Chemotherapy, the use of cytotoxic drugs to kill cancerous cells remains the most common approach for cancer therapy. In conventional chemotherapy most of the anti-cancer drugs administered systemically using i.v. injection. Most of the drug content is released soon after administration and distributed between normal and cancer cells leading to unacceptable side effects. Due to the short period of actions, repeated injections are often required, which can lead to exacerbation of side effects and poor patient compliances. Due to these limitations localized sustained delivery of anti-cancer drugs has been widely studied and shown lot of potential for cancer treatment.

Cancer chemotherapy offers drug delivery specialists one of the greatest challenges and opportunities to increase the therapeutic index and ultimately deliver the drug to target cancer cells whilst minimizing off-target side effects. As the scientists continue to find innovative ways to treat cancer, drug delivery specialists have the task of making sure that those treatments reach the correct site in the body, in the required concentration, and at the right time. The Indian Pharma industry is gearing up to introduce oncology drugs/innovative technologies in the international market. The global oncology drug market is growing at the rate of 17% annually.

Paclitaxel is commonly used anti-cancer drug and approved by the (USFDA) for the treatment of breast, ovarian, lung cancers and skin sarcoma in AIDS related Kaposi’s sarcoma. Breast cancer is the most frequently diagnosed cancer among women. Surgery, radiotherapy, immunotherapy and chemotherapy are the common strategies used to treat breast cancer. The best available treatment for breast cancer is surgery but this is associated with the risk of recurrence of the disease stemming from residual malignant cells. Other options available for breast cancer therapy are administration of local radiotherapy or systemic chemotherapy. Paclitaxel has been shown to be having impressive activity in both first and second line setting for breast cancer. It interacts with
tubulin dimers in the G2 mitotic phase of cell division. Paclitaxel’s success to date is largely due to its unique mechanism of action against tumors and its ability to work in combination with other anti-cancer drugs. Paclitaxel is also highly efficacious against many skin cancers, such as classical and HIV-associated forms of Kaposi’s sarcoma and basal cell carcinoma.

However, the usefulness of paclitaxel for the treatment of skin cancer is limited due to the serious adverse effects associated with the i.v. administration. Paclitaxel is a high molecular weight drug and has poor water solubility (<2 µg/mL). Presently, Cremophor EL (Polyethoxylated castor oil) and dehydrated ethanol (50:50 % v/v) are used as solubilizing agents for the preparation of paclitaxel i.v. concentrate injectable formulation (6 mg/mL). Cremophor EL is a pharmacologically active compound. Its use is associated with a number of side effects. Such as vasodilation, labored breathing, lethargy, hypersensitivity, cardio-toxicity, life threatening anaphylaxis, aggregation of erythrocytes, nephrotoxicity and neurotoxicity in many patients. Hence, the development of an improved paclitaxel drug delivery system without Cremophor EL as vehicle is required for the safe delivery of paclitaxel.

In addition to the side effects associated with the presence of Cremophor EL, the i.v. formulation of paclitaxel leads to very severe cardiotoxicity, myelosuppression and mucositis, when it distributes into vital organs like heart, liver and kidney. In addition to this, very little amount of drug reaches the target tumor tissue like breast and skin cancer, resulting in very poor therapeutic efficiency.

For the reasons mentioned above, there is a pressing need to explore a safe carrier for delivering paclitaxel and to alter its biodistribution in such a way that a greater fraction of the dose reaches the target site. This can be achieved by the development of topical formulations of paclitaxel for localized delivery into viable skin layers. Localized delivery of paclitaxel can maintain desired tissue concentration by noninvasive zero order delivery, which would enhance its efficacy for the treatment of skin and breast cancers, with high patient compliance. In spite of the obvious advantages associated with the cutaneous delivery of paclitaxel, currently no topical formulation is commercially
available. Breast cancer treatment also requires localized delivery of paclitaxel. Localized delivery of paclitaxel can maintain desired tissue concentration by noninvasive zero order delivery, which would enhance its efficacy for the treatment of skin and breast cancers, with high patient compliance.

However, high molecular weight of paclitaxel makes it difficult for it to penetrate through a dense and hydrophobic stratum corneum at a rate sufficient to achieve therapeutic efficacy. One strategy to achieve the high local drug concentration at tumor vicinity and also sustain the drug release is to encapsulate paclitaxel in the deformable lipid based vesicular drug carrier, elastic liposomes that was introduced for the effective topical site-specific delivery of number of low and high molecular weight drugs. Elastic liposomes are basically modified liposomes and developed to increase the skin permeation of encapsulated drug. Elastic liposomes are of several orders of magnitude, more deformable than the conventional liposomes and thus well suited for the skin penetration and localized delivery.

Intracellular transport of anti-cancer drugs is one of the key problems in cancer chemotherapy. Poor intracellular uptake is a major contributor to the failure of cancer chemotherapy. This limitation highlights the need to develop a drug delivery system, which enhances the cellular uptake of anti-cancer drugs in tumor cells. This type of drug delivery can overcome certain important limitations of cancer chemotherapy such as development of multidrug resistance. Vesicular carriers, such as elastic liposomes, are known to have a better intracellular uptake than other carriers like conventional liposomes in cancer cells due to the preferential uptake.

In the present study an attempt was made to develop a paclitaxel formulation with the objectives of removing Cremophor EL and enable the localization of high drug concentration at tumor site. To achieve these objectives two approaches were studied. In the first approach, elastic liposomal formulation was prepared and extensively characterized in vitro, ex vivo and in vivo. The results obtained were compared against the marketed paclitaxel formulation. The safety profile of the elastic liposomal formulation was studied by conducting acute, sub-acute toxicity and histopathology studies. In the
second approach, *in situ* thermosensitive elastic liposomal hydrogel formulation was studied. Cytotoxicity of formulation was determined by MTT assay using A549 cell line. Finally anti-cancer activity of formulation was determined by using Ehrlich ascites cell model in mice and Soft agar colony formation assay (Cytoselect 96-well cell transformation assay, Cell Biolabs, USA). Attempt has also been made for evaluating the intracellular uptake of paclitaxel elastic liposomal and marketed formulations using Fluorescence activated cell sorting assay (FACS).

### 3.1 OBJECTIVES OF THE STUDY

1. To develop Cremophor EL free elastic liposomes based formulation of paclitaxel for its localized delivery, which would serve to target the paclitaxel to its target organ, reduce its toxicity and provide the sustained release of the drug.
2. To prepare, characterize and optimize the elastic liposomes based formulation of paclitaxel.
3. To study the effect of different formulation and process variables.
4. To compare intracellular uptake, anti-cancer activity and cytotoxicity of elastic liposomes based formulation with marketed formulation.

### 3.2 PLAN OF WORK

#### 3.2.1 Identification and Characterization of Paclitaxel

- UV spectrophotometry
- HPLC
- IR spectroscopy

#### 3.2.2 Preformulation Studies

- Determination of solubility in different solvent systems
- Determination of partition coefficient
- Preparation of calibration curve using UV spectrophotometry in
  - Methanol
  - 1% SLS in PBS (pH 7.4)
  - Methanol : PBS (pH 7.4) [1:1]
- Preparation of calibration curve by using HPLC method in
3.2.3 Preparation of Elastic Liposomal Formulation

3.2.4 Microscopic Studies
- Optical microscopy
- Transmission electron microscopy

3.2.5 Characterization of Elastic Liposomal Formulation
- Shape
- Vesicle size distribution studies
- Drug entrapment efficiency
- Drug content
- Elasticity measurement
- Qualitative method
  - Filter paper vesicle interaction study using TEM and SEM
- Quantitative method
  - Extrusion method
- Zeta potential
- Vesicle population
- Turbidity measurement

3.2.6 Optimization of Formulation and Process Variables
- Amount of drug loaded in formulation
- Surfactant type and concentration
- Hydration media
- Sonication time

3.2.7 Skin Permeation and Deposition Study

3.2.8 Evaluation of Mechanism of Better Skin Permeation of Vesicular Formulation (Vesicle Skin Interaction Study)
Chapter 3 - Need for present study

- Fourier transform infrared spectroscopy (FTIR)
- Fourier transform infrared attenuated total reflectance spectroscopy (FTIR-ATR)
- Transmission electron microscopy of treated skin
- Scanning electron microscopy of treated skin
- Quantitative estimation of skin cholesterol and triglycerides content

3.2.9 Determination of Skin Localization Index

- Fluorescence microscopy
- Confocal laser scanning microscopy (CLSM)
- Quantitative determination of skin localization

3.2.10 Preparation, Optimization and Characterization of Elastic Liposomal In Situ Thermosensitive Hydrogel Formulation

3.2.11 Stability Study

3.2.12 Ex Vivo Study Against the Marketed Paclitaxel Formulation

- Cytotoxicity assay of dummy and drug loaded formulation
- Determination of IC_{50}
- Intracellular uptake study using fluorescence activated cell sorting assay (FACS) and fluorescence microscopy

3.2.13 In Vivo Study Against the Marketed Paclitaxel Formulation

- Single dose acute and repeated dose 28 days sub-acute toxicity assay
  - Determination of MTD (Maximum tolerated dose)
  - Determination of LD_{50}
  - Effects on hematological parameters
  - Effects on biochemical parameters
  - Histopathology study
  - Relative organ weight study
- Determination of anti-cancer activity in comparison to marketed formulation using
Chapter 3- Need for present study

- Ehrlich ascites carcinoma model in mice
- Soft agar colony formation assay using Cytoselect 96-well cell transformation assay
  - Pharmacokinetic study
  - Biodistribution study
  - Skin irritation study
    - Draize test
    - Transepidermal water loss (TEWL) measurement

3.2.14 Compilation, Analysis of Data and Interpretation of Results