Synthesis and Characterization of Gel/HPMC-HA and Gel/HPMC-TCP Nanocomposites for Biomedical Applications

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Dedication

Dedicated to my respected parents

Acknowledgement

All praises to Allah Almighty, the origin of all knowledge, the Knower of everything, and with Whom rests all power. The favors He has bestowed upon me are uncountable, and blessed I am to have completed my work by His Will and Honor.

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Finally, I would like to thank my family members for their support, praise, and prayers.

Abstract

Polymers used for biomedical applications do not solely possess all the desired properties. Thus, polymer blending approach is used to combine two or more polymers to enhance their properties. Individual gelatin doesn't possess all the characteristics that are suitable for its potential use as a scaffold in tissue regeneration. This work is based on preparing the gelatin blend with HPMC, to achieve desired properties, by using solution casting method. HA and TCP were added as fillers to mimic the polymeric scaffold for tissue regeneration. The resulting membranes were subjected to XRD, FTIR, SEM, TGA and Mechanical Testing. XRD peaks confirms the presence of all the components in the final membranes. FTIR confirms the presence of functional groups for all the components in membranes. SEM analysis shows the presence of both the fillers at the polymeric matrix's surface. TGA analysis indicates that the thermal degradation and weight loss of the membranes containing gelatin as major component. The mechanical properties were analyzed through Universal Testing Machine and the results show that the mechanical properties of Gel/HPMC have been enhanced for both HA and TCP nanocomposites with the increase in filler contents. The results and properties in this study indicates the potential application of Gel/HPMC-HA and Gel/HPMC-TCP networks as scaffolds for tissue regeneration.

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Abbreviations

NMs	Nanomaterials
NPs	Nanoparticles
HA	Hydroxyapatite
TCP	Tricalcium Phosphate
Gel	Gelatin
HPMC	Hydroxypropyl Methyl Cellulose
XRD	X Ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
TGA	Thermal Gravimetric Analysis
EAB	Elongation at Break

Chapter 1 Introduction

1.1 Nanotechnology; an Introduction and History

"Nanotechnology" or "Nanoscience" is a multidisciplinary field that involves the synthesis, characterization and applications of materials, devices and implants whose dimensions, or at least 1 dimension, lies in the range of nanometer scale [1]. One nanometer is bilionth of a meter i.e., 1nm = 10^{-9} m or one nanometer (nm) is almost the length equal to ten H or five Si atoms aligned in a line [2].

According to "US NSTC" report tilted as "National Nanotechnological Initiatieve: Leadings to the Next Industrial Revolutions", the range of 1-100nm was taken as defining range for a matter to be in nanoscale. In this range or at this level, the physico-chemical and biological properties of the materials change significantly as compared to those of the bulk material. Nanotechnology integrates the atoms and molecules to form the devices at nanoscale. Nanoscale fabricated devices can improve the applications and efficiency of the resulting products. As all industries rely on materials and devices made up of atoms or molecules, however at nanoscale, properties of these materials can be enhanced without increasing the cost [3].

Scientists observed the structures present in mother nature to mimic the nanostructures. For example, Gecko has nanostructures present in their toes that allows them to stick with the wall. The cellulose fibers present in cotton are arranged at nanometer scale which gives the strength and durability to cotton. Mollusks creates shells around themselves, that are strong enough, by fabricating the calcium carbonate at nanoscale. Many centuries ago, Chinese make use of gold nanoparticles (NPs) to give red color to ceramic porcelain and Roman make use of metallic NPs to make glass artifacts beautiful in color [2, 4].

In 1925, Chemistry Nobel Laureate, Richard Zsigmondy explained a term "nanometer" for the very first time. He measured the particle size (such as Au colloids) with the aid of microscope. Physics Nobel Laureate, Richard Feynman in 1959 present a talk in American Physical Society at Caltech, titled as "There's Plenty of Room at the Bottom" where he introduces the manipulation of matter at the atomice level. For this novel and new idea, he is considered as the father of modern Nanotechnology. Later, another Japaneese scientist, Norio Taniguchei, first used the

term ‘‘nanotechnology’’ to describe semiconeductor processes occurring at the order of a NM. He proposed that nanotechnology consists of separating, consolidating, and deformation of materials by one atom or a molecule. 1980s are believed to be the golden phase of nanotechnology when Kroto, Smalleiy, and Curle discovered fullerenes and Taniguchi’s termed “nanotechnology” in his 1986 book [5].

1.2 NMs and their Characteristics

Any materials with 1 dimension in the range of 1-100nm is said to be a nanomaterial (NM). However, there are certain characteristics that are associated with the NMs when they are processed from bulk materials [3]. Those properties are as follow:

- Electromagnetic forces dominate and gravitational forces become negligible at nanometer scale.
- Instead of moving freely, electrons and atoms are confined at nanometer scale due to smaller size.
- Nanomaterials possess larger surface area because they have extremely small sizes and maximume number of atoms are present on the surface. This makes nanomaterials highly efficient.
- Quantization of energy effect occurs in NMs because electrons can exist in discrete energy levels and excitation can exist in all three dimensions e.g., in quantum dots.

1.3 Classifications of Nanomaterials

Based on the dimensions, NMs are classified into four main categories, as follow:

- **0-Dimensional Nanomaterials:** materials having have all 3 dimension in NM range for example NPs.
- **One-Dimensional Nanomaterials:** materials having 2 dimensions in NM range for example nano-rods, nanotubes, and nanowires. Such materials have needle like shape.
- **2-Dimensional Nanomaterials** are those having two dimensions out of nanometer scale and one in nanometer scale for example thin films and nano-layers. These nanomaterials have plate like shape.

- **3-Dimensional Nanomaterials** have no dimension in nanometer range. They can be agglomerates of any of 0 D, 1D, 2D materials.

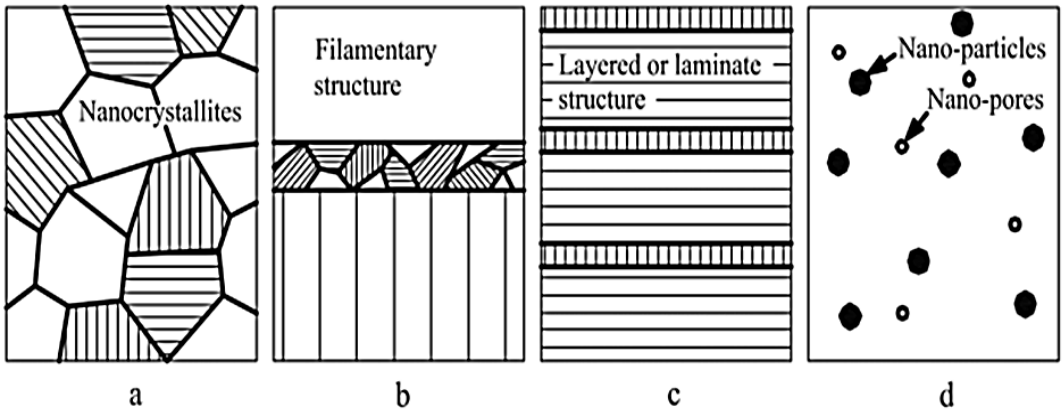

Figure 1.1**:** Different Types of Nanomaterials (a) 3-Dimensional NM (b) 2-Dimensional NM (c) 1-Dimensional NM (d) 0-Dimensional NM [2]

1.4 Synthesis of Nanomaterials

Generally, the methods of synthesizing nanomaterials are categorized into two main categories, as follow:

- Bottome-up approache
- Top-down approache

1.4.1 Bottom-up approach

In bottome-up approach, the small components up to atomic size arrange themselves in specific patterns to form nanostructures. If the process parameters are well controlled this synthesis method gives very fine nanostructures, individual NPs, and nano shells etc. with fine size distribution. However, scaling up is difficult in this approach. Bottom-up approach is further divided into following categories.

- Sol-Gel Synthesis
- Solvothermal/Hydrothermal
- Templated Synthesis
- Self-Assembly
- Molecular Beam Epitaxy (MBE)

1.4.2 Top-down approach

In top-down synthesis approach large components are divided into smaller pieces until nanometer scale. It is helpful in creating bulk nanostructures rather than individual NPs like bottom-up approach. Scaling up is easy in top-down synthesis approach. Top-down synthesis approach is divided into following types i.e.

- Physical Vapor Deposition (PVD)
- Chemical Vapor Deposition (CVD)
- Lithography
- Sputtering

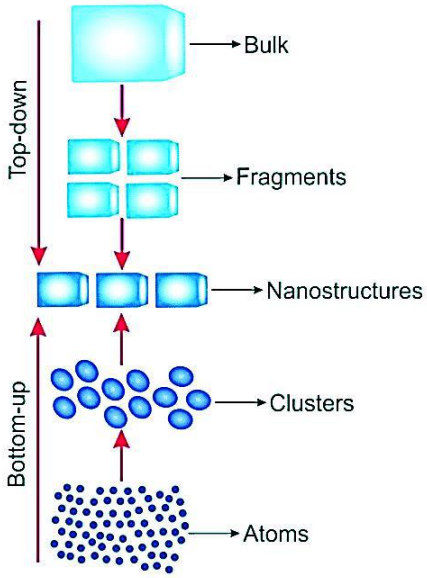

Figure 1.2: Schematic representation of fabrication of nanostructures using Top down and Bottom-Up approaches

1.5 Applications of Nanomaterials

Nanomaterials play significant role in our daily life. Few important areas where NMs have found their application useful, are described as follow:

1.5.1 Energy:

Nanotechnology provides substantial resourcese to solve the issues associated with energy. Since the developing materials are lower than 100 nm, thus they provide the new ways to capture, store and exchange energy. Daily, the sun radiates enough energy which is produced through nuclear fusion process. Among a number of devices, the solar collectorsr, the fuele cells, and

solare photovoltaics have employed the NMs to enhance the efficiency. It is observed that by using NMs, the incident radiation can get increased by 9 times and the efficiency of the solar collector is 10% higher as compare to that of a conventioonal flat plates solar collectors [6].

1.5.2 Food and Agriculture:

The rapidly developing nanotechnology has enabled the production of smart and actives packaging, nano-pesticides, and nano-fertilizers. Nanomaterials are engineered such as they possess unique properties and offer great benefits for food processing ingredients or supplements. Inorganic oxides which includes MgO (E530), TiO_2 (E171), and SiO_2 (E551) permitted by the U.S. FDA as anti-caking agent, food flavor carrier, and food color additives. Titania is widely used as additive in foods such as gum, white sauces, cake icing, candy, and puddings.

In agriculture, NMs are used to enhance the food production with greater nutritional value. The most essential strategies to enhance agricultural output are to utilise fertilisers, insecticides, herbicides, and plant growth stimulants. Nanocarriers such as silica NPs and polymers based NPs have also been created as modified release systems for pesticide delivery. Nano carriers can be used to ensure flawless delivery and delayed release of these specieas. The strategy of improving crop yield without damaging soil and water is known as “precision farming”[7].

1.5.3 Environmental Nanotechnology

The rapidly increasing world population causes the larger consumption of energy and materials, which has consequently led to the increased solid waste production, increased air pollution, and contamination of surface and water. Nanotechnology has the ability to enhance the environment in 2 ways: directly by making use of nanomaterials to remove contaminants, and indirectly by using improved industriale designe processees and manufacture of products that are environmentally friendly. The generation of dangerous air pollutants such as NOx, SOx, CO, and others are increasing as human cultures become more industrialized. Nano-sensors, nano-catalysts, nanocomposites, nano-

filters, and nano-biomaterials are examples of nanotechnology being used in environmental challenges to minimize pollutants in the air [8].

1.5.4 Biomedical

Nanotechnology has ushered in a revolution in biomedical sciences. Several novel nanorobotics devices are being developed that not only aid in in-vivo diagnostics but also in medication transfer within the body. Because of nanotechnology, in vivo imaging with minimal miniaturization is now achievable. These micro devices aid in the opening of narrow arteries, the removal of stones from various bodily areas, and a variety of other tasks. Nanotechnology aids in the effective on-the-spot detection of illnesses. NMs have also found widespread application in cancer therapy, artificial bone grafting, tissue engineering, and wound dressings [3].

1.5.5 Sports

For many years, there has been a desire for sport products that improve performance and efficiency, and nanotechnology opened novel avenues for the development of functionale sportswear. Sporting goods incorporating various nanoparticles have improved performance, greater flexibility, and durability, as well as being lighter. The athletes' performance is influenced by the equipment, which also protects them from danger. CNTs have been used to lower the weight of tennis and badminton racquets while also increasing their flexibility, durability, and hand feel. Graphite and carbon fibers make up most of these racquets. Tennis balls coated with nanoparticles, such as nano clays, have a much longer services life because of the gas barrier effect, which slows the rate of air penetrations into the ball [9].

1.6 Biomaterials

Biomaterials are definede as “Materials, natural or synthetic, that can be incorporated within the living organisms to replace or treat organs, tissues or functioning of body are considered as biomaterials”. The artificial materials, e.g., artificial limbs and

hearing aids, are not considered to be as biomaterials because they are in contact with the skin only and are not incorporated into the body [10, 11].

A biomaterial is designed to interact with biological systems at their interface. It can also be utilized as a medication or biological factor delivery device. Biomaterials are created to meet specific application requirements. Going all the way back to the dawn of civilization, Gold was utilized in dental applications by the Romans, Aztecs, and Chinese. The Mayans were discovered to have made dental implants out of seashells. Biomaterials are utilized to supplements, repair, or replaces any bodily tissues, organes, or functiones that has been lost due to trauma, illness, or damage. Recent medical practice frequently employs **autograft** tissue reconstruction, in which a tissue graft or organ transplant from one location to another of the same human is performed. However, donors site morbiditey, and, most importantly, the necessity for a 2nd operation limits their use. On the other hand, **allograft**, which is a tissue based graft or organ i.e., transplanted from a donor of the same species as the recipient, is a viable option. Another option is **xenograft**, which is a tissue based graft or organe transplants from a donor of a different species than the recipiente. Because of the immunogenic reaction, both allograft and xenograft usage are relatively limited, and they may impose poor biocompatibility in patients' bodies. These considerations direct our focus to biomaterials derived from other sources, which may be synthetic or natural [12, 13].

1.6.1 Properties of Biomaterials

For any material to be considered as biomaterial, it must possess certain properties or characteristics that make them eligible of biomaterials. A good biomaterial must be biocompatible, which means that its existence and implantation do not affect the host organisms. As a result, the implanted biomaterial must have the following characteristics [14, 15]:

1) Blood and host tissue compatibility is required for biomaterials.
2) The degradation of scaffold or implanted biomaterial must be in accordance with the regeneration of tissue i.e., the implanted materials must heal the damage before degradation.
3) Biomaterials should not be able to disrupt or influence the tissues and organs that surround them.

4) When used in a drug delivery systeme, targeted drugs deliveryi, or the destruction of malignant cells, a biomaterial should be non-toxice and non-hazardouse.
5) In nature, a biomaterial should be non-inflammatoriey, non-carcinogenic, non-allergic, non-mutagenic, and non-pyrogenice.
6) Some biomaterials are incorporated into live cells to improve mechanical and biological characteristics as well as compatibility with living tissues.
7) For a biomaterial to be an ideal, the biomaterial's characteristics might alter depending on where it's implanted and the patient's past medical history.

1.6.2 Classification of Biomaterials

Mostly used biomaterials used in medicine are generally classified into following two categories [14-16]:

1.6.2.1 Resorbable Biomaterials*:*

Resorbable materials are materials that, after being implanteed in the humane body, gets degrade, and are reabsorbed by the body's tissues. These types of materials degrade or decay slowly, releasing mass in tiny forms into the surroundings environments in the bodies, which is then absorbed by the body's fluids.

1.6.2.2 Non-resorbable Biomaterials*:*

Non-resorbable materials are biomaterials that do not disintegrate after being implanted in the human body and are not resorbed by the body's tissues.

1.7 Types of Biomaterials

Biomaterials having a range of characteristics are required for surgical applications. Depending upon the material characteristics, biomaterials are classified into several categories, as mentioned below:

1.7.1 Metallic Biomaterials:

Metallic biomaterials are manufactured systems that are utilized in joints replacementes, dentale implanets, orthopedics, and the stents to offer internal support to biological tissues. The most widely utilized bio-inert metals for

fracturee fixations, angioplastie, and bone remodelings are surgical SS (316L), cobalt-chromiume (CoCr) alloys, and titaniume (Ti) alloys. This is because to their long-terme stability in highliy reactivee in-vivo settings as well as their superior mechanical characteristics [15]. Commonly used metals based on Titaniume, Cobalte and steel are sensitive for load bearings functions. Their resistance to corrosione provide perfect long-term stability and reliable mechanicale strengthe with minimum toxicitiy to host. These materials possesses excellent tensile strength (TS), fatiguee stress and fracturee toughness and thus found applications in orthopedics, dental implants, and stents [17, 18].

The usage of implanted metals comes with several significant drawbacks. Controlling biodegradation kinetics, for example, is essential for resorbable metals like Mg or Fe, because early breakdown might result in premature loss of mechanical strength before the tissue or function has fully healed or recovered. Long-term exposure to metals such as steel, Co-Cr, or Ti alloys in the body, on the other hand, is linked to an increased risk of cutaneous and systemic hypersensitivity reactions, whereas a relatively high modulus of these metals compared to natural bone tissue causes stress shielding and, as a result, osteopenia. Any implant has the danger of infection and inflammation, which can drastically reduce the implant's function and result in considerable tissue loss in the vicinity of the implant. In the case of load-bearing implants, septic or aseptic loosening of the implant may interfere with force transmission, such as improper transference of the bitinge forces to the dental implants and surrounding bones, resulting in mechanicaul failures. After the dental implant's fatiguee strength is achieved, it will fail [19, 20].

Figure 1.3: Biomedical devices and implants made of titanium alloys [21]

1.7.2 Polymeric Biomaterials

Polymers have been used in medicine since nearly the beginning of the discipline of polymer research; nearly every early synthetic polymer made their ways into experimentale surgicale investigations soon after the development, and so many have remained to become clinical practices standards. Nylone sutures were first described in the mid-1940s, review papers on the use of polymers in surgery such as nylon, poly (methyl methacrylate) (PMMA), Dacrone polyesters, and PVC began to appear in important medical publications.

Synthetic polymeric materials are frequently utilized in dentals materials, implants, dressinigs, encapsulanits, polymerics drug deliveryi systems, tissuee engineered products, and orthodosees such as those with metal and ceramic substituentse. Polymers based biomaterials must have the same qualities as conventional biomaterials, including biocompatibility and sufficient mechanicale and physical propertiees, and manufacturability [22]. Aside from tissue regeneration, polymeric biomaterials have a variety of significant applications. Polymers such as poly (ethylene glycol) are frequently employed to extend further the half life of certain medicines in circulation [23].

1.7.3 Ceramic Biomaterials

Ceramics used as biomaterials are referred to as bioceramics [24]. Ceramics are becoming increasingly essential because to their biocompatibility, corrosion

resistance, and, most importantly since mineral phases constitute a major component of bones themselves.

Ceramics includes a wide range of biomaterials, including Ca and C phosphates, as well as aluminae. Surgeons and researcheers expressed a strong interest in them, although markets applications are still being sought. This category is dominated by hydroxyapatite (HA), which is utilized as a bone replacement and as a covering for metal and carbon implants in oral and maxillofacial surgery. HA can be found in many regions of body as a component of various types of calcifiede tissues. Calcified tissue comprises dental enamel (HA), dentin (HA), and cement; bone has an organic component and an inorganic component (70% HA and tri calcium phosphate). The mineral component of bone is mostly composed of calcium phosphate microcrystals, the most important of which is hydroxyapatite, whose chemical formula is $Ca_{10}(PO_4)_6(OH)_2$. Other minerale phases are di-calciume phosphate, di-basic calciume phosphaate, tri-calciume phosphaate, and a number of amorphouse calcium phosphates [25].

In general, ceramics have good biocompatibility, strong corrosion resistance, high compression resistance, and low electrical and thermal conductivities. These features make them ideal candidates for implants [26].

1.7.4 Composite Biomaterials

The creation of smart biomaterials for tissue regeneration has piqued the interest of many researchers. The composite method of integrating biomaterials in the form of biopolymers and/or bio ceramics, either synthetic or natural, opens more possibilities [27]. The creation or identification of materials capable of encouraging desired cellular and tissue behavior is a key problem in tissue engineering. Given that few biomaterials have all the essential qualities to operate optimally, engineers and physicians alike have sought the creation of hybrid or composite biomaterials to synergize the positive attributes of various biomaterials elements into an improved matrix. The mixing of natural and synthetic polymers with a variety of different materials has been shown to improve cellular contact, stimulate integration into host tissue, and give adjustable material characteristics and degradation kinetics.

The ideal tissue engineered scaffold is porous interconnected structure that supports cell growth, cell proliferation and cell differentiation. To achieve this, superposition of two or more materials is required. Ceramic-polymer composites are gaining popularity as possible fillers for bone deformities. Tricalcium phosphate and hydroxyapatite, two of the most widely used calcium phosphate ceramics, have proven acceptable biocompatibility as well as osteoconduction and osseointegration. Bioceramic glasses, such as 45S5 Bioglass, have also been shown to stimulate bone bonding and vascularization. However, these ceramics are deemed too stiff and brittle to be utilized on their own. The mixing of a ceramic to a polymer scaffold offers numerous advantages i.e., combining the inorganic phase's osteoconductivity and bone-bonding potential with the porosity and interconnectivity of the polymer [28] .

Nanocomposites enable for larger quantities of ceramics to be used, resulting in improved mechanical characteristics such as increased tensile strength, bending strength, impact energy, and moduli closer to the order of natural bone while preserving an interconnected architecture [29].

Table 1: Table showing the common biomaterials with their characteristics and applications [25]

Materials	**Advantages**	**Disadvantages**	**Applications**
Polymers: Teflone, Dacrone, Nylone, PMMA, PE, Silastic, Polytetrafluorethylene	Less density, Easy to fabricate	Lower mechanicale resistance, easy to degrade	nose, heart valves, lenses, veins, tendon
Metals: Steels 316, 316L, Vitallium, Tantaluum Cobalte F-75 and Ti, Cr+CO, Cr+Co+Mo alloys	Ductilile	Lesser biocompatibility, corrosion in a physiological environment	articulation prosthesis, tooth implants, and meshes for face reconstructions, Staples
Ceramics: AlO, Ca aluminates, TiO, CaPO, Bio glass	Corrosion resistance, higher resistance to compression, inertness, lower thermal and electricals conductivity, high biocompatibility	Lower impact resistance	Dentale parts, bonee fillings, endoscopy, otlogic implants, , coatings and medicale tools
Composites: Metals with ceramics coating, material coated with carbons	inert	hard to reproduce during fabrication	Kneee implants, artificiale articulations, Heart valves, hipe implants

1.8 Objective

This work aims to:

- Synthesize Hydroxyapatite (HA) by using wet chemical precipitation method
- Synthesize pristine gelatin and Gel/HPMC composite films
- Synthesize Gel/HPMC-HA and Gel/HPMC-TCP composite films with varying ratios of HA and TCP
- Optimize the mechanical properties and analyzing the composite films through various characterization techniques

Chapter 2 Literature Review

Marcela P. Tedesco et al. prepared the films containing gelatin and HPMC and their blends as matrices for oral disintegrating films. The casting technique was used to synthesize the blends and films. They studied the properties of these films through various characterization techniques such as SEM, FTIR, mechanical properties and contact angle. The concentration of HPMC was varied in the gelatin matrix from 0% to 100%. SEM images shows the smooth surfaces in case of gelatin and HPMC films. On the other hand, for blends, the surface was observed to be more heterogeneous due to increased HPMC concentration. FTIR results indicates that the polymers blends did not favor the formation of additional interaction between the two polymers. Increased concentration of HPMC in gelatin caused the tensile strength to decrease from 51.52 MPa to 29.54 MPa and EAB to increase from 2.82% to 12.67%. The higher contents of HPMC in gelatin matrix decreases the contact angle from 104.53° to 54.3°, hence making the blends more hydrophilic [30].

Sara Esteghlal et al. prepared Gelatin/HPMC water-in-water emulsions for the bio base packaging material to prevent the phase separation in two polymers. HPMC droplets were dispersed, with concentration varying from 5% to 30%, in the continuous gelatin network through entrapment. The physicochemical and mechanical properties of emulsion base films were studied and compared with the individual polymer films. Mechanical and swelling properties of the films were studied and analyzed. Mechanical results revealed that tensile strength remains unchanged at 13.5 MPa, due to the absence of attractive forces between the two polymers. However, the incorporation of HPMC in gelatin, significantly decreases the EAB values from 26.75% to 19.55% and with the increase in amount of HPMC, the EAB values increases from 19.55% to 25.63%. Swelling analysis reveals that the Swelling % increases from 314.16% to 467.99% with the increase in HPMC content from 0% to 30%, respectively [31].

Xingxun Liu et al. synthesized the Gelatin/HPMC blends through solution casting method with varying ratio of both the polymers. This study was aimed at analyzing the compatibility and phase composition of Gelatin/HPMC blends through Synchrotron Micro-infrared Spectroscopy (μST-FTIR) and confocal Raman Spectroscopy based chemical imaging. The results from Raman spectroscopy shows that HPMC particles easily aggregated to form

continuous phases on smaller detected zones. For 50:50 (Gelatin /HPMC), the μST-FTIR results shows only the bands of gelatin and HPMC and no newly formed bands appear. Thus, it suggests the system is with separated phases. This study concludes that chemical mapping techniques are also significant to investigate the compositional properties of polysaccharide-gelatin blend materials [32].

Zhili Ji et al. has synthesized Gelatin/HPMC blends to study the pH-controlled gelation behavior. This study investigates the properties of Gel/HPMC through UV spectrophotometer, fluorescence microscope (FM) and environmental scanning electron microscopy (ESEM). The study shows that at pH less than 4.5, the HPMC shows continuous phase, gelatin shows separated phase distribution in HPMC matrix as small spherical domains. At pH=5, the phase transition happens, and gelatin shows continuous phase and HPMC appeared as separated droplets that are dispersed in gelatin matrix. Many interphasic regions were noticed in the blend system which indicates that gelatin and HPMC are partially miscible and are compatible to a certain degree [33].

Ali Samadikuchaksaraei et al. prepared scaffolds containing HA/Gelatin through layer solvent casting combined with the freeze-drying and lamination techniques. This study was aimed at investigation of the effects of osteoblast-conditioning upon mechanical behavior, biodegradation, biocompatibility, and osteo-inductive properties of HA/gelatin. The scaffolds were subjected to culture of osteoblasts on their surface and their removal by a freeze-thawing process, thus scaffolds become HA/Gelatin/OC. Results suggests that the invitro biological properties and mechanical properties were not affected by the conditioning process. However, the in-vivo studies suggests that osteoblast-conditioning improved and enhanced the biocompatibility and osteo-inductivity of the nanocomposite scaffold. Also, it was observed that the osteoblast conditioning accelerates the collagen content during the bone healing process. Thus it can be concluded that the osteoblasts conditioning is viable technique for the development of bone tissue engineering scaffolds [34].

Hae-Won Kim et al. prepared nanofibrous structure of Gelatin/HA nanocomposite using electrospinning method. The precipitated HA/gelatin was lyophilized and dissolved in an organic solvent to generate a continuous nanofiber with a diameter of the order of hundreds

of nanometers (nm). HA was embedded in gelatin at 20% and 40%. Results shows that the nanocrystals of ultrafine HA were homogeneously distributed in gelatin network. As compared to pure gelatin, the nanofibrous mesh produced this way has improved, significantly, the osteoblastic cellular activity. These electro-spun nanofibrous membranes of Gelatin/HA can strongly be applied in the field of tissue regeneration [35].

MJ Hossan et al. prepared Gelatin and HA scaffold by using solvent casting process. The raw material was first mixed and then molded into petri dishes. Samples were dried both naturally and in oven. These scaffolds were prepared to mimic mineral and organic component of natural. Thermal and mechanical properties of the composite films were investigated. HA concentration was increased in the gelatin matrix. The results suggests that tensile strength and microhardness of the films increases with the increase in HA concentration. The naturally dried composites show higher tensile strength as compared to the over dried because in naturally dried composites more crosslinking occurred. However, elongation of both, naturally and oven dried, decreases with increase in HA content. These results suggests that Gelatin/HA films with different ratios of HA are good to apply in tissue regeneration [36].

Mehdi K. Narbat et al. synthesized the Gelatin and HA composite scaffolds using the solvent casting method with freeze drying process. All the precursors were compounded, and the resulting composite was molded into cylindrical shape. Glutaraldehyde was added as crosslinker. Different weight fraction of HA (30%,40% and 50%) were added in gelatin matrix. FTIR, SEM and light microscopy were used to investigate the mechanical and structural properties. The results of these studies suggested that scaffolds have an open and interconnected porous structure with pore size of 80-400 μm which helps in cell proliferation. Gelatin/HA with 50% has tensile strength of 32MPa and compressive modulus of 10GPa. It was also found that the addition of HA content can reduce water absorption and porosity. These results suggest that the synthesized scaffolds has potential application in bone regeneration [38].

Kimberly T. Hunter et al. fabricated the composite of hydroxyapatite, chitosan and gelatin (HCG) by using solution casting method for guided tissue regeneration. HA, chitosan, and

gelatin have chemical similarity to the structural components of natural bone and their composites have been tested as bone scaffolds. This study involves the investigation of dynamic interaction of hMSC with the composite HCG. The association of HCG produced a biodegradable membrane with approximately 60 weight % water and the initial stiffness of approximately 20 kPa. Preconditioning in the media that contains serum results in the formation of nanopores in HCG membranes and enhances the extracellular matrix (ECM) protein adsorption. Further results indicates that these membranes possess the desired mechanical, structural , and biological properties necessarily required for application as barrier membranes in guided bone tissue regeneration [39].

He Lian et al. fabricated the series of HA/Gelatin membranes with varying HA to Gelatin ratios by using the solution casting method. The ratio of HA/Gelatin were kept at 100/0, 70/30, 50/50 30/70, 0/100. Results suggest that the varying ratios has significant impact on biological and physico-chemical properties of the resulting composites. HA/Gelatin-70/30 has fibrous and porous morphology whereas 50/50 and 30/70 membranes possess non-porous and rough surfaces. Mechanical testing indicates that increase in HA content enhances the tensile strength of HA/Gelatin-70/30 to 114±8 MPa. Increase in gelatin content decreases the Young's modulus by 25% (from 4.7 to 3.5 GPa). Results indicates that the HA/gelatin composite films exhibit good cell adhesion and proliferation. Osteogenic differentiation of mouse bone marrow mesenchymal stem cells on the 70/30 membrane was greater to that of the remaining films, as evident by alkaline phosphatase activity, calcium deposition, and gene expression. Thus, the HA/gelatin 70/30 membrane prepared has potential as a bone substitute for bone regeneration [40].

Chhavi Sharma et al. successfully fabricated the scaffold, in the form of bead, consisting of natural polymers i.e., chitosan, gelatin, alginate and a nano-hydroxyapatite by using simple foaming method. Combining HA with natural polymers can enhance the mechanical properties and provide the biological properties that mimic the natural bone. SEM analysis reveals the presence of interconnected pores that are spread uniformly on the surface of scaffold. The scaffold exhibit porosity of 82% with an average pore size of 112 ± 19.0 µm. Swelling and degradation studies shows that scaffolds have excellent properties of

hydrophilicity and biodegradability. Mechanical testing shows no rupturing of scaffold under physiological conditions which proves that scaffolds have good mechanical stability. In vitro cell culture studies showed good cell adhesion, proliferation rate and viability. In light of these properties, authors concluded that the scaffold containing these natural polymers has the paramount importance for application in the field of tissue engineering [41].

A Bigi et al. investigated mechanical, thermal, swelling and release properties of glutaraldehyde (GTA) crosslinked gelatin films in order to examine the influence of GTA on the stability of the films. Gelatin films were air dried and then subjected to treatment with GTA solution at different concentrations ranging from 0.05 to 2.5 wt%. The crosslinking degree was determined by trinitrobenzene sulfonic acid assay. At smallest GTA concentration, the crosslinking degree amounts to 60% and it increases nearer to 100% for concentrations greater than 1 wt%. Simultaneously the deformability of the films decreases whereas Young's modulus and stress at break increases. According to Differential Scanning Calorimetry results, crosslinking greatly affects the thermal stability of the films. Swelling analysis shows that, crosslinking degree of 85% obtained using 0.25wt%, is enough to prevent gelatin release in buffer solution and to provoke a significant decrease of swelling in physiological solution [42].

Haffsah Iqbal et al. developed scaffold containing Chitosan/HA where HPMC was added as crosslinker by using freeze drying method. Different concentrations of HPMC were added in the CS/HA/HPMC scaffold to evaluate the effect of HPMC on CS/HA scaffold. TGA, SEM, EDS, DSC and swelling studies were carried out to analyze the resulting properties of the scaffolds. Higher concentrations of HPMC in CS/HA scaffold shows better thermal stability as compared to those with low concentrations because of the strong crosslinking of HPMC with CS/HA. This crosslinking was also observed through FTIR spectra. The increase in HPMC concentration also results in increased swelling. The increase in HPMC and HA concentration lessen the pores and hence scaffold become softer. Scaffolds with optimum concentration of HPMC and HA had maximum compressive strength of 14 MPa/cm^3. These results suggests that presence of crosslinker in different amounts effects the properties of scaffolds. Scaffolds with 25% HPMC exhibits more viable cells and good cell adhesion

whereas the one with 10% HPMC demonstrated high pre-osteoblast differentiation potential [43].

Chapter 3 Materials and Methodology

3.1 Materials Required

Calciumhydroxide Ca $(OH)_2$ (CAS No. 1305-62-0) was bought from VWR International (USA), Phosphoric acid (H_3PO_4) (CAS No. 7664-38-2) was bought from Honeywell (USA), Gelatin (CAS No. 9000-70-8) and Tricalcium Phosphate (TCP) (CAS No. 7758-87-4) were bought from DAEJUNG (Korea), HPMC (CAS No. 9004-65-3) and Glutaraldehyde (CAS No. 111-30-8) were bought from Sigma Aldrich (Germany).

3.2 Synthesis of Hydroxyapatite (HA)

HA was synthesized using wet precipitation method. The precursors used for the synthesis of HA were Ca $(OH)_2$ and H_3PO_4.

Ca $(OH)_2$ was allowed to stir in deionized water at 100^{o}C for 2 hours. After complete dissolution of Ca $(OH)_2$ in water, 9.1 ml acidic solution of H_3PO_4 was added in Ca $(OH)_2$ at the rate of 5ml/5minutes. The pH was measured after every alternate interval. Initial pH was 13 and after some intervals of adding acidic solution, the pH tends to decrease. This indicates that both the solutions are soluble. Further addition of acid in Ca $(OH)_2$ was stopped when the pH becomes 10. However, Ca $(OH)_2$ and H_3PO_4 were allowed to mix for another 4 hours under constant stirring. The final product was centrifuged immediately and washed for 5 times at 40,000 rpm for 10 minutes in each round. After centrifugation, the final product was allowed drying in oven at 100^{o}C for 24 hours, followed by the calcination in muffle furnace at 950^{o}C for 4 hours.

3.3 Synthesis of Gelatin/HPMC Film

The Gel/HPMC film was prepared by solution casting method. Gelatin (95%) solution was prepared for 2 hours in 10 ml of deionized water at room temperature. After 2 hours, the temperature was increased to 60^{0}C for 30 minutes. Separately, HPMC (5%) was dissolved in 10 ml of hot water and stirred for 30 minutes. After stirring HPMC for half an hour in 10 ml of hot water (at 80^{0}C), the temperature was brought to room temperature for complete dissolution. At this stage, both the Gelatin and HPMC solutions, were mixed gently and allowed to stir for 1 hour at 55^{0}C. The temperature

was then lowered to room temperature and glutaraldehyde (3µL) was added as crosslinker, followed by stirring for just 30 seconds. The resulting solutions were casted on petri dish and kept overnight for air drying.

3.4 Synthesis of Gelatin/HPMC Films with HA and TCP as fillers

The films were prepared through solution casting method. Gelatin (95%) solution was prepared in deionized water and stirred for 2 hours at room temperature. After 2 hours, the temperature was increased to 60 ^{0}C for 30 minutes. Separately, HPMC (5%) was dissolved in hot water and stirred for 30 minutes. After stirring HPMC for half an hour in hot water, the temperature was brought to room temperature. HA and TCP were sonicated for 4 hours before incorporating as filler in Gel/HPMC matrix. At this stage all the 3 solutions i.e., Gelatin, HPMC and HA/TCP were mixed, gently and allowed to stir for 1 hour at 55 ^{0}C. The temperature was then lowered to room temperature and glutaraldehyde (3 µL) was added as crosslinker at this stage followed by stirring for just 30 seconds. The resulting solutions were casted on petri dish and kept overnight for air drying.

Through this synthesis method, following samples with varying filler ratios were synthesized.

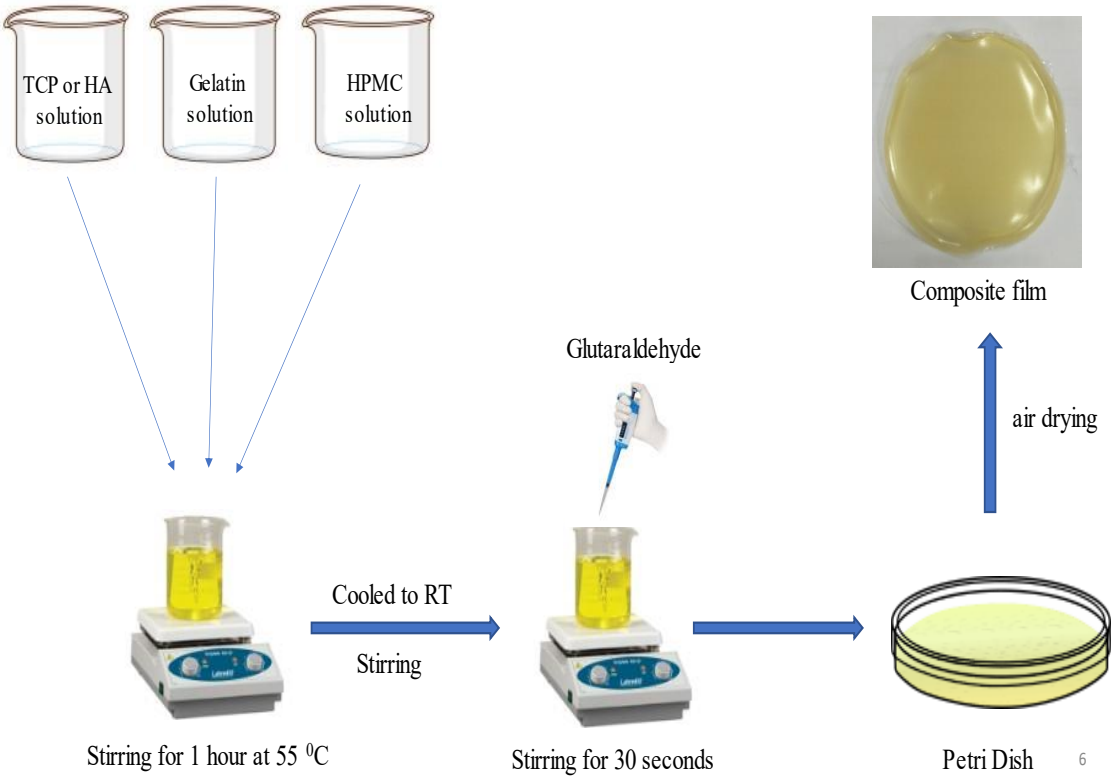

Figure 3.1: Schematic showing the synthesis route for Gel/HPMC films with HA and TCP as fillers

Table 2: Table showing all the prepared samples

Sample Name	**Gelatin**	**HPMC**	**HA**	**TCP**
Pristine Gelatin	100%	-	-	-
Gel/HPMC	95%	5%	-	-
Gel/HPMC-HA 0.1%	95%	5%	0.1%	-
Gel/HPMC-HA 0.5%	95%	5%	0.5%	-
Gel/HPMC-HA 1%	95%	5%	1%	-
Gel/HPMC-HA 3%	95%	5%	3%	-
Gel/HPMC-HA 5%	95%	5%	5%	-
Gel/HPMC-TCP 0.1%	95%	5%	-	0.1%
Gel/HPMC-TCP 0.5%	95%	5%	-	0.5%
Gel/HPMC-TCP 1%	95%	5%	-	1%
Gel/HPMC-TCP 3%	95%	5%	-	3%
Gel/HPMC-TCP 5%	95%	5%	-	5%

3.5 X-ray Diffraction (XRD)

X-Ray Diffraction (XRD) technique was used to analyze the structural information of all the samples through Siemens D5005 STOE & Cie GmbH (Darmstadt, Germany) at an angle 2θ in the range of 10° to 60°.

X-ray diffraction (XRD) is an analytical technique which is used to determine the material's structure and degree of crystallinity. It is a non-destructive technique that enables us to determine the characteristics and structure at the atomic level. With the help of XRD, we can determine the crystalline nature of pure and composite materials, phase purity, bond length and angles, and crystallinity index.

The XRD apparatus consists of following components:

- X-ray tube
- Monochromator (Collimator)
- X-ray detector
- Sample holder

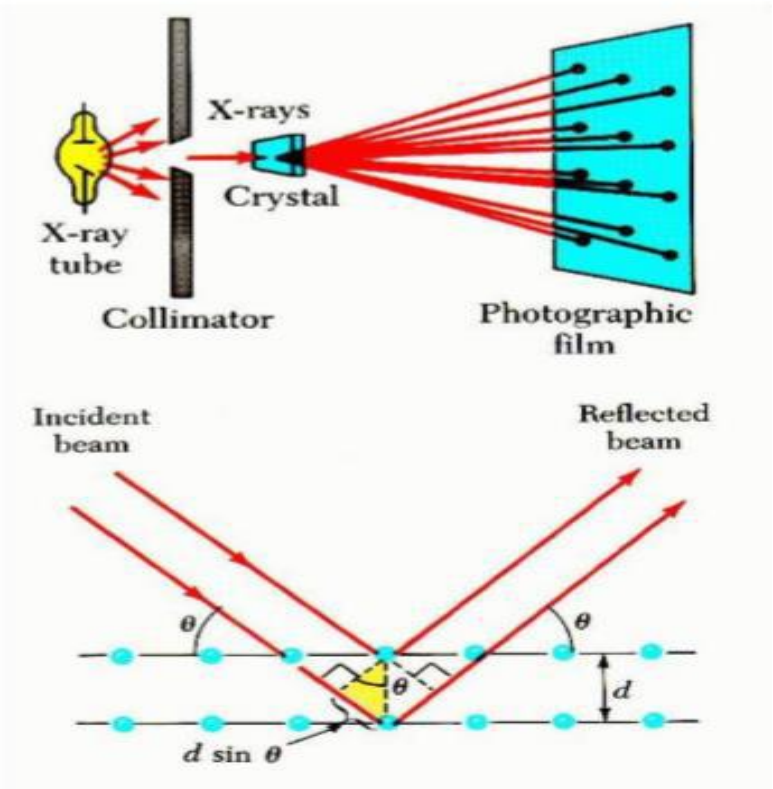

Figure 3.2: Basic schematic of XRD and Bragg's Law [44]

X-rays are produced by heating filament of cathode in x-ray tube. These x-rays are accelerated toward sample crystal after passing through monochromator. As sample crystal is made of atoms arrange to form regular planes. Some of the x-rays are absorbed and some of it having same wavelength as that of planes are reflected with the same angle of incident. That results in diffraction which is described by Bragg's law. Mathematically, the Bragg's law is:

$$\mathbf{2d \sin \theta = n \lambda}$$

Where,

d = interplanar distance

θ= angle formed by crystal plane and incident beam

n = order of reflection

λ = wavelength

The Bragg's law is satisfied for the constructive interference. The Bragg's reflections provide information on interlayer spacing, which may be used to calculate unit cell dimensions, lattice misfit, and dislocations [44].

3.6 FTIR Spectroscopy

FTIR Analysis was done on PerkinElmer, SpectrumTM100 spectrophotometer using Potassium Bromide (KBr) pellets with dried piece of membrane samples in the range 400cm^{-1} to 4500cm^{-1}.

The FTIR is an investigative technique used to find functional groups in organic materials and sometimes inorganic materials as well. This method uses infrared radiation to fall on sample to observe its chemical properties. Every atom in a molecule above absolute temperature is in continuous vibration. So different functional groups absorbed IR radiations with respect to their characteristic frequencies. Using functional groups, we can identify different compounds. In infrared spectroscopy, when IR radiations are directed on the sample, the frequency of vibration of a molecule in a sample when ties with the infrared radiation frequency, it absorbed that radiation. There are mainly two different vibrations i.e., stretching and bending.

The IR spectroscopy uses interferometer to provide the resulting infrared spectrum. The interferometer consists of three main parts:

- Source
- Detector
- Beam splitter

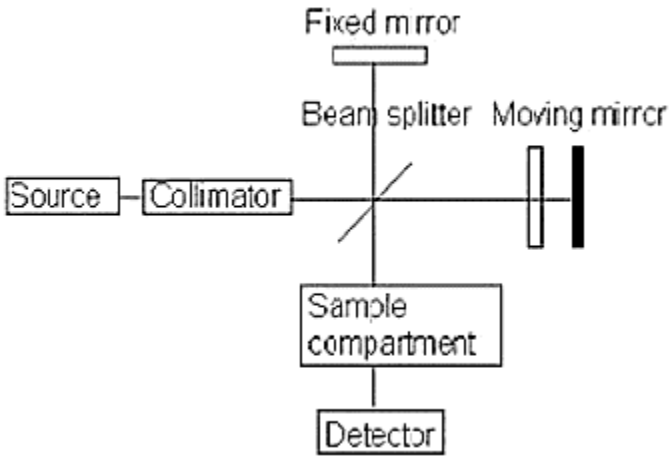

Figure 3.3: Schematic of FTIR spectroscopy

In this technique infrared, light is directed toward the beam splitter present in the interferometer as shown in figure, the moving and mixed mirror reflect the infrared beams to beam splitter, these beams recombine constructively and destructively at beam splitter and directed towards the sample which is detected as an interferogram. This interferogram is converted into IR spectrum using Fourier Transform. The resulted spectrum is shown a light output (% transmittance) as a function of wavenumber.

3.7 SEM

SEM was performed using JEOL JSM-6490A (Tokyo, Japan) at 10kV. All the samples were gold sputtered before testing.

SEM is used to study the interaction of electrons with matter to find the composition, particles size, topography, and phase mapping of nanomaterials. When a high energy electron beam strikes a material, many interactions occur between them which can be used for the analysis of sample. The sample surface is scanned by using beam of electrons. And the electrons that got scattered are collected back by the detector which gives information about morphology, particle size and topography. When electron beam interacts with matter surface x-rays, auger electron, back scattered electrons and secondary electrons are generated. Secondary electrons are most used for analysis in SEM. This is because they give very fine image of even small particles of 1 nm. The only requirement for the SEM sample is it should be conductive. The instruments required for the SEM are:

- Electron generation source
- Magnetic lenses
- Scanning coil for detection of electrons
- The sample chambers
- High processing computers for imaginations of scanned images

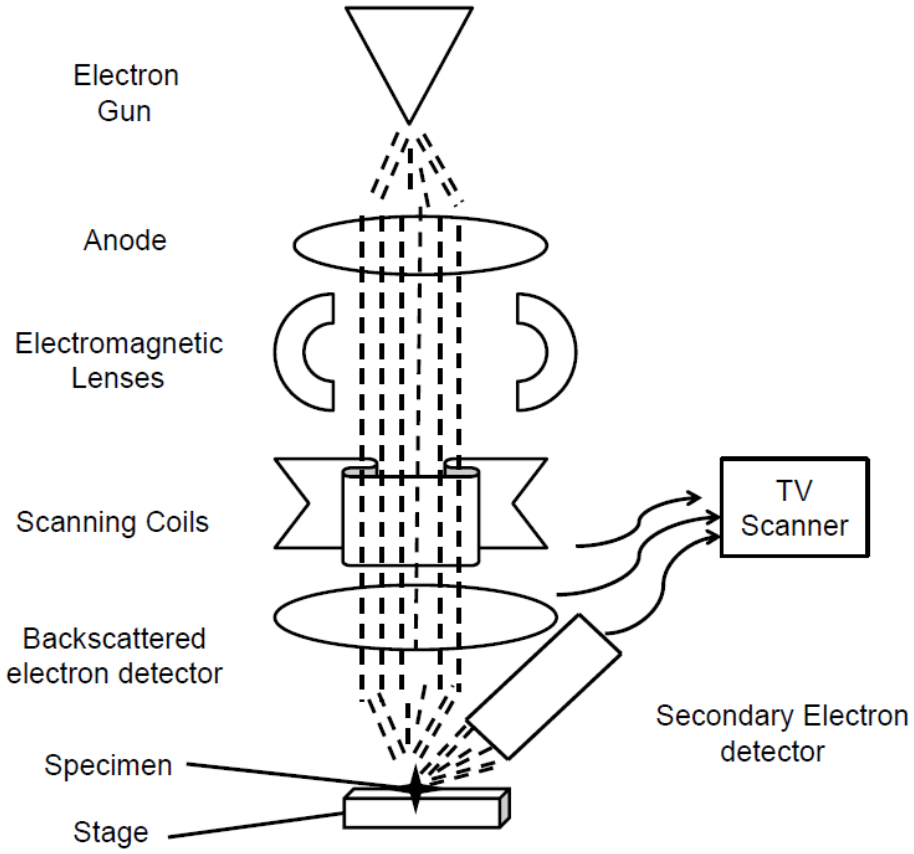

Figure 3.4: Schematic diagram of Scanning Electron Microscopy [45]

The electrons are produced commonly by using thermionic emission method. A set of different type of detectors is used for the collection of emitted electrons and photons when sample interacts with the electron beam. The rastering of electron beam is done by adjusting the X and Y of cathode ray with the X and Y of voltages. As a result of this image is obtained which is saved and used for the topographical and particle size analysis of sample [46].

3.8 Thermal Gravimetric Analysis (TGA)

TGA was done under nitrogen atmosphere through detector DTG-60H (serial no. C30575300507TK). The initial mass taken was 1.797mg and the gas flow rate was 50 ml/min.

This approach is used to estimate the mass change of materials when subjected to heat processes such as oxidation, decomposition, and reduction. TGA is a method that records changes in the weight of a material with respect to the temperature. The sample is affected by temperature changes because the mass of the sample changes. Thermobalance is the instrument used in TGA. TGA data is represented as a curve,

which is referred to as a "thermogram."." The furnace raises the temperature as high as 1000 ^{0}C and it is constructed of quartz.

TGA can be classified into three categories.

- **Dynamic TGA:** The sample temperature is continuously linearly increased with regard to time in this approach.
- **Isothermal TGA:** The temperature of the sample is held constant over a length of time in this sort of examination, and the change in weight is measured.
- **Quasistatic TGA:** When the temperature is increased in a sequence, the sample is heated until the constant weight is reached.

The sample is put into the microbalance using an auto sampler. Thermocouple is positioned directly above the sample. The mass change caused by the temperature is then measured. The thermocouple is used to monitor the temperature of a sample as well as changes in the temperature. The weight is calculated by determining the deflection of the beam. During the whole process of putting the sample in platinum pan, direct contact with the thermocouple is avoided. TGA quantifies changes in sample mass, and it is scanned in a carefully controlled environment. The weight of the sample is measured using a thermobalance as a function of temperature. The sample is held with balance in a furnace, and it is thermally separated from the furnace [47].

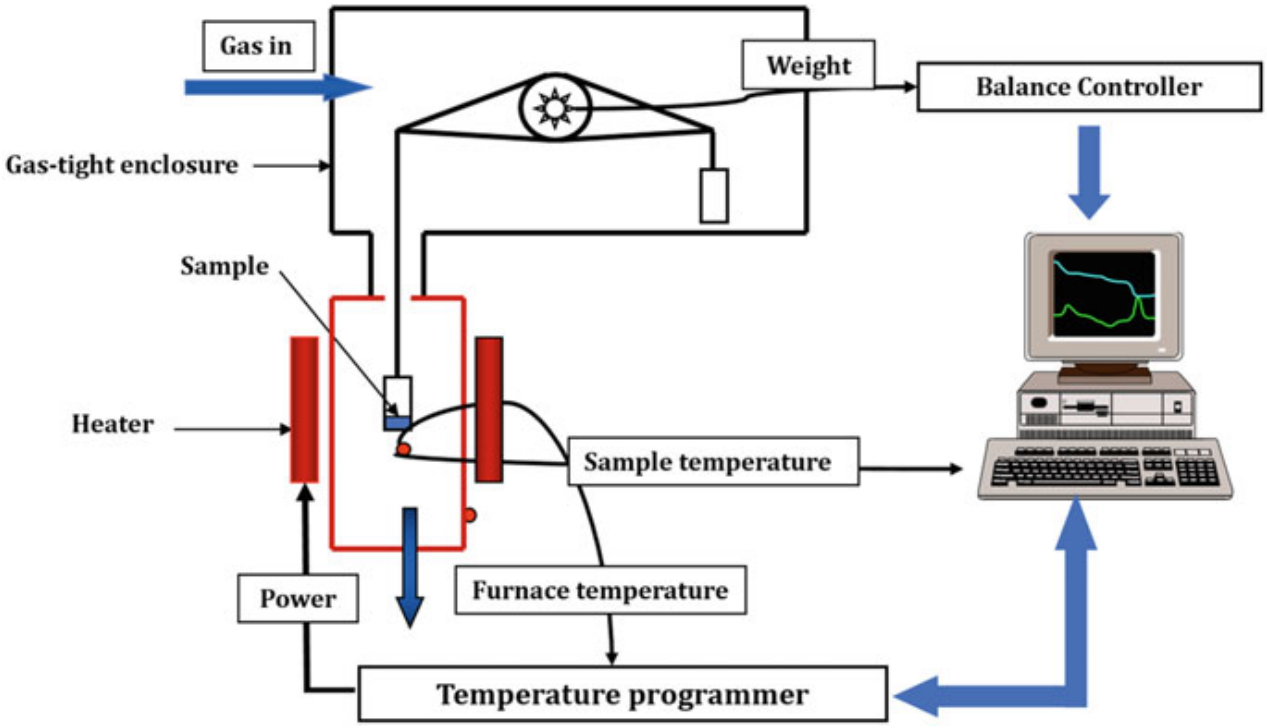

Figure 3.5: Schematic representation of components of thermo gravimetric analysis [47]

3.9 Mechanical Testing

The tensile properties of all the composites were tested using the apparatus ASTM D882 on a UTM (Shimadzu AGX Plus, Kyoto, Japan and using a load cell of 20 kN. The size of the tensile specimen were kept 0.7 cm * 2.6cm. Three specimens from each sample were averaged to provide the tensile strength data and the results are calculated as Mean ± SD.

The capacity of any material to withstand fracture is measured. When a force is applied to a material, it either plastically or elastically elongates or fractures. It calculates the percentage elongation of a material by stretching it until it fractures and the percentage compression by pushing it until it fractures. Plastic deformation occurs before fracture in ductile materials, whereas fracture occurs instantly in brittle materials. Through mechanical testing from UTS machine, we can obtain stress-strain curve, tensile strength, fracture point, strain at break, percentage elongation, toughness and young's modulus.

The sample is clamped in the machine's jaws and a particular rate of stress is applied to it until fracture occurs. The bottom end of the specimen is held in place by a stationary holder, while the top end is held in place by a moving holder. The sample lengthens till it fractures. This sample elongation is referred to as gauge length. The report is generated automatically by software and includes information on how much stress the investigated material can bear before fracture [48].

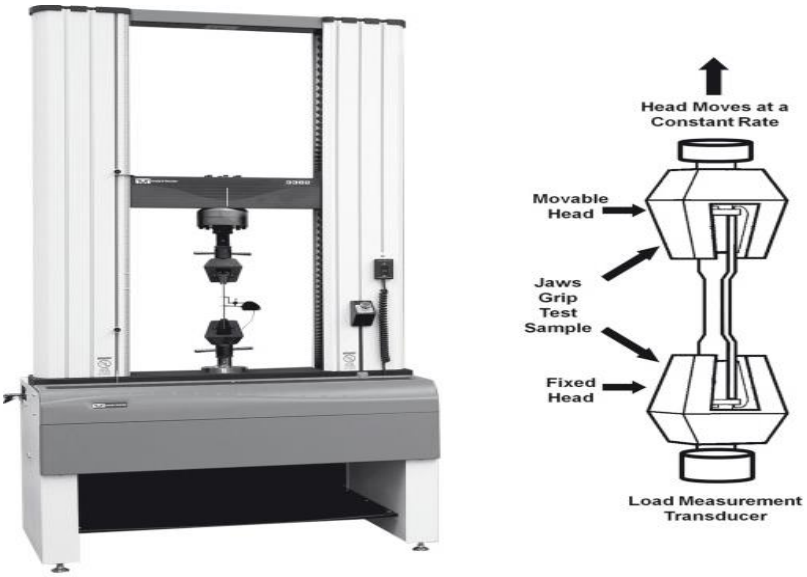

Figure 3.6: **:** Schematic illustration of Universal testing Machine [49]

3.10 Swelling Analysis

Swelling analysis is used to determine the water uptake or water absorption ability of membranes. All the samples were cut into equal piece of 1 x 1 cm^2. All the pieces of samples were weighed (initial weight, W_i) before immersing in glass vials which contains the PBS buffer solution and water, separately. The PBS solution was used to measure the swelling tendency of the nanocomposite membranes because it constitutes the major component of the body fluids. The pH of the PBS solution was 7.4. The samples were kept for 24 hours at ambient conditions. The membranes were removed from the fluid after 24 hours (equilibrium time) and then blotted while using cellulose paper to remove excess water content that waspresent and weighed again (final weight, W_f).

The degree of Swelling can be found using a formula:

$$\boldsymbol{Degree\ of\ Swelling}\ (\%) = \frac{\boldsymbol{W_f - W_i}}{\boldsymbol{W_i}}\ \mathbf{X\ 100}$$

The mean value was calculated using three replicates for each sample.

3.11 Contact Angle Measurements

A Kruss DSA 25 Goniometer (Hamburg, Germany) was used to measure the contacts angles of all the prepared samples using a seessile drop (water) at room temperature (20°C) for 0–20 seconds. The contact angle data were acquired by averaging three specimens of each sample and presented as the mean SD.

In contact angle measurements, the wettability of solid substrates is measures through the liquid droplets. Wettability is frequently defined by the contact angle created between a liquid drop and a solid surface. This measurement is thought to be a relatively simple and sensitive method for measuring solid surface energy. Furthermore, surface roughness may be determined using contact angle measurements.

If the characterized solid surfaces are ideal (i.e., smooth, and chemically homogeneous) then the contact angle measurement and its interpretation would be straightforward. In this scenario, the observed contact angle would be the same as the ideal contact angle calculated using Young's equation.:

$$\boldsymbol{\gamma_{sv} = \gamma_{sl} + \gamma_{lv}.cos\theta}$$

However, practically the surfaces are heterogeneous to a certain extent. The only quantifiable value on these surfaces is the apparent contact angle, θ_{ap}, which could be different from the ideal contact angle [50].

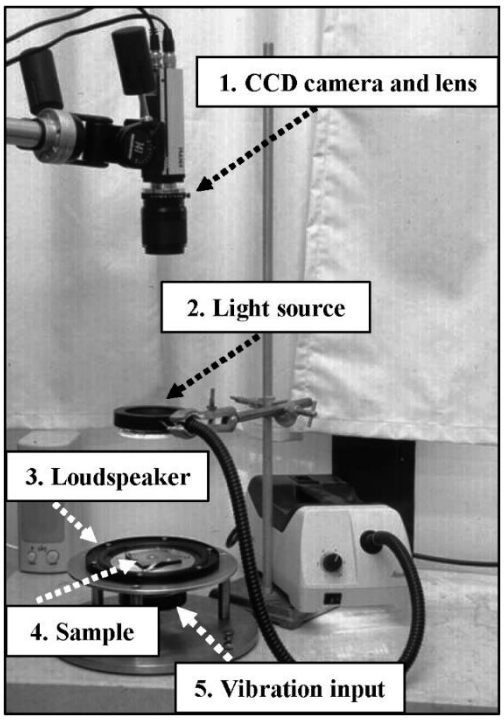

Figure 3.7: Schematic showing the working of contact angle measurement [50]

3.12 Optical Profilometry

The instrument used to obtain the roughness of the membranes was from Nanovea (Model PS50).

Optical Profilometry is used to determine step heights on surfaces, and surface roughness. Optical profilometry was used to measure the roughness of all the nanocomposite membranes. Profilometry is a method of extracting data from a surface. A single point scan, line scan, or a full 3 Dimensional scan are all possible options.

Chapter 4 Results and Discussions

4.1 XRD Analysis

4.1.1 XRD of HA Powder

The XRD spectra for HA, before and after annealing is shown below.

Before annealing, the less sharpness in peaks was observed. After heat treatment, the peaks become sharp and hence the crystallinity of HA increases. The structure of hydroxyapatite particles was hexagonal. The diffraction peak, for HA, matches the JCPDS Card No = 09-0432 [51].

The characteristic peaks for HA i.e., (002), (211), (300), (202), (213) were observed through XRD.

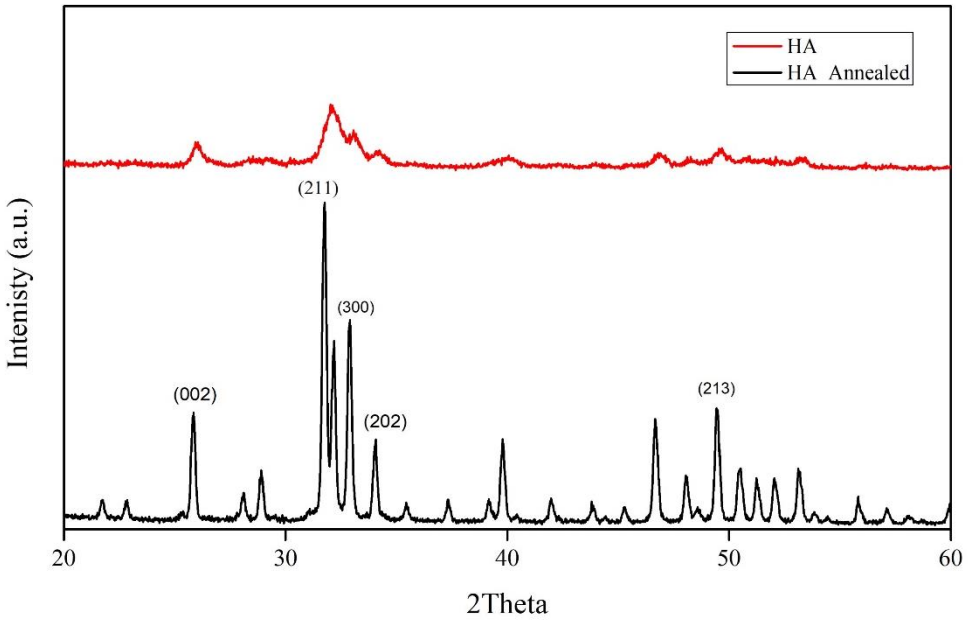

Figure 4.1: Showing XRD of HA and Annealed HA

4.1.2 XRD of Membranes

All the samples prepared were subjected to XRD analysis to study the crystalline nature.

The Gel/HPMC film has broad peak at around rather than sharp peak, which indicates that Gel/HPMC blend possess amorphous nature [52]. Both, the gelatin and HPMC possess characteristic peaks at around 20°.

However, with the increase in HA contents, the HA characteristic peaks become visible which confirms the presence of HA in the Gel/HPMC blend. The peaks for 3% HA and 5% HA, shows more intensity. This is due to the higher HA content in the blends.

Similarly, in case of TCP, the characteristic peak appears in blends with higher quantity of TCP at 31.8° i.e., for 3% and 5%. This shows the presence of TCP content in Gel/HPMC blends.

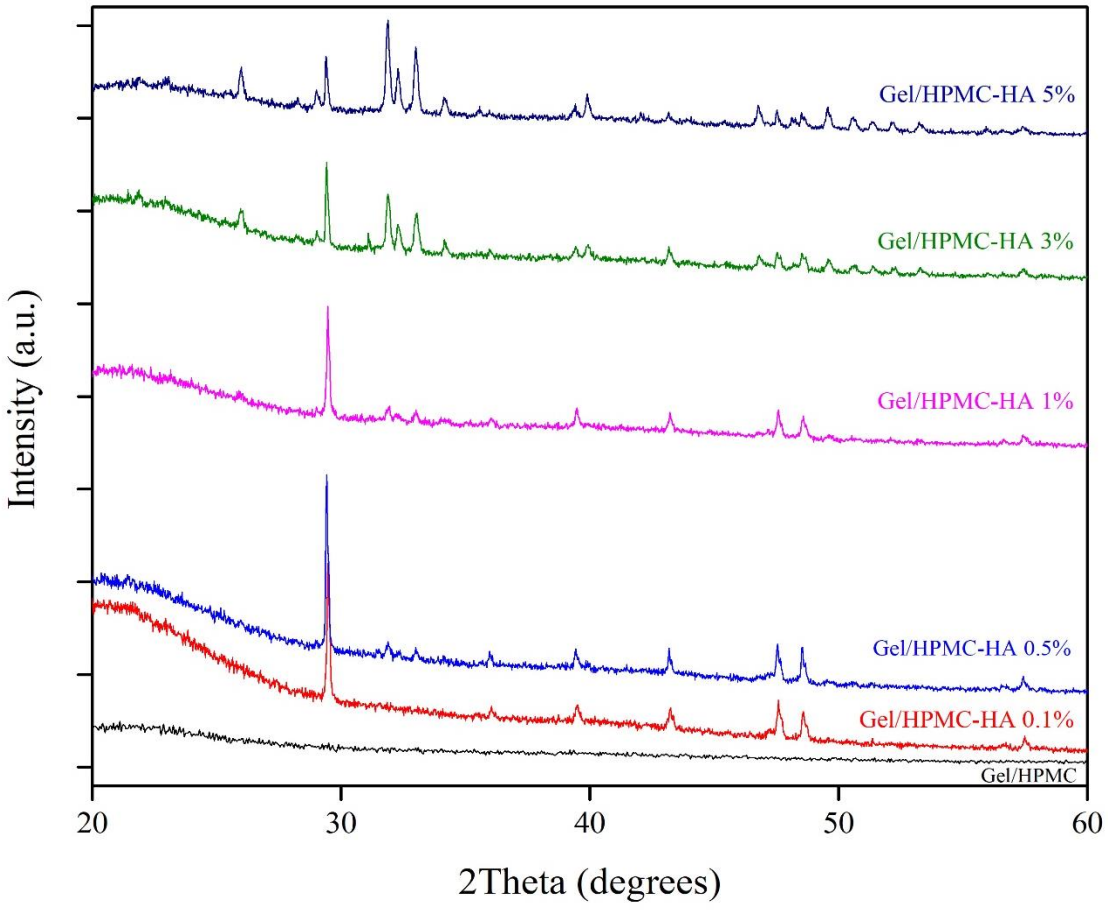

Figure 4.2: XRD of Gel/HPMC composites containing HA as filler

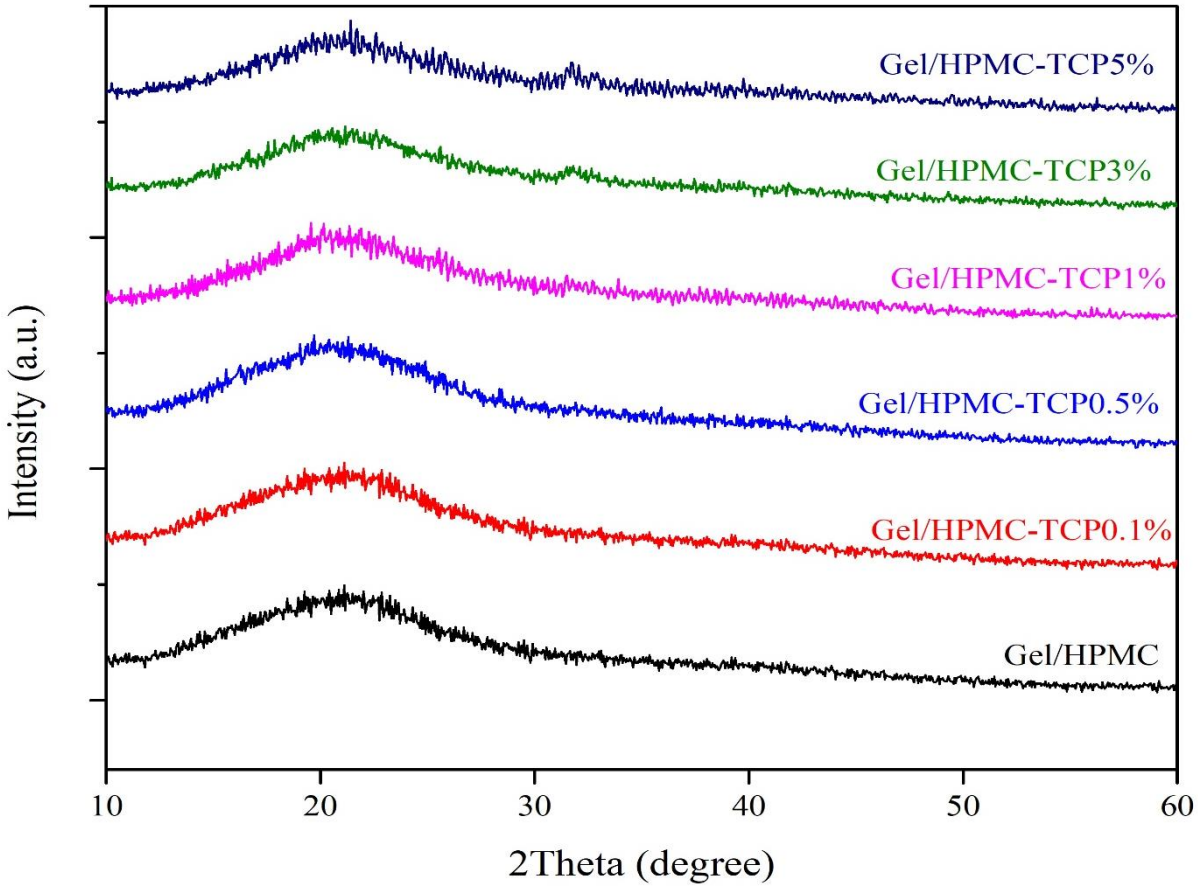

Figure 4.3: XRD of Gel/HPMC composites containing TCP as filler

4.2 FTIR Analysis

For pure blend Gel/HPMC, the characteristic bands were observed for both gelatin and HPMC with relatively larger intensity. Gelatin shows characteristic bands at 3430 cm^{-1} (Amide A) and 1630 cm^{-1} (Amide I) [53]. And for HPMC, the characteristic bands was observed at 1051 cm^{-1} and the region between 900 cm^{-1} and 1400 cm^{-1} corresponds to the fingerprint region for HPMC [30].

For gelatin, Amide-I (1630 cm^{-1}) band corresponds to the stretching vibration of C=O bonds and Amide-II (1535 cm^{-1}) was due to coupling of N=H bond bending and C=N bond stretch. For HPMC, band around 3444 corresponds to O-H vibrational stretching. [32].

With the addition of both the fillers, these characteristic bands were also observed but with lower intensity.

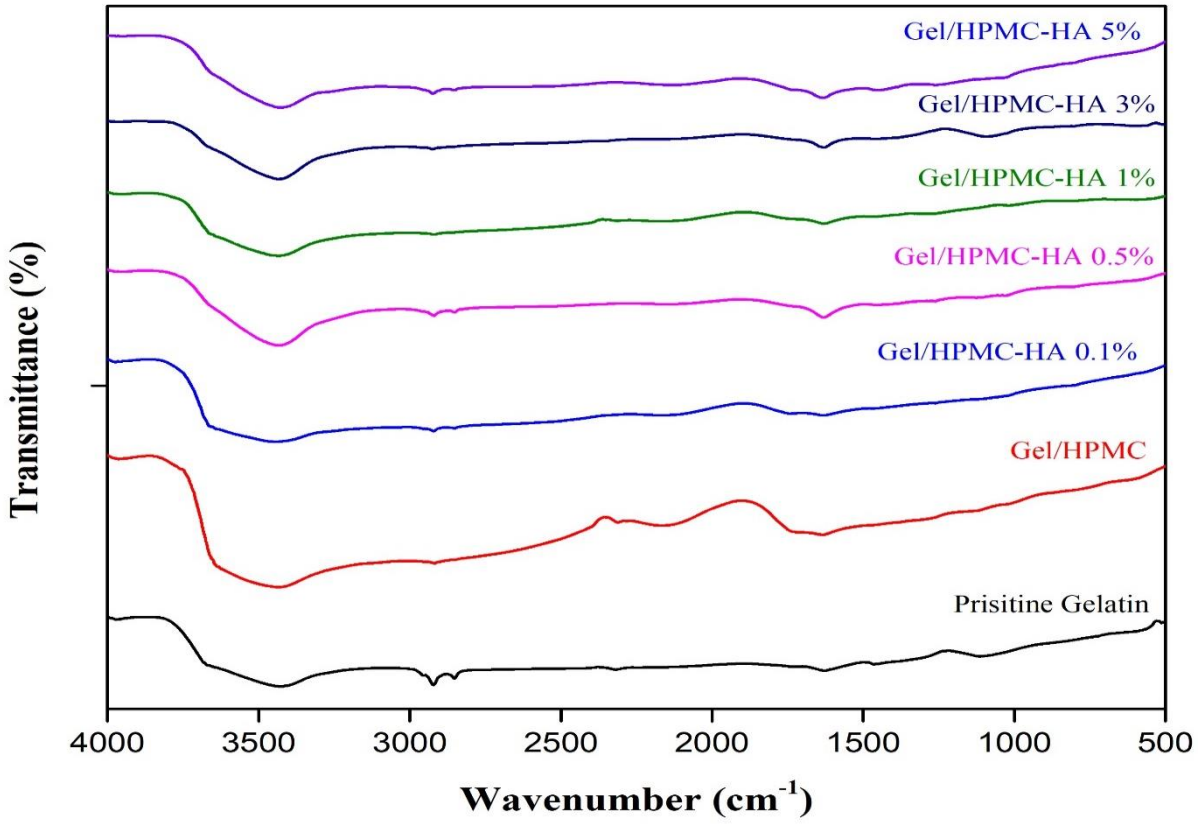

Figure 4.4: FTIR of pristine gelatin, Gel/HPMC composite and Gel/HPMC composites with HA as filler

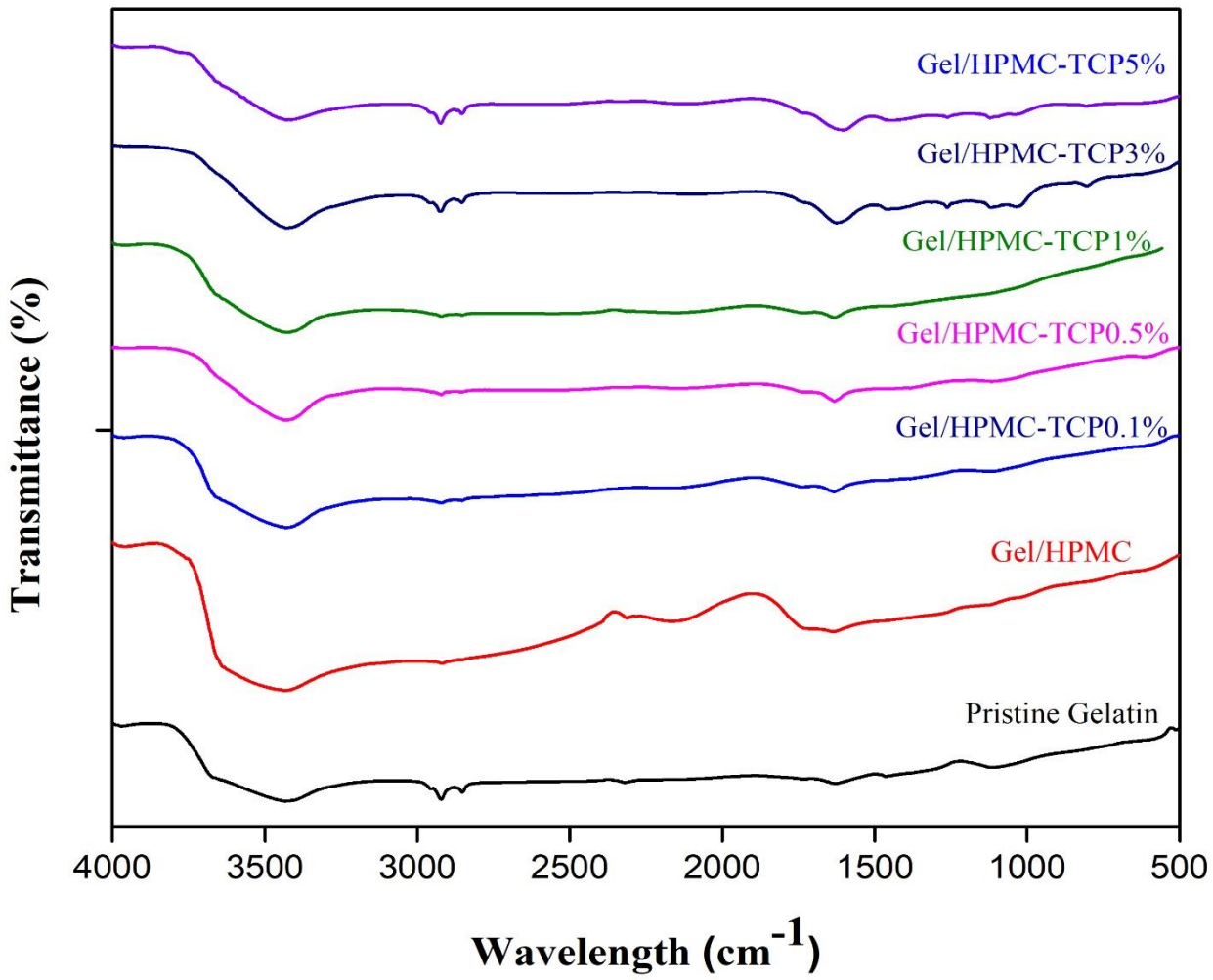

Figure 4.5:FTIR of pristine gelatin, Gel/HPMC composite and Gel/HPMC composites with TCP as filler

4.3 SEM Analysis

SEM images of the surface of Pure and Gelatin/HPMC films containing HA are shown in the figure below. As observed, the pure film containing only Gelatin and HPMC has smooth and homogeneous surface.

However, with the increase of HA and TCP content in the Gelatin/HPMC matrix (from 0.1% to 5%), the surface tends to become rough. Higher the HA and TCP content, more the degree of dispersion was observed in films, and thus rougher the blend surface becomes. At higher concentrations, the filler particles spread out

uniformly throughout the Gel/HPMC matrix. This indicates that the both the fillers are incorporated homogeneously in the Gel/HPMC matrix. However, for TCP at 3% and 5%, the agglomeration of TCP occurs.

These results are also in accordance with the results obtained through optical profilometry, whereby the roughness increases linearly with the increase in both the fillers content.

The bright spots are attributed to the HA and TCP particles. The particle of both the fillers shows spherical morphology and are distributed uniformly through the Gel/HPMC blends.

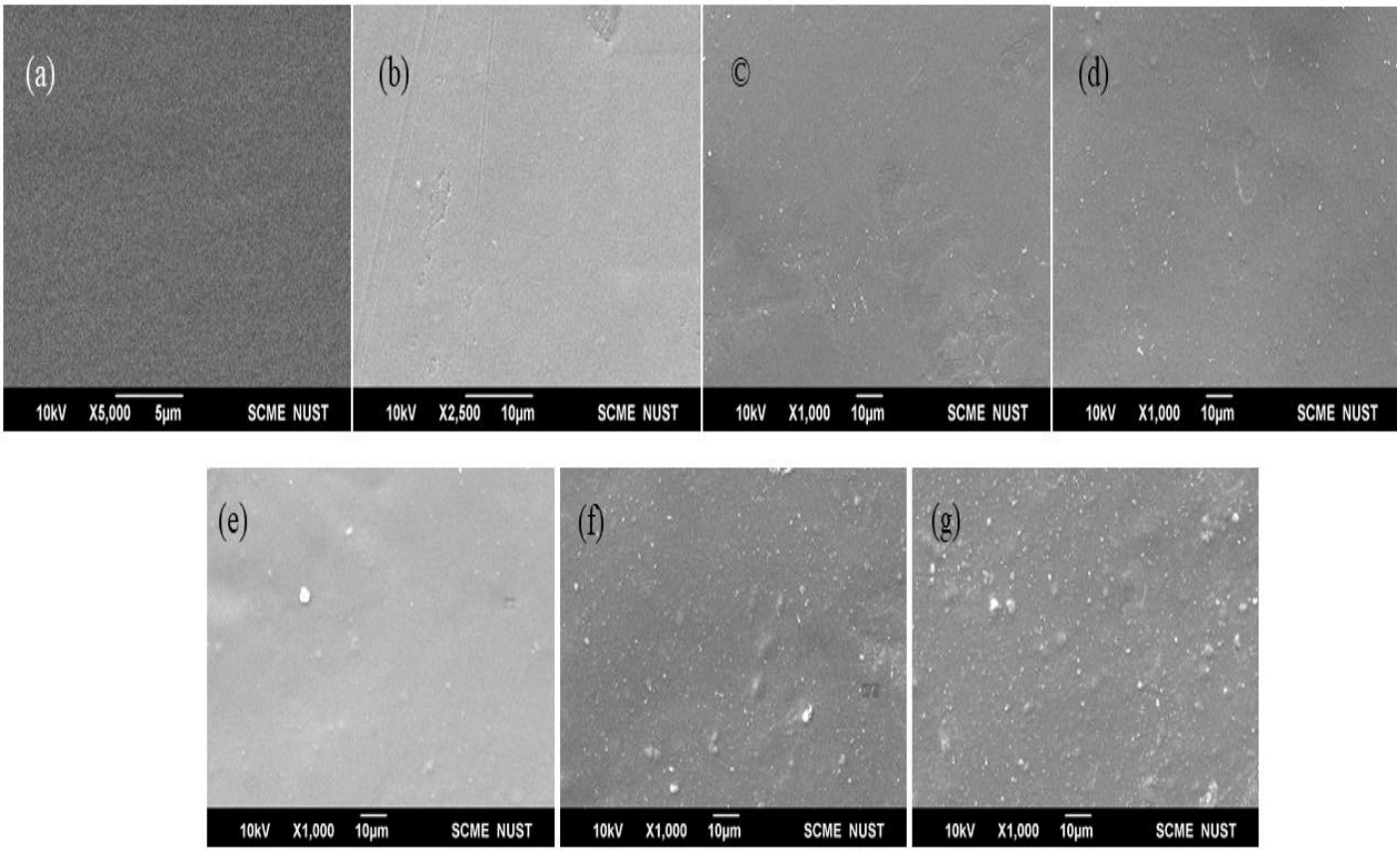

Figure 4.6: Showing SEM images for (a) Pristine Gelatin, (b) Gel/HPMC, (c) Gel/HPMC-HA0.1%, (d) Gel/HPMC-HA0.5%, (e) Gel/HPMC-HA1%, (f) Gel/HPMC-HA 3%, and (g) Gel/HPMC-HA5%

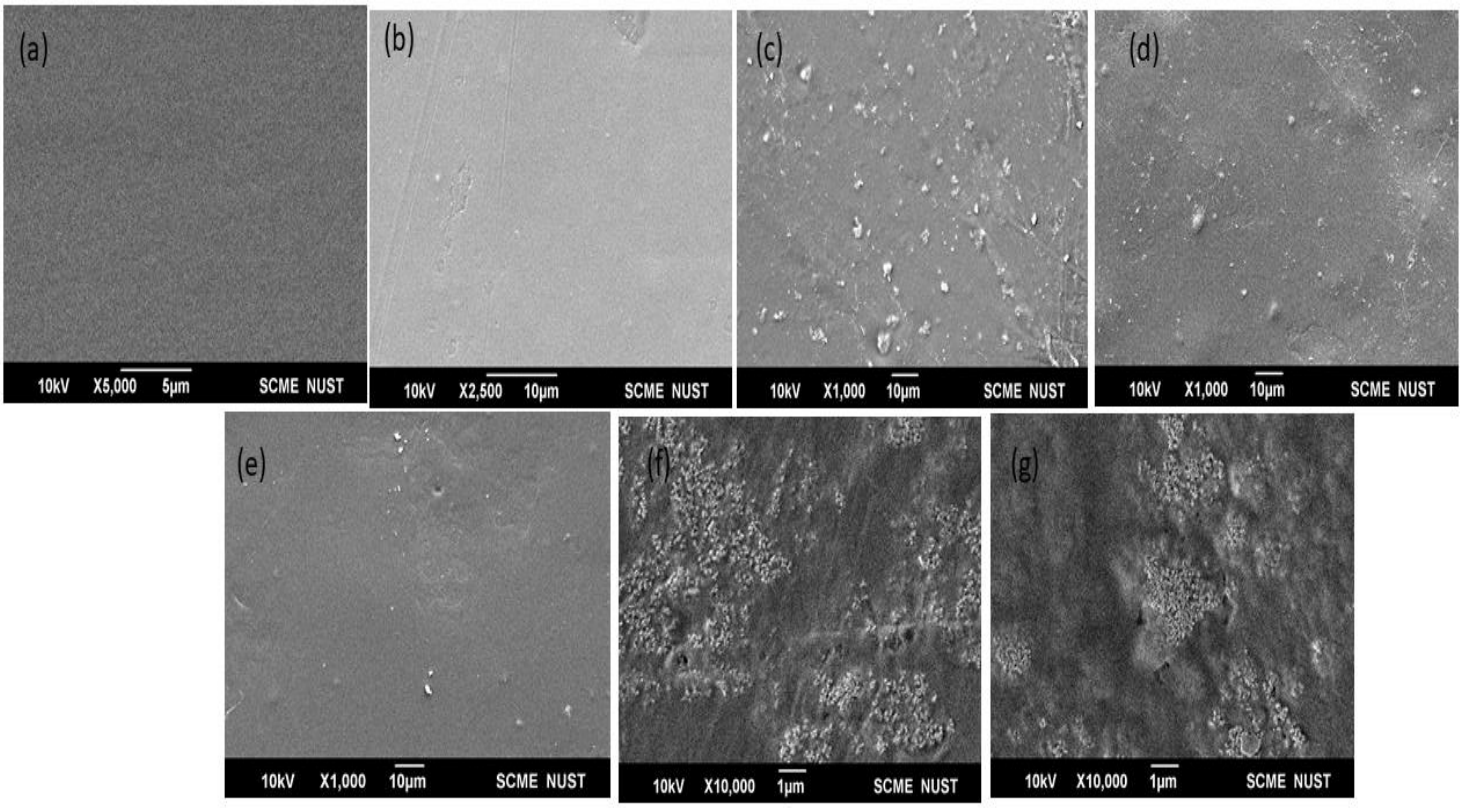

Figure 4.7: Showing SEM results for (a) Pristine Gelatin, (b) Gel/HPMC, (c) Gel/HPMC-TCP0.1%, (d) Gel/HPMC-TCP0.5%, (e) Gel/HPMC-TCP1%, (f) Gel/HPMC-TCP3%, and (g) Gel/HPMC-TCP5%

4.4 Thermal Gravimetric Analysis (TGA)

Samples of pristine Gelatin membrane, Gel/HPMC, Gel/HPMC-HA5% and Gel/HPMC-TCP5% were analyzed through TGA Analysis.

TGA curves of Pristine Gelatin and Gelatin composites indicates that they losses weight in three phases. The first phase occurs at temperatures ranging from ambient temperature to 200 to 220^{o}C, with a weight loss of almost 10 to 12%. This occurs because of the loss of adsorbed water acquired during synthesis. The second degradation phase, which is linked with protein degradation, occurs between 220^{o}C and 400^{o}C. This is due to the loss of lower molecular weight proteins. During this phase, Gelatin, and blended gelatin losses majority of their weight i.e., 52 to 55%, approximately. The third phase, which occurs with the increase in temperature from approximately 420^{o}C and onwards, relates to the thermal breakdown of the gelatin network in gelatin and blended gelatin membranes. The weight loss occurred is from nearly 28% to 6% [54-56].

All the membranes, within temperature range of 25^{o}C to 800^{o}C, loses almost 94% weight as compared to their initial weight. The TGA curves indicates that Gel/HPMC-HA5% and Gel/HPMC-TCP5% shows less weight loss as compared to Pristine Gelatin and Gel/HPMC membranes.

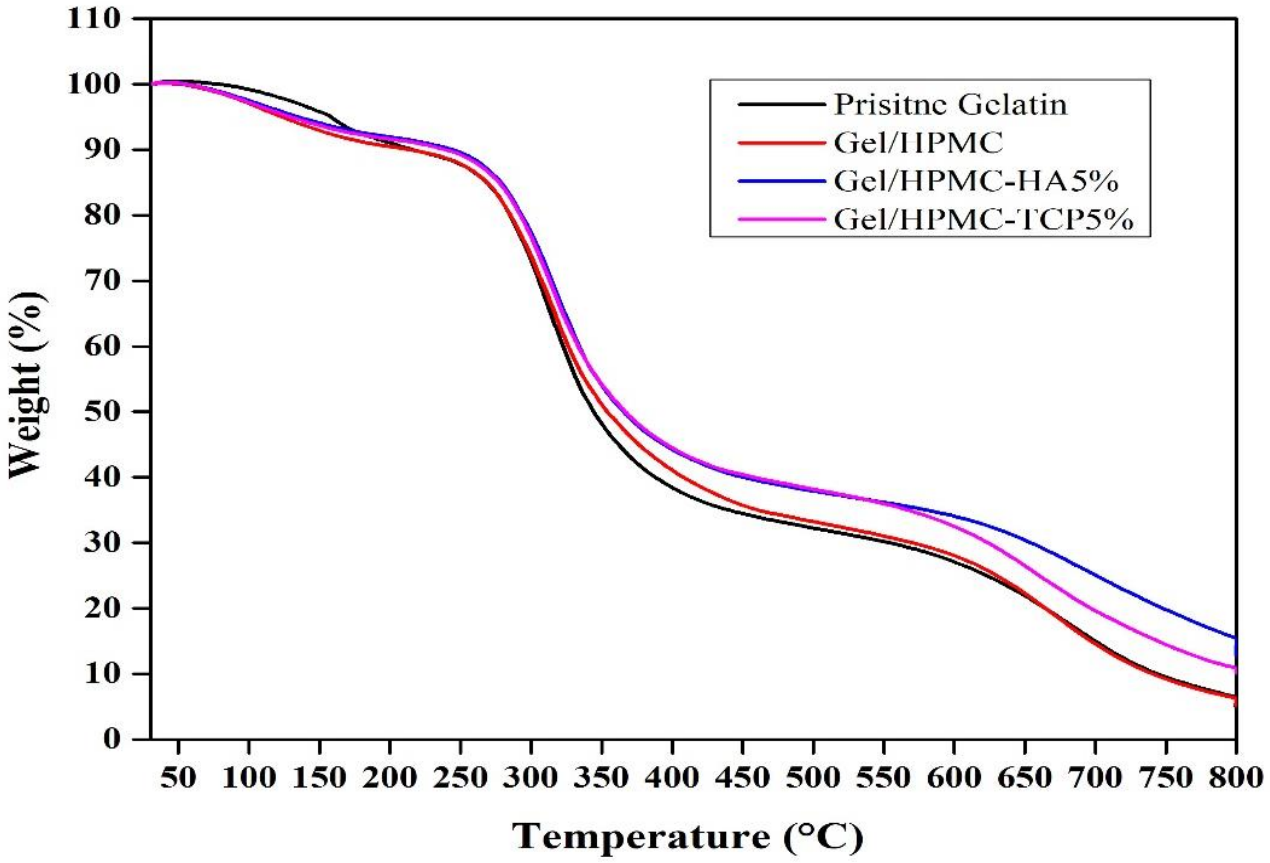

Figure 4.8: TGA curves for pristine gelatin, Gel/HPMC, Gelatin/HPMC-HA5% and Gel/HPMC-TCP5%

4.5 Mechanical Testing

Polymeric blends must exhibit enough strength to withstand or afford any damage that can occur during the handling and application. Usually, higher tensile strength values and lower elongation values shows the brittleness of the material.

Mechanical tests shows that the overall tensile strength of Gel/HPMC increases with increase in the concentration of both the fillers.

Pure Gel/HPMC have tensile strength of 78.55 MPa. Initially, for HA-0.1%, the tensile strength of the Gel/HPMC film decreases to 76.32 MPa for Gel/HPMC-HA0.1% and 75.45MPa for Gel/HPMC-HA0.5%. However, from Gel/HPMC-HA0.5% and onwards, it increases linearly with the increase in HA content in the Gel/HPMC matrix [36]. The maximum tensile strength was obtained to be 110.22 MPa for Gel/HPMC matrix with HA-5%. In case of TCP, the tensile strength increases linearly with the increase in TCP content. The maximum value observed was for TCP-5% which is 108.41 MPa.

This enhancement of tensile strength, with the increase in HA and TCP content, can be due to the presence of increased electrostatic forces. Also, can be due to the establishment of stable network between Gel/HPMC matrix and Hydroxyl group of HA and phosphate group of TCP [57].

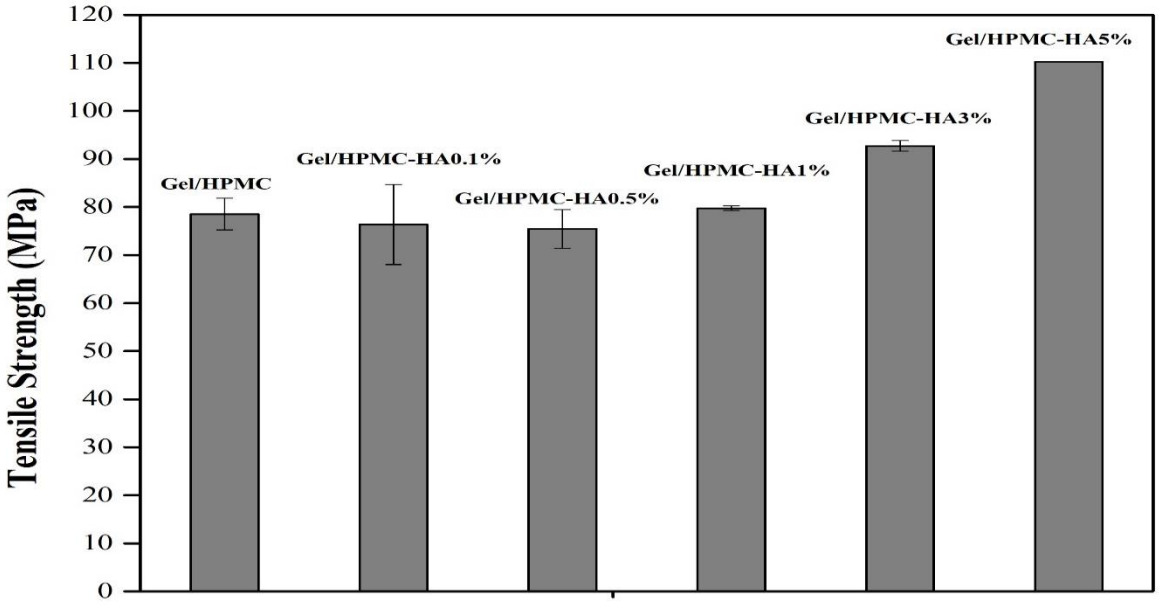

Figure 4.9: Tensile Strength of Gel/HPMC and Gel/HPMC composites with HA as filler

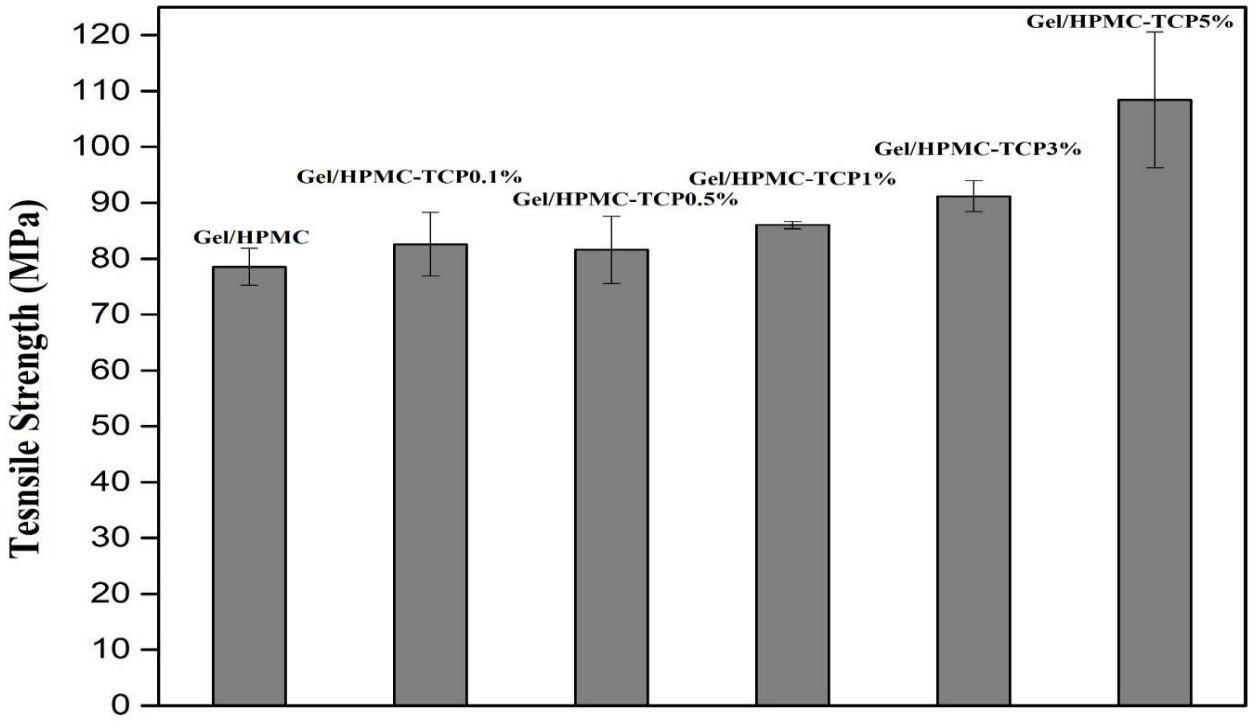

Figure 4.10: Tensile Strength of Gel/HPMC and Gel/HPMC composites with TCP as filler

Table 3: Table showing values of tensile strength for all samples

Samples	**Tensile Strength (MPa ± SD)**
Gel/HPMC	78.55 ± 3.3
Gel/HPMC-HA0.1%	76.32 ± 3.3
Gel/HPMC-HA0.5%	75.45 ± 8.34
Gel/HPMC-HA1%	79.4 ± 4.03
Gel/HPMC-HA3%	92.72 ± 1.13
Gel/HPMC-HA5%	110.22 ± 0.07
Gel/HPMC-TCP0.1%	82.59 ± 5.69
Gel/HPMC-TCP0.5%	81.58 ± 6.02
Gel/HPMC-TCP1%	86.01 ± 0.65
Gel/HPMC-TCP3%	91.18 ± 2.75
Gel/HPMC-TCP5%	108.41 ± 12.16

The elongation at break is the measure of ductility of the materials. It represents that how much a material can change its length after facing the load as compared to the initial length [30].

The results for both the fillers, HA and TCP, suggests that there is no significant increase and decrease in EAB. Pure membrane shows EAB (%) values as 4.83 ± 0.03 %. Among HA samples the maximum EAB value was shown by HA 3% and for TCP, it was TCP 0.1% which shows maximum value. However, these maximum values are not significant enough to be distinguished from other samples.

These low values of EAB for Gel/HPMC blends are because gelatin is a brittle in nature. Since the majority (95%) of the blend is composed of gelatin, thus these EAB values are largely controlled by gelatin.

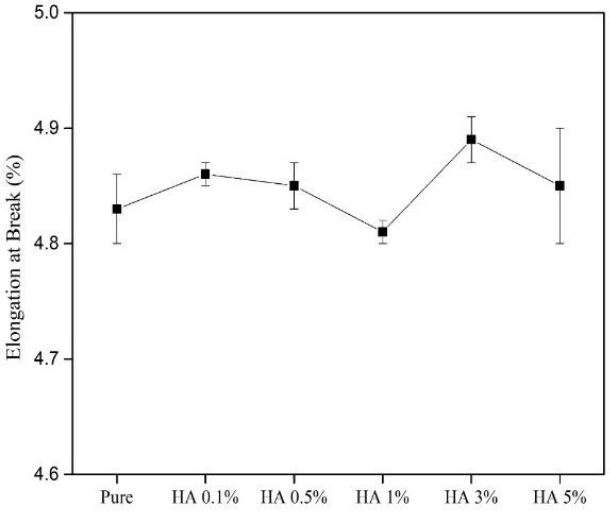

Figure 4.12: EAB graph for HA samples

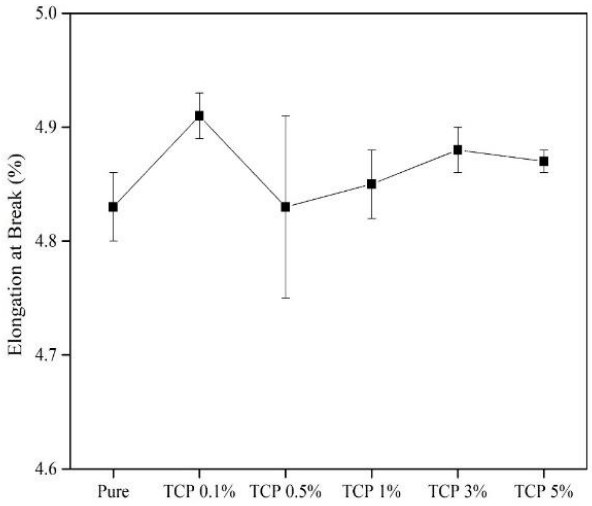

Figure 4.11: EAB graph for TCP samples

Table 4: Table showing EAB values of all the samples

Samples	**EAB (% ± SD)**
Gel/HPMC	4.83 ± 0.03
Gel/HPMC-HA0.1%	4.86 ± 0.01
Gel/HPMC-HA0.5%	4.85 ± 0.02
Gel/HPMC-HA1%	4.81 ± 0.01
Gel/HPMC-HA3%	4.89 ± 0.02
Gel/HPMC-HA5%	4.85 ± 0.05
Gel/HPMC-TCP0.1%	4.91 ± 0.02
Gel/HPMC-TCP0.5%	4.83 ± 0.08
Gel/HPMC-TCP1%	4.85 ± 0.03
Gel/HPMC-TCP3%	4.88 ± 0.02
Gel/HPMC-TCP5%	4.81 ± 0.01

4.6 Swelling Analysis

The water uptake ability of Gel/HPMC-HA and Gel/HPMC-TCP films after 24h of incubation in PBS solution were examined.

The pure (Gel/HPMC) film shows swelling up to 820% and Gel/HPMC-5% shows the maximum water uptake ability of 970%. With the increase in HA content in Gel/HPMC blend, the degree of swelling increases. This shows that the HA, being hydrophilic in nature, is controlling the water uptake ability of the blends. Higher contents of HA cause the surface roughness and larger pore size, due to which the PBS solution can easily infiltrate in the membranes [58]. After incubating membranes for 24 hours, the membranes become flexible and shows very less brittleness and thus maintaining the stable swelling ratio [59].

In case of TCP, the significant increase in the swelling was not observed. TCP1% shows maximum swelling at 730% and TCP-0.1% with the least swelling at 723%. This indicates that the TCP amounts in Gel/HPMC blends do not significantly control the water uptake ability.

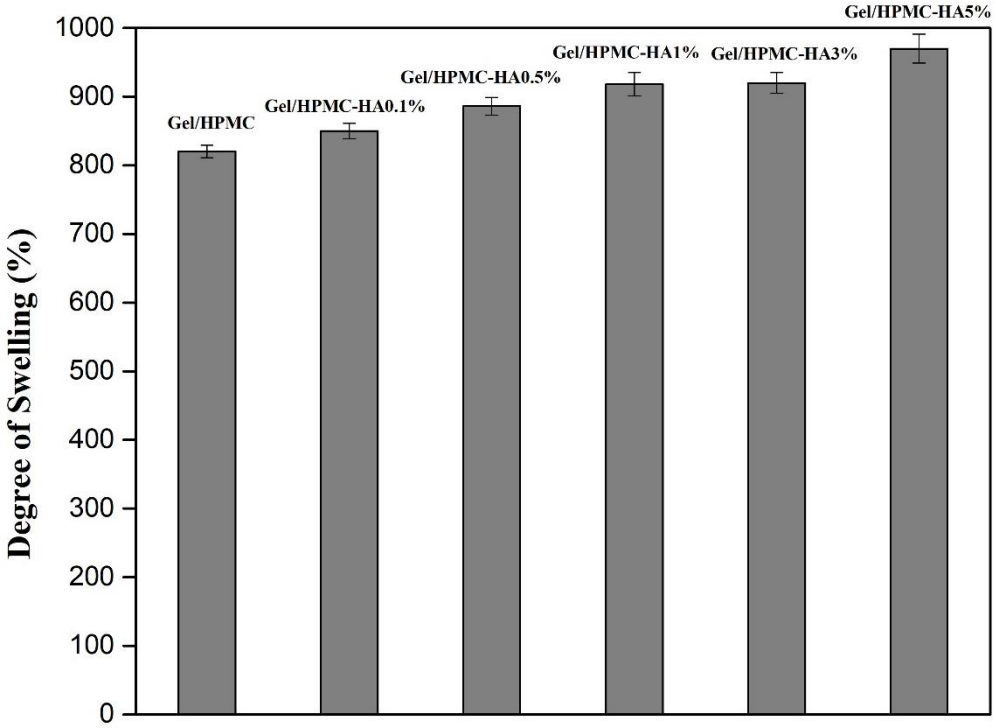

Figure 4.13: Tensile Strength of Gel/HPMC composites with HA as filler

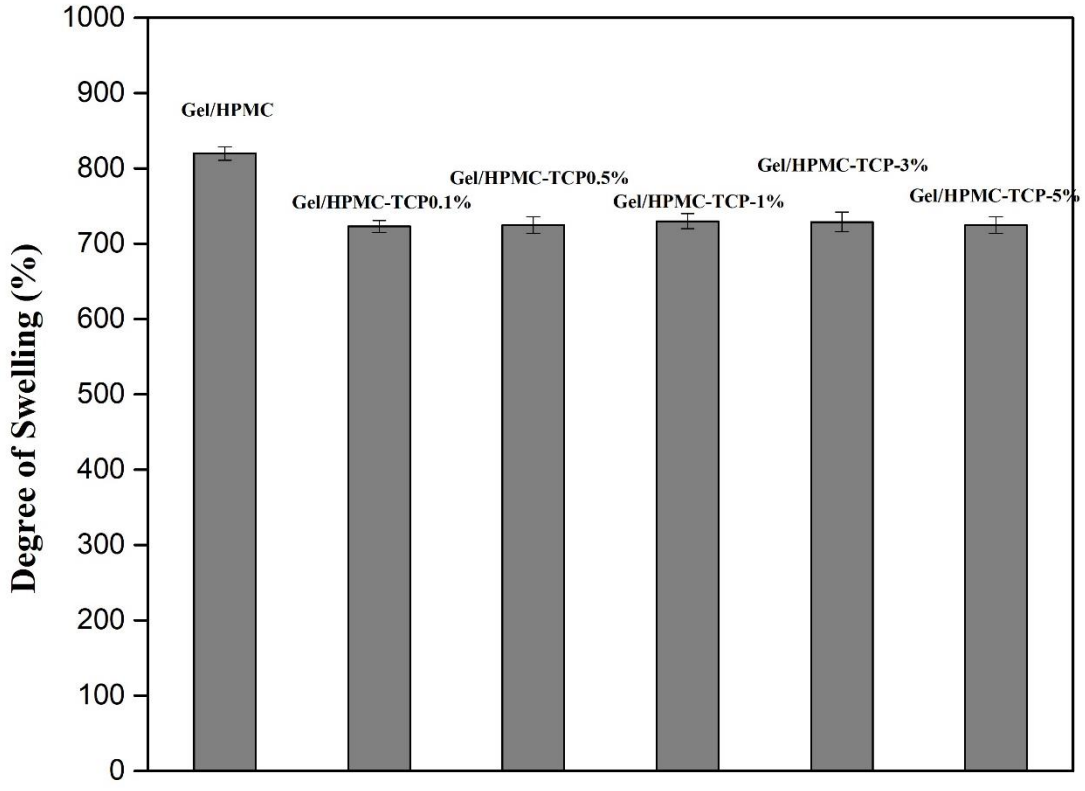

Figure 4.14: Tensile Strength of Gel/HPMC composites with TCP as filler

Table 5: Table showing values of swelling for all samples

Samples	**Degree of Swelling (% ± SD)**
Gel/HPMC	820 ± 9
Gel/HPMC-HA0.1%	850 ± 11
Gel/HPMC-HA0.5%	886 ± 13
Gel/HPMC-HA1%	918 ± 17
Gel/HPMC-HA3%	920 ± 15
Gel/HPMC-HA5%	970 ± 21
Gel/HPMC-TCP0.1%	723 ± 8
Gel/HPMC-TCP0.5%	725 ± 11
Gel/HPMC-TCP1%	730 ± 10
Gel/HPMC-TCP3%	729 ± 13
Gel/HPMC-TCP5%	725 ± 11

4.7 Contact Angle Measurements

For cell adhesion, surface hydrophilicity plays an important role. Pure membrane shows the contact angle value at 82.65 ± 0.45. This indicates that the Gel/HPMC film is hydrophilic in nature. HPMC is more hydrophilic as compared to gelatin because polar groups in HPMC are more representative than in gelatin [30].

Results shows that the increase in HA content in Gel/HPMC matrix, initially for 0.1%, 0.3% and 1%, the contact angle decreases linearly. This is due to the hydrophilic nature of HA. But at higher HA contents, for 3% and 5%, the contact angles were increased to 79.81 ± 0.50 and 85.15 ± 0.15, respectively. This increase in contact angle or decrease in relative hydrophilicity can be attributed to the fact that more rough the surface is, greater will be the contact angle [60]. Thus, for HA 3% and 5%, the heterogeneity of the surfaces played their role in increasing contact angle.

For TCP, the random trend was observed in the contact angle values. This means the hydrophilicity in case of TCP samples, is not significantly driven by the TCP. Gel/HPMC-TCP1% is more hydrophilic with least contact angle value of 77.85% whereas Gel/HPMC-TCP3% shows maximum value and is considered as least hydrophilic among all TCP samples.

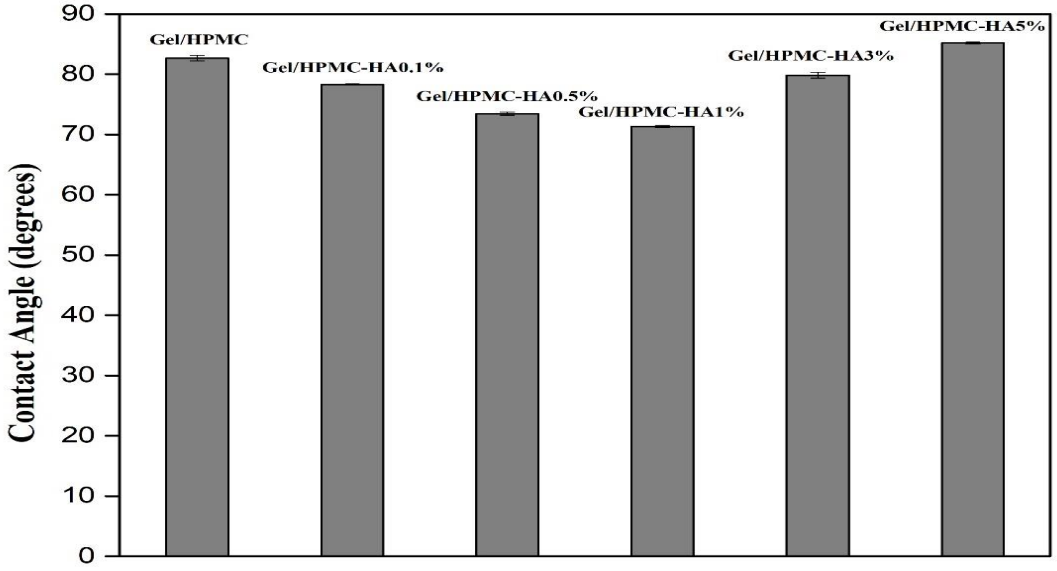

Figure 4.15: Contact Angle Values for Gel/HPMC composites with HA as filler

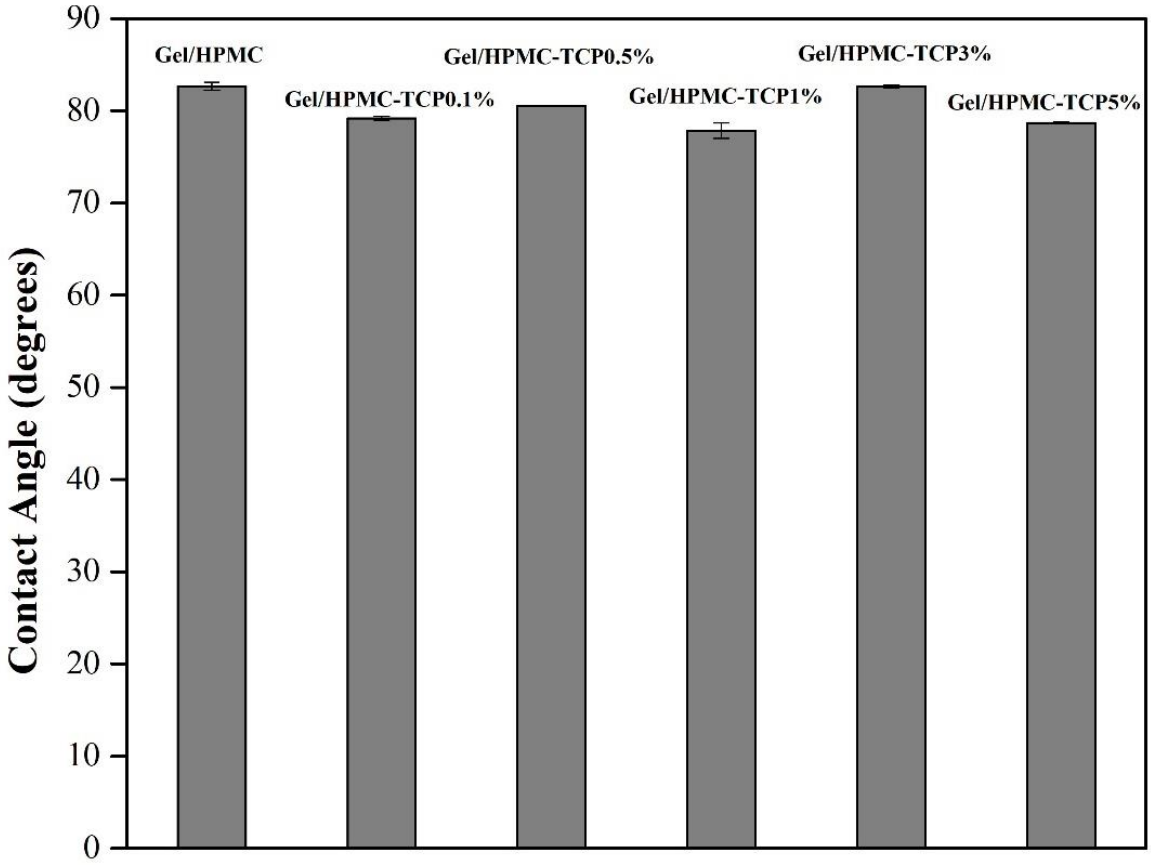

Figure 4.16: Contact Angle Values for Gel/HPMC composites with TCP as filler

Table 6: Table showing values of contact angle for all samples

Samples	**Contact Angle (Degrees ± SD)**
Gel/HPMC	82.65 ± 0.45
Gel/HPMC-HA0.1%	78.3 ± 0.1
Gel/HPMC-HA0.5%	73.45 ± 0.25
Gel/HPMC-HA1%	71.3 ± 0.2
Gel/HPMC-HA3%	79.8 ± 0.5
Gel/HPMC-HA5%	85.15 ± 0.15
Gel/HPMC-TCP0.1%	79.2 ± 0.2
Gel/HPMC-TCP0.5%	80.55 ± 0.05
Gel/HPMC-TCP1%	77.85 ± 0.85
Gel/HPMC-TCP3%	82.65 ± 0.15
Gel/HPMC-TCP5%	78.7 ± 0.1

4.8 Optical Profilometry

The pure film shows the average arithmetic roughness ($\boldsymbol{R_a}$) value of 0.0406μm. With the increase in both the HA and TCP content, the $\boldsymbol{R_a}$ value increases. In case of samples with HA, the maximum roughness is shown by Gel/HPMC-HA5% i.e., 0.791 μm.

For TCP, the maximum roughness was shown to be 1.15 μm by the Gel/HPMC-TCP5%.

These results are in accordance with the SEM results, where higher concentrations from both the fillers exhibits maximum roughness.

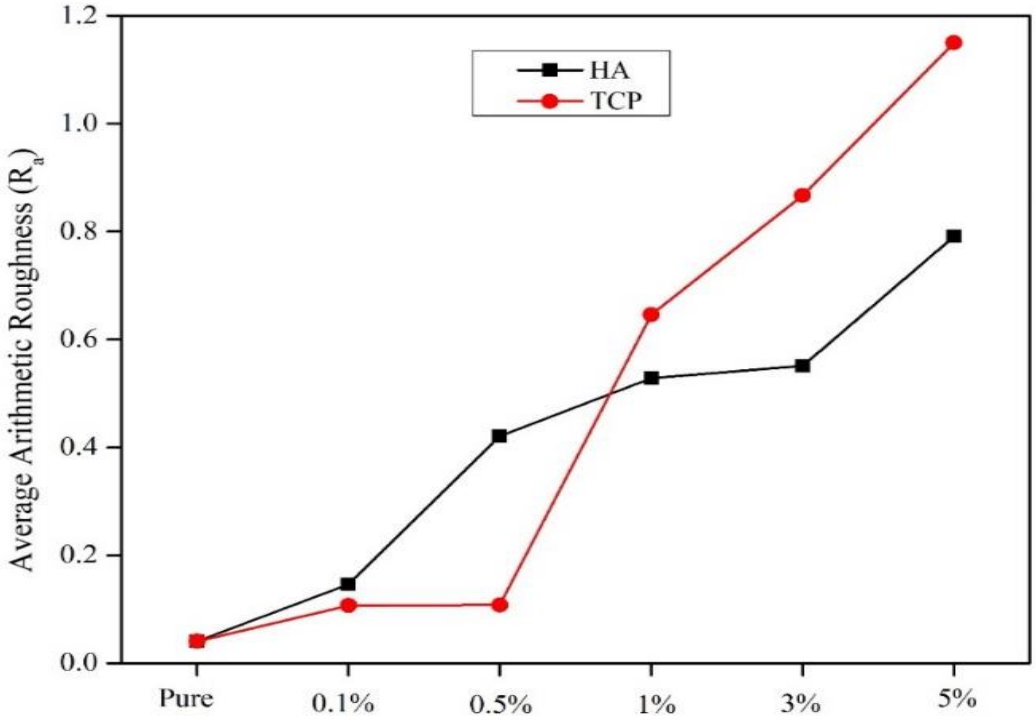

Figure 4.17: Roughness graph for all samples

Table 7: Average Roughness Values for all samples

Samples	**Average Roughness (µm)**
Gel/HPMC	0.046
Gel/HPMC-HA0.1%	0.146
Gel/HPMC-HA0.5%	0.421
Gel/HPMC-HA1%	0.528
Gel/HPMC-HA3%	0.551
Gel/HPMC-HA5%	0.791
Gel/HPMC-TCP0.1%	0.107
Gel/HPMC-TCP0.5%	0.108
Gel/HPMC-TCP1%	0.646
Gel/HPMC-TCP3%	0.867
Gel/HPMC-TCP5%	1.15

Conclusion

Hydroxyapatite was successfully synthesized using wet chemical precipitation method. Series of samples containing Gelatin/HPMC matrix, with varying ratios of HA and TCP as fillers, from 0.1% to 0.5% each, were successfully synthesized using solution casting method. The synthesized membranes were studied using various characterization techniques. XRD analysis confirms the formation of network for Gel/HPMC-HA and Gel/HPMC-TCP composites with the characteristic peaks appearing for all the components in the matrix. For Gelatin and HPMC, the broad peak was observed confirming the amorphous nature of both the polymers and sharp peaks for HA were observed confirming its crystalline nature. FTIR results shows the presence of desired functional group because of blending of polymers. SEM images depicts the presence of both the fillers i.e., HA and TCP, on the surface of the Gel/HPMC membranes. Mechanical Testing results reveals the increase in tensile strength with the increasing filler content for both HA and TCP. For Gel/HPMC-HA0.1%, the tensile strength was 76.32 MPa and it increases to 110.22 MPa for Gel/HPMC-HA5%. Similarly for Gel/HPMC-TCP0.1%, the tensile strength was observed to be 82.59 MPa and it increases to 108.41 MPa for Gel/HPMC-TCP5%.

The thermal degradation curves were studied by performing TGA analysis. TGA curves indicates the weight loss of approximately 94%, in three stages, for all the gelatin containing membranes. Swelling of all the samples were performed in PBS solution with pH= 7.4 and it reveals the maximum swelling of Gel/HPMC-HA5% to be 970%. The contact angle measurements showed that the Gel/HPMC membranes with HA as filler are hydrophilic and their contact angle range was from 71.3 ± 0.2 to 85.15 ± 0.15. Similarly for Gel/HPMC membranes with TCP as filler has contact angle range from 77.85 ± 0.85 to 82.65 ± 0.15. These contact angle ranges for both the fillers indicates that the samples with both the fillers are hydrophilic. The optical profilometry results indicates that roughness increases linearly with the increase in filler contents for both HA and TCP. Under the light of these properties, we can conclude that Gel/HPMC membranes, with HA and TCP as fillers, has potential application in tissue regeneration.

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