the elimination half-life averages 2.5 hours (AHFS Drug Information, 2005). Paclitaxel is extensively metabolized in the liver by the isoenzymes cytochrome P450 (CYP) 2C8 and CYP3A4.

The clearance of the drug appeared to be linear in early studies of prolonged infusions, but clearance may be nonlinear or saturable when the drug is infused for shorter periods, with both peak plasma concentrations and drug exposure increasing disproportionately with increasing doses (Brown et al., 1991; Rowinsky et al., 1992; Sonnichsen et al., 1994; Gianni et al., 1995). Despite extensive binding to plasma proteins (95 to 98 percent), paclitaxel is readily cleared from plasma. Paclitaxel and its metabolites are principally excreted in the feces via biliary elimination with minimal urinary excretion; unchanged drug in urine typically accounts for less than 10% of the administered dose (AHFS Drug Information, 2005).
Table 3-3. Pharmacokinetic Properties of Paclitaxel*

<table>
<thead>
<tr>
<th>Duration of Infusion</th>
<th>Model</th>
<th>Half-Life</th>
<th>CL</th>
<th>VDss</th>
<th>Cpeak</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alpha/Beta/Gamma</td>
<td>ml/min/m²</td>
<td>liter/m²</td>
<td>µmol/liter (dose)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hr</td>
<td>Triphasic Saturable</td>
<td>0.20</td>
<td>1.4</td>
<td>14.4</td>
<td>294</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Saturable</td>
<td>0.27</td>
<td>2.3</td>
<td>18.8</td>
<td>212</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Biphasic</td>
<td>0.36</td>
<td>6.4</td>
<td>-</td>
<td>195</td>
<td>59</td>
</tr>
<tr>
<td>24 hr</td>
<td>Biphasic</td>
<td>0.39</td>
<td>3.3</td>
<td>-</td>
<td>359</td>
<td>119</td>
</tr>
<tr>
<td>24 hr</td>
<td>Triphasic Saturable</td>
<td>0.09</td>
<td>2.2</td>
<td>49.8</td>
<td>363</td>
<td>657</td>
</tr>
<tr>
<td>24 hr</td>
<td>Saturable elimination and distribution</td>
<td>0.14</td>
<td>2.0</td>
<td>23.6</td>
<td>393</td>
<td>269</td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>161</td>
<td>123</td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>437</td>
<td></td>
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<tr>
<td>96 hr</td>
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</tbody>
</table>

*Mean values are given. CL-systematic clearance, VDss-volume of distribution at steady state, and Cpeak-peak plasma concentration.
3.4 PLAN OF WORK

The work was undertaken on following lines:

1. Literature survey
2. Selection and procurement of lipids and paclitaxel
3. Characterization and Identification of paclitaxel
   a. Physico-chemical characterization
   b. Spectral characterization
4. Development of analytical methodology for paclitaxel
   a. Analytical method for routine analysis
   b. Analytical method for \textit{in vitro} studies
   c. Analytical method for plasma analysis
5. Preparation of SLN using different methods:
   a. Microemulsion technique
   b. Solvent injection technique
      Attempt would be made to develop new method of preparation modification of accepted production techniques.
6. Optimization of formulation based on:
   a. Influence of various process parameters
   b. Influence of lipids
   c. Influence of emulsifiers
7. Incorporation of paclitaxel into SLN
8. Determination of entrapment efficiency
9. Characterization of SLN
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   c. Influence of emulsifiers

7. Incorporation of paclitaxel into SLN

8. Determination of entrapment efficiency

9. Characterization of SLN
Objective & Plan of Work

a. Surface morphology
   i. Scanning electron microscopy
   ii. Transmission electron microscopy
b. Particle size measurements
c. Polydispersity measurements
d. Zeta potential measurements
e. Differential scanning calorimetry
f. X-ray diffraction

10. *In vitro* release kinetics of paclitaxel from SLN

11. Stability studies of the optimized formulation

12. *In vivo* performance of optimized SLN formulation
   
a. Plasma profile

   b. Organ/ tissue uptake