INVESTIGATIONS ON THE BIODEGRADABLE POLYMERIC AND INORGANIC SUBSTRATES FOR CONTROLLED DRUG DELIVERY AND BONE AND CARTILAGE REPAIR

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INVESTIGATIONS ON THE BIODEGRADABLE POLYMERIC AND INORGANIC SUBSTRATES FOR CONTROLLED DRUG DELIVERY AND BONE AND CARTILAGE REPAIR

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ABSTRACT

INVESTIGATIONS ON THE BIODEGRADABLE POLYMERIC AND INORGANIC SUBSTRATES FOR CONTROLLED DRUG DELIVERY AND BONE AND CARTILAGE REPAIR

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Tissue engineering is an interdisciplinary field that seeks to address the needs by applying the principles of chemistry, biology and engineering for the development of viable substitutes that restore and maintain the function of human bone and cartilage tissues. In tissue engineering, scaffolds play an important role as temporary supports for the transplantation of specific cells and tissues. In this study, poly(ester-urethane)urea (PEUU) and poly(caprolactone) (PCL) scaffolds were fabricated. Scaffolds were characterized by SEM. Porosities of scaffolds vary from 67 % to 80 %.

Controlled drug delivery systems release drugs at predetermined rates for extended periods. In this study; firstly poly(lactic-co-glycolicolide/tricalcium phosphate) (PLGA/TCP) and poly(L-lactide)/tricalcium phosphate (PLLA/TCP) composites loaded with Gentamicin or Vancomycin were prepared as controlled drug delivery systems for the local treatment of osteomyelitis. The release behavior of drugs were monitored by UV-VIS spectrometer. It was shown that, Vancomycin loaded samples released higher amounts of drug than the samples loaded with Gentamicin.

Secondly, porous ceramic samples were coated with PLGA and PLLA and they were loaded with dexamethasone. The release behavior of samples were monitored by UV-VIS spectrometer.The cubic ceramics released higher amounts of dexamethasone than cylindrical ceramics. When the mechanical properties of porous ceramic samples were concerned, PLLA coated samples had better mechanical properties.

Keywords: Tissue Engineering, Porosity, Controlled Drug Delivery Systems, Tricalcium Phosphate, Biodegradable Polymer

ÖZ

KONTROLLÜ İLAÇ SALIMI VE KEMİK VE KIKIRDAK DOKU ONARIMLARI İÇİN KULLANILACAK BİYOBOZUNUR POLİMERİK VE İNORGANİK SUBSTRATLAR HAKKINDA İNCELEMELER

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Doku mühendisliği, insan kıkırdak ve kemik dokularını korumak ve iyileştirmek için kimya, mühendislik ve biyoloji bilimlerinin prensiplerini uygulamaya ihtiyaç duyan disiplinlerarası bir alandır. Doku mühendisliğinde iskeleler, belirli hücrelerin ve dokuların nakli esnasında geçici olarak desteklenmesi için çok önemli rol oynarlar. Bu çalışmada poli(ester-uretan)ure (PEUU) ve poli(kaprolakton) (PCL) dan oluşan iskeleler üretilmişlerdir. Hazırlanan iskeleler SEM ile karakterize edilmişlerdir. İskelelerin porozite değerleri % 67 ile % 80 arasında değişmektedir.

Kontrollü ilaç salım sistemleri, ilaçları uzatılmış periyotlarda daha önceden belirlenmiş hızlarla salarlar. Bu çalışmada öncelikle Gentamisin veya Vankomisin yüklü poli(laktit-ko-glikolit)/trikalsiyum fosfat (PLGA/TCP) ve poli(L-laktit) /trikalsyium fosfat (PLLA/TCP) dan oluşan kompozitler osteomiyelitin bölgesel tedavisinde kontrollü ilaç salımı yapmak amacıyla hazırlanmıştır. Salım özellikleri

UV VIS spektometresi ile belirlenmiştir. Vankomisin yüklü kompozitler, gentamisin yüklü kompozitlerden daha fazla miktarda ilaç salımı yapmışlardır.

İkinci olarak ise poroz yapıdaki seramik numuler deksametazonla yüklenmiş ve PLLA veya PLGA ile kaplanmışlardır. Numunelerin salım özellikleri UV VIS spektrometresi ile belirlenmiştir. Küp şeklindeki numuneler silindir şeklindeki numunelerden daha fazla miktarda deksametazon salmışlardır. Poroz seramiklerin mekanik özellikleri incelendiğinde, PLLA kaplı numunelerin daha iyi mekanik özelliklere sahip oldukları belirlenmiştir.

Anahtar Kelimeler Doku Mühendisliği, Porozite, Kontrollü İlaç Salım Sistemleri, Trikalsiyum Fosfat, Biyobozunur Polimer

To my dear family

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Lastly, I wish to express my love to “him” who is always with me, loves me and supports me.

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LIST OF SYMBOLS

PLGA Poly(lactide-co-glycolide)

PLLA Poly(L-lactide)

PCL Poly(caprolactone)

PEUU Poly(ester-urethane)urea

MSC Mesenchymal Stem Cell

PGA Poly(glycolide)

TCP Tricalcium Phosphate (Well-known Name of Calcium Phosphate in Ceramic Industry)

HA Hydroxyapatite

VEGF Vascular Endothelial Growth Factor

MRSA Methicilin Resistant Staphlococcus Aureus

SEM Scanning Electron Microscope

DSC Differential Scanning Calorimetry

NMR Nuclear Magnetic Resonance

ATR Attenuated Total Reflectance

DMSO Dimethyl Sulfoxide

CHAPTER 1

INTRODUCTION

Biomaterials have been used for tissue engineering and controlled drug delivery systems. Biomaterial is a synthetic or natural material used to replace part of a living system or to function in intimate contact with living tissue. Biodegradable polymers are suitable for the manufacturing of medical devices or delivery systems, able to operate in a specific application by interacting with the biological systems, without carrying on their function on the human body or in its inside through mechanisms of pharmacological, immunological or metabolic type. On the other hand they do not require surgical removal and hence are preferred for drug delivery applications.

Tissue engineering is an emerging interdisciplinary field that seeks to address the needs by applying the principles of chemistry, biology and engineering to the development of viable substitutes that restore and maintain the function of human bone and cartilage tissues. In tissue engineering, scaffolds play an important role as biologically active, temporary supports for the transplantation of specific cells and tissues. An ideal scaffold should be three-dimensional, highly porous (high pore-to-volume ratio and surface area) and possess a permeable structure with a uniformly distributed and interconnected open pore network.

Osteomyelitis is an infection of bone or bone marrow, usually caused by pyogenic bacteria or mycobacteria. It can be usefully sub classifed on the basis of the causative organism, the route, duration and anatomic location of the infection. Conventional or

local antibiotic administration methods are used in osteomyelitis treatment. Conventional administration methods which the drug is absorbed into blood results in a generalized non site-specific action of the drug. An alternative method in osteomyelitis treatment is controlled drug delivery systems. Controlled delivery systems allow for maintenance of the drug level within a desired range and reduce the number of administration.

CHAPTER 2

BACKGROUND INFORMATION

2.1 Biomaterial

A biomaterial is a synthetic or natural material used to replace part of a living system or to function in intimate contact with living tissue. They are intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body (1). Biomaterials must be chemically inert and free of leachable impurities (2). They are capable of being in contact with bodily fluids and tissues for prolonged periods of time, while eliciting little if any adverse reactions key factors in a biomaterial usage are its biocompatibility, biofunctionality, and availability to a lesser extent (3).

Biomaterials can be classified as bioinert, bioresorbable, or bioactive according to tissue responses.

i. Bioinert refers to any material that once placed within the human body has minimal interaction with its surrounding tissue, e.g stainless steel, titanium, alumina, ultra high molecular weight polyethylene.

ii. Bioactive refers to a material, which upon being placed within the human body interacts with the surrounding bone and in some cases, even with the soft tissue, e.g synthetic hydroxyapatite, glass-ceramic.

iii. Bioresorbable refers to a material that upon placement within the human body starts to dissolve (resorbed) and is slowly replaced by advancing tissue (such as bone), e.g tricalcium phosphate, and polylactic-polyglycolic acid copolymers (4).

2.2 Polymers

Polymers have been widely used as biomedical materials such as prosthetic materials, dental materials, implants, dressings, pacemakers, polymeric drug delivery systems, tissue engineered products. Especially biodegradable polymers have gained much attention in industrial and medical applications in the past decades. They are suitable for the manufacturing of medical devices or delivery systems, able to operate in a specific application by interacting with the biological systems, without carrying on their function on the human body or in its inside through mechanisms of pharmacological, immunological or metabolic type (5).

Biodegradable polymers on the other hand do not require surgical removal and hence are preferred for bimedical applications. However, since they degrade to smaller absorbable molecules, it is important to make sure that the monomers are non-toxic in nature (6). Biodegradable polymers offer the ability to control surface as well as mechanical properties and degradation kinetics (7).

2.2.1. Poly(ά-hydroxy acids)

Poly (α-hydroxyacids) were found to be bioabsorble and biocompatible in the 1960s (8). Polyglycolide (PGA) and polylactide (PLA) homopolymers and their copolymers poly(lactide-co-glycolide) (PLGA), as well as polylactic acid stereocopolymers produced using L-, D- or DL-lactides and rasemic polymer copolymer PLDLA are all poly (α-hydroxyacids) (9). Structures of PGA, PLLA and PLGA are given in Figure 2.1.

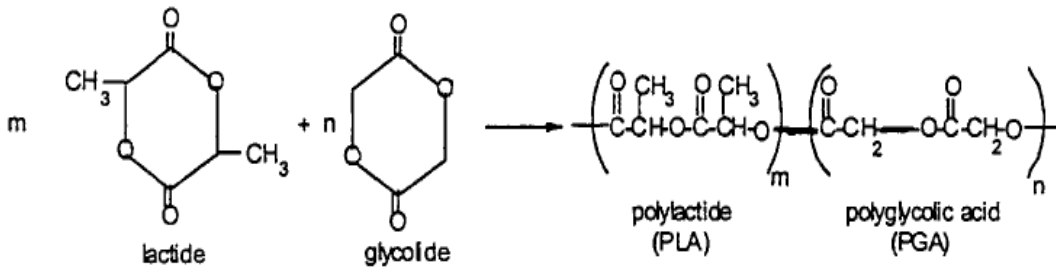

Figure 2.1 Structures of lactide, glycolide and PLGA

One of the most famous biodegradable polymers is PGA, which has been used for three decades as suture and reinforcement material in surgical operation (10). PGA is the simplest linear aliphatic polyester. Because PGA is highly crystalline, it has low solubility in organic solvents and a high melting point (11). The application of PGA is extremely limited because of its rapid degradation. In an attempt to overcome this disadvantage, new biodegradable polymers which possess longer biodegradation periods have been developed. One of them is PLA (10) which is a chiral molecule and therefore it exists in two stereoisomeric forms, D and L; a racemic form, D,L-PLA, is also available. The polymers derived from the optically active D and L monomers are semicrystalline materials, while the optically inactive D,L-PLA is always amorphous (11). Poly(DL-lactide-co-glycolide) (PLGA) is the most widely investigated biodegradable polyester and is widely used as a carrying agent. The reasons for the widespread use of PLGA are obviously its proven biocompatibility and favourable regulator status (12).

2.2.2 Polycaprolactone

Polycaprolactone (PCL) is a semi-crystalline biodegradable aliphatic polyester with a low melting point of around 60°C and a glass transition temperature of about −60°C. PCL can be prepared by ring opening polymerization of ε-caprolactone using a

catalyst such as stannous octanoate (Figure2.2) (13). The polymer's biocompatibility with soft and hard tissue, coupled with ease of processing has led to its widespread investigation for drug delivery and tissue engineering (14**).**

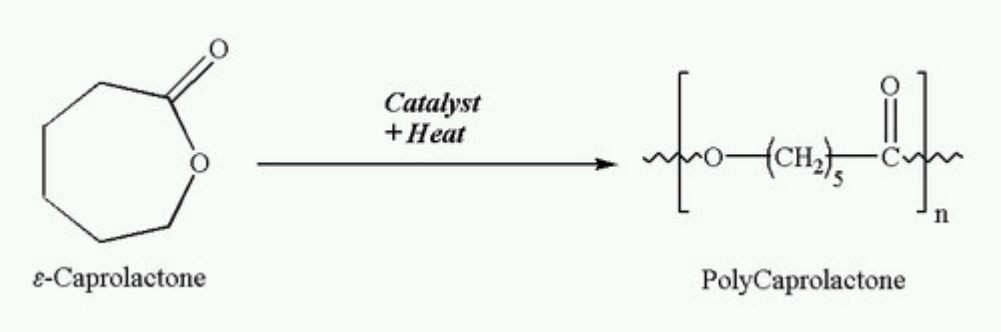

Figure 2.2 Ring opening polymerization of ε-caprolactone to polycaprolactone

2.2.3 Poly(ester-urethane)urea

Aliphatic polyesters and their copolymers often possess mechanical properties best suited for hard tissue engineering because of their relatively higher glass transition temperatures and high modulus. For engineering of soft tissues, elastic scaffolds are desirable (15**).** Thermoplastic or thermoset polyesters and polyurethanes with polyester containing soft segments as well as cross-linked polymers with polyester functionality are used in soft tissue engineering (16).

Poly(ester-urethane)ureas (PEUU) are members of biodegradeble polyurethane family. They are made of soft segments based on polyester and hard segments based on the reaction of diisocyanate and diamine chain extender (17). They are highly flexible and strong, and could be fabricated into flexible scaffolds using a variety of techniques (15).

2.2.4 Biodegradation of Polymers

Biodegradation of the lactide/gylcolide copolymers occurs by bulk erosion and involves random hydrolysis (Figure 2.3) into lactic acid and glycolic acid, which are then incorporated into the tricarboxylic acid cycle and excreted (11). These natural metabolites are ultimately converted to water and carbon dioxide through the action of enzymes in the tricarboxylic acid cycle and are excreted via the respiratory system (7).

PCL possesses a similar biocompatibility of poly(ά-hydroxy acids), although it exhibits a much slower degradation rate. This has led to heightened interest in PCL as a long-term drug delivery system (18).

PEUUs are subject to hydrolytic degradation (19,20). Degradation rates can be accelerated by introducing hydrolytically labile segments into the polymer backbone. Polyester soft segments, such as polylactides, polycaprolactone, and their copolymers, can be used to impart this reactivity (21). The degradation behavior of PEUUs do not show evidence of an autocatalytic effect during the monitored degradation process. This degradation behavior is different from that of poly(α-hydroxyester)s such as PLLA and PLGA (15).

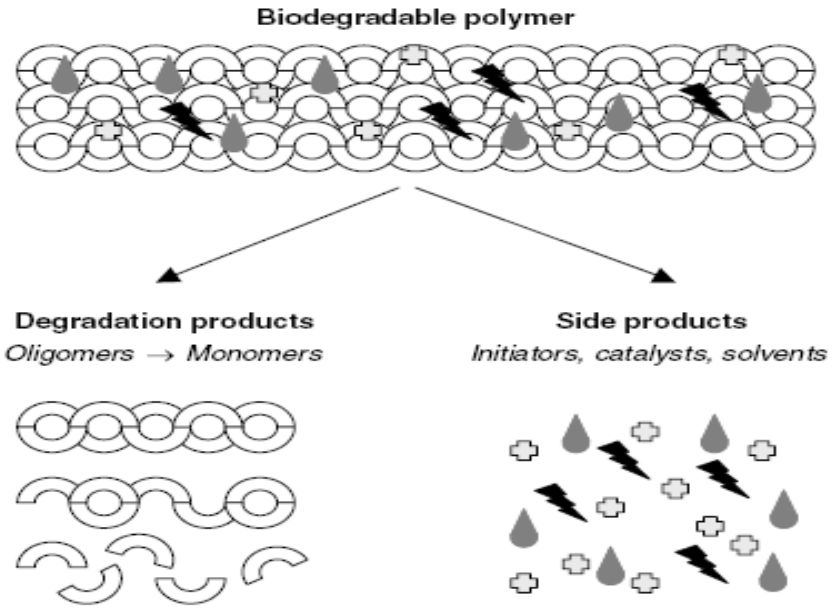

Figure 2.3 Degradation products of the biodegradable polymer

2.3. Ceramics

Hydroxyapatite (HA) and tricalcium phosphate (TCP) are the most widely used ceramic materials. Ceramics are brittle and have poor tensile strength. Their use in clinical situations requiring significant torsional, impact, or shear stress is limited (10).

Calcium phosphate is one of the main combustion products of bone. TCP is a compound with formula $Ca_3(PO_4)_2$. It has an alpha and a beta crystal form, the alpha state being formed at high temperatures (22).

HA is a naturally occurring form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$. HA can be found in teeth and bones, within the human body. Therefore, it can be used as a filler to replace amputated bone or as a

coating to promote bone in growth into prosthetic implants. Coral skeletons can be transformed into hydroxylapatite by high temperatures; their porous structure allows relatively rapid in growth at the expense of initial mechanical strength (23). HA is a slowly resorbing calcium phosphate ceramic. Due to the insoluble and inert structure of crystalline HA, remodeling is extremely slow. Large amounts of HA may remain in the body for 10 years. TCP is the rapidly resorbable calcium phosphate ceramic and is reabsorbed 10 to 20 times faster than HA. Because the porosity of bulk TCP implants is too small, bone ingrowth within the matrix material becomes difficult. Due to increased porosity of the matrix and the bioavailable surface, granules of TCP may be more effective than bulk TCP (10).

TCP have been explored as potential drug delivery system. However, the inability of hydroxyapatite to swallow up in vivo limits the natural remodeling and replacement of bone while restricting the full release of the drug load. Conversely, TCP systems are often challenged by degradation at a rate that is too fast to control over drug release rates and results in an inability to maintain release over the entire treatment period (24).

2.4 Bone-Cartilage Tissue Engineering

The main goal of tissue engineering is to produce new tissue where it is needed. Therefore, knowledge of the structure and functional limits of the regenerated tissue is essential (25). Polymers play a pivotal role in tissue engineering. To fulfill the diverse needs in tissue engineering, various polymers, copolymers, polymer blends, or polymeric composite materials are used.

2.4.1 Bone

Bone is a complex, highly organized living organ undergoing continuous remodeling throughout life. It contains a large amount of organic material (40%). Some of this

organic material is collagen, predominantly type. Most of the inorganic matrix or mineral phase of the bone is the crystal of an apatite of calcium and phosphate (10).

Organ or tissue failure or loss is an expensive problem in health care. The treatment of fracture non-unions and bone loss associated with trauma and musculoskeletal disease remains a significant challenge in the field of orthopedic surgery (26). Currently, major approaches to fracture non-unions and bone loss are surgical reconstruction, transplantation, and artificial prosthesis (10). Bone grafts have been used to fill bone defects caused by disease or trauma, such as bone fractures, infections, and tumors (27). Autografts have the distinct advantage of histocompatibility without the risks of disease transfer and are still the best material for bone repair. The graft possesses a surface favorable for cell attachment (osteoconductive), releases proteins and growth factors which promote bone growth (osteoinductive), and contains cells that may contribute to bone regeneration (osteogenic) (26). Autografs generally have poor morphologies and interconnectivities that are dissimilar to cancellous bone. They also have limited resource that leads to donor site morbidity. This may impair their population with cells and vascularization. Allograft bone can also be a successful procedure. Allograft bone has a porous structure and contains some growth factors such as insulin-like growth factor, transforming growth factor-, platelet derived growth factor (10). But, allografting introduces the risk of disease and/or infection; it may cause a lessening or complete loss of the bone inductive factors (28).

2.4.2 Bone Tissue Engineering

Over the past decade, the main goal of bone tissue engineering has been to develop biodegradable materials as bone graft substitutes for filling large bone defects. These materials should maintain adequate mechanical strength, be osteoconductive, and degrade at a controlled rate to provide space for the formation of new bone (27). Tissue engineering of bone requires three important elements. These are cellular components, extracellular matrix scaffolds, and growth and differentiation factors.

Cells can be either obtained from an exogenous source or they can be recruited from the local environment. Growth and differentiation factors guide the appropriate development of the cellular components (10).

2.4.3 Cartilage

Cartilage is a type of dense connective tissue. It is composed of collagen fibers and/or elastin fibers, and cells called chondrocytes (29). There are no nerves or blood vessels in cartilage, and when damaged, it does not heal readily (30).

Current surgical techniques for cartilage repair rely on either autogenous composite tissue grafts or on the placement of artificial prosthetic implants. Each of these techniques have limited clinical utility. Harvesting autologous tissue, which is limited in supply, results in donor site morbidity, is difficult to shape, and can undergo unpredictable resorption over the long term. Prosthetic metallic or plastic implants undergo migration, extrusion, and unknown long-term side effects (10).

2.4.4 Cartilage Tissue Engineering

Cartilage has several characteristics that make it particularly well suited for cell transplantation and tissue engineering. It is a relatively simple tissue in that it contains only one cell type chondrocytes (10). Cartilage tissue engineering provides a potential method for the production of 3-dimensional scaffolds (31) (which are seeded with an appropriate cell source, and are loaded with bioactive molecules to promote cellular differentiation and/or maturation (32). The scaffolds should permit cell adhesion, promote cell growth, and allow retention of differentiated cell function, biocompatibility, biodegradability, highly porosity, mechanically strength, and also malleability into desired shapes (10). These 3-dimensional scaffolds which can be implanted into defects to provide a natural repair will become integrated with the patient's tissues (33).

2.4.5 Scaffolds and Fabrication Methods

In tissue engineering, scaffolds play an important role as biologically active, temporary supports for the transplantation of specific cells and tissues (34). An ideal scaffold should be three-dimensional, highly porous (high pore-to-volume ratio and surface area) and possess a permeable structure with a uniformly distributed and interconnected open pore network (18) and act as temporary guide for the regeneration and formation of new tissue, and be completely degraded and eliminated when the need for the artificial support has diminished (35). In general, a high porosity and a high interconnectivity of the scaffold is desired to minimize the amount of implanted material and to increase the specific surface area for cell attachment and tissue ingrowth (34). Interconnected pores larger than the dimensions of the cells are essential for allowing infiltration of the cells into the scaffold, whereas smaller pores may positively influence the exchange of nutrients and cellular waste products (34, 36).

Numerous techniques for constructing porous scaffolds have been employed, where the outcome is a three dimensional structure with large surface area and high porosity (35). Particulate leaching involves the casting of a mixture of a polymer solution and porogen in a mold, drying the mixture, followed by a leaching-out of the porogen, usually with water, to generate the pores. The disadvantage is remaining of residual porogen and solvent (18).

Freeze-drying has been used frequently in the preparation of porous polymeric structures (34). Liquid–liquid phase separation gives rise to isotropic pores of 1–30 mm in diameter, depending on the process parameters and the thermodynamics of the polymer/solvent system. Solid–liquid phase separation (with crystallisation of the solvent) leads to ladder- or sheet-like anisotropic morphologies, which strongly depend on the quenching rate (36).

Melt processing is often used in the biomaterials field to produce solid implants of biodegradable polymers. Fixation systems such as plates, rods, and screws used in orthopedics are often fabricated using extrusion or injection molding techniques (18).

In replication technique; highly porous materials with controllable pore sizes have been prepared from inorganic materials, and polymer materials (34).

Phase separation approaches based on polymer solutions require that the solution be taken through a concentration or temperature induced miscibility gap. Removing the final traces of solvent from a polymer that has been in solution can be a difficult procedure (18).

Another method to fabricate a porous scaffold without organic solvents is the gas foaming technique. In this process, the compression and release of carbon dioxide in polymers results in the formation of porous scaffolds (37).

Fiber bonding generates scaffolds through the entanglement of fibrous structures. This provides a large surface area, but the scaffold lacks structural stability and has only been developed for polyglycolide fibers (18).

2.4.6 Mesenchymal Stem Cells

Adult mesenchymal stem cells (MSC) have the potential to differentiate into chondrocytes, osteoblasts, adipocytes, fibroblasts, marrow stroma, and other tissues of mesenchymal origin (38). These cells, also found in the bone marrow (39). MSCs play an important role in bone modelling and remodelling where they give rise to the osteoblasts necessary for bone formation**.** In normal fracture healing, MSCs differentiate into osteoblasts and chondrocytes to form a fracture callus that calcifies extracellular matrix and serve as scaffold for bone formation (40).

The differentiation of MSCs into osteoblasts and chondral cells is regarded as a critical step in the formation of bony and cartilaginous tissues in defect (41, 42). An understanding of the cellular and molecular events of osteogenic differentiation of MSCs provides the foundation for the emergence of a new therapeutic technology for cell therapy (43).

2.4.7 Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF), the best-characterized angiogenic factor, plays an important role in bone growth (44). It is a specific mitogen for endothelial cells and directs development of blood vessels (45).

During bone formation and fracture healing, there is a cross-talk between endothelial cells and osteoblasts in which VEGF plays a key role: osteoblast-like cells produce while VEGF enhances osteoblast differentiation (46). Blocking VEGF leads to a decrease in ngiogenesis (the growth of new capillary blood vessels), callus mineralization and bone healing (44,46). A combination of PLGA microspheres embedded in a PLGA matrix has been used for rapid delivery of VEGF from a PLGA matrix. Improved growth of stable blood vessels around tissue engineering constructs was attributed to this combination of matrix and microsphere delivery of multiple growth factors (45).

2.4.8 Dexamethasone

Dexamethasone is a member of the glucocorticoid class of hormones. This means they are steroids but, unlike the anabolic steroids that we hear about regarding sports medicine, these are "catabolic" steroids (47). MSCs should undergo osteogenic differentiation to form bone tissue. Dexamethasone, either alone or in combination with bone morphogenetic proteins (BMPs) are osteogenic inducers for MSCs (48).

Dexamethasone stimulates the devolopment of phenotypic features of human osteoblasts and enhances a more differentiated osteoblast phenotype: validation of an in vitro model for human bone marrow-derived primary osteoblasts (49). The structure of dexamethasone is given in Figure 2.4.

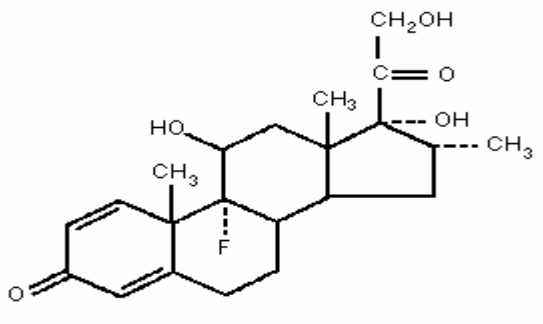

Figure 2.4 Structure of dexamethasone

2.5 Controlled Drug Delivery Systems

The earliest drug delivery systems, first introduced in the 1970s, were based on polymers formed from lactic acid. Today, polymeric materials still provide the most important avenues for research, primarily because of their ease of processing and the ability of researchers to readily control their chemical and physical properties via molecular synthesis (50).

Generally, drugs are toxic at high doses however, they must be present in the circulation for a time sufficient to reach therapeutically useful results. This is commonly achieved by repeated drug administrations, which give rise to peak and valley drug concentration. Such a method of administration has two disadvantages: on the one hand the low compliance by the patient and, on the other, the risk of reaching toxic levels (51).

A solution to these problems, an alternative method has been developed. The controlled drug delivery systems aim to minimize the disadvantages of the conventional method. Firstly, the local application of conrolled drug delivery system to the target site provides a decrease in the total dose need for the treatment. This would also maximize the efficiency at the target site and minimize the side effects in the body (52).

A controlled drug delivery system is consist of two parts: a drug and a carrying device. Drug choise can be changed according to disease to be treated. Controlled delivery systems allow for maintenance of the drug level within a desired range and reduce the number of administration (Figure 2.5). In addition to tight regulation of the effective drug release period, controlled drug delivery formulations constitute realistic options for targeted, localized administration of active compounds (7).

The controlled drug delivery systems can be classified in three main categories: controlled, targeted, and prolonged or sustained delivery. Controlled systems are designed in order to release proper drug amounts continuously or under specific physiological, chemical, or physical conditions. Targeted delivery is defined as a strategy to get the drug to the site of action. Prolonged or sustained drug delivery systems act by maintaining constant therapeutic levels in the body for a long time (53).

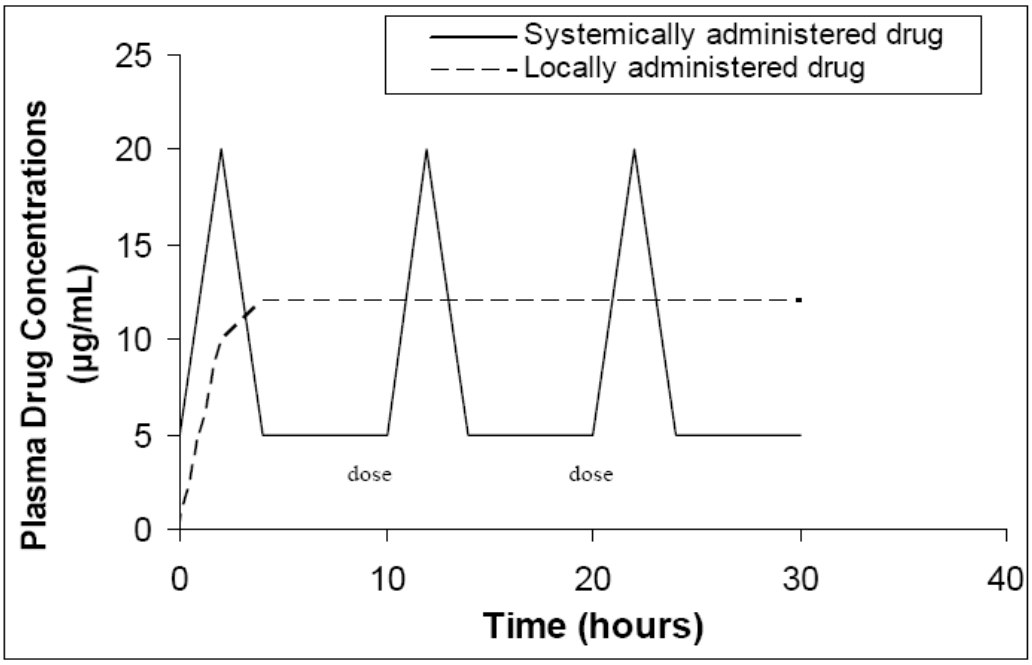

Figure 2.5 Plasma drug concentration versus time profile of systemically and locally delivered drug

2.5.1 Mechanisms of Drug Release

There are three general mechanisms by which drugs are delivered from the carrier systems (53).

∗ Diffusion of the drug species from or through the system.

∗ Chemical (hydrolytic) or enzymatic degradation of the system.

∗ Solvent activation either through osmosis or swelling of the system.

The drug will be released over time either by diffusion out of the polymer matrix or by degradation of the polymer backbone. The continuous release of drugs from the polymer matrix could occur either by diffusion of the drug from the polymer matrix,

or by the erosion of the polymer (due to degradation) or by a combination of the two mechanisms. Several reviews have been presented on the mechanisms and the mathematical aspects of release of drugs from polymer matrices (54). For a given drug, the release kinetics from the polymer matrix are governed predominantly by three factors, the polymer type, polymer morphology and the excipients present in the system (6).

2.5.1.1 Diffusion Controlled Systems

The controlled release of the drug or active agent to the environment is obtained by the diffusion of the molecules that are embedded within a polymeric or ceramic carrier. There are two types diffusion controlled systems, namely reservoir and matrix systems.

In matrix system (Figure 2.6) also referred as monolytic system; a polymer and active agent have been mixed to form a homogeneous system. Diffusion occurs when the drug passes from the polymer matrix into the external environment. As the release continues, its rate normally decreases with this type of system, since the active agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release (2). However, in this case, the release kinetics of the drug from these formulations is not constant and depends on the volume fraction of the agent within the matrix. The greater the concentration of dissolved agent within the matrix, greater the release rate from the system (55). Therefore, a first order release kinetics is obtained from these systems.

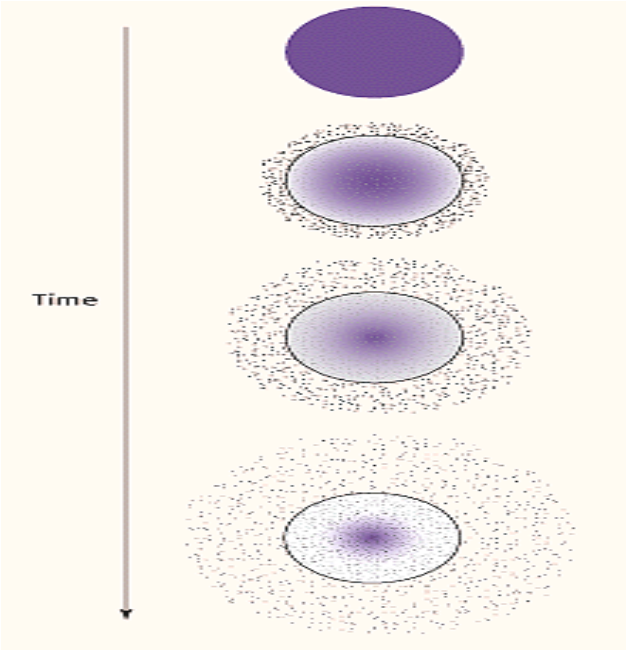

Figure 2.6 Drug delivery from a typical matrix drug delivery system.

The reservoir type device (Figure 2.7) consists of a compact drug core surrounded by a permeable membrane. The rate at which drug is released is determined by thickness and permeability of the membrane (56). The drug delivery rate can remain fairly constant. Since this permeable coating is essentially uniform and of a nonchanging thickness, the diffusin rate of the active agent can be kept fairly stable throughout the lifetime of the delivery system (2). The release kinetics of this type of systems suggest a zero order release kinetics of the drugs (57).

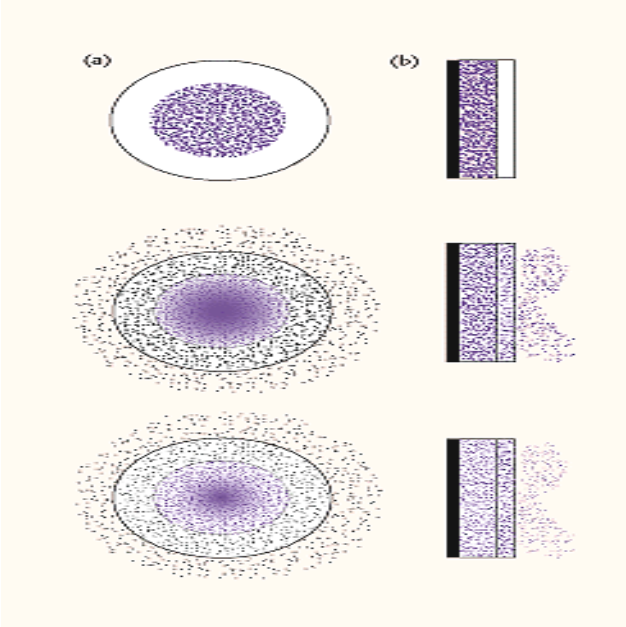

Figure 2.7 Drug delivery from typical reservoir devices: (a) implantable or oral systems, and (b) transdermal systems

2.5.1.2 Degradation Controlled Systems

The polymeric controlled delivery systems (Figure 2.8) can be classified into two main categories on the basis of their architecture: monolithic and reservoir devices.

Monolithic devices are basically constituted by a single polymeric matrix that contains the drug molecularly dispersed or suspended in a fine state and controls its release. The release occurs by diffusion mechanisms, following the second Fick's law, and therefore the drug must be partially soluble in the polymeric network (51).

Reservoir devices are complex systems obtained by assembling two or more polymeric layers. The layers provide for different functions and must therefore be prepared with polymers possessing different physicochemical, mechanical, and

biological properties. An inert matrix containing the drug in a molecularly dispersed or aggregated state constitutes the reservoir layer (51).

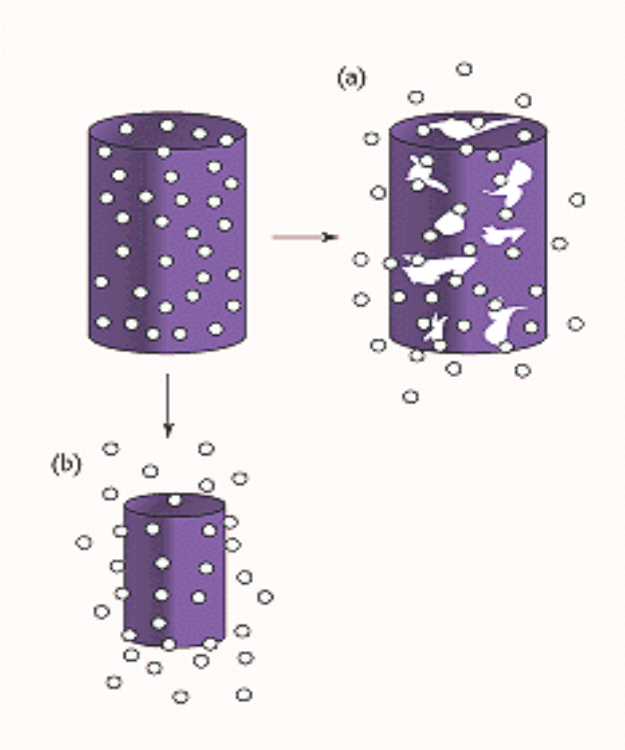

Figure 2.8 Drug delivery from (a) bulk-eroding and (b) surface-eroding biodegradable systems.

2.5.1.3 Swelling Control Systems

Another mechanism for drug delivery is based on swelling controlled release systems (Figure 2.9) which is capable of releasing the agent via swelling of the carrier after being placed in solution.

They are initially dry and, when placed in the body, will absorb water or other body fluids and swell. The swelling increases the aqueous solvent content within the

formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into the external environment (2). Hydrogels which are hydrophilic networks which are able to swell rapidly and retain large volumes of water in their swollen structure are mostly used in swelling control systems (58).

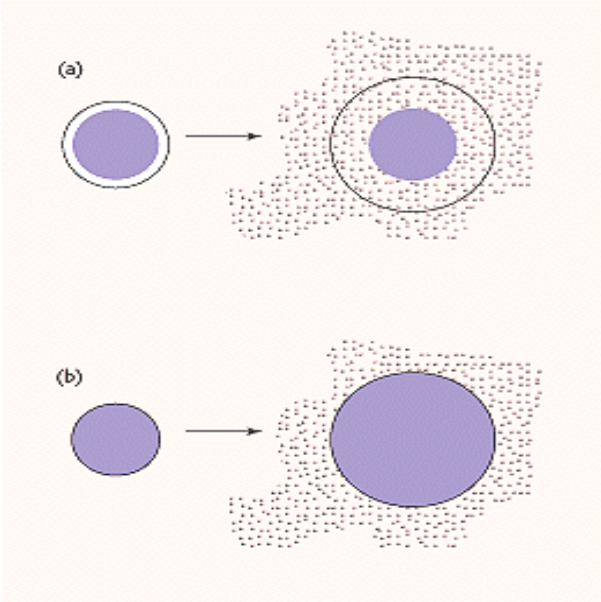

Figure 2.9 Drug delivery from (a) reservoir and (b) matrix swelling-controlled release systems.

2.5.2 Kinetics of Drug Release

In order to study the mechanism of controlled drug delivery systems; the in vitro release data obtained are fitted into various kinetic equations; Zero order (G.M. Khan,

2001), First order (D.M. Morkhade et al, 2006), Higuchi square root (T. Higuchi, 1963), Korsmeyer-Peppas equation (Korsmeyer et al., 1983). The criteria for selecting the most appropriate model was chosen on the basis of goodness of fit test (59).

Korsmeyer-Peppas model (Eq. 1) has been used often to describe drug release behavior from polymeric systems (Korsmeyer et al., 1983):

$$\mathbf{Log\ (M_t/M_\infty) = Log\ k + n\ Log\ t} \qquad \mathbf{(2.1)}$$

where M_t/M_∞ is the fraction of drug released at time t, k is the release constant incorporating the properties of the polymeric system and the drug, n is the release exponent dependent on structural and geometric characteristics (Eq.1) (60). The Higuchi's model which described release by Fickian diffusion through a porous matrix is given by Eq. (2):

$$\mathbf{M_t/M_o = Kt^{1/2}} \qquad \mathbf{(2.\ 2)}$$

where M_t is amount of drug release at time t, M_o is the total amount, t is time and K is the release constant (Eq. 2) (61).

Zeroth order rate equation (3) and first order rate equation(4) can be applied.

$$\mathbf{M_t = M - kt} \qquad \mathbf{(2.3)}$$

$$\mathbf{M_t = M\ e^{-kt}} \qquad \mathbf{(\ 2.4)}$$

where, M_t is the amount of drug release at time t, M is total amount of drug and k is release constant (62).

2.6 Osteomyelitis

Osteomyelitis is defined as an inflammation or an infection in the bone marrow and surrounding bone caused by pyogenic bacteria or mycobacteria. It can be usefully subclassifed on the basis of the causative organism, the route, duration and anatomic location of the infection. It is an infective process which encompasses all of the bone components, including the bone marrow. The disease is characterized by a bacterial plaque formation around the infected area and is classified as either acute or chronic depending on the duration of the infection or persistance of symptoms. The symptoms include pain, swelling, warmth in the bone, drainage of pus through the skin, excessive sweating, chills, etc (63).

When a bone becomes infected, bone marrow usually swells. This swollen tissue applies pressure against the rigid outer wall of the bone, and blood vessels may become compressed and blood supply to bone could reduce. Without an adequate blood supply, parts of the bone may die. The presence of an open fracture is not sufficient to cause osteomyelitis alone. In most cases the body's immune system is capable of preventing the colonization of pathogens. The timing and extent of the treatment are critical in determination whether the infection develops. The likelyhood of developing osteomyelitis increases with impaired immune function, extensive tissue damage and reduced blood supply to the affected area (63).

2.6.1 Treatment

Conventional or local antibiotic administration methods are used in osteomyelitis treatment. Conventional administration methods which the drug is absorbed into blood results in a generalized non site-specific action of the drug. It is distributed in the body to various organs and tissues perfused with blood, and only a relatively small amount reaches its target tissue (64). To maintain a high concentration of antibiotic at the site of infection for an extended period of time is very important and necessary in

osteomyelitis treatment (65). Controlled drug delivery systems are an alternative method for the treatment of osteomyelitis.

2.6.2 Gentamicin

Gentamicin is an aminoglycoside antibiotic used in the treatment of infections caused by E. coli, Klebsiella, Enterobacter, Staphylococcus aureus, Staphylococcus epidermidis and other microorganisms (66). Like all aminoglycosides, when Gentamicin is given orally, it is not effective. This is because it is absorbed from the small intestine, and then travels through the portal vein to the liver, where it is inactivated. Therefore, it can only be given intravenously, intramuscularly or topically (67,68). The structure of Gentamicin is given in Figure 2.10.

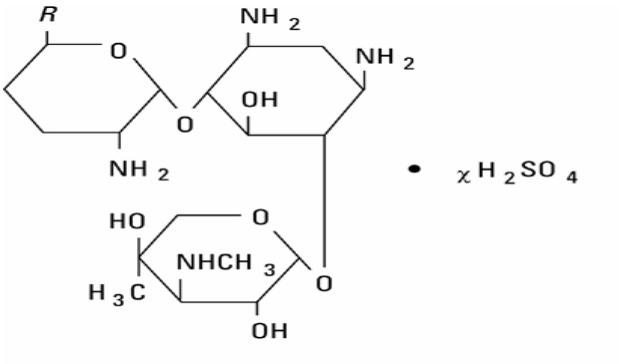

Figure 2.10 Structure of Gentamicin sulfate

2.6.3 Vancomycin

Vancomycin is a glycopeptide antibiotic which is used to treat serious infections of many gram positive bacterias such as MRSA (methicilin resistant staphlococcus aureus) (69,70). It has been used clinically for over 50 years (71). The halflife of

Vancomycin is between 5-11 hours for patients with normal renal functions (72) and Vancomycin demonstrates a post antibiotic effect lasting between 1 to 6 h. The structure of Vancomycin is given in Figure 2.11.

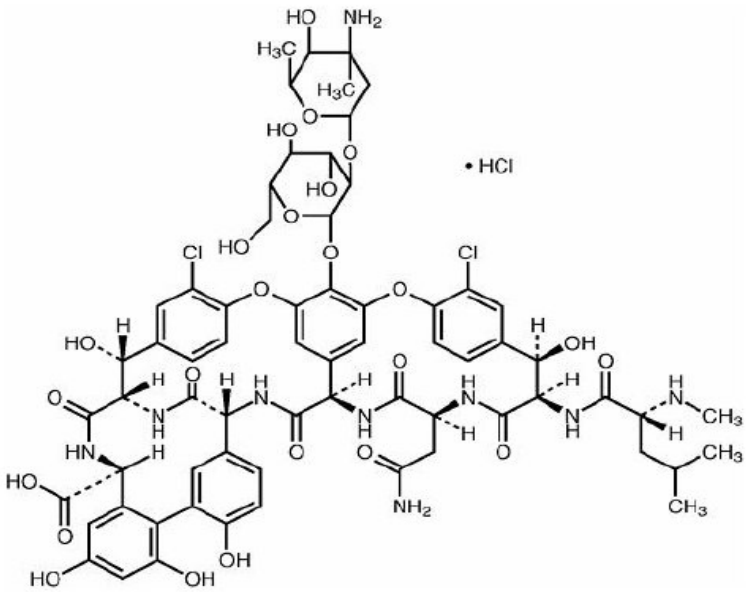

Figure 2.11 Structure of Vancomycin hydrochloride

2.7 Recent Studies

Benoit et al. (Antibiotic-loaded plaster of Paris implants coated with poly lactide-co-glycolide as a controlled release delivery system for the treatment of bone infections ;M.-A. Benoit, B. Mousset, C. Delloye, R. Bouillet, J. Gillard) have studied local drug delivery system for the treatment of bone infections. Plaster of Paris, dried calcium sulphate hemihydrate ($CaSO_4.1/2\ H_2O$) as antibiotic carrier, Vancomycin as antibiotic and PLA45GA10 (MW=95,000) composed of 10% (w/w) polyglycolic acid and 90%

(w/w) racemic poly(D,L-lactic acid) are used. In vitro release of implants were performed in a test tube containing 2 ml phosphate buffered saline (PBS). Four types of plaster of Paris implants with Vancomycin (60 mg/g) are prepared. Vancomycin retaines its antimicrobial effect after incorporation into plaster of Paris as the type of implants change and the release concentration of Vancomycin into medium is enough to inhibit bacterial growth during 20 days, The antibiotic release is inversely proportional to the thickness of the coating,being faster without coating and slower with 6 layers of polymer.

Silverman et al. (Release of Gentamicin from a Tricalcium Phosphate Bone Implant) have studied controlled delivery of Gentamicin for the treatment of osteomyelitis. Naturally forming clot that occurs within a porous tissue scaffold when combined with autologous blood or bone marrow aspirate (BMA) is a new method for achieving controlled drug delivery. The results show that impregnating Gentamicin in porous TCP scaffolds using clotted blood plus thrombin or BMA as a binder significantly slows drug release as compared to simple aqueous preparations over the course of 3 to 5 days. Furthermore, modified tests simulating a restricted diffusion path with clotted blood or tissue surrounding the implant produce release profiles that extend up to 2 weeks. The porous TCP acts as a scaffold for new bone formation.

Joosten et al. (Effectiveness of hydroxyapatite-Vancomycin bone cement in the treatment of Staphylococcus aureus induced chronic osteomyelitis) have studied hydroxyapatite cement (HAC) as a carrier for Vancomycin in the treatment of chronic osteomyelitis due to Staphylococcus aureus strains with various mechanisms of resistance. Powdered hydroxyapatite is mixed with Vancomycin in three different concentrations (80, 160 and 240 mg/g). The released quantities of Vancomycin are measured by an agar diffusion test and the microbiological assay is performed to estimate the active amount of Vancomycin released during the in vitro dissolution test. The release period of Vancomycin is over a period of 20 days. The released quantities

are initially 1512.12±318.45 mg/ml (80 mg/g Vancomycin) up to 1936.6±335.85 mg/ml (240 mg/g Vancomycin).

Richardson et al. (The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on PLLA scaffolds Stephen M. Richardsona, Judith M. Curranb, Rui Chenb, Anne Vaughan-Thomasc, John A. Huntb, Anthony J. Freemonta, Judith Alison Hoylanda;) have studied the differention of bone marrow mesenchymal stem cells on three dimensional poly(L-lactide) scaffolds. The majority of this study has concentrated on tissue engineering of articular cartilage using biodegradable scaffolds and hydrogels for the intervertebral disc (IVD) regeneration. A potential system for tissue engineering of the nucleus pulposus (NP) of the severely degenerate IVD has been developed. While cells from degenerate discs are not suitable for tissue engineering, bone-marrow derived mesenchymal stem cells offer a potential source of cells. These cells were seeded on porous, biodegradable three-dimensional (3D) poly-L-lactic acid scaffolds. The predifferentiation of MSCs in monolayer and seeded onto PLLA scaffolds have been studied both. Using SOX-9 transfection and culture in a specialised medium it was feasible to differentiate human MSCs into chondrocytic cells in vitro and that when these cells were seeded onto PLLA scaffolds they retained their phenotype.

Kim et al. (Poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering) have studied fabrication of biodegradable polymer/bioceramic composite scaffolds for bone tissue engineering. Poly(D,L-lactic-co-glycolic acid)/nano-hydroxyapatite (PLGA/HA) composite scaffolds were fabricated by the gas forming and particulate leaching (GF/PL) method without the use of organic solvents. The GF/PL scaffolds showed interconnected porous structures without a skin layer and exhibited superior enhanced mechanical properties. The scaffolds were seeded with rat calvarial osteoblasts and cultured in vitro or were subcutaneously implanted into athymic mice for eight weeks. The GF/PL scaffolds exhibited significantly higher cell growth, alkaline phosphatase activity, and mineralization

compared to the solvent casting-particulate leaching (SC/PL) scaffolds in vitro. Results show that the biodegradable polymer/bioceramic composite scaffolds fabricated by the novel GF/PL method enhance bone regeneration compared with those fabricated by the conventional SC/PL method.

Nam et al. have studied (Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation) to fabricate various porousbiodegradable scaffolds suitable for tissue engineering and drug delivery based on a thermally induced phase separation (TIPS) technique. The coarsening effect of pore enlargement affected by controlling the quenching temperature was used for the generation of a macroporous open cellular structure with pore diameters above 100 mm. The fabricated porous devices loaded with recombinant human growth hormone (rhGH) were tested for the controlled delivery of rhGH, as a potential additional means to cell delivery. As a result these biodegradable scaffolds could be potentially applied for tissue regeneration that requires effective cell seeding through the macropores in the matrix.

Kim et al. have studied (In vivo bone formation by human marrow stromal cells in biodegradable scaffolds that release dexamethasone and ascorbate-2-phosphate) release kinetics of dexamethasone and ascorbate-2-phosptahe for in vivo bone formation. Porous poly(D,L-lactide-co-glycolide) (PLGA) scaffolds that released biologically active dexamethasone (Dex) and ascorbate-2-phosphate (AsP) have been fabricated. Dexamethasone (Dex), either alone or in combination with ascorbate-2-phosphate (AsP), and bone morphogenetic proteins (BMPs) are osteogenic inducers for MSCs. However, the fabricated scaffolds released Dex and AsP continuously over 3 months. The release kinetics of Dex followed almost zero-order release, which is the ideal release pattern for maintaining a constant concentration of a drug. The release kinetics of AsP also followed almost zero-order release after an initial burst over the first 7 days.

Sherwood et al. have studied (A three-dimensional osteochondral composite scaffold for articular cartilage repair) the fabrication of three dimensional scaffolds for articular cartilage defects. Tissue engineering of cartilage using a cell-scaffold approach has demonstrated potential to offer an alternative and effective method for treating articular defects. A osteochondral scaffold using the TheriFormTM three-dimensional printing process have been developed. The upper, cartilage region was 90% porous and composed of D,L-PLGA/L-PLA, with macroscopic staggered channels to facilitate homogenous cell seeding. The lower, cloverleaf-shaped bone portion was 55% porous and consisted of a l-PLGA/TCP composite, designed to maximize bone ingrowth while maintaining critical mechanical properties. The transition region between these two sections contained a gradient of materials and porosity to prevent delamination. Chondrocytes preferentially attached to the cartilage portion of the device, and biochemical and histological analyses showed that cartilage formed during a 6-week in vitro culture period.

2.8 Scope of Study

One of the aims of this study was to produce a polymer-tricalcium phosphate composite structure capable of releasing drugs at predetermined rates for extended periods for the local treatment of osteomyelitis. For this purpose, PLLA/TCP and PLGA/TCP composites containing Gentamicin or Vancomycin were prepared. In vitro release kinetics of Vancoymin and Gentamicin from polymer/TCP composites were studied. To these end release profiles of the drugs in time were established to give a path for experiments on animals.

One other aim of the study was to coat the porous ceramic surfaces with a suitable biodegradable polymer to facilate growth factor release, make cell attachment possible and improve the mechanical properties of ceramics. For this purpose, porous ceramics composed of siliconized hydroxyapatite, tricalcium phosphate powders, frit and wollastonite were coated with PLLA and PLGA by dip coating method.

Dexamethasone was used instead of growth factor in vitro release studies due to its chemical similarity. Finally a totally polymeric, porous structure was produced to be used as a substrate for cartilage repair by inoculation by stem cell cultures. PCL and PEUU were used as polymers for the production of porous polymeric substrate for cartilage repair.

CHAPTER 3

EXPERIMENTAL

3.1. Materials

3.1.1 Polymers

Poly(DL-lactide-co-glycolide) (PLGA) (Resomer RG 504) with a lactic acid:glycolic acid ratio of (50:50) and Poly(L-lactide) (PLLA) (Resomer L 209 S) were purchased from Boehringer Ingelheim (Germany). Polycaprolactone (PCL) was purchased from Aldrich Chem. Co. (USA).

Table 3.1 Properties of polymers

Polymer	Appearance	Molecular Formula	Melting point	Inherent viscosiy
PLGA	White to off-white powder	$[(C_6H_8O_4)x (C_4H_4O_4)y]n$	52,24 ˚ C	0.45 - 0.60 dl/g
PLLA	White to off-whitegranulate	$-(C_6H_8O_4)n$	174, 94 ˚C	2.6 - 3.2 dl/g
PCL	Pellets	$(C_6H_{10}O_2)_n$	56,63 ˚C	0.55-0.75 dl/g-

3.1.2 .Drugs

Vancomycin (500 mg) was obtained from Mayne Pharma. Plc. (UK). The active substance was Vancomycin hydrochloride. The other ingredient was EDTA disodium. Gentamicin (160 mg) was obtained from I.E Ulugay Co. (Turkey). The active substance was Gentamicin sulfate. The other ingredients were Nipagin M, Nipazol M, sodium metabisulphite, EDTA disodium. Dexamethasone (96%) was purchased from Acros Organics (Belgium).

Table 3.2 Properties of drugs

Drugs	**Appearance**	**Molecular weight**	**Molecular formula**	**Melting point**
Vancomycin	White crystalline powder	1449.25	$C_{66}H_{75}Cl_2N_9O_{24}$	Decomposes
Gentamicin	Clear solution	477.596	$C_{21}H_{43}N_5O_7$	105°C
Dexamethasone	White crystalline powder	392.46	$C_{22}\ H_{29}\ F\ O_5$	255°C to 261°C

3.1.3. Ceramics

β-tricalcium phosphate (TCP) was obtained from METU Metallurgical and Materials Engineering Dept. Prof. Dr. Muharrem Timuçin's laboratory.

Table 3.3 Properties of tricalcium phosphate

Ceramic	Appearance	Molecular weight	Molecular formula	Melting point
Tricalcium Phosphate	White amorphous powder	310.18	$Ca_3(PO_4)_2$	Liquifies under high pressure

3.1.4 Other Chemicals

Chloroform, ethanol (99.5%) (Absolute alcohol), and isopropanol (2-propanol) were purchased from J.T.Baker (USA). Dimethyl sulfoxide (DMSO) (99.5%) was purchased from Lab-scan Limited (Ireland) Poly(caprolactone) diol and 1,6-diisocyanatohexane (98%) was purchased from Aldrich Chem. Co. (USA). Putrescine (1,4-diaminobutane) (98%) was purchased from Acros Organics (Belgium). Stannous octoate (95%) was purchased from Sigma Chem. Co (USA).

Table 3.4 Properties of chemicals

Chemicals	**Appearance**	**Molecular weight**	**Molecular formula**	**Melting point**
Chloroform	Colorless liquid	119.4	$CHCl_3$	-63.5 °C
Isopropanol	Colorless liquid	60.10	C_3H_8O	-89 °C
Ethanol	Colorless clear liquid	46.07	C_2H_5OH	–114.3 °C
Dimethyl sulfoxide	Colorless clear liquid	78.13	$(CH_3)_2SO$	18.5 °C
1,6-diisocyanato Hexane	Liquid	168.19	$OCN(CH_2)_6NCO$	-67 °C
Stannous octoate	Liquid	405.12	$[CH_3(CH_2)_3CH(C_2H_5)CO_2]_2Sn$	-
1,4-diamino butane	Solid at room temp.	88.1516	$C_4H_{12}N_2$	27°C
Polycaprolactone Diol	Solid (waxy)	Average M_n~2000	$HO(C_6H_{10}O_2)_nOH$	SoftingTem. 50°C

3.2 Methods

3.2.1 Polymer/Ceramic Composites For Bone Tissue Engineering

3.2.1.1 Preparation of Ceramic Samples

Porous ceramic substrates were prepared at METU Metallurgical and Materials Engineering Dept. Prof. Dr. Muharrem Timuçin's laboratory. They were composed of siliconized hydroxyapatite, tricalcium phosphate powders, frit and wollastonite.

3.2.1.2 Preparation of Polymer/Ceramic Composites

Porous ceramics were coated with PLLA and PLGA to illustrate the organic component of bone and to facilate the growth factor release for regeneration of new bone tissue. Dexamethasone was used instead of growth factor in vitro release studies due to its chemical similarity. Growth factor is much more expensive than dexamethasone. Dexamethasone is osteogenic inducer for stem cells which undergo osteogenic differentiation to form bone tissue.

3 % solution of PLLA in chloroform and 5 % solution of PLGA in chloroform were prepared. 50 mg dexamethasone was mixed with polymer solution containing 450 mg PLLA or PLGA. Homogeneous dispersion of dexamethasone and polymer was formed (90 % polymer, 10 % dexamethasone). Porous ceramic samples were loaded with this mixture by dip coating method (Figure 3.1). Dip coating method involves;

*Dipping a sample into a liquid solution

* Dipping a sample vertically and waiting for the surface to attain motionless status.

* Pulling up a sample vertically with an optimal speed.

* Withdrawal speed controls the film thickness by means of the liquid viscosity and gravity.

* The pendant drop formed after total withdrawal from the bath is absorbed gently to a tissue paper.

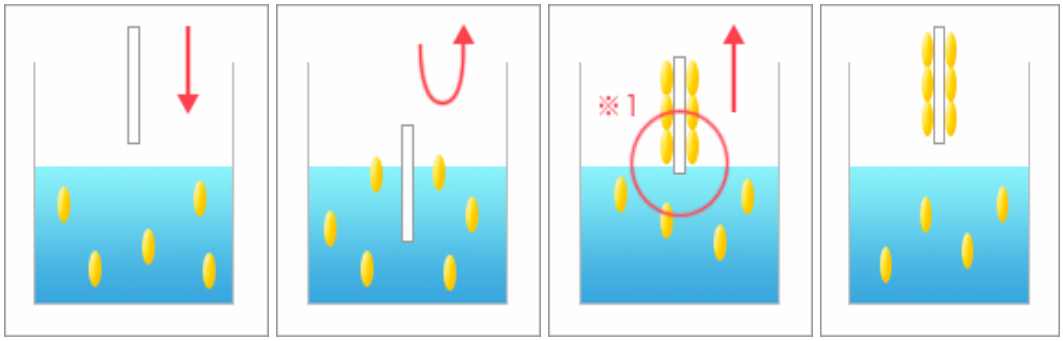

Figure 3.1 Schematic view of dip coating method

Chloroform was evaporated in vacuum drier at 37 °C . Ceramic samples were coated with a thin polymer film. The photographs of porous ceramic samples are given in Figure 3.2.

Figure 3.2 Photographs of porous polymer/ceramic composites

3.2.1.3 Release Studies of Porous Polymer/Ceramic Composites

Calibration of dexamethasone was performed by using Shimadzu 160 UV-VIS spectrometer. Dexamethasone showed a maximum absorption at 242 nm. Calibration curve of dexamethasone is given in Appendix A.

Samples were put in 20 ml de-ionized water (pH 7.0) and were stored at 37 °C. Daily and weekly changes were monitored by Shimadzu 160 A UV-VIS spectrometer for controlled drug release. After every measurement, the solution medium was withdrawn and replaced with equal volumes of fresh de-ionized water.

3.2.2 Polymer/TCP Composites For Osteomyelitis Treatment

3.2.2.1 Preparation of Drug/TCP Mixture

8 % solution of Vancomycin/Gentamicin in de-ionized water was prepared. Tricalcium phosphate powder was mixed into drug solution. This mixture was dried at 37 °C. 10 % of the mixture was Vancomycin or Gentamicin, 90 % was tricalcium phosphate. The antibiotic drugs are basically water soluable. In order to mix the drugs with organic phase soluable PLLA or PLGA, a composite system was made use of.. The antibiotic was absorbed on TCP powder surface from an aqeous solution and the water was evaporated in vacuum oven at 37 °C In the previous step, powder was easily mixed with the polymer solution in chloroform that results in homogeneous distribution of polymer and drugs in the final structure. Secondly, TCP is similar to bone structure and easily absorbed by the bone.

3.2.2.2 Preparation of Polymer/TCP Composites

3 % solution of PLLA in chloroform and 5 % solution of PLGA in chloroform were prepared. Vancomycin loaded samples were prepared by mixing 800 mg

TCP+Vancomycin powder composed of 90 % TCP (720 mg) and 10 % (80 mg) Vancomycin with 1600 mg 5 % (w/w) solution of PLGA in chloroform containing 80 mg PLGA and 2670 mg 3 % (w/w) solution of PLLA in chloroform containing 80 mg PLLA. The paste was located into teflon mold and was dried at 37 °C for 12 hours. The height of the samples loaded with Vancomycin was 15 mm and the radius was 8 mm. Gentamicin loaded samples were prepared by mixing 270 mg TCP+Gentamicin powder composed of 90 % TCP (243 mg) and 10 % (27 mg) Gentamicin with 900 mg 3 % (w/w) solution of PLLA in chloroform containing 27 mg PLLA and 540 mg 5 % (w/w) solution of PLGA in chloroform containing 27 mg PLGA.. The paste was located into teflon mold and dried at 37 °C for 12 hours. The height of the samples loaded with Gentamicin was 5 mm and the radius was 10mm. The photographs of polymer/TCP composites are given in Figure 3.3.

Some Gentamicin loaded samples were coated with thin a polymer film by dip coating method (Figure 3.1). 3 % PLLA solution in chloroform and 5% PLGA solution in chloroform were used in coating process.

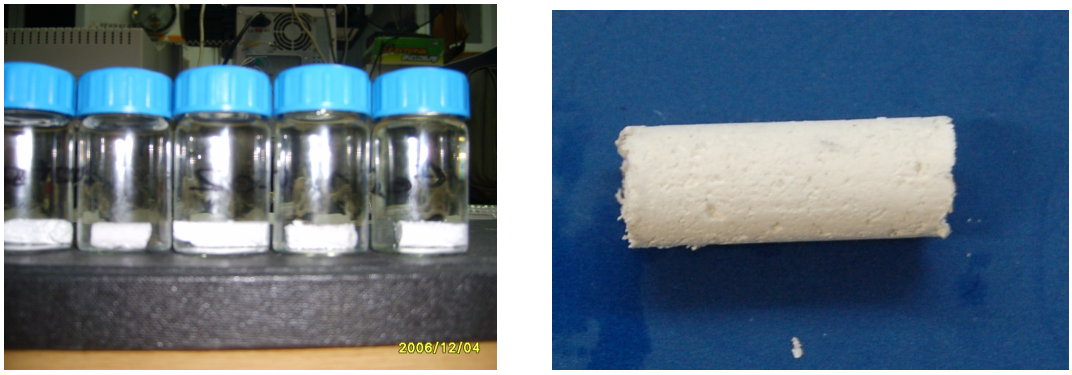

Figure 3.3 Photograph of drug loaded polymer/TCP composites

3.2.2.3 Release Studies of Polymer/TCP Composites

The calibration of Gentamicin and Vancomycin were performed by using Shimadzu 160 UV-VIS spectrometer. Gentamicin showed a maximum absorption at 256 nm and Vancomycin showed a maximum absorbtion at 281 nm in UV spectrum. Calibration curves are given in Appendix A.

Vancomycin loaded samples were placed in 20 ml de-ionized water (pH 7.0) and Gentamicin loaded samples were located in 10 ml de-ionized water (pH 7.0). All samples were stored at 37 °C. The drawn solutions were analyzed spectrophotometrically at 256 nm and at 281 nm in order to determine the amount of Gentamicin and Vancomycin release by using Shimadzu 160 UV-VIS spectrometer. After every measurement, the solution medium was withdrawn and replaced with equal volumes of fresh de-ionized water.

3.2.3 Synthesis of Poly(ester-urethane)urea Polymers

Two-step solution polymerization method was used to synthesize poly(ester-urethane)urea (PEUU) (15). The stoichiometry was 2:1:1 of 1,6-diisocyanatohexane: polycaprolactone-diol: 1,4-diaminobutane (putrescine).

Firstly; 15 % 1,6-diisocyanatohexane solution in DMSO was continuously mixed with 25 % polycaprolactone-diol solution in DMSO. Two drops of stannous octoate (0.2 final wt %) were added. Synthesis was carried out in a three necked flask under a dry nitrogen atmosphere at 80 ° C for 3 hours. The pre-polymer solution was cooled to room temperature. Secondly, 5 % putrescine solution in water was added to pre-polymer solution dropwise under stirring at room temperature for 18 hour. The polymer solution was precipitated in distilled water. The wet polymer was immersed in isopropanol for 1 day to remove the unreacted monomers. Finally, the polymer was dried under vacuum at 50 °C for 24 hours. Synthesis of PEUU is given in Figure 3.4.

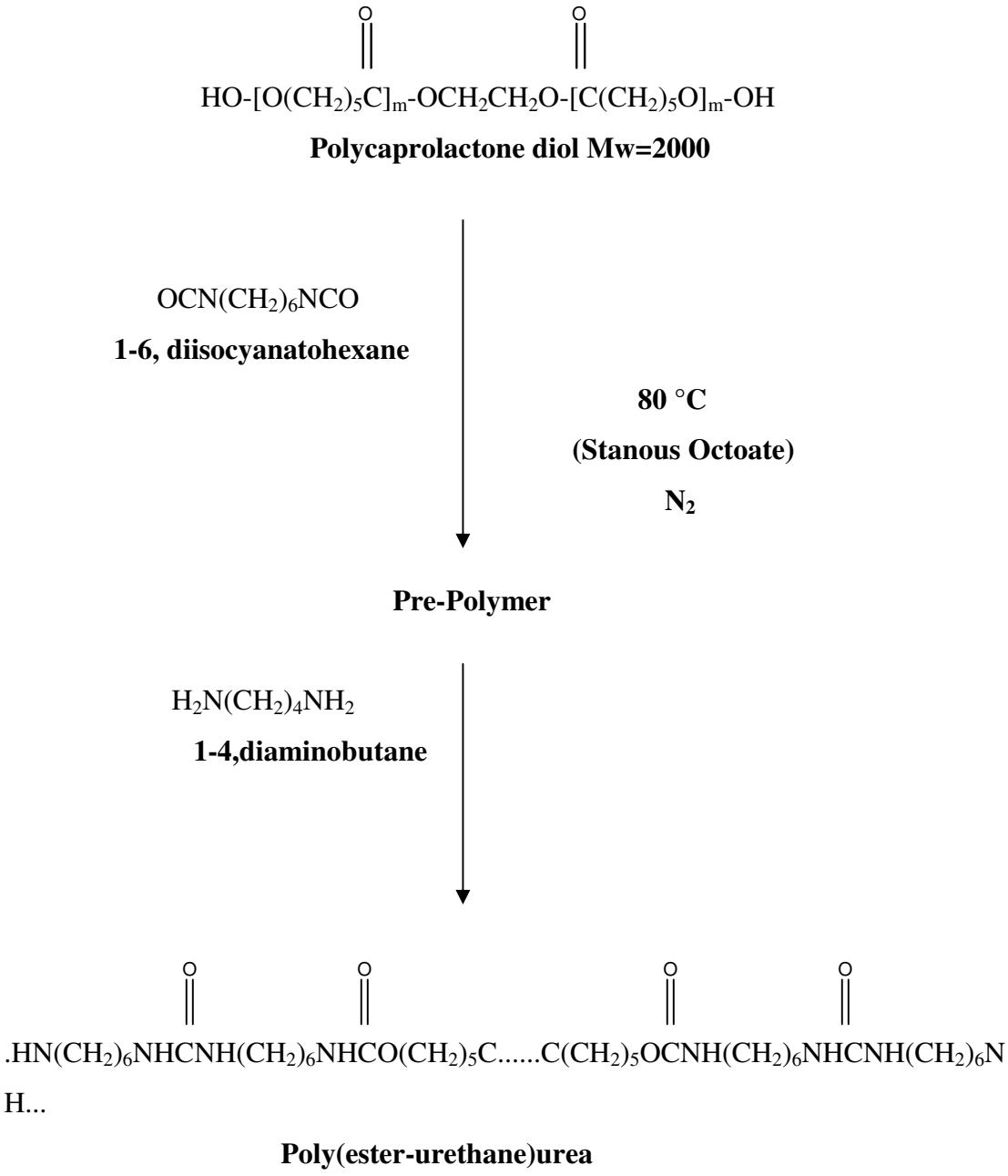

Figure 3.4 Synthesis of PEUU

3.2.4 Porous Scaffolds For Cartilage Tissue Engineering

3.2.4.1 Preparation of PEUU Porous Scaffolds

Thermally induced phase separation and subsequent solvent extraction method was used to fabricate PEUU scaffolds. 5% solution of PEUU in DMSO was injected into a glass mold which consisted of two glass cylinders equipped with two rubber stoppers. The outer diameter of the inner cylinder was 20 mm, the inner diameter of the outer cylinder was 25 mm and the height of cylinders was 5 cm. The mold was put into a freezer at -20 °C for 24 hours. Then the mold was placed in absolute alcohol at -20 °C for 7 days to remove the DMSO from scaffold. Lastly, scaffolds were air dried and vacuum dried (15). The photograph of PEUU scaffold is given in Figure 3.5.

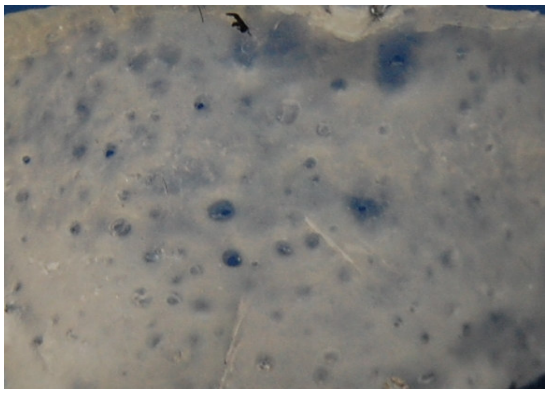

Figure 3.5 Photograph of porous PEUU scaffold

3.2.4.2 Preparation of PCL Porous Scaffolds

Poly(caprolactone) (PCL) fibres were prepared from molten PCL manually. The fibres were pressed into a teflon mold and was wetted with a good solvent-poor solvent

(50/50 ethanol-chloroform (v/v) solvent mixture) solution. The important point is that the fibers adhered to each other at their contact points without dissolving in the solvent mixture. The mold (Figure 3.6) was dried at 37 °C for 4 hours.

Figure 3.6 Photograph of PCL scaffolds

3.3 Characterization

3.3.1 Morphological Analysis

3.3.1.1 Scanning Electron Microscopy (SEM)

Samples were examined by a JEOL JSM-6400 Scanning Electron Microscope. Before SEM photographs were taken, samples were coated with gold to obtain a conductive surface and to reduce the incidence of charging, which is due to high negative charges accumulating on the sample surface.

3.3.2 Structural Analysis

3.3.2.1 Attenuated Total Reflactance (ATR) Spectroscopy

Samples were examined by Bruker Vertex 70 ATR spectroscopy. ATR spectroscopy is a popular IR surface-analysis technique for soft polymers.

3.3.2.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

Samples were examined by Bruker-Spectrospin Avance DPX 400 Ultra-Shield NMR spectroscopy**.** D-DMSO was used as a solvent.

3.3.3 Mechanical Analysis

3.3.3.1 Compression Test

Samples were tested by Shimadzu AGS-J compression test device under 1 ton load. The cross-head speed was 1 mm per minute.

3.3.4 Thermal Analysis

3.3.4.1 Differential Scanning Calorimetry (DSC)

DSC is a good method for determining a polymer's melting temperature, glass transition temperature, degree of crystallinity. Samples were examined by DSC 910 S TA Instrument.

3.3.5 Determination of Porosity

The porosities of PCL scaffolds were calculated by using Formula 3.1 given below.

$$\text{Porosity} = 1 - \frac{\text{Weight of the sample}}{\text{Calculated weight of sample assuming no porosity}} \qquad (3.1)$$

The porosities of PEUU scaffolds were determined using a liquid displacement method (15). Ethanol was used as displacement liquid. Scaffolds were submerged in a known volume of ethanol (V_1). They were kept in ethanol for a while and then pressed to allow the ethanol to penetration into the pores of scaffolds. The total volume of ethanol and ethanol submerged scaffolds was $V_{2.}$ The scaffolds were taken out from ethanol and the residual ethanol was $V_{3.}$ The porosity of scaffolds were calculated from the Formula 3.2

$$p = (V_1 - V_3)/(V_2 - V_3) \quad . \qquad (3.2)$$

where p represents porosity.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Polymer/Ceramic Composites For Bone Tissue Engineering

Two types of polymers (PLLA and PLGA 50:50) were used in vitro release studies of dexamethasone from polymer/ceramic composites. Porous ceramics were in cylindrical and cubic forms. They were fabricated at METU Metallurgical and Materials Engineering Dept. Prof. Dr. Muharrem Timuçin's laboratory.

Table 4.1 Weights of porous ceramics

Sample	Initial weight (gr)	Final weight after polymer coating (gr)	Percentage of coated polymer and dexamethasone
PLLA coated cylinder	0.9929	1.0596	6.71 %
PLGA coated cylinder	1.0596	1.1490	7.58 %
PLLA coated cube	1.5582	1.7694	13.5 %
PLGA coated cube	1.4330	1.6937	18.1 %

Dexamethasone percentage of coated ceramic samples (w/w) were calculated as 0.6 %, 0.8 %, 1.35 % and 1.8 % for PLLA coated cylinder, PLGA coated cylinder, PLLA coated cube and PLGA coated cube, respectively. (Dexamethasone/ polymer=0.1)

Table 4.2 Porosities of ceramics

Sample	Porosity % before polymer coating	Porosity % after polymer coating
PLLA coated cylinder	60.91	58.31
PLGA coated cylinder	54.07	50.19
PLLA coated cube	75.72	72.43
PLGA coated cube	72.54	67.55

The porosities of samples were calculated according the Formula 3.1. Cubic ceramics have higher porosities than cylindircal ceramics. Polymer coating decreased the porosities of ceramics.

4.1.1 In Vitro Release Studies

PLGA coated cylindrical sample released 0.857 mg dexamethasone and PLLA coated cylindrical sample releasing 0.638 mg dexamethasone on the first day. On the third day; PLGA coated sample released higher amount of dexamethasone (1.04 mg). But PLLA coated sample released less amount of dexamethasone (0.367 mg) compared to first day. After one week, PLGA coated sample almost released 35 % of dexamethasone while PLLA coating sample released 23 % of dexamethasone. Both of the two samples maintained their high release rate through four weeks. On the fourth week, PLGA coated sample released 70 % of dexamethasone and PLLA coated sample released 59% of dexamethasone. But after four weeks, the amounts of

dexamethasone released from PLGA coated cylindrical sample and PLLA coated cylindrical sample were decreased. The release period was 8 weeks for PLGA coated sample and 7 weeks for PLLA coated sample. Release behavior of dexamethasone from cylindrical polymer/ceramic composites is given in Figure 4.1. Release efficiencies of the samples were calculated as 70.54 % and 75.27 % for PLLA coated and PLGA coated, respectively. (Table 4.3)

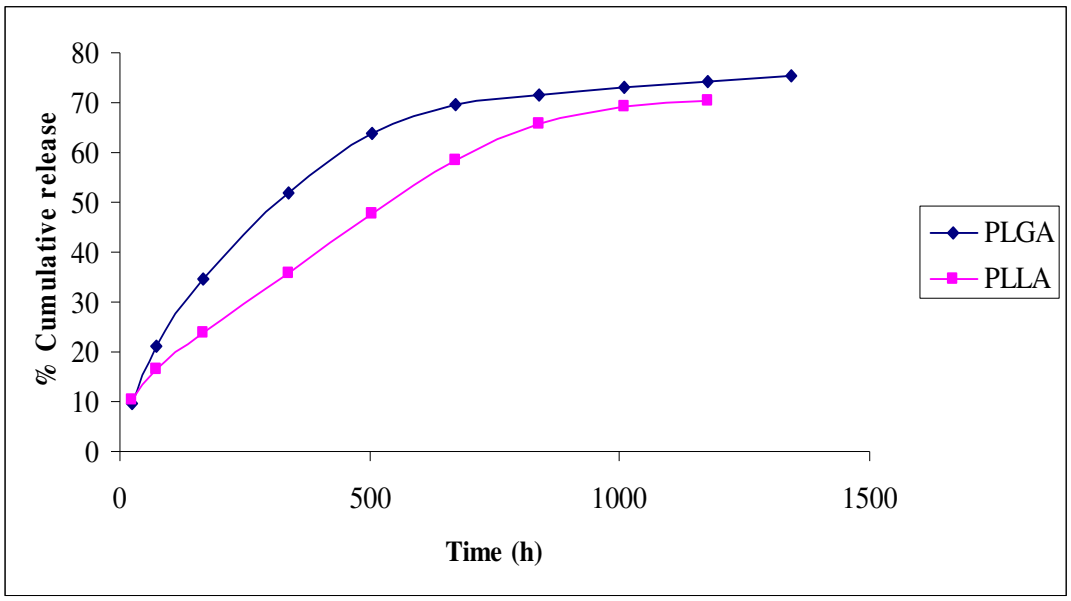

Figure 4.1 Cumulative release of dexamethasone from cylindrical polymer/ceramic composites

Table 4.3 Entrapment values of cylindrical polymer/ceramic composites

Sample	**Maximum Release**	**Entrapped Amount**	**Release Efficiency**
PLLA	4.28 mg	6.07 mg	70.54 %
PLGA	6.73 mg	8.94 mg	75.27 %

PLGA coated cubic sample released 1.97 mg dexamethasone and PLLA coated cubic sample releasing 1.47 mg dexamethasone on the first day. On the third day; PLGA coated cubic sample released higher amount of dexamethasone (1.35 mg) than the amount of PLLA coated cubic sample released (1.27 mg). After one week both samples released 20 % of dexamethasone. On the second week, release percentages of samples were closer to each other (PLGA coated 27 % and PLLA coated 25 %). After second week, PLGA coated sample usually released higher amount of dexamethasone compared with PLLA coated sample. The release period was 11 weeks for both PLLA coated sample and PLGA coated sample. Release behavior of dexamethasone from cubic polymer/ceramic composites is given in Figure 4.2. Release efficiencies of the samples were calculated as 87.64 %, 90.98 % for PLLA coated and PLGA coated, respectively.

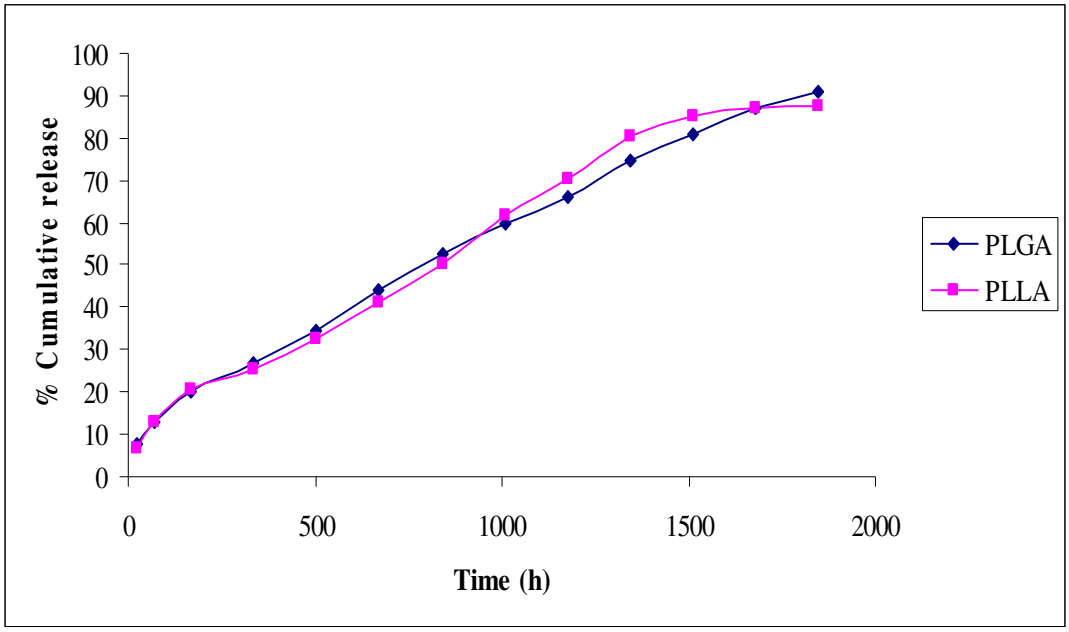

Figure 4.2 Cumulative release of dexamethasone from cubic polymer/ceramic composites

Table 4.4 Entrapment values of cubic polymer/ceramic composites

Sample	**Maximum Release**	**Entrapped Amount**	**Release Efficiency**
PLLA	18.51 mg	21.12 mg	87.64 %
PLGA	23.73 mg	26.07 mg	90.98 %

Cubic samples released higher amounts of dexamethasone compared to cylindrical samples and their release period was relatively longer. Because the percentage of dexamethasone loaded to cubic samples were higher than cylindrical ones.

4.1.2 Release Kinetics

In order to understand the type of release kinetics from polymer/ceramic composites, the data was fitted into the standart release equations (zeroth order, first order, Higuchi and Krosmeyer equations).

For PLGA coated cubic sample best fit was observed with Zeroth order model and for PLLA coated cubic sample best fit was observed with Higuchi model. According to R^2 values; PLGA and PLLA coated cylindrical samples fitted to Korsmeyer model. The plot of kinetic data in accordance with release models is given in Figure 4.3. and Figure 4.4.

Table 4.5 Release kinetics of polymer/ceramic composites

Sample	**Zero Order**		
	K_0	**R^2**	
PLLA coated cubic sample	0.0471	0.9735	
PLGA coated cubic sample	0.0459	**0.9922**	
PLLA coated cylindrical sample	0.0546	0.9494	
PLGA coated cylindrical sample	0.0461	0.7935	
Sample	**First Order**		
	K_1	**R^2**	
PLLA coated cubic sample	0.0012	0.8548	
PLGA coated cubic sample	0.0012	0.8560	
PLLA coated cylindrical sample	0.0015	0.8393	
PLGA coated cylindrical sample	0.0012	0.6373	
Sample	**Higuchi**		
	K_H	**R^2**	
PLLA coated cubic sample	2.234	**0.9854**	
PLGA coated cubic sample	2.264	0.9801	
PLLA coated cylindrical sample	2.230	0.9879	
PLGA coated cylindrical sample	2.129	0.9277	
Sample	**Korsmeyer-Peppas**		
	K_{kp}	**R^2**	**N**
PLLA coated cubic sample	0.92	0.9816	0.627
PLGA coated cubic sample	1.02	0.9887	0.587
PLLA coated cylindrical sample	1.85	**0.9911**	0.520
PLGA coated cylindrical sample	2.27	**0.9636**	0.511

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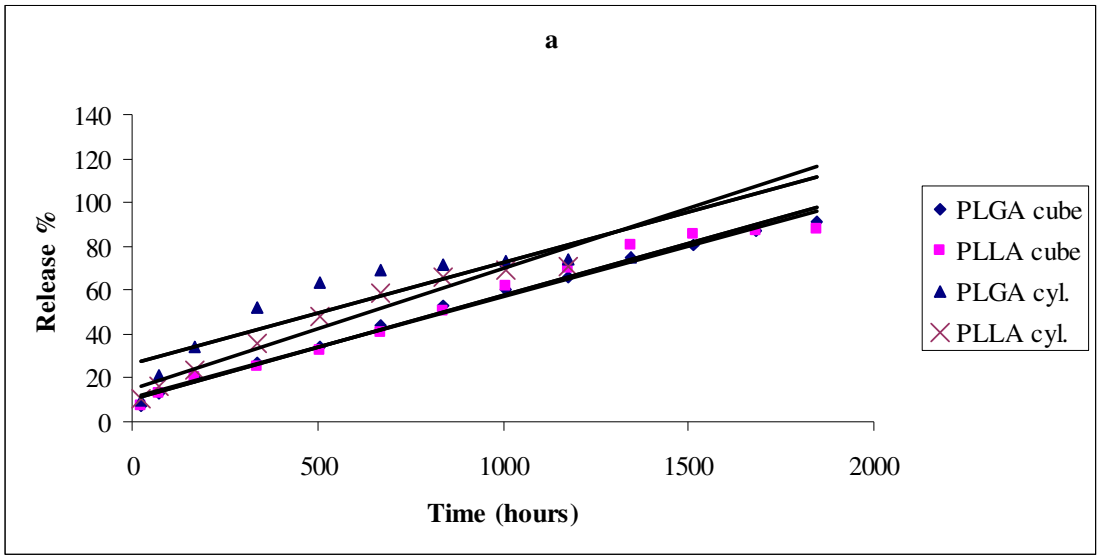


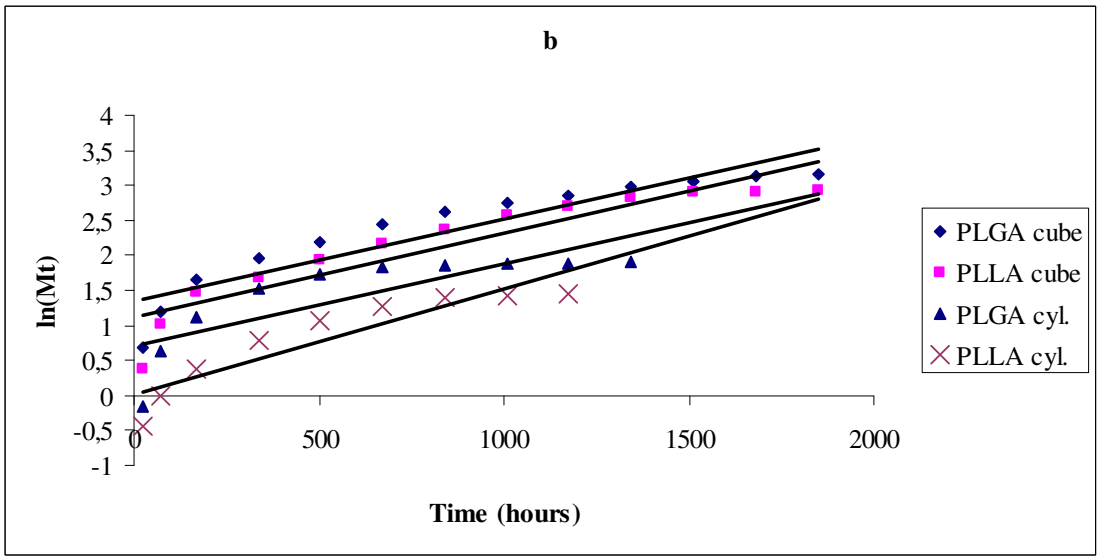
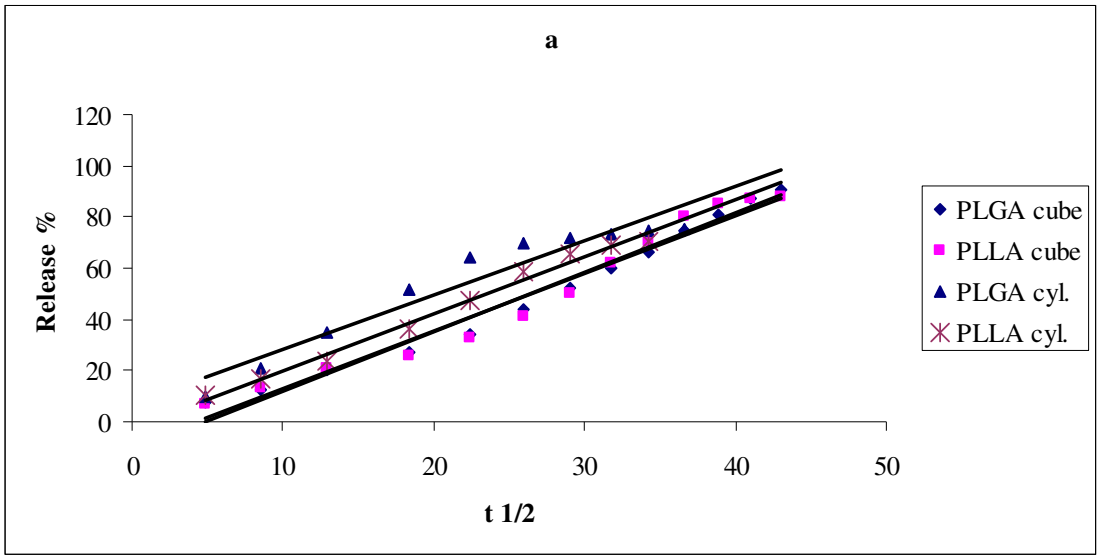


Figure 4.3 Plot of kinetic data in accordance with release models (a) zeroth, (b) first order



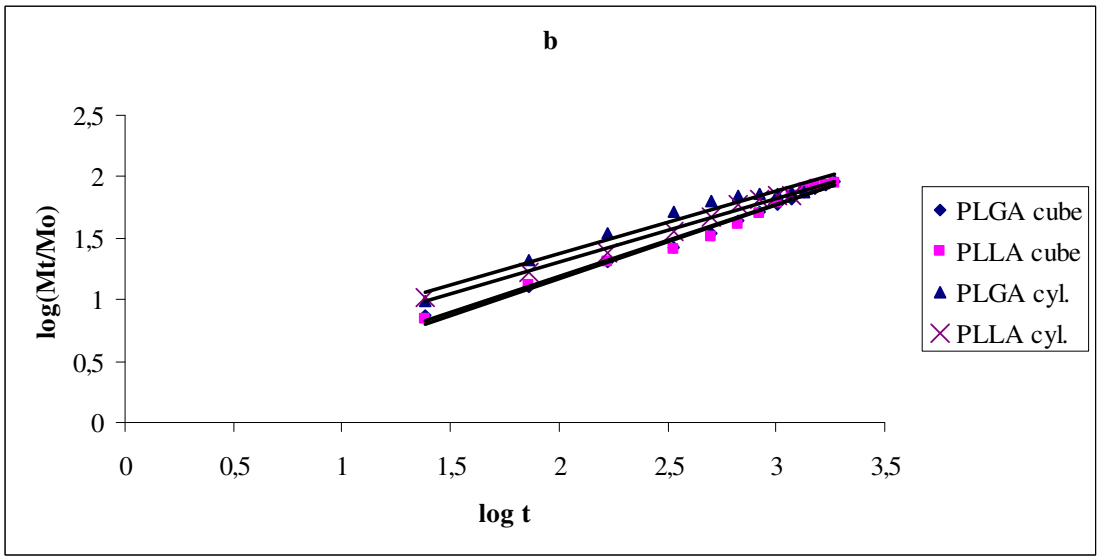


Figure 4.4 Plot of kinetic data in accordance with release models , (a) Higuchi and (b) Krossmeyer models

4.1.3 Mechanical Tests

Compression tests were performed with cylindrical porous ceramics. Samples were coated with PLGA and PLLA by dip coating method. They were compressed along their axes by placing them in between the platens of the machine. The cross-head speed was kept at 1mm/min. The comprresive strength of the samples were calculated from breaking load and the original cross section of the cylindir samples. The average ultimate compressive strength value of samples with PLLA coating, PLGA coating and without coating were found as 9.03 MPa, 7.61 MPa and 5.53 MPa respectively (Figure 4.5).

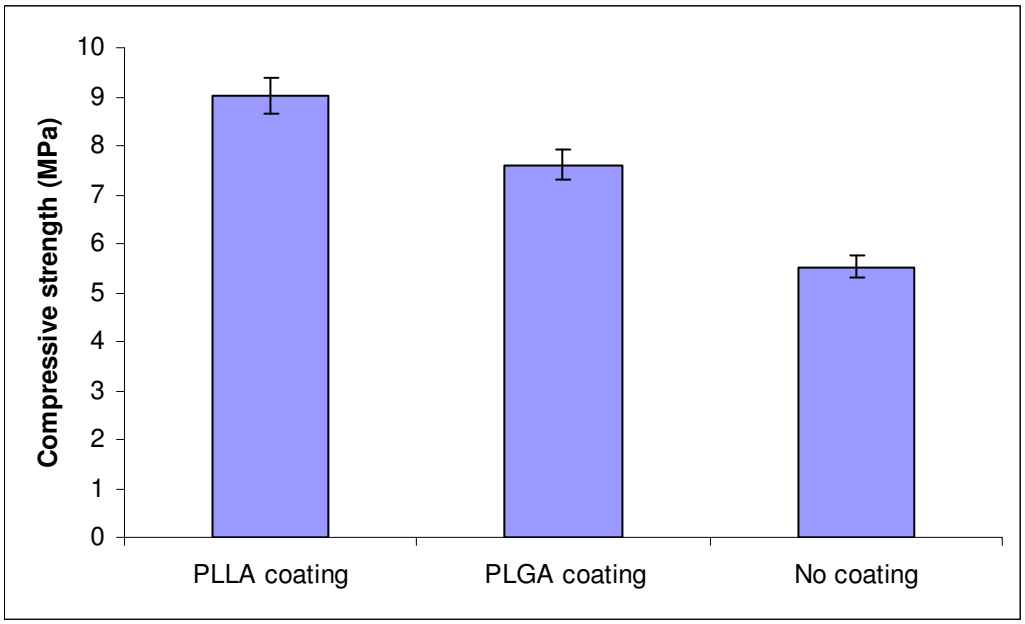


Figure 4.5 Average compressive strength of polymer/ceramic composites

Compressive modulus of samples are given in Figure 4.6. The modulus of PLLA coated samples is 91,8 MPa, the modulus of PLGA coated samples is 103,3 MPa and the modulus of samples without coating is 101,4 MPa which implies that bioceramic has obtained s tougher and more flexible morphology with polymer coating. PLLA coated samples have the highest compressive strength so a low compressive modulus for PLLA coated samples means that it can absorb stress much better than the other two (PLGA coated and uncoated). PLGA coated samples have the highest compressive modulus. Samples without polymer coating have the lowest compressive strength value. Because bioceramics with a porous form have low mechanical properties, which have restricted their use to low- or non-load-bearing applications. Biodegradable polymer/bioceramic composites scaffold can overcome the limitation of conventional ceramic bone substitutes such as brittleness and difficulty in shaping and combine the strength and stiffness of an inorganic compound with the flexibility, toughness and resorbability of an organic phase.

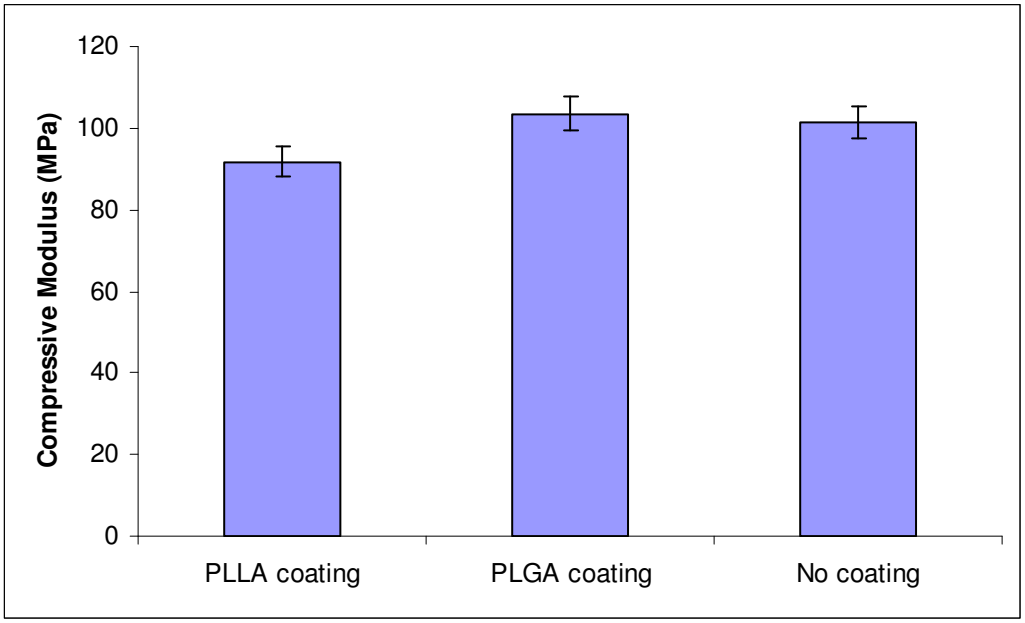


Figure 4.6 Compressive modulus of polymer/ceramic composites

In order to improve the mechanical properties such as compressive strength and compressive modulus and maintain the desirable bioactivity, porous ceramics were coated with PLLA and PLGA. According to results, the mechanical properties of the porous composites were found to improve significantly.

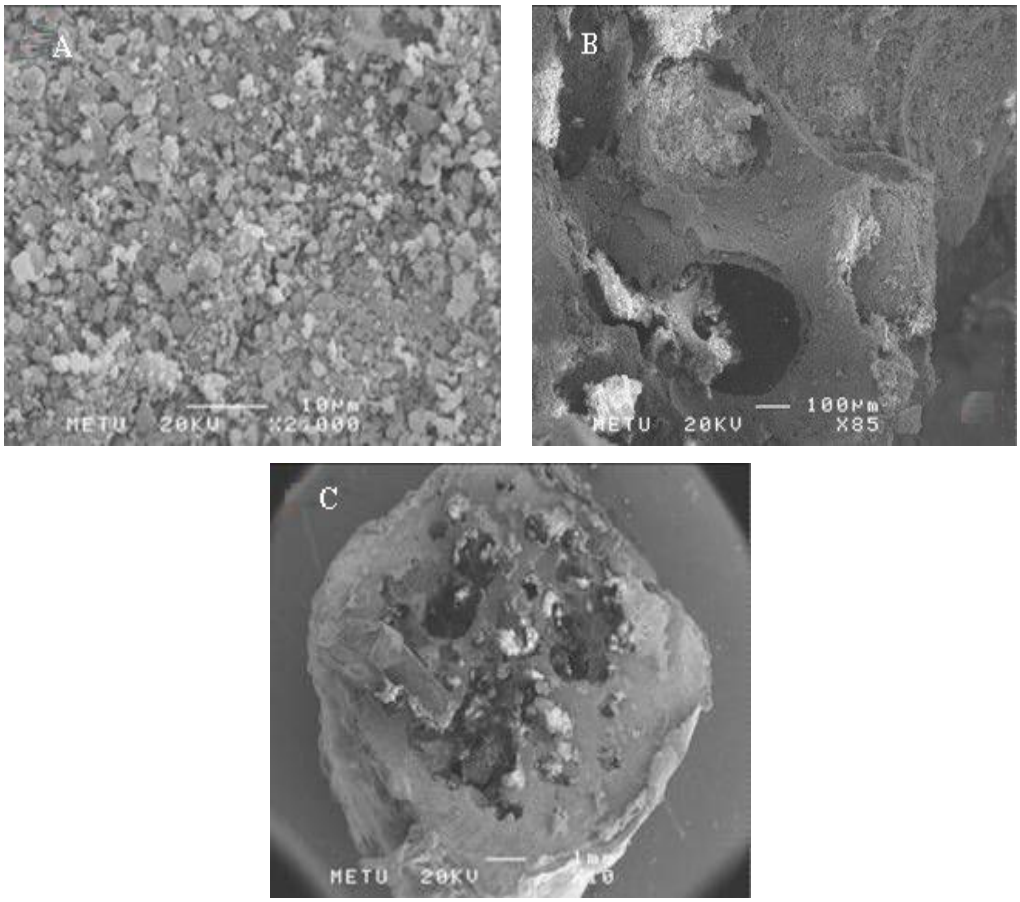


Figure 4.7 SEM micrographs of sliced surfaces of polymer/ceramic composites (A) x 2000, (B) x 85, (C) x 10

The morphology analysis performed by SEM on porous structure are presented in Figure 4.7. From Figure 4.7.B, some pores were coated with a thin polymer film and

some pores were filled with polymer. Polymer coating improved the mechanical properties of ceramics and also it would provide to facilate growth factor release and make cell attachment possible. Larger pores which can be seen from Figure 4.7.C, are longitudinal coloums to illustrate the Haver's canals of bone. They were canalized at micro CNC tool. The pore structures were dependent on the production method of the porous ceramics. Sintering process also affects the properties of the ceramics.

The average compressive strength of PLLA coating samples were higher compared to PLGA coating ones. This difference may be explained by degree of crystalliniy of polymers. PLLA is linear polyester constituted by macromolecular chains resulting from the structural unit represented in Fig. 2.1. The presence of an asymmetric chiral carbon atom in the polylactide structure leads to the existence of stereoisomers, as D-, L-, and DL-lactic acid. Stereoregular polylactides result from polymerization of optically pure lactides. Optically pure polylactides, poly (L-lactide) (PLLA), and poly (D-lactide) (PDLA), are therefore usually found to be semicrystalline. On the other hand, PLGAs is an amorphous polymer rather than crystalline. Crystallinity influences many of the polymer properties (hardness, modulus, tensile, stiffness, melting point). A higher degree of crystallization means lower content of free volume and therefore an increase of stiffness generally follows. Highly crystalline polymers are rigid, high melting, and less affected by solvent penetration. Since both physical and mechanical properties of polymers are strongly dependent on the extent of crystallization and morphology, crystallization kinetics are important to understand on the relationship between the structure, and the mechanical properties of samples.

4.2 Polymer/TCP Composites for Osteomyelitis Treatment

4.2.1 Influence of Polymer Type on Release Kinetics from Polymer/TCP Composites

In order to study the influence of polymer type on release behavior from polymer/TCP composites, two polymers (PLLA and PLGA 50:50) were used. Vancomycin was used as drug in this part of study. Effect of polymer type on the release behavior was studied by comparing the in vitro drug release kinetics of composites loaded with Vancomycin.

4.2.1.1 In Vitro Release Studies

PLGA containing composite released higher amount of Vancomycin (54.18 mg) in the first day (approximately 83 % of total release). PLLA containig composite released similar amount of Vancomycin (38.24 mg) in the first day (approximately 82 % of total release). The initial releases can be named as 'burst' effect.

On the second day PLGA containing composite released higher amount of Vancomycin again when compared to PLLA containing composite. The release of Vancomycin was higher with PLGA containing composites than with PLLA containing composites. It was also observed that the amount of Vancomycin released on third day was less than that on the second day. The release period was seven days for both PLLA and PLGA containing composites. Total amount of Vancomycin released from PLGA/TCP composite was 64.68 mg and total amount of Vancomycin released from PLLA/TCP composite was 46 mg.

Table 4.6 Entrapment values polymer/TCP composites loaded with Vancomycin

Sample	**Maximum Release**	**Entrapped Amount**	**Release efficiency**
PLLA	46 mg	80 mg	57.5 %
PLGA	64.68 mg	80 mg	80.85 %

Release efficiencies of the samples were calculated as 57.5 % and 80.85 % for PLLA containing sample and PLGA containing sample, respectively. Release behavior of Vancomycin from polymer/TCP composites is given in Figure 4.8

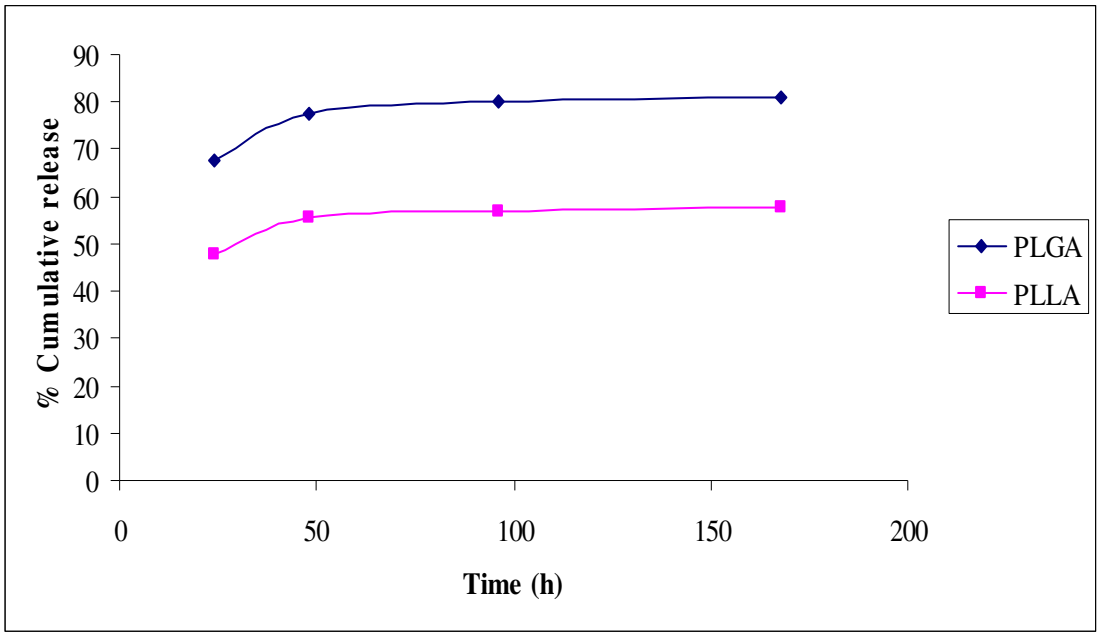


Figure 4.8 Cumulative release of Vancomycin from PLLA/TCP and PLGA/TCP composites

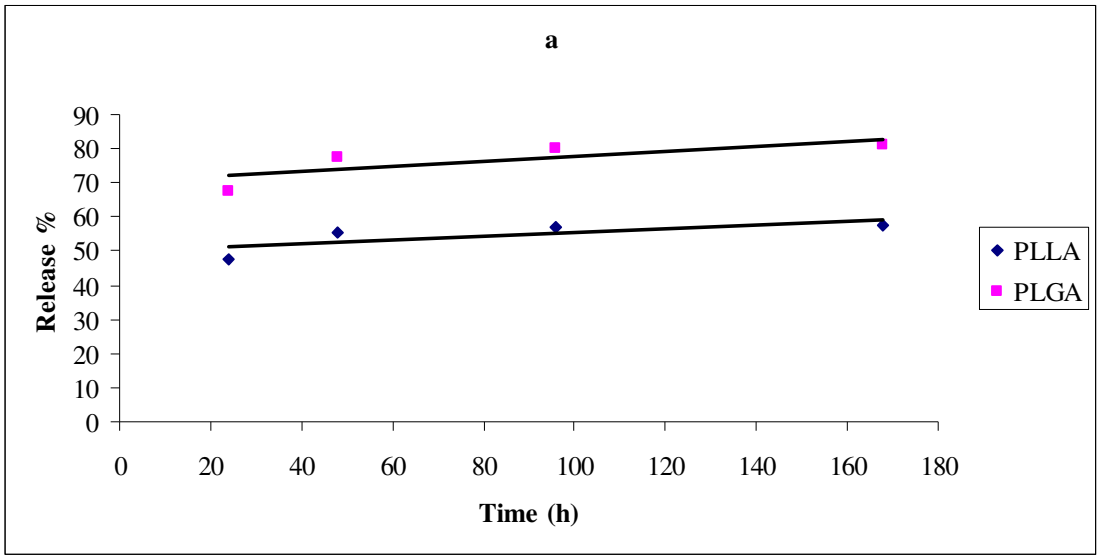
The difference between release behavior may be explained in terms of the properties of the polymers. PLGA is more sensitive to moisture and temperature because of its hydrophilic structure. PLGA has high amounts of amorphous regions in the bulk which are susceptible to hydrolytic degradations. PLLA has semicrystalline structure and extra methyl in repeating unit in comparison to PLGA. The hydrophobic methyl group reduces the molecular affinity to water and leads to a slower hydrolysis rate of PLLA. When compared to PLLA, PLGA undergo hydrolytic degradation very rapidly.

4.2.1.2 Release Kinetics

The release kinetics of Vancomycin loaded polymer/TCP composites were calculated according to the standart release equations (zeroth order, first order, Higuchi and Krosmeyer equations). For both PLGA containing sample and PLLA containing sample best fit was observed with Korsmeyer model. The plot of kinetic data in accordance with release models is given in Figure 4.9 and 4.10.

Table 4.7 Release kinetics of Vancomycin loaded polymer/TCP samples

Sample	**Zero Order**		
	K_0	**R^2**	
PLLA containing sample	0.0528	0.5559	
PLGA containing sample	0.0749	0.6113	
Sample	**First Order**		
	K_1	**R^2**	
PLLA containing sample	0.001	0.5266	
PLGA containing sample	0.001	0.5951	
Sample	**Higuchi**		
	K_H	**R^2**	
PLLA containing sample	1.044	0.6666	
PLGA containing sample	1.472	0.724	
Sample	**Korsmeyer-Peppas**		
	K_{kp}	**R^2**	**N**
PLLA containing sample	37.15	**0.7625**	0.0909
PLGA containing sample	52.48	**0.8154**	0.0889



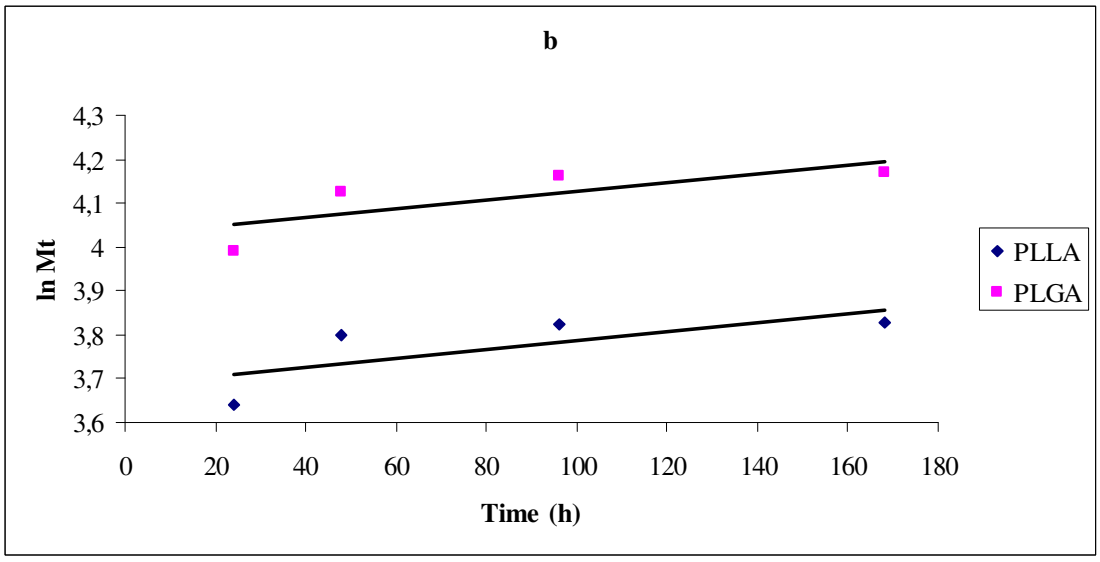
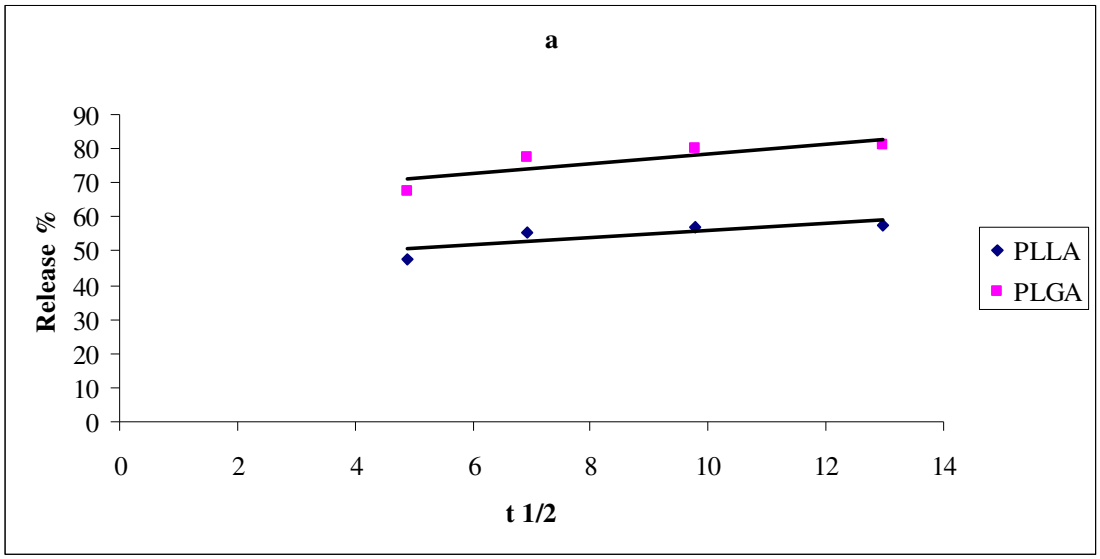


Figure 4.9 Plot of kinetic data in accordance with release models (a) zeroth, (b) first order



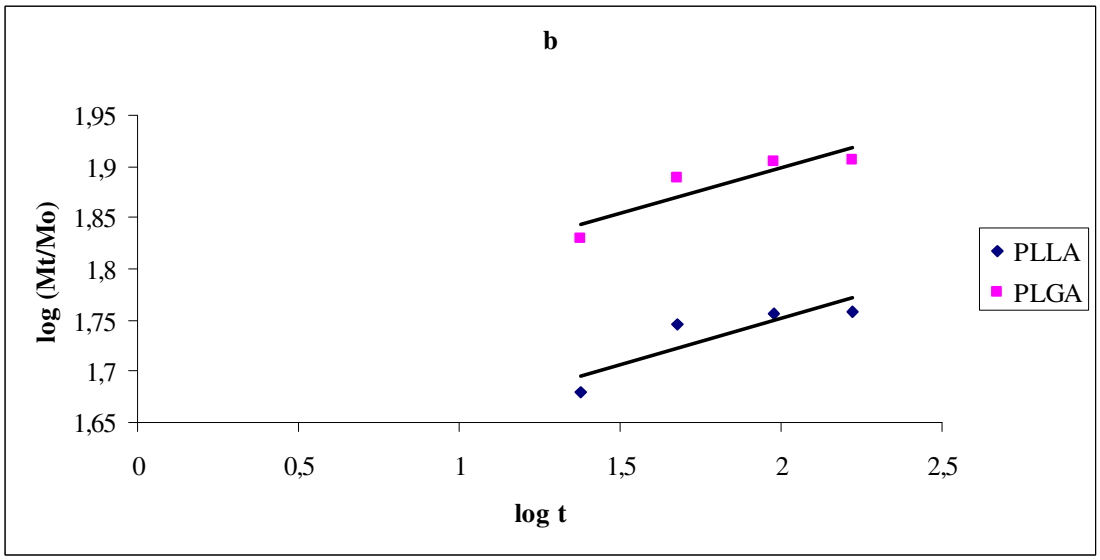


Figure 4.10 Plot of kinetic data in accordance with release models order, (a) Higuchi and (b) Krossmeyer models

4.2.2 Influence of Polymer Coating on Release Kinetics from Polymer/TCP Composites

Effect of polymer coating on the release behavior was studied by comparing the in vitro drug release kinetics of polymer/TCP composites loaded with Gentamicin. PLLA and PLGA were used as polymers and Gentamicin was used as a drug in this part of the study.

4.2.2.1 In Vitro Release Studies of PLLA/TCP Composites

Experiments were performed with three PLLA/TCP composites, uncoated sample, one layer PLLA coated sample and two layers PLLA coated sample.

Uncoated PLLA/TCP sample released 4.16 mg Gentamicin on the first day. On the third day sample released lower amount of Gentamicin (0.93 mg). This decreasing release character continued in the following days. The release period was two weeks. Sample released 5.59 mg Gentamicin during two weeks.

One layer PLLA coated sample released 3.77 mg Gentamicin on the first day. Sample released 1.09 mg Gentamicin on the third day. Beginning from third day, release profile of sample decreased compared to first day. The release period was three weeks. Sample released 5.74 mg Gentamicin during three weeks.

Two layers PLLA coated sample released 4.42 mg Gentamicin on the first day. On the third day, the amount of antibiotic released from sample was decreased (1.87 mg). On the seventh day, sample released 0.9 mg dexamethasone. The release period was five weeks. Sample released 8.38 mg Gentamicin during five weeks.

Release efficiencies were calculated as 20 %, 21 % and, 31 % for uncoated sample, one layer PLLA coated sample and two layers PLLA coated sample, respectively**.**

Table 4.8 Entrapment values of PLLA/TCP composites loaded with Gentamicin

Samples	**Maximum release**	**Entrapped Amount**	**Release Efficiency**
Uncoated	5.59 mg	27 mg	20 % (14 days)
One layer PLLA coated	5.74 mg	27 mg	21 % (21 days)
Two layers PLLA coated	8.38 mg	27 mg	31 % (35 days)

The total amount of Gentamicin released from PLLA/TCP composites were 5.59 mg, 5.74 mg and 8.38 mg for uncoated sample, one layer PLLA coated sample and two layers PLLA coated sample respectively. The release behavior of Gentamicin from PLLA/TCP composites is given in Figure 4.11.

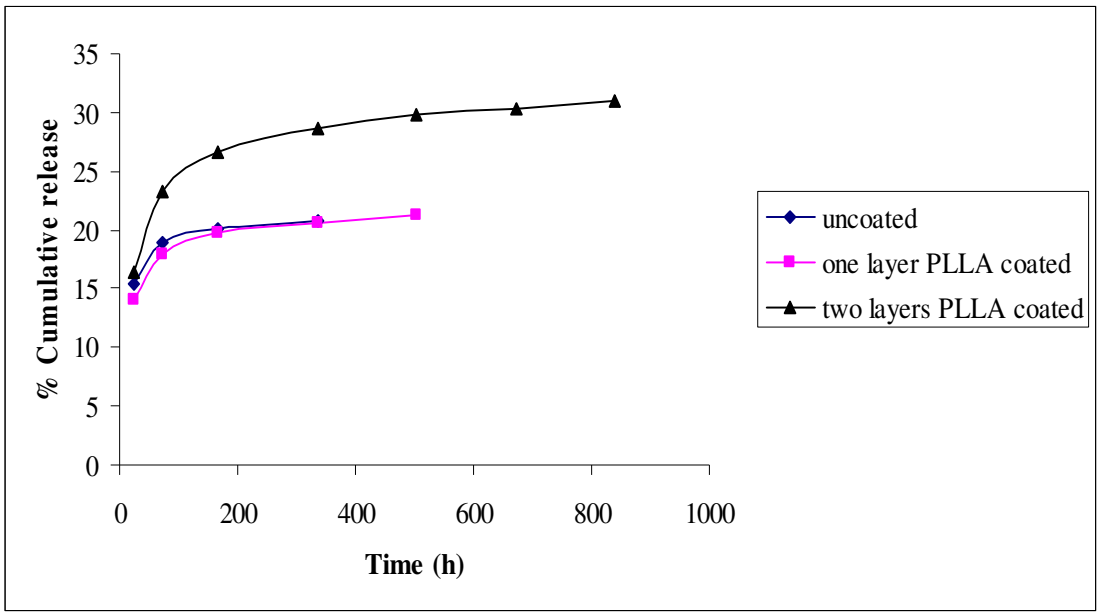


Figure 4.11 Cumulative release of Gentamicin from PLLA/TCP composites

The release of Gentamicin from the uncoated PLLA/TCP sample was characterized by a `burst effect' with 74 % (w/w) of the total amount of drug detected during the first day. Sample coated with one layer of PLLA released Gentamicin during 21 days, with 65 % (w/w) of the initial concentration of drug released during the first day. Sample coated with 2 layers PLLA released 53 % of total amount of Gentamicin on the first day. The percentage of drug released on the first day was lower but the amount of drug was higher when compared with uncoated sample and one layer PLLA coated sample.

4.2.2.2 In Vitro Release Studies of PLGA/TCP Composites

Experiments were performed with three PLGA/TCP composites, uncoated sample, one layer PLGA coated sample and two layers PLGA coated sample.

Uncoated PLGA/TCP sample released 5.9 mg Gentamicin on the first day. On the third day sample released 2.35 mg Gentamicin. Sample released lower amount of Gentamicin on the seventh day (0.89 mg). In the following days, this decreasing release character continued. The release period was three weeks. Sample released 9.62 mg Gentamicin during three weeks.

One layer PLGA coated sample released 5.55 mg Gentamicin on the first day. The amount of Gentamicin released from the sample decreased to 2.27 mg on the third day. On the seventh day sample released lower amount of Gentamicin (1.16 mg) compared first and third day. The release period was three weeks. Sample released 9.51 mg Gentamicin during three weeks.

Two layers PLGA coated sample released 5.37 mg Gentamicin on the first day. On the third day, the amount of antibiotic released from sample was decreased (1.86 mg). On the seventh day, sample released 1.16 mg Gentamicin. The release period was four weeks. Sample released 9.23 mg Gentamicin during four weeks.

Release efficiencies were calculated as 35 %, 35 % and, 34 % for uncoated sample, one layer PLGA coated sample and, two layers PLGA coated sample respectively. The release behavior of Gentamicin from PLGA/TCP composites is given in Figure 4.12.

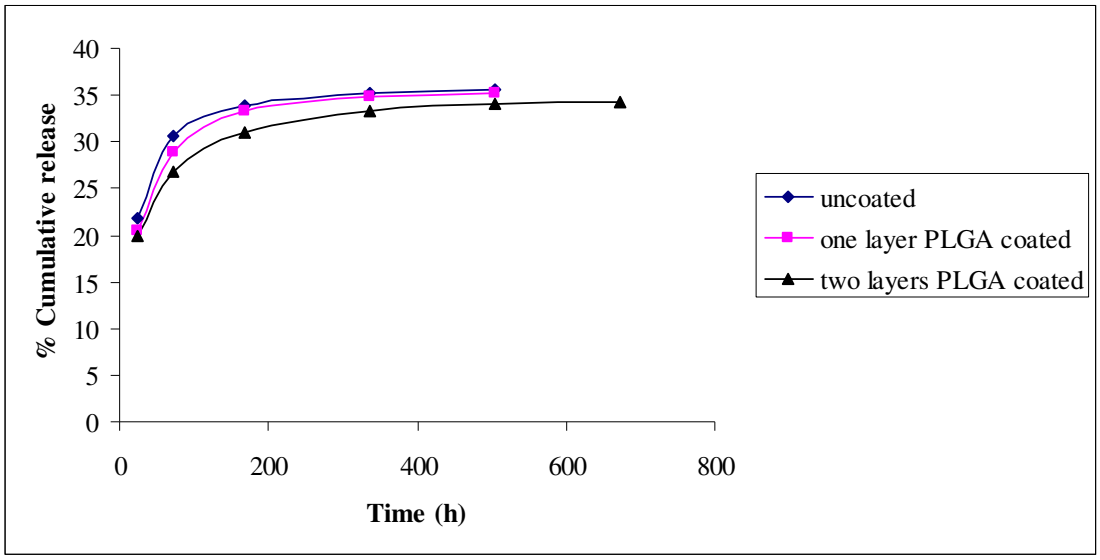


Figure 4.12 Cumulative release of Gentamicin from PLGA/TCP composites

Uncoated sample released 9.62 mg Gentamicin during 21 days, one layer PLGA coated sample released 9.51 mg Gentamicin during 21 days and two layers PLGA coated sample released 9.23 mg Gentamicin during 28 days.

The release efficiencies of PLGA/TCP composites are very close to each other. Their release periods are also closer to each other, only PLGA/TCP composites with two layers PLGA coating released Gentamicin for four weeks. Uncoated sample released higher amount of Gentamicin on the first day compared to other samples. Two layers PLGA coated sample released lowest amount. It can be said that, PLGA coating did not affect the release efficiencies of samples, but coating process decreased the amount of Gentamicin released from PLGA/TCP composites and increased the release period. From the results of in vitro release studies, it can be said that the coating process was not successful. Most probably the original polymer layer was damaged during coating.

Table 4.9 Entrapment values of PLGA/TCP composites

Samples	Maximum release	Entrapped Amount	Release Efficiency
Uncoated	9.62 mg	27 mg	35 % (21 days)
One layer PLGA coated	9.51 mg	27 mg	35 % (21 days)
Two layers PLGA coated	9.23 mg	27 mg	34 % (28 days)

4.2. 2.3 Release Kinetics

The release kinetics of Gentamicin loaded PLLA/TCP and PLGA/TCP composites were calculated according to the standart release equations (zeroth order, first order, Higuchi and Krosmeyer equations). For all samples best fit was observed with Korsmeyer model. The plots of kinetic data in accordance with release models are given in Figures 4.13, 4.14, 4.15 and 4.16.

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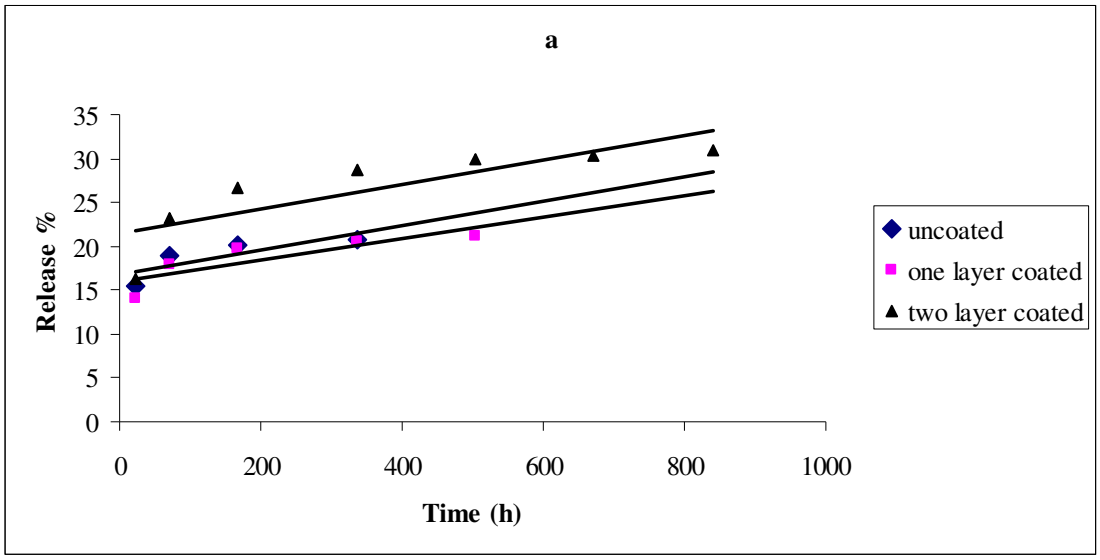
Table 4.10 Release kinetics of Gentamicin loaded PLLA/TCP samples.

Sample	**Zero Order**		
	K_0	**R^2**	
Uncoated	0.0141	0.6666	
One layer PLLA coated	0.0122	0.6945	
Two layers PLLA coated	0.014	0.6877	
Sample	**First Order**		
	K_1	**R^2**	
Uncoated	0.0008	0.6362	
One layer PLLA coated	0.0007	0.6426	
Two layers PLLA coated	0.0006	0.6145	
Sample	**Higuchi**		
	K_H	**R^2**	
Uncoated	0.368	0.8073	
One layer PLLA coated	0.3723	0.8326	
Two layers PLLA coated	0.5229	0.8352	
Sample	**Korsmeyer-Peppas**		
	K_{kp}	**R^2**	**N**
Uncoated	13.8	**0.9868**	0.0698
One layer PLLA coated	8.55	**0.8929**	0.1539
Two layers PLLA coated	14.45	**0.979**	0.1139



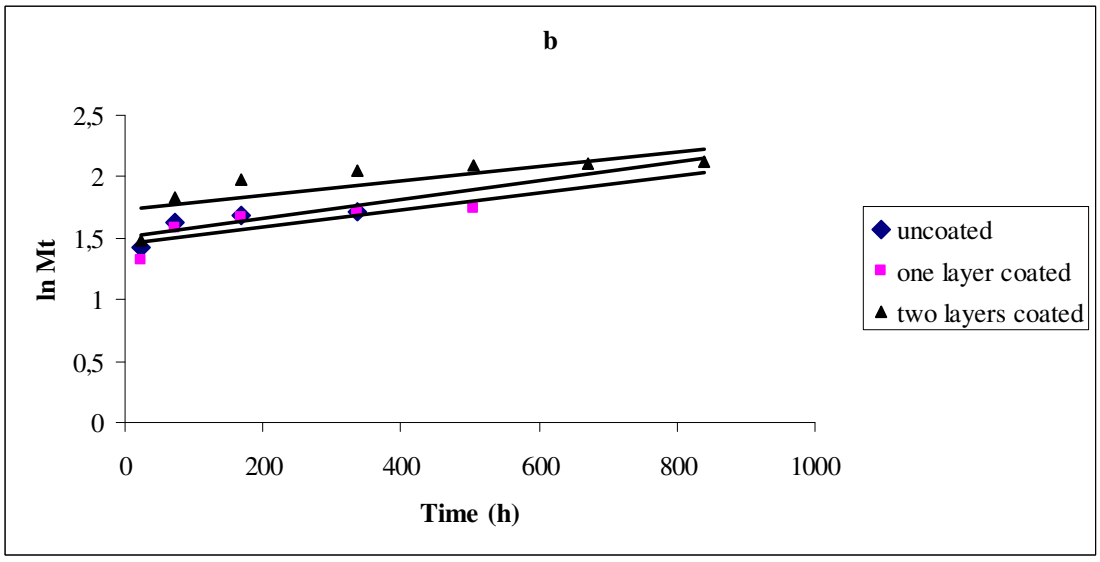
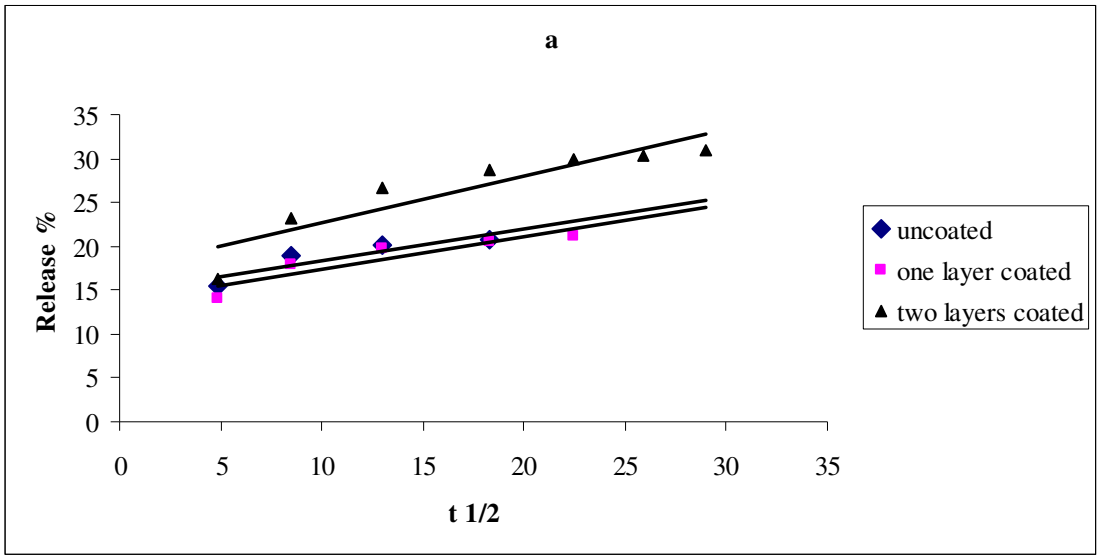


Figure 4.13 Plot of kinetic data in accordance with release models (a) zeroth, (b) first order,



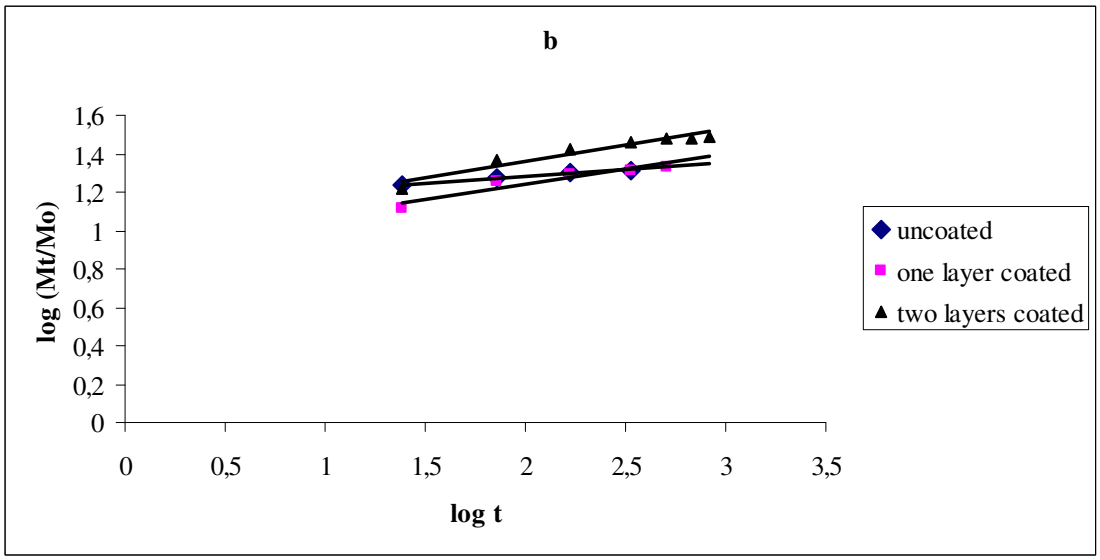
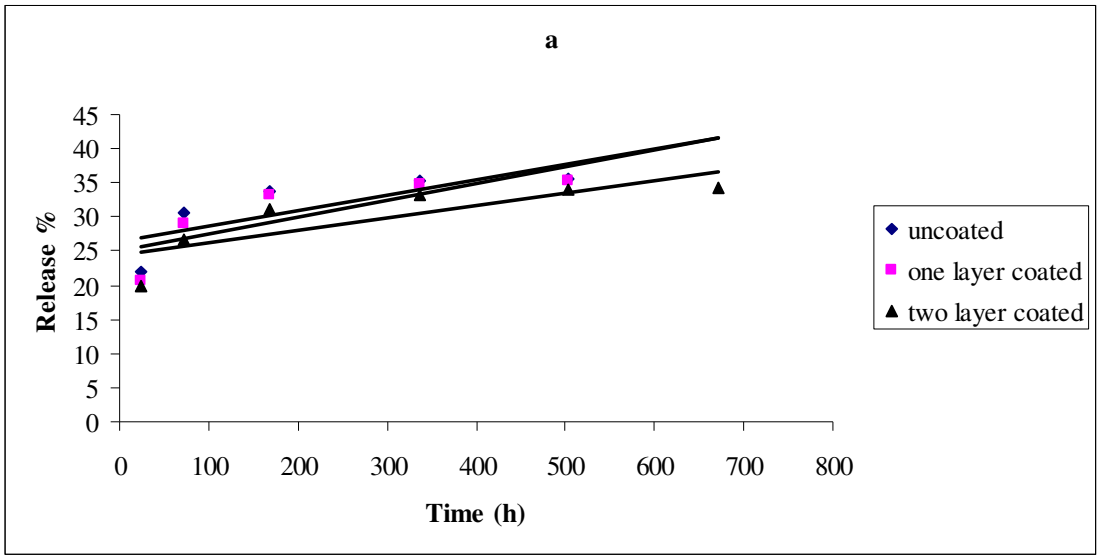


Figure 4.14 Plot of kinetic data in accordance with release models (a)Higuchi and (b) Krossmeyer models for PLLA/TCP composites

Table 4.11 Release kinetics of Gentamicin loaded PLGA/TCP samples.

Sample	**Zero Order**		
	K_0	**R^2**	
Uncoated	0.0223	0.6022	
One layer PLGA coated	0.0246	0.638	
Two layers PLGA coated	0.018	0.669	
Sample	**First Order**		
	K_1	**R^2**	
Uncoated	0.0008	0.5567	
One layer PLGA coated	0.0009	0.5871	
Two layers PLGA coated	0.0006	0.6156	
Sample	**Higuchi**		
	K_H	**R^2**	
Uncoated	0.6967	0.755	
One layer PLGA coated	0.7621	0.7895	
Two layers PLGA coated	0.6238	0.8216	
Sample	**Korsmeyer-Peppas**		
	K_{kp}	**R^2**	**N**
Uncoated	1.43	**0.8743**	1.65
One layer PLGA coated	1.49	**0.8984**	1.49
Two layers PLGA coated	1.44	**0.9266**	1.1



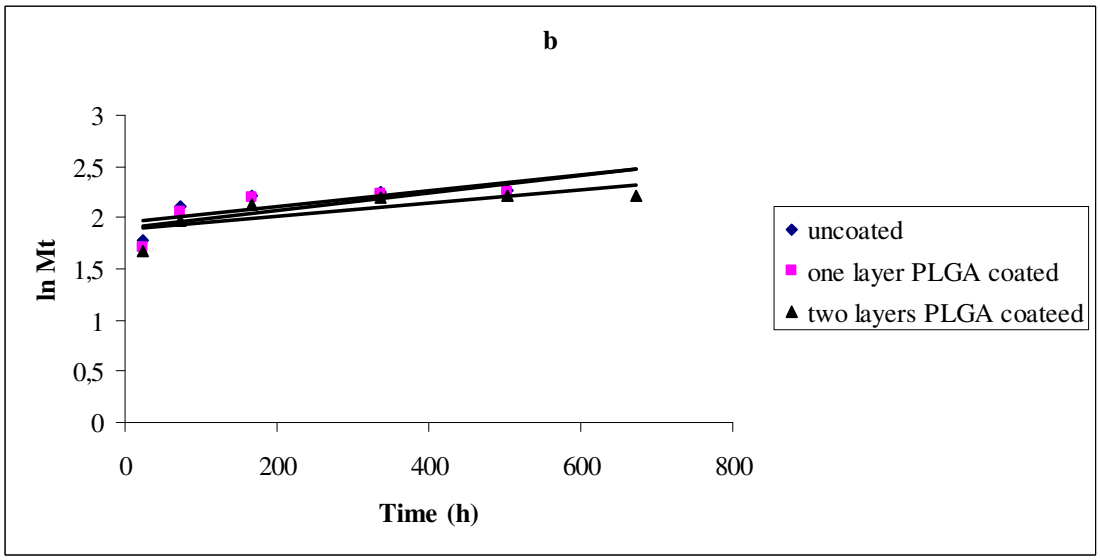
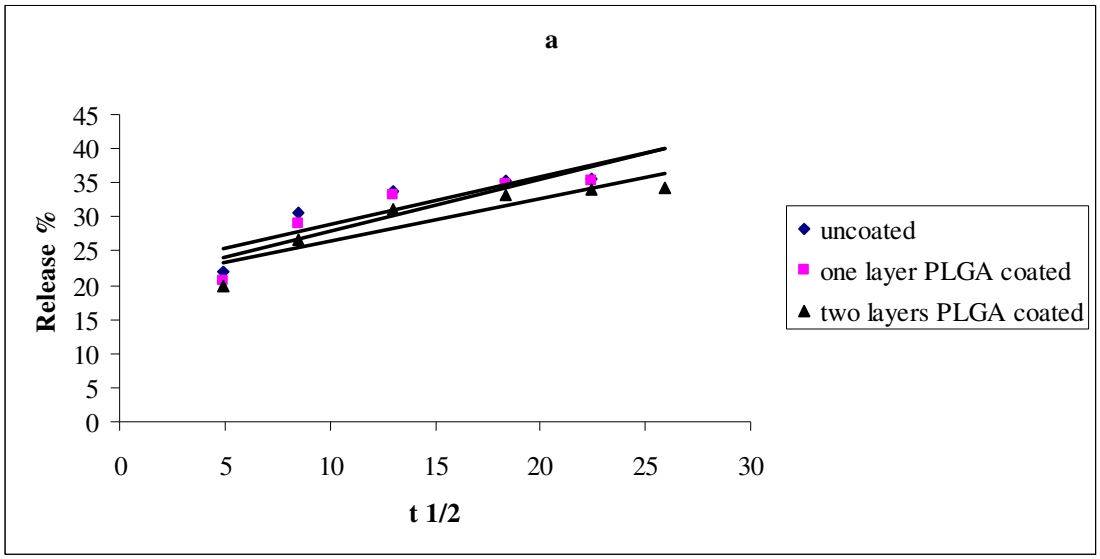


Figure 4.15 Plot of kinetic data in accordance with release models (a) zeroth, (b) first order for PLGA/TCP composites



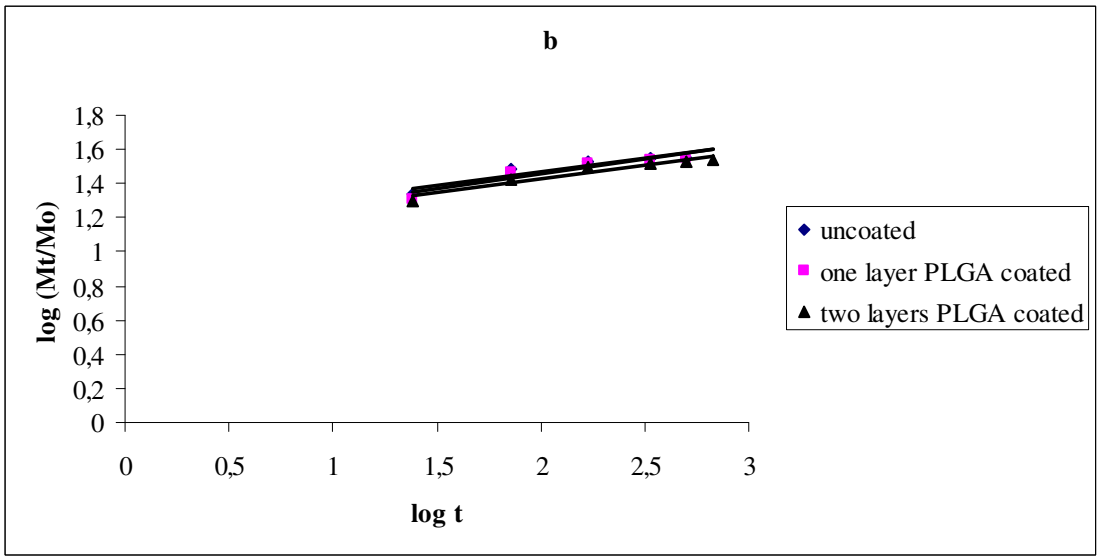


Figure 4.16 Plot of kinetic data in accordance with release models , (a) Higuchi and (b) Krossmeyer models for PLGA/TCP composites

4.3. Characterization of PEUU

4.3.1 ATR Spectroscopy

The ATR spectrum confirmed the PEUU structure with the pronounced carbonyl peak of ester group at 1722 cm^{-1}. The shoulder peak at lower wavelength was attributed to hydrogen bond associations of the urethane, urea, or amide groups. The absorbance at 2942 cm^{-1} was attributed to C-H streching and the absorbance at 1461 cm^{1} was attributed to C-H bending. The urethane and urea groups showed absorbance between 3500-3300 cm^{-1} as N-H streching The signal corresponding to isocyanate groups (2300-2200 cm^{-1}) is absent in this spectrum. This can be explained by a lack of unreacted isocyanate groups in PEUU polymer. The ATR spectrum of PEUU is given in Figure 4.17.

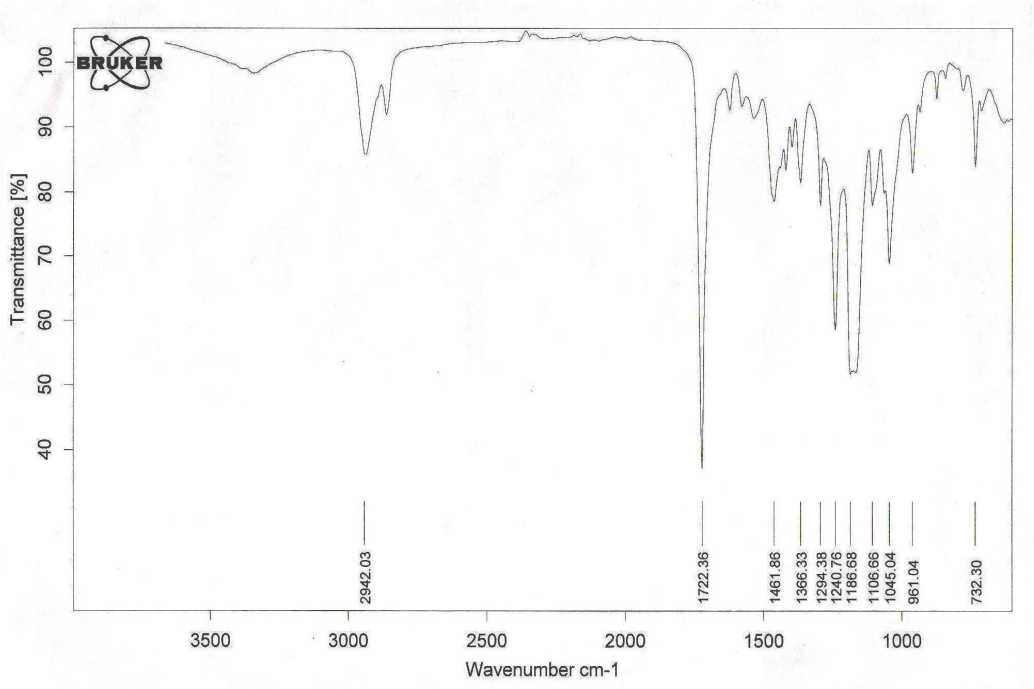


Figure 4.17 ATR spectroscopy of PEUU

4.3.2 NMR Spectroscopy

The ^{1}H NMR spectrum of PEUU is shown in Figure 4.18. The characteristic peaks of methyl protons of PCL segment were seen at 4 ppm (A) , between 1.2-1.4 ppm (B), between 1.5-1.6 ppm (C), and between 2.4-2.6 ppm (D). The characteristic peaks of PEG segment were seen at 4.2 ppm (E), and between 3.2-3.4 ppm (F). The hard segment showed the characteristic peaks of the 1,6-diisocyanato hexane (peaks G and C). The chemical formula of PEUU is given in Figure 4.19.

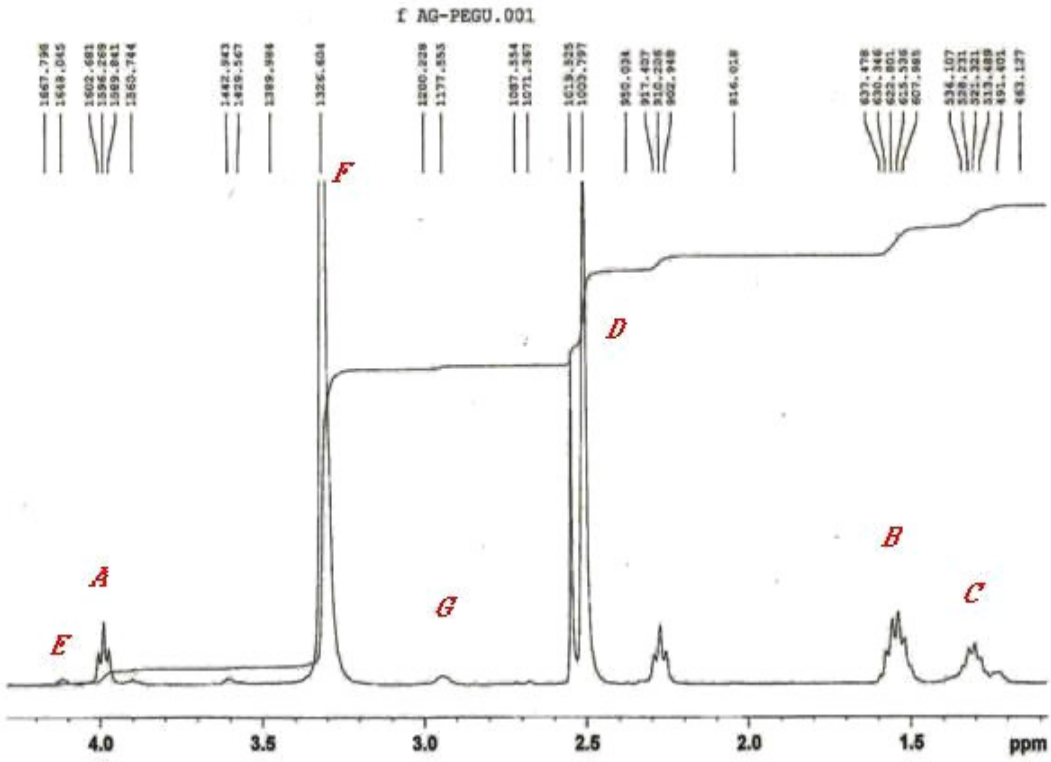


Figure 4.18 ^{1}H NMR spectroscopy of PEUU

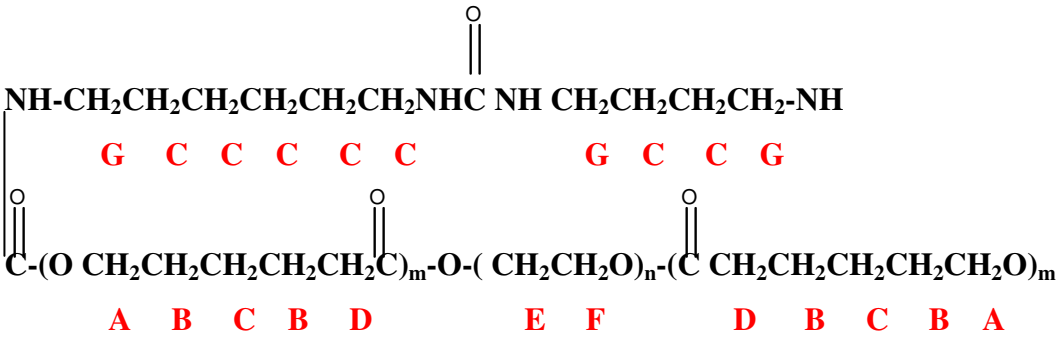
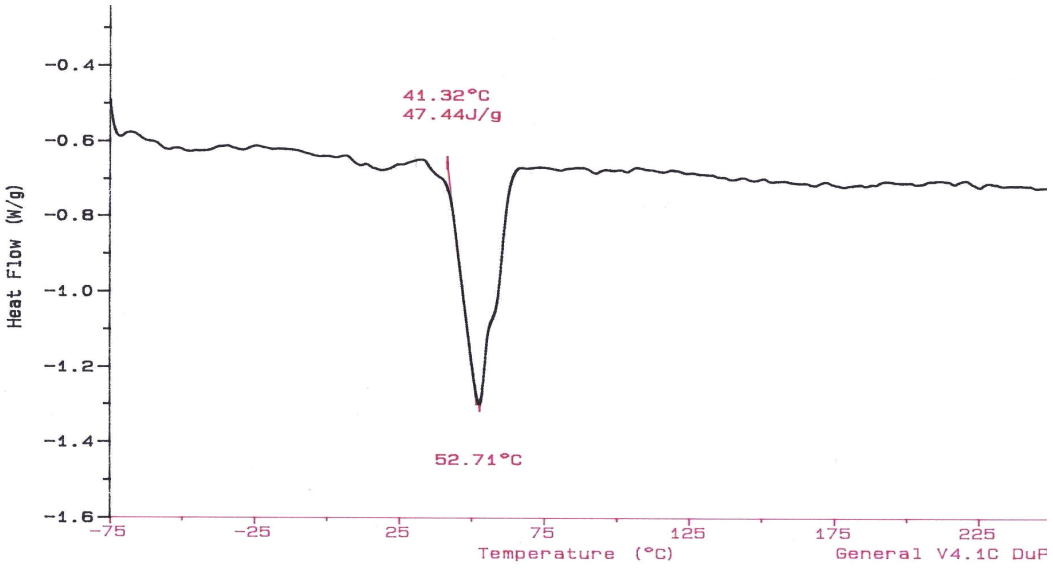


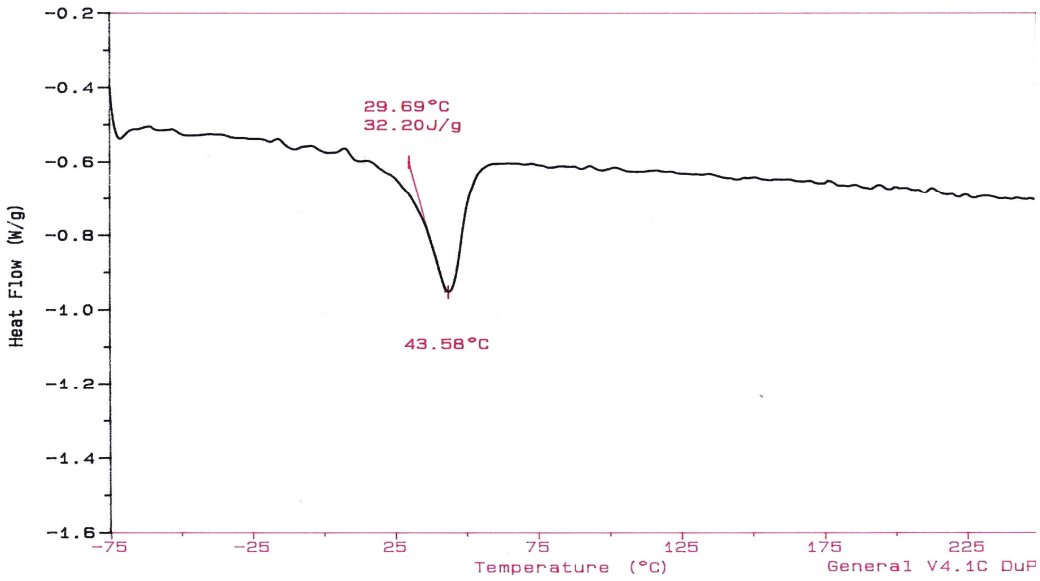
Figure 4.19 Chemical formula of PEUU

4.3.3 DSC Analysis

From DSC curve (Figure 4.20), melting point of PEUU was found 52.71 ˚C at first run and 43.58 ˚C at second run. The polyurethanes (PU)s exhibited soft segment transitions over the temperature range of -100 to 200 ˚C. All of the PUs had glass transition temperatures <-54 °C. The glass transition temperature decreased with increasing soft segment molecular weight (16).



(A)



(B)

Figure 4.20 DSC curve of PEUU (A) first run (B) second run

4.4 Porous Scaffolds For Cartilage Tissue Engineering

4.4.1 PEUU Scaffolds

Porosity of PEUU scaffolds were determined according to liquid displacement method. Ethanol was used as displacement liquid.

Table.4.12 Porosity results of PEUU scaffold according to liquid displacement method

Sample	V_1	V_2	V_3	Porosity %
1	5 ml	5.4 ml	3.8 ml	75
2	5 ml	5.5 ml	4 ml	67
3	5 ml	5.4 ml	4 ml	71.4
4	5 ml	5.4 ml	4.2 ml	67
5	5 ml	5.2 ml	4.2 ml	80
6	5 ml	5.5 ml	3.8 ml	70.5

Porosity percentages of PEUU scaffolds vary from 67 % to 80 %. SEM micrographs of PEUU scaffolds are given in Figure 4.21.

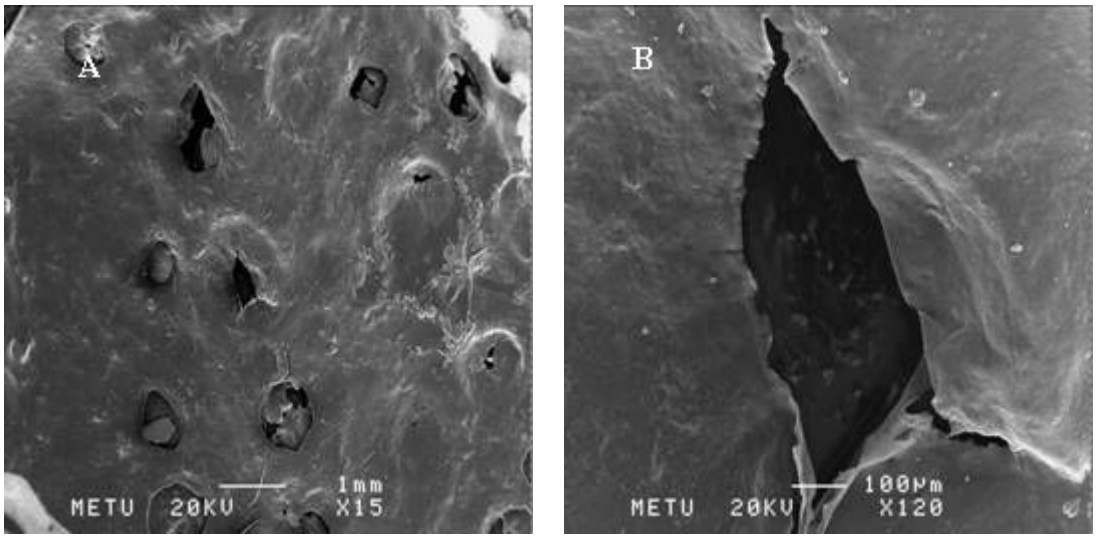


Figure 4.21 SEM micrographs of PEUU scaffolds (A) X 15, (B) X 120

4.4.2 PCL Scaffolds

The porosity results were given in Table 4.13. The porosities of samples are between 66.3 % and 75.8 %. This difference can be explained by diameters of fibers

Table 4.13 Porosities of PCL scaffolds

Sample	Weight	Theoretical weight	Porosity %
1	0,3587 gr	1.079 gr	66.7
2	0.28 gr	0.899 gr	68.9
3	0.2824 gr	0.899 gr	68.6
4	0.2823 gr	1.169 gr	75.8
5	0.335 gr	1.349 gr	69.5
6	0.5956 gr	1.798 gr	66.9
7	0.3039 gr	0.899 gr	66.3
8	0.3021 gr	0.899 gr	66.4
9	0.2485 gr	0.899 gr	72.35

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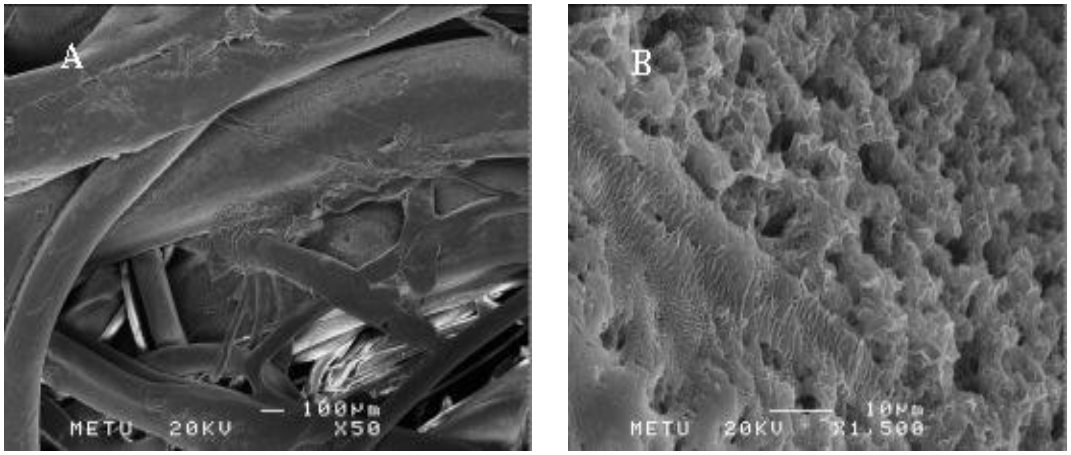

Figure 4.22 SEM micrographs of PCL scaffolds (A) x 50, (B) x 1500

As seen in the SEM micrograhps (Figure 4.22), fibers were not uniform. This non uniform structure of fibers affected the porosity of scaffolds.

Compression tests were performed with PCL scaffolds which were 10 mm in height and 9 mm in diameter. Samples were not fractured under load, they were buckled under 2.24 MPa load. The average deformation under load was 16.5 %.

CHAPTER 5

CONCLUSION

In this study, porous scaffolds were fabricated from poly(ester-urethane)urea (PEUU) and poly(caprolactone) (PCL) for tissue engineering to generate bone and cartilage-like
tissues to be used in an interdisciplinary study. The porosities of PEUU scaffolds varied between 67 % and 80 % and the porosities of PCL scaffolds varied between 66.3 % and 75.8 %.

Secondly, porous ceramics were coated with PLLA and PLGA and they were loaded with dexamethasone instead of growth factor in order to study in vitro release studies, because growth factor is much more expensive than dexamethasone. Cubic samples released higher amounts of dexamethasone when compared to cylindrical samples. Biodegradable polymers, PLLA and PLGA were used to improve the mechanical strength of porous ceramics. PLLA coated samples had better mechanical properties than PLGA coated samples, while both were superior to that of uncoated samples. Polymer coating and impregnating also improved the integrity and reduced dust formation of the ceramics.

The controlled release of Vancomycin and Gentamicin from polymer/TCP composites is also studied in conjunction with osteomyelitis treatment. Vancomycin loaded samples including PLGA released higher amounts of drug than PLLA/TCP samples. Release period was seven days for both two types of samples. Gentamicin loaded samples release efficiencies were lower than Vancomycin loaded samples. Samples with extra inert polymer coating released Gentamicin for longer periods.

As a conclusion, it can be said that biomedical applications for bone and cartilage tissue engineering can take the advantages of biocompatibility and biodegradability of certain polymers. The use of TCP as a carrier medium for antibiotics and other types of drugs turned out to be a feasible method. The use of inert coating is also found to be an effective way of adjusting and prolonging the release period of the active agent.

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APPENDIX A

CALIBRATION CURVES OF DRUGS

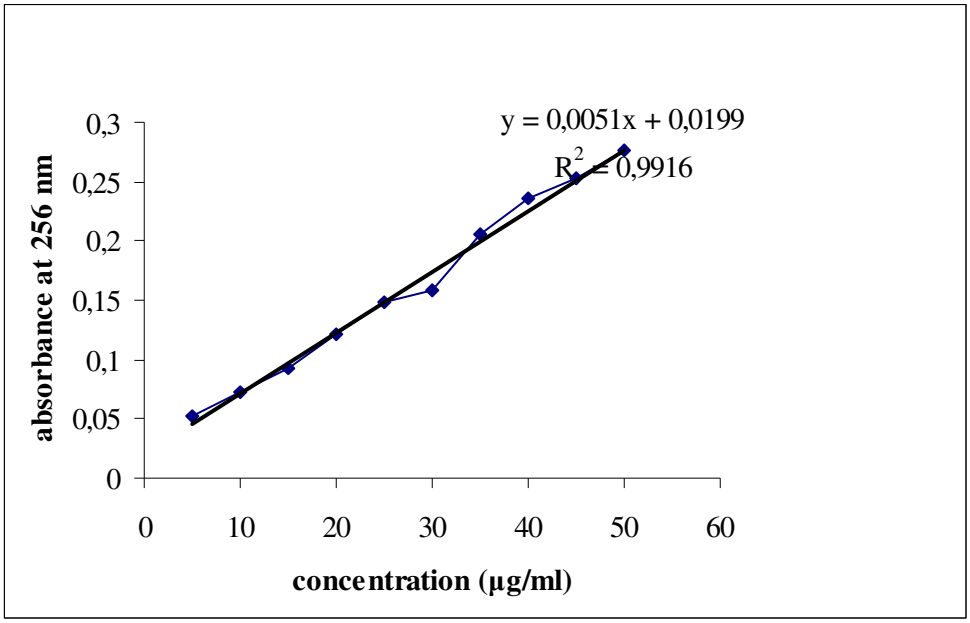

Figure A.1 Calibration curve for Gentamicin (at 256 nm)

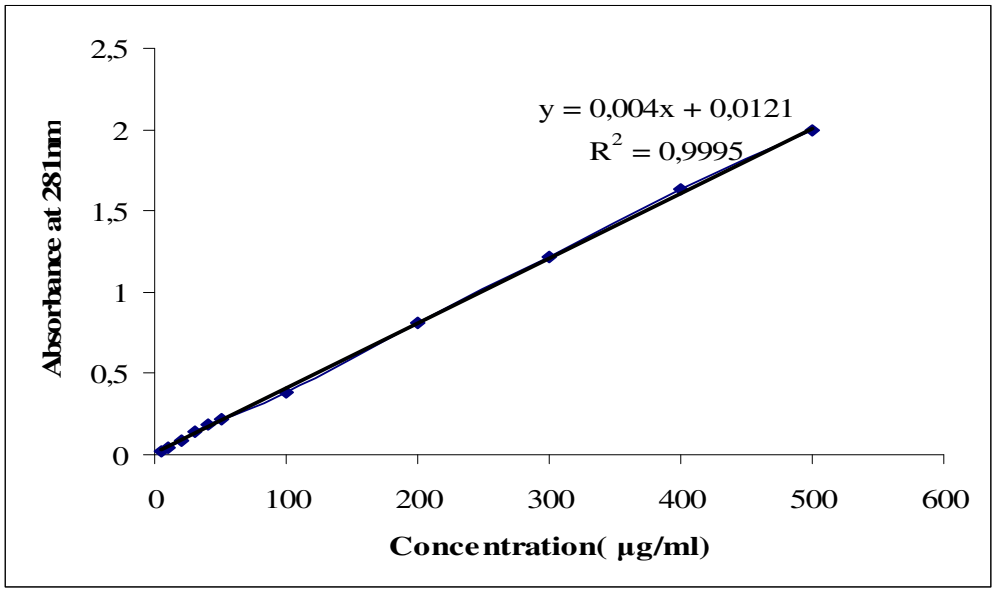

Figure A.2 Calibration curve for Vancomycin (at 281 nm)

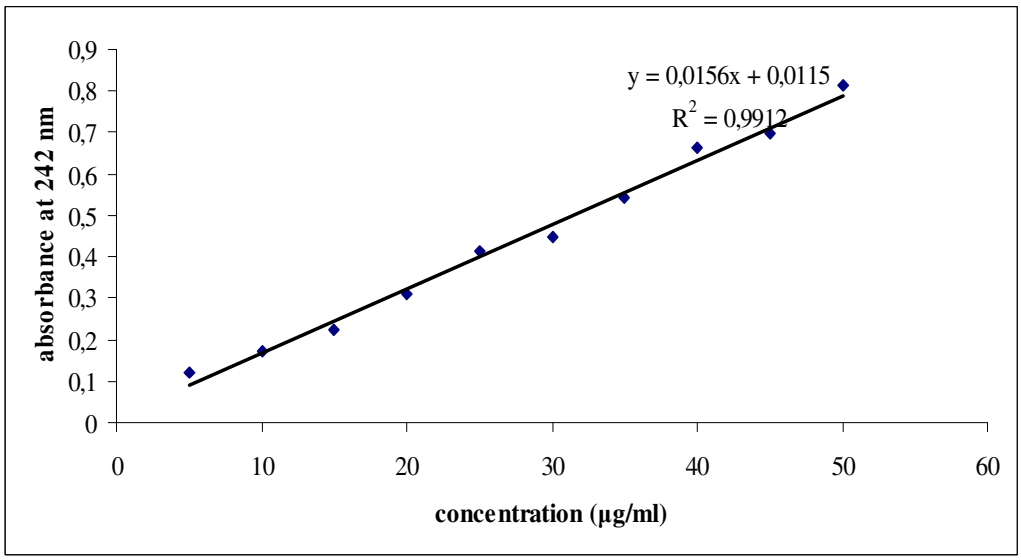

Figure A.3 Calibration curve for dexamethasone (at 242 nm)

APPENDIX B

UV SPECTROSCOPY SAMPLES OF POLYMER/CERAMIC COMPOSITES

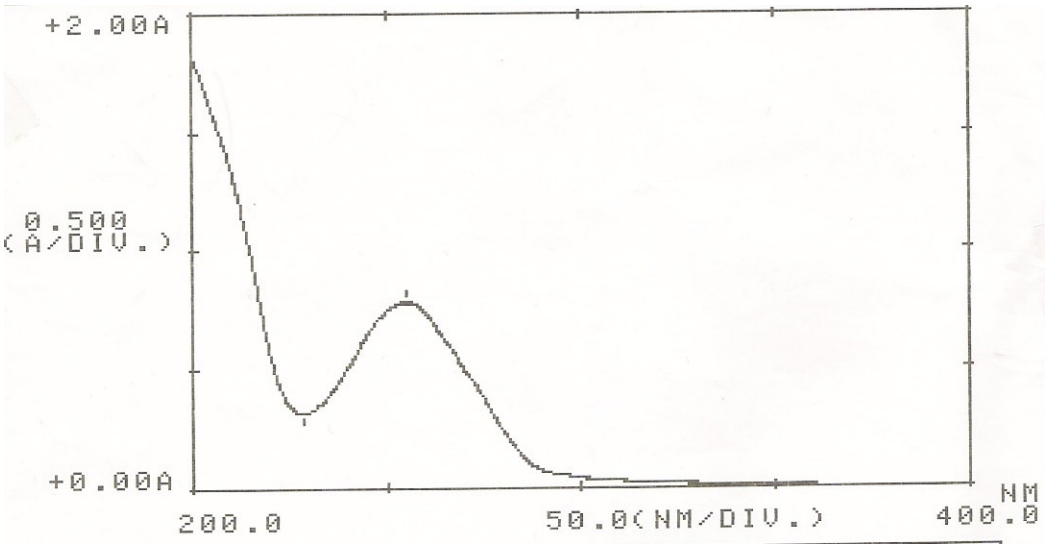

Figure B.1 UV spectroscopy of Gentamicin loaded polymer/TCP composite

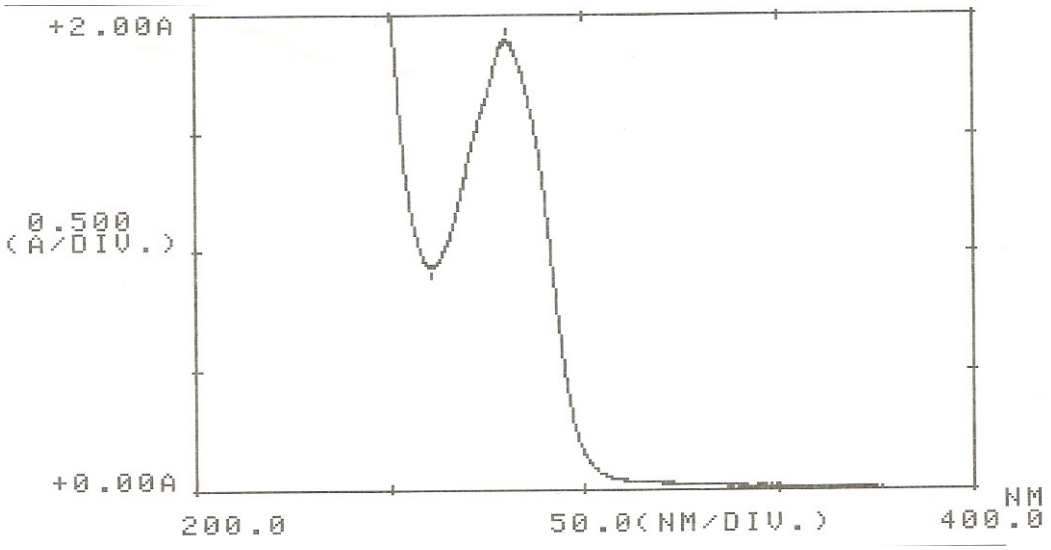

Figure B.2 UV spectroscopy of Vancomycin loaded polymer/TCP composite

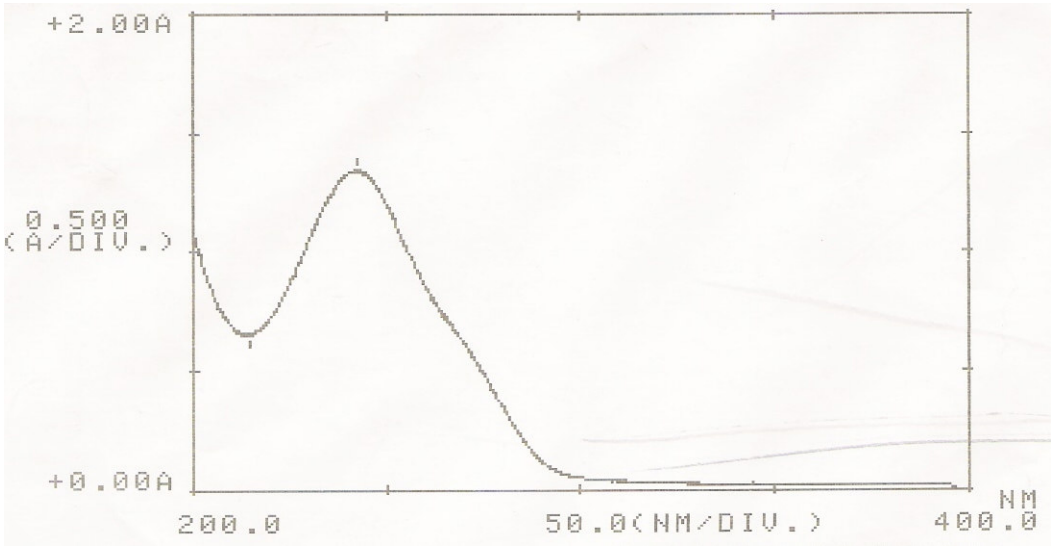

Figure B.3 UV spectroscopy of Dexamethasone loaded polymer/ceramic composite

APPENDIX C

DSC CURVES OF POLYMERS

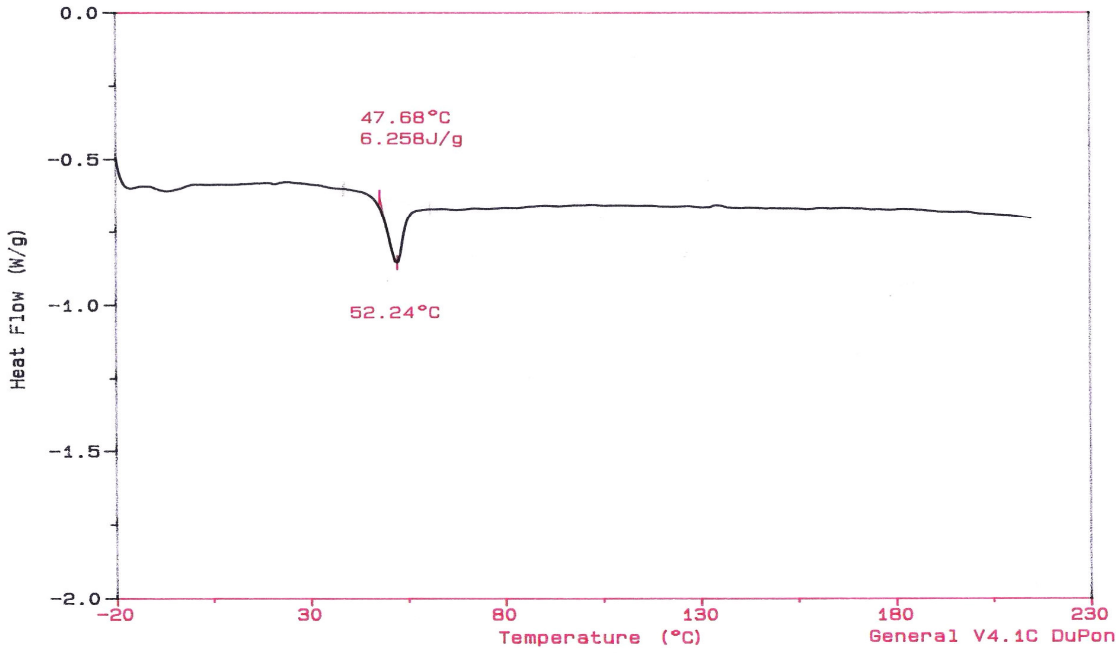

Figure C.1 DSC curve of PLGA

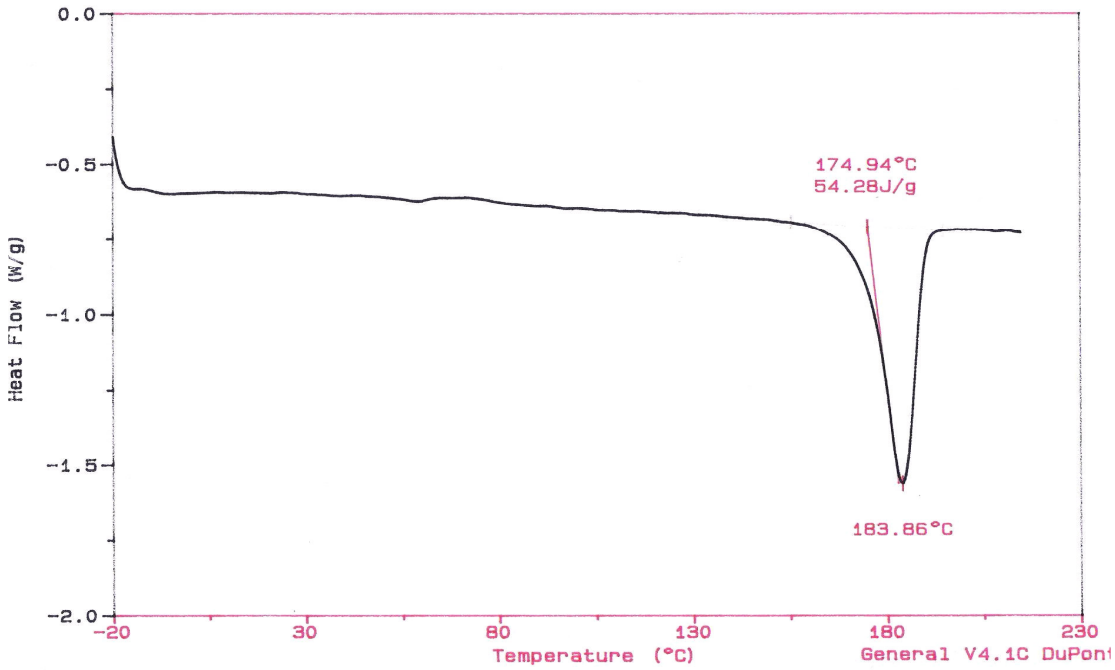

Figure C.2 DSC curve of PLLA

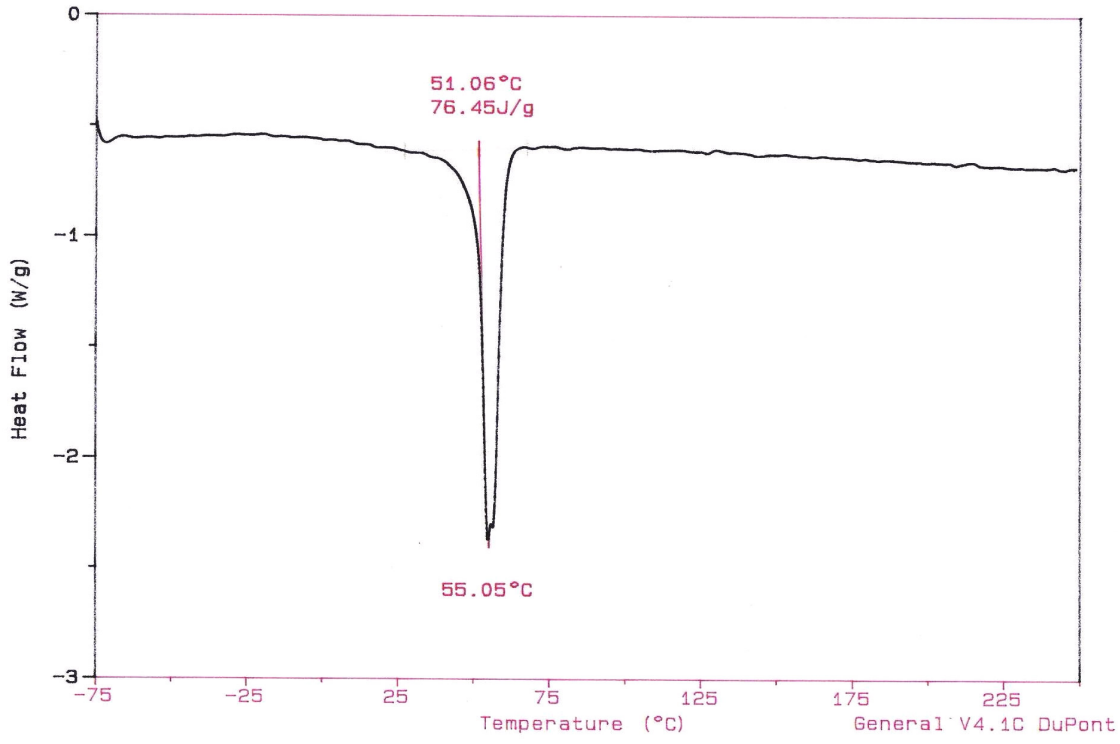

Figure C.3 DSC curve of PCL

APPENDIX D

COMPRESSION TEST RESULTS OF POLYMER COATED CERAMICS

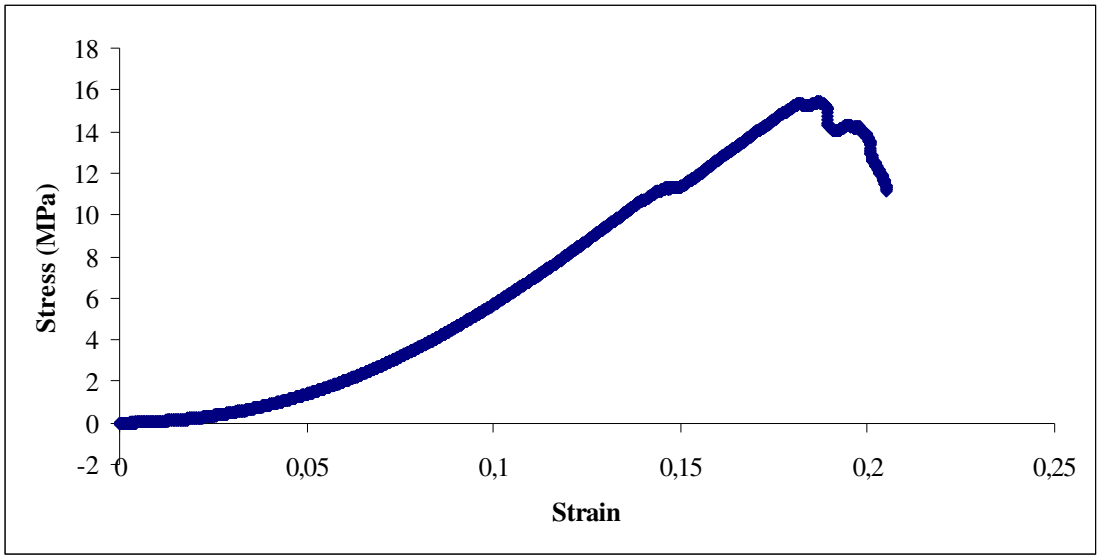

Figure D.1 Compression test results of sample 1 (PLLA coated)

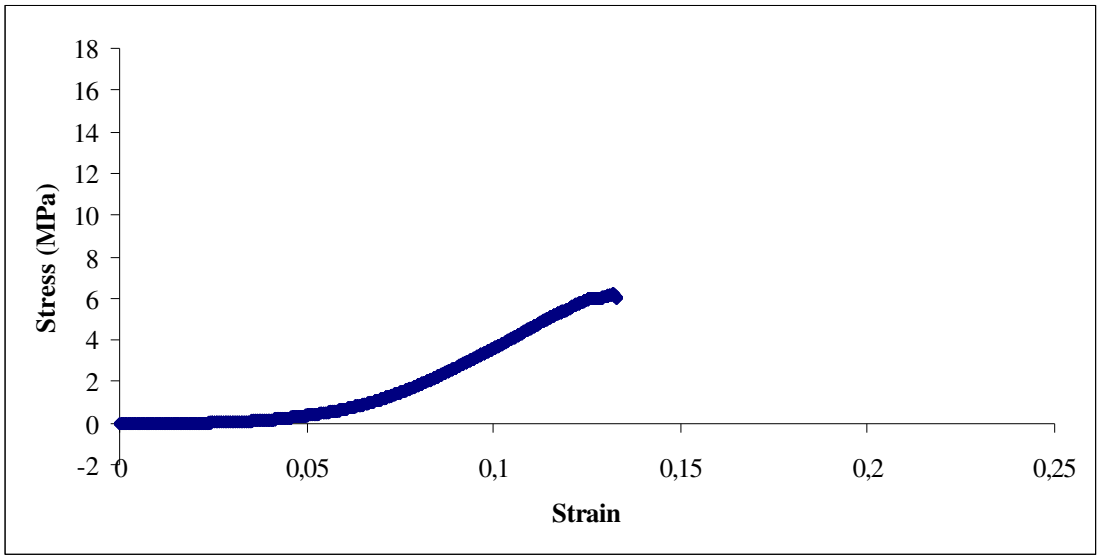

Figure D.2 Compression test results sample 2 (PLLA coated)

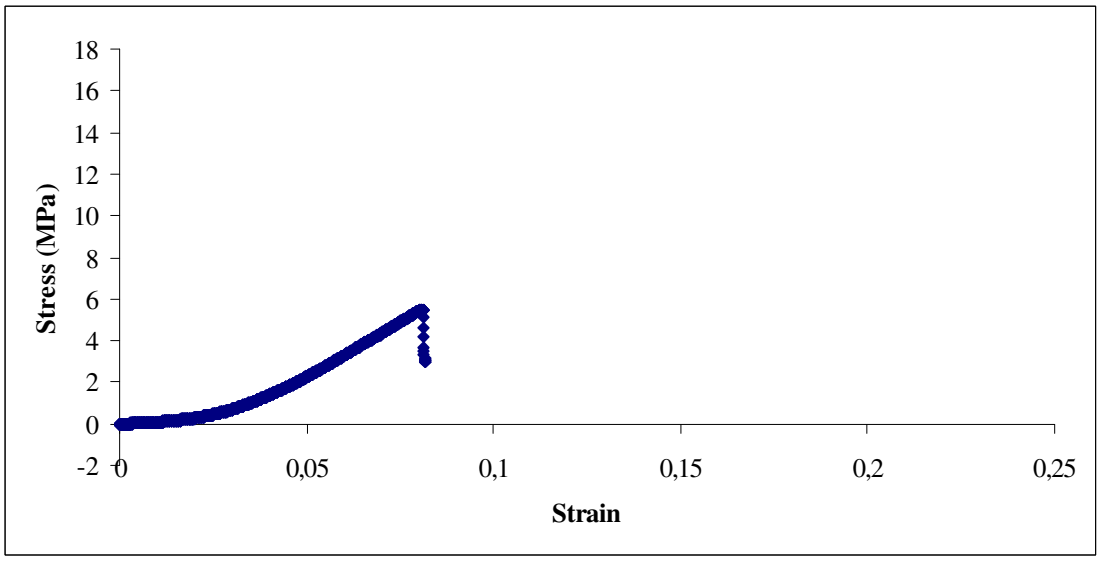

Figure D.3 Compression tesr results of sample 3 (PLLA coated)

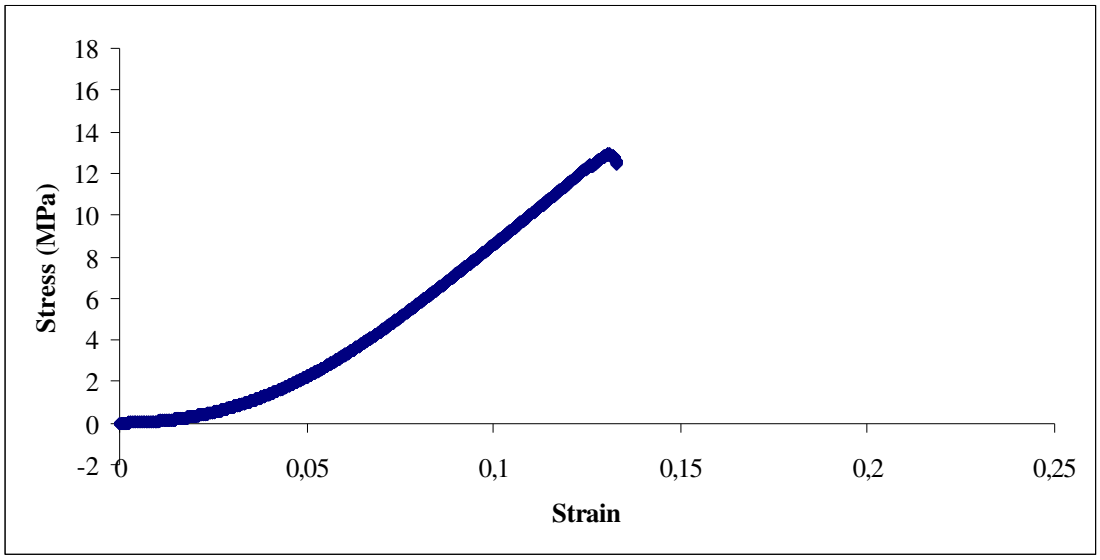

Figure D.4 Compression test results of sample 4 (PLGA coated)

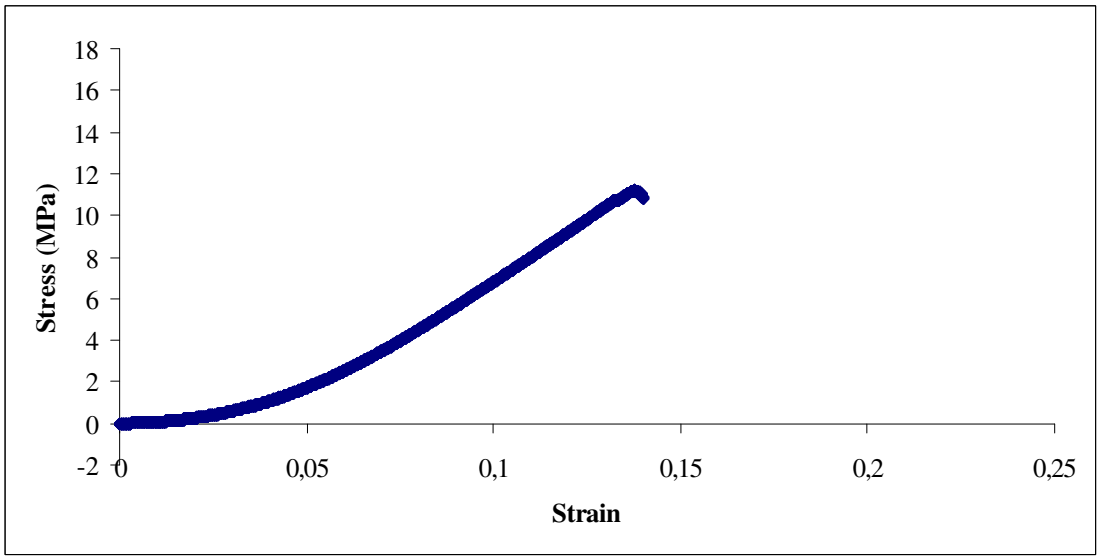

Figure D.5 Compression test results of sample 5 (PLGA coated)

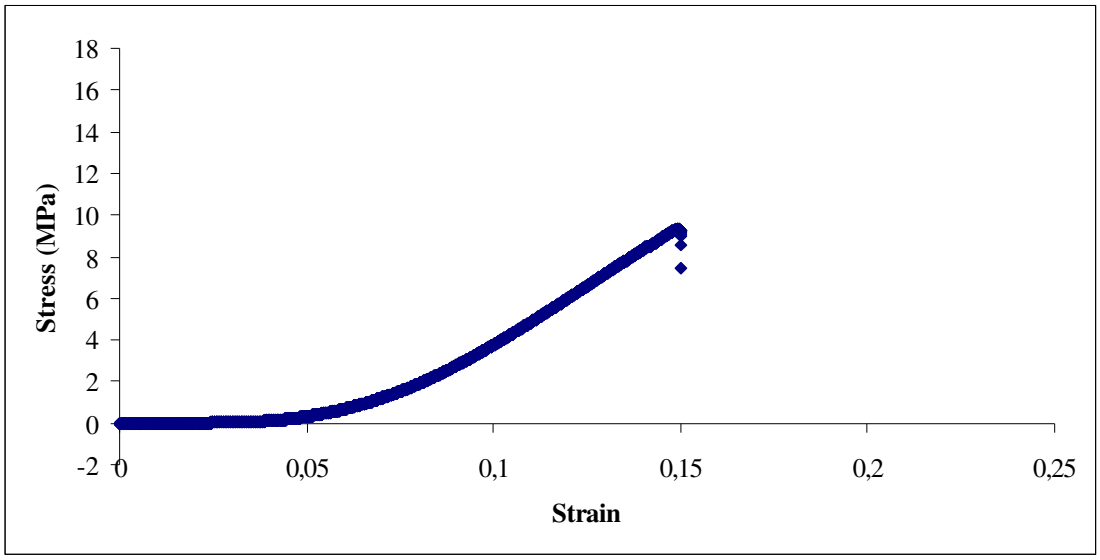

Figure D.6 Compression test results of sample 6 (PLGA coated)