CONSTRUCTION OF DRUG-ELUTING POLYMERIC BIODEGRADABLE STENT

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Submitted to the Institute of Graduate Studies in
Science and Engineering in partial fulfillment of
the requirements for the degree of
Master of Science
in
Chemical Engineering

Yeditepe University
2011

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DATE OF APPROVAL: 18/01/2011

ACKNOWLEDGEMENTS

Foremost, I would like to express my deep and sincere gratitude to my advisor, Assist. Prof. Dr. Seyda Bucak, for providing guidance and encouragement that helped a good basis for thesis. I also appreciate my co-advisors, Assist. Prof Dr. Erde Can and Assoc. Prof. Dr. Gamze Torun Köse for their significant comments and support throughout this work. Writing this thesis would have been impossible without precious helps of my advisors.

Furthermore, special thanks are due to Ministry of Industry and Commerce, and Alvimedica, who support this project with the name of SANTEZ 00155.STZ.2007-2 grant.

I am heartily thankful to my great friends, my project partner İrem Ayşe Kanneci, my lab partners Merve Yüksel and Binnaz Coşkunkan for their encouragements and supports during the study. I also thank to my colleagues Görke Gürel, Gülçin Cem and Melek Şekerci.

Many thanks goes to Emre Kınacı and Sebla Önder due to their precious efforts to continue the project.

Lastly and most importantly, I wish to thank my family for their very special encouragement and confidence throughout my life. Without their love and understanding, it would be impossible for me to finish this work. Moreover, I am deeply grateful to my best friends, Emre Elitok, Sencer Anagün, Ahmet B. Oğuz and Pelin Eren for their moral supports.

ABSTRACT

CONSTRUCTION OF DRUG ELUTING BIODEGRADABLE POLYMERIC STENT

Coronary Artery Disease (CAD) is the most widespread disorder of heart disease for adults. With the progressive aging of the population, cardiovascular disease has become more definitely the main cause of death and coronary heart disease the major form of fatal cardiac disease. Without treatment of the disorder of coronary arteries can lead to serious problems and even death.Treatment of coronary artery disease is aimed at controlling symptoms and slowing or stopping the progression of disease. The aim of this project is to demonstrate stent implantation, the most important and beneficial way of CAD treatment.

The ultimate aim of this study was to construct drug eluting biodegradable polymeric stent for the treatment of coronary artery disease. The intended design of stent consists of two layers (inner and outer), where an appropriate polymer matrix embedding a drug, namely paclitaxel releases the drug over a desired period of time, such as one month. For the inner layer, biodegradable polymer blends were prepared via melt blending to obtain required mechanical properties with degradation in about 12 months.

The release profiles of the drug (paclitaxel) from PLLA-PCL blends were determined by using HPLC and these blends were found to release the drug depending on the film thickness and blend composition.The degradation studies were carried out by pH and weight measurements and degradation times were shown to depend on polymer blend composition. The crystallinity of polymer blends were determined by using Differential Scanning Calorimetry (DSC) and the DSC analysis of the PLLA-PCL blend solution cast films indicated that these blends are all phase separated. In the mean time, Scanning Electron Microscopy (SEM) was used to investigate the morphology of polymer blends where again the pahse separation was evident. As complementary experiments, in vitro studies of polymer blends were studied to examine the biocompatibility and all blends were found to be biocompatible, thus suitable for the desired application.

ÖZET

İLAÇ SALAN BİYOBOZUNUR POLİMERİK STENT ÜRETİMİ

Koroner arter hastalığı, yetişkinlerde en yaygın şekilde görülen kalp rahatsızlığıdır. Kardiyovasküler hastalıklar nüfusun hızla yaşlanmasıyla başta gelen ölüm sebeplerinden biri olmuştur. Koroner damarlardaki sorunun tedavi edilmemesi ölüme kadar varabilecek ciddi sağlık sorunlarına yol açabilmektedir. Koroner arter hastalığının tedavisinde amaç, semptomların kontrol edilmesi ve hastalığın ilerleyişinin yavaşlatılması ya da durdurulmasıdır. Bu tedavi hayat tarzının değişimi, ilaç tedavisi veya cerrahi müdahale şeklinde olablir. Bu projenin amacı, koroner arter hastalıklarının tedavisinde en önemli ve yararlı yol olan stent emplantasyonunun uygulanmasıdır.

Projenin esas amacı, koroner arter hastalığının tedavisinde kullanılmak üzere ilaç salan biyobosunur polimerik stent üretimidir. İlaç salan biyobozunur polimerik stent üretimi amacıyla başlanan proje iki bölümden oluşmaktadır. Birinci kısım üst tabakayı oluşturan, 1 ay sürecinde ilaç salımını sağlayan polimer matriksinin belirlenmesidir. İkinci kısım ise, iç tabaka için hazırlanan polimer karışımlarının mekanik özelliklerinin incelenmesi ve stent dizaynının yapılmasıdır.

İlk olarak PLLA ve PCL (%5, %10, %15 ve %20 PCL) karışımları çözücü uçurma metodu ile paklitakselli ve paklitakselsiz olmak üzere iki şekilde hazılandı. Paklitakselli hazırlanan örneklerin ilaç salım profilleri HPLC cihazı kullanılarak belirlendi. Paklitakselsiz hazırlanan örnekler için de degradasyon testi uygulandı. PLLA-PCL karışımlarının termal analizlerini ve kristallinitelerini incelemek amacıyla DSC cihazı kullanılmıştır. SEM cihazı kullanılarak çözücü uçurma metodu ile hazırlanan polimer karışımlarının gözenek boyutları belirlendi ve gözenek boyutu için dağılım grafiği çizildi. Ayrıca, stent çatısının biyouyumluluğunu incelemek amacıyla MTS ve Calsein testleri uygulanmıştır.

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1. INTRODUCTION

Coronary artery disease (CAD) is a narrowing of the blood vessels that supply oxygen and blood to the heart and is a major cause of illness and death making CAD treatment extremely important. Although there are many ways to treat CAD, stent implantation is the most effective method amongst them. Percutaneous transluminal cardiovascular angioplasty (PTCA) followed by stent insertion has become the therapeutic procedure of choice for treating cardiovascular occlusion. There are two broad types of stents, namely bare metal stent (BMS) and drug eluting stent (DES).

Due to the drawbacks of bare metal stents drug eluting stent made of polymers have been generated in the field of biomaterials. Biomaterials are subject to an extended set of requirements in order to establish safe application. These requirements mainly include acceptable biocompatibility and safe degradation characteristics.

A variety of natural, synthetic, and biosynthetic polymers are biocompatible and environmentally degradable. A polymer based on a C-C backbone tends to resist degradation, whereas heteroatom containing polymer backbones confer biodegradability. Biodegradability can, therefore, be engineered into polymers by the judicious addition of chemical linkages such as anhydride, ester, or amide bonds, among others. The usual mechanism for degradation is by hydrolysis or enzymatic cleavage of the labile heteroatom bonds, resulting in a scission of the polymer backbone. Microorganisms can uptake and, sometimes, digest polymers, and also initiate a mechanical, chemical, or enzymatic aging . Biodegradable polymers with hydrolyzable chemical bonds are researched extensively for biomedical, pharmaceutical, agricultural, and packaging applications.

In order to be used in medical devices and controlled drug release applications, the biodegradable polymer must be biocompatible and meet other criteria to be qualified as biomaterial-processable, sterilizable, and capable of controlled stability or degradation in response to biological conditions. The chemical nature of the degradation products, rather than of the polymer itself, often critically influences biocompatibility. Poly (esters) based on polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), and their copolymers

have been extensively employed as biomaterials. Controlled drug delivery takes place when a polymer is reasonably combined with a drug that is released from the material in a predesigned manner. The release of active ingredient can show different properties such that it may be constant over a long period, it may be cyclic over a long period or it may be activated by the external events or environment. The aim of controlled drug delivery is to obtain more effective therapies while removing the potential for under and overdosing.

The ultimate aim of this study was to construct drug eluting biodegradable polymeric stent for the treatment of coronary artery disease. The intended design of stent should be consisted of two layers (inner and outer), where the inner layer serves as a backbone and the outer layer elutes the drug.

For the outer layer PLLA and PCL polymer blends were prepared via solvent casting method. The amount of released drug (paclitaxel) from polymer blends was determined by using HPLC. Degradation of appropriate polymer blends were also studied. Moreover, the thermal transitions of these polymer blends were analyzed by Differential Scanning Calorimetry (DSC) and the morphologies of the polymers were characterized by Scanning Electron Microscopy (SEM). Along with these studies, the biocompatibility study of polymer blends was done with the method of MTS and Calcein AM assay by Genetics and Biotechnology Department.

For the inner layer, polymer blends including PLLA, PCL and PBS, with different compositions were prepared by using a microcompounder. This project continues with the biodegradability studies of these polymer blends prepared via melt blending method. Thermal and thermomechanical properties will be analyzed by Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analyzer (DMA) respectively. In the first chapter of this thesis, general information is given about biodegradable polymers used in drug delivery and related topics. The following chapter presents experimental set up, chemicals and methods. In the third chapter, results are given in detail. At the end of results section, by using the information and data that were collected during the experiments, in a separate chapter, necessary discussions, comments and recommendations are done.

2. THEORITICAL BACKGROUND

2.1. CORONARY ARTERY DISEASE

The heart receives its own supply of blood from the coronary arteries. Coronary arteries provide oxygen-rich blood to the heart muscles. Plaque inside the coronary arteries which builds up atherosclerosis hardening of the arteries causes a malfunction in heart called coronary artery disease (CAD). CAD is the most widespread disorder of heart disease for grown-up people. With the progressive aging of the population, cardiovascular disease has become more definitely the main cause of death and coronary heart disease the major form of fatal cardiac disease. Without treatment, the disorder of coronary arteries can lead to serious problems and even death [1].

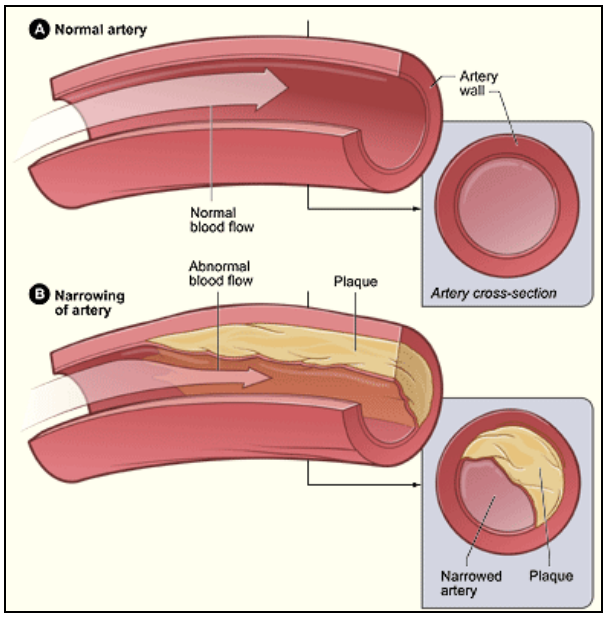


Figure 2.1. Schematic representation of normal artery (A) and narrowing of artery (B) [2]

Genetic factors, dietary factors including cholesterol, sugar, fats and calories, hypertension, smoking cigarettes and lack of physical exercises have been assigned major reasons of coronary heart disease [1].

Atherosclerosis is by far the common lesion of the coronary arteries. Sooner or later, in varying degrees, most hearts are affected. During atherosclerosis the plaque narrows coronary artery and the thickness of arterial wall increases steadily. Blood clot and heart attack can suddenly appear because of the plaques tearings [3].

2.2. CORONARY ARTERY DISEASE TREATMENT TECHNIQUES

The treatment of coronary heart disease is essentially that of its specific designation such as lifestyle changes, drug treatment or surgical intervention.

2.2.1. Lifestyle changes

Weight loss for overweight patients, giving up smoking, physical exercises are some of the ways to reduce the risk of coronary artery disease. The main purpose of the weight loss is to reduce the hazardous factors such as cholesterol and fat which cause the formation of plaque inside the coronary arteries. The effect of smoking is narrowing the arteries and building up the tendency for blood to clot. Physical activity reduces the occurrence of coronary heart disease [4, 5].

2.2.2. Drug Treatment

Another way to treat CAD is the commonly utilized medication method. . During the intake, some of drugs reduce the blockages and provide lowering the pulse rate and blood pressure. Dissolved drugs taken into blood decrease the primary materials such as cholesterol which deposits on the coronary arteries [6]. Medication is used regularly as a supplement.

2.2.3. Surgical Intervention

Surgical intervention is the most important treatment of CAD for certain cases. Coronary angioplasty is a surgical procedure that is used to widen blocked or narrowed coronary arteries. Angioplasty involves using a flexible tube to insert a stent into the coronary artery. Stents are short and hollow, usually with a mesh-like structure. The stent

is collapsed to a small diameter and placed over a balloon flexible tube (crimping). Stent will be placed into the artery where plaque is, by inserting this tube though the leg. After the placement of stent in the correct location, the balloon is inflated for expanding the stent and it sticks to the walls of the artery to form a scaffold with the aim of holding the artery open as shown in Figure 2.2 [7].

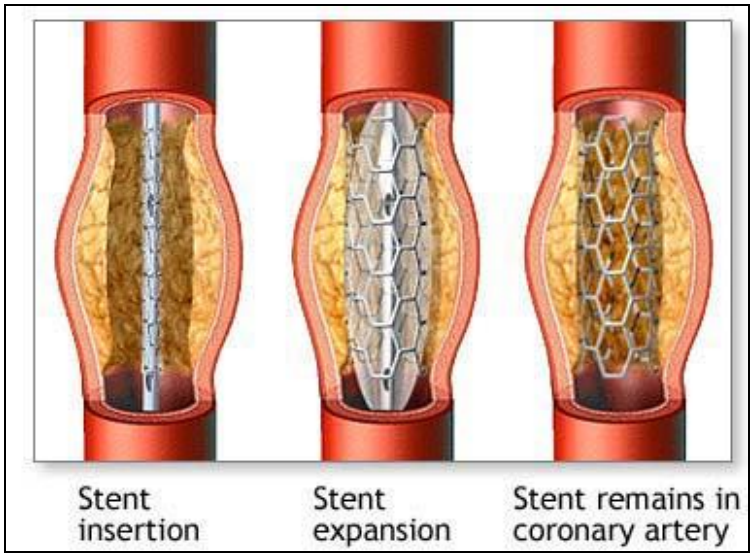


Figure 2.2 Stent Placement into a coronary artery [8]

2.3. STENTS

As early as 1912, glass tubes described by Alexis Carrel were used in canine aortae to maintain luminal patency and this invention encouraged scientists to improve such devices for clinical cases. Charles Dotter implanted simple metal spirals in animal arteries in the year of 1968. Senning Maas *et al*. suggested a similar concept in the late 1970s and his work led to the development of a large diameter spiral prosthesis made from surgical stainless steel alloys. [9]

In 1986, Puel and Sigwart invented the first coronary stent to prevent vessel closure percutaneous transluminal coronary angioplasty (PTCA) which is therapeutic technique applied to the stenotic coronary arteries. Bare metal stents (BMS), which are the first-generation stents, have shown beneficial characteristics such as formation of scaffold in the vessel and prevention of acute collapse. However, bare metal stents caused some problems

such as restenosis, endothelial injury and inflammation. Due to the drawbacks of bare metal stent, drug eluting stent was invented in 2001 to minimize restonosis. Paclitaxel and sirolimus are used as drugs to reduce the risk of revascularization [10]. Stents which can elute drugs to block cell proliferation are called drug eluting stents.

Stents used in coronary artery are made of different metals including nitinol, stainless steel and cobalt chromium or polymers that are biostable or biodegradable [11].

2.3.1. Drug Delivery

In controlled drug delivery, the active compound is released from the biomaterial in a predesigned way. Controlled drug delivery provides two important potential applications as controlled release and site-directed for the use of polymers in the effective management of medical drugs in the body. On the other hand, site-directed drug delivery through a polymer serves as a carrier to bring a drug to a specific site in the body [12].

The rate of drug release can be controlled by diffusion, reaction or solvent. In diffusion control, the driving force for diffusion is the concentration gradient across the delivery device. The drug is encapsulated by a polymeric membrane and release rate will decrease with time as the distance the drug has to travel from within the matrix increases due to the depletion of drug concentration shown in Figure 2.3a. Diffusion is dependent on porosity, which means that drug goes to through the pores of polymer matrix. Another diffusion controlled system is the reservoir systems to control the rate of drug release. For the reservoir system polymers are coated with rate controlling materials including polyethylene-co-vinyl acetate (PEVA) and poly n-butyl methacrylate (PBMA) that contain active agent like sirolimus. The release of drug is controlled by the diffusion of the drug from the rate controlling barrier illustrated in Figure 2.3b [13].

In reaction controlled systems,the drug can be dispersed in the polymer, which degrades in the body as a result of hydrolysis or enzymatic attack. Drug can be chemically linked to a polymer chain by a group that provides a biodegradable link. Drug release is proportional with degradation rate shown in Figure 2.3c [13].

In solvent-controlled delivery, drug release is regulated by the permeation of water through the polymer. Polymer swelling process is to increase aqueous solvent content within the formulation and drug can release through swollen polymer into the environment. According to Figure 2.3c, another type of solvent activated system for controlled release uses polymeric hydrogels, which are water-swollen cross-linked polymer networks [13].

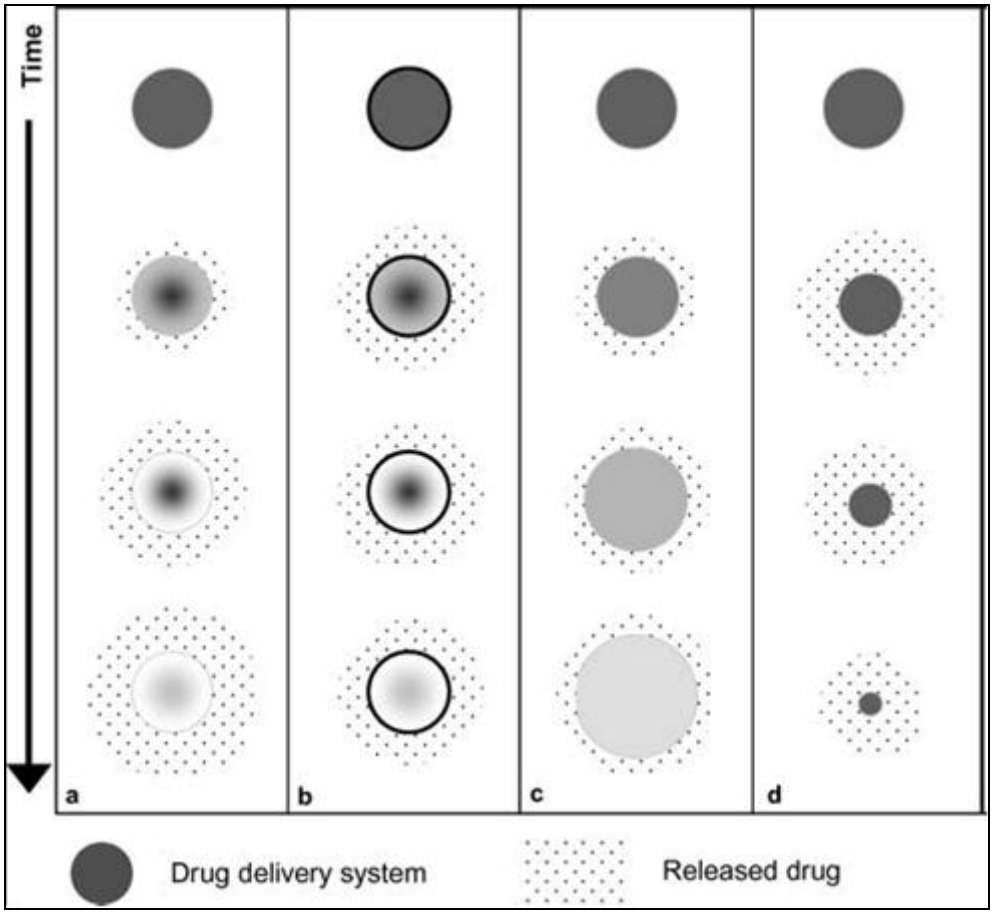


Figure 2.3. Drug release mechanisms [12]
(a-b diffusion, c swelling, d degradation or erosion)

In polymer coated stents, the drug is dispersed in a polymer matrix which is applied on the stent surface. The release profile of drug from stent surface is determined by the stent surface area, polymer and binding agent coating, matrix fabrication, and characteristics of matrix design [13].

2.3.1.1. Paclitaxel

Paclitaxel is one of the most efficient antiploriferative agents for treatment of a wide range of cancers. Paclitaxel inhibits cellular replication and ultimately causes cellular death. The chemical structure of paclitaxel is given in Figure 2.4. The coating of paclitaxel on stents can be classified into two types like polymer based and non polymer based. Heldman *et al*. coated paclitaxel on the stent surface by dipping stent in the paclitaxel solution. The disadvantage of dip coating is the loss of a certain amount of drug during the stent placement. This drawback led the scientists to improve polymer coating stent technology as polymer coatings can carry higher loads of drug compared to direct drug adsorption on the metal stent [14].

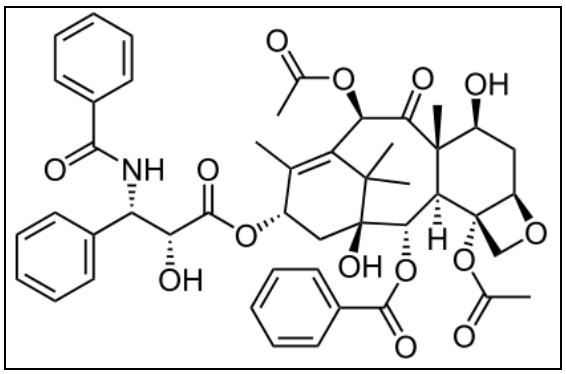


Figure 2.4 Paclitaxel [15]

Sirolimus is another efficient antiploriferative agent used in stent treatment. Sirolimus is also known as rapamycin [16].

2.3.2. Metal Stent

Generally, metals commonly used for manufacturing stents are 316L stainless steel (316L SS), platinum-iridium alloy (Pt-Ir), tantalum (Ta), nitinol (Ni-Ti), cobalt-chromium (Co-Cr) alloy, Titanium (Ti), pure iron (Fe), and magnesium alloys (Mg) [15].

Metallic characteristics, bulk and surface properties design and chemistry are all important factors to consider in the conception of an optimal stent. The metal stent must have enough plasticity to remain at the required size when deployed [17].

In 1994 Johnson and Johnson produced the first bare metal stent approved by Food and Drug Administration (FDA). After Johnson and Johnson production, several companies have investigated to construct new metal stents using different kind of materials. Through this investigation metal stents are produced with better flexibility and durability. Chromium and titanium are used to prevent the corrosion of stent [18]. A typical metal stent is shown in Figure 2.5.

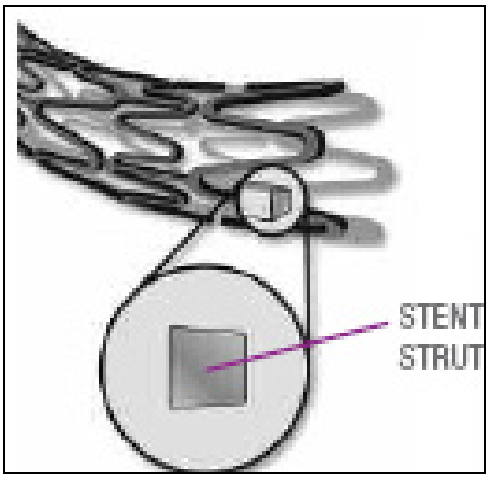


Figure 2.5 Schematic of Bare-metal stent [19]

The main function of bare metal stent is to prevent the formation of plaque which narrows the artery and keep the artery open. After lots of surgical interventions bare metal stents do not perform their missions and blockage will be formed again. Due to the drawbacks of the bare metal stent, drug coated metal stent was developed to release drug for prevention of blockage formation.

Drug coated stent has also same properties of bare metal stent like prevention of late vessel modeling, formation of scaffold in the vessel and decrease in restenosis ratio. Coating materials such as inorganic compounds including silicon carbide or iridium oxide, synthetic and biological polymers such asPLLA, polyurethane, and polyester, and drugs change the chemistry of the stent surface. [12]

The FDA approved sirolimus coated stent CYPHER^{TM} is made of 316L SS and the metallic stent is coated with polymers as polyethylene-co-vinyl acetate (PEVA) and poly

n-butyl methacrylate (PBMA). The CYPHER stent releases 50% of its sirolimus content during the first week after implantation and 85% of the drug over 30 days [20].

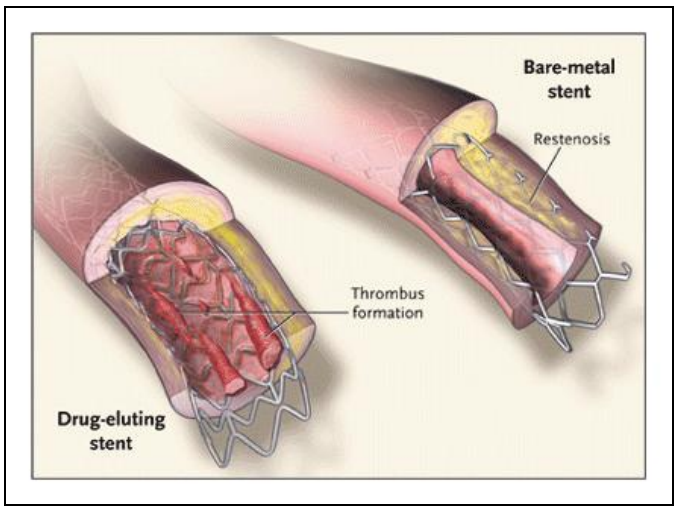


Figure 2.6. Drug Eluting stent versus bare metal stent [20]

Figure 2.6 shows that drug eluting stent not only prevents restenosis, but also provides thrombus formation.

As mentioned above, drug eluting coated metal stents have certain advantages over bare metal stents. However, still they have the deficits of hypersensitivity reactions, late thrombosis and delayed endothelization risks. Due to these drawbacks, scientists developed stents from different materials, known as polymer stents.

2.3.3. Polymer Stent

Polymers used in stent coating can be classified as biostable (non-biodegradable) polymers, biodegradable polymers, copolymers and biological polymers. Although a wide range of polymers have been used to coat stent, only a few, like polyethylene terepthalate, poly-L-lactic acid and poly-L-glycolic acid have been tested as a lone stent material [21].

The principle of biostable polymeric stent is very similar to metallic stent. It should be biocompatible and should not initiate inflammatory reactions. The most common material used in stent is polyethylene terepthalate (PET).

Biodegradable polymers are widely used as polymeric materials for polymer stents. Polymer stents have advantages such as high biocompatibility, fully degradation after healing, controllable degradability and possibility for repeating the operation. The drug delivery system of polymer stent is more likely than metal stent and the release of drug can be easily controlled. Biodegradable polymers are not only used as coating on metal stents for drug delivery but also the main structure of stent is produced from biodegradable polymers [22].

The Duke stent was the first biodegradable stent made of poly L-lactic acid (PLLA) which was discovered in 1980s. The mechanical behavior of these stent was also investigated. In spite of the controversies of using biodegradable stents, PLLA stents were implanted in clinical trial [21].

However the first FDA approved biodegradable stent was designed from Igaki-Tamai the first certified bioabsorbable stent which was implanted in humans. Igaki-Tamai stent that has a zigzag helical coil design was also produced from PLLA and used for people who reacted to metallic materials. The structure of Igaki Tamai Stent is shown in Figure 2.7 and its properties are summarized in Table 2.1. [23]

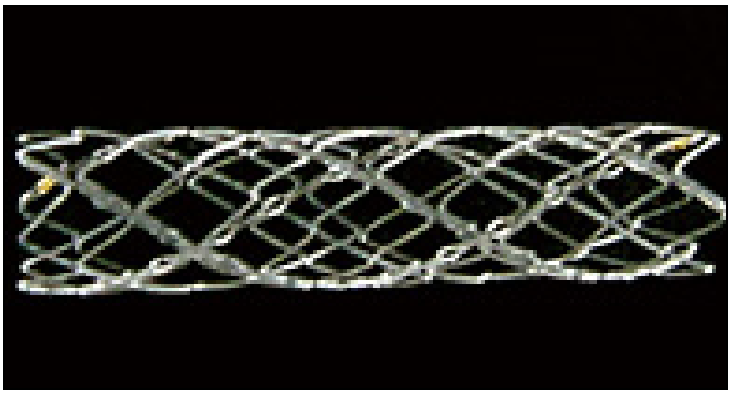


Figure 2.7. Igaki Tamai Stent [23]

Table 2.1 Stent Specification of Igaki-Tamai Stent [24]

Material composition	PLLA medical grade
Stent design	Zig zag hellical coil

Strut thickness	0.24 mm
Available length	36 mm
Available diameter	5.0,6.0,7.0,8.0 mm
Expansion range	7 mm (stent type at 5 and 6 mm) 9mm (stent type at 5 and 6 mm)
Polymer cross-sectional area	0.68 mm2
Percentage shortening on expansion	<1.6 % (at 5 mm), <1.9 % (at 6 mm) <3.2 % (at 7 mm), <3.7 % (at 8 mm)
Polymer surface area	21.17 % (at 5 mm), 17.69 % (at 6 mm) 17.22 % (at 7 mm), 15.27 % (at 8 mm)
Longitudinal flexibility	Excellent

PLLA stent has been loaded mostly with paclitaxel to reduce restenosis and to prevent the proliferation of smooth muscle cells. PLLA is not the only used polymer but also PGA/PLGA, polycaprolactone (PCL), polyhydroxybutyrate valerate and poly(butylene terephtalate) (PBT) can be used as polymer materials for stent production. Different polymer compositions can be used to get the best mechanical properties of polymer stent [25].

The mechanical properties, dimension and the dynamical properties of the stent do not correspond to the properties of the vessel or generally of tissue where this stent is introduced. For this reason, scientists want to identify and to describe these relations between the mechanical properties of stents and tissues. [26]

2.4. POLYMERS

The word polymer is derived from the classical Greek words ‘poly’ meaning many and ‘meres’ meaning parts. A polymer is a large molecule, a macromolecule, built up of repetition of smaller chemical units, monomers. According to the thermal behavior of polymers there are two main groups of polymers; thermoplastics and thermosets [13].

Thermoplastic polymers which can be heat softened, have weak Van der Waals forces and can be recovered by using heat and pressure. Above the glass transition

temperature thermoplastics are flexible and they are liquid at high temperatures. Polystyrene is the most common thermoplastic (Figure 2.8) [13].

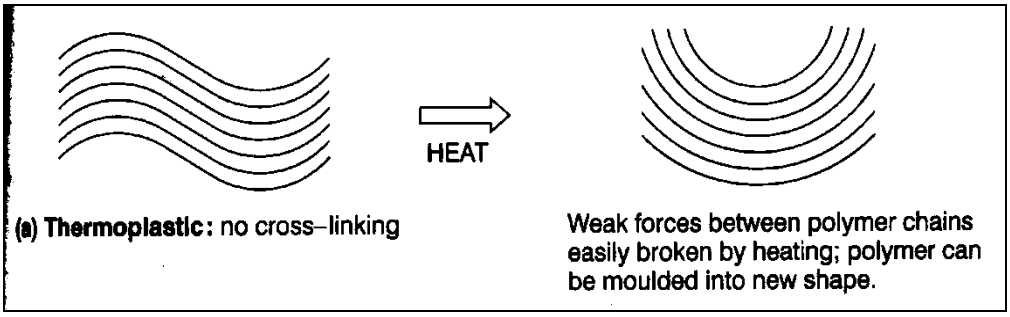


Figure 2.8. Thermoplastics [27]

Thermosets are polymers whose individual chains have been chemically linked by covalent bonds during polymerization. Once formed, these cross-linked networks resist heat softening and solvent attack and cannot be thermally processed. Such properties make thermosets suitable materials for composites, coatings and adhesive applications. Important thermosets include phenolics, ureas, melamines, epoxies, polyesters, silicones, rubbers, and polyurethanes (Figure 2.9) [13].

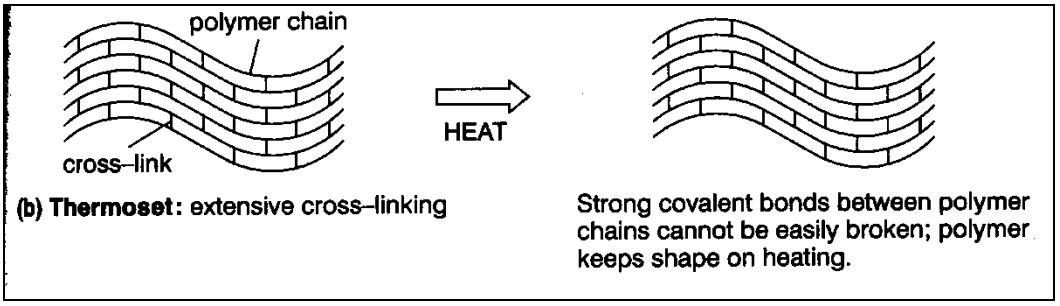


Figure 2.9. Thermosets [27]

2.5. BIODEGRADABLE POLYMERS

Biodegradation is a process carried out by bacteria in nature. During biodegradation molecules are ultimately converted into carbon dioxide, water and other elements. The rate

of degradation depends on factors such as concentration, pH and temperature and among the factors temperature is the most pronounced [28].

According to their chemical, physical, mechanical and biological properties, biodegradable polymers are useful for various applications like medical, agriculture, drug release and packaging fields. Biodegradable polymers which are used as biomaterials should not have toxic response when implanted in the body and the degradation time of the material should match the healing or regeneration process. Moreover the degradation products that can be quickly metabolized by microorganisms should be non-toxic [29].

As summarized in Table 2.2 biodegradation of polymers depend on many factors such as chemical structure, processing conditions and physical factors. Ultimately, polymer degradation is shown to be chiefly governed by molecular weight, crystallinity, susceptibility to hydrolysis and device architecture considerations whilst maintaining its thermodynamic equilibrium.

Table 2.2 General factors Affecting Polymer Biodegradation [12]

Chemical composition and structure • Repeat unit distrubition in multimers • Molecular weight • Morphology(crystallinity, microstructures) • Physicochemical factors • Adsorbed and absorbed (body) compounds (water, lipids, proteins, air, etc.)
Processing conditions (force, solvents, catalyst, etc.)
Sterilization process (affecting crystallinity)
Physical factors (changes in size, shape, diffusion and mechanical stress)
Implantation site (mechanical and biological enviroment)

Generally, natural polymers are more biodegradable than synthetic polymers. Among synthetic polymers, polyurethanes are sensitive to biological degradation. Polymers containing ester function, especially aliphatic polyesters are biodegradable and the most important group of polyesters is poly(β-hydroxyalkanoates). These polymers can be used as biomaterials as well as disposable plastic packaging materials. They are produced in

nature by bacterial fermentation of sugar. Poly(β-hydroxybutyrate) (PHB), poly(β-hydroxyvalerate) (PHV) and their copolymers are the most commercial members of poly(β-hydroxyalkanoates). Another important biodegradable polymer group is polyesters. The members of polyesters are poly(lactic acid) (PLLA), poly(lactide-co-glycolide) (PLGA) and polycaprolactone (PCL) [16].

Controlling the mechanical properties and degradation kinetics make synthetic biodegradable polymers including polyesters, polyanhyrides, polyorthoesters, polyphosphazenes and polyurethanes available for lots of applications such as drug delivery system, resorbable sutures and orthopedic fixation devices. Especially, polyesters are used because of their degradation through hydrolysis of the ester linkage. The degradation products include oligomers and monomers whereas the side products include initiators, catalysts and solvents as shown in Figure 2.10 [30].

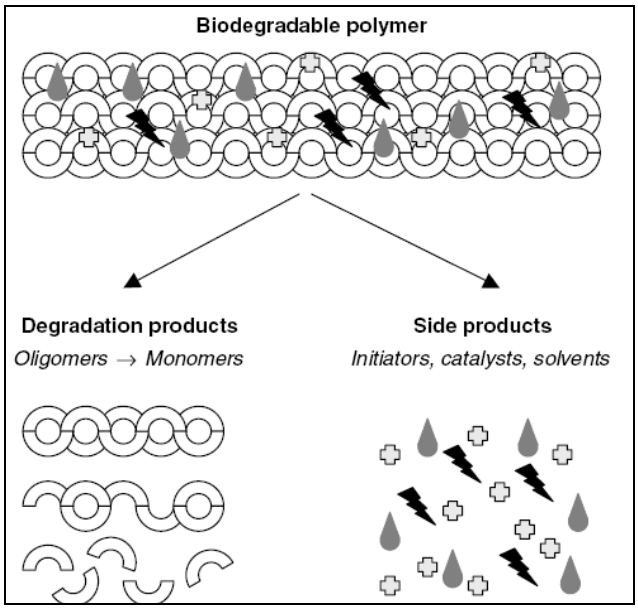


Figure 2.10. Degradation products of a biodegradable polymer [30]

In the first stage, biodegradable polymers are broken down into oligomers and afterwards oligomers go to pieces of monomers ultimately. Side products will occur within the breaking bonds on polymers.

2.5.1. Poly (L-lactic acid)

Poly lactic acid can be polymerized from lactic acid, by fermentation of carbohydrates such as glucose, sucrose, or lactose [31].

Lactide is a chiral molecule and exists in two optically active forms; L-lactide and D-lactide and by using lactide as a monomer, poly lactic acid is synthesized via the polymerization reaction shown in Figure 2.11.

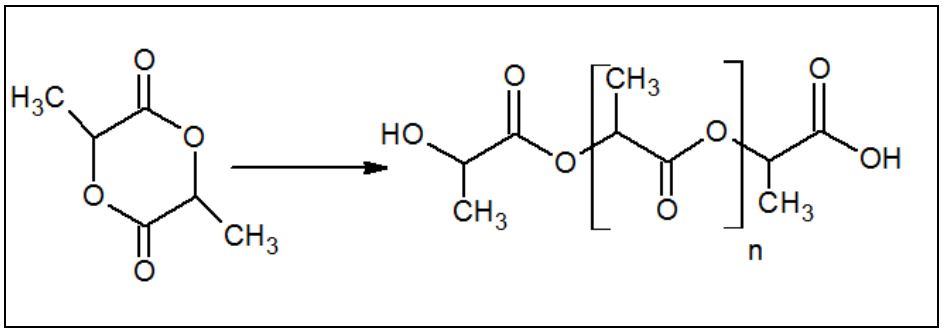


Figure 2.11 Polymerization reaction of poly lactic acid

PLLA has nearly 37% crystallinity which means that PLLA is predominantly amourphous but its degree of crystallinity that has big influence on hardness, density and diffusion depends on molecular weight of the polymer. The glass transition temperature of PLLA is approximately 60-65 ^{o}C and PLLA has melting temperature of nearly 175 ^{o}C. PLLA has good tensile strength, low extension and a high modulus and hence, has been used as an ideal material for biomedical applications such as drug delivery systems, sutures and agricultural applications due to its biodegradability and biocompatibility [32].

Degradation products of polylactide consist of lactic acid. PLLA can easily be formed into desired shapes with high mechanical properties and desired degradation rates. Factors affecting degradation rate depends on degree of crsytallinity, molecular weight, and stress factors [29].

2.5.2. Polycaprolactone

PCL is generally prepared from the ring-opening polymerization of ε-caprolactone as shown in Figure 2.12.

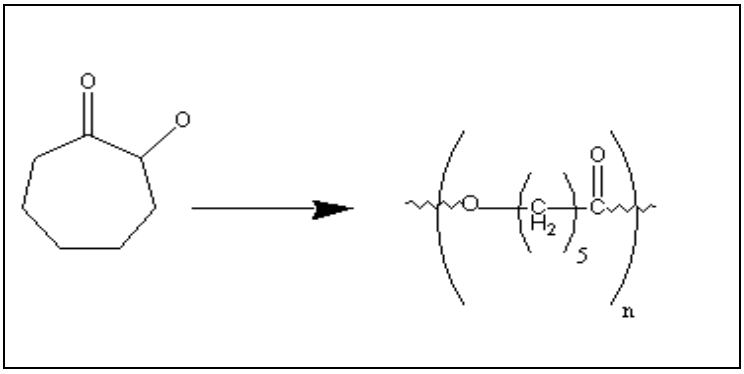


Figure 2.12 Polymerization reaction of Polycaprolactone

PCL is highly processible as it is soluble in a wide range of organic solvents, has a low melting point of about 60 C^o and glass transition temperature of -60 C^o. It has the ability to form miscible blends with a wide range of polymers. The polymer undergoes hydrolytic degradation due to the presence of hydrolytically labile aliphatic ester linkages. The tensile strength of PCL is lower than PLLA and PCL has high elongation at breakage.

Polycaprolactone (PCL) has been thoroughly studied as a substrate for biodegradation and as a matrix in controlled-release systems for drugs. PCL is degraded by biological systems but their applications have been limited because of their relatively low molecular weights and poor physical strengths [29].

2.5.3. Polybutylene succinate

Poly(butylene succinate) (PBS) is an aliphatic polyester known as with the trade name of Bionolle. PBS is produced by polycondensation of glycols such as ethylene glycol and 1,4-butanediol with dicarboxylic acids as shown in Figure 2.13. PBS has enough toughness and lower melting temperature than PLLA, recorded as approximately 120 C^o. It has high flexibility, excellent impact strength, and thermal and chemical resistance. PBS can be used for several applications such as packaging, agriculture and medical [33]

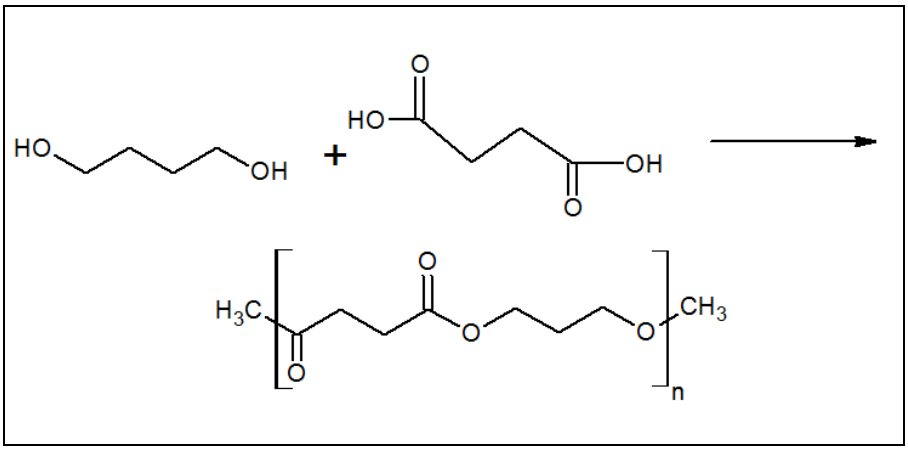


Figure 2.13 Polymerization reaction of PBS

In our experiments Poly(1,4-butylene succinate), extended with 1,6-diisocyanato hexane whose structure is shown in Figure 2.14 was used.

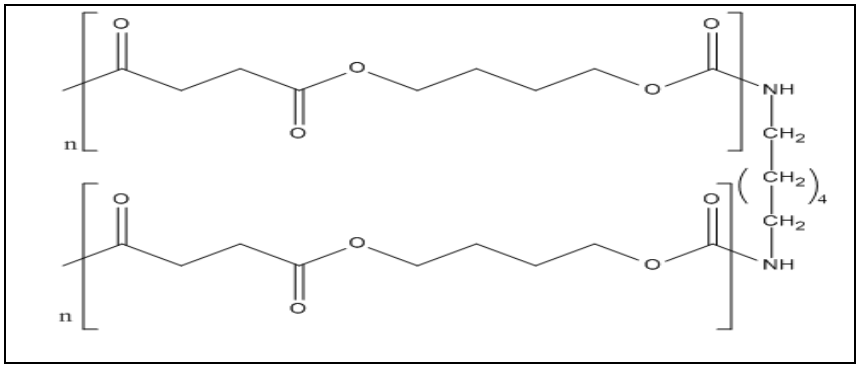


Figure 2.14 Structure of Poly(1,4-butylene succinate), extended with 1,6-diisocyanatohexane

2.6. BIOCOMPATIBILITY

Generally, the term biocompatibility describes the availability of the material when it is placed into the body. It is the ability of a material to perform with an appropriate response in a specific application and is very dependent on the particular circumstances. A material will be considered biocompatible in a specific application if it allows the body to

function without any complications. Biocompatible materials prevent lots of complications such as extended chronic inflammation at the contact point, cytotoxicity and cell disruption [34].

Biodegradable polymers are natural or synthetic in origin and are degraded *in vivo*, either hydrolytically or enzymatically to produce biocompatible, toxicologically safe by-products that are further eliminated by the normal metabolic pathways. Since the last two decades, synthetic biodegradable polymers have been increasingly used as scaffolds in tissue engineering to direct specific cell growth and also to deliver drugs.

2.7. SURFACTANTS

The abbreviation of surface active agent is surfactant that is characterized by its tendency to absorb at surfaces and interfaces. Interface indicates a boundary between two immiscible phases. The main function of surfactant is to reduce surface tension and the free energy of boundary phases. Classification of surfactants depends on their charge of polar head group such as anionics, cationics, non-ionics and zwitterionics [35].

2.7.1. Anionic Surfactants

The polar groups in anionic surfactants are carboxylate, sulfate, sulfonate and phosphate. The most common surfactant class is anionic because of the ease and low cost of their manufacture. The main usage area of anionic surfactants is detergent formulations. They are generally not compatible with cationics as over a wide range of mixing ratios, precipitation may occur [35].

2.7.2. Cationic Surfactants

Cationic surfactants are generally based on nitrogen atom carrying the cationic charge. Both amine and quaternary ammonium based products are very common. Permanently charged quaternary ammonium cations are not pH sensitive. On the other hand, cationics having amines cannot be used at high pH [35].

2.7.3. Non-ionic Surfactants

Second largest surfactant class is non-ionic surfactants and the polar group of non-ionics is either a polyether or a polyhydroxyl. Usually non-ionics are compatible with other surfactants. Nonionic surfactants are typically less foaming surfactants than anionic surfactants. The main common non-ionic surfactant is pluronic surfactants, which have a wide ratio range of hydrophobic and hydrophilic chains including polypropyleneoxide (PPO) and polyethyleneoxide (PEO). It is used to increase the miscibility of two substances with different hydrophobicities due to their good availibility of enhancement of water solubility. According to the properties of pluronic surfactants they are used in lots of applications such as cosmetics, pharmaceuticals and drug delivery [35].

2.7.3.1. Polyethylene oxide

Polyethylene oxide is nonionic, water soluble, and highly hydrophilic. PEOs are characterized with their flocculent, thickening, sustained-release, lubrication, dispersing, and water-retention properties. They are liquids or low-melting solids, depending on their molecular weights shown in Figure 2.15 [36].

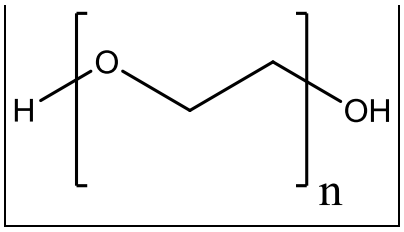


Figure 2.15. Polyethylene oxide [36]

2.7.3.2. Polypropylene oxide

Polypropylene oxide has similar properties with polyethylene oxide. It is usually liquid at room temperature. Solubility in water decreases rapidly with increasing molar mass. PPO is less toxic than PEO, so PPO is more useful at biotechnological applications [36].

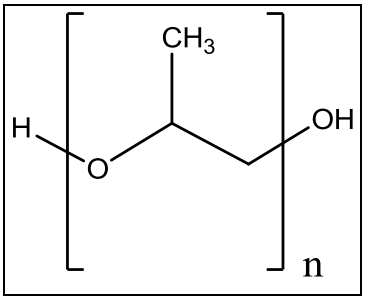


Figure 2.16. Polypropylene oxide [36]

2.7.4. Zwitterionic Surfactants

Zwitterionics contain two charged groups of different signs. The positive charge is most frequently ammonium, the source of negative charge may vary, although carboxylate is the most common [35].

2.8. COPOLYMERS

Polymers synthesized with more than one kind of monomer are called copolymers. Two monomers can be made into a copolymer in many different ways. When the two monomers are arranged in an alternating fashion, the polymer is called, an alternating copolymer. In a random copolymer, the two monomers may follow in any order. In a block copolymer, all of one type of monomer is grouped together, and all of the other is grouped together. And finally homopolymer side branches of one type may be grafted to homopolymer main chains that are composed of a different mer; such a material is termed a graft copolymer [37].

The most common copolymer that can be used as a biomateral is Poly(lactic-*co*-glycolic acid) (PLGA) that was approved by FDA due to its biodegradability and biocompatiblity. PLGA is synthesized by two different monomers as glycolic acid and lactic acid. PLGA is linear, aliphatic polyester and it was used as a biomaterial for several applications [32].

2.9. SOLID STATE PROPERTIES OF POLYMERS

Molecular shape and the way molecules are arranged in a solid are important factors in determining the properties of polymers. From polymers that crumble to the touch to those used in bullet proof vests, the molecular structure, conformation and orientation of the polymers can have a major effect on the macroscopic properties of the material [38].

2.9.1. Amorphous State

An amorphous solid is a solid in which there is no long-range order of the positions of the atoms. Most classes of solid materials like polymers, ceramics and cotton can be found or prepared in amorphous form.

Completely amorphous polymers like polystyrene exist as long, randomly coiled, interpenetrating chains that are capable of forming stable, flow-restricting entanglements at high molecular weight. An amorphous solid is formed when the chains have little orientation throughout the bulk polymer. The glass transition temperature is the point at which the polymer hardens into an amorphous solid. This term is used because the amorphous solid has properties similar to glass. In the glassy state, at temperatures below T_g, the only molecular motions that can occur are short-range motions of several contiguous chain segments [38].

2.9.1.1. Glass transition temperature

Glass transition temperature, T_g, can be defined as the temperature at which an amorphous system changes from the glassy to the rubbery state [39].

At low temperatures the amorphous regions of a polymer are in the glassy state. In this state the molecules are frozen in place. They may be able to vibrate slightly, but do not have any segmental motion in which portions of the molecule wiggle around. In the glassy state, the motion of the red segment in the schematic diagram on the right in Figure 2.17 would not occur. When the amorphous regions of a polymer are in the glassy state, it generally will be hard, rigid, and brittle [39]

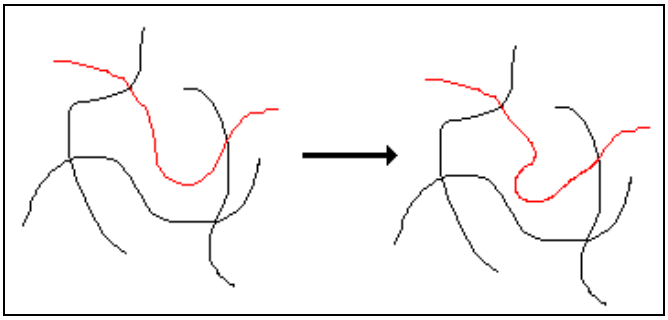


Figure 2.17. Motion of red segment in the glassy state [40]

Many physical properties change at the glass transition temperature including coefficient of thermal expansion, heat capacity, refractive index, mechanical damping and electrical properties. All of these are dependent on the relative degree of freedom for relative motion within a given polymeric material and each can be used to monitor the point at which the glass transition occurs [41].

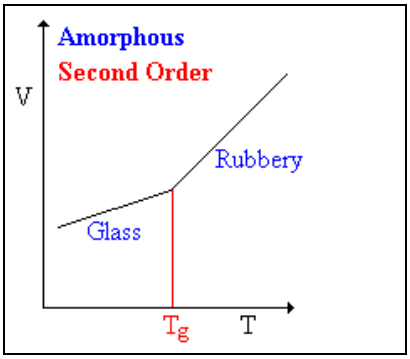


Figure 2.18. Plot of specific volume versus temperature for a typical polymer passing through its glass transition [40]

Briefly, the following features are known to influence the glass transition temperature:

- The presence of groups pendant to the polymer backbone, since they increase the energy required to rotate the molecule about primary bonds in the main polymer chain. This is especially true for side chains or branches.
- The presence of inherently rigid structures in the backbone of the molecules

- Cross-linking
- Hydrogen bonds between polymer chains
- Relative molar mass, which influences T_g because higher molar mass polymers have less ease of movement and more restrictions in their overall molecular freedom than polymers of lower molar mass.
- The presence of plasticisers

The effects of these different factors can be seen in the T_g values of some typical polymers. A number of these values are shown in Table 2.3, together with a brief note about what feature particularly contributes to the relative level of the glass transition temperature [41].

Table 2.3 Glass transition temperatures of some typical polymers [41]

Polymer	**T_g (^oC)**	**Contributing feature**
Poly(ethylene)	-20	Flexible backbone
Poly(propylene)	5	CH_3 group inhibits freedom of rotation
PVC	80	Strong polar attraction between molecules
PTFE	115	Very stiff backbone

Copolymers generally exhibit a single T_g value which lies in a position intermediate with respect to the T_g of the constituent homopolymers. In block copolymers, where the blocks are large enough to phase separate, two glass transitions can be obtained. If phase separation is complete, the T_g lie at the temperatures of the corresponding homopolymers. For immiscible polymer blends two T_gs result and are located the T_gs of the individual polymers. If, on the other hand, the pair of constituent polymers are completely miscible, as for the block copolymer case, there will be only a single T_g. [42]

2.9.1.2. Methods of Determining Glass Transition Temperature

DSC and DMA are the most common method for determining glass transition temperature of many polymers.

Differential Scanning Calorimetry (DSC) measures the change of the difference in the heat flow rate to the sample and to a reference sample while they are subjected to a controlled temperature program [44].

DSC allows reaction heats and heat of transition, or heat flow rates and their changes at characteristic temperatures, to be quickly measured on small sample masses, in wide temperature ranges and with an accuracy which is usually sufficiently high for respective purpose. DSCs are applied in the following fields; characterization of materials, stability investigations, evaluation of phase diagrams, purity determinations, determination of heat capacity. A schematic diagram of DSC is given in Figure 2.19 [44].

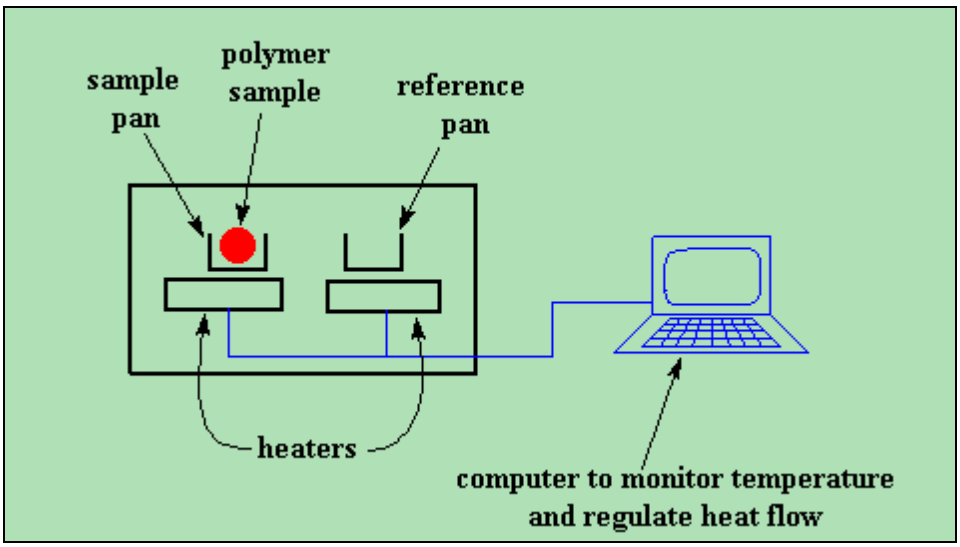


Figure 2.19. Schematic representation of working principle of DSC [44]

In place of differential power, values of specific heat capacity, C_p, may be obtained from the recorded heat flow rate by calibrating with a pure compound for which C_p is known precisely at different temperatures from calorimetry measurements [13].

Dynamic Mechanical Analyzer, otherwise known as DMA, is a technique where a small deformation is applied to a sample in a cyclic manner. This allows the materials response to stress, temperature, frequency and other values to be studied [45]. A schematic diagram of DSC is given in Figure 2.20.

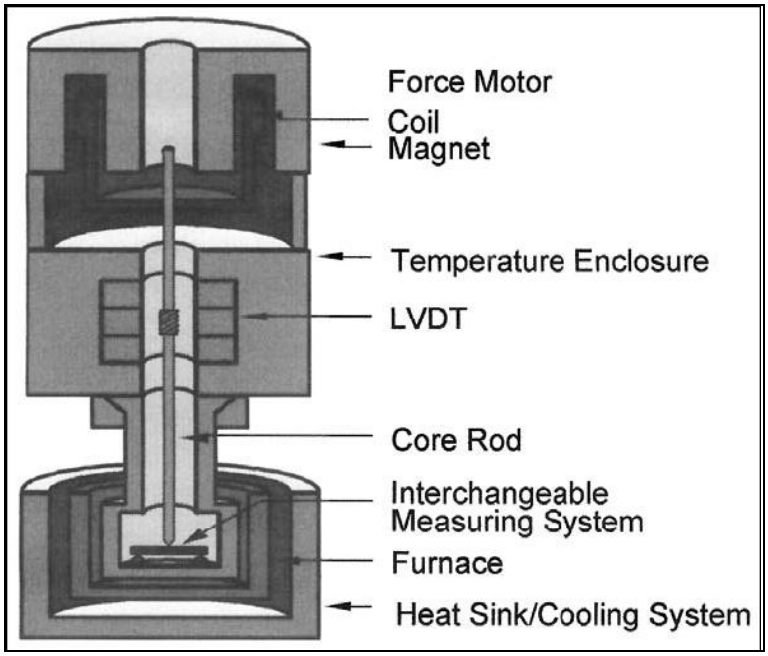


Figure 2.20. Schematic representation of DMA basic principle [45]

DMA works by applying a sinusoidal deformation to a sample of known geometry. The sample can be subjected by a controlled stress or a controlled strain. For a known stress, the sample will occur several deformation such as tensile, torsion, compression, flexure and shear [45].

The T_g of cured materials or thin coatings is often difficult to measure by other methods, and more often than not the initial cost justification for a DMA is in measuring a hard-to-find T_g. While estimates of the relative sensitivity of DMA to DSC vary, it appears that DMA is 10 to 100 times more sensitive to the changes occurring at the T_g [45].

2.9.1.3. Melting Temperature

The melting of polymer crystals corresponds to the transformation of a solid material having an ordered structure of aligned molecular chains to a viscous liquid in which the structure is highly random; this phenomenon occurs upon heating at the melting temperature, T_m [46].

There are several features distinctive to the melting of polymers that are not normally observed with metals and ceramics; these are consequences of the polymer molecular

structures and lamellar crystalline morphology. First of all, melting of polymers takes place over a range of temperatures. In addition, the melting behavior depends on the history of the specimen, in particular the temperature at which is crystallized [47].

2.9.2. The Crystalline State

Under favorable conditions, some polymers cooled from the melt can organize into regular crystalline structures which is a unique arrangement of atoms or molecules in a crystalline liquid or solid. Such crystalline polymers have less perfect organization than crystals of low molecular weight polymers crystallized from the solution. For some polymers crystallized from the melt or from concentrated solution crystallites can organize into larger spherical structures called spherulites. Each spherulite contains arrays crystallites that are typically oriented with the chain axis perpendicular to the radial direction of the spherulite. A spherulite is schematically represented in Figure 2.21, containing both amorphous and crystalline regions [48].

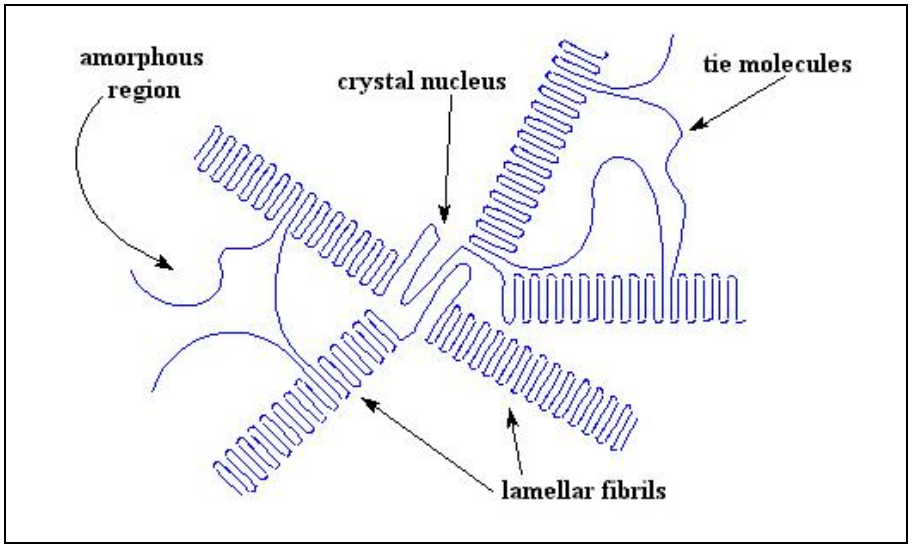


Figure 2.21. A polymer crystalline spherulite [48]

However, no polymer is completely crystalline, even the most crystalline polymers. Crystalline polymers may exhibit, therefore, both a T_g corresponding to long-range segmental motions in the amorphous regions and a crystalline-melting temperature or T_m at which crystallites are destroyed. Specific volume versus temperature for amorphous and crystalline polymers is shown in Figure 2.22 [47].

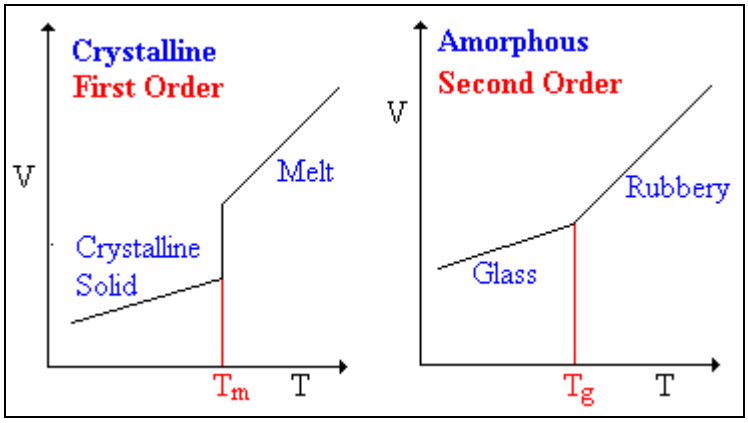


Figure 2.22. Specific volume vs. temperature for amorphous and crystalline polymers [47]

Table 2.4 summarizes there are several differences between glass transition temperature and melting temperature.

Table 2.4. Comparison of glass transition with melting [47]

Glass Transition	Melting
Property of the amorphous region	Property of the amorphous region
Below T_g: Disordered amorphous solid with immobile molecules	Below T_m: Ordered crystalline solid
Above T_g: Disordered amorphous solid in which portions of molecules can wiggle around	Above T_m: Disordered melt
A second order transition	A first-order transition

Melting and glass transition temperatures are important parameters relative to in-service applications of polymers. They define, respectively, the upper and lower temperature limits for numerous applications, especially for semi-crystalline polymers. The glass transition temperature may also define the upper use temperature for glassy amorphous materials [48].

2.10. MECHANICAL PROPERTIES OF POLYMERS

To understand and describe how materials deform as a function of applied load, time, temperature, and other conditions are needed first to discuss standard test methods and standard language for mechanical properties of materials. The bulk properties of a polymer are those most often of end-use interest.

The term mechanical property is commonly used to denote stress-strain relationship for polymer systems. Unlike many more familiar materials where these relationships depend essentially only on temperature, in polymeric systems time dependence is also of importance. This time dependence necessitates very careful definitions of parameters such as moduli and compliances which result from experiments involving discontinuous stress or strain levels. In addition, the time dependence may be explored using oscillatory perturbations as is done when investigating dynamic mechanical properties or dielectric relaxation in polymers [49].

2.10.1. Stress-Strain Behavior

Stress is the average amount of force exerted per unit area. It is the internal resistance a material offers to being deformed and is measured in terms of the applied load as shown in Figure 2.23. The effects of mechanical stress on a polymer can be measured using dynamic mechanical analysis [50].

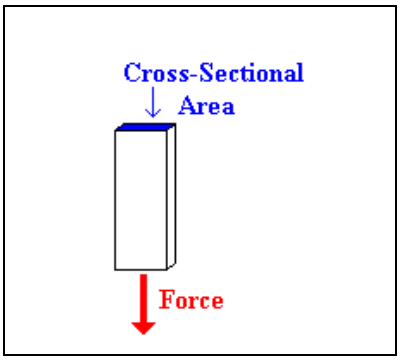


Figure 2.23 Stress [50]

Strain is a geometrical measure of deformation representing the relative displacement between particles in a material body, i.e. a measure of how much a given displacement differs locally from a rigid-body displacement, as shown in Figure 2.24 [50].

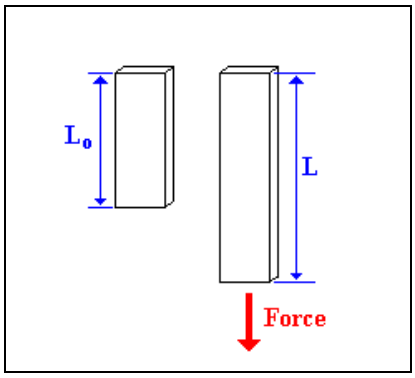


Figure 2.24. Strain [50]

When the stress is directly proportional to strain the material is said to obey Hooke's law, which is an approximation of elasticity that states the extension of a spring in direct proportion with the load added to it as long as and this load does not exceed the elastic limit. A typical stress-strain curve is presented in Figure 2.25.

The slope of the straight line portion of curve is equal to the modulus of elasticity. The maximum stress point on the curve, up to which stress and strain remain proportional, is called the proportional limit (point P) [50].

Most materials return to their original size and shape, even if the external load exceeds the proportional limit. The elastic limit represented by the point E in Fig. 2.25 is the maximum load which may be applied without leaving any permanent deformation of the material. If the material is loaded beyond its elastic limit, it does not return to its original size and shape, and is said to have been permanently deformed. On continued loading a point is reached at which the material starts yielding. This point is known as the yield point, where an increase in strain occurs without an increase in stress. It should however be noted that some materials may not exhibit a yield point. The point B in Fig. 2.26 represents break of the material [50].

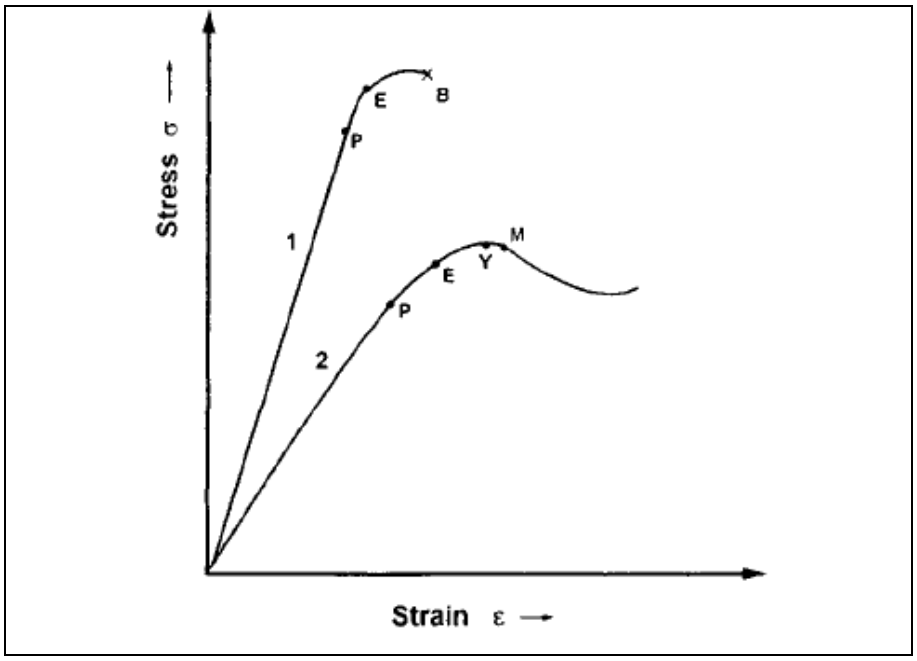


Figure 2.25. Stress-strain curve [51]

2.10.2. Strength

Strength of a material is its ability to withstand an applied stress without failure whether the applied stress may be tensile, compressive or shear. Tensile strength refers to strength upon pulling which is important for a material that is going to be stretched or under tension. Compression strength is strength against compression whereas flexural strength is against bending.

If the tensile strength is to reach the high value, the stress-strain curve must assume a high slope and this step rise must be maintained up to the range of the breaking stress. If manifestation of a high slope in the stress-strain curve requires crystallization in a given polymer system, then one may look for a correlation between crystallinity and tensile strength. These observations show that the development of tensile strength in polymers is related to their crystallization [48].

2.10.3. Modulus

In order to know how well a material resists deformation, its ‘modulus’ is measured. To measure tensile modulus, strength and ultimate elongation are measured. This time stress exerted on the material is measured, just like measuring tensile strength. The amount of stress is increased slowly, and then the elongation the sample undergoes at each stress level is measured until the sample breaks [50].

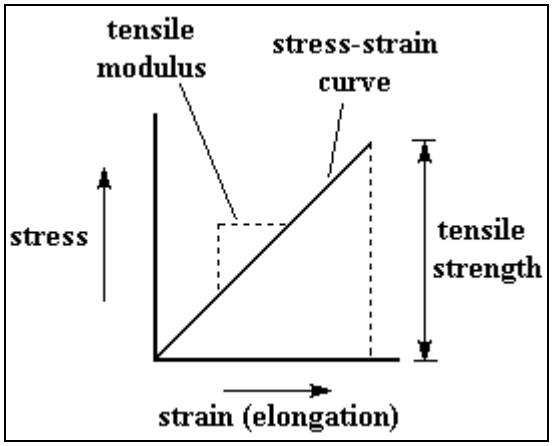


Figure 2.26. Stress – Strain – Tensile Strength Graph [50]

As can be seen in Figure 2.26, if the slope is steep, the sample has a high tensile modulus, which means it resists deformation. If the slope is gentle, then the sample has a low tensile modulus, which means it is easily deformed.

The storage modulus measures the stored energy, representing the elastic portion, and the loss modulus measures the energy dissipated as heat, representing the viscous portion. The tensile storage and loss modulus are defined as follows [13].

- Storage Modulus (*E’*)

$$E' = \frac{\sigma_0}{\varepsilon_0} \cos\delta \tag{2.1}$$

- Loss Modulus (*E’’*)

$$E'' = \frac{\sigma_0}{\varepsilon_0} \sin\delta \tag{2.2}$$

- Phase angle, tan (δ)

$$\tan\delta = \frac{E''}{E'} \tag{2.3}$$

Where δ is phase lag between stress and strain, σ_0 is the amplitude of the stress response and ε_0 is the amplitude of applied strain.

2.10.4. Elongation

Elongation is the increase in length of tensile test specimen, usually expressed as a percentage of the original length. Percent elongation is equal to the length the polymer sample is after it is stretched (L), divided by the original length of the sample (L_0), and then multiplied by 100 [50].

$$\frac{L}{L_0} \times 100 = \%\ elongation \tag{2.4}$$

The tensile stress is divided by the rate of elongation for elongational flow. Except for a Newtonian fluid, the elongational viscosity is not constant, but is dependent on the rate of elongation. It is important whenever a polymer melt is stretched, as in many polymer melt processing, the rate of shear at which such tension thinning or tension thickening occurs is the critical elongation rate [33].

Elastomers are polymers with the ability to stretch a long distance and still come back to its original state. Most of them can stretch from 500 to 1000 per cent elongation and return to their original lengths without any trouble [50].

2.10.5. Toughness

Plot of stress versus strain given in Figure 2.27 can give another very valuable piece of information. If one measures the area underneath the stress-strain curve, colored red in Figure 2.29, gives the property called toughness [49].

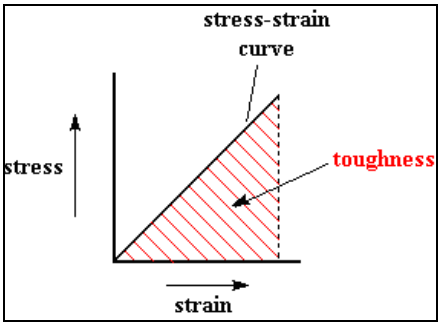


Figure 2.27. Stress vs. strain curve [49]

Toughness is a measure of the energy a sample can absorb before it breaks. If the height of the triangle in the plot is strength, and the base of the triangle is strain, then the area is proportional to strength times strain. Since strength is proportional to the force needed to break the sample, and strain is measured in units of distance (the distance the sample is stretched), then strength times strain is proportional is force times distance which is energy [49].

Toughness is different from strength. For example if a material is strong, it is not necessarily going to be tough as well [49].

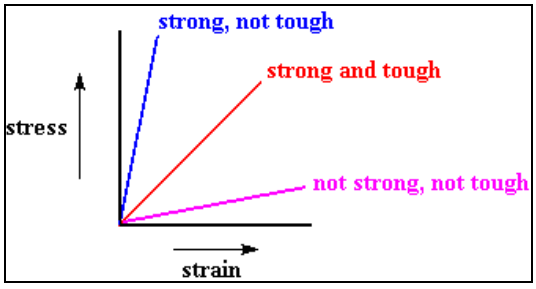


Figure 2.28. Strength vs. strain graph [49]

In Figure 2.28; the blue plot is the stress-strain curve for a sample that is strong, but not tough. It takes lots of force to break this sample, but not much energy, as the area underneath the curve is not large. Likewise, this sample can not stretch very far before it breaks. A material like this which is strong, but can not deform very much before it breaks is called brittle [49].

On the other hand, the red plot is a stress-strain curve for a sample that is both strong and tough. This material is not as strong as the sample in the blue plot, but the area underneath its curve is larger than the area under the blue sample's curve. So it can absorb more energy than the blue sample can. The red sample elongates more before breaking than the blue sample does. Deformation allows a sample to dissipate energy. If a sample can not deform, the energy will not be dissipated, and will cause the sample to break [49].

The blue sample has a much higher modulus than the red sample. While it is good for materials in a lot of applications to have high moduli and resist deformation, in the real world it is a lot better for a material to bend than to break [49].

The ultimate aim of this project is the construction of a biodegradable drug-eluting polymeric stent for treatment of coronary artery diseases, which have the required mechanical properties

As mentioned in previous sections, the term biocompatibility describes the availability of the material when it is placed into the body and the biocompatibility of stent is extremely important. Chosen polymers that were used for stent construction have to be highly biocompatible to prevent chronic inflammation and cell disruption. Besides, stent should be constructed from biodegradable polymers such as PLLA, PCL and PBS and the time for full degradation of stent is anticipated as nearly 12 months. Also the drug should be released during the first month after implantation of stent into the body to prevent the restonosis.

The intended design of stent should be consisted of two layers (inner and outer). For the outer layer PLLA and PCL polymer blends were prepared via solvent casting method. The amount of released drug (paclitaxel) from polymer blends were determined by using HPLC and also degradation study of appropriate polymer blends were carried out.

Moreover, the thermal transitions of these polymer blends were analyzed by Differential Scanning Calorimetry (DSC) and the morphologies of the polymers were characterized by Scanning Electron Microscopy (SEM). The biocompatibility study of

polymer blends was done with the method of MTS and Calcein AM assay by Genetics and Biotechnology Department.

For the inner layer, polymer blends including PLLA, PCL and PBS, with different compositions were prepared by using a microcompounder. As an ongoing project, the biodegradability studies of these polymer blends prepared via melt blending method are continuing while thermal and the thermomechanical properties are being analyzed by Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analyzer (DMA) respectively. The biocompatibility study of these polymer blends are also going to be carried out with the method of MTS and Calcein AM assay by Genetics and Biotechnology Department. The following chapter presents experimental set up, chemicals amd methods used in this study.

3. MATERIALS AND METHODS

3.1. CHEMICALS

For the preparation of the solution cast polymer blends and the analysis of degradation and drug release from the prepared polymer blends, different chemicals were used as shown in Table 3.2. Paclitaxel whose structure is shown in Table 3.3 was used as the drug component of the stent coating. .

Table 3.1. Polymers used for preparing different polymer blends

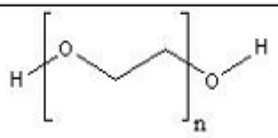
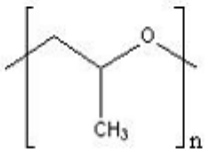
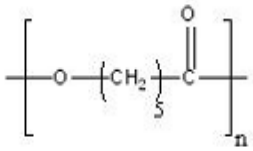
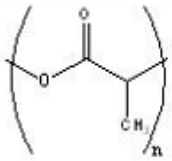
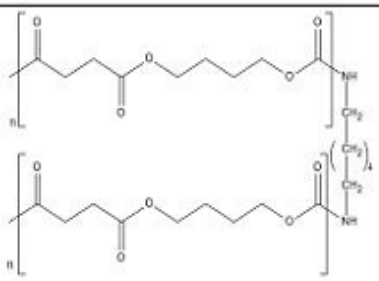
Polymer Name	Formula	Structure	Provider	Purity
Polyethylene oxide	$H[OCH_2 CH_2]nOH$		BASF	N.A.
Polypropylene oxide	$[OCH CH_3CH_2]n$		BASF	N.A.
Polycaprolactone (PCL)	$(C_6H_{10}O_2)_n$		Aldrich	N.A.
Poly-L-lactic acid (PLLA)	$(C_3O_2H_4)_n$		Fluka	90%
Poly(1,4-butylene succinate) (PBS)	$[C_{10}H_{15}O_4]n - C_6H_{14}N_2 - [C_{10}H_{15}O_4]n$		Aldrich	N.A.

Table 3.2. Chemicals used for the preparation of the solution cast polymer blends and the analysis of degradation and drug release from different polymer blends

Chemical Name	Formula	Structure	Provider	Purity

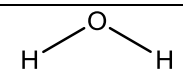
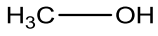
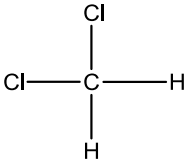
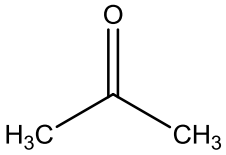
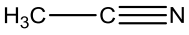
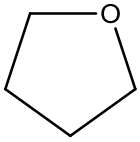
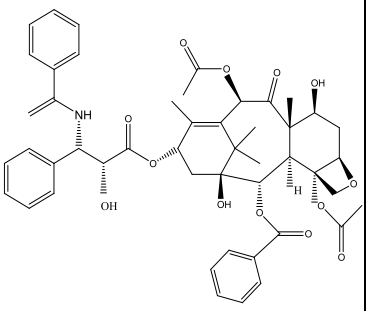
Water	H_2O		-	-
Methanol	CH_3OH		Aldrich	99%
Dichloromethane	CH_2Cl_2		BDH	99.8%
Acetone	C_3H_6O		Aldrich	99 %
Acetonitrile	CH_3CN		Aldrich	97%
Tetrahydrofuran (THF)	$(CH_2)_4O$		Aldrich	99.9 %

Table 3.3. Paclitaxel used as the drug component of the stent coating

Chemical Name	**Formula**	**Structure**	**Provider**	**Purity**
Paclitaxel	$C_{47}H_{51}NO_{14}$		LC	99.5 %

3.2. METHODS

3.2.1. High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a chromatographic technique that is used to separate a mixture of compounds, to identify, quantify and purify the individual components of the mixture [52]. HPLC was used to determine the amount of drug release from different polymer blends. An image of the HPLC used in this study is given in Figure 3.1.

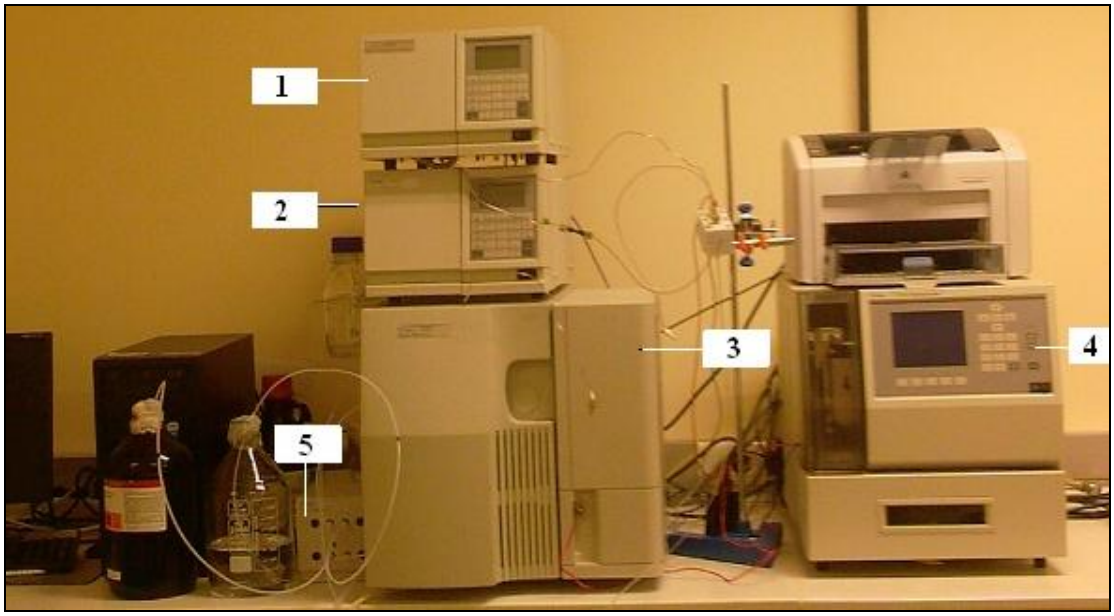


Figure 3.1 High Performance Liquid Chromatograph used in the study

The High Performance Liquid Chromatograph used in this study consists of the following five components:

1. Multi λ Fluorescence Detector
2. UV/ Visible Detector
3. Binary Pump
4. Autosampler
5. In Line Degasser AF

HPLC utilizes different types of stationary phase (typically, hydrophobic saturated carbon chains), a pump that moves the mobile phase and analyte through the column, and a detector that provides a characteristic retention time for the analyte. The detector may also provide other characteristic information. Analyte retention time varies depending on the

strength of its interactions with the stationary phase, the ratio/composition of solvent used, and the flow rate of the mobile phase [52].

With HPLC, a pump provides the higher pressure required to propel the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography [52].

The sample to be analyzed is introduced in small volumes to the stream of mobile phase. The analyte's motion through the column is slowed by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. How much the analyte is slowed depends on the nature of the analyte and on the compositions of the stationary and mobile phases. The time at which a specific analyte elutes is called the retention time; the retention time under particular conditions is considered a reasonably unique identifying characteristic of a given analyte [52]

The use of smaller particle size column packing increases the linear velocity giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combination of water or various organic liquids. Water may contain buffers or salts to assist in the separation of the analyte components, or compounds such as trifluoroacetic acid which acts as an ion pairing agent [52].

3.2.2. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry was used to determine the thermal transitions such as the melting, glass transition and the crystallization temperature of the components of the prepared polymer blends. Moreover, through the enthalpies of melting and crystallization of the blend components, the crystallinity of the polymer blends was also evaluated. The ability to determine transition temperatures and enthalpies makes DSC a valuable tool in producing phase diagrams for various polymers.

The Differential Scanning Calorimeter and its accessories used during the experimental study can be seen in Figures 3.2 and 3.3.

Figure 3.2. Differential Scanning Calorimeter

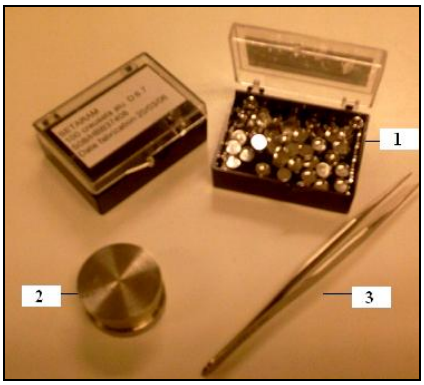


Figure 3.3. Accessories used for DSC

1. Creusets
2. Crimping Tool
3. Tweezers

3.2.3. Scanning Electron Microscopy (SEM)

The morphology of the prepared polymer blends was analyzed using SEM. The scanning electron microscope is a type of electron microscope that images the sample

surface by scanning it with a high energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

4. EXPERIMENTAL STUDY

4.1. EXPERIMENTAL PROCEDURES

4.1.1. Preparation of Polymer Films

Polymer blends of different PLLA and PCL contents were prepared by solution casting. About 0.1 g of the polymer mixture was solubilized in dichloromethane (10 mL). Solutions were transferred to petri dishes with the dimensions of 100 mm diameter and 15 mm height. Solutions were kept under the hood to allow the evaporation of dichloromethane overnight at room temperature. Polymer films were then kept for one hour in a vacuum oven (Memmert VO 400, Germany). Drug containing films films were prepared in a similar manner by dissolving 0.00819 mmol (7 mg) of paclitaxel and 0.1 g of PLLA/PCL at different blend ratios (95/5, 90/10, 85/15, 80/20 wt/wt).

4.1.2. DSC Analysis and Determination of Crystallinity of PLLA/PCL Blends

The thermal behavior of thin films of PLLA/PCL blends at different weight ratios (95/5, 90/10, 85/15, 80/20) prepared via solution casting method, was investigated by differential scanning calorimetry (DSC) (Setaram DSC 131, France). Each film sample (5-8 mg) was scanned from 25 to 300 ^{o}C at a heating rate of 5 ^{o}C/min under nitrogen atmosphere. Neat PLLA film sample was also prepared via solution casting method similar to that of the preparation of the blend films and scanned under the same conditions for comparison.

Crystallinity of the PLLA phase in PLLA/PCL blends was determined from DSC analysis by measuring the integrated area under the PLLA melting peak (175 ^{o}C-180 ^{o}C) and the PLLA crystallization peak (84-87 ^{o}C) to determine the corresponding enthalpy of melting, $dH_{m,PLLA}$, and crystallization of PLLA $dH_{c,PLLA}$. The crystallinity of PLLA for each of the PLLA/PCL blend samples were then evaluated using the $dH_{m,PLLA}$ and $dH_{c,PLLA}$ values according to the following equation:

$$x_{c,PLLA}(\%) = \frac{100 \times (dH_{m,PLLA} + dH_{c,PLLA})}{93 \times X_{PLLA}} \tag{4.1}$$

where $x_{c,PLLA}(\%)$ is the percent crystallinity of PLLA, X_{PLLA} is the weight fraction of PLLA, and 93 J/g stands for the enthalpy of fusion of PLLA having infinite crystal thickness.

4.1.3. Degradation Test for Different Polymer Blends

Polymer films were obtained as described in section 4.1.1. The total mass of the films were recorded to be approximately 0.3 gram. The films were cut into 8 almost equal pieces and all of the pieces were weighed. The polymers were put in PBS buffer (DPBS 10X PAN BIOTECH GmbH) at pH 7.4. All the polymers with the contact solution were placed in the orbital shaker at 37^{o}C and 120 rpm. The pH values of the solutions were measured on the first, fourth and seventh days of the week. The contact solutions were refreshed every seven days for the duration of the experiments. Every week one piece of each polymer was taken from the contact solution, washed with water and freeze dried to remove water. The dry sample was weighed and the difference between the initial and final weight was taken to show the weight loss of the polymer.

4.1.4. Drug Elution of Polymer Blends

The thin film (thickness about 0.4-0.6 mm) was divided into three pieces and each piece was weighed. Each piece was put into a different test tube and PBS solution at 7.4 pH was added into the test tubes. Then the test tubes were placed in an orbital shaker and temperature was kept at 37°C with a shaking rate of 120 rpm. The contact solution was drawn out every other day. The amounts of drug in these solutions were measured by using HPLC in order to obtain drug elution profiles from each blend. For this purpose, 1 ml dichloromethane was added to the drawn out solution to extract Pactitaxel into the dicholoromethane phase. Upon phase separation, dichloromethane phase was taken and then placed in petri dishes. Dishes were left at room temperature and upon evaporation of dicholoromethane, paclitaxel was observed to recrystallize. The crystallized drug was solubilized in 1 ml acetonitrile: water (1:1) solution to be analyzed by HPLC.

4.1.5. Determination of the Released Drug Amount

Paclitaxel standards were diluted into five different concentrations with different volumes of methanol. Used amount of paclitaxel and methanol and the calculated concentration of paclitaxel standards are shown in Table 4.1.

Table 4.1. The amount of paclitaxel and methanol

Paclitaxel (mg)	Methanol (ml)	Concentration (mg/ml)
1	2.5	0.4
2	2	1
3	1.5	2
4	1.3	3
5	1.25	4

The whole system was purged to clean the system of air bubbles and possible contamination. C-18 5 μm Column was connected to the pump. The mobile phases flow from pump A (Acetonitrile) and B (Distilled Water) were adjusted to deliver the fluids with a rate of 1 mL/min for 20 minutes. 0.5 mL of all samples and standards were put into the vials. Then, vials were placed into the autosampler inside the HPLC. Computer Program with the name of Empower, HPLC was started.

Sample set and location of the vials were recorded to the computer program. The run time of each sample was 50 minutes, injection volume 100 μL and the number of injections was two. The two mobile phases in the gradient study consisted of 100% acetonitrile and 100% double distilled water. (0-8 min 25% acetonitrile, 8-20 min 44% acetonitrile, 20-45 min 80% acetonitrile, 45-50 min 25% acetonitrile) The flow rate was held constant at 0.5 ml/min. The drug concentrations were determined by measuring peak areas, which were compared to a linear calibration curve of known standard concentrations.

4.1.6. Determination of the Porosity and Phase Morphology of Polymer Blends

The porosity of polymer blends effects the degradation rate and also the drug release profile. Because of that scanning electron microscope was used to determine the porosity of polymer films.

Moreover, polymer blends prepared with the method of solution casting were put into the liquid nitrogen to break the films. After that, the fracture surfaces of the broken films were exposed to THF vapor for nearly 10 minutes. This procedure is expected to extract only PCL from the blend and allow observation of PCL domains in the blend.

4.1.7. Preparation of Polymer Blends with the Method of Melt Blending

Totally 8 grams of polymers and 1% pluronic surfactant were put into the microcompounder. The temperature was fixed according to the melting point of the polymers. The polymer blend components were mixed for about 30 minutes at a rotor speed of 150 rpm by using double screws shown in Figure 4.1. After 30 minutes, the melt polymer blend was taken into the DMA sample mold shown in Figure 4.2.

Table 4.2. Compositions of Polymer

Polymer Content	Compositions of Polymers
PBS-PCL	90-10
PBS-PCL	85-15
PBS-PCL	80-20
PBS-PCL	70-30
PBS-PCL	60-40
PBS-PCL	50-50
PBS-PLLA	90-10
PBS-PLLA	80-20
PBS-PLLA	70-30

For the inner layer of the stent body, PBS-PCL and PBS-PLLA polymer blends were prepared via melt blending using a mircocompounder. The prepared polymer blends are tabulated in Table 4.2.

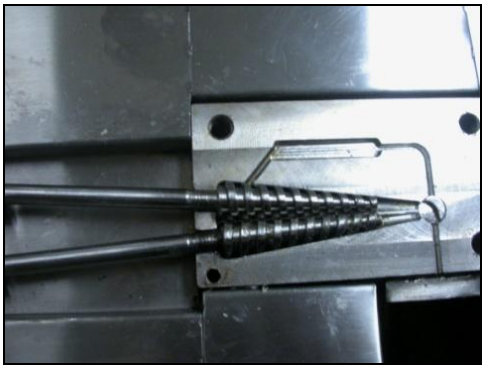


Figure 4.1. A picture of the double screw in the mixing chamber of the microcompounder

Figure 4.2. A picture of the DMA mold

5. RESULTS AND DISCUSSIONS

5.1. DETERMINATION OF THE POROSITY AND PHASE MORPHOLOGY OF POLYMER BLENDS

Figure 5.11 shows the SEM images of the surface of PLLA/PCL and Paclitaxel loaded PLLA/PCL solution cast films at 4000 magnification. The pore size distribution of the solution cast PLLA/PCL films as determined from SEM analysis is displayed in Figure 5.12. The surface SEM pictures of the PLLA/PCL solution cast films clearly show the porous morphology due to solvent evaporation process.

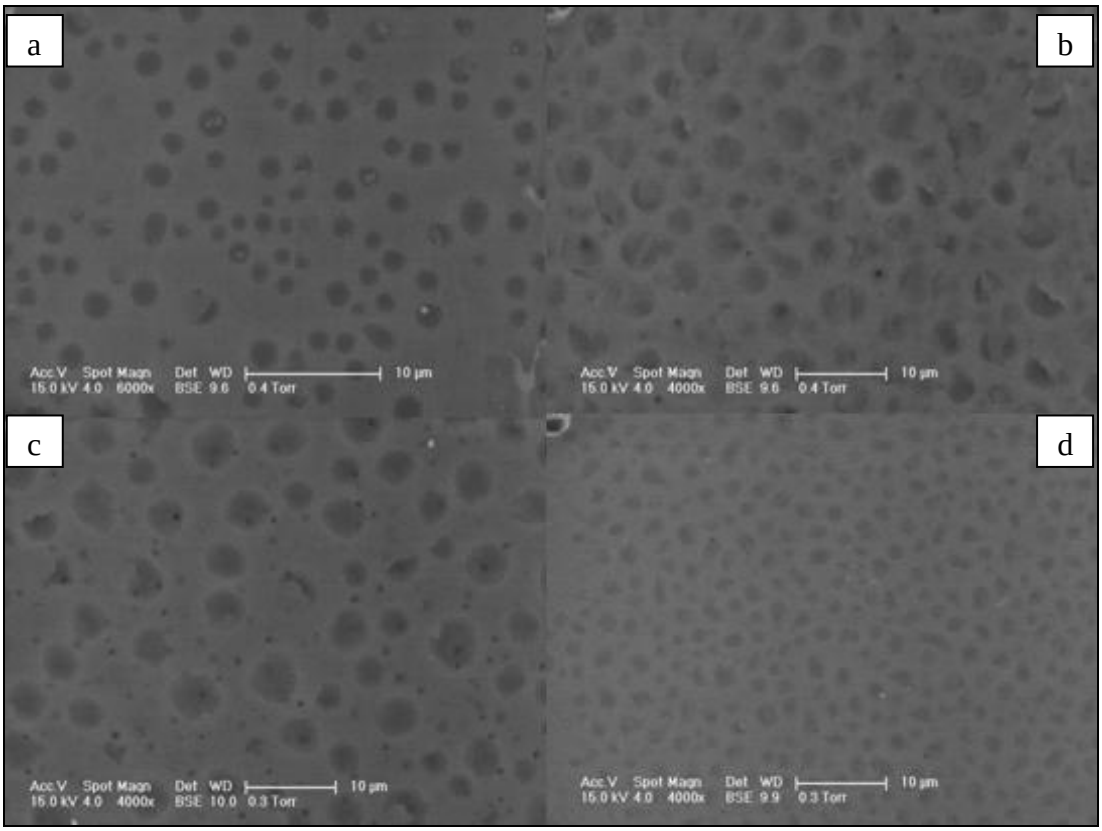


Figure 5.1 SEM images of PLLA-PCL and PLLA/PCL / Paclitaxel solution cast films: (a) PLLA-PCL (80-20) (b) PLLA-PCL (85-15) / Paclitaxel (c) PLLA-PCL (90-10) / Paclitaxel (d) PLLA-PCL (95-5) / Paclitaxel at 4000x magnification.

In Figure 5.1 small black dots represent paclitaxel and paclitaxel is seen to be well dispersed throughout the polymer blends. Figure 5.2 shows that, the pore size diameters range from 1 to 5.4 µm for all films of PLLA-PCL blends. These pores are not related with the phase separation of the two polymers, instead form as a result of solvent eveporation during film formation.

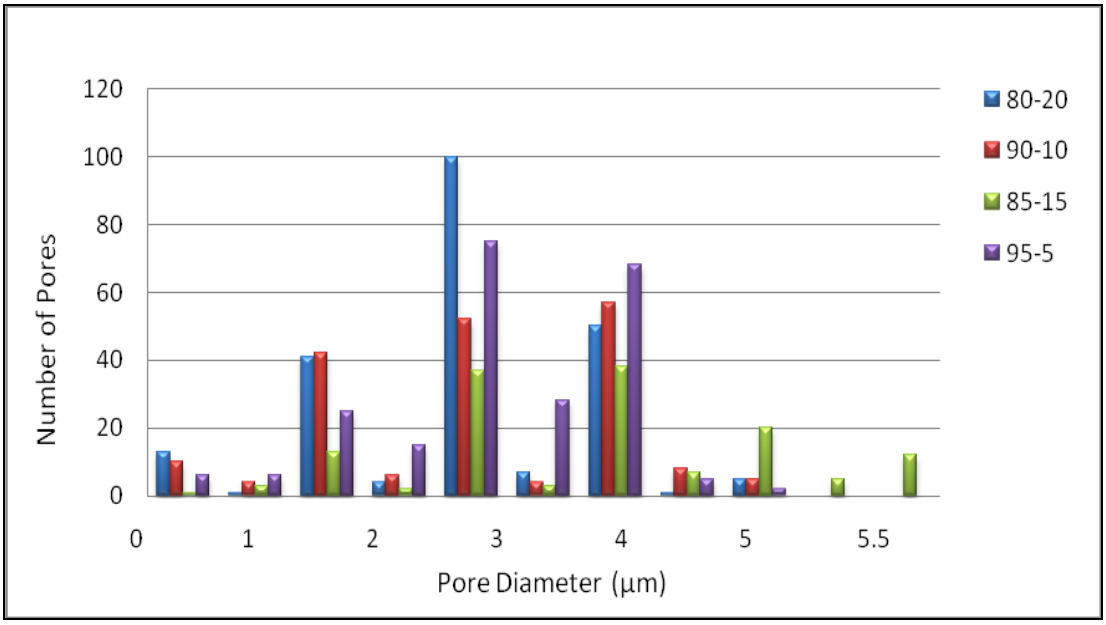


Figure 5.2 Pore size distribution of polymer films

Second part of the SEM analysis involves the analysis of PCL phase in the polymer blends. For this purpose, frozen polymer films with different PLLA/PCL contents were broken in liquid nitrogen and the fractured surfaces were etched with THF vapor to extract PCL from the blends. SEM images of the THF etched fracture surfaces of the PLLA-PCL blends with changing PCL contents are shown in Figure 5.3.

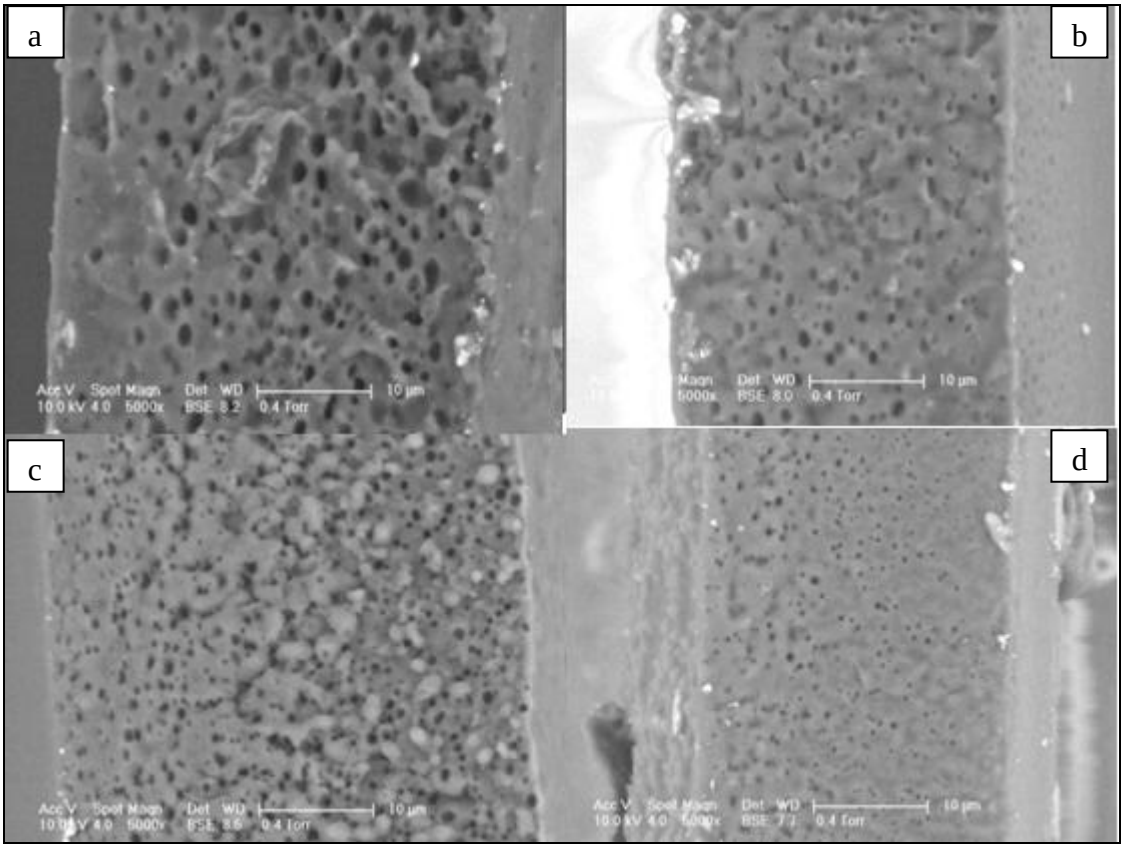


Figure 5.3 SEM images of etched polymer films with THF vapor (a) PLLA-PCL (80-20) (b) PLLA-PCL (85-15) (c) PLLA-PCL (90-10) (d) PLLA-PCL (95-5)

As can be seen, the PCL is dispersed homogenously in the PLLA matrix as spherical domains. From these images, it can clearly be seen that these PLLA-PCL blends are phase separated. The SEM images shown in Figure 5.3 clearly indicates that the pore diameters of the PCL domains significantly decrease as the PCL content decreases from 20 wt% PCL to 5 wt% PCL (from 0.5-2 μm size range to 0.1-0.4 μm size range). The reportedly immiscible PLLA-PCL pairs have relatively different solubility parameters thus they tend to demix from each other during solvent evaporation and coalesce in separate domains to reduce the interfacial area. The extent of coalescence in the binary PLLA-PCL blends prepared via melt blending is reported to increase with increasing concentration of the dispersed phase which is in agreement with the SEM pictures of PLLA-PCL blends at various PCL contents presented in Figure 5.3 [53]

Solvent casting is a good method of mixing immiscible polymers like PLLA, PCL and PBS homogenously. Melt blending is the other method to get uniform polymer blends. Polymer blends of PBS and PCL with different ratios were prepared by melt blending technique using a microcompounder. SEM images of the fracture surfaces of PBS-PCL blends fractured in liquid nitrogen and etched with THF vapor are shown in Figure 5.4.

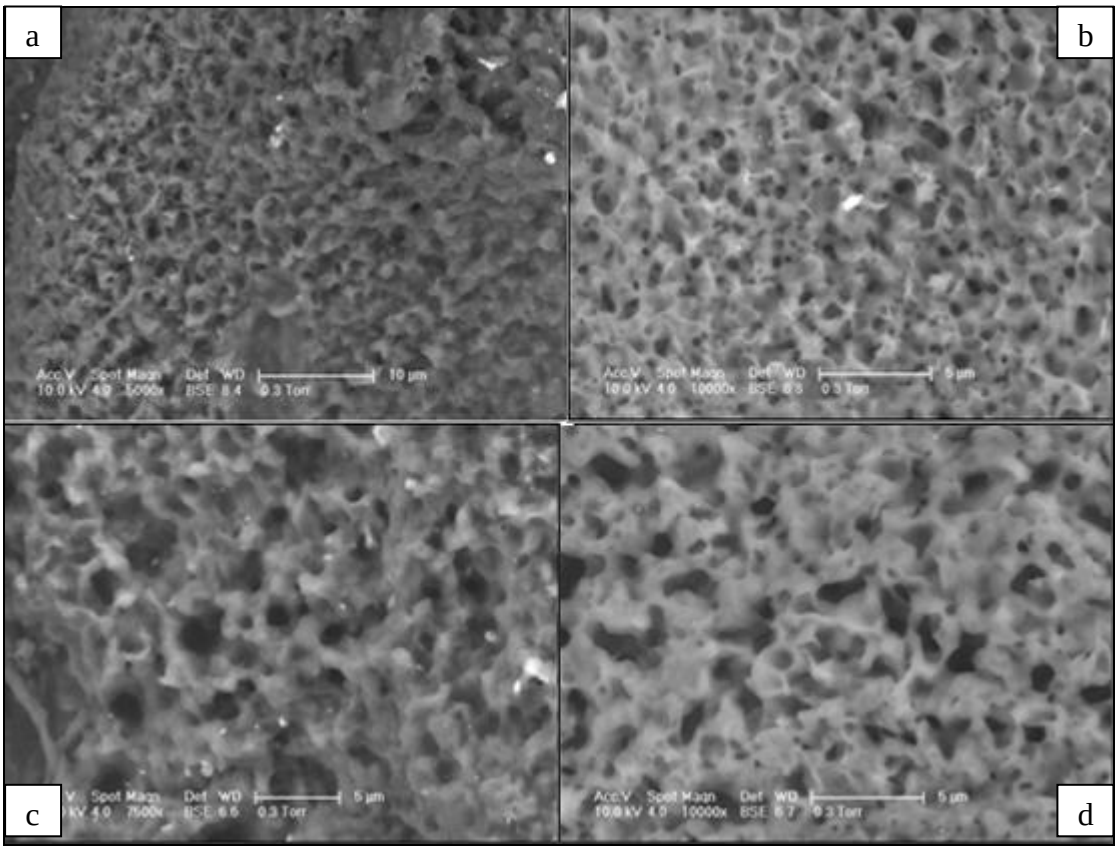


Figure 5.4 SEM images of fracture surfaces of PBS-PCL polymer blends etched with THF vapor (a) PBS-PCL (70-30) (b) PBS-PCL (80-20) (c) PBS-PCL (85-15) (d) PBS-PCL (90-10)

As shown in Figure 5.4, the PBS-PCL blends like the PLLA-PCL blends ,show a phase seperated structure where PCL exists in the form of spherical domains.

5.2. DSC ANALYSIS AND DETERMINATION OF CRYSTALLINITY OF PLLA/PCL BLENDS

Differential Scanning Calorimetry (DSC) was used to determine the thermal behaviour of solvent cast films of PLLA-PCL polymer blends with different ratios (95-5, 90-10, 85-15,80-20).

DSC measures the heat flows associated with transitions in materials as function of time and temperature in a controlled atmosphere. These measurements provide quantiative and qualitative information about physical and chemical changes that involve endothermic or exothermic processes or changes in heat capacity.

One of the most widely used techniques to measure T_g and T_m is Differential Scanning Calorimetry. This method uses individual heaters to maintain identical temperatures. During heating of polymers, additional crystallization may occur at temperatures between T_g and T_m as illustrated by the crystallization exotherm of polymers.

PLLA-PCL blends (95-5, 90-10, 85-15, 80-20) were prepared via solvent casting method to see thermal behaviour of polymer films and also heat flow vs. temperature graph of these polymers were generated by using DSC results. Due to Figure 5.15, the glass transition temperature of pure PLLA film was determined nearly 64 ^{o}C. Pure PLLA has an exothermic peak around 90 ^{o}C which is known as crystallization of PLLA. Melting temperature of PLLA was recorded as 177 ^{o}C by observing endothermic peak of PLLA.

As can be seen, PLLA-PCL blends have also exothermic peak around 84-87 ^{o}C that indicates the crystallization of PLLA. Moreover, the DSC spectrum PLLA-PCL blends has two endothermic peaks like melting peak PLLA (175-177 ^{o}C) and melting peak of PCL (64-66 ^{o}C). However, that was impossible to determine the glass transition temperature of PLLA of polymer blends due to the overshadow of melting peak of PCL.

The percent crystallinity of PLLA for each blend was calculated with the formula shown below as described in Section 4.1.2.

$$x_{c,PLLA}(\%) = \frac{100 \times (dH_{m,PLLA} + dH_{c,PLLA})}{93 \times X_{PLLA}} \quad (5.1)$$

The values of enthalpy of melting, $dH_{m,PLLA}$, and crystallization of PLLA, $dH_{c,PLLA}$ were determined with reference to the melting and crystalliztion peak areas. The percentage values of crystallinity and all obtained data through DSC analysis are tabulated in Table 5.1.

As seen in Table 5.1, the melting temperature of PLLA and the PCL melting temperatures for the PLLA/PCL blends are not significantly altered with changing PCL contents. The melting enthalpy of PLLA ($\Delta H_{m(PLLA)}$) as determined from the endothermic melting peak area of the PLLA matrix is decreased slightly with the introduction of PCL as compared to pure PLLA. In a similar manner, the enthalpy of PLLA crystallization ($\Delta H_{c(PLLA)}$) decreases with increasing PCL contents.

The percent PLLA crystallinity values obtained from these data on the other hand, exhibit an increase from 34% for pure PLLA to about 40% at 15 wt% PCL content, followed by a decrease to 26% at 20 wt% PCL content as shown in Figure 5.6. The overall increase in percent PLLA crystallinity, $x_{c,PLLA}(\%)$ with increasing PCL contents (until 15 wt% PCL) indicates that the enthalpy of melting, $\Delta H_{m(PLLA)}$ for the blends decreases due to the decrease in the enthalpy of PLLA crystallization ($\Delta H_{c(PLLA)}$) during the heating process.

Thus, although PCL decreases the enthalpy of crystallization for PLLA during heating, the presence of PCL helps PLLA crystallization from solution. Increasing PCL content above 15 wt% seems to disturb the formation of ordered (crystalline) structure of PLLA chains that crystallinity of PLLA starts to decrease above this content of PCL [53].

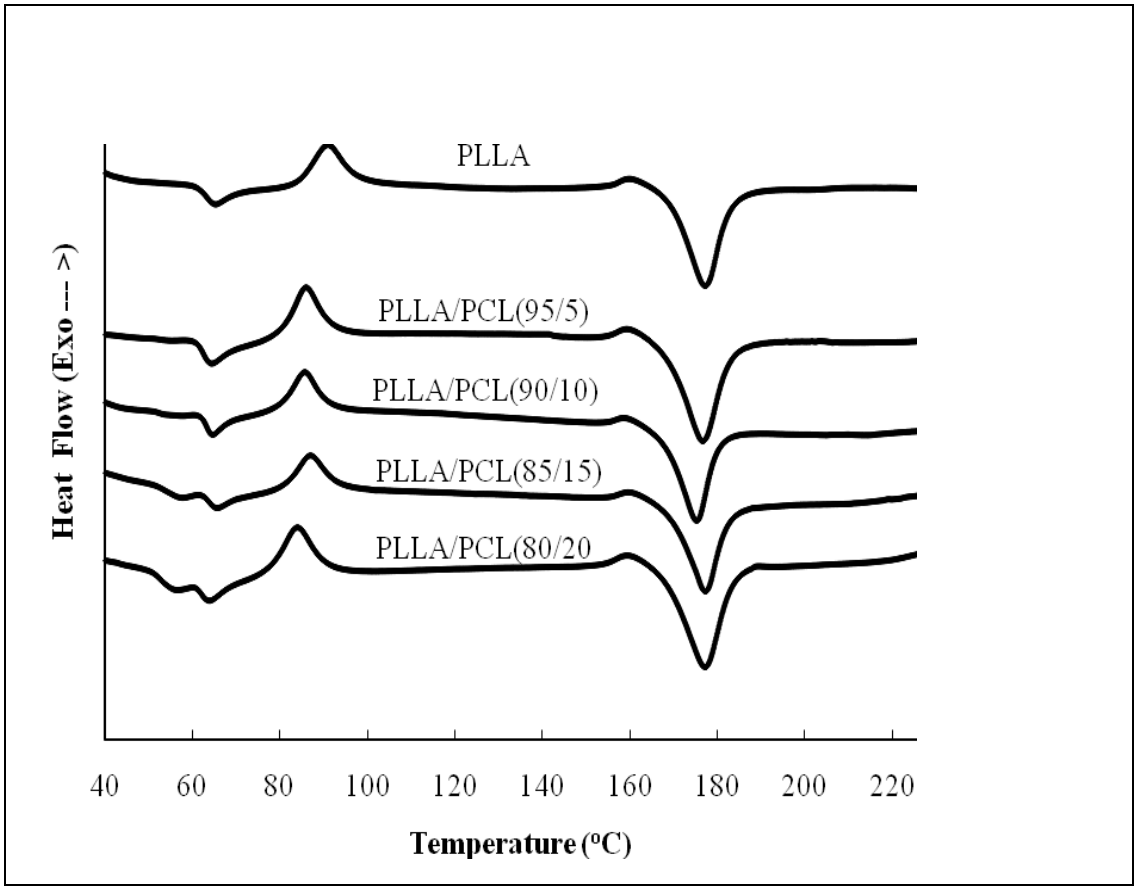


Figure 5.5 DSC thermograms of PLLA and PLLA-PCL blends

Table 5.1. DSC Results of PLLA/PCL Blends

Composition (PLLA/PCL)	$T_{m(PLLA)}$ (°C)	ΔH_m (Jg^{-1})	T_c (°C)	ΔH_c (Jg^{-1})	$T_{m(PCL)}$ (°C)	**%PLLA Crystallinity**
100/0	177.2	46.9	90.4	-15.29	-	34.01
95/5	176.7	46.5	85.9	-14.05	64.4	36.7
90/10	175.2	44.2	85.7	-11.7	64.5	38.8
85/15	177.3	42.8	87.0	-11.3	65.6	39.8
80/20	177.1	27.9	83.8	-8.5	63.7	26.2

The presence of well separated melting peaks for PLLA and PCL and the fact that PLLA and PCL melting temperatures are unaffected by changing PLLA/PCL ratios show that these two polymers are phase separated as also confirmed by SEM analysis. On the other hand, the increase in PLLA crystallinity with increasing PCL contents indicate that

there is a certain amount of interaction between PCL and PLLA at least in the presence of solvent and that the phase separation occurs during the solvent evaporation process. The results also indicate that although the presence of PCL enhances PLLA crystallization from solution, above 15 wt% PCL content the presence of PCL domains disturbs the PLLA crystalline structure.

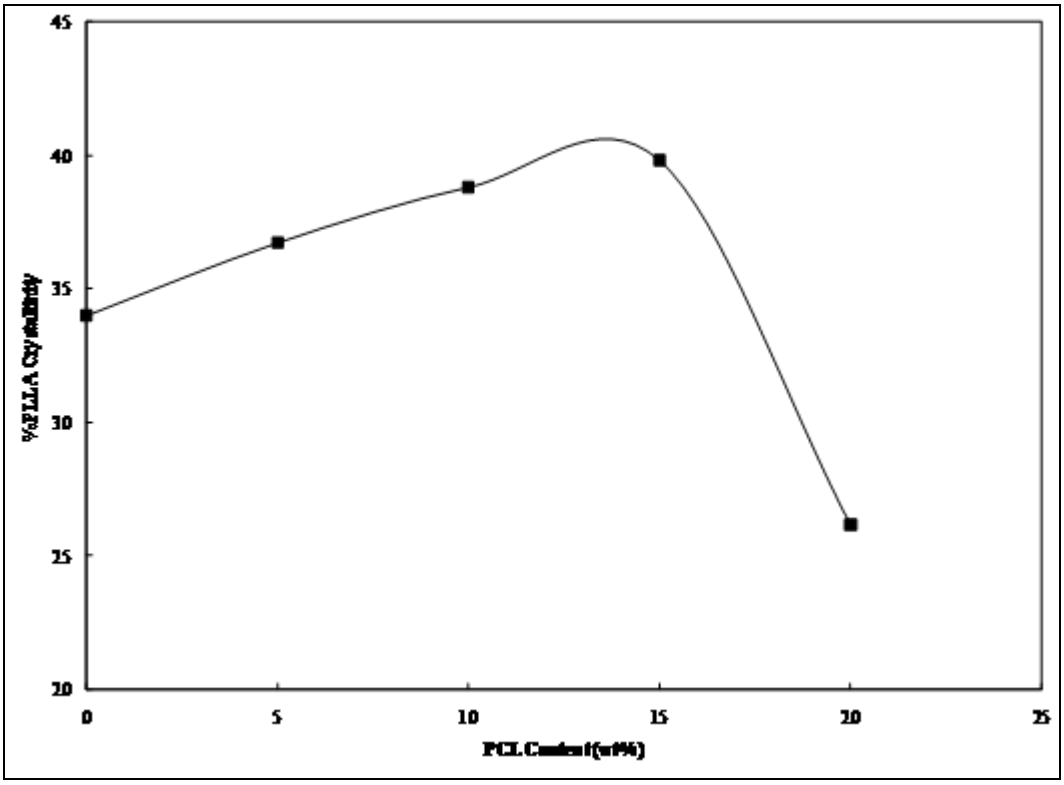


Figure 5.6 Change of %PLLA crystallinity with PCL content (wt%)

5.3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY RESULTS PACLITAXEL RELEASE FROM PLLA-PCL BLENDS

HPLC (High Performance Liquid Chromatography) was used to determine the amount of paclitaxel released from different polymer blends.

Following the completion of the HPLC runs, the amount of paclitaxel released from polymer blends was determined by measuring peak areas according to the calibration curve prepared with known standard concentrations.

First of all, 7 mg paclitaxel was mixed in with pure PLLA (0.1 g) and the contact solution was drawn out everyday for 11 days. The amounts of the released paclitaxel are tabulated in Table A.1 in Appendix A.

The cumulative amount of the released paclitaxel versus time graph was plotted to see release profile of 7 mg paclitaxel in 0.1g PLLA as shown in Figure 5.7. As summarized in Table.1, the amount of released paclitaxel at the end of 11 days was determined to be nearly 4.5 mg.

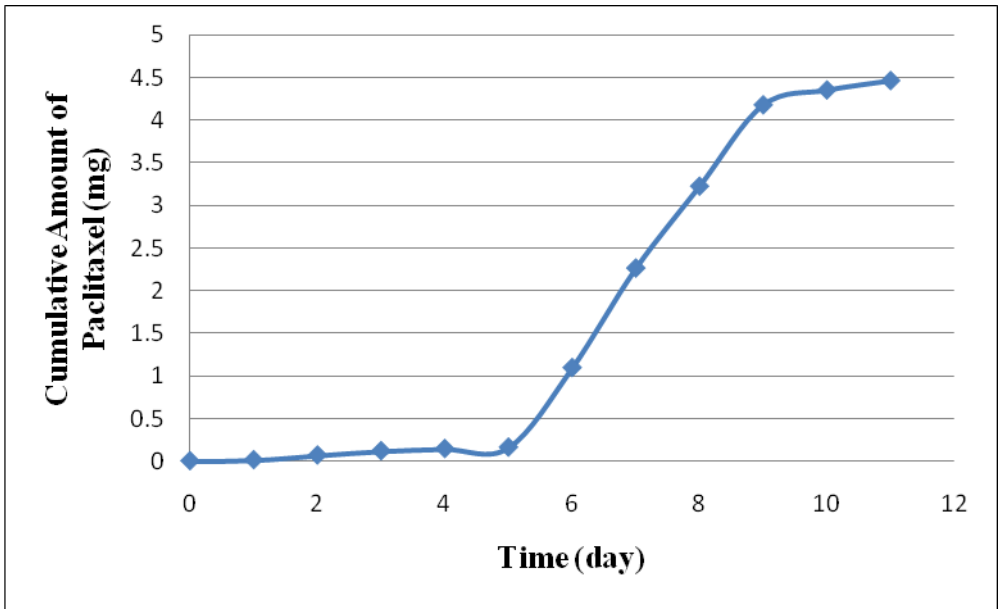


Figure 5.7 Cumulative amount of paclitaxel released vs. time graph of paclitaxel in 0.1 g PLLA

According to Figure 5.7 most of paclitaxel was released within nine days after a lag period of five days. However, the aim of this project is to obtain a polymer blend that can release the drug slowly in about 30 days. That is to say this profile is not desirable for our application.

As it was shown that PLLA is not convenient to obtain the desirable drug release profile, PLLA-PCL blends with different weight contents were tested for paclitaxel release.

0.1 gram polymer film with the ratio of 90-10 PLLA-PCL was prepared and 7 mg paclitaxel was mixed into the polymer blend. The film thickness of polymer blends was measured to be nearly 0.5 mm. The amounts of paclitaxel released from 90-10 PLLA-PCL blend at different time intervals are shown in Table A.2 in Appendix A.

Cumulative amount of released paclitaxel vs. time graph was created to obtain the release profile for the 90.10 PLLA-PCL blend.

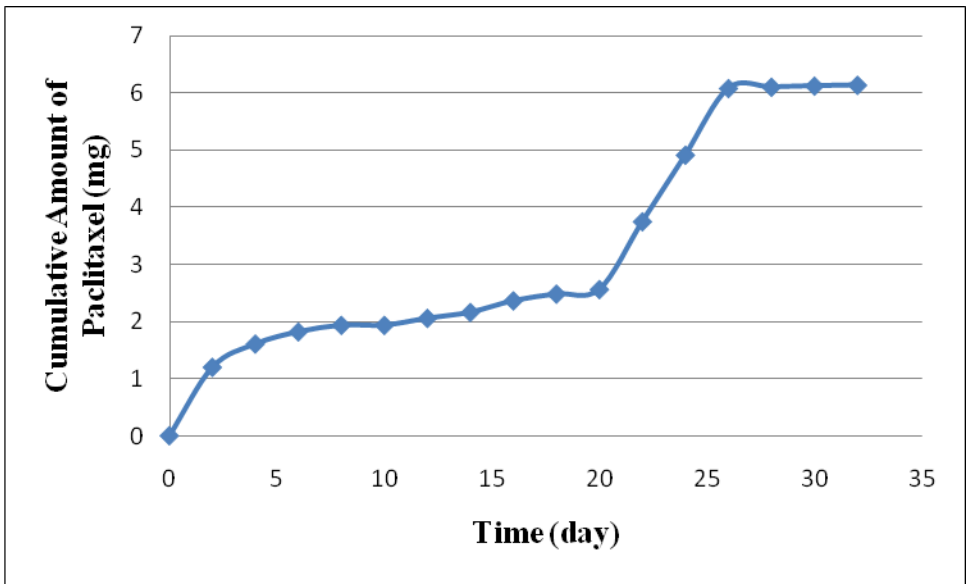


Figure 5.8 Cumulative amount vs. time graph of paclitaxel (7 mg) in 90-10 PLLA-PCL blend (totally 0.1 g)

Almost all paclitaxel (5.6 mg of 7 mg) was released in about 25 days from 90-10 PLLA-PCL blend. This release profile appeared to be ideal for the application which requires the drug to be gradually released in about 30 days.

Moreover, 3 times thick polymer film was prepared to see the effect of thickness on the drug release profile. The drug/polymer ratio was held the same. Samples with 0.3 g polymer blend with the ratio of 95-5, 90-10, 85-15 and 80-20 PLLA-PCL containing 21 mg paclitaxel were prepared. The tabulated amounts of released paclitaxel from each blends are given in Table A.3 in Appendix A.

The cumulative amount of released paclitaxel versus time graph was plotted so as to see release profile of paclitaxel from 0.3 g PLLA-PCL blends. The effect of film thickness and high polymer content on the drug release was determined through this experiment. The total amount of drug is released in 80 days versus 25 in the case of a film 3 times thicker. Results showing the paclitaxel release profiles from all prepared polymers with different amounts of embedded paclitaxel (7 mg and 21 mg) are shown in Figures 5.9 and 5.10.

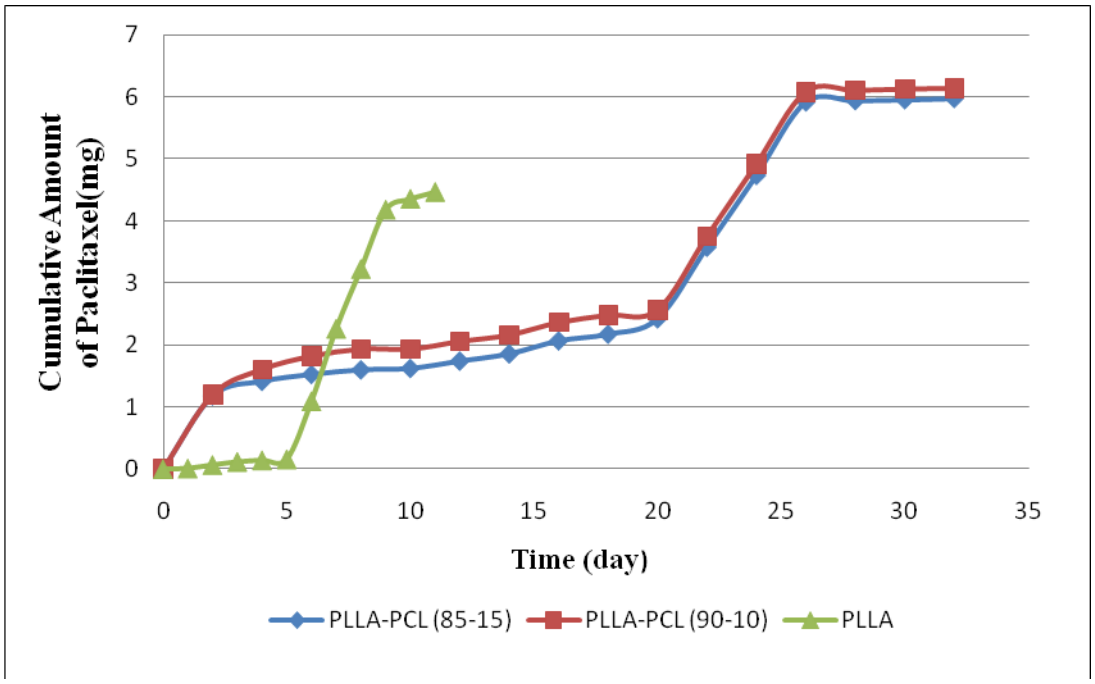


Figure 5.9 Cumulative amount vs. time graph of 7 mg paclitaxel in 90-10 and 85-15 PLLA-PCL polymer blends and PLLA polymer alone

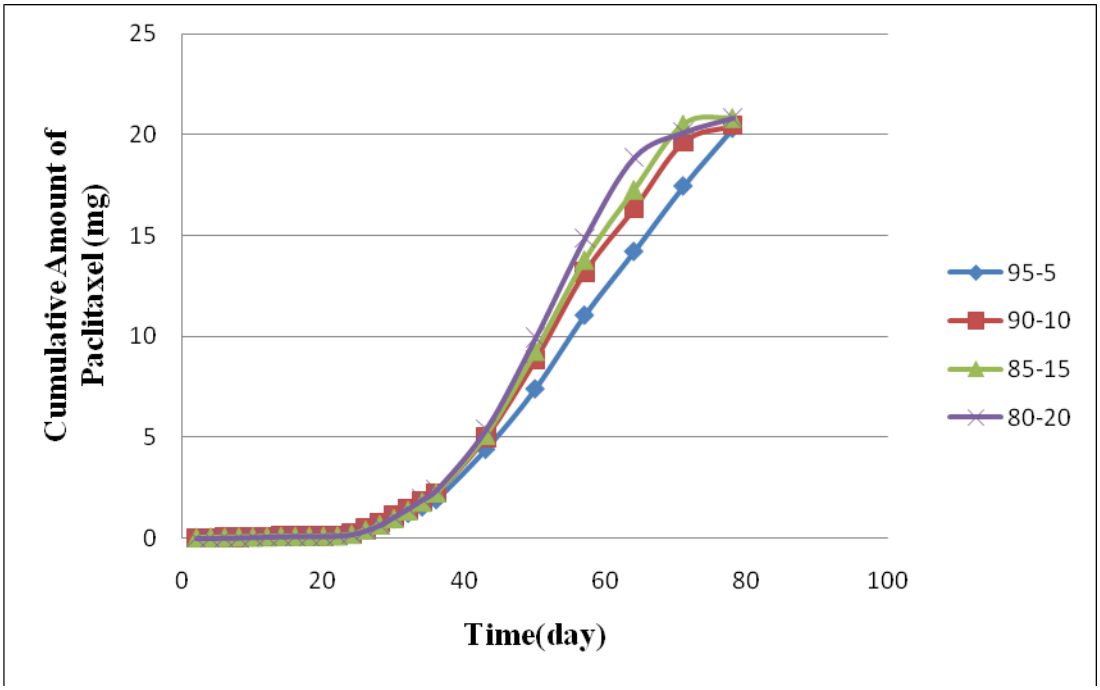


Figure 5.10 Cumulative amount vs. time graph of 21 mg paclitaxel in all PLLA-PCL polymer blends

Although the drug/polymer ratio was kept the same, the film thicknesses were about 3 times more in the case of high polymer amount. The total amount of drug was released in 80 days versus 25 in the case of a film 3 times thicker as it can be seen in Figure 5.10, which corresponds to an almost three fold increase in the release time.

As described in the theory section 2.3.1, the drug release from a polymer matrix can take place by two mechanisms such as degradation (erosion) of the polymer or by diffusion of the drug through the polymer matrix. The degradations results which will be discussed in the next section revealed that degradation is much slower than the release, suggesting a predominantly diffusion controlled mechanism.

5.4. DEGRADATION RESULTS OF THE PLLA-PCL BLENDS

Degradation time is very important for the application of biodegradable polymers and has a big effect on the release of the drug; therefore drug-free films of different PLLA and PCL contents were prepared for degradation studies.

8 separate polymer films of each blend were prepared and the weight of each part was determined and all the values were recorded. Polymer films were put into buffer solutions and pH values of polymers were determined. Three different samples were prepared for the same blend composition and the average values were tabulated and presented in Appendix A.

For pH measurements pH data were recorded for the first, fourth and seventh days per week and during degradation studies, acidic species released from hydrolysis of polymers resulted in a decrease of pH over time. Figure 5.11 shows the pH profiles for the different PLLA-PCL polymer blends.

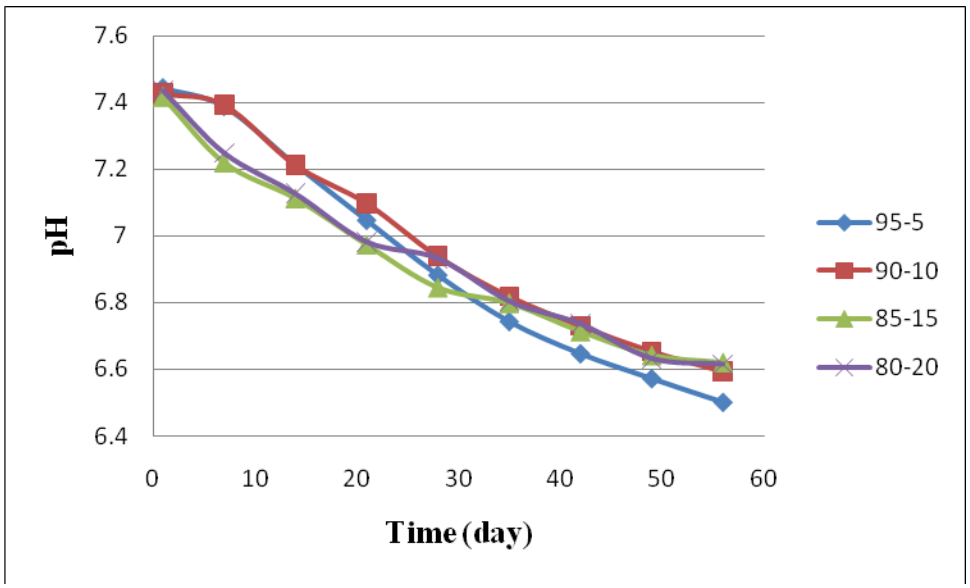


Figure 5.11 pH versus Time graph of PLLA-PCL blends

According to Figure 5.11, pH values of all polymers started at 7.4 and after 8 weeks the pH values were recorded nearly as 6.6 due to release of acidic species during the degradation of PLLA and PCL. Using both the weight loss and pH measurement methods, although similar trends were observed, weight loss studies revealed more of a distinction between different polymer blends in terms of degradation rate.

The percentages of weight loss calculated from weight loss data of the PLLA-PCL blends are tabulated in Table.5 in Appendix. The weight loss percentage versus time plot is shown in Figure 5.12.

The degradation of polymer blends were notably accelerated by increasing PCL content because of the contact surface of PLLA-PCL blends. That is to say, pores diameter of PCL increase with increase of PCL content in polymer matrix and also contact surface get smaller. Whether contact surface get smaller, degradation rate of polymer blends will increase. Because of that, water access to PLLA may be facilitated resulting in the observed increase in degradation rate [53].

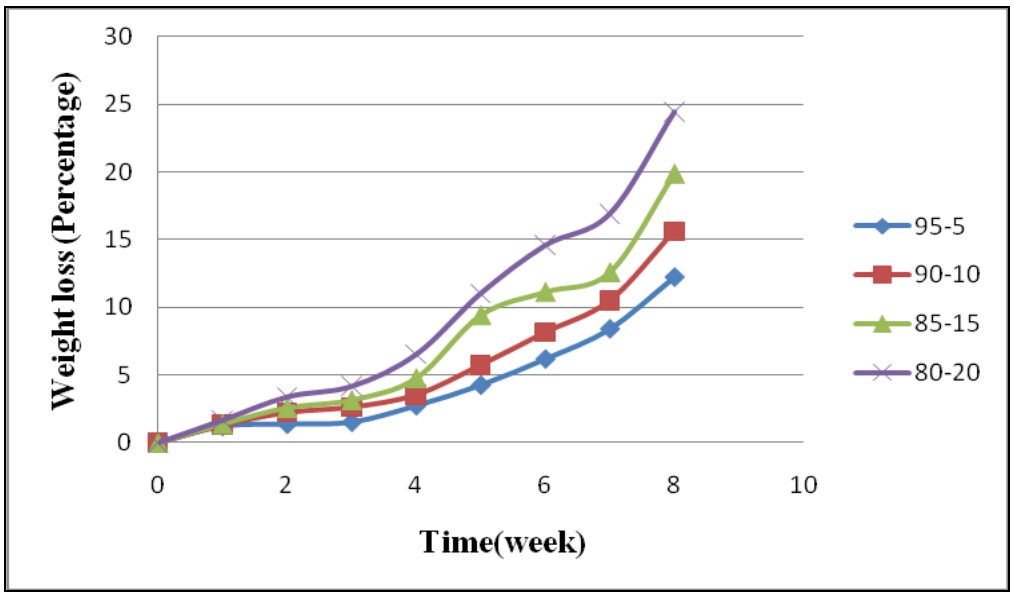


Figure 5.12 Weight Loss versus Time Graph

Assuming all drug to be evenly distributed in the polymer matrix and that it gets released with the degraded polymer, a drug release data set is generated using the weight loss data as shown in Table.6 in Appendix. The drug release data calculated from the weight loss data for different PLLA-PCL polymer blends versus time plots are shown in Figure 5.13. This figure clearly shows the initial stage of drug release to be degradation controlled followed by a new phase of faster drug release which we attribute to diffusion controlled release.

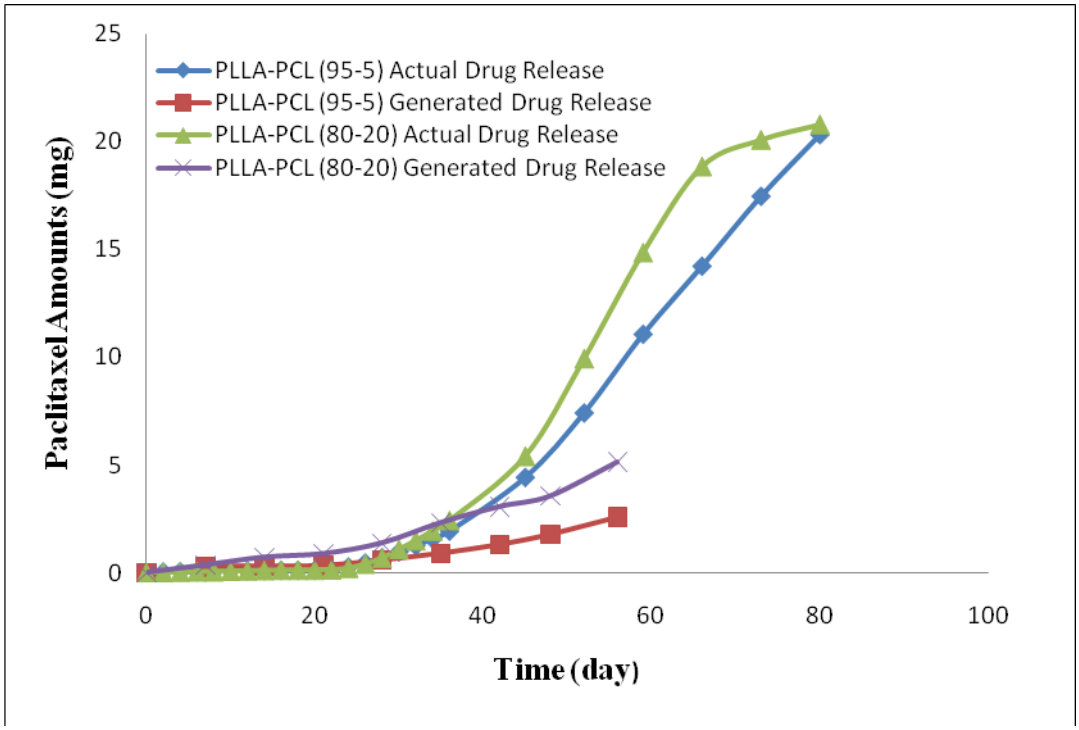


Figure 5.13 Generated drug release versus time for 95-5 and 80-20 PLLA-PCL

5.5. BIOCOMPATIBILTY STUDIES OF POLYMER BLENDS

Appropriate polymer blends were chosen for *in vitro* study to determine the biocompatibility of drug eluting stent coating polymers. Different polymeric blends of PLLA/PCL (95/5, 90/10, 85/15, 80/20 wt/wt) prepared via solution casting were seeded with HUVECs and tested by MTS assay in order to determine the biocompatibility of the films. MTS assay of polymer blends with different compositions were done at the end of 1, 7, 14 days. These experiments were performed by Ayse Irem Kanneci as a part of her MSc Project and for further details, her thesis should be referred [54].

According to Figure 5.14, polymeric films mainly have the same cell number at the defined time intervals. Cell growth rates of the PLLA-PCL blends were almost the same with the control (OC) which means that, all polymer blends are highly biocompatible.

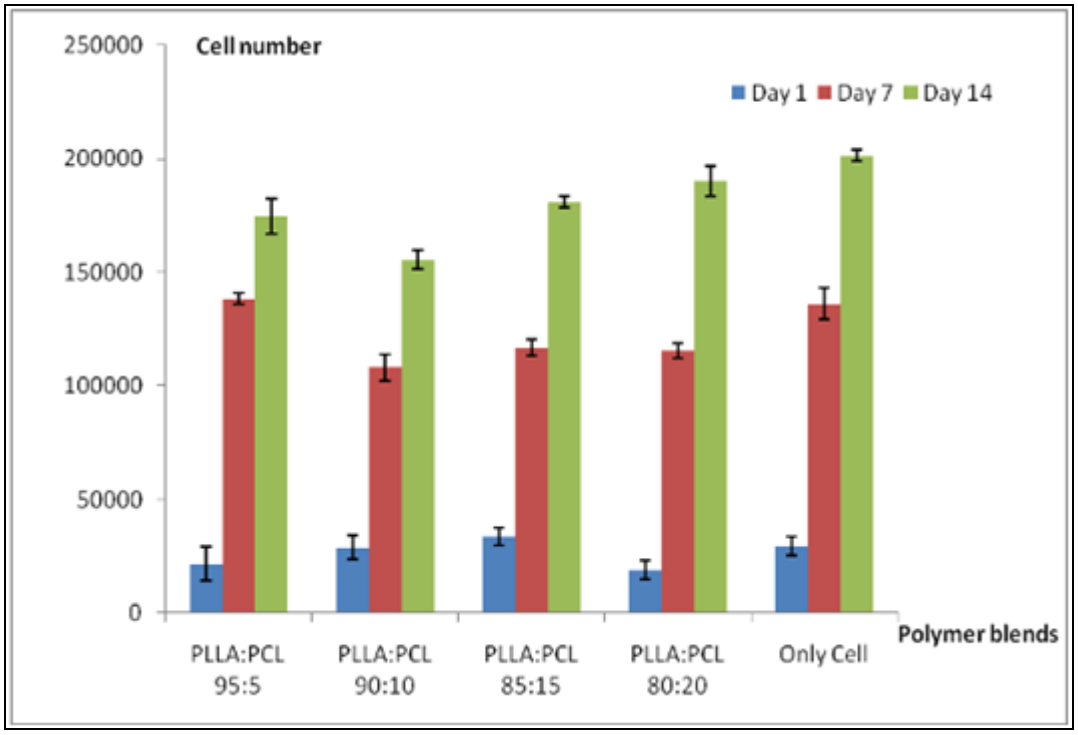


Figure 5.14 HUVEC cell growth determination on PLLA/PCL films

Moreover, apoptosis assay known as the process of programmed cell death (PCD) that may occur in multicellular organisms, was prepared to determine the apoptotic effect of paclitaxel release from different compositions of PLLA/PCL films. The assay was performed due to paclitaxel elution from different polymer blends at the end of 1, 4, 7 days [54].

Figure 5.15 shows that there was an increase in cell death in all blends of PLLA/PCL films throughout 7 days of incubation, the paclitaxel loaded films exhibiting a higher cell death than unloaded polymer films as expected. According to the results, cell death was increased as PCL ratio increases in paclitaxel loaded blends. In the paclitaxel loaded 80-20 PLLA-PCL blend, nearly 53% decrease in cell viability was observed comparing to its control due to paclitaxel release at the end of day 7 [54].

Meanwhile, 35%, 38% and 44% decreases were determined in cell viability in 95-5, 90-10 and 85-15 PLLA-PCL paclitaxel loaded blends, respectively. Degradation accelerated by increasing PCL contents in the blend as shown in degradation studies should lead to a higher paclitaxel release which may cause the higher cell death observed.

The results of this study proved that efficient drug release can be supplied by using the PLLA-PCL blend [54].

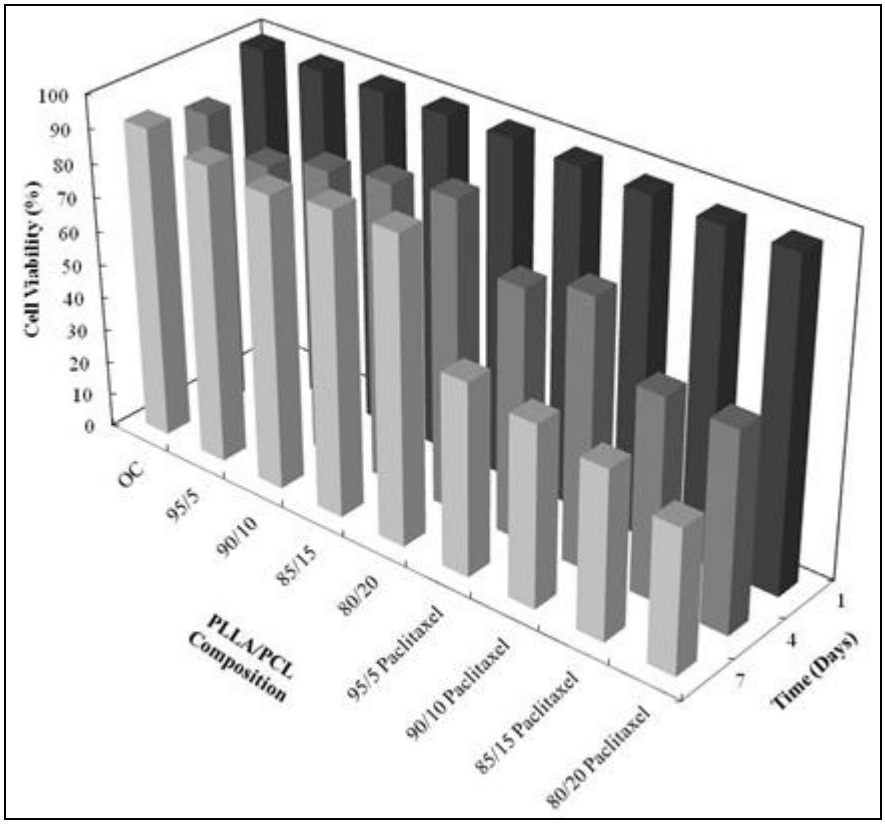


Figure 5.15. Cell viability percentages of HUVECs due to paclitaxel elution from PLLA-PCL blend

6. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The aim of this project was to design a drug eluting biodegradable polymeric coating material for a stent for the treatment of coronary artery disease. The results of this study formed the first goal of the ultimate aim of the construction of the drug eluting polymer stent: the construction of the drug eluting coating material. In addition, preliminary studies and recommendations for the construction of the inner body of the polymer stent is also presented.

In this study, PLLA/PCL blends were analyzed as drug (paclitaxel) releasing coating materials for stents. For this purpose, PLLA/PCL blends with different ratios were prepared to determine the properties such as crystallinity, porosity and degradation, along with their biocompatibility and drug release profiles.

Differential Scanning Calorimetry was used to obtain the crystallinity results of polymer blends. Through the DSC analysis, all polymer blends (PLLA content from 95 to 80 wt %) prepared via solvent casting were found to be phase separated. The crystallinity of PLLA increased as PCL content (until 15 wt %) increased. Above 15 wt % PCL content crystallinity of PLLA decreased due to the disturbance on PLLA chains.

Scanning Electron Microscopy was used to determine the porosity of polymer blends and determine phase morphology. During the solvent evaporation pores were formed on the surface of polymers. SEM pictures of the polymer blends showed that the pore size diameters ranged from 1 to 5.4 μm for all PLLA/PCL blend films and that paclitaxel was dispersed homogenously in the polymer matrix. The SEM analysis of the PLLA/PCL solution cast films etched with THF revealed that PCL was dispersed homogenously in the PLLA matrix as spherical domains and that the pore diameters of the PCL domains significantly increased as the PCL content increased.

The degradation of polymers was shown by the decrease of pH values due to the release of acidic byproducts of polymer hydrolysis. The pH values of the solutions were measured on the first, fourth and seventh days of the week. The weight loss data showed that, as the concentration of PCL increased in the blends, the degradation rate also increased. Moreover, thickness of the films was found to have an effect on degradation rate, as expected.

In order to determine the amount of paclitaxel release from the prepared polymer blends, High Performance Liquid Chromatography (HPLC) was used. Although a different release profile was observed for different blends, for all components the total drug was released within 30 days. In order to observe the difference in release profiles, the amount of polymer and drug was increased with increasing the thickness of the films. Release time of paclitaxel changed depending on thickness of films; thicker films leading to slower drug release. The drug release profiles from the PLLA/PCL blend solution cast films indicated that the drug release was first degradation controlled followed by a faster diffusion controlled drug release.

The biocompatibility of the PLLA/PCL blends was shown by cell proliferation on PLLA/PCL blends as determined by MTS assay. Apoptotic effect of PLLA/PCL blends with and without paclitaxel was tested by Caspase-3 apoptosis assay. The results of the apoptosis assay showed an increase in cell death throughout 7 days of incubation for all blends. Drug loaded blends caused more cell death than those without through 7 days of incubation as expected and the cell death was increased with increasing PCL contents.

As a conclusion, the PLLA/PCL polymer blends prepared in this study, with all presented properties have a big potential for being used to coat the outer layer of a constructed stent, whether the stent be metal or of another polymeric material.

6.2. RECOMMENDATIONS

As a future work, the biodegradability studies of the polymer blends prepared via melt blending method will be carried out to ensure complete degradation at the end of 12 months. Thermal and mechanical properties will be analyzed by Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analyzer (DMA) respectively to determine the most suitable polymer. SEM analysis will also be used to see the effect of melt blending method on phase-separation. The biocompatibility study of polymer blends will also be completed with the method of MTS and Calcein AM assay. After the designing of stent, drug eluting stent will be implanted to laboratory animal to complete the *in vivo* studies.

APPENDIX A

Table.A.1 Cumulative amount of released paclitaxel from 0.1 g PLLA

Time (day)	Cumulative amount of released paclitaxel (mg)
0	0
1	0.0122
2	0.0672
3	0.1172
4	0.1434
5	0.1596
6	1.0926
7	2.2593
8	3.2193
9	4.1723
10	4.3473
11	4.4573

Table.A.2 Cumulative amount of released paclitaxel (7 mg) from 90-10 PLLA-PCL blend (totally 0.1 g)

Time (day)	**Cumulative amount of release paclitaxel (mg)**
0	0
2	1.19799
4	1.60499
6	1.81799
8	1.93279
10	1.93279
12	2.05479
14	2.15899
16	2.36099
18	2.47899
20	2.55999
22	3.74699
24	4.91199
26	6.07899
28	6.10149
30	6.12149
32	6.13549

Table.A.3 Cumulative amount of released paclitaxel (21 mg)
from PLLA-PCL blends (totally 0.3 g)

Time	**95-5**	**90-10**	**85-15**	**80-20**
0	0	0	0	0
2	0.0259	0.01273	0.01343	0.00846
4	0.03604	0.0217	0.02254	0.01725
6	0.04292	0.03057	0.03895	0.03309
8	0.06016	0.05184	0.04646	0.04349
10	0.0648	0.06039	0.05736	0.06604
12	0.08111	0.07939	0.06894	0.07664
14	0.08808	0.08602	0.07755	0.10008
16	0.09641	0.09809	0.08578	0.10867
18	0.10001	0.10433	0.09107	0.11231
20	0.1064	0.1144	0.09577	0.11675
22	0.13564	0.13284	0.10938	0.13354
24	0.25857	0.24852	0.19032	0.20252
26	0.44192	0.52614	0.39495	0.39674
28	0.68981	0.78256	0.65819	0.67728
30	0.95615	1.10674	0.97112	1.07276
32	1.23849	1.44634	1.36174	1.48165
34	1.55747	1.82274	1.77819	1.9286
36	1.91523	2.22681	2.24032	2.41373
43	4.4082	5.00128	5.11441	5.40416
50	7.40479	8.84895	9.26713	9.93704
57	11.0539	13.1993	13.7885	14.864
64	14.2121	16.3619	17.2755	18.8652
71	17.4542	19.6642	20.5201	20.1029
78	20.3136	20.4585	20.8347	20.8141

Table.A.4 Average pH measurements for different PLLA/PCL blends

Time	95-5	90-10	85-15	80-20
1	7.443579	7.42728	7.417166	7.436109
7	7.388162	7.392834	7.220569	7.24905
14	7.214132	7.212735	7.112942	7.126898
21	7.047109	7.098027	6.974235	6.982283
28	6.882433	6.938484	6.845346	6.932754
35	6.743688	6.818904	6.799197	6.80454
42	6.647237	6.730675	6.714307	6.736497
49	6.572552	6.653652	6.642578	6.632603
56	6.501517	6.59409	6.620623	6.615378

Table A.5 Percentage of Weight Loss for PLLA-PCL Blends

Week	95-5 (%)	90-10 (%)	85-15 (%)	80-20 (%)
0	0	0	0	0
1	1.2251	1.3141	1.3986	1.6783
2	1.386963	2.236422	2.583587	3.394625
3	1.533742	2.635229	3.159851	4.172876
4	2.750809	3.545232	4.778157	6.545961
5	4.266212	5.747126	9.424084	11.04651
6	6.198347	8.152866	11.13139	14.59227
7	8.421053	10.50228	12.5937	16.92708
8	12.23565	15.621	19.85621	24.44444

Table.A.6 Generated drug release due to degradation from PLLA-PCL blends

Week	95-5 (mg)	90-10 (mg)	85-15 (mg)	80-20 (mg)
0	0	0	0	0
1	0.257271	0.275961	0.293706	0.352443
2	0.291262	0.469649	0.542553	0.712871
3	0.322086	0.553398	0.663569	0.876304
4	0.57767	0.744499	1.003413	1.374652
5	0.895904	1.206897	1.979058	2.319767
6	1.301653	1.712102	2.337591	3.064378
7	1.768421	2.205479	2.644678	3.554688
8	2.569486	3.28041	4.169804	5.133333

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