CHAPTER - IV
OBJECTIVES OF THE STUDY

4.1 BACKGROUND

Cancer is the second leading cause of morbidity and mortality and the incidences are expected to continue on an upward trend for at least the near future. For over half a century, extensive research has been undertaken for the control of cancer. However, success has been limited to certain malignancies and surgical intervention is potentially curative for early stage patients. For the majority of patients with advanced stage of cancer, the treatment is limited to chemotherapy or radiation. Chemotherapy in particular has limitations due to the lack of selectivity with severe toxicity.

In particular, current anticancer drug therapy results in systemic side effects due to non specific uptake by normal healthy noncancerous tissues. Many anticancer drugs have marginal selectivity for malignant cells because they target the replicative apparatus in cells with high proliferation rates. Thus, anticancer drugs having this mechanism of action also have high toxicity against rapidly dividing normal cells. The side effects associated with chemotherapy limit the dose or cumulative doses that can be administered to patients, which can lead to relapse of the tumour and often to the development of drug resistance. Under these circumstances, tumour targeted delivery of anticancer drug is perhaps one of the most important steps for cancer chemotherapy.

There have been numerous investigations aimed at developing more efficient systems for the site-specific delivery of drugs. Nanocarrier mediated delivery has emerged as a successful strategy to enhance delivery of therapeutics and imaging agents to tumours, thereby increasing the potential for diagnosis at an earlier stage or for therapeutic success or both. Drug targeting to a specific organ or tissue selectively and quantitatively would not only improve
therapeutic efficacy but also enable a reduction of the amount of drug that must be administered to achieve a therapeutic response, thus minimizing side effects.

Despite this advantageous effect, a drug delivery device must be present in the circulation for a long enough time to reach its intended target tissue. Plasma proteins, known as opsonins, can bind circulating drug delivery devices, including nano carriers and remove them from the circulation within seconds to minutes through the RES.

Recently, polymeric micelles composed of amphiphilic block copolymers have shown much advantage in drug delivery and attracted lots of interest both in theory and application. Generally, amphiphilic block copolymers can self assemble to form nano sized spherical structures consisting of hydrophilic outer shell and hydrophobic cores can be used to incorporate lipophilic drugs and released them in a controlled manner at a later stage, while the hydrophilic shell provides stabilization for the polymeric micelles with no need of additional stabilizers. The size of these polymeric micelles is normally in the range of 10-200 nm, small enough to survive the filtration of lung and spleen. In addition if the hydrophilic shell is composed of flexible polymers such as PEG and its derivatives, the outer shell can also help these polymeric micelles escape from the RES uptake after intravenous administration by preventing the adsorption of opsonins and suppressing the complement activation. Therefore, such drug carriers may stay in the circulation, maintaining a required blood level of a pharmaceutical agent for extended time intervals, resulting in better drug availability and convenience. It has also been confirmed that if particulate drug carriers stay long enough in the circulation, they may slowly accumulate in pathological sites, including tumours, inflammations and infarcted areas through the enhanced permeability and retention (EPR) effect known as passive targeting.

Thus, amphiphilic block copolymer based polymeric micelles would be an ideal candidate as antitumour drug carriers by utilizing the so-called passive
targeting, which may in turn improve the drug efficacy and reduce the high toxicity to normal cells that accompanies most chemotherapy treatments.

Camptothecin (CPT), a cytotoxic alkaloid first isolated from *Camptotheca acuminata* is a promising antitumour agent that targets the nuclear enzyme topoisomerase I and inhibits the relegation of the cleaved DNA strand, which leads to the death of tumour cells. CPT demonstrated its antitumour activity towards a wide range of experimental tumours. However, poor solubility in water and in physiological acceptable organic solvents limits their practical use. Moreover, the opening of the labile E-ring at physiological pH and above, which may render the drug much less active and highly toxic, represents a bigger obstacle to the wide clinical application of CPT.

Among the many ways to solve the solubility problem associated with the delivery of water insoluble CPT, employing the polymeric micelles drug delivery system might be one of the simplest and most promising in terms of drug loading capacity, formulation stability, biocompatibility and the passive targeting ability. As a promising antitumour drug carrier, polymeric micelles have attracted much attention for the delivery of CPT.

Numerous attempts to overcome the poor water solubility of camptothecin by liposomal or nanoparticular lipid carriers formulations are described in various literatures. But, the results of all these approaches may be hampered by incomplete separation of free CPT from the formulations. Also the literature reviews shown that the formulation of polymeric micelles by emulsification solvent evaporation method and *in vivo* antitumour activity has not been investigated in detail and the present work is attempted to address all these issues.

Hence in this research trial, an investigation on the feasibility of using amphiphilic block co polymer based PMs was tried,
Chapter 4

Objective of the Study

1. To develop a novel drug delivery system of camptothecin using amphiphilic block co polymers as the building material.
2. To study the influence of co polymer chain length and concentration in organic phase.
3. To develop formulations that allows continuous delivery and protection of CPT.
4. To characterize the prepared CPT-PMs and to study the *in vitro* release.
5. To study the stability of CPT-PMs at room temperature and refrigeration temperature.
6. To study the tissue distribution of the polymeric micelles of camptothecin.
7. To investigate the *in vivo* antitumour effect of CPT-PMs in tumour bearing mice.

4.2 RATIONALE BEHIND THE SELECTION OF RESEARCH TOPIC

1. **Reason for Selection of Cancer as a Disease**
   
   The death rate of major cancers such as lung, breast, colon, prostate and pancreas at advance stage has not changed much in the last half century. In the past 40-50 years, low molecular weight anticancer drugs have been the main treatment modality for many cancers of advanced stage, but have offered no improvement in the cure rate. The biggest limitation of these therapeutic agents is overwhelming toxicity due to lack of selectivity. Scientists realized this fact finally towards the end of 20th century and thus selective targeting became one of the most important goals. Cancer still remains a major cause of death in most developed countries and the lack of effective control of many cancers is becoming increasingly burdensome on the health care system.

2. **Reason for Selection of Polymeric Micelles as Drug Delivery System**
   
   Block co polymer micelles have been drawing significant attention as promising carriers in drug delivery systems. The block copolymer micelles composed of amphiphilic block co polymers were utilized as a carrier of various
hydrophobic drugs including anticancer agents. The aim of utilizing the block copolymer micelles is mainly to solubilize hydrophobic and poorly water soluble drugs as well as to modulate pharmacokinetics of these drugs. Indeed, polymeric micelles with the appropriate size and surface properties showed prolonged circulation in the blood compartment. With this propensity, micelles eventually demonstrated their utility, especially in cancer therapy because of their promoted accumulation in solid tumours through the EPR effect.

Thus amphiphilic block copolymer based PM would be an ideal candidate as antitumour drug carriers by utilizing the so called passive targeting, which may in turn improve the drug efficacy and reduce the high toxicity to normal cells that accompanies most chemotherapy treatments.

3. **Reason for Selection of CPT as a Model Drug for Polymeric Micelle Delivery**

CPT inhibits DNA topoisomerase I, subsequently stabilizing the DNA topoisomerase complex resulting in apoptosis of cancer cells. Clinical application of CPT against cancer cells has been limited by its formulation and delivery problems. Parenteral administration of CPT is hampered largely by poor water solubility of its active lactone form. CPT lactone form is also unstable under physiologic conditions and converts to the inactive carboxylate form. Finally CPT can cause number of side effects to normal tissues.

4.3 **PLAN OF WORK**

1. Formulation of polymeric micelles containing various drug: polymer ratios of Camptothecin by emulsification solvent evaporation method using Methoxy polyethylene glycol block Polycaprolactone of different molecular weights (Me PEG$_{5000}$-b-PCL$_{13000}$ and Me PEG$_{5000}$-b-PCL$_{5000}$).
2. Optimization of formulation variables by trial and error method.
   ♦ Speed of centrifuge
   ♦ Rotation of rotary flash evaporator
   ♦ Sonication time

3. Characterization of polymeric micelles
   ♦ Morphological characterization using Scanning electron microscopy and transmission electron microscopy
   ♦ Particle size determination using light scattering technique
   ♦ Zeta potential, Polydispersity index
   ♦ Determination of Encapsulation efficiency
   ♦ Critical micellar concentration (CMC) determination by fluorescence spectrophotometer.

4. *In vitro* release study.

5. Tissue distribution study of camptothecin polymeric micelles in tumour bearing mice.

6. Pharmacokinetic studies of camptothecin polymeric micelles in rabbits.

7. Antitumour activity in tumour bearing mice [Ehrlich Ascites Carcinoma (EAC) cells and Daltons Lymphoma Ascites (DLA) cells].