Chapter 1
Introduction

1.1 Introduction

Cancer continues to be one of the dreaded and killer diseases despite the concerted research work all over the world to understand and conquer it. The most deadly aspect of cancer is its ability to spread, or metastasize. Cancer cells initially group together to form a primary tumor. Once the tumor is formed, cells may begin to break off from this tumor and travel to other parts of the body and this process is called as metastasis. In particular, solid tumors have posed many challenges to systemic therapy. Major barriers to drug penetration in solid tumors include heterogeneous vascular supply and high interstitial pressures within tumor tissue, specifically in necrotic zones. The most active current drugs against cancer are the anthracyclines, taxanes (Paclitaxel) (Dorr and Von Hoff, 1994), platinum derivatives, folic acid derivatives and camptothecins (Irinotecan) (Conti et. al, 1996: DeVore et. al., 1999). Though some what effective, these drugs lead to side effect due to the bio-distribution to organs other than target organ. There are other issues too. For instance, irinotecan, being a water-soluble drug, poses the problem of hydrolytic conversion into less active carboxylate isomers. Another example is paclitaxel which is poorly soluble in aqueous solutions. Consequently this is formulated with vehicles Cremophor EL and Ethanol. Such a formulation is highly allergenic, requires extensive premedication, and is responsible for numerous acute toxicities observed with taxane therapy.

The aim of the present study was to develop delivery systems containing anti-cancer drugs with prolonged circulation time, slow systemic delivery and increased accumulation in the tumor afflicted areas. The colloidal drug delivery systems offer the potential to enhance the therapeutic index of these anticancer agents, either by increasing the drug concentration in tumor cells and or by decreasing the exposure in normal host tissues. The delivery systems can even worsen these due to the slow diffusion of macromolecular agents through the tumor tissue. However, delivery systems have been developed to exploit a feature of tumor microphysiology which is often referred to as the **'Enhanced permeability and retention effect'** (Matsumura and Maeda, 1986). This effect is a consequence of the dysregulated nature of tumor angiogenesis, which

characteristically involves structural and physiologic issues leading to hyperpermeability. Macromolecular agents with highly restricted volumes of distribution and the capacity for enhanced circulation will preferentially extravasate from these abnormal vessels and accumulate in the tumor tissues. In recent years, liposomes have been increasingly utilized to deliver drugs, enzymes, antisense oligonucleotides (Fattal et. al., 2004), and genes to various therapeutic targets (Drummond et. al. 2000; Simoes et. al., 2004). Presently a number of drugs such as Doxil® (Alza Corp.; Palo Alto, CA), Evacet® (Liposome Co.; New Brunswick, NJ), Daunoxome® (Nexstar Pharm.; Boulder, CO), and conventional liposomal vincristine VincaXome®, all of which based on liposomal preparation, are available in the market. Significant advances have been made in overcoming many of the barriers associated with liposomal drug delivery; an elusive problem has been the ability to selectively increase the bio-availability of the drug at the target tissue, while maintaining stability in the circulation.

A recent work has exploited the pH of the tumor in order to release the contents into the cytoplasm. This approach relies on selective destabilization of liposomes following acidification of the surrounding medium. The initial rationale for the design of pH-sensitive liposomes was to exploit the acidic environment of tumors to trigger destabilization of liposomal membranes (Yatvin et. al., 1980). However, the sites of greatest acidity in tumors are often the most distant from the tumor microvasculature, where liposomes may fail to reach (Huang et. al., 1992; Dellian et. al., 1996; Helmlinger et. al., 1997). In addition, the pH of the tumor interstitium rarely declines below pH 6.5 and therefore, makes it technically difficult to engineer liposomes that become disrupted in such a narrow window of pH. Endosomes and lysosomes, on the other hand, can reach values below 5.0 (Ohkuma and Poole, 1978; Tycko and Maxfield, 1982; Daleke et. al., 1990) and liposomes can be internalized by cells on the tumor periphery. Few pH-sensitive liposomes have been designed to circumvent this problem by releasing their contents prior to reaching the lysosomes and at least partly, into the cytosol, where they can diffuse to their cytosolic or nuclear targets. Endosomes and lysosomes are acidified by proton-translocating ATPases (Tycko and Maxfield, 1982; Anderson and Orci, 1988) to an average pH of approximately 5.0 (Tycko and Maxfield, 1982), but which can be as

low as 4.6 in macrophages (Ohkuma and Poole, 1978; Daleke et. al., 1990). pH-Sensitive liposomes release their contents into the cytosol by a single or a combination of several potential mechanisms. These liposomes can be induced to undergo a pH-induced fusion of liposomal membranes with endosomal membranes, directly releasing liposomal contents into the cytosol. Alternatively, liposomes can become destabilized and cause the destabilization of endosomal membranes, resulting in leakage of the drug or liposomal "cargo" into the cytosol. Studies show that pH sensitive liposomes can be targeted to areas of the body such as primary tumors and metastases or sites of inflammation and infection in which pH is low (Yatvin et. al., 1980). Recently pH sensitive liposomes were analyzed for targeting hepatocytes (Wen et. al., 2004) and breast cancer cell lines (Cardone et. al., 2005).

1.2 Objectives of the present work:

The prime goal of the study is to formulate stable (lyophilized) intravenous preparation of anticancer drugs- Paclitaxel (PCL) and Irinotecan Hydrochloride Trihydrate (IH) for effective treatment of cancer. The objectives that would lead to the above goal are listed below:

a. To optimize formulation variables and process variables of pH sensitive, serum stable, long circulating liposomes of anticancer drugs, Paclitaxel and Irinotecan Hydrochloride Trihydrate using suitable methods such as Film Hydration, Ethanol Injection or Reverse Phase Evaporation.

b. To characterize the prepared pH sensitive, serum stable, long circulating liposomes for its physicochemical characteristics such as i) Particle size, ii) Entrapment efficiency, iii) Zeta potential, iv) Solid-state analysis (DSC and XRD), v) Transverse Electron Microscopy (TEM).

c. To carry out the *in-vitro* release studies of the optimized formulations using appropriate methods.

d. To optimize and evaluate the process variables for lyophilization of the prepared pH sensitive liposomes and carryout the stability studies under various environmental conditions.

e. To carryout preclinical *in-vitro* characterizations of the pH sensitive liposomes using cancer cell lines (B16 F1 and B16F10 melanoma cells).

f. To evaluate the *in-vivo* characteristics such as biodistribution and undertake scintigraphy studies using radiolabeling of plain drug and prepared pH sensitive liposomal formulations using Technetium (^{99m}Tc) in mice models. The study will also include comparison with the plain drug and conventional liposomes.

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