1. INTRODUCTION

1.1 Overview of cancer

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells that might affect almost any tissue of the body (Rath, 2001). The spreading of the cancerous cells is called ‘metastasis’. It can result in death, if the spread is not controlled. Once considered a mysterious disease, cancer, however, has been eventually revealed to investigators (Trichopoulos et al., 1996). Disease development begins from a genetic alteration (mutation) of a cell within a tissue. The genetic alteration allows the cell to proliferate at a very high rate and finally form a group of fast reproducing cells with normal appearance called hyperplasia. Some hyperplastic cells mutate and produce abnormally looking descendants called dysplasia. Further mutations of dysplastic cells will eventually lead to the formation of a tumor, which can either remain localized at its place of origin, or invade neighbouring tissues called malignant tumor and establish new tumors (metastases).

There are several of cancer types such as prostate cancer, lung cancer, colorectal cancer, bladder cancer, cutaneous melanoma, pancreatic cancer, leukemia, breast cancer, endometrial cancer, ovarian cancer, brain cancer, non-Hodgkin lymphoma, etc. General classification of cancer includes Carcinoma, Sarcoma, Lymphoma, Leukemia, Germ cell tumor, Blastic, tumor, etc.

Cancer cells have some unique properties that help them to compete successfully against normal cells:
• Under appropriate conditions cancer cells are capable of dividing almost infinitely whereas normal cells have a limited life span and do not divide infinitely (Hayflick & Moorhead, 1961).

• Normal cells adhere both to one another and to the extracellular matrix. The insoluble protein mesh promotes this adherence and fills the voids between the cells whereas cancer cells fail to adhere. In addition, cancer cells possess the ability to migrate from the site where they began, invade nearby tissues and form masses at distant sites in the body through the bloodstream known as metastasis. Melanoma cells migrates to the lung, colorectal cancer cells to the liver and prostrate cancer cells to bone.

All cancers are almost caused by the abnormalities in the genetic material of the transformed cells. These genetic abnormalities in cancer affect two types of genes, namely Proto-oncogenes and tumor Suppressor genes. These two genes play a major role in cancer. Proto-oncogenes encourage growth of tumor cells, whereas tumor suppressor genes inhibit it. The coordinated action of these two gene classes normally prevents cells from uncontrolled proliferation. However, when mutated, oncogenes promote excessive cell division, while inactivated tumor suppressor genes fail to block the division mechanism. On a molecular level, control of cell division is maintained by the inhibitory action of various molecules, such as pRB, p15, p16, p21 and p53 (Meijer et al., 1997). Under normal conditions, deregulation of the cell control mechanism leads to cellular suicide, the so-called apoptosis or programmed cell death. Cell death may also result from the gradual shortening of telomeres, the DNA segments at the ends of chromosomes. Most tumor cells, however, manage to preserve telomere length due to the presence of the enzyme telomerase, which is absent in normal cells.
1.2 Cancer prevalence, causes and risk factors

Cancer is a disease of worldwide importance. Its incidence in the developed countries is rising and its mortality occupies second rank in the order of cause for death (Jemal et al., 2010, 2011). About 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008 worldwide, with 56% of the cases and 64% of the deaths in the economically developing and undeveloped countries. According to American Cancer Society, 7.6 million people died from cancer all over the world during 2007 and about 1.4 million new cancer cases were expected to be diagnosed in the year 2008. The National Institute of Health has estimated overall costs of cancer in 2007 at $219.2 billion: $89.0 billion for direct medical costs (total of all health expenditures); $18.2 billion for indirect morbidity costs (cost of lost productivity due to illness); $112.0 billion for indirect mortality costs (loss of productivity due to premature death). By the year 2050, the global burden is expected to grow to 27 million new cancer cases and 17.5 million cancer deaths simply due to the growth and ageing of the population (Siegel et al., 2011).

Breast cancer in females and lung cancer in males are the most frequently diagnosed cancers (Althuis et al., 2005). Lung cancer is preceded by prostate cancer as the most frequent cancer among males in economically developed countries. These cancers were followed by stomach and liver cancers in males, cervix and lung cancers in females in economically developing countries and by colorectal and lung cancers in females and colorectal and lung or prostate cancers in males in the economically developed world (Hanahan and Weinberg 2000).

The two factors that cause cancer are the external factors and the internal factors. The external factors include; tobacco smoking, chemicals, radiation, infections, alcohol, poor
diet, lack of physical activity and overweight, whereas the internal factors include; inherited mutations, hormones, growing older and immune conditions (Danaei et al., 2005). These factors are said to be the most common risk factors for cancer. Many of these risk factors can be avoided and several of these factors may act together to cause normal cells to become cancerous. The chemicals that cause cancer are called carcinogens and the chemicals that cause cancer through mutations in DNA are called mutagens (Griffiths et al., 2000). All mutagens are carcinogens, but all carcinogens are not mutagens. They cause rapid rates of mitosis of the cells and thus inactivate the enzyme that does the DNA repair. One of the most important carcinogens is tobacco. Smoking and its related disease remains the world’s most preventable cause of death and so is the cancer. According to National Cancer Institute (NCI), each year, more than 180,000 Americans die from cancer that is related to tobacco use. Tobacco smoking accounts for at least 30% of all cancer deaths and 87% of lung cancer deaths. The risk of developing lung cancer is about 23 times higher in male smokers and 13 times higher in female smokers compared to non-smokers.

Prolonged exposure of radiation such as ultra violet radiation from the sun, sun lamps and tanning booths causes early ageing of the skin and skin damage that can lead to skin cancer. Ionizing radiation usually causes cell damage that leads to cancer. This kind of radiation comes from the rays that enter the earth’s atmosphere from outer space, radioactive fallout, radon gas, x-rays and other sources. The radioactive fallout can come from accidents at nuclear power plants or from the production, testing or use of atomic weapons. People exposed to fallout may have an increased risk of cancer, especially leukemia and cancer of thyroid, breast, lung and stomach (Sankpal et al., 2012).
Radon is a radioactive gas that we cannot see, smell or taste. People who work in mines may be exposed to radon. People exposed to radon are at increased risk of lung cancer. The risk of cancer from low dose x-rays is very small and that from the radiation therapy is slightly higher. Being infected with certain viruses or bacteria may increase the risk of developing cancer. HPV (Human papillomavirus) infection is the main cause of cervical cancer. It also may be a risk factor for other types of cancer. Hepatitis B and Hepatitis C viruses can cause liver cancer after many years of infection. Infection with HTLV-1 (Human T-cell leukemia/lymphoma virus) increases a person’s risk of developing lymphoma and leukemia. HIV (Human Immunodeficiency Virus) virus causes AIDS. People who possess HIV have a greater risk of having cancer such as lymphoma and a rare cancer called ‘Kaposi’s sarcoma’. EBV (Epstein-Barr Virus) infection can cause lymphoma. Human herpesvirus 8 (HHV8) is a risk factor for kaposi’s sarcoma. Helicobacter pylori bacteria can cause stomach ulcers. It can also cause stomach cancer and lymphoma in stomach lining. The viruses are responsible for about 15% of the cancers worldwide (Carrillo-Infante et al., 2007). The hormonal imbalance causes cancer due to the hormones acting in the same manner as the non-mutagenic carcinogens. Hormones imbalance may increase the risk of breast cancer, heart attack, stroke or blood clot (Tinelli et al., 2008; Nahleh, 2011). The immune system malfunction and heredity causes may also causes cancer to a greater extent (Abdulla & Gurber, 2000; Strate & Syngal, 2005; Lynch et al., 2002).

1.3 Cancer treatment

The treatment for cancer varies based on the type of cancer and its stage. The stage of a cancer refers to how much it has grown and whether the tumor has spread from its
original location. Cancer can be treated by many methods such as; surgery, radiation therapy, chemotherapy, immunotherapy, targeted therapy, hormonal therapy, etc. The goal of the treatment is the complete removal of the cancer without damage to the rest of the body (Eckhardt, 2002).

Surgery is one of the method to treat cancer in the respective location by physical operation prior to metastatized cancer. It is normally used to remove small cancers and those that are not metastasized. The goal of the surgery is the removal of either the tumor alone or the entire organ. When cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible (DeVita et al., 2001).

Radiation therapy is the use of ionizing radiation to kill cancer cells and shrink tumors. It can be administered externally or internally. The effects of radiation therapy are localized and confined to the region being treated. Radiation therapy injures or destroys cells in the area being treated by damaging their genetic material, making it impossible for these cells to continue to grow and divide. Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly. The goal of radiation therapy is to damage as many cancer cells as possible, while limiting harm to nearby healthy tissue. Radiation therapy may be used to treat almost every type of solid tumor, including cancers of the brain, breast, cervix, larynx, lung, pancreas, prostate, skin, stomach, uterus and soft tissue sarcomas. Radiation is also used to treat leukemia and lymphoma (Lee, 2003).

Chemotherapy is the treatment of cancer with anticancer drugs which can destroy cancer cells. In current usage, the term "chemotherapy" usually refers to cytotoxic drugs which affect rapidly dividing cells. Chemotherapy drugs interfere with cell division in various
possible ways, like with the duplication of DNA or the separation of newly formed chromosomes. Chemotherapeutics generally target all rapidly dividing cells and are not specific for cancer cells, although some degree of specificity may come from the inability of many cancer cells to repair DNA damage while normal cells generally can. Hence, chemotherapy has the potential to harm healthy tissue especially those tissues like intestinal lining that have a high replacement rate. To overcome the side effects associated with chemotherapeutics mostly chemotherapeutic drugs are given in a combination called "combination chemotherapy" (Carrick et al., 2009).

Further, cancer can be treated by targeted therapy, using specific agents that can deregulate proteins of cancer cells (for example tyrosine kinase inhibitors). Monoclonal antibody therapy is another strategy in which the therapeutic agent is an antibody which specifically binds to a protein on the surface of the cancer cells and inhibit cell proliferation, for example anti-HER2/neu antibody trastuzumab, Herceptin, used in breast cancer, and the anti-CD20 antibody rituximab, used in a variety of B-cell malignancies (Cobleigh et al., 1999; Hurwitz 2004; Tokuda et al., 1999; Baselga, 2001).

Cancer immunotherapy is another method for treating cancer especially bladder cancer, renal carcinoma and melanoma patient. In this therapy a person induces own immune response to destroy the tumor (Mitchell, 2003).

Further, the growth of some cancers can be inhibited by providing or blocking certain hormones, namely hormonal therapy. For example in the case of breast and prostate cancers, administration of hormone agonists may provide additional therapeutic benefits (Lee, 2003; Kanoh & Okudaira, 1993).
Angiogenesis inhibitors prevent the extensive growth of blood vessels (angiogenesis) that tumors require to survive and thus it can also be considered as a treatment for cancer. Some inhibitors, such as Bevacizumab, have been approved and are in clinical use. One of the main problems with these anti-angiogenesis drugs is that they target only one factor and other factors continue to stimulate blood vessel growth. Further, the route of administration, maintenance of stability, activity and targeting at the tumor vasculature are problematic (Shih & Lindley, 2006; Ellis et al., 2001; Han et al., 2013).

1.4 Cancer chemotherapy and its evolution

Chemotherapy refers to “treatment with drugs or chemicals” to destroy the cancer cells by interfering with their life cycle. Cancer cells are more sensitive to chemotherapy than healthy cells because they divide more frequently. Healthy cells can also be affected by chemotherapy, especially the rapidly dividing cells of the skin, the lining of the stomach, the intestines and the bladder (Lowenthal & Eaton, 1996). Chemotherapy is often the first choice for treating many cancers. In chemotherapy the drug travels throughout the body to reach cancer cells wherever they may have spread (Wright, 1984). More than 100 drugs are used today for chemotherapy, either alone or in combination with other drugs for treatment. As research continues, more drugs are expected to become available.

Chemotherapeutic drugs can be divided into several groups based on factors such as how they work, their chemical structure, and their relationship to another drug. The common types of chemotherapeutic drugs are as follows;

**Alkylation agents**: Alkylation agents are used to treat a wide range of cancers, including acute and chronic leukemia, lymphoma, Hodgkin disease, multiple myeloma, sarcoma, as well as cancers of the lung, breast, and ovary. The different alkylating agents include
nitrogen mustards such as Mechlorethamine, Chlorambucil, Cyclophosphamide (Cytoxan), Ifosfamide, and Melphalan, nitrosoureas which include Streptozocin, Carmustine (BCNU), and Lomustine, alkyl sulfonates that include Busulfan, triazines such as Dacarbazine (DTIC), and Temozolomide (Temodar), ethylenimines such as Thiotepa and Altretamine (hexamethylmelamine). The platinum drugs, namely Cisplatin, Carboplatin, and Oxalaplatin are important drugs of alkylating agents. They directly damage DNA to prevent the cancer cell from reproducing (Heidelberger 1969).

**Antimetabolites:** Antimetabolites are a class of drugs that interfere with DNA and RNA growth by substituting for the normal building blocks of RNA and DNA. These agents damage cells during the S phase. They are commonly used to treat leukemias, tumors of the breast, ovary, and the intestinal tract, as well as other cancers. Examples of antimitabolites include 5-Fluorouracil, Capecitabine (Xeloda) and 6-Mercaptopurine (Heidelberger 1969).

** Anthracyclines:** Anthracyclines are antitumor antibiotics that interfere with enzymes involved in DNA replication. These agents work in all phases of the cell cycle. Thus, they are widely used for a variety of cancers. A major drawback associated with this class of drugs is that they adversely affect the functioning of the heart. Examples are Daunorubicin, Doxorubicin (Adriamycin), Epirubicin, Idarubicin, Methotrexate, Gemcitabine (Gemzar) (Alimta) Cytarabine (Ara-C), Fludarabine and Emetrexed (Hortobágyi, 1997).

**Other antitumor antibiotics:** They include Actinomycin-D, Bleomycin, and Mitomycin-C. Mitoxantrone is an antitumor antibiotic that is similar to Doxorubicin in many ways, including the potential for damaging the heart. This drug also acts as a
topoisomerase II inhibitor, and used to treat leukemia. Further, Mitoxantrone is used to
treat prostate cancer, breast cancer, lymphoma, and leukemia.

**Topoisomerase inhibitors:** These drugs interfere with enzymes called topoisomerasases,
which help separate the strands of DNA so they cannot be copied. They are used to treat
certain leukemias, as well as lung, ovarian, gastrointestinal, and other cancers. Examples
of topoisomerase I inhibitors include Topotecan and Irinotecan (CPT-11). Examples of
topoisomerase II inhibitors include Etoposide, Teniposide and Mitoxantrone (Sinha,
1995).

**Mitotic inhibitors:** Mitotic inhibitors are often plant alkaloids and other compounds
derived from natural products. They can stop mitosis or inhibit enzymes from making
proteins needed for cell reproduction. These drugs works during the M phase of the cell
cycle but can damage cells in all phases. They are used to treat different types of cancers
including breast, lung, myelomas, lymphomas, and leukemias. These drugs are known for
their potential to cause peripheral nerve damage, which can be a dose-limiting side effect.
Examples of mitotic inhibitors include the taxanes like Paclitaxel (Taxol), Docetaxel
(Taxotere), epothilones like Ixabepilone (Ixempra), the vinca alkaloids such as
Vinblastine (Velban), Vincristine (Oncovin) and Vinorelbine, and estramustine like
(Emcyt), (Navelbine) (Deep & Agarwal, 2008; Chan et al., 2012).

**Corticosteroids:** Steroids are natural hormones and hormone-like drugs that are useful in
treating some types of cancer (lymphoma, leukemias, and multiple myeloma) as well as
other illnesses. When these drugs are used to kill cancer cells or slow their growth, they
are considered chemotherapy drugs. Corticosteroids are also commonly used as anti-
emetics to help prevent nausea and vomiting caused by chemotherapy. Examples include
Prednisone, Methylprednisolone (Solumedrol) and Dexamethasone (Decadron) (Stiefel et al., 1989).

1.5 **Barriers encountered in cancer chemotherapy**

The following are four main barriers encountered in cancer chemotherapy which gives rise to increased side effects;

**Solubility:** Solubility has been identified as a critical parameter in cancer chemotherapy. The drug administered either intravenously or orally has to be soluble in the blood or should have a better oral absorption. Since most of the anticancer drugs are hydrophobic, they have a very low solubility, which results in poor therapeutic effect.

**Macrophages uptake:** Macrophages are white blood cells within tissues produced by the division of monocytes. Human macrophages are about 21 micrometers in diameter. The important role of macrophages is to find the foreign materials that enter the blood, engulf them and digest them. It is a protective system to prevent the body from attack of pathogens that enter the blood. This is considered to be a barrier for chemotherapy, because the anticancer drugs can be recognized as foreign particles and can be digested by the macrophages, which results in very poor treatment.

**Multi drug resistance (MDR effect):** The MDR is defined as the resistance of tumor cells to the cytostatic or cytotoxic actions of multiple, structurally dissimilar and functionally divergent drugs commonly used in cancer chemotherapy (Gottesman, 1993; Gottesman et al., 2002; Safa, 1996). The most studied mechanism of MDR is that resulting from the overexpression of ATP-binding cassette (ABC) transporters, localized in the cell membrane, which cause MDR by extruding a variety of chemotherapeutic agents from tumor cells. Three major ABC transporters are involved in MDR, namely P-
glycoproteins (P-gp), ABC-G2 protein and the multidrug resistance associated proteins (Perez-Tomas, 2006). P-glycoproteins are the most important transporters resulting in decreased anticancer activity of the drugs. P-glycoproteins are large glycosylated membrane proteins which localize predominantly to the plasma membrane of the cell. They confer drug resistance by active, ATP-dependent extrusion of cytotoxic drugs from the cell thus resulting in low drug accumulation in the cancer cells (Schinkel, 1997). MDR include a range of widely used anticancer drugs, such as Anthracyclines, Vinca alkaloids, Epipodophyllotoxins and Taxanes, etc.

**Stability and absorption in small intestine:** The stability and the absorption in small intestine is one of the barriers in delivering drugs to cancer cells.

**1.6 Side effects associated with chemotherapy**

Chemotherapy is a very complicated procedure that gives rise to a high or low risk making it an ineffective or effective therapy, respectively. The risk is due to the high toxicity of the chemotherapeutic drugs that leads to side effects. The side effects of chemotherapy are usually caused by its effects on healthy cells. Some of the most common side effects of chemotherapy are listed below (Tipton, 2003).

**Blood-related side effects:** One of the most important side effects of chemotherapy is its effect on blood cells, namely RBCs (Red Blood Cells), WBCs (White Blood Cells) and Platelets. Normally blood cells are the most rapidly dividing cells in the body, and therefore, the most sensitive to chemotherapy. Chemotherapeutic agents may usually decrease temporarily the levels of these blood components and occurs one to two weeks after the chemotherapy had begun.
Hair loss: Another side effect of chemotherapy is hair loss also called “alopecia”. Cells in the hair follicles are responsible for hair growth and maintenance. These cells divide rapidly and hence are affected by chemotherapeutic drugs.

Nausea and vomiting: Some chemotherapeutic agents can lead to nausea and vomiting.

Sore throat: The cells lining the inside of the mouth and throat divide rapidly. They are also continuously exposed to infections from the food. Chemotherapy can cause inflammation and infections inside the mouth known as “stomatitis” makes swallowing difficult and painful.

Diarrhea: Because the cells lining the intestines and colon divide constantly, they can be affected by chemotherapy. This can cause diarrhea.

Constipation: It is also sometimes caused by chemotherapy.

Effect on the skin: Cells lining the skin divide fairly and rapidly and hence they are susceptible to chemotherapy. This can cause skin dryness and increased reaction to the sunlight.

Fertility and sexuality: Chemotherapy may cause inhibition of gonadal cells, oligozoospermia and impotence in males. Chemotherapy can also cause inhibition of ovulation and amenorrhoea in females leading to infertility.

Other possible side effects: Besides the common side effects of the chemotherapy, there are other side effects depending on the type of cancer, the type of chemotherapy treatment and the patient’s medical condition. These side effects are due to certain factors such as dosage form, pharmacokinetics, and toxicity associated with the drug and the drug resistance by the cancer cells. The drug resistance is of three categories, namely pharmacokinetic resistance (due to low concentration of drug), kinetic resistance (small
fraction of cells in susceptible state) and genetic resistance (due to biochemical resistance). The dosage form of the anticancer drug is also a factor for the side effects. Mostly anticancer drugs are hydrophobic in nature. They have to be made hydrophilic in order to make them to be soluble in blood and available for cancer cells. For this purpose, adjuvants are added to the drugs, which cause the side effects. In the case of anticancer drug Paclitaxel, Cremophor EL is added as an adjuvant in order to improve its availability to cancer cells and to improve its solubility. This has been found to have serious side effects like hypersensitivity, nephrotoxicity, cardiotoxicity, etc.

1.7 Engineering aspects of cancer chemotherapy

The sustained prevalence of cancer, therefore, continues to motivate investigators to look for newer therapies. Several investigations in drug delivery systems have, therefore, been carried out so as to target anticancer drugs only to the site of action without affecting the healthy organs and tissues. Polymers have become an important component of these targeting approaches.

1.7.1 Polymeric delivery of chemotherapeutics

Polymers have recently gained attention as carriers capable of improving chemotherapeutic delivery to tumors (Duncan, 1992 & 2006). Polymer conjugates for drug delivery are typically large hydrophilic molecules linked to therapeutic agents that improve the solubility, increase plasma half-life, reduce toxicity, evade multidrug resistance, and add functionality (Duncan, 1992; Jain, 2001; Langer, 1998; Putnam & Kopecek, 1995). Many polymers have been investigated as candidates for the delivery of anticancer drugs (Brochini & Duncan, 1999). In general, an ideal polymer for drug delivery should have characteristics like biodegradability or adequate molecular weight
that allows elimination from the body to avoid progressive accumulation *in vivo*, low polydispersity to ensure an acceptable homogeneity of the final drug formulations and longer residence time either to prolong the drug action or to allow distribution and accumulation in respective body compartments.

### 1.7.2 Polymers used for drug delivery applications

**Synthetic polymers:** Polyethylene glycol (PEG), N-(2-hydroxypropyl)-methacrylamide copolymers (HPMA), poly(ethyleneimine) (PEI), poly(acroloylmorpholine) (PAcM), poly(vinylpyrrolidone) (PVP), polyamidoamines, divinylethermaleic anhydride/acid copolymer (DIVEMA), poly(styrene-co-maleic acid/anhydride) (SMA) and polyvinylalcohol (PVA) have been used for drug delivery applications.

Vinyl polymers can bring about high drug loading due to the reactive pendant groups and thus acts as polymeric carriers. They are usually non biodegradable and high molecular weight polymers.

HPMA is one of most widely studied synthetic polymers (Kopecek & Bazilova, 1973; Lloyd et al., 1983). Its derivative with the antitumor drug namely, Doxorubicin was the first drug-conjugate which is in phase II clinical trials (Duncan & Vicent, 2013). HPMA copolymer was also used in conjugating other anticancer drugs that suffer from low solubility namely, Campothecin (Schoemaker et al., 2002), Paclitaxel (Meerum Terwogt et al., 2001) and Pt-malonate conjugation (Gianasi et al., 2002; Rademaker-Lakhai et al., 2004).

SMANCS is a hydrophobic copolymer which is obtained from maleic anhydride and styrene. Neocarcinostatin, SMANCS, is a well known drug conjugate which exhibits cytotoxicity against mammalian cancerous cells. The SMANCS conjugate has entered

PEG is another important synthetic polymer used for drug conjugation purposes. It has unique properties such as lack of immunogenicity, antigenicity and toxicity, high solubility in water and in many organic solvents, high hydration and flexibility of the chain, low polydispersity, prolonged pharmacokinetic properties of drugs and approval by FDA for human use (Pasut & Veronese, 2007). Some of the conjugates prepared with PEG are PEG-camptothecin, PEG-Doxorubicin, etc are in phase II clinical trials.

**Pseudosynthetic polymers:** It includes polyglycolic acid (PGA), poly(L-lysine), poly(malic acid), poly(aspartamides) and poly((N-hydroxyethyl)-L-glutamine) (PHEG). These polymers are easily synthesized, biodegradable and have high capacity for drug loading. PGA with a 17,000 Da molecular weight has been conjugated to Paclitaxel through an ester bond with better drug loading (Singer et al., 2003). It has been approved for clinical trials.

**Natural polymers:** Natural polymers have also been widely studied for drug delivery purposes. Their applications range from delivery of small drugs to preparation of protein conjugates (Mehvar, 2003). Natural polymers are biodegradable, biocompatible and have low toxicity. This makes them suitable for use in biomedical and pharmaceutical applications. It includes starch, cellulose and its derivative, chitosans, dextrin, hyaluronic acid and proteins (Danhauser-Riedl et al., 1993).

1.8 Prodrugs

1.8.1 The concept of prodrug

A prodrug is a form of a drug that remains inactive during its delivery to the site of action and is activated by the specific conditions in the targeted site. Prodrugs are developed
mainly to overcome the drawbacks of the drugs such as site specificity, permeability, resistance and hydrophobicity. Albert and his coworkers were the first to suggest the concept of prodrug approach for increasing the efficiency of drugs (Albert, 1958). The prodrug approach has been one of the most promising means of site-specific drug delivery. Currently, 5-7% of the drugs approved worldwide can be classified as prodrugs and approximately 15% of all new drugs approved in 2001 and 2002 were prodrugs (Rautio et al., 2008). The conjugation of a drug with a polymer leads to a ‘polymeric prodrug’ (Hoste et al., 2004).

The use of polymeric prodrug can be reasoned as due to their advantages that include an increase in water solubility of low soluble or insoluble drugs and thus enhancement of drug bioavailability, protection of drug from deactivation and preservation of its activity during circulation, transport to targeted organ or tissue and intracellular trafficking (an improvement in pharmacokinetics) a reduction in antigenic activity of the drug leading to a less pronounced immunological body response, the ability to provide passive or active targeting of the drug specifically to the site of its action, and the possibility to form an advanced complex drug delivery system, which in addition to drug and polymer carrier, includes several other active components that enhance the specific activity of the main drug (Takakura & Hashida, 1995) Figure 1.
1.8.2 Ringsdorf model for design of polymeric prodrugs

Design of polymeric prodrugs is one of the approaches developed for improved use of drugs for therapeutic applications. Helmut Ringsdorf proposed a model that describes the ideal polymeric prodrug concept for the polymer-low molecular weight drug conjugates (Ringsdorf, 1975). This model includes a polymer chain as a carrier a spacer and a targeting moiety for anticancer agents. The polymer chain can be a homo-polymer or a heteropolymer depending on the constituents of the carrier polymer. The spacer should assist mild drug fixation. The spacers are classified as permanent and temporary spacers. Permanent spacers are those that interfere in the biological activity of the drug and temporary spacers are those that do not interfere in the biological activity of the drug. The drug must be covalently bonded to the polymer and must remain attached to it until the macromolecule reaches the desired site of action. The drug also must detach from the parent polymer at the site of action by hydrolysis or by specific enzymatic cleavage of the drug-polymer bond (Figure 2).
There are some limitations for the choice of drugs;

- only potent drugs can be used because there is a restriction on the amount of drug that can be administered.
- the drug should have a functional group by which it can be covalently bound to the polymer backbone directly or by means of a spacer.
- the drug must be sufficiently stable and should not be excreted until it is released at the desired site.

![Figure 2: Ideal polymeric prodrug model (Ringsdorf H, 1975)](image)

1.8.3 Polymer-drug conjugates

Investigation on polymer-drug conjugates started in 1975 after Ringsdorf’s model for idealized polymer chemistry for drug conjugation and Trouet and De Duve’s concept of “lysosomotropic drug delivery” pathway for targeting drugs (Trouet et al., 1972; De Duve et al., 1974). Further, the biological rationale for their design and mechanism of action were well documented by Duncan and Kopecek (Duncan & Kopecek, 1984; Duncan et al., 1992; Duncan 1992 & 2009). In a polymer-drug conjugate, the drug is covalently attached to a polymeric carrier via a biodegradable linker. The rationale for
polymer-drug conjugate is the possibility to achieve improved drug targeting to the tumor, to reduce drug toxicity and to overcome the mechanisms of drug resistance, in addition to providing prolonged half-life to the therapeutically active agents by increasing their hydrodynamic volume and hence decreasing their excretion rate. The advantages of polymer-drug conjugates are thus increased water solubility, enhanced bioavailability and prolonged plasma half-life, decreased systemic toxicity, protection towards degrading enzymes, ability to overpass some mechanisms of drug resistance, prevention or reduction of aggregation, immunogenicity and antigenicity and specific accumulation in organs, tissues and cells, by active or passive targeting (Matsumura & Maeda, 1986; Maeda et al., 2000; Iyer et al., 2006).

Several polymer-drug conjugates have been investigated and tested clinically (Table 1) (Kopecek & Bazilova 1973; Kopecek et al., 2000 & 2001; Khandare & Minko 2006; Pasut & Veronese, 2007).

Table 1: Polymer-drug conjugates in the market and clinical development (Duncan & Vicent, 2013)

<table>
<thead>
<tr>
<th>Polymer therapeutics (Class)</th>
<th>Name</th>
<th>Composition</th>
<th>Status</th>
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<tbody>
<tr>
<td>Polymeric drugs</td>
<td>Copaxone</td>
<td>Glu, Ala, Tyr copolymer</td>
<td>Market</td>
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<tr>
<td></td>
<td>Vivagel</td>
<td>Lysine based dendrimer</td>
<td>Market</td>
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<td>PEGylated proteins</td>
<td>Cimzia</td>
<td>PEG-anti-TNF Fab</td>
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<td>Mircera</td>
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<td>Uricase-PEG 20</td>
<td>PEG-Uricase</td>
<td>Market</td>
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<td></td>
<td>ADI-PEG 20</td>
<td>PEG-Arginine deaminase</td>
<td>Phase III</td>
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<tr>
<td>Polymer-drug conjugate</td>
<td>Opaxio</td>
<td>PGA-Paclitaxel</td>
<td>Phase I</td>
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<td></td>
<td>Prolindac</td>
<td>HPMA-Copolymer-DACH Platinate</td>
<td>Phase II</td>
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<td>PEG-SN 38</td>
<td>PEG-Camptothecin</td>
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<td></td>
<td>XMT-1001</td>
<td>Polyacetal-Camptothecin</td>
<td>Phase I</td>
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Polymers such as N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, poly(ethylene glycol) and poly(L-glutamic acid) (PGA) have been used as carriers for anticancer drugs such as Doxorubicin, Paclitaxel, Camptothecin and Gemcitabine (Greenwald et al., 2003; Chytil et al., 2006; Pasut et al., 2008). HPMA copolymer conjugates have been extensively studied. Anticancer agents have been bound to the HPMA copolymer using peptidyl spacers designed for cleavage by lysosomal thiol-dependent (cytsteine) proteases. Further, polymeric conjugates like PEG-Paclitaxel, PEG-Camptothecin, PEG-Methotrexate, PLA-Paclitaxel, PEG-Doxorubicin, PLGA-Paclitaxel have also been developed for targeted delivery (Maeda et al., 1992; Li et al., 1996; Riebeseel et al., 2002; Veronese et al., 2005).

### 1.9 Drug delivery

Drug delivery to cancer cells is generally categorized as either passive or active targeting depending on the presence or absence of site-directing ligands (Allen, 2002; Willis & Forssen 1998) (Figure 3).

![Drug Delivery Diagram](image)

**Figure 3: Drug delivery**

**Active targeting:** Active targeting requires site-directed ligands to bind and interact with the surfaces at the target site. Various targeting moieties or ligands against tumor-cell-
specific receptors have been immobilized on the surface of drug carriers to deliver them within cells via receptor mediated endocytosis (Lu et al., 1999 & 2002; Tarek et al., 2005).

**Passive targeting and EPR effect:** This can be attributed to the unique physiology of growing tumors and alternatively enhanced permeability and retention (EPR) effect. Matsumura and Maeda have explored the concept of EPR for passively targeting anticancer drugs to the specific sites (Matsumura & Maeda, 1986). The EPR effect can be attributed to two factors, namely the disorganized pathology of angiogenic tumor vasculature with its discontinuous endothelium that provides hyperpermeability to circulating macromolecules and the lack of effective tumor lymphatic drainage, which leads to subsequent macromolecular accumulation (Figure 4).

Figure 4: Representation of EPR effect for drug delivery to tumors (MacEwan et al., 2010)
Apart from this, the heterogeneous distribution of blood vessels combined with its aberrant branching and tortuosity, increases drug release into tumor interstitium. Further, the pressure in the interstitium is high relative to the vasculature, which retards the extravasation of drugs from the tumor site. The EPR effect along with application of external hyperthermia is, therefore, ideal for selective delivery of anticancer drugs to tumor site for better therapeutic efficacy. It involves heating of tumor tissues above the body temperature (40°C-42°C) which enhances drug accumulation at tumor sites and kills cancer cells. In addition, it has been observed that hyperthermia can also affect the biological function of cancer cells by inhibiting DNA synthesis and repair mechanism (Jain, 1987; Nakayama & Okano, 2011). Mild hyperthermia also promotes high blood flow and high vascular permeability of therapeutic agents at tumor site (Ponce et al., 2006).

1.10 Lysosomotropic delivery of polymer-drug conjugates

The well-known concept for targeted delivery of polymer drug-conjugates, namely ‘lysosomotropic’ drug delivery system was advocated more than two decades ago by Trouet et al. (Trouet et al., 1972; De Duve et al., 1974). Hydrophilic polymer–drug conjugates administered intravenously diffuse rapidly into circulation. The drug which is covalently bound to the polymer by a linker is stable in the circulation and hence mostly prevented for uptake by normal tissues. Initially the polymer-drug conjugate target passively due to the enhanced permeability and retention effect (EPR effect) into the tumor interstitium. On arrival in the tumor interstitium, they are internalized by tumor cells through either fluid-phase endocytosis (pinocytosis) or receptor-mediated endocytosis. The internalized drug conjugate is then trafficked via the endosomal
compartments to the lysosomes (Figure 5). The drug is then released intracellularly on exposure to the lysosomal enzymes at their acidic pH. For example, Gly-Phe-Leu-Gly and polyglutamic acid spacers are susceptible to cleavage by cathepsin B whereas the hydrazone linkage degrades in lysosomes at their acidic pH (Christie & Grainger, 2003; Nakayama & Okano, 2011).

![Diagram of Lysosomotropic delivery of polymer drug conjugate](Duncan, 2003)

In the past significant research efforts have been directed towards targeting anticancer drugs to tumors using specialized drug carriers. Biopolymers have become an important component of these targeting approaches (Dreher et al., 2003). In this context, thermally responsive elastin like polypeptides (ELPs) have also been examined.

1.11 The origin and the properties of Elastin like polypeptides (ELPs)

ELPs are derived from elastin proteins. Elastin, the elastomeric protein, is one of the main components of the extracellular matrix which provides structural integrity to the tissues and organs of the body. This highly cross linked and insoluble protein is the
essential element of elastic fibers, which induce elasticity to the tissues of lung, skin, and arteries. These elastin fibers form the internal core, which is interspersed with non-elastin components, namely microfibrils that includes fibrillins, fibulins and microfibril associated glycoproteins (Vrhovski & Weiss, 1998). Nearly 90% of the elastic fibre consists of elastin protein, which is a polymer of the monomeric precursor, tropoelastin and is the dominant contributor to fibre elasticity (Muižnieks et al., 2010). The protein, tropoelastin is the fundamental building component of all elastins. The most important feature of the primary tropoelastin sequence is a distinct arrangement of alternating hydrophobic and cross-linking domains which is highly conserved in elastins from all species (Chung et al., 2006; He et al., 2007). Further, tropoelastin has an ability to self-assemble under physiological conditions and exhibits an inverse temperature transition (ITT) behavior. This specific property has led to the development of a new class of polypeptides, namely elastin like polypeptides (ELPs), that mimic elastin in its composition.

ELPs derived from elastins can be genetically engineered and expressed in mammalian and bacterial cells. Genetic engineering allows regulation of the ITT of these ELPs through modification of several their characteristics like length, guest residue, molecular weight and hydrophobicity (Chilkoti et al., 2002a; Meyer & Chilkoti, 2004; Urry, 1997). ELPs have several attractive features for biomedical applications. ELPs are naturally occurring pentapeptide repeats of Val-Pro-Gly-Xaa-Gly amino acids (VPGXaaG)ₙ, where Xaa is a guest residue (except Proline) and n may vary from 10 to 200 (Meyer et al., 2001a; Raucher & Chilkoti, 2001). The repetitive ELP phenomenon was inspired from W4 domain of human tropoelastin that consists of repetitive VPGG, VPGVG, and
APGVGV peptides (Jensen et al., 2000; Rapaka & Urry, 1978; Toonkool et al., 2001). These polymers are biocompatible, biodegradable, non immunogenic and can retain in blood circulation for longer times. Biocompatibility, biodegradable and non immunogenic properties of ELPs suggest their suitability for *in vivo* applications (Mackay & Chilkoti, 2008). Another important feature of ELPs is their capability to undergo inverse temperature transition (ITT) behavior in response to temperature change (Urry, 1992; Urry, 1997; Li et al., 2001) which has attracted attention for targeting cancer therapeutics to solid tumors by the application of external hyperthermia to induce tumor localization of these ELPs. They are soluble in aqueous solutions at temperatures below their ITT but become insoluble and aggregate at temperatures above their ITT (Meyer & Chilkoti, 2002). The ITT of ELPs can be precisely tuned between 0-100°C with a precision of a few degrees Celsius and optimized for specific applications like drug delivery, protein purification, etc. This phase transition behavior of ELPs is sensitive to numerous variables, including molecular weight, concentration, co-solute, presence of guest residue, hydrophobicity, mole fraction, etc. For example, it has been observed that hydrophobic guest residues lower the ITT whereas hydrophilic residues increase the ITT. Further, the distribution of the guest residues along the polypeptide chain, incorporation of residues with ionizable side chains at the fourth position of ELPs can also alter their ITT (Urry, 1997). The exact placement and nature of each amino acid is also important for its ITT behavior. This ITT behavior, namely the tunable ITT of ELPs between 37°C-42°C, can be exploited for delivering drugs to solid tumors by the application of external hyperthermia. Hyperthermia promotes more uptakes of ELPs within the solid tumor. Hyperthermia mediated drug delivery is thus ideal for targeting cancers using ELPs as
carriers. It involves heating of tumor tissues above body temperature (40°C-42°C) to kill cancer cells and/or enhance drug accumulation. Mild hyperthermia also promotes high blood flow and high vascular permeability of therapeutic agents at tumor site (Ponce et al., 2006). In addition, it has been observed that hyperthermia can also affect the biological function of cancer cells by inhibiting DNA synthesis and repair mechanism (Jain 1987, Nakayama & Okano, 2011). ELPs also have longer plasma half-lives, reduced normal tissue toxicity and improved cytotoxicity in multi-drug resistant cell lines compared to free drugs (Raucher & Chilkoti, 2001). In addition to passively targeting to tumors by the enhanced permeable and retention (EPR) effect (Matsumura & Maeda, 1986), they also show improved cytotoxicity in multidrug resistant cell lines compared to the free drug because of their longer plasma half-lives (Liu et al., 2006).

The ability to synthesize ELPs with a precise molecular weight and low polydispersity using genetically engineering techniques, favorable pharmacokinetics, the potential biocompatibility and their controlled degradation make them interesting delivery vehicles for systemic drug delivery (Meyer & Chilkoti, 2002, Duncan, 2006). They are degradable by proteolysis which reduces their adverse side effect due to long term accumulation in the body (Mackay & Chilkoti, 2008). Further, it has been shown that ELPs as drug carriers do not cause any inflammation at the site of injection. It is non toxic to mouse fibroblast, do not produce systemic toxicity, dermal irritation, systemic antigenicity and hemolysis (Urry et al., 1991; Cappello et al., 1998). ELPs can be used for facile attachment of chemotherapeutics via precise positions for conjugation purposes to provide for specific release of drugs (Dreher et al., 2003).
Together these properties help in targeting anticancer drug to specific sites using ELP as carrier.

### 1.12 Drug delivery applications of ELPs

Several investigations have been carried out using ELPs for drug delivery purposes (Chilkoti et al., 2002b; Meyer et al., 2001a; Furgeson et al., 2006; Dreher et al., 2003). ELP based drug delivery systems no doubt have several possible advantages over the parent free drug like improved drug pharmacokinetics, passive tumor targeting by the EPR effect, decreased toxicity, increased solubility in biological fluids, site specific drug targeting and prolonged biological half-life thus leading to less frequent administration to the patient and programmed profile of drug release.

### 1.13 Doxorubicin

Doxorubicin (trade name Adriamycin; also known as hydroxydaunorubicin) is a well known anticancer drug (Figure 6) widely used in all types of cancer. It is an anthracycline antibiotic, closely related to the natural product Daunomycin, and like all anthracyclines it act by intercalating DNA. It is commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas. The drug is administered intravenously in the form of hydrochloride salt. It is photosensitive and it is often covered by an aluminum foil to prevent its decomposition from light (Blum & Carter 1974).
Its IUPAC name is \((8S,10S)-10-(4\text{-amino}-5\text{-hydroxy}-6\text{-methyl}-\text{tetrahydro-2H}\text{-pyran-2-yloxy})-6,8,11\text{-tri}-\text{hydroxy}\text{-8-(2\text{-hydroxyacetyl})-1\text{-methoxy}}-7,8,9,10\text{-tetrahydrotetracene-5,12-dione}\). It has a molecular mass of 543.52 g/mol with 5% oral bioavailability and 12-18.5 h half-life. Doxorubicin is a 14-hydroxylated version of Daunorubicin, the immediate precursor of Doxorubicin in its biosynthetic pathway (Binaschi et al., 2001).

Daunorubicin is more abundantly found as a natural product because it is produced by a number of different wild type strains of *streptomyces*. Further, in 1969 Arcamone et. al., found a non-wild type species, namely *streptomyces peucetius* subspecies *cesius* ATCC 27952, which is also capable of producing Doxorubicin but not in good quantity (Arcamone et al., 2000).

**Mechanism of Action:** The mechanism of action of Doxorubicin is complex and still somewhat unclear, though it is thought to interact with DNA by intercalation (Gewirtz, 1999). Doxorubicin is known to interact with DNA by intercalation and inhibits the progression of the enzyme topoisomerase II, which unwinds DNA for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication (Liu et al., 1983; Tewey et al., 1984). As evidenced by
several crystal structures, the planar aromatic chromophore portion of the molecule intercalates between two base pairs of the DNA, while the six-membered daunosamine sugar sits in the minor groove and interacts with flanking base pairs immediately adjacent to the intercalation site, thus inhibiting the DNA replication (Figure 7) (Pigram et al., 1972; Frederick et al., 1990).

![Figure 7: Mechanism of action of Doxorubicin (Intercalation of Doxorubicin with DNA)](http://en.wikipedia.org/wiki/File:Doxorubicin%20DNA_complex_1D12.png)

**Limitations and side effects:** Although Doxorubicin is one of the most effective chemotherapeutic agents with most frequent usage, its clinical use is limited due to the acute side-effects that include nausea, vomiting, and heart arrhythmias. It can also cause neutropenia (a decrease in white blood cells) as well as complete alopecia (hair loss). When the cumulative dose of Doxorubicin reaches 550 mg/m², the risks of developing cardiac side effects, including congestive heart failure, dilated cardiomyopathy, and death, dramatically increase (Petit, 2004; Minotti et al., 2004). Additionally, some patients may develop Palmar plantar erythrodysesthesia, or, "Hand-Foot Syndrome,"
characterized by skin eruptions on the palms of the hand or soles of the feet, characterized by swelling, pain and erythema. Due to these side effects and its red color, Doxorubicin has earned the nickname "red devil" or "red death". Doxorubicin can also cause reactivation of Hepatitis B. Besides these side effects, it has another limitation, namely multi-drug resistance (MDR) (Luqmani, 2005; Krishna & Mayer 2000). MDR are expressed in many tumor cells like liver, kidney and colon cells, as well as malignant cells. Doxorubicin also has a short half-life and low therapeutic efficiency (Links & Brown, 1999).

**Drug delivery aspects of Doxorubicin:** Various researchers have studied to target Doxorubicin delivery to cancer tissues or to diminish the side effects (Kalyanaraman et al., 2002). To overcome the limitations and side effects of Doxorubicin, different formulations have been developed. The Doxorubicin can be delivered to the cancer cells by drug delivery systems that include nanoparticles, prodrugs, micelles, liposomes, etc. The nanoparticles, especially polymeric nanoparticles, are said to have better delivery of Doxorubicin to cancer cells due to its smaller size and encapsulation of the drug by the polymer which results in a sustained release. Over the past decade, polymeric micelles have received much attention to deliver anticancer drugs. Micelles are used for improving the delivery of Doxorubicin due to its size, which is less than 100 nm, and escape from renal exclusion and reticulo-endothelial system giving them enhanced vascular permeability (Noguchi et al., 1998). Biodegradable polymeric micelles of Doxorubicin were also extensively utilized for passive as well as active targeting to solid tumors (Yokoyama et al., 1992; Yoo & Park, 2001 & 2004; Yoo et al., 2002). Prodrugs were developed to deliver the anticancer drug with reduced side effects by increasing the half-
life of the drug for example HPMA-Doxorubicin conjugate (Shiah et al., 2001), Doxorubicin-PEG-folate conjugate, Doxorubicin-cephalosporin prodrug (Veinberg et al., 2004), N-(phenylacetyl) Doxorubicin (Zhang et al., 2006), PEG-Doxorubicin conjugates (Rodrigues et al., 1999; Veronese et al., 2005), etc. Doxorubicin loaded liposomes have enhanced efficiency in some solid tumors compared with free Doxorubicin, because they passively target solid tumors through the EPR effect, resulting in increased drug payloads delivered to tumors (Gaber et al., 1995; Laginha et al., 2005).
1.14 Literature review on drug delivery applications of ELPs

The literature on drug delivery applications of ELPs as drug conjugates will now be reviewed.

Meyer et al., (2001a) have synthesized a thermally responsive ELP with an ITT of 41°C and thermally unresponsive control ELP using genetic engineering techniques. *In vivo* fluorescence video microscopy and radiolabel distribution studies revealed that hyperthermic targeting of the thermally responsive ELP shows a 2-fold increase in tumor localization compared to the unresponsive ELP in SKOV-3 ovarian carcinoma and D-54MG glioma implanted in nude mice. They observed aggregates of the thermally responsive ELP within the heated tumor and concluded that accumulation is caused by its ITT behavior. Further, the results of window chamber studies also revealed the preferential accumulation of the thermally responsive polypeptide in heated tumors is due to the combined effect of EPR and hyperthermia.

Meyer et al., (2001b) have also performed a study using a genetically engineered ELP and a synthetic and thermally responsive copolymer of N-isopropylacrylamide (NIPAAm) and showed a two fold increase in the accumulation of the drug in ovarian tumors implanted in dorsal skin of nude mice with local hyperthermia. The results thus reveal that enhanced delivery of anticancer drugs to solid tumors can be achieved by conjugating drugs to thermally responsive natural as well as synthetic polymers with mild hyperthermia.

Raucher & Chilkoti (2001) have examined the quantitative uptake of a fluorescence-labeled and thermally responsive ELP as a function of its concentration between 5 and 15 μm in solution in response to hyperthermia by three cultured cancer cell lines. Flow
cytometry of the fluorescein-ELP conjugate showed that hyperthermia enhances the cellular uptake of the thermally responsive ELP in human ovarian carcinoma, SKOV-3 and HeLa cells at a concentration of 10 µm or higher as compared to the uptake of a thermally inactive ELP control. In FaDu cells, hyperthermia stimulated the uptake of the thermally responsive ELP at all solution concentrations of the ELP between 5 and 15 µm. In particular, a >2-fold greater uptake of thermally responsive ELP compared to the thermally inactive control ELP was observed for FaDu cells at a solution concentration of 15 µm in heated cells. Further, confocal fluorescence microscopy of tumor cells incubated with a rhodamine conjugate of the thermally responsive ELP showed that the cytoplasm was uniformly stained below its ITT. Above its ITT fluorescent, particles were observed in the cytoplasm, suggesting that these particles are aggregates of the thermally responsive ELP.

Chilkoti et al., (2002a) have reviewed the recombinant DNA methods for the design and synthesis of ELPs for targeted delivery of radionuclides, chemotherapeutics and biomolecular therapeutics to tumors and concluded that ELPs as drug carriers enhance the localization of ELP-drug conjugates within solid tumors when external hyperthermia is applied.

In an effort to understand the behavior of ELPs within the tumor, Dreher et al. (2003) have synthesized a thermally responsive ELP by genetic engineering technique and conjugated Doxorubicin through an acid labile hydrazone bond to enable its release in the acidic environment of the lysosomes. The results of confocal fluorescence microscopy revealed that the ELP-Doxorubicin conjugate is endocytosed by squamous cell carcinoma cells (FaDu) and trafficked into the lysosomes. Results also revealed that both the free
drug and ELP-Doxorubicin conjugate exhibit near equivalent \textit{in vitro} cytotoxicity, although their subcellular localization was significantly different. The free drug was largely concentrated in the nucleus whereas the ELP-Doxorubicin conjugate was dispersed throughout the cytoplasm with limited nuclear accumulation.

Bidwell & Raucher (2005) have shown the application of an ELP as a delivery vehicle for a short peptide capable of inhibiting the transcriptional function of a specific oncogene. The sequence for the ELP was modified by the addition of the membrane translocating sequence, penetratin, and a peptide derived from helix 1 of the helix-loop-helix region of c-Myc (H1S6A, F8A) known to inhibit c-Myc transcriptional function. The designed polypeptide, Pen-ELP-H1, was then expressed and purified from \textit{Escherichia coli}. The results of the flow cytometry analysis revealed that the cellular uptake of Pen-ELP-H1 is enhanced by both the penetratin sequence and by the hyperthermia-induced phase transition. Further, the results of the immunofluorescence and reverse transcription-PCR studies revealed that the Pen-ELP-H1 is able to disrupt the nuclear localization of c-Myc and inhibit transcriptional activation by c-Myc. Cell proliferation studies showed that Pen-ELP-H1 inhibits growth of MCF-7 cells. Further, the use of hyperthermia was found to increase the antiproliferative effect of the thermally responsive Pen-ELP-H1 by 2-fold when compared with a nonthermally responsive control polypeptide. Through these studies they showed that genetically engineered ELP carriers may provide a new way to thermally target specific oncogene inhibitors to solid tumors.

Herrero-Vanrell et al., (2005) have studied the self-assembled Poly(VPAVG) micro-and nanoparticles as vehicles for the controlled release of Dexamethasone phosphate (DMP).
Poly (VPAVG) was prepared as stable particles with a size below 3 µm above its transition temperature (~30°C). These self-assembled particles were able to encapsulate significant amounts of the drug when self-assembling was carried out in a co-solution polymer-DMP. The results of study revealed that sustained DMP release and confirmed that the ELPs have the potential for releasing the drugs at specific sites.

Furgeson et al., (2006) have synthesized a thermoresponsive and genetically engineered ELP containing a C-terminal cysteine residue and conjugated Doxorubicin to the ELP through different pH-sensitive and maleimide activated hydrazone linkers. The results revealed that the ELP-Doxorubicin conjugates with longer linkers exhibit slower transition kinetics compared to the ELP-Doxorubicin conjugates with shorter linkers. The aim of the study was to deliver high concentrations of the drug from the ELP-Doxorubicin conjugate to solid tumors in a controlled manner.

Kaufmann & Weberskirch (2006) have investigated an elastin mimetic polypeptide-drug conjugate for targeted delivery of Doxorubicin and demonstrated that elastin mimetic polypeptides, (EMM)$_7$ [GRDPSS(VPGVG VPGKG VPGVG VPGVG VPGEG VPGIG)$_7$], can be used as potential drug carriers for cancer chemotherapy. They used an efficient synthetic method for conjugating (EMM)$_7$ to Doxorubicin thus avoiding the generally used multi-step synthesis of drug conjugation with higher cost and lower efficiency.

Liu et al., (2006) have synthesized a genetically engineered ELP and examined its preferential accumulation in solid tumors upon mild hyperthermia. They investigated the biodegradability, pharmacokinetics, tumor localization and tumor spatial distribution of a C$^{14}$-labeled ELP. The tumor accumulation and spatial distribution of the intravenously
administered C\textsuperscript{14}-labeled ELP and a control ELP (that was designed to remain soluble in heated tumors) were examined in both heated (41.5\textdegree{}C) and unheated tumors. The C\textsuperscript{14}-labeled ELP was found to accumulate in significantly higher concentrations in heated tumors than in unheated tumors. Quantitative autoradiography of tumor sections provided similar tumor accumulation results as the whole tumor analysis. In addition the C\textsuperscript{14}-labeled ELP had a more homogeneous distribution in heated tumors and a greater concentration in the tumor center than the control treatment.

Bidwell et al., (2007) have investigated the multidrug resistance problems in cancer cells, a major obstacle for successful anticancer therapy. The major mechanism of resistance involves cellular drug efflux by the expression of P-glycoprotein (P-gp), a membrane transporter, with a wide variety of substrates. In this context, they pointed that anthracyclines, a class of antitumor drugs, are especially prone to induction of resistance by the P-gp mechanism. To overcome the problem associated with multidrug resistance they synthesized novel analogs of drugs conjugated to macromolecular carriers in order to circumvent the multidrug resistance. Further, they also investigated hyperthermia mediated cytotoxicity of the free drug, Doxorubicin, and ELP-Doxorubicin conjugate. The study thus revealed that both cytotoxicity and apoptosis were enhanced by hyperthermia in the Doxorubicin resistant cells. The results obtained suggest that ELP-Doxorubicin conjugates may provide a means to thermally target Doxorubicin to solid tumors and overcome drug resistance in cancer cells.

Dreher et al., (2007) have studied a new methodology to target a temperature responsive ELP to solid tumors. They used a dorsal skin fold window chamber model and intravital laser scanning confocal microscopy for their study and showed that ELP forms micron-
sized aggregates and adhere to the tumor vasculature when hyperthermia is applied. Their results further revealed that thermal cycling of tumor increases the exposure of tumor cells to the ELP drug carrier. The study thus highlights a new approach, tumor thermal cycling, to exploit stimuli-responsive polymer in vivo to target to tumor vasculature with high specificity.

Massodi et al., (2009) have investigated a thermally responsive ELP fused to a Lactoferrin-derived peptide for the treatment of pancreatic cancer. They showed that the well characterized peptide derivative of bovine lactoferrin L12 possesses anticancer properties in multiple cell lines with some limitations. However, adverse side effects in normal tissues and poor plasma kinetics of this L12 peptide hindered its clinical effectiveness. To overcome these limitations, they developed Tat-ELP-L12 drug conjugate for targeted delivery of the L12 peptide and found that the conjugate is soluble in aqueous solution at 37°C but aggregates near 41°C, which makes this conjugate ideal for targeting to solid tumors. The results of their study revealed that under hyperthermic conditions the designed Tat-ELP-L12 drug conjugate possesses 30 fold increased cytotoxic properties in MIA PaCa-2 pancreatic adenocarcinoma cells.

Mackay et al., (2009) have developed a recombinant chimeric polypeptide (CP), namely ELP-Doxorubicin conjugate nanoparticle formulation and evaluated the same for its in vivo efficacy in a murine tumor model. The results revealed that the ELP spontaneously self assemble into sub-100 nm size upon conjugation with Doxorubicin. They concluded that ELP-Doxorubicin conjugate has a four-fold higher accumulation in solid tumor when compared to the free drug. They also concluded that nanoparticles based formulation of CP can be used as multifunctional nanomedicines.
Bidwell & Raucher (2010) have developed ELP fused therapeutic peptides for thermally targeted delivery of cell penetrating peptides to enhance their intracellular delivery in various cancers. The study results revealed that the ELPs are promising carriers for delivery of therapeutic peptides, namely cell penetrating peptides.

Liu et al., (2010) have synthesized and evaluated ELPs of molecular weight approximately 49 kDa using genetic engineering techniques. An ELP\textsubscript{1} radio-conjugate was designed to spontaneously undergo a soluble-insoluble phase transition between room temperature and body temperature upon intratumoral injection and on ELP\textsubscript{2} radio-conjugate was designed to remain soluble upon injection as a control. The study results revealed that after intratumoral administration, ELP\textsubscript{1} radio-conjugate retained in the tumor for significantly longer times when compared to the ELP\textsubscript{2} radio-conjugate. The study further revealed that ELP\textsubscript{1} radio-conjugate has low systemic toxicity even at high radionuclide doses, delay tumor growth and improve survival when compared to ELP\textsubscript{2} radio-conjugate. They concluded that ELPs exhibiting ITT behavior are promising carriers for intratumoral drug delivery when compared to thermally unresponsive ELPs.

MacEwan et al., (2010) have shown that ELPs as carriers enhance the efficacy of antitumor therapeutics when compared with the administration of free drugs by three mechanisms, namely increasing the overall accumulation within solid tumors, providing a homogeneous spatial distribution in tumor tissues and increasing the intracellular localization of anticancer therapeutics. Their report highlights recent developments in 'smart' stimulus responsive peptides and lipid drug carriers designed to enhance the localization and efficacy of therapeutic payloads as compared to free drugs.
Walker et al., (2012) have demonstrated that ELPs passively accumulate in solid tumors and aggregate in tumor tissues when external hyperthermia is applied. They conjugated Doxorubicin, and three different cell penetrating peptides (CPP) to an ELP. Fluorescence microscopy studies in MCF-7 breast carcinoma cells demonstrated that the three different CPP-ELP-Doxorubicin conjugates deliver Doxorubicin to the cell nucleus. Under hyperthermic conditions, tumor inhibition with CPP-ELP-Doxorubicin was found to be 2-fold higher than under therapy with free Doxorubicin at the equivalent dose.

Moktan et al., (2012) have investigated the thermal targeting of an acid-sensitive ELP-Doxorubicin conjugate and evaluated its therapeutic efficacy in vivo against the free drug, Doxorubicin. The results revealed that the ELP aggregates in response to mild hyperthermia accumulate in solid tumors but remain soluble under normal physiological conditions. They evaluated the therapeutic potential of ELPs in delivering Doxorubicin in the E0771 syngeneic mouse breast cancer model.

The literature review reveals that several investigations have been carried out using naturally occurring ELPs for drug delivery purposes. In all these investigations genetic engineering techniques have been used for synthesizing the naturally occurring ELPs. This synthetic technique is, however, a cumbersome and expensive process. Further the stability of these ELPs and conjugating drug to the ELPs are also problematic.

Further, naturally occurring long ELPs are polydisperse and have high molecular weights, properties that are not ideal for their pinocytotic uptake by the cells. The presence of reactive side chain in these ELPs also makes them unstable over long periods of time and at higher temperatures. Synthetic short ELPs, however, are monodisperse and low molecular weight peptides, properties that are ideal for their pinocytotic uptake by
the cells. They offer possibility of higher amount of drug conjugation. Further, it has been shown that synthetic short ELPs also undergo ITT behavior like naturally occurring long ELPs thus showing that pentameric repeat units are not always necessary for this behavior (Nicolini et al. 2004; Nuhn and Klok 2008). Short ELP-drug conjugate can, therefore, be used for hyperthermic drug targeting. A detailed literature survey, however, revealed that such short synthetic ELP-drug conjugates have not been investigated so far for targeting purposes. The objective of the present investigation was, therefore, to synthesize a short ELP, namely GVGVPGVG, and evaluate its potential for targeted delivery of anticancer drugs by in vitro method.