DESIGN OF A 5-FLOUROURACIL COPOLYMERIC NANOGEL DRUG DELIVERY SYSTEM FOR THE TREATMENT OF BREAST CANCER

WITS
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A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the partial requirements for the degree of Master of Science in Medicine with specialisation in Pharmaceutical Affairs.

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DECLARATION

I, Ramokgopa Ofentse Grace, declare that this Dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the field of Pharmaceutical Affairs in the Faculty of Health Sciences as a research component at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Ofentse Ramokgopa

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Signature

On this ……..22nd .....day of ……August…… 2024

DEDICATION

This dissertation is dedicated to my daughter, my motivation and purpose. Thank you for never giving up on me. We can struggle through the journey, but we never give up – Never give up Olehlogonolo – Love Mama

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ABSTRACT

The present study investigates the development of a 5-fluorouracil (5-FU) loaded copolymeric nanogel for breast cancer treatment. Breast cancer remains the leading cause of cancer-related mortality among women globally, with current therapies often limited by adverse effects such as nausea, vomiting, fatigue, alopecia, and fertility issues. The standard administration method, intravenous (IV) injection or infusion of 5-FU, typically spans several hours or days, contributing to low patient compliance and potential drug resistance. This study aims to address these challenges by developing a nanogel formulation capable of sustained drug release over a two-week period, thereby reducing administration frequency and enhancing patient compliance. Nano emulsions offer promising potential as alternative cancer therapies due to their biocompatibility and high drug-loading capacity. This research focuses on formulating a nano emulsion into a nanogel with a small particle size to improve absorption in cancer cells and prolong circulation time. The mechanical and physical properties of the formulation were characterized using Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and rheological analysis. The study successfully formulated a copolymeric nanogel loaded with 5-FU, achieving an average particle size of 75 nm and a polydispersity index (PDI) of 0.464, confirming an effective nanogel drug delivery system. The drug-loaded nanogel demonstrated a sustained release of 60% over 14 days. FTIR analysis identified key functional groups, confirming the chemical integrity of the formulation, while DSC indicated thermal stability. Rheological studies revealed the nanogel's elastic and flow behaviour, supporting its suitability as a drug delivery system. These findings suggest that the developed 5-FU-loaded copolymeric nanogel represents a promising step toward alternative drug delivery systems for cancer therapy.

Keywords: nano emulsion, nanogel, cancer, therapy, treatment.

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BCT	Breast Conserving Therapy
BRCA	Breast Cancer Gene
CSCs	Cancer Stem Cells
DCIS	Ductal Carcinoma in Situ
DNA	Deoxyribonucleic Acid
DSC	Differential Scanning Colorimetry
HER 2	Human Epidermal Growth Factor Receptor 2
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared Spectroscopy
5-FU	5-flourouracil
GEP	Gene Expression profiling
LBIS	Lobular in Situ
LCST	Lower critical solution temperature
MSCs	Mesenchymal Stem Cells
mTOR	Mammalian target of rapamycin
MW	Molecular weight
ODN	Oligonucleotides
OSM	Oligosulfamethaxozole
PBS	Phosphate Buffered Saline
PEG	Polyethylene glycol
PLGA	Poly Lactic co Glycolic acid
PVA	Poly Vinyl Alcohol
RPM	Rotations per minute
SD	Standard Deviation
SEM	Scanning Electron Microscopy
TNF	Tumour necrosis factor
Tween 80	Polysorbate 80
UCST	Lower critical solution temperature
UV/Vis	Ultraviolet/Visual
UVB	Ultraviolet B-ray
WHO	World Health Organization

Chapter 1: Background and introduction

1.1 Introduction

A tumour is an abnormal growth of cells at a specific area of the body. A tumour can be benign (meaning that is localized to a certain area of the body and controlled) or metastatic meaning it has migrated to various organs (Kirandeep et al., 2016). According to the World Health Organization 2019, cancer has been listed as one of the five diseases that are to be treated with priority. Its complex mechanisms of genetics and abnormal cell growth that are caused by various sources make it difficult to treat with drugs available on the market. Cancer therapy will remain a huge area of research in the pharmaceutical sector because of new disease-related and drug-related discoveries (Kirandeep et al., 2016). The death of cancer cells is apoptosis and involves a process of shrinking of cells and nuclear fragmentation (Lin and Lowe., 2000). This apoptotic process involves changes in proliferation of a tumour and regression of the tumour cells which is the ultimate cause of apoptosis. Studies have shown that cell loss resulting from apoptosis affects not only cancer cells but also surrounding tissues (Perez-Alvarez et al., 2019).

Cancer treatment is clinically performed in three ways, including surgical resection, radiotherapy and chemotherapy. Surgical resection and radiotherapy are invasive methods and are highly beneficial for localised tumours. Chemotherapy is utilized locally but can also target metastasising cells (Lin and Lowe., 2000). The use of chemotherapy that target cancer systemically is ideal however the risk of intolerable side effects is also increased (Yang et al., 2018). Owing to the aggressive and invasive nature of surgical resection and radiotherapy, chemotherapy is then a preferred method of treatment (Yang et al., 2019).

The molecule 5-Fluorouracil (5-FU) is a cytotoxic drug which acts by inhibiting microtubule depolymerization. 5-flourouracil is a highly potent drug with high affinity to the tubule and presents with toxicity symptoms (Lee et al, 2009). According to the Biopharmaceutics classification system (BCS), 5-Flourouracil is a class IV agent, having low permeability and low solubility (Wang et al., 2014). 5-flourouracil is the model drug in the treatment of breast cancer, due to its potency and efficiency in chemotherapeutics. Thus, there is a need in development of methods of delivery that can increase permeability, solubility and decrease toxicity (Jain et al., 2016). The administration of 5-flourouracil parenterally is thus ideal for bypassing the challenges associated with oral regimes (Jain et al, 2016).

Cancer can be alleviated or completely eradicated if detected early, hence focus should be on attempts of early detection and initiation of therapy in these early stages of cancer development. Targeted cancer therapy includes prevention of growth, progression, and migration of cancer cells to other tissues (Thambi et al., 2017). Targeted cancer therapy is mainly accomplished by incorporation of active pharmaceutical nanoparticles, including nano emulsions, liposomes, micelles and dendrimers. Amongst these nanoparticles, nanoemulsions are preferred for targeted cancer chemotherapy due to their physical properties. These nanoparticles have an average particle size of about 5-100 nm (Hanafy et al., 2018). Nano emulsions improve solubilization, enhance drug circulation and bioavailability of cytotoxic therapeutic agents (Gao and Lo., 2018). Chitosan based nano emulsions are ideal as they possess low immunogenicity and further decrease tumour activity by interfering with cell metabolism to inhibit cell growth, resulting in apoptosis (Jain et al., 2016). Chitosan is preferred in nanoemulsion formulations, due its abundant amino and hydroxyl groups (Jain et al., 2016).

1.2 Study Rationale and Motivation

Conventional cancer treatment has been known to cause undesired and toxic side effects when administered either intravenously or intraperitoneal. Most cancer treatment methods include the disruption of cancerous and non-cancerous DNA synthesis resulting in apoptosis of cancer cells and surrounding tissues. Targeted cancer is aimed at specific sites of the disease and thus, provides improved treatment with less toxicity and few side effects leading to patient compliance with improved prognosis (Wang et al., 2014). Drugs such as docetaxel, cisplatin and paclitaxel have been used for many years in the cancer chemotherapeutics. These drugs are effective, however possess limitations such as resistance, low solubility and thus, require solubilisation with co-solvents (Norouzi et al.,2016). The use of nano emulsions in the present study, in targeted cancer therapy will be ideal due to their nature and composition.

A study conducted by Shanmuganathan et al., (2019) showed that by administering the desired active pharmaceutical ingredient (API) as a prodrug with encapsulation within nanoparticles, can provide sustained release to the body. The evidence of this study was supported by combining the prodrug of paclitaxel, paclitaxel palmitate with polyethylene glycol (PEG), with results demonstrating extended release of the API into the system over a period of 14 days. *In-situ* forming injectable hydrogels are used in implanting polymeric drug loaded nanoparticulates into the body. These gels are responsive to chemical and physiochemical stimuli and upon injection, form a non-flowing viscoelastic gel that enable sustained release of the required pharmaceutical agent at the target site (Hanafy et al.,2017). A study conducted

by De Graaf et al., (2012), demonstrated pentablock copolymer synthesis and end coupling reaction of carboxyacid terminated oligosulfamethoxazole (OSM) and triblock copolymer poly (CL-CO-A) polyethylene glycol (CL CO LA) (PCLA -PEG-PCLA). This study showed the reversible sol-to-gel phase transition of the copolymer by modifications in temperature and pH (Thambi et al., 2017).

The purpose of this study was to design a 5-flourouracil loaded nanogel, as seen in Figure 1, for the treatment of breast cancer. This *in situ* nanogel will have the ability to provide sustained 5-flourouracil release into the human breast carcinoma over extended periods of time. The aim of the nanogel system is to cause cell death in a targeted manner with least side effects associated with chemotherapy.

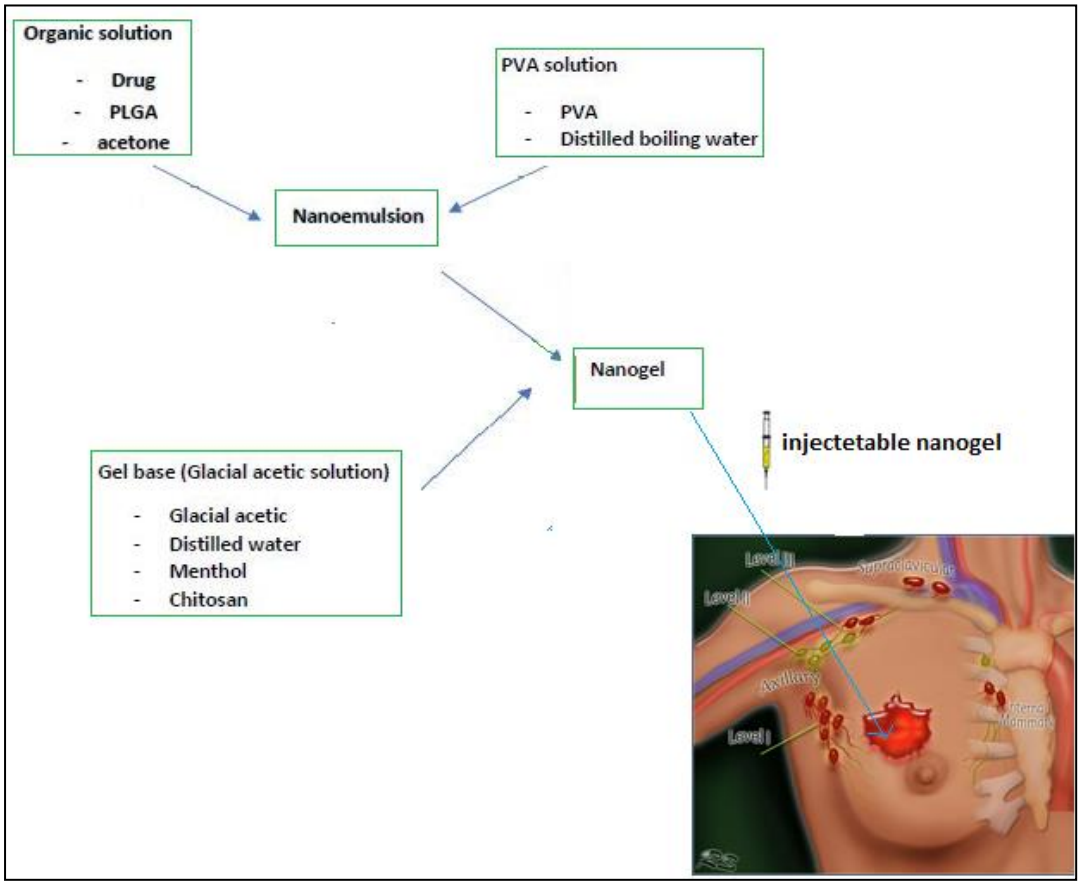

Figure 1: A Representation of the synthesis of a nanogel drug delivery system for human breast carcinoma treatment, employed as an injectable therapeutic.

1.3 Aim and Objectives

This study Intends to develop a drug delivery system for targeted and sustained delivery to the treatment site.

The following objectives were conducted and completed:

1. To formulate a drug loaded nano emulsion by emulsification-solvent evaporation method. The size of the nano emulsion will be confirmed by a zeta-sizer.
2. The presence of 5-FU and polymers used in the formulation will be confirmed through FTIR.
3. To assess physio-chemical and mechanical properties using DSC and Rheometer.
4. To quantify the drug release behaviour from nanogel employing UV-spectroscopy.

1.4 Overview of this Dissertation

Chapter 1 serves as the introduction of the dissertation and describes pathophysiology of cancer and the challenges faced by scientists and physicians. An overview of Current therapies for the breast cancer and the challenges including toxic side effects, challenges with patient compliance, delay of diagnosis because of delayed screening. Furthermore, this chapter briefly presents nanogel systems as novel drug delivery platforms for the treatment of benign breast cancer and advantages thereof. Literature from previous studies involving breast cancer is presented as evidence for the efficacy of nanoparticles treatment of localized breast cancer. Finally, the aim and objectives of the study are outlined in this chapter.

Chapter 2 is a critical study of nanogel systems in the treatment of cancer. The drug delivery systems developed for this purpose are elaborated on, in a systematic manner. Current studies explore the limitations and advantages that can assist in the development of an ideal drug that could have a better prognosis. This chapter also investigates the use of nanogels and their advantages and limitations in comparison with conventional therapy.

Chapter 3 describes the design, materials and methods used, as well as results and discussion for the synthesis of a 5-Flouroutacil loaded nanogel system. It explains the characterization of the nanogel, conducted utilizing the FTIR spectrometer, Zeta sizer, and Thermal analysis. Drug release profiles were determined by dissolution and UV/Vis Spectroscopy. Rheological studies of the nanogel were evaluated using the HAAKE MARS III Rheometer

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Chapter 2: A review of literature on advanced nanogel therapeutics in breast cancer

2.1 Introduction

Tripple negative breast cancer which is the most common in Africa is characterized by lack of expression of oestrogen receptors, progesterone receptors as well human growth receptor in malignant cells (Zerdan et al., 2022). Triple negative breast cancer is aggressive and is leading in poor prognosis and cancer related deaths in females worldwide (Chikara et al, 2023). Targeted treatment is ideal for this type of breast cancer as current treatment remains challenging due to lack of specificity. Currently live macrophage delivered doxorubicin loaded liposomes have proven to be effective (Zerdan et al., 2022).

Breast cancer is made up of four stages, namely stage I-IV. Stage I is where the tumour is localised and less than 2 cm, stage II is the stage where they may be metastasis of the cancer to the lymph nodes and the tumour may be 2-5 cm. At stage III cancer the tumour is usually more than 5 cm and there is metastasis to axillary lymph nodes and at stage IV cancer the tumour can be any size with metastasis to distant organs such as bones, brain or even the liver. Understanding the different stages of cancer is important to consider as it determines tumour targeting mechanism which are ideal as therapy (Yap et al, 2021). Breast cancers can further be classified into biologically and clinically meaningful subgroups according to histological grade and histological type. The term grade refers to the extent of differentiation. (Yap et al., 2021).

There are certain risk factors which can assist in early detection and a better prognosis of breast cancer, the risk factors for breast cancer can be categorised into non-modifiable and modifiable risk factors. Non-modifiable risk factors include female sex hormones, genetic mutations, ethnicity, pregnancy and breastfeeding, menstrual period and menopause, density of breast tissue and previous history of breast cancer. Modifiable risk factors include hormonal replacement therapy, diethylstilbesterol, physical activity, obesity, alcohol intake, smoking, intake of processed food and exposure to chemicals (Lukasiewicz et al., 2021).

Breast cancer is measured by the number of confirmed diagnoses within a specified period. The number of new cases and the rates at which they occur assists in determining the risk factors for a diagnosis (Mayrovitz et al, 2022). The potential rate of growth of breast cancer is determined by the developmental stage of a country. The availability of resources such as screening methods such as breast self-examination and mammographs are not easily

accessible in countries like the United States of America and the United Kingdom have shown to have a less incidence of breast cancer compared to other countries (Mayrovitz et al., 2022).

Breast cancer is often discovered when an individual notices a lump or changes in normal breast tissue and sometimes cancer is discovered during screening. The lump associated with cancer is usually abnormal and is accompanied by dimpling of the skin, changes in the shape of a breast, nipple discharge not associated with infection and discoloration of the skin (Laronga et al., 2023). The prognosis of breast cancer is dependent on advanced screening methods, early diagnosis, and drug therapy (Nounou, 2015). Breast carcinoma can be classified into situ carcinoma and invasive carcinoma, the two categories can further be classified to ductal carcinoma or lobular cancer situ (Nounou et al., 2015). Nanomedical formulations assist in increased drug delivery to sites of action of chemotherapeutic agents while mitigating side effects (Chikara et al., 2023).

2.2 Breast Cancer Overview

2.2.1 Epidemiology of breast cancer

The number of new breast cancer cases are still seen as rising in many populations across the world. The term breast cancer incidence is a measurement of the number of breast cancer cases that occur within a specific period, divided by the number of persons at risk for breast cancer during that period (Azarnova and Mayrovitz., 2022). The growth of breast cancer depends in the risk factors of a population. Understanding the risk factors may assist in a better understanding of the disease, which can assist in planning and prioritization of treatment options (Azarnova and Mayrovitz, 2022). There are certain risk factors that predispose individuals to cancer. The most common risk factors are identified as gender, age, and the degree of development of an economy. (Smorlaz et al, 2022). Most cases of cancer occur in women. In very few cases, cancer can occur in males, the onset of cancer in males is usually associated with obesity (Smolarz et al, 2021). Hormonal contraceptives are a risk factor for women who start menstruation early or late, women who are post-menopausal, women on hormone replacement therapy and women who are using contraceptives (Smolarz et al, 2021). Certain risk factors are related to physiological and health status, family history and being over 35 years of age in women who have had ovarian and endometrial cancer increases the risk of breast cancer (Smolarz et al, 2021). Other factors that predispose one to cancer include, previous infection with Epstein-bar virus or Hodgkin's lymphoma, a diet composed of excessive fats, low intake of vitamins could increase the incidence of cancer (Smolarz et al, 2021).

2.2.2 Classification of breast cancer risk factors

Risk factors for breast cancer can be classified as non-modifiable, as mentioned above as well as modifiable. The factors such as age, female sex, family history is classified as non-modifiable. There are also several genes that predispose to cancer, namely, BRCA1, BRCA2, TP35, CDH1 and PTCN, these genes are usually responsible for DNA repair and cell cycle but are often linked to mutations that cause carcinogenesis, which is the development of cancer cells (Lukasiewicz et al, 2021). It is important to also identify the modifiable risk factors, this will eventually assist reduction in the incidence of breast cancer (Lukasiewicz et al, 2021). Obesity as well as ahigh body mass index are associated with the probability of cancer in women who are post-menopausal, the kind of cancer linked to this risk is oestrogen receptor positive breast cancer. (Lukasiewicz et al, 2021). factors such as excessive alcohol intake is not only linked to cancer in the gastrointestinal tract but also an increase in breast cancer, The carcinogens that are found in tobacco can be transported to breast tissue, vitamins such as vitamin C, E and folic acid have shown to be beneficial in protection against cancer. More studies are required to understand the mechanism of protection of vitamins against cancer.

2.3 Treatment of Breast Carcinomas

There are two approaches to treating cancer depending on the stage of the cancer, namely localized therapy, or systemic therapy. The survival rate of cancer has shown a significant increase up to 90 % due to molecular classification and personalized treatment (Zhai et al ,2022). Cancer is treated according to the cancer type, where the cancer is located and the progression of the cancer (Attama et al., 2022). Treatment of cancer discovered in the early stages' cancer may be treated through localized therapy which involves a mastectomy or a lumpectomy, in surgery a lymph node called the sentinel lymph is biopsied and removed as it would be the node that would receive lymphatic treatment from the primary tumour causing it to spread.

A mastectomy is a complete removal of the breast whereas a lumpectomy is also referred to breast conserving therapy (BCT). A lumpectomy is characterised by a wide excision, quadrantectomy or a partial mastectomy (Laronga et al., 2023), Radiation therapy is often used alongside a lumpectomy to reduce the chances of the regrowth of cancer cells. Radiation therapy around surrounding areas such as the lungs and lymph nodes are also recommended for patients who have had a mastectomy. Once cancer has advanced it is treated at a systemic level, therapy can be initiated before or after surgery. Neoadjuvant therapy is referred to cancer treatment initiated before surgery and adjuvant cancer therapy refers to treatment given after surgery (Laronga et al, 2023). Systemic treatment is usually given to females who are stage 1 and 2 breast cancer. Three types of systemic therapy can be identified, namely

endocrine therapy, anti-her2 therapy and chemotherapy. Systemic cancer treatment includes chemotherapy, targeted therapy, endocrine therapy, and immunotherapy (Zhai et al., 2022).

Chemotherapeutic agents work by enforcing apoptosis by slowing tumour progression by killing off the ability of the cancer cells to multiply. The toxic effect of chemotherapy to the patient is occasioned by increasing the susceptibility to host diseases and effect on bone marrow cells. (Attama et al., 2022). Chemotherapeutic agents target tumour cells by causing disruption which will result in either death or inhibition of the growth of the cells (Trayes and Cokenakes.,2021). Chemotherapeutic agents cannot differentiate between normal cells and cancer cells and because of this non-selectiveness normal cells are also affected. The result is undesired adverse effects such as compromised immune system, hair loss, vomiting, nausea, weakness, complicated infections or even death (Trayes and Cokenakes.,2021).

There are many chemotherapeutic agents that are available in the treatment of cancer. The five most used drugs include alkylating agents, antimetabolites, anthracyclines, mitotic inhibitors, and hormonal chemotherapeutic agents. Alkylating agents are usually used in the treatment of lymphoma, leukaemia, and diseases such as Hodgkin's lymphoma by causing direct DNA damage which stops the division of cancer cells. Unwanted side effects such as damage to the bone marrow and leukaemia are associated with alkylating agents (Attama et al., 2022).

Anthracyclines work by targeting DNA replication enzymes affecting cells in all cell cycle phases. Examples of anthracyclines include doxorubicin, idarubicin and daunorubicin. The disadvantage of anthracyclines is that these drugs a permanently damage the heart (Attama et al., 2022). Antimetabolites act by incorporation and the inhibition of the growth of DNA and RNA. This group of anticancer drugs affect the S phase of the cell in particularly and is used for the treatment of ovarian cancers, leukaemia, intestinal tract and other forms of cancer, examples include 5-flourouracil, methotrexate, pentostatin and 6-mecaptopurine. There are also topoisomerase inhibitors which act by unwinding DNA to stop replication, topoisomerase drugs usually used for lung cancer, leukaemia and ovarian cancer and some examples include etoposide and mitoxantrone (Attama et al., 2022). Mitotic inhibitors such as plant alkaloids are naturally derived products which function by stopping cell division and the mitotic phase of cell cycle as well other phases of cancer too (Attama et al., 2022).

Radiation therapy uses high energy x-rays to kill cancer cells. Radiation therapy can be categorised into external radiation therapy where radiation is sent toward the area of the body

with cancer and internal radiation therapy where if discovered in the early stage's cancer may be treated through localized therapy which involves a mastectomy or a lumpectomy. Radiation therapy is also defined as local treatment and is often used in metastatic or unresectable breast cancer. The type of radiation depends on the presented clinical situation. Techniques include breast radiotherapy which after breast cancer, chest wall therapy which is after a mastectomy and breast boost which is a boost of high dose radiotherapy. Side effects associated with radiation therapy include fatigue, lymphoedema, radiation therapy besides its side effects has been associated with improved survival rates and lowered recurrence (Lukasiewicz et al., 2021).

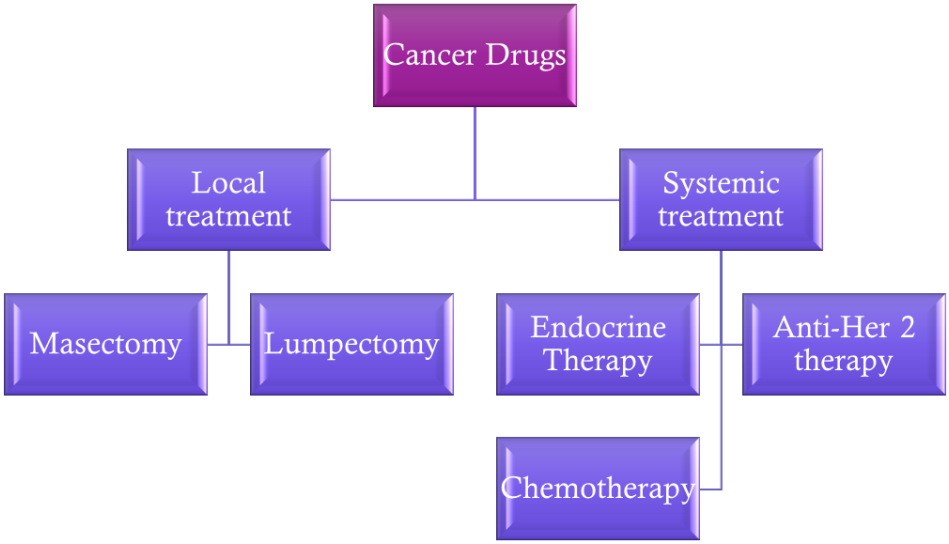

Figure 0-1 An illustration of current traditional treatment options for cancer, divided into local and systemic treatment (Rabie et al, 2020)

2.4 Advanced therapy in treating breast carcinomas

There are several advanced therapies that have been developed to overcome obstacles associated with cancer therapy, the aim of advanced and innovative therapy is eliminate drug resistance as chemotherapeutic agents have shown susceptibility to reduced drug reuptake and increased drug efflux. The table below summarizes some of the newer and innovative drug treatment. Innovative drug treatments of cancer are a potential area of interest as their use may give alternatives to conventional therapy, there are side effects associated such as tumour regenesis, drug toxicity and drug resistance, incidence of viral infections, increased immune responses and autoimmunity (Debele et al, 2021).

Table 0-1 A table illustrating innovative stem cell therapy for breast cancer.

Types of stem cell treatment	Mechanism	Applications	References
Stem Cell Therapy	Undifferentiated cells present in the bone marrow (BM) with the ability to differentiate into any type of blood cell. Stem cell therapy is one of the treatment options considered safe.	Mesenchymal stem cells (MSCs) are used in trials that delivered from BM, fat, and connective tissues, MSCs regenerate damaged tissue. Stem cells have a variety of uses such as Immunomodulatory role in wound environments, umbilical cord matrix-stem cells.	(Jin et al, 2020)
Pluripotent Stem cells	Embryonic stem cells which are isolated from the inner mass cells of the embryo possess the flexibility to administer cells within the placenta.	Induction of effector T cells and natural killer cells Anti-tumour vaccine preparation. Human induced pluripotent stem cells are used to for modelling of arrythmias, inherited cardiomyopathies and can be used as a tool for drug screening.	(Nakao et al, 2020)
Adult stem cells	Give rise to specialized cell types of tissues and organ. The	Primary and metastatic breast cancer; Examples include	(Chu et al, 2020)

	treatment with adult stem cells uses hematopoietic stem cells, mesenchymal stem cells and neural stem cells.	Hematopoietic stem cells and MSCs and b=neutral stem cells. MSCs are found in many tissues and organs which play an important role in tissue repair and regeneration in osteocytes, adipocytes, and chondrocytes. NSCS can self-renew and generate new neurons and glial cells.	
Cancer stem cells	CSCs are generated into normal cells by epigenetic mutations m CSCs are responsible for tumour growth, metastasis, and recurrence. CSCs posess a self-renewal and differentiation capacities	Treatment of solid tumours, CSCs change their characteristics in both genetic and protein role which is important in preventing metastasis.	(Liang et al, 2023)

2.5 Targeted therapy in the treatment of breast cancer

Targeted therapy is the use of drugs or substance to particularly attack specific cancer cells. Biomarkers are usually used to identify the cancer. There is various treatment option for targeted therapy namely, monoclonal antibodies where immune system proteins engineered in a lab are used to target specific cancer cells, monoclonal antibodies can attack cancer cells, inhibit their growth, inhibit malignancy, and ultimately result in necrosis of the tumour. Tyrokinase inhibitors block the signalling pathways required for tumour cells to grow. Tyrokinase inhibitors are seldom used as sole therapy but rather adjuvant therapy to other treatment options. Cyclin dependant kinase inhibitors block the cyclin dependent kinase which is required for a tumour to grow. Mammalian target if papamycin (mTOR) inhibitors block mTOR which prevents the growth of new blood cells required for a tumour to grow. PARP inhibitors block DNA repair of cancer which could lead death of the cancer cells, this type of therapy is particularly useful in the treatment of BRAC1 or BRAC2 related mutations. There are various drugs available as target therapy, the table illustrated below gives examples of available targeted therapy.

Table 0-2 Table illustrating drugs used in targeted breast cancer therapy.

Targeted pathway	**Examples**	**Area of Target**	**Reference**
Monoclonal Antibodies	margetuximab pertuzumab sacituzumab govitecan trastuzumab trastuzumab deruxtecan	Area around the cancer cells or tissues, send toxic substances right into cancer cells	(Behl et al, 2023)
Tyrokinase Inhibitors	lapatinib neratinib tucatinib	Target tyrokinase inhibitors that play a role in cancer example HER-2 receptors	(Iancu et al, 2022)
Cyclin-dependent kinase inhibitors	abemaciclib alpelisib palbociclib ribociclib	Inhibit proliferation of Rb positive tumour cells, target abnormal CDK activity in cancer cells	(Reddy et al, 2022)

Mammalian target of rapamycin (mTOR) inhibitors	Everolimus	Key regulator in cell proliferation and growth in cellular processes of uncontrolled growth of cancer cells	(Lo Russo PM. 2012)
Poly (ADP-ribose) polymerase PARP Inhibitors	Olaparib Talazoparib	Stop PARP from doing repair work, interfere with BER and DNA repair in the treatment of breast cancer and ovarian cancer	(Weil et a, 2011)

2.6 Leading nanotherapeutics in breast cancer

The treatment of breast cancer can be considered difficult due to the tumour microenvironment (TME), the TME is a complex environment and therefore current therapies are unable to target tumour cells specifically and the result thereof is undesired side effects. (Gadi et al.,2022). A rapidly developing field for the treatment of cancer is nanotechnology. Nanotechnology brings promising opportunities to human cancer diagnosis and better prognosis. The use of nanoparticulate-based platforms overcomes biological barriers and results in prolonged blood circulation time, specific tumour targeting and enhanced accumulation of drugs in tumours (Tang et al., 2017).

Nanoparticles are of particular interest in cancer due to their physical and chemical characteristics which ensure effective drug delivery, non-toxic nature, and increased biocompatibility. Nanogels are gels with a size of a 100 nm (Ali et al., 2022). Some nanoparticles have been observed to cause autophagy which results in cell degradation, such nanoparticles include gold nanoparticles, quantum dots and titanium oxide nanoparticles (Tang et al., 2017). Current nanomedicines as approved by the FDA or are under phase 2 clinical trials include pegylated liposomal doxorubicin agents, albumin bound paclitaxel, non-pegylated doxorubicin, Peg -polyaspartate paclitaxel. liposomal paclitaxel, peg-polyglutamic acid, paclitaxel poliglumex, heat activated liposomal doxorubicin, liposome semisynthetic doxorubicin analogue annamycin (Gadi et al, 2022).

Liposomes are self-assembling closed spherical vesicles composed of an aqueous core and a membranous lipid layer, liposomes are used to entrap drugs, hydrophilic drugs, genes, and siRNA can be encapsulated with core hydrophobic drugs within the lipid bilayer to reduce systemic side effects and increase circulation time to achieve longer in vivo circulation of liposomes, bio inert components such as polyethylene glycol are coated on the surface of liposomes to form a protective layer and to decrease recognition by the reticulo endothelial system(De Oliviera et al, 2021). Liposome based drug delivery systems are improved via enhanced permeability and the retention effect. Doxorubicin pegylated liposomes was one of the first drugs to be used as approved by the FDA (Tang et al, 2017). Doxorubicin intercalates between base pairs of DNA strands by inhibiting DNA synthesis and transcription. Doxorubicin is associated with cardiotoxicity which was seen to significantly improve when in the form of liposomal doxorubicin (Tang et al, 2017).

2.7 Current challenges for the treatment of breast cancer

Breast cancer still has a high death rate despite the many advances that have emerged over the years. Prolongation of life and maintenance of quality of life is the goal when treating metastatic breast cancer (Wang and Wu., 2023). Providing access to breast cancer management drugs is important to a higher cure rate. A combination of early detection and adequate treatment is likely to decrease mortality. Accessibility to surgery, radiation and systemic therapy is a vital step after a diagnosis has been confirmed. (Barrios., 2022) Research has shown that in low resource scenarios, the push should not necessarily be for the latest antibody, but for having surgery, providing patients to access to radiation therapy as well as standard adjuvant or neoadjuvant chemotherapy. The approach will improve advances and technology incorporation will be possible and implemented in a much more organized and efficient care system. There are several possible resistance mechanisms to anti-HER2 therapies that have been identified that ultimately lead to the reactivation of HER2 mediated or its downstream signalling pathways, via path redundancy or activation of alternative survival pathways (Barrios., 2022).

2.8 Overview of Nanogels in the management of breast cancer

The term nanogel refers to 3-dimensional hydrogel materials in nanoscale size formed by cross linked swellable polymer networks with a high capacity to hold water without dissolving the water into aqueous media. Nanogels may be composed of synthetic or natural polymers that either anionic, cationic, or natural depending on their bonded groups Nanogels are a growing area of therapeutic interests because their macromolecular systems are designed to achieve a long circulation half-life of their systems, nanogels depending on their route of administration which could be oral, pulmonary, intraocular, or transdermal are able to overcome barriers that are usually occurred with cancer drugs (Soni et al., 2016). Polymeric

nanogels act to prolong drug circulation by preventing fast clearance due to their small particle size. Nanogels also prevent degradation of biomolecules. Nanogels also possess a pegylation property which gives them “stealth characteristics which leads to a more hydrophilic surface., this property allows for steric hindrance by shielding a charge that the core (Soni et al., 2016).

2.8.1 Synthesis of Nanogels

Polymerization of monomers is a method is when there is colloidal suspension of the polymer is made by the standardised nucleation of water-soluble monomers that are used to produce stabilized nanogels, in this method particle size is controlled. Nano-gels of smaller particle size can be produced by adding an ionic surfactant, which also increases the colloidal stability of the formulations (Sharma et al., 2014). Physical assembly is where nanogels are synthesised using the physical self-assembled polymers with amphiphilic polymers, the inter-action between the drug and solvent occurs by the Van der Waals’ interaction, hydrogen bonding. Micro- and macromolecules are entrapped within the nanogels’ structure during self-assembly. Chemical cross linking is used for the synthesis of large-particle-size nanogels. In cross-linking polymerisation method, nanogel are prepared by oil-in-water emulsion, followed by solvent evaporation method where PEG is conjugated to a branched polyethyleneimine in aqueous media (Sharma et al.,2014).

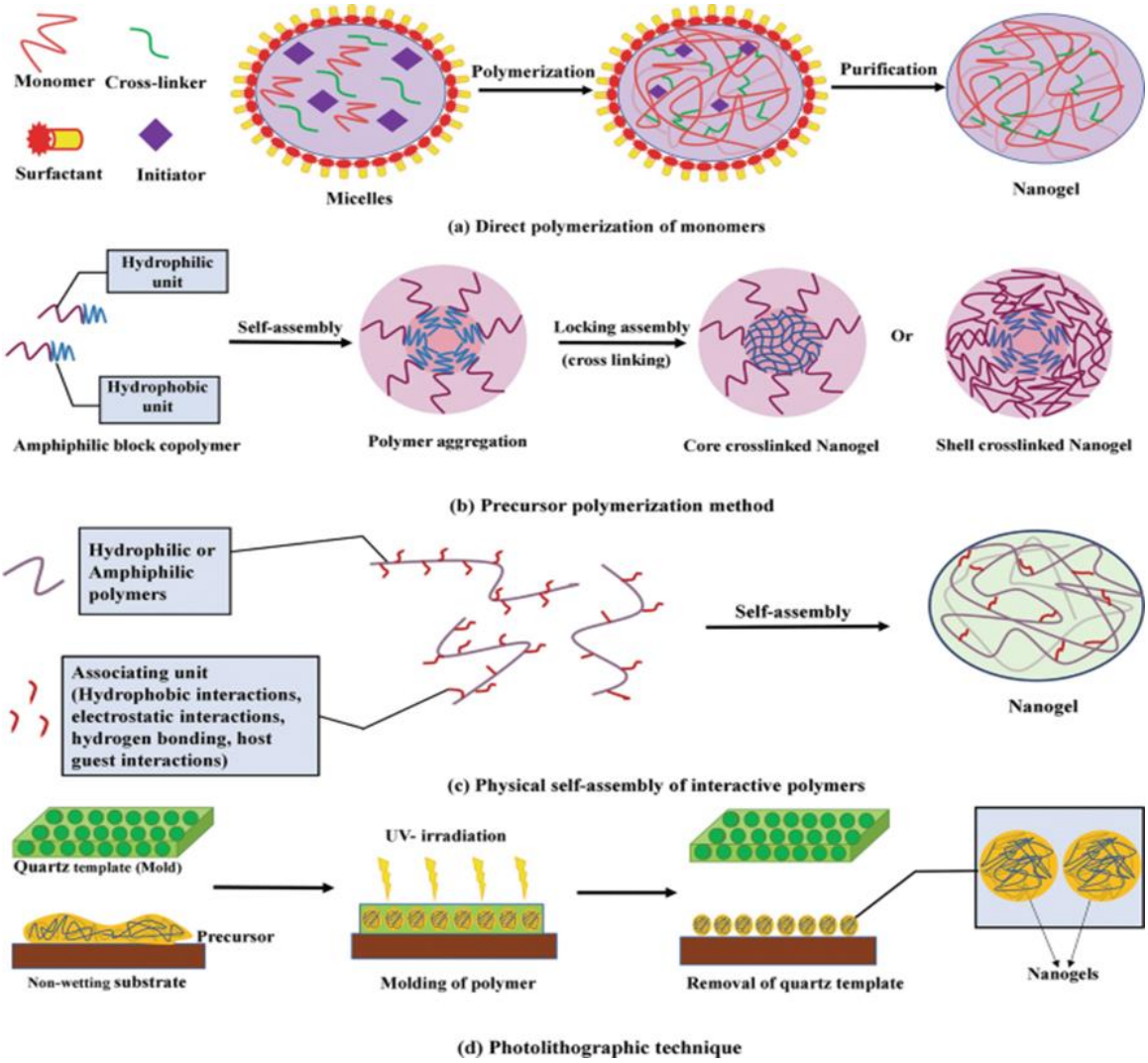

Figure 0-2 An illustration of synthesis of nanogels through 4 different types of methods (Sethi et al., 2023)

2.8.2 Classification, advantages, and disadvantages of nanogels

Classification

Nanogels are classified according to either response to stimuli or cross linking as illustrated in figure 2.5. A nanogel can be classified as responsive or non-responsive, responsive nanogels will swell according to an environmental condition such as pH, magnetic field, temperature, or ionic strength (Critchley L, 2019). Non-responsive nanogels swell due to water absorption. Nanogels can also be classified by the kind of the type of cross linking where they can be physically cross linked or chemically cross linked. Physically cross linked nanogels are held together by weak intramolecular interactions such as hydrogen bonds and van der Waals forces whereas chemically cross linked nanogels are chemically bonded to monomer chains through strong covalent bonds (Critchley, 2019).

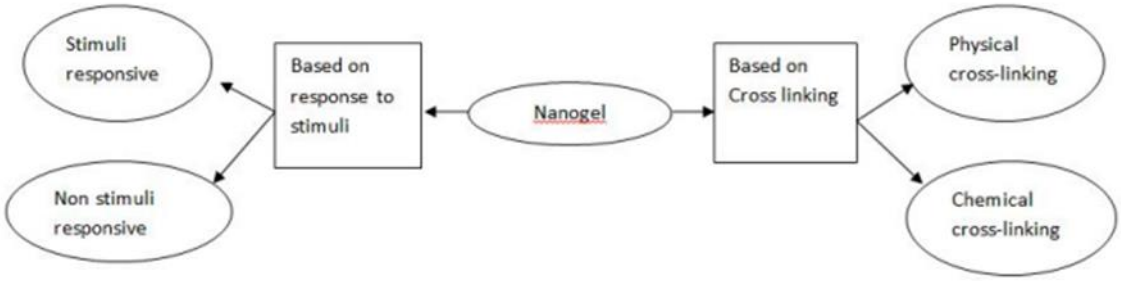

Figure 0-3 A schematic representation of classification of nanogels according to schematic representation (Critchley, 2019)

Advantages

Nanogels have proven to be advantageous due to properties such as the ability to cross the blood brain barrier due to their small size, a high biocompatibility due to having a high-water content as well as a porous nature that assist in incorporating drugs. They are soft, biodegradable with a high surface area which is ideal in drug development (Ali et al, 2022). This kind of gel can quickly respond to stimuli due to their small size and is able to respond to stimuli that change the nanogel structure. They are inert when administered directly to the blood stream. These formulations have an enhanced permeability capability and can also avoid rapid renal exclusion which results in a prolonged half-life and are also able to avoid clearance by phagocytic cells and reticular endothelial uptake. These gels also possess an ability to cross the blood brain barrier (Yadav et al, 2017).

Disadvantages

Nanogels are usually diffusion based and that may result in a rapid drug release rate (Shoge, 2022). They have proven to be expensive, nanogels sometimes collapse the structure formulated because of the strong interaction between the polymer used and the active pharmaceutical ingredient (Ghaywat et al., 2021). Certain nanocarriers have proven to have limitations such as liposomes, PLA-GLA polymer NPs are known for their large size and poor dispersion, dendrimers are known to cause toxicity and biopolymers are known for their hydrophilicity (Zhang et al., 2022).

2.9 Nanocarriers for treatment with 5-FU

The molecule 5-flourouracil has been used in the treatment of cancer for many years. Nanocarriers have demonstrated improved bioavailability *in vivo* and *in vitro*. The combination of nanoparticles and 5-FU molecule allows the ability of the drug to be delivered at various sites (De Oliviera et al ,2021. The combination is used for drugs to be delivered intravenous, orally, and topical delivery. The use of cytotoxic agent such as 5-flourouracil is associated with unwanted adverse effects (De Oliviera et al ,2021). Currently 5-FU is available as creams and lotions for topical use in certain cancers, 5-FU exerts its cytotoxic mechanisms through inhibition of thymidylate synthesis (Chandran et al.,2017). Nanocarriers of 5-Fu have several advantages that may be beneficial in treating breast cancer. The combination of 5-FU with solid nanoparticles gives a high dispersibility and decreased cost, extended release, and high entrapment (Chandran et al ,2017). The combination of 5-FU with chitosan has also been used in various applications, chitosan because of its low toxicity can be used in combination with 5-Fu for oral, venous and ocular uses (Chandran et al ,2017).

2.10 Current challenges for the treatment of breast cancer with 5-flourouracil

There are several side effects associated with drug treatment of 5-flourouracil, the non-specificity of 5-FU causes side effects such as skin irritation, flaking, pain and burning sensation. Treatment with 5-FU has also can also cause skin erosion which can lead to bacterial infections and sepsis (De Oliveira et al ,2021). Resistance, a short half-life and non-specificity are shortcomings that have been identified with the use of 5-FU in the treatment of cancer.

2.11 Nanogel drug delivery

The delivery of cancer drugs through a nanogel system can be achieved in three ways, namely diffusion, pH-responsiveness and chemosensitivity. Diffusion a drug release mechanism where a drug is physically encapsulated in the gel. The diffusion of the drug is dependent upon the sizes of the open spaces of the nanogel polymer networks to control steric interactions between the formulated drug and the polymer network (Botha et al., 2023). pH responsiveness occurs when there is de-swelling of the nanogel due to weakly basic or acidic groups in their structure because of pH (Botha et al., 2023). An important factor to consider with pH responsive is due to pH gradient that exists between normal cells and cancer cells. Thermosensitive nanogels incorporate polymeric networks that are sensitive to changes in temperature. Thermo-responsive polymers are sensitive to changes in temperatures, the polymers that exist in the nanogels are classified into a lower critical solution temperature (LCST) and upper critical solution temperature (UCST), this is because of a delicate balance between hydrophilic and hydrophobic sections of the polymer chains (Botha et al., 2023).

2.12 Therapeutic applications of nanogels in different disease states

The use of nanogels is increasingly growing as therapeutic agents for certain medical conditions, they are said to be a promising area of treatment for neurological conditions as they deliver oligonucleotides, this is ideal for the treatment of neurodegenerative illness (Zhang et al., 2022). Their ability to be encapsulated with a negatively charged oligonucleotide, creates a stable liquid electrolyte dispersion complex with a small size particle size that is ideal to cross the blood brain barrier. They are also used in transdermal drug delivery as their ideal porous characteristic and sustained drug delivery (Zhang et al., 2022). Another application is for their ability to overcome drawbacks associated with current conventional therapy is associated with a limited therapeutic window, toxic side effects and poor solubility which decrease the chances of a good prognosis with a disease as serious as cancer (Vashist et al., 2018).

An ideal feature of nanogels as a formulation is the ability to release intra- or extracellularly through pH sensitivity. Chitosan based nanogels are examples of ph. responsive nanogels that though external stimuli will release the loaded drug as desired (Shoge MO, 2022). Nanogels loaded with 5-FU are considered positively charged nanogels which through their amplified acidity in endosomes will result in the release of the drug from the nanogel system. The ability to encapsulate molecules such as gold and platinum make for a good drug delivery system as this enhances the biological impact of x-irradiation and delivery of the hydrophobic photosensitizer (Shoge MO, 2022). Other uses include, control of bleeding through proteins, the self-assembling properties of the micronized sacchachitin promotes wound healing, diabetes to release insulin by responding to aldohexose, vaginal drug delivery in treating infections and in ocular problems through Polyvinyl pyrrolidone – poly (acrylic acid) (PVP/PAAc) nanogel and nanogels have been used a s vehicle for the immunosuppressive drug mycophenolic acid (MPA) in ocular problems through Polyvinyl pyrrolidone – poly (acrylic acid) (PVP/PAAc) nanogel and nanogels have been used as vehicle for the immunosuppressive drug mycophenolic acid (MPA). (Shoge MO, 2022). Nanogels are usually developed from natural polymers of natural origin such as chitosan, hyaluronic acid, and alginate. The most common used polymer used for nanogel development is polyethylene glycol (PEG). Pegylated nanoparticles usually have a particle size of 20-200nm, this small particle size is ideal for size penetration (Ghaywat et al., 2021; Siafaka et al ,2023). The desired size of nanoparticles is between 20-200nm, factors such as surface charge and encapsulation allow for targeted delivery using conjugated ligand against breast cancer cells (Thakur et al., 2019).

Table 0-3 Table illustrating the applications of a nanogel (Yadav et al, 2017)

Targeted area	Mechanism of delivery	References
Transdermal Drug delivery	Nanogels may be used for delivery of drugs to the stratum corneum, transdermal drug delivery is ideal as it can penetrate drugs into their site of action as well as bypass first pass metabolism. Transdermal nanogels may be used as an anti-pyretic drug, the nanosized dispersion of the drug through the solvent emulsification method has shown porosity properties, stability, and sustained drug release. Aclofenac incorporated with Carbapol 940. .	(Yadav et al, 2017)
Diabetes	Oppositely charged nanoparticles are used to attract each other which keeps the gel phase and prevents the nanoparticles from separating once injected into the body.	(Yadav et al, 2017)
Anticancer therapy	Nanogels prepared by cross linking pf polyethyleneimine and PEG are known to be less toxic, chemotherapeutical medicine into the nanogel not solely will increase the bioavailability however additionally increased permeability and retention.	(Yadav et al, 2017)
Diagnostic and Imaging or	Properties such as high-water content, structural flexibility, fluid-like transport, biocompatibility, and biodegradability give nanogels an ability to probe various images an contrast media that can be used in diagnostics.	(Soni et al, 2016)
Neurological Conditions (CNS Drug delivery)	delivering oligonucleotides (ODN) to the brain for diseases such as Alzheimer's. There are limitations of (ODN) in neurological conditions because of their inability to penetrate the blood brain barrier and their rapid renal excretion. A nanogel crosslinked with polyethylene glycol and polyethyleneimine, was found to have the to have the ability to form a stable aqueous dispersion of polyelectrolyte. complex by charged of the drug.	(Yadav et al, 2017)
Vaginal drug delivery	Vaginal nanogel having antiretroviral medication could decrease the severity of HIV infection among ladies.	(Yadav et al, 2017)

2.13 Conclusion and future aspects of nanogels

An important aspect to consider when it comes to targeted breast cancer therapy is the in vivo and the in vitro stability of the dosage form. Nanogels have proven to be stable and are advantageous in treating breast cancer because when administered intravenously the gels are able to provide the necessary payload required to effectively treat cancer (Attama et al., 2022). The hydrogel component of the nanogel means a biocompatible drug delivery system that can evade the reticuloendothelial system and morph nuclear phagocytic system ensuring a longer circulation time (Attama et al., 2022). Existing cancer therapy has proven to be limited due to the homogenous treatment of approach.

Cancer is a heterogenous disease that has over the year has developed resistance to chemotherapy (Ali et al., 2022). Another limiting factor in cancer therapy is he non-specificity that results in damage to adjacent normal cells leading to unwanted side effects and toxicity (Ali et al., 2022). The ideal cancer agent would be multifactional by completely specific or tumour targeting to eliminate resistance and non-specificity (Ali et al, 2022). An approach is using stimuli that specifically targets drug release such as example is using an enhanced drug permeability (EPR) effect. Nanogels can respond to two kinds of stimuli, firstly intrinsic and endogenous stimuli and the second group is external stimuli such as temperature or ultrasound, stimuli sensitive nanogels would be ideal as future treatment for their ability to resist premature release of the drug (Ali et al., 2022).

Nanogels are gaining considerable interest in advanced drug delivery. The ability of nanogels to actively target tumour cells and reduce non specificity is ideal in ensuring a better prognosis for breast cancer worldwide. The prognosis of cancer is highly dependent of the agents used during treatment and with current growing resistance to conventional therapy which as previously proven to be effective against cancer, a new approach is ideal in decreasing mortality of breast cancer patients. Nanogels may be applied in different medical conditions such as skin conditions, pain management and brain therapy; however, cancer remains a huge area of concern and therefore advanced drug delivery platforms for cancer therapy should thoroughly be explored.

Chapter 3: Design of a copolymeric Nanogel Drug Delivery System for the treatment of Breast Cancer

3.1 Introduction

Breast carcinoma remains the second leading cause of death in females in Africa despite many years of research, discoveries, neoadjuvant and adjuvant systemic therapies. Past studies have shown that nearly 40% of all women with breast cancer will experience recurrence. Conventional chemotherapy such as radiation and coactive drugs are not target specific and result is systemic toxic effects. Traditional cancer therapy has been linked to side effects such as chronic toxicity which means that these effects are irreversible, those include alopecia, thrombocytopenia (affecting blood cells) and mucositis. Targeted drug delivery to specific tumour sites has proven to a beneficial as early treatment for breast carcinomas and has a higher patient tolerance as side effects will be introduced. Standard treatment approaches for breast cancers are surgery together with chemotherapy or radiotherapy. The return of localized breast Cancer after mastectomy is considered as being a poor prognostic predictor (Alshreeda et al., 2023).

The drug 5-flourouracil (5-FU) is widely used in the treatment of different kinds of cancers including breast carcinomas, 5-FU is an analogue of pyrimidine analogue which prevents tumour cell growth and has shown harness the host immune system to prevent cancer. This drud sensitises tumour cells to natural killer cells and CD8 driven cytotoxicity. 5-FU is converted to fluorodeoxyuridine monophosphate (FdUMP), which forms a stable complex with thymidylate synthase (TS), which inhibits the production of deoxythymidine monophosphate (dTMP). The dTMP analogue is essential for DNA replication and repair and its depletion thereof causes cytotoxicity (Zhang et al., 2008).

Nano emulsions are a formulation with a droplet size of a 100 nm. A nano emulsion is composed of an oil phase and a water phase with the addition of an emulsifier. The purpose of the emulsifier is to create small sized droplets decreasing the interfacial tension between the oil and the water phase. The interfacial tension can be described as the surface energy per unit area (Gupta et al., 2016). Nano emulsions have been proven to be a good vehicle for poorly aqueous soluble drugs which are usually difficult to formulate in conventional dosage forms (Ganta et al.,2014). The particle size and larger surface area of the nano emulsions allow for physical properties that have proven to be advantageous in overcoming anatomical and physiological barriers associated with drug delivery in cancer (Ganta et al.,2014).

Nanogels are formulated with natural, or synthetic polymers or a combination of natural and synthetic polymers (Sultan et al., 2021). Natural polymers are obtained from nature. Some examples of natural polymers used in nanogels include cellulose, chitosan, gelatin, pullulan, and hyaluronic acid. Natural polymers have desirable characteristics of being biodegradable, non-toxic, biocompatible, and abundant in nature. Chitosan is a biodegradable and biocompatible polysaccharide derived from chitin, that has shown extensively a promising nanovehicle for cancer therapy. Chitosan has benefits such as good dissolving properties, drug stability, and providing controlled-release drug control, with a multifunctional platform for targeting, stimulus-responsive release, or use in image-guided medicine (Herdiana et al., 2023).

Nanogels can lead to a high specific surface area because of the nanoscale size and consequently increase the solubility and pharmacokinetics of the conjugated drug molecules (Li et al., 2011). Nanogels can regulate the drug release and pharmacokinetic properties and have a great potential in drug delivery systems. Nanogels are tri dimensional networks of chemically or physically cross-linked polymers entrapping a large volume of water, hydrogels can be designed as nano sized particles combining the advantages of hydrogels and nanoparticles (Ou Bs et al., 2022). Localised cancer therapy is receiving significant attention in the treatment of breast cancer. The aim of localised cancer therapy is to increase bioavailability of the drug at the site of action while decreasing associated systemic side effects (Wolinsky et al., 2012). Polymer delivery vehicles includes sustained release drug delivery systems for implantation intra-tumorally or adjacent to the cancerous tissue, this type of technology has been embodied in a variety of form-factors such as drug-eluting films, gels, wafers, rods, and particles and feature predictable and prolonged drug release kinetic (Wolinksy et al, 2012). Hydrogels are proving to be effective due to their diverse characteristics such as biocompatibility, biodegradability, and flexibility (Alshareeda et al, 2023).

Nanogels are designed to have several properties at the target site that will allow active targeting of cancer cells, the onset and rate of drug release must be properly controlled. There are three major methods of achieving this: (1) drug release via gel degradation, (2) drug release via gel expansion and drug diffusion, and (3) drug release via the breakage of interactions between the drug and the nanogel. In addition, a nanogel can be designed to be either a programmed pulsed-release system (PPRS) or an intelligent pulsed-release system (IPRS). The release mode of a PPRS is achieved by the predetermined gel structure, with the lag time and duration of drug release being controlled by the polymer degradation profile (Li et al., 2021).

The purpose of this study was to synthesize a 5-FU loaded nanogel, consisting of a nano emulsion containing an aqueous phase using PEG 4000 in poly vinyl alcohol and a chitosan-based nanogel. 5-FU is a poorly soluble drug with potential toxic effects. The incorporation of 5-FU into a nanogel can decrease the unwanted side effects associated with chemotherapeutic agents, while affording a sustained release of the drug. Nanocarriers have unique properties such as high ratio of surface to volume, quantum effects and an ability to carry therapeutically active compounds to the targeted site due to their nano size (El-Sattar et al., 2022).

3.2 Materials and Methods

3.2.1 Materials and Equipment

The following chemicals were sourced and utilized throughout this study: 5-fluorouracil. (5-FU), polyvinyl alcohol, polyethylene glycol (PEG 4000), Acetone, Soybean, polysorbate 80 (Tween 80), Glacial acetic acid, distilled water, Polyvinyl Alcohol (PVA), water (dH_2O). All chemicals utilized were purchased from Sigma (Sigma-Aldrich, Missouri, USA). DSC [Mettler Toledo, DSC1, STARe Instrument (Schwerzenback, Switzerland)], FTIR [PerkinElmer Spectrum 100, Beaconsfield, United Kingdom], TGA [PerkinElmer TGA 4000, Beaconsfield, United Kingdom], Zeta sizer [Zeta-sizer Nano-ZS machine (Malvern Instruments, Worcestershire, United Kingdom).

3.2.2 Preparation of a 5-FU loaded Nanogel

Preparation of 5-FU loaded nanoemulsion

An amount of 500 mg of 5-FU and PEG 4000 was dissolved in 15ml of acetone at room temperature to make the organic solution, as illustrated in figure 3.1. The aqueous phase was prepared by dissolving 3 g of PVA solution in 100 ml boiled water, 95 °C using a magnetic stirrer to make a 3 % m/v PVA solution. The PEG -5-FU mixture was added dropwise to the PVA solution at room temperature to make the nanoparticle solution. The solution containing PEG400, 5-FU and PVA was homogenised at 10000 rpm for 2 minutes. A mixture of soybean and tween 80 were added at room temperature to make the nano emulsion (Marihno et al., 2020).

Preparation of a chitosan based nanogel.

A 1 % glacial acetic acid solution was prepared by dissolving 1 ml of Glacial acetic acid in 99 ml of water to make a 1% glacial acetic acid solution. 4 grams of chitosan was added to the glacial acetic for 2 hours at 6000 rpm to form a chitosan gel. The gel was subjected to sonification for 15 minutes to remove entrapped bubbles. The prepared nano emulsion was

added to the chitosan solution at room temperature under constant stirring, the mixture was then homogenised to give a viscous gel.

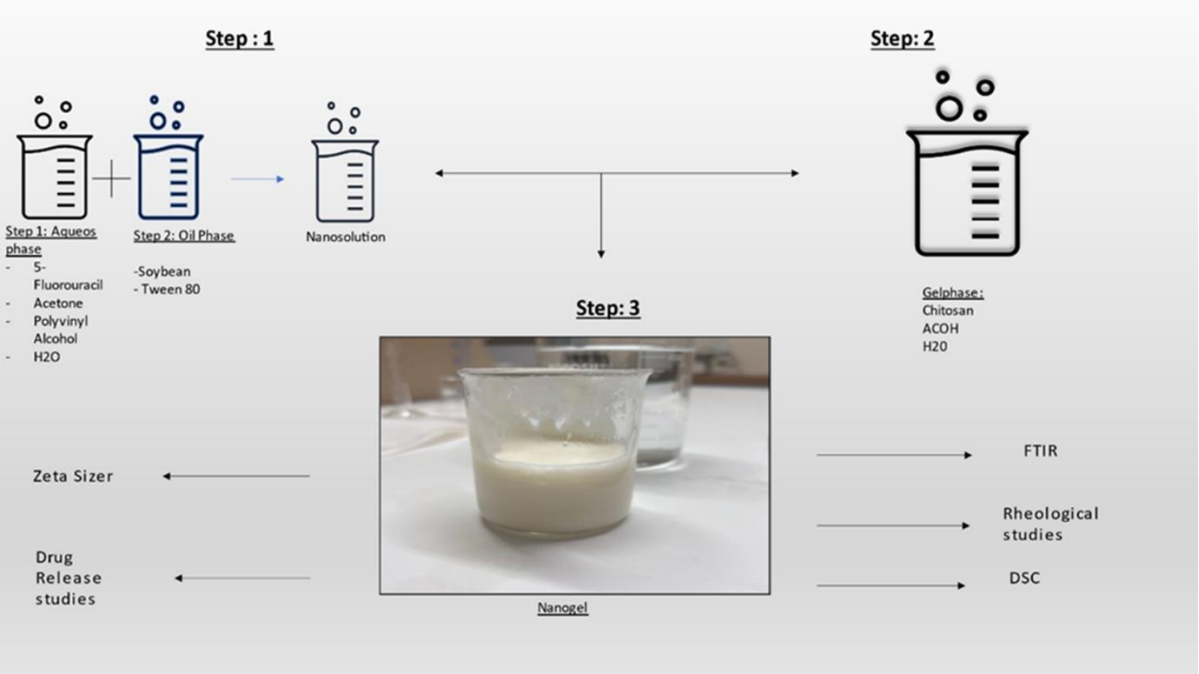

Figure 0-1 Illustration of the preparation of a 5-FU nanogel system, the figure demonstrates loading of 5-FU into a nano emulsion for loading into the hydrogel.

3.2.3 Determination of the size of the nanoparticles using Zetasizer

Partize size distribtuuion and PDI of nanoparticles are importatnt parameters in the evaluation of drug-loaded nanoparticles. PDI specifies the uniformity of nanoparticles where a PDI value of 0.1-0.25 indicates narrow size particles distribution and a PDI of 0.5 and above indicates a broader size of distribution. Determining the PDI and particle size of a formulation tells us how stable the formulation is , the lower the PDI and particle size the more stable a formulation is (Hoseini et al,2023). The particle size distribution of the 5-flourouracil nanoemulsion was determined using a Zetasizer NanoZs analyser (Malvern Instruments Ltd. UK). The measurements were carried out in triplicate. The samples were diluted and sonicated before measurement, with the sample temperature maintained at 25 °C throughout the analyses. A 2 ml sample of the nanoparticle suspension was filtered through 0.22 µm filter and placed in disposable cuvettes. Particle size, and zeta potential values were analysed.

3.2.4 Characterization using FTIR Spectroscopy

Fourier transform infrared spectroscopy (FTIR) was carried out to determine compatibility between the 5-flourouracil nanogel and the polymers (Figure 3-3), (Kamaraj et al., 2017). The peaks were analysed to confirm the molecular vibrations of key functional groups of the drug

and polymers in the formulations. The Perkin Elmer Spectrum 2000 FTIR Spectrometer was employed, applying a single-reflection diamond MIRTGS detector (Perkin Elmer Spectrum 100, Llantrisant, Wales, UK).

3.2.5 Rheological properties of 5-FU loaded nanogel

Rheological properties assist in looking at the deformation and flow materials or gelling properties. The rheological analysis of the 5-FU loaded nanogel gel, PEG 4000, 5-FU and Chitosan was carried out using the Elastosens Bio 2, Haake Modular advanced rheometer system (Thermo Fischer Scientific), using a cone plate geometry (rotor c35/1, Dmm, 1 titan. The nanogel was stored overnight in a freezer at 4 °C before the evaluation of the rheological properties. The rheological properties of the materials included 4 types of tests namely sheer stress, yield stress, sweep stress and thixotropy. The yield stress for the 5-FU nanogel was performed at 0.01-100 Pa. The thixotropy was analysed for all materials 0.01 and 100 Pa (Mndlovu et al., 2019)

3.2.6 In vitro drug release studies

To study the *in vitro* release studies 5-FU nano emulsion a PBS Buffer was prepared by using 3 PBS tablets in 600 ml distilled water at room temperature. The mixture was left on a stirrer at room temperature until all 3 tablets were completely dissolved. The PBS media were prepared by adding a 100 ml of PBS solution in a suitable glass container (Shang et al., 2022) The sample was prepared by placing 2 ml of the 5-FU loaded nanogel in a snakeskin membrane and 1 ml of the buffer solution. Each solution was placed in an orbital shaker for 1 hour at 50 rpm, the first reading was taken at 1 hour and then at 2-hour intervals. A volume of 3 ml was extracted from the solution and placed on a cuvette to measure the absorbance. 3 ml of the PBS solution was added to the solution to maintain the stock solution at the 1 set hour and the 2 hourly after the first reading. A UV Spectrophotometer was used to measure the absorbance at a wavelength of 254 nm (Yang et al., 2021).

3.3 Results and Discussion

3.3.1 Zeta size of the 5-FU loaded nanogel

A zetasizer Nano ZS instrument (Malvern Instruments (Pty) Ltd. was used to determine the particle size of the nano emulsion. The sample was sonicated at 80 % amplification for two minutes. An amount of 0.1 ml of the sample in 4 ml distilled water in to determine the particle size. Figure 3-2 illustrates the particle size and distribution of the nano emulsion, with an average particle size of 75 nm and a PDI of 0.464. A particle size of less than 100 nm indicates a small particle size that could be beneficial to the penetration of cancer cells. The PDI also indicates that the particles are uniform, and that the nano emulsion is stable. The PDI on the nano emulsion was 0.464 which indicates that the formulation was a good emulsion (Ziaei et al. 2023).

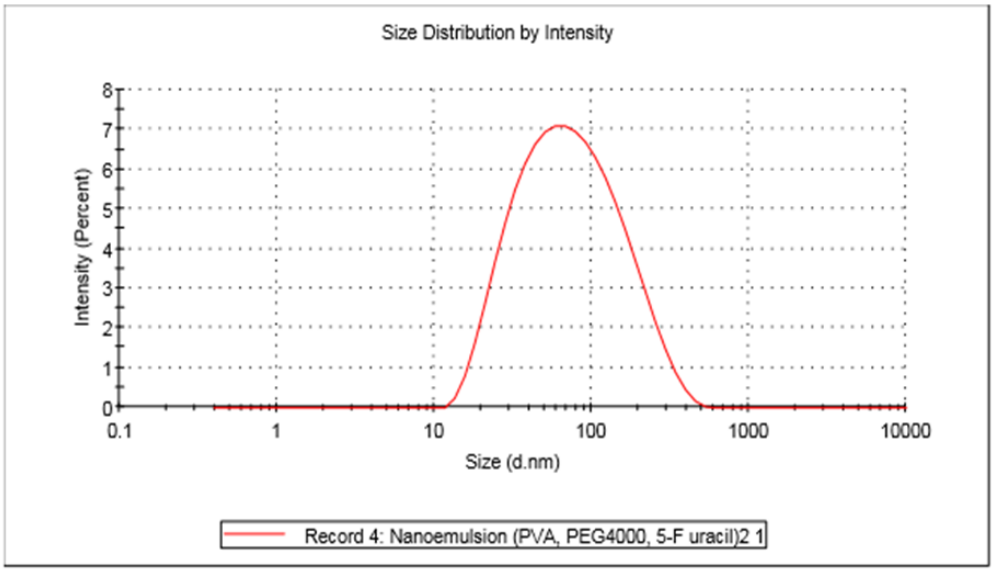

Figure 0-2 Illustration of the zetasizer size distribution of a 5-flourouracil nanogel

3.3.2 Characterization of 5-FU nanogel using FTIR Spectroscopy

FTIR was used to determine structural between the drugs and the polymers. (Kamaraj et al, 2017). The molecular composition and characteristics of the 5-FU nanogel and polymers was evaluated for its chemical characteristics. Peaks were analysed to confirm the molecular vibrations of the polymers in the formulations. The Perkin Elmer Spectrum 2000 FTIR Spectrometer was employed, applying a single-reflection diamond MIRTGS detector (Perkin Elmer Spectrum 100, Llantrisant, Wales, UK).

The 5-FU Nanogel gave rise to a peak at 3279.80 cm^{-1} indicating sharp and strong OH stretching and a hydrogen bonding (Naranjo et al.,2022), the second peak was observed at 2925.65 cm^{-1} indicating C-H stretching, the peak observed at 2858.7cm^{-1} indicated N-H strong stretching. A peak was observed at 1647 cm^{-1} indicating possible N-H_2 scissoring absorptions, while the peak absorbed at 1106.07 cm^{-1} indicated C-N vibrations and the signal at 1040.23

cm^{-1} showed C-O stretching. Signals at 984.28 showed C-C bending, the peak at 831 cm^{-1} showed C-H bending (Satapue et al., 2017). The results depicted in Figure 3-3 graphically illustrate the comparison of the drug loaded nanoemulsion against the blank (non-loaded) nano emulsion and the individual starting, materials 5-FU, Chitosan, PVA and PEG 4000 (Liu et al., 2016).

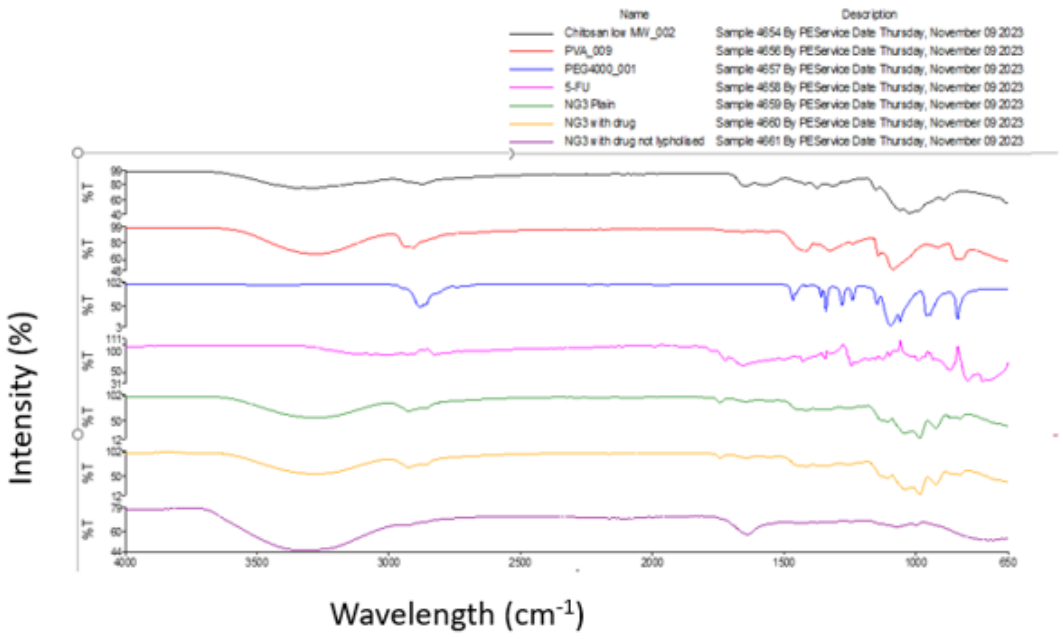

Figure 0-3 Illustration of FTIR Spectra for 5-flourouracil Nanogel, including FTIR for polymers PVA, PEG 4000 and chitosan.

3.3.3 Drug release sudies for the 5-FU nanoemulsion with the cumulative drug release calculated by the formula:

Cumulative drug release (%) = CiVi + ∑n i=1 (Ci− 1Vs) mt × 100.

Equation 1: Calculation of cumulative drug release

Ci is the concentration of 5-flououracil released from Nanogel at time t, Vi is the total volume of the release medium, Vs is the specified volume removed from the release medium, and mt is the total initial weight of the 5-flourouracil (Zaire et al., 2023). The calibration curve (Figure 3.4) was used to determine the amount of drug released. The coefficient determination, R^2, indicates how well the data points fit the line, as observed in, figure 3-4, is 0.9985 and confirms a positive linear relationship. A high coefficient determination indicates that the data obtained is accurate (Bewick et al., 2023).

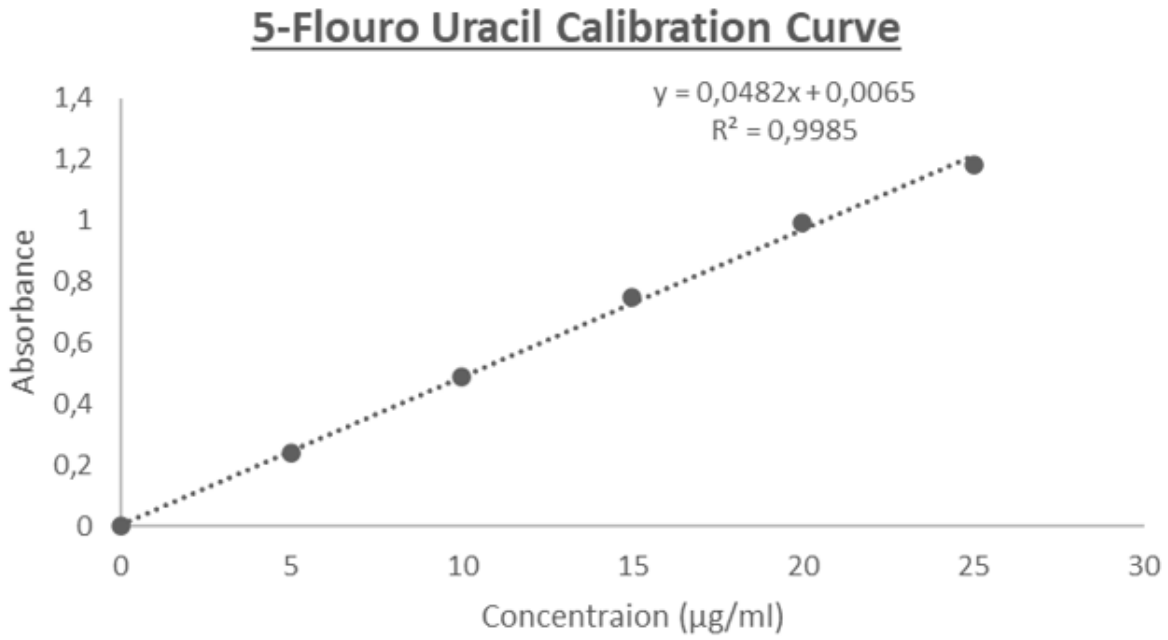

Figure 0-4 Illustration of the Calibration curve for 5-flourouracil.

3.3.4 In vitro drug release for 5-FU nanogel

The term drug release refers to the process where drug molecules move from their initial location within a membrane to their exterior surface. The drug release behaviour of the 5-FU nano emulsion was studied over 14 days. (Allaoui et al., 2023) The burst release behaviour of the nano emulsion can be due to the osmotic pressure difference between the concentration of 5-FU in the formulation and the release media (Ashna et al., 2020). The release is characterised by an incline with the highest percentage of 69 % reached at 10 hours, followed by sustained release up to 60 % for 14 days. According to studies, the defined categories of release are bolus release, which has a 30 min drug release, sustained release, is release observed over a period of four hours and controlled release. The third drug type of drug release is controlled release, in this type of release medication is delivered from a system by pore-forming erosive actions and diffusion. (Allaoui et al, 2023). The release behaviour the 5-FU nanogel is suggestive of a controlled release mechanism. The aim of the study was to formulate a drug system with controlled release to minimise the toxic side effects of 5-FU or typically any cancer treating drug. The observations of the drug release behaviour support the aim of the study. The drug could not be released up to a 100 percent due blockage by the release media. The constant release of 5-FU could be due to enhanced cross linking of the hydrogel network in the 5-FU loaded nanogel (Shan et al., 2020). The release behaviour of the 5-FU loaded nano emulsion is presented by two phases, the first phase is a major phase characterised by burst release that happened at the first 6 hours followed by a controlled

release phase that lasted for 14 days (Guo et al., 2016). The drug release is shown by figure 3.5.

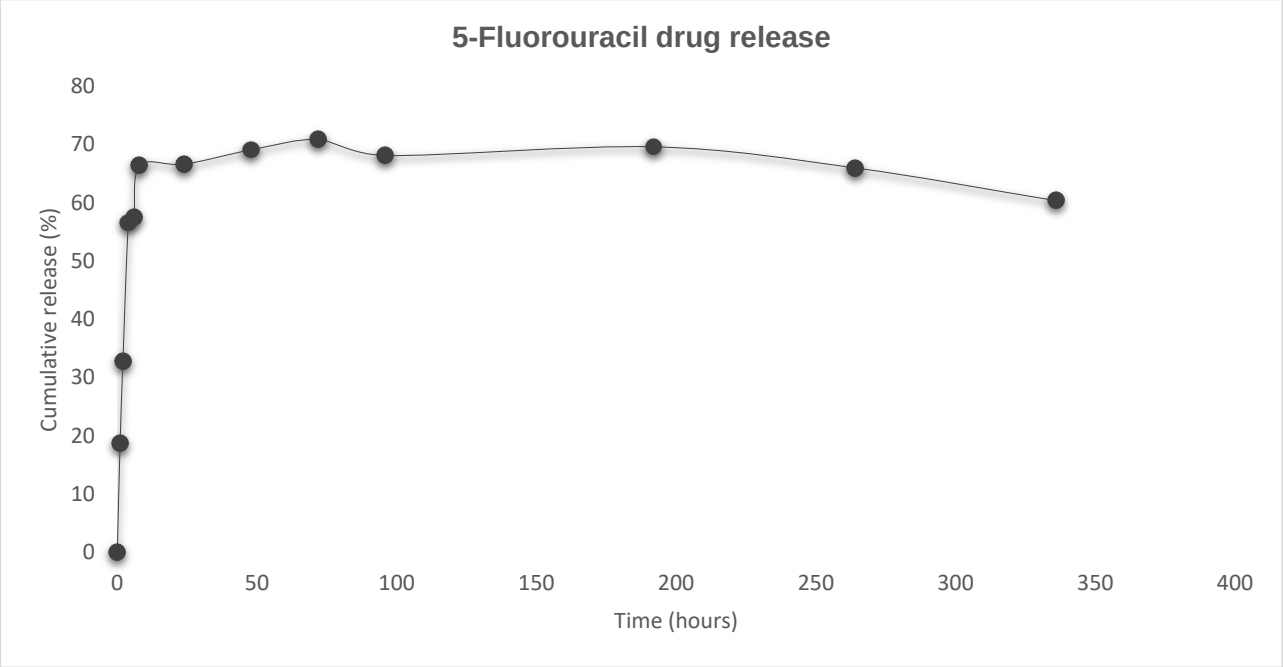

Figure 0-5 Cumulative release analysis of 5-flourouracil.

3.3.5 Rheological Study of the 5-FU loaded nanogel.

Rheology is the study of the flow and deformation behaviour of materials. The analysis of the rheological studies of the 5-FU nanoemulsion is crucial as the formulation that is intended to be developed in the study is an injectable, viscosity allows for assessment of the ability to deliver formulations as injectables, (Reid E., 2021). The rheological properties of PEG 4000, Chitosan, PVA and 5-flourauracil were investigated using the HAAKE MARS III Rheometer (Thermofisher Scientific, Rheology, USA equipped with a stainless-steel parallel plate (P35 TiL LI507) probe and 0.5mm gap, operated by Rheo Win Data management software (Munoz et al, 2010). The experiments were conducted sheer stress, sweep test, yield stress and thixotropy. Figure 3.6 (A) (illustrates) the stress sweep of the nanogel shows that G' is higher than G" which supports the gel properties that the gel is more elastic than it is viscous, figure 3.6 (B) shows an increase in both G' and G" is observed with applied frequency indicating a more rigid and polymeric shift network, the cross over frequency is also observed which indicates the ability of the material to form a reversible network (Stojkov et al.,2021). Figure 3.6 (C) illustrates the thixotropy of the 5-FU, an increase in enthalpy is seen and with a decrease viscosity over time, these properties confirm that the 5-FU loaded nanogel remails in gel state with increased stress (Zhou et al, 2022). Figure 3-6 (D) illustrates the yield stress of a the 5-FU nanogel, the viscosity of the nanogel decreases and plateaus with increased pressure which confirms plastic flow of the gel when under stress and confirms the structural strength of the gel (Dabbashi et al., 2021).

Figure 3-6 (A) 5-FU Nanogel stress sweep

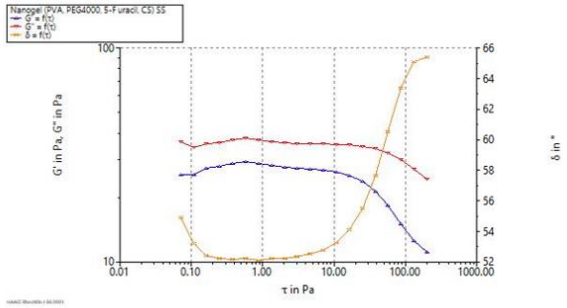

Figure 3-6 (C) 5-FU Thixotropy

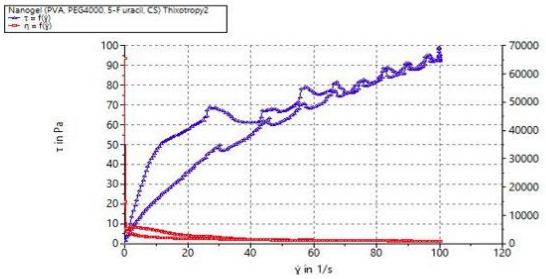

Figure 3-4 (B) 5-FU Sweep cross over

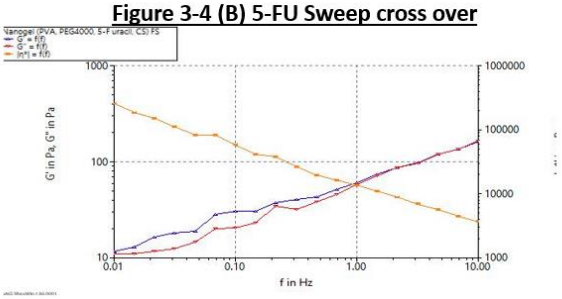

Figure 3-6 (D) 5-FU Yield stress

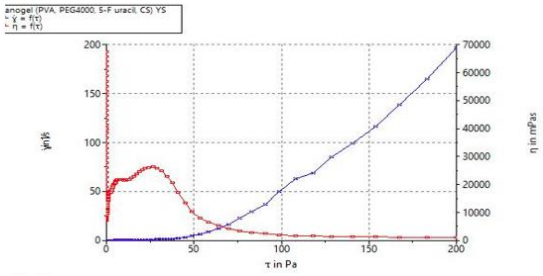

Figure 0-6 Illustration of 5-flourouracil loaded Rheology studies, (A) Nanogel stress sweep test , (B) Nanogel Sweep cross over Test, (C) Nanogel Thixotropy test , (D) Nanogel Yield stress test

3.3.6 Differential scanning calorimetry (DSC) analysis of 5-FU loaded nanogel.

The term calorimetry refers to a technique that measures the thermal characteristics of materials to determine the relationship between temperature and physical properties of a substance by measuring the change in the physical properties of a sample with change, along with temperature and time (Gill et al., 2010). In this, study was performed using the Perkin Elmer 4000. DSC in this study was used to examine the starting materials. The DSC thermogram of the 5-FU loaded nanogel showed a sharp peak at 190.31 °C and another peak at 231.99 °C, DSC thermographs of excipients showed peaks at 58.19 °C for PEG, 226.51 °C for PVA and 283.43 °C for 5-FU. No endothermic peak was observed for 5-FU in the drug loaded nanogel which indicates that the drug was incorporated into PEG 4000 (Padya et al,2023). The peaks around 190 °C indicated an endothermic reaction and the peak at 231 °C indicated an endothermic reaction by melting point of PVA (Gill et al, 2010). The peak observed on the PEG DSC graph coincides.

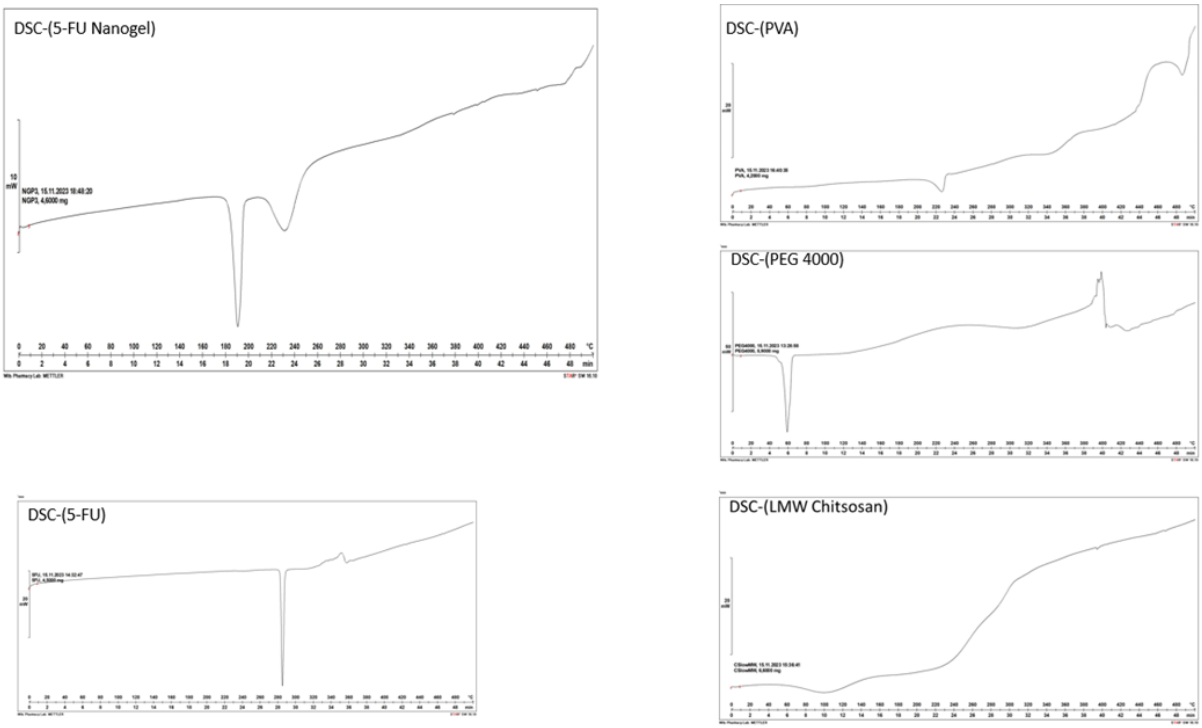

Figure 0-7 An illustration of thermal analysis for 5-flourouracil loaded nanogel.

Chapter 4: Conclusion and future recommendations

Breast cancer is a complex disease to treat due to the current challenges and resistance mechanisms associated with current conventional therapy. Nanogels are a promising drug delivery method not only for targeted therapy but also ensuring tolerance of anti-neoplastic and therefore better therapeutic outcome. This study evaluated the potential benefits associated with the treatment of breast cancer with nanogels to overcome factors such as insufficient specificity, lack of access to drugs at the site of metastasis, drug resistance at cellular and tumour microenvironment level (Afzal et al, 2021). The objective of the study was to improve bioavailability of the drug at the site of action while considerably reducing side effects associated with the use of chemotherapeutic agents, the aim was also to formulate a gel that would have sustained release. A 5-FU loaded nanogel was synthesized successfully through the preparation of a nano emulsion consisting of an aqueous phase consisting of 5 - FU, an PVA phase and a mixture of soybean and tween 80 to stabilize the solution. The nanogel depicted a narrow size distribution of less than 0.5 which indicates a good particle size distribution. The nanoparticles were 75 nm in size which is favourable in formulating. FTIR characterization confirmed the integration of 5-FU, chitosan, PVA.

5-FU Nanogel presented with sustained release at a two-week timeframe which would assist in the dosing of cancer which is 340-370-400 mg/m^2 given at a daily dose (Marini et al, 1985). The 5-FU loaded nanogel presented sustained release of 5-FU conventional 5-FU which has mean plasma half-life of only 15 minutes, with toxicity associated with frequent dosing the nanogel could be useful in breast cancer. The nanogel formulation protects 5-FU from rapid elimination and rapid distribution associated with the conventional dosing of the drug. A cumulative percentage release 60 % of the drug was released in two weeks, meaning that less of the drug molecule is degraded and free drug can circulate to ensure optimal therapeutic treatment. There are several disadvantages associated with 5-FU in the treatment of breast cancer such as resistance therefore new and innovative ways of administering the drug should be explored as it has been proven to be effective against cancer for many years. The prognosis of cancer is also dependent on patient tolerability of the side of the drugs which include serious GI effects, fatigue, and fertility problems. The formulation of 5-FU to a nanogel could have better tolerance as with higher doses free drug circulates leading to chances of experiencing uncomfortable side effects. The pharmacokinetic profile of 5-FU will be greatly improved by the formulation as the local delivery method prevents expected non-linear and erratic kinetics. 5-FU is associated with lower clearance at higher doses so therefore for consideration of extended-release higher doses can still be eliminated while minimizing side effects.

The drug release of the nanogel showed a promising feature of nanogels that needs to be studied further, it would be ideal to have a drug that release up to 100 % in two weeks and so that patients can get the drug every 14 days. Polymers which provide further extended release should be studied, therapeutic levels of the drug over a period of 14 days should be studied. A local drug is ideal as it treats cancer at the area of target therefore awareness should be prioritised in the early treatment of breast cancer, the drawback such as costs of testing for BRCA 1 and 2 genes will limit such awareness initiatives but investments and area of focus should be prioritized worldwide due to the high rates of cancer deaths and poor prognosis due to lack of access to healthcare in undeveloped countries.

The area of nanogel therapeutics could be a possible solution to alleviate adverse events associated with 5-flourouracil, there are limited studies or information of such gels. Studies and focus should be put into looking at localized treatment with a drug that has been proven to work such as 5-flourouracil and alternative ways such as a nanogel system to ensure better patient compliance and a possible better outcome of treatment.

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