Literature Review

Unit A: Disease-related literature survey

2.1 Cancer

The word cancer is derived from Greek word karkinoma used for crab, the origin of this word credited to Hippocrates in 460-370 BC. Later Celsus (28-50 BC) translated karkinoma into a Latin word known as cancer [Sarode et al., 2014]. It is a broad term for a large group of diseases distinguished as tumors (neoplasm or new growth), but tumors are not necessarily cancer. The branch of medicinal science that deals with cancer is known as oncology. The Greek word "oncos" was first used by Galien (130-200 AD) refers to tumor or growth while "logy" means study [Pasipoularides, 2014]. The earliest known descriptions and surgical treatment of cancer appear in Egypt around 1600 BC. According to WHO factsheet, cancer has become the second most likely cause of death worldwide accounting for 8.2 million deaths after cardiovascular diseases [Ferlay et al., 2015].

Cancer is a highly heterogeneous disease occurs as a result of mutation or abnormal changes in the gene, categorized by the uncontrolled and fast growth of abnormal cells that invade surrounding tissues and metastasize to distant sites inside the body [Rejniak, 2007]. Normally, cells grow, divide and die in a controlled manner because of the process of apoptosis and replace old cells with new ones by the orderly process of cell growth. Here, if normal cells come into contact with like cells they stop dividing, this mechanism is known as Contact Inhibition, but in cancer due to mutations in certain genes process of cell growth goes wrong. Thus, cells keep dividing without control order and lead to disturbing the balance between cell

growth and death; producing more new cells when the body doesn't need them, results in the formation of a mass of tissue called growth, lump or tumor.

Whether the cell is normal or cancerous, the process of cell division occurs through the cell cycle, where it goes from resting phase, through active growing phases and then to mitosis. Cancer is difficult to diagnose and treat because it grows from the heart of the organs and is not identified as a foreign body by our immune system [Jackson and Bartek, 2009]. The chance of being cured of cancer increases with early detection and treatment.

2.1.1 Breast

Normally breast is a modified skin appendage that contains narrow ducts (tiny tubes that carry milk from lobules to the nipple), lobules (milk-producing glands), nipple, stroma (fatty or adipose tissue and connective tissue), pectoralis major muscle and chest wall or rib cage. The glands and ducts are supported by connective tissue formed up of fat and fibrous material. The lymphatic system of the breast is a part of an immune system and also one of the ways which help in spreading of breast cancer as it runs throughout our body. This system includes lymph nodes which are connected to lymphatic vessels (same like the vein) that convey clear fluid lymph away from the breast. The connection of lymphatic vessels and lymph nodes named differently depending on where its connection located like axillary nodes (under the arm), internal mammary nodes (inside the chest) and supraclavicular or infraclavicular nodes (above or below the collarbone). Lymph comprises tissue fluid, waste products and immune system cells for fighting infections [Ramesh, 2013].

2.1.2 Breast cancer

The world's oldest case of breast cancer hails from ancient Egypt in 1500 BC. Incident of carcinoma of the breast is higher in premenopausal women group of developed countries because of increase in urbanization and life expectancy. Among women, it is the 2nd leading cause of cancer deaths after lung cancer, and overall it holds 5th position after lung, liver, stomach and colorectal cancer accounting about 508000 deaths each year worldwide [Jemal et al., 2008].

Breast cancer belongs to a malignant tumor that starts and develops in the tissue of breast due to the genetic abnormality. It usually begins with the formation of a small lump (confined tumor) or calcium deposits (microcalcifications) and arises from the ductal epithelium (tube-like passages that drain milk from lobules to the nipple) in 90% cases while the remaining 10% originate from the lobular epithelium (milk-producing glands). Sometimes it also begins in stromal tissues, includes fatty and fibrous connective tissues. Over time, if untreated it can grow and invade nearby healthy tissue and then underarm lymph nodes by entering through lymphatic vessels and further gotten into the blood stream from where it spread to different parts of the body, referred as metastatic or advanced breast cancer [Kopans, 2007].

2.1.3 Etiology and cause of breast cancer (IARC, WHO)

Cancer formation takes place from a single cell when a cell's DNA is damaged. DNA is the chemical present in each cell that makes up our genes which control the process of growth, division, and death. Genes that are responsible for cell division in organize manner are Protooncogenes and in an unorganized way are Oncogenes.

Genes that cause cell death to control cell division are called tumor suppressor genes. But due to abnormal changes or mutations in DNA, activation of oncogenes and inhibition of tumor suppressor genes takes place that leads to cancer.

Inherited gene mutations: Scientists identified tumor suppressor genes that are BRCA genes (BRCA1 and BRCA2) which are inherited from the parent when one of these genes is mutated development of cancer takes place. Other less common genes that are discovered is PTEN or TP53.

Acquired gene mutations: Due to factors like radiation or cancer-causing chemicals DNA mutation take place, this leads to activation of HER2 oncogenes. Healthy HER2 (human epidermal growth factor receptor-2) help in managing breast cell growth, division, and repair, but after mutation, it makes an excess number of copies of itself (HER2 gene amplification process). Then, these additional genes initiate the cells to create too many HER2 receptors (HER2 protein overexpression process) and finally results in uncontrolled growth of breast cells. These changes are the result of the interaction between a person's genetic factors and the following external agents [MacMahon et al., 1973].

- Physical carcinogens - ultraviolet and ionizing radiation like x rays, α, β, γ-rays, radioactive isotopes, protons or neutrons.

- Chemical carcinogens - asbestos, aflatoxin (group of chemicals produced by Aspergillus flavus fungi during food contaminant), arsenic (a drinking water contaminant), coal tar, fossil fuel, mineral oil, azodyes, vinyl chloride, betel nuts,

saccharin, actinomycin D, mitomycin C, metals (nickel, lead, cobalt, chromium) and components of tobacco smoke.

- Biological carcinogens - viruses include DNA virus (EBV, HPV, HBV & poxviruses) and RNA virus (HCV, HTLV-I & II), bacteria like Helicobacter pylori and parasites (Schistosoma haematobium).

2.1.4 Breast cancer therapeutic approaches

The type of applied cancer treatment is usually selected based on the kind and stages of cancer. There are various conventional cancer treatment methods applied in the hospitals. Chemotherapy is mainly used to reduce the size of the tumor or to make it disappear.

- Surgery and Chemotherapy
- Radiotherapy and Immunotherapy
- Hormonal and Gene Therapy

All of these treatments have undesired side effects, they are normally not available all the time, and they are expensive.

2.1.4.1 Chemotherapy treatment

In chemotherapy we use cytotoxic anti-cancer medicines to weaken and kill cancer cells by halting or stopping the process of cell division as if the cells are unable to divide, they will die. The drugs that affect cells when they are dividing are called cell-cycle specific, and the drugs that affect cells at resting are called cell-cycle non-specific. Usually, the drugs work by damaging the RNA or DNA by copying itself in

the process of cell division. Chemotherapy is applied to treat both early-stage invasive breast cancer as well as advanced-stage breast cancer to destroy or damage the cancer cells [Vlastos and Verkooijen, 2007]. The scheduling of chemotherapy is set based on; the type of cells, the rate at which they divide, and the time point at which a delivered drug is expected to be effective. This is why the chemotherapy is typically given in cycles. The faster the cells are dividing, the most probably chemotherapy will kill the cells, causing the tumor to shrink. They also induce cell self-death or apoptosis.

2.1.4.2 Adverse effect during chemotherapy

Unfortunately, chemotherapy does not know the difference between the cancerous cells and the normal cells; it kills all the cells that are rapidly dividing. Normally, cells grow and divide with order in control and precise manner, but some healthy cells do divide quickly which get affected by chemotherapy includes, the blood, nails, bone marrow, digestive tract, stomach, bowel, hair follicles and the cells in the mouth; resulting in low blood counts, mouth sores, nausea, diarrhea and hair loss [Alley et al., 2002].

In addition, chemotherapeutic agents are also hampered by barriers such as solubility, macrophage uptake, and multidrug resistance. The solubility of anticancer drugs is also a critical factor as they exhibit poor water solubility causing to low therapeutic effects. Although the investigation of chemotherapeutic agents via nano-size carriers enhances the stability and cellular uptake of the drug, its application is limited due to immune system response of the body. This process results in accumulation of the drug in unspecific site leading to severe damages of the liver.

The other negative side of chemotherapy is resistance developed by most of the cancer cells to cease the action of drug which termed as multidrug resistance. In most of the tumors there are the different population of cells, partly are responsive to drug and others are resistance. Anticancer agents can only kill cells which are sensitive to the drug [Gottesman, 2002]. Hence, drug resistance cells remain alive and continue to grow faster than killing process. To enhance the activity of chemotherapeutic agents, they are objected to internalize to cancer cells following to target to the tumor site. Targeting of the chemotherapeutic agent as a combination system is a primary strategy to fulfill internalization of the delivery vehicle and intracellular release of cancer treatment agent.

Table 2.1 Some nanoparticulate dug-carrier system developed for breast cancer management

S. No.	Drug	Formulation	Inferences	Ref.
1.	Paclitaxel	Albumin nanoparticles (NPs)	Formed nanoparticles demonstrating superior efficacy and reduced toxicity compared with pure paclitaxel in preclinical studies.	[Gradishar et al., 2005]
2.	Doxorubicin	Mesoporous silica nanoparticles	Comprehensive analysis of the impact of heterogeneity in the tumor microenvironment confirmed the efficacy of siRNA delivery *in vivo*.	[Meng et al., 2013]
3.	Doxorubicin	Lipid hybrid nanoparticles	Polymer–lipid hybrid nanoparticle was developed that can efficiently load water-soluble anticancer drug and enhanced its toxicity against multidrug-resistant cancer cells.	[Wong et al., 2006]

4.	Doxorubicin	Iron oxide nanoparticles	Substantial tumor growth inhibition was observed after intratumoral injection of the nanoparticles. *In vivo* and the proliferative activity of the tumor tissue was found to be reduced.	[Kossatz et al., 2015]
5.	Oxaliplatin	Chitosan nanoparticles	The expression of Bax, Bik, cytochrome C, caspase 9 and 3 was significantly up-regulated during the Bcl-2 and survivin were hindered in breast cancer MCF-7 cells for prepared NPs.	[Vivek et al., 2014]
6.	Paclitaxel	Nanoparticle-stabilized nanocapsules	The resulting combination showed higher cytotoxicity than the single agent with synergistic action established using combination index values.	[Kim et al., 2015]
7.	Rapamycin & piperine	PLGA nanoparticles	Pharmacokinetic studies showed better absorption profile of drug from polymeric nanoparticles corresponded to its suspension and increased bioavailability of 4.8 folds in combination with a chemosensitizer. An *in vitro* cell line study indicated higher efficacy of nanoparticles compared to free drug solution.	[Katiyar et al., 2016]

8.	Docetaxel & tamoxifen	PLA-TPGS nanoparticles	The authors validated the enhanced cellular uptake of drugs delivered by nanoparticles and concluded the concept of protecting drugs from metabolism in the form of DDNPs have the potential in the treatment efficacy of tumor as it reduces drug antagonism.	[Tan et al., 2014]
9.	Epigallocatechin gallate & paclitaxel	PLGA-casein nanoparticles	The subsequent release of Epigallocatechin gallate followed by paclitaxel from nanocarrier sensitized drug resistant MDA-MB-231 cells to paclitaxel-induced their apoptosis, inhibited NF-κB activation and down-regulated the key genes associated with angiogenesis, tumor metastasis, and survival.	[Narayanan et al., 2015]
10.	Paclitaxel	Magnetoelectric nanoparticles	Nanoparticles Conjugated with HER2-neu antibodies, loaded with paclitaxel were administrated intravenously. The mice treated with PTX-loaded nanoparticles were completely cured, as confirmed through infrared imaging and post-euthanasia histology studies.	[Rodzinski et al., 2016]

11.	Cisplatin, doxorubicin & 5-fluorouracil	PCL-PEG nanoparticles	The MTT assay confirmed that cisplatin, doxorubicin, and 5-fluorouracil-loaded PCL-PEG NPs improved cytotoxicity and drug delivery in T47D and MCF-7 breast cancer cells. However, the IC_{50} value of doxorubicin was below the IC_{50} values of both cisplatin and 5-fluorouracil.	[Eatemadi et al., 2016]
12.	Tamoxifen	PLGA nanoparticles	A sustained drug release pattern was witnessed for the entire period of study up to 60 days. Further, nanoparticles were internalized well in the cytoplasm confirmed by MCF-7 breast cancer cells.	[Maji et al., 2014]
13.	MiR-34a & doxorubicin	Hyaluronic acid-chitosan nanoparticles	The delivery of doxorubicin and miR-34a according to authors could deliver synergistic impacts on tumor suppression and chemotherapeutic agents.	[Deng et al., 2014]
14.	Docetaxel	PLGA nanoparticles	The results demonstrated that the cholic acid conjugate, docetaxel-loaded PLGA nanoparticles combined with autophagy inhibitors such as 3-MA and CQ could significantly enhance the therapeutic effects both *in vitro* and *in vivo.*	[Zhang et al., 2014]

15.	Sorafenib	Cyclodextrin-porous silicon nanoparticles	Nanoparticles showed a significantly increased interaction with breast cancer cells and also sustained the drug release. Furthermore, the sorafenib-loaded nanoparticles efficiently inhibited cell proliferation of the breast cancer cells.	[Correia et al., 2015]

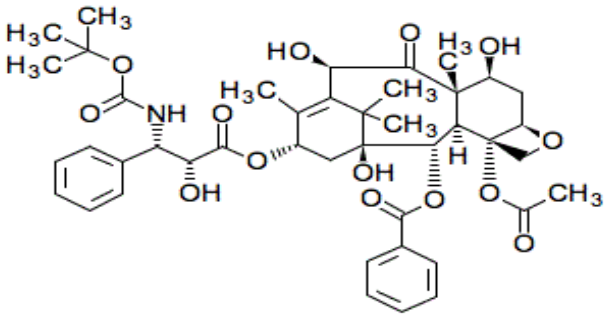
Unit B: Drug and excipient related literature survey

2.2 Drug and Excipients

2.2.1 Docetaxel

In 1986, docetaxel was discovered from the collaborative effort between Rhone-Poulenc and the Institut de Chimie des Substances Naturelles, France [Seibel and Reaman, 1996]. Docetaxel was extracted from the needles of the European Yew tree, 'Taxus baccata'. It is primarily used for the treatment of breast, ovarian, lung, prostate, head and neck cancer. The physicochemical properties, pharmacology, toxicology and pharmacokinetic profiles of this drug are discussed below.

Chemical Structure:



Mol. Wt. : 807.89 g/mol

Mol. Formula : $C_{43}H_{53}NO_{14}$

Half-life : 11 h

State : Solid white to off white crystalline powder

pKa : 10.96 (acidic) -3 (basic)

Log P : 2.59

Log S : -4.8

Log D : 3.54 (pH 7.4)

Log Kow : 2.83

Bioavailability : Complete (IV), 8% (Oral)

Melting point : 232 ^{0}C

Solubility : Insoluble in water, n-hexane; sparingly soluble in acetonitrile; soluble in methanol, ethanol, acetone, and ethyl acetate.

Water solubility : 0.274 mg/L (25 ^{0}C)

Protein binding : >98%

Routes : IV

Trade names : Taxotere, Docefrez

Absorption : 3- Compartment model

Elimination : Fecal and renal

Renal clearance : 21 L/h/m^2

Vd : 113 L

Dosage form : Injection (IV), Solution (20, 80, 160 mg)

UV absorbance : 230 nm

Metabolism : Hepatic by CYP3A4 isoenzyme

Toxicity : Bone marrow suppression, mucositis, peripheral neurotoxicity, neutropenia, mild asthenia, mild paresthesia, neurosensory disturbances, anaemia, alopecia, myalgia, hepatotoxicity, hypersensitive reactions and fluid retention [Bank, 2002].

Mechanism: Docetaxel binds to microtubules reversibly with high affinity and has a maximum stoichiometry of one-mole docetaxel per mole tubulin in microtubules. It improves the assembly of microtubules from tubulin dimers and stabilizes microtubules by inhibiting depolymerization. This stability results in the repression of the normal dynamic rearrangement of the microtubule interface that is needed for vital interphase and mitotic cellular functions. Also, docetaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis. This damages the cell's ability to use its cytoskeleton flexibly [Hamel, 2003].

Table 2.2 Some docetaxel-loaded nanoparticulate carrier based drug delivery system

S. No.	Carrier	Inferences	Ref.
1.	PLGA-PEG nanoparticles	Docetaxel NPs contributed an increased blood residence time of docetaxel fulfilled its role as a long-circulating sustained-release drug delivery system. Furthermore, confirmed the role of PEG in helping NPs evade from Cl mechanisms present in the systemic circulation and the body.	[Rafiei and Haddadi, 2017]
2.	PLGA-TPGS nanoparticles	The mannitol-functionalized star-shaped M-PLGA-TPGS NPs was prepared and demonstrated cellular uptake investigation that could be internalized by human breast carcinoma cells (MCF-7) and the *in vivo* anti-tumor efficacy indicated that formed NPs exhibited significantly superior anti-tumor effect compared with commercially available Taxotere® formulation.	[Tao et al., 2013]
3.	Interleukin nanoparticles	*In vivo* imaging, cell apoptosis, growth inhibition of tumor spheroids and TUNEL assay demonstrated the better glioblastoma target effect of ILNP and its distribution in blood vessels, macrophages, and intracellular endosomes of tumor cells.	[Gao et al., 2014]
4.	Amphiphilic dendritic nanoparticles	The dendrimer nanoparticles formed were of higher drug entrapment efficiency, faster drug release, higher cellular uptake and cytotoxicity than the linear PLA NPs and PLA-b-TPGS NPs. The *in vitro* and *in vivo* antitumor effects of the DTX-loaded H40-PLA-b-TPGS NPs were found to be significantly superior.	[Zeng et al., 2015]

5.	PEGylated CMC nanoparticles	The formed nanoparticles exhibited significantly increased efficacy compared with that of DTX in a taxane-resistant breast tumor model and demonstrated 6.5 times lower IC_{50} compared with that of native DTX.	[Roy et al., 2014]
6.	PLGA nanoparticles	The relative bioavailability of nanoparticles co-administered with R7 was enhanced about 5.57 and 9.43 fold, respectively and demonstrated maximum cytotoxicity against MCF-7 cells in MTT assay.	[Bu et al., 2015]
7.	PEG-Pep-PCL nanoparticles	The author found that radiosensitization of DOC-NPs was improved significantly in all three gelatinase overexpressing GC cells. They concluded that gelatinase-mediated nanoscale delivery system could serve as a potential strategy possessing both universality and selectivity for radiosensitizers.	[Cui et al., 2014]
8.	Folate-targeted nanoparticles	Docetaxel-loaded Chol-PEG1000-FA nanoparticles were prepared, and it's *in vitro* results showed a higher anti-tumor efficiency of the Chol-PEG1000 nanoparticles than docetaxel solutions. The IC_{50} values of DCT-Sol, DCT-NPS, and DCT-FA-NPS were found to be 1.6260, 0.3772 and 0.0171 μg/mL, sequentially, for 4T1 cancer cells after 48 h of treatment.	[Han et al., 2016]
9.	pH-sensitive nanoparticles	Here, copolymer TPGS-b-PBAE, TP was synthesized and formulated for overcoming multidrug resistance. The formed nanoparticles increased the cell cytotoxicity against both drug-sensitive human ovarian A2780 and drug-resistant A2780/T cells and their IC_{50} of DTX-loaded TP against A2780/T cells and were 100 fold lower than that of commercial DTX.	[Zhao et al., 2013]

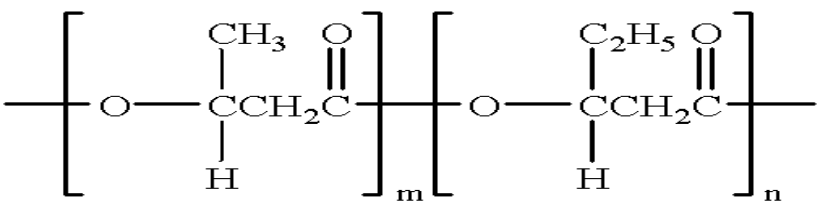
10.	Peptide-conjugated nanoparticles	Docetaxel-loaded PLGA nanoparticles were prepared and covalently conjugated with Bombesin. Nanoparticles showed the sustained drug release and 12 times more cytotoxicity than pure drug.	[Kulhari et al., 2014]

2.2.2 Polymers

Polymers are formed by chemical reaction in which a large number of molecules called monomers are joined sequentially to form a large molecule having higher molar masses. Polymers used in preparation should be biocompatible with the body concerning adaptability (non-toxicity and non-antigenicity) and should be biodegradable. Biodegradable natural polymers (Protein & Polysaccharides) and biodegradable synthetic polymers will be used in the formulation methodology as they breakdown into monomers easily by contacting with body fluids, enzymes and microbial flora [Nair and Laurencin, 2007]. Antineoplastic drugs have a narrow therapeutic index with greater potential for causing side effects. So, the selection of matrix materials should be made depending on the size of nanoparticles (NPs) required and drug release profile desired to ensure safety and efficacy of the drug.

2.2.2.1 Poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) [PHBV]

Chemical Structure:



Polyhydroxybutyrate-co-hydroxyvalerate (PHBV) polymer is novel, biodegradable, biocompatible, nontoxic hydrophobic polyester and economical with low-cost production. It is rigid, and a crystalline polymer having lower glass transition temperature and produced naturally by bacteria. It is obtained from copolymerization of polyhydroxybutyrate and hydroxyvalerate belonging to the hydrophobic family [Gumel et al., 2013]. It has been intensively reported as a biomaterial for recent biodegradable tissue engineering development and controlled release drug delivery systems. The advantages of PHBV over attractive polymers like PLGA and PLA are that it is economical with a low production cost and provides longer encapsulation time for anticancer agents to release once it enters the tumor cells. PHBV has shown high effectiveness as a sustained release polymer in protecting the drug from premature degradation, and microorganisms produce it whereas, PLA and PLGA are prepared under high-temperature conditions with use of catalysts.

Table 2.3 Some PHBV based nanoparticulate carriers in drug delivery

S. No.	Drug	Inferences	Ref.
1.	Ellipticine	The *in vitro* cell toxicity studies indicated good biocompatibility with no significant effect on % inhibition of the cancer cell line A549. While the % inhibition for EPT-PHBV nanoparticles was approximately two-fold higher in comparison to EPT.	[Masood et al., 2013]
2.	Ceftiofur	In this work, authors developed and characterized polymeric nanoparticles based on PHBV functionalized with SPIONs and the antibiotic ceftiofur. These	[Solar et al., 2015]

		nanoparticles could serve for the diagnosis and treatment of cancer and its associated bacterial infections. Its cytotoxicity was assessed in HepG2 cells which showed low cytotoxicity with IC_{50} > 10 mg/mL nanoparticles.	
3.	Medroxy-progesterone	PHBV prepared by supercritical fluid extraction of emulsions were consistently found to be smaller than those developed by the conventional emulsion solvent evaporation technique. The *in vitro* release kinetics of this system indicated mean time for 35% release in 18 h and its cellular viability indicated low toxicity.	[Giufrida et al., 2016]
4.	Teriparatide	PHBV/PLGA blend nanoparticles containing teriparatide were loaded in hyaluronic acid/jeffamine (HA-JEF ED-600) hydrogel for prolonged delivery of Teriparatide. MTT assay was carried by using NIH3T3 cell line. *In vivo* studies demonstrated effectively increase serum calcium level after subcutaneous injection in mice.	[Javan et al., 2017]
5.	Zein	The bioactive MPZ ternary composite was prepared by the solvent casting method. The MPZ presented excellent hydrophilicity, *in vitro* degradability and promote cell adhesion, proliferation, and differentiation.	[Qian et al., 2016]
6.	Curcumin	Curcumin efficiently encapsulated into PHBV nanoparticles, exhibited very low cytotoxicity and was efficiently internalized and displays anti-inflammatory activity on activated human endothelial cells by suppressing the phosphorylation of p38MAPK.	[Simion et al., 2013]

7.	Progesterone	The formed PHBV NPs demonstrated narrow size distributions, high encapsulation efficiency, and high drug loading, indicated for the proposed application.	[Leimann et al., 2015]
8.	Nafarelin	Nafarelin-PHBV with sodium alginate/poloxamer 407 in situ gel was prepared. The % cumulative release from NPs was found significantly lower than Nafarelin released from PHBV NPs which demonstrated a promising candidate for long-lasting formulation.	[Alizadeh et al., 2017]
9.	Teriparatide	PHBV and PLGA blend nanoparticles with teriparatide were prepared by double emulsion solvent evaporation technique. The release from optimized NPs led to 64.4% release over 30 days, showed a promising candidate for designing a controlled release formulation.	[Bahari Javan et al., 2016]
10.	Gentamicin	They fabricated PHBV / Hydroxyapatite (HA) composite microsphere. The initial burst of the composite microspheres was found to be very low, while the sustained release can last more than ten weeks, which makes it as a long-term drug delivery system.	[Wang et al., 2007]

Unit C: Formulation related literature survey

2.3 Polymeric Nanoparticles

In pharmaceutical drug delivery system, a series of different biomaterials have been discovered as carriers of drugs and fabricated as nanoparticles, including lipids, polymers, metals, and ceramics [Hasirci et al., 2006]. Natural and synthetic biodegradable polymers constitute the most important type of biomaterials for use as nanoparticulate drug delivery systems. Nanoparticles are submicron colloidal solid particles or particulate dispersion within the size range of 1-1000 nm. Polymeric nanoparticles are prepared from biocompatible and biodegradable polymers where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. In the formulation of polymeric nanoparticles, methods of preparation will decide whether it is nanocapsule (drug within the cavity surrounded by a polymeric membrane), nanospheres (drug dispersed in matrix system) or other nanoformulation [Soppimath et al., 2001]. Polymeric nanoparticles have been broadly considered as particulate drug carriers in the pharmaceutical and medical fields, because of their controlled and sustained release characteristics, subcellular size, high drug loading capacity, prolonged circulation, greater stability and good biocompatibility with tissue and cells. The properties of NPs should optimize depending on the particular application. To achieve the properties of interest, the method of preparation plays an important role.

2.3.1 Methods for preparation of nanoparticles

a) Solvent evaporation

b) Nanoprecipitation / Solvent displacement method

c) Emulsification / Solvent diffusion (Modified solvent evaporation method)

d) Salting out (Modified emulsification / Solvent diffusion method)

e) Dialysis

f) Supercritical fluid technology (SCF)

i) RESS (Rapid expansion of supercritical solution)

ii) RESOLV (RESS into liquid vehicle)

g) Polymerization of monomers

i) Emulsion

ii) Mini-emulsion

iii) Micro-emulsion

iv) Interfacial polymerization

v) Controlled / Living radical polymerization (C/LRP)

vi) Dispersion polymerization

h) Ionic gelation or coacervation of hydrophilic polymers

2.3.2 Drug release from polymeric nanoparticles

The polymeric drug carriers deliver the drug at the tissue site by any one of the three general physicochemical mechanisms [Nagavarma et al., 2012]:

1. By the swelling of the polymer nanoparticles (hydration followed by release through diffusion).

2. By an enzymatic reaction resulting in rupture/cleavage/degradation of the polymer at the site of delivery, thereby delivering the drug from the entrapped inner core.

3. Dissociation of the drug from the polymer and its release from the swelled nanoparticles (higher the Molecular weight of the polymer, slower the *in vitro* release due to anti-adhesive properties).

However, *in vitro*, the rate of drug release from polymeric vessels can exceed the rate of copolymer degradation, and so polymer degradation is often ruled out as one of the primary mechanisms of drug release. Thus, diffusion becomes the principle mechanism of drug release from nanoparticles. The three most important factors which influence the release of drug from polymeric nanoparticles are the characteristics of the drug, the properties of the core-forming block and the degree of polymer-drug compatibility [Letchford and Burt, 2007].

One of the major issues in the use of polymeric nanoparticles as drug delivery systems is their hydrophobicity. Hydrophobic nanoparticles are rapidly recognized by the body as foreign and cleaned up from the systemic circulation by the macrophage phagocytic system (MPS). The hydrophobicity of the core can also affect the permeability of the core to aqueous media. Particles with a highly

hydrophobic core will likely have a slow rate of drug release due to poor water penetration into the core in comparison to particles that have a more hydrophilic core. The physical state of the core can also affect the diffusion of the drug from nano carriers [Singh and Lillard, 2009]. Polymers that are more crystalline or have a high Tg or bulky groups present in their backbone have limited flexibility since the ordered alignment of the polymer chains and bulky side groups lower the free volume. Finally, the degree of polymer-drug compatibility can significantly influence the rate of drug release from the polymeric vessels. Drugs that are miscible with the core-forming block possess good polymer-drug compatibility. However, an increase in the extent of interaction between a drug and a polymer can result in a slower release.

Table 2.4 Commercially approved nanoparticulate drug delivery system

S. No.	Product & Manufacturer	Drug	Indication	Delivery system & route
1.	Abelcet (Enzon & Sigma-Tau)	Amphotericin B	Fungal infections	Lipid particles; Injection (Intravenous route)
2.	AmBisome (Gilead & Astellas)	Amphotericin B	Fungal and protozoal infections	Liposome; Injection (Intravenous route)
3.	Amphotec (Alkopharma)	Amphotericin B	Antifungal and aspergillosis	Micelles; Injection (Intravenous route)
4.	Abraxane (Abraxis & AstraZeneca)	Paclitaxel	Breast Cancer	Albumin Nanoparticles; Injection (i.v.)

5.	Adagen (Enzon)	Adenosine deaminase	Immuno-deficiency	Nanoparticles; Injection (Intramuscular route)
6.	Copaxone (TEVA)	Glatiramer Acetate	Multiple sclerosis	Nanoparticles; Injection (Intravenous route)
7.	Caelyx (Schering-Plough)	Doxorubicin	Breast Cancer, Kaposi sarcoma	Liposome; Injection (Intravenous route)
8.	DaunoXome (Galen & Gilead)	Daunorubicin	Kaposi sarcoma	Liposome; Injection (Intravenous route)
9.	Doxil (Jonssen & Sequus)	Doxorubicin	Breast Cancer, Kaposi sarcoma	Liposome; Injection (Intravenous route)
10.	DepoCyt (Pacira & Skye Pharma)	Cytarabine	Cancer	Liposome; Injection (Intravenous route)
11.	Diprivan (Fresenious kabi)	Anesthesia	Propofol	Lipid emulsion; Injection (Intravenous route)
12.	DepoDur (Pacira, Skye & Endo)	Morphine	Postsurgical pain	Liposome; Injection (Epidural route)
13.	Docetaxel-PNP (Samyang)	Docetaxel	Breast Cancer	Polymeric Nanoparticles; Injection (Intravenous route)

14.	DEP-Docetaxel (Starpharm)	Docetaxel	Breast Cancer	Dendrimer Nanoparticles; Injection (Intravenous route)
15.	Epaxal (Berna Biotech & Crucell)	Hepatitis A Vaccine	Hepatitis A	Virosome; Injection (Intradermal route)
16.	Estrasorb (Novavax & Medicis)	Estradiol	Menopausal therapy	Micelles Nanoparticle; Topical route
17.	Exparel (Pacira)	Bupivacaine	Analgesia	Liposome; Injection (Epidural & intrathecal route)
18.	Emend (Elan & Merck)	Aprepitant	Antiemetic	Nanocrystal; Oral delivery
19.	Genexol-pm (Samyang)	Paclitaxel	Breast Cancer	Micelles Nanoparticles; Injection (Intravenous route)
20.	Inflexal V (Crucell & Berna)	Influenza antigen	Influenza	Virosome; Injection (Intradermal route)
21.	Marqibo (Talon)	Vincristine	Cancer	Liposome; Injection (Intravenous route)
22.	Myocet (Cephalon & Zeneus)	Doxorubicin	Acute lymphomatous Leukemia	Liposome; Injection (Intramuscular & subcutaneous route)

23.	Neulasta (Amgen)	Granulocyte colony-stimulating factor (GCSF)	Neutropenia	PEG Nanoparticles; Injection (Intravenous & subcutaneous route)
24.	Oncasper (Enzon)	Asparaginase	Leukemia	PEG Nanoparticles; Injection (Intramuscular & intravenous route)
25.	Pegasys (Nektar & Roche)	Interferon-α 2a	Hepatitis C	PEG Nanoparticles; Injection (Intravenous & subcutaneous route)
26.	PEG-Intron	Interferon-α 2b	Hepatitis C	PEG Nanoparticles; Injection (Intravenous & subcutaneous route)
27.	Renagel (Genzyme)	Sevelamer HCL	Chronic kidney disease	Cross-linked poly(allylamine) resin; Oral delivery
28.	Rapamune (Elan & Wyeth)	Sirolimus	Immunosuppressant	Nanocrystal; Oral delivery
29.	Tricor (Abbott & Elan)	Fenofibrate	Anti-hyperlipidemic	Nanocrystal; Oral delivery
30.	ThermoDox (Celsion)	Doxorubicin	Primary hepatocellular carcinoma	Nanoparticles; Injection (Intravenous route)

31.	Visudyne (QLT & Novartis AG)	Verteporfin	Age-related macular degeneration	Liposome; Injection (Intravenous route)
32.	Xyotax (Cell)	Paclitaxel	Breast Cancer	Nanoparticles; Injection (Intravenous route)

2.3.3 Drug Delivery

The purpose of drug delivery systems is to maximize the therapeutic effect of the drug and minimize the related adverse effects. Drug carriers not only influence pharmacokinetic of the drug but also improve its biodistribution in the body. The ideal drug delivery system possesses a broad range of properties such as biocompatibility, ease of fabrication, high loading capacity, and low cost. Controlled and sustained release systems have been designed to achieve sufficient drug concentration for an extended period [Huang and Brazel, 2001]. In drug administration via traditional formulation ways, the drug level in the blood rises after each administration and then decreases until the next dose managed. In this case, the concentration of drug in the blood may go over the toxic level subsequently after drug administration and reduces to below of effective dose during the time. To prevent the concentration to go to toxic level, the applied dose can be kept low, but in this case, the number of applications should be high. Getting an oral dose or injection in every two or three hours is not suitable. Therefore, controlled and sustained drug delivery systems have been designed for long-term administrations. For this, the drug concentration in the blood is kept constant in an effective dose which is between minimum and maximum of the therapeutic range. Various delivery

systems and formulations, either particles or devices have been produced aiming an effective drug delivery [Parveen et al., 2012]. To achieve a controlled release, the drug can be entrapped in a matrix, encapsulated within a thin polymeric membrane or directly bonded to polymer chains. Polymeric carriers either natural or synthetic are known as promising materials for preparation of controlled and targeted delivery systems. These systems can release the drug over an extended period or at a specific moment and the specific region.

2.3.4 Nano-size drug delivery

In the last few decades, numerous types of submicron drug delivery systems have been developed due to their unique advantages such as relatively high intracellular uptake, high efficiency in targeting to objective tissue and not requiring any injury during the application [Vasir et al., 2005]. Submicron particles demonstrate numerous benefits in comparison to micro-size particles. For instance, they exhibit relatively high intracellular uptake over microparticles. Nanoparticles provide an opportunity to administer of poorly water soluble drugs in aqueous medium [Merisko-Liversidge et al., 2003]. Nanoscale delivery vehicles may be classified into some main categories due to their different properties. However, the major classification is related to the particle shapes coming from preparation process. They may be prepared as a capsule or sphere. Nanospheres are solid matrices containing molecules adsorbed at the surface of the sphere or entrapped within the particle. Nanocapsules are a class of submicron particles composed of a core either liquid or solid surrounded by a solid polymeric wall. The main advantage of nano-size delivery vehicles is their possibility to target the active agent to the proper tissue

after intravenous administration [Serda et al., 2011]. This is very important in the case of therapy with cytotoxic agents such as anti-cancer drugs. Chemotherapy agents using in cancer treatment exhibit serious side effects coming from their non-selectivity actions [Coates et al., 1983]. Therefore, the advanced approach in drug delivery is concentrated on the design of intelligent delivery systems for targeting a biochemical or chemically active agent to the specific site either actively or passively. By this way, a drug would be accumulated around the objective tissue and would be prevented to distribute in the rest of the body parts. Targeting of chemotherapeutic drugs directly to tumor site leads more effect, and as a result, administration of a small amount of drug will have a promising effect of improving the patient's life and its quality.

Table 2.5 Some polymeric nanoparticulate carriers in drug delivery system

S. No.	Drug	Carrier	Inferences	Ref.
1.	Metformin	Alginate nanoparticles	The results of *in vitro* release studies and *in vivo* efficacy tests of MLANs confirmed enhanced efficiency (3 times higher) comparable to pure metformin.	[Kumar et al., 2017]
2.	Bortezomib	Magnetite nanoparticles	Formed Magnetite Fe_3O_4 NPs and its synergistic combination with polydopamine established these core–shell NPs as a versatile platform for multiple applications.	[Liu et al., 2013]
3.	5-Flurouracil	PLA/PLGA-PEG nanoparticles	Cumulative percentage drug release was observed between 90% and 94.4% in all NPs by the end of 72 h, indicated the 5-FU	[Ocal et al., 2014]

			polymeric nanoparticles as matrix controlled diffusion release.	
4.	Ag85A (Mycobacterium tuberculosis)	Guar-gum nanoparticles	The developed nanoparticles demonstrated high antigen entrapment efficiency for oral vaccine delivery. The acid protection assay, Peyer's patch uptake study and *in vitro* antigen study confirmed the formulations could protect the antigen from the gastric environment and could safely deliver it to the intestinal region.	[Kaur et al., 2015]
5.	Loperamide	PLGA-PEG Nanoparticles	The developed nanoparticle system for drug delivery across the blood–brain barrier consisting of loperamide was prepared by the nanoprecipitation method. The nanoparticles accumulation in brain tissue showed much higher concentration for PEP coated by surfactant than both the PEP and the PN.	[Chen et al., 2013]

2.3.5 Targeting drug delivery

During last decades, many challenges have been established to improve cancer diagnosis and treatment by targeting drug delivery. The main purpose of targeting drug delivery is improving the therapeutic index as well as minimizing the side effects of drugs. The nonselective toxicity of anticancer drugs has limited their clinical application near to their maximum tolerated dose. For all chemotherapy

cases, the success of treatment depends on the antineoplastic agent's ability to target and to kill the cancer cells as well as to cause less damage to healthy cells [Allen, 2002]. Targeting drug delivery carriers have been merged to overcome the lack of selectivity of cytotoxic anticancer drugs. Targeted carriers enhance the accumulation of drug in the tumor site and inhibit drug distribution in the whole body [Bae and Park, 2011].

Active targeting drug delivery system uses smart molecules such as antibodies, proteins and targeting ligands which can find cancerous cells, recognize tumor-specific or tumor-associated antigens and bind to related specific receptors. A receptor is a protein molecule that interferes the internalization of legends through a process called receptor-mediated endocytosis. It mainly depends on specific interactions between drug carrier and the target cells [Das et al., 2009]. Hence, to design this system, first, we should know the tumor type, location, and properties.

In passive targeting, the stimuli-responsive delivery system is developed to show their bioactivity against properties or physiological changes occurring in objective site [Bogdanov Jr et al., 1997]. This physical targeting is presented by a complex drug delivery vehicle that can circulate in the body and target itself to proper tissue by responding abnormal conditions of the body like variations in pH or temperature. Most tumors possess few unique pathophysiologic characteristics like tortuous and poorly defined vasculature system. It is known that the pH of the blood is 7.4, but in the tumor area, it shifts to the acidic region around 4-5. To accomplish the demand of oxygen, anaerobic glycolysis is the main pathway which supplies energy and produces lactic acid as an end product, resulting in a decrease in pH. On the other

hand, fast budding of the cells in the tumor area shifts the temperature from the normal body of 37 ^{0}C to about 41 ^{0}C. The faster and continuous replication of cells results in high glycolytic flux and its higher metabolic activity raises the temperature of tumor cells [Vaupel et al., 1989]. By this way, the chemotherapeutic compound can be entrapped or accumulate to extensive angiogenesis system (high vascular permeability) due to lack of functional lymphatic, which is designed responsive to these variations and sent to the objective tumor by the mechanism called EPR (enhanced permeation retention) effect.

Table 2.6 Some passively targeted nanoparticulate carriers in drug delivery

S. No.	Drug	Carrier	Inferences	Ref.
1.	Paclitaxel	PLGA	Authors demonstrated active targeting NPs are better for accumulation into the tumor tissue with an 8 fold increase compared to NPs with passive targeting.	[Schleich et al., 2014]
2.	Paclitaxel	Pegylated polyelectrolyte nanoparticles	The surface of the NPs was pegylated to prolong the persistence of nanocarriers in the circulation. The multi-layer polyelectrolyte NPs retained its biological activity against cancer cell lines CT26-CEA and 4T1.	[Szczepanowicz et al., 2016]
3.	Doxorubicin	Nanohybrid liposome	Cellular uptake of drug from the nanohybrid liposome was found to be higher than that from the conventional liposome. *In vivo* targetability of the developed liposome	[Park et al., 2014]

			was confirmed indicating the prolonged circulation in the blood stream.	
4.	Docetaxel	Shrapnel nanoparticles	The encapsulation efficiency of prepared NPs was found to be 99.02%. The cellular uptake and cytotoxicity of nanoparticles in 4T1 cells after the pre-treatment of activated MMP-9 were significantly enhanced.	[Xu et al., 2015]
5.	Cisplatin	Polysilsesquioxane nanoparticles	The developed nanoparticle comprised of poly- silsesquioxane polymer crosslinked with cisplatin prodrug and was evaluated for chemoradiotherapy *in vitro* and *in vivo*. The result demonstrated significantly higher therapeutic efficacy when compared with cisplatin alone.	[Della Rocca et al., 2015]

2.3.6 Stimuli-responsive drug delivery

Stimuli response strategy has been used to design intelligent delivery systems that have a natural reflex and can respond to either internal physiological changes such as temperature, acidity and extrinsic conditions (magnetic field, light, heat, and ultrasound). It is known that temperature sensitive polymers and polypeptides display low critical solution temperature transition. They are water soluble below their transition temperature (Tt), and they exhibit an aggregation causing to water insolubility above their Tt. By this property, various thermo-responsive systems

have emerged for application between body temperature (37°C) and the temperature approved for clinical hyperthermia (42°C) [Chilkoti et al., 2002]. Considering the rapid development of tumor vasculature by abnormal and poorly controlled angiogenesis leads porous-wall vessels with the pore size between 200 nm to 2 μm, and an average of 400 nm have been observed in most of the solid tumors. This property causes to remain of nanoparticles in the tumor site for a long time. This phenomenon has been characterized and termed the tumor-selective enhanced permeability and retention (EPR) effect of nanoparticles. This system regarded as a 'gold standard' in the design of nano-sized anticancer agent delivery systems. There are many types of research regarding the preparation of nano and micro-size particles investigated for different types of therapy [Ganta et al., 2008]. Some of them have been approved by United State Food and Drug Administration (FDA) and used in drug delivery systems.

2.3.7 pH-responsive drug delivery

pH sensitive drug delivery strategy is used to design the formulations which respond towards pH shifting in the body. Based upon the pH value of most tumors in patients and tumor growth rate, it was observed that pH of tumor site shifted to acidic region due to the production of the excess amount of lactic acid while normal blood pH remains constant at 7.4. This pathophysiology of tumors has been considered as an ideal trigger to the delivery of anticancer agents via pH-sensitive delivery systems [Mok et al., 2010].

Poly (L-histidine) or PLH, a kind of polyamino acid, exhibits high potential to cell membrane fusion after protonation of the imidazole groups in the acid medium (pH

below 6). The imidazole side chain of histidine has a pKa of around 6, and overall the amino acid has a pKa of 6.5. This suggests that, below a pH of 6, the imidazole ring is mostly protonated leading to hydrolysis of the polymer [Li et al., 2015]. Therefore poly (L-histidine) is known to have an endosomal membrane disruption activity [Lee et al., 2005]. There are various researches focused on the application of poly (L-histidine) (PLH) for either drug or gene delivery [Benns et al., 2000]. Although poly (L-histidine) is a promising pH-sensitive compound, some limitation such as difficulty in blocking of an imidazole group, controlling the molecular weight of polymer during the synthesis and its low solubility in organic solvents have limited the production and investigation of this pH-sensitive polymer. However, still, there are many studies which are going to overcome these limitations by either making copolymers or combining with other molecules to design pH-responsive drug carrier systems.

Table 2.7 Some pH-responsive nanoparticulate carrier in drug delivery

S. No.	Drug	Carrier	Inferences	Ref.
1.	Doxorubicin	Chitosan nanoparticles	The doxorubicin release profiles showed a pH-dependent and slow release pattern. The CS MNPs released most of the doxorubicin at pH 4.2, while the nanoparticles are quite stable at pH 7.4.	[Unsoy et al., 2014]
2.	Doxorubicin	Dendrimers	The anticancer drug doxorubicin was conjugated to the dendronized heparin block via an acid–labile hydrazone linkage and this conjugate based nanoparticle showed a pH-	[She et al., 2013]

			sensitive drug release property. It also showed promising biosafety and high tumor inhibition which was confirmed by *in vitro* IC_{50} study and *in vivo* tumor growth curves, immune histochemical assessment and histological analysis.	
3.	Oxaliplatin	Peptide dendrimer	The formed dendrimer conjugates achieved higher *in vivo* antitumor efficacy than the clinically approved agent oxaliplatin® at equal doses. This conjugate demonstrated decrease toxic side effects and tolerated dose have low toxicity as measured by changes in body weight and histological analysis.	[Pan et al., 2014]
4.	Paclitaxel	Cyclodextrin	The developed pH-sensitive NPs leads to Nanotherapeutics with significantly improved cytotoxic activity against various tumor cells. These nanoparticles with pH-modulated hydrolysis and pH-triggered drug delivery ability showed good biocompatibility *in vitro* and *in vivo*.	[He et al., 2013]
5.	Docetaxel	LPH-PEG	Here, docetaxel was incorporated into the lipid core and then shielded with the pH-responsive block co-polymer polyethylene glycol-b-polyaspartic acid. The *in vivo* anticancer effect of nanoparticles was affirmed by the elevated	[Tran et al., 2015]

levels of caspase 3 and poly ADP ribose polymerase observed in the tumors after treatment.