1. INTRODUCTION

The emergence of nanotechnology has made significance in clinical therapeutics for the last two decades. Nanoscale drug carriers such as polymeric nanoparticles are proven safer and more efficient for delivery of a myriad of drugs. Advantages in nanoparticles drug delivery, particularly at systemic level, include longer circulation half lives, least side effects, reduced dosing frequency and patient compliance. In cancer treatment, nanoparticles can further rely on the enhanced permeability and retention effect caused by leaky vasculature for better accumulation at tumor sites. These advantages have made therapeutic nanoparticles a promising candidate to replace traditional chemotherapy, where intravenous administration of toxic drug poses serious threat to healthy tissue and results in dose-limiting side effects.

Breast cancer is the most commonly occurring cancer in women and it comprises of almost one third of all malignancies in females. It is second to lung cancer as a cause of cancer mortality and it is the leading cause of death for American women between the ages of 40 and 55. The lifetime risk of a woman developing invasive breast cancer is 12.6 %\(^1\).

Treatments of breast cancer include surgical removal of breast, excision of tumor mass and radiation therapy to residual breast tissue. Radiation adjuvant therapy is routine after breast surgery to prevent recurrence of cancer in the breast. Radiation therapy causes risk of damaging heart, lungs, change of skin erythema and chances of transient lymphedema. Hormone adjuvant therapy helps to prevent recurrence by blocking the effect of estrogen, which is known to stimulate cancer cell growth e.g. Tamoxifen. Other hormonal therapeutic agents include aromatase inhibitors, which play a critical role in production of estrogen in postmenopausal woman e.g. Anastrozole, Letrozole, Exemestane. Chemotherapeutic agents are used in combination to treat patient with breast cancer, which are mainly used in adjuvant chemotherapy (after surgery to reduce risk of recurrence) for breast cancer.

The major problems in cancer chemotherapy are

1. Toxic drug effects on normal cells
2. Rapid clearance of the drugs from tumor tissues
Although anticancer drugs are of many different classes of chemicals and act by different mechanisms, they are specifically toxic to actively proliferating cells, regardless of whether they are malignant or normal, and rapidly eliminated from circulating blood by enzymatic degradation or urinary excretion. In many cases, the toxic drug effects on normal cells are more profound and long lasting than any therapeutic effect on tumor cells. Effects have been, therefore, directed towards increasing the drug selectivity by means of selective drug administration into the tumor feeding arteries. Three drugs are selected which act by different mechanism at different stages of cancer.

**Docetaxel** is a clinically well-established anti-mitotic chemotherapy medication (i.e., it interferes with cell division). It is used mainly for the treatment of breast, ovarian, prostate, and non-small cell lung cancer. Docetaxel is biopharmaceutical classification system (BCS) class II drug. It is only available for intravenous (IV) administration in the form of solution, powder for solution and solution for infusion. Usual dose of solution is 20mg /0.5 ml. It is insoluble in water, soluble in dimethyl sulphoxide and ethanol.

**Colchicine** is an anti-mitotic agent which inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore Colchicine effectively functions as a "mitotic poison" or spindle poison. It is used for the treatment cancer. It has low therapeutic index which demands the need for targeted delivery systems for delivering the drugs to the tumor site. Colchicine is biopharmaceutical classification system (BCS) class III drug, which is difficult to formulate. Limited number of marketed products belongs to this class since passive diffusion is the rate limiting step for the oral absorption of this type of drugs. Oral route of administration is not fruitful to develop. Colchicine is lipophilic, partially blocked by intestinal barrier with mean bioavailability of 40-45% so; intravenous route of administration is preferred to overcome above problems. IV administration is reported to non-toxic for single dose of 2mg / day. IV administration of colchicine is effective but patient should not receive colchicine by any route for continues seven days so: need to develop controlled release formulation. Initially colchicine was used in the treatment of gout but it is extremely poisonous, and later it has been investigated as antitumor agent. Usual dose of colchicine is 0.3 to 2 mg depending on
age and condition of patient (renal impairment or hepatic dysfunction). Colchicine is freely soluble in alcohol or chloroform and insoluble in petroleum ether.

**Anastrozole** is an aromatase inhibiting drug, approved for the treatment of breast cancer after surgery as well as for metastasis in pre and post menopausal woman. Anastrozole is a biolopharmaceutical classification system (BCS) class I drug. It is administrated orally in tablet dosage form with the dose of 1 mg/day. It is used in post menopausal woman having surgical removal of breast due to breast cancer. Anastrozole is freely soluble in methanol, acetone, and ethanol and very soluble in acetonitrile. It is soluble in water (0.5mg/ml).

Above mentioned anticancer drugs have severe side effects of myelosuppression, alopecia and general side effects which again require suitable drug delivery system which again need targeted drug delivery system. Nanoparticulate drug delivery systems are effective drug delivery system to overcome the drawback of side effect of these anticancer drugs by specific targeting to the solid breast tumors.

Drug targeting is a specific form of drug delivery where the pharmacological agent is directed selectively to its site of action in organ or cell. Drug entrapped in the nanoparticles goes to the site where it is needed and other tissues are not exposed to possible harm. Hence, it leads to reduction of side effects and adverse reactions. To achieve better targeting potential different modification, like PEGylation, are possible in the nanoparticulate system are applied. PEGylation of polymer is specially-targeted delivery vehicle, aims to increase effective levels of chemotherapy for tumor cells while reducing effective levels for other cells. This should result in an increased tumor cells kill and/or reduced toxicity. Specially-targeted delivery vehicles have a differentially higher affinity for tumor cells by interacting with tumor-specific or tumor-associated antigens. In addition to their targeting component, they also carry a payload - whether it is a traditional chemotherapeutic agent, or a radioisotope or an immune stimulating factor. Specially-targeted delivery vehicles vary in their stability, selectivity, and choice of target but in essence, they all aim to increase the maximum effective dose that can be delivered to the tumor cells. Reduced systemic toxicity means that they can also be used in weak patients,
and that they can carry new chemotherapeutic agents that would have been far too toxic to deliver via traditional systemic approaches.

**PEGylation** is a process of attaching the strands of the polymer PEG to molecules most typically polymers, peptides, proteins, and antibody fragments that can help to meet the challenges of improving the safety and efficiency of many therapeutics. It produces alterations in the physiochemical properties including changes in conformation, electrostatic binding, hydrophobicity etc. These physical and chemical changes increase systemic retention of the therapeutic agent. Also, it can influence the binding affinity of the therapeutic moiety to the cell receptors and can alter the absorption and distribution patterns.

PEGylation increases the molecular weight of a molecule which can impart several significant pharmacological advantages over the unmodified form such as:

1. Improved drug solubility
2. Reduced dosage frequency without diminishing efficacy with potentially reduced toxicity
3. Extended circulation time systematically
4. Increased drug stability
5. Enhanced protection of drugs from proteolytic degradation

PEGylated drugs have the following commercial advantages also:

1. Opportunities for new delivery formats and dosing regimens
2. Extended patent cycle of previously approved drugs

Poly (ethylene glycol)-5000 monomethyl ether (MPEG) was used for PEGylation.

For more than two decades, uses of polymeric materials to deliver drug are attention of investigators throughout the scientific community. Many of pioneering researches have been employed in developing non-biodegradable polymers. However, a contribution of drug release cannot be achieved from a non-biodegradable polymer based controlled
release device. Hence, biodegradable polymers are being widely studied for many applications. Biodegradable polymers have properties of degrading in biological fluids with progressive release of dissolved or dispensed drug. The use of natural polymers to deliver drugs looks to be an active area of research due to obvious reasons of compatibility, inexpensiveness and ready availability than synthetic polymers. Out of many natural polymers investigated, chitosan, gelatin, albumin, polysaccharides like dextran and starch showed promising potentialities. Chitosan and Gelatin have been investigated for the formulation of drug delivery systems in the form of nanoparticles, microspheres, films or cross linked hydrogels.

**Chitosan** is a polysaccharide with a structure comparable to cellulose. Both chitosan and cellulose are made by linear β-(1→4)-linker monosaccharide. The essential difference from cellulose is, chitosan is composed of 2-amino2-deoxy-β-D-glucan combined by glycosidic linkage. The primary amino groups lead to special properties that render chitosan very interesting for pharmaceutical properties.

**Gelatin** is a heterogeneous mixture of single or multi-stranded polypeptides, each with extended left-handed proline helix conformations and contains 50 - 1000 amino acids. Chemical cross-links can be introduced, to alter the gel properties, using transglutaminase to link lysine to glutamine residues or by use of glutaraldehyde to link lysine to lysine. There are two types of gelatin dependent on whether or not the preparation involves an alkaline pre-treatment, which converts asparagine and glutamine residues to their respective acids and results in higher viscosity. Acid pre-treatment (Type A gelatin) uses pig skin whereas alkaline treatment (Type B gelatin) makes use of cattle hides and bones.

**Mechanism of tumor targeting**

**Passive tumor targeting**

Almost all anticancer chemotherapeutic agents are not specific to tumor cells; they are randomly distributed in the body. Conventional chemotherapeutic agents have low therapeutic index. Because of this reason solid tumors are difficult to treat with chemotherapy. Polymeric carriers are conjugated or entrapped with drug molecules are
used to improve tumor targeting. These polymeric nanoparticles alter pharmacokinetic properties at drug and cellular level\textsuperscript{2}.

Tumor blood vessels have high proportion of proliferating cells, pericyte deficiency, aberrant basement formation and increased tortuosity. So, tumor blood vessels vascularise rapidly which require more oxygen and nutrients, which results in decrease lymphatic drainage and increase permeability to macromolecules. Because of poor lymphatic drainage the permanent macromolecules are not removed easily and retained in the tumor cell. This passive targeting mechanism is called as “enhanced permeability and retention (EPR) effect”\textsuperscript{3}.

Optimum size for nanoparticles for the effectiveness to tumor is not decided precisely but based on study of liposomes and nanoparticles, the cut-off size of pore in tumor vessel ranges between 200-1.2 µm\textsuperscript{4-5} and direct observation demonstrated a tumor dependant pore cut-off size ranges from 200 nm -2 µm\textsuperscript{6-7}.

![Figure: 1 Mechanism of EPR (Enhanced Permiability and Retention) effect](image)

**Selection of methods of preparation of nanoparticles**

**Ionic gelation method**

Chitosan, gelatin and sodium alginate are hydrophilic biodegradable polymer, and polymeric nanoparticles prepared by ionic gelation method for such polymers. For chitosan
nanoparticles, ionic gelation method was used by Calvo and co-workers\textsuperscript{8-9}. In this method the mixture of two phases used, one is positively charged chitosan and other is negatively charged poly anion sodium tripolyphosphate. The method involves interaction between positively charged amino group of chitosan interacts with negatively charged tripolyphosphate to form coacervates with nanomer range of particles.

**Ethanol precipitation method**

This method is used to prepare nanoparticles by adding a non-solvent, salting out, or adjusting the pH to the isoelectric point of gelatin. In this work, Gelatin nanoparticles are prepared by adjusting pH to the isoelectric point of gelatin\textsuperscript{10}. 
