Fabrication and Characterization of Polymer Composite for Biomedical Applications

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CERTIFICATE

This is to certify that work in this thesis has been carried out by **Aamna Hameed, Rameez Riaz** and **Salaha Ali** and completed under my supervision in Functional Materials Laboratory, Mechanical Testing Laboratory and Heat Treatment Laboratory, School of Chemical and Materials Engineering, National University of Sciences and Technology, H-12, Islamabad, Pakistan.

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DEDICATED

TO

MY LOVING MOTHER

Who is my heaven

Her prayers, guidance and help

Made possible everything

Her hands are always raised for

My success and well-being

MY DEAR FATHER

Whose love is more precious than pearls and diamonds

By the virtue of his prayers

I have been able to reach at this position

MY LOVING SISTER AND BROTHER

Who are the world for me

Their love and prayers encouraged me

At every step

MY KIND TEACHERS AND FRIENDS

Whose efforts and guidance

Made possible for me to reach at this position

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We set our humble thanks before Him, who is one and only, the Creator of all skies and earth and Lord of all worlds The Almighty ALLAH, the omnipotent and the omnipresent. All acclamations and praises to Him, who blessed us with good health, zeal of doing work, talented teachers and an opportunity to add a little part to the sphere of knowledge in this world. The humblest and the deepest obligation are also paid, with great honor and esteem to the Holy Prophet Muhammad (PBUH), the cause of creation of Islam, the most perfect in this universe, who is forever, a beacon of perfect guidance and knowledge for humanity as a whole. Who enlightening our conscience with the essence of faith in Allah. Converging all this kindness mercy upon him who guided the mankind to the path of education and research.

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ABSTRACT

By

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Synthetic polymeric bio ceramic scaffolds are employed for tissue regeneration, which involves the fabrication of functional structures. We synthesized the bio ceramic filler hydroxyapatite (HA) which enhances the mechanical properties and cell adhesion properties of scaffolds. By using wet chemical Analysis technique, Hydroxyapatite is synthesized. To obtain the optimum particle size we annealed HA at three different temperatures, 500℃, 900℃ and 1200℃. SEM results of Hydroxyapatite depict that by increasing annealing temperature the particle size of HA also increases and porosity decreases due to sintering that occur among the ceramic powder particles. The XRD results show that the crystalline peaks of HA are sharper at high temperatures. After the synthesis of ceramic filler, we synthesized four different composites of Polycaprolactone (PCL) and four different composites of TOPAS (cyclic olefin copolymer) with hydroxyapatite (HA) as the ceramic filler by solution casting method. We are aiming to study the enhancement in mechanical properties of PCL by adding HA. The samples were designed according to ASTM standard for tensile testing. By comparing the tensile results of PCL/HA composites it is observed that mechanical properties of PCL are significantly enhanced by adding hydroxyapatite. We also analyzed the dispersion of hydroxyapatite in the composite films of PCL and TOPAS by carrying out scanning electron microscopy (SEM) analysis.

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ABBREVIATIONS

PCL- Polycaprolactone

HA-Hydroxy Apatite

XRD-X-Ray Diffraction

UTM-Universal Testing Machine

SEM-Secondary Electron Microscopy

$Ca\ (NO_3)_2$-Calcium Nitrate

$(NH_4)_2HPO_4$ -Ammonium Phosphate

NH_4OH -Ammonium Hydroxide

pH-Power of Hydrogen

$CaCO_3$ -Calcium Carbonate

TOPAS- Cyclic olefin copolymer

FDA- Food and Drug Administration

PLA- Polylactic Acid

PLGA- Poly lactide coglycolide

SDS- Sodium dodecyl sulfate

CHAPTER 1

INTRODUCTION

Our project is on the synthesis of "Scaffolds for tissue regeneration". The most important field in tissue engineering is the design and manufacture of scaffolds. Scaffolds should have specific appropriate mechanical and biological properties as compatible for the desired application in the human body for the support and repair of organs. The porosity of scaffold is also a crucial property for its application. Therefore, the materials incorporated for the design of scaffolds have an essential role to play in tissue repair and regeneration.

1.1 Problem statement

A suitable polymeric bio-ceramic composite film is to be synthesized by simpler methods, as a suitable material for scaffolds used in tissue regeneration.

1.2 Our objective

- Synthesis and characterization of Hydroxyapatite as the ceramic filler with optimum particle size
- Study the effect of annealing temperature on the particle size of Hydroxyapatite
- Synthesis and characterization of polymeric bio-ceramic composite scaffolds using Polycaprolactone (PCL) and TOPAS as the polymers
- Investigation of mechanical properties of Polycaprolactone (PCL)/Hydroxyapatite composite

1.3 Our purpose of study

Our aim is to locally develop a suitable biomaterial composite for tissue regeneration, having appropriate mechanical properties by using simpler methods. We are using Polycaprolactone (PCL) and TOPAS as our two polymers for the synthesis of two different composites using Hydroxyapatite (HA) as the ceramic filler. This composite is synthesized to give suitable combination of toughness and stiffness by the incorporation of Hydroxyapatite which is used to increase mechanical strength and cell adhesion for the support and repair of body tissues. The effect of different annealing temperature for obtaining hydroxyapatite particles of optimum particle size shall be studied by carrying out SEM and XRD analysis. Four different combinations of compositions shall be employed for synthesizing composites of PCL/HA and TOPAS/HA. Synthesis and characterization of the polymeric-bio ceramic composite films to study analyze the effect on porosity and dispersion of different compositions.

The framework of the introduction is planned to direct the reader about important aspects of history, background, material, and synthesis of the scaffolds. The following section describes the process of tissue regeneration, synthesis, and processing techniques of scaffolds.

1.4 Significance of our study

To contribute to the field of biomaterials and tissue regeneration by synthesizing composites with the ceramic filler being added to the bio-polymer in various proportion in order to investigate the flexibility of mechanical properties such as elasticity, tensile strength and stiffness. This study allows the synthesis of scaffolds with the desired mechanical and biological properties. The synthesis of ceramic filler of optimum particle size with simpler methods is crucial for use in such bio-

composites. Typically, the biomaterials belonging to the group of synthetic polymers, natural polymer and ceramics were widely being used as an individual component of scaffolds. However, one property or the other such as strength, elasticity, biocompatibility, biodegradability, or stiffness needs to be compromised when such materials are used individually. Therefore, the study on the synthesis and characterization of such composites allows the fabrication of scaffolds with the desired properties. The revolutionizing field of tissue engineering can facilitate then numerous surgeries that are being performed frequently to support, replace or repair the human body tissues. These scaffolds being highly porous interact with the human body cells when incorporated inside the body to help regenerate and promote the growth of damaged tissues.

1.5 Organization of the study

This thesis has been divided into the following five different chapters:

- ✓ The first chapter constitutes the introduction to our thesis which describes the purpose of study and our objectives. We have included the background of the field of tissue regeneration which is a subfield of tissue engineering and how the discovery of biomaterials have revolutionized the study of the supportive structures called scaffolds. The second chapter outlines the information we have attained from the existing literature on the study of scaffolds for tissue regeneration. It includes the characteristics and specifications that are desired in scaffolds such as the mechanical and biological characteristics. The materials that have already been studied and employed in scaffolds are mentioned. We have also summarized the materials that we will be employing for our project.
- ✓ The third chapter describes the methodology and experiments that we have performed for the synthesis of hydroxyapatite, PCL/HA composite and TOPAS/HA composite.

- ✓ The fourth chapter consists of analysis and discussion of the results we have retrieved by performing scanning electron microscopy, x-ray diffraction and tensile testing on our samples of hydroxyapatite, PCL/HA and TOPAS/HA composite films.
- ✓ The fifth chapter constitutes of conclusion of our research which summarizes the study we have performed.

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1.6 Tissue engineering and tissue regeneration

The field of Engineering and Technology is not static, it is changing dynamically every minute. This advanced knowledge alters our understandings about knowledge itself. Providing us new means and methods of organizing and utilization of that knowledge. And ultimately results in advancement of different fields and emergence of various opportunities in research. In a biomedical sense, the word "tissue engineering" simply refers to a clear, effective and insightful central concept: the development of living tissues for medicinal functions. [10]

Tissue engineering includes the combination of engineering, material science and biological science to replace or remove the biological tissues. It is one of the vital fields of biomaterial in which tissue scaffolds of different biomaterials are used in

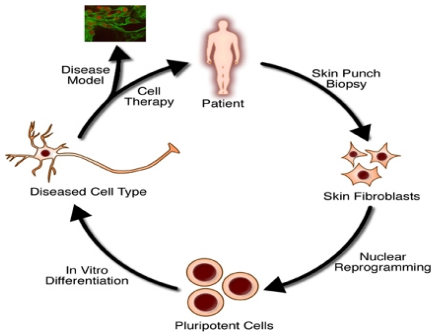


FIGURE 1. 1 GENERAL METHODS USED IN REGENERATIVE MEDICINE

the regeneration of new tissues used in therapeutic procedures. [3]

Tissue engineering's aim is to create functional structures that repair, sustain, or recover damaged tissue or entire organ. Cartilage and artificial skin are cases of engineered tissues which are approved by the FDA but, they have restricted use in human patients at present. Groups of cells generally create and conceal their own support frameworks, known as extra-cellular matrix. This cellular matrix, or scaffold, supports cells as well as acts as a monitoring station for various gesticulating molecules. Therefore, cells collect information from different sources that are present in the local environment. By studying about the response of different cells to this information and how these cells organize themselves into tissues and organs, the scientists have been able to maneuver these functions to mend or even create new tissues that were damaged. The cycle starts with a scaffold being assembled from a wide range of possible materials e.g. proteins or plastics. When scaffolds are formed, cells can be added with or without a growth factor. If the atmosphere is suitable, then a tissue can grow. Sometimes, all cells, growth factors and scaffold are blended at the same time, allowing "self-assembly" of the tissue. The use of human tissue to help test candidates for medication could speed progress and provide key resources to promote therapeutics while saving money and reducing the number of animals used for testing. [7]

Regeneration means re-growth of a part of the remaining tissue's affected or lost organs. Some organs, such as the liver, may regenerate from animals. If, due to infection or injury, a part of the liver is damaged, the liver grows back to its initial size, but not in its actual shape or structure. Regenerative medicine is a modern scientific discipline that attempts to improve the course of fatal diseases and, in many cases, regrows organs structures that malfunction due to age, illness, injury or genetic disorders.

Tissue regeneration studies ' main objective is to gain information that will improve the advanced scope of regenerative medicine. This knowledge consists of scientific proves to stimulate stem cell activity, better scaffold structural engineering or the precise instigation of programs for biological regeneration. [8]

1.7 Scaffolds for tissue regeneration

Scaffolds are 3D porous structures manufactured to perform following functions:

1. Helps in interaction between biomaterial and cell.

2. Promote extra-cellular matrix deposition and cell adhesion

3. For cell proliferation, survival and differentiation scaffolds allow proper transport of nutrients, gases, and other essential factors.

4. The degradation rate is controlled and same as tissue regeneration rate under specific biological conditions.

5. Non-toxic in nature and cause no or very less inflammation in vivo.

Developing scaffolds with desirable properties, including their strength, degradation rate, microstructure, porosity, as well as their sizes and shapes, are regulated in polymeric composite scaffolds quite conveniently. Biological scaffolds are made from polymers or from natural tissues like animal tissues, and human tissue. In 1974, the very first biologically responsive scaffold was designed; its degradation properties and unusually low in vivo antigenicity, including its in vitro thromboresistant actions were explained. In 1977 the initial patent covering certain scaffolds was issued. Principles for the synthesis of a biologically active scaffold were described in detail in 1980, comprising the crucial importance of the degradation rate. (1)

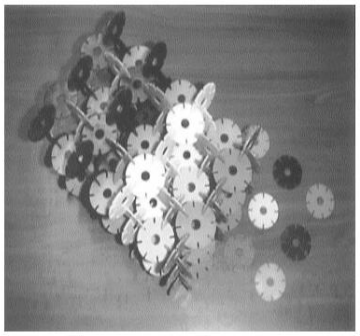


FIGURE 1. 2 POLYMER DISKS WITH A DIAMETER OF 500 MM AND 40 MM THICKNESS

1.8 Role biomaterials in tissue engineering

In tissue engineering, biomaterials play an important role by behaving as synthetic structures, described as matrices, scaffolds, or constructs. The designing of biomaterials is continuously evolving. Materials used in biomedical fields have become increasingly important in recent years. [11]

As Hench and Polak mentioned in their main article [10] published in 2002, biomaterials have developed over the last fifty years and today can be believed "third generation biomaterials." Biomaterials were initially selected due to the reason that they were biologically inert, the objective was to decrease the immune response of the body to foreign implanted material. Although this objective is still effective today, scientists have come to realize that complete biological inertness is synonymous with the body's failure to recognize it. This dearth of biological understanding is frequently followed by fibrous encapsulation of tissue and fatal inflammation, that in effect jeopardizes the mechanical efficiency and long-term prosthesis biocompatibility. Therefore, biomaterials of the second generation were designed to modify or improve biological detection to improve the interface between the biomaterials and the body. Biomaterials of the second generation used bioactive components which could induce monitored action and reaction in the physiological environment. Synthetic hydroxyapatite and Bioglass® are two very typical examples of those components. The problem of the biomaterial-body interface was also solved by using resorbable materials, removing the interface between them completely. The principal example of these resorbable materials are resorbable polymers, like polylactic acid (PLA) and polyglycolic acid (PGA) which degrade hydrolytically into water and Carbon dioxide. These are used as screws in orthopedics, sutures, and in delivery systems with controlled drug discharge. Biomaterials of the third generation are currently being developed, extending the idea of biological recognition toward specific biological recognition. While biomaterials of the third generation strive to induce cellular responses: association with different integrins, cell differentiation stimulation or initiation of specific genes. It is also important to stress how the design of these biomaterials is

underway. In other words, biomaterials from third generations are no longer derived from present materials and tailored to a therapeutic purpose. [13]

CHAPTER 2

LITERATURE REVIEW

2.1 Requirements for scaffolds

Regenerative medicine and Tissue engineering approaches for tissue and organ recovery or replacement, differ widely but usually involve using a scaffold material in vitro and/or in vivo to facilitate the transmission and/or development of the cells. [14]

Improving the acceptance of clinical applications of such tools requires the incorporation of certain biological features as well as clinical and mechanistic characteristics which can play a role in theoretical and practical application. A suitable scaffold must be efficient enough of repairing body tissues with minimal needs for vascularization, cell growth, proliferation, and host incorporation, and lastly, materials should be degraded naturally during or after the recovery procedure. [15]

Multiple scaffolds developed from a wide range of biomaterials and produced utilizing a variety of manufacturing practices were applied in the area in efforts to regenerate various tissues and organs within the body. Some characteristics which should be considered while developing scaffolds are:

2.1.1 Biological characteristics:

The biological characteristics of scaffolds include their properties in terms of biocompatibility and nontoxicity. To produce a new matrix, cells grown in scaffolds need to be able to reproduce and differentiate freely without interference. A scaffold is therefore regarded as an ultimate scaffold for Tissue Engineering applications if it can imitate the Extra Cellular Matrix properties of tissues for optimal and maximum regeneration. [14]

2.1.1.1 Biocompatibility:

For tissue engineering, the most important requirement of any applicable scaffold is that it must show biocompatibility, means, the cells should adhere, work absolutely

normal, and transfer to the surface and ultimately through the scaffold and continue to proliferate before establishing a new matrix. Upon implantation, the scaffold or tissue-engineered structure must induce a minor immune response to avoid it from triggering such a serious provocative reaction that it may minimize healing or cause body's negative response. [15]

Biocompatibility allows new tissue to grow concurrently, along with matrix degradation. The cellular matrix should not be harmful, so it can be disposed of by the system without affecting other members. A scaffold's biological characteristics are a major modulating problem as they influence scaffold contact with tissues and organs. Because of the low capacity of biological material to communicate with the environment, work have been done to integrate bioactive scaffolds to facilitate the proper cellular activity, replication or differentiation, tissue information, and host integration, and to practice bioactive scaffolds to evade unwanted processes such as blemishing. [14]

2.1.1.2 Biodegradability

The important goal in the field of tissue engineering is to enable the body's own cells to ultimately replace the scaffold or tissue-engineered construct implanted over time. Scaffolds are not meant to be everlasting implants. Therefore, the scaffold must be biodegradable, so that cells can produce their own extracellular matrix. The final by products formed in the result of degradation should not be toxic and capable of exiting the body with no interfering with other organs.[15]

2.1.2 Mechanical characteristics:

The scaffold should have mechanical properties that are compatible with the functional site in which it is to be inserted and must be robust enough from a practical perspective to require surgical treatment in the course of implantation. Although this is essential in all tissues, it specifically poses some challenges for cardiovascular and orthopedic applications. One of the major problems in trying to engineer bone or cartilage is the development of scaffolds with appropriate mechanical characteristics. The implanted scaffold must have adequate mechanical reliability for these tissues to operate well from the time of implantation until the

remodeling process is complete. It is important that mechanical strength as well as porosity for proper vascularization should be incorporated in scaffold designs. [15]

2.1.3 Structural Characteristics:

Biological tissue has an extremely complicated 3D structure along with intricate mechanical functions connected with the properties of mass transport. Hence Tissue Engineering's crucial objective is to shorten this structural intricacy and function by using scaffolds that offer tissue regeneration cells, proteins, and genes. It is evident that biological materials and assemblies cannot reproduce multifaceted tissue surroundings, comprising many types of cells that corelate with various of cytokines to create extracellular matrices inside cells with ordered properties that demonstrate high nonlinearity and two-phase mechanical operation. [14]

Scaffolds should consist of an interconnected porous arrangement with elevated porosity rate to guarantee cellular penetration and enough nutrient diffusion to the cells within the scaffold as well as to the extra-cellular matrix that these cells create. [5]

2.2 Materials used in scaffolds:

2.2.1 Polymeric materials:

For over fifty years polymers have found common use in biomedical applications. Polymers recognize biomaterials as the main class. These also offer the benefits of biodegradability and fast processing comparative to ceramic or metal biomaterials. All, natural polymers and synthetic polymers are used for medical use. Natural polymers might be of plant as well as animal origin. Cellulose, natural rubber or sodium alginate is some examples of natural polymers extracted from the plants. Types of animal-derived ones are collagen, or chitin. Natural polymers give the benefit of biological sensitivity which decreases issues like platelet attachment and unwanted protein adsorption. This makes them perfect candidates for the development of cardiovascular tissue, where these problems are critical. Nevertheless, they also need physical or chemical pretreatment to improve their

material features, enhance their resistance to oxidative or chemical biodegradation and decrease immunogenicity. In comparison, synthetic polymers offer great reproducibility and the capacity for wide-scale production, as well as regulated mechanical and biodegradation properties. However, they have very less biological activity, and may be highly hydrophobic. Few examples of synthetic polymers are polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL) etc.

2.2.2 Ceramic materials:

Ceramics contain a wide array of inorganic and non-metallic materials. While their use in tissue engineering is new, for centuries now ceramics have been used in medical science as eyeglasses, medical tools, chemical ware, endoscopy fiber optics, etc. Ceramics are used especially as biomaterials to replace strong connective tissues. Ceramic biomaterials can be classified by their comparative chemical activity which relates with the rate of interfacial bond formation with the body. In fact, implant malfunctions every so often result from movement taking place between the interface of tissue and biomaterial or caused by poor interfacial bond. To solve interfacial problems, careful selection of the implantation sites and fits is required. For example, in the case of approximately inert bio ceramics, an exceptionally close fit minimizes the encapsulation of fibrous tissue and thus ensures successful implant. A biological fixation can be formed in the case of porous implants, if the host tissue starts growing into the pores. Bioactive ceramics form a good interfacial bond that can even withstand considerable mechanical forces. Bioactive ceramic includes Bioglass ®, calcium phosphate compounds and glass ceramics. Bioactive ceramics grow on their surface a carbonated hydroxyapatite layer that forms an interface with the neighboring tissue.[12]

2.3 Reported Materials for Scaffolds

2.3.1 PCL and PCL/PLA scaffolds for tissue engineering and their characterization

Polycaprolactone is a biopolymer that has high biodegradability and biocompatibility along with having excellent mechanical properties and structural stability. Therefore, PCL is being used extensively for tissue regeneration in order to produce scaffolds. There are a few limitations in using PCL because it has a low bioactivity and high hydrophobicity resulting in lower surface energy, resulting in smaller cell affinity and lower rates of tissue regeneration. A study has been conducted by combining Polylactic acid (PLA) with PCL to reduce the limitations of PCL to generate fewer hydrophobic structures with variable degradability and assumed mechanical characteristics.

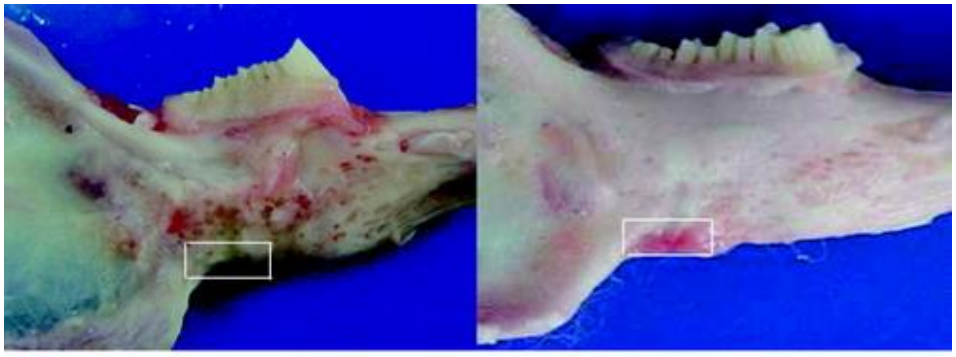
Two methods were used to synthesize the PCL/PLA mixtures: solvent casting and melt blending. Which were used to produce the scaffolds which then underwent the biological and morphological characterization. The characterization results depicted that the scaffolds synthesized of PCL/PLA blends exhibit better mechanical and morphological properties and biological behavior. It was also concluded from the results the scaffold roughness depends upon the blending method used and roughness determine the proliferation and cell adhesion. PCL/PLA scaffolds synthesized from blends produced by solvent casting, represent a lowered pore size, which also improves cell proliferation.

PCL is a polyester with semi-crystalline linear aliphatic structure. PCL can be hydrolyzed and is also susceptible to slow degradation by auto catalyzation. PCL can be easily processed due to its high elasticity at room temperature as it has a low melting point a low glass transition temperature of 60 degree Celsius. PCL also maintains elasticity at body temperature. PLA is Also characterized as a aliphatic polyester which is biocompatible and resorbable. However, it has high melting temperature of 160 degree Celsius and glass transition temperature of 60 degree Celsius. [13]

2.3.2 The bone formation in vitro and mandibular defect repair using PLGA porous scaffolds

Poly lactide coglycolide (PLGA) scaffolds have been synthesized by the Solution-Casting/Salt-leaching method to produce highly porous scaffold that are biodegradable and biocompatible. To study the in vitro degradation characteristics of PLGA scaffold, the measurement of water absorption, Normalized weight, molecular weight, and pH of the scaffold were taken. Three dimensional PLGA scaffolds were incorporated with Mesenchymal stem cells (MSCs) which were seeded and cultured to form in vitro tissue engineering bone. The deposition of mineralized matrix, cell morphology and cell number were studied and observed. Experimental results were produced in order to study the proliferation of seeded Mesenchymal stem cells and their differentiated function.

Two other mandibular defect repairs were made using control PLGA scaffold and blank without scaffold to show the efficacy of the PLGA / MSCs scaffolds installed in the rabbit to repair the mandibula defect. Histopathologic methods were used to approximate the restorative functions. The results of histopathologic methods that were utilized to determine the restorative functions of scaffolds suggest that it is practical to regenerate bone tissue in vitro using PLGA foams with pore size ranging from 100.1–250.1 μm. The results proved that using PLGA/MSCs tissue engineering bone it was possible to repair the defects after 3 months because they promote cell growth by the transplantation of MSCs to improve the healing. Whereas, with blank PLGA scaffold it was impossible to repair the defect. This research proved that PLGA/MSCs tissue engineering bone has considerable prospects as suitable



(a) (b)

FIGURE 2. 1 THE MACROSCOPIC ANALYSIS OF BONE DEFECT REPAIR

substitution for fortunate repair of bone defect. (a) the bone defect still retain after 12 weeks of healing for the blank group (without scaffold); (b) the defect has been filled with new bone for the PLGA/MSCs scaffolds group.[19]

2.3.3 Hydroxyapatite/TOPAS hybrid composite for bone tissue engineering applications

Repair and regeneration of bone tissues is the most essential processes in the field of tissue engineering. The novelty materials hydroxyapatite (HA) and cyclic olefinic copolymer (COC) which is also known as TOPAS are used to make hybrid composites of HA and TOPAS to investigate their bioactivity and mechanical properties for use in applications of bone tissues regeneration. Nanoparticles of HA, which are spherical in shape and in the size range of about 60nm are synthesized. HA of different concentrations in the range of 1 to 30% are added to TOPAS along with sodium dodecyl sulfate (SDS) which is utilized as a coupling agent in order to uniformly disperse HA which is hydrophilic with TOPAS that is known to be hydrophobic. Characterization of hybrid composite using Scanning electron microscope (SEM) allows us to study the morphology of the scaffold films and depicts the even dispersion of HA in less than 10 weight% in TOPAS whereas at higher concentrations, greater than 10 weight%, agglomeration occurs in the hybrid composites.

It is possible to control the mechanical properties such as compressive modulus and strength of the scaffolds by the addition HA to TOPAS in varying percentages; the compressive strength and modulus around 135 percent from 6.4 to 15.3 MPa and 180 percent from 0.26 to 0.74 MPa, respectively. This modification of mechanical properties takes place as the polymeric chains of TOPAS interact and anchor much more effectively in the proximity of the dispersed HA nanoparticles which help to improve the interfacial interactions and dispersion of polymeric chains.

Also, the biological characteristics such as cell adhesion and proliferation of hybrid TOPAS/HA composites are better than the scaffolds synthesized by utilizing pure TOPAS.TOPAS / HA hybrid composites proliferation and cell density are enhanced

by 9 and 3 folds, respectively, after 1 day of pre osteoblast cultivation. In addition, cell morphology has shifted from spherical to flattened spread morphology, clearly displaying the cell movement for the development of interconnected cellular network. Furthermore, cytocompatibility has been shown in hybrid composites as very few dead cells are present. Overall, TOPAS / HA's hybrid composites displayed superior strength and rigidity as well as improved cytocompatibility for bone tissue engineering applications.[21]

2.3.4 Biodegradable poly (lactic acid)-based scaffolds

The most suitable polymer for making scaffolds is stated to be synthetic PLA. PLA synthesized by melt / solution polycondensation polymerizations creates finished product properties comparatively simple to tailor. Nevertheless, their processing reactions are influenced by multiple factors such as temperature or time of polymerization, strain, catalysts, and solvent polarity. In addition, for example ethylene glycol (EG) a hydrophilic monomer is used to regulate equilibrium reactions. Those factors can have a major impact on PLA's final properties. Therefore, knowing the impact of operating parameters during the polymerization cycle is indispensable. Optimizing conditions for synthesis can be achieved by decreasing side-reactions. Moreover, this can be accomplished by racemizing by using chain extensors to build high molecular weight and increase thermal stability. Due to the nature of the degradation rate and mechanical characteristics comparable to those of proteins in soft and hard tissue engineering, PLA has many advantages in creating synthetic vascular and bone scaffolding. By using the reinforced materials, the mechanical properties of the PLA matrices can be modified and enhanced. The porous structure of PLA promotes the propagation and metabolism of cells. However, porosity effects the mechanical properties which is compensated by the use of reinforcement. Porosity can result in a decrease in mechanical properties, like elasticity modulus (Young's modulus), tensile strength and flexural modulus. Thus, by modifying parameters such as the weight content of

the reinforced elements and the composition of the polymer matrices a class of biomaterials with an eclectic variety of mechanical properties will be produced.

Initial polymers	Reinforce element	Porosity % (pore size)	Methods	Process condition	Strength (MPa)	Modulus (MPa)	Ref
PLLA	None	87.37 (64 μm)	Thermally induced, phase separation	Freeze-dry at 200 °C for 72 h	1.8	2.2	[85]
PLA	Nano-HA	85.06	Solvent casting	160 °C	8.7	14.9	[86]
PLLA (70)	AB (30)	92 (62 μm)	Supercritical CO_2	1,4-Dioxane (15 MPa, 35 °C)	0.05	NA	[87]
PLA	Nanoclay	73	Screw extruder injection molding and leaching method	200–220 °C, 100–200 rpm	NA	3.5	[88]
PLA	MFC	90	Compression molding at 180 °C and 10 MPa	High-speed blender at 20,000 rpm for 60 min	38.7	3.1	[89]
PLA	Epoxy	74	Electrospinning	Dissolved in chloroform methanol (2:1)	4.3	2.7	[90]
PLA	HA fibers (70 wt%)	NA	Hot pressing	175 °C, 10 MPa	40	11	[91]
PLA	BCN (5%)	92	Solvent casting and freeze-drying	1,4-Dioxane (1:19) at 45 °C for 8 h	1.03	36.84	[92]
PDLLA	HA/β-TCP	69 ± 5	CO_2 foaming with a solvent infiltration method	Room temp under vacuum	0.36	0.22	[93]

AB ammonium bicarbonate, *MFC* microfibrillated cellulose, *BCN* bacterial cellulose nanowhiskers, *NA* not available

TABLE 2. 1 PHYSICAL AND MECHANICAL PROPERTIES OF PLA SCAFFOLDS SYNTHESIZED WITH REINFORCED ELEMENTS:

2.3.5 Biocompatible composites of polyaniline nanofibers and collagen

Various ratios of nanofibers of polyaniline to collagen are employed to synthesize a latest hybrid composite material. The analysis of the composite was designed to make sure that the nanofibers were uniformly distributed in the matrix obtained by scanning electron microscopy (SEM). Thus, polyaniline is a conductive substance, so the nanofibre – collagen composite scaffold film remained conductive. Flash welding, a method used to combine polyaniline nanofibers with a light pulse, did not substantially impact the composite, possibly because of the discontinuous nanofibers in the composite matrix; only minor substantial alterations were detected. The contact angles are comparatively high (about 81 °) and are independent of the polyaniline quantity of the composite, suggesting that collagen is the primary surface material of the composite. Porcine skeletal muscle cells were

developed on composite films and reference collagen samples, implying that the composite material is ideal for biomedical uses.[22]

2.3.6 Gelatin-bioactive glass composites scaffolds

In this study bio ceramics and biopolymers are combined to synthesize a bio composite which has the required mechanical characteristics and biological properties to be used as a scaffolding material for bone tissue regeneration. Among the numerous ceramics bioactive glass has been selected as the most suitable bio ceramic which has an excellent ability to for bond with the bone through osteo condition. As a biopolymer gelatin is utilized which is a natural polymer derived from collagen. So bioactive glass/gelatin composite scaffolds have been synthesis with controlled macro porosity. Porosity of the scaffold is the major parameter that affects cell seeded, proliferation and osteo conduction. The composite scaffold exhibit superior mechanical properties such as strength, stiffness, and toughness.

The shapes of scaffolds can be tailored using different molds. The scaffolds are porous with bioactive glass powder distributed in gelatin. Scanning electron microscopy is done to perform characterization of the scaffold to analyze the morphology and structure of scaffold along with its porosity.[18]

2.4 Our approach pertaining to literature

We are using the materials Polycaprolactone and Hydroxyapatite in order to synthesize the suitable scaffolds for tissue regeneration with the required combination of mechanical properties.

2.4.1 Hydroxyapatite

Nanocomposites are being used widely in the field biomedical engineering due to the unique properties they depict. Nanocomposites are especially being applied for many applications of tissue engineering. Hence, Nano-Hydroxyapatite has been subjected to meticulous research and study. It is the most common bio ceramic that

has composition like bone hence has high biocompatibility and osteo conductivity. There are various types of Hydroxyapatite (HA) bio ceramics that can be synthesize for use in bone transplant, which are- ceramic matrix composites, metals matrix composites and polymer matrix composites.

The various types HA that can be formed allows its properties to be tailored according to the application requirement. HA is non-toxic and no inflammatory. It is bioactive, osteoconductive and biocompatible.

Hydroxyapatite (HA) has stoichiometric composition that is alike bone. HA is calcium phosphate with the Ca/P ratio of 1.670 and has hexagonal structure. HA is the most appropriate material for use in the human body as it is the most stable thermodynamically stable calcium phosphate when exposed to conditions of temperature, pH and composition of body fluids.

Furthermore, Nanotechnology has a great impact in the field of material science in biomedicine technology. It has been observed that by adding nanocrystalline hydroxyapatite to any type of synthetic and natural polymer the mechanical properties have been improved over a wide range of value.

2.4.2 Polycaprolactone

PCL is polyester that is biodegradable and biocompatible. It has high elasticity and easy processing due to its low melting point of 60 degree Celsius. PCL has a glass transition temperature of around -60 degree Celsius. PCL is often employed as an additive in polymers such polyurethane, polyvinyl chloride as it helps to improve their properties and make them easy to process because it can act as a plasticizer and also exhibits great resistance to solvents such as water, oil or chlorine. The PCL is synthesized by ring-opening polymerization of ϵ-caprolactone which leads to the formation a hydrophobic semi crystalline polymer. The catalyst used for the polymerization is stannous octoate.

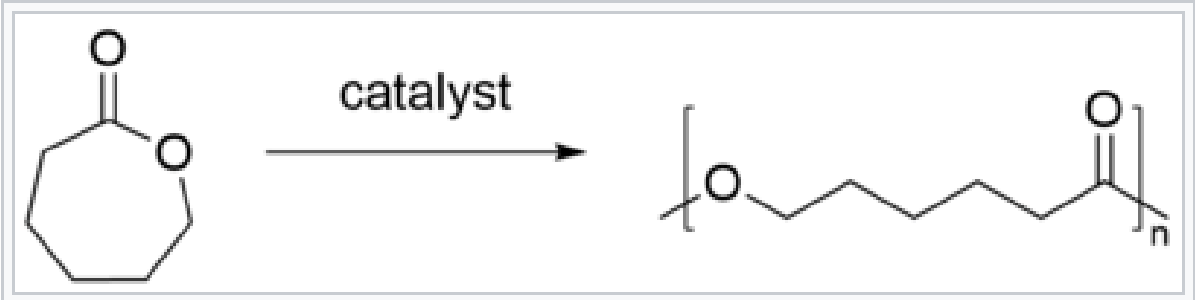


FIGURE 2. 2 RING-OPENING POLYMERIZATION OF ϵ-CAPROLACTONE

However, as homopolymer PCL takes a long time to degrade of about two years. For this reason, further research has been occurring to produce polymers that are resorbed inside the body at a faster rate which has been accomplished by the synthesis of the copolymers of ϵ-caprolactone incorporation dl-lactide. The copolymers are much more flexible to be used as scaffolds. Also, PCL is highly permeable to most agents which makes it applicable to application of drug delivery along with tissue regeneration.[20]

2.4.3 CYCLIC OLEFIN COPOLYMER (TOPAS)

TOPAS (cyclic olefin copolymer) is an amorphous, crystal clear copolymer built on ethylene and nor borne polymerization of metallocene catalysts. The property balance can be differed by changing the chemical makeup while polymerization over a broad range. There is a complex mix of properties in this class of copolymers whose efficiency advantages include

• It's Glass transition temperature Tg>180 °C.

• TOPAS acts as Exceptional smell and moisture barrier

• It has high yield strength and young Modulus.

• It can be easily extruded and thermoformed.

• By enhancing polyolefin property, it becomes resistant to hydrolysis, acids, polar organics, and alkalis

• It is glossy and shows high transparency.

• Wide-ranging global food and healthcare controlling compliance.

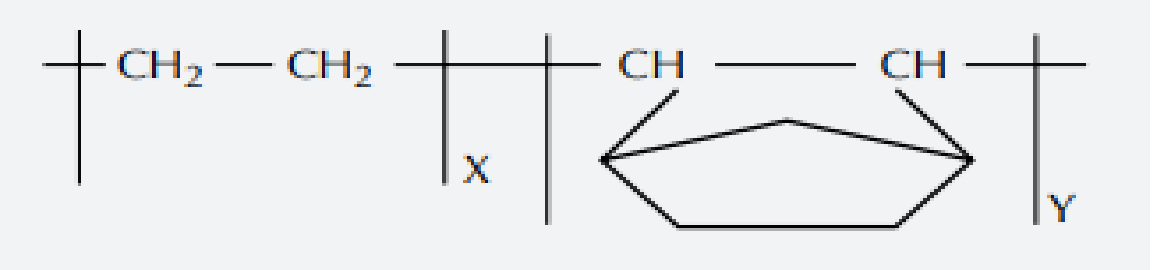


FIGURE 2. 3 THE STRUCTURE OF TOPAS

CHAPTER 3

METHODS AND EXPERIMENTS

3.1 Methods used

The methods we used in our experiments are as follow:

3.1.1 Wet chemical analysis

For the synthesis of Hydroxyapatite, we used wet chemical analysis method which is defined is defined by M. Khalid et al, for the synthesis of HA nanoparticles.[5]

In this process the precipitation reaction takes place in the solution containing Calcium nitrate ($Ca(NO_3)_2$)and Diammonium hydrogen phosphate ($(NH_4)_2H\,PO_4$) as the precursors and distilled water was used as solvent media and Ammonium Hydroxide NH_4OH was used for adjusting the pH. The solution was the refluxed, filtered, and dried to get the dried and pure particles of HA. The HA was then crushed to obtain the fine powder.

3.1.2 Annealing

After the synthesis of powdered HA particles, they were annealed to homogenize the microstructure of HA particles. Annealing is the thermal process of heating the powdered ceramic at suitable temperature and cooling it gradually. Annealing usually homogenize the microstructure and distribute the stresses and improve the ductility of ceramics. Annealing also helps in decreasing the porosity of Hydroxyapatite.

3.1.3 Solution casting

For the synthesis of Hybrid composites solution casting was used. It is one of the easiest ways for the synthesis of thin composite films. This process is based on the Stokes' law principle. The polymer and ceramic filler are mixed in a suitable solvent

and after homogenized mixing the mixture is casted and dried. In this way polymer matrix dissolves completely in the solvent and ceramic filler disperse in the mixture. Depending on various properties of filler you can also disperse the filler separately and then intermix both polymer and filler solutions. In this process the correct solvent is very important. We used chloroform for both PCL/HA and TOPAS/HA films.

3.2 Synthesis of hydroxyapatite

The procedure for the synthesis of HA nanoparticles was followed. [5]

3.2.1 Materials and Apparatus:

For synthesis of HA by wet chemical method following materials are required:

- Distilled water
- Calcium nitrate ($Ca\ (NO_3)_2$)
- Diammonium hydrogen phosphate ($(NH_4)_2HPO_4$)
- Ammonium Hydroxide NH_4OH

Following apparatus is required in the synthesis of HA

- 500ml beakers
- Alumina boat
- Steel medium sized spatula
- Digital Weighing balance
- 2 medium sized magnetic stirrers
- Hot plate
- Glass rod stirrer
- Rough filter fiber
- Glass condenser

- Oil bath
- 1000ml round bottom flask
- Separating funnel

3.2.2 Method and Procedure:

Experiment 1:

- First take 2 glass beakers and add 250ml distill water in each beaker.
- Now add 30g of Calcium nitrate $(Ca\ (NO_3)_2)$ in one beaker and 10g of Diammonium hydrogen phosphate $((NH_4)_2HPO_4)$ in other.
- Stir both solutions.
- Now the pH of calcium nitrate solution is 5.6 and pH of Diammonium hydrogen phosphate is 8.42
- Add Ammonium hydroxide NH_4OH in both solutions to achieve pH >10. The pH of calcium nitrate rose to 11.5 and pH of Diammonium hydrogen phosphate rose to 11.
- Use separating funnel to add solution of Diammonium hydrogen phosphate into solution of calcium nitrate at the speed of 30drops/min.
- The pH or reaction mixture should be above 10.
- When addition is completed the reaction is stirred to 24hrs.
- The pH of solution after mixing was 10.3
- Now pour the solution in round bottom flask for refluxing. Refluxing of the solution is done by using glass condenser at 100℃ for 2hrs.
- The resulting solution was then filtered by distilled water until its pH dropped below 8.

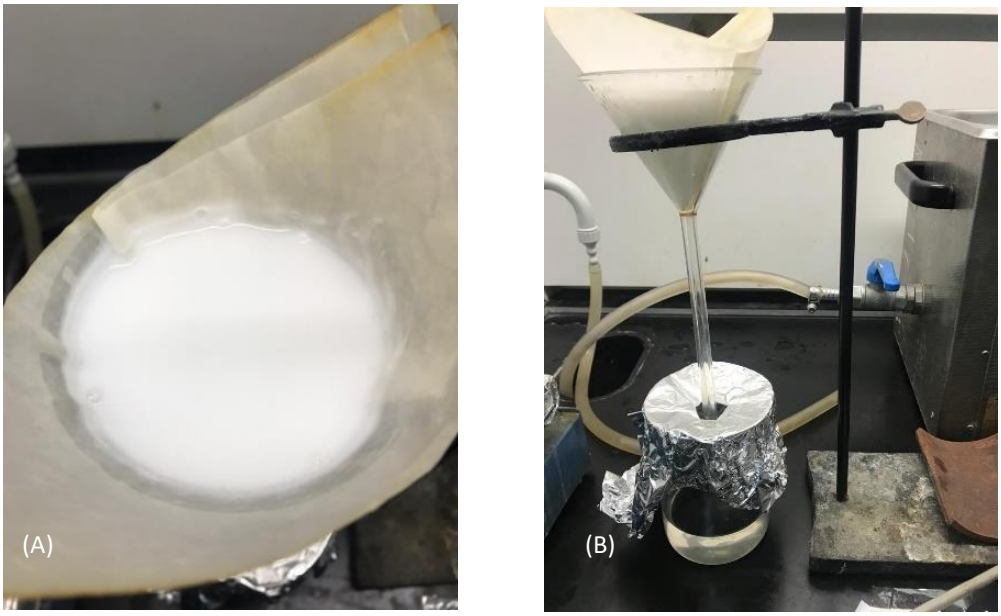


FIGURE 3. 1 (A) AND (B) SHOWS THE FILTERING OF HA

- The wet powder obtained was dried in a microwave at 100°C for 24 hrs.

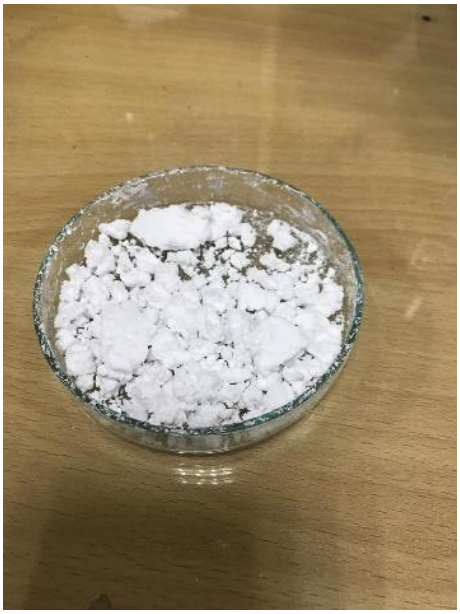


FIGURE 3. 2 DRIED HA

- Dry pellets were obtained which were crushed to powder in a mortar
- Resulting powder was annealed for 2 hrs. at 900°C with temperature increasing at 10 °C/min in a muffle furnace

- The powder was characterized through XRD and SEM confirming the pure HA powder.

Experiment 2:

Above procedure is followed but instead of annealing the powdered HA at 900℃ we are annealing it at 500℃ and 1200℃ to study the effect of annealing temperature on microstructure and morphology.

3.3 Synthesis of PCL/HA hybrid composite

For achieving a good dispersion of HA in PCL, the solution casting method was used. PCL and HA were dried for 5 h at 50 °C before mixing to minimize the moisture content. We are preparing composites with four different compositions i.e. PCL/HA= 98/2, PCL/HA= 95/5, PCL/HA= 90/10, PCL/HA= 80/20.

3.3.1 Materials and apparatus:

Following materials are required for synthesis PCL/HA film

- Polycaprolactone (PCL)
- Nanoparticles of Hydroxyapatite (HA)
- Chloroform
- SDS as a coupling agent

Equipment required are as follow

- Media bottles
- Magnetic stirrers
- Weighing balance
- Glass petri dish

3.3.2 Method and Procedures

Following procedure is followed for preparing PCL/HA composite film

- 1g, 0.98g, 0.95g, 0.9g and 0.8g PCL in 5 different media bottles

FIGURE 3. 3 PCL IN CONTAINER

- Add 20ml of chloroform in each media bottle and stir for 24hrs.

FIGURE 3. 4 CHLOROFORM IN BOTTLE

- Now take 0.02g, 0.05g, 0.1g and 0.2g of HA in 4 separate media bottles and add 0.01g of SDS in each bottle
- Now add 10ml of chloroform in each bottle and stir for 24hrs.
- After stirring mix 0.98g solution of PCL with 0.02g solution of HA, 0.95g PCL with 0.05g of HA, 0.9g of PCL with 0.1g of HA and 0.8g pf PCL with 0.2g HA solution and stir these four mixtures for 24hrs.

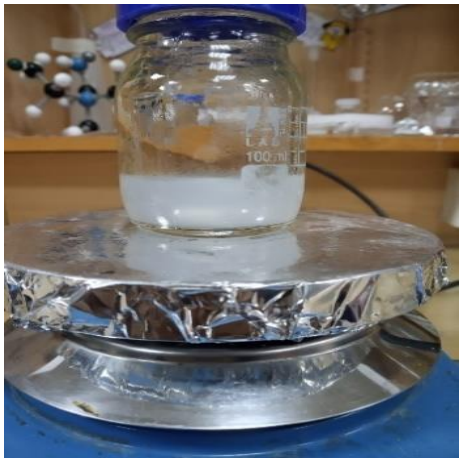


FIGURE 3. 5 MIXING OF PCL AND HA SOLUTIONS

- Now pour these mixtures in four different petri dishes.
- Dry these solutions for 4hrs at 50°C.
- Four films with different composition are prepared and their characterization results are discussed.

FIGURE 3. 6 PCL/HA FILMS

3.4 Synthesis of TOPAS/HA hybrid composite

For achieving a good dispersion of HA in TOPAS, the solution casting method was used. TOPAS and HA were dried for 5 h at 50 °C before mixing to minimize the moisture content. We are preparing composites with five different compositions i.e.

TOPAS/HA= 100/0, TOPAS/HA= 98/2, TOPAS /HA= 95/5, TOPAS /HA= 90/10 and TOPAS /HA= 80/20.

3.4.1 Materials and apparatus:

Following materials are required for synthesis TOPAS /HA film

- TOPAS
- Nanoparticles of Hydroxyapatite (HA)
- Chloroform
- SDS as a coupling agent

Equipment required are as follow

- Media bottles
- Magnetic stirrers
- Weighing balance
- Glass petri dish

3.4.2 Method and Procedures:

Following procedure is followed for preparing TOPAS /HA composite film

- 1g, 0.98g, 0.95g, 0.9g and 0.8g TOPAS in 5 different media bottles
- Add 20ml of chloroform in each media bottle and stir for 24hrs.
- Now take 0.02g, 0.05g, 0.1g and 0.2g of HA in 4 separate media bottles and add 0.01g of SDS in each bottle
- Now add 10ml of chloroform in each bottle and stir for 24hrs.
- After stirring mix 0.98g solution of TOPAS with 0.02g solution of HA, and 0.9g of TOPAS with 0.1g of HA, 0.95g with 0.05 and 0.8g of TOPAS with 0.2g of HA solution and stir these four mixtures for 24hrs.
- Now pour these mixtures in different petri dishes
- Dry these solutions for 4hrs at 50℃.

- 5 films with different composition are prepared and their characterization results are discussed.

CHAPTER 4

RESULTS AND CHARACTERIZATION

4.1 Results of Hydroxyapatite

4.1.1 SEM Analysis

Comparing SEM results show that the particle size is increasing by increasing annealing temperature. At 1200℃ there is more agglomerates and particle size are bigger as shown in fig.4.3. As we increase the temperature, agglomeration among the ceramic powder particles are increasing, and porosity is decreasing in fig.4.3. The reduction in porosity at higher temperatures is due sintering which causes the ceramic particles to coalesce.

HA-900 have bigger particles as compared to HA-500 and particle size of HA at 500℃ is less than 100 micrometers as shown in fig.4.1.

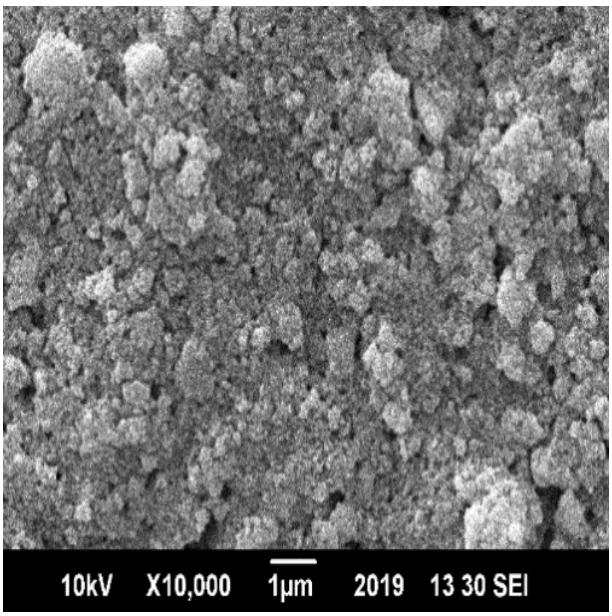


FIGURE 4. 1 HA AT 500°C

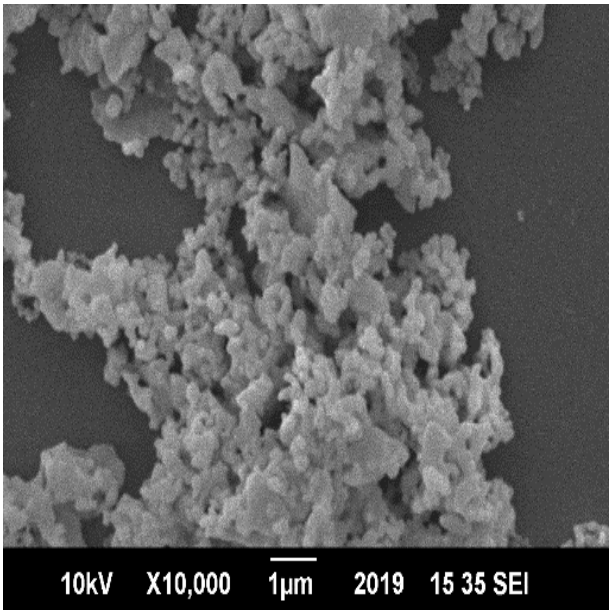


FIGURE 4. 2 HA AT 900°C

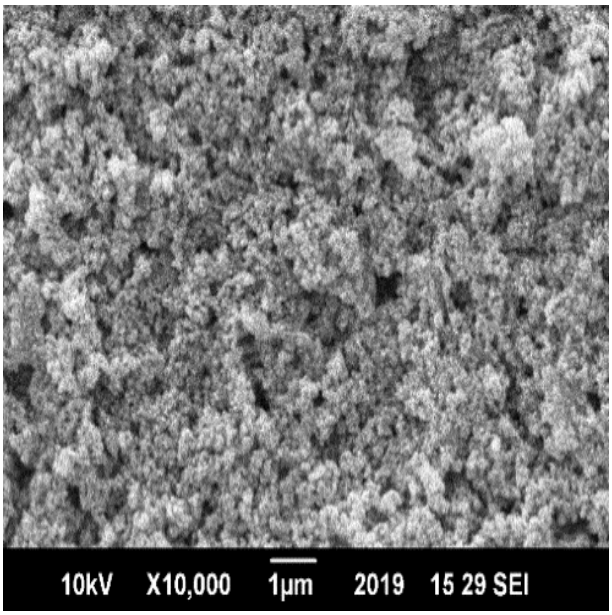


FIGURE 4. 3 HA AT 1200°C

The commercially prepared nano particle of hydroxyapatite has similar morphology and microstructure fig.4.4. as of HA-500 fig.4.1. but HA-1200 has more agglomerates.

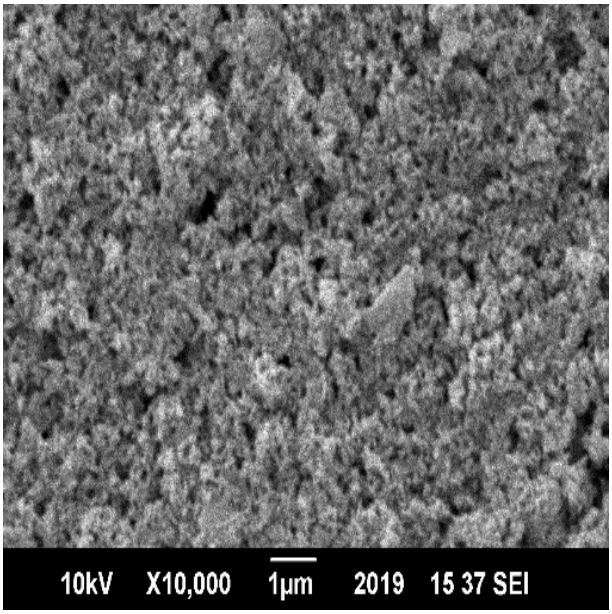


FIGURE 4. 4 HA NANOPARTICLES COMMERCIALLY PREPARED

The calculation of particle size of HA annealed at 500°C and HA annealed at 1200°C is shown in Table.4.1. The following scaled SEM images were used to calculate the average particle size:

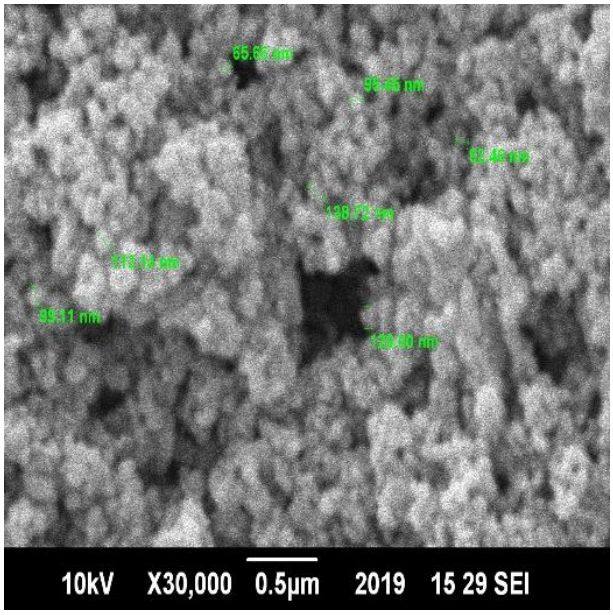


FIGURE 4. 5 SHOWS THE PARTICLE SIZE OF HA ANNEALED AT 1200°C

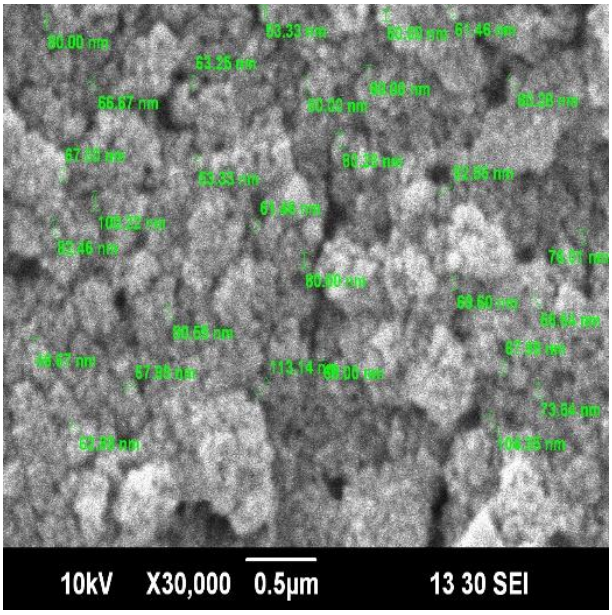


FIGURE 4. 6 SHOWS THE PARTICLE SIZE OF HA ANNEALED AT 500°C

TABLE 4. 1 PARTICLE SIZE CALCULATION OF HA-1200 AND HA-500

	Particle size at 1200°C(nm)	**Particle size at 500°C(nm)**
	82.46	53.33
	99.11	46.67
	95.45	60
	113.14	61.46
	120	66.67
	138.72	67
	65.66	69.9
Average Particle Size (nm)	**102.08**	**60.72**
Standard Deviation (nm)	24.32	8.29
Standard Error	9.19	3.13

4.1.2 XRD analysis:

XRD results of HA prepared at different temperatures are as follow:

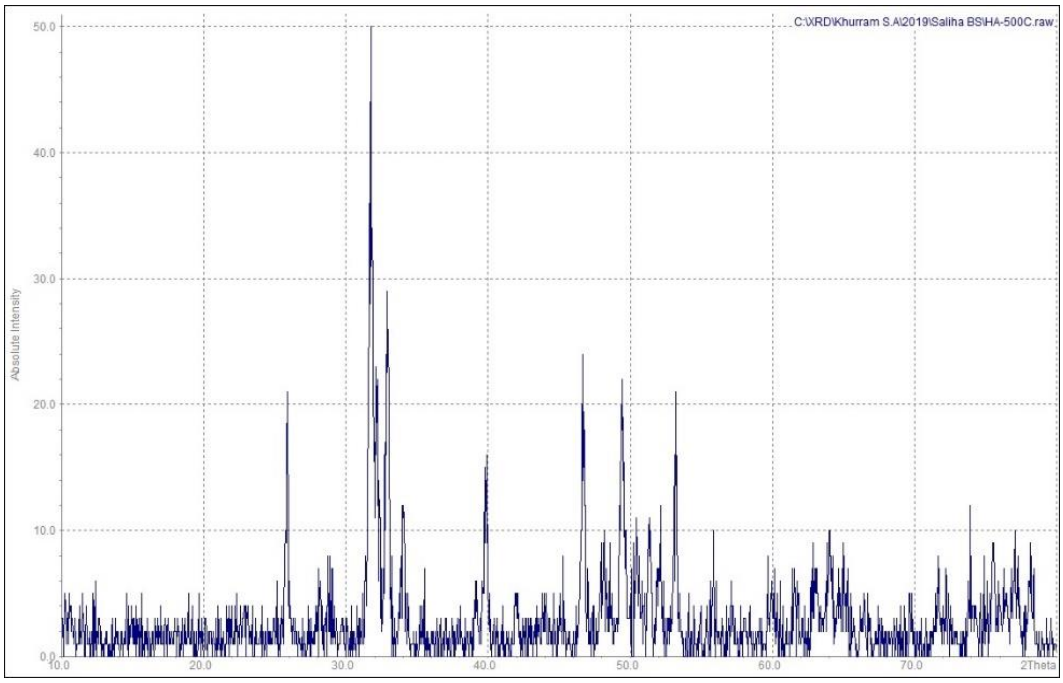


FIGURE 4. 7 XRD RESULT OF HA ANNEALED AT 500 °C

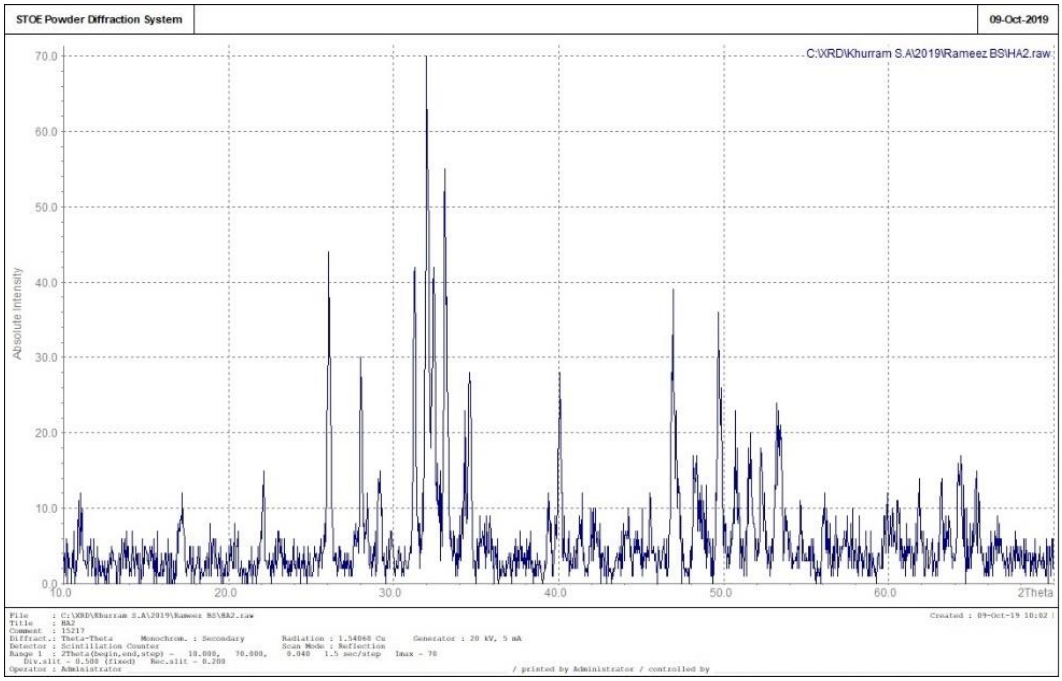


FIGURE 4. 8 XRD RESULT OF HA ANNEALED AT 900 □

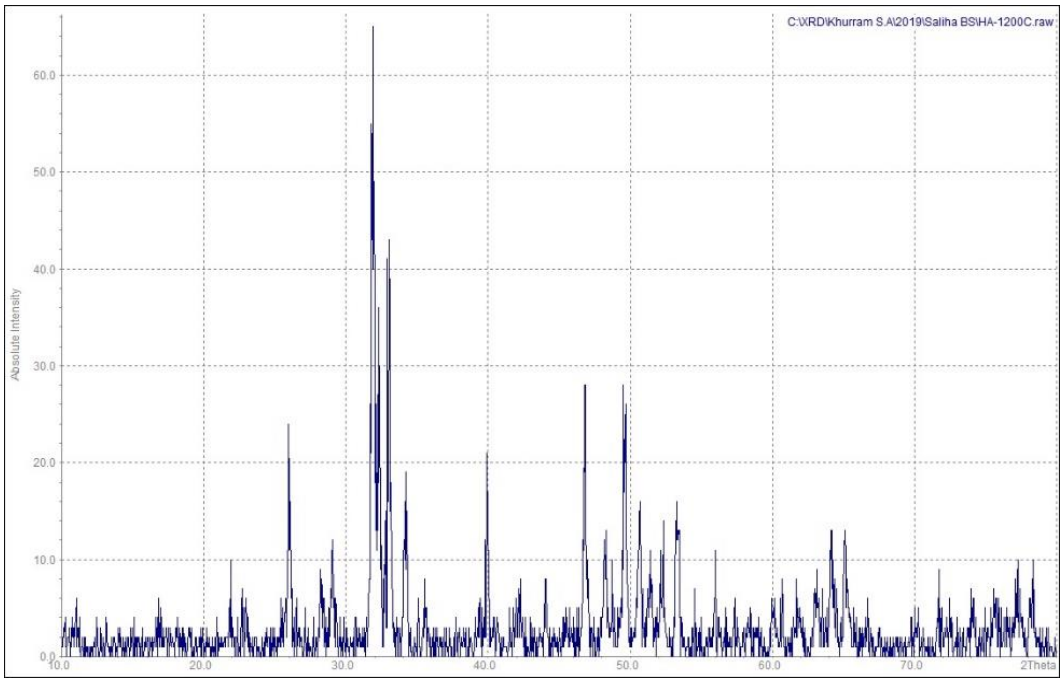


FIGURE 4. 9 XRD RESULT OF HA ANNEALED AT 1200 ⍰

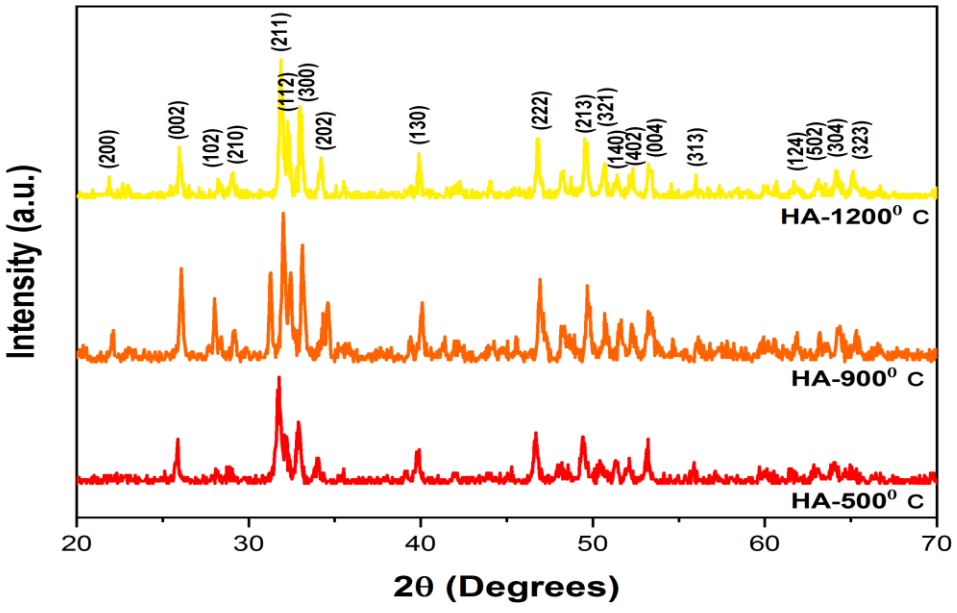


FIGURE 4. 10 COMPARISON OF XRD RESULTS OF HA-500, HA-900 AND HA-1200

By analyzing and comparing XRD results of HA-500, HA-900 and HA-1200 as shown in fig.4.10, it is visible that the HA shows most intense peaks at 211 planes which also match with the standard data of hydroxyapatite. Annealing of HA makes it more crystalline. By increasing temperature of annealing from 900˚C to 1200˚C, there was visible rise in the intensity of peaks. At 1200˚C annealing temperature, the development of HA was in ideal condition which is at highest intensity. The principal HA phases were detected in the range of 31.72degrees - 31.82degrees (2 theta) for all annealed HA corresponding to 211 planes. At low intensity, several peaks of HA were also observed. Even With the alteration of annealing temperature, all annealed samples of HA still show almost same XRD pattern. The standard corresponding plane for HA (viz. 100,101, 200, 002, 211, 202, 301, 130, 131, 113, 203, 222, 132, 321, 004, 240, 241, 502, 323, 511) are well noticed in case of all the manufactured powders.

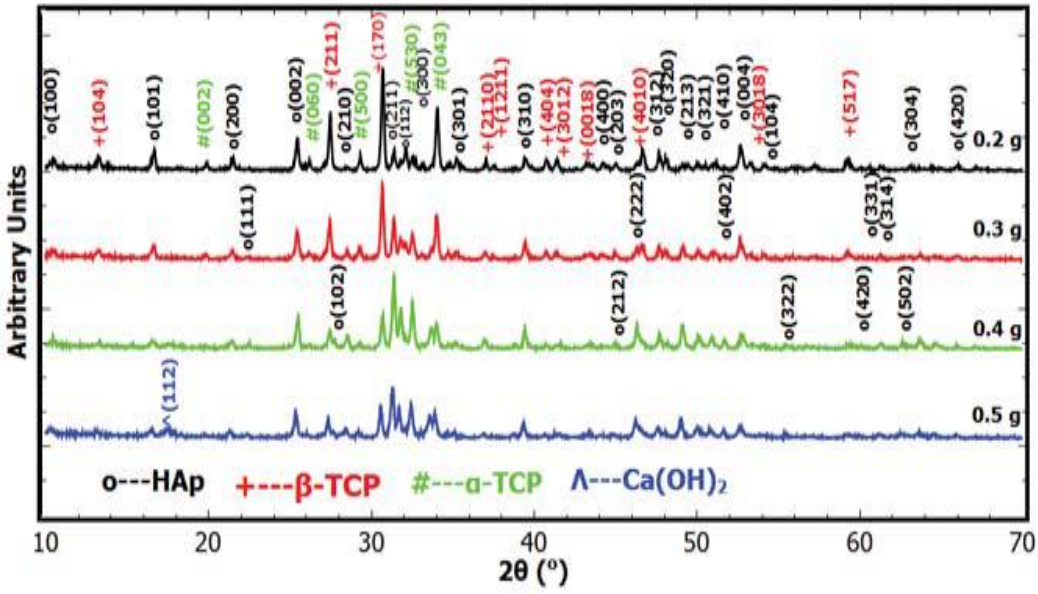
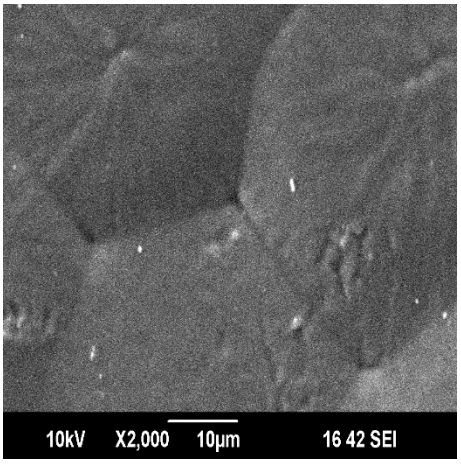


FIGURE 4. 11 REFERENCE GRAPH FOR HA NANOPARTICLES IN LITERATURE

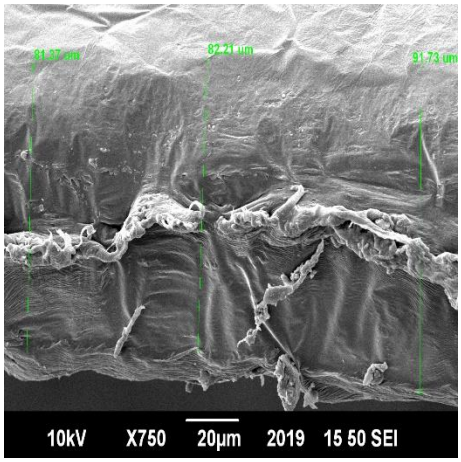
4.2. Results of PCL/HA films

4.2.1. SEM Analysis results:

The SEM analysis shows the internal microstructure of PCL/HA composite synthesized at different compositions i.e. PCL/HA= 100/0, PCL/HA= 98/2, PCL/HA= 95/, PCL/HA= 90/10, PCL/HA= 80/20. All the synthesized films have porous morphology and very less agglomeration of HA. Although, PCL/HA film of 80/20 composition as shown in fig.4.16, shows some agglomeration due to increased amount if HA. The SEM images show uniform dispersion of HA in films.

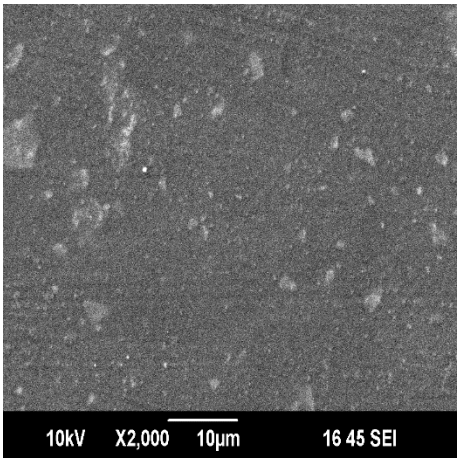


(A)

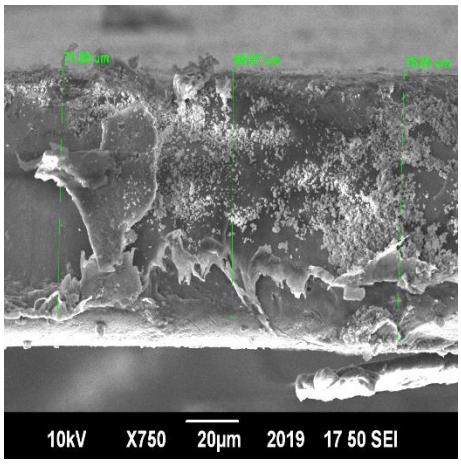


(B)

FIGURE 4. 12 (A)SEM OF PURE PCL (B) CROSS-SECTIONAL VIEW

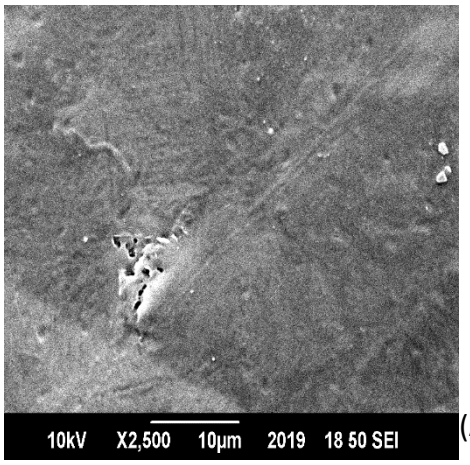


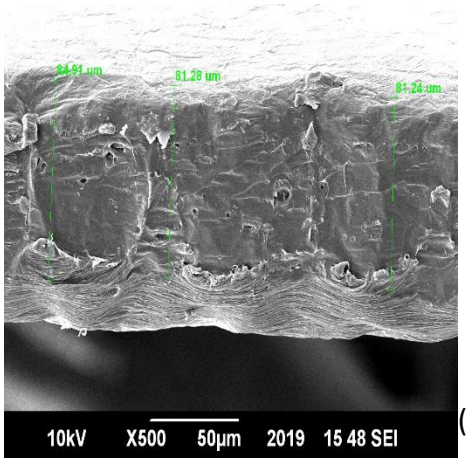
(A)



(B)

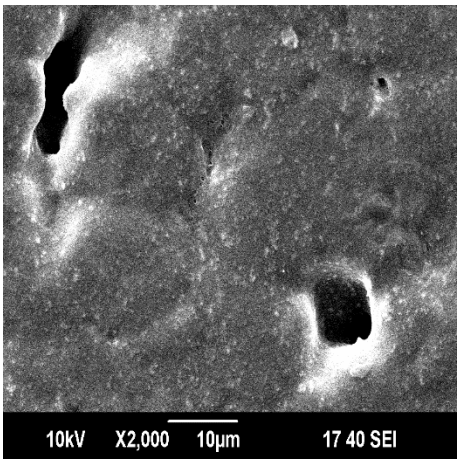
FIGURE 4. 13 (A) SEM OF PCL WITH 2% HA (B) CROSS-SECTIONAL VIEW

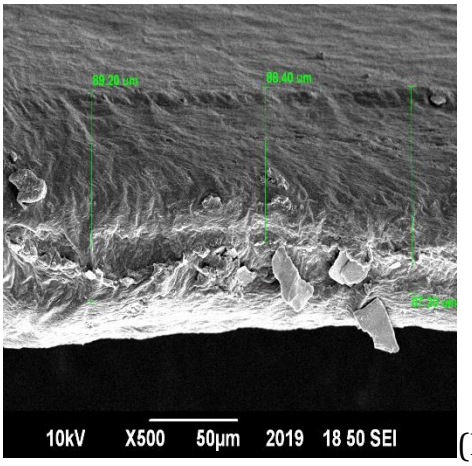


(A)


(B)

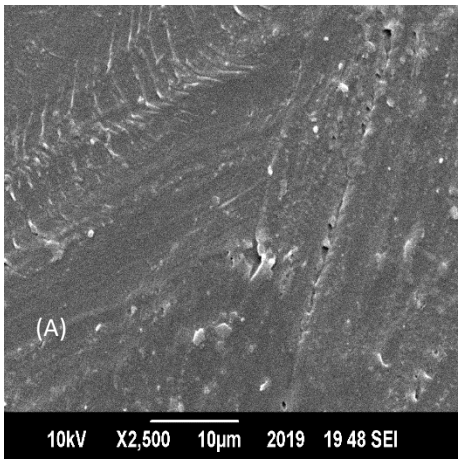
FIGURE 4. 14 (A) SEM OF PCL WITH 5% HA (B) CROSS-SECTIONAL VIEW

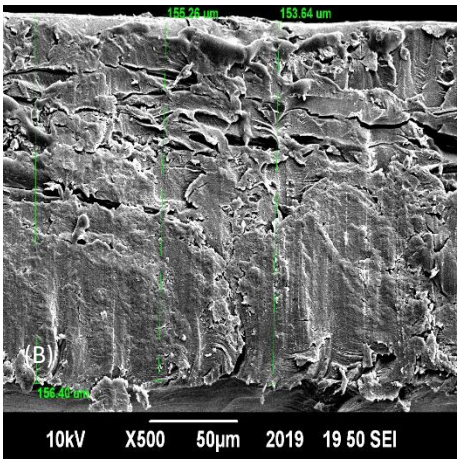


(A)


(B)

FIGURE 4. 15 (A) SEM OF PCL WITH 10% HA (B) CROSS-SECTIONAL VIEW



(A)


(B)

FIGURE 4. 16 (A) SEM OF PCL WITH 20% HA (B) CROSS-SECTIONAL VIEW

4.2.2. XRD Analysis:

Following are the XRD results of different PCL/HA films

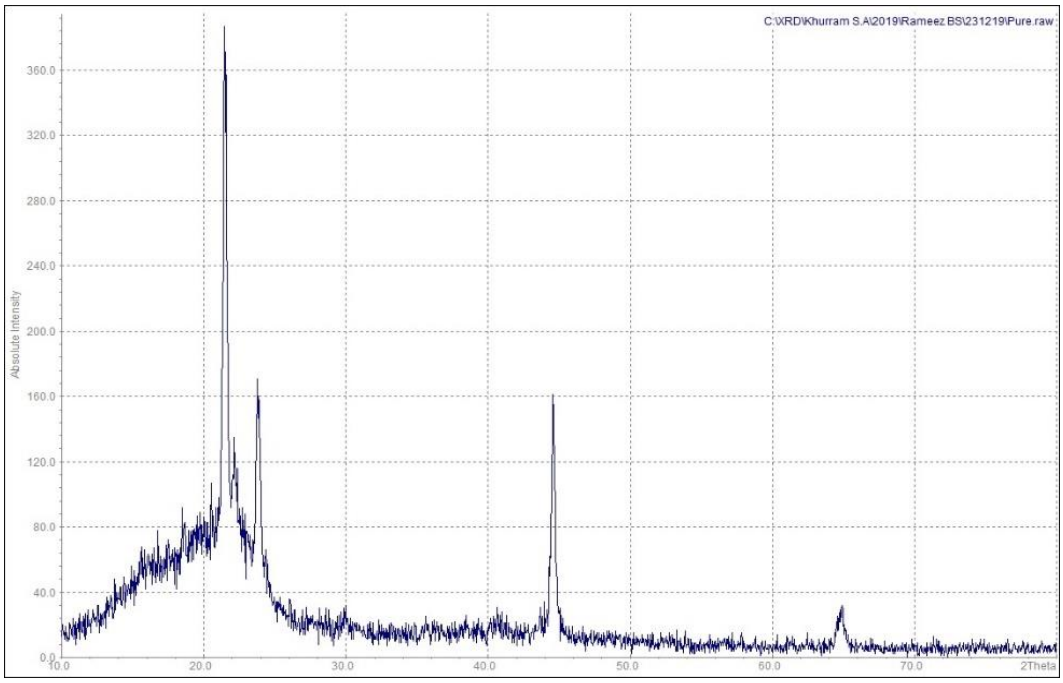


FIGURE 4. 17 XRD RESSULT OF PURE PCL FILM

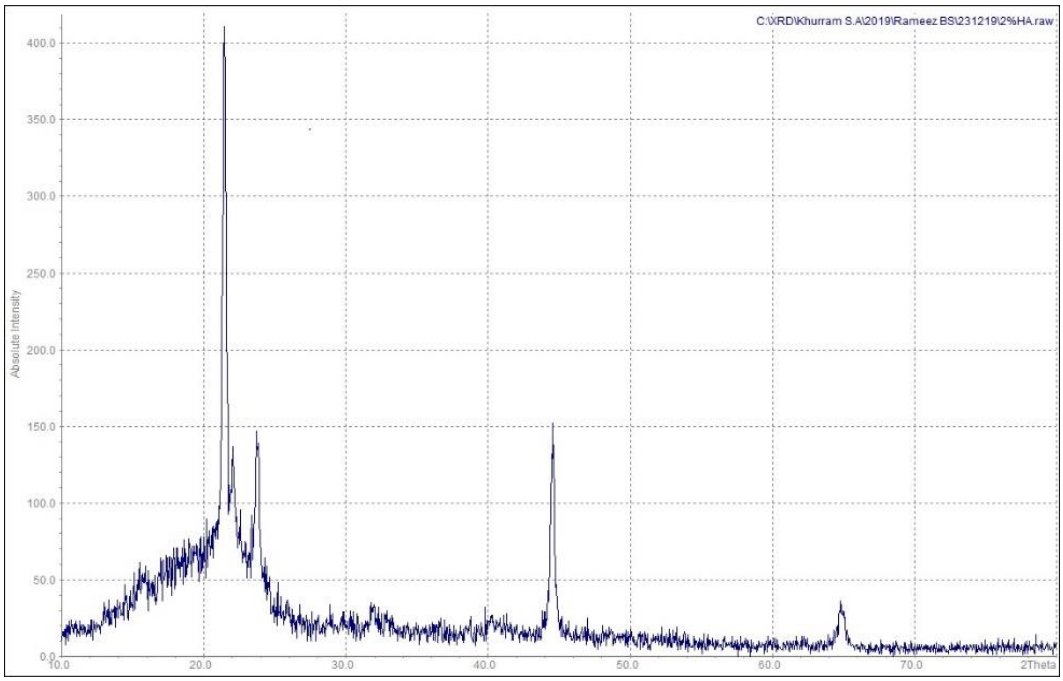


FIGURE 4. 18 XRD RESULT OF 2% HA IN PCL

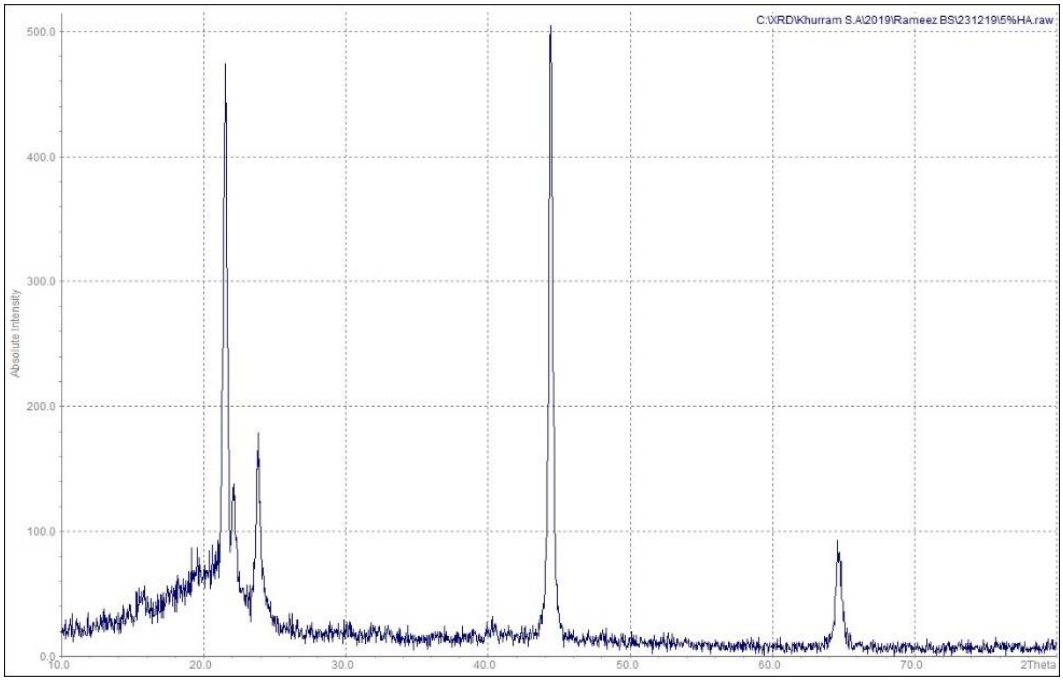


FIGURE 4. 19 XRD RESULT OF 5% HA IN PCL

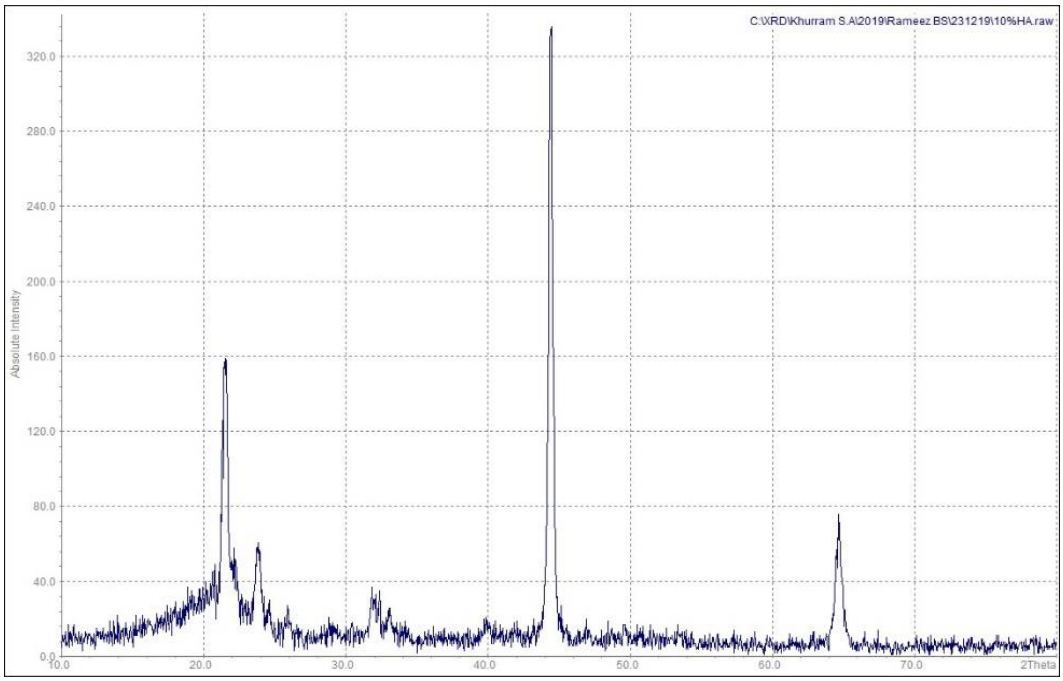


FIGURE 4. 20 XRD RESULT OF 10% HA IN PCL

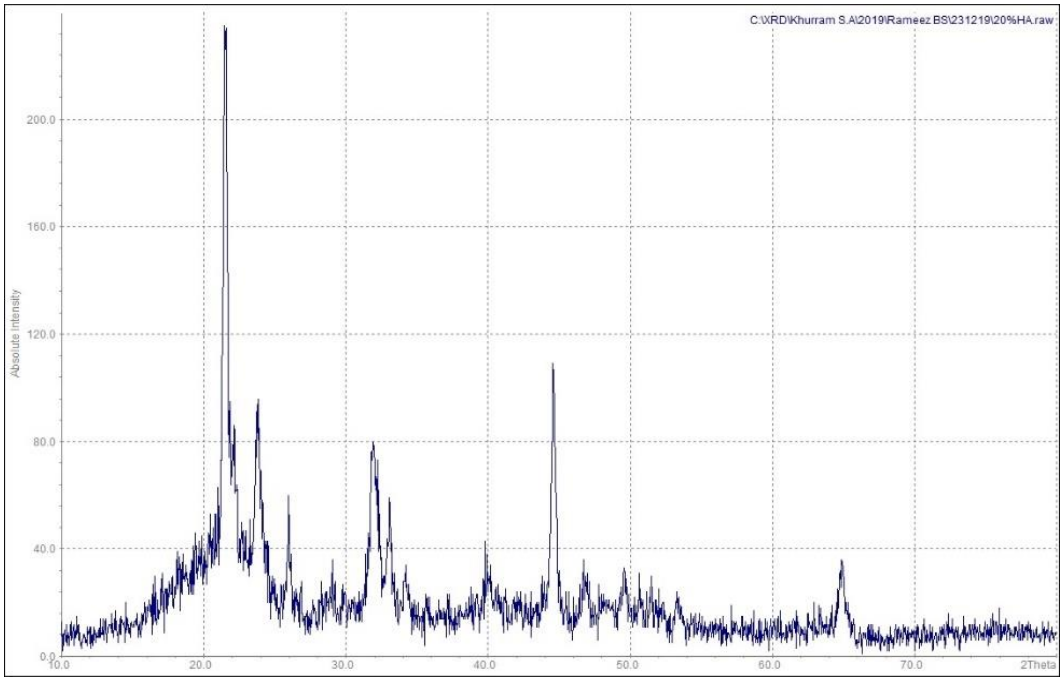


FIGURE 4. 21 XRD RESULT OF 20% HA IN PCL

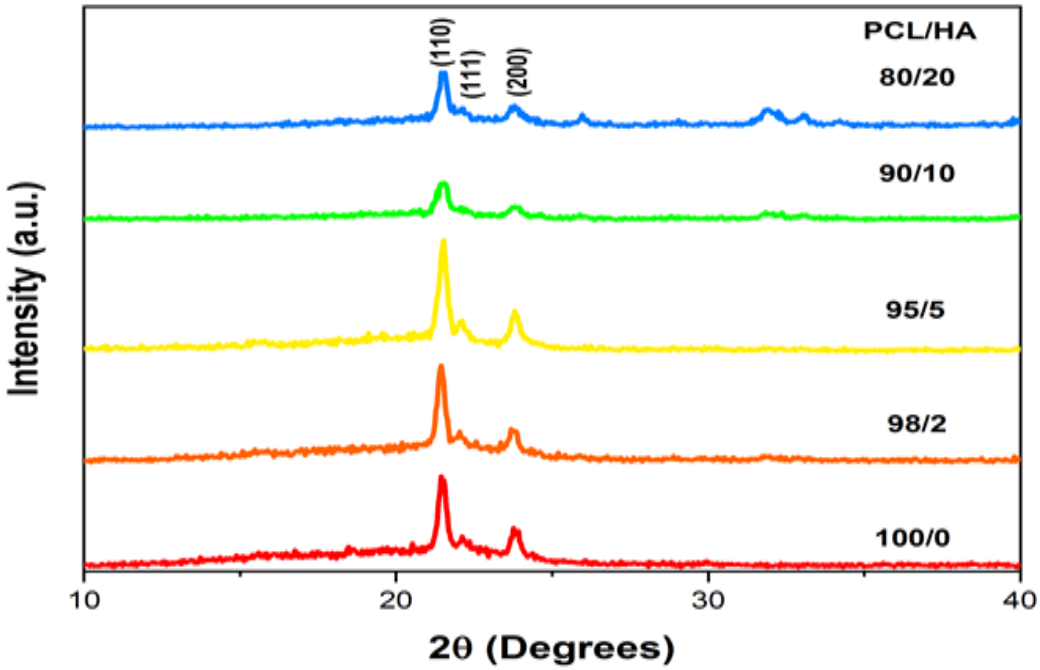


FIGURE 4. 22 XRD RESULTS COMPARISON OF PCL/HA FILMS

The XRD results of these PCL/HA films are compared. Before the addition of HA, the PCL polymeric film shows its main crystalline PCL phase peak at (110) plane between angle 21-22. By adding HA, the additional crystalline phase peaks of HA are observed in the range of 31.72degrees - 31.82degrees (2 theta) corresponding to 211 planes. By increasing the amount of HA, the intensity of these peaks is increasing as shown in fig.4.22.

4.2.3. Tensile results of PCL/HA films:

In this study we mainly focused on the improvement in tensile properties of polycaprolactone by adding hydroxyapatite. For this purpose, we performed tensile testing of our 5 samples having 5 different composition of PCL/HA i.e. PCL/HA= 100/0, PCL/HA= 98/2, PCL/HA= 95/5, PCL/HA= 90/10, PCL/HA= 80/20 on UTM.

The ASTM standard we followed is D882 which is used for thin plastic sheets. The thickness of our samples was 0.08mm, width was 1cm and the length of the sample was 5cm. The figure below shows the comparison in ultimate tensile strength of PCL/HA films of different composition.

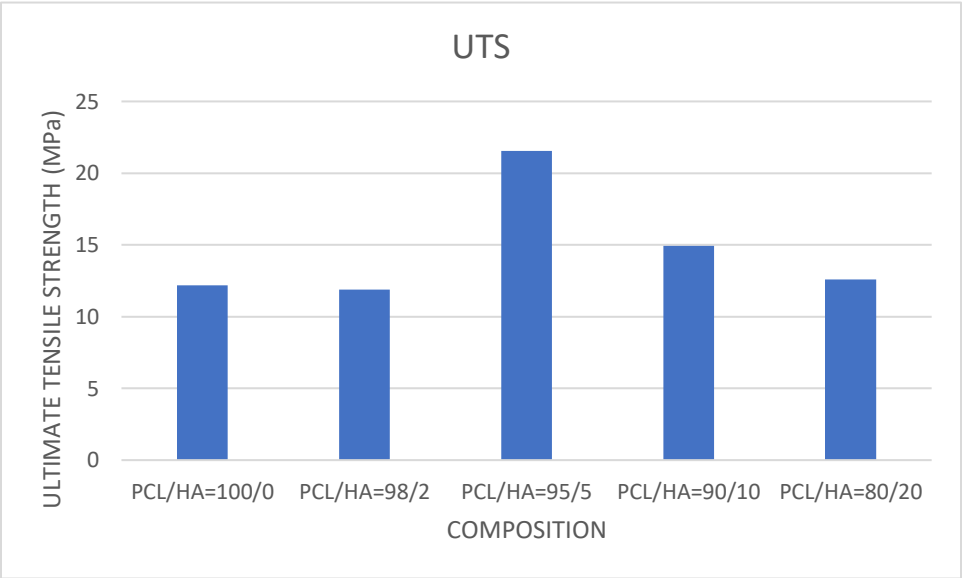


FIGURE 4. 23 COMPARISON IN ULTIMATE TENSILE STRENGTH OF PCL/HA FILMS OF DIFFERENT COMPOSITION.

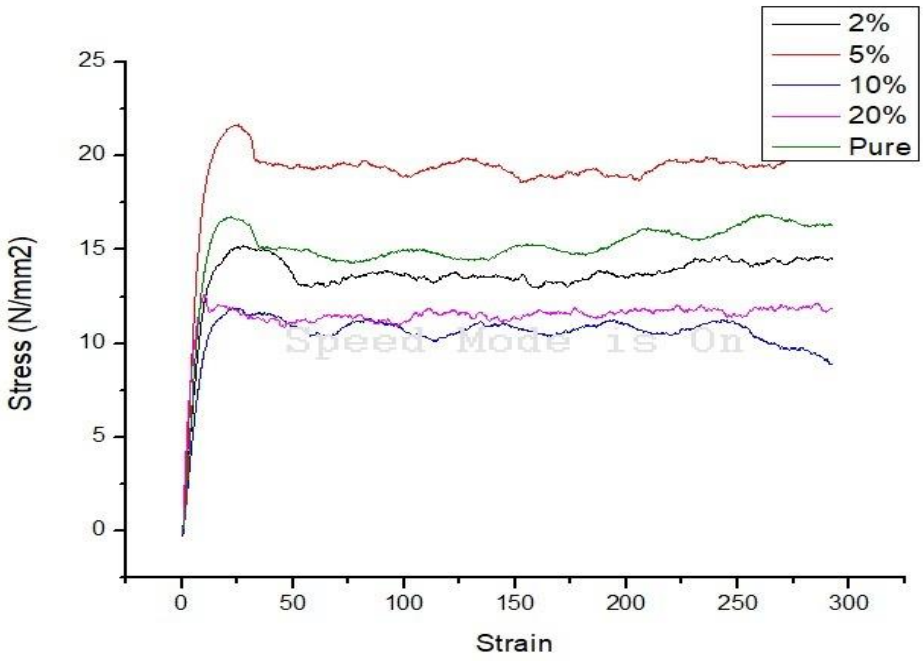


FIGURE 4. 24 COMPARISON IN TENSILE STRENGTH OF PCL/HA FILMS OF DIFFERENT COMPOSITION

According to fig.4.23 it is visible that by adding HA in PCL the ultimate tensile strength significantly increases. The maximum strength of PCL incorporated with HA is given at composition of PCL/HA 95/5. Means by adding 5% HA in PCL maximum strength is obtained and by exceeding the amount of HA more than 5% in PCL films the tensile strength starts decreasing. This behavior is due to the agglomeration and the stress is concentrated in those agglomerated parts which ultimately results in the failure of film and the tensile strength decreases. That is why the film of composition PCL/HA 80/20 has lowest tensile strength.

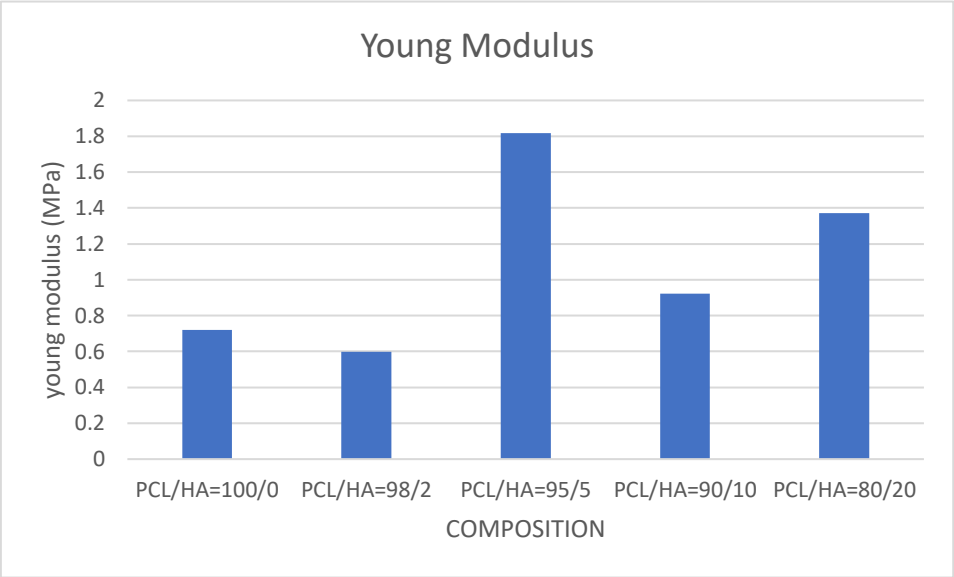


FIGURE 4. 25 COMPARISON IN YOUNG MODULUS OF PCL/HA FILMS OF DIFFERENT COMPOSITION

The exact ultimate tensile strength of polymer films is determined manually from the graphs plotted. The figure 4.25 shows that PCL/HA composition with 5% HA in PCL have higher young modulus. This means by adding HA, the young modulus of PCL also increases until a limit, after that agglomeration causes decrease in Modulus but increase in brittleness. To find the yield strength of each sample we used offset method. The most used offset is 0.2%. This systematic methodology was recognized by the research industry as one tool for evaluating the yield strength of products that do not have a readily discernible yield point.

4.3. Results of TOPAS/HA films

4.3.1. SEM Analysis results of TOPAS:

The SEM analysis shows the internal microstructure of TOPAS/HA composite synthesized at different compositions i.e. TOPAS/HA= 100/0 (fig.4.26), TOPAS/HA= 98/2 (fig.4.27), PCL/HA= 90/10 (fig.4.28). All the synthesized films have porous morphology which can analyze to determine the dispersion of HA in films of different composition and the tendency of ceramic powders to form agglomerates in the composite films.

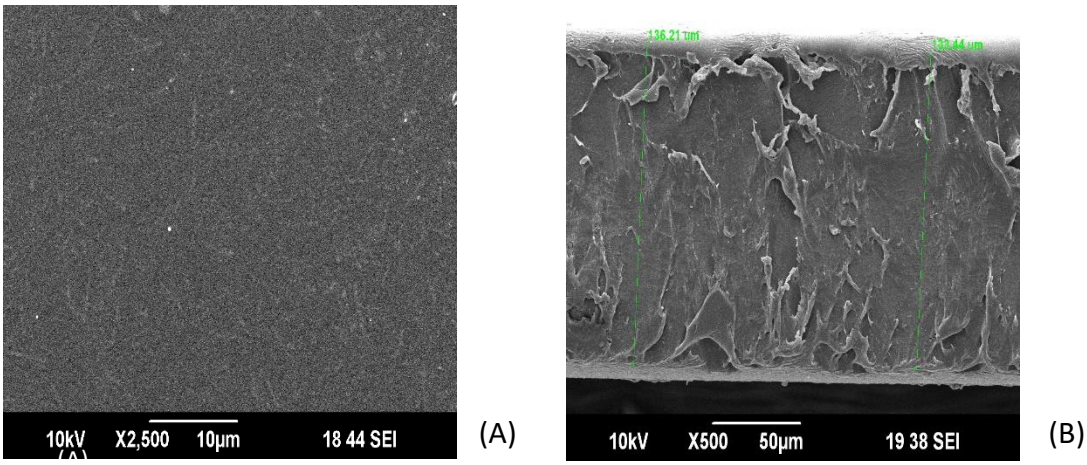


FIGURE 4. 26 SEM OF PURE TOPAS (B) CROSS-SECTIONAL VIEW

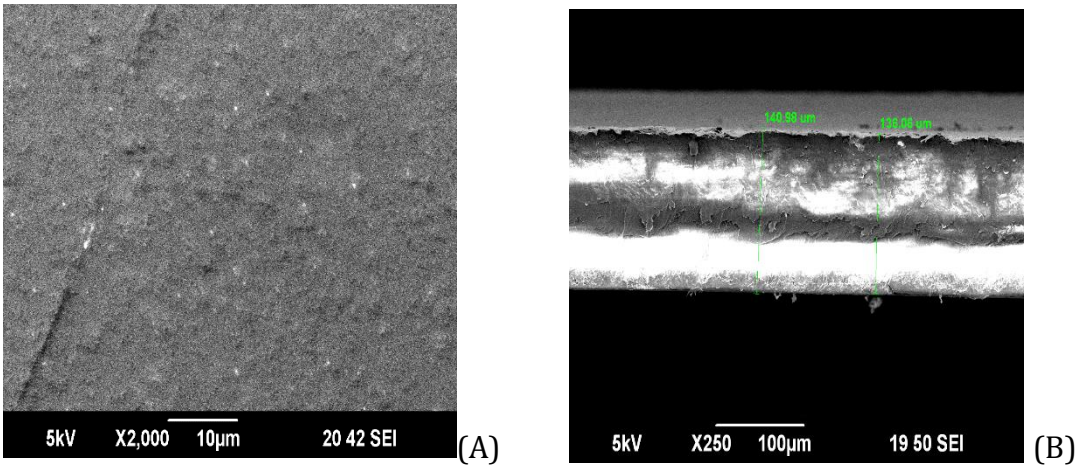


FIGURE 4. 27 SEM OF 2% HA IN TOPAS (B) CROSS-SECTIONAL VIEW

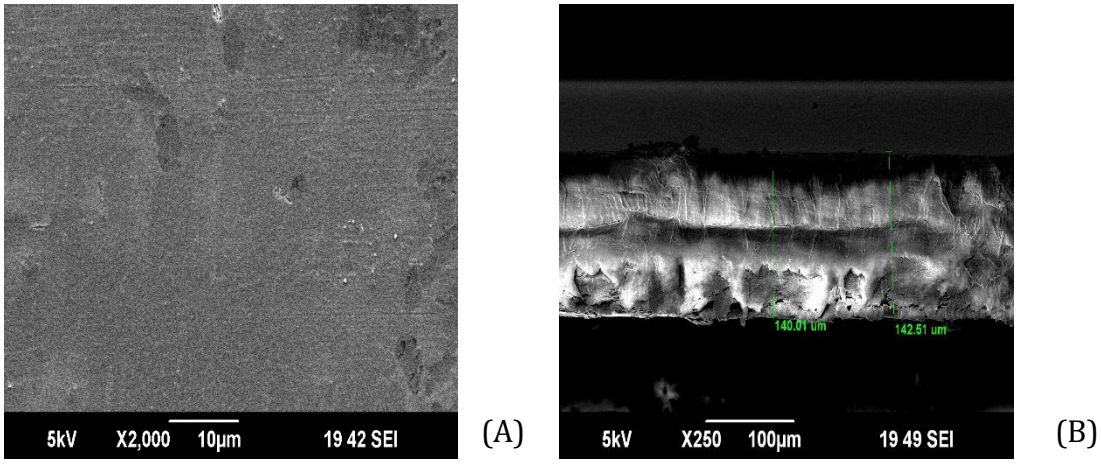


FIGURE 4. 28 (A) SEM OF 10% HA IN TOPAS (B) CROSS-SECTIONAL VIEW

CONCLUSION

Our objectives regarding the synthesis and characterization of polymeric bio-ceramic composite films were achieved. We first synthesized HA nanoparticles by a simpler and cheaper method to be used as a ceramic filler in our composite films. After the synthesis of HA, we annealed the ceramic powders as a final step to obtain homogenized particles with relieved internal stresses. To study the effect of annealing temperature on the particles of HA, we performed annealing at three different temperatures. By studying the morphology of ceramic particles from the SEM results, we successfully investigated and concluded the effect of increasing the annealing temperature of HA. As stated in literature our results depict that increasing annealing temperature increases the particle size of HA particles due to sintering which causes a reduction in porosity. Therefore, HA annealed at 500°C produces nanoparticles close to standard particle size. Moreover, the addition of HA to PCL enhanced the mechanical properties of PCL leading to the formation of a composite film with a combination of properties from the two constituent materials (polymer and ceramic). Tensile testing of the composites films with different percentages of HA were performed and results from the stress vs strain graphs were analyzed. results concluded that maximum tensile strength is achieved with 5% HA in PCL/HA composite films. This behavior is due to agglomerates formation at higher percentage of HA and the stress is concentrated in those agglomerated parts which ultimately results in the failure of film and the tensile strength decreases. Furthermore, TOPAS/HA composite of various composition was also synthesized, and SEM analysis was performed to study the morphology of the composite's microstructure to analyze the dispersion for different composition of HA in TOPAS and the porosity of composite films.

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