Preparation and Characterization of Hydroxyapatite and Polymer Composite Biomaterials

By

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ABSTRACT

In the thesis, the preparation and characterization of polylactide-Hydroxyapatite(HA) composite films for biomaterial applications have been studied. The effects of number of parameters such as polymer type, HA loading, surface modification and its concentration on the mechanical, thermal microstructural and hydrolytic degradation properties of the composites were investigated. Four different types of polymers, Poly (L-lactide)(PLA_1), 96/4 L-lactide,D-Lactide Copolymer ($PDLA_1$), Poly (L-Lactide)(PLA_2), and 67/23 Poly (L-Lactide-co-D,L-Lactide)($PDLA_2$), have been used. In this study, PolyLactide-HA composite films have been prepared by solvent-casting technique.

The HA powder was synthesized by precipitation technique. Interfacial interactions between HA and polylactide polymer were modified to improve filler compatibility and mechanical properties of the composites by surface treatment of the HA with two different silane coupling agents; 3-aminopropyltriethoxysilane (AMPTES) and 3-mercaptopropyltrimethoxysilane (MPTMS) at three different concentration.

Silane treatment indicated better dispersion of HA particles in the polymer matrix and improvements in the mechanical properties of the composites compared to the untreated HA loaded polylactide composites. Tensile test results showed that the maximum improvement in the mechanical properties of the composites was obtained for the PLA composites containing 1 wt % aminofunctional silane treated HA and 0.5 wt % mercaptopropyltrimethoxy silane treated HA for PDLA composites. Scanning electron microscopy studies also revealed better dispersion of silane treated HA particles in the polymer matrix. Thermal degradation kinetics of the composites was investigated and it was found that addition of HA into polymer matrix decreased the thermal degradation temperature and also slowed down the degradation rate.

In this study, the hydrolytic degradation of poly (L-Lactide)(PLA), poly (L-Lactide-co-D-Lactide) (PDLA) and their hydroxyapatite (HA) loaded composites (10-50-w/w %) were investigated in simulated body fluid (SBF) at 37 ^{0}C and at pH 7.4 by in vitro static testing. Using different techniques, namely weighting to quantify water absorption monitored the hydrolytic degradation and weight loss, scanning electron microscopy (SEM) to observe morphological changes occurred at the surface of the films over time. At the end of the 150 days, only 12.5 wt % and 9.5 wt % of weight

PLA_1 and PLA_2 were lost respectively. Degradation of the copolymers was faster than PLA_1 and PLA_2 and weight loss data of $PDLA_1$ and $PDLA_2$ were found to be nearly same with 17.5 wt % and 17 wt %, respectively. The changes of pH on all polymer were stable at 7.4, because of simulated body fluid indicates buffer solution properties. Degradation rate of PLA and PDLA composites containing 10 wt % HA decreased, and also water absorption of these samples increased. Weight loss decreased approximately from 12 wt % to 5 wt % and water absorption increased from 10 wt % to 13 wt % for PLA composites containing 10 wt % HA. The change of microstructural properties of obtained composites has been determined in simulated body fluid as a function of time. It was found that the surface of polymer composite films was coated with the calcium phosphate layer. This coating was increased with HA loading and ageing time.

ÖZ

Bu çalışmada, polilaktid ve hidroksiapatit kompozit filmlerinin hazırlanması ve karakterizasyonu çalışılmıştır. Bir çok parametrenin; polimer tipi, hidroksisapatit yükü, yüzey modifikasyonu ve konsantrasyonun, mekanik, termal, mikroyapısal ve hidrolitik bozunma özellikleri üzerine etkisi incelenmiştir. Dört farklı tip polimer, Polilaktid (PLA_1), 96/4 L-Laktid,D-Laktid kopolimeri ($PDLA_1$), Polilaktid (PLA_2), ve 67 /23 Poli(L-Laktid- ko-D,L-Laktid) kopolimeri ($PDLA_2$) kullanılmıştır. Bu çalışmada, polilaktid ve hidroksiapatit kompozit filmleri çözücü yöntemi ile hazırlanmıştır.

Hidroksiapatit (HA) tozları kimyasal çöktürme yöntemi ile sentezlenmiştir. Polilaktid-HA kompozitlerinin mekanik özelliklerini ve dolgu maddesi ile polimer arasındaki yüzeyi geliştirmek amacıyla, HA tozlarının yüzeyi iki farklı silan bağlayıcıyla, üç farklı oranda ; 3-aminopropiltrietoksisilan (AMPTES) ve metioksisilan (MPTES) kaplanmıştır.

Silan bağlayıcıları ile modifiye edilmiş HA tozları ile yapılan polilaktid kompozitlerinde işlem görmemiş HA tozları ile yapılan kompozitlere göre, HA tozlarının polimer matriksdeki dağılımı ve mekanik özelliklerinde gelişme gözlenmiştir. Çekme testi sonuçlarında, mekanik özelliklerindeki maksimum iyileşme, PLA kompozitleri için, kütlece % 1 oranında AMPTES silan ve PDLA kompozitleri için ise kütlece 0.5 % oranında MPTES silan ile kaplanmış HA tozlarında, elde edilmiştir. Taramalı elektron mikroskopu ile yapılan çaılşmalar silan ile modifiye edilmiş HA tozlarının polimer matriks içinde daha iyi dağıldığını göstermiştir. Kompozitlerin termal bozunma kinetiği incelenmiştir ve polimer matrikse HA ilavesinin hem termal bozunma sıcaklığını düşürdüğü hem de bozunma hızını yavaşlattığı bulunmuştur.

Bu çalışmada, Polilaktid (PLA_1), 96/4 L-Laktid,D-Laktid kopolimeri ($PDLA_1$) ve onların HA kompozitlerinin (kütlece %10-50 HA yükü içeren) hidrolitik bozunma özelliği yapay vücut sıvısı ortamında 37 ^{0}C de 7.4 pH da statik test yöntemi ile araştırılmıştır. Zaman içerisinde, kompozitlerin su tutma kapasitesi, ağırlık kaybı ve kompozit yüzeyindeki morfolojik değişimlerin gözlenebilmesi için taramalı elektron mikroskopunun kullanılması gibi farklı teknikler kullanılarak hidrolitik bozunma özelliği gözlenmiştir. 150 günün sonunda, saf PLA_1 ve PLA_2 filmlerinde sırasıyla kütlece % 12.5 ve 9.5 kütle kaybı kaydedilmiştir. Kopolimerlerin bozunma hızları homopolimerlerden daha hızlıdır. $PDLA_1$ ve $PDLA_2$ için kütle kaybı değerleri oldukça

yakın değerlerde, sırasıyla kütlece % 17.5 ve 17 olarak bulunmuştur. Bu zaman içerinde tüm polimerlerin bulunduğu ortamın pH değeri stabilitesini yaklaşık olarak 7.4 de korumuştur. Bunun nedeni yapay vücut sıvısının tampon çözelti özelliği göstermesidir. Kütlece % 10 HA içeren PLA ve PDLA kompozitlerinde bozunma hızının yavaşladığı ve bu örneklerde su tutma kapasitesinin arttığı görülmüştür. Kütlece % 10 HA içeren PLA kompozitleri için kütle kaybı değeri %12 den % 5'e düşmüş ve su tutma kapasitesi ise %10 dan %13'e yükselmiştir. Bu kompozitlerin yapay vücut sıvısında zamanla mikro yapısal özelliklerindeki değişim incelenmiştir. Polimer kompozit filmlerin yüzeyinin kalsiyum fosfat ile kaplandığı bulunmuştur. Bu kaplama, HA yükü ve bozunma zamanın artmasını sağlamıştır.

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CHAPTER 1

INTRODUCTION

Thousands of patients throughout the world have the quality of their lives improved with the aid of some kind of implanted device. Diseases and accidents do damage human bodies. The materials from which implanted devices are constructed include metals, polymers, ceramics and an array of composites. As we reached the end of twentieth century, the success of using these materials increased, because of both improved surgical skill and better understanding of how the body interacts with such devices. In general, materials of natural or man-made origin that are used supplement, or replacing living tissues of human body are defined as biomaterials, that have two basic criteria: biocompatibility and biofunctionality. Success interaction between biomaterial and host is achieved when both surface and structural compatibilities are met. The success of a biomaterial in the body also depend on many factors such as surgical technique, degree of trauma imposed during implantation, sterilisation methods, health condition and activities of patients.

Biomedical research and industrial area have been widely developed in the world in last forty years. Figure 1.1 indicates overview of the world market of biomaterials / biodevices area in % [1].

Bone fractures and damages are serious health problems, which result in more than 1.3 million surgical procedures each year in the United States [2]. In the field of orthopaedic surgery, bone substitutes are often required to replace damaged tissue due to disease, trauma or surgery. Current bone substitutes do not exhibit the physiological and mechanical characteristics of the true bone. The development of artificial bone would seem to solve these problems, although it may be cause other problems. Up to the present, various kinds of materials such as ceramics, metals, polymers and their composites have also been used as artificial bone to fill bone defects or replace bony structure. Metallic materials, which are inert, have been beneficial in orthopaedic surgery. For example, metals or metal alloys render them valuable as load bearing implants as well as internal fixation devices in large part for orthopaedic applications as well as dental implants. 316 L stainless steel, titanium alloys, and cobalt alloys when processed suitably contribute high tensile, fatigue and yield strengths; low reactivate and good ductility to the stems of hip implant devices. One complication that can occur from the use of metals in orthopaedic

applications is the phenomenon of stress shielding. Lack of stress causes bone density to decrease as bone tissue resorbs, eventually baring the location and causing complications in the implant/tissue interface. This is known as the "use it or lose it" phenomenon and it applies to more than just bone tissue, including the brain.

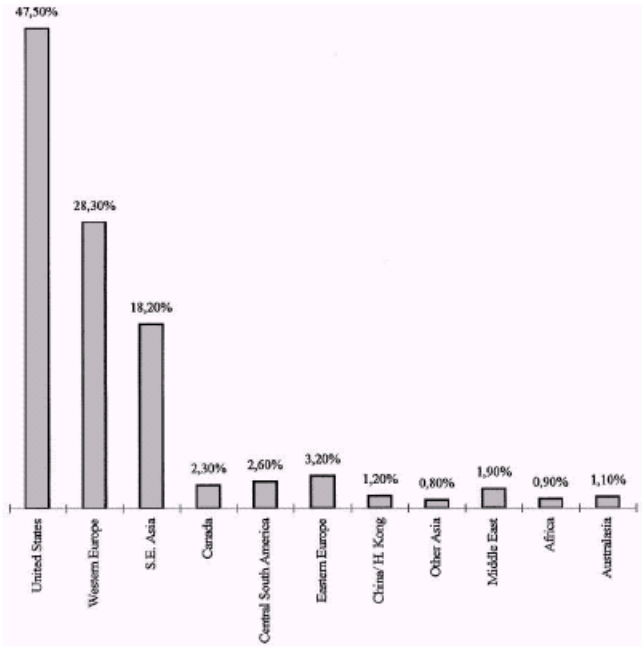


Figure 1. 1 Overview of the World Market of Biomaterials/ Biodevices Area in %

Since the discovery that the bone tissue of mammals contains 69% calcium hydroxyapatite and remaining part is collagen protein, which are composite, great efforts have been made to develop phosphate ceramics as a potential implant material. Besides being biocompatible and non-toxic, this material exhibits unique osteoconductive properties. However, up to now, hydroxyapatite did not have mechanical properties necessary for this type of application. Low toughness of HA limited the application of this material as implants. Similarly, polymeric materials are used in biomedical applications, but mechanical and physical properties of polymers are not sufficient as an implant material and can cause some problems. One of the solutions of the problems is the development of the biocomposite. Nowadays, the developing of mechanical properties of the polymer/ceramic composite as the biomaterials have been investigated. Recently polymer/ceramic composite with polylactide (PLA) as the polymer phase has attracted great attention due to favourable characteristics of polylactide. PLA is a biodegradable polymer. These types of composites are partially

resorbable. The combination of a bioactive ceramics (HA) and bioresorbable polymer (PLA) is expected to result in a promising composite because of its bone–bonding potentials and ability to resorb. The polymeric part is metabolized and ceramic part is assimilated in the body. This composite has possible prospects for application as implant material in restricted load areas [3-7].

Many researchers have examined the preparation and characterization of hydroxyapatite/PLA composites in the last two decades.

Ignjatovic et al [8] have studied the synthesis and properties HA/Poly-L-Lactide composite biomaterials. This study described optimization of the procedure for the production of HA/PLA composites using solvent casting technique. Designing of the material was achieved by cold and hot pressing at pressure ranges of 49-490 MPa and temperature ranges of 20-184 ^{0}C. The material obtained at optimum process parameters, had a density of 99.6%, maximum porosity of 0.4%, maximum compressive strength of 93.2 MPa and Elastic modulus of 2.43 GPa was obtained.

Kasuga et al [9] have examined preparation and mechanical properties of polylactic acid composites containing hydroxyapatite fiber. Solvent casting technique was used for preparation of the composites that were designed by hot pressing. HA fiber was found to be successfully integrated into the PLA matrix phase. The modulus of elasticity of the composite increased with increasing fiber content. The modulus of elasticity values has been obtained in the range of 5- 10 GPa for the 20-60-wt % fiber loaded composites.

Verheyen et al. [6,10] studied also physico-chemical properties of HA/PLA composites that were prepared by mixing HA particles with prior to L-Lactide polymerization. Three different buffer solutions were used; 0.1 M citric acid buffer 0.2 M Gomori's buffer and 0.2 M phosphate buffer saline. HA/ PLA composites with 30 or 50 wt % HA showed linear release of calcium and phosphate ions and L-Lactide when incubated into different buffers within the tested incubation period of 24 weeks at pH of 7.2. The composites were used as drug carriers because of the linear releases of the tested constituents.

Higashi et al. [11] have combined poly(DL-Lactide) with HA in a 1:1 ratio to develop biodegradable artificial bone fillers where high mechanical strength is not required composites in vivo and in vitro. Composites were prepared by mixing before L-Lactide polymerization. Degradation studies were done in a distilled water medium at 37 ^{0}C and pH 6.8. Specimens were analysed according to pH level, calcium

concentration and phosphorous concentration. The addition of hydroxyapatite showed a very slight increase in pH level with time. On the other hand, HA/PLA composite became acidic, pH level reached to 3.4 in a 1 week.

Bleach et al [12] studied effect of filler content on mechanical properties of biphasic calcium phosphate (BCP) and polylactide composite films that were produced by solvent casting technique. The mixtures were stirred for at least 24h before casting onto clean glass slides. Homogeneous distribution of BCP particles in the films were observed but some agglomeration and void formation was seen in the composites containing larger volume fractions of BCP. In the composite all elongation arises from the polymer since the BCP is rigid relative to the PLA.

In spite of the promising results obtained so far in PolyLactide-HA composite system, nobody has studied on the improvement of the interfacial interaction and adhesion between polymer and filler. The interaction and adhesion between ceramic filler and polymer matrix have a significant effect on the properties of the particulate filled reinforced materials, being essential to transfer the load between two phases and thus improve the mechanical properties. To improve the mechanical properties, it is necessary to render the surface of the filler and the polymer compatible, which can be achieved using several types of surface coupling agents. [13] Various methods have already been developed to improve the interfacial interactions between HA and a particular polymeric matrix: silane coupling agents [13,14,15], organic isocynates [14] and polyacids [15] are good examples.

The objective of this study is to prepare and characterize PolyLactide-HA biocomposites for biomedical applications. The effects of number of parameters such as polymer types, HA loading, surface modification of HA and its concentration on the mechanical, thermal, microstructural and biodegradation properties of the biopolymer-bioceramic composites have been studied.

In this thesis, preparation and characterization biopolymer hydroxyapatite composites for biomaterials are outlined. Chapter 2 presents general information on biomedical materials, related terms, bone properties and structure. In chapter 3, the literature review of the polylactide and hydroxyapatite composites is given. Chapter 4 deals with degradation mechanism of polylactide, its copolymer and Polylactide-HA composites. In chapter 5 and 6, the experimental study and the results and discussions are given. Finally, Chapter 7 presents the conclusion of this study with recommendations for future studies.

Chapter 2

BIOMEDICAL MATERIALS, PRODUCTION AND THEIR APPLICATION

2.1 Biomaterials and Their Developments

Biomaterials are the materials of natural or man-made origin that are used to direct, supplement, or replace the functions of living tissues of human body are defined as biomaterials [4]. At the European Society of Biomaterials Consensus was agreed a new simple definition: “Biomaterial-a non-viable material used in a medical device intended to interact with biological system “ A synonymous term is “biomedical materials” [16].

Biomaterials in the form of implants; such as sutures, bone plates, joint replacements, ligaments, vascular grafts, heart values, intraocular lenses, dental implants and medical devices, such as pacemakers, biosensors, artificial hearts, blood tubes are widely used to replace and/or restore the functions of traumatised or degenerated tissues or organs, to assist in healing, to improve function, to correct abnormalities and thus improve the quality of life of the patients. The use of certain materials as constituent of surgical implants is not new. Artificial eyes ears and noses were found on Egyptian mummy [3].

For centuries, when tissues became diseased or damaged a physician had little recourse but to remove the offending part, with obvious limitations. The discovery of antiseptics, penicillin and other antibiotics, chemical treatment of water supplies, improved hygiene, and vaccination all contributed to a major increase in human survivability in developed countries. The revolution in medical care began with the successful replacement of tissue [4].

In the early days all kinds of natural materials such as wood, glue and rubber and manufactured materials such as gold, iron, zinc and glass were used as implants based on trial and error. Some materials were tolerated by the body whereas others were not. Unfortunately, a science in which materials other than these were considered as substances suitable for implantation was not developed until the mid-nineteenth century. Over the last 30 years considerable progress has been made in understanding the interactions between the tissues and the materials. For the next millennium, a working hypothesis should be Long-term survivability of prosthesis will be increased by the use

of biomaterials that enhance the regeneration of natural tissues. Historical development of biomaterials is indicated in Figure 2.1 [4].

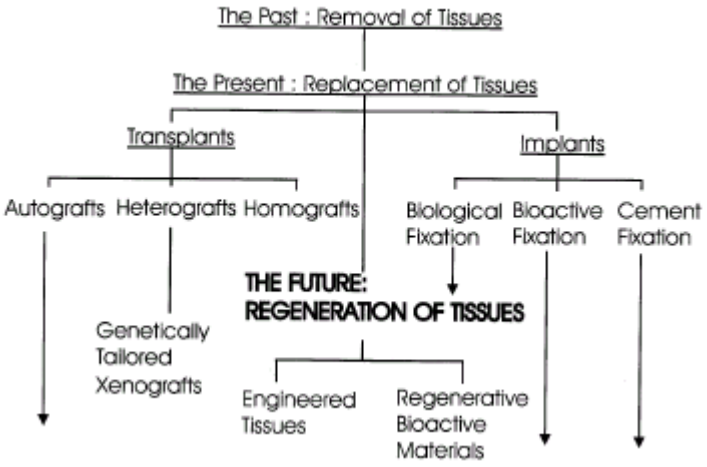


Figure 2.1. Biomaterials Historical Development and Forecast for the Future [4].

2.2 Biocompatibility and Biofunctionality

Two basic criteria; biocompatibility and biofunctionality control the performance of the biomedical material. Biomaterials must fulfil these criteria. Biofunctionality may be considered in relation to a set of properties, which allow a device to perform a function effectively and as long as necessary in or on the body while biocompatibility refers to the ability of device to continue to perform function [17].

Biocompatibility means that the material must not elicit an unresolved inflammatory response nor demonstrate extreme immunogenicity or cytotoxicity. In addition, because it degrades in vivo, this must be true not only for the intact material and any of its unreacted components, but also for the degradation products. The implant should be compatible with tissues in terms of chemical, surface and pharmacological properties. At the simplest level, it could be stated that biocompatibility means a total absence of interaction between material and tissue.

The environment of the body is an aqueous medium, extremely well buffered so that pH is maintained at around 7.4 and it is held constant temperature 37 ^{0}C. The saline solution is an excellent electrolyte and facilitates electrochemical mechanism of

corrosion and hydrolysis. There are also many molecular and cellular species in the tissues, which have the ability to catalize certain chemical reactions or rapidly destroy certain components identified as foreign. Degradation of materials such as metals and polymers takes place in this aggressive environment. The response of the body is a complex issue, which could be dealt with in a number of ways. The important parameters that influence the response of the tissues include the type of tissue that is in contact with the material, the physical and chemical characteristics of the material and the general status of the host. There are different types of responses that are seen with varying distance from the surface [18].

In order to implant a material in the tissue, some surgical intervention is required. This surgical procedure will itself result in a wound healing processes. The tissue response to the material can therefore be seen as a modification of the wound healing process. The tissues will response to damage through a well-defined procedure involving two phases. The first phase, inflammation, is the initial reaction of the body to injury that involves localized change to microvasculature and the cellular composition of the tissue. The second phase is the repair phase in which the tissue attempts to restore the damage. The events that occur at the material-tissue interface could be controlled. The possibility of modifying such materials by using bioactive material interfacial reactions is especially significant and may be controlled. One of the best examples to this possibility is the use of HA and other calcium based materials to actively encourage bone regeneration at implant surfaces. Modification of polymer surfaces to improve their compatibility with blood is another significant example.

Other characteristic of biomaterials is biofunctionality that is important in the function of an implant device made of biomaterials. Mechanical properties must be as similar as possible to those of the tissue that is to be regenerated. As well as providing proper support in the early stages of healing, graded load transfer is needed later in the process for creation of replacement tissue that is identical to the original. While many mechanical properties should be considered for materials to be used in orthopaedics, including those in compression, tension and torsion, compressive properties are the most relevant for replacement of cancellous bone, while tensile properties are important for cortical bone. The functional requirements of the materials include:

Load transmission and stress distribution: The main point of the issue is the particular need for bone replacement or augmentation devices to be “iso-elastic” with

the adjacent bone. Cortical bone has a Young's modulus in the region of 20 GPa as an example. This is not easily achieved in high strength materials.

Articulation: All joint replacements require low friction, low wear, and articulating surfaces to allow movement.

The control of blood and other fluid flow, simple space filling, generation and application of electrical stimuli, transmission of light and sound, the handling of drugs and other substances are some of the other important functional requirements.

Besides these two terms, the bioinert, biointegration, biodegradation, bioresorption and bioactivity are important topics in biomaterials research.

A non-viable materials used for medical purposes and interacting non-adversely with the living system. When no interaction occurs, the material is called " bioinert"[4]. The integration of a biomaterial to bone involves essentially two processes; interlocking with bone tissue and chemical interactions with bone constituents. The direct bonding of orthopedic biomaterials with collagen is rarely considered, however several non-collagenic proteins have been shown to adhere to biomaterial surface. Biodegradation, that is, gradual break down of a material mediated by specific biological and/or biochemical activity. Biodegradation is determined by physical-chemical factors and cell behaviour and is also affected by surgery. Bioresorption, that is, the removal process through cell activity (directly by phagocytosis or indirectly by enzymatic action) and/or through dissolution by continuous ionic diffusion of the material continuous ionic diffusion of constituting the device body, when placed in a biological environment. Bioactivity, that is, the behaviour of a material designed to induce a specific biological activity. The biological activity of most orthopedic biomaterials is related to their ability to promote the formation of a formed layer of carbonate apatite crystals analogous to bone mineral, this layer also associates specific bone proteins and is the starting point of bone reconstruction [16].

2.3. Types of Biomaterials and Applications

The various materials used in biomedical applications may be grouped into metals, ceramics, polymers, and composites and can be classified according to the material type or their applications. A classification based on type of biomaterials is given in Table 2.1 [19].

Table 2.1 Biomedical materials and applications [19].

Material	Application
Nondegradable Synthetics-Commodity Polymers	
Polymides	Suture
Polyesters	Vascular Grafts
Polyformaldehyde	Heart Valve Stents
Polyolefins	Sutures, mesh for hernia repair
Polyvinly choloride	Tubing, blood bags
Nondegradable Synthetics- Value-Added Polymers	
Fluorocarbons	Vascular grafts
Hydrogels	Contact lenses, catheter coatings
Polyolefin elastomers	Tubing, artificial heart bladder
Polyurethanes	Catheters, artificial hearts bladders
Silicones	Soft tissue reconstruction, tubing
Ultrahigh molecular weight polyethylene	Acetabular cup
Biodegradables	
Albumin, cross-linked	Vascular graft coatings, Cell encapsulation
Collagen/gelatin, cross-linked	Soft tissue reconstruction, Vascular graft coatings
Polyamino acids	Controlled release, Cell adhesion peptide
Polyanhydrides	Controlled release
Polycaprolactones	Controlled release, bone plates
Polylactic/glycolic acid copolymers	Sutures, bone plates
Polyhydroxybutyrates	Controlled release, bone plates
Polyorthoesters	Controlled release, bone plates
Biologically Derived Materials	
Bovine carotid artery	Vasular grafts
Bovine ligaments	Ligaments
Bovine pericardium	Pericardial substitute, heart valves
Human umbilical vein	Vascular grafts

Porcine heart valve	Heart valves

Bioderived Macromolecules

Chitosans	Experimental, wound dressing, Controlled release
Collagen	Soft tissue injectables, coatings, Wound dressing
Elastin	Experimental, coatings
Gelatin, cross-linked	Artificial heart bladder coating
Hyaluronic acid	Coating, wound dressing, Surgical non-adhesion

Tissue Adhesives

Cyanoarcylates	Wound closure, microsurgery
Fibrin glue	Vascular graft coating
Molluscan glue	Enhancement of cell adhesion, Vascular stents

Metal and Metallic Alloy

Cobalt chorome molybdenum alloys	Heart valve stents
Nitrol alloys (shape memory alloys)	Orthopedic wire
Stainless steels	Orthopedic wire
Titanium and titanium alloys	Artificial heart housing, Heart Valve Stents

Ceramics, Inorganics and Glasses

Aluminum, calcium, and phosphorous	Degradable bone filler, enhanced bone growth
Bioglass	Bioactive phosphorous calcium glass,orthopedic coating
Glass Ceramic	Encapsulation of implantable, medical electronics
High density alumina	Acetabular cup, ball of hip prosthesis
Hydroxyapatites	Bioactive ceramic, orthopedic coatic bone fillers
Glassy Carbons	Fiber for orthopedic composites
Pyrollytic(low temperature isotropic)carbon	Heart valves, dental implants
Ultra low temperature isotropic carbon	Coatings on heat sensitive polymers

Passive Coatings

Albumin	Thromboresistance
Alkyl chains	Adsorbs albumin for thromboresistance
Fluorocarbons	Reduced drag for catheters, thromboresistance

Hydrogels	Reduced drag for catheters, thromboresistance
Silica-free silicone	Thromboresistance, improved wound healing for soft tissue reconstruction

Bioactive Coatings

Anticoagulants, e.g, heparin and hirudin	Thromboresistance
Bioactive Ceramics and glasses	Bone adhesion and formation; soft tissue adhesion
Cell adhesion peptides	Enhanced cell adhesion, epithelium,
Cell adhesion proteins	Enhanced cell adhesion, epithelium,
Negative Surface Charge	Thromboresistance
Thrombolytics	Thromboresistance

2.3.1. Metals and Alloys

Metals and alloys are successful as biomaterials that have excellent mechanical durability. Metals are known for high strength, ductility and resistance to wear. Shortcomings of many metals include low biocompatibility, corrosion, too high stiffness compared to tissues, high density and release of metal ions that may cause allergical tissue reaction.

Usage of metals as biomaterials include that [20].

1. Stainless steel (nickel chrome molybdenum alloy, 316L): Type 316L stainless steels. It is the first material used to produce an artificial bone. It is cheaper and easily cast into different shapes, but is not necessarily durable.
2. Cobalt chromium alloy: This alloy is less susceptible to corrosion, fatigue and wear the stainless steel, but heavy, any large prostheses need to be hallows.
3. Titanium alloy (Ti-6Al-4 Va): Titanium alloys are lighter than others. For this reason, these alloys are used for prostheses to replace large joints such as the hip and the knee. In addition it has an excellent biocompatibility. This alloy is the most widely used one in the last two-decade.

Use of metallic material is a common practice in traumatology and orthopedic surgery, leading to good clinical results by stable fixation of the bony fragments. But the deficiency of load transmission during the process of bone healing, due to the stress protection by the rigid metallic plates and some possible disadvantages of long lasting metallic fixation described above such as inflammative reactions of the surrounding

tissues or allergic reactions, caused by corrosion products and finally migration of screws, seen occasionally after surgery, require the surgical removal of metallic implants after fracture healing [21].

2.3.2. Ceramics

Ceramic consists of inorganic, non-metallic compounds that exhibit a variety of combinations of ionic and covalent bonding, which are the oldest man-made materials. Since ancient times, ceramic materials have found extended application in products serving human hygiene such as sewage pipe systems and many kinds of sanitary ware [22].

During the last forty years a revolution has occurred in the use of ceramics to improve the quality of human life and in some cases the length of the life. This revolution is the development of specially designed and fabricated ceramics for the repair and reconstruction of diseased, damaged or worn out parts of the body. Ceramics used for this purpose are called as bioceramics. Bioceramics are generally used as implants usually hard tissue applications such as bones and tooth. They can be bioinert (e. g. alumina), resorbable (e. g. tricalcium phosphate), bioactive (e. g. Hydroxyapatite, bioactive glass) or porous for tissue ingrowth (e. g. metals coated with HA).

In restorative dentistry, the classical silicate ceramics have been widely used. Porcelains and multilayered enamels with colours and other carefully adjusted properties allow the reconstruction of tooth crowns, the bridging of gaps above missing teeth and for cosmetically acceptable partial and complete dentures. For implants, however, silicate containing classical ceramics can not be used because of their solubility in body fluids [22].

There are many types of bioceramics available at present for biomedical applications and they fall into two main categories: the alumina and calcium phosphate ceramics.

Alumina (Al_2O_3) Ceramics: Alumina is extracted from bauxite by heating and hydrolysis. Precious stones such as rubies and sapphires are natural single crystals of alumina. However, the usage of alumina in artificial bones and joints and as filling for bony defects left after the excision of lesions.

Calcium Phosphate Ceramics: Those mode of $Ca_{10}(PO_4)_6(OH)_2$ or hydroxyapatite (HA) are the most well-known type of ceramic in this group. The

structural formula of HA is the same of that for the inorganic component of bone, and synthetic HA can be regarded as equivalent to bone itself. Its affinity for bone is far higher than that alumina. Consequently, this material is ideal as filling for bony defects. However, low thougness of HA limited its application in high load bearing applications. In order to overcome the problem of brittleness of HA, a number of attempts have been made to increase durability HA ceramic materials.

The use of bioceramics depends on achieving a stable attachment to the connective tissue and a match of mechanical behaviour of the implant with the tissue to be replaced. Survivability of a bioceramic requires the formation of a stable interface with living host tissue. The mechanism of tissue attachment is directly related to the type of tissue response at the implant interface. The four types of response as summarised in Table 2.2 allow different means of achieving attachment of prostheses to the musculo-skeletal system [23].

Table 2.2 Types of Implant–Tissue Response [23]

If the materials toxic, the surrounding tissue dies.
If the material is non-toxic and biologically inactive (almost inert),
A fibrous tissue of variable thickness forms.
If the material is non-toxic and biologically active(bioactive),
An interfacial bond forms.
If the material is non-toxic and dissolves, the surrounding tissue replaces it.

Some mechanical properties of metallic and ceramic materials are summarised in Table 2.3. Much interest is still focused on those ceramics, which resemble more or less closely the mineral phase of bony tissue like hydroxyapatite.

Although bioceramics are known for their good biocompatibility, corrosion resistance, drawbacks of ceramics include brittleness, low fracture strenght, difficult to fabricate, low mechanical reliability and high density. Polymer and its composites materials provide alternative choice to overcome many shortcomings of homogeneous materials mentioned above such as ceramics or metals. The advantages of the polymer and its composites are highlighted in the following sections.

Table 2.3 Some mechanical properties of metallic and ceramic material [3].

Material	Modulus (GPa)	Tensile Strength (MPa)
Stainless Steel	190	586
Co-Cr Alloy	210	1085
Ti-Alloy	116	965
Amalgam	30	58
Alumina	380	300
Zirconia	220	820
Bioglass	35	42
Hydroxyapatite	95	50

2.3.3. Polymers

Polymers have very long chain molecules, which are formed by covalent bonding along the backbone chain. The long chains are held together either by secondary bonding forces such as Van der walls and hydrogen bonds or primary covalent bonding forces through cross links between chains. Their structural and chemical features control properties of polymers. Foremost among these is the high molecular weight that arises from the repetitive linking of the repeating unit or units to form long-chains, the length of which has a profound effect on the properties of the polymer. The physical properties of polymers can be affected in many ways. In particular, the chemical composition and arrangement of chains will have a great effect on the final properties.

Polymers have many advantages [24]

- are available with a wide variety of mechanical and physical properties
- are relatively readily formed into the desired shape
- are considered inert toward the host tissue
- are available at reasonable cost

Polymer scientists, working closely with those in the device and medical fields, have made enormous advances over the past 30 years in the use of synthetic materials in the body. Synthetic polymeric materials have been widely used in medical disposable supplies, prosthetic materials, dental materials, implant, dressings, extracorporeal devices, encapsulate, polymeric delivery system and orthopedic devices [24]. Although

hundreds of polymers are easily synthesised and could be used as biomaterials, only ten to twenty polymers are mainly used as medical device fabrications.

There are two types of polymers according to degradation in the body. Non-resorbable polymers such as polyethylene, polypropylene, polymethylmetacrylate have been widely used as biomedical application for a long time. Resorbable (or biodegradable) polymers such as polyglycolic acid, polylactic acid, polycarbonate, chitosan have been also used.

The ideal polymer for an application would have the following properties:

- Does not evoke an inflammatory/toxic response, disproportionate to its beneficial effect,
- Is metabolized in the body after fulfilling its purpose leaving no trace,
- Is easily processed into the final product form,
- Has acceptable shelf life, is easily sterilized.

The mechanical properties match the application so that sufficient strength remains until the surrounding tissue has healed. Table 2.4 gives some mechanical properties of polymeric biomaterials. As seen in the Table 2.4, the mechanical properties of the polymers are too weak to meet the mechanical property demands of certain applications e. g. as implants in orthopaedic surgery. Therefore the composite materials provide alternative choice to overcome the disadvantages of homogeneous materials.

Table 2.4 Some mechanical properties of polymeric materials [3].

Material	Modulus (GPa)	Tensile Strength (MPa)
Polyethylene (PE)	0.88	35
Polyurathane (PU)	0.02	35
Polytetrafluoroethylene	0.5	27.5
Polyacetal (PA)	2.1	67
Polymethylmethacrylate (PMMA)	2.55	59
Polyethyleneterephtalate (PET)	2.85	61
Polyetheretherketone	8.3	139
Silicone rubber	0.008	7.6
Polysulfone	2.65	75

2.2.4 Composites

Composite materials are solids that contain two or more distinct constituent materials or phases, on a scale larger than the atomic. [24] There are three important points to be included in the definition of composite material:

- It consists of two or more physically distinct and mechanically separable materials.
- Mixing the separate materials in such a way can make that the dispersion of one material in the other can be done in a controlled way to achieve optimum properties.
- The properties are superior, and possibly unique in some specific respects, to the properties of the individual components.

The properties of composite materials depend very much on their structure. Composites differ from homogeneous materials in that considerable control can be exerted over the larger-scale structure and hence over the desired properties. In particular, the properties of a composite material depend on the shape of the inhomogeneities, the volume fraction occupied by them, and the interface among the constituents.

The methods for producing a composite material by compounding different materials with each other can be classified according to the reinforcing systems by which the dispersive material (reinforcing material) is distributed in the matrix as follows [25]:

(i) Dispersion-strengthened composite materials,

(ii) Particle-reinforced composite materials, and

(iii) Fiber-reinforced composite materials.

Figure 2.2 illustrates the classification of composite materials according to reinforcing system [25].

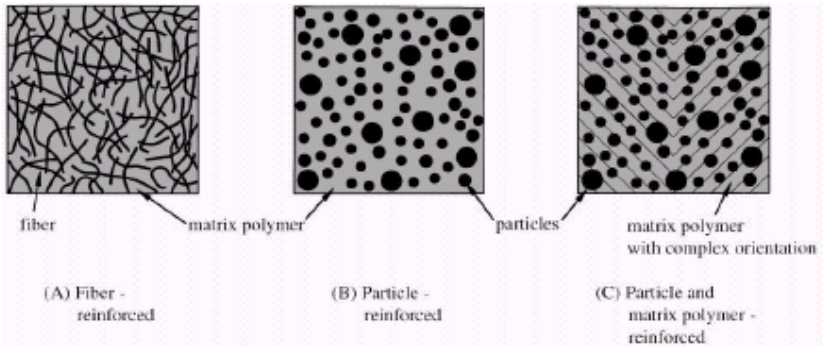


Figure 2.2. Comparison of Morphologies in Composite Materials [25]

Natural biological materials tend to be composites. Bone, dentin, cartilage, skin are natural composites which exhibit hierarchical structures in which particulate, porous and fibrous structural features are seen on different microscales. Composite materials offer a variety of advantages when compared to homogeneous materials. Composite systems can be divided into various classes based on the type of the components: Polymer/ceramic composite, ceramic/metal composites, ceramic/ceramic composite and so on.

Ceramics have been used in biomedical material. Besides being biocompatible and non-toxic, this material exhibits unique osteoconductive properties. However, ceramics did not have mechanical properties necessary for some types of application. They are hard and brittle and not suitable for handling and processing into different forms as structural biomaterials. For example, low toughness of ceramics limited the application of this material as implants. Similarly, polymeric materials are used in biomedical applications, but they have some problems associated with mechanical and physical properties for load bearing applications. Recently, attention has been given to the applications of bioceramic in combination with biopolymers. The idea of using polymers to produce composites with improved handling and retention characteristics and to overcome the problem of brittleness associated with ceramic bone repair implants has been evaluated as an attractive approach. There are also other reasons of choosing polymer/ceramic composite biomaterials:

Absence of corrosion and fatigue failure of metal alloys and release of metal ions such as Ni or Cr which may cause loosening of the implant, patient discomfort and allergic skin reactions and low fracture toughness of ceramic materials which make them a difficult choice for load bearing applications. In addition, composite biomaterials have several other advantages. Metal alloys and ceramics are radio opaque and sometimes they undesirable artifacts in x-ray radiography. However, polymer composite materials can be made radio transparent. Polymer composite materials are fully compatible with modern diagnostic methods such as computed tomography (CT) and magnetic resonance imaging (MRI).

Biopolymer/ bioceramic composites are widely used in biomedical application. Composite systems comprised of inorganic (bioceramic) fibers or particles, and organic polymers can be divided into three classes as follow [25].

- both non-bioresorbable reinforcing and matrix components such as CaO - $P_2 O_5$ – SiO_2 – Al_2O_3 (CPSA) glass fibers with polymethylmethacrylate (PMMA),

carbon fibers (CF) with epoxy resingraphite fibers with polyetheretherketone (PEEK) or polyester

- bioactive particles for reinforcement with a non-bioresorbable polymer as matrix such as sintered hydroxyapatite (s-HA) particles with high density polyethylene (HDPE), bioglass particles with polysulphone (PS)
- bioactive but not totally resorbable reinforcing components with a bioresorbable matrix. Such as; Calcium metaphosphate (CMP) fibers with Poly D,L- Lactide, HA particles with polyhydroxybutylate (PHB), HA Particles with poly L Lactide.

Polymer composite biomaterials are particularly attractive for their suitable manufacturing process and biological and mechanical properties comparable to those of the host tissues. Various applications of different polymer composite biomaterials are summarised in Figure 2.3 [3].

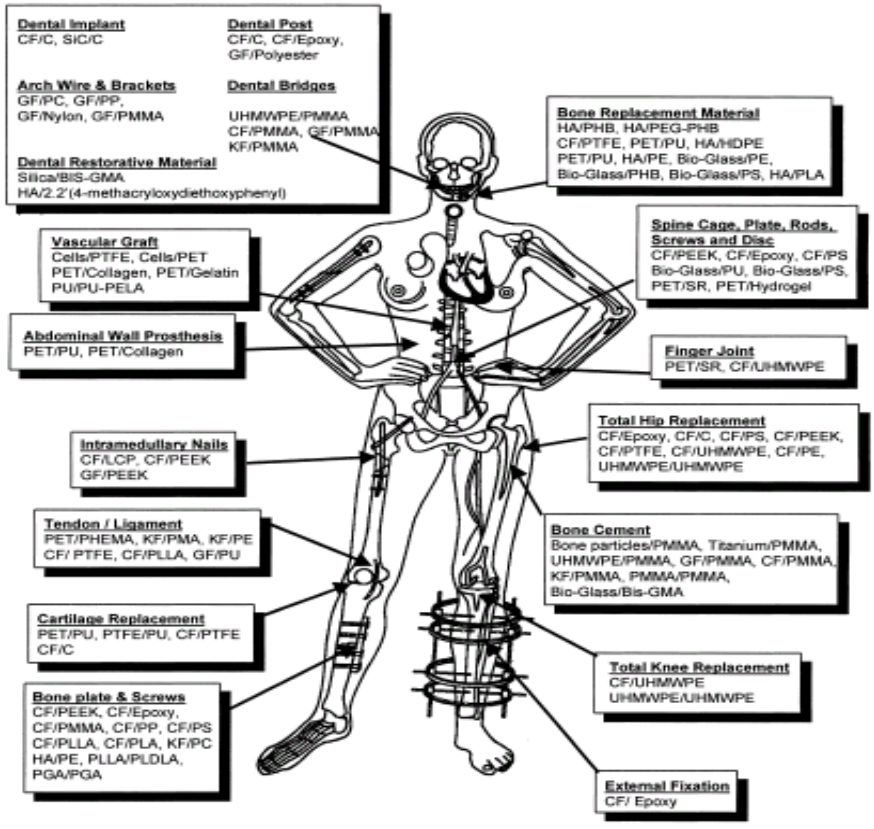


Figure 2.3. Various Applications of Different Polymer Composites [3].

Ramakrishna and co-workers [3] classify composite materials into subgroups as shown in figure 2.4. A composite material made of avital (non-living) matrix and reinforcement phases, is called 'avital/avital composite'. Alternatively, a composite material comprising of vital (living) and avital materials is called 'vital/avital composite'. The avital/avital composites are analogous to polymer composites known to engineers. The avital/ avital composites are further divided into non-resorbable, partially resorbable and fully resorbable composite biomaterials.

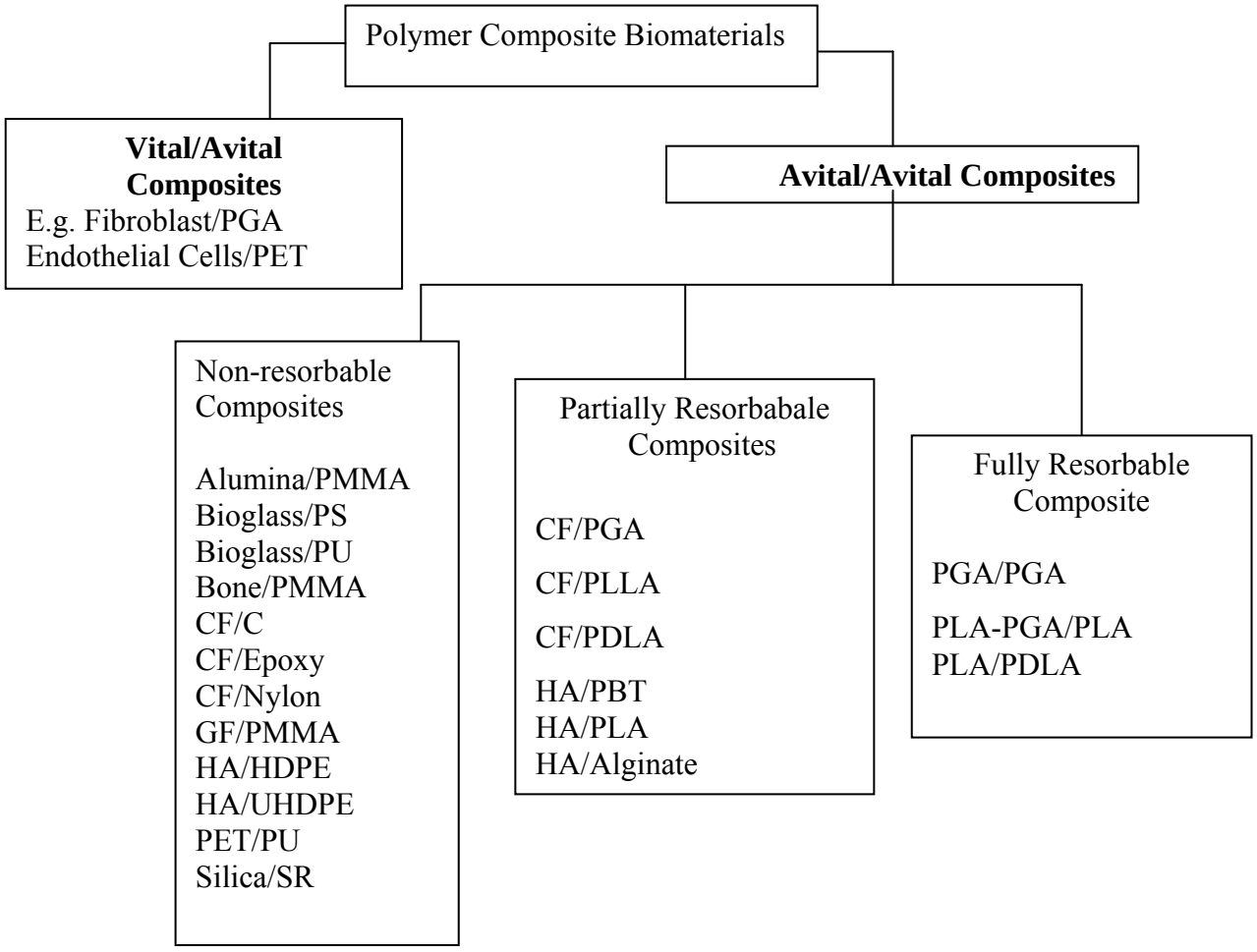


Figure 2.4. Classification of Man-made Polymer Composite Biomaterials.

One of the most interesting approaches to improve the reliability and to decrease the stiffness of the HAp biomaterials is the fabrication of HAp/polymer composites.

Bonfield and co-workers [26,27,28] have developed HAp/high density polyethylene (HDPE) composites since the early 1980s as an analogue for bone replacement. With increasing HAp content, both Young's modulus and bioactivity of the composites increase, while ductility decreases. The HAp/polyethylene composites

exhibit brittle/ductile transition at a HAp volume content of 40-45%. Their Young's modulus is in the range of 1-8 GPa, which is quite close to the Young's modulus of bone. As compared to the cortical bone, the composites have superior fracture toughness for HAp concentrations lower than 40% and similar fracture toughness in the 45-50% range. Unfortunately, The HAp/polyethylene composites are not biodegradable. Moreover, the presence of bioinert polyethylene decreases the ability to bond to the bone.

Other studies of polymer composites with ceramic fillers have also been investigated over last two decades. Mikos et al., reported the results of a poly(propylene fumarate) (PPF) biodegradable bone cement that can be combined with a leachable component and injected into osseous defects [29]. The injectable nature of the material allows it to fill irregular osseous defects, and the leachable component allows room for bone ingrowth. This particular material also has potential as a drug delivery system. Composite systems can be formed with tricalcium phosphate (TCP) to improve mechanical integrity [29]. Similarly, Bennett et al., showed that a poly-dioxanone-*co*-glycolide based composite reinforced with HA or TCP can be used as an injectable or moldable putty [30]. Greish and co workers studied the effect of bioactive glass on the mechanical properties of Hydroxyapatite-Ca polyacrylate composites. They found that the mechanical properties of these composites were enhanced by the addition of bioactive glass [31].

Jansen et al. [32] prepared a sheet of poly (ethyleneglycolterephtalate) / poly(butyleneterephthalate) coated with HA powder and found good properties for tissue regenaration membrane. However, these kinds of composite were not easily cast: for this reason, their applications were limited.

Bleach et al [12] studied effect of filler content on mechanical properties of biphasic calcium phosphate (BCP) and polylactide composite films that were produced by solvent casting technique using chloroform as a solvent. The mixtures were stirred for at least 24h before casting onto clean glass slides. Homogeneous distribution of BCP particles in the films were observed but some agglomeration and void formation was seen in the composites containing larger volume fractions of BCP. In the composite all elongation arises from the polymer since the BCP is rigid relative to the PLA.

Shikinami and co-workers [25] have examined ultra high strength resorbable implants that were made from bioactive ceramic particles/PLA composites. The small granules uniformly distributing HA microparticles within a PLA matrix which were

obtained by precipitating polymer solution, dropping ethanol into dichloromethane-polymer solution, were extruded to make a thick billet. Thereafter, the billet was formed into a thin billet by compression molding at 103 ^{0}C. The composites were filled with 20,30,40 and 50 weight fractions of HA particles. Effects of u-HA contents on the mechanical properties of u-HA / PLA were investigated in their study. The gradual decrease in tensile strength was inversely proportional to the fraction of HA particles, but a high enough strength of over 100 MPa was maintained and this was sufficient for practical use as bone fixation devices. The high mechanical strength, the high bending, shear, and impact strengths and the high bending modulus of these composites depend on the mechanical interlocking between the HA particles and PLA matrix.

2.4. Structure and Properties of Bone

There are many types of bone fractures depending on the crack size, orientation, morphology, and location. Bone fractures are treated anatomic reduction in different ways and they may be grouped in to two types namely external fixation and internal fixation. The external fixation does not require opening the fracture site whereas the internal fixation requires opening the fracture site. In the internal fixation approach the bone fragments are held together by different ways using implants such as wires, pins, screws, plates and intramedullary nails [3].

The physical, chemical and mechanical properties of bone are important parameters for successful production of artificial bone replacement implants and bone fixation devices. Hard tissue, are often used as synonyms for bone when describing the structure and properties of bone.

2.4.1. Composition of Bone

Bone is anisotropic, heterogeneous, inhomogeneous, nonlinear, thermorheologically complex, viscoelastic material. It's function is "load carrying". Bone is one of the most interesting materials known in terms of structure property relationship.

Bone in human and other mammal bodies is generally classified into two types:

1) Cortical bone, also known as compact bone
2) Trabecular bone, also known as cancellous or spongy bone.

These two types are classified as on the basis of porosity and the unit microstructure. Cortical bone is much denser with porosity ranging between 5% and 10%. Cortical bone primarly is found in the shaft of long bones and forms the outer shell around cancellous bone at the end of joints and the vertebrae. Figure 2.5 shows hierarchical level of structure in human femur [33].

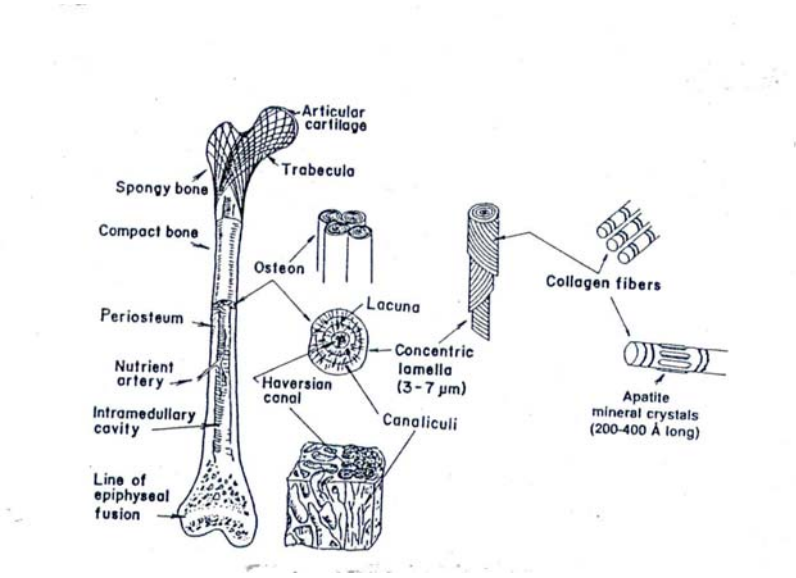


Figure 2.5 Organisation of Typical Bone [33].

The composition of bone depends on a large number of factors, the species, which bone, the location from which the sample is taken, and the age, sex, and type of bone tissue, e.g., cancellous, cortical. But a rough estimate for overall composition by volume is two-third hydroxyapatite, nearly one-third collagen and other organic components, and water. It is indicated that bone is a composite material. Some data in the literature for the composition of adult human bone is given in Table 2.5.

Table 2.5 Composition of the human bone [33].

Components	**Amount(wt%)**
Mineral (apatite)	69%
Organic Matrix Collagen Others	22% 90-96% of organic matrix 4-10% of organic matrix
Water	9%

The process of bone mineralization is complex and for the most part still unknown. The large quantities of cations and complex anion groups that are found under chemical analysis of bone, are Ca^{+2}, PO_4^{-3}, CO_3^{-2}. Other ions are present in smaller quantities such as: Mg^{+2}, Fe^{2+}, F^-, Cl^- and also present are very small amounts of Na^+ and K^+ ascorbic acid, citric acid, polysaccharides in the two-third inorganic part.

Calcium and phosphate ions lead to formation of salts, primarily calciumtriphosphate and hydroxyapatite, is in a crystalline form, which constitutes the framework providing the principal mechanical characteristics of bone. The apatite crystals are formed as needles, 20-40 nm in length by 1.5-3 nm in thickness, in the collagen matrix. The mineral phase present in the bone is not a completely discrete aggregation of calcium phosphate mineral crystals. It is made up of a continous phase.

Organic part of the bone contains 90-95 % of collagen protein fibres that is chemically characterised by a high content of aromatic amino acid. Collagen fibres are surrounded in bone by a supporting substance referred to as ‘cement’. This organic cement, along which bone mineralises, fills the spaces between fibrils [7].

2.4.2. Mechanical Properties of Bone

It is important to examine some characteristic properties of bone not normally taken into consideration. The mechanical properties of bone depend on the humidity, rate of loading and direction of the applied load. During daily activities bones are subjected to a stress of approximation 4 MPa. The mean load on a hip joint is up to 3 times body weight and peak loading during jumping can be as high as 10 times body weight. More importantly, these stresses are repetitive and fluctuating depending on the activities such as standing, sitting, jogging, and climbing. The effect of rate of loading on the bone is shown in Figure 2.6 [33].

Organic components of bone (mainly collagen) themselves would behave as a compliant material with high thougness, low modulus, and other property characteristic for polymers. Inorganic components, i.e., HA crystals, provide appropriate stiffness to the bone. As a ceramic-organic composite, bone exhibits high toughness and relatively high modulus. High toughness is related not only to the presence of collagen, but also to the complicated fibrous microstructure [23].

Figure 2.6 represents a linear elastic region, followed by a flat plastic region at about 0.8% strain. Failure occurs at strains up to 3 %. It is necessary to mention that

bone is a tough material at low strain rates. The slope of the stress strain curve, i.e., the stiffness of the bone, increases with increasing mineral content. Bone exhibits excellent toughness (at low strain rates) mostly due to its hierarchical structure, which stops cracks after little propagation. The main toughening mechanisms seem to be microcracks, which appear in the plastic region of the stress-strain curve [23]. As can be seen in the Figure 2.6. The Young Modulus, ultimate compressive and yield strength increase with increased rate of loading. However, failure strain and fracture toughness of the bone reach a maximum and then decrease. This implies that there is a critical rate of loading. The mechanical properties of human compact bone are summarised in the Table 2.6 [7].

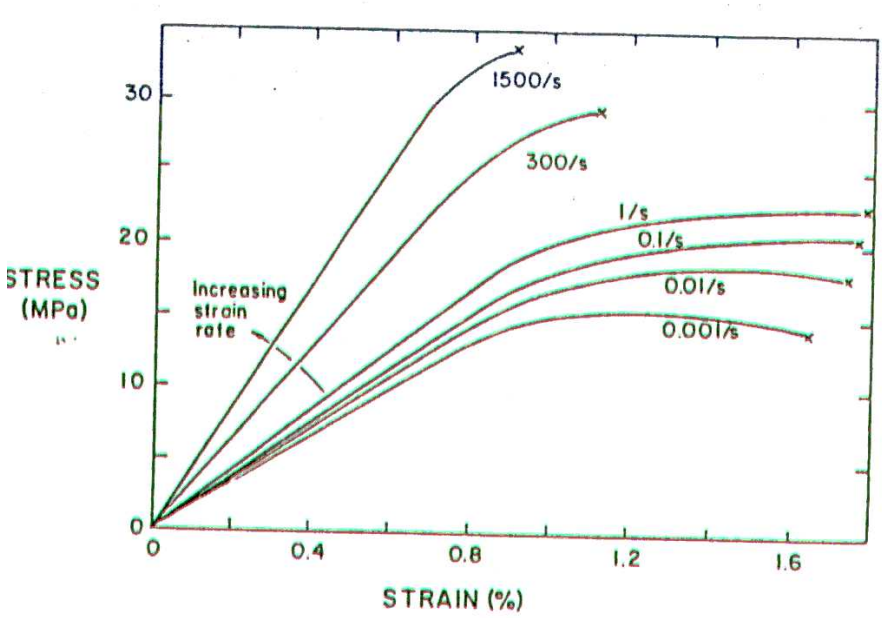


Figure 2.6 Stress as a Function of Strain and Strain Rate for Human Compact Bone.

Table 2.6 Mechanical Properties of a Compact Human Bone.

Properties	Test direction related to bone axis	
	Parallel	Normal
Tensile Strength	124-174 MPa	49 MPa
Compressive Strength	170-193 MPa	133 MPa
Bending Strength	160 MPa	
Shear Strength	54 MPa	
Young's Modulus	17-18.9 GPa	11.5 GPa
Work of fracture	6000 (low strain rate) 98 (high strain rate)	
Ultimate Tensile Strain	0.014 - 0.031	0.007
Ultimate Compressive Strain	0.0185-0.026	0.028
Yield tensile strain	0.007	0.004
Yield compressive strain	0.010	0.011

CHAPTER 3

POLYLACTIDE HYDROXYAPATITE COMPOSITES

Bioabsorbable devices which are new in biomedical applications for internal fixation of fractures, osteotomies, ligament and meniscal injuries, support the fixation, decompose gradually, and the stresses are transferred to the healing tissue during healing. Bioabsorbable devices do not require a removal operation so that decreasing the total cost of treatment when compared to inert material. These devices have been clinical use only for 15 year. Applications of bioabsorbable devices will continue to increase in orthopedic surgery. These materials will have a more significant part in modern surgical technique.

Ceramic/polymer composites have some advantages over pure ceramics and polymers. Recently ceramic/polymer composite with polylactide(PLA) as the polymer phase has attracted great attention due to favourable characteristics of polylactide. PLA is a biodegradable polymer. These types of composites are partially resorbable. The combination of a bioactive ceramics (HA) and bioresorbable polymer (PLA) is expected to result in a promising composite because of its bone –bonding potentials and ability to resorb. The polymeric part is metabolized and ceramic part is assimilated in the body. This composite has possible prospects for application as implant material in restricted load areas. In this study, Poly (L- Lactide) and Poly (L-Lactide-DL–Lactide) copolymer were used as the matrix material and hydroxyapatite was used as the filler.

3.1.Hydroxyapatite

Calcium Phosphate Ceramics (CPC) are ceramic materials with varying calcium to phosphate ratios. They have considerable potential as bone substitute materials. These ceramics have been used in medicine and dentistry for nearly 30 years. Different phases of CPC's are used depending upon whether resorbable or bioactive material is desired. CPC's with Ca/P ratio in the 1.5-1.67 range are the most interesting and useful materials. Ca/P ratio of Tricalcium phosphate (TCP) is 1.5. Ca/P ratio of Calcium hydroxyapatite (HA) is 1.67. These materials have been widely investigated [34].

Hydroxyapatite is a major component of the inorganic compartment of the bone. Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (Ca/P=1.67) and tricalcium phospahate $Ca_3(PO_4)_2$ (Ca/P= 1.5) are widely used in biomedical application for load bearing implants and the dental industry due to excellent biocompatibility and bioactivity. Unfortunately, mechanical properties of pure HA ceramics are poor. Most members of this group are characterised by a high Young's modulus, very low elasticity, and a hard, brittle surface [4].

Hydroxyapatite (HA), specifically, calcium hydroxyapatite, has a definite crystallographic structure, belongs to the hexagonal system. An ideal weight percentage of HA is 39.9 % Ca, 18.5 % P, 3.38 % OH. [18] Schematic crystal structure of HA is showed in Figure 3.1 [34]. The ideal Ca/P ratio of HA is 5:3 and the calculated density 3.219 g/cm^3. The differences in structure, chemistry and composition of apatite come from the differences in material preparation techniques, time and temperature and medium. Powder processing, forming, and densification of HA have been understood quite well, allowing control of chemical composition and microstructures of both dense and porous HA Ceramics.

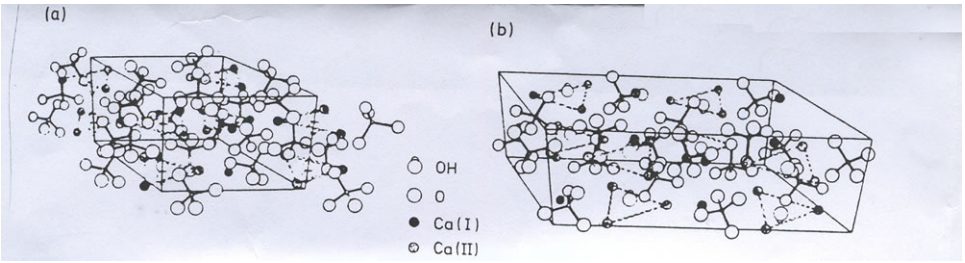


Figure 3.1. Schematic of Crystal Structure of Hydroxyapatite (a) hexagonal, (b) monoclinic.

3.1.1.Preparation of HA Powder

Many techniques have been used for preparation of HA powders as reviewed in literature. There are two main methods for preparation of HA which are wet methods and solid-state reaction methods. In the case of HA production, the wet methods can be divided into three groups: precipitation, hydrothermal technique and hydrolysis of other calcium phosphates. Depending upon the technique, materials with various morphology, stoichiometry, and level of crystallinity can be obtained. Moreover, the properties of

HA powders depend on the preparation technique conditions that are starting materials, pH, temperature, aging time, and calcination conditions.

Precipitation methods are commonly used ones in production of HA powder [7]. Rathje's method consisted of drop wise addition of phosphoric acid, $H_3(PO_4)_2$, to suspension of calcium hydroxide, $Ca(OH)_2$ in water under stirring at room temperature. By this method, powders with different Ca/P ratio can be produced by changing the conditions (such as weight of solution and concentration)

$$10\ Ca(OH)_2 + 3\ H_3(PO_4)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18\ H_2O$$

This method is modified by addition of ammonium hydroxide, NH_4OH, to keep the pH of the reaction very alkaline at about 11 to insure the formation of HA, to obtain the powder with Ca/P ratios in the range of 1.6-1.73 [7].

HA powders can also be prepared by using other starting materials through precipitation. A $(NH_4)_2\ HPO_4$ aqueous solution 200 ml (11.4 wt %) was slowly dropped into a stirred, 400 ml $Ca(NO_3)_2$ aqueous solution (16.8 wt %). The pH for both solutions was 10-12, adjusted with ammonium hydroxide, NH_4OH and reaction was occurred at room temperature. After precipitation, slurry was put into an autoclave and hydrothermal treated at 140 ^{0}C and 0.3 MPa for 5 h. Finally, obtained slurry was dried. The Ca/P ratio of resultant HA powder were determined as 1.61 by an atomic absorption spectrophotometer. HA powders were rod-like particles with length between 40-80 nm and the width between 20-40 nm. [35].

Ignjatovic and co-workers [8] investigated the synthesis of HA/Poly-L-Lactide composite biomaterials. In their study, HA powder was prepared by precipitation technique. A solution of $(NH_4)_2HPO_4$ was added to solution of $Ca(NO_3)_2$ for 180 min and mixed by magnetic stirrer with rate of 100 rpm/min. The pH of the solution was held above 10 by adding ammonia due to stability of HA. The obtained suspension was heated to boiling, and the precipitate, being held in the starting solution for 18 h, was separated by filtration using Bihner's funnel. After filtering, the resultant filter cake was washed with warm distilled water until the ammonia removed. After the obtained filter cake was dried in vacuum at room temperature, dried granules were calcined at 1100 ^{0}C for 6 h. The size of these granules was obtained in the range of 0.3-0.13 mm. The precipitation of HA powder was obtained according to the reaction given below;

$$5\ Ca(NO_3)_2 + 3\ (NH_4)_3PO_4 + NH_4OH \xrightarrow{pH>10} Ca_5(PO_4)_3PO_4 + 10\ NH_4NO_3$$

In another study, Furukawa et al. [25,36] studied biodegradation behaviour of ultra-high-strength HA/ Poly(L-lactide) composite rods for internal fixation of bone fractures. Two types of synthetic HA were used as reinforcing particles in this study; calcined HA (c-HA) and uncalcined HA (u-HA). U-HA was produced by hydrolysis of pure calcium hydrogen phosphate and calcium carbonate by heating their aqueous solution at 90 ^{0}C. They were matured for 5 h and fully dried for 10 h after filtering. The chemical reaction is given below

$$6\ CaHPO_4 + 4\ CaCO_3 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 4\ CO_2 + 2\ H_2O$$

The molar ratio of Ca/P by chemical analysis was 1.69 and very close to that of pure HA, which is 1.67. The particle size was limited within a range of 0.3-20 μm.

There are various preparation techniques used the HA powder production such as flux method, electrocrystallization, spray-pyrolysis, freeze- drying, microwave irradiation, emulsion processing.

3.1.2. Surface Modification of Hydroxyapatite

Many kinds of fillers used for incorporation into polymers are treated with various surface modifiers in order to modify the characteristics of the filler matrix interface. Surface modification of the filler prevents the agglomerations of the particles by decreasing the strength of the interactions between particles. This way lead to improvements in the dispersion of the fillers during preparation and the mechanical properties of filled polymer composites [37].

Since modification of the filler changes the surface free energy of the filler, it effects on both particle-particle and particle-matrix interactions. Surface treatment causes the filler surface to become hydrophobic and moisture adsorption of particulate filled polymer composites is significantly reduced during storage inorganic fillers mostly contain OH group on the surface owing to reactions with atmospheric water. The hydrophilic nature of inorganic filler makes it's easy for atmospheric water to

accumulate at interface by diffusing through matrix. As a result, interfacial bonds between polymers-filler are either weak or deterioratable on ageing, when composites exposed to humid environment. For this reason, the most of the time, the fillers are coated with coupling agents to form hydrophobic nature. A wide range of coupling agents has been used in particulate filled polymer composite [38].

Coupling agents are biofunctional molecules containing organic and inorganic ends improve mechanical properties and chemical resistance of composites by enhancing across polymer-filler interface. The most widely used coupling agents are silane and titanate based compounds whose chemical composition allows them to react with both the surface of filler and the polymer matrix.

3.1.2.1. Silane Coupling Agents

The general formula of silane coupling agents is $Y\ (CH_2)_n\ Si\ (OR)_3$, where R stands for most frequently methyl, ethyl or isopropyl group while Y denotes a functional group capable of interaction with polymers such as amino, mercapto or vinyl group. The modification reaction on the filler develops as follow [39].

Hydrolysis reaction

$$Y\ (CH_2)_n\ Si\ (OR)_3 + 3H_2O \longrightarrow Y\ (CH_2)_n\ Si\ (OH)_3 + 3\ ROH$$

Condensation Reaction

$$\equiv Si\text{-}OH + (OH)_3\ Si\ (CH_2)_n\ Y \longrightarrow \equiv Si\text{-}o\text{-}Si\ (CH_2)_n\ Y + H_20$$

The presence of water in the system is one of most significant parameters in the reaction water molecules cause hydrolysis and formation of silanols (≡Si-OH). Silanols can be combined to form of siloxane linkage (≡Si-O-Si≡) between two silane molecules and production of a new water molecule. The formation of siloxane group allows the bonding of the coupling agent with filler. Commonly used silane coupling agents are listed in Table 3.1.

Table 3.1 Commonly Used Silane Coupling Agents.

Silane Coupling Agents	Chemical Formula
(3-methacryloxypropyl)trimethoxy silane	CH_2=C-CO-O-CH_2-CH_2-CH_2-Si-(O-CH_3)$_3$$CH_3$
(3-aminopropyl)triethoxy silane	NH_2-CH_2- CH_2- CH_2-Si-(O- CH_2-CH_3)$_3$
Vinyl triethoxysilane	CH_2=CH-Si-(O- CH_2-CH_3)$_3$
(3-mercaptopropyl)trimethoxy silane	HS- CH_2- CH_2-Si-(O- CH_3)$_3$

Dupraz et al. [13] focused on the coupling agent as a coating on the HA powder's surface in their study. Investigations have been performed to select an appropriate silane with regard to its ability to achieve a stable coverage the of HA particles. To ensure a uniform coverage of the HA surface, silane coupling agents were applied from an aqueous alcohol solution. Depending on the molecular structure of the silane, different times were allowed for hydrolysis and silanol formation. The silanized powders were dried at room temperature to improve the stability of the coating and then heated at 100-120 ^{0}C to strengthen the coating by polysiloxane network structures.

They found that silane coupling agents were able to bond chemically of the HA surface because of a thin coating remained after washing powder with water. The water stability of this bond was evaluated and aminofunctional silane was found to be most effective one among the others used. And the presence of the silane coatings on HA did not impair the minerals'tendency to dissolve partly in water or to take up calcium and phosphate from simulated physiological solutions.

The treatment of HA has received little attention in literature for biopolymer-ceramic composite systems. Vaz et al [38] studied the surface modification of HA coupling agents for starch EVOH/HA composite system to improve mechanical properties of the composites. Significant improvements were obtained in modulus about 30% increase when using zirconate-coupling agent. 3-Aminoproplymethyl timethoxysilane also enhanced stiffness of composites to about 30%. But composites rapidly lose strength and stiffness when exposed to an aqueous environment. All composites containing coupling agent should be tested in terms of cytotoxic behaviour, if they will be used for biomedical applications.

3.2. Biodegradable Polymers

Using of biodegradable polymeric biomaterials have increased irregularly during the past two-decade. The total US revenues from commercial products developed from absorbable polymers in 1995 was estimated to be over $300 million with over 95% of revenues generated from the sale of bioabsorbable sutures. The other 5% is attributed to orthopedic fixation devices in the forms of pins, rods and tacks, staples for wound closure, and dental applications [40]. Biodegradable polymeric devices have some advantages that non-biodegradable material do not have [25].

- No need operation for removal
- No restriction of one growth owing to the gradual decrease in mechanical strength
- No tissue reaction caused by metallic corrosion
- No generation of artifacts on computed tomography
- To be able to regenerate tissues

Although the earliest and most commercially significant biodegradable polymeric biomaterials originated from linear aliphatic polysters such as polyglycolide and polyactide from poly (α-hydroxyacetic acids), recent introduction of several new synthetic and natural biodegradable polymeric biomaterials extends the domain beyond this family of simple polyesters. These new commercially significant biodegradable polymeric biomaterials include poly (orthoesters), polyanhydrides, polysaccharides, poly(ester-amides), tyrosine-based polyarylates or polyiminocarbonates or polycarbonates, poly (D, L-Lactide-urethane), poly (β-hydroxybutyrate), poly(ε-caprolactone) ,poly(bis(carboxylatopheneoxy) phosphazene), poly(amido acids), pseudo-poly (amino acids) and copolymers derived from amino acids and nonamino acids.

The factors that affect the mechanical performance of biodegradable polymers are those that are well known to the polymer scientist. These factors are monomer selection, initiator selection, process conditions, and the presence of additives. These factors in turn influence the polymer's hydrophilicity, crystallinity, melt and glass transition temperatures, molecular weight, molecular weight distribution, end groups, sequence distribution (random versus blocky), and the presence of residual monomer or additives.

[40] In addition, the polymer scientist working with biodegradable polymers must also evaluate each of these variables for its effect on biodegradation. Table 3.2 indicates properties of synthetic absorbable polymers.

Biodegradation polymeric biomaterials could be generally divided into eight groups based on their chemical origin [3].

1. biodegradable linear alipathic polyesters (e.g. polyglycolide, polylactide, polycaprolactone and their copolymer within the aliphatic polysters family such as poly (glycalide-L-lactide copolymer)
2. biodegradable copolymer between linear alipathic polyesters and monomers other than linear alipathic polyesters (e.g. poly(L-lactic acid-L-Lysine) copolymer, poly (e.g. (D-L-lactide urethane)copolymer)
3. polyandhydrides
4. polylorthoesters
5. poly (ester-ethers) like poly-dioxanone
6. biodegradable polysaccharides
7. polyamino acids (such as poly-L-glutamic acid and poly-L-lysine)
8. inorganic biodegradable polymers (such as polyphosphazene)

The polymers used in this study come from the group 1, linear aliphatic family which are Poly (L-Lactide) and Poly (L-Lactide-DL- Lactide) copolymer. Next section presents the information about them.

3.2.1.Polylactide and its copolymer

Lactide is the cyclic dimer of lactic acid, which exists two optical isomers, D and L. Lactide, is the naturally occurring isomer, and DL-Lactide is synthetic blend of solid state through ring-opening polymerization due to their thermal instability and should be melt processors at the lowest temperature [24]. Figure 3.2 indicates synthesis of Polylactide.

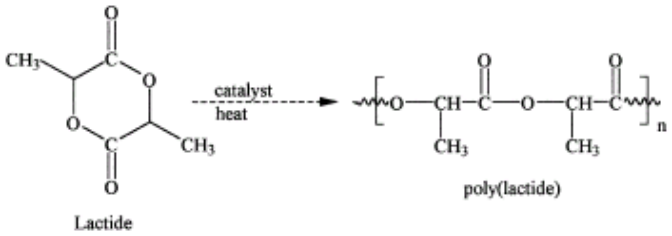


Figure 3.2 Synthesis of Polylactide [40].

The homopolymer of L-lactide (PLA) is a semicrystalline polymer with melting temperature, T_m, 170 ^{0}C and glass transition temperature, T_g, 56^0 C. This high T_g is mainly responsible for the observed extremely slow biodegradation rate at body temperature reported in the literature [40]. PLA exhibit high tensile strength and low elongation and have a high modulus that makes them more applicable than amorphous polymers for load-bearing applications such as in orthopaedic fixation.

The polymer made from optically inactive racemic mixture of D and L enantiomers, poly-DL-Lactide is an amorphous polymer. This material has lower tensile strengths and higher elongation and much faster degradation time making it more suitable as a drug delivery system. Physical, mechanical and degradation properties of selected biodegradable polymers and comparison with bone and steel as reference materials are summarised in Table 3.2 [40]. Consequently, devices prepared from PLA should biodegrade less rapidly than devices prepared from racemic PDLA.

Table 3.2. Physical, Mechanical and Degradation Properties of Selected Biodegradable Polymers.

Polymer	T_m(^{0}C)	T_g(^{0}C)	Modulus(GPa)	Elongation (%)	t_d(months)
PGA	225-230	35-45	7.0	15-20	6 to 12
PLA	173-178	60-65	2.7	3-10	>24
PDLA	Amorphous	55-60	1.9	3-10	12 to 16
PCL 85/15	58-63	60-65	0.4	300-500	>24
DLPLG 65/35	Amorphous	50-55	2.0	3-10	5 to 6
DLPLG 50/50	Amorphous	45-50	2.0	3-10	4 to 5
DLPLG	Amorphous	45-50	2.0	3-10	3-4
Bone			10-20		
Steel			210		

T_m : Melting Point

T_g :Glass Transition Temperature

3.3. HA Reinforced PolyLactide Based Composites

Development of the HA reinforced polylactide based composites has been studied extensively in the literature. Next sections focus on the literature review of the preparation techniques and characterization of the Polylactide-HA composites.

3.3.1. Preparation Techniques

There are many different techniques used according to application area and the properties needed for preparation of this composite in the literature: solvent casting, [8, 9, 12, 25, 35] thermal kneading, heating mortar, [41] mixing ceramic powder before polymerization [6, 10] and phase separation [42, 43]. After these techniques, compression molding has been widely applied to give a form.

Ignjatovic et al [8] investigated the preparation of HA / Poly(L-Lactide),(PLA), composites by solvent casting technique. HA and PLA were synthesised chemically. PLA was completely dissolved in chloroform at 20 ^{0}C for 1 h. HA powder was added to PLA solution, and the mixture was stirred at 20 ^{0}C for 20 min. After homogenisation, the mixture was poured into molds, then heated in a vacuum drying oven at 40^{0}C until complete evaporation of chloroform. Pressing in cylindrical molds shaped the obtained HA/ PLA composite biomaterial. In their study, effect of mass fraction of HA in the composite on the mechanical properties has been studied.

Preparation and mechanical properties of PLA composites containing HA fibers have been examined in another study [9], 10 g PLA was soaked in 100 ml of methylene chloride at room temperature. After soaking for 24 h, PLA was dissolved completely. HA fiber was added to PLA solution and the mixture was stirred for 30 min. The mixture was cast into a stainless steel die of 38-mm diameter and was dried for 8 h at room temperature. Hot-pressed uniaxially was applied to give a form at 180 ^{0}C under a pressure of 40 MPa.

Shikinami and co-workers [25] examined ultra high strength resorbable implants that were made from bioactive ceramic particles/PLA composites. The small granules uniformly distributing HA microparticles within a PLA matrix which were obtained by precipitating polymer solution, dropping ethanol into dichloromethane-polymer solution, were extruded to make a thick billet. Thereafter, the billet was formed into a

thin billet by compression molding at 103 ^{0}C. The composites were filled with 20,30,40 and 50 weight fractions of HA particles.

Zhang and Ma investigated [42,43] Poly (α-hydroxyl acids)/HA porous composites for bone-tissue engineering. Poly (L-lactic acid) and Poly (D,L-lactic acid-co-glycolic acid; 75/25) (PLGA) polymers were used in the study. To prepare the composite, firstly PLA or PLGA was dissolved completely in dioxane and mixed HA homogeneously. After preparation of polymer-HA mixture, thermally induced phase separation and subsequent sublimation of solvent prepared composite foam. Thermally induced phase separation technique was used to create the highly porous scaffolds for bone tissue engineering. Varying polymer concentration controlled the microstructure of the foams.

Deng et al [35] studied nanocomposite production of PLA/HA composites. These composites were prepared through solvent-cast technique. But this preparation method was different from the others. First, HA powder was dispersed in solvent, which is dimethyl formamide (DMF), using ultrasonics for 30 min. The dispersion was filtered for removal of large aggregates. Pre-weighted PLA was added into a flask that contained HA powder. PLA then totally dissolved into the sol. The resulting mixture was poured into mould and dried under vacuum.

Bleach and co-workers [12] examined effects of filler content on mechanical properties of biphasic calcium phosphate (BCP)/PLA composites. BCP consisted of a mixture of 70 wt % β-TCP and 30 wt % HA. Solvent casting technique was used to produce composite films.

In another study, Kikuchi et al [41] studied preparation of calcium phosphate/ copoly-L-Lactide(CPLA) with fatty polyesters composites. They synthesised composites using three different methods.

1) Solvent Casting Method; A calcium phosphate powder was added into a chloroform solution of copoly-L-Lactide and dried at 293 K in vacuum.
2) Heating mortar Method; CPLA was put in a mortar and heated at 453 K for 30 min. Calcium phosphate also heated at the same temperature, and mixed with melted CPLA for 15 min. After mixing, the composite was removed from the mortar and cooled at room temperature.
3) Thermal kneading Method; Mixture of CPLA and calcium phosphate was kneaded for 10 min at 20-50 rpm at 453 K.

These three types of composites were formed into plates by thermal pressing. Finally thermal-kneading method was found to be better than the other preparation methods. The other method used for the production of polymer- ceramic composite is based on mixing of ceramics with monomers prior to polymerization.[6,10] Appropriate weight by weight mixture of D-lactide monomer and hydroxyapatite (0,30,50 wt %) were heated to 105 ^{o}C in rotating vacuum sealed ampules for 72 h. Stannous octoate was used as a catalyst to initiate the polymerization.

3.3.2. Characterization of Polylactide based HA Composites

In literature, different characterization techniques were employed to understand the mechanical, physicochemical and morphological properties of the composites

3.3.2.1. Mechanical and Microstructural Characterization

The complete mechanical characterisation of composite is achieved using mechanical and viscoelastic analysis. Measurements of the elastic and viscoelastic components of the modulus characterise the material. And also knowledge of the values of resistance to deformation and fatique is important in selecting a material. Mechanical tests have been standardised and are described in the publications of American Society for Testing and Materials (ASTM). The tests are defined to obtain about the final end use performance. Mechanical properties of composites are affected by many factors, such as, filler loading, homogeneous dispersion of filler, preparation technique and conditions. Tensile strength, Young modulus, yield stress, ultimate elongation are generally used to characterise the mechanical properties of the composites. Young's Modulus is the ratio of stress to strain below the elastic limit, gives information about rigidity of the composite. Tensile yield stress is very important property of the composites, gives information on the maximum allowable load before plastic deformation occurs.

Microscopy techniques such as optical, transmission and scanning electron microscope are useful as analysis technique, particularly in the case of morphological structure of composites. Optical Microscopy and scanning electron microscopy (SEM) give information about the state of dispersion of particles in the polymer matrix and effects of surface treatment on the fillers. Optical microscopy is of limited value in

examining the interface region. For this reason electron microscopy, which has resolutions to several nanometers, is used to study details of especially fracture surface.

Mechanical and microstructural properties of polyLactide based HA composites have been investigated in literature.

Kikuchi and co-workers [41] investigated effects of calcium phosphate type, tricalcium phosphate (TCP) and hydroxyapatite (HA) and preparation technique (Chloroform- solvent method, Heating mortar method, Thermal kneading method) on the mechanical properties of calcium phosphate/ copoly-L-Lactide (CPLA) composites. TCP/CPLA and HA/CPLA composites prepared by the thermal kneading method were the most homogeneously dispersed without colouring. TCP/CPLA composites had better mechanical properties and thermoplasticity than HA/CPLA. Especially, Young's modulus was improved by strong adhesion between TCP and CPLA. In their study, SEM images showed HA particles were not well covered with CPLA although the primary particle size of HA was smaller than that of TCP. On the other hand, in TCP/CPLA composites, CPLA formed a homogeneous dispersion and covered TCP particles well.

Kasuga et al. [5] examined preparation of bioresorbable poly-L-lactic acid (PLLA) composites containing β-Ca $(PO_3)_2$ fibers (CPF and CPF_t) treated with dilute NaOH solution. PLLA dissolved by using methylene chloride was mixed with the fibers. The strength of PLLA/ CPF decreased with increasing the fiber content, while that of PLLA/ CPF_t was almost independent of the content. The modulus of PLLA/CPF composite increased with increasing CPF content up to 20 wt %, and then decreased when the fiber content was over 35 wt%. On the other hand, the modulus of PLLA/ CPF_t increased monotonously with increasing the fiber content. Their study showed that the fiber surface modification with dilute NaOH was essential for the preparation of the PLLA composites. The calcium orthophosphate phase on the surface of the fiber formed by the NaOH treatment was believed to be linked chemically with PLLA.

The preparation and mechanical properties of nanocomposites of Poly (D,L-Lactide) with Ca-deficient HA nanocrystals were examined by Deng and co-workers.[35] Nanocomposites were prepared by solvent casting technique using dimethylformamide. The obtained nanocomposites are semitransparent films with the thickness about 0.1 mm. The microstructural observation indicates close contact between the polymer matrix and the filled nanocrystals. The dispersion of nanocrystals in the polymer matrix is homogeneous at a microscopic level. The relationship between

the HA particles loading and tensile modulus for HA/ PLA composites has been investigated in their study. For the composite containing 10.5 v % HA, the modulus even reaches to 2.47 GPa as compared to 1.66 GPa for pure PLA. Tensile modulus for composite increases with HA loading. Yield stress for the composites varies irregularly with HA loading.

Bleach and coworkers [12] investigated effect of filler content on mechanical properties of bicalcium phosphate(BCP)/polylactide composites. Adding ceramic particles to the polymer increased the modulus and yield stress. In addition, modulus and yield stress were maintained over a 12-week in vitro period when filler particles were added to the composite a significant decrease in modulus and yield strenght at 12 week. In the composite, all the elongation arises from the polymer since the BCP is rigid relative to the PLA. Increasing the amount of filler decreases the amount polymer available for elongation and reduces the failure strain. Following preparing the composite, preliminary tests using DSC showed that the cast films representing all BCP were free from crystallinity. The morphological structure of BCP particles in the composite films was investigated in their study. Scanning electron Microscopy showed a homogeneous distribution of BCP particles in the films, which were a maximum of 50 μm thick. However some agglomeration was seen in the composites containing larger volume fractions of BCP.

Poly (α-hydroxyl acids)/ hydroxyapatite porous composites for bone tissue engineering were prepared by thermally induced phase separation technique [42]. The microstructure of the pores and walls was controlled by varying the polymer concentration, HA content, quenching temperature, polymer and solvent utilized in their study. The composite foams showed a significant improvement in mechanical properties over pure polymer foams. Both compressive modulus and the compressive yield strength of the composite foams are significantly higher than those of the PLLA foam. The mechanical properties data showed the positive effects of the addition hydroxyapatite in enhancing the mechanical performance of the foams.

Shikinami et al [25] studied preparation and characterization of HA / PolyL-Lactide composites. The composites were prepared by forging process. Uncalcined and unsintered HA (u-HA) was used as filler in their study. Effect of u-HA loading on the mechanical properties of u-HA / PLA was investigated. Mechanical properties of these composites as a function HA loading were given in Table 3.3. The gradual decrease in tensile strength was inversely proportional to the fraction of HA particles, but a high

enough tensile strength of over 100 MPa was maintained and this was sufficient for practical use as bone fixation devices. It was confirmed that the composites generally showed the highest mechanical strenght among this type of reinforced bioceramic particles or fibers- biopolymer composites known to date. The high bending, shear, and impact strengths and the high bending modulus of these composites were found to be very dependent on the mechanical interlocking between the HA particles and PLA matrix.

Table 3.3 Effects of u-HA Contents on the Mechanical Properties of u-HA / PLA.

Mechanical Properties	PLA	u-HA 20	u-HA 30	u- HA 40	u-HA 50
HA/PLA Ratio(wt)	0/100	20/80	30/70	40/60	50/50
M_v (K Da)	220	215	210	208	202
S_b (MPa)	258.5	252.2	269.2	270	267.5
E_b (GPa)	6.5	7.0	7.6	9.1	12.3
S_t (MPa)	154.1	152.8	121.5	110.0	103.0
E_t (GPa)	1.3	2.0	2.3	2.3	2.4
S_c (MPa)	123.5	144.5	106.7	107.3	115.3
E_c (GPa)	4.8	5.3	5.6	6.1	6.5
S_s (MPa)	93	127.2	126.4	127.6	143.6
T_s (kg.cm)	6.6	6.8	6.6	6.0	4.0
S_I (kj/cm^2)	76.3	ND	166.1	90.9	30.2
H_v	20.2	20.8	22.9	23.8	26.3

M_v; viscosity avr. Molecular weight, S_b; bending strength, E_b; bending modulus, S_t; tensile strength, E_t; tensile modulus, S_c ; compressive strength, E_c ; compressive modulus, S_s ; shear strength, T_s ;torsional strength, S_I; impact strength, H_v; vickers hardness

Chapter 4

DEGRADATION OF POLYMER CERAMIC COMPOSITE

The term biodegradation is frequently loosely associated with materials that could be broken down by nature either through hydrolytic mechanisms without the help of enzymes and/or enzymatic mechanism. Other terms, such as absorbable, erodible and resorbable have also been used in the literature to indicate biodegradation. The interest in biodegradable polymeric biomaterials for biomedical applications has increased dramatically during the past decade.

Why would a medical practitioner want a material to degrade? There may be a variety of reasons, to have advantages. For example, a fractured bone, fixated with a rigid, non-biodegradable stainless a device, which can be used as an implant and will not necessitate a second surgical event for removal. In addition to not requiring a second surgery, the biodegradation may offer other steel implant, has a tendency for re-fracture upon removal of the implant. The bone does not carry sufficient load during the healing process, because the load is carried by the rigid stainless steel. However an implant prepared from biodegradable polymer can be engineered to degrade at a rate that will slowly transfer load to the healing bone. Another exciting application for which biodegradable polymers offer tremendous potential is the basis for drug delivery, either as a drug delivery system alone or in conjunction to functioning as a medical device. In orthopedic applications, the delivery of a bone morphogenic protein may be used to speed the healing process after a fracture, or the delivery of an antibiotic may help prevent osteomyelitis following surgery [40].

There are different types of polymer degradation such as photo, thermal, mechanical and chemical degradation. All polymers share the property that they erode markedly under the influence of UV light or γ-radiation. For polymeric biomaterials, such effects are of minor importance, unless they are subjected to γ-sterilisation, after which a significant loss of molecular weight can be observed. Thermal degradation plays a greater role for non-degradable polymers. Mechanical degradation affects those biodegradable polymers that are subjected to mechanical stress, such as non-degradable polymers or biodegradable polymers used as fixture or suture material. All biodegradable polymers contain hydrolysable bonds. Their most important degradation mechanism is therefore chemical degradation via hydrolysis or enzyme-catalysed

hydrolysis. The latter effect is the degradation mediated at least partially by a biological system. Polymer degradation is the key process of erosion. There are two principal ways which polymer can cleave bonds: passively by hydrolysis or actively by enzymatic reaction. The latter option is only effectively available for naturally occurring biopolymers like polysaccharides, proteins (gelatin and collagen) and poly (α-hydroxy acids) where appropriate enzymes are available. For most, biodegradable materials, especially artificial polymers, passive hydrolysis is the most important mode of degradation [44].

There are several factors that influence the speed of this degradation reaction: the type of chemical bond, pH, copolymer composition, temperature and water uptake are the most important factors. Chemical and physical changes go along with the degradation of biodegradable polymers, like the crystallisation of oligomers and monomers or pH changes. Some of these factors can have a substantial feedback effect on the degradation rate. The most important parameter for monitoring degradation is molecular weight change. Besides loss of molecular weight, other parameters have been proposed as a measure for degradation into monomers or monomer release. All of these are related need not necessarily obey the same kinetics. For example, complete degradation of poly (L-lactic acid) is known to take substantially more time than the loss of tensile strength. Aqueous solutions of lactic acid form spontaneously poly (lactic acid) oligomers that might affect molecular weight measurements and monomers from copolymers need to be released with identical kinetics during erosion. The specific relation between the erosion parameters varies according to the type of polymer. There are, however, basic principles, according to which degradation proceeds, and how degradation can be influenced [45].

In general, for most synthetic biodegradable polymers degradation is considered to be a hydrolytic process. The cleavage of an ester bond yields a carboxyl end group and a hydroxyl one. Thus, the formed carboxyl end groups are capable of catalysing hydrolysis of other ester bonds. This phenomenon is called as autocatalysis [46].

There are two main types of degradation: Bulk erosion and Surface erosion.

Bulk erosion occurs when the rate at which water penetrates the device exceeds that at which the polymer is converted into water-soluble materials. The polylactide and polyglycolide commercially available devices and sutures degrade by bulk erosion [40]. The degradation rate at the surface of large lactide-glycolide implants is slower than the degradation in the inside. Initially, degradation does occur more rapidly at the surface

due to the greater availability of water. The degradation products at the surface are rapidly dissolved in the surrounding fluid and removed from the bulk polymer. In the inside of the device the inability of large polymeric degradation products to diffuse away from the bulk device results in a local acidic environment in the interior of the implant. The increased acidic environment catalyses further degradation resulting in accelerated hydrolysis of the ester linkages in the interior.

There is a second type of biodegradation called surface erosion when the rate at which the polymer penetrates the device is slower than the rate of conversion of the polymer into water-soluble materials [44]. Surface erosion results in the device thinning over time while maintaining its bulk integrity. Polyanhydrides and polyorthoesters are examples of this type of erosion when the polymer is hydrophobic, but the chemical bonds are highly susceptible to hydrolysis. In general, this process is referred to in the literature as bioerosion rather than biodegradation.

The morphology of a polymeric material (ie, amorphousness or semicrytallinity) plays a critical role in the degradation process. Chain orientation in crystalline and amorphous regions could also play an important role in the degradation of polymers.
The semicrystalline polymers of degradation occur in two phases:
In the first phase, water penetrates the bulk of the device, preferably attacking the chemical bonds in the amorphous phase and converting long polymer chains into shorter, finally water-soluble fragments can be occurred. A decrease in physical properties as water begins to fragment the device follows the decrease in molecular weight. In the second phase, enzymatic attack of the fragments occurs. The metabolising of the fragments results in a rapid loss of polymer mass.

The degradation-absorption mechanism is the result of many interrelated factors, including:

- the chemical stability of the polymer backbone,
- the presence of catalysts,
- additives,
- impurities or plasticisers,
- the geometry of the device,
- the location of the device.

The balancing of these factors to tailor an implant to slowly degrade and transfer stress to the surrounding tissue as it heals at the appropriate rate is one of the major

challenges facing the researchers today. The factors, which accelerate polymer degradation, are the following:

- More hydrophilic monomer.
- More hydrophilic, acidic end groups.
- More reactive hydrolytic group in the backbone.
- Less crystallinity.
- Smaller device size.

4.1.Hydrolytic Degradation of Polylactide Polymers and Polylactide Based Composites Containing Hydroxyapatite

The in vivo and in vitro degradations of polylactide and its copolymers have been extensively investigated during the past two decades. [47-50]

PolyLactide (PLA) is aliphatic polyesters that contain flexible ester bonds in particular appear to be the most promising because of their excellent biocompatibility and variable degradability. It was established that carboxyl end groups formed by chain cleavage and amorphous regions are preferentially degraded faster than crystalline regions.

Since PLA is a semicrystalline polymer, the effect of crystallinity on the degradation rate should be examined, Li investigated the degradation rate of both amorphous and crystalline PLA in order to examine the influence of the initial crystallinity on the degradation rate [46]. In the semicrystalline PLA, a small weight loss was detected after 7 weeks, which was earlier than in amorphous PLA. At the end of the 110 weeks, only 26% of the material were lost. Therefore, the crystallinity reduced the overall degradation rate of PLA in terms of weight loss.

Cha et al. [51] studied the effect of sample preparation techniques on the biodegradation properties of Poly(ε-caprolactone) (PCL), Poly(L- lactic acid) (PLA), Polyglycolic acid-co-L-lactic acid (PGLA). Three different methods; compression mouldings, coprecipitation, and solvent evaporation of methylene chloride in water emulsion of the polymers were used for the preparation of the polymers. The rates of hydrolytic chain of each component of the blends were determined in phosphate buffer, pH 7.4, at 37 ^{0}C, for up to 3000 h. The observed rates were dependent on the

preparation method. For compression moulded blends, the rate of degradation PGLA was decreased and that of PCL and PLA increased.

Furukawa et al [36] investigated the biodegradation behaviour of ultra-high strength hydroxyapatite/ poly (L-Lactide) composite rods for internal fixation of bone fractures. The biodegradation of the composite rods in subcutis and medullary cavities of rabbits were evaluated were prepared histologically. Two kinds of composite materials were prepared using uncalcinated and calcinated HA as reinforcing filler in their study. A bending strength higher than 200 MPa were maintained at 25 weeks in the subcuits.

Greish et al [31] investigated the effects of the addition of a bioactive glass to Ca-polyacrylate in ratios up to 50% by weight on the mechanical and the microstructural properties of HA_p-Ca-polyacrylate-bioactive glass composites. The behaviour of these composites in Simulated Body Fluid (SBF) revealed an apatite layer formed on the surface of the bioactive glass. This was in accordance with the changes in the ionic concentrations of the calcium, phosphorous and silicate ions in the SBF.

Verheyen et al [6] examined the physico-chemical behaviour of hydroxyapatite/ poly (L-lactide) composites in the tests solution. The polymer PLA, the composites 30 wt % and 50 wt % HA/PLA and one- side HA-coated PLA were evaluated. Specimens were incubated in various acellular aqueous buffer solutions; citrate, Gomori's and phosphate- buffered saline up to 24 weeks. HA/ PLA composites with 30 or 50 wt % HA showed linear release of calcium and phosphate ions and L-Lactide when incubated into different buffers within the tested incubation period of 24 weeks at pH of 7.2. In this study in vitro results showed that solubility of calcium phosphates is not only dependent on the surrounding medium, but also the material itself.

Higashi et al. [11] have combined poly (DL-Lactide) with HA in a 1:1 ratio to develop biodegradable artificial bone fillers where high mechanical strength is not required in the composites. They investigated biodegradation behaviour of the composites tested both in vivo and in vitro. Degradation studies were done in a distilled water medium at 37 ^{0}C and pH 6.8. pH measurements, calcium and phosphorous concentration determinations were done for each specimen. It was reported that Hydroxyapatite showed a very slight increase in pH level with time. On the other hand HA/PLA composite became acidic, and pH level reached to 3.4 value in a one week. The pH level in vivo does not decrease as much as that in vitro because of the buffer action mechanism.

Shikinami et al [52] investigated the change of bending strength of HA/PLA composite by degrading in vitro in phosphate buffered saline (PBS) solution having the pH value of 7.4 at 37 ^{0}C and the bonding osteointegration ability of the composite to bone in simulated body fluid (SBF) at 37 ^{0}C. In Vitro bioactivity tests showed that generous apatite crystals began to form on the surface of the composite after 3-6 days in SBF and covered the whole surface with a fairly thick layer after 4 weeks. The apatite deposition may lead to bond a device to bone at the beginning in vivo, and then to reduce a bone hole due to both degradation and resorption of PLA by replacing with the surrounding tissues.

Marra et al [53] studied the degradation rates of HA/Poly (caprolactone) and HA/ Poly (D,L-lactic-co-glycolic acid) in vitro for applications in bone tissue engineering. Weight loss during storage at 37 ^{0}C in phosphate-buffered saline (pH 7.4) was determined for specimens. The buffer solution was changed every 2 weeks. The weight loss of the composites over an 8-week period was determined by gravimetric analysis. 10-50 wt % weight loss was observed.

Zang and Ma [42, 43] investigated the highly porous poly (L-lactic acid)/ apatite composites were prepared through in situ formation of carbonated apatite onto poly (L-lactic acid) foams in simulated body fluid. The highly porous polymer foams were prepared by solid liquid phase separation. The foams were immersed in the SBF at 37 ^{0}C to allow the in situ apatite formation. After 30 days, a large number of microparticles with a diameter up to 2 μm was formed on the surfaces of the PLA pore walls. The EDS spectrum showed that the main elements of the incubated PLLA foam were carbon, oxygen, calcium and phosphorus. Carbon and oxygen could be from both PLA and the particles but calcium and phosphorus could only be from the particles. These results suggested that the particles formed in the PLA foams might be similar to HA. The film surfaces were completely covered with microparticles after 15 days of incubation. The particle number and size were affected by several factors such as incubation time, ionic concentration of the SBF, water treatment and polymer surface area.

As for the in vitro studies, many types of environment such as phosphate buffer solution, saline solution, ringer solution and simulated body fluid can be used These solutions can be prepared at different conditions, such as pH, temperature and so on.

4.2. Simulated Body Fluid

SBF is known to be as metastable buffer solutions [54], and even a small, undesired variance in both preparation steps and storage temperatures, may drastically affect the phase purity and high-temperature stability of the produced HA powders, as well as the kinetics of the precipitation processes.

It is believed that synthetic HA ceramic surfaces can be transformed to biological apatite through a set of reactions including dissolution, precipitation and ion exchange. Following the introduction of HA to simulated body fluid, a partial dissolution of the surface is initiated, causing the release of Ca^{+2}, HPO_4^{-2} and PO_3^{-3} and increasing the supersaturation of the environment with respect to stable phase. A hydroxy-carbonate apatite (HCA) layer can form with calcium and phosphate ions released from partially dissolving ceramic HA and from biological fluids, which contain other electrolytes, such as CO_3^{-2} and Mg^{+2}. This polycrystalline HCA phase is equivalent in composition and structure to mineral phase of bone.

Protocol for the preparation of Simulated Body Fluid (SBF) having ion concentrations nearly equal to those of human blood plasma which is buffered at pH 7.40 with 50 mM trishydroxymethylaminomethane and 45 mM hydrochloric acid at 36.5 ^{0}C. After washing SBF solutions were prepared by dissolving appropriate quantities of the chemicals given in Table 4.1 in deionised water. Reagents were added, one by one after each reagent was completely dissolved in 700 ml of water, in the order given in Table 4.1 [55].

Synthetic (simulated) body fluids (SBF) were first used by Kokubo et al. [54] The solutions can be prepared to the identical chemical compositions of human body fluid, with ion concentrations nearly equal to those of the inorganic constituents of human blood plasma. And also they proved the similarity between in vitro and in vivo behaviour of certain glass-ceramic compositions. Table 4.2 indicates the ionic concentrations (mM) of SBF in comparison with those of and bloods plasma.

Table 4.1 Reagents Used for the Preparation SBF (pH 7.4, 1 L).

Order	Reagent	Amount
1	NaCl	7.996 g
2	$NaHCO_3$	0.350 g
3	KCl	0.224 g
4	$K_2HPO_4.3H_2O$	0.228 g
5	$MgCl_2.6H_2O$	0.305 g
6	1N-HCl	40 mL
(About 90% of total amount of HCl to be added)		
7	$CaCl_2$	0.278 g
8	Na_2SO_4	0.071 g
9	$NH_2C(CH_2OH)_3$	6.057 g

Table 4.2. Ion Concentrations (mM) of SBF and Human Blood Plasma.

Ion	Simulated body fluid	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

Chapter 5

EXPERIMENTAL

5.1. Materials

Polymeric materials used in this study were Polylactide and their copolymers purchased from PURAC Biochem and Boehringer Ingelheim. The properties of four different polymers used in this study are listed in Table 5.1. Two different types of Hydroxyapatite powders (HA) were used; commercial HA and laboratory synthesized HA. Commercial HA powder was purchased from Aldrich Chemical Company. In Table 5.2 the properties of the commercial hydroxyapatite are listed. Chloroform was used as a solvent for the preperation of polymer solution. The properties of the solvent are given in Table 5.3.

Table 5.1. Properties of the Polymers Used in This Study.

Polymers	**Inherent Viscosity**	**Melting Temperature**
*Poly(L-Lactide) (PLA_1)	1.75 dl/g	170-200 ^{0}C
*96/4 L-Lactide/D-Lactide copolymer ($PDLA_1$)	5.68 dl/g	amorphous
**Poly(L-Lactide) (PLA_2)	3.6 dl/g	172-195 ^{0}C
**Poly(L-Lactide-co-D,L-lactide) ($PDLA_2$)	6 dl/g	amorphous

* purchased from PURAC

** purchased from Boehringer Ingeltheim

Table 5.2. Properties of Commercial Hydroxyapatite.

Chemical Formula	$Ca_{10}(PO_4)_6(OH)_2$
Molecular Weigth (g/mol)	1004.6
Density (g/ cm^3)	3.16

Table 5.3. Properties of Chloroform.

Chemical Structure	$CHCl_3$
Molecular Weight (g/ mol)	119.38
Density (g/ cm^3)	1.47
Purity, min(%)	99

Two different silane-coupling agents were used for the surface modification of Hydroxyapatite particles. These agents were (3-aminopropyl) triethoxysilane (Fluka) and (3 Mercaptoprophy trimethoxysilane) (Merck).

Simulated body fluid (SBF) was prepared by dissolving reagent grade chemicals of NaCl, $NaHCO_3$, KCl, Na_2HPO_4, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, and Na_2SO_4 in deionised water. The solution was buffered at physiological pH 7.4 and at 37 ^{0}C with 50 mM trishydroxymethyl aminomethane [$(CH_2OH)_3CNH_2$] (THAM) and 36.23 mM HCl acid. Sodium Azid was also added into SBF for preventing microbial effects.

5.2. Methods

Experimental methods can be summarised in six groups:

- Size reduction of hydroxyapatite
- Synthesis of hydroxyapatite
- Surface treatment of hydroxyapatite
- Preparation of Polylactide based composite films containing hydroxyapatite particles
- Characterisation of hydroxyapatite
- Characterisation of composite films

5.2.1. Preparation of Hydroxyapatite Powders

5.2.1.1. Size Reduction of Hydroxyapatite

Particle size analysis of the HA was done in order to determine the average particle size. Although HA was purchased as nearly as 1 μm particles, hydrophilic HA particles may form agglomerate in the presence of moisture causing an increase in the average particle size. Therefore, the HA powder was milled using Multifix Ball Mill under wet condition. Zirconia balls were used as grinding media during ball milling. HA powders were ground at a speed of 120 rpm for 8 h. diameters.

Particles less than 1 μm in size were separated according to Stoke's Law in a hydroxyapatite-water suspension. Stoke's Law [56] given by Equation 5.1, states that under fixed condition; the time taken for a particle to settle to a fixed depth is inversely proportional to the square root of its spherical

$$V = l / t = a^2 (D_p - D_l)\, g / 18\, \eta \qquad (5.1)$$

Where V: Velocity (cm/s)

D_p: Particle Density (g/cm^3)

D_l: Liquid Density (g/cm^3)

g: Gravitational force ($g.cm / s^2$)

a: Particle diameter (cm)

η: Viscosity of liquid (cm^3/g)

l: a distance (cm)

t: time for particle to settle a distance (sec)

D_p and D_l were taken as 3.16 g/cm^3 and 1 g/cm^3 respectively. The time required for 1μm HA particles to settle down could be easily determined using equation 5.1.

After sedimentation process, the suspension was taken separated from the solids settled at the bottom of the container. This suspension was washed by ethanol and used for obtaining soft powder. The slurry was dried in an oven at 120 ^{0}C for 8 h.

5.2.1.2. Synthesis of Hydroxyapatite

In this study, Hydroxyapatite was synthesised by precipitation technique. A batch mixture with Ca/P=1.67 molar ratio was prepared using starting materials Ca (NO_3). 4 H_2O and $(NH_4)_2HPO_4$. Ca (NO_3). 4 H_2O aqueous solution of 1000 ml (3.78 wt %) was slowly dropped into stirred, 1000 ml $(NH_4)_2HPO_4$ aqueous solution (1.3 wt %) for 150 min. The pH of both solutions was kept at around 10-11, with adjusting NH_4 OH addition and reaction was carried out at room temperature. Solution pH was held above 10 by adding ammonium hydroxide due to stability of HA. The precipitate of calcium hydroxyapatite was obtained according to the reaction given below;

$$10\ Ca(NO_3).4\ H_2O + 6\ (NH_4)_2HPO_4 + 8\ NH_4\ OH \rightarrow Ca_{10}\ (PO_4)_6\ (OH)_2 + 20\ NH_4NO_3 + 6\ H_2O$$

The obtained suspension was heated and mixed for 24 h that was aging time. The precipitate was separated by filtration using Bihner's funnel. The obtained filter cake was washed with deionised water until the ammonia smell disappeared. After washing deionised water, filter cake was washed with ethanol to obtain mild powder.

The filter cake was dried in oven at 90^{0}C-100 ^{0}C and mixed regularly. Then the dried powder was calcined at 400 ^{0}C for 3 h.

5.2.1.3. Surface Treatment of Hydroxyapatite

Surface modification of hydroxyapatite was carried out to decrease the hydrophilic nature of its surface and to make it more compatible with the Polylactide (PLA) and its copolymer. Agglomeration of hydroxyapatite particles can be avoided in

polylactide composites by surface modification of particles, and a homogeneous dispersion of particles in composites can be obtained.

HA powder, which was synthesised in laboratory, was treated with two different silane coupling agents: (3-aminopropyl)triethoxysilane (AMPTES) and 3-merkaptopropyltrimethoxysilane (MPTMS). Chemical structures of surface modifiers used are tabulated in Table 5.4

Table 5.4.Chemical Structures of Surface Modifiers.

Surface Modifier	Chemical Formula	Producer
AMPTES	NH_2-CH_2- CH_2- CH_2-Si-(O- CH_2-CH_3)$_3$	Fluka
MPTMS	SH- CH_2- CH_2- CH_2-Si-(O-CH_3)$_3$	Merck

To ensure a uniform coverage of the HA surface, silane coupling agents were applied from an aqueous ethanol solution. HA powder was added to a solution of silane coupling agent (0.5, 1, and 2 wt %) in aqueous ethanol solution (90 v %). HA to solution ratio was taken as 1:2 on weight / volume basis. Mixing of silane ethanol solution carried out hydrolysis reaction. The slurry was stirred for 1 h by magnetic stirrer and then kept for 2 h at room temperature for treatment of the HA with hydrolysed solution to improve the stability of the coating. Then, the resulting slurry was dried at 100-120 ^{0}C to form silanol structure. The powder was then stored in desiccator until use. The sequence of surface treatment process of Hydroxyapatite powder with silane coupling agents is shown in Figure 5.1. Y denotes organofunctional group of silane coupling agent.

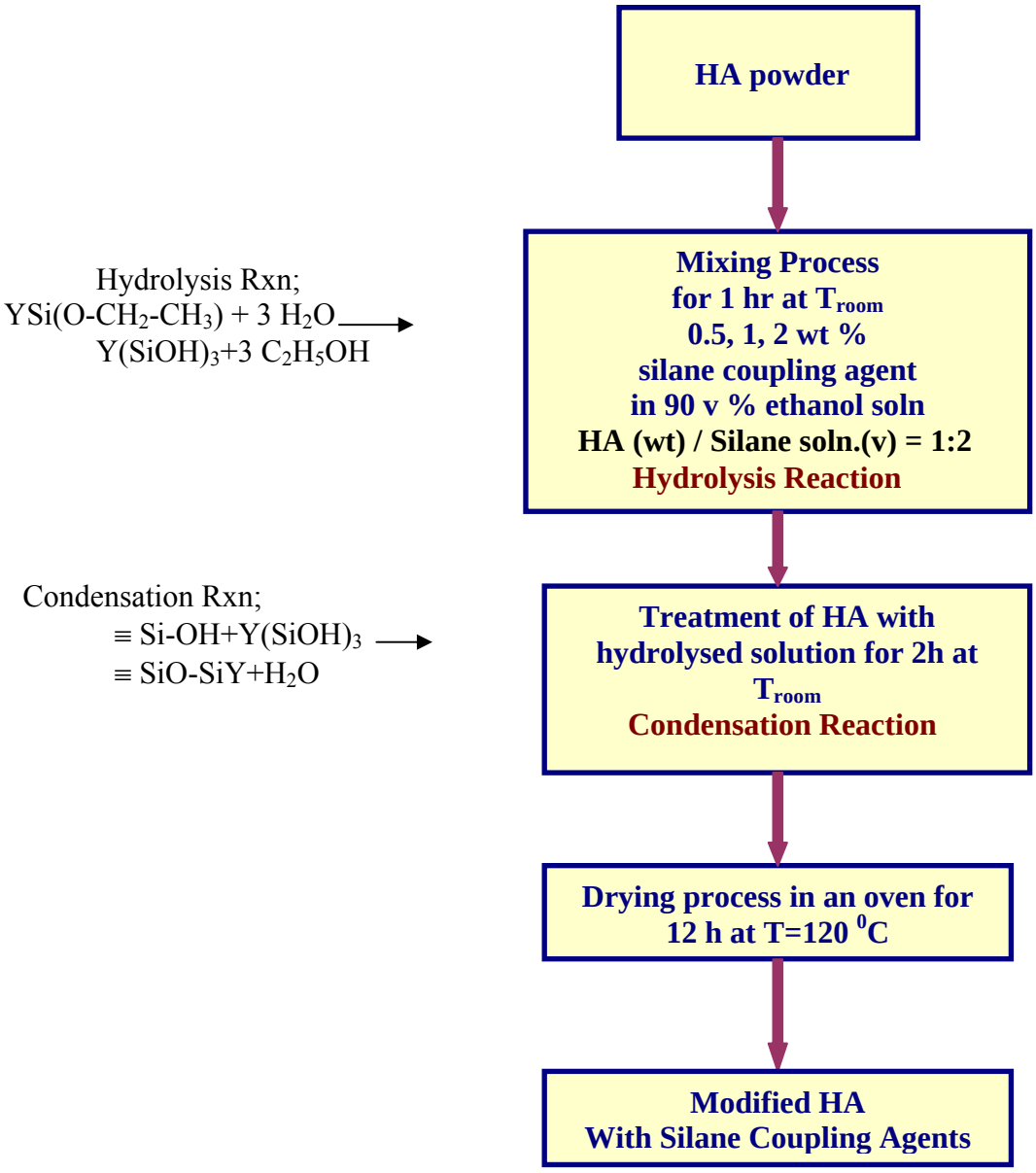


Figure 5.1. Surface Treatment Process of HA with Silane Coupling Agents

5.2.2. Preparation of HA/PLA Composite Films

The poly (L-Lactide) (PLA) and poly (L-Lactide-D,L-Lactide) and poly (L-Lactide-D-Lactide) copolymers (PDLA) composites containing 10 to 50 wt % untreated and treated Hydroxyapatite were prepared by using solvent casting technique. In this technique, chloroform was used as a solvent to prepare polymer solution.

In the process of the experimental study, two different methods were used to prepare composite films. In the first method, PLA or PDLA was weighed accurately in to a Pyrex jar and measured volume of chloroform was added to the Pyrex jar to make a solution with desired concentration. PLA/ solvent ratio and PDLA / solvent ratio were in the range of 5-10 % (w/v) and 3-7 % (w/v), respectively. The mixture was stirred at room temperature for 1-2 h to obtain a homogenous polymer solution. HA powder was added into the prepared solution to make a polymer HA mixture. The PLA or PDLA / solvent / HA mixture was stirred for 12 h on the magnetic stirrer until HA was totally dispersed in the solution. In the second method, HA powders were dispersed in chloroform in ultrasonic bath for 1 h. and then, PLA or PDLA were added to the solution. Mixing time and temperature conditions were the same in both methods.

The resulting mixture was then poured on glass surface using film applicator. Figure 5.3 indicates simple film applicator used in this study. It was dried overnight at room temperature, then finally dried under vacuum oven at 40 ^{0}C for 3 h. The dried films were gently removed from the glass surface. PLA or PDLA / HA composite films were stored in desiccator until they use. Flow sheet of the preparation of composite films is shown in Figures 5.2, 5.3, 5.4 and 5.5, respectively.

Figure 5.2. Mixture of PLA or PDLA –HA Composites on Magnetic Stirrer at T_{room} .

Figure 5.3. Simple Film Machine.

Figure 5.4.Prepared PLA or PDLA- HA Composite Films.

Figure 5.5. Separation of PLA or PDLA Composite Films from the Glass Surface.

5.2.3. Characterization of Hydroxyapatite Powders

Hydroxyapatite powder was characterised by particle size analysis, Fourier Transform infrared spectroscopy (FTIR), thermal gravimetric analysis (TGA), scanning electron microscopy (SEM) and SEM-EDX.

5.2.3.1. Particle Size Measurement of Hydroxyapatite

Particle size measurement provides the determination of the size distribution of particles. Particle size analyses of untreated commercial and synthesised HA are measured in the range of 10-1μm by Micromeritics X-ray Sedigraph 5100.

5.2.5.2. FTIR Analysis of Hydroxyapatite

FTIR analysis gives information about the chemical structure of Hydroxyapatite and the chemical reactions between silane coupling agents and hydroxyapatite. Untreated and treated hydroxyapatites with different silane coupling agents were analyzed by KBr

pellet technique. The pellets were prepared by pressing of 5 μg hydroxyapatite samples with 0.2 g of KBr. IR spectra were taken in the range of 400-4000 cm^{-1} with a Shimadzu FTIR 8201 Model.

5.2.3.3. TGA Analysis of Hydroxyapatite

TGA of hydroxyapatite was carried out using Shimadzu Thermal Gravimetric Analyzer (TGA-51) from room temperature to 500 ^{0}C at a heating rate of 10 ^{0}C/min. The analyses were performed in a dry nitrogen atmosphere. N_2 flow rate was 40 ml/ min and kept constant through out the experiments.

5.2.3.4. XRD Analysis of Hydroxyapatite

X-Ray Diffraction of HA powder was performed on to determine the crystalline phase(s) present in the powder.

5.2.3.5. SEM and EDX of Hydroxyapatite

Scanning Electron Microscopy (SEM) was used to determine the particle morphology of the HA powder by using Philips XL- 305 FEG, because SEM and EDX analysis allow one to obtain information about the micro structure and elemental composition of HA powder. Energy Dispersive X-Ray (EDX) detected Ca/P ratio of untreated and treated Hydroxyapatite.

5.2.4. Characterization of Composite Films

5.2.4.1. Mechanical Characterization of the Composites

Tensile testing of polymer composites containing (10, 20, 30, 40,wt %) untreated and treated hydroxyapatite were performed on a Testometric Universal Testing Machine. Load cell was 5 kgf, crosshead speed at 10 mm/min and the gauge length was 50 mm. Tensile tests were applied at room temperature. Tensile test specimens were prepared as strips of 10-mm width according to ASTM D-882. At least three specimens

were tested for each composite film, the mean values were reported on the basis of the three specimens.

5.2.4.2. Microstructural Characterization of HA/PLA Composites

Optical micrographs of the composites with different surface modifiers, HA loading and polymer type were taken using transmission mode with a camera fitted to Olympus BX-60 microscope at 20X magnification.

SEM analysis in composites allows one to obtain information about the micro structure and interaction between filler and matrix of a composite. Surface microscopy of the composites loaded with untreated and treated HA and fracture surface microscopy of tensile tested composites were observed with Philips XL-30S FEG scanning electron microscope (SEM). The samples were gold coated before use. The in vitro microstructural changes of the composites were also analyzed by means of SEM

5.2.4.3. TGA Analysis of Composites

Thermal analyses of pure PLA_1 and $PDLA_1$ films and their composites loaded with untreated HA were done using Shimadzu Thermal Gravimetric Analyzer (TGA,51). The experiments were carried out from room temperature up to 500 ^{0}C at heating rates 5, 10, 20 ^{0}C/min in order to determine the thermal degradation kinetics. The analyses were performed in a dry nitrogen atmosphere. N_2 flow rate was 40-ml/ min and kept constant throughout the experiments.

5.2.4.4. FTIR Analysis of Composites

The IR Spectrums of the composite films were taken by placing the samples on the way of the beam using the transmission technique. IR Spectroscopy was used to identify molecules and the types of bonds inside the polymer composite structure, the presence of possible solvents. All the samples spectra were taken between 400 cm^{-1} to 4400 cm^{-1} with a Shimadzu FTIR 8201 Model.

5.2.5. Hydrolytic Degradation of PLA, PDLA and Their HA Composites

Weight loss and water absorption during storage at 37 ^{0}C in simulated body fluid (pH 7.4) were determined for all pure polymer films (PLA_1, PLA_2, $PDLA_1$, $PDLA_2$) and two different polymer (PLA_1 and $PDLA_1$) composites loaded with untreated HA (10, 20,30,50 wt %). The effect of surface treatment on HA was also investigated on the water absorption and weight loss of the composites. The samples, which were nearly 80-100 mg, were immersed into a glass bottle containing 25 ml SBF. The bottles were allowed to stand in a thermo-stated oven for predetermined periods of time. After being incubated for various period of time, the specimens were removed from the fluid and rinsed with distilled water. After wiping, the specimens were weighed and dried at room temperature for 3 h and then dried at 37 ^{0}C until the weight is constant. Each data point is the mean of two measurements. The pH of sample fluid was also monitored by Metrohm pH-meter as a function of time. Experimental set up for the biodegradation studies in simulated body fluid was shown in Figure 5.6.

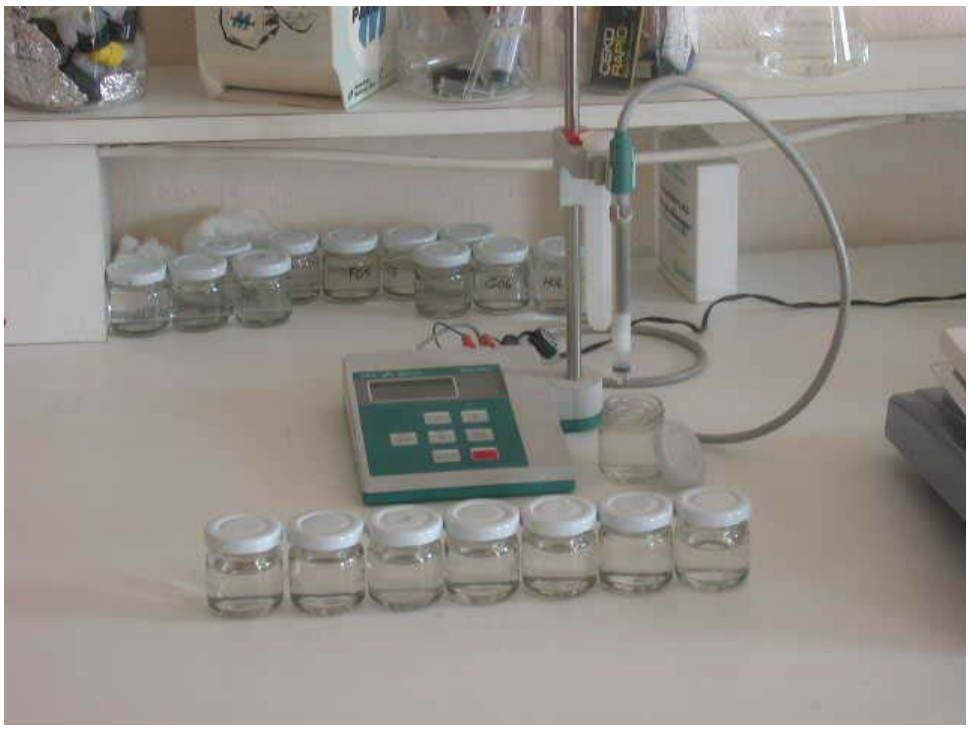


Figure 5.6. Experimental Set-up for the Biodegradation of the Films in Simulated Body Fluid.

5.2.5.1 Preparation of Simulated Body Fluid (SBF)

Simulated body fluid was prepared according to procedure in the literature [18] by dissolving reagent grade chemicals of NaCl, $NaHCO_3$, KCl, Na_2HPO_4, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, and Na_2SO_4 in deionised water and they were added to the solution in the order they are listed. The simulated body fluid solution was buffered at physiological pH 7.4 and at 37 ^{0}C with 50 mM trishydroxymethyl aminomethane [$(CH2OH)_3CNH_2$] (THAM) and 36.23 mM HCl acid. HCl was added before calcium chloride and THAM was the last reagent added to the solution. And also Sodium Azid (4 g) was added into SBF for preventing microbial effects. The amounts of reagent grade chemicals were listed in Table 5.5.

Table 5.5. Reagents Used for the Preparation of SBF (pH 7.4, 2 L)

Order	Reagent	Amount
1	NaCl	13.094 g
2	$NaHCO_3$	4.5360 g
3	KCl	0.7440 g
4	Na_2HPO_4	0.2480 g
5	$MgCl_2.6H_2O$	0.6100 g
6	1 M HCl	Nearly 80 ml
7	$CaCl_2.2H_2O$	0.7360 g
8	Na_2SO_4	0.1420 g
9	trishydroxymethyl aminomethane	12.114 g

Chapter 6

RESULTS AND DISCUSSION

In this study, the preparation and characterization of hydroxyapatite-polylactide composite films for biomedical applications were investigated. Poly(L-Lactide) and Poly(D-Lactide-L-Lactide) copolymer were used as a polymer matrix and HA was used as a filler for the preparation of the composites. Surface treatment of the filler was carried out with two different silane coupling agents: 3-aminopropyltriethoxysilane (AMPTES) and 3-mercaptopropyltrimethoxysilane (MPTMS) to improve dispersion of particles in the matrix and adhesion between polymer and filler. In the scope of this study, the effects of a number of parameters such as type of polymers, hydroxyapatite (HA) type, HA loading, surface modification of HA and its concentration on the mechanical, micro structural, thermal and hydrolytic degradation properties of the composite films have been investigated and reported.

6.1. Characterization of Hydroxyapatite Powder

Commercial HA and synthesized HA, which were untreated and treated, were characterized using different methods to examine particle size distribution, crystal phases, the chemical structure and the chemical reactions between silane coupling agents and HA, microstructure and Ca/P ratio of HA.

6.1.1. Particle Size Measurement of HA

Particle size measurement provides the determination of the size distribution of particles. Although the particles size of the commercial HA (HA_1) is nearly 10 μm, very hydrophilic HA particles may form agglomerates in the presence of moisture causing an increase in the average particle size. Therefore, commercial HA was ground. After size reduction process of HA, milled HA (HA_2) was obtained with an average particle diameter nearly as 3 μm. The average particle size of hydroxyapatite which was synthesized by precipitation technique was found as 0.28 μm. The particle size distributions of commercial HA powder (HA_1), milled HA powder (HA_2), and

synthesized HA powder (HA_3) were given in Table 6.1. The most fine particle size was obtained for the synthesized HA, (HA_3) as seen in Table 6.1.

Table 6.1. The Particle Size Distribution of Commercial HA Powder (HA_1), Milled HA Powder (HA_2), and Synthesis HA Powder (HA_3).

HA_1		HA_2		HA_3	
Low Diameter	Cumulative Mass %	Low Diameter	Cumulative Mass %	Low Diameter	Cumulative Mass %
10.00	81.7	10.00	87.3	5.000	99.6
5.000	69.1	5.000	76.5	2.000	95.1
2.000	46.9	2.000	63.2	1.500	91.4
1.500	40.8	1.500	59.5	**1.000**	**85.3**
1.000	**43.5**	**1.000**	**54.4**	0.500	73.5
0.500	27.0	0.500	47.0	0.100	54.6

6.1.2. FTIR Analysis of HA

The FTIR spectra of untreated HA powder in Figure 6.1 shows that the stretching and vibration band of OH^- appear at about 3572 cm^{-1} and 572-632 cm^{-1} respectively. Phosphate peaks can be seen at 1408, 1091, 603 and 571 cm^{-1} [57,58]. The IR spectrum at 1620 cm^{-1} represents the H_2O bending. As a result, FTIR spectra determined in this work matches with those reported previously in the literature for the HA powder. Figure 6.1 also shows the comparison of FTIR spectra of HA powder treated with 1 wt % aminofunctional silane (silane1) and mercaptosilane (silane 2). The silane deformation and siloxanes (Si-O-Si) bond formed as a result of reaction between HA and silane coupling agents absorbs at 1030 and 1130-1000 cm^{-1}, respectively. These characteristics group indicating the reaction between the silane and HA were not observed in the silane treated HA. This may be due to presence of the strong vibration of HA in the range of 1200-900 cm^{-1} and also amount of silane concentration used on HA modification.

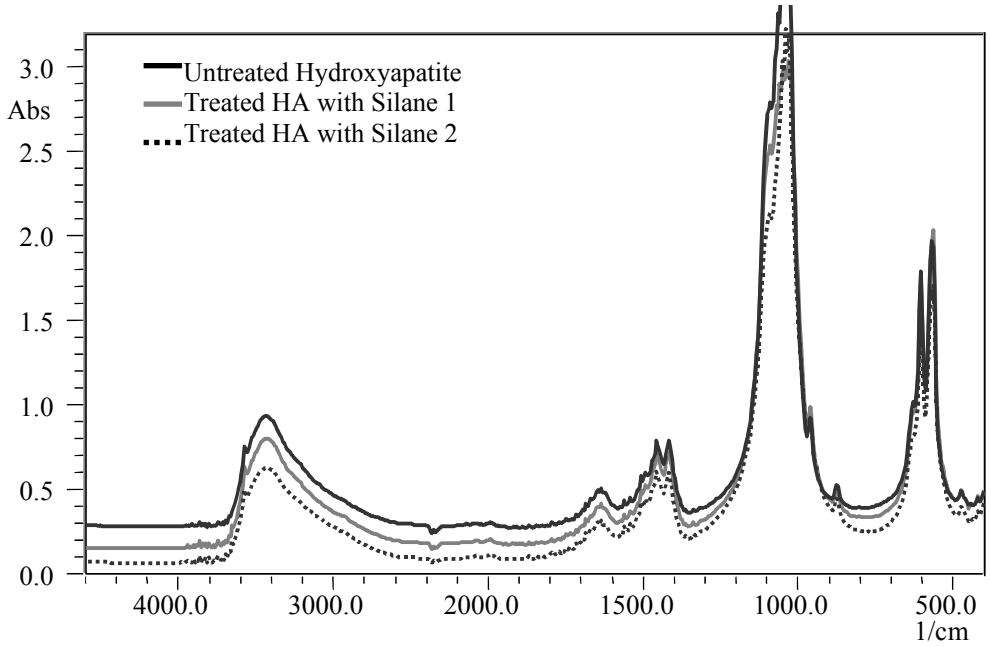


Figure 6.1. FTIR Spectra of Untreated Hydroxyapatite, Treated HA with Silane 1 and Silane 2.

6.1.3. X-Ray Diffractometry (XRD) of HA

The phase composition and structure of the synthesized HA powder was characterised by XRD. X-ray diffraction provides information about the crystal thickness in mats of single crystals. Wide-angle X-ray diffraction can be used to determine the crystal size. Small crystals give broad Braggs reflections and a simple way of assessing the crystal thickness (D_{hkl}) perpendicular to a given set of (hkl) planes is through the Scherrer equation [59].

$$D_{hkl} = K\lambda / \beta \cos \theta \qquad (6.1)$$

where;

K : the Scherrer shape factor

λ : the wavelength of the x-ray

β : the breadth of the diffraction peak associated with the hkl planes.

Figure 6.2 illustrates XRD patterns of synthesized HA powders. The result showed that XRD pattern of synthesized powder HA obtained in these work matches with these reported previously in the literature [60]. Crystallographic particle size of HA that was determined by using Equation (6.1) was found as 27 nm.

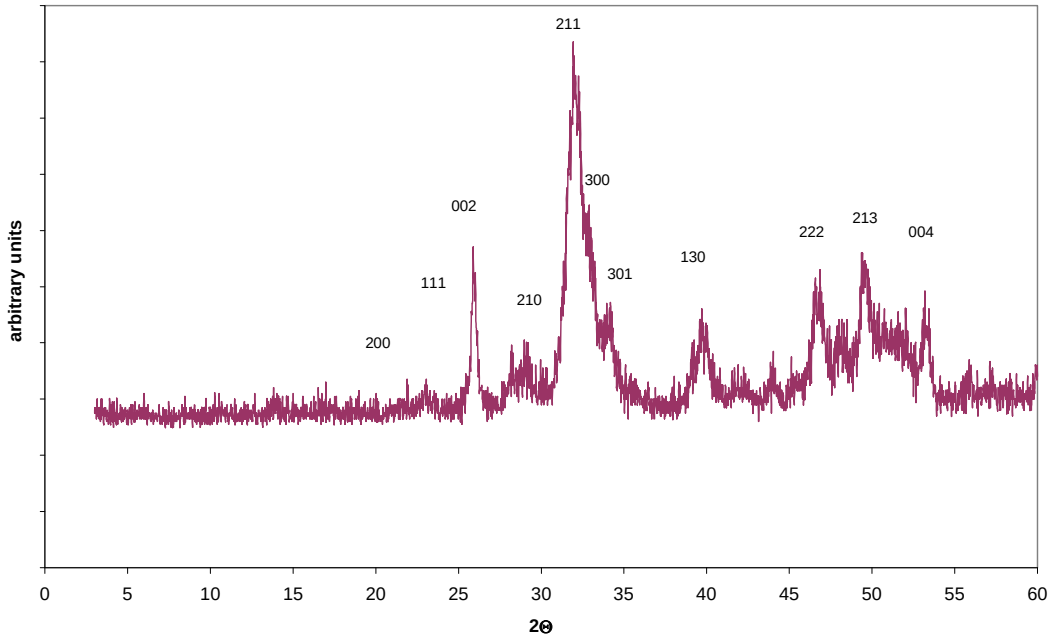
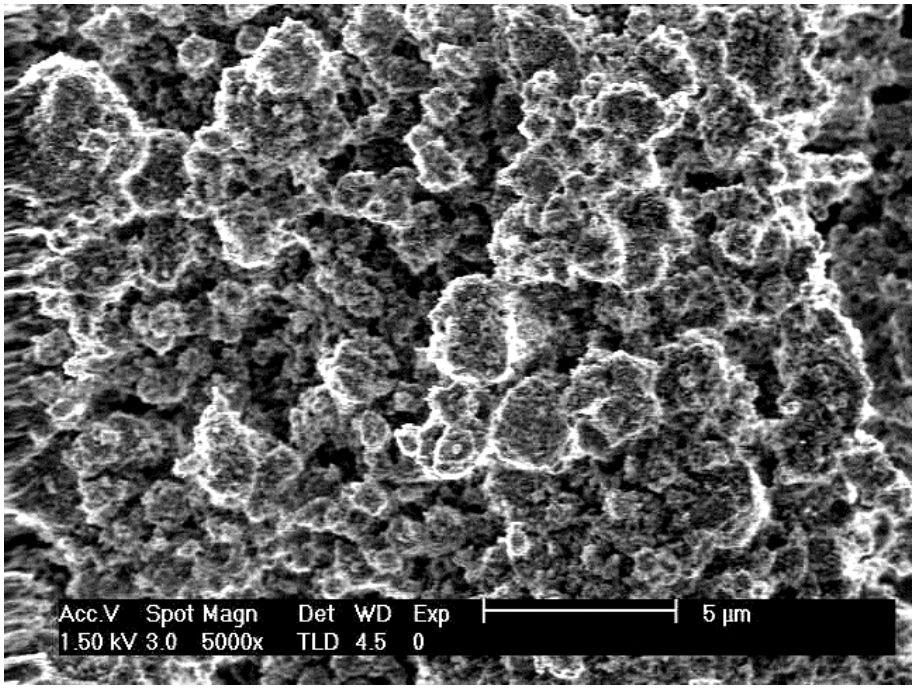


Figure 6.2.The X-ray Diffraction Pattern of HA Powder

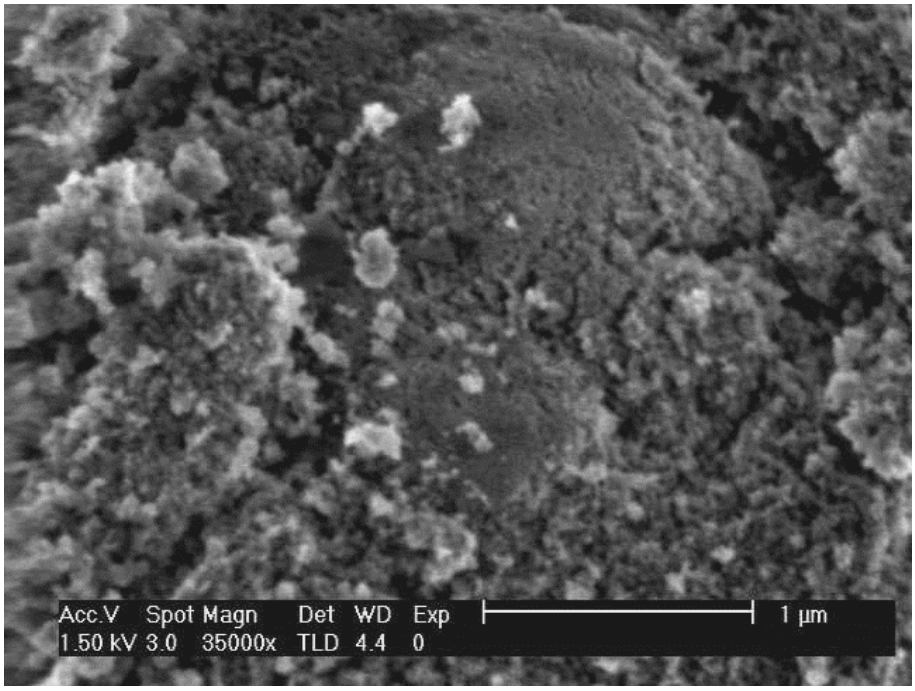
6.1.4. Microstructural Properties of HA

Figure 6.3 (a) and (b) show SEM micrographs of untreated and treated HA with 1 wt % aminofunctional silane coupling agent, respectively. As shown in Figure 6.3(b), the surface modification of HA with silane coupling agent reduces the agglomerate size of HA particles significantly.

Figure 6.4(a) and (b) illustrate EDX analysis results of the untreated and treated HA with 1 wt % aminofunctional silane. Ca/P ratio of the untreated HA powders was determined by SEM-EDX analysis and found as approximately 1.6. EDX studies showed that the major surface components of untreated HA surface were Ca, P and O. As seen in Figure 6.4 (b), Si and N atoms were observed in the spectra of treated HA powders which indicated the presence of silane coating on HA.

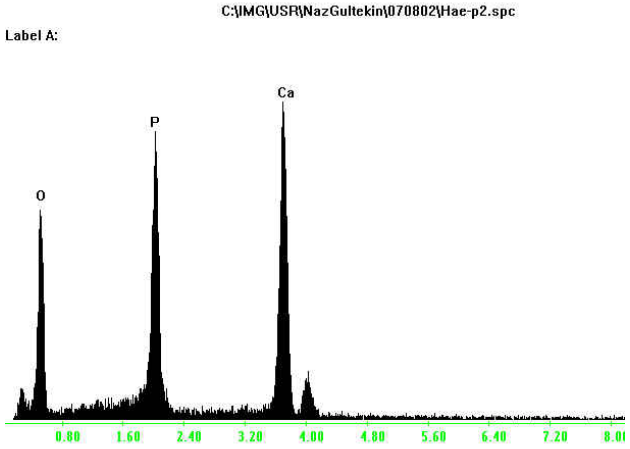


(a)

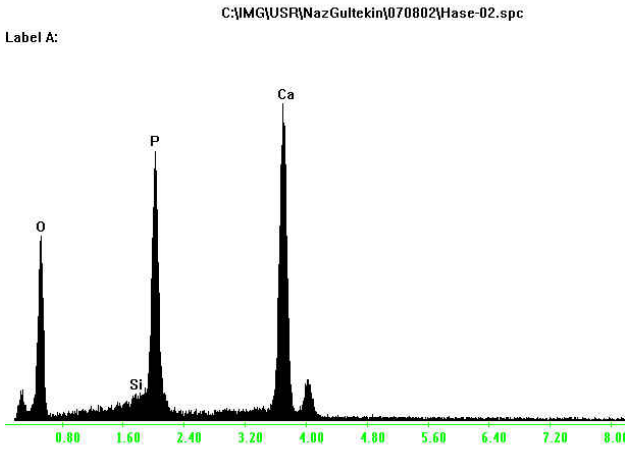


(b)

Figure 6.3 SEM Micrographs of Hydroxyapatite Prepared by Precipitation Technique. (a) Untreated HA (b) Treated HA with Silane Coupling Agents.



(a)



(b)

Figure 6.4 EDX spectra of the untreated and treated HA with aminofunctional silane coupling agent.

6.2. Characterization of HA/ PLA Composite

The PLA-Hydroxyapatite composite films were prepared by solvent casting techniques. In the initial stages of the work, the optimum polymer/solvent ratio for all polymers studied were determined. Chloroform was used as a solvent in all polymer-solvent solution procedure. The visual observations on the properties of the four different films were given in Tables 6.2-6.5, for PLA_1, $PDLA_1$, PLA_2, and $PDLA_2$, respectively. During preparation of polymer –solvent solution, other process variables were kept constant. The optimum polymer /solvent ratio for each polymer was

determined and found as 0.1, 0.05, 0.05 and 0.04 for PLA_1, $PDLA_1$, PLA_2, and $PDLA_2$, respectively and fixed for the rest of this work.

Table 6.2. Effect of Polymer/ Solvent Ratio for PLA_1/Chloroform System

PLA-1/Chloroform (g/cc)	Final Structure
0.07	Nonuniform and thin thickness,
0.1	Uniform thickness
0.12	Highly viscous paste

Table 6.3. Effect of Polymer/ Solvent Ratio for $PDLA_1$/Chloroform System

PDLA-1/Chloroform (g/cc)	Final Structure
0.07	Highly viscous paste
0.05	Uniform thickness
0.03	Nonuniform and thin thickness,

Table 6.4. Effect of Polymer/ Solvent Ratio for PLA_2/Chloroform System

PLA-2/Chloroform (g/cc)	Final Structure
0.07	Highly viscous paste
0.05	Uniform thickness
0.03	Nonuniform and thin thickness,

Table 6.5. Effect of Polymer/ Solvent Ratio for $PDLA_2$/Chloroform System

PDLA-2/Chloroform (g/cc)	Final Structure
0.05	Highly viscous paste difficult solving
0.04	Uniform thickness,
0.03	Very thin, Uniform thickness

In the preparation of polylactide-hydroxyapatite composites by the solvent technique, two different preparation methods were carried out. In the first method, firstly, polymer-solvent mixture is prepared and then, HA is added (PLA or PDLA + solvent + HA). Second method follows firstly the dispersion of HA in the solvent, and then polymer is added (HA + solvent + polymer) PLA-HA composites prepared by

second method gave more homogeneous dispersion of HA in polymer matrix than those prepared method by first method.

6.2.1. Mechanical Testing of HA/PLA Composites

Tensile tests of Poly(L-Lactide) and Poly (D-Lactide-L-Lactide) copolymer composites filled (10, 20, 30, and 50 wt %) with the untreated and treated hydroxyapatite were conducted to determine how mechanical properties were influenced by the particle size of HA, the presence of surface modifiers (AMPTES and MPTMS) and their concentrations, polymer types and HA loading. Experimental tensile test results of these samples were given in Table A.1 in the Appendix. Young's Modulus, yields stress, tensile fracture stress and fracture strain values of the PolyLactide composites as a function of HA were measured.

The effects of polymer type, preparation method, HA loading, surface treatment of HA on the Young's modulus of the Polylactide-HA composites were studied and shown in Figures 6.5-6.12.

Figure 6.5 indicates modulus of elasticity of different PolyLactide polymers used in this study. As expected and seen in the Figure 6.5, since poly-L-Lactide polymers, PLA_1 and PLA_2 are semicrystalline polymers, their modulus should be higher than Poly L- Lactide-D Lactide copolymers ($PDLA_1$ and $PDLA_2$) which have amorphous structure. Elastic Modulus of PLA_1 was found to be the highest among all. Among the $PDLA_1$ polymers, $PDLA_2$ has higher elastic modulus.

Figure 6.6 shows the Young Modulus of PLA_1 composites containing 10 wt % HA which were prepared by two different methods. Young Modulus of composites that was prepared by the second method was greater than those prepared by the first method. This is due to fact that the better dispersion of HA in polymer matrix and less agglomeration of the HA particles in the polymer matrix were obtained. Since the homogeneous dispersion plays an important role in improvement of the mechanical properties, the better mechanical properties were obtained for the composites prepared by second method. Table 6.6 shows the mechanical properties of the PLA-HA composite containing 10 wt % HA prepared by both first and second methods.

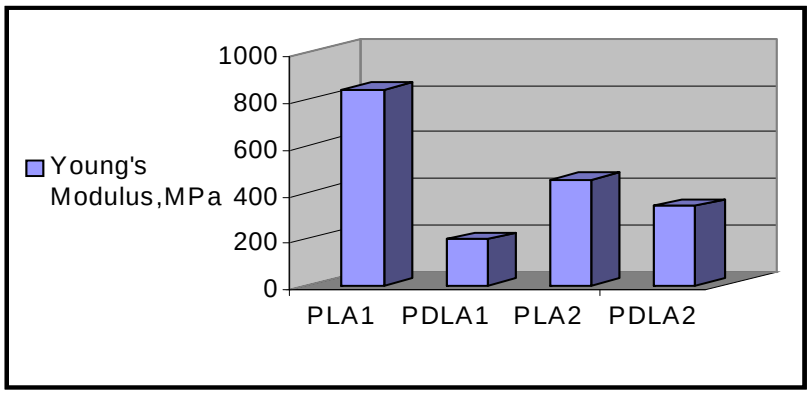


Figure 6.5 The Young's Modulus of Polymers

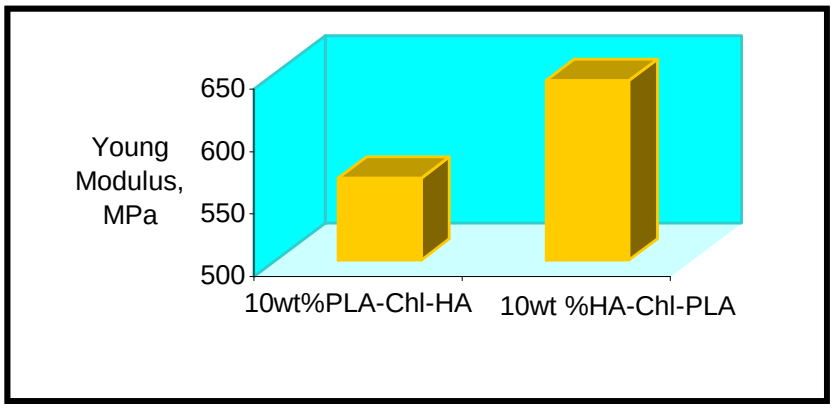


Figure 6.6 Effect of Preparation Methods on Young Modulus Value of the 10 wt % HA Loaded PLA_1 Composites.

Table 6.6. The Comparison of the Mechanical Properties of the 10 wt % Loaded PLA_1 Composites Prepared by two Different Preparation Methods.

Mechanical Properties	**First Method**	**Second Method**
Young's Modulus, MPa	565.8	644.4
Yield Stress, MPa	23.1	28.1
Stress at break, %	4.7	5.1
Elongation at break, mm	3.2	3.7

The effect of particle size of hydroxyapatite powder on the mechanical properties of the PLA_1 composites containing 10 wt % HA particles were investigated and shown in Table 6.7. As shown in Table 6.7, Young's Modulus and yield stress increased as the particle size of HA reduced. On the other hand, stress at break and elongation at break values decreased with decreasing particle size.

Table 6.7 Effect of Particle Size of Hydroxyapatite on Mechanical Properties of PLA_1 Composites

Mechanical Properties	HA_1/PLA_1	HA_2/PLA_1	HA_3/PLA_1
Young's Modulus, MPa	644.2	676.5	877.7
Yield Stress, MPa	28.14	30.9	39.2
Stress at break, %	5.10	4.8	4.8
Elongation at break, mm	3.7	3.9	3.4

Figure 6.7 shows typical stress-strain curve of the PLA_1 composite containing 10 wt % HA obtained from Tensile Test Machine, as described above. The slope of the initial linear part of the curve gives the "Young's Modulus". The maximum stress values (indicated with bars) are defined as the "yield point". These values for the composites were measured and given in Appendix 1-A.

The effects of HA content and polymer type on Young Modulus (Elastic Modulus) of PLA-HA composites were studied and shown in Figure 6.8. Young's Modulus clearly had a positive correlation with the mixing ratio of composites. Thus, Young's Modulus of the composites depends on the loading of HA. It was observed that Young's Modulus of the composites increased as HA content increases. Up to 40 wt % HA loading for both polymer types then, started to decrease. This could be due to the increase in agglomeration size of the HA particles in the polymer matrix which could cause the weak adhesion between polymer and filler.

In addition to this, it was decided by the composite effect of Young Modulus of both HA and polymer type. Young Modulus of PLA_1 and its composites are found to be significantly higher than that of $PDLA_1$. This is again due to the fact that PLA is a semi crystalline polymer and its mechanical strength should be higher compared to the amorphous PDLA polymer as expected.

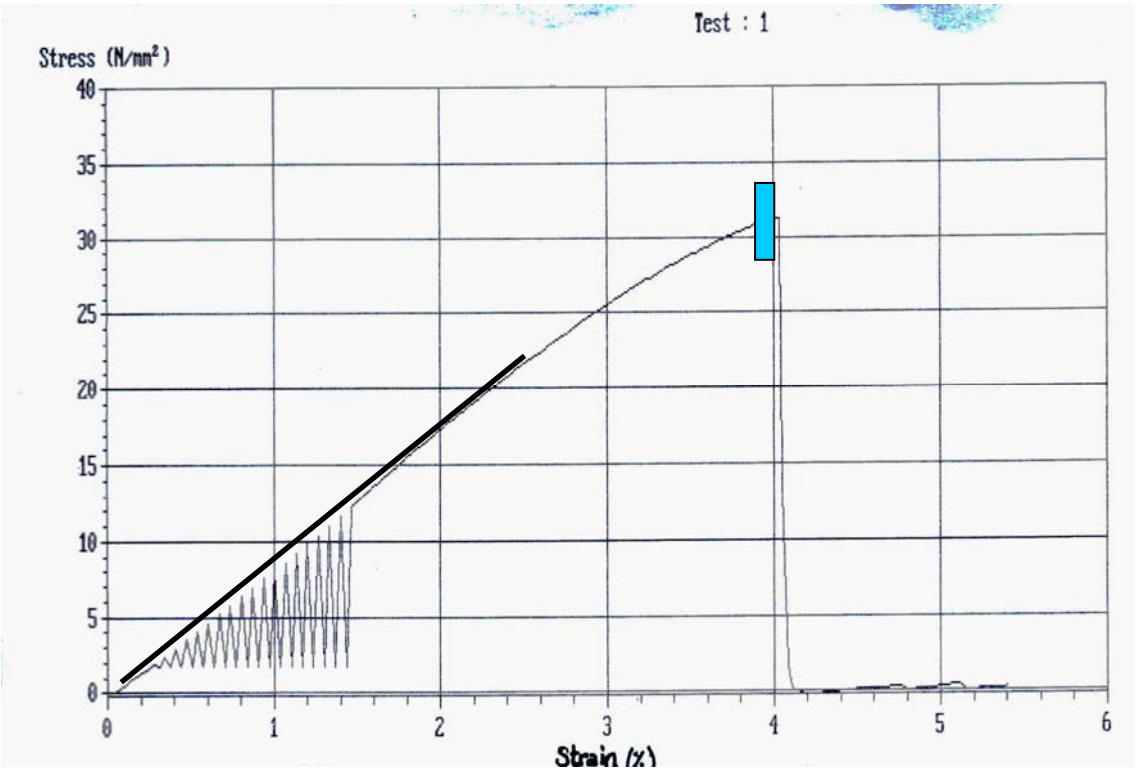


Figure 6.7 Typical Stress-Strain Curves of the PLA_1 Composite Containing 10 wt % Untreated HA.

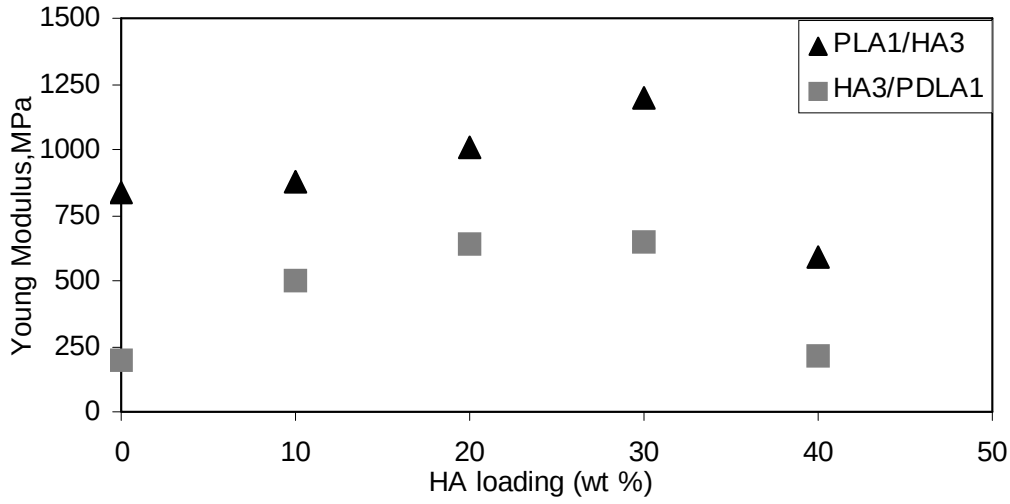


Figure 6.8 Young's Modulus of PLA_1/HA_3 and $PDLA_1/HA_3$ Composites with Respect to HA_3 Content.

The effect of surface treatment of the HA filler on Young's Modulus of the composites was investigated. Figure 6.9 and 6.10 show the influence of the surface modifier concentration of silane coupling agents on Young's modulus of composites containing 20-wt% hydroxyapatite for PLA_1 and $PDLA_1$, respectively. Young's moduli of composites were measured for three different types of silane concentration, which was 0.5, 1, and 2 wt %. As seen in the figures, silane treatment leads to increase in Young Modulus due to the improvement of adhesion between polymer and filler and dispersion of filler in the polymer matrix. Although 1 wt % AMPTES (Silane1) coupling agent concentration shows maximum on Young Modulus of the PLA composites, 0.5 wt % MPTMS (Silane2) coupling agent concentration shows a maximum for the PDLA composites as seen in Figures 6.9 and 6.10. For both composites, Young Modulus decreases with increasing silane concentration after the maximum point. The maximum Young's Modulus values for 1 wt % silane1 coupling agent concentration show the maximum strength of interaction between polylactide and hydroxyapatite. Therefore, 1 wt % silane1 concentration was choosen for PLA composites. On the other hand, the maximum Young's Modulus values for PDLA composites were seen at 0.5-wt % silane2 coupling agent concentration. The PDLA composite modified with silane 2 is always stiffer than the one containing untreated HA and modified with silane1. For this reason, surface modification of HA was done 1 wt % silane 1 concentration, and 0.5 wt % silane 2 concentration for PLA and PDLA composites, respectively.

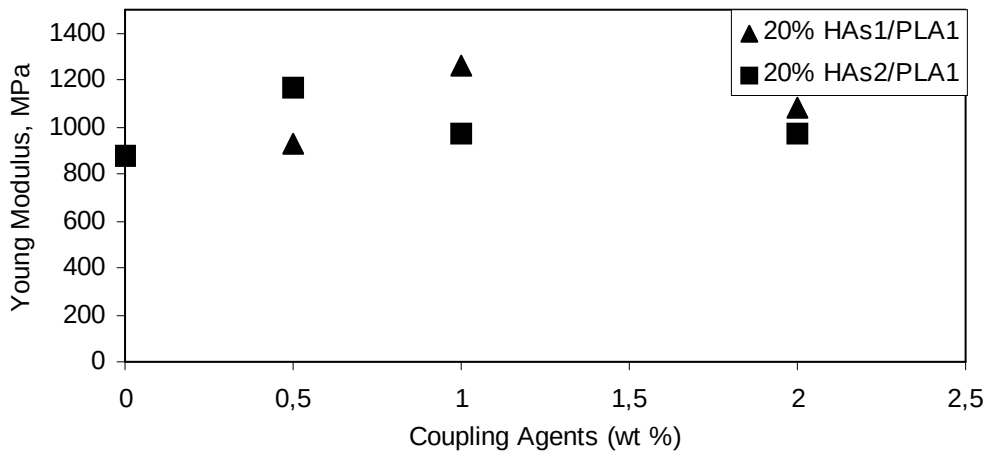


Figure 6.9 Effect of Silane Coupling Agents Concentration on the Young's Modulus of PLA_1 Composites Containing 20 wt % HA.

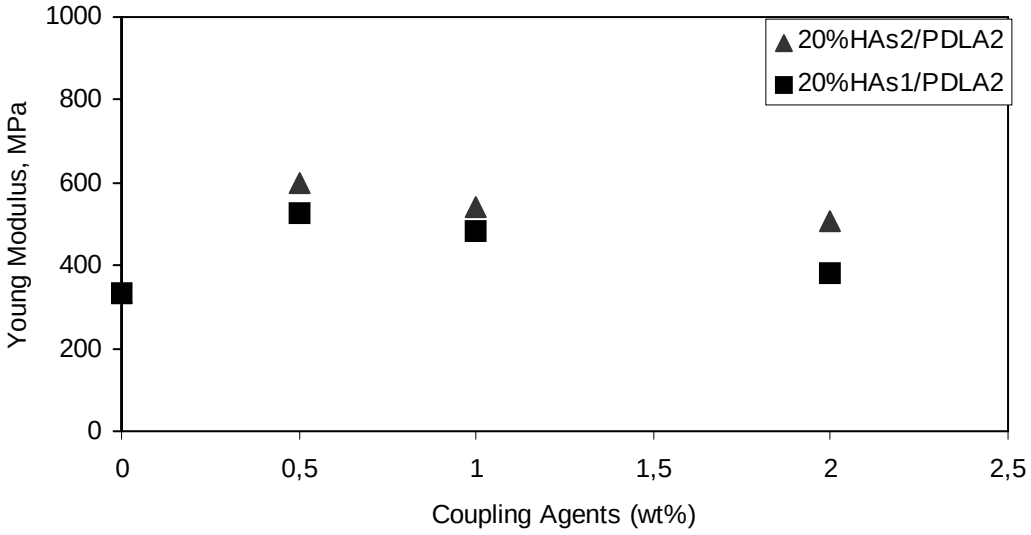


Figure 6.10 Effect of Silane Coupling Agents Concentration on the Young's Modulus of $PDLA_2$ Composites Containing 20 wt % HA.

Deng et al [35] studied the preparation and mechanical properties of nanocomposites of Poly (D,L-Lactide) with Ca-deficient HA nanocrystals which were prepared by solvent casting technique using dimethylformamide. The obtained nanocomposites are semitransparent films with the thickness about 0.1 mm. The relationship between the HA particles loading and tensile modulus for HA/ PLA composites has been investigated in their study. For the composite containing 10.5 v % HA, the modulus even reaches to 2.47 GPa as compared to 1.66 GPa for pure PLA. Tensile modulus for composite increases with HA loading. Yield stress for the composites varies irregularly with HA loading. In the other study, the preparation and characterization of HA / PolyL-Lactide composites were examined by Shikinami and coworkers [25]. The composites were prepared by forging process. Uncalcined and unsintered HA (u-HA) were used as filler in their study. Effect of u-HA loading on the mechanical properties of u-HA / PLA was investigated. The gradual increase in tensile modulus was proportional to the fraction of HA particles.

Figure 6.11 and 6.12 show modulus of elasticity of PLA composites containing untreated and treated HA with 1 % AMPTES and PDLA composites containing untreated and treated HA with 0.5 % MPTMS, respectively. The modulus of composites increased with increasing untreated and treated HA content up to 30-wt % and then decreased. This may be due to the increase in the agglomeration size with an increase in

HA loading which could cause poor adhesion between polymer and HA. The adhesion between polymer-filler plays an important role in the improvement of mechanical properties. As seen in these Figures, Young's Modulus values of all PLA and PDLA composites loaded with treated HA were greater than those loaded with untreated ones. This result indicated that the better dispersion HA into polymer matrix and interfacial enhancement between hydroxyapatite and poly-L-Lactide or poly D,L-Lactide matrix were achieved by the treatment of HA with silane coupling agents.

According to the other mechanical test results given in Appendix 1A, two important points can be discussed. Yield stress of the composite decrease with an increase in HA content for both polymer types. The percent elongation becomes much smaller than in the composites with higher HA contents as expected. PDLA copolymer and its composites are relatively softer, weaker, and much more flexible compared to the PLA homopolymer and its composites.

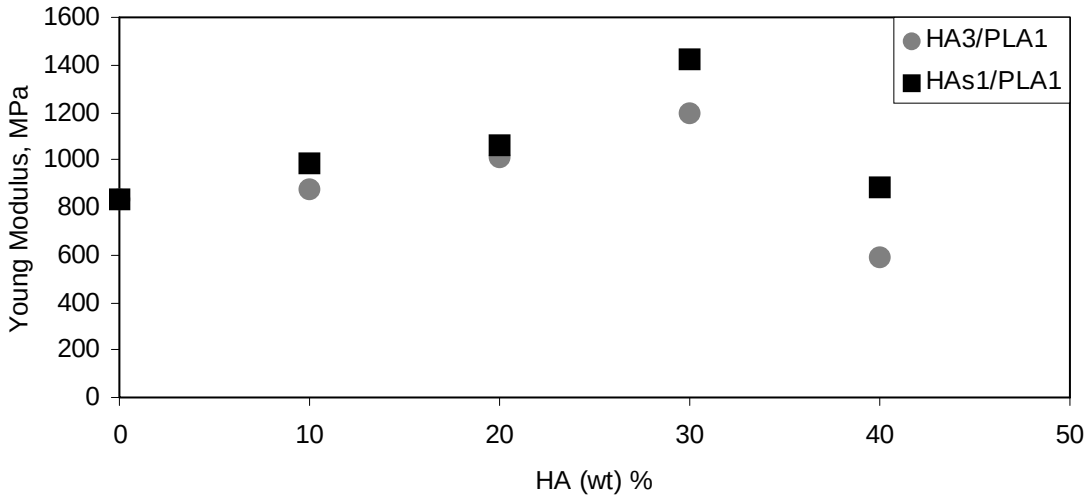


Figure 6.11 Effect of Coupling Agents on Young Modulus of PLA Composites with Respect to HA Contents.

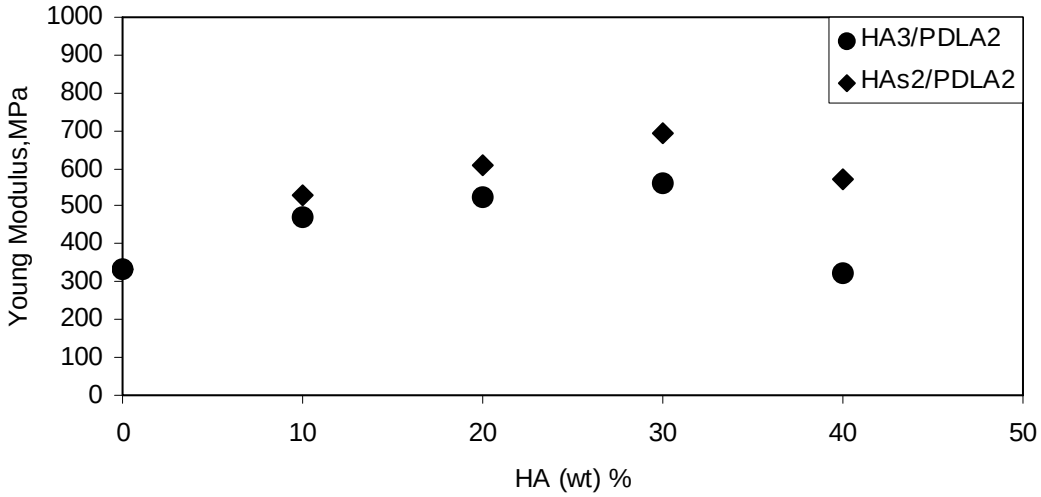


Figure 6.12 Effect of Coupling Agents of Young Modulus of PDLA Composites with Respect to HA Contents.

6.2.2. Microstructure Analyses

The effect of preparation methods on microstructural properties of PLA composites containing 10 wt % HA was examined by their optical micrographs. In the second method, HA was dispersed in chloroform using ultrasonics for 45 min prior to PLA dissolved. Optical micrographs of PLA composites prepared by first method and second method for 10 wt% untreated as-received commercial HA composites are shown in the Figures 6.13 and 6.14, respectively. As seen in these figures, HA is better-dispersed in PLA composite, which were prepared by second method, compared to the composites prepared by first method. Although there are still agglomeration of HA particles in PLA matrix in Figure 6.13, the sizes of the agglomerations are much lower.

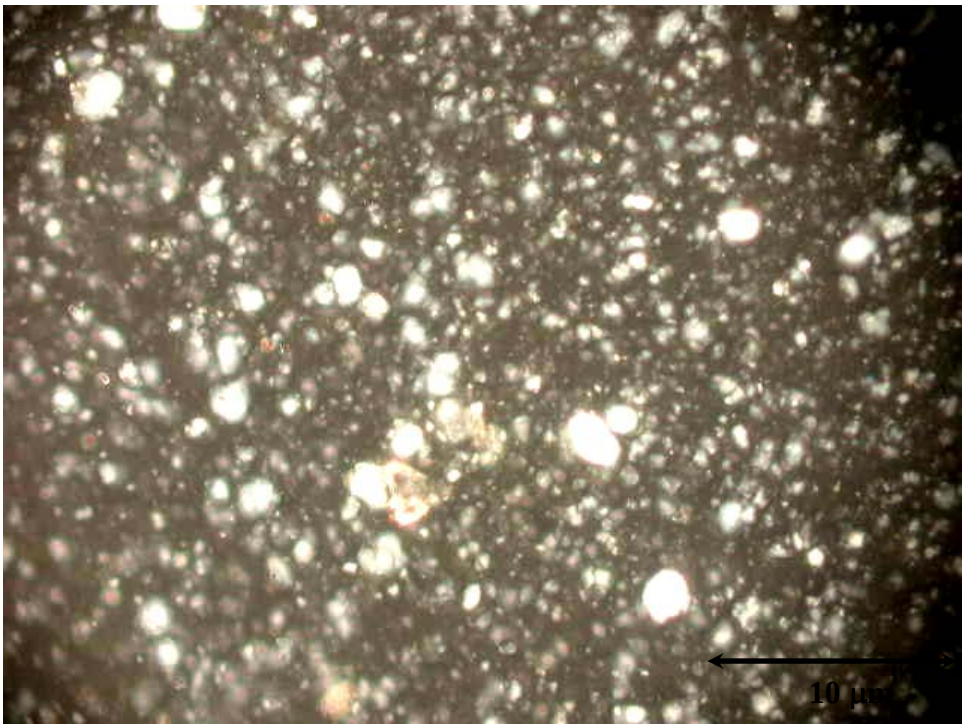


Figure 6.13. Optical Micrographs of PLA-HA Composite (First preparation method)(20X)

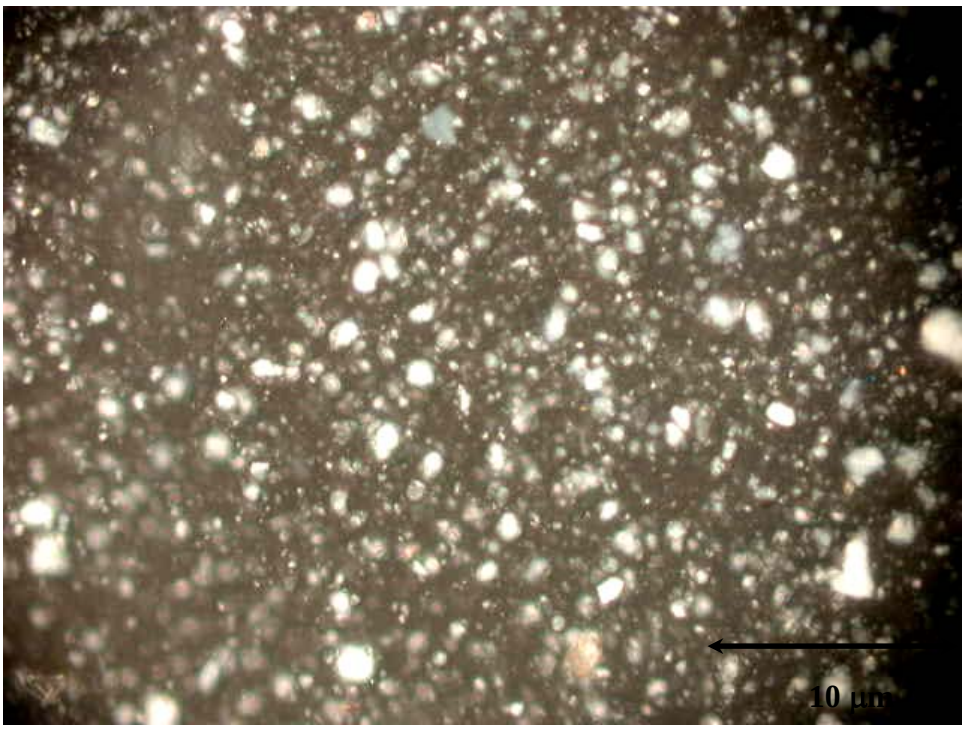


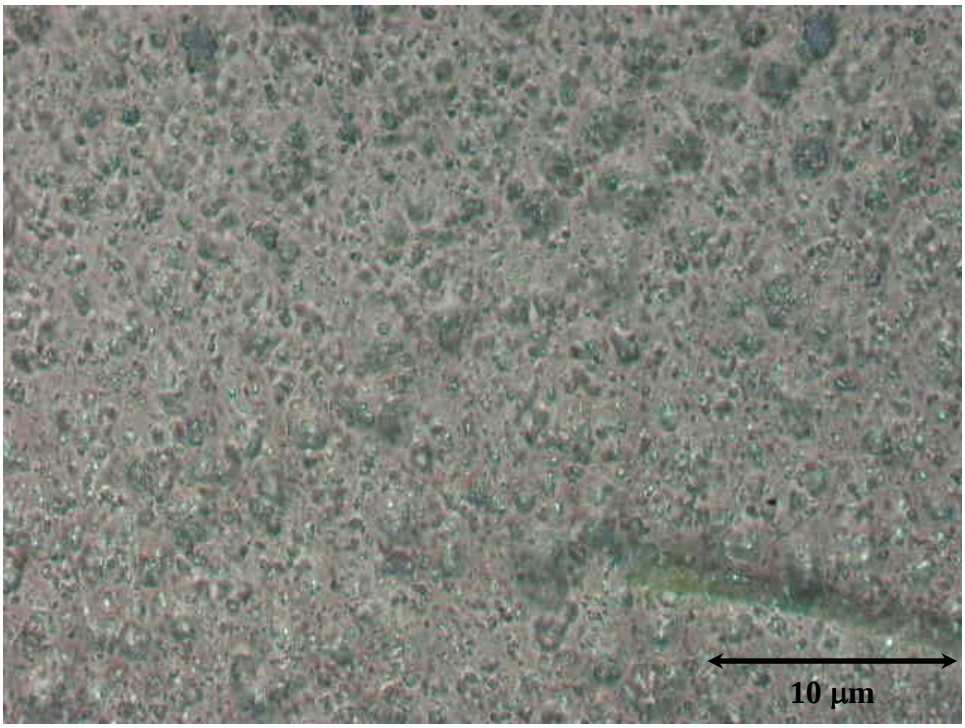
Figure 6.14. Optical Micrographs of PLA-HA Composite (2nd preparation method)(20X)

The effect of the surface modifier concentration of silane coupling agents for different polymers (PLA, PDLA) on surface structure of composites containing 20-wt% hydroxyapatite was examined by the optical micrographs. Figure 6.15 shows the optical micrographs of PLA composites containing treated HA with 0.5, 1 and 2 wt % AMPTES. As seen in the Figure 6.15 (b), 1 wt % AMPTES treated HA shows more homogeneous dispersion of HA particles in the PLA matrix. Since good dispersion of filler in the matrix is required in order to obtain composites having satisfactory mechanical properties, 1 wt % AMPTES was selected as optimum silane coupling agent concentration for PLA matrix composites.

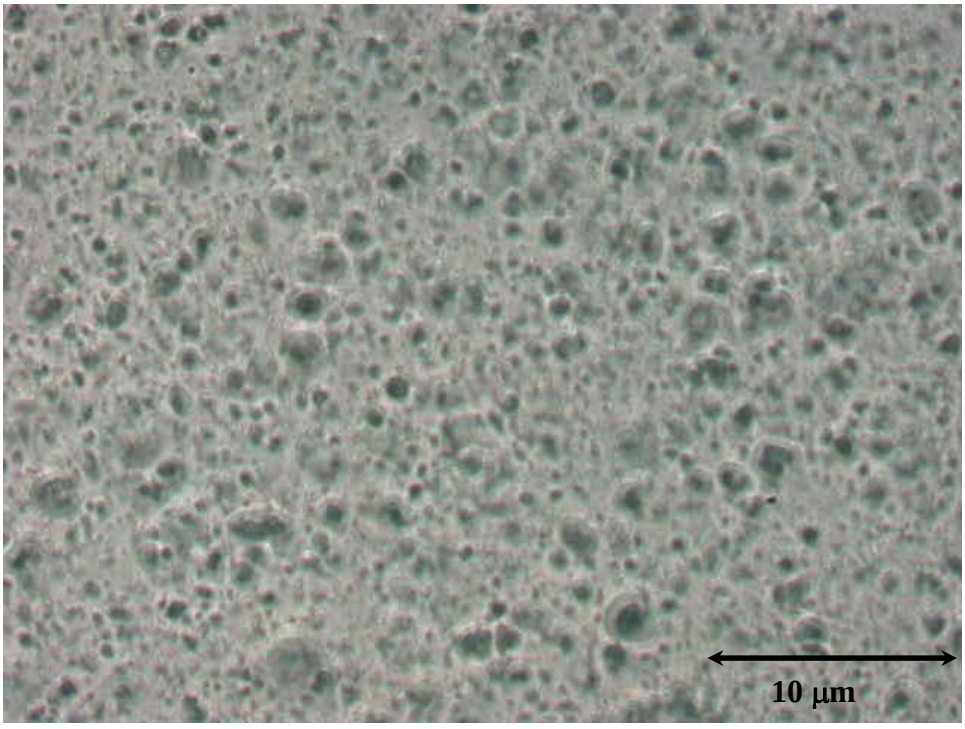
The optical micrographs of PLA composites consist of treated hydroxyapatite with 0.5, 1, 2 wt % MPTMS are illustrated in Figure 6.16. As seen in the figure, as a result of agglomeration and voids, non-homogeneous distribution of treated HA particles in the PLA matrix. The morphology of these composites was compared to those of corresponding the PLA composite containing treated HA with 0.5, 1, 2 wt % AMPTES. Mechanical and microstructural properties of PLA composite containing treated HA with 1 wt % AMPTES gave the mechanical results. Therefore, 1 wt % AMPTES was choosen as optimum silane coupling agent concentration for PLA composites.

The effect of the surface modifier concentration of silane coupling agents for PDLA on surface structure of composites containing 20-wt% hydroxyapatite was also examined by the optical micrographs. Merkapto silane coupling agent was used for the modification HA in the PDLA composite because treated HA with 0.5 wt % MPTMS gave the better dispersion of HA in the PDLA matrix, as well as better good mechanical properties. Figure 6.17 shows optical micrograph of PDLA composite containing 20 wt % modified HA with 0.5 wt % MPTMS.

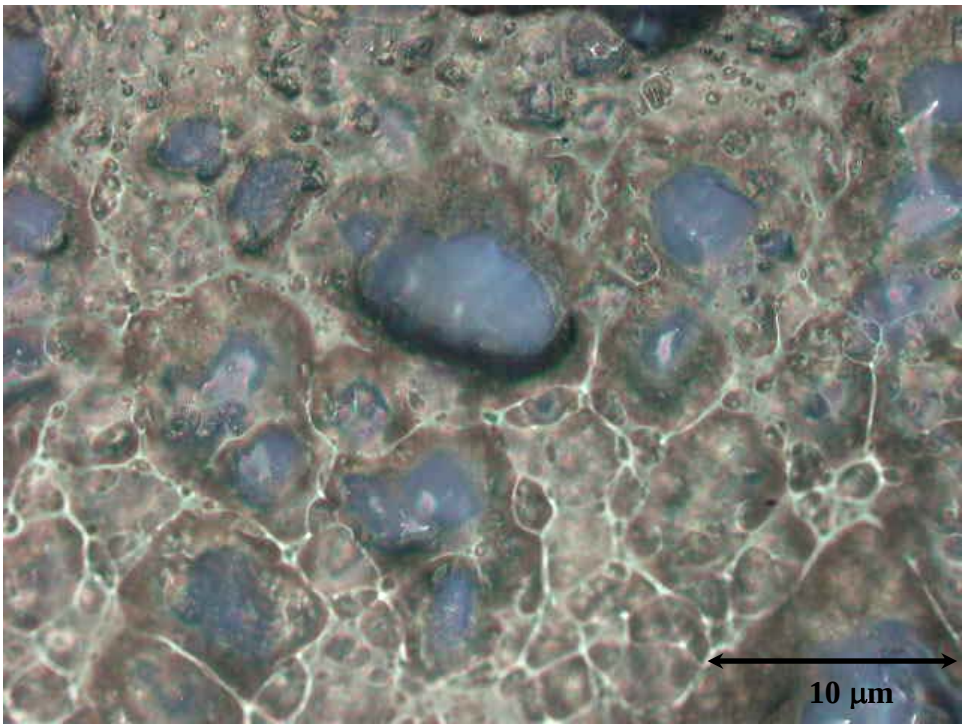
The effect of particle size of HA on the microstructural properties was also studied. Figure 6.18 indicates the SEM images of PLA composites containing 10 wt % untreated HA prepared by using three different particle size of HA. Larger HA particles affect appearance of deformation of the polymer composites. On the other hand, aggregation tendency of small particles is higher than that of large particles. As shown in the Figure 6.18 (c), the dispersion of HA in the polymer matrix is much better in PLA composites containing smallest particle size of HA.



(a)

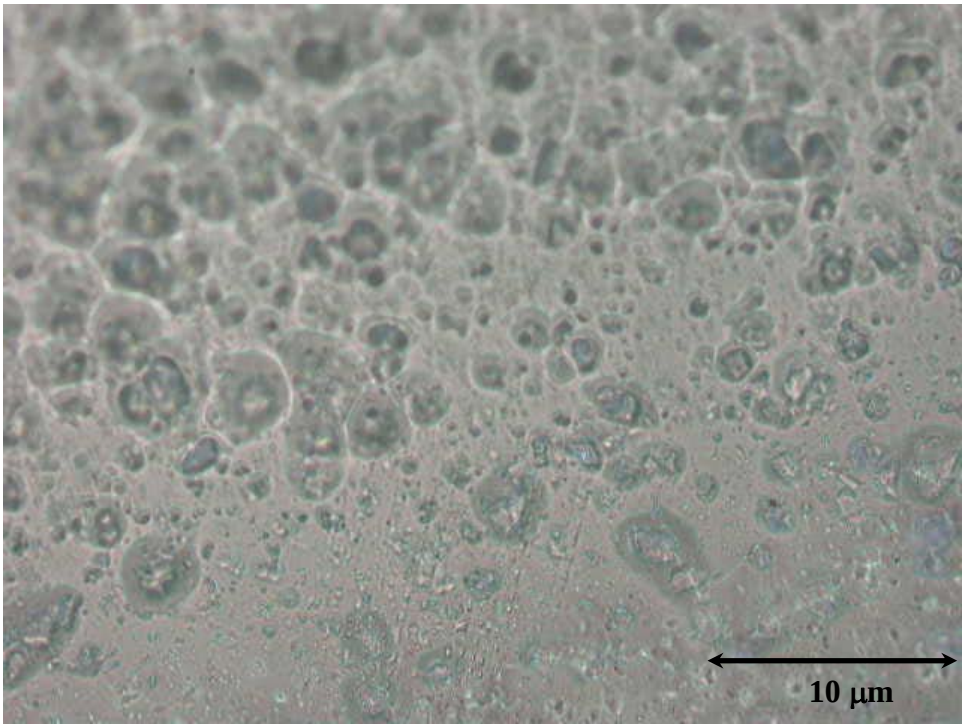


(b)

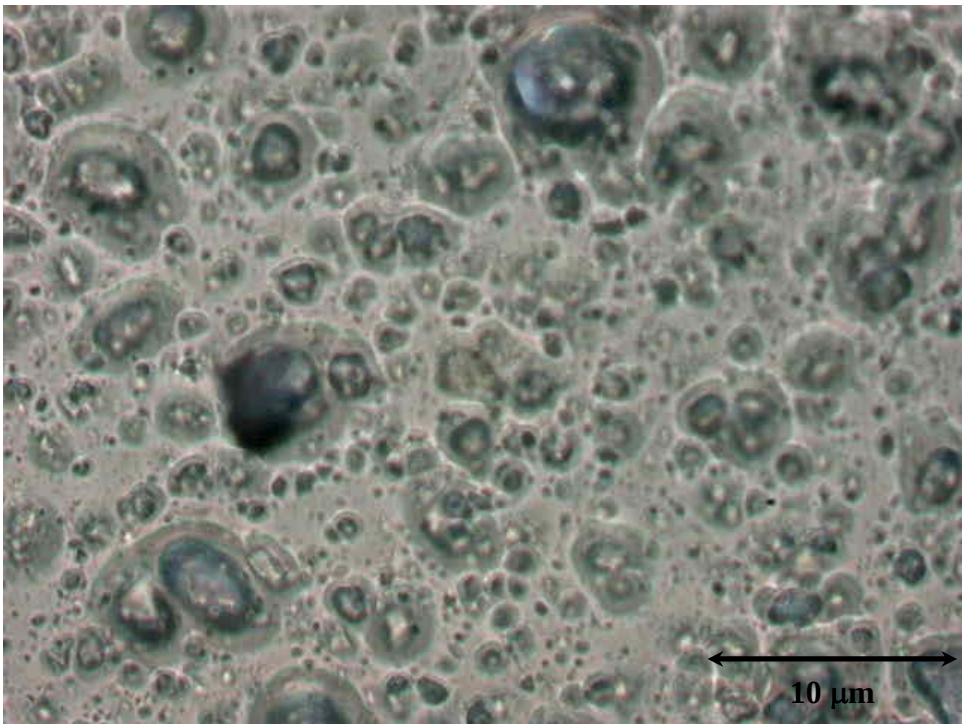


(c)

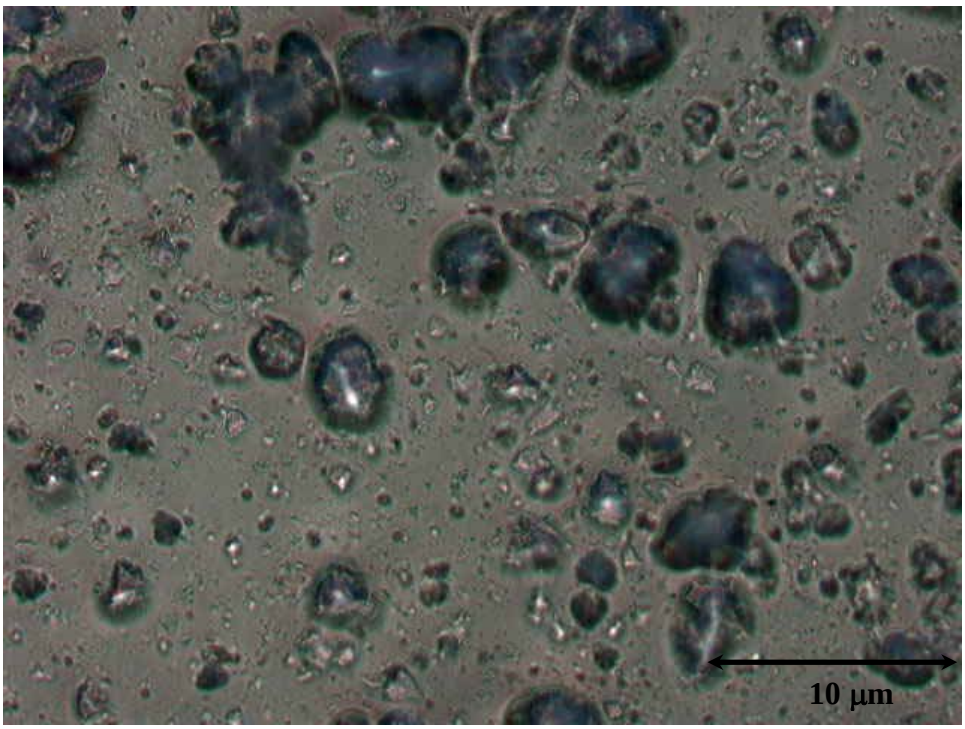
Figure 6.15 Optical Micrographs of PLA Composites Containing 20 wt % Treated HA with (a)0.5 wt % AMPTES/HA, (b)1 wt % AMPTES/HA, (c) 2 wt % AMPTES/HA



(a)



(b)



(c)

Figure 6.16 Optical Micrographs of PLA Composites Containing 20 wt % Treated HA with (a) 0.5 wt % MPTMS/HA, (b) 1wt% MPTMS/HA, (c) 2 wt % MPTMS/HA

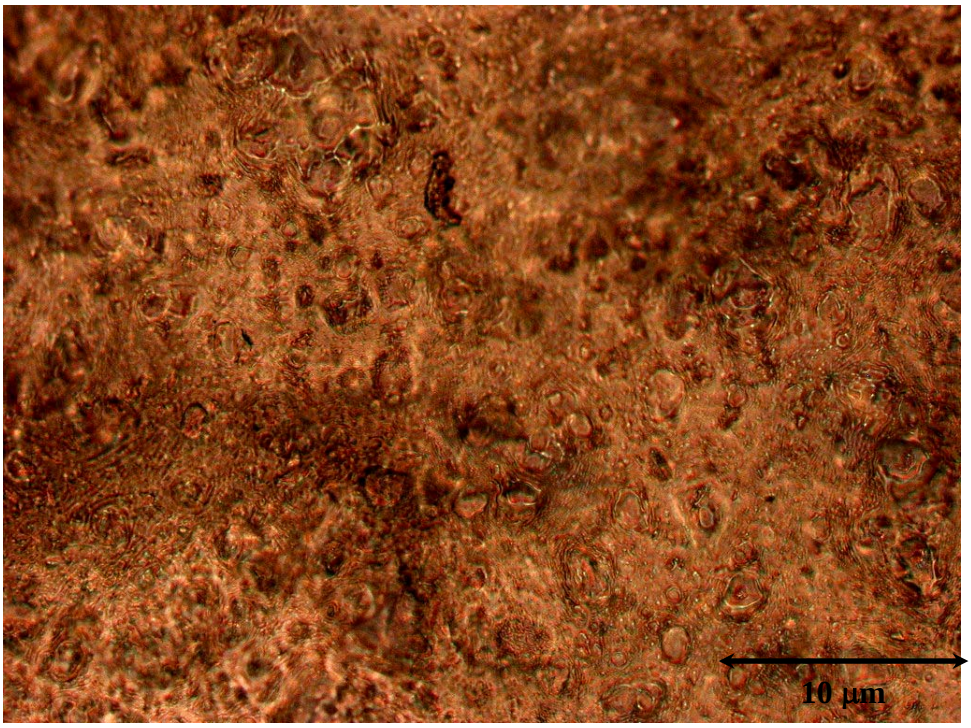
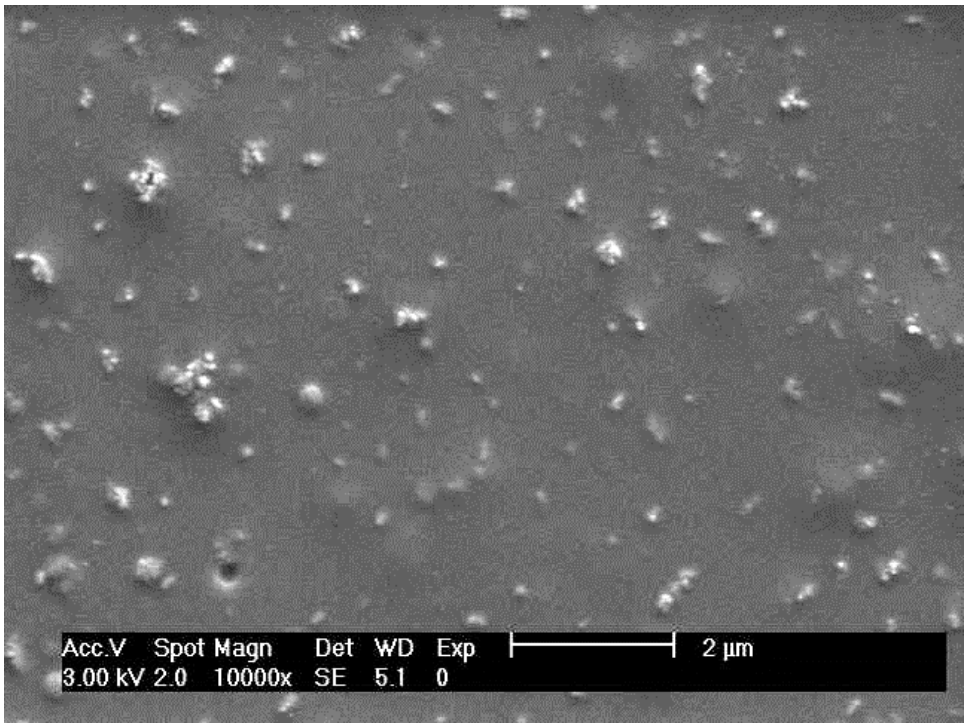


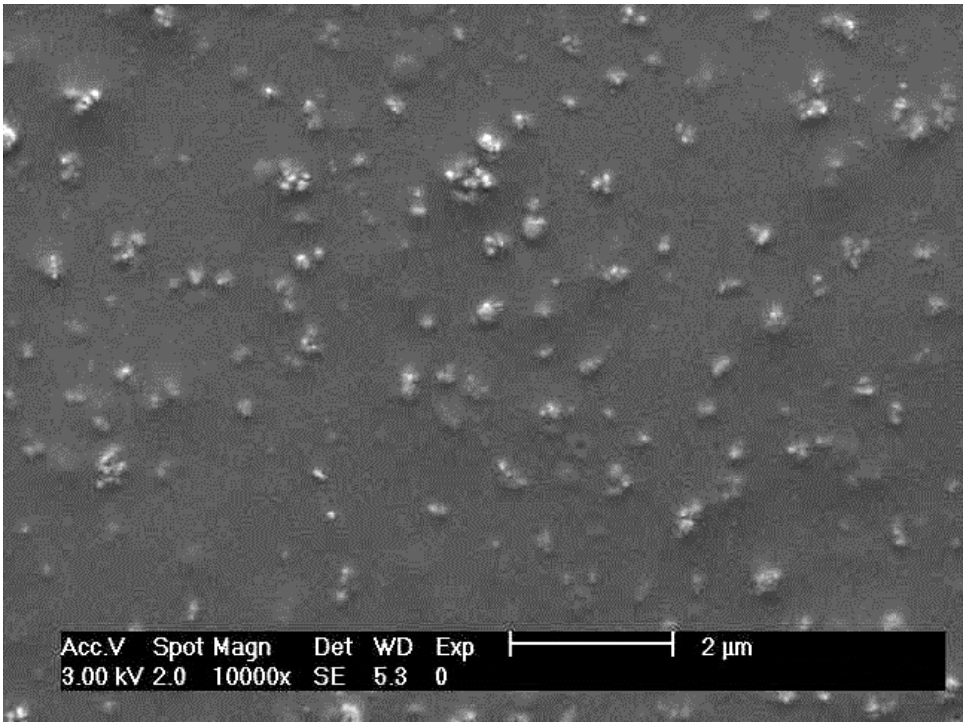
Figure 6.17 Optical Micrograph of PDLA Composites Containing Treated HA with MPTMS.

SEM micrographs of tensile fracture surfaces for PLA composites with two different particle size of HA are shown in Figure 6.19. As seen in the Figure 6.19(b), although the agglomerate size of synthesized HA (HA_3) in the PLA matrix was higher than that of the commercial HA (HA_1), better dispersion of HA in PLA matrix was obtained by using the synthesised HA.

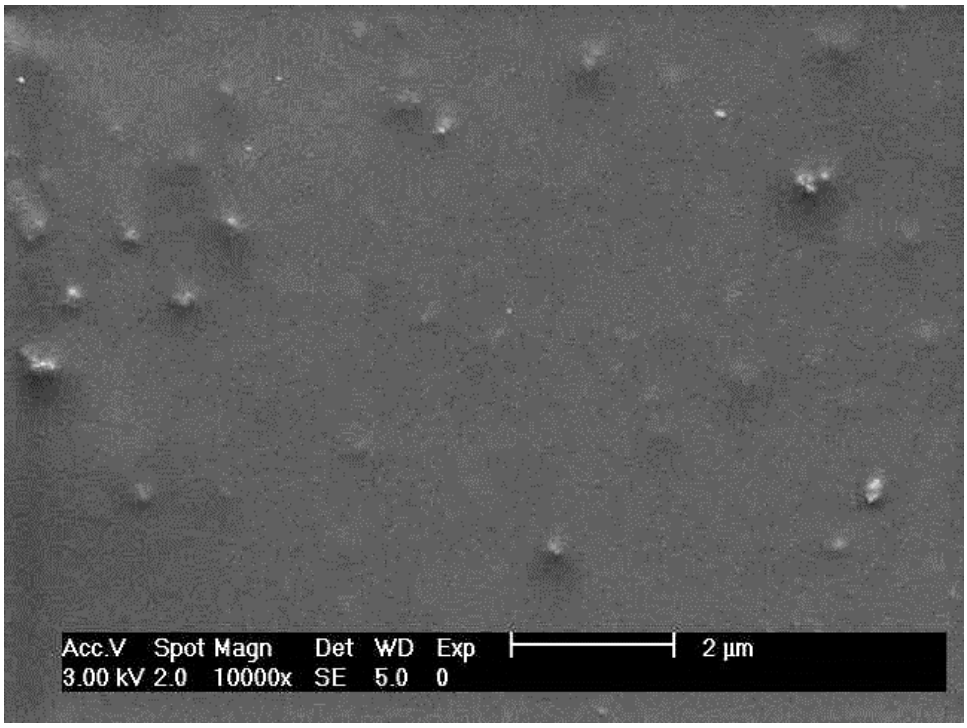
Particularly, good dispersion of HA particles in the polymer matrix is required in order to obtain the composites having satisfactory mechanical properties. Figure 6.20 shows the SEM micrographs of PLA composites loaded 20 wt % untreated and treated HA with 1 wt % AMPTES. The agglomerate size of the treated HA with amino functional silane coupling agents in the matrix was found to be lower than that of the untreated HA. This showed that silane coupling agent improved the dispersion of HA in the polymer matrix.


Acc.V Spot Magn Det WD Exp 2 µm
3.00 kV 2.0 10000x SE 5.1 0

(a)


Acc.V Spot Magn Det WD Exp 2 µm
3.00 kV 2.0 10000x SE 5.3 0

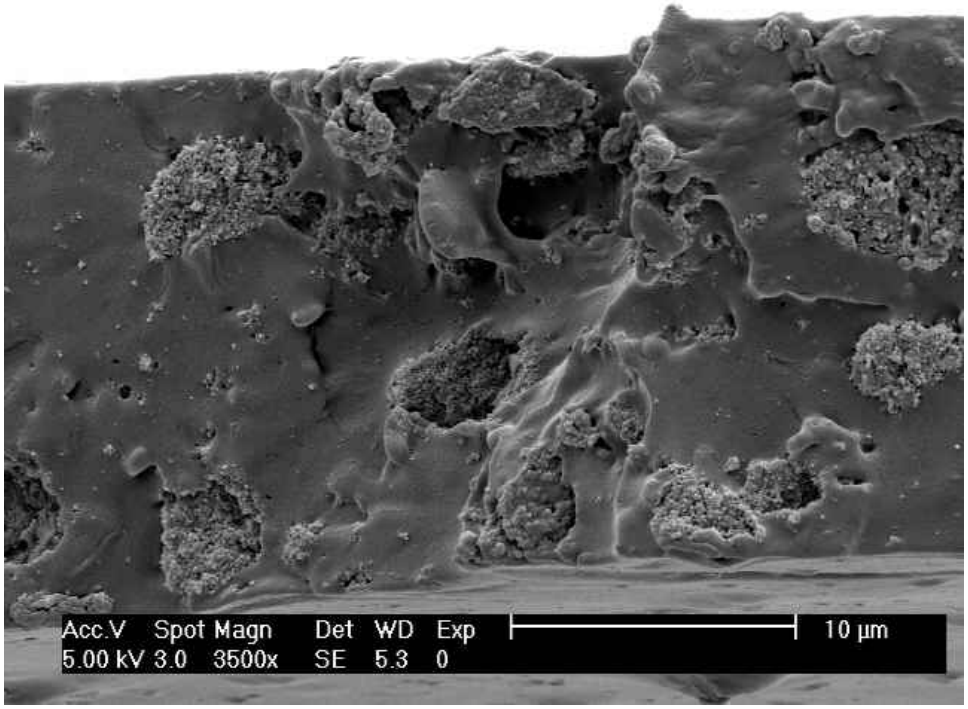
(b)



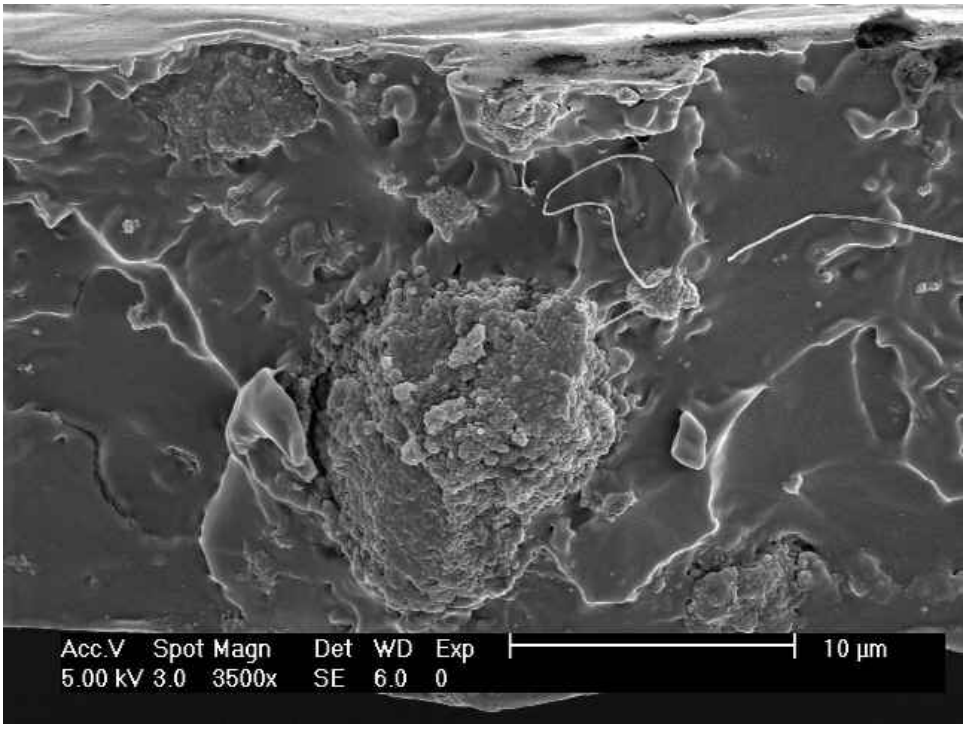
(c)

Figure 6.18 SEM images of PLA Composites Prepared by Using Three Different Particle Size of HA (a) HA_1/PLA Composite (b) HA_2/PLA Composite (c) HA_3/PLA Composite.

SEM was also used to examine the effects of the surface treatment of HA powder on the interface between polymer and HA, and HA loading on the microstructural properties of the composites. Figure 6.21 shows SEM images of surface of the composites containing 10, 20, and 30 wt % treated HA with 1 wt % AMPTES. SEM showed more homogeneous distribution of treated HA particles in the polymer matrix compared to the untreated HA particles, which had a maximum 10 µm agglomeration size in the polymer matrix. However, as the HA weight percentage of HA in the polymer was increased from 10 to 30 wt %, the agglomeration size of treated HA particles was slightly increased. Although it is shown that more homogeneous distribution of HA with silane treatment HAs1 at microscopic level was observed, agglomeration increased with an increase in HA loading.

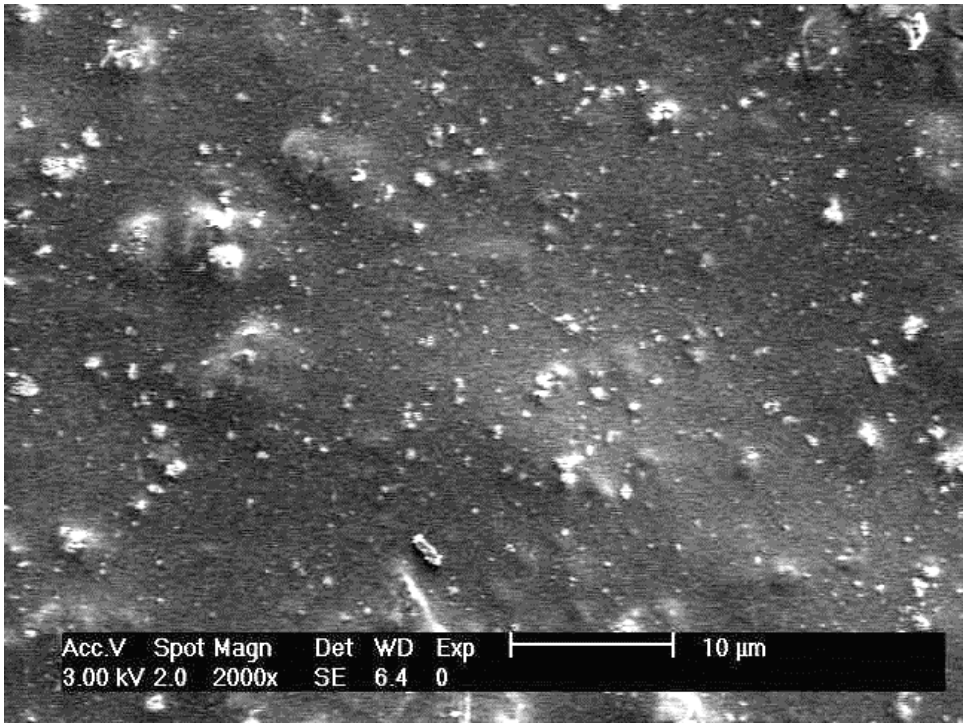


(a)

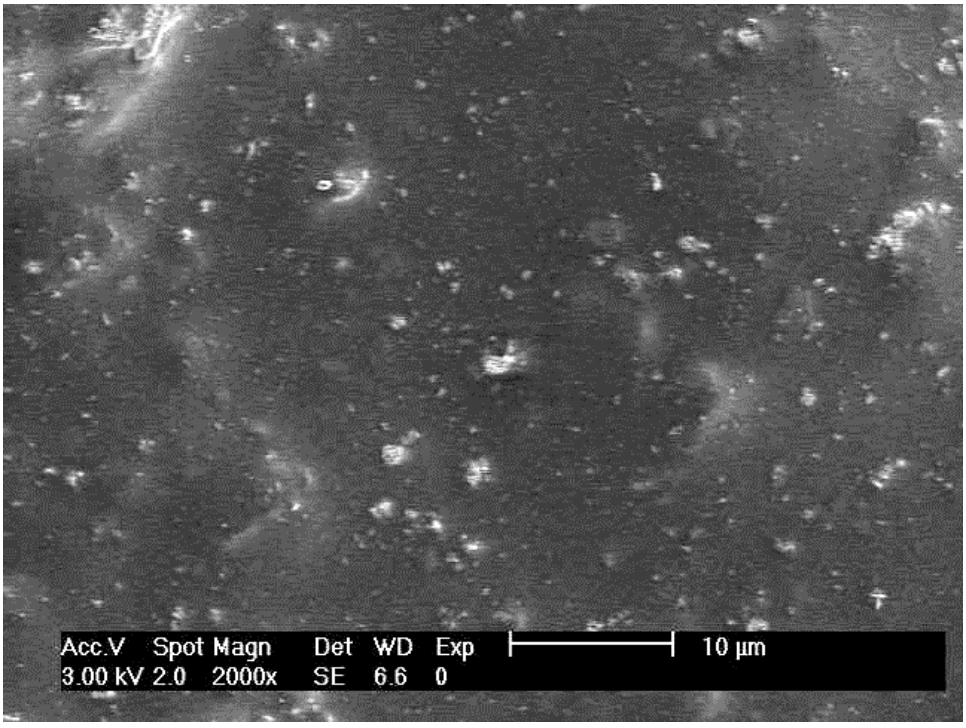


(b)

Figure 6.19 SEM Micrographs of Tensile Fracture Surfaces of PLA Composites Prepared by Two Different Particles Size of HA (a) HA_1/PLA Composite (b) HA_3/PLA Composite

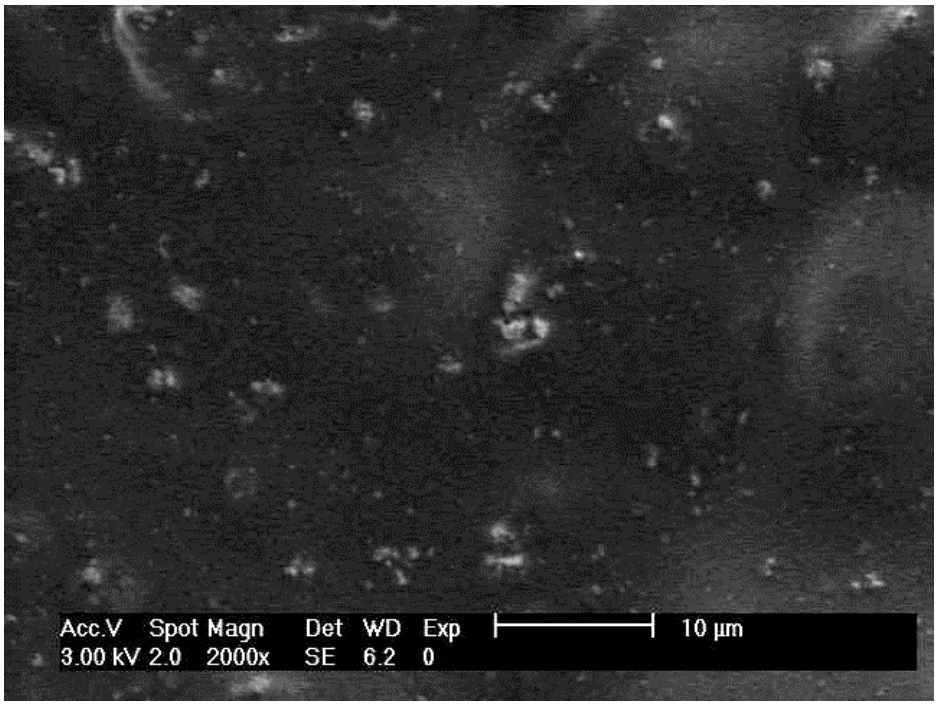


(a)

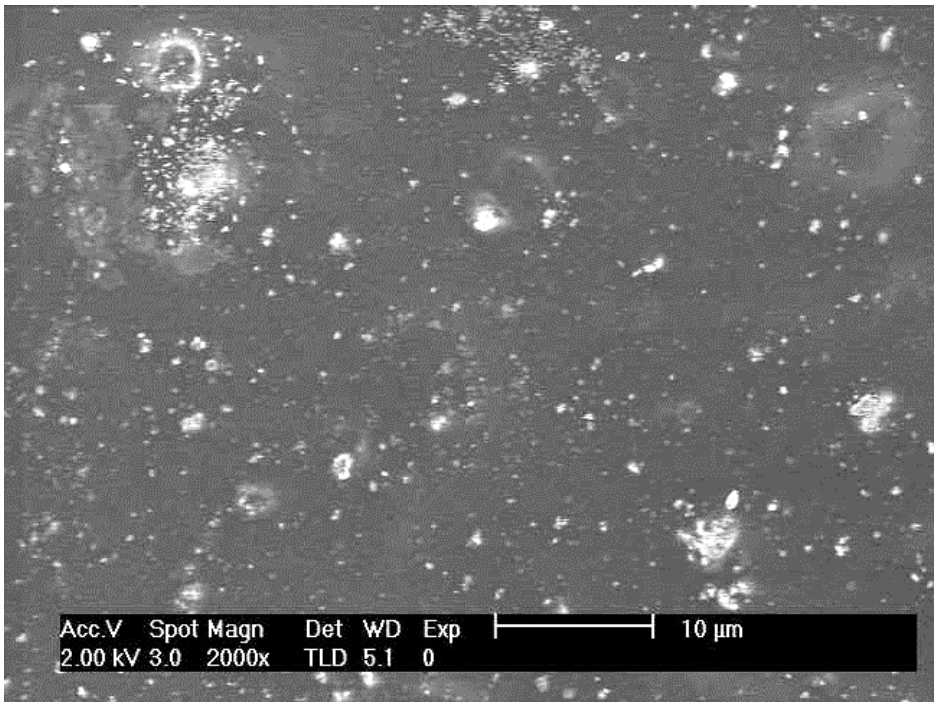


(b)

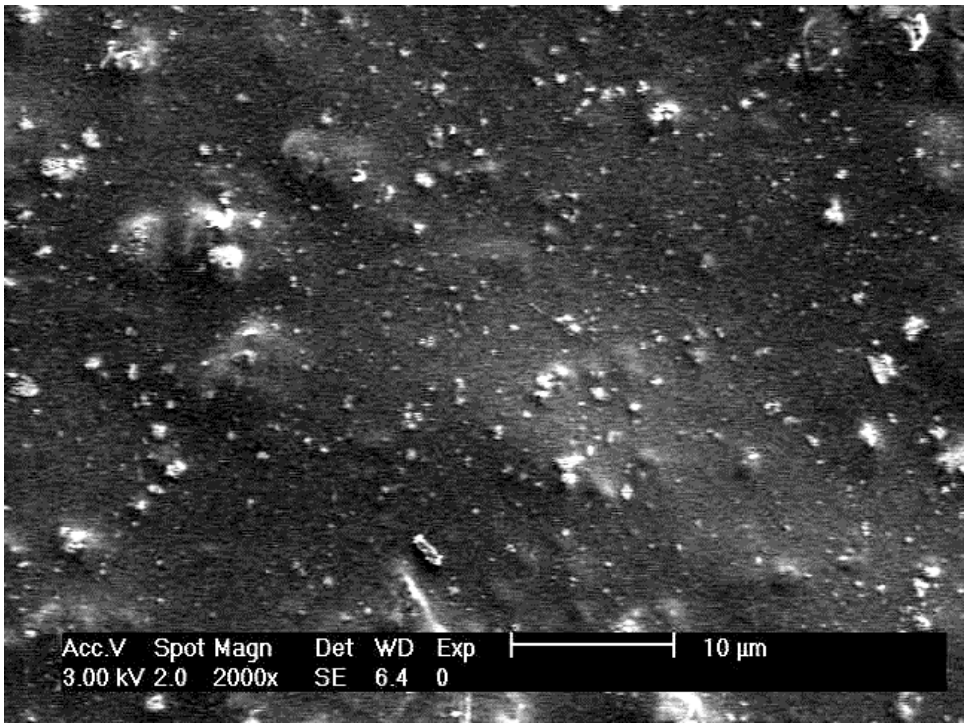
Figure 6.20 SEM micrographs of 20 wt % PLA composites consist of untreated and treated HA with 1 wt % AMPTES.


Acc.V Spot Magn Det WD Exp
3.00 kV 2.0 2000x SE 6.2 0
10 µm

(a)


Acc.V Spot Magn Det WD Exp
2.00 kV 3.0 2000x TLD 5.1 0
10 µm

(b)

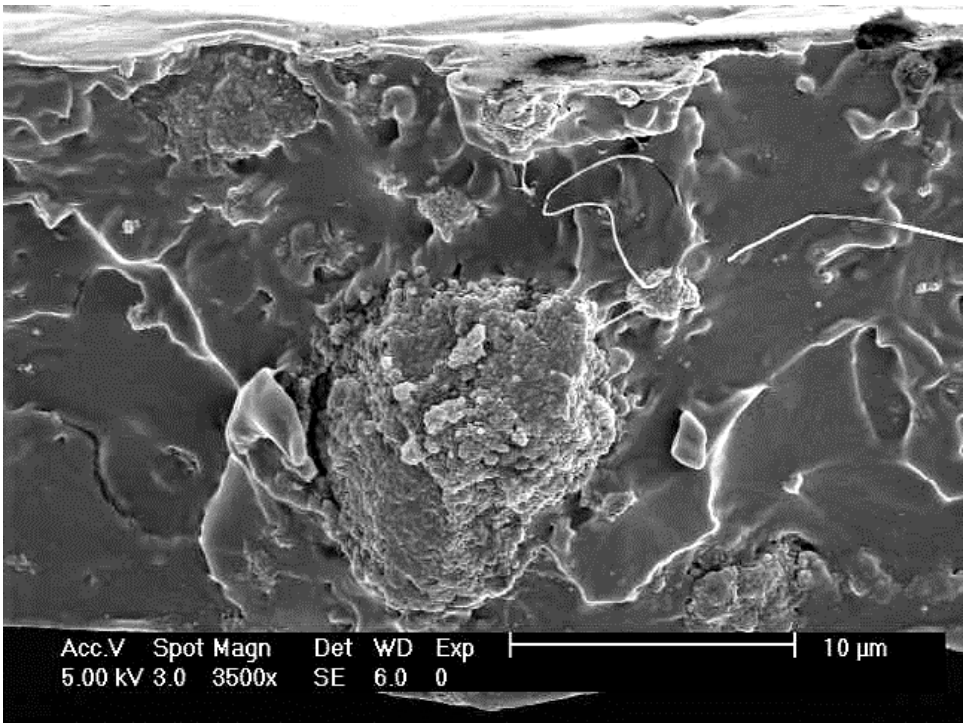


(c)

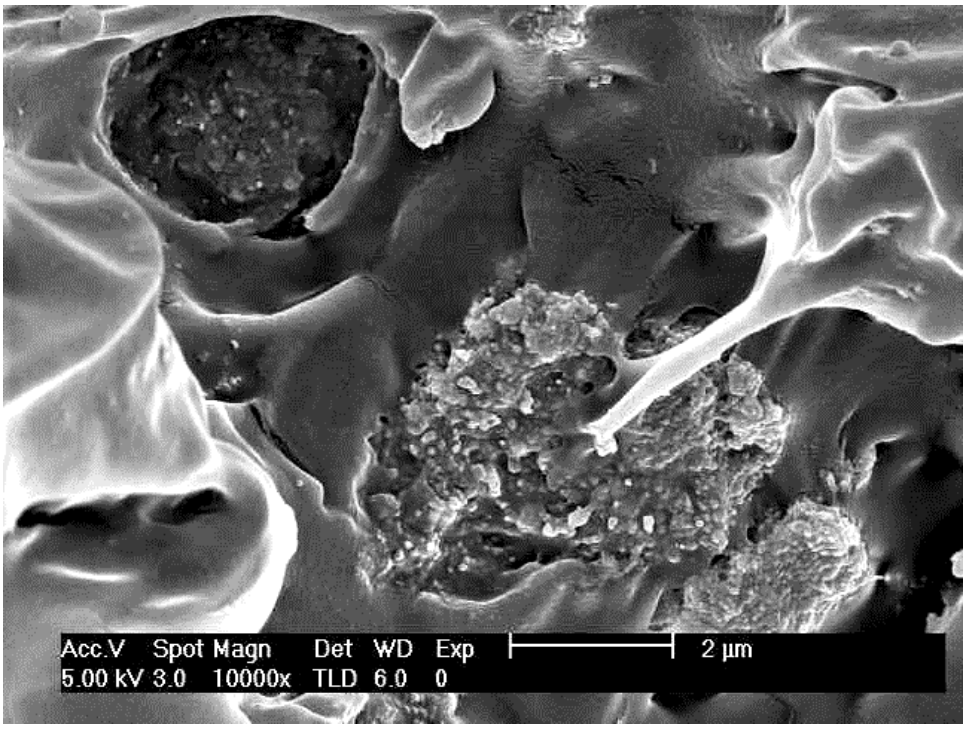
Figure 6.21 PLA Composite Films Containing (a) 10 %, (b) 20 %, (c) 30 % wt treated HA with amino functional silane coupling agent.

The effect of surface treatment on the interface between PLA matrix and HA powder was investigated by examining the fracture surface of tensile tested composites with SEM. Figure 6.22 and 6.23 show SEM micrographs of the fracture surfaces of PLA composites containing 10 wt % unmodified and modified HA with 1 wt % amino functional silane coupling agent at two different magnifications, respectively. Although the adhesion between polymer and HA was not bad in Figure 6.22, the agglomeration size of HA particles was higher than that of treated HA particles.

As seen from the Figure 6.23, the treatment of HA with silane coupling agent improved the dispersion of HA particles in to the matrix and the reduced agglomeration size. The micrograph on the Figure 6.23 shows that the enhanced modification of AMPTES treated composite interface compared to untreated one. HA particles are not seen very clearly due to covering of HA particles by polymer matrix. This indicates wetting of HA particles with the matrix due to improvement of adhesion and dispersion.

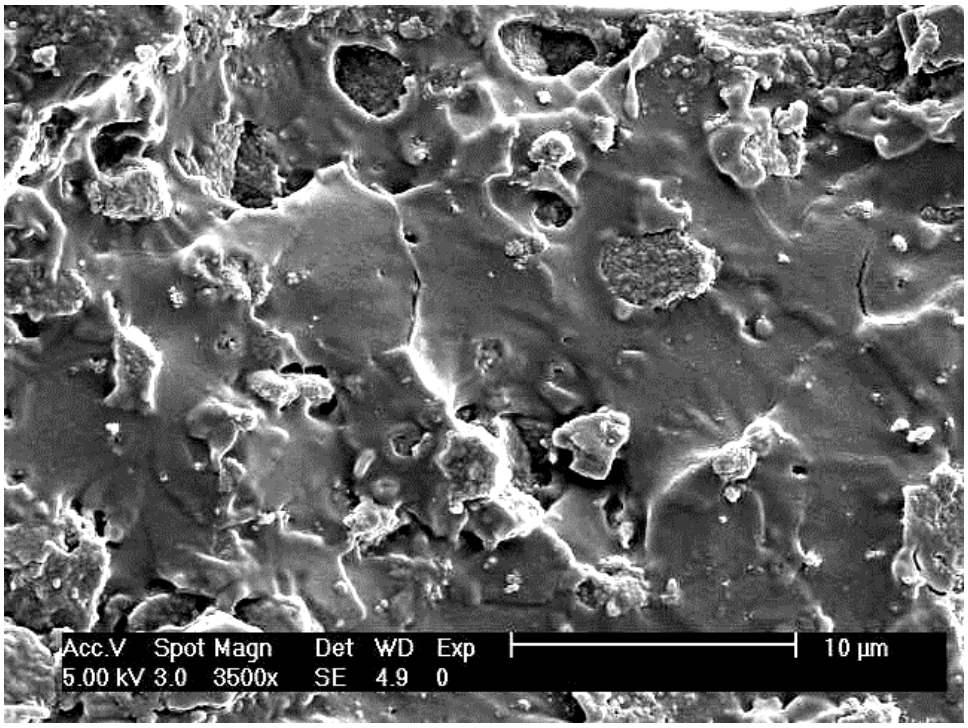


(a)

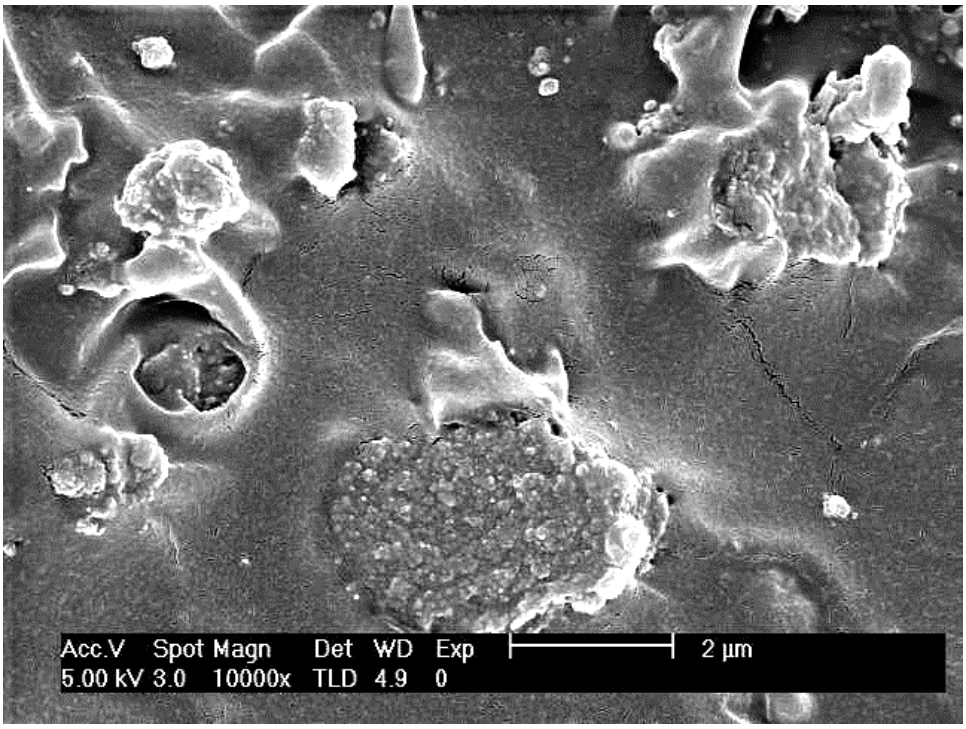


(b)

Figure 6.22. SEM Micrographs of Fracture Surfaces of the PLA_1 Composites Loaded with 10 wt % Untreated HA



(a)



(b)

Figure 6.23. SEM Micrographs of Fracture Faces of the PLA_1 Composites with Treated HA.

Figure 6.24 showed the SEM micrographs of the poly-L-Lactide (PLA_1) composites containing 40 wt % treated HA with 1 wt % AMPTES silane coupling agent. As seen in Figure 6.24, PLA composites containing 40 wt % HA do not show adhesion. Poor interfacial adhesion was obtained for the 40 wt % HA containing composites. Void was present between filler and polymer. That's why the mechanical properties of the composites were weakened.

Figure 6.25 indicates the SEM micrographs of PDLA composites loaded with 10 wt % modified HA with 0.5 wt % MPTMS at different magnification. As seen from the figure, modification of HA with silane coupling agent does not sufficiently improve the interface between HA and PDLA and poor interfacial adhesion between two phases was clearly seen. This explains why the Young's Modulus of the 40 wt % HA loaded composites had a lower value than 30 wt % HA loaded composites.

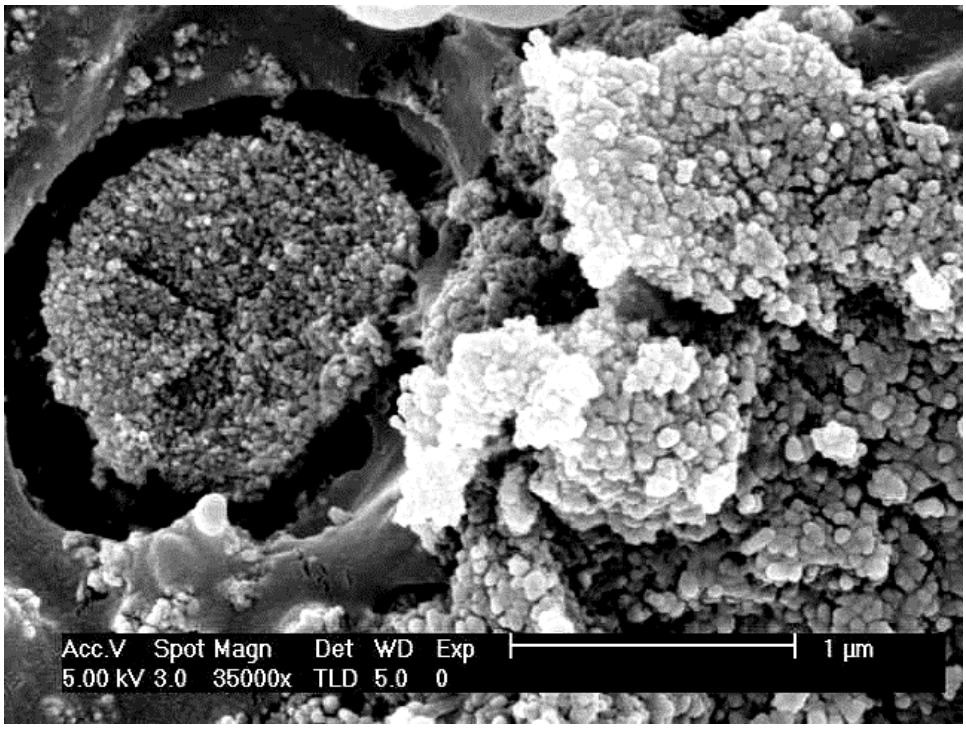
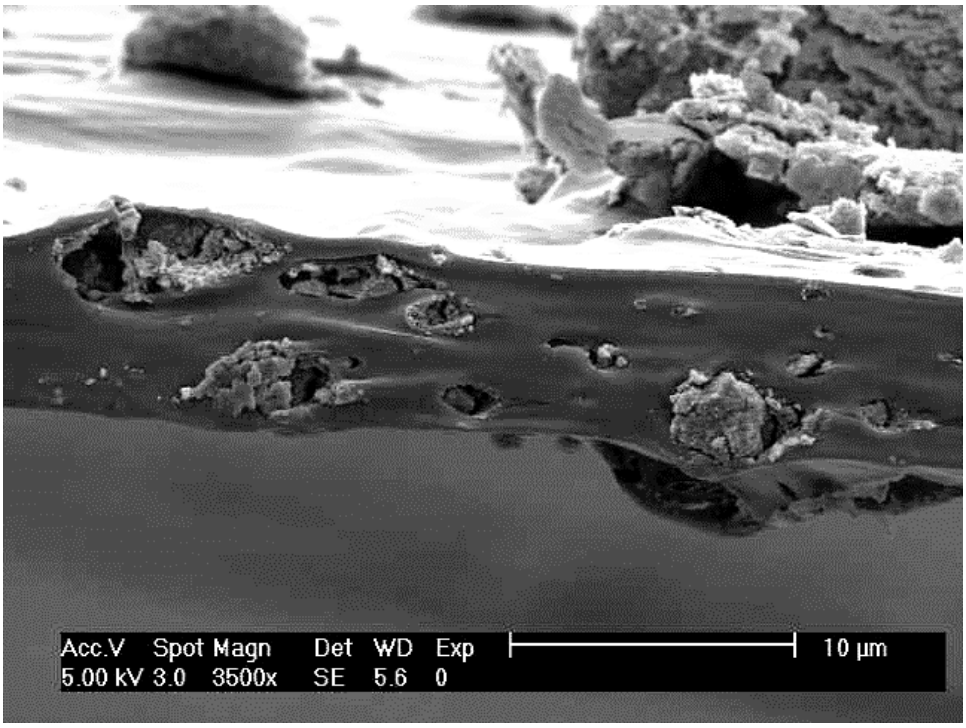
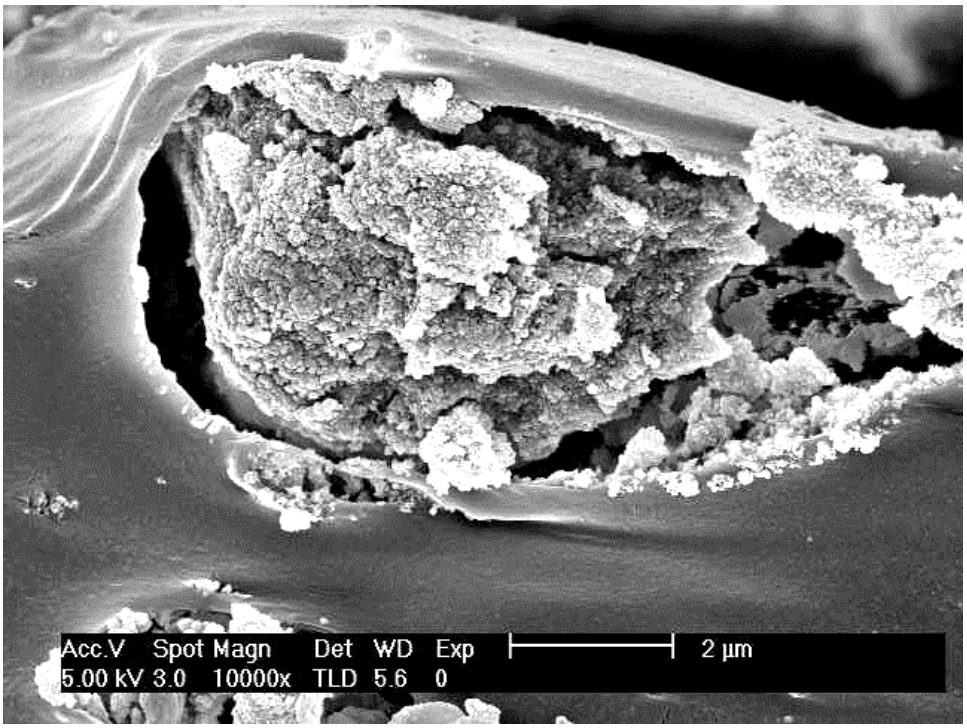


Figure 6.24 SEM Micrographs of the poly-L-Lactide (PLA1) Composites Containing 40 wt % Treated HA with 1 wt % AMPTES Silane Coupling Agent.



(a)



(b)

Figure 6.25 SEM Micrographs of PDLA Composites Prepared by 10 % Modified HA with 0.5 wt % MPTMS for Different Magnification.

6.2.3. Results of Thermal Analyses

In this study, TGA and DSC were used for the thermal characterization of the PLA and PDLA and their composite films consist of HA. The melting, glass transition and degradation temps of the polymers were studied by DSC. TGA was used to determine the thermal degradation behaviour of polylactide-HA composites as well as HA content in the composites.

Figures 6.26 shows the DSC curve of PLA polymer at a heating rate of 10 ^{0}C/min in a stream of dry nitrogen gas. As seen from DSC curve in the Figure 6.26, the glass transition, melting, and the degradation temperatures of the PLA, were found to be 178.8 ^{0}C, and 366.8 ^{0}C, respectively. The heat of fusion of the sample came out to be 42.28 kj/kg. The energy of the second endothermic that was attributed to degradation of PLA was found as 671.4 kj/kg.

Figure 6.26. DSC curves of Poly-L-Lactide.

Figure 6.27 shows the TGA curves of PLA_1 and $PDLA_1$ analysed with a heating rate 10 ^{0}C/min in a nitrogen atmosphere. The weight loss started at around 220 ^{0}C and at terminates about 380-400 ^{0}C. The mass loss occurs in two steps. The first and the sharp decrease in the mass occurred at between 220-380 ^{0}C. The second and the slow mass loss step was observed around 380-480 ^{0}C. As seen in the figure, there is not much difference obtained between thermal degradation curves of PLA and PDLA polymers.

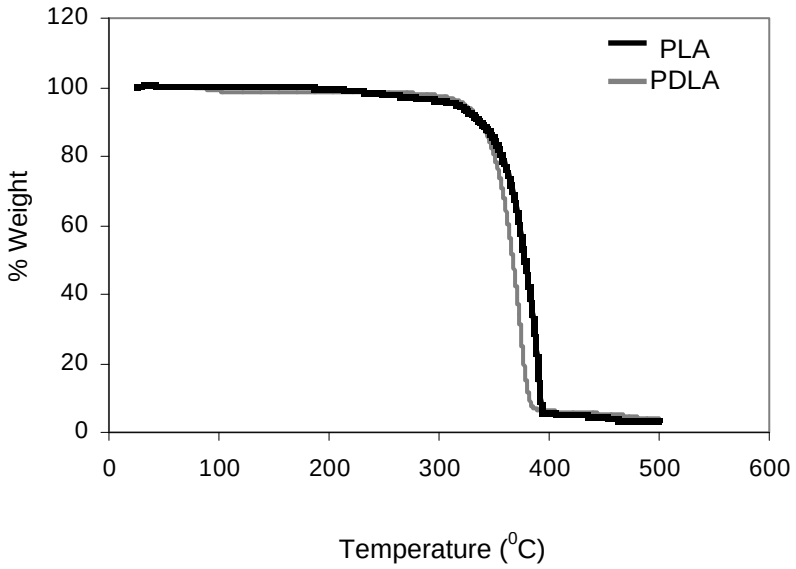


Figure 6.27. TGA curves of PLA and PDLA

Figure 6.28 shows the effect of heating rate on the degradation of Poly-DL-Lactide composite containing 10 % untreated HA. With increase in heating rate, while the thermograms shifted towards the right, the onset of degradation temperature increased and also termination of degradation temperatures increased. That is, the onset of degradation, and the termination of degradation values for heating rates of 5, 10, and 20 ^{0}C/min were about 300 ^{0}C, 325 ^{0}C, 345 ^{0}C, and 385 ^{0}C, 390 ^{0}C, 410 ^{0}C, respectively.

The Poly (L-Lactide) (PLA) and its composites loaded with 20 wt %-50 wt % untreated HA prepared by the second method were analyzed by TGA with a heating rate of 10 ^{0}C/min. Figure 6.29 shows the TGA curves of these composite samples. As shown on the TGA curve, weight loss of PLA increased rapidly above 270 ^{0}C and weight losses of all composite samples increased nearly above 310 ^{0}C. Samples containing 20 and 30 wt % untreated HA behaved similar to each other. Their onset

and termination of degradation temperatures were very close each other as indicated in Figure 6.29. As shown in figure, with the increase HA loading, the onset of degradation temperatures of composite shifted towards left, but termination degradation temperature shifted towards the right and degradation was slow down. This indicates that the addition of HA into polymer matrix slowed down thermal degradation of the polylactide polymers.

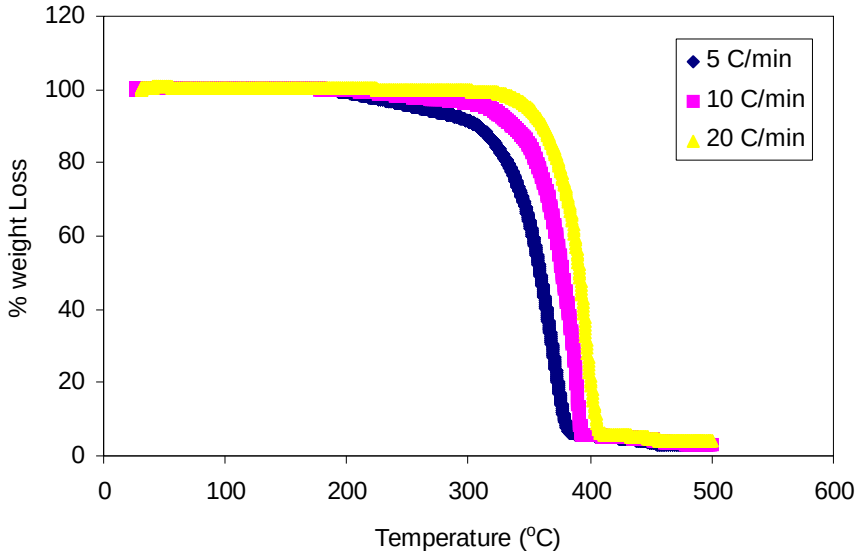


Figure 6.28. Effect of heating rate on the degradation behaviour of PDLA composites containing 10 wt % HA.

The kinetic analysis was performed at heating rates of 5, 10 and 20 ^{0}C/min for the PLA and PDLA and their composite samples. Kinetic analyses of these samples were done with in a temperature range 300-420 C for fast mass loss step using Shimadzu 51 TGA kinetic analysis software according to the Ozawa methods. Equation used for the analysis of the experiments was given below;

$$\ln (d\alpha/dt)x[1/(1-\alpha)]^{n} = \ln (A/\beta)^{-E/RT} \qquad (6.1)$$

where: α: rxn rate

A: frequency factor;

E: Activation Energy;

β: Heating rate;

t: time;

T: temperature;

r: rxn order

The Ozawa plot shows the logarithm of heating rate versus 1/T at constant x values (reaction percent) for different conversions (weight losses). From the slope of the lines, the activation energy, E, is calculated for different weight losses and the averaged by the software. Reaction rate constant for the degradation reaction was determined using Arrhenius equation given below at 300 ^{0}C.

$$k = A\, e^{-E/RT} \qquad (6.2)$$

The kinetic analysis results of the samples were shown in Table 6.9. The kinetic energies and related parameters obtained for all samples were given in the Table. As seen in Table 6.8, both activation energy and reaction rate constant decrease with increasing HA loading. This showed that thermal decomposition rate slows down with the addition of HA in PLA_1 and $PDLA_1$ matrix.

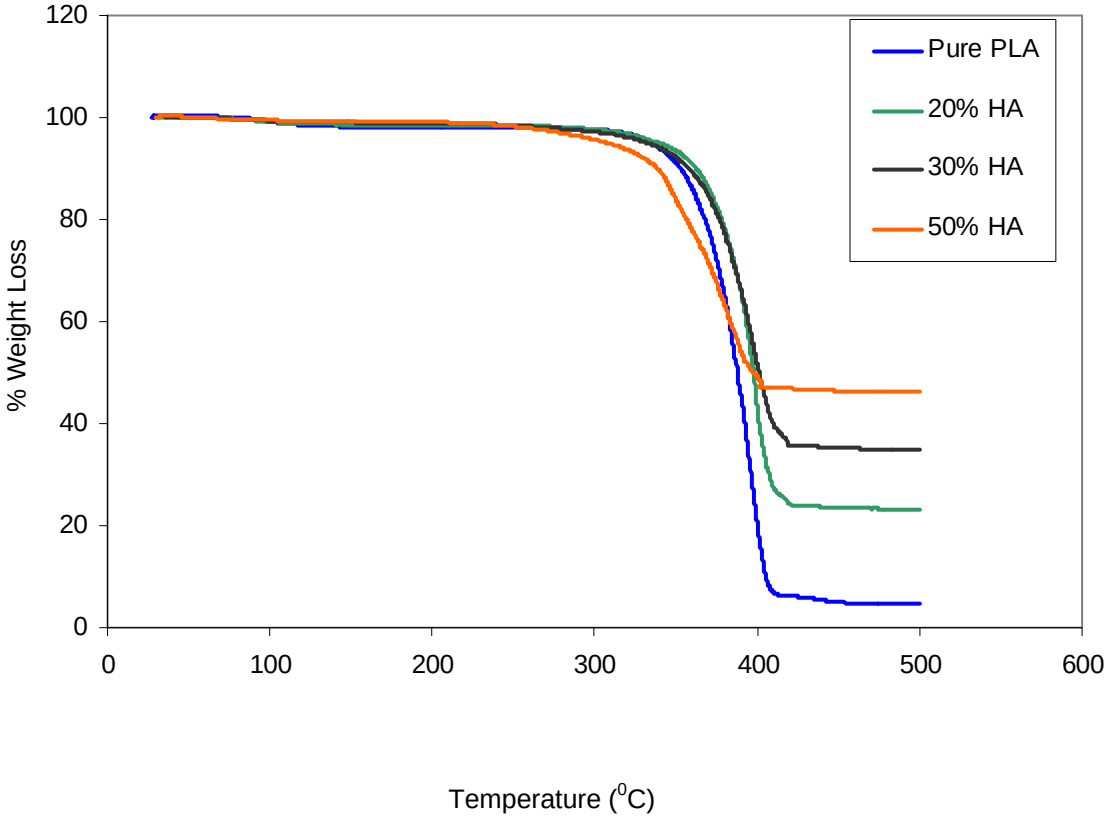


Figure 6.29 TGA curves of PLA and PLA composites

Table 6.8. Kinetic Analysis Results of Poly(L-Lactide), Poly(D,L-Lactide) and Their Composites

Samples	HA Content (%)	Activation Energy E (kj/mol)	Frequency Factor A (min^{-1})	k @ 300 ^{0}C (min^{-1})
Poly (L-Lactide)	0	184.1	2.96×10^{14}	2.85×10^{14}
Poly (L-Lactide)	10	157.1	3.12×10^{12}	3.02×10^{12}
Poly (L-Lactide)	20	139.6	5.38×10^{10}	5.22×10^{10}
Poly (L-Lactide)	30	132.6	1.70×10^{10}	1.65×10^{10}
Poly (L-Lactide)	50	132.5	6.80×10^{10}	6.61×10^{10}
Poly (D,L-Lactide)	0	104.6	1.05×10^{8}	1.03×10^{8}
Poly (D,L-Lactide)	10	171.5	5.30×10^{13}	5.11×10^{13}
Poly (D,L-Lactide)	20	145.1	3.94×10^{11}	3.82×10^{11}
Poly (D,L-Lactide)	30	156.8	1.70×10^{12}	1.64×10^{12}
Poly (D,L-Lactide)	50	124.5	4.38×10^{9}	4.26×10^{9}

6.2.4. FTIR Spectroscopy Results

Infrared (IR) as well as Fourier transform infrared (FTIR) spectroscopy has been widely used as powerful methods in characterization of biomedical materials. FTIR spectroscopy was used to investigate the chemical composition of the poly-L-Lactide and poly-D,L-Lactide and their composites loaded with untreated and treated HA films. Film samples were used for the analysis of the polymer and composite. The spectral range of 400-4000 cm^{-1} was used.

The FTIR Spectra of PLA and PDLA that are used throughout this study are given in Figures 6.30 and 6.31, respectively. As shown from the figures, all characteristic peaks of Poly(L-Lactide) and Poly (D, L-Lactide) are nearly at the same wavelenght in their spectra and matched with the literature.[58,59] Characteristic absorption bands at 1760 cm^{-1} and at about 2900 cm^{-1} are attributed to stretching vibrations of the C=O and C-H group of PLA, respectively. The peaks at 1450 cm^{-1} and 1370 cm^{-1} come from $–CH_3$ bending and the 2930 cm^{-1} peak in the figures indicate -CH_3 stretch.

The spectrum of Poly-L-Lactide composites with untreated HA loading of 10 %, and 20 wt % samples were given in Figures 6.32 and 6.33 respectively. An absorption band at 3570 cm^{-1} as well as a triplet of absorption bands at 572-632 cm^{-1} come from the OH groups from HA, while characteristic absorption bands with maxima at 1050 and 1090cm^{-1} arise from the phosphate groups from HA. The peaks at 1760 cm^{-1} and 2900 cm^{-1} come from the C=O and C-H group of PLA, respectively. As shown in the figures the peak intensity at 572-632 cm^{-1} was increased with the increasing hydroxyapatite content in the composites.

The FTIR spectrum of poly(L-Lactide) composite containing 20 wt % HA treated with 1 wt % with aminofunctional silane was given in Figure 6.34. The characteristic peaks of poly (L-Lactide) and HA were observed in the spectra of the composites, however, silanol formation band, which absorbs at 1130-1000 cm^{-1} was not seen. This could be due to the low vibration intensity of silane coupling agent.

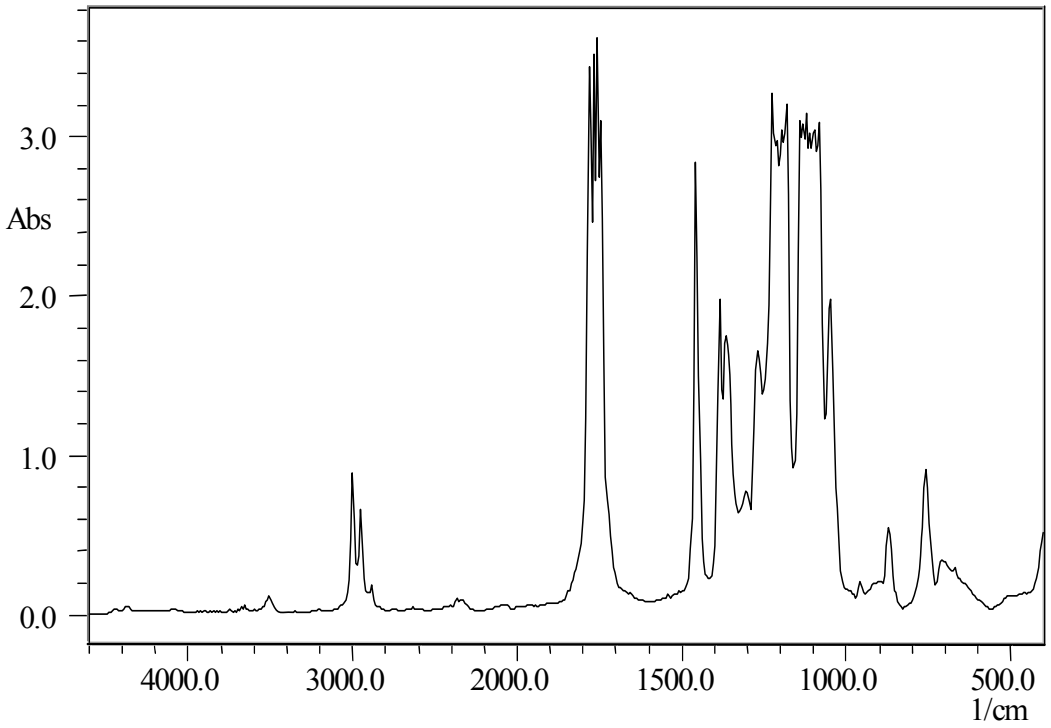


Figure 6.30. FTIR Spectrum of Poly-L-Lactide.

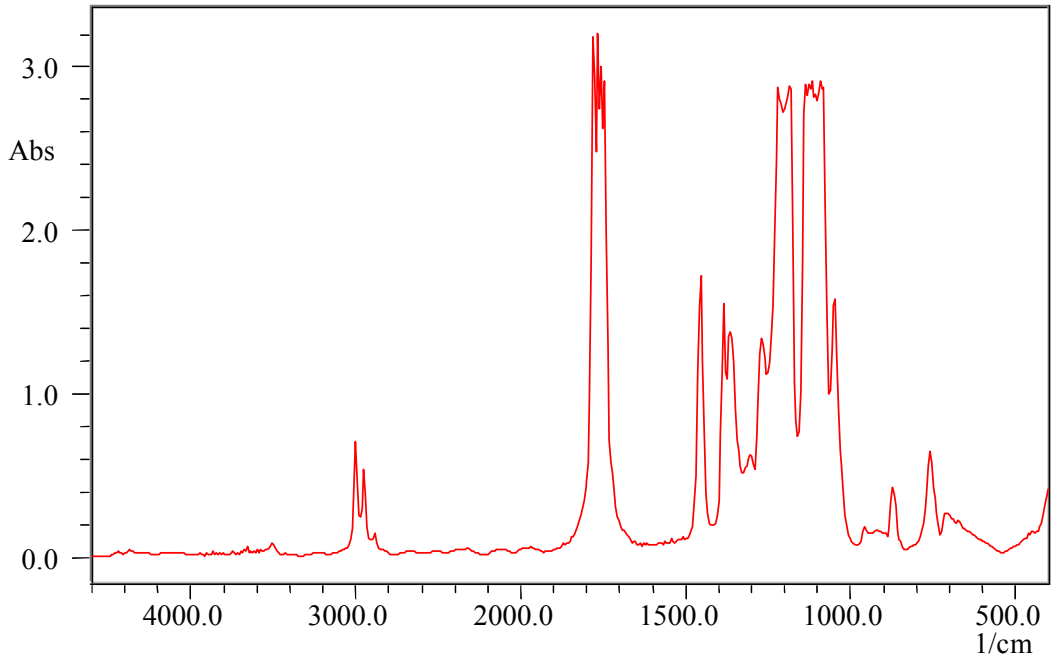


Figure 6.31. FTIR Spectrum of $PDLA_1$

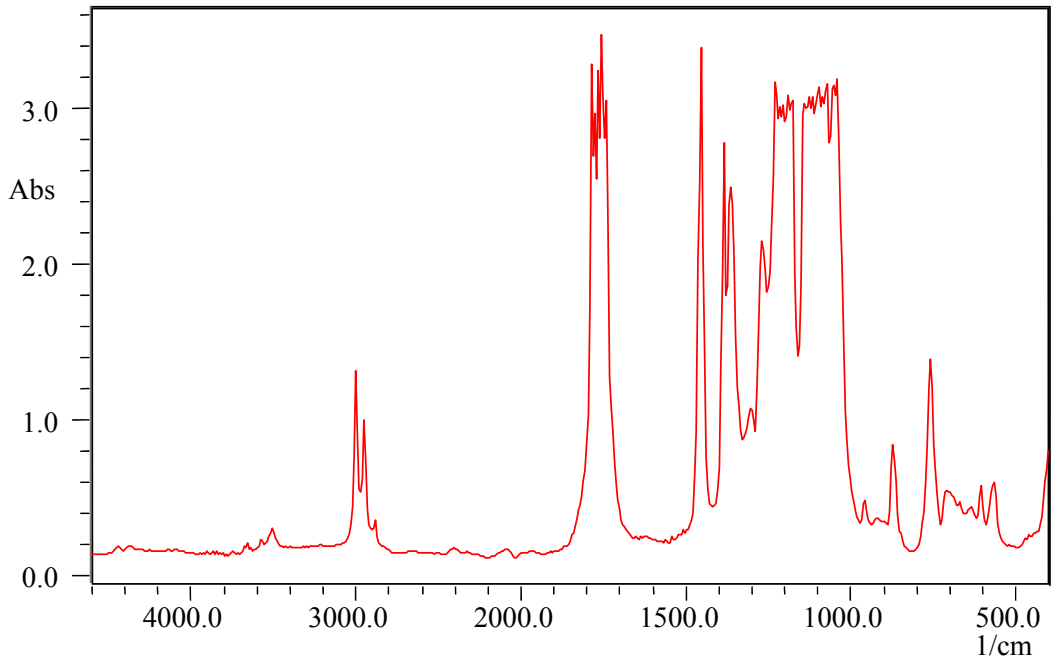


Figure 6.32. FTIR Spectrum of Poly(L-Lactide) Composite Containing 10 wt % Untreated HA.

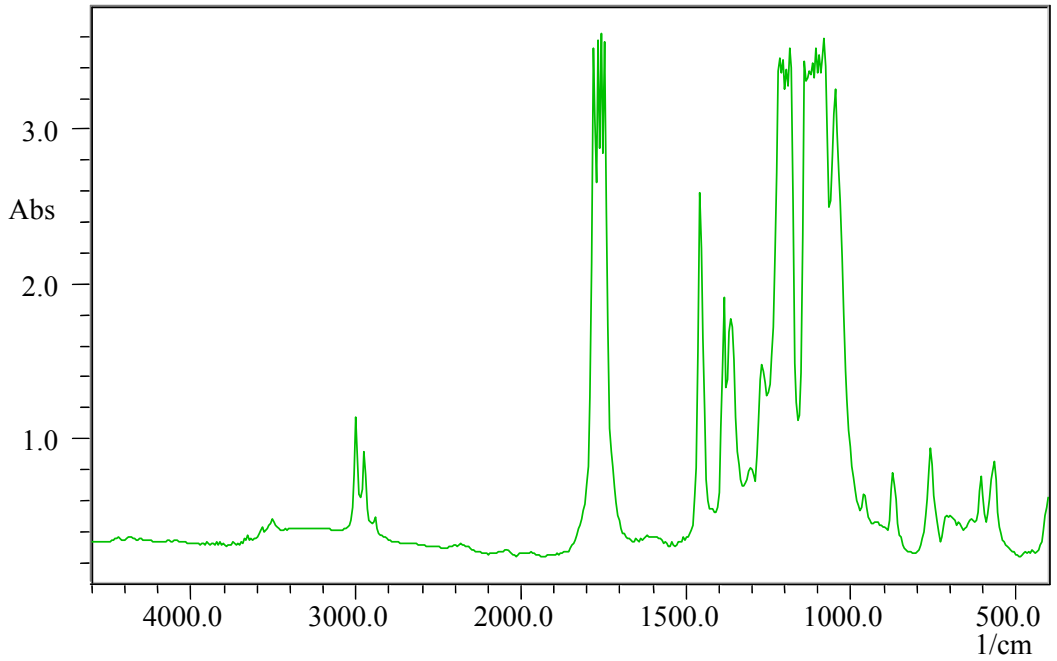


Figure 6.33. FTIR Spectrum of Poly(L-Lactide) Composite Containing 20 wt % Untreated HA.

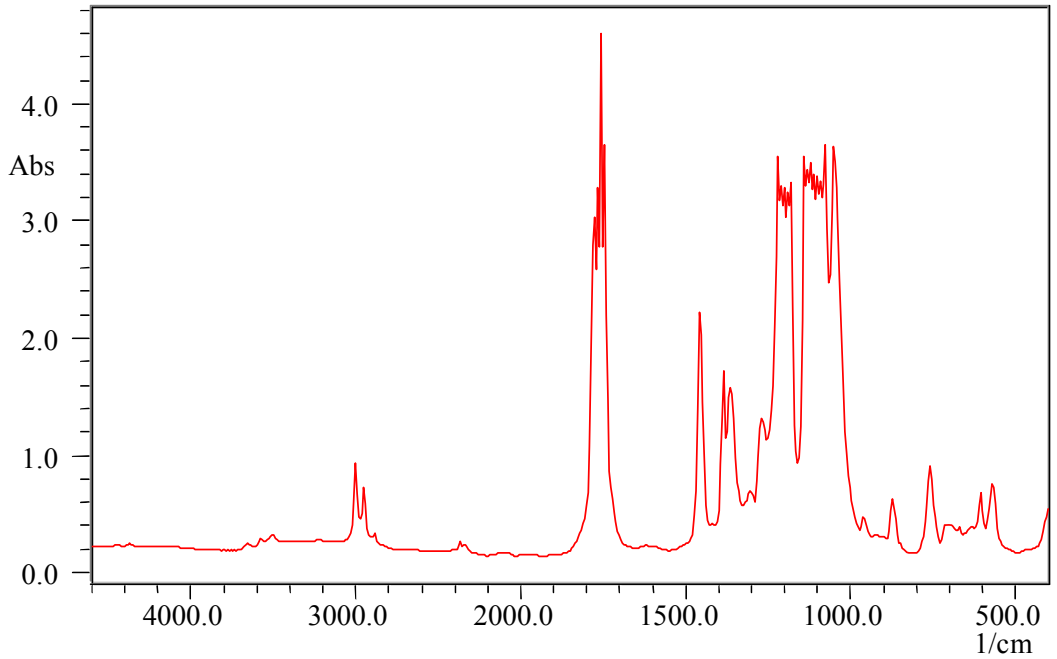


Figure 6.34. FTIR Spectrum of 20 wt % Treated HA with Aminofunctional Silane – Poly(L-Lactide) Composite.

6.3. Hydrolytic Degradation of Poly (L-Lactide), Poly(D,L-Lactide) and their Composites

For most biodegradable materials, for especially artificial polymers, chemical degradation via hydrolysis is the most important mode of degradation. There are several other factors such as the type of chemical bond, pH, copolymer composition and water uptake affects the rate of this reaction. Therefore, in this study water absorption, percent weight loss and pH changes of homopolymers, copolymers and ceramic/ polymer composites have been investigated during the hydrolysis reaction with respect to time. Since the simulated body fluid (SBF) represents the chemical environment of blood plasma, *in vitro* degradation mechanism of polymers were tested in SBF solutions at the physiological pH of 7.4 and at temperature of 37 ^{0}C. Specimens subjected to *in vitro* study were the pure polymer films (PLA_1, PLA_2, $PDLA_1$, $PDLA_2$) and polymer (PLA_1 and $PDLA_1$) composites loaded with untreated HA (10, 20, 30, 50 w/w %). The weight loss and water absorption values of these pure polymer films and their composites over an 8-week and 16-week period were determined by gravimetric analysis. The samples held in an aqueous solution (simulated body fluid) were examined by Scanning Electron Microscopy (SEM) at different time intervals to observe the morphological changes occurred at the surface as a result of hydrolytic degradation process.

Figure 6.35 illustrates the changes in the pH of the SBF solution over time for the polymers under investigation subjected to static tests. In static degradation test the SBF solution used for aging was not replaced over the whole testing period. As seen from this figure, the pH of the medium containing polymers in all cases did not change significantly and stayed stable at around 7.4 up to 150 days. The pH of the SBF solutions containing PLA_1 and PLA_2 increased to 8.2 at the day of 30.

Taylor et al [61], studied the *in vitro* degradation of Poly (L-Lactide) in a buffer solution of pH 7.4 at 37 ^{0}C. The pH of the aging medium was reduced from 7.4 to 3.0 after 40 weeks. Up to 40 weeks period, the pH value was nearly the same at the value of 7.4. Mainil-Varlet and co-workers [62] examined the effect of *in vitro* degradation in the phosphate buffer solution, pH 7.4 at 37 ^{0}C for various low molecular weight polylactides. The changes of pH, crystallinity and mechanical properties were investigated in the static *in vitro* aging tests. The pH of the medium-containing poly (L-Lactide) decreased from 7.4 to 6.9 after 40 weeks and reached to a value of 5.4 after 1 year. For poly (L/D-Lactide) and poly (L/DL-Lactide) polymers, the pH of the

phosphate buffers solution decreased over the whole experiment period, approaching the value of 3.2 after 5 weeks.

In this study, since the *in vitro* static testing in SBF solution for polymeric materials were performed within approximately 22 weeks period, no change in pH was observed as pointed out in Taylor's work and results were similar to Mainil-Varlet's study for Poly(L-Lactide). The stability of pH at 7.4 may be because of the good buffering effect of SBF solution and the ions present in the medium. Another reason may be due to the medium sterilization, therefore, no microbial attack could be the cause of any degradation. The change of pH would have been obtained if specimens were left in SBF solution over longer periods, because it is assumed that polylactides degrades at low rates and that degradation of polyhydroxyacids in the aqueous media proceeds via a random, bulk hydrolysis of ester bonds in the polymer chain. The carboxylic acids, which are products of the degradation, catalyze the degradation process.

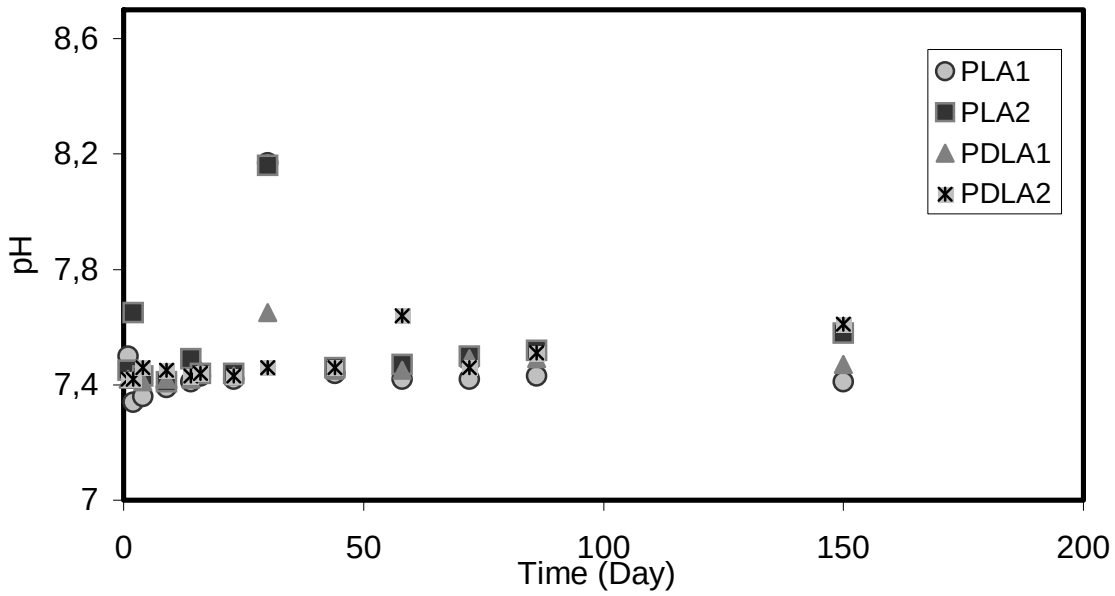


Figure 6.35 The Change of pH During Degradation Period of Time for PLA_1, PLA_2, $PDLA_1$, $PDLA_2$.

Figure 6.36 displays the weight loss of polymers during 150 days period. Weight loss and water absorption values were determined from the Equations 6.3 and 6.4.

$$\% W_L = [(W_I - W_r)/W_I] \times 100 \quad (6\text{-}3)$$

$$\% W_A = [(W_w - W_I)/ W_I)] \times 100 \quad (6\text{-}4)$$

where;

W_I is initial weight, W_w is wet weight and W_r is remaining weight of the samples. Weight loss data showed that during 150 days period, the degradation rates of PLA_1 and PLA_2 were much slower than those of $PDLA_1$ and $PDLA_2$. As shown in the figures, at the end of the 150 days, only 12.5 and 9.5 % weights (PLA_1 and PLA_2) were lost, respectively. As expected, degradation of the copolymers was faster than degradation of homopolymers and weight loss data of $PDLA_1$ and $PDLA_2$ were nearly found to be similar with the values of 17.5% and 17%, respectively.

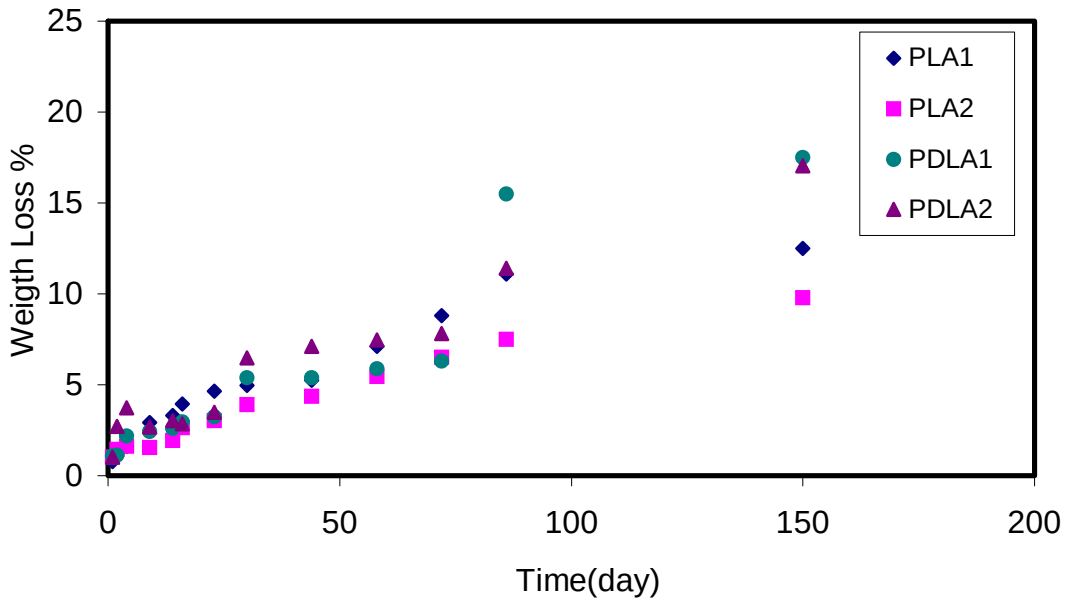


Figure 6.36 Weight Loss Change as a Function of Time for Different Types of Biodegradable Polymers (PLA_1, PLA_2, $PDLA_1$, $PDLA_2$) in Simulated Body Fluid, pH 7.4 at 37 ^{0}C.

Li [46] studied the hydrolytic degradation characteristics of aliphatic polyesters derived from Lactic and Glycolic Acids in pH 7.4 phosphate buffer at 37 ^{0}C. After 40 weeks, nearly 8 %, 10 % of the weight was lost for PLA and PDLA, respectively.

The morphology of a polymeric material (i.e., amorphousness and semicrystallinity) plays a critical role in the degradation process. It is known that degradation of semicrystalline polyester in aqueous media occurs in two stages. The first stage consists of water diffusion into the amorphous regions with hydrolytic

breaking of ester bonds. The second stage starts when most of the amorphous regions are degraded. PLA_1 and PLA_2 are intrinsically semicrystalline polymers and $PDLA_1$ and $PDLA_2$ are amorphous. The composition of polymer chains, which was the content in L-LA and D-LA, effect the degradation rate of PLA polymers.

Water absorption data for polymers as a function of degradation time was indicated in Figure 6.37. As seen from the figure, the uptake of water by PLA_1 was slower and lower compared to PLA_2, especially in 50 days. After the 90th day, both homopolymers showed the similar trend and absorbed the same amount of water (~ 11-12 %) at the day of 150.

It can be seen in Figure 6.37 that copolymers $PDLA_1$ and $PDLA_2$ had higher water absorptions, the difference between the two polymer being around 5%. At the day of 150 $PDLA_1$ and $PDLA_2$ have water absorption values of 16 % and 20 %, respectively. It was found that the water absorption of copolymers with lower LA content was comparatively higher than those of higher LA content.

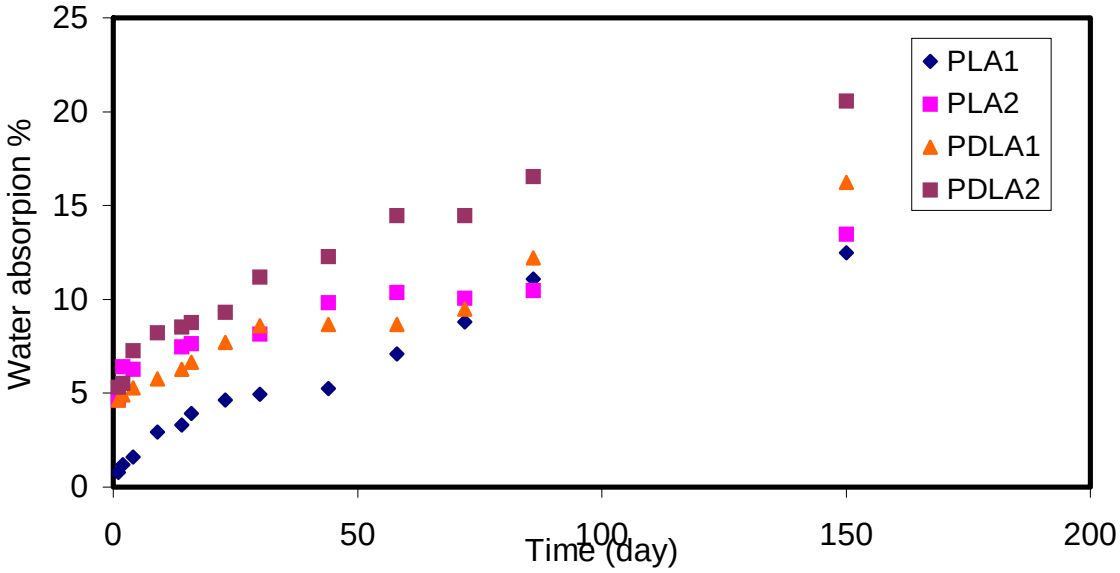


Figure 6.37 Water Absorption Behaviour of Polylactide (PLA_1, PLA_2, $PDLA_1$, $PDLA_2$) as a Function Degradation Time.

The effect of HA loading on the degradation behaviour of the polymer composites was also investigated. The degradation rates of the PLA_1 and $PDLA_1$ composite containing 10, 20, 30 and 50 % untreated HA were studied. Figure 6.38 (a),

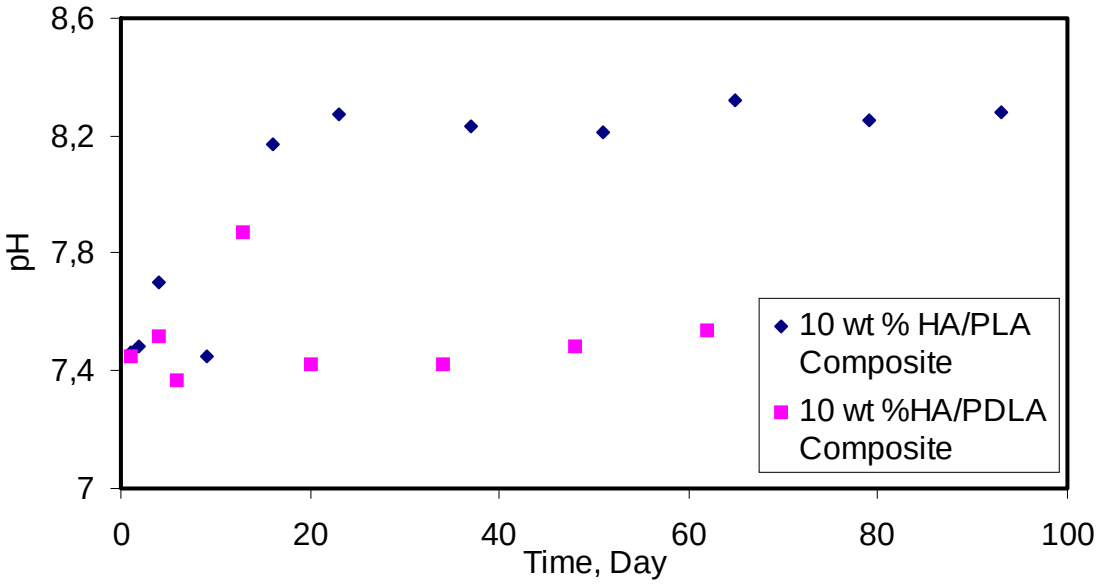
(b), (c) shows the changes in the pH of the SBF solution, the % weight loss and water absorption of the PLA_1 and $PDLA_1$ composite incorporated with 10% untreated HA respectively. The *in vitro* static testing in SBF solution for PLA and PDLA composite materials were performed within approximately 13 and 9 weeks period, respectively. As seen in Figure 6.38(a), while the pH of the solution containing 10 w/w % HA/PDLA composite did not change, pH increase was observed in the case of solutions containing 10 w/w % HA/PLA composites. The increase in pH was recorded during the first 20 days, after this period pH remained at around 8.2. This change may be the reason of the hydroxyapatite content of polymeric material. HA alone tend to increase the pH's of the SBF solutions due to its composition. It contains PO_4^{-2} and Ca^{+2} ions; and ion transfers may occur both from the material side to the solution and vice versa. In 20 days time; pH raised from 7.4 to 8.2, which can be attributed to the equilibration reaction between the material and solution. This type of behaviour can not be seen in case of HA/PDLA composites even with this polymer having the same amount of HA. The difference may be due to the fact that PDLA and PLA polymer structures; their crystallinity, homogeniety and making different chemical bonds with HA. Figure 6.38 (b) shows the weight loss data for HA/PLA and HA /PDLA composites loaded with 10 w/w % HA. For both polymer composites, weight loss curves tend to increase with time; at the day of 50 similar values were recorded and approximately 6 % weight losses were obtained. The weight loss values are not shown here after 50 days for HA/PDLA composite due to the lack of experimental data, the weight loss values stayed stable at 5 % for HA/PLA composite material.

HA/ PLA and HA/PDLA composites have approximately similar weight loss values in Figure 6.38 (b); when compared with the pure polymer materials (Figure 6.36) at the day of 50; again nearly the same weight losses were obtained for both polymers and polymer /HA composites. Prolonged time periods caused increases in weight loss values for PDLA polymers than those of PDLA /HA composites and PLA polymers than those of PLA /HA composites. This may be due to the slower degradation of HA in SBF when compared with pure polymer materials in the same solutions. Weight loss decreases approximately from 9.5% to 5 % when PLA's were loaded with 10 % HA at the end of 90 days.

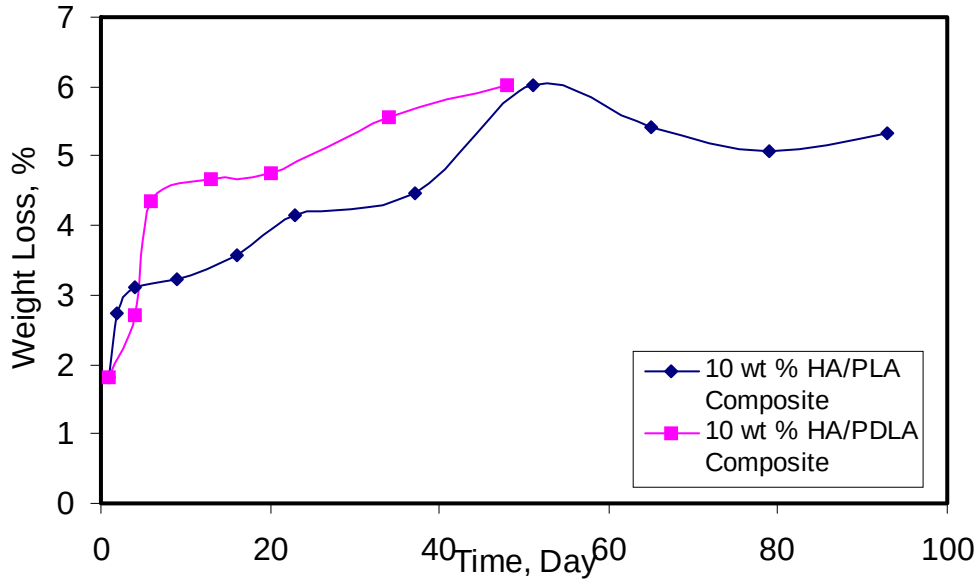
Water absorption data for HA/polymer composites can be seen in Figure 6.38 (c). Initial rates of water absorption for both HA/PLA and HA/PDLA composites showed similarities up to the day of 10. The uptake of water by HA/PDLA was higher

than that of HA /PLA composite and reached to a value of 15 % at the day of 60, while water absorption value was found to be around 10 % for HA/PLA composite material. This value increased up to 13.5 % at the day of 90 for HA/PLA composite, but no data is available at this period for HA/PDLA composite for comparison.

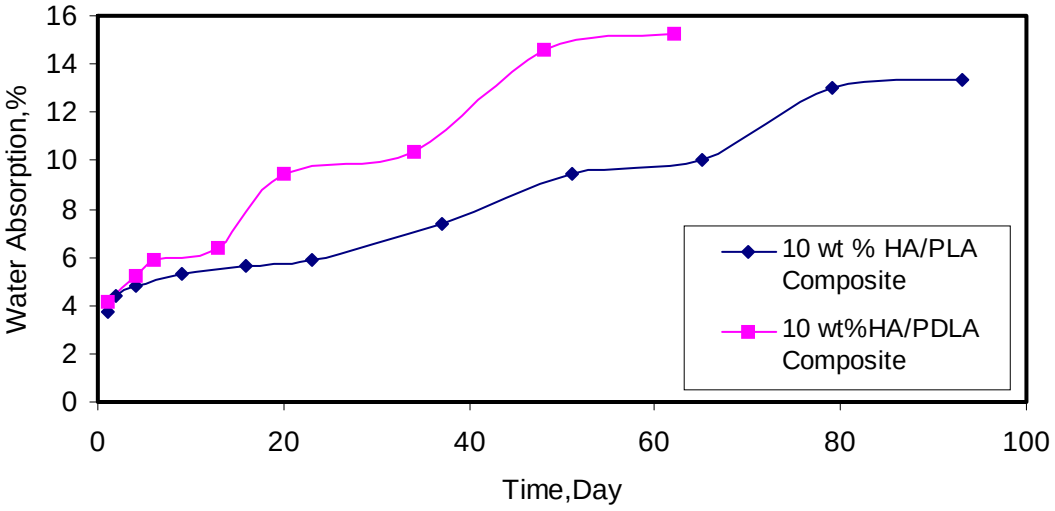
Water absoption behaviour of pure polymer and polymer composites can be compared from the Figures 6.37 and 6.38 (c). It can be said that water uptake increased from 10 % to 13 % for PLA when loaded with 10 % HA and water absorption of these samples increases. This may be due to the high watering absorption capability of HA granules. It is thought that water molecules, which penetrate within the polymeric matrix, are responsible for the hydrolytic decomposition of the polyester chains. Most probably, HA granules were able to absorb the degradation products, monomers and oligomers that further reduced the autocatalytic degradation of the polymer phase. Degradation of the composites is much slower especially for the composites with higher HA content.



(a) Change of pH



(b) Weight Loss



(c) water Absorption

Figure 6.38 Change of pH, Weight Loss and Water Absorption of PLA and PDLA Composites with 10 wt % HA with Respect to Degradation Time.

Shikinami et al [25] studied the hydrolytic degradation of PLA and its composites in thin slices of rods. Degradation behaviour of HA particles/PLA composites was examined in a solution such as phosphate buffer solution or simulated body fluid without the addition of the cells. Generous apatite crystals began to form on the surface of composites (40 wt % HA/PLA composites) after 3-7 weeks in SBF at 37

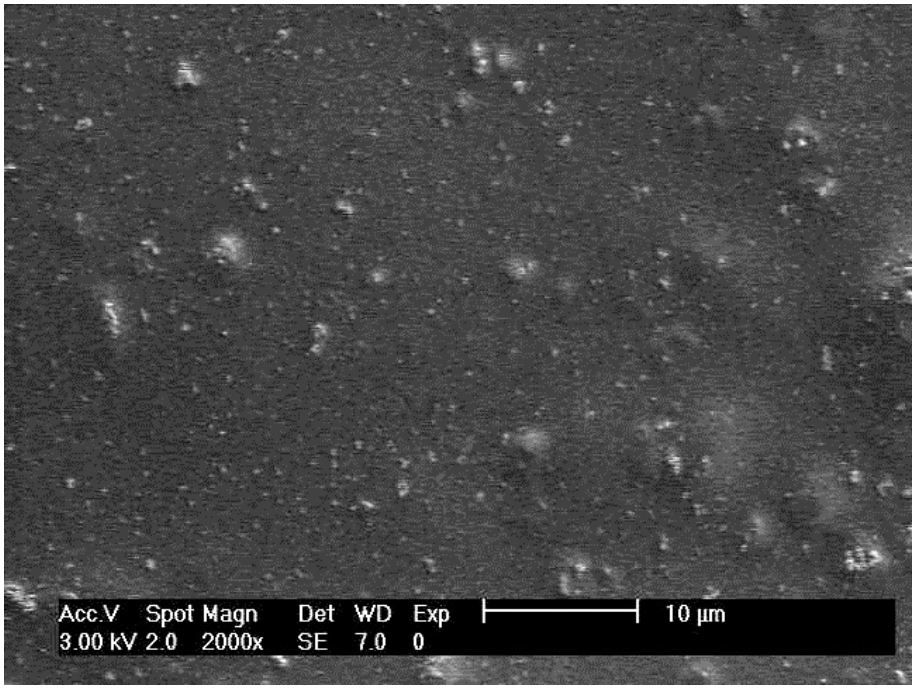
^{0}C and covered the whole surface with fairly thick layer in 7 weeks. The deposition of calcium phosphate crystals after immersion in SBF indicated the bonding capability of HA particles to surrounding bone. In other words, the apatite deposition may lead to bond a device to bone at start *in vivo*, and then to reduce a bone hole due to both degradation and resorption of PLA by replacing with the surrounding tissue.

As it is pointed out in Shikinami et al [25]'s work; even higher HA contents (more than 10 w/ w % HA) are desired when preparing polymer composites. Higher HA content may lead to better osteointegration of polymeric implants with bone since the biodegradability (or resorbability) of polymers are high.

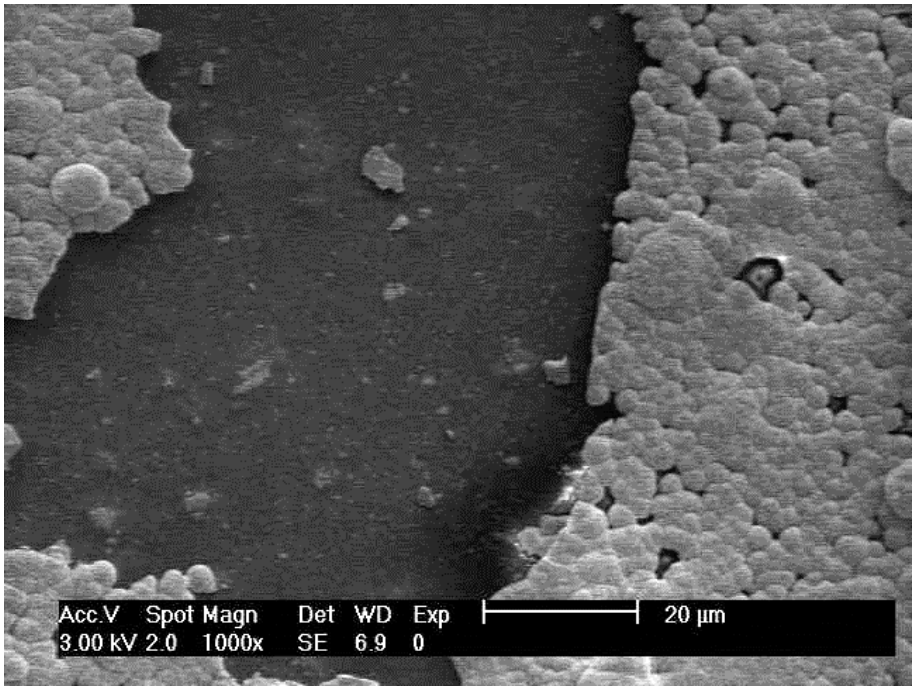
Therefore, composites containing 20-50 w/w % HA were also studied. Since dry weight of these samples was found higher than the initial weight, the weight loss of PLA and PDLA composites loaded with 20-50 w/w % HA could not be determined. The values obtained from these type of samples are shown in Appendix 2-A. It can be observed that composites containing 20-50 % HA showed different degradation trend at the end of 8th weeks.

In order to support our findings of degradation studies, the morphological changes occurred in PLA composites loaded with 10 and 30 w/w % HA before and after 8 weeks incubation in SBF and also HA powder were examined using SEM.

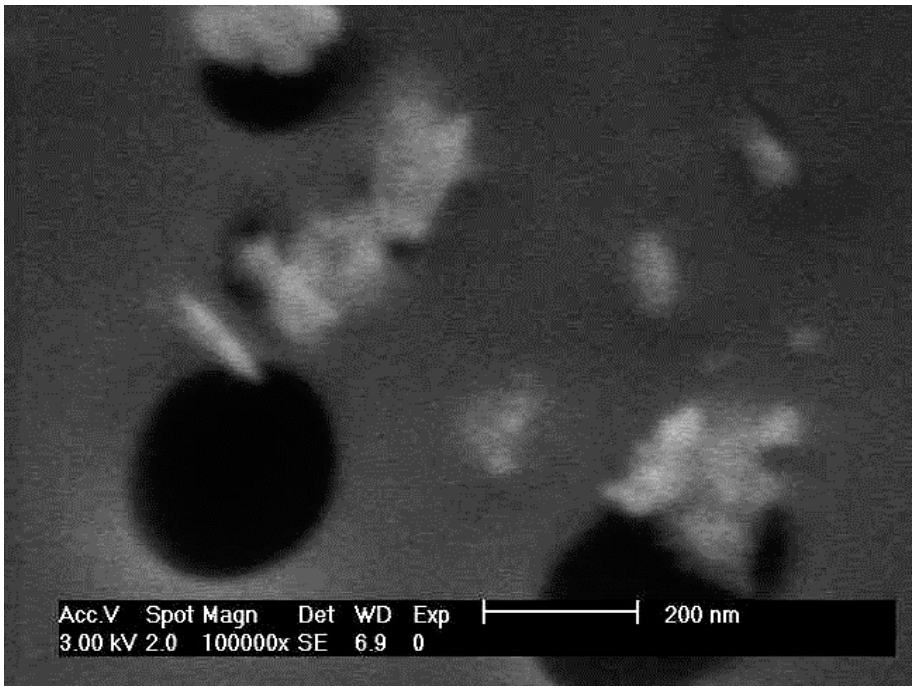
Figure 6.39 shows SEM images of surfaces that contain 10 w/w % untreated HA-PLA composites before and after being placed in simulated body fluid. Figure 6.39 (a) indicated that surface of the Polylactide composite, which contains 10 w/w % untreated HA. On scanning electron micrographs, it appeared that the dissolution of the HA coating on PLLA and PDLA composite is rather abrupt in nature. A possible explanation is that the precipitation of calcium in simulated body fluid is coated into the polymer composites. At the end of 8 weeks, Ca^{2+} and PO_4^{3-} come from simulated body fluid or deposition of HA, disappearance of the HA coating on the polymer surface as seen in the scanning micrographs in Figure 6.39 (b). It is also likely that dissolved calcium (Ca^{+2}) and phosphate (PO_4^{-3}) ions may be utilized in new bone formation and also these types of composites have good bone bonding capability. PLA and PDLA composites have osteogenic potential. On the other hand, bulk degradation of the polymer matrix was observed at the end of 8 weeks as seen in SEM images in Figure 6.39(c). It was observed that void of surface on polymer has caused the degradation of the matrix starting from the inner parts of the material. During this period, morphology of the hydroxyapatite was changed as shown in Figure 6.39 (d).



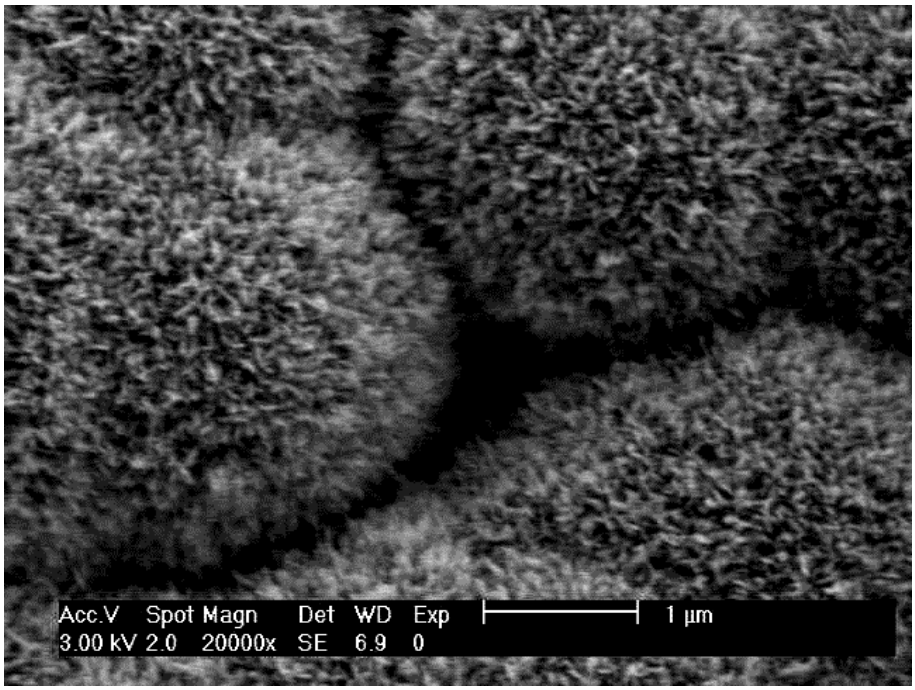
(a) PLA Composites content 10 % HA loading before SBF



(b) PLA Composites Content 10 % HA Loading after 8 Weeks in SBF



(c) PLA Composites Content 10 % HA Loading after 8 weeks in SBF

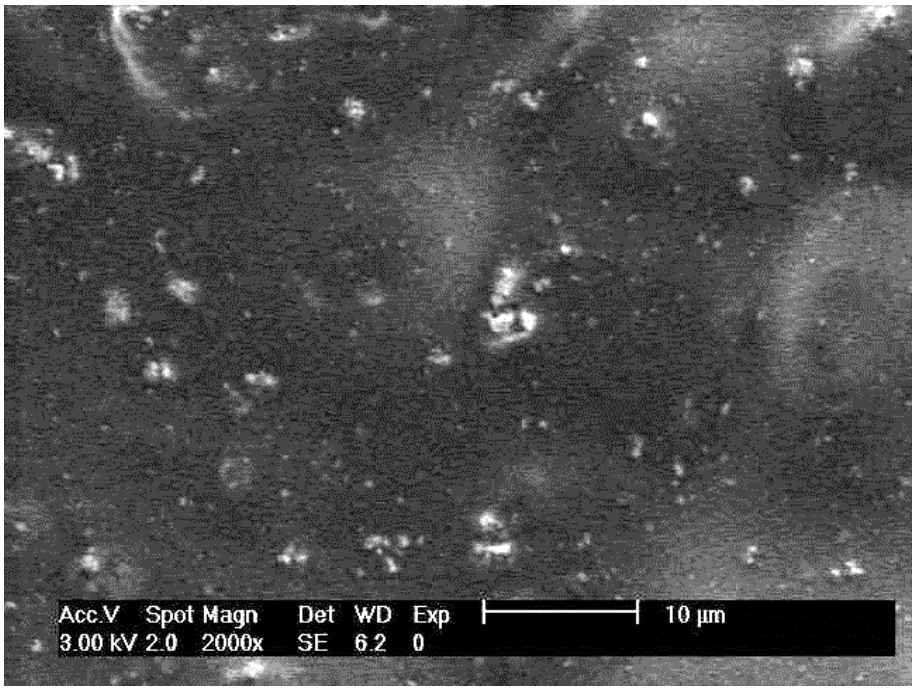


(d) Morphology of HA Powder after 8 weeks in SBF

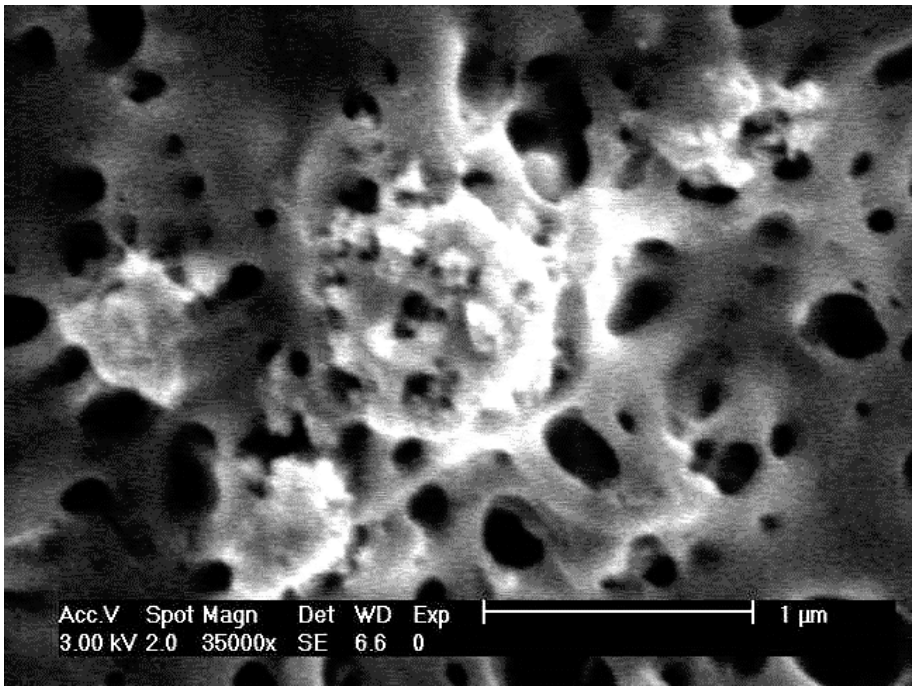
Figure 6.39 SEM Images of Surfaces that Contain 10 wt % Untreated HA-PLA Composites Before and After Placed in Simulated Body Fluid.

Verheyen and co-workers [6] studied the degradation behaviour of HA/Poly (L-Lactide) composites in three different aqueous buffer solutions; Citrate, Gomori's and phosphate buffered saline up to 24 weeks. PLA composites containing 30 w/w % and 50 w/w % HA showed a significant dissolution of HA coating on PLA in all buffer solutions and citrate buffer none. This study supported that the *in vitro* solubility of calcium phosphates is as dependent on the surrounding medium as on the composites. And also, increasing the HA content in the composites increased deposition of calcium and phosphate ions.

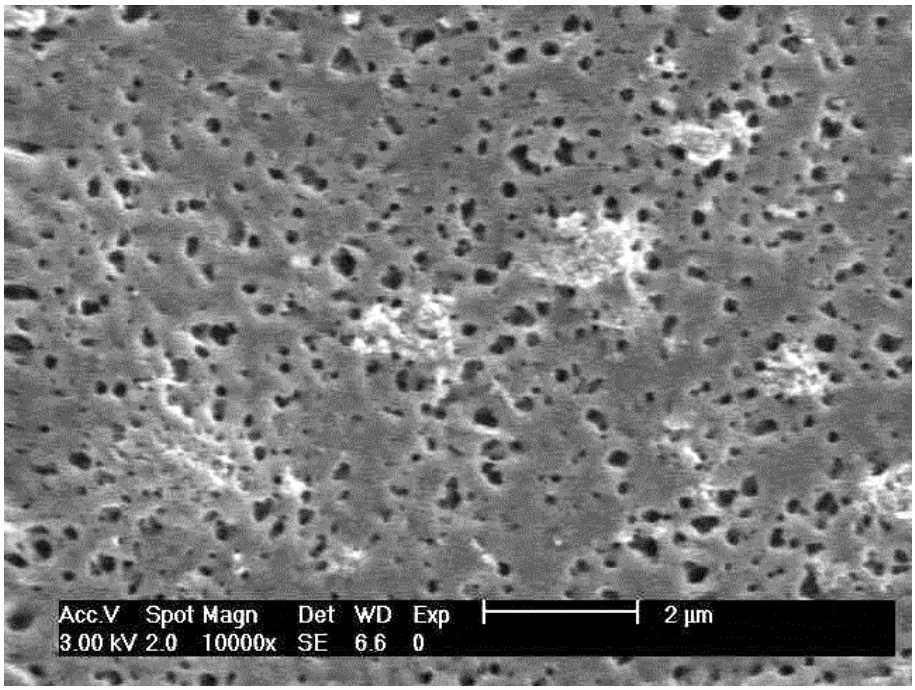
Coatings of the composites with calcium layer are much higher especially for the composites with higher HA content. In addition, SEM images demonstrated that the porosity of polymer matrix increased rapidly in relation to HA content. Figure 6.40 indicated that SEM photographs of surfaces that contain 30 w/w % untreated HA-PLA composites before and after being placed in simulated body fluid during 8 weeks.



(a) PLA Composites content 30 % HA loading before SBF



(b)PLA Composites Content 30 % HA Loading after 8 weeks in SBF



(c) PLA Composites Content 30 % HA loading after 8 weeks in SBF

Figures 6.40 SEM Images of Surfaces that Contain 30 wt % Untreated HA-PLA Composites before and after Being Placed in Simulated Body Fluid.

Chapter 7

CONCLUSIONS AND RECOMMENDATIONS

In the scope of this study, the preparation and characterization of Hydroxyapatite-Poly-L-Lactide and Poly-L-Lactide-D-Lactide copolymer composites as biomaterial were studied. The effects of polymer types, Hydroxyapatite loading, surface modification of Hydroxyapatite and their concentrations on the mechanical, microstructural, hydrolytic and thermal degradation properties of the composites were investigated.

In the preparation of composites, Poly-L-Lactide and Poly-L-Lactide-D-Lactide copolymer were used as a matrix and Hydroxyapatite was used as filler. Solvent casting technique was used to obtain the polymer composite films. Hydroxyapatite was synthesized by the precipitation technique. To improve the dispersion of filler in the polymer and the interfacial adhesion between polymers and fillers, the surface of this powder has been treated with two different silanes as coupling agents, which were 3-aminopropyltriethoxysilane (AMPTES) and 3-mercaptopropyl-trimethoxysilane (MPTMS).

Mechanical test studies indicated that Poly-L-Lactides (PLA_1 and PLA_2) are semicrystalline polymers and their Young's Modulus were found to be higher than poly-D-Lactide-L-Lactide copolymers which have amorphous structure. The effect of particle size of HA on the Young Modulus values of the composites was also determined in this study. It was found that, Young Modulus value of composite with 10-wt % HA loading increased from 644.24 MPa to 877.72 MPa as the particle size of HA was reduced. The Young Modulus increased with HA loading increase except for the 50 wt % HA loaded composite. This might be due to the increase agglomeration size of HA.

Surface treatment of HA with silane coupling agent improved the dispersion of HA in the polymer matrix as well as the mechanical properties of the composites. According to the tensile test results, the maximum improvement in the mechanical properties of the composites was obtained for PLA composites containing 1 wt % AMPTES treated HA and 0.5 wt % MPTMS treated HA for PDLA composites. Young's modulus, yield stress values of the PLA composites containing 10 wt % treated

HA with 1 wt % AMPTES were found as 982,8 MPa, and 42,1 MPa, higher than those of 10 wt % untreated HA loaded composites.

The particle size and surface treatment of HA with silane coupling agents affected the morphology of the PLA and PDLA composites. The surface treated and small particle size of HA was dispersed more homogeneously in polymer matrix. This may be due to the decrease in the agglomeration size with surface modification of HA by the strenght of interactions between particles.

In the FTIR studies, the characteristic peaks of Polylactide and HA was observed in the spectra of the composites. However, silane-coupling agents did not significantly affect the peaks corresponding to both hydroxyapatite and Polylactide composite films. This could be due to the low vibration intensity of silane coupling agent.

Thermal analysis studies indicated that the addition of HA into the polymer matrix decreased the thermal degradation temperature and degradation rates. The information obtained from TGA studies about the hydroxyapatite content of the samples did nearly match with actual amount of HA present in the composites. This also showed that HA was dispersed homogeneously in the polymer matrix.

The results of hydrolytic degradation properties of the pure polymers and their composites showed that the hydrolytic degradation depend on polymer type, the HA loading and ageing conditions. The pH of the medium containing polymer or composites in all cases did not change significantly end stayed. Weight loss data of pure polymers showed that the degradation rates of PLA1 and PLA_2 were much slower than those of $PDLA_1$ and $PDLA_2$. At the end of 150 days, only 12.5% and 9.5 % of weight loss were obtained for PLA_1 and PLA_2, respectively. Weight loss data of $PDLA_1$ and $PDLA_2$ were found to be nearly the same being 17.5 wt % and 17 wt %, respectively. On the other hand, PLA and PDLA composites loaded with 10 wt % HA degraded slowly while water absorption values increased. Weight loss decreased approximately from 12 wt % to 5 wt % and water absorption increased from 10 wt % to 13 wt % for PLA with loaded 10 % HA. At the end of 8 weeks, weight loss of PLA and PDLA composites loaded with 20-50 wt % HA were not determined, because dry weight of samples increased. In order to support our findings of degradation studies, the morphological changes occurred on PLA composites loaded with 10-50 wt % HA before and after incubation in SBF was examined using SEM. On scanning electron micrographs it appeared that the dissolution of the HA coating on PLLA and PDLA

composite is rather abrupt in nature. A possible explanation of this is that the precipitation of calcium in simulated body fluid is coated into the polymer composites and also the dissolution of HA in the polymer matrix. This showed the new bone formation and also composites have good bone bonding capacity. It was also found that the coating of polylactide composites with calcium phosphate layer was increased with the increase HA loading and ageing time.

In the future studies, the polylactide-HA composites can be prepared by phase separation technique in order to see the effects of preparation method on the mechanical, microstructural and hydrolytic degradation properties of the composites. The effect of solvent and HA type can also be studied. In the hydrolytic degradation studies, ageing conditions of the SBF such as pH, temperature can be changed in order to see the effects on degradation behaviour. Mechanical properties of the samples aged in SBF can also be monitored.

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APPENDIX

Table A.1. Tensile Test Results of Polylactide-Hydroxyapatite Composites

	Young Modulus N/mm^2	Stress @ Peak N/mm^2	Strain @ Peak %	Elongation @Break mm
PLA1	833.9	27.7267	3.6793	3.4506
PDLA1	194.9	9.381	2.4553	1.8383
PLA2	450.2	17.141	4.4926	3.373
PDLA2	335.5	4.8731	1.7247	2.313
MN1	877.7	39.272	4.849	3.423
MN2	1006.9	24.381	2.8993	2.0753
MN3	1198.7	27.3133	2.8453	2.0093
MN4	589.3	7.8967	2.444	1.727
CD1	982.8	42.118	4.7	3.227
CD2	1062.4	27.1423	3.41	2.607
CD3	1420.8	18.89.43	2.1553	1.7253
CD4	880.2	13.11	1.974	1.475
DM1	501.3	13.212	4.2327	2.7287
DM2	643.2	15.1467	3.996	2.4986
DM3	650.3	16.421	6.369	3.89
DM4	214.9	14.003	2.8273	2.1746
HJ1	621.9	18.876	3.3820	2.7156
HJ2	708.9	14.446	3.2718	2.4586
HJ3	762.3	15.379	4.4080	2.9886
HJ4	638.2	14.439	3.3006	2.5196
AB1	417.2	18.911	3.7753	2.6590
AB2	463.9	12.577	4.3880	2.8943
AB3	762.7	18.600	4.1690	2.5830
AB4	508.7	12.971	2.7080	1.8916
EF1	632.9	23.4880	4.9770	3.0355
EF2	843.055	24.2485	3.6150	2.3190
EF3	1082.20	19.1885	2.6320	1.9575
Ef4	725.340	15.8380	4.3067	2.7480
BC1	472.96	12.865	2.9436	2.8086
BC2	525.51	12.213	2.9075	2.0885
BC3	561.896	10.991	3.6346	2.4126
BC4	321.98	9.309	1.8535	1.3550
KL1	528.736	16.934	5.964	4.216
KL2	607.26	15.439	5.034	3.7593
KL3	692.55	14.89	5.0527	3.651
KL4	571.325	7.5735	2.062	1.548
C21	565.81	23.1856	4.7705	3.2717
C12	644.24	28.142	5.1093	3.7416
P12	676.56	30.991	4.892	3.997
CD20	929.7	20.970	3.1784	1.9868
CD22	1084.0	20.754	2.5533	1.7527
CD21	1263.4	28.771	3.2113	3.0100

GH20	1167.0	32.593	4.2555	2.5257
GH21	972.5033	19.56	2.8426	2.959
GH22	967.8	21.814	2.8185	1.728
EF20	462.06	18.62	4.0860	4.509
EF21	843.055	24.2485	3.6150	2.3190
EF22	586.32	21.869	4.4413	2.7963
FG20	528.575	16.1185	4.7	3.053
FG21	485.22	18.539	5.5067	3.459
FG22	382.56	10.946	4.4705	2.7215
KL20	597.203	12.77	3.557	2.26
KL21	541.52	11.081	3.847	2.594
KL22	508.185	13.133	3.861	2.749

Table A.2. Hydrolytic Degradation Data for Polylactide Composite containing 20 wt %-30 wt % HA.

Time, Day		Wi (mg)	Wf (mg)
	MN1		
1		107	106
2		113	111
4		124	121
9		102	100
16		111	108
23		137	132
38		135	129
52		116	109
66		120	114
80		106	101
94		135	127
	MN2		
1		104	102
2		97	95
4		88	85
9		88	85
16		83	79
23		102	104*
38		88	94*
52		109	114*
66		76	82*
80		87	94*
94		92	99*
	MN3		
1		98	96
4		108	106
7		98	94
14		105	100
21		107	113*
35		106	114*
49		103	112*
63		95	109*
	MN4		
1		162	158
4		154	150
7		142	138
14		140	136
21		146	154*
35		150	156*
49		151	159*
63		160	169*
	DM1		
1		55	54
4		45	43

7		46	44
14		46	44
21		49	47
35		44	40
49		45	40
63		46	39
	DM2		
1		49	48
4		57	55
7		53	49
14		48	46
21		46	51*
35		43	47*
49		42	45*
63		40	46*
	DM3		
1		46	45
4		43	42
7		47	45
14		45	43
21		45	49*
35		36	39*
49		44	49*
63		47	53*
	DM4		
1		98	96
4		76	74
7		71	69
14		41	48*
21		61	69*
35		45	56*
49		53	61*
63		63	72*

NOMENCLATURE

PLA1: Poly L-Lactide from PURAC

PDLA1: 96/4 L-Lactide, D-Lactide Copolymer from PURAC

PLA2: Poly L-Lactide from Boehringer Ingelheim

PDLA2: 67/23 Poly (L-Lactide-co-D,L-Lactide) from Boehringer Ingelheim

HA_1: Commercial Hydroxyapatite powder

HA_2: Reduced particle size of commercial Hydroxyapatite powder

HA_3: Synthesized Hydroxyapatite powder in laboratory

HA_{s1}: Treated synthesized Hydroxyapatite powder in laboratory with AMPTES

HA_{s2}: Treated synthesized Hydroxyapatite powder in laboratory with MPTMS

MN1: 10 wt % HA_3 /PLA1 Composite

MN2: 20 wt % HA_3 /PLA1 Composite

MN3: 30 wt % HA_3 /PLA1 Composite

MN4: 40 wt % HA_3 /PLA1 Composite

CD1: 10 wt % HA_{s1} /PLA1 Composite

CD2: 20 wt % HA_{s1} /PLA1 Composite

CD3: 30 wt % HA_{s1} /PLA1 Composite

CD4: 40 wt % HA_{s1} /PLA1 Composite

DM1: 10 wt % HA_3 /PDLA1 Composite

DM2: 20 wt % HA_3 /PDLA1 Composite

DM3: 30 wt % HA_3 /PDLA1 Composite

DM4: 40 wt % HA_3 /PDLA1 Composite

HJ1: 10 wt % HA_{s2} /PDLA1 Composite

HJ2: 20 wt % HA_{s2} /PDLA1 Composite

HJ3: 30 wt % HA_{s2} /PDLA1 Composite

HJ4: 40 wt % HA_{s2} /PDLA1 Composite

AB1: 10 wt % HA_3 /PLA2 Composite

AB2: 20 wt % HA_3 /PLA2 Composite

AB3: 30 wt % HA_3 /PLA2 Composite

AB4: 40 wt % HA_3 /PLA2 Composite

EF1: 10 wt % HA_{s1} /PLA2 Composite

EF2: 20 wt % HA_{s1} /PLA2 Composite

EF3: 30 wt % HA_{s1}/PLA2 Composite

EF4: 40 wt % HA_{s1} /PLA2 Composite

BC1: 10 wt % HA_3 /PDLA2 Composite

BC2: 20 wt % HA_3 /PDLA2 Composite

BC3: 30 wt % HA_3 /PDLA2 Composite

BC4: 40 wt % HA_3 /PDLA2 Composite

KL1: 10 wt % HA_{s2} /PDLA2 Composite

KL2: 20 wt % HA_{s2} /PDLA2 Composite

KL3: 30 wt % HA_{s2} /PDLA2 Composite

KL4: 40 wt % HA_{s2} /PDLA2 Composite

C21: 10%wt HA_1/PLA1 Composite (PLA1+SOLVENT+HA1)

C12: 10%wt HA_1/PLA1 Composite (HA1+SOLVENT+ PLA1)

P12: 10%wt HA_2/PLA1 Composite (HA1+SOLVENT+ PLA1)

CD20: 20%wt HA_{s1} /PLA1 Composite with 0,5 wt % AMPTES/HA_3

CD21: 20%wt HA_{s1} /PLA1 Composite with 1 wt % AMPTES/HA_3

CD22: 20%wt HA_{s1} /PLA1 Composite with 2 wt % AMPTES/HA_3

GH20: 20%wt HA_{s2} /PLA1 Composite with 0,5 wt % MPTMS/HA_3

GH21: 20%wt HA_{s2} /PLA1 Composite with 1 wt % MPTMS/HA_3

GH22: 20%wt HA_{s2} /PLA1 Composite with 2 wt % MPTMS/HA_3

EF20: 20%wt HA_{s1} /PLA2 Composite with 0,5 wt % AMPTES/HA_3

EF21: 20%wt HA_{s1} /PLA2 Composite with 1 wt % AMPTES/HA_3

EF22: 20%wt HA_{s1} /PLA2 Composite with 2 wt % AMPTES/HA_3