PACLITAXEL LOADED POLYLACTIDE-CO-GLYCOLIDE ACIDS FOR CORONARY ARTERY DISEASE AND CANCER TREATMENT

A Thesis

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DEDICATION

I dedicate this work to Jesus for his guide and strength that I enjoyed during my master program. Also, to my mum, Mrs. Funmilayo Bakare-Adedeji, for her support, understanding and encouragement.

ABSTRACT

The number one killer disease in the world is Cardiovascular Disease[1]. The most serious element of Cardiovascular Disease is Coronary Artery Disease. Currently, the main treatment for Coronary Artery Disease is stent placement surgery. However, its long time safety and efficiency are the major setbacks, as it is associated with late restenosis and late thrombosis. These are set caused by the durable polymeric film used as the drug carrier. To improve on the current treatment (stent placement surgery), a biodegradable drug delivery system was synthesized. Paclitaxel was successfully entrapped into the polylactide-co-glycolide acids microspheres via single solvent microencapsulation evaporation method. Optical Microscopy was used to study the shape and morphology of the microspheres. The particle sizes were found to range of the microspheres is 15. 18 – 40.11 μm, with a mean size of 29.415 μm, a p-value 0.986 and a z-value-0.02. The results suggest that the encapsulated drug loaded microspheres can be used as biodegradable drug eluting structures for localized vascular-targeted therapy for the treatment of coronary artery disease. This is a presented as potential alternative to stent placement surgery. The potential extensions to localized cancer treatment are also elucidated.

ACKNOWLEDGEMENT

My heart-full gratitude goes to God the father, the Son and the Holy Spirit for being my shade, my shelter, my strength and my wisdom. Thank you father, for you have made me a show piece of your glory.

My profound gratitude goes to Prof. Wole Soboyejo, my supervisor, an ideal picture of a supervisor, lecturer and father, who lives for impact and legacy. You showed me how a teacher should be and for this I am honored to be your student. I know I can't pay you for hours of teaching, supervision, advice, counsel, encouragement and care, but I pray that your children will enjoy God's help at every junction of their life. You taught me materials science and engineering and it is now a tool of transformation of Nigeria and Africa at large. Thank you sir, I am forever grateful.

Also, I want to appreciate Dr Shola Odusanya, my co-supervisor, the man that believes every research is possible. Your push and effort in this research cannot be overemphasized. I really appreciate all your input and support; may your effort be crowned with greater glory in Jesus name (Amen).

Sincere appreciation goes to Godwin Etuk-Udo, Mrs Onogu, Ogbu Chinyere Joy, AUST Academics and AUST Account Department, AUST drivers, John Obayemi, Yiporo Danyo, Emmanuel Arthur Kwesi and class of 2013 (materials science and engineering) for your contributions towards the success of this research work and I pray that God will be your very help in need. See you all at the climax.

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Chapter One

1.0 Background and Introduction

1.1 Statement of the problem (Heart Disease)

Cardiovascular disease (CVD) is caused by disorders of the heart and blood vessels, which includes coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure [1,2]. The first two are the major killer diseases in the whole world.

In 2002, it was estimated that 29% of deaths worldwide (16.7 million deaths) were due to CVD and that 43% of global morbidity and mortality, measured in disability-adjusted life years (DALYs), and was caused by CVD [1]. Furthermore, 78% of global mortality and 86% of mortality and morbidity from CVD occurs in developing countries.

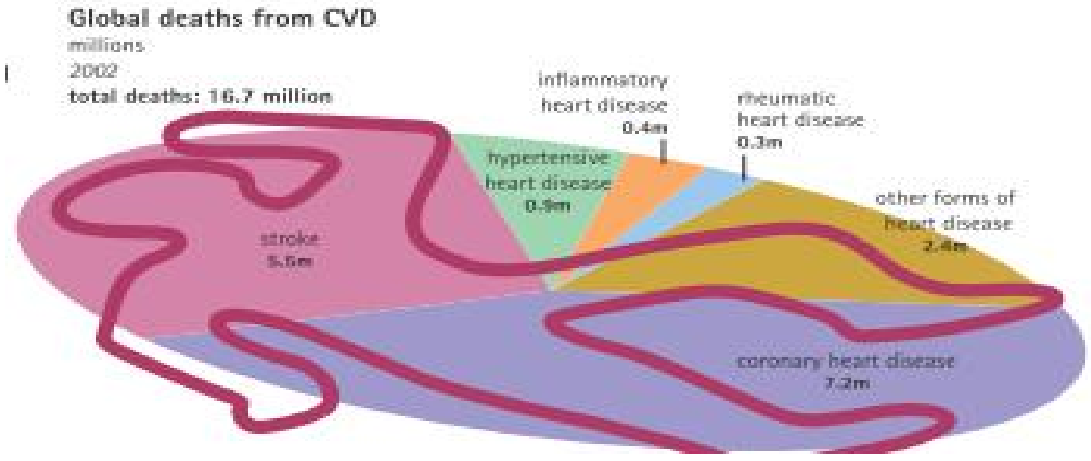


Figure 1.1.1 Pie chart representation of mortality rate of different types of cardiovascular disease in 2002

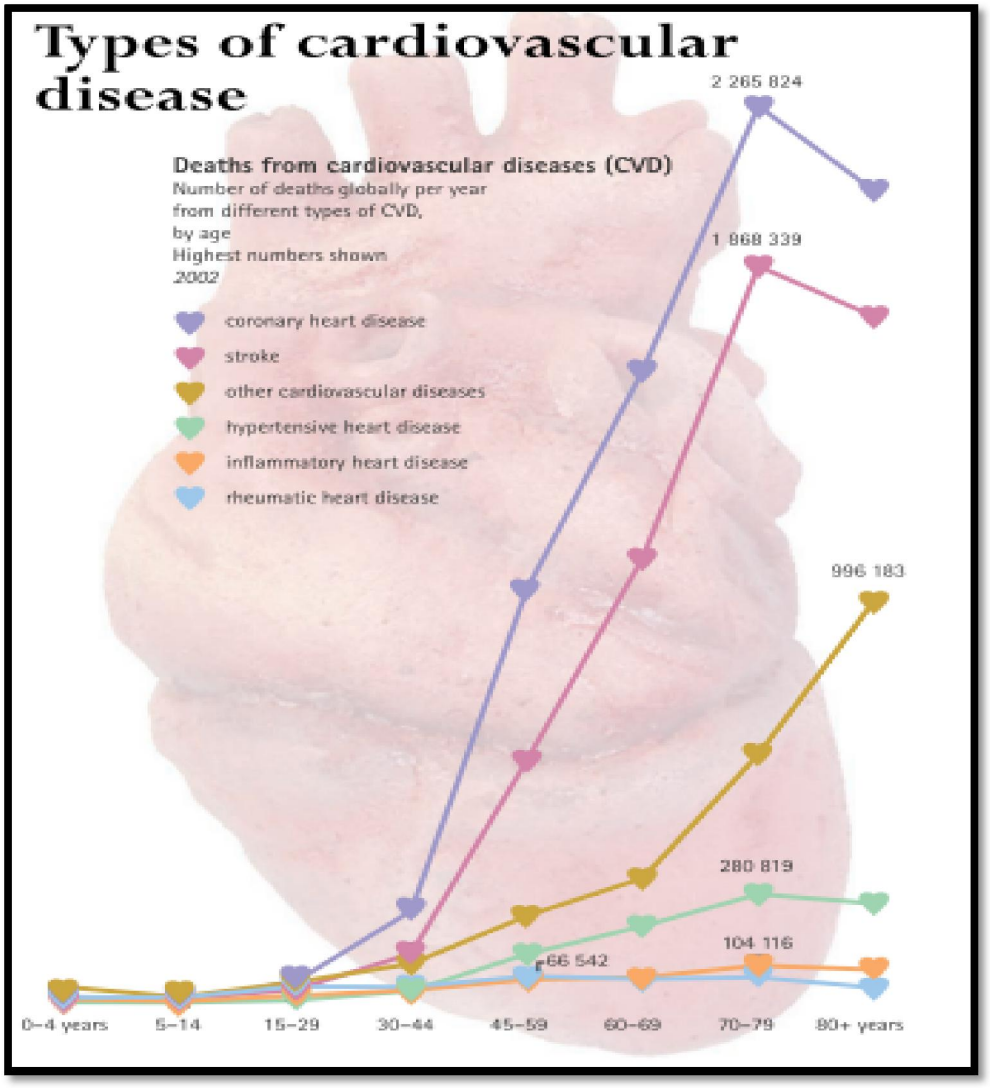


Figure 1.1.2 Fact sheet of cardiovascular disease for 2002

In 2008, about 17.3 million people died as a result of cardiovascular disease. These represent 30% of the global death toll 2008. Out of this figure, 7.3 million were due to coronary heart disease (Atherosclerosis), 6.2 million were due to stroke and 5.8 million were jointly caused by hypertension, high blood pressure, diabetes, and heart failure. 80% of cardiovascular disease, that is 5.8 million deaths, occurs in low and medium income countries. Furthermore, Coronary Artery Disease (CAD) is the number one causes of

death in both males and females in the low-income and high-income countries [3]. There is, therefore, a need for improved methods for the treatment of CAD in both developed and developing countries [3].

1.2 Coronary Artery Disease

Coronary artery disease is the narrowing or blockage of the coronary arteries. It is caused by atherosclerosis. Atherosclerosis (sometimes called “hardening” or “clogging” of the arteries) is the buildup of cholesterol and fatty deposits (called plaques) on inner walls of the arteries. These plaques can restrict blood flow to the heart muscle by physically clogging the artery or by causing abnormal artery tone and function. Without an adequate blood supply, the heart becomes starved of oxygen and the vital nutrients that needs to work properly. This can cause chest pain called angina. If blood supply to a portion of the heart muscle is cut off entirely, or if the energy demands of the heart become much greater than the blood supply, a heart attack (injury to the heart muscle) may occur [4].

1.2.1 Mechanism of Coronary Artery Disease (Atherosclerosis)

Coronary arteries are hollow tube-like blood vessels that supplies oxygenated blood to the heart muscles. The muscular walls of the coronary arteries are normally smooth and elastic. They are lined with a layer of cells called the endothelium. The endothelium provides a physical barrier (protective layer) between the blood stream and the coronary artery walls, while regulating the function of the artery by releasing chemical signals in response to various stimuli.

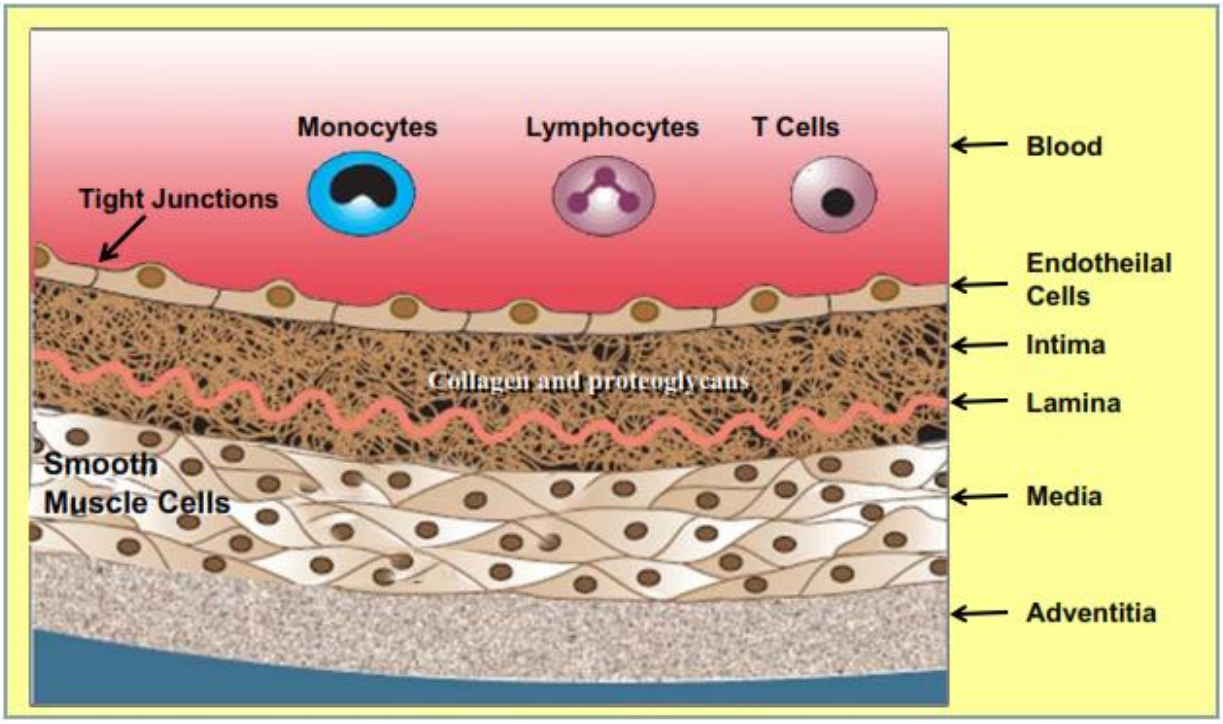


Figure 1.2 Structure and layers within coronary artery

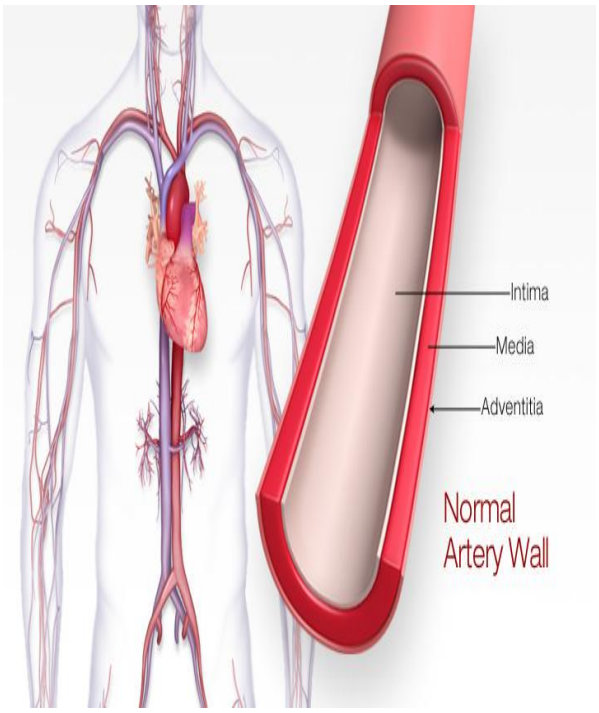
A large artery consists of three morphologically distinct layers. The intima, the innermost layer, is bounded by a monolayer of endothelial cells on the luminal side and a sheet of elastic fibres, the internal elastic lamina, on the peripheral side. The normal intima is a very thin region (size exaggerated in this figure) and consists of extracellular connective tissue matrix, primarily proteoglycans and collagen. The media, the middle layer, consists of smooth muscle cells (SMCs). The adventitia, the outer layer, consists of connective tissues with interspersed fibroblasts and SMCs [5].

The pathogenesis of coronary artery disease (Atherosclerosis) is associated with the buildup or accumulation of cholesterol (low-density lipoprotein), fatty cells, calcium and other blood nutrients on the walls of coronary artery . Atherosclerosis starts when the endothelium becomes damaged, allowing low-density lipoprotein cholesterol to accumulate in the artery wall. The body sends macrophage white blood cells to clean up the cholesterol, but sometimes the cells get stuck there at the affected site.

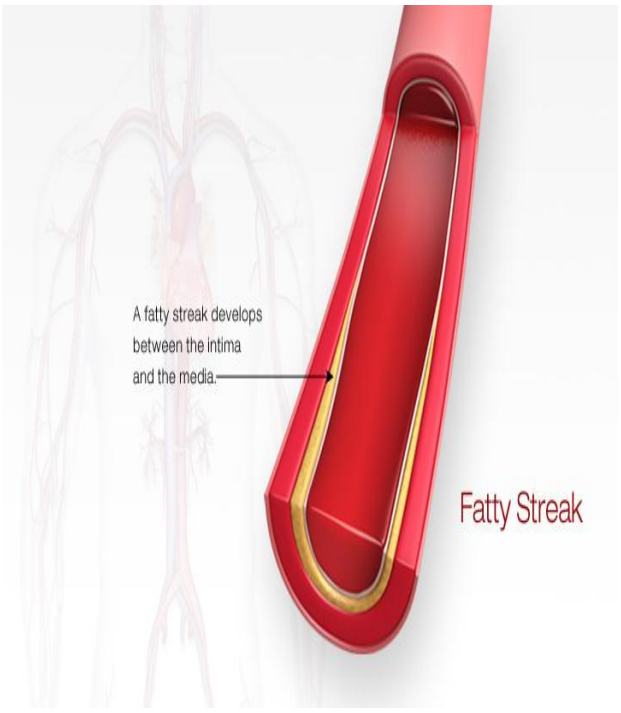
Over time this results in plaque being built up, consisting of bad cholesterol (LDL cholesterol) and macrophage white blood cells [7].

The early stage of Atherosclerosis; Fatty streak, which is common in infants and young children, is a pure inflammatory lesion consisting of monocytes-derived macrophages and T- lymphocytes [6] (Figure 1.2.1 (a)) [7]. As one advances in age, the fat keeps accumulating and damage the protective layer of the wall; endothelial cells. This is called endothelial dysfunction. Consequently, some of the fat, calcium and other nutrients within the blood escape in to the smooth muscles (Figure 1.2.1(b)) and affects the dilation and contraction of the smooth muscles which controls the flow of blood stream in the coronary artery.

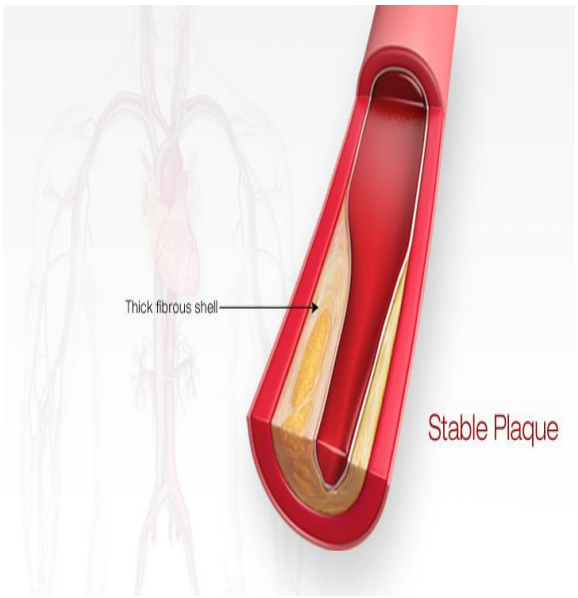
Over time, the inside of the arteries develop plaques of different sizes (c). Many of the plaque deposits are soft on the inside with a hard fibrous “cap” covering the outside. If the hard surface cracks or tears, the soft, fatty inside is exposed (d). Platelets (disc-shaped particles in the blood that aid clotting) come to the area, and blood clots form around the plaque (e-f). The endothelium can also become irritated and fail to function properly, causing the muscular artery to squeeze at inappropriate times. Thismy causes the artery to narrow even more. Sometimes, the blood clot breaks apart, and blood supply is restored. In other cases, the blood clot (coronary thrombus (g)) may suddenly block the blood supply to the heart muscle (coronary occlusion (h)), causing one of three serious conditions, and called acute coronary syndromes [4].



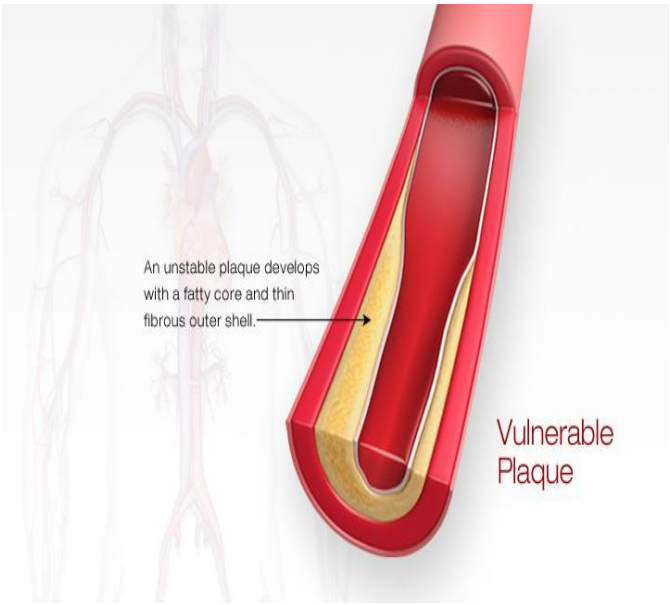
(a) Normal Artery



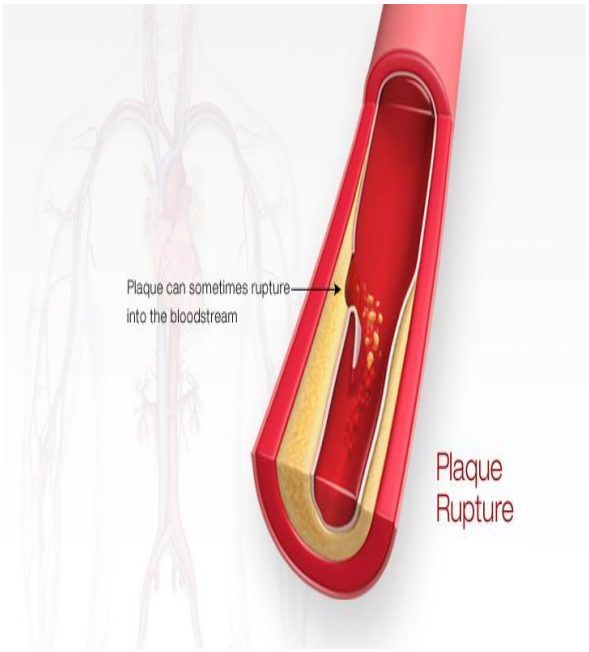
(b) Fatty Streak



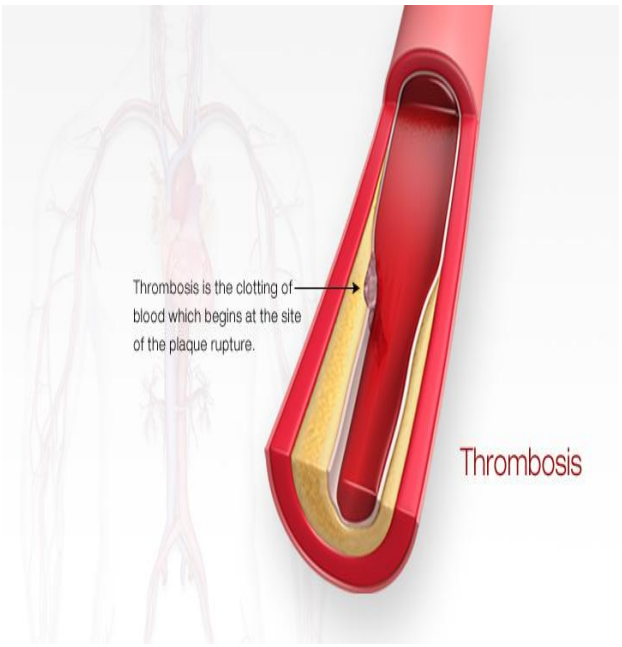
(c) Stable growth of the plaque



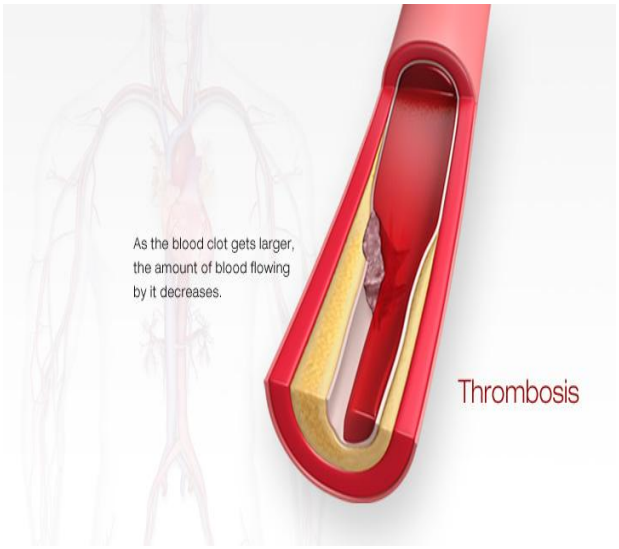
(d) unstable stage of plaque



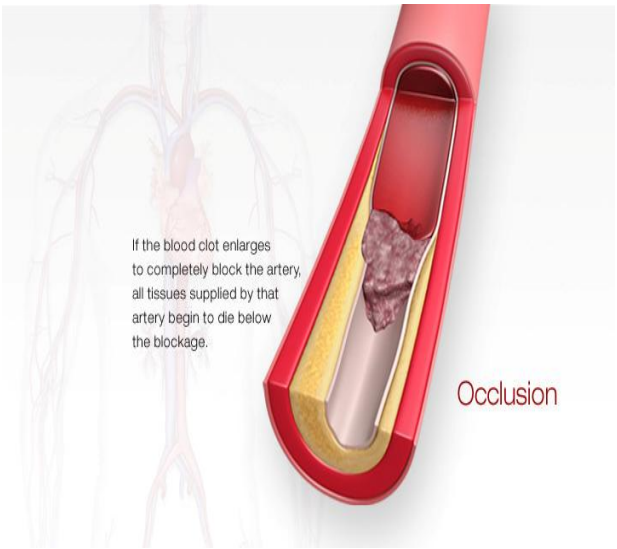
(e) Rupture of plaque



(f) Thrombosis



(g) Growth of thrombosis



(h) Occlusion

Figure 1.2.1 Progression of plaque of Atherosclerosis

1.2.2 Past Treatment and Current treatment on Coronary Artery Disease (Atherosclerosis)

The first attempt was made by Dr Andreas Gruentzig on September 14, 1977 at University Hospital in Zurich, Switzerland on a 38 years old man [8]. He used an approach known as Percutaneous Transluminal Coronary Angioplasty (PTCA). Percutaneous means the procedure is done through the skin, Transluminal means the procedure is done inside the artery, Coronary means the artery of the heart, Angioplasty is the technique of widening the blocked artery with a balloon. This is otherwise known as a balloon procedure [5]. The limitation of this procedure is restenosis, which is the re-clogging treated artery caused by the scar tissue formation, uncontrolled proliferation of smooth cells and thrombosis. To reduce the restenosis problem associated with the percutaneous transluminal coronary angioplasty, cardiologists, materials scientist and engineers and biomedical engineering developed drug eluting stents in late 1990's. Drug eluting stents have reduced early-restenosis to a minimum level. However, late or long time- restenosis is still a challenge that has attracted the attention of many scientists in the last decade.

1.3 Unresolved Issues

The first-generation Cypher Sirolimus-eluting stent (SES) (Cordis, Johnson & Johnson, NJ, US), the Taxus Express paclitaxel-eluting stent (PES) (Boston Scientific, MS, US) and the Taxus Liberté PES (Boston Scientific), and the second generation Endeavor zotarolimus-eluting stent (ZES) (Medtronic Vascular, CA, US) and Xience-V everolimus-eluting stent (EES) (Abbott Vascular, CA, US) have all been effective at treating early restenosis [6].

However, in the late 2000, major concerns arose over the long-term safety of first and second generation of drug eluting stents. They occured when a number of clinical and observational studies

reported significant increased risk of mortality in patients treated with drug eluting stent beyond 12 months [6].

Today, the primary concern with long-term drug eluting stents safety is stent thrombosis, a potentially fatal adverse event that can leads to myocardial infarction and/or death [6]. It has been shown that the non-erodible polymer coatings employed by DESs (particularly the first-generation Cypher SES and Taxus ExpressPES) impair stent strut endothelialisation. They also induce late hypersensitivity reactions and subsequent stent thrombosis [6]. As a result of these findings, research in this area is currently centered on the development and evaluation of improved DESs which maintain the impressive clinical benefits observed with currently approved devices, while eradicating long-term safety concerns such as stent thrombosis.

Furthermore, the adhesive strength between polymeric carrier (either erodible or non erodible) and stent reservoirs can determine whether thrombosis occur or not [6]. There is, therefore a need to understand the adhesion levels that are needed for the robust application of stents.

1.4 Scope of Work

This work explores the adhesion of polylactide-co-glycolide acid (PLGA) loaded with anti-proliferative agent on the stent platform in new generation of Drug Eluting Stents (DES) [NEVO^{TM}] coronary drug eluting stent, using an Adhesion-push-out tester. The potential degradation of the adhesion is also explored after exposure to environment and fatigue loading. Finally, the swelling behavior and drug release kinetics is elucidated for polylactide and polylactide-co-glycolide acid (85:15, 75:25, 65:35, and 50:50) loaded with prodigiosin or taxol.

The Implication of the results will be discussed for the design a bio-erodible/bio-absorbable stents with enhanced adhesion and degradation resistance.

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Chapter Two

2.0 Literature Review

2.1 Stents and Drug Eluting Stents

The first attempt to clear-off plaques within the coronary artery was made by Dr. Andreas Gruentzig in 1977 at University hospital, Zurich, Switzerland [1, 2]. The method he employed is called percutaneous transluminal coronary angioplasty procedure. This treatment involves threading of bare-metal mesh-like material into the coronary artery through the femoral or radial artery [3]. The bare-metal is in a collapsed form onto a catheter balloon and expanded at the narrowed area of the coronary artery. The bare-metal expands and clears off the plaque and the coronary artery becomes wider (Figure 2.1).

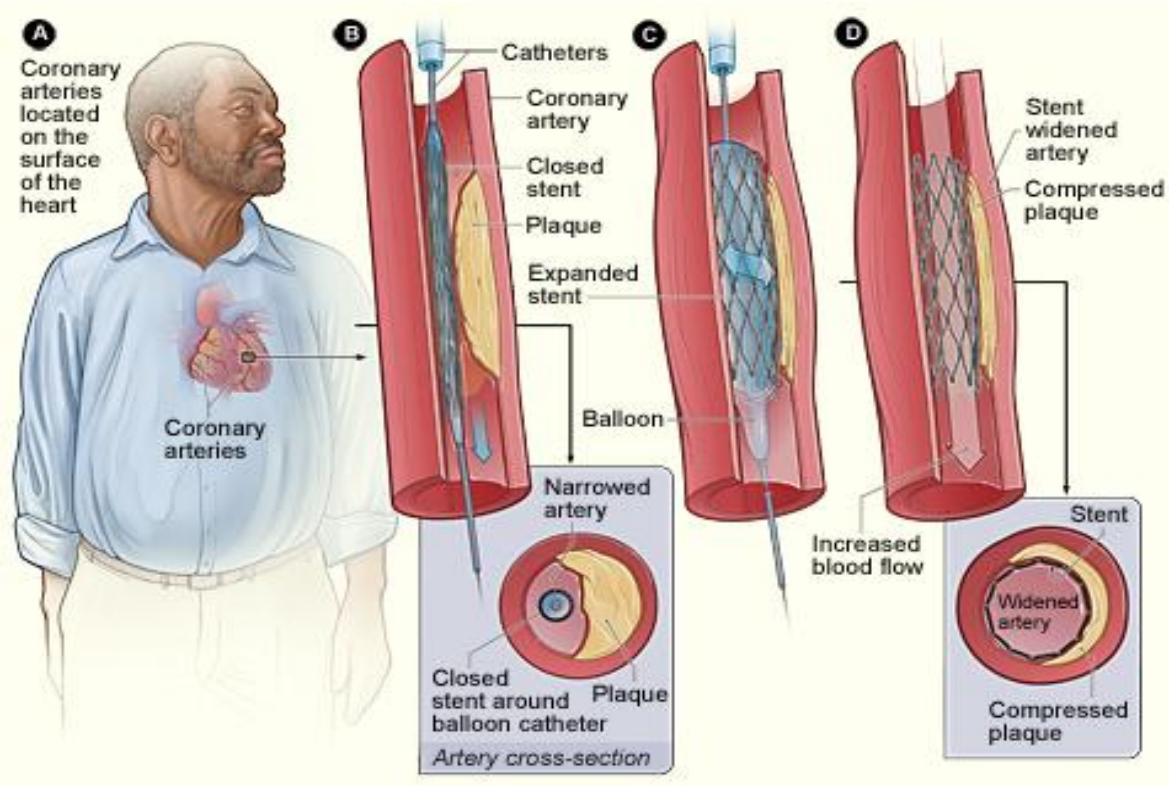

Figure 2.1 Percutaneous transluminal coronary angioplasty (PTCA) procedures [3]

This was a good idea and positive approach to reduced the death mortality and disability caused by coronary artery disease. For two decades, it prevailed on atherosclerosis but later it was discovered that the patients that undergo percutaneous transluminal coronary angioplasty tends to develop angina after 6-12 of PTCA [1, 9]. This limitation is called restenosis. The restenosis rate after PTCA has been reported to vary from 17% to 61%, depending on the nature of the lesion and the patient's subgroup, such as diabetics and uremia [1]. Restenosis is considered as local vascular manifestation of the general biologic response to injury [1]. These results from the compressive forces exerted on the plaques by the inflated balloon catheter which is transferred to the walls of the coronary artery [6]. In the course of crushing the plaque, both intima and the media of the arterial wall are disrupted [7]. In response to the wound, inflammatory cells-monocytes, macrophages, lymphocytes e.tc, infiltrate the site of injury [8]. Platelets are activated within the intravascular blood clot and releases cytokines, such as platelet-derived growth factor (PDGF) [8]. PDGF stimulates the growth of smooth muscle cells and myofibroblast, which results into neointimal proliferation [1]. Within months, the healing site resembles a fibrous plaque, which is the beginning of restenosis [7].To inhibit the proliferation of the injured smooth muscle cells, an anti-restenotic therapeutic agent was introduced (as part of the structure) as an attachment to the wall of bare metal stents (figure) and this new structure is called Drug Eluting Stents (DESs) [9].

Drug-eluting stents with controlled local release of anti-proliferative agents have consistently reduced the risk of repeat revascularization [10], as compared with bare metal stents [10-12]. The robustness of different DESs and their success in combating restenosis will be discussed in the next section.

2.2 Drug Eluting Stents

Generally, Drug Eluting Stents comprises of three components which are; stent platform, drug delivery mechanism and an anti-restenoic therapeutic agent [2, 9]. Most of the stents platforms are modular and slot-tube configurations, which are highly flexible to pass through the tortuous passage associated with cardiovascular system [9]. In prior years, materials such as gold, platinum, tantalum, nitinol (Ni-Ti) alloy, stainless steel, and Co-Cr alloy have been used to manufacture the stents platform [8,10]. It is expedient for the stents platform to possess the following properties; optimum vessel coverage, high radial strength (such that it undergoes minimal radial recoil and achieves a final diameter consistent with that of the host vessel upon unloading); good corrosion resistance; good fatigue resistance; non-sticking of blood onto the surfaces; non-separation of the blood constituents; good adhesion to the walls of the artery and homogeneous distribution of drug reservoirs around the stents [2, 9, 10]. The balance of all these properties drives the research and development of new cardiovascular stents with optimum properties in the biomedical industry.

Furthermore, the second component is drug delivery system. This is mostly a polymeric film (erodible or non-erodible) that serves as anti-restenotic carrier. Its response to the physiological conditions controls the elution of the drug release [2, 9]. To date, the most successful method of facilitating drug adhesion and delivery from a stent has involved the use of permanent synthetic polymer coating materials such as polyethylene-co-vinyl acetate (PEVA), poly-n-butyl methacrylate (PBMA), and the tri-block copolymer poly (styrene-b-isobutylene-b-styrene) (SIBS) [9]. By carefully mixing anti-restenotic drugs with these materials, a drug-polymer matrix may be formed and applied to the surface of the stent platform [2, 9]. Upon deployment, drug-delivery is driven by diffusion from the matrix and the rate of this diffusion is dictated by the type, composition and number of polymers used in the drug–polymer matrix [2].

In recent years these permanent polymers have been superseded by advanced biocompatible permanent polymers such as phosphorylcholine (PC) and the co-polymer poly (vinylidenefluoride-co-hexafluoropropylene) (PVDF-HFP). These advanced polymers mimic the phospholipids on the outer surfaces of red blood cells resulting in a stent platform that induces minimal thrombus formation upon deployment and has minimal adverse clinical effect on late healing of the vessel wall [9].

The robustness of the above drug carriers depends on their ability to withstand deformations of stent crimping and expansion, good adhesion to the stent platform, good durability and surface finish, and stability [7]. The current research in this area is currently centered on the assessment of biocompatible and bioabsorbable polymer coating materials and on the development of novel mechanisms of drug-release. An example of this system is NEVO™ Sirolimus-eluting Coronary Stent (NEVO SES, Cordis Corporation, Spring House, PA), which has polylactide-co-glycolide acid (bioerodible polymer) as the drug carrier.

Lastly, the anti-restenotic therapeutic agent that inhibits the proliferation of the scar tissue formation as a result of the injury induced during deployment of stents. The common anti-restenotic agents used so far in drug eluting stent are sirolimus, zotarolimus, everlimus and paclitaxel [2, 9]. Sirolimus, zotarolimus and everolimus, potent immunosuppressive agents, inhibit smooth muscle cells proliferation in response to cytokine and growth factor stimulation by binding to the cytosolic FK binding protein 12 (FKBP12) [2, 9]. This prevents the activation of the mammalian target of rapamycin (mTOR) and leads to interruption of the cell-cycle in the G1-S phase [2, 9].

Paclitaxel is a strong anti-proliferative agent. It suppresses neointimal growth by binding with and stabilizing microtubules [9]. The stability of these microtubules inhibits their disassembly and renders them non-functional, resulting in cell-cycle arrest in the G0–G1 and G2–M phases (Fig. 2.2) [2].

As robust as this system is, it is important to understand the penetration depth of the anti-restenoic drugs (diffusion length), the uptake of the drug by the surrounding cells and tissue, adhesion of the polymeric carrier to the stent reservoir, the optimal dose required for restenosis, and drug release profile. All these determine the overall efficiency of the drug eluting system.

The safety and long term effectiveness of the first generation and second generation drug eluting stents is discussed shortly along with their shortcomings as observed in clinical trials is also discussed.

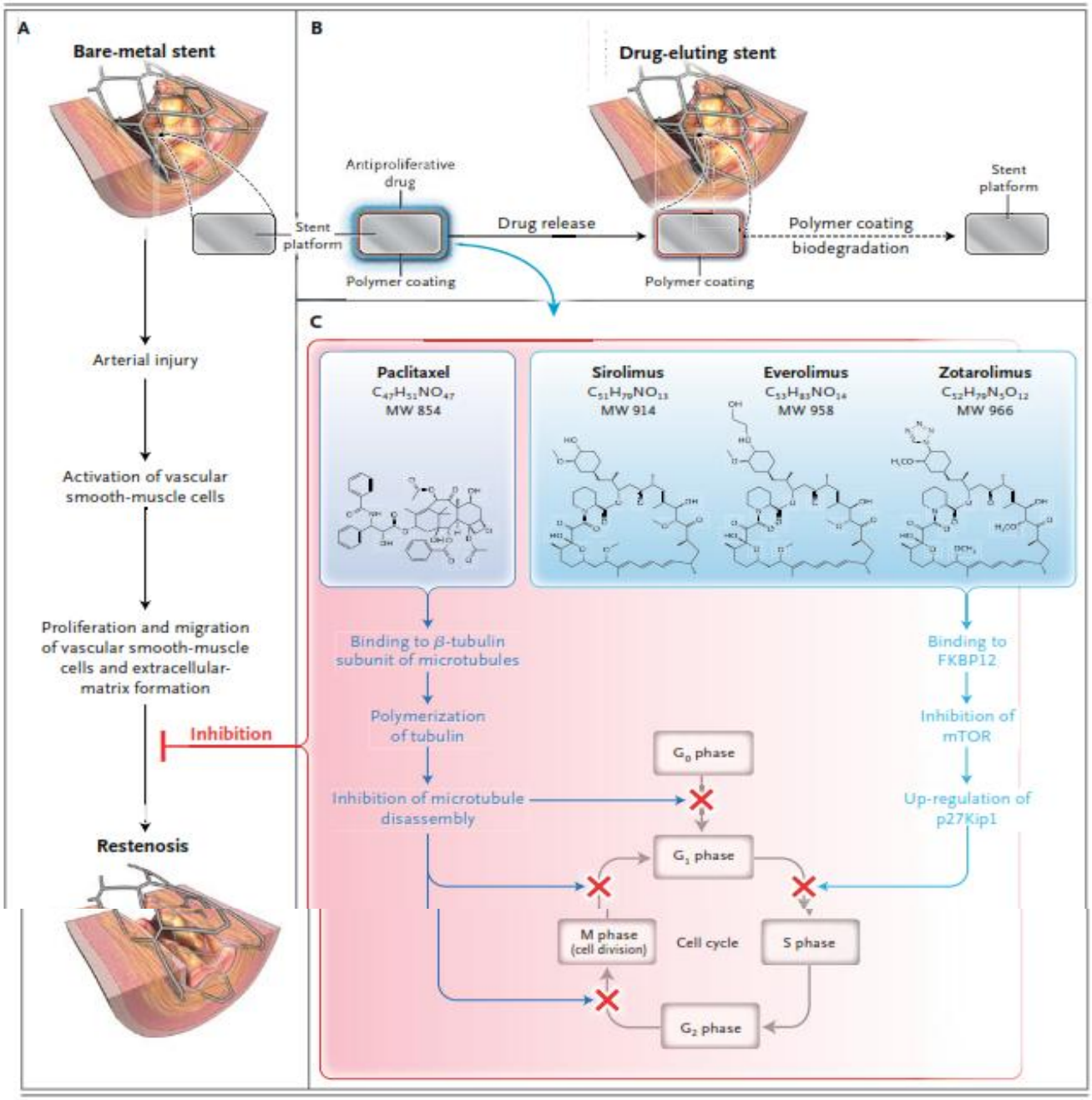

Figure 2.2 Components and Mechanisms of action of Bare Metal and Drug Eluting Stents [2]

2.2.0 First Generation Drug Eluting stents

2.2.1 Cypher-Sirolimus Drug Eluting stents (SES)

Cypher SES consists of Bx-velocity bare metal stent(BMS) (a product of Johnson & Johnson) and the anti-restenotic agent are embeded in two durable and non-erodible polymers poly(butyl methacrylate) (PBMA) and polyethylene-co-vinyl acetate (PEVA)[9, 6]. The ratio of copolymer is 33% PBMA and 67% PEVA.

The stent platform, Bx-velocity BMS, is manufactured from 316L stainless steel, of series of sinusoidal strut-segments joined by N-shaped, flexible link-segments but of thicker struts.

The drug–polymer formulation is evenly-distributed over the whole surface of the stent platform (with equal standard concentration of 140 µg of sirolimus per cm^2) [9]. 80% of release is expected within the first 30days after stent deployment.

Five different clinical trials were conducted to ascertain the long term safety of Cypher . The FIRST IN MAN (FIM) trial (which involved 45 patients) shows minimal in-stent neointimal proliferation, with fast and slow release of sirolimus in a 4-month follow up after deployment [8]. The RAVEL trial, which is a random trial of 238 patients confirmed the superiority of the Cypher- Sirolimus Drug Eluting Stent over the Bare metal stent, in terms of in-segment late loss at 6 months [14]. The SIRIUS, C-SIRIUS and E-SIRIUS trials were randomised trials involving a total of 1510 patients, with more complex lesions than those enrolled in the RAVEL and FIM trials. The superiority of the Cypher SES over the Bx-Velocity BMS was further demonstrated in these trials, with markedly lower rates of target lesion revascularization and adverse clinical events observed in patients treated with the Cypher SES [10, 11, 12]. The Cypher SES was the first DES to receive both CE and FDA approval in April 2002 and April 2003, respectively.

From the outcome of the above trials, the supremacy of Cypher SES over Bare Metal Stent was established. However, late restenosis and target revascularization were not fully eradicated.

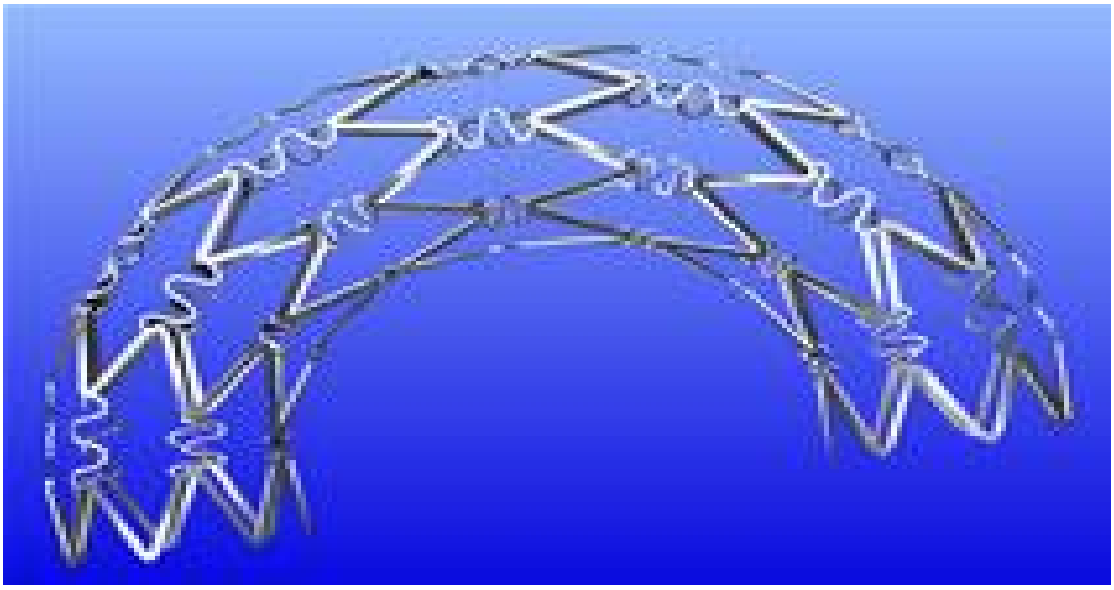

Figure 2..2.1 Cypher – Sirolimus Drug Eluting Stents[16]

2.2.2 The Taxus-Paclitaxel-Drug Eluting Stents (PES)

Taxus DES consists of bare metal stents coated with the formulation of paclitaxel and a permanent copolymer SIBs (styrene block polymer). Its stent platform (express BMS) is a closed-cell modular tube that is produced from 316L stainless steel (the same material as Cypher SES). Its design and configuration is a series of sinusoidal strut-segments, joined by straight articulations to short, narrow strut-segments (Figure 2.2.2). The importance of the design and configuration is for the purpose of adhesion and integration with the wall of the coronary artery. The drug-polymer formulation is evenly-distributed over the surface of the bare stents, with standard concentration of 100 µg of paciltaxel per cm^2 of stent surface area [9].

The release of paciltaxel is in two stages; the first 40hrs after deployment is associated with burst release, which is followed by the slow release of the next 10 days after the first 40 hrs. To test for the efficacy and long time safety of the Taxus express, three different clinical trials were conducted.

The first was a randomized trial (Taxus 1), which involved 61 patients. This showed a no-binary in-stent restenosis at 6 months and minimal adverse clinical events compared to BMSs at 12 months []. The second trial was also a randomized trial with 536 patients (more patients than the first trial).

This also established the superiority of the Taxus express over Bare Metal Stent (BMS) in terms of stent volume obstructed by neointimal proliferation at 6 months . The third clinical trials involve 1314 patients with complex lesion demonstrated the superiority of Taxus Express over Bare metal stents in the area of in-stent late loss, binary in-stent restenosis and target-lesion revasculation at 9 months [15] .

In May 2002 and 2004, Taxus express received CE and FDA approval respectively. After this, a new stent platform know as Liberte stent was fabricated to replace the Taxus express stent platform, but with the same drug-polymer coating. It was manufactured from the same material (316L stainless steel) but with thinner struts compared to Taxus express.

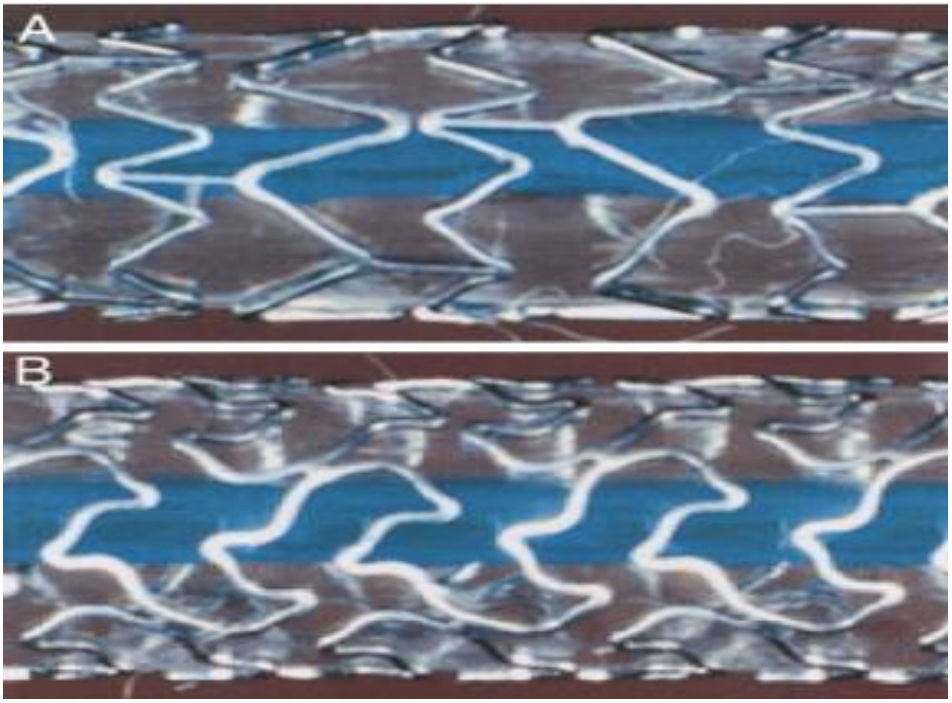

Figure 2.2.2 (A) Taxus Exxpress PES (B) Taxus Liberte PES [9]

To fully improve the performance of the first generation drug eluting stents, the second generation drug eluting stents were developed with better mechanical properties, thinner shruts and improved corossion resistaance.

2.3 The Second Generation Drug eluting stents

2.3.1 The Endeavor ZES

The Endeavor ZES consists of stents platform known as Driver BMS(Medtronic Vascular) manufactured from MP35N cobalt- chrome alloy with thinner strut. This is coated with formulation of zotarilimus and biocompactible polycarbonate copolymers (drug carrier) [9]. The drug–polymer coating is homogeneously distributed over the stent surface area with a standard concentration of 100 µm of zotarolimus per cm length of the strut. It is expected to release about 95% of zotarolimus within the first 15 days after stent placement[1]. The following clinical trials establish the safety and efficacy evidence of Endearvor ZES [9].

The non-randomised trial (Endeavor 1) involved 100 patients . The result from this trials showed that binary in-stent restenosis occurs within 4-12 months of stent placement [1].

The ENDEAVOR III trials was a randomised trial involving 1197 patients. It revealed superemacy of Endeavor ZES over Driver Bare Metal Stent. These suprimacy is justified by lower rate of binary in-stent restenosis and target-vessel revasculation within the period of 8 months and 9 months, respectively [6]. The ENDEAVOR III AND ENDEAVOR IV trials were randomised trials that involved 436 and 1548 patients respectively. They established the edge and superiority of the Endeavor ZES over the Cypher sirolimus and Taxus Xpress drug eluting stents [9]. In these two trials, the short term follow up showed that Endervor ZES is associated with higher rates of targeted lesion revasculation and significantly higher rates of in-stent late loss [9, 39] .

Figure2.3.1ZotarolimusDrugElutingStents[http://www.medgadget.com/2008/02/medtronics_endeavor_drugeluting_stent_approved_by_fda.html]

2.3.2 The Xience-V EES

This consists of multi-link vision bare metal stent coated with formulation of everolimus , poly n-butyl methacrylate (PBMA) and a permanent biocompatible co-polymer ,PVDF-HFP . The multi-link vision bare metal stent is fabricated from the L605 cobalt-chrome alloy. It consists of series of corrugated zig-zag struts with thiner struts [9].The drug-polymer is applied to the entire strut surface with a standard concentration of 100 µm of everlimus per cm^2 of stent surface area . It is designed to release 80% of everlimus within 30 days of stent placement [16]. The safety and efficacy of Xience – V EES was established through four different clinical trials.

The SPIRIT FIRST trial was a randomised trial involving 60 patients that shows the superiority of Xience-V EES over multi-link vision bare metal stent in terms of in-stent restenosis and in-stent late loss at 6 months [16]. The SPIRIT II trial was a randomised trial involving 300 patients. It revealed the superiority of Xience- V EES over Taxus Express PES in terms of in-stent late loss at 6 months [17]. The SPIRIT III trial was a randomized trial involving 1002 patients, it demonstrated the significantly reduced in-segment late loss and non-inferior rates of target-vessel failure in patients treated with a

Xience-V EES, compared to the Taxus Express2 PES at 12 months [25]. The SPIRIT IV trial is a randomized trial involving 3687 patients that has demonstrated the superiority of the Xience-V EES over the Taxus Express2 PES in terms of target-lesion failure and target-vessel revascularization at 12 months [19]. Interestingly, following three year follow-up of the SPIRIT II and III trials, investigators observed an increase in the absolute difference in target-vessel failure and adverse clinical events in favor of the Xience-V EES [20, 21]

Figure 2.3.2 Xience – V EES drug eluting stent

The problems of late thrombosis, late restenosis, and local drug toxicity were associated with the first generation stents and second generation stents. This is associated with the permanent presence of durable polymer that causes inflamatory response and local toxicity in preclinical analysis[12].Furthermore, the durable polymers that were used in first and second generation stents are associated with mechanical complications such as polymer delamination and webbed polymer surface leading to stent expansion issues (through which plasticity sets in) and nonuniform coatings resulting in erratic drug distribution. Hence, in recent years, the focus of clinical research has been on the

development of novel drug carrier systems.These include: absorbable (or biodegradable) polymers and nonpolymeric stent surfaces. Additional improvements include the development of more modern platforms (e.g, better deliverability, radiopacity, flexibility, and radial strength), as well as the use of novel antiproliferative agents or reduced doses of current approved antiproliferative drugs

2.5 NEVO™ Sirolimous Coronary stent

Late stent thrombus and inflammatory response have been associated to the durable polymer carrier system [9]. To eradiacate this challenges, absorbable/erodible/ biodegradable polymeric system that release drugs in a controlled manner and erodes out of the body through metabolic processes [49].

Nevo™ Sirolimous Coronary Stent is an example of a drug eluting stent with an absorbable polymeric drug carrier. This is a cobalt-chromium stent dotted, with "reservoirs" that can be loaded with 1 or more drugs and polymers to release drug more specifically, potentially in various doses or formulations.

In the case of the Nevo, the reservoirs are filled with a biodegradable polymer impregnated with sirolimus. As a potential advantage, the stent reduces tissue-polymer contact by more than 75% and total polymer load by approximately 80% [24].

The stents platform is an open cell design made from cobalt-chrome alloy with thin struts, that promote drug distribution and scafolding. The sculpted reservoirs on the Nevo stent are filled with sirolimus in a dose similar to the Cypher stent .The release of the drug is controlled by the biodegradable polymer [24]. In the first 10 to 15 days, the release of sirolimus is somewhat slower than in the Cypher stent. However, similar to Cypher, 100% of the drug is released by the 90th day after the procedure [9 ,24]. The polymer degrades in a similar time course, so that by the third month, there is a complete reversion of Nevo to a bare metal chromium-cobalt stent [24].

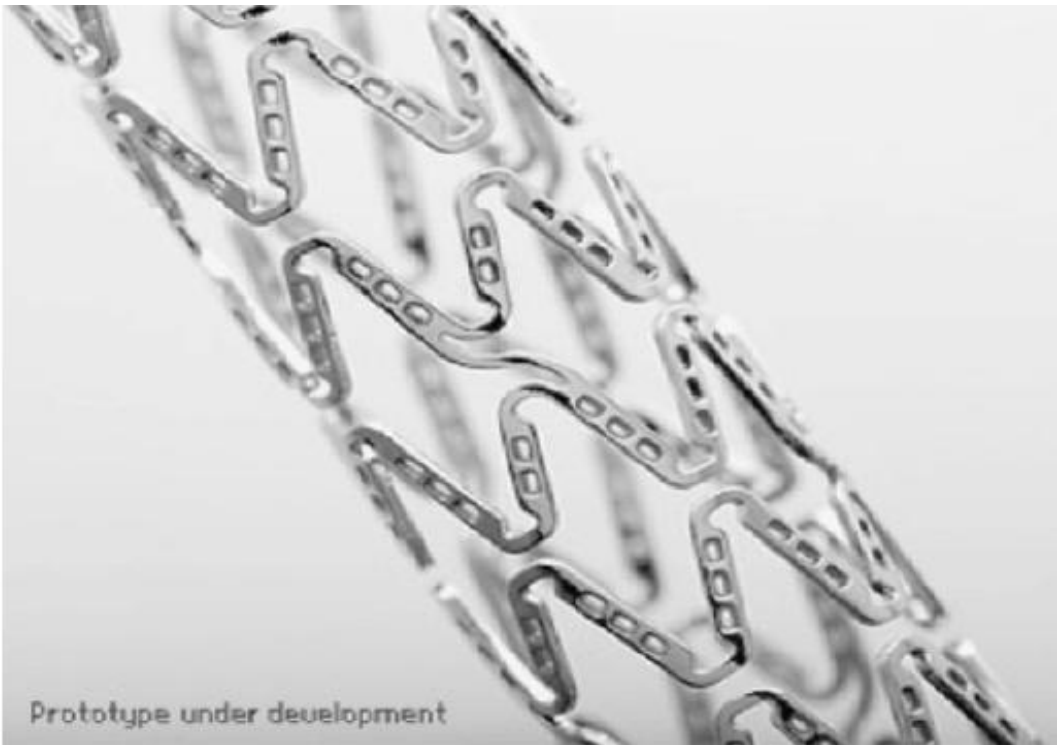

Figure 2.5.1 NEVO^{TM} Sirolimus Coronary Stents [24]

2.6 Polylactide –co-glycolide acids (PLGA)

Polylactide-co-glycolide acid has had great success in applications in drug delivery devices and scaffolds for tissue engineering [25].

Biodegradrable polymers occurs naturally of sythesised in the laboratory. The degradation process are either enzyme-mediated degradation or non-enzyme mediated degradation or both. The by-products of the degradation process are non-toxic and biocompactible (self accepting). They also leaves the body through normal metabolic processes [26]. The suitability of natural biodegradable polymer (such as Bovine Serum Albumin (BSA), Human Serum Albumin (HSA), Collagen, Gelatin and heamoglobin) for drug delivery systems is limited by their cost and questionable purity [25]. This creates a need for the

synthesis biodgradable polymers that meet the biocompatibility and biodegradability requirements with controlled and sustained drug release for localized drug delivery devices [27].

Examples of synthesised biodegradable polymers are poly(amides), poly (akyl-α-cyano acrylates), poly (esters), poly (orthoesters), poly (urethanes), and poly (acryl amides).

Out of all these polymers, thermoplastic aliphatic polyesters e.g polylactide, polyglycolide and their copolymers- polylactide-co-glycolide acids, have exhibited an outstanding biocompatibility and biodegradability [49]. They have, therefore, been established as the reference points for all other synthesised biodegradable polymers [27].

Polylactide-co-glycolide acid is a thermoplastic aliphatic polyester with good biocompatibility, good mechanical properties and biodegradability. It is easily formulated into different devices for the loading of peptides, DNA, RNA,and other macromolecules [25]. Polylactide-co-glycolide acid is also well established and popular biodegradable polymer amongst other biodegradable polymers [] because of its long clinical experiences, hydrolytic degradation process and sustained drug release [] .

The understanding of the physiochemical and biological properties will help in choosing the right microencapsulation process for drug loading and drug release from polylactide-co-glycolide acid [28]. Polylactide exists in two forms; L-polylactide and D,L-polylactide.L-polylactide is semicrystalial activne and optical active, while D,L-polylactide is amorphous and exist in an optically inactive recenic form [29-36]. The D,L- polylactide is preferable for biomedical and drug delivery systems. This is because it enables even and homogeneous dispersions of therapeutic agents within the polymeric matrix that serves as the drug carrier [36, 37].

Polyglycolide is more crystalline and less hydrophobic (more hydrophilic), compared to polylactide. Therefore, lactide-rich polyglycolide is less hydrophilic, absorbs less water and subsequently degrades more slowly [25, 29, 37].Polylactide-co-glycolide acid, which is the copolymer of lactide and glycolide,

can be processed into any shape, size and it can encapsulate molecules of virtually any size [28]. It is soluble in wide range of common solvents, including chlorinated solvents, tetrahydofuran, acetone or ethyl acetate [33, 35].

2.7 Micro encapsulation of therapeutic agent within the PLGA (Single Emultion Process)

Drugs and proteins are the most rapidly growing class of pharmaceuticals for which controlled or targeted release is used to increase specificity, lower toxicity and decrease the risk associated with treatment [38]. In order to increase the shelf live, reduce the hydrolytic degradation and promote the long half life of peptides, proteins and therapeutic agents, solid formulations are required [38, 39].

In many biomedical applications, such as the treatment of prostate cancer, atherosclerosis and breast cancer, injectable biocompatible and biodegradable PLGA microspheres, nanospheres, microcapsules and nanocapsules will aid the treatment of disease and reduce the use of surgical implantable devices. The release of therapeutic agents (e.g paclitaxel, prodigiosin) is controlled by the diffusion and bulk erosion of the PLGA [38]. Solvent encapsulation or extraction methods have been widely used in the encapsulation of drugs. This is especially true for the encapsulation of hydrophobic drugs into biodegradable and biocompatible polymers. This can be done without reducing the bioactivity of the therapeutic drugs and improving the long term delivery of the therapeutic drug [41]. Single emulsion processes will be used in fabricating Paclitaxel-loaded PLGA microspheres. In this work, it is important to review the prior in this area. In this case, the polymer (which in this case is polylactide-co-glycolide acid) of a specific amount is first dissolved in an immiscible, volatile organic solvent (e.g. dichloromethane, ethyl acetate, acetone, methyl acetate) [41, 42]. The therapeutic agent is either dissolved or dispersed into the drug solution. The drug solution is added or suspension is added into a continuous phase, which is an aqueous solution that contains an emulsifier (e.g poly vinyl alcohol

(PVA) [43], Carbopol® 951 [44], methyl cellulose [45]), with an appropriate stirrer and temperature [38, 41]. The organic solvent in the dispersed phase is removed either by evaporation or extraction.

In the solvent evaporation process, hardening of the emulsion into microspheres occurs when the volatile organic solvent in the dispersed phase leaches into the continuous phase and evaporates from continuous phase at atmospheric pressure. This process becomes faster at moderate temperature or in vacuum [41]. It is much more applicable for long term delivery with lesser pores.

In the solvent extraction process, the emulsion is transferred to a large amount of water or other quenching medium. The extraction of organic solvent occurs faster than in the solvent evaporation process [41]. Thus, microspheres produced by solvent extraction process are more porous than the ones produced by solvent evaporation. The porous structure usually results in faster release of peptide/protein drugs [41]. Finally, the microspheres are obtained by centrifugation at moderate revolution per minute (rpm).

This is followed by filtration or sieving. The microspheres are washed in distilled water or de-ionised water and freeze-dried using lyophilizer [41, 46-48]. All the stages explained aforementioned are shown in Fig.2.7.

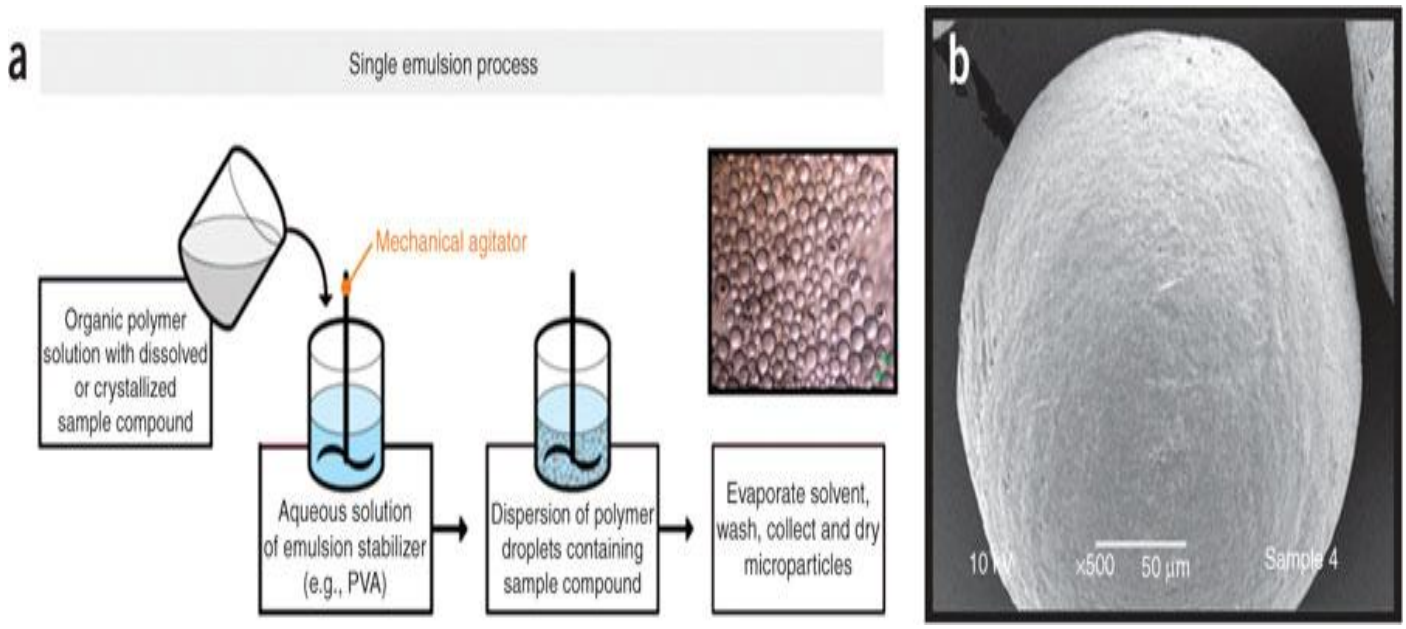

Fig 2.7 (a) Stages in single emulsion process (b) Scanning electron microscopy of the microsphere produced by single emulsion process[www.wikipedia.org]

This method will be employed in synthesing paclitaxel/prodigiosin loaded polylactide-co-glycolide acids (PLGA) , which will find its application in treating cancer and atherosclerosis lesion.

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Chapter Three

3.0 Materials and Methods

The major challenge in combating cancerous tumor is the lack of selectivity and specificity. This results into severe side effect, affecting the normal cells and reducing the therapeutic index of the drugs [1]. Several medical interventions in combating cancers (such as radiation therapy, chemotherapy, hyperthermia e.t.c) have only succeeded in prolonging the life of cancer patients for a short period and new tumors replace the old ones, resulting into revisiting therapies[1] . To fully takes care of this challenges, Our approach towards the treatment of cancer (breast cancer, prostate cancer and cervical cancer) and an optimal formulation for atherosclerosis, is through injectable biodegradable microspheres(which takes care of selectivity), which are loaded with anti-poliferative /anti-restenotic . These microspheres are expected to deliver therapeutic agent to the tumor site and degrades through hydrolysis or biodegradable (when body fluid or enzymes acts catalyse for degradation). The sizes of the microspheres correlate with that of tumor size , which will enhance localized delivery of anti-cancer drugs, with no cytotoxic effects on the normal cells and effective delivery of therapeutic agents.

3.1 Materials for Single Emulsion Encapsulation and Water Absorption

Polylactide-co-glycolide of different ratios of lactide to glycolide compositions and polylactide (50:50, 75:25, 65:35 and molecular weight 30,000-60,000, 66,000-107000, 40,000-75,000, and 75,000-120,000 respectively) were obtained from Sigma Aldrich, St Louis, MO,USA. The paclitaxel (anti-poliferative/ anti-restenotic/anti-cancer drug) was obtained from LC Laboratories, Woburn, MA, USA. For the dissolution of PLGAs, dichloromethane, ethyl acetate, and acetone were obtained from BDH Chemicals, Poole Dorset, England. Poly vinyl alcohol $(C_2H_4O)n$ of 300g mass was obtained from BDH Chemicals,

Poole Dorset, England. Sodium dihydrogen Orthophosphate (NaH_2PO_4), Sodium Chloride (NaCl), Potassium chloride (KCl), Potassium hydrogen Orthophosphate (KH_2PO_4), Tween 20 were obtained from BDH Chemicals, England.

3.2 Water Adsorption by Polylactide-co-glycolide Acids (PLGAs)

The water absorption of polylactide-co-glycolide acid (PLGAs) is controlled by the ratio of lactide to glycolide. The lactide-rich polylactide-co-glycolide copolymers are more hydrophobic.This means that such copolymers are less hydrophilic [21]. To fully understand the hydrophobicity, stages of degradation and the behavior of PLGA in water, swelling experiment were used to study the water uptake of PLGA. The experiments were designed to mimics the behavior of PLGAs with different composition in the body fluid.

3.2.1 Experimental Methods

The water bath (Fisher Scientific Isotemp Water Bath, model 2321, Marietta, OH, USA) was filled with 10 litres of water and set to 37ºC. The reason for this value of temperature is to mimics the body temperature. Microparticles of each of the PLGAs (50:50, 75:25,100:0) were measured using weighing balance (OHAUS, Analytical model AR3130, Nanikon, Switzerland) and the corresponding value was recorded as the initial mass.

The measured masses were kept in a labeled falcom tubes. Total of twenty-four (24) samples (two samples for each hour) were soaked with two samples for durations between 1-12 hours.

Figure 3.1 Set up for Water Absorption Experiment

3.3 Single Emulsion Micro-Encapsulation Method

The Single Emulsion Micro-encapsulation (SEM) method is a common method for the entrapment of hydrophobic drugs into polylactide-co-glycolide acids (PLGAs) [2]. It involves an oil-in-water (o/w) emulsification process. The single emulsion micro-encapsulation process involves the dispersion or dissolution of drugs in a polymer solution [12]. The method yields polymeric microspheres with low porosity and a long term release of drugs [2]. The Stages involved in the single emulsion micro-encapsulation method are: the dissolution of PLGAs in organic solvent; the dispersion and dissolving of drugs in polymer solution; the transfer of drug-polymer solution into emulsifier solution, the sonication of the compound solution, and the transfer of the compound solution into a large volume of emulsifier solution (under a mechanical agitation) for the removal of organic phase (organic solvent) [13-17]. This method was used to produce paclitaxel loaded PLGAs microspheres in this work.

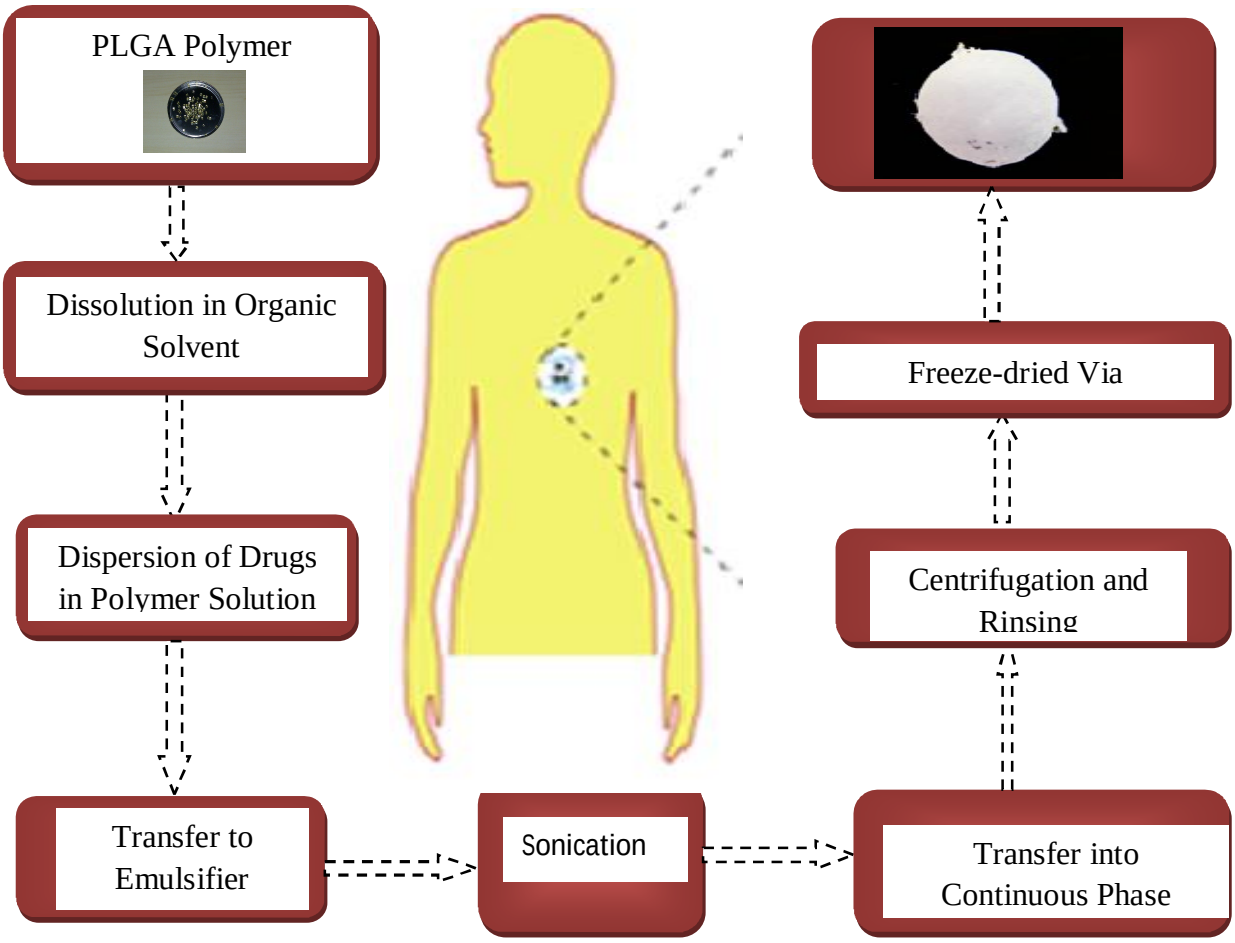

Figure 3.2 Stages in Single emulsion micro-encapsulation process [designed by Adedeji, Abimbola Feyisara]

3.3.1 Role of Poly vinyl alcohol Stabilizer

Stabilizers or surfactants are amphiphilic molecules that posses both hydrophilic and hydrophobic parts. The hydrophilic moiety is called the head and the hydrophobic part the tail (or tails) [5]. The hydrophobic part may consist of a single chain or may have up to four chains [5]. The head can be a charged or uncharged polar group. Depending on the nature of the head groups, stabilizers are classified into anionic, cationic, non-ionic and zwittterionic (amphoteric) [5, 6].

The type of the drug dissolved and the conditions of the target site will determine the type of surfactant used to carry the medicine. It serves as surfactant, stabilizer, or emulsifier in preparation of PLGA microspheres. The groups of surfactants that have proven results in stabilizing and preventing coalesce of the microspheres are polyvinyl pyrrolidone (PVP), Tween 80, Fluonic 127 (poloxamer 407), Fluonic 68 (poloxamer 188), didodecyl dimethyl ammonium bromide (DMAB), carbopol (prop-2-enoic acid) , polyvinyl alcohol (PVA), Tocopheryl polyethylene glycol 1000 succinate (TPGS) [2, 3, 7].

They induce specific zeta potentials, which are the electrical potentials that exist across the interfaces of all solids and liquids . The value of zeta potential is a very important characteristic of any particle. The zeta potential also has a significant influence on the stability of the particle. With the control of the specific zeta potentials, PVA (or PVP) can reduce the agglomeration of particles because particles of the same charge are not attracted to each other [8, 9].

Furthermore, the concentration of poly vinyl alcohol (PVA) controls the size of the microspheres and the release kinetics of the entrapped drugs [4]. In addition, it improves the surface chemistry of the PLGA microscopes. PVA and PVP create negatively charged PLGA particles [8].

To study the role of poly vinyl alcohol (PVA), different concentrations, 0.2%, 0.5%, 1.0%, and 2% w/v aqueous PVA were prepared for the emulsification process.

3.3.2 Preparation of Aqueous PVA solution

Three Pyrex containers with a volume of 1 liter were filled with tap water. They were then heated up to temperature of 80-90 ºC. The measured poly vinyl alcohols were sprinkled into the water maintained at 90 ºC under continuous stirring using magnetic stirrer (JENWAY 1000). The process was continued for 30-45 minutes until a viscous, clear, colorless solution was formed.

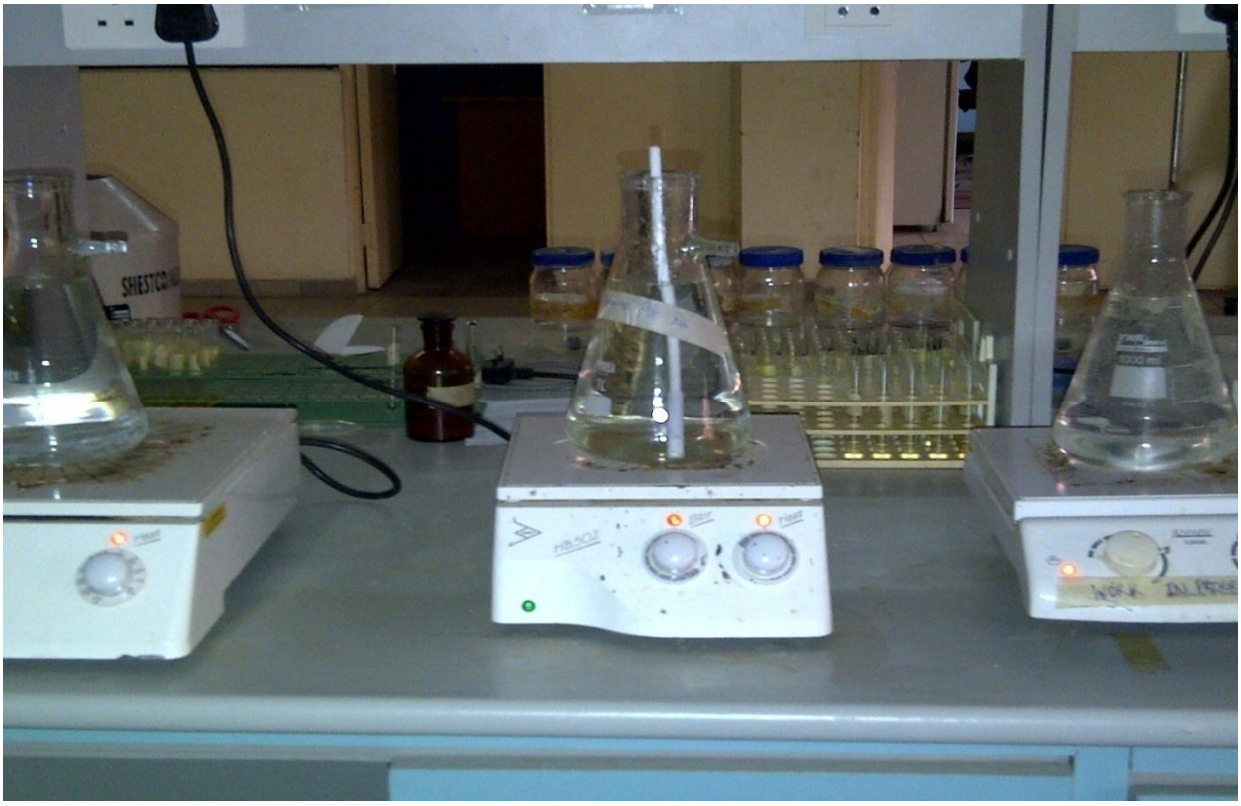

Figure 3.3 Dissolution of PVA in hot tap water.

3.3.3 Dissolution of polylactide-co-glycolide acid

Generally, Organic solvents like acetone, dichloromethane, methylene chloride, ethyl acetate, chloroform, benzyl alcohol, are good dissolving solvent for polylactide-co-glycolide acids. However, these solvents are toxic and may cause inflammation [10, 11].

To promote biocompatibility and low toxicity, ethyl acetate is a choice. It has low toxicity, low boiling point (77 °C) but is inflammable [12].

Experimental Procedure

For each mixture of PLGA (500 mg) was measured and added into a 10 ml stirrer. The solution of the polymer was transferred into 50 ml of 0.5% (1%,2%) w/v of aqueous PVA solution and sonicated for 10 minutes. The emulsion formed was slowly poured into a large volume of aqueous PVA solution under continuous stirring.

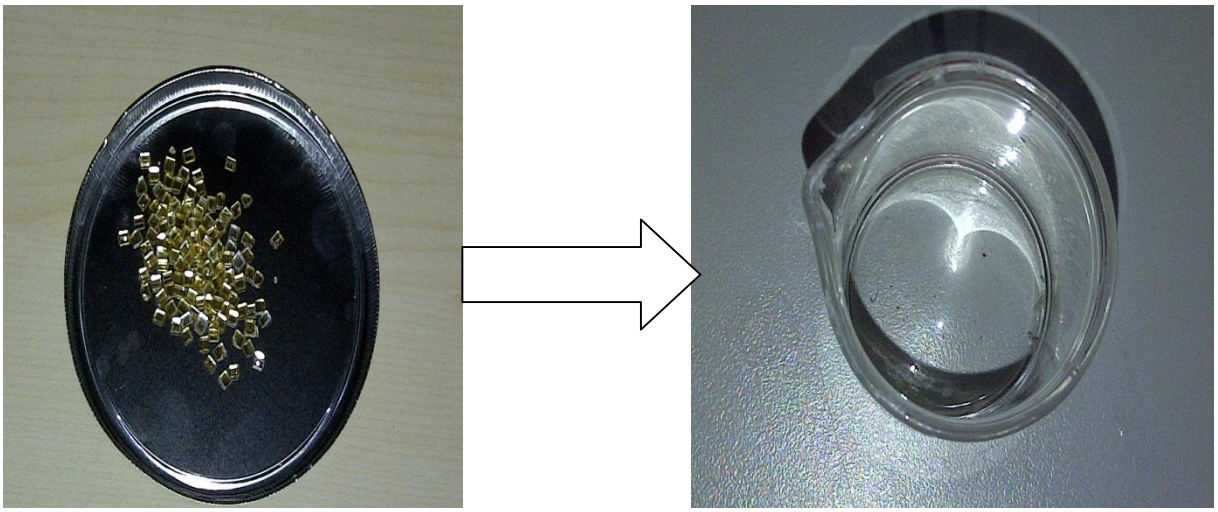

Figure 3.3 From solid phase to liquid phase by dissolution in Organic solvent

Figure3.4 Organic solvents used for dissolution of PLGAs

Figure3.5 Ultrasonic water-bath sonicator

3.3.4 Hardening of the microspheres and removal of organic solvent

A large volume of emulsifier solution (300 ml) that contained the micro-droplets was stirred continuously for 12 hours (over-night). This was done to remove the organic solvent that was used in dissolving the PLGAs and hardened the microspheres. To sort out the microspheres, the compound solution was centrifuged at 2500 rpm for 10 minutes at 15ºC. The microspheres were collected and rinsed three times with distilled water to remove the excess PVA emulsifier [19]. The rinsed microspheres were kept in petri dishes and pre-frozen in a -80ºC freezer over-night [3, 19]. The frozen microspheres were lyophilized (freeze-dried) for 16 hours at a temperature -41ºC and a pressure of 0.101325MPa [17, 18, 19]. The resulting freeze-dried samples were then kept at 4±1ºC in a vacuum dessicator [3, 19].

Figure 3.6 Magnetic Stirrer for continuous stirring

Figure 3.7 Centrifugation for sorting out the microspheres

Figure 3.7 Lyophilizer for freeze-drying

Figure 3.8 Desicator for storage of paclitaxel loaded PLGAs microspheres

3.4 Experiments on Drug Loading Efficiency

It is very important to quantify the total amount of paclitaxel that gets entrapped within the microspheres formed for different PLGAs compositions. To determine the drug contents within the microspheres, the UV absorbance was measured at 250nm using a UV/VIS Spectrophotometer for different standard paclitaxel solutions were obtained. The standard paclitaxel solutions require dimethyl sulphoxide (DMSO) and Phosphate Buffer Solution as solvents. The former was purchased from BDH Chemicals, Poole Dorset, England, while the later was prepared in the laboratory.

3.4.1 Preparation of Phosphate Buffer Saline Solution

Pyrex flask with a volume of 1litre was filled with 800ml of distilled water. It was then placed on a magnetic stirrer and stirred at 200-400 rpm. The stirring was done at a minimum revolution per minute to avoid the introduction of oxygen into the solution. 8g of Sodium Chloride (NaCl), 0.2g of Potassium Chloride (KCl), 1.44g of Sodium hydrogen Orthophosphate (Na_2HPO_4) and 0.25g of Potassium Hydrogen Orthophosphate ($KHPO_4$) were added to the distilled water during stirring. All the solute dissolves in about 5 minutes. The pH of the solution was then checked using a pH meter (Jenway 3510) . Instantaneously, droplets of 1M Hydrochloric acid were added until the pH value reached 7.4. An Additional 200 ml was added to the solution to produce a volume of 1 litre. The solution was transferred to the autoclave. The autoclave was set at atmospheric pressure (15lb/in^2 or 0.1034 MPa) and the duration of the autoclave was 20 minutes. The solution was subsequently cooled and stored at room temperature. The role of phosphate buffer saline solution was used to mimic blood whose pH is 7.4.

Figure3.9 Set up for PBS preparation and Autoclave

3.4.2 UV absorbance for standard solutions of paclitaxel

Paclitaxel (10 mg) was accurately weighed and dissolved in 30 ml of dimethyl sulphoxide (DMSO). The volume was made up to 100 ml with 30:70 dimethyl sulphoxide (DMSO): PBS to give a stock solution of 100μg/ml. Aliquots of 100 μg/ml solution were transferred into different 10 ml volumetric flasks. The volumes were then adjusted with 30:70 dimethyl sulphoxide (DMSO): PBS to give final concentrations of 2.0-22.0μg/ ml [3, 20]. The absorbance was measured at 250 nm against 3 ml dimethyl sulphoxide (DMSO) made up to 10 ml with 30:70 dimethyl sulphoxide (DMSO): PBS as a blank. Furthermore, 25mg of paclitaxel-loaded PLGA microspheres were dissolved in 3ml of dimethyl sulphoxide (DMSO). Then, adjust up to 10 ml, 30:70 dimethyl sulphoxide (DMSO): PBS was added. A volume of 3ml of this solution was then poured into a cuvette (1cm path length). The absorbance was then measured at the same wavelength using the UV/VIS .

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Chapter Four

4.0 Results and Discussion

4.1 Water Absorption of polylactide-co-glycolide acids

The degradation of polylactide-co-glycolide acids (PLGAs) is controlled by hydrolysis [1]. This is the most important mode of chemical degradation in which the organic materials (e.g polymer) break down into oligomers and monomers as the water penetrates into the bulk materials [2]. There are three different stages associated with degradation of polylactide-co-glycolide acids in body physiological condition [2].

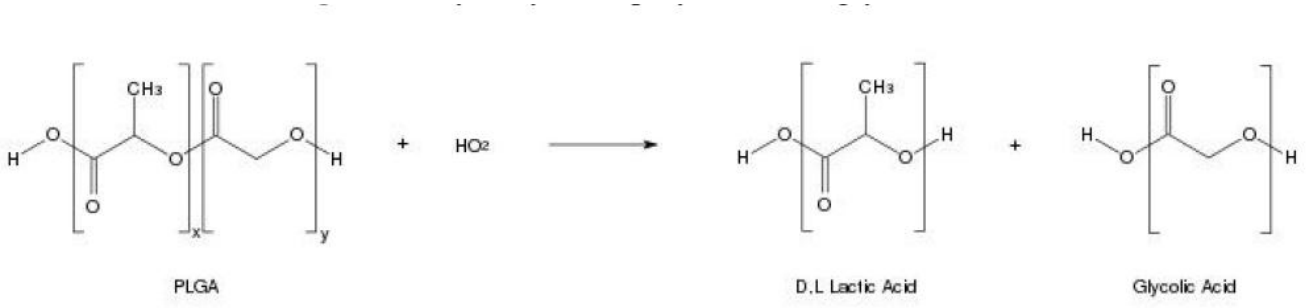

Figure 4.1.1 Hydrolysis reaction of polylactide-co-glycolide acids

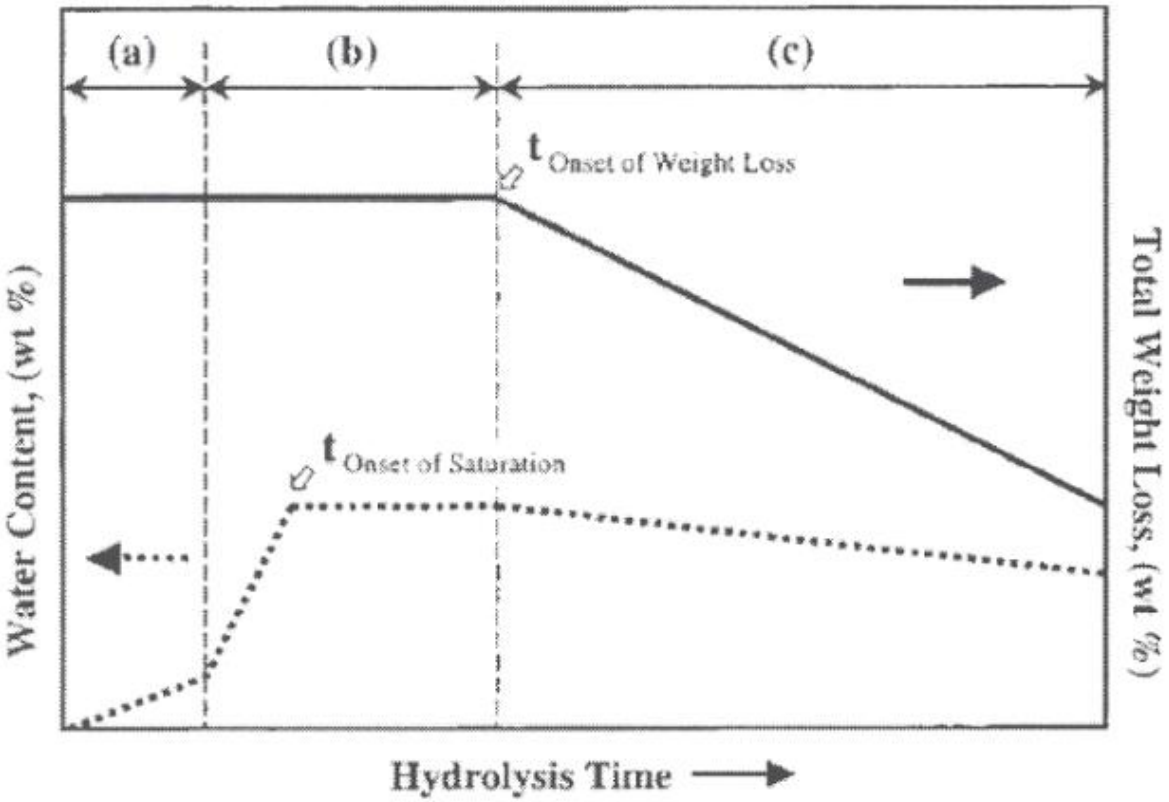

Figure 4.1.2 Stages in the degradation of polylactide-co-glycolide acids [2].

The stages are (Figure 4.1.2);

(a) Water penetrates deeply into the interior areas;

(b) The functional groups in polymer chains hydrolyze and absorb the water;

(c) Water reacts with the polymer resulting in cleavage of covalent chemical bonds. This leads to break down of polymer into oligomers and monomers (transported from the polymer bulk controlled by diffusion).The release of degradation products leads to the mass loss which is characteristic of erosion.

Based on the results obtained from the water absorption experiment, the first stage (stage a) was attained in the experiment.

Figures 4.1.3, 4.1.4 and 4.1.5 show the weight gain graphs of different PLGAs (different lactide content), the graphs shows an increase in the mass of PLGAs as the water penetrates the interior areas of the polymer. There are some few deviations identified with 5 hours, 3 hours, and 3 hours of hydrolysis time for PLGAs 50:50, PLA , PLGA 75:25 respectively. The swelling ratio decreased (highlighted in the tables) and deviate from the straight line relationship. This might be as result of measurement errors while taken the readings for initial mass of the PLGAs. The expression used to calculate the swelling ratio, r , is given by;

$$r = \frac{M_t - M_0}{M_0} \times 100 \text{---------- (4.0.0)}$$

In addition, the rate at which the water penetrates into the interior part of the PLGAs is very slowi as observed in the data for each composition. This is consistent with the prior work [1p] that shows that PLGAs are highly hydrophobic. Hence, as the lactide content increases, the water absorption capacity of the copolymers (PLGAs) decreases. To break the covalent bonds in PLGAs, longer times in water or aqueous environments are required.

Time (hr)	Initial masses (g)	Final masses (g)	Change in mass (g)	Swelling ratio (%)	Av.swelling ratio (%)
1	0.0124	0.01253	1.30E-04	1.048	6.87E-01
	0.01225	0.01229	4.00E-05	0.325	
2	0.01268	0.0128	1.20E-04	9.46E-01	8.93E-01
	0.01309	0.0132	1.10E-04	8.40E-01	
3	0.01315	0.01329	1.40E-04	1.065	2.199
	0.0132	0.01364	4.40E-04	3.333	
4	0.01378	0.01398	2.00E-04	1.451	2.159
	0.11395	0.01435	4.00E-04	2.867	
5	0.014	0.01421	2.10E-04	1.5	1.5
	0.01417	0.01418	1.00E-05	7.06E-02	
6	0.01435	0.01523	8.80E-04	6.132	4.288
	0.01514	0.01551	3.70E-04	2.444	

Table 4.1.1 Initial mass, final mass and swelling ratio of polylactide-co-glycolide acids 50:50

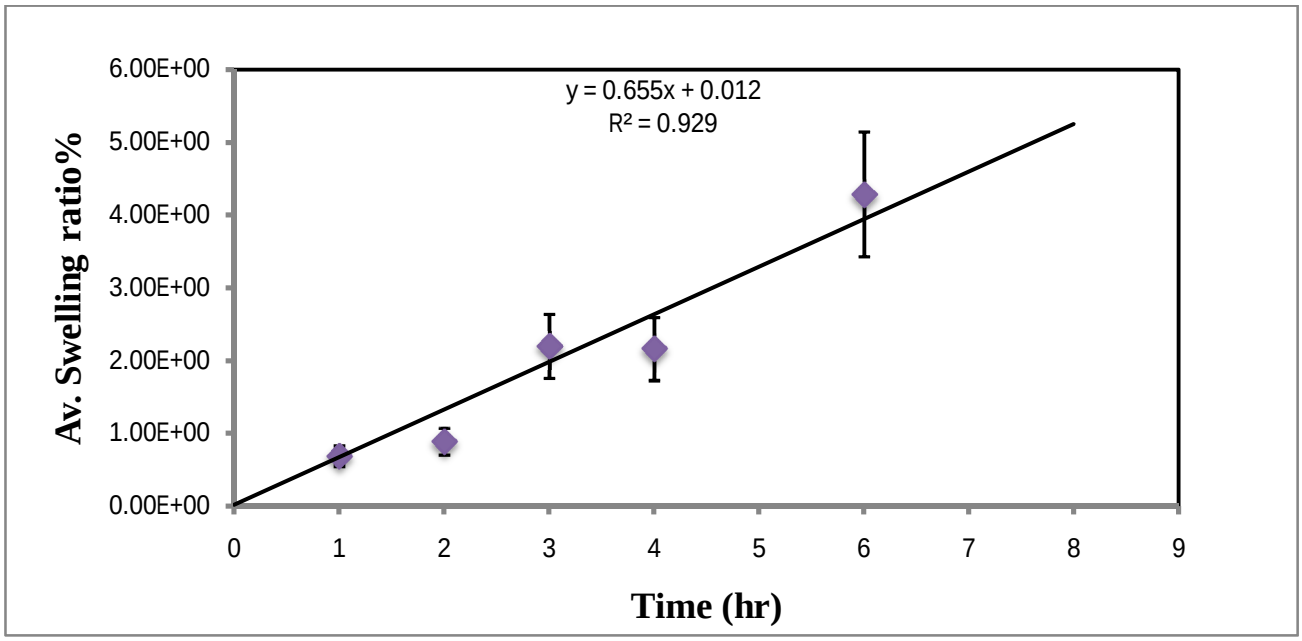

Figure 4.1.3 Plots of average swelling ratio against soaking time for PLGAs 50:50

Time (hr)	Initial mass(g)	Final mass (g)	change in mass (g)	Swelling ratios %	Av.Swelling ratio %
	0.01566	0.01607	4.10E-04	0.02618	2.55E+00
1	0.01567	0.01606	3.90E-04	0.02489	
	0.01431	0.01448	1.70E-04	1.19E-02	1.468
2	0.01431	0.01456	2.50E-04	1.75E-02	
	0.0137	0.01386	1.60E-04	0.011679	1.1679
3	0.01423	0.01423			
	0.01702	0.01811	1.09E-03	0.06404	9.487
4	0.01679	0.0189	2.11E-03	0.1257	
	0.01436	0.01621	1.85E-03	0.1288	9.94
5	0.01457	0.01559	1.02E-03	0.07	

Table 4.1.2 Initial mass, final mass and swelling ratios of polylactide acids

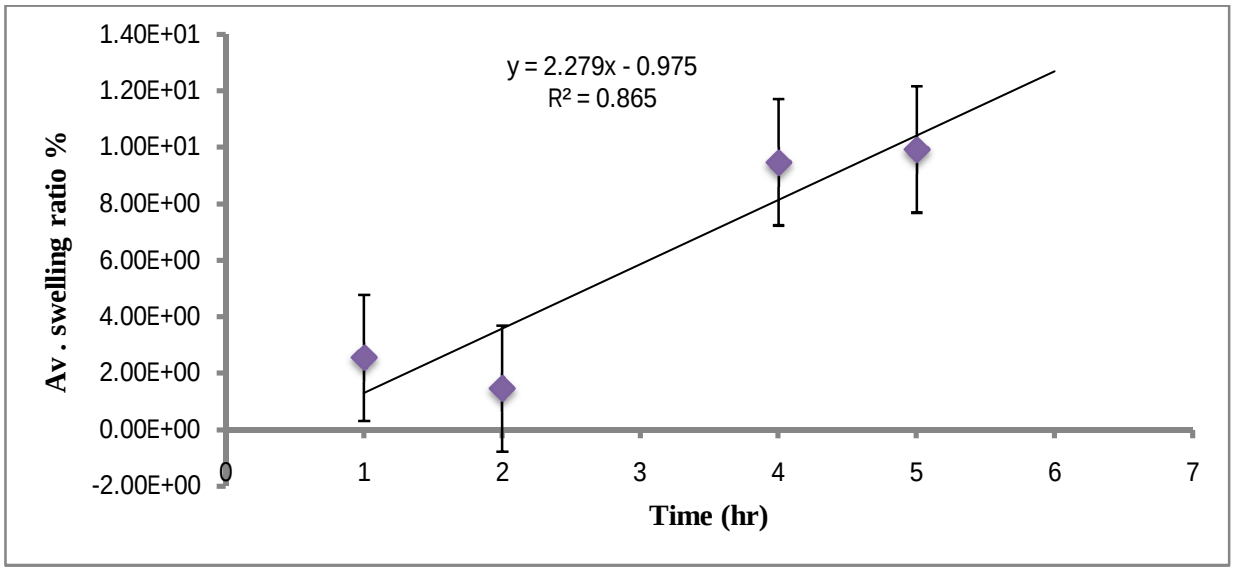

Figure 4.1.4 Graph of average swelling ratio against soaking time for polylactide at 37°C

Time (hr)	Initial masses	Final mass	Change in mass	Swelling ratio (%)	Av. Swelling ratio %
	0.02311	0.02324	1.30E-04	5.63E-03	4.62E-01
1	0.01938	0.01945	7.00E-05	3.61E-03	
	0.02472	0.02494	2.20E-04	8.90E-03	6.09E-01
2	0.0244	0.02448	8.00E-05	3.28E-03	
	0.02666	0.02672	6.00E-05	2.25E-03	2.29E-01
3	0.02585	0.02591	6.00E-05	2.32E-03	
	0.02885	0.02932	4.70E-04	1.63E-02	8.89E-01
4	0.02687	0.02691	4.00E-05	1.49E-03	
	0.02926	0.02952	2.60E-04	8.89E-03	8.66E-01
5	0.02846	0.0287	2.40E-04	8.43E-03	

Table 4.1.3 Initial mass, final mass and swelling ratio of polylactide-co-glycolide acids 75:25 at 37°C

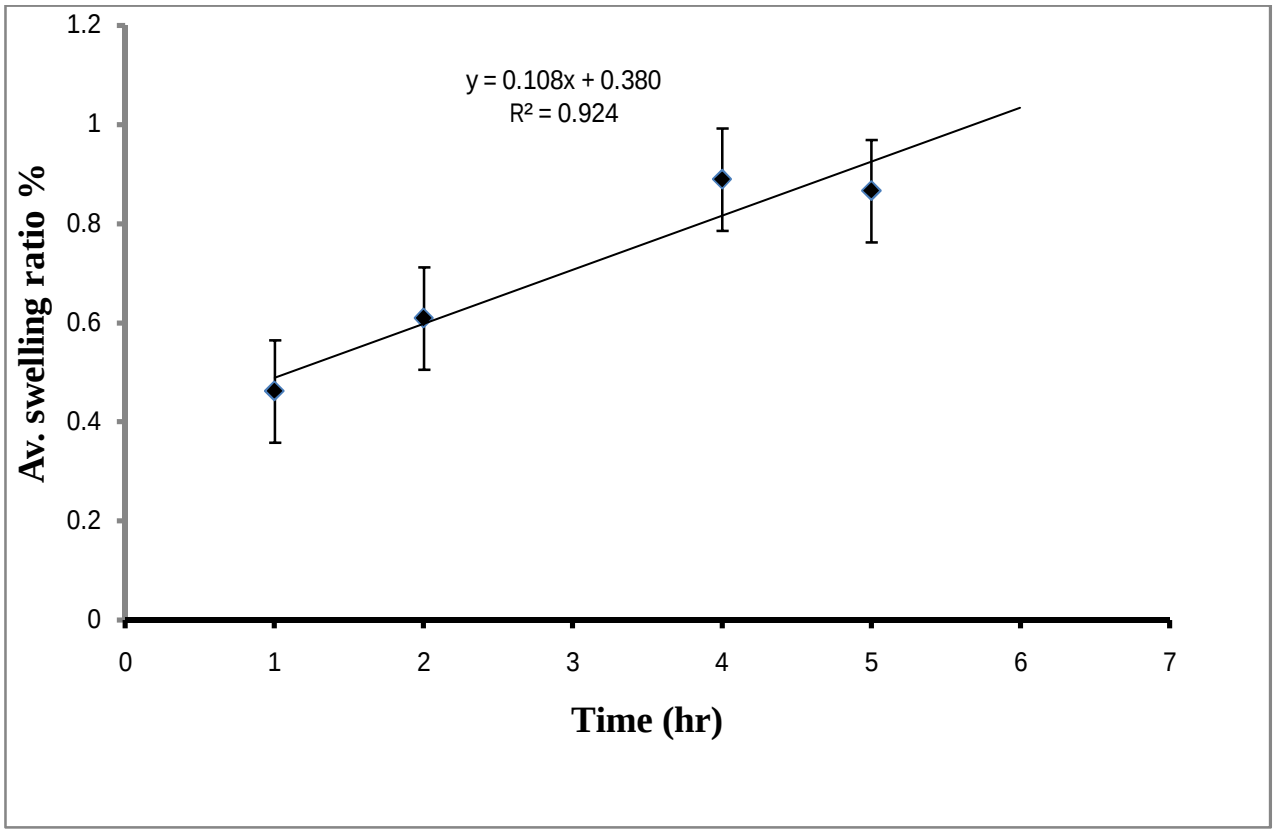

Figure 4.1.5 Graph of average swelling ratios against soaking time at 37°C

4.2 Particle Size, Shape and Morphology

The morphologies and shape of paclitaxel loaded polylactide-co-glycolide acids/ polylactide acids microspheres were obtained using Celestron Digital Microscope (Model #44345LCD Deluxe Digital Microscope) of resolution 320X240 pixels and magnification of 1640 (164X).

Figure 4.2.1 Celestron Digital Microscope (Model #44345LCD Deluxe Digital Microscope) [3]

Most of the microspheres are not perfect spheres but some are near perfect spheres. This might be due to the rapid evaporation of the organic phase at the solvent removal steps. In addition, tiny pores are distributed on the surface of the spheres; these have an advantage and a disadvantage. The advantage is

that the pores serve as the passage through which the drug elutes into the neighboring cancerous cells and atherosclerosis lesion.

The disadvantage is that a large volume of pores can cause a very burst release with no proper control [3].

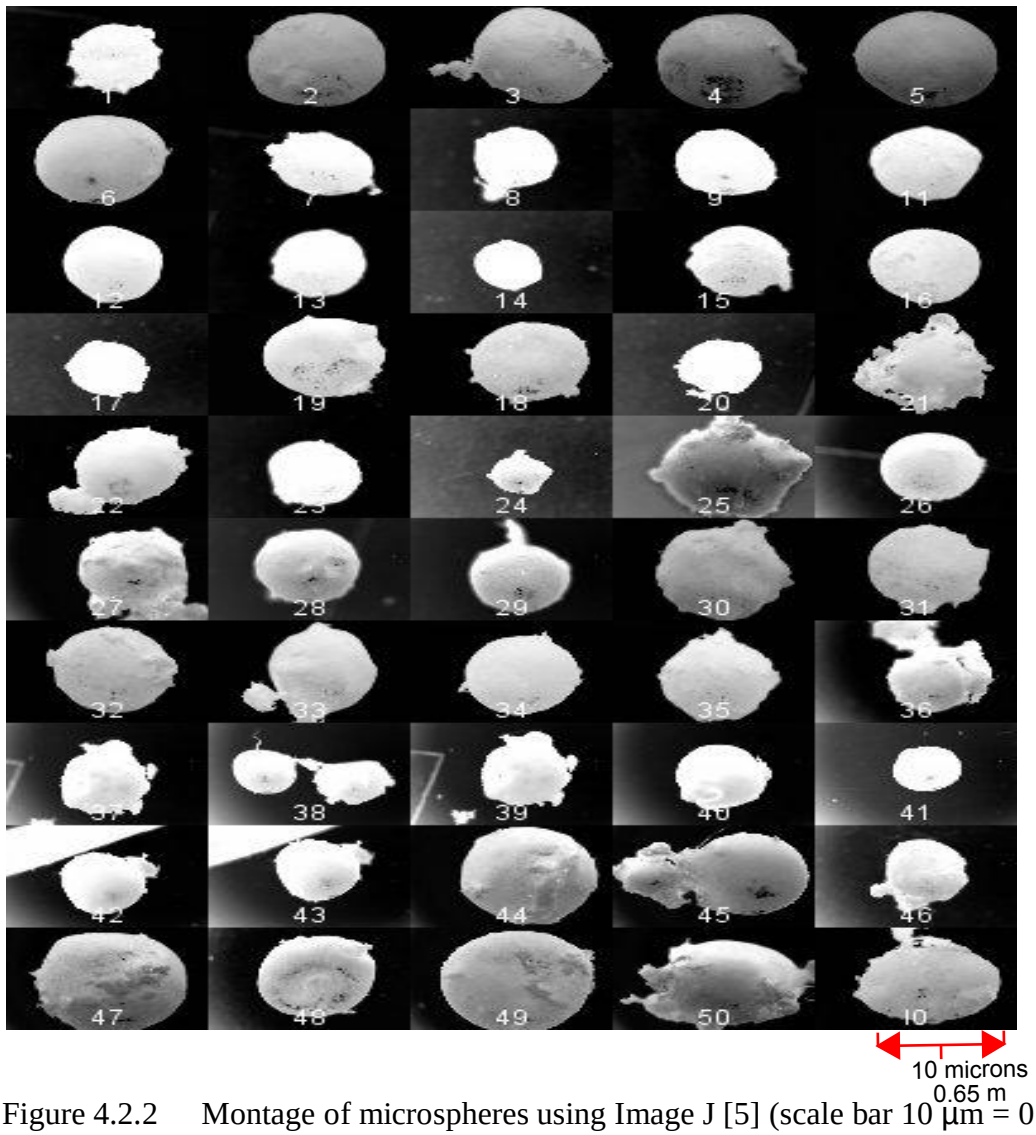

Figure 4.2.2 Montage of microspheres using Image J [5] (scale bar 10 µm = 0.65 in = 0.65m (note 1in = 0.0254 m))

4.3 Particle Size Analysis

Out of the population of microspheres images, 50 microspheres were measured using pdf – xchange viewer (Figure 4.3.2). Due to the irregularity associated with the spherical shape of the microspheres, four different diagonals for each microsphere were measured. The measuring tools in pdf-xchanger viewer measures in inches (with scale ratio of 1in = 1 m), so conversion was done to have the image sizes in SI units (m).

1 inches = 0.0254 meters (4.4.1)

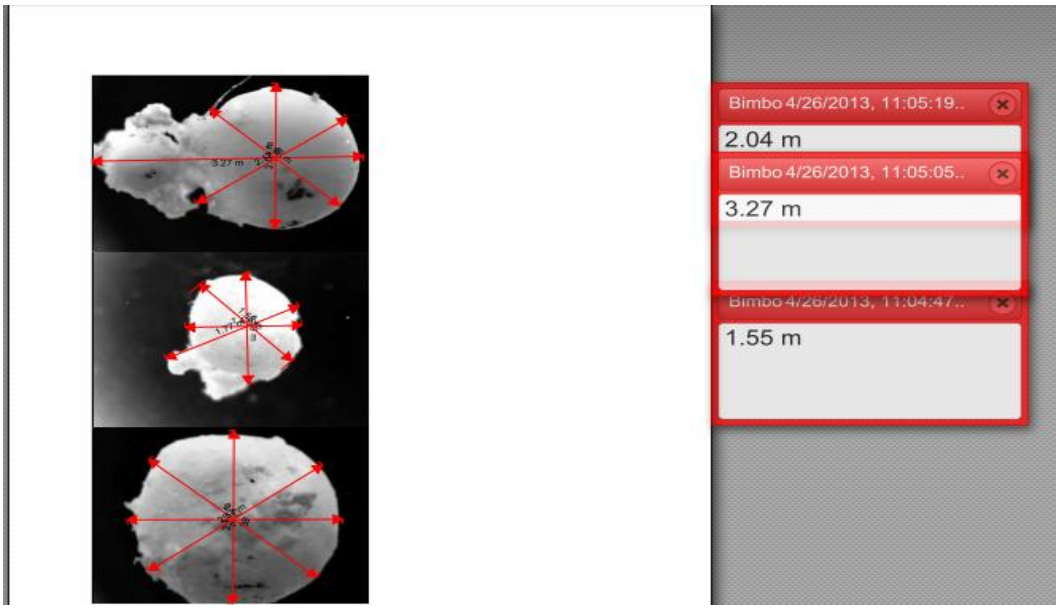

Figure 4.3.2 Pdf – xchanger viewer environment

The values obtained for each sphere is shown in table 4.3.1.

Image No	Diagonal 1(inches)	Diagonal 2(inches)	Diagonal 3(inches)	Diagonal 4(inches)	Av.size(inches)	Av.size in (m)	object size(µm)
1	1.53	1.84	1.68	1.54	1.65	0.04191	25.56
2	2.34	2.29	2.24	2.31	2.3	0.05842	35.62
3	2.41	2.22	2.41	2.31	2.34	0.05944	36.24
4	2.29	2.31	2.42	2.36	2.35	0.05969	36.4
5	2.45	2.25	2.39	2.27	2.34	0.05944	36.24
6	2.18	2.26	2.21	2.3	2.24	0.0569	34.7
7	1.44	1.88	1.73	1.69	1.69	0.04293	26.18
8	1.86	1.57	1.37	1.43	1.56	0.03962	24.16
9	1.63	1.77	1.63	1.54	1.64	0.04166	25.4
10	1.89	1.89	1.78	1.85	1.85	0.04699	28.65
11	1.8	1.72	1.65	1.87	1.76	0.0447	27.26
12	1.91	1.68	1.72	1.83	1.79	0.04547	27.73
13	1.71	1.6	1.68	1.68	1.67	0.04242	25.87
14	1.2	1.09	1.06	1.13	1.12	0.02845	17.35
15	1.75	1.8	1.98	1.7	1.81	0.04597	28.03
16	1.89	1.86	1.84	1.86	1.86	0.04724	28.81
17	1.4	1.34	1.3	1.42	1.37	0.0348	21.3
18	1.96	2	2.16	2.15	2.07	0.05258	32.06
19	1.98	2.04	2.11	1.99	2.03	0.05156	31.44
20	1.51	1.55	1.37	1.31	1.44	0.03658	22.31
21	2.49	2.24	1.99	2.13	2.21	0.05613	34.23
22	2.61	1.68	1.95	1.77	2	0.0508	30.98
23	1.61	1.56	1.64	1.54	1.59	0.04039	24.63
24	0.93	0.91	1.11	0.92	0.98	0.02489	15.18
25	2.48	2.73	2.4	2.24	2.46	0.06248	38.1
26	1.79	1.82	1.67	1.62	1.73	0.04394	26.79

27	1.81	1.78	1.79	1.94	1.83	0.04648	28.34
28	2.39	1.72	1.72	1.7	1.88	0.04782	29.16
29	2.43	2.3	2.19	2.3	2.31	0.05867	35.77
30	2.35	2.04	2.17	1.99	2.14	0.05436	33.15
31	1.98	2.22	2.09	2.2	2.12	0.05385	32.84
32	2.54	1.84	2.47	2	2.31	0.05867	35.77
33	2.03	1.97	2.02	1.85	1.97	0.05004	30.51
34	2.34	2.21	2.02	1.92	2.12	0.05385	32.84
35	2.25	1.78	1.88	2.39	2.08	0.05283	32.21
36	1.92	1.6	1.91	1.74	1.79	0.04547	27.73
37	1.31	1.17	1.39	1.14	1.25	0.03175	19.36
38	1.1	1.08	1.23	1.17	1.15	0.02921	17.81
39	1.9	1.65	1.82	1.75	1.78	0.04521	27.57
40	1.73	1.2	1.58	1.7	1.67	0.04242	25.87
41	1.23	1.46	1.33	1.18	1.24	0.0315	19.21
42	1.65	1.39	1.69	1.54	1.59	0.04039	24.63
43	1.56	2.31	1.65	1.52	1.53	0.03886	23.7
44	2.43	2.08	2.43	2.34	2.38	0.06045	36.86
45	3.29	1.76	2.71	2.12	2.55	0.06477	39.49
46	1.63	2.08	1.51	1.56	1.62	0.04115	25.09
47	2.43	2.58	2.6	2.67	2.57	0.06528	39.81
48	2	1.97	2.28	1.98	2.06	0.05232	31.9
49	2.53	2.56	2.67	2.52	2.57	0.06528	39.81
50	2.25	2.9	2.67	2.52	2.59	0.06579	40.11

Table 4.3.1 Measurement of diagonal, average size and object size of the PLA microspheres

To calculate the object size, the relationship between lateral magnifications, object size/height, and image size/height was used.

$$\text{Magnification} = \frac{\text{Image Height/size}}{\text{Object Height/size}} \quad \text{---------- (4.3.2)}$$

From this expression, the object size, which is the actual size of the spheres were calculated.

The microspheres size range is 15.18 - 40.11 µm and the mean particle size is 29.42 µm.

The particle size distribution is shown in Figure 4.4.3 and 4.4.4.

Bins	Freq
15.18	1
18.18	2
21.18	2
24.18	4
27.18	9
30.18	9
33.18	9
36.18	5
39.18	5
42.18	4

Table 4.3.2 Bins size and frequency of the PLA microsphere sizes

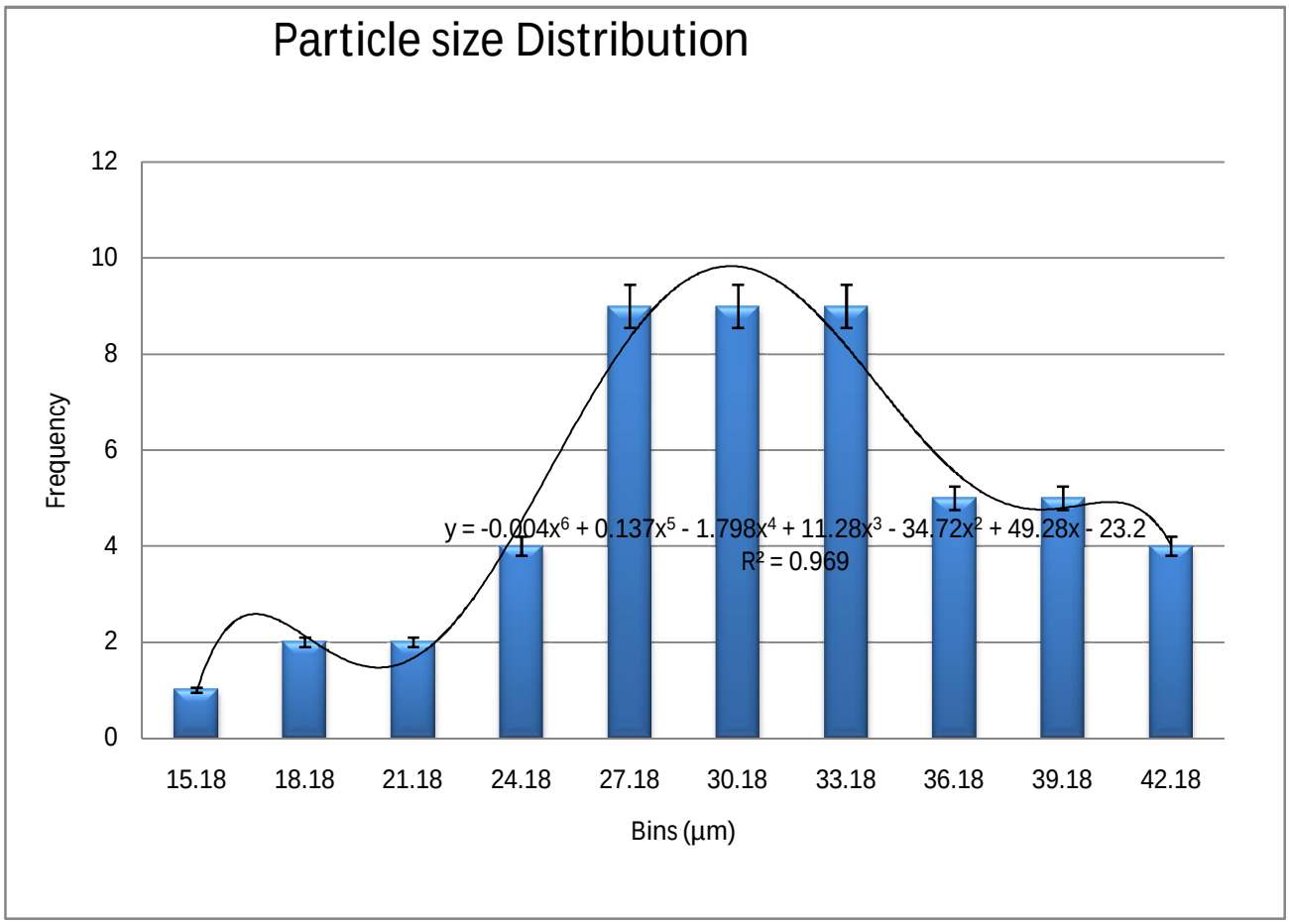

Figure 4.3.3 Histogram of microspheres size distribution using Microsoft Excel

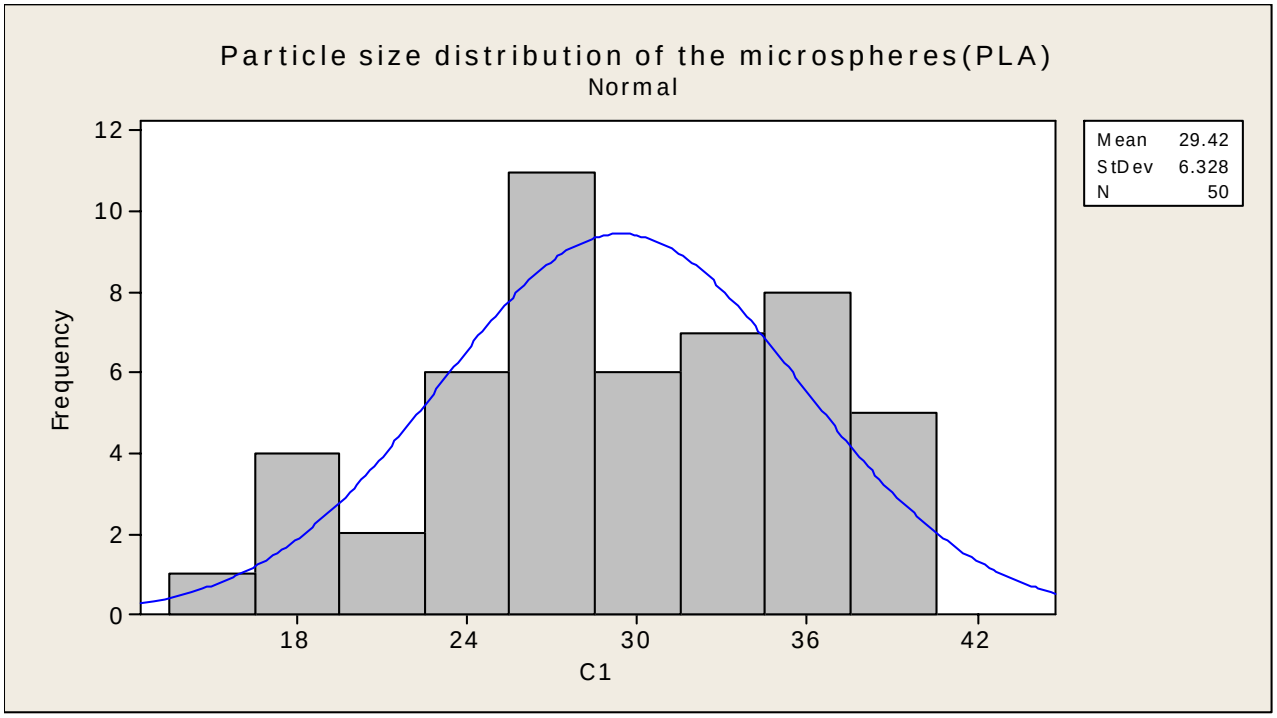

Figure 4.3.4 Histogram of microspheres size distribution using Minitab 16

4.4 Hypothesis Test

Experimental data are associated with an inherent variability which makes the outcomes of the experiment to vary even when the same process is used in million times. This is the case of the particle sizes obtained in single emulsion microencapsulation processes. According to Table 4.3.1, the size of the microspheres ranges from 15.18 µm to 40.11 µm. To characterize the variability associated with the population from which the sample was extracted, the probability distribution function will be used with an appropriate probability model. This will yield information about the unknown parameter of the population as sample data serves as basis for that information.

Hypothesis testing is carried out on the sample to obtain parameters associated with the population and validate the null hypothesis or fail to reject the null hypothesis. The hypothesis statements are written bellow;

Ho: $\Theta_0 = 29.4$ µm, the null hypothesis (status quo) for the population of the sizes of PLA microspheres.

Ha: $\Theta_a \neq 29.4$ µm, the alternative hypothesis

To carry out the hypothesis testing, Minitab 16 was used and the programming steps are written below;

Statistics > Basic statistics > 1-Sample Z > sample in column > hyposynthesis mean > option > confidence level (95%) . The result obtained is summarized in the table 4.4.1below;

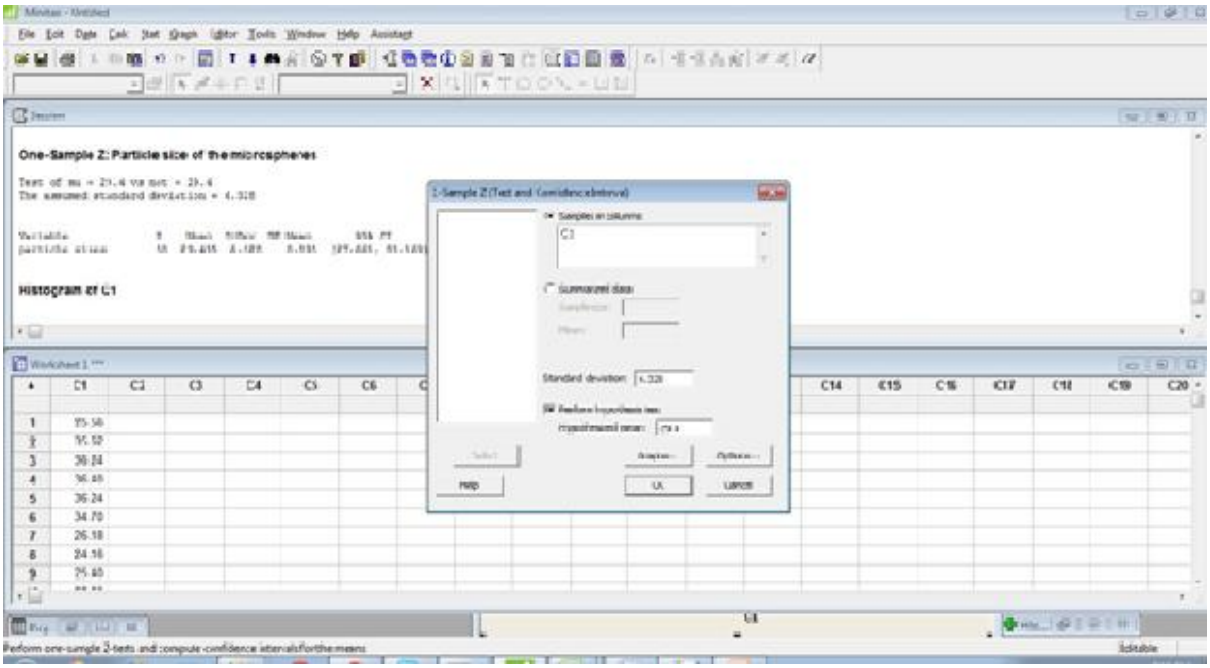

Figure 4.4.1 Minitab 16 Environments

One-Sample Z: Particle size of the microspheres

Variable	N	Mean	St Dev	SE Mean	95% CI	Z	P
Particle sizes	50	29.415	6.328	0.895	(27.661,31.169)	0.02	0.986

Table 4.4.1 Minitab results for hypothesis test

From the results in table 4.4.1, figure 4.4.2, 4.4.3, 4.4.4, there are two main parameter that guilds the decision on whether to accept the null hypothesis for the population or to reject the null hypothesis and accept the alternative hypothesis [4]. These are obsersved significance level (p-value) and the critical region associated with the Z-statistics [4].

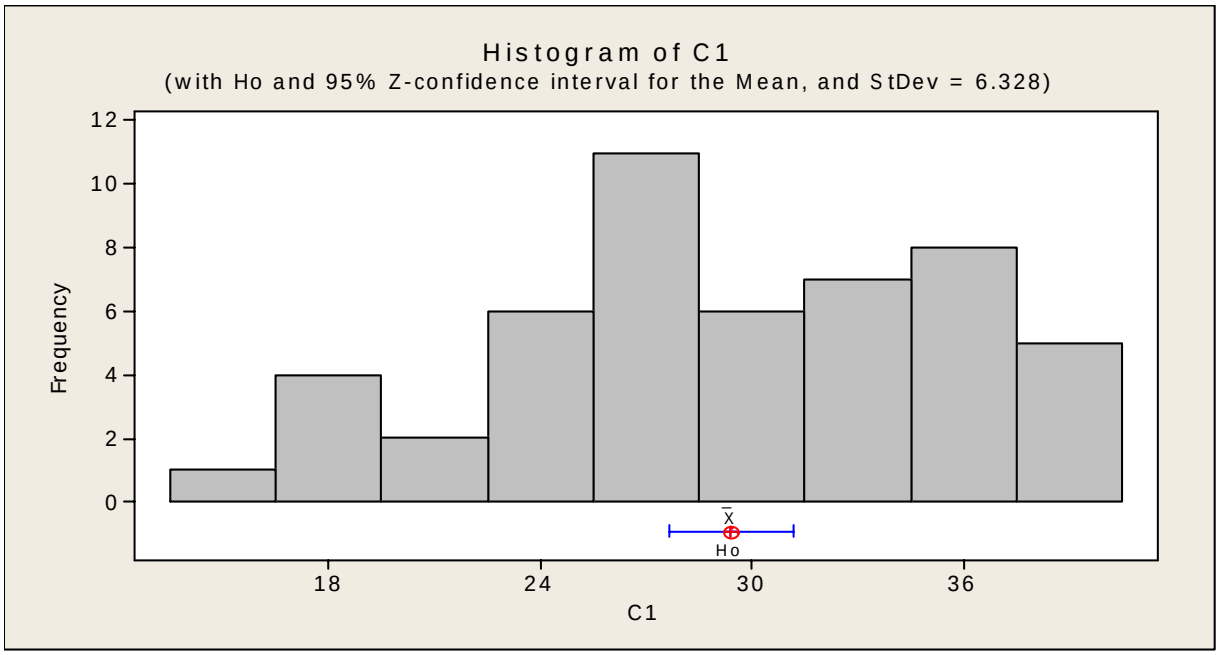

Figure 4.4.2 Histogram showing the frequency distribution of PLA microsphere sizes with hypothesized mean

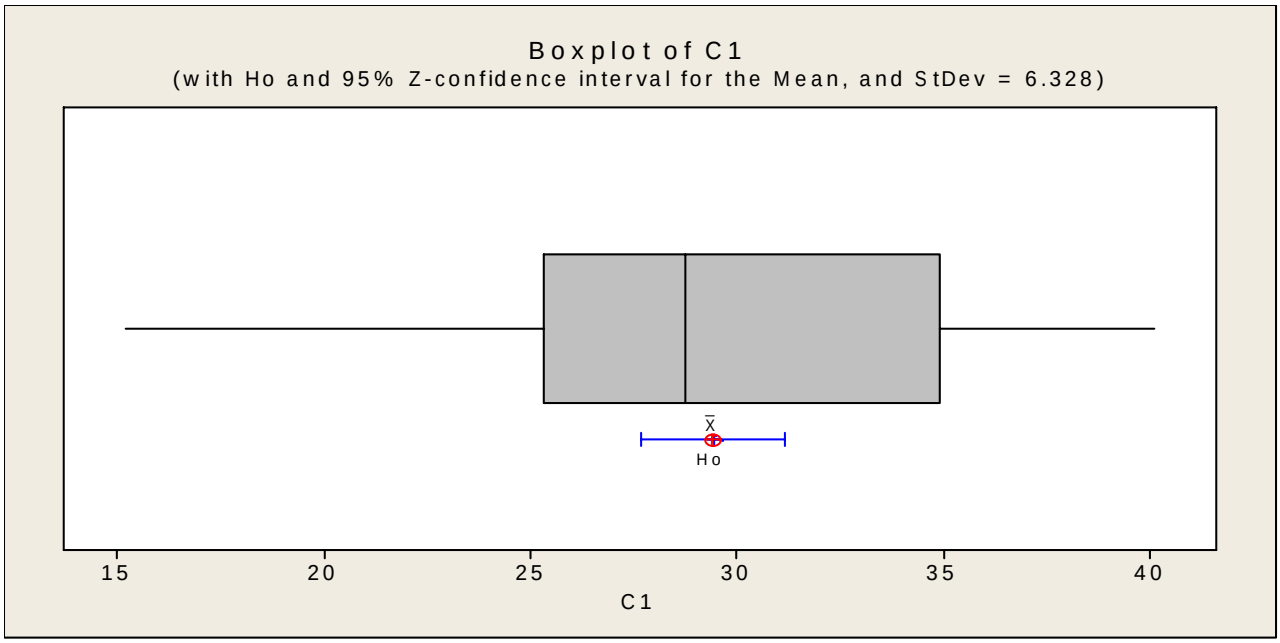

Figure 4.4.3 Box plot for PLA microsphere particle size with hypothesized mean for the population of PLA microspheres

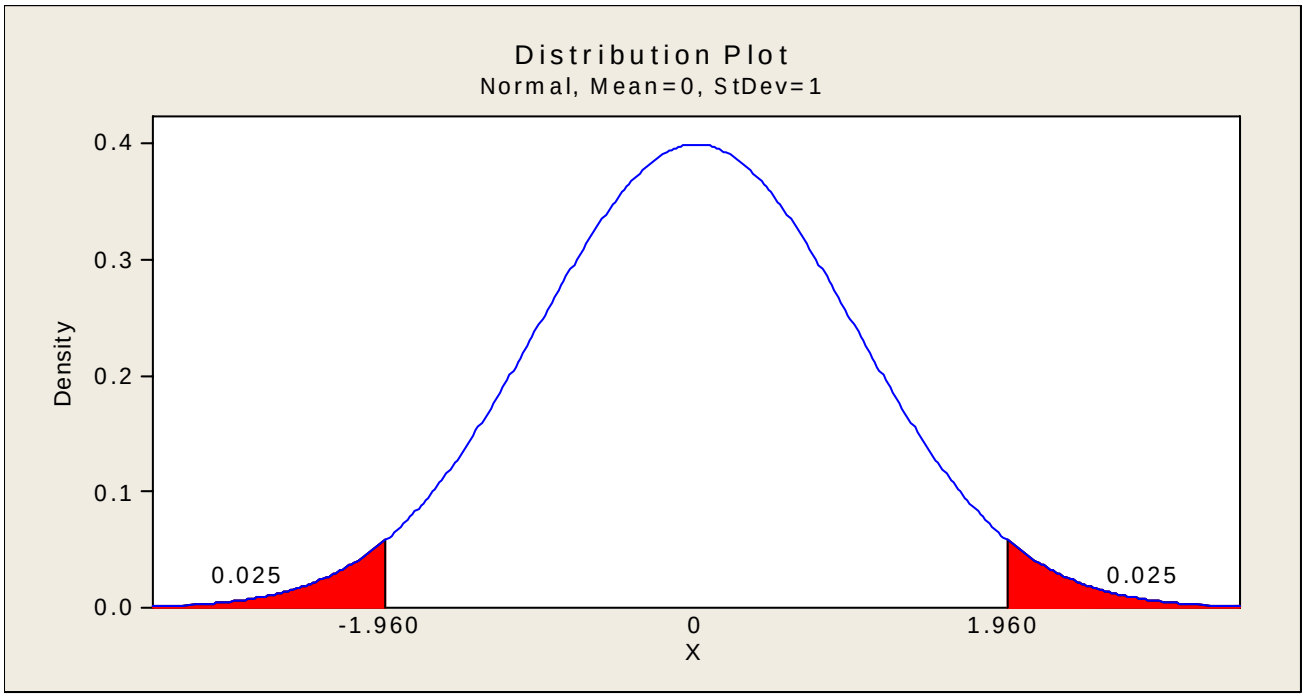

Figure 4.4.4 Critical region plot for Z-statistics (two-sided) characterizing the population

The p-value is 0.986 which is far greater that the significance level, i.e, p>0.05. This is the first evidence to fail to reject the null hypothesis. In addition, the Z- statistics critical region is $z > 1.96$ or $z < -1.96$, meaning,

RC = { $z \mid z < -1.96; z > 1.96$ } ; or $|z| > 1.96$ where RC is the critical region

↔ If $z > 1.96$ or $z < 1.96$, then H_0 will be rejected or if otherwise, we fail to reject H_0.

From Table 4.4.1, Z=0.02 which is not within the critical region. Based on these two evidences, I fail to reject the null hypothesis (H_0). Hence, the mean of the population of the paclitaxel loaded polylactide microspheres is 29.4 µm.

References

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Chapter Five

5.0 Conclusions and Recommendations

5.1 Conclusions

Paclitaxel was successfully loaded into polylactide-co-glycolide acid microspheres via a single solvent microencapsulation evaporation method. The temperature of the aqueous emulsifier solution at solvent removal step was found to influence the shape and the pore distributions in the microspheres. The size range of the microspheres is 15 - 40 µm. The mean of the microsphere's population is 29.4 µm. This is supported by the hypothesis testing results.

In addition, the swelling ratio of polylactide-co-glycolide acid increased linearly with the hydrolysis/ soaking time. This corresponds to the first stage of degradation of polylactide-co-glycolide acids.

5.2 Recommendations

The following recommendations are made:

Degradation of unloaded PLGA/PLA particles for longer hydrolysis time should be studied, so as to have a full understanding of the degradation process.

Degradation of paclitaxel loaded PLGA microspheres in deionized water and phosphate buffer saline solution should be studied.

Drug loading efficiency and entrapment efficiency of paclitaxel loaded PLGA microspheres. This will give the amount of paclitaxel entrapped in each of the sphere.

Release kinetics of different formulation microspheres. The results will guides in diagnosis and treatment of cancer and coronary artery disease