Insights into the Pharmaceutical and Clinical Applications of Nanoparticles in Cancer Therapy

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Edited by

Sheba R. David and Rajan Rajabalaya

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CHAPTER 1

CURRENT NANODRUG DELIVERY SYSTEMS USED FOR COLORECTAL CANCER

RAJAN RAJABALAYA[1] AND SHEBA RANI DAVID*[2]

[1]PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Brunei Darussalam
[2]School of Pharmacy, University of Wyoming, Laramie, WY 82071, USA

***Corresponding author:**
Dr Sheba Rani David
Assistant Professor
School of Pharmacy
University of Wyoming
1000 E. University Avenue
Laramie, Wyoming, 82071
United States of America
Email: sdsheba@gmail.com
Phone: +1- 307-766-6482

Abstract

Colorectal cancer is one of the five most widely diagnosed cancers among humans worldwide. This high ranking among different diseases presents an opportunity to treat colorectal cancer by maximising the therapeutic efficacy of anticancer drugs while reducing possible toxicities and side effects. Advancements in cancer drug delivery have led to nanosized particles as drug vehicles to improve therapeutic drug outcomes by translocating drugs to the cancerous targeted sites. This chapter presents different nanodrug delivery systems in cancer therapy, emphasising colorectal cancer. This chapter also summarises critical information on various current nanodrug delivery strategies developed to treat colon cancer, including examples of treatments and their preparation procedures.

Abbreviations

5-FU	5-fluorouracil
anti-EGFR	Anti-epidermal growth factor receptor
anti-HER2	Anti-human epidermal growth factor receptor2
anti-VEGF-A	Anti-vascular endothelial growth factor-A
APC	Adenomatous polyposis coli
AVEX	Avastin in the elderly with Xeloda
CEA	Carcinoembryonic antigen
CYP	Cytochrome
Doxo	Doxorubicin
DR5-NP	Death receptor 5–specific antibodies
Dtxl	Docetaxel
FAP	Familial adenomatous polyposis
FDA	Food and Drug Administration
HNPCC	Hereditary nonpolyposis colorectal cancer
LPS	Lipopolysaccharides
mAb	Monoclonal antibodies
NLC	Nanostructured lipid carriers
OS	Overall survival
PEG	Polyethylene glycol
PFS	Progression-free survival
PLGA	Polylactic glycolic acid
PlGF	Placental growth factor
PNP	Polymeric nanoparticles
Ptxl	Paclitaxel
RES	Reticuloendothelial system
RESOLV	Rapid expansion of a supercritical solution into a liquid solvent
RESS	Rapid expansion of a supercritical solution
ROS	Reactive oxygen species
RR	Resection rate
SLN	Solid lipid nanoparticle

1. Introduction

Cancer is defined as an uncontrolled replication or expansion (neoplasm) of cells that form an abnormal tissue mass known as a tumour [1]. Tumours have abnormal morphologies and/or functions compared to normal cells. They are classified into benign or malignant tumours. Notably, cancer results from a somatic mutation that alters the physiology of normal cells to form malignant tumour cells [2]. Tumour cells found at the outer edge of a mass have the best access to nutrients compared to inside cells. Cells in the inner region rely on diffusion to deliver nutrients and eliminate metabolic waste products. Eventually, cells in the inner region of a tumour mass will die because of an inadequate nutrient supply, resulting in a necrotic core within the tumour. A cancer cell that grows nearby a healthy tissue multiplies faster than other cells, necessitating a higher demand for nutrients from the bloodstream. Healthy tissues cannot compete with cancer cells for nutrient supply in the presence of a tumour. Oxygen, glucose and amino acids are examples of substrates required for the functioning of tumour cells. When a tumour grows in an environment with a limited supply of nutrients, the maximum size of tumour mass reaches approximately 2 mm. Angiogenesis must occur to expand beyond the size of 2 mm, where blood vessels form at the tumour growth site. Angiogenesis is an essential process for the continuous development of a tumour mass. The expansion of a tumour mass above 2 mm may take years to occur. A tumour will reach a steady state if the rate of proliferation of cells equals the rate of cell death [3].

2. Colorectal cancer

Colorectal cancer is the fourth most diagnosed cancer among humans worldwide [4]. The incidence rate is significantly higher in the United States of America and Europe than in Africa and Asia [5]. Colorectal cancer is defined by the growth of a malignant tumour in the mucosa of the colon or rectum [4]. Most large bowel cancer cases occur within the pre-existing polyps. About 50% of cases occur in the rectum and 20% in the sigmoid colon. Signs and symptoms of colorectal cancer are the presence of bloody stool, irregular bowel habits, low appetite, reduced body weight, perforation or blockage in the colorectal region [6]. The staging of cancer depends on the size of the primary tumour (T stage), involvement of lymph nodes (N stage) and the incidence of metastases (M stage) [5]. It is categorised into five stages: Stage O, Stage II (IIA, IIB and IIC), Stage III (IIIA, IIIB and IIIC) and Stage IV [4]:

- **Stage 0:** Stage 0 is the early stage of colorectal cancer [7]. Stage 0 indicates that a polyp or a benign tumour has grown on the mucosal layer [4].
- **Stage I:** Stage I indicates the invasion of a tumour into the submucosa and muscularis propria layers [4].
- **Stage II:** Stage II demonstrates the spread of cancer cells beyond the colon but not to the lymphatic system via metastasis [7]. Stage II comprises three sub-divisions (IIA, IIB and IIC). Stage IIA indicates the invasion of a tumour into the peri-colorectal tissues through the muscularis propria. Stage IIB indicates the invasion of a tumour into the visceral peritoneum. Finally, Stage IIC indicates the invasion and adherence of a tumour to other bodily structures or tissues [4].
- **Stage III:** Stage III depicts the spread of cancer cells throughout the colon wall and surrounding lymphatic nodes [7]. Similar to Stage II, Stage III of colorectal cancer has three sub-divisions, namely, IIIA, IIIB and IIIC. Stage IIIA indicates the invasion of a tumour into the muscularis propria layer and its dispersion in one to three lymphatic nodes or surrounding tissues. Another criterion for Stage IIIA is the tumour invasion into the submucosal layer and its dispersion in four to six lymphatic nodes. Stage IIIB is either indicated by the invasion of a tumour into the muscularis propria and its dispersion in more than six lymphatic nodes or the invasion of a tumour through the muscularis propria into the peri-colorectal tissues and its dispersion in four to six lymphatic nodes. Another criterion for Stage IIIB is the invasion of a tumour into the visceral peritoneum and its dispersion in one to three lymphatic nodes or surrounding tissues. The advanced Stage III cancer, Stage IIIC, is indicated by the invasion of a tumour into the visceral peritoneum and its dispersion in four to six lymphatic nodes. Another criterion for Stage IIIC is the invasion of a tumour into the peri-colorectal tissue and its dispersion in more than six lymphatic nodes or tumour adherence to other bodily structures with tumour dispersion in at least one lymphatic node [4].
- **Stage IV:** The final stage of colorectal cancer, Stage IV, is indicated by the dispersion of tumour cells at one site, such as in the liver, lungs, ovaries or a non-regional lymphatic node [4].

2.1 Pathophysiology and common risk factors of colorectal cancer

Colorectal carcinogenesis manifests in the mucosal lining of the intestinal lumen. If it is left untreated, cancer cells can spread into the muscular layers

underlying the lining and the intestinal wall. At the early stage of colorectal cancer, a polyp can develop into a tumour and eventually enter the mucosal layer's inner lining. Most colorectal cancer cells have overexpressed carcinoembryonic antigen (CEA) on the cell surface in comparison to normal cells in the colon and biliary epithelial layer. Risk factors of colorectal cancer are age, personal history, lifestyle, race and ethnic group [7]. For instance, an individual's lifestyle based on the Western lifestyle, frequent consumption of red meat from beef or pork and alcohol are often linked to an increased risk of colorectal cancer [5]. Further, environmental conditions, exposure to chemicals, infectious agents, and radiation can contribute to carcinogenesis. Genetic mutation, immune system dysregulation and hormonal factors can also trigger carcinogenesis in an individual. Hereditary predisposition syndrome involves the adenomatous polyposis coli (APC) gene mutation and can cause colorectal cancer. Patients with familial adenomatous polyposis (FAP) gene inherit a mutated *APC* gene that increases their risk of developing colorectal cancer [7]. The *APC* gene defect is caused by mismatched DNA formed during repair [5]. Additionally, hereditary nonpolyposis colorectal cancer (HNPCC) is linked with DNA gene mutation, including *MLH1*, *MSH2* and *MSH6* genes, accounting for 5% of colorectal cancer cases [5–7]. *H. Pylori* also shares a positive association with the risk of colon cancer [8]. The loss of regulation of *COX-2* expression, a tumorigenesis rate-limiting step in tumorigenesis, occurs early in carcinogenesis [9]. Lipopolysaccharides (LPS) and gastrin are protumorigenic and stimulate the inflammatory pathways that contribute to neutrophil extracellular traps formation [10].

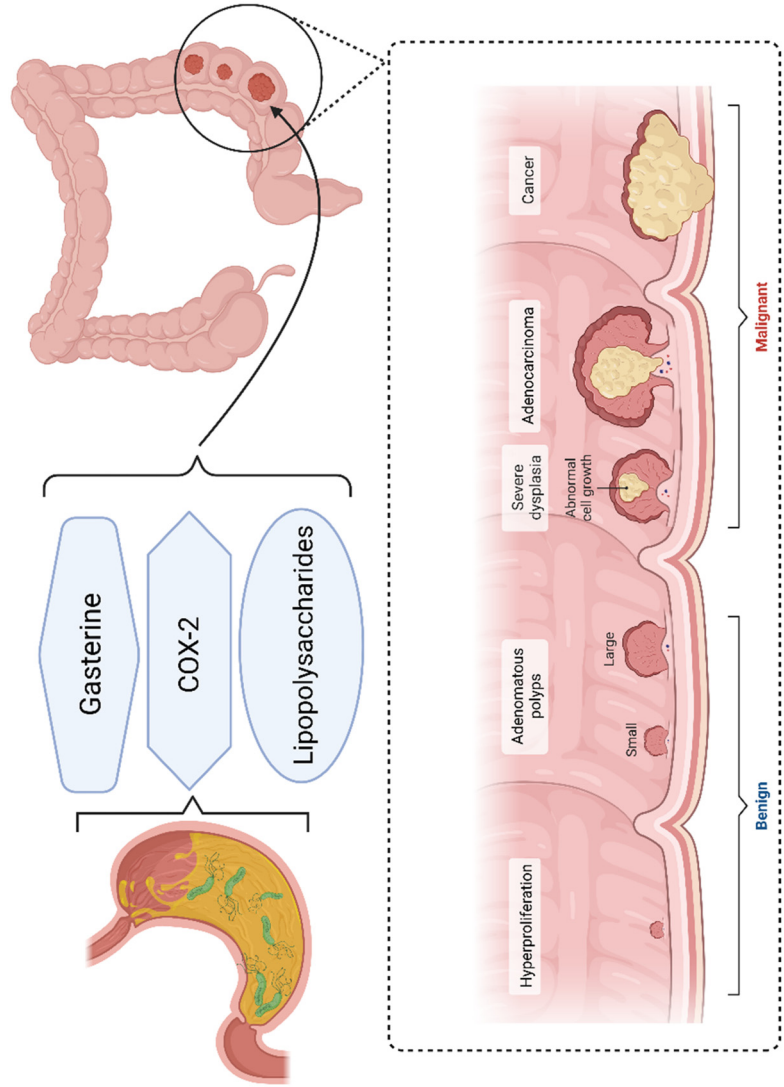

Figure 1-1: Colon cancer pathogenesis and stages of benign and malignant carcinoma.
Created with BioRender.com

2.2 Management and treatment of colorectal cancer

The possibility of a cure and the survival rate of patients with colorectal cancer is determined by the stage of colorectal cancer [5]. There are various interventions for treating colorectal cancer, such as tumour-removal surgery, radiation therapy, chemotherapy and targeted therapies. A polypectomy procedure can eradicate polyps during colonoscopy in patients diagnosed with Stage 0 colorectal cancer. After the procedure, it would lead to a survival rate of above 90% [7]. After undergoing the primary surgery, a patient with cancer may receive additional therapy, known as adjuvant therapy [11].

Patients with Stage I and Stage II colorectal cancer are expected to have a five-year survival rate post-operation even without administering adjuvant chemotherapy [5]. There is an 80–95% chance of a five-year survival rate for patients with Stage I colorectal cancer. The chance of a five-year survival rate may decrease based on the patient's prognostic factors, involvement of nodes and the extent of invasiveness of tumour cells [6]. Adjuvant therapy is usually administered to patients diagnosed with colorectal cancer from Stage III to Stage IV to lower the risk of cancer recurrence [5]. Adjuvant therapy with an oral administration of fluoropyrimidine has demonstrated improvement in survival rate after surgical intervention [6]. However, fluoropyrimidine and oxaliplatin or irinotecan co-administration is the standard treatment for patients with metastases. An increase in five-year survival rate by 2–3% by capecitabine or 5-fluorouracil (5-FU) has been shown in patients with Stage II who exhibit no risk factors for colorectal cancer. Additionally, the co-administration of oxaliplatin to capecitabine achieved a gain of 4% to a 3-year disease-free survival rate. Patients with a poor prognosis may be administered bevacizumab and capecitabine or infusional 5-FU to increase their overall survival rate. An AVEX (Avastin in Elderly With Xeloda) study including the older population has shown an improvement in the overall survival rate of elderly patients administered with capecitabine (Xeloda) [5]. Capecitabine inhibits the formation of thymidine monophosphate, an essential substrate in DNA synthesis, by the de novo pathway [12].

Patients with Stage III colorectal cancer treated with 5-FU–based regimens have demonstrated an increase in their five-year survival rate by 10–15% [5]. Fluorouracil-based therapy helps improve the survival rate of patients with Stage III colorectal cancer. The administration of the FOLFOX regimen, comprising fluorouracil, folinic acid and oxaliplatin, can improve

the disease-free survival rate without affecting the overall survival rate [6]. For patients at the final stage of colorectal cancer, Stage IV, their treatment options include tumour-removal surgery, chemotherapy and radiation therapy [7]. Patients with Stage IV colorectal cancer have less than a 10% chance in their five-year survival rate; they have a poor prognosis. In a second-line treatment for colorectal cancer, bevacizumab is added to the FOLFOX therapy to improve the survival rate of patients. An antiangiogenic drug, aflibercept, can target various growth factors, such as VEGF-A, VEGF-B and PlGF. The addition of aflibercept to the FOLFIRI therapy (5-FU and irinotecan) shows an overall survival rate after a failure in the initial therapy with oxaliplatin [5].

In a later-line treatment, the foremost objective is to prolong the survival of patients with regimens with minimal toxicity. Although more research is required, studies including new treatments with TS-102 and TS-114 compounds that disrupt thymidylate metabolism may benefit patients in later-line treatment [5]. Fluorouracil infusion is recommended in conventional palliative treatment. An alternative for fluorouracil is its prodrug: capecitabine [13]. Adding folinic acid to the therapy increases its therapeutic efficacy [14]. The main objective of treatment is to reduce the growth and replication of tumour cells. However, it produces a wide range of side effects (e.g., nausea, hair loss and vomiting) because of its poor specificity to target cells. Therefore, multiple cytotoxic drugs are simultaneously administered to patients to reduce side effects.

The cancer therapy regimen depends on the patient's general health condition and requirements [4]. Other essential factors to consider when choosing the proper regimen are the location or type of cancer, patients' age, consent, preference and stage of cancer [3, 5]. The choice of therapy in a patient with cancer is a critical element to consider by the healthcare provider because each cancer cell responds to each treatment method differently [7]. Each patient's regimen varies with the medicinal dose, medicinal dosage form, frequency of therapy and cycle duration [4]. In rectal cancer, capecitabine has demonstrated an improvement in the survival rate of patients compared to the 5-FU administration [5].

A significant challenge in cancer drug delivery is an efficient process to the desired cancer tissue without affecting the healthy tissues [15]. Therefore, developing a site-specific delivery system for chemotherapeutic agents would increase the concentration of drugs at the desired or target site. This action would increase the drug's efficacy while reducing the side effects because only low doses are required [7]. Despite the globally high

prevalence of colorectal cancer, it could be prevented by maintaining a diet rich in minerals containing calcium, vitamin D, curcumin and quercetin [7]. Other primary prevention techniques are increasing the intake of whole grains, fruits and vegetables. Maintaining a proper weight through regular physical activity can help to reduce the colorectal cancer risk in patients with obesity [5].

2.3 Therapeutic approach in metastatic colorectal cancer

Approximately 50% of patients with colorectal cancer will develop metastases, contributing to a high mortality rate for colorectal cancer. Fluorouracil treatment (5-FU) has been the first-line treatment for colorectal cancer. The co-administration of 5-FU with cytotoxic agents (e.g., irinotecan and oxaliplatin) has prolonged the survival of patients with metastatic cancer. Intravenous 5-FU can be substituted for capecitabine either as a single agent or in a combination regimen with oxaliplatin. The treatment goals for patients with metastatic colorectal cancer are increasing the survival length of patients, curing patients, alleviating symptoms related to malignancy, and stopping the progression of tumours. The priority established for first-line treatment is an immediate control of tumour growth in patients to decrease metastases before a surgical intervention. The medical intervention of metastatic colorectal cancer also aims to provide a higher resection rate (RR), a more prolonged progression-free survival (PFS) and better overall survival (OS) [16]. The OS rate is the period of survival from the date a patient is diagnosed with the disease [17].

3. Nanodrug delivery systems

A drug delivery system delivers the necessary drug to the target site directly or via the systemic circulation in a controlled manner. An advanced drug delivery system functions in a controlled manner to create a personalised treatment for a specific disease while maintaining the drug level within the therapeutic window. Several strategies have been developed to regulate the essential parameters (e.g., rate of drug release, time of drug release and site-directed drug delivery) that determine the treatment efficacy and mark the beginning of the drug delivery system [18]. Nanodrug delivery systems comprise submicron-sized particles with one or more therapeutic agents dispersed and adsorbed into vesicles, capsules or polymer matrices [15]. Submicron-sized particles are nanoparticles in the nanometre range of 10–1,000 nm [19]. The diameter of entrapped drugs lies between 10 and 200 nm, ideal for drug uptake and efflux. The most common nanodrug delivery

systems are inorganic nanodrug delivery, lipid-based and polymer nanodrug delivery systems [20]. Nanotechnology has opened the doors to advancing therapeutic and diagnostic strategies, such as selective drug delivery systems, molecular imaging techniques and potential theranostic agents in cancer therapeutics [15]. Theranostic agents can be used to diagnose and treat cancers. Theranostics involve monitoring and assessing drug outcomes in the tumour cells. Hence, the clinical applications of theranostic agents hold great potential for an individualised treatment [13]

4. Clinical application of nanoparticles in cancer therapy

The nanoparticle drug delivery system applications are becoming popular because of their modified and site-specific drug release properties [20]. The development of site-specific or targeted drug delivery systems led to greater efficacy of cytotoxic drugs than non-targeted conventional drug delivery systems. Moreover, the nanoparticle drug delivery system enhances the bioavailability of drugs because only a low dose is required to produce an optimal therapeutic effect. For instance, oncology therapy's current available drug delivery systems are based on polymer nanoparticles, lipid nanoparticles, or liposomes. Drug loading into nanoparticles effectively targets tumour sites by targeting moieties or ligands. Moieties or ligands should be specific to overexpressed cancer cell receptors. Site-specific delivery increases the drug uptake by the tumour, resulting in improved chemotherapeutic efficacy. Examples of moieties with a targeting property are antibodies, peptides, oligonucleotides and other small molecules (e.g., folic acid), transferrin and integrin molecules. The first generation of drugs approved by the Food and Drug Administration (FDA) are liposomal doxorubicin (Doxil®) and albumin-bound (i.e., polymer-drug conjugate) paclitaxel (Abraxane®) [15].

5. Characteristics of nanoparticles

Nanoparticle formulations should be stable and non-reactive to plasma components in the systemic circulation. The nanoparticle drug vehicle should preserve drug components from degradation in the blood [15]. Small nanoparticles can persist in the leaky defective blood vessels more than large nanoparticles [13]. The ideal size of a nanoparticle for targeted drug delivery is 100 nm or less [3]. Drug uptake into cancerous cells can be enhanced by optimising the size of nanoparticles. The morphology of nanoparticles can influence nanoparticle uptake into tissues because of changes in fluid dynamics. The polarity of nanoparticles can affect their

stability and dispersion in the blood [13]. Nanoparticles have a hydrophilic surface that can resist plasma proteins while allowing the adsorption of surfactants to their surface. Hydrophilic nanoparticles can be prepared from polyvinyl pyrrolidone [3]. The interactions between plasma proteins and nanoparticles can affect the biodistribution and biocompatibility of nanoparticles in the body. The creation of a protein-nanoparticle complex can alter the surface chemistry and size of the nanoparticle. This action will result in nanoparticle identification by macrophages, inducing phagocytosis and eliminating nanoparticles from the bloodstream [21]. Positively charged nanoparticles have demonstrated a targeting property to tumour vessels; however, the neutralisation of nanoparticles after extravasation enables rapid diffusion into the tumour tissue.

Hydrophilic nanoparticles can increase the bioavailability of non-water-soluble drugs for effective drug delivery [13]. Additionally, particles with a hydrophobic surface will be subjected to uptake into the liver, spleen and lungs [3]. The reticuloendothelial system (RES) recognises hydrophobic compounds as foreign and removes them from the circulatory system [14]. Moreover, monocytes and macrophages will easily recognise hydrophobic substances coated with opsonin proteins, thus activating phagocytosis. Consequently, nanoparticles cannot penetrate the tumour cells to exert a therapeutic action [13]. The sustained release property of nanoparticles causes drug accumulation at the desired site, enhancing the therapeutic effect [20].

6. Modification of nanoparticles

Modifications in nanoparticle formulation are the early steps for transforming (customising) nanoparticles for clinical use [15]. Targeted nanoparticles can be produced using passive or active mechanisms to enhance the chemotherapeutic efficacy of anticancer drugs. The passive targeting mechanism uses the increase in permeability and retention effects from nanoparticle accumulation without altering the surface of nanoparticles. The accumulation of nanoparticles will enable increased therapeutic action at the site of interest. The active targeting mechanism uses the binding of specific ligands to the nanoparticle surface. The surface of nanoparticles can be manipulated with specific moieties or ligands that can prolong retention time and enhance the uptake of nanoparticles into cancerous tissues. Ligands attached to the surface of nanoparticles can interact with specific receptors and antigens expressed on the surface of cancerous cells. Some ligands that can be used are transferrin, folic acid,

antibodies and macromolecules (e.g., proteins and carbohydrates). The ligands' density should be considered to prevent their elimination by the reticuloendothelial system and protein binding [13]. Nanoparticles conjugated with polyethylene glycol (PEG) have been used to prolong the retention time of nanoparticles in the circulatory system and reduce renal clearance rates [15]. The reduction in renal clearance enhances the bioavailability and increases the nanoparticle half-life to prevent drug degradation before reaching the tumour site. PEG is a hydrophilic and non-ionic polymer that can conceal nanoparticles' hydrophobicity and does not affect the function of charged molecules (e.g., DNA) [13].

Nanoparticles that can transport a large number of drugs are more desirable to produce an optimal therapeutic effect. Some examples of chemotherapeutics that can be conjugated to polymers are paclitaxel (Ptxl), docetaxel (Dtxl) and doxorubicin (Doxo) [22]. Stimuli-responsive nanoparticle drug delivery systems have also been developed to stimulate the drug release upon exposure to certain stimuli. pH level, redox reaction, ionic charges and stress are some examples of physiological and intracellular stimuli. Temperature, light exposure, ultrasound waves, and magnetic and electric fields are some examples of physical and external stimuli. For instance, blood vessels become more permeable at temperatures between 37ºC and 42°C, improving drug delivery by nanoparticles. Lipoprotein-delivered benzoporphyrin derivative mono-acid (BPD) verteporfin is an example of light-responsive nanoparticles. Although BPD is biocompatible, it can form reactive oxygen species (ROS) that can destroy DNA, causing the death of cells [13]. Although there are several advantages of multifunctional nanoparticles, issues related to reproducibility and toxicity due to their complicated nature continue to remain a challenge. Therefore, before clinical testing, the pharmacodynamics of the nanoparticle system should be studied thoroughly [15].

7. Types of nanodrug delivery systems

7.1 Liposome

A liposome-based nanodrug delivery system comprises vesicles consisting of eight phospholipid bilayers that envelop an inner aqueous phase [15]. The phospholipid bilayer has both hydrophilic and hydrophobic properties that support the encapsulation of water-soluble and lipid-soluble drugs [20]. Water-soluble cytotoxic agents dissolve in the aqueous phase of the liposomes, whereas lipid-soluble cytotoxic agents are entrapped in the lipid bilayer [15]. Liposomes have a special targeting property that can be presented by two

approaches: the passive or active targeting mechanism [2]. A wide range of applications of liposomes is present in gene delivery. The following are examples of lipid reagents used for gene delivery: 1,2bis[oleoyloxy]-3-[trimethylammonio]propane; dioctadecylamido-glycylspermine, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride and 3β[N-(N ',N'-dimethylaminoethane)-carbamoyl]cholesterol [15].

The liposomes are classified based on the number of bilayers and size of liposomes. The terms used to describe the number of bilayers is unilamellar (i.e., one phospholipid bilayer) and multilamellar (i.e., more than one phospholipid bilayer). Liposomes may be formulated into the size range between 25 nm and 2.5 micrometres. However, drugs that loaded into liposomes with a diameter less than 400 nm can build up in tumour cells more efficiently. However, drugs loaded into liposomes with a diameter of less than 400 nm can build up in tumour cells more efficiently [2]. Because lipids have a high density of cations, they are combined with adjuvant lipids (e.g., cholesterol) to reduce the overall energy required to separate the ionically linked DNA and cationic lipid molecules [15]. Liposomes have a greater cellular retention capacity in the systemic circulation. This trait alters the pharmacokinetics and distribution of P-glycoprotein inhibitors, enabling the saturation of anticancer drugs in tumour cells and enhancing the chemotherapeutic effects. Liposome-based nanodrug delivery systems exhibit no specific targeting. However, this can be achieved by modifying the system using various ligands, including folic acid or anti-transferrin monoclonal antibodies (mAb). Specific cells may detect the infusion of ligands into the phospholipid bilayer of liposomes, rendering selective and modified nanoparticles. The composition of liposomes can be optimised by adding adjuvant lipids or PEG to reverse the incidence of multidrug resistance [20].

7.2 Treatments with liposome-based nanoparticle

Doxorubicin restricts the synthesis of nucleic acids within cancer cells [3]. Drug-loaded liposomes are produced in a size that allows their penetration into tumour tissues through pores found in the tumour microvasculature [2]. The liposomes release the doxorubicin that has been entrapped in the cancer cells. Disadvantages of the doxorubicin therapy can be associated with undesirable side effects, such as cardiotoxicity and suppression of bone marrow (i.e., myelosuppression). These effects result in a low therapeutic window for doxorubicin [4]. Conjugating doxorubicin with dextran in chitosan nanoparticles is a strategy to diminish adverse effects by generating a targeted delivery [3]. Liposomal drug delivery can also enhance the

bioavailability of platinum drugs. Examples of cisplatin drugs delivered via the liposome-based nanodrug delivery system for colorectal cancer therapy are LiPlaCis® and Aroplatin® [15]. Cisplatin restricts DNA synthesis by forming intrastrand covalent bonds with DNA bases, which eventually causes DNA damage [23].

7.3 Preparation liposome-based nanoparticle

In water, the dispersion of amphiphilic lipids (such as phospholipids) creates liposomes. Liposomes are structurally stable and not bonded covalently compared to polymeric nanoparticles [2]. For example, Doxorubicin (Doxil®) has a lipid bilayer comprising a PEG-modified 1,2,-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt, hydrogenated soy phosphatidylcholine and cholesterol [15]. Myocet® comprises doxorubicin citrate surrounded by egg phosphatidylcholine and cholesterol. Doxorubicin is placed in an incubator with liposomes with an acidic core at a neutral pH. A non-ionic drug diffuses down its concentration gradient across the liposome and becomes protonated. Liposomes were initially created using a citric acid buffer (300 mM) to produce an acidic interior. Meanwhile, its exterior is coated with a buffer solution using sodium carbonate and sodium phosphate [24].

7.4 pH-sensitive liposome

pH-sensitive liposomes provide another progressive liposomal nanodrug application with great potential in cancer therapy. They are stable at normal physiological pH of 7.4. However, after the internalisation of liposomes in the endosomes of cancer cells, the structure of liposomes becomes less stable because of the acidic pH (e.g., pH: 6.0–6.5). The pH-sensitive liposomes comprises zwitterionic oligopeptide lipid molecules and amino acid-based lipids (e.g., 1,5-dioctadecyl-l-glutamyl 2-histidyl-hexahydrobenzoic acid and 1,5-distearyl N-(N-alpha-(4-mPEG2000) butanedione)-histidyl-l-glutamate)).

Another advantage of pH-sensitive liposomes is that high concentrations of anticancer agent/cytotoxic agents (e.g., 5-FU) can be incorporated into pH-sensitive liposomes compared to conventional liposomes [15]. Poly(styrene-co-maleic acid) (SMA) is the main component in pH-responsive liposomes that promotes the delivery of 5-FU within the HT-29 cells of colon cancer [25]. 5-fluorouracil has been shown to interfere with DNA synthesis during the S phase by restricting the action of the thymidylate synthetase enzyme

[26]. Banerjee et al. demonstrated that polystyrene-co-maleic acid undergoes a conformational shift at low pH, causing the destabilisation of the liposomal structure and drug release. Drug-loaded pH-sensitive liposomes fuse with the endovascular membrane because of the acidic pH in cellular endosomes, resulting in a conformational change of the polymer chain. SMA is a copolymer that transforms from a random coil to a collapsed non-ionic globular conformation at a low pH. The conformational change causes the destabilisation of liposomes following the formation of channels within the membrane [25]. This action stimulates the release of drug contents from the core of the liposomes. The extracellular environment of solid tumours has an acidic nature, signalling liposomal drug release [15]. The outcome is an increase in intracellular drug availability [25].

7.5 Antibody-coated liposomes

Conjugating nanoparticles have developed a site-specific liposomal nanoparticle drug delivery system for cancer therapy with antibodies to produce immunoliposomes. The mechanism of immunoliposomes includes their localisation to the tumour site, where antibodies on the nanoparticle surface bind specifically to overexpressed receptors on the tumour cells. The interaction between tumour cells and immunoliposomes induces enhanced intracellular delivery of the nanoparticle. Examples of antibody moieties used to target specific tumour cells via surface antigen interaction are anti-human epidermal growth factor receptor 2 (anti-HER2), anti-epidermal growth factor receptor (anti-EGFR), anti-CD19 and GAH $F(ab)_2$ goat anti-human monoclonal antibody [15]. Other targeted substances are anti-vascular endothelial growth factor-A (anti-VEGF-A), antiangiogenic multi-kinase inhibitor (e.g., Regorafenib), antiangiogenic compound (e.g., Aflibercept), antibodies including bevacizumab, cetuximab and panitumumab [5].

7.6 Preparation of temperature-sensitive liposome

A COOH-PEG_{3400}-PLGA copolymer (w/w) and camptothecin drug are mixed before forming an emulsion. The emulsion is combined with PLGA RG502H, followed by evaporation. The carboxyl groups of copolymer allow the conjugation of DR5-specific antibodies, conatumumab, via their amino groups [27]. An example of a temperature-sensitive liposomal nanodrug delivery system is ThermoDox®. It is formulated with liposomal doxorubicin, releasing the cytotoxic agent when the temperature reaches 39.5°C [15].

7.7 Treatment antibody-coated liposomes

For instance, Fay et al designed polylactic glycolic acid (PLGA) nanoparticles coated with conatumumab DR5-NP (death receptor 5–specific antibodies). The antibodies can target the colorectal HCT116 cancer cell model because cancer cells exhibiting DR5 receptors can be targeted by DR5-NP antibodies [7]. Once DR5 receptors become stimulated, they induce apoptosis, leading to the death of tumour cells . The interactions between antibodies and DR5-expressing tumour cells activate the caspase 8 enzyme, which enhances the cytotoxic activity of camptothecin drugs. Schmid et al. demonstrated that conatumumab antibodies stay on the nanoparticle surface for 96 hours while activating caspase 8 [27]. The clearance of antibody-liposome conjugates occurs more readily than Fab (antigen-binding fragment)?-liposome conjugates due to the inadequate Fc region of an antibody [15].

8. Lipid nanoparticle

Lipid nanoparticles are assembled using synthetic lipids as a matrix or drug reservoir for cytotoxic drugs [20]. Stearic acid, lecithin and triglycerides are examples of the lipid materials used in this process [7]. Various routes of administration are available for nanodrug delivery systems that use lipid nanoparticles [20]. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are two types of common lipid nanoparticles with a solid matrix [7–28]. The particle size of lipid nanoparticles ranges from 50 to 1000 nm. The advantages of lipid-based nanoparticles include good compatibility with biological substances and good stability. Moreover, the drug released from its matrix can be controlled to avoid leakage and degradation [20]. Another benefit of using lipid nanoparticles in vivo is their minimal toxicity, making them a suitable drug carrier. NLC is often referred to as the second generation of SLN . Similar to nanoemulsions and liposomes, SLNs are made up of lipids and fatty acids with good biocompatibility and non-toxicity. However, SLN has a solid core instead of a liquid core in nanoemulsions. In SLN, the motion of drug particles in the solid core is restricted, further enhancing the controlled release property of loaded drug particles [28].

Additionally, SLN has a solid matrix wherein the drug is distributed between fatty acids of glycerides that protect the drug against chemical degradation. The SLN’s stability can be improved by adding a coat of surfactant. NLCs comprise many lipid molecules in liquid and solid states. Although liquid lipid is present, the matrix of NLC is solid at room

temperature. The solid matrix of NLCs can restrict the mobility of drugs and prevent the formation of coalescence compared to nanoemulsions. The advantages of NLCs are good biocompatibility, biodegradability, controlled-release properties and the absence of organic solvent in its formulation [28].

8.1 Preparation lipid nanoparticle

A high-pressure homogenisation (100–2000 bar) is used for the large-scale production of SLN and NLC. The high-pressure application causes the fluid to levitate above a speed beyond 1000 km/h, resulting in the breakdown of particles to submicron size. The strong turbulence resulting from the mechanical shear coupled with a strong cavitation force and low pressure across the homogeniser valves are the essential steps in forming nanoparticles. The high-pressure homogenisation can be performed at a high temperature (hot homogenisation or low temperature (cold homogenisation)). The drug is added to the melted lipid at 5–10°C higher than its melting point [28].

8.2 Hot homogenisation procedure

The procedure is conducted at temperatures higher than the melting point of the lipid used. Lipids and drugs can melt at this temperature and mix with a liquid surfactant. A high mechanical shear device can be used for pre-emulsion. Next, a piston-gap homogeniser or a jet-stream homogeniser transforms the pre-emulsion into a hot colloidal emulsion. Crystals of hot colloidal emulsion droplets (nanoemulsion) are formed at room temperature to produce SLNs or NLCs. The droplets should be a few micrometres in size. The low viscosity of the droplets' inner region at higher temperatures gives rise to smaller particle sizes. About 3–5 homogenisation cycles at 500–1,500 bar are enough to complete the process. However, repeated cycles often produce a greater particle size because of a high particle kinetic energy [28].

8.3 Cold homogenisation procedure

Issues associated with the hot homogenisation procedure have led to the development of the cold homogenisation procedure. Examples of these challenges are drug degradation, drug distribution into the liquid phase during the homogenisation process and complications that arise from the crystallisation step of the nanoemulsion and result in alterations and/or supercooled melts. The solid lipid is heated first, followed by the addition

of drug molecules into the matrix of melted lipids. The drug-loaded lipid is converted into a solid form using dry ice or liquid nitrogen. The cooling process is done rapidly to stimulate a uniform distribution of the drug within the lipid. The solid is ground to form microparticles via milling process. Next, the microparticles are incorporated into a cold solution containing surfactant(s) with a high-pressure homogenisation to produce SLNs. In hot homogenisation, solid lipids are homogenised in cold homogenisation instead of homogenising molten lipid [28].

Camptothecin-based drugs, specifically irinotecan (Camptosar) and topotecan (hycamptin), are frequently used with 5-FU [3]. Irinotecan drugs loaded into lipid-based nanoparticles with a size between 100 and 375 nm have been produced using the SN-38 irinotecan analogue [3]. Irinotecan binds to topoisomerase-1 DNA complex reversibly to restrict the formation of double-stranded DNA after a single-strand break. Thus, the DNA becomes damaged, leading to cell apoptosis [29]. A study on mice injected with irinotecan demonstrated an increase in the survival length of mice (65 days) compared to mice treated with encapsulated irinotecan (48 days). However, some challenges still exist in drug delivery due to the hydrophobicity of camptothecin-based drugs [3]. A few limitations of SLNs are low drug-loading capacity, risk of dose dumping after polymorphic shift during storage and high amount of water of the dispersions (e.g., 70–99.9%). Additionally, some methods can increase the oral bioavailability of drugs. These include increasing the solubility, preventing the formation of precipitates in the intestine, improving membrane permeability, inhibiting drug removal by protein efflux transporters, lowering cytochrome (CYP) liver enzymes level, and increasing chylomicron production and lymphatic drainage [28].

9. Polymeric nanoparticle

A polymeric nanoparticle is described as a spherical particle with a hydrophobic core and a hydrophilic shell. The assembly of amphiphilic block copolymers forms the hydrophilic envelope via the aqueous or microencapsulation approach [4]. Polymer nanoparticles is a term used to represent nanosized spheres and capsules [19]. Polymeric nanoparticles can be formulated into matrix-based nanoparticles (i.e., nanospheres) or nanocapsules, where therapeutic agents are entrapped, adsorbed or encapsulated [15]. Nanospheres are matrix particles wherein the drug molecules are adsorbed to the surface sphere or entrapped within the spherical particle. Nanocapsules act as a reservoir wherein drug molecules

become entrapped in a liquid phase comprising either oil or water. QA solid material envelops the liquid phase [19].

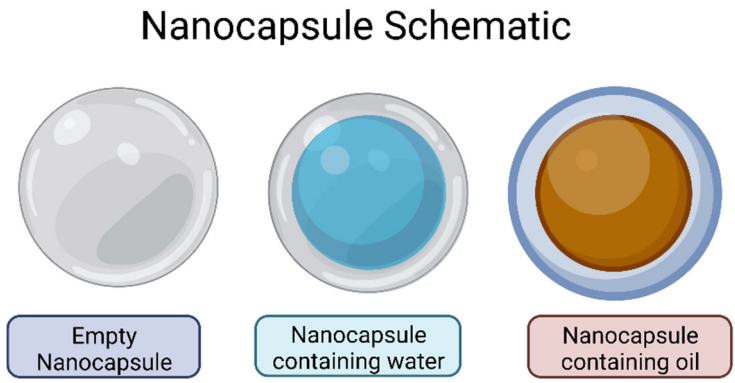

Figure 1-2: Schematic of nanocapsule containing water and oil
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There are two types of polymer nanoparticles: natural and synthetic polymers. Gelatin, albumin, chitosan and alginate are some examples of natural polymers. Polylactic acid, PLGA, polyhydroxyalkanoate and polymethyl methacrylate are some examples of synthetically produced polymers [15]. Flexibility is present in manipulating specific properties or the chemical composition of synthetic polymer for a desired biological application [18].

9.1 Natural polymers

Natural polymers or biopolymers are proteins with a high molecular weight. A protein comprises building blocks called amino acids bonded together by peptide linkages. Collagen, gelatin and albumin are examples of natural polymers. Collagen plays a larger role in the composition of bone, cartilage and skin. Moreover, incomplete hydrolysis of collagen produces gelatin, a highly soluble protein with inadequate mechanical properties. Collagen is highly biocompatible, degradable and non-teratogenic in the body. Apart from the strengths of using natural polymers, they also present various limitations, such as antigenicity, an increased likelihood of viral infection, and a non-homogenous property between product batches [18].

9.2 Synthetic polymers

The synthetic polymer's repeatability enables the production of polymeric drug delivery systems with homogenous properties. Synthetic polymers can be modified to produce polymers with specific interfacial, biological and mechanical properties. However, because many synthetic polymers cannot be broken down in the body, they are eliminated from the body by renal clearance. Hence, the molecular weight of synthetic polymers should be within the threshold of renal clearance. Acrylic polymers, such as polymethyl methacrylate and Polyhydroxyethylmethacrylate, are some examples of non-biodegradable polymers. Ester, ortho-ester, amide, urea or urethane are some examples of components found in a biodegradable polymer. Biodegradable polymers produce normal metabolites of the body that can be eliminated from the body. It is possible to mix the biodegradable and non-toxic properties of a natural polymer and the mechanical properties of a synthetic polymer during product formulation to obtain the desired properties [18].

The presence of functional groups on the surface of polymer nanoparticles allows modification with ligands to increase the nanoparticles' specificity [15]. The polymeric-based drug delivery system is divided into four categories: diffusion-controlled system, chemically controlled system, solvent-activated system, osmotically controlled system, and magnetically controlled system. The diffusion-controlled system can be distinguished by the release of drugs via the diffusion process. It comprises two sub-systems: reservoir and matrix systems. The reservoir system consists of a polymeric membrane that coats the drug's inner region, whereas the matrix system is based on a polymeric matrix where the drug is dispersed uniformly. Nevertheless, the poor polymeric membrane resistance of the reservoir system can lead to the sudden destruction of the system, which will result in dose dumping [18].

A chemically controlled system includes a polymer-drug conjugate, where drug molecules are connected to a polymer by a molecule. The linkage between the polymer and drug is cleaved by two processes: hydrolysis or by an enzymatic activity. Chain cleavage will result in either the biodegradation or bioerosion of the polymer-drug conjugate. Biodegradation refers to when the polymer's weight is reduced after chain cleavage. Bioerosion is defined as reducing the polymer-drug conjugate system due to the erosion of the polymer surface. Polymeric matrix disruption is responsible for the drug's release from the system. Hence, the control of the drug release depends on the matrix's degradation rate [18]. Polymer-drug conjugates should only

produce non-teratogenic and biocompatible metabolites. Ideally, polymers should be degradable in the body. Stimuli-responsive nanoparticles are produced to counteract barriers in the microenvironment of the tumour cells and enhance chemotherapeutic efficacy. Many polymer-drug conjugates have linkages between the drug molecule and polymer that control the drug release. Drugs will only be released at the tumour site upon exposure to physiological stimuli (e.g., light, heat or pH) [22]. For instance, in the pH-sensitive polymeric nanoparticle system, nanoparticle uptake into cells is controlled by stimuli because the pH-sensitive nanoparticles are responsive in acidic and alkaline mediums [15].

The diffusion of water controls the solvent-activated system into the hydrophilic polymer chain, which does not dissolve the polymer[18]. Water entry into the polymeric system can cause the system to swell. The increased osmotic pressure inside the system pushes the drug into the external environment. Hence, drug release control depends on the amount of water in the matrix that determines the osmotic pressure [18].

Osmotically controlled systems use a device that comprises a semi-permeable membrane across which a solvent flows to a chamber containing drugs. The increased osmotic pressure inside the chamber pushes the drug through the device's orifice [18].

A magnetically controlled system involves the combination of a polymer with magnetic particles controlled by an externally applied magnetic field . The movement of drug particles depends on the final force from the combination of the magnetic and haemodynamic forces of the systemic circulation. However, the external magnetic force should be greater than the haemodynamic force to affect the motion of drug particles. Widely used magnetic nanoparticles include nickel, cobalt and iron, possessing a high magnetic force at room temperature [18].

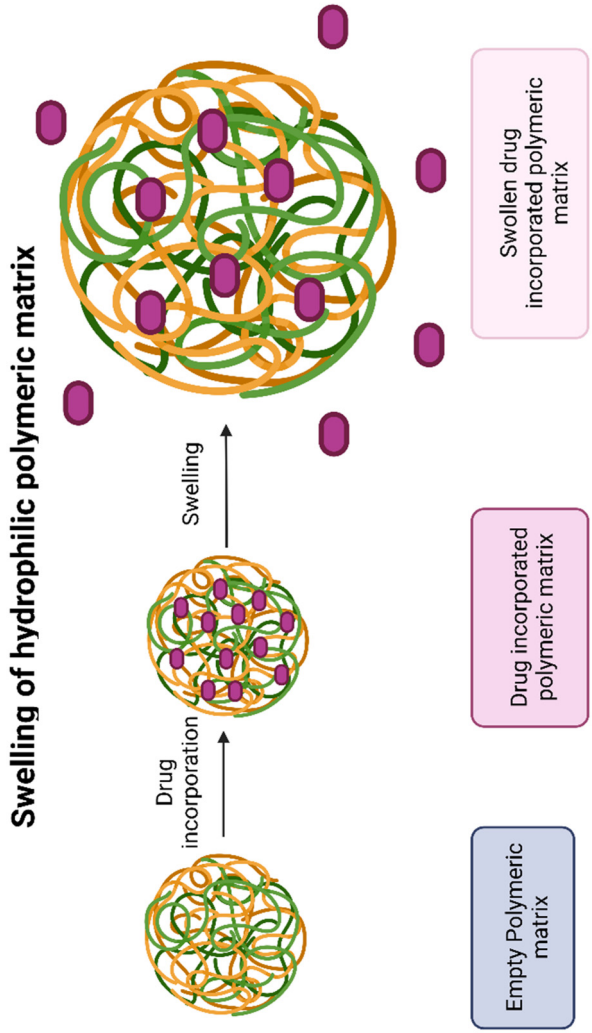

Figure 1-3: Schematic diagram of drug release that follows the swelling of a hydrophilic polymeric matrix [18]
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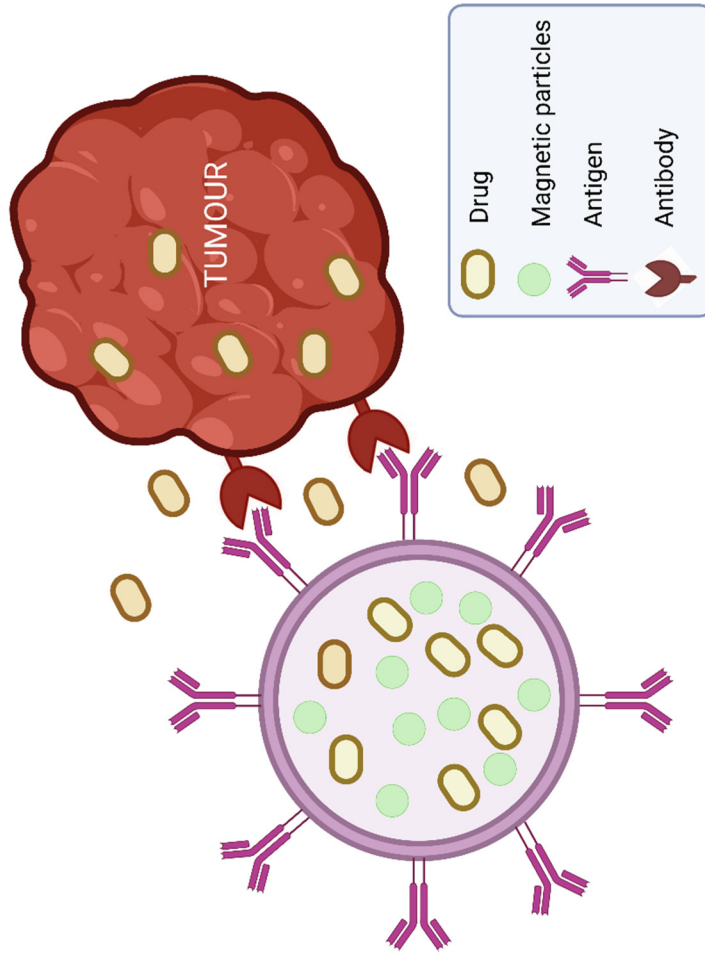

Figure 1-4: Schematic diagram of drug particles loaded into a magnetic particle with specific antibodies on the surface of the particle used in cancer therapy Created with BioRender.com

9.3 Factors to be considered in polymer-drug conjugate preparation

9.3.1 Molecular weight

The molecular weight of polymer impacts the bioavailability of hydrophilic polymers. Polymers with higher molecular weight will increase half-life and slower polymer clearance from the body. For example, polyHPMA-Doxo polymer-drug conjugate, with a molecular weight of 1230 kDa, has a longer half-life by 29 times. Its clearance rate from cancer cells was slower than single-agent Doxo by 25 times [22].

9.3.2 Architecture

The polymer architecture plays a pivotal role in the pharmacokinetic activity of the polymer-drug conjugate. Notably, the polymer architecture influences the renal clearance of polymers. Polymers with a high molecular weight have lower flexibility and more chains at the ends of polymers, resulting in reduced polymer removal and increased retention effect of the polymer in the bloodstream [22].

9.3.3 Composition of block copolymer

The ratio of copolymer composition influences the final spherical shape of nanoparticles. For instance, star micelles will be formed when the molecular weight of the hydrophilic block is greater than the hydrophobic block. The size of nanoparticles can also be affected by the concentration of copolymers in a solvent. Polymers that comprise zwitterions (e.g., cations and anions) have high water solubility to increase the circulation time of nanoparticles [22].

9.3.4 Preparation of polymeric nanoparticles (PNP)

Polymeric nanoparticles (PNP) can be produced by two procedures involving pre-formed polymers and monomers, respectively. The first procedure is the synthesis of nanoparticles from pre-formed polymers by dispersion methods, such as evaporation of solvent, salting-out, dialysis and supercritical fluid technology. The second procedure requires the polymerisation of monomers by various techniques, such as microemulsion, mini emulsion, additives-free emulsion and interfacial polymerisation methods. This chapter will discuss only two methods from each category. The PNP production method depends on the type of polymetric system, application site and nanoparticle size. Nanoparticles should not contain