Fabrication of Biodegradable Implantable Devices for Sustained Localized Drug release

A Thesis

Submitted to the Graduate Faculty

In partial Fulfillment of the requirements for the award of

Master of Science in

Materials Science and Engineering

African University of Science and Technology, Abuja- Nigeria

By

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2016

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DEDICATION

This work is dedicated to the memory of those whose precious lives were cut short by cancer:

Prof. Dora Akuyuli, Steve Jobs, Bob Marley, and so on. Your memory lives on.

ABSTRACT

This thesis is focused on fabrication of biodegradable implantable devices for extended localized drug release. Paclitaxel (PT) was used as cancer drug in the study. Poly lactic –co- glycolic acid (PLGA) is a polymer used for drug elution. In this work, the role of enzymes on the degradation of PLGA, Effect of different pH on the degradation of PLGA and the kinetics of drug release was elucidated. PLGA ratios of 75:25 and 85:15 were used. The enzyme used in the study is lipase enzyme. The pH used was 4.0, 6.0, 6.5, 7.0 and 7.4. From the study, it was observed that lipase enzyme increased the rate of polymer degradation and thus the rate of drug release from PLGA. This experiment also shows that PLGA degrades faster in acidic medium. This also caused the kinetics of drug release to be higher in acidic medium than in alkaline or neutral medium.

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Chapter One

1.0 Introduction

In 2008, the World Health Organization (WHO) estimated all global deaths arising from cancer to be up to 84 million (WHO, 2008). In recent years, the increasing incidence of cancer has been associated with high cancer mortality rates across the globe (WHO, 2014). It was also reported that the different types of cancer causes more deaths than those due to HIV/AIDS, tuberculosis and malaria all combined (WHO, 2014). In any case, early detection and improved treatment are crucial for a successful management of cancer (Anand P *et al*., 2008). However, it is difficult to detect breast cancer at the early stages. This causes late detection and reduces the chances of effective treatments especially for cases in which the metastatic stage, before detection.

Furthermore, the current cancer treatment methods such as bulk systemic chemotherapy (American Cancer Society (ACS), 2013; Kushi *et al*., 2012; Parkin *et al*., 2011; WHO, 2014) and radiotherapy (Gotzsche and Jorgensen, 2013; National Cancer Institute (NCI), 2014) have severe side effects. Such severe side effects can be reduced by a sustained and controlled release of cancer drugs into regions containing cancer cells/tissue (NCI, 2014; WHO 2014). There is, therefore, a strong interest in the localized delivery of cancer drugs from implantable drug delivery systems (NCI, 2014; WHO 2014WHO, 2014; Dubas and Ingraffea, 2013). Recent work focused on the development of implantable non-resolvable systems for cancer drug delivery (ACS, 2014). However, such systems remain in the body, or require surgical removal, after drug release. Hence there is a need for resorbable structures for the controlled release of cancer drugs (Cakir *et al*., 2012; Jemal *et al*., 2011; ACS, 2013; WHO, 2014) to tumor regions. Such resorbable structures have been studied over the past decade (ACS, 2014), using biodegradable polymers that facilitate the controlled release of cancer drugs. These include polymers, such as

poly (lactic-acid) (PLA) and poly(glycolic-acid) (PGA), and their copolymers (PLGA)

Biodegradable microparticles have also been formulated from PLA or PLGA for controlled drug release (National Cancer Institute, NCI, 2013). PLA or PLGA have also been shown to be biocompatible and biodegradable (NCI 2013; Hanahan and Weinberg, 2000). Furthermore by altering their molecular weight, sample size and surface morphologies (Hanahan and Weinberg, 2011) well-defined degradation rates can be achieved and used to control the release of encapsulated therapeutic agents. This will be explored in the current study of minirods of PLGA that encapsulate PT.The degradation and drug release kinetics are studied using a combination of optical microscopy and UV-Vis spectrophotometry. The implications of the results are also discussed for the development of resorbable/implantable devices for multipulse cancer drug delivery

1.1 Motivation

The use of biodegradable systems are proposed to overcome localized tumours which may lead to the down regulation of receptors or the development of tolerance, while sparing the host tissue from harmful drug concentrations (Jeong and Gutowska., 2002; Soppimath *et al*, 2002). Such systems would not require any surgical removal once the drug supply is exhausted. The fabrication techniques would involve solvent casting molding of polymeric materials with drug encapsulation. Solvent casting molding method has the advantages of controlling the porosity of samples (scaffolds) (Jeong and Gutowska., 2002) and it is also recommended for heat sensitive drugs (Rabin *et al*., 2008). Moreover, it is simple to fabricate devices at room temperature as compared to other methods such as compressing molding/melting molding (Yang *et al*., 2009).

1.2 The goal of the project

The major goal of this thesis is the fabrication of biodegradable devices to achieve cancer drug delivery for an extended time.

1.3 The Specific Objectives

In order to achieve the above goal, the research would be carried out in the following steps:

- Fabricate drug loaded PLGA-based minirods using solvent casting method by applying slight compression.
- Study drug encapsulation procedures of PLGA-based minirods for controlled and sustained drug release.
- Study the kinetics of drug release
- Studied the degradation mechanisms of PLGA minirods: photodegradation, chemical degradation (biological), thermal degradation (DSC), weight loss experiments in a phosphate buffer saline solution with incubation conditions such as pH 7.4, 37˚C, 60 rpm and carry out FTIR analysis.
- Study the activities of enzymes in biodegradation and drug release.
- Study the effect of different pH on the degradation and the kinetics of drug release.
- Discuss the implications of the result for the fabrication of a novel implantable biomedical device for an extended localized breast cancer drug delivery.

1.4 The Scope of the work

Chapter one of the thesis presents a brief statement of the subject matter relating to cancer incidence and challenges.

In addition, the motivation behind the new idea, the goal of project and specific objectives are part of the discussion in chapter one.

The chapter two of the work presents the literature survey on existing issues relating to cancer statistics, possible solutions based on conventional treatments methods, current status in control drug delivery and theories on drug release kinetics.

Moreover, the materials and methods section are presented in chapter three. Detailed materials used and experiments conducted are properly reported with enough details for reproducibility. Methods of characterizations conducted are also reported in this chapter.

The results from the study are clearly discussed under chapter four. Implications of the result are clearly discussed for the development of a multi-layered degradable device for localized breast cancer drug delivery.

Recommendations and conclusions on the current work are presented in chapter five of the thesis.

Chapter Two

2.0 Literature Review

2.1 Cancer and Tumor Types

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body (WHO 2014, NCI 2014). Tumors are either malignant or benign. Benign tumors are not cancerous; they do not spread to other parts of the body. They can often be removed, and in most cases, they do not come back (NCI 2014). Malignant tumors are cancerous and are made up of cells that grow out of control. The cells in these tumors can invade nearby tissues and spread to other parts of the body. They are known as metastasis or secondary cancer (Wilkins *et al*, 2009). The possible signs and symptoms associated with cancer include new lump, abnormal bleeding, prolonged cough, unexplained weight loss, change in bowel movement (NCI 2014). Though these symptoms indicate cancer, they may also occur due to other issues (WHO 2014). There are over 100 different known cancers that affect humans (NCI 2014).

2.2 Recent Development in Drug Delivery

Danyuo *et al* (2015) used poly (N-isopropylacrylamide) (PNIPA) based gels encapsulated in poly-di-methyl-siloxane (PDMS) packages to deliver localized heating (hyperthermia) and controlled concentrations of prodigiosin. The drug release rates were found to be governed by an earlier time approximation, while the diffusion rates of drug molecules were strongly influenced by temperature. However, drug released from the current study was limited by extended duration for a complete treatment of cancer.

Liu *et al* (2008) showed that paclitaxel,a cancer drug, conjugated to branched polyethylene glycol chains on Single walled Carbon Nanotubes (SWCNT) via a cleavable ester bond, has higher efficacy in suppressing tumor growth than clinical Taxol in a murine 4T1 breast cancer.

Their studies showed that due to prolonged blood circulation, paclitaxel uptake when delivered by Single walled Carbon Nanotubes (SWCNT) is 10 fold higher because of enhanced permeability and retention than when paxitaxel is delivered alone. They showed that nanotube drug delivery is promising for high treatment efficacy and minimum side effect with low drug doses. This work is motivated by using PLGA-based minirods loaded with controlled concentration of paclitaxel, to study the possibility of developing cancer treatment with sustain drug release.

Wong *et al* (2013) showed that carbon nanotubes CNTs can also be used as either the main carrier or adjunct material for delivery of various non anti-cancer drug. In- vivo and in-vitro results from functionalized carbon nanotubes (f-CNTs) are promising for the development of unique delivery systems of anticancer drugs (Lay *et al*, 2011). Functionalization of CNTs involves drug loading of carbon nanotubes using covalent attachment and/or other physical approach. However, the mechanism of cell uptake of CNT is not well defined.

In vivo analysis using a human breast cancer xenograft mice model confirmed improved drug efficacy with conjugated lipid molecule of PT loaded onto CNTs by a hydrophobic interaction (Shao W *et al* ., 2013) . The targeted SWNT-lipid-PT was found to be non-toxic following biochemical analysis of blood samples and the histological analysis of major organs.

Hampel *et al* (2008) showed that CNTs are feasible carrier for carbonplatin, a therapeutic agent for cancer treatment. This research showed the potential of CNTs in drug delivery.

2.3 Cancer Statistics

Tobacco use caused 22% of cancer death (WHO, 2014), while 10% of cancer death is due to obesity, poor diet, lack of physical activity, and consumption of alcohol (WHO 2014, NCI 2014).

Other factors include certain infections, exposure to ionization, and environmental pollutants (Anand P, 2008). In the developing world, nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C, and human papillomavirus (HPV) (WHO 2014).

Approximately 5 – 10% of cancers are due to genetic defects inherited from parents.(ACS, 2013). Cancer can be detected by certain signs and symptoms or screening tests (which includes blood tests, Urine tests), biopsy or medical imaging (WHO 2014)

Cancer is often treated with some combination of radiotherapy, surgery, chemotherapy and targeted therapy (WHO, 2014; NCI, 2014). Pain and symptom management are important part of the care. Palliative care is given to those with advanced diseases (WHO, 2014). The extent of survival depends on the type of cancer and the extent of diseases at the start of treatment (WHO, 2014).

In 2012, about 14.1 million new cases of cancer occurred globally (not including skin cancer other than melanoma) (WHO, 2014). This caused about 8.2 million deaths (14.6% of all human death). If skin cancer other than melanoma were included in total new cases each year, it would account for around 40% of cases (Dubas and Ingraffea, 2013; Cakir *et al*, 2012). Lymphoblastic leukaemia, brain tumors and non-Hodgkin lymphoma (for Africans) are most common in children (WHO, 2014). The risk of cancer increases significantly with age and cancers are more common in developed countries (WHO, 2014).

2.4 Malignant Cells

Cancer is a type of neoplasm. A neoplasm (figure 2.1) is a group of cells that have undergone unregulated growth which often forms lump that may be distributed diffusely (WHO, 2014; NCI, 2013). The six characteristics that cancer cells needs to produce malignant tumor (known as the six hallmarks of cancer) include (Hanahan and Weinberg, 2000)

- Cell growth and division without proper signal to do so
- Continuous growth and division even when there are signals telling them to stop
- Avoidance of programmed cell death
- Limitless number of cell divisions
- Promoting blood vessel construction
- Invasion of tissues and formation of metastases (Hanahan *et al*., 2000)

The progression from normal cell to cells that can form detectable mass and then to cancer involves multiple steps known as malignant progression (Hanahan *et al*., 2000; 2011).

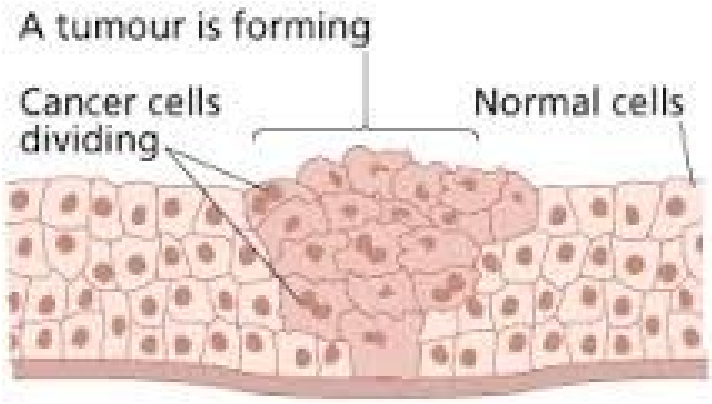


Figure 2.1 Cancer cell mutation Source: www.drfadi.org

2.5 Signs and Symptoms of Cancer

The symptoms of cancer (fig 2.2) are grouped into localized, systemic and metastasis

2.5.1 Localized Symptoms

The following symptom occurs due to the presence of the mass of tumor at locations where it is found. The symptom includes:

(i) Lump from lung cancer cause blockage of the bronchus resulting in cough or pneumonia.

(ii) Mass effect from oesophageal cancer can cause narrowing of the oesophagus, making it difficult or painful to swallow.

(iii) Lumps from colorectal cancer may lead to narrowing or blockages in the bowel.

(iv) Mass in breasts or testicles causes discomfort as the can easily be felt.

(v) Ulceration causes bleeding. If it occurs in the lung, it will lead to coughing of blood. If it occurs in bladder, it will lead to the presence of blood in the urine. Some cancer causes a build up of fluid within the chest or abdomen (Hanahan *et al*., 2000).

2.5.2 Systemic Symptoms

These are symptoms that result from distant effects of cancer. They include unintentional weight loss, fever, being excessive tired. (O'Dell and Micheal, 2009)

2.5.3 Metastasis Symptoms

These occurs when cancer spread from its original site by to lymph nodes or by blood (**haematogenous spread**) to distant sites. The symptoms depend on the location of the tumor.

They include enlarged lymph nodes, enlarged liver or enlarged spleen (can be felt in the abdomen) pain or fracture of the affected bone and neurological symptoms (Hanahan *et al*., 2000).

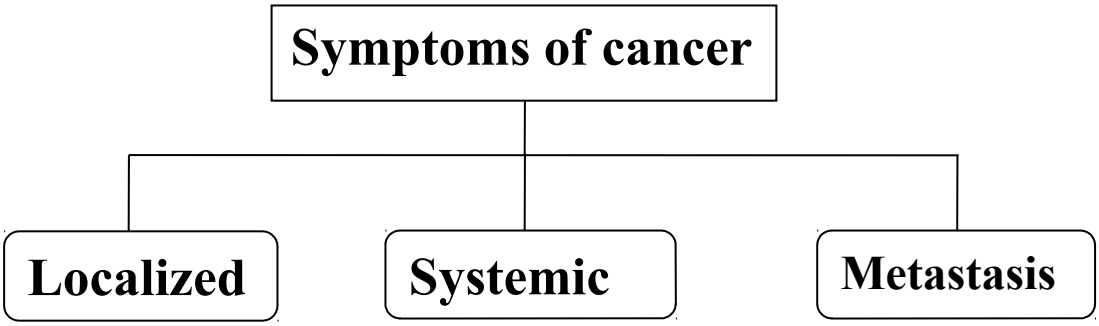


Figure 2.2: Schematics illustrating the different symptoms

2.6 Causes of Cancer

The real cause of cancer is not well understood due to cancer incidence emanating from different sources. However, cancer can be caused by environmental factors (90-95% cases) and inherited genetics (Anand P *et al*., 2008). The common environmental factors include tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), Alcohol (Kravchenko J. *et al*, 2009), radiation (both ionizing and non-ionizing up to 10%), stress, lack of physical activity and environmental pollutants (Anand P *et al*., 2008).Hereditary cancers are primarily caused by an inherited genetic defect. For example, certain inherited mutations in the genes BRCA 1and BRCA 2 posse 75% risk of breast cancer and ovarian cancer (Biesalski *et al*, 1998).

2.7 Classification of Cancer

Cancers are classified by the type of cell that the tumor cells originate from. These include:

2.7.1 Carcinoma

These are cancers derived from epithelial cells. This group includes many of the most common cancers, particularly in the aged. Examples include nearly all those cancer developing in the breast, prostate, lung, pan crease, and colon (Strebhardt and Ullrich, 2008)

2.7.2 Sarcoma:

These are cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develops from cells originating from mesenchymal cells outside the bone marrow (Waldmann, 2003).

2.7.3 Lymphoma and leukemia

These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively. Leukemia is the most common type of cancer in children accounting for about 30% (Varrichchio and Claudette, 2004)

2.7.4 Germ cell tumor

These are cancers derived from pluripotent cells, most often present in the testicle or the ovary (seminoma and dysgerminoma, respectively) (Danaei *et al*, 2005)

2.7.5 Blastoma

These are cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults (Danaei *et al*, 2005).

Cancers are usually named using *-carcinoma*, *-sarcoma* or *-blastoma* as a suffix, with the Latin or Greek word for the organ or tissue of origin as the root. For example, cancers of the liver parenchyma arising from malignant epithelial cells is called *hepatocarcinoma* (Wicki and Hagmann, 2011), while a malignancy arising from primitive liver precursor cells is called a hepatoblastoma (Wicki and Hagmann, 2011), and a cancer arising from fat cells is called a *liposarcoma* (Rothwell *et al*, 2011).

2.8 Prevention of cancer

Cancer prevention is the means of providing active measures to decrease the risk of cancer (Mayo Clinic, 2008). The vast majority of cancer cases are due to environmental risk factors, and many, but not all, of these environmental factors are controllable lifestyle choices. Thus, cancer is considered a largely preventable disease (Danaei *et al*, 2005). Between 70% and 90% of common cancers are due to environmental factors and therefore possibly preventable (Wu *et al*, 2015).

Greater than 30% of cancer deaths could be prevented by avoiding risk factors including: tobacco, overweight / obesity, an insufficient diet, physical inactivity, alcohol, sexually transmitted infections, and air pollution (WHO, 2011). Not all environmental causes are controllable, such as naturally occurring background radiation, and other cases of cancer are caused through hereditary and genetic disorders in which case prevention may become inevitable.

2.8.1 Use of Diet

Dietary recommendations for cancer prevention typically include an emphasis on vegetables, fruit, whole grains, fish, and avoidance of processed and red meat (beef and pork), animal fats, and refined carbohydrates (Kushi *et al*, 2012; Tobio *et al*, 1998).

2.8.2 Medication.

Cancer medication involves the use of drugs to prevent cancer. Some medication has been found to reduce the risk associated with cancer. They include Aspirin, (Rothwell *et al*, 2011), tamoxifen (Thomsen and Kolesar, 2008) paclitaxel (American Society of Health-System Pharmacists, 2015), prodigiosin (Brito *et al* 2008), doxorubicin (Bray *et al*., 2012)

2.8.3 Vaccines.

Some vaccines have been developed to prevent infections by some carcinogenic viruses. Human papillomavirus vaccine decreases the risk of developing cervical cancer (NCI, 2008). The hepatitis B vaccine prevents infections with hepatitis B viruses and thus decreases the risk of liver cancer (NCI, 2008).

2.9 Cancer Treatment

There are various treatment methods available for cancer. This includes Surgery (Subotic *et al*, 2012), Chemotherapy (Perry, 2011), Radiotherapy (Wilkins, 2009), Immunotherapy (Perry, 2011), Targeted Therapy (Cappellani *et al*, 2012) and palliative care (Hill *et al*, 2014). The type of treatment used depends on the type, location, grade of cancer, Patient‘s health and wish. Treatment may be given with or without curative intent.

2.9.1 Chemotherapy

Chemotherapy treatment uses chemical substances such as anti-neoplastic or anticancer drug which are cytotoxic in nature. Chemotherapy acts by killing rapidly dividing cells (cancerous cells). They also harm cells that divide rapidly under normal circumstances for example cells in the bone marrow, cells in digestive tract, and hair follicles are usually affected during chemotherapy treatment (NCI, 2012). This results in the most common side effect of chemotherapy: myelosuppression (decreased production of red blood cells, hence immunosuppression), mucositis (inflammation of the lining of the digestive tract), and hair loss (alopecia) (Linda, 2008). Chemothrapy is used on breast cancer, colorectal cancer, pancreatic cancer and so on (Subotic, 2012).

2.9.2 Targeted therapies

Targeted therapy is a form of chemotherapy that targets specific molecular differences between cancer and normal cells (NCI, 2012).For example the first targeted therapies blocked the estogen receptor molecule, thereby inhibiting the growth of breast cancer. Another example is the use of a class of Bcr-Abl inhibitors to treat chronic myelogenous leukaemia (CML) (NCI, 2012). It has a significant impact in the treatment of some types of cancer, and is currently a very active research area.

2.9.3 Radiation

Radiation therapy (Radiotherapy) involves the use of ionizing radiation in an attempt to either cure or improve the symptoms of cancer (Wilkins, 2009). It works by damaging the

Deoxyriboucleic acid (DNA) of cancerous tissue leading to cellular death.As with chemotherapy, different cancers respond differently to radiation therapy (Wilkins, 2009).

Radiation uses low energy x-rays to treat skin cancers while higher energy x-ray beams are used in the treatment of cancers within the body (Hill *et al*, 2014). Radiation can be used alone or typically used in combination to surgery and or chemotherapy for certain types of cancer, such as early head and neck cancer (Wilkins, 2009). For painful bone metastasis, radiation has been found to be effective in about 70% of people (Wilkins, 2009).

2.9.4 Surgery

Surgery is the primary method of treatment for most isolated solid cancers and may play a role in palliation and prolongation of survival (Cappellani *et al*, 2012). It is typically an important part of making the definitive diagnosis and staging the tumor as biopsies are usually required. Localized cancer surgery typically attempts to remove the entire mass along with, in certain cases, the lymph nodes in the area. For some types of cancer this is all that is needed to eliminate the cancer (Cappellani *et al*, 2012)

2.9.5 Palliative Care

Palliative care refers to treatment that attempts to make the person feel better and may or may not be combined with an attempt to treat the cancer. Palliative care includes action to reduce the physical, emotional, spiritual, and psycho-social distress experienced by people with cancer. Unlike treatment that is aimed at directly killing cancer cells, the primary goal of palliative care is to improve the person's quality of life (ACS, 2012).

People at all stages of cancer treatment should have some kind of palliative care to provide comfort. In some cases, medical specialty professional organisations recommend that people and physicians respond to cancer only with palliative care and not with cure-directed therapy (ACS, 2012).Thus, people with low performance status (showing no strong evidence that treatment would be effective), and have limited ability to care for themselves and people who received no benefit from prior treatments. Palliative care is used only when people approach end of life (ACS, 2012).

2.9.6 Immunotherapy

Immunotherapy is the use of the immune system to treat cancer. These immunotherapeutic approaches exploit the fact that cancer cells often have subtly different molecules on their surface that can be detected by the immune system known as cancer antigens. Cancer antigens are often proteins or other macromolecules (e.g. carbohydrates) (Couzin- Frankel, 2013). Immunotherapy is categorized as active, passive, or combinatory (active and passive) (Strebhardt and Ullrich, 2008). Active immunotherapy is used to provoke the immune system into attacking the tumor cells by targeting tumour-associated antigens (TAAs) (Strebhardt and Ullrich, 2008). Passive immunotherapies are intrinsically functional and include monoclonal antibodies, lymphocytes, and cytokines (Strebhardt and Ullrich, 2008). Among these, antibody therapies are the most successful to date and treat a wide range of cancers (Waldmann, 2003).

2.10 Biodegradable polymers

Biodegradable materials are materials that degrade *in vivo* by enzymatic and non enzymatic means, to produce biocompatible, toxicologically safe by – products which are further eliminated normal metabolic pathways. The basic category of biomaterials used in drug delivery

can broadly be classified into **synthetic biodegradable polymers**, which includes relatively hydrophobic materials such as the α - hydroxyl acids (e.g PLGA) and polyanhydrides (Bouissou *et al*, 2006), and **naturally occurring polymers (**Jain, 2000) such as complex sugars (hyaluronan, chitosan) and inorganics (hydroxyapatite) (Uhrich *et al*, 1999; Nair and Laurencin, 2007; Anderson and Shive, 1997). Biocompatibility of materials is not an intrinsic property of a material, but it depends on the biological environment and the tolerability that exits with respect to specific drug-polymer interactions (Anderson and Shive, 1997).

2.11 Poly Lactic-co-Glycolic Acid (PLGA)

PLGA is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA). It is the best defined biomaterial available for drug delivery with respect to design and performance. Poly lactic acid contains an asymmetric α-carbon which is typically described as the Dextro (D) or Laevo (L) form in classical stereochemical terms and sometimes as R and S form, respectively. The enantiomeric forms of the polymer PLA are poly D-lactic acid (PDLA) and poly L-lactic acid (PLLA). PLGA is generally an acronym for poly D, L-lactic-co-glycolic acid where D- and L- lactic acid forms are in equal ratio (Mohamed and Van der Walle, 2008).

2.11.1 Physco-Chemical Properties of PLGA

A good understanding of the physical, chemical and biological properties of PLGA is needed for a better design a better controlled drug delivery device. The physicochemical properties of optically active PDLA and PLLA are nearly the same. In general, PLA can be made in highly crystalline form (PLLA) or completely amorphous (PDLA) due to disordered polymer chains. PGA does not have methyl side group. Thus PGA shows highly crystalline structure in contrast to PLA. PLGA can be processed into almost any shape and size, and can encapsulate molecules

of virtually any size. It is soluble in wide range of common solvents including chlorinated solvents, tetrahydofuran, acetone or ethyl acetate (Uhrich *et al*, 1999; Nair and Laurencin , 2007).

PLGA biodegrades by hydrolysis of its ester linkages (Figure 2.3) when present in water. Presence of methyl side groups in PLA makes it more hydrophobic than PGA and hence lactide rich PLGA copolymers are less hydrophilic, absorb less water and subsequently degrade more slowly. Parameters, that are considered invariant for a solid formulation, change with time for PLGA due to hydrolysis. These parameters include transition temperature (Tg), moisture content and molecular weight. The effect of these polymer properties on the rate of drug release from biodegradable polymeric matrices has been widely studied. The change in PLGA properties during polymer biodegradation influences the release and degradation rates of incorporated drug molecules. The physical properties of PLGA depends on the initial molecular weight, the ratio of lactide to glycolide, the size of fabricated device, exposure to water (surface shape) and storage temperature (Houchin and Topp, 2009). The mechanical strength of PLGA depends on the molecular weight of the polymer and the polydispersity index. The physical and mechanical properties of PLGA also affect its ability to be formulated as a drug delivery device and may control the device degradation rate and hydrolysis. Studies have shown that the type of drug also affect the release rate of the drug (Siegel *et al*, 2006). The degree of crystallinity of PLGA influences the mechanical strength, swelling behaviour, capacity to control hydrolysis and biodegradation rate. The crystallinity of polymer depends on the type and molar ratio of the individual monomer component (PLA and PGA) present in the copolymer chain. When crystalline PGA is copolymerized with PLA, the degree of crystallinity is reduced with a consequent increase in the rate of hydration and hydrolysis. Thus as a rule, higher content of

PGA leads to quicker rates of degradation. The exception to this rule is the 50:50 ratio of PLA/PGA which exhibits the fastest degradation. The degree of crystallinity and melting point of the polymers are directly related to the molecular weight of the polymer. The Tg (glass transition temperature) of the PLGA copolymers are reported to be above the physiological temperature of 37 ◦C and hence are glassy in nature, thus exhibiting fairly rigid chain structure. It has been further reported that Tg of PLGAs decrease with a decrease of lactide content in the copolymer composition and with a decrease in molecular weight (Passerini and Craig, 2001). Commercially available PLGA polymers are usually characterized in terms of intrinsic viscosity, which is directly related to their molecular weights.

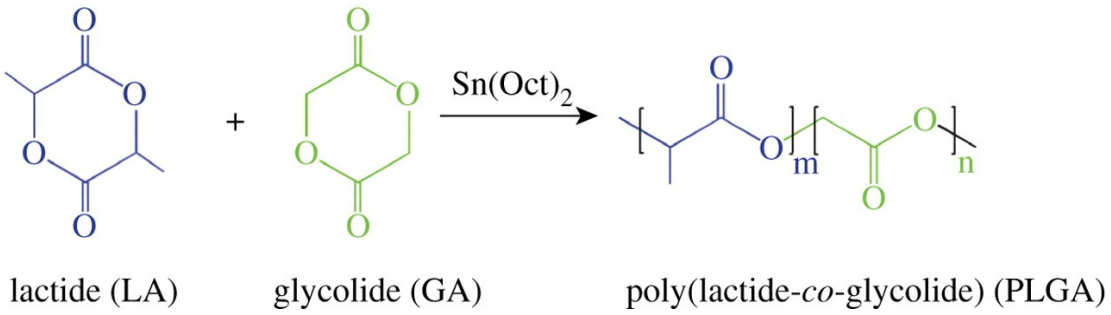


Figure 2.3: Structure of poly lactic-co-glycolic acid (n is the number of lactic acid units and m is number of glycolic acid units)

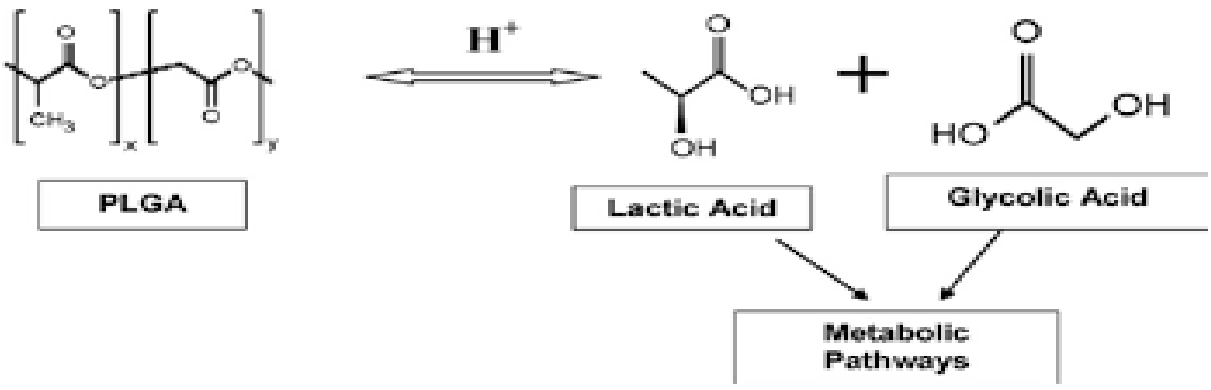


Figure 2.4: Hydrolysis of poly lactic-co-glycolic acid

2.11.2. Pharmacokinetic and Biodistribution Profile

The drug delivery device/ vehicle (PLGA) must be able to deliver drugs with appropriate duration, biodistribution and concentration for the intended therapeutic effect. Thus, in the design of the delivery device (e.g PLGA), the material type, geometry and location are considered and they determine the mechanism of degradation, the clearance of the drug vehicle after drug delivery as well as active pharmaceutical ingredients (API). Biodistribution and pharmacokinetics of PLGA follows a non-linear and dose-dependent profile (Yang *et al*, 2001). Furthermore, prior studies suggest that both blood clearance and uptake by the mononuclear phagocyte system (MPS) may depend on dose and composition of PLGA carrier systems (Panagi *et al*, 2001). The degradation of the PLGA carriers is quick on the initial stage (around 30%) and slows down eventually to be cleared by respiration in the lung (Bazile *et al*, 2001). To address these limitations, studies have investigated the role of surface modification, suggesting that incorporation of surface modifying agents can significantly increase blood circulation half-life (Esmaeili *et al*, 2008).

2.11.3 Fabrication Techniques for PLGA Carriers.

Drug and protein delivery devices like PLGA are used for targeted or controlled release in order to increase specificity, lower toxicity and decrease the risk associated with treatments with such drugs and proteins. However, the stability and delivery challenges associated with these agents have limited the number of marketed products. Maintaining adequate shelf-life of peptide and protein drugs often requires solid-state formation to limit hydrolytic degradation reaction (Houchin and Topp, 2008). Drug delivery of peptides and proteins may also require parenteral formulations to avoid degradation in the digestive tract and first pass metabolism and reduce

dosing frequency. PLGA are both biocompatible and biodegradable and thus can be used to avoid the inconvenient surgical insertion of large implants. PLGA can be used for controlled drug release. Drugs formulated in such polymeric devices are released either by diffusion through the polymer barrier, or by degradation of the polymer material, or by a combination of both diffusion and degradation mechanisms. PLGA is mainly used for drug delivery due to its biocompatibility, drug compatibility, suitable biodegradation kinetics, mechanical properties easy processing and fabrication. The fabrication techniques of PLGA controlled drug delivery device is discussed below

2.11.3.1 Solvent Evaporation Method

Solvent evaporation method are of two types based on the nature of the drug dissolved in the polymer. They are single emulsion process and Double/ multiple emulsion process.

(1) **Single emulsion process**

An example of this method is Oil-in-water emulsification processes. This method is suitable for water insoluble drugs, for example, steroids. Polymer in the appropriate amount is first dissolved in a water immiscible, volatile organic solvent (e.g., dichloromethane (DCM)) in order to prepare a single phase solution. The drug of particle size around 20–30 μm is added to the solution to produce a dispersion in the solution. This polymer dissolved -drug dispersed solution is then emulsified in large volume of water in presence of emulsifier (polyvinyl alcohol (PVA) etc.) in appropriate temperature with stirring. The organic solvent is then allowed to evaporate or extracted to harden the oil droplets under applicable conditions. The resultant solid microspheres are then washed and dried under appropriate conditions to give a final injectable microsphere formulation (Arshady, 2001; King and Patrick, 2001; Rosca *et al*, 2004; Sah, 1997).

(2) **Double (Multiple) emulsion process**

Water-in-oil-in-water emulsion methods are best suited to encapsulate water-soluble drugs like peptides, proteins, and vaccines, unlike single emulsion methods which is ideal for water-insoluble drugs like steroids. First, an appropriate amount of drug is dissolved in aqueous phase (deionised water) and then this drug solution is added to organic phase consisting of PLGA solution in DCM or chloroform with vigorous stirring to yield a water-in-oil emulsion which is then added to PVA aqueous solution and further emulsified for around a minute at appropriate stress mixing conditions. The organic solvent is then allowed to evaporate or is extracted in the same manner as oil-in-water emulsion techniques. In double emulsion processes, choice of solvents and stirring rate predominantly affects the encapsulation efficiency and final particle size (Arshady, 2001; Chaisri *et al*, 2009; Mao *et al*, 2007).

2.11.4. Implant Preparation Techniques

2.11.4.1. Solvent-Casting and Compression Molding

Solvent casting is a method to fabricate a macroscopic millimeter size formulation which can be implanted or inserted for long term medication (Widmer, 1998). Large size, macroscopic formulations act as a reservoir for drug that can be delivered over a longer interval. In this method, a polymer and drug mixture is dissolved in a common solvent (e.g., acetone or DCM) in the desirable proportion, and the solvent is cast at around 60 ◦C until complete evaporation. Their resultant structure is a composite material of the drug together with the polymer. The solvent cast material is then compression molded into its desired geometry at around 80 ◦C and 25,000 psi to final density of 1 g/cc. This implant can be subcutaneously delivered in the body. These implants have the ability to manage adverse physiological conditions since they are biodegradable and can

revert back to their initial state (monomer units (Siegel *et al*, 2006; Widmer, 1998). However, Solvent-casting methods are not ideal for industrial scale up for many reasons. First, the process requires large amounts of organic solvent to dissolve PLGA and the active pharmaceutical agent (API) to combine the drug and polymer for pellet fabrication. Such systems can lead to the denaturation of drugs because of large use of organic solvents. Denatured drugs are therapeutically inactive and can cause unpredictable side effects, such as immunogenicity or other toxicity. Second, the method requires a long time to completely remove solvents from the resulting material. Third, solvent casting method and compression molding are not continuous process, which may increase batch-to-batch variation in the composition of implants as well as cost of manufacturing (Wang *et al*, 2010)

2.11.4.2. Extrusion

Unlike solvent-casting, extrusion is a continuous process of drawing polymer-drug mixture through a die to create implants of fixed cross-sectional profile without any use of solvent. The process requires an extruder and polymer-drug mixture with required micron size feed material. During the process, the polymer-drug mixture is heated to semi-liquid state by a combination of heating elements and shear stress from the extrusion screw. The screw pushes the mixture through the die. The resulting extrudate is then cooled and solidified before cutting into desired lengths for implants or other applications (Yang *et al*, 2005). One disadvantage of the extrusion method is the exposure of the drug to high temperature which can lead to denaturation. This disadvantage limits the use of the method for some drug based on their melting point, polymorph stability and chemical interaction with PLGA.

2.11.5. Multi-Drug Delivery Devices

A pulsated drug release profile is sometimes preferred over the continuous presence of the drug because the continuous process may lead to down regulation of receptors or the development of tolerance for the drug. Novel multi-pulsatile delivery devices have developed in which there is a predetermined off period followed by rapid and transient drug release in a cycle until the device is degraded. Such devices have also been shown to be capable of releasing multiple drugs for a sequence of cycles. PLGA is also an attractive candidate for devices with multi-drug delivery and multi-pulsed delivery applications because of its desirable and tunable properties (Grayson *et al*, 2005; Stubbe *et al*, 2004; Grayson *et al*, 2003; Koushik and Kompella, 2004). Such systems can be extended to achieve programmed delivery of multiple drugs in a predetermined sequence of pulses from a single device (Grayson *et al*, 2003). For example, a single biodegradable polymeric microchip can be constructed of PLGA and/or PLLA in combination with multiple drugs to achieve pulsatile drug delivery over a long period of time (Koushik and Kompella, 2004).

2.11.6 Drug release behaviour

2.11.6.1 Biphasic Release

PLGA copolymer undergoes degradation by hydrolysis or biodegradation through cleavage of its backbone ester linkages into oligomers and, finally monomers. This has been demonstrated in both *in vivo* and *in vitro* for various drug types and proteins with different polymer ratios (Amann *et al*, 2010; Faisant*et al*, 2002). The degradation of PLGA copolymer is a collective process of bulk diffusion, surface diffusion, bulk erosion and surface erosion. Since there are many variables that influence degradation process, the release pattern of drug from the

copolymer is often unpredictable. The biodegradation rates of PLGA copolymers are dependent on the molar ratio of the lactic and glycolic acid in the polymer chain, molecular weight of the polymer, the degree of crystallinity, and the Tg of the polymer.

The release of drug from the homogeneously degrading matrix is more complicated. A biphasic curve for drug release as a result of PLGA biodegradation has been shown to display following pattern: (Figure 2.5) (Amann *et al*, 2010; Faisant *et al*, 2002; Crotts nd Park, 1998). Drug release from the polmers stages as explained below:

(1) Initial burst of drug release is related to drug type, drug concentration and polymer hydrophobicity. Drug on the surface, in contact with the medium, is released as a function of solubility as well as penetration of water into polymer matrix. Random scission of PLGA decreases molecular weight of polymer significantly, but no appreciable weight loss and no soluble monomer product are formed in this phase

(2) In the second phase, drug is released progressively through the thicker drug depleted layer. The water inside the matrix hydrolyzes the polymer into soluble oligomeric and monomeric products. This creates a passage for drug to be released by diffusion and erosion until complete polymer solubilization. Drug type also plays an important role here in attracting the aqueous phase into the matrix.

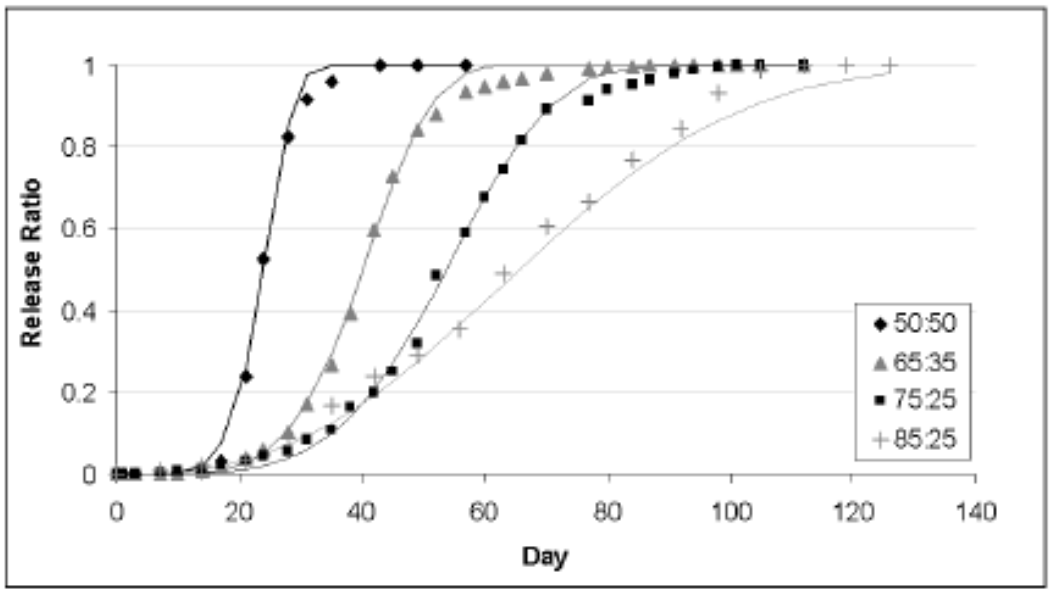


Figure2.5 Modelled *in vivo* release profiles *for 50:50, 65:35, 75:25 and 85:15 PLGA. (Notation 65:35 PLGA means 65% of the copolymer is lactic acid and 35% is glycolic acid. A biphasic release profile with an initial zero release period followed by a rapid drug release has been observed. The profiles also show increase in release rate with decrease in lactide to glycolide proportion).*

The role of enzymes in any PLGA degradation is unclear. Most literature indicates that degradation is purely through hydrolysis. However, some investigators have suggested an enzymatic role in PLGA breakdown based upon the difference in the *in vitro* and *in vivo* degradation rates.

The PLGA polymer biodegrades into lactic and glycolic acids. Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbondioxide and water (Kranz *et al*, 2000). Glycolic acid is either excreted unchanged in the kidney or it enters the tricarboxylic acid cycle and is eventually eliminated as carbondioxide and

water. PLGA polymers used for drug delivery have considerable mechanical strength which helps to withstand the physical stress that they are constantly subjected to. The physical stress influence he mechanical breakdown of the drug delivery device and also alter surface area and hydrolysis (Lu *et al*, 1999).

2.12 Factors affecting degradation

A good knowledge of these factors will help to design a device that is more efficient and efficacious.

2.12.1 Effect of Composition

Composition of the polymer delivery material determines the rate of degradation and hydrophilicity of the delivery matrix. Studies of polymer composition with its degradation has shown that polymers with higher proportion of glycolic acid than lactide in the oligomer has faster degradation and weight loss (Park, 1995; Alexix, 2005). The results show that PLGA 50:50 (PLA/PGA) exhibited a faster degradation than PLGA 65:35 due to higher proportion of glycolic acid and thus higher hydrophilicity. Subsequently PLGA 65:35 shows faster degradation than PLGA 75:25 and PLGA 75:25 than 85:15 (Tsuji *et al*, 2000). Thus, the absolute value of degradation rate increases with the glycolic acid proportion. Hence composition can be considered as the most important factor affecting degradation of polymers.

2.12.2 Effect of Crystallinity (or Tg)

Composition of copolymer determines the glass transition temperature and its crystallinity. Polymers that have higher crystallinity have lower rate of degradation (Liggins, 2001). Some groups proposed that the crystallinity of lactide acid (PLLA) increases the degradation rate

because the degradation of semi-crystalline polymer is accelerated due to an increase in hydrophilicity (Park, 1994).

2.12.3 Effect of Weight Average Molecular (M_w)

Polymers with high molecular weight have long polymer chains and hence require more time to degrade than small polymer chains (Frank *et al*, 2005). However, for PLLA, the higher the molecular weight, the lesser the crystallinity and the lesser the rate of degradation.

2.12.4 Effect of Drug Type

The type of drug in the polymer matrix determines the mechanism of polymer-drug matrix degradation and the parameters of drug release rate (Grizzi *et al*, 1995). The drug type may change the degradation mechanism from bulk erosion to surface degradation and as well affect the rate of matrix degradation (Siegel *et al*, 2006). The drug release profile, defined as the time required for 100% release, and the steady-state rate also varies significantly.

2.12.5 Effect of Size and Shape of the Matrix.

The ratio of surface area to volume is a significant factor for the degradation of large devices. Higher surface area ratio leads to higher degradation of the matrix. Studies have shown that bulk degradation is faster than pure surface degradation for PLGA. Thus PLGA with high surface area to volume ratio releases drug faster from the device (Liggins 2001; Holy 1999; Zolnik and Burgess 2007).

2.12.6 Effect of pH

The *in vitro* biodegradation/hydrolysis of PLGA showed that both alkaline and strongly acidic media accelerate polymer degradation (Ghahremankhani *et al*, 2007). However, the difference

between the slightly acidic and neutral media is less pronounced due to autocatalysis by the carboxylic end groups (Li *et al*, 2001).

2.12.7 Effect of Enzymes

There are conflicting results published on the effect of enzymes on degradation mechanisms (hydrolytic versus enzymatic cleavage) partially due the difference in degradation between *in vivo* and *in vitro* test (Schliecker *et al*, 2003). It has been proposed that PLGA degrades primarily through hydrolytic cleavage but it has also been suggested that enzymatic degradation may play a role in the process. The difference in degradation between the invivo and invitro test is proposed to be as a result of the activities of enzymes (Eniola and Hammer, 2005; Dailey *et al*, 2006).

2.12.8 Effect of Drug Load.

The amount of drug loading in the drug delivery matrix plays a significant role on the rate and duration of drug release. Matrices having higher drug content possess a larger initial burst release than those having lower content because of their smaller polymer to drug ratio. However, this drug content effect is attenuated when the drug content reaches a certain level depending upon drug type (Sundback *et al*, 2005).

2.13 Toxicology

Toxicological studies with PLGA devices suggest that local tissue reaction at the site of application may occur (Olivier *et al*, 1999; Berchane *et al*, 2007). These reactions are generally mild and PLGA has been shown to be extremely safe for macroscopic and microparticle systems

2.14 Cancer Drugs

2.14.1 Paclitaxel ™

Paclitaxel ™ is an anti-cancer or cytotoxic chemotherapy drug which can be produced from the bark of the pacific yew tree (Taxus brevifolis).It is classified as “plant alkaloid”, “a taxane”, and an “antimicrotubule agent”. Paclitaxel is used for treatment of breast, ovarian, lung, bladder, prostrate, melanoma, esophageal, and other solid tumour cancer (ACS, 2015). It has also been used in Kaposi‘s sarcoma (Saville *et al*, 1995).

Pacitaxel ™ is given intravenously. It is an irritant and can cause tissue damage if it escapes from the vein. There is no pill form of it. The side effects of paclitaxel and their severity vary and depend on how much of the drug Is given and/or the schedule in which it is given. The common side effect includes low blood counts, hair loss, Arthralgais and myalgais (pain in the joints and muscles, peripheral neuropathy (numbness and tingling of the hands and feet) Nausea and vomiting, diarrhoea and mouth sores (ACS, 2015).

Paclitaxel acts by inhibiting the microtubule structures within the cell (Brito *et al*, 2008). Microtubules are part of the cells apparatus for dividing and replicating itself. Inhibition of these microtubules ultimately results in cell death. Paclitaxel can also induce cell suicide (self death or apoptosis) (Bharadwaj *et al*, 2004; Brito *et al*, 2008).

Chapter Three

3.0 Materials and Methods

3.1 Introduction

This chapter presents the experimental procedure that was used for the preparation of drug solution, dissolution of polymer and fabrication of polymer-drug device. The rate of degradation and drug release from the fabricated device was studied. The degradation of the device was carried out in phosphate buffer saline of pH 7.4 and at temperature of 37 ℃ to simulate the pH of blood and normal human body temperature. The effects of different pHs on the degradation and drug release from the delivery device were also carried out. PBS solutions with pH 4.0, 6.0, 6.5, 7.0, 7.5 and at temperature of 37 ℃ were used for the study.

3.2. Materials

The materials that was used in the formulation of the drug delivery device includes PLGA 75:25 (procured from sigma Aldrich, St Louis, MO, USA), paclitaxel™ (PT) (anti-cancer drug that was procured from LC Laboratories Woburn, MA, USA)], dimethylsulfoxide, $(CH_3)_2SO$ (DMSO) that was purchased from BDH Chemicals (Poole Dorset, England), Dichloromethane (DCM) and polyvinylpyrrolidone (PVP) were purchased from BDH Chemicals (Pool Dorset, England). Ethyl acetate (EA) (purchased from BDH chemicals Ltd, pools, England), Probe scope HR 640 (Bodelin Technologies, Oswego, USA) equipment to observe and monitor structural changes during degradation process.

Probe scope image analyzing software (HR 640, Bodelin Technologies, Oswego, USA) was used to scale and identify features observed on the micrographs.

3.3 Preparation of Drug Concentrations

0.05g of PT was weighed with an analytical balance (Maxcap 310g, OHAUS Corp, China) and was dissolved with 2ml DMSO to give a stock solution of 25mg/ml which is later adjusted with PBS to give a final concentration of 1.25mg/ml. The reaction of DMSO and PBS produced turbid solution which was filtered twice to give a clear solution.

Table 3.1: Preparation of Drugs Solutions into Working Concentrations.

Drug/sample	Initial dissolution	Final dissolution (Topped up with)	Final Concentration
0.05 g of Paclitaxel	2 ml DMSO	38 ml PBS	1.25 mg/ml (DMSO:PBS) (5:95 %)

3.4 Dissolution of Polymers for Degradable Matrix

1g of PLGA (75:25) was dissolved with 2ml ethyl acetate in an air tight container to prevent the DCM from evaporating because of its volatility at room temperature for 15mins. The sample was gently stirred with a spatula till a homogeneous polymer blend was formed.

Table 3.2 Ratios of Polymer, Drug and PVP Used in PLGA-Based Minirods Formation.

	Polymer	Drug	PVP
Amount (g)	1	0.05	0.2
Percentage (w/w%)	80	4	16

3.5 Formation of PLGA-Based Minirods

1 ml of drug solution was added to the dissolved PLGA (75:25) as discussed above. This resulted in a mixture consisting of PLGA-PT formulations. 1 ml of PVP (formed from dissolving 0.2g of PVP in 1 ml of EA) was added to the PLGA-PT formulations as a cross linker. The resulting mixture of PLGA-PT-PVP was vigorously mixed and then cast into molds at room temperature. The samples were dried at 37 ℃ for 12 hours. . Different samples of PLGA-drug cast were used for the experiments and their descriptions are given in table 3.2 below

Table 3.2 shows the sample codes and composition (containing 5ml PBS at pH 7.4) used for the experiments

Sample Code	Concentration of Lipase Enzyme
A	No enzyme.
B	5μ g/ml
C	5μ g/ml
D	10μ g/ml
E	10μ g/ml
F	0.1mg/ml
G	0.5mg/ml
H	1mg/ml

3.6. Characterization of Samples

3.6.1. Optical Imaging

Prior to optical imaging, samples were washed thoroughly with distilled water to remove soluble products, salts or other impurities and then dried under vacuum conditions until a constant weight was achieved. Then, Probe scope HR 640 (Bodelin Technologies, Oswego, USA) equipment to observe and monitor structural changes during degradation process.

Probe scope image analyzing software (HR 640, Bodelin Technologies, Oswego, USA) was used to scale and identify features observed on the micrographs.

3.6.2 Ultra Violet Visible spectrophotometric (UV-Vis)

Ultra violet visible spectrophotometric (CECIL 7500) was to determine the absorbance of drugs released at a wavelength of 200-700 nm. The PBS in the test tube, stored at $37℃$, was changed at regular time interval of two days and the absorbance of the collected sample was measured. The collected samples were stored in the refrigerator until the time for measuring the absorbance and wavelength for PT. The peak absorbance was found to 232nm. From Beer-Lambert law, the concentration of drug released into the PBS solution at different time intervals were obtained. Beer-Lambert law is given as:

$$\varepsilon = \frac{A}{LC} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where A is the absorbance, L is the path length of the quartz cuvette used (1cm / 0.01m) and C is the concentration of the drug used (1.25g/ml). The concentration of each sample of known absorbance is obtained from the formula below.

$$c = \frac{A}{L\varepsilon} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

3.7. Degradation and Drug Release

3.7.1. Polymer Degradation

1. The initial weights of the samples (casts of PLGA-PVP-PT) were determined before immersing in 5ml PBS (pH 7.4). The PBS containing the weighed samples was then incubated at an incubator shaker at 37˚C. The PBS is changed at an interval of two days and stored in the refrigerator for measuring the absorbance. The samples were also removed at an interval of 2 days during the change of PBS and then thoroughly washed and dried for about 10 minutes before weighing. Polymer degradation is characterized by weight loss when incubated in PBS as well as studying the structural changes using Probe scope HR 640 (Bodelin Technologies, Oswego, USA) equipment.

$$\frac{M_t}{M_i} = k\sqrt{t} \quad \text{..... (3)}$$

Where M_t is the mass of the sample at time t, M_i is the initial mass of the sample, and t is the degradation time. The rates of polymer erosions are determined from the absorption of PBS for the different samples:

$$\frac{dm}{dt} = -mk \quad \text{..... (4)}$$

Where dm is the change in mass at time, t and k is the kinetic rate constant of degradation.

m is the initial mass.

3.7.2 Determination of Drug Release

Examine the amount of drugs released with the UV-Vis. The rate of drug released is estimated from the reaction kinetics:

$$\frac{dC}{dt} = -k C^n \qquad (3)$$

Where k is the reaction rate constant, n is the order of reaction and C is the concentration of drug released at time t. The order of reaction is estimated by model equations below:

Model	Equation	R^2
Zero-order	$C_t = C_o + k_o t$	
First-order	$\ln C_t = \ln C_o + k_o t$	
Second-order	$\frac{1}{C_t} = \frac{1}{C_o} + \frac{1}{kt}$	
Huguichi	$C_t = C_o + k_o t^{\frac{1}{2}}$	

The initial concentration of drug release may also be is characterized by an exponential time dependent function (eg. first order):

$$C(t) = C_o e^{-kt} \qquad (4b)$$

The drug loading content (DL %) in addition to the drug encapsulation efficiency (DEE %) are given, respectively by:

$$DL = Wd/(Wd + Wp) x\, 100 \qquad (5a)$$

$$DEE = (Wd/Wi) x\, 100 \qquad (5b)$$

Where W_d is the amount of drug loaded, W_i is the initial mass of drug and W_p is the mass of the polymer incorporated. Thud DL% = $\frac{0.05}{1.05} \times 100 =$¿ 4.761%

DDE% = $\frac{0.05}{1.25}$ x 100 = 4%

3.8 Effect of pH on polymer degradation

PBS at different pH (4.0, 6.0, 6.5, 7.0, and 7.4) was used to study the rate of drug release and polymer degradation. The PBS was incubated at 37˚C in an auto shaker. The PBS of different pH was changed at daily interval. The samples were thoroughly washed and weighed on analytical balance on a daily interval.

Chapter Four

4.0 Results and Discussion

4.1 Introduction

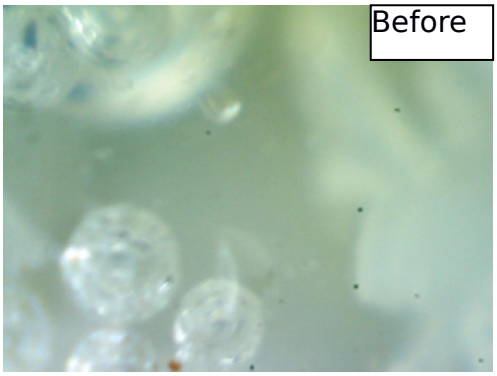
This chapter presents and discusses the implication of the results. PLGA based minitablets were used as the delivery device for paclitaxel ™. The rates of drug release from the polymer (PLGA), the rates of degradation and the effects of different pH on the polymer degradation and drug release were studied. Optical characterization and mass loss were used to ascertain the polymer degradation.

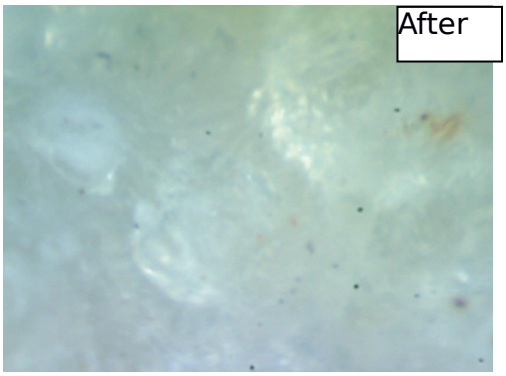
4.2. Characterization result

4.2.1. Surface Morphology

The sample images were taken with Probe scope HR 640 before putting the samples in PBS solution and also at the end of the experiments.

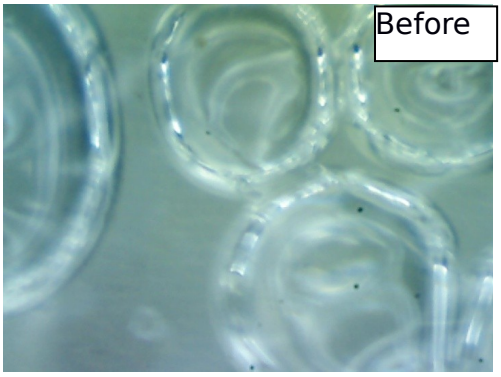
(a) (b)





(c)

(d)



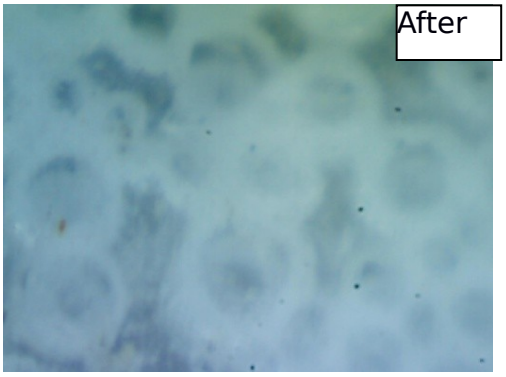
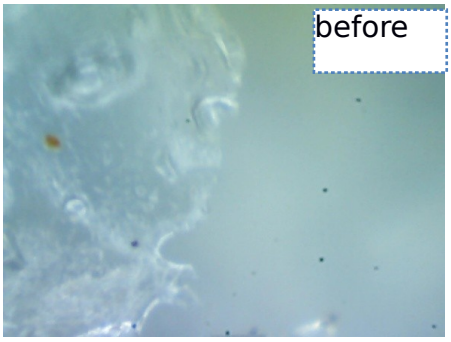


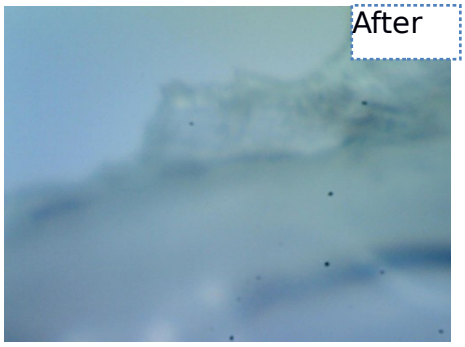
Figure 4.1 Probe scope images of samples (a) Samples before immersing in pH 4.0 (b) Samples after day 7 in pH 4.0 (c) samples before immersing in pH 7.4 (d) samples after immersing in pH 7.4

The samples above show degraded samples in PBS solution at different pH. The degradation is higher in acidic than in alkaline medium.

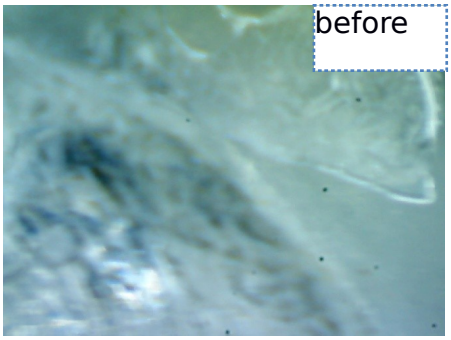
(e)

(f)

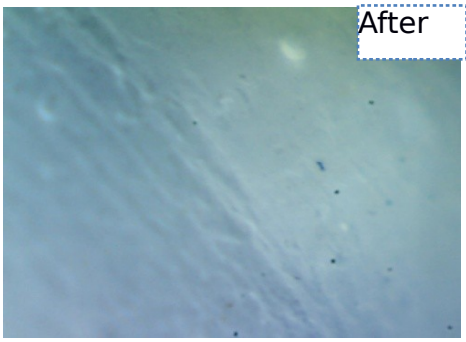




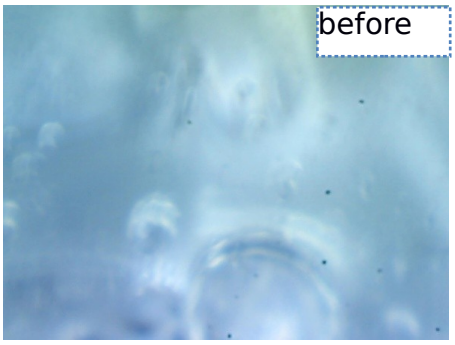
(g)



(h)



(i)



(j)

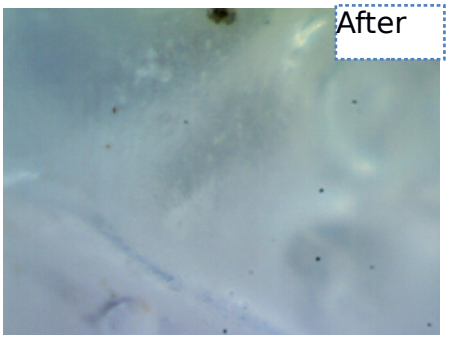


Figure 4.2 Probe scope images of samples before immersion and after 6 days, in PBS containing different concentrations of enzymes. The descriptions have been given in table 4.1 (e) A before (f) A, after (g) B, before (g) B, after (i) H, before (j) H, after.

These probe scope Images also show that samples degrade in PBS solution. It can also be seen that there is more degradation in presence of enzyme.

4.2.2 Standard curves

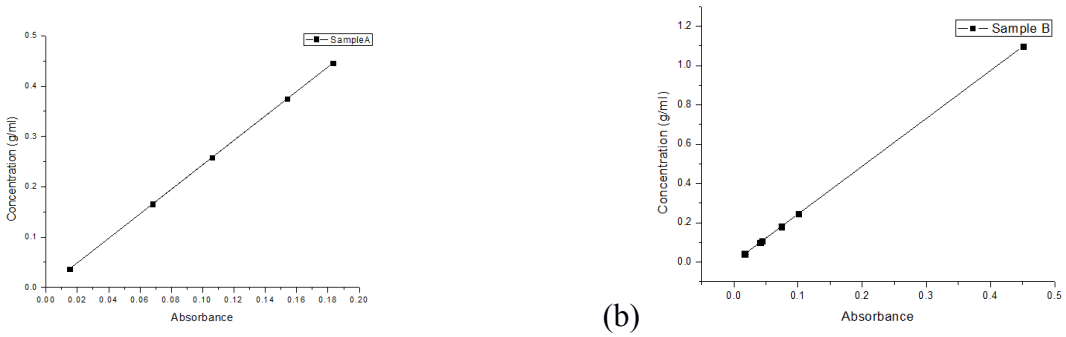
The graph of absorbance against concentration (fig 4.3) gives a straight line graph with a slope equal to $\frac{1}{L\varepsilon}$, where L is path length (1cm) and ε is absorptivity coefficient (ml/mg cm).

The graphs of absorbance against concentration for all samples give a straight line that can be extrapolated from origin from the origin. The graphs of absorbance against concentration

(standard curves) for samples pH 4.0, 6.0, 6.5, 7.0 and 7.4 all gave straight line that could be extrapolated from the origin. Results for pH 4.0 and 6.0 are compared (Fig 4.4).

4.2.3 Absorbance versus Concentration

The graphs of Absorbance against Concentration (standard curve) for samples are shown below.



(a) (b)

Figure 4.3 Standard Curve of Drug Release (a) for Sample A (no enzyme) (b) for sample B (containing 5 $\mu g/ml$ of lipase enzyme).

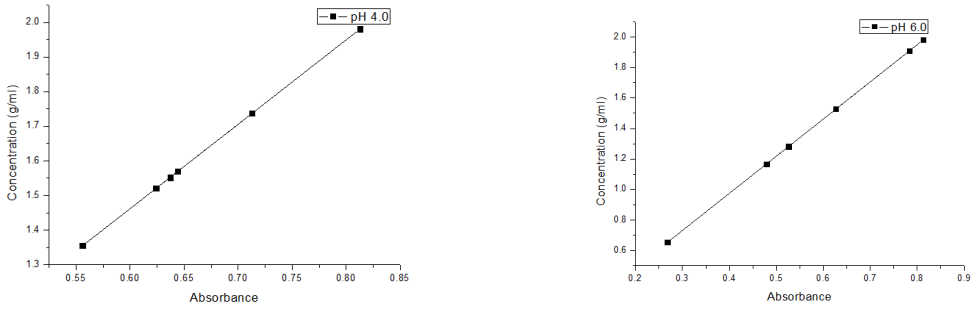
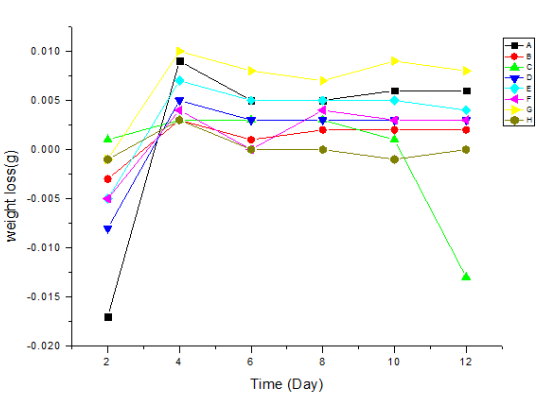
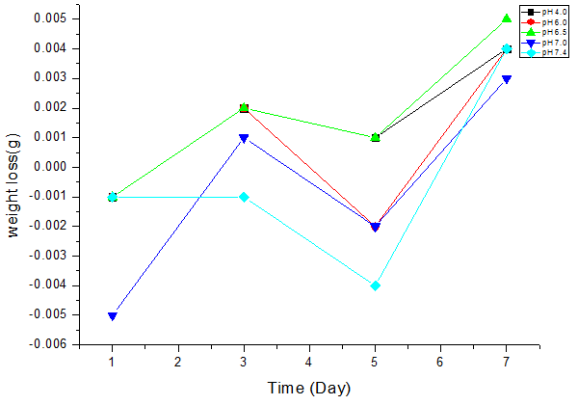


Figure 4.4 Standard curve for samples at pH 4.0 and at pH 7.4, respectively.

4.2.4 Weight loss versus time



(a)



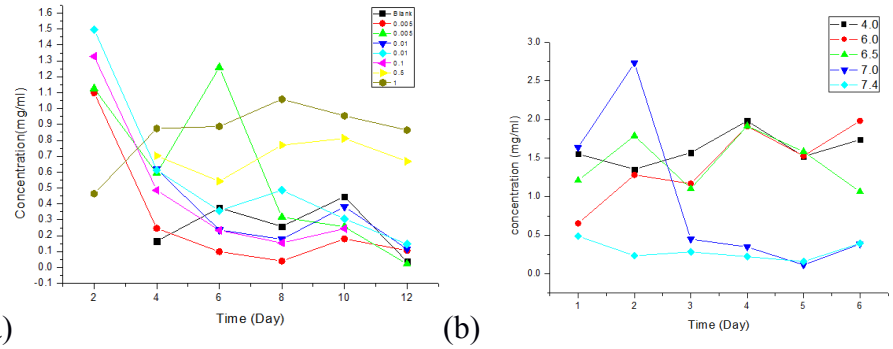
(b)

Figure 4.5 weight loss of different samples Vs time (a) samples in different enzyme conditions (b) samples in different pH

From plot (a) shown above, it can be seen that there is an initial increase in weights loss of the samples, followed by a rapid decrease, and then, a slight increase which is followed by a slight decrease. The initial increase in weight of samples is as a result of the swelling of the samples when immersed in PBS solution. The initial sharp decrease is as a result of degradation of the polymers in the sample mixture. This is evidenced by weight loss of the PLGA.The subsequent slight decreases as seen from the graph are also as a result of degradation of the delivery device (PLGA). This is also seen as weight loss of PLGA. From the graph, the highest degradation after the initial swelling is seen in Sample H (containing 0.1mg/ml of enzyme).This is followed by sample G (containing 0.5mg/ml of enzymes). This is in turn followed by sample E (containing 10μ g/ml of enzymes) and then sample C ($containing 5\mu g/ml of enzymes \wedge$ then sample A (containing no enzyme). The experiment shows that enzymes (lipase) increases the rate of degradation of PLGA used for the delivery of paclitaxel. The graph also shows that the higher the concentration of enzymes, the faster the rate of degradation.

From plot (b), there is increase in weight loss, followed by a sharp decrease in weight loss for each sample in different pH. The weight loss is higher for samples in acidic medium (pH 4.0, 6.0 and 6.5) than when compared to samples in alkaline medium (7.4) and neutral medium (pH 7.0).

The amount of drug released from the samples was quantified from absorbance by routinely changed PBS solution. Using Beer Lambert law, it was possible to relate the amount of drug release to the concentration (Fig 4.4). The graph shows that as the concentration of enzymes increases, the concentration of drug released from the polymer also increased. Hence, enzyme (lipase) has an effect on the rate of drug released from PLGA. The absorbance was measured using UV-Vis spectrophotometer.



(a) (b)

Figure 4.6 Plot of concentration of different versus time (a) For different enzyme concentration (b) For different pH

The graph (a) shows that as concentration of enzymes increases, the concentration of drug released from the polymer also increased. Hence, enzyme (lipase) affects the rate of drug released from PLGA. It also showed that the higher the concentration of enzymes, the faster the rate of drug released from PLGA.

For (b), the concentration of drug released in the first two days is in descending order of 7.0, 4.0, 6.5, 6.0 and 7.4. After the first two days the concentration of drug released into PBS is in descending order of 4.0, 6.0, 6.5, 7.0 and 7.4. The result showed that there is a faster rate of drug release from acidic medium than in alkaline medium. The result also shows that the more acidic the solution containing the PLGA-Drug cast, the higher the concentration of drug released.

CHAPTER FIVE

5.1 Conclusion

An extended and controlled localized drug delivery has been proposed in this study. An improved way of treating localized cancer can be achieved with a biodegradable implantable device which can be capable of delivering multi-pulse drugs to cancer cells or tissue. Biodegradable polymers such as PLGA were used for this study because of its biocompatibility. Biodegradable devices will eliminate the need for surgical removal after drug delivery as degradable products will be managed by the body's natural metabolism.

One of the objectives in this research was to determine the effects of enzyme (lipase) on the degradation rates of PLGA-based miniparticles as well as determine the drug release kinetics for localized cancer drug delivery. The concentration of the lipase increased the rate of degradation of the PLGA and increased the concentration of drug released from the sample (PLGA-PT casts). The study showed that the Drug Loading content (DL %) is 4.761%. The Drug Encapsulation Efficiency is 4%. This knowledge aids in our calculation for determining the amount of drug and PLGA required for achieving a desired effect over a stipulated period of time. The study also revealed that the higher the amount of enzyme, that can break down PLGA, the faster the rate of

degradation and drug release from drug delivery device. Hence the knowledge of the type and quantity of enzyme surrounding the tissues is very important in determining the life span of the biodegradable delivery device of choice and the quantity of drug released.

Another objective of the study was to determine the effect of pH on the PLGA used for delivery PT. It was observed that PLGA degrades faster in acidic medium (pH 4.0) than in neutral (pH 7.0) or in alkaline medium (pH 7.4). The implication of the result is that if PLGA is used to deliver drugs in tissues that are more acidic (for example stomach), it will degrade faster than when present in tissues that are more alkaline (mouth, blood, e.t.c). This knowledge will aid in the calculation of the right amount of drug and PLGA required for achieving desired effects in targeted areas.

5.2 Future Work

An extended study and proper studies on drug release kinetics are expected in the future including computational models to validate experimental result. Cell viability test are recommended with PLGA-PT based mini particles to determine the cytotoxicity of the drug concentrations required for cancer treatment,

Animal experiments with the drug delivery device should also be carried out in order to determine the feasibility and efficacy of environment and conditions that mimics the physiological conditions.

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