DEPARTMENT OF APPLIED CHEMISTRY

DELHI TECHNOLOGICAL UNIVERSITY, DELHI-42

CERTIFICATE

This is to certify that the dissertation entitled **MECHANICAL STUDIES ON NANO BIOACTIVE GLASS EMBEDDED GELATIN-PECTIN NANOFIBERS** is being submitted in partial fulfillment for the award of the degree of Master of Technology, Delhi Technological University, is a record of an authentic work done by Pragya Rai (2K15/PTE/07) under my supervision and guidance.

Dr. Deenan Santhiya
Assistant Professor

Dr. Archana Rani
Head of Department
Department of Applied Chemistry

Declaration

I hereby declare that all the information in this document has been obtained and presented in accordance with academic rules and ethical conduct. This thesis is my own, unaided work. I have fully cited and referenced all material and results that are not original to this work. It is being submitted for the degree of Master of Technology in Engineering at Delhi Technological University. It has not been submitted before for any degree or examination in any other university.

Pragya Rai

2K15/PTE/07

Polymer Technology

Department of Applied Chemistry

Acknowledgement

I would like to express my sincere gratitude to my Head of Department Dr.Archana Rani for her support and motivation. I would like to thank my project supervisor, Asst. Prof. Dr. Deenan Santhiya, for inviting me to work in her laboratory and her aspiring guidance, motivation and support help me throughout the extent of the project.

I would like to thank PhD scholar Ms. Nidhi Gupta and Mr. Chandramohan Srivastava for her precious suggestions, support and technical help during the course of this project.

Last but not the least, I want to thank my parents, for inculcating ethos, as a result of which I am able to do my post graduation from such an esteemed university. I would like to thank my friends for believing in my abilities and for always showering their invaluable support and constant encouragement.

Pragya Rai

2K15/PTE/07

Polymer Technology

Department of Applied Chemistry

Table of Contents

Table of Figures

List of Abbreviations

HA – Hydroxyapatite Layer

ECM – Extracellular Matrix

GTA – Glutaraldehyde

ESP – Electrospinning

PBS – Phosphate Buffered Saline

BAG – Bioactive glass

SEM - Scanning Electron Microscopy

FTIR – Fourier Transform Infrared Spectroscopy

TGA – Thermogravimteric Analysis

UTM – Universal Testing Machine

UV - Ultraviolet

ABSTRACT

Ultrafine fibers and fibrous mats are produced by electrospinning of aqueous solution of Gelatin and Pectin. The process used in this method avoids the use of synthetic polymer or non aqueous solution for making the fibers by using the natural polymers. Pectin is polyelectrolyte in nature that's why the electrospinning of pectin was not possible. Earlier the blends of pectin and poly (ethyleneoxide) (PEO) were for electrospinning for making ultrafine pectin fibers. The main aim of my investigation is to search for the alternatives of the PEO for electrospinning of proteins and polysaccharides. On performing electrospinning by using only pectin electro spraying occurs, no fiber formation takes place. So there is need of some carrier polymers so that jet formation of pectin starts. So in the process of making the pectin fibers gelatin with formic acid is used as a carrier polymer. Then bioactive glass solution is used in the aqueous solution of gelatin and pectin. The parameters of electrospinning are optimized so that formation of fibers takes place. When all the parameters are optimized then drug, vitamin-D precursor has been loaded and its release is performed in PBS. The various characterization of the fibers are done like SEM, FTIR, TGA, tensile testing for mechanical properties. The SEM results shows the fiber structure of pectin, gelatin, gelatin-pectin, gelatin-pectin-BG which shows that nanofibers are formed. The tensile strength increases when pectin is added and further increases when BG is added. FTIR shows the peaks of various functional groups present in the fiber.

CHAPTER 1

INTRODUCTION

Nanofibers are readily used in the fields of biomedical sciences as the nanometers range makes it able to form highly porous and surface area to volume ratio is also very high for this type of materials [1]. This thesis show the glimpse of the drug loaded nanofibers utility in bone healing. The electrospun fibers have many applications in different fields like in defense, environmental, biomedical fields, energy storage etc. In Nanofibers the porous structure of the fibers makes it more versatile. The electrospinning is the method for making good quality fibers ranging from nm to μm range. This method is very easy to handle because there is need to feed only proper parameters of spinning which must be optimized. Any polymers which are soluble in some kind of solvent, so that polymeric solution can be made, are used for electrospinning. Nowadays the biomedical fields are moving towards the use of natural polymers as the use of synthetic polymers for clinical purpose is of no use. So the main focus is towards the use of biodegradable and biocompatible

polymers in biomedical science. This thesis focuses on the use of only natural polymers which is to be used for bone healing. So the various applications of Nanofibers are shown below.

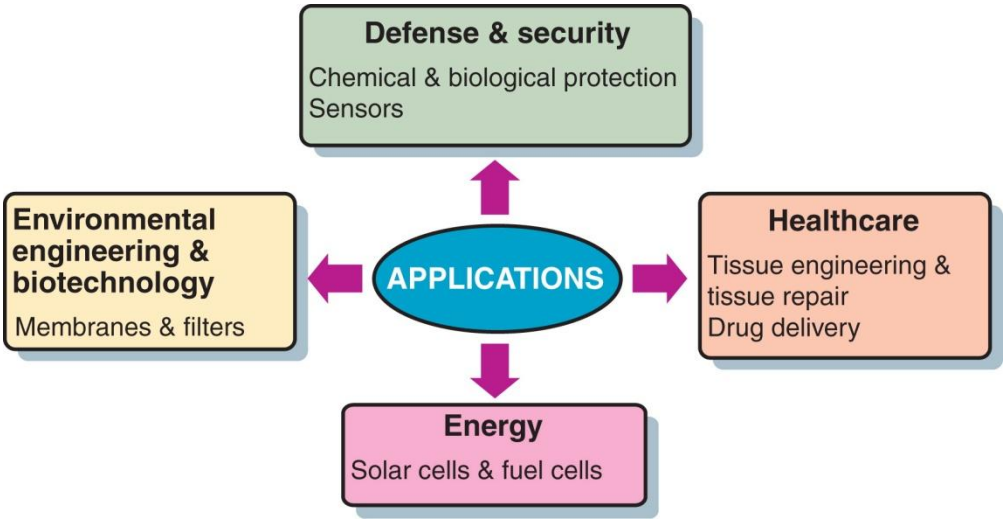


Figure 1 Nanofibers applications [1]

The synthetic polymers used for bone healing don't interact in an active manner so this material cannot aid in the healing process around the implant site. Only natural polymer fibers do not possess higher strength to be used in bone healing so some kind of biomaterials need to be used. A biomaterials is inert substance used for implantation within living system so that it support living tissue functioning.

The Bioactive materials are the important materials for the production of the synthetic scaffold. Bioglass is a biomaterial consists of ceramic having network of amorphous silica having phosphate groups and calcium oxide attached [2]. Bioglass is a very good and remarkable material as it can be used in biomedical sciences because it has bone healing capability. Bioglass has ability to form apatite layer (bone inducing biomaterial) but for the formation of this layer there is requirement for the simulated body fluid, bioglass can support cell adhesion, support growth, induce the formation of bone.

Natural polymers are used instead of synthetic polymer in this work due to its good compatibility and biodegradability. Gelatin and pectin are two famous natural polymers which are used in this work. As the availability of these polymers is plenty and their degradation is also easy that's why they are used. Natural polymers molecular weight is high and because of its molecular structure there is some problem in electrospinning of nanofibers. The choice of solvent for gelatin is very important at it is not stable below 30^0C.

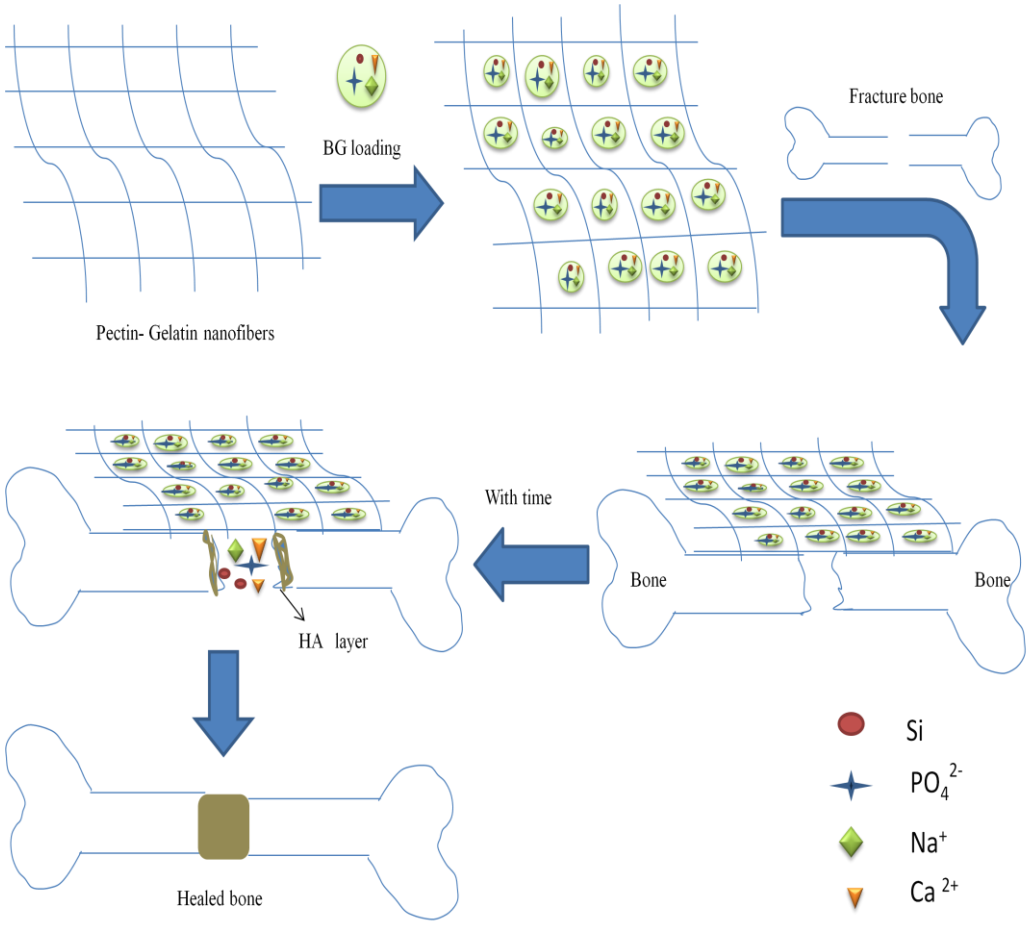


Figure 2 Mechanism of bone healing by using BAG fibers

The use of pectin in the formation of the Nanofibers is beneficial in medical field. The pectin is hydrophilic in nature so it easily dissolves in water. Nanofibers

produced from pectin behave like extracellular matrix (ECM) in structure, size, composition, and mechanical properties due to which it promotes cell proliferation, communication, and behavior. The pectin does not form the fibers so gelatin has been used for making the fibers of pectin. For bone healing the blend of gelatin, pectin, and bioactive glass is used and this solution is then electrospun to make nanofibers. Further work has been done with this fibers which is drug loading in the fibers and releasing of drug is performed. The releasing behavior of drug is determined by UV spectrophotometer. For bone strengthening the use of Vitamin D is necessary which is used in the blend of gelatin, pectin, and bioglass. The vitamin D is used in the form of precursor i.e. 7-dehydrocholestrol. The Nanofibers made will be used in the bone healing (in case of small fracture like hairline frature). This thesis contains the results of Nanofibers like SEM, FTIR, TGA, Tensile Testing, drug release.

CHAPTER 2

LITERATURE REVIEW

The most complex heterogeneous polysaccharides in nature is pectin which is composed of (98%) linear chain of (1, 4)-linked-d-galacturonic acid. Pectin is considered as a good gelling in the industries of processing and food technology. The pectin has very limited application just because of the inconsistencies in chemical structure because of the extraction method of the pectin. There are many applications of the pectin which is of non food type like in making hydrogels, films/coatings for drug release in controlled manner. There are many applications of the pectin some of them are non toxicity that's why it is used in food grade applications, abundance, cheaper, biodegradable in nature easily soluble in water, bioadhesive property, pectin has a reactive or /and charged functional groups. Pectin is very interesting and attractive natural materials which can be used in many fields like biomedical, pharmaceutical, electrochemical device applications

2.1) Pectin

Pectin is basically found in the plant which is used to provide shape and structural support and the pectin exists in between the terrestrial plant cells primary cell wall. Pectin is one of the major constituents of citrus by products and has good gelling properties [3]. The polysaccharides found naturally in plant are composed of D-galactoronic acid units connected through α-(1, 4) glycosidic linkage [4, 5]. The hydrophilic nature of this complex is provided by carboxyl and hydroxyl side chains. The hydrogel is produced in which the pectin molecule is crosslinked with each other. This process takes place in presence of calcium ions (Ca^{2+}) or oligochitosan. On comparison with synthetic polymers, pectin is more biocompatible and biodegradable. Nanofibers produced from pectin behave like extracellular matrix (ECM) in structure, size, composition, and mechanical properties due to which it promotes cell proliferation, communication, and behavior [6]. Pectin was first described in 1825. The color of the pectin is white to brown and it is extracted from citrus fruits, and it can be used in fruit jam as a gelling agent. It can be used as dietary fiber source and a stabilizer fruit juice

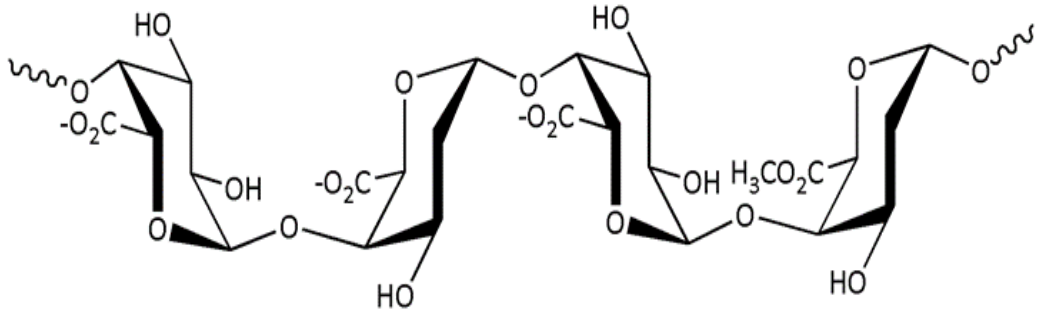


Figure 3 Structure of Pectin

RAW MATERIALS:-

The sources of the pectin are apple pomace; refuse from vinegar mill, Citrus peels, beet pulp, and carrots. The commercial sources of pectin which can be used in making products are citrus fruits or apple [8].

Table 1. percentage of pectin in various raw materials

MATERIALS	PECTIN IN FRESH MATERIALS PERCENT
Apple Pomace	1.5 to 2.5
Lemon Pulp	2.5 to 4.0
Orange	3.5 to 5.5
Beet Pulp	1.0
Carrots	0.62

2.2) Gelatin

The famous protein from animals is gelatin. Gelatin can be used in many applications like in weight loss and in many more applications in medical field related to bones like in osteoarthritis, rheumatoid arthritis, and brittle bones. Gelatin can be used for strengthening purpose in the body parts like in bones, joints, fingernails. In the field of manufacturing, gelatin can be used in preparation of cosmetics, foods, medicines. The biodegradability and biocompatibility of the natural polymers is better than the synthetic polymer that is why it is used in biomedical applications. Natural polymers molecular weight is high and because of its molecular structure there is some problem in electrospinning of nanofibers. The denatured form of collagen is gelatin [9, 10].

The collagen is the naturally occurring component which is the most abundant form of protein family in the body which can be used in the tissue engineering. Collagen mainly found in the extra cellular matrix [11-13]. The process of obtaining pure collagen is very costly because of the complicated manufacturing process. So the denatured form of the collagen that is gelatin is used as its cost is very less as compared to the collagen manufacturing process.

Gelatin is less expensive as well as biocompatible but it forms a gel below 25^0C from aqueous solution. Due to this limitations of gelatin the applications and use of gelatin reduces. The aqueous solution of gelatin viscosity deceases in a very short time. So the stability of gelatin solution becomes important for electrospinning of nanofibers below 30^0C. The selection of solvents for the electrospinning of gelatin plays a vital role in electrospinning. The solvent which is used for the electrospinning of gelatin should not be toxic that's why formic acid is used as solvent for dissolving the gelatin. The electrospun nanofibers structure of gelatin is readily soluble in water. This property also reduces the applications of gelatin. The main problem of gelatin is cross linking which can be achieved by Glutaraldehyde [14]. With the help of this Glutaraldehyde the gelatin nanofibers are cross linked and exhibit lower water sorption capability. The main advantages of using GTA are higher thermal stability and young's modulus. The gelatin is dissolved in the formic acid as it is volatile in nature so easily evaporate during electrospinning.

Structural unit :-

Gelatin has many glycine, proline and 4-hydroxyproline residues. The structure of gelatin can be defined as heterogeneous mixture of multistranded polypeptides.

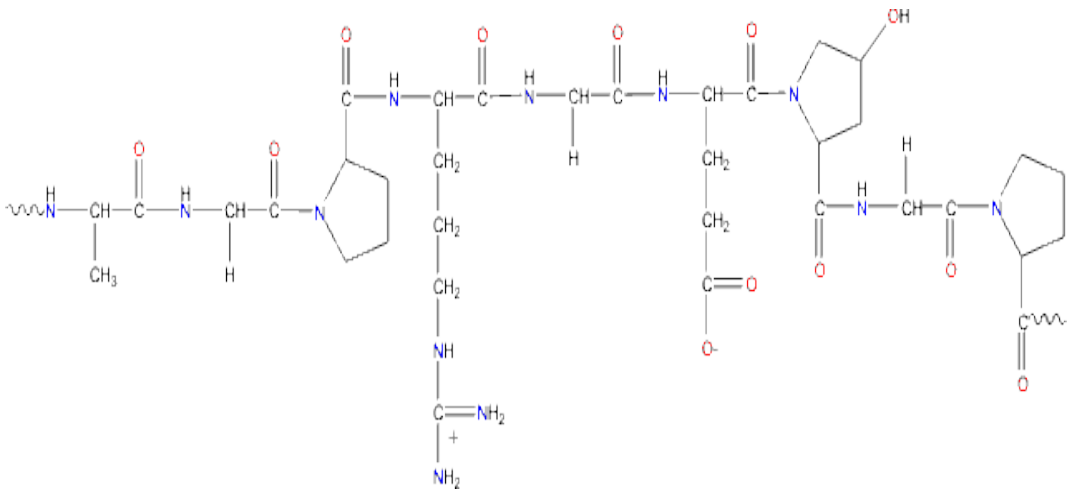


Figure 4 Structure of Gelatin

2.3) Bioactive glass

There are many diseases which affects the different areas and parts of bone. The ordinary function of the bone is affected by these diseases and can results in weakening of the bone which results the trauma during ordinary function reconstructive surgery need arises, and bone replacement may also be performed [15, 16]. The very latest engineering known as tissue engineering is used for these entire problem this engineering can be used as a solution to the current therapies. The important function of the tissue engineering is to design a synthetic scaffold, creation of biocompatible scaffold structure that mimic the extra cellular matrix functions. The extracellular matrix support the cell growth and helps in segregating different tissue types and also cells gets nutrients as it transport the nutrients.

The Bioactive materials are the important materials for the production of the synthetic scaffold. Bioglass is a biomaterial consists of ceramic having network of amorphous silica having phosphate groups and calcium oxide attached. Bioglass is a very good and remarkable material as it can be used in biomedical sciences because it has bone healing capability [17]. Bioactive glass has ability to form apatite layer (bone inducing biomaterial) but for the formation of this layer there is requirement for the simulated body fluid, bioglass can support cell adhesion, support growth, induce the formation of bone [18]. In the past years, one of the most exploited and most used materials in the biomedical science is Bioactive glass, where the plenty of research is going on in order to increase the area of application and variety of research papers are published and publications are increasing by leaps and bound. Bioactive glass is a material which has capability which induces biological activity with the help of surface reactions. When this material is implanted into the body, it starts the formation of HA-like layer which forms the bond between hard and soft tissues [19]. The ability of the material to form the bond which is strong in nature shows that it is biocompatible. There are two techniques for the formation of the bioactive glass, melt quench and sol gel techniques.

Bioactive glass is biomaterials whose morphological and structural properties decide the performances and applications of the BAGs. There exists large nano porosities in the bioactive glass and the deposition of HA layer takes place due to their high surface area. BAGs have 3D structure which is interconnected and form macro porous network is favorable material for bone tissue engineering scaffold.

Bioactive glasses are the material which can be used for the bone regeneration because it exhibits strong interfacial bonding and its biocompatibility. Bioactivity is the good quality of bioactive glass leads to the formation of hydroxyapatite layer which is very similar to the mineral part of bone. Bonding between the BAG and bone depends upon the rate of formation of the hydroxyapatite layer. There are several multiple reactions taking place in a sequence between the fluids and tissue present in the body, the implanted material. The three step mechanism for the formation of Hydroxyapatite Layer when incorporated bioactive glass comes in contact with physiologic fluids: ion exchange, dissolution, and precipitation mentioned in *Hench et al* [20-22]. On the surface of bioactive glass ion exchange takes place: H^+ ions present in the solution and Na^+ and Ca^{2+} present in the bioglass exchange ions with the ions present in the surrounding solution. By the action of hydroxyl ions (OH^-) the breaking of Si-O-Si bonds takes place which is the reason for network dissolution. On the surface of the glass there is formation of hydrated silica (Si-OH) and this hydrated silica with the neighboring silanol undergoes rearrangement by polycondensation, which forms the gel layer which is silica rich. A Ca^{2+} and PO_4^{2-} ion which is released from glass the precipitation takes place.

Nowadays there is very high demand in the field of clinical for the purpose of scaffolds in bone tissue repairing. For this purpose the bioactive glass is preferred because of its controllable degradation and its bioactivity. Bioglass material form strong bond with the bone by forming the hydroxyapatite layer. Bioactive glass consists of silicate network in which Ca^{2+} and PO_4^{2-} is incorporated which shows the enhanced bioactivity due to the presence of nano porosity and relatively loose silicate network structure. In the fabrication of scaffolds care should be taken such that it resembles or mimics the extracellular matrix. The extracellular matrix

structure is fibrous in nature and composed of proteins. For making the same kind of structure there is one method known as electrospinning techniques which can be used for generating the fibers which is similar to the structure of native extracellular matrix. Bioactive glass is electrospun in non woven nanofibers which have large surface area and interconnected structure are formed which is 3D in structure. The advantages of large specific area are rapid release of ions and absorb a large amount of protein, due to which its applications in the field of biomedical sciences increases.

In my work the electrospinning techniques is used for the preparation of the nanofiber of BAGs. These BAGs have two main advantages. One is the high specific surface areas of the nanofibers because of the ultrathin diameter. The Nanofibers can be arranged into the 3D membranes with an interconnected network which is macroporous because of its super long length. This is the reason that it shows the excellent scaffold for the bone repairs. The optimal structure of the BAGs fibers are not formed due to the lack of nano porosity.

2.4) 7-dehydrocholestrol

Human requirements for the need of vitamin D can be fulfilled by the exposure to sunlight. Vitamin D can regulate the calcium homeostasis in the body and can help in mineralization of bone in order to avoid problem of rickets in children and osteomalacia in adults. 7-dehydrocholestrol in the skin absorbs solar UV B photons, which leads to the transformation of vitamin D precursor to vitamin D_3 [23]. Children and adults suffer from the deficiency of vitamin D. Due to the lack of vitamin D in adult osteomalacia disease is caused. There are many more diseases which occurs due to the lack of vitamin D like cancers, type I diabetes, arthritis. Sun exposure for 5-10 min for 2-3 times per week and increase the supplement of vitamin D leads to the sufficiency.

Vitamin D is known as sunshine vitamin. Phytoplankton and zooplankton present in the ocean when exposed to sunlight produces vitamin D. The need for vitamin D is not well known during that time >500 million years ago. Vitamin D_3 is known

as cholecalciferol. This vitamin is not soluble in water it is soluble in fat which is necessary in promoting normal growth of bone of animals and human beings. Vitamin D_3 is not active, but it can become important if hydroxylation is performed [25]. This hydroxylation occurs in the liver and form 25-hydroxyvitamin D_3, which helps in coordinating Ca and P homeostasis, and also help in healthy mineralization, growth of bone. Vitamin D_3 naturally exists in food and fish and on the exposure to ultraviolet light it produced on the skin vertebrates. Photo conversion process can be used for obtaining vitamin D_3. 7-dehydrocholestrol is the precursor of vitamin D_3 which can be photolyzed at wavelength (270-300 nm) by UV light, after this previtamin D_3 is produced and the conversion from previtamin D_3 to vitamin D_3 takes place spontaneously. Photoconversion process is neither pure nor crystalline Vitamin D_3. It is a mixture of many byproducts like tachysterol, lumisterol. So there is need to isolate the vitamin D_3 and purification need to be done.

It is to some degree unexpected that vitamin D, through a verifiable mischance, wound up plainly named a 'vitamin', attributable to the way that vitamin is expectedly characterized as 'fundamental thing required in the eating routine'. 'Vitamin D' is that eating regimen in essence is typically poor in vitamin D with the exception of cod or other fish oils or nourishment invigorated with this vitamin. Vitamin D is really a fat-dissolvable prohormone steroid that has endocrine, paracrine and autocrine capacities. The endocrine impacts of vitamin D are predominantly required in serum calcium homeostasis. Vitamin D and calcium are regularly utilized as a part of the same sentence since they work firmly together, vitamin D's essential part is to control the levels of calcium found in the circulation system by continually permitting calcium and phosphate assimilation from the digestive tract or taking calcium from bones. Moreover, vitamin D is empowering specialists that, when show in ideal focuses, has no detectable impact on calcium assimilation in its own particular right; be that as it may, it allows or encourages adaptable physiologic reaction to fluctuating calcium require. The paracrine and autocrine impacts of vitamin D rely on upon hereditary interpretation, novel to the kind of cell communicating atomic vitamin D receptors. These potential impacts

incorporate restraint of cell expansion, advancement of cell separation, what's more, apoptosis which may thusly have parts in disease, invulnerability, and numerous organ frameworks. The potential horde impacts of this vitamin in human well being and ailment have prompted a heightening enthusiasm for vitamin D deficiency and the best strategies to standardize imperfect levels. Sources of vitamin D [26-28] are

1. Sunlight

The most surely understood sources of vitamin D is by means of amalgamation in the skin prompted by sun presence. The principal reference to the physiological impact of daylight on vitamin D was delineated by the Greek history specialist Herodotus. He went by the war zone where Cambyses (525 BC) defeated the Egyptians, and investigated the skulls of killed Persians and Egyptians. He noticed that the Persian skulls were fragile to the point that they made back the initial investment when struck with a stone, though those of the Egyptians were solid furthermore, could barely be earned back the original investment when hit with a stone. The Egyptians' clarification to Herodotus was that they went bareheaded from youth presenting their heads to daylight, while Persians secured their heads with turbans shading them from the sun bringing about skull bone shortcoming. Afterward on, in the mid seventeenth century Francis Glisson, Professor of Physics at Cambridge University, in his treatise on rickets watched that the ailment was regular among babies and youthful offspring of nation ranchers who ate well, and whose weight control plans were known to incorporate eggs and spread, yet who lived in blustery, cloudy parts of the nation and who were kept inside amid long serious winters [29].

2. Vitamin D synthesis in the skin

According to the Commission Internationale de l'Eclairage *CIE. Action spectrum for the production of previtamin D3 in human skin. Technical report 174; 2006*, action spectrum describes the vitamin D effective radiation (i.e. vitamin D synthesis in skin at each wavelength) [30, 31]. The spectrum range lies between (255-330 nm) with maximum value at about 283nm. When the whole body is

exposed to UVB radiation for 15-20 min can produce up to 250µg vitamin D. Precursor of vitamin D (7-dehydrocholestrol) within the plasma membranes of each epidermal basal and suprabasal keratinocytes and dermal fibroblasts is converted to previtamin D_3 [32]. Vitamin D_3 which is synthesized is released from plasma membrane and this previtamin enters into systemetic circulation bound to vitamin D-binding protein.

3. Dietary sources and supplements

Two types of vitamin D is available one of it is ergocalciferol (vitamin D_2) and second one is cholecalciferol (Vitamin D_3). Sunlight exposure provide only the vitamin D_3 but the food supplements i.e. dietary sources provides both type of vitamin which are interchangeable [33-35]. Natural source of vitamin D includes cheese, egg yolks, salmon, tuna fish, cod liver oil, beef liver. To gain an adequate amount of vitamin D from natural dietary sources is not easy for individuals that's why many countries enhances the sources of vitamin D through foods like milk, orange juice, yogurt, and cereals with vitamin D.

2.5) Electrospinning

In electrospinning technique, electrostatic forces are used to produce fine fibers by using polymeric solution and the formed fibers have a diameter from nanometer to micrometer range and fibers have very high aspect ratio i.e. larger surface area which is higher than conventional spinning process [36]. In order to produce the fibers from this method DC voltage is required in the order of several tens of KVs. Currently two standards of electrospinning techniques are present, horizontal and vertical. This method of making the fibers are used more frequently but several research group have made many other system that can make more complex nano fibrous structure in an efficient and controlled manner. There are no specific requirements of temperature and atmosphere to perform electrospinning; it can be done at room temperature with atmospheric conditions. The set up of electrospinning is shown in the figure. Major components of electrospinning

system consist of a high voltage power supply, a spinneret, and a collecting plate. The high voltage is used to inject charge of certain polarity into a polymer solution and this solution move towards the oppositely charge collector plate. For dissolving polymers some kind of solvent is used so that after dissolving polymers it forms polymeric solution. This formed solution is then filled into the capillary tube for electrospinning. This process of making the fibers is conducted within the chamber because some polymers may emit harmful or unpleasant smells. Polymeric solution in capillary tube is held at its end in the form of drop because of surface tension of the liquid, the electric field is applied at the capillary tube due to which electric charge is induced on the liquid surface and jet will form only when the surface tension of the liquid is overcome by repulsive electrical forces. At the tip of the capillary tube a taylor cone is formed and a solution which is charged is ejected from this and the evaporation of solvents occurs in the space between the collector and capillary, the jet is not stable in between these two points. The stability of the jet is disturbed in between the space of capillary and collector plate as jet is stable only at the tip of the spinneret.

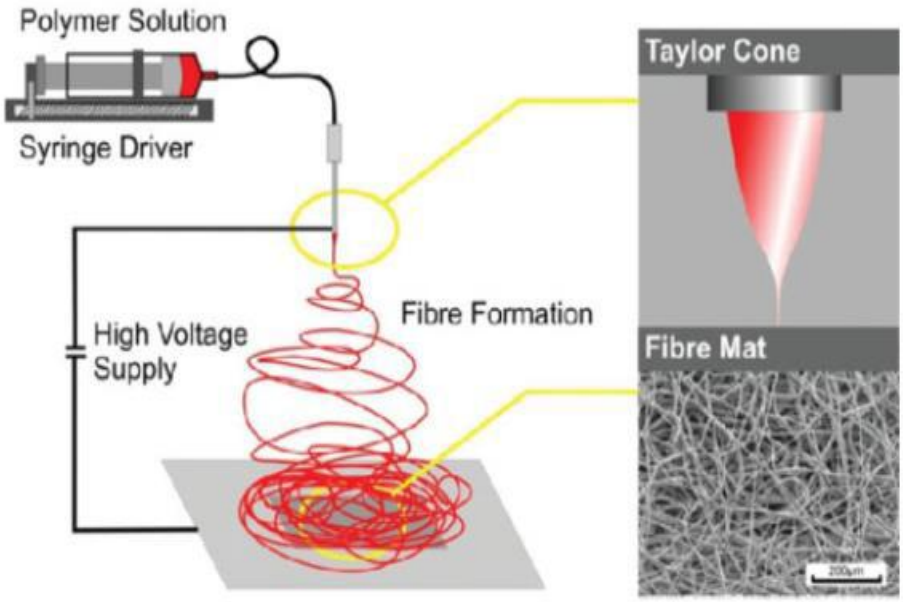


Figure 5 ESP set uo

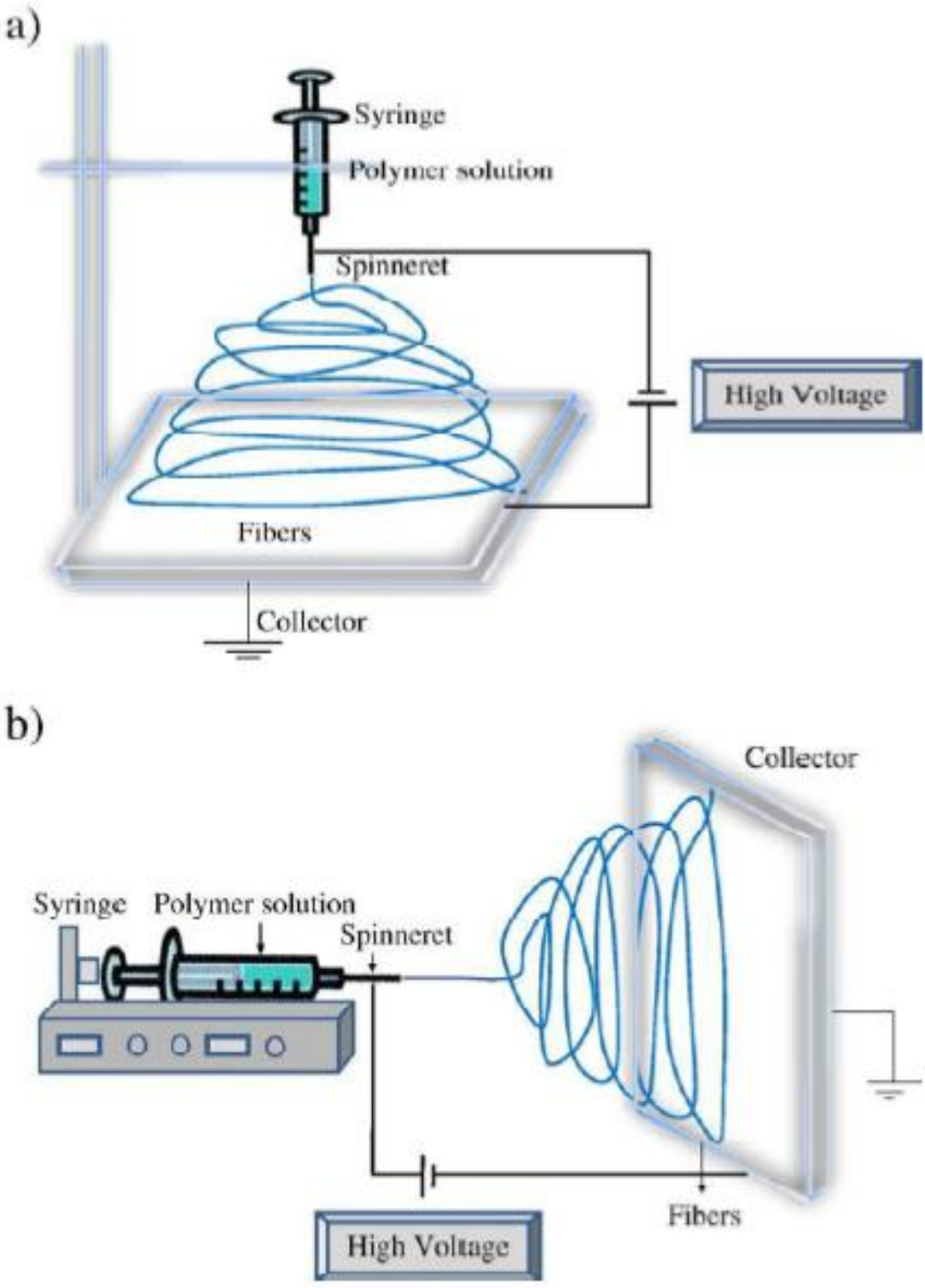


Figure 6 Set up of ESP

2.5.1)Electrospinnable polymeric materials

Based on the applications of the Nanofibers variety of the polymers is used in the electrospinning techniques in order to form the fibers in the nm to μm range. Nanofibers are formed by many polymers like natural polymers, synthetic polymers, or blends of natural and synthetic polymers including proteins, nucliec acid and polysaccharides.

2.5.1.1) Natural polymers

Nowadays variety of polymers are used for making the Nanofibers by electrospinning techniques from polymeric solution and this can be used in variety of field based on the applications like tissue engineering scaffolds, filtration membranes, and in biomedical fields. When compared with synthetic polymers naturally occurring polymers have good biocompatibility and immunogenicity when uSed in biomedical applications [38]. The ability of the natural polymers for binding cells is the main reason for using natural polymer for ESP as they carry specific protein sequences. Natural polymers used for scaffolds fabrication show better clinical functionality. The following are the natural polymers used in electrospinning.

2.5.1.1.1) Gelatin

It is a natural polymer produced by the controlled hydrolysis of the collagen which have variety of applications in the field of biomedical sciences because of its biocompatibility and biodegradability in physiological environment. Two types of gelatin exists: Type A and Type B this classification of the gelatin is based on the hydrolysis condition of isolation from collagen. The nature of the gelatin is polyelectrolyte due to which the fiber formation is not easy as it is coupled with its strong hydrogen bonding. Apart from this problem, one more problem exists in gelatin that is related with solubility, at lower temperature it form gel (i.e. at room temperature), so gelatin is not a good choice for tissue engineering applications. It

can be used only when cross linking is used with gelatin. The blends of gelatin with some other materials are used for electrospinning and then mechanical characterization has been done for tissue engineering scaffolds.

2.5.1.1.2) Collagen

This is the protein which is found in the body in abundant and can be extensively used in the field of tissue engineering. In extracellular matrix the main structural unit is type I and type III collagen. It is non immunogenic, highly conserved polymer to be used in tissue engineering applications. Tissue is structurally supported by the collagen and it also required for tissue maintenance and regeneration. This is the reason it is used for the tissue scaffolds as it is ideal material for it. The main issue associated with the electrospinning of the collagen is the choice of the solvents that dissolves the collagen with suitable concentrations and it should also be volatile enough so that rapid drying of mats takes place. This electrospun mats can be used for wound dressing and preliminary vascular tissue engineering as it closely mimics the native collagen network.

2.5.1.1.3) Silk Fibrion

From thousands of years silk obtained from silkworm (Bombyx mori and Antheraea mylitta) and spiders has been extensively used in the textile industries. Two types of silk proteins are found namely, hydrophobic fibroin and hydrophilic sericin. Silk fibroin is mainly used in biomedical field because of its various properties like biodegradability, biocompatibility, minimal inflammatory reaction and outstanding mechanical properties. Silk nanofibers are used in many fields like tissue engineering, wound dressing and also in drug delivery because of the good specific surface area and its strength, surface energy, thermal and electrical conductivity also increases.

2.5.1.1.4) Chitosan

This also comes under the category of the natural polymers which can be used in the field like biomedical and also in cosmetics. Its physiological properties make the chitosan very interesting materials like its solid state structure and dissolving state conformation.

2.5.1.1.5) Fibrinogen

This is another important polymer which naturally occurs can be used for the fabrication of the scaffolds to be used for tissue and also in haemostatic and wound dressing. Fibrinogen can be used for making the mats of fibers by electrospinning which can be used as scaffolds for tissue as its properties makes it suitable for scaffolds like biodegradability, non immunogenic and promote cell migration. The fibers thus formed have very high surface area and volume ratio available for clot formation, so this fibers mat can be used for wound dressing. The mats of fibers formed easily removed from the collector because it posses good surface integrity.

2.5.2) Effects of various parameters on electrospinning

The process of electrospinning is influenced by many parameters which affect the fibers formation ability of the polymeric solution are classified as solution parameters, process and ambient parameters. There are many parameters which is included in the solution parameters like the solution viscosity, molecular weight, conductivity, surface tension. Similarly process parameters have certain parameters like applied field, tip to collector distance, flow rate. Each of these parameters affects the fibers morphology, diameters and by the proper control of these parameters desired fibers diameters and morphology can be obtained [39].

2.5.2.1) Parameters of solution

2.5.2.1.1) Concentration

This parameter affects the fiber formation of polymeric solution so optimum solution concentration is required. There will be formation of beaded fibers if the concentration in the solution is very low but as the concentration increases the uniform fibers formed as the beads shape of the fibers changes from spherical shape to spindle like shape. So the optimization of the concentration is very important as low concentration leads to the formation of beaded fibers while the high concentration prohibit the formation of continuous fibers as the solution will not be able to flow continuously from the tip of the needle so leads to the formation of the larger fibers.

2.5.2.1.2) Molecular Weight

The properties related to the molecular weight are rheological and electrical properties which include the surface tension, viscosity, conductvity, and dielectric strength. This parameter is one of the important parameter which needs to be optimized as it affects the morphology of the fibers. In the electrospinning techniques high molecular weight polymer is used for making polymeric solution as high molecular weight provide the desired viscosity for the fiber generation. The beads formation occur when the molecular weight of the polymer is very low and the large average diameter fibers are formed when the molecular weight is very high. Entanglements of the polymeric chains are the direct reflection of the molecular weight and thus affect the viscosity. So in the electrospinning chain entanglements plays important role.

2.5.2.1.3) Viscosity

Fiber size and its morphology can be determined with help of viscosity of solution during the spinning. There should be continuous fibers formation; if the viscosity is low then this continuity breaks and if the viscosity is very high then it will be very

difficult for the polymeric solution to be ejected out of the needle, thus the proper optimization of the viscosity plays an important role in the fibers morphology.
There is range of viscosity for the polymeric solution of different polymers. After many research the maximum viscosity achieved is 1 to 215 poise. The three terms are interrelated to each other they are viscosity, concentration and molecular weight. The viscosity and the concentration of the solution are strongly related to each other. The uniformity in the fiber diameter is obtained when the viscosity of the solution or the concentration of the solution is increased. The range of concentration can be determined from the viscosity of the solution which helps in the continuous fibers formation in electrospinning. The other factor such as surface tension plays an important role in determining the surface morphology. Surface tension become dominant when the viscosity is very low so beaded fiber will form but there will be continuous fiber when the concentration is above a critical value.

2.5.2.1.4) Surface Tension

The surface tension also affects the fibers formation as it the function of solvent compositions of the solution. When the surface tension of the solution is low then there is formation of fibers without beads. There is variation in surface tension for different solvents. High surface tension is not preferred for electrospinning because it inhibits the electrospinning due to reason of instability of jets and as a result of this spraying of solution occurs. So the formation of fibers, beads, or droplets are directly related to the surface tension. At lower surface tension spinning of solution occurs even at very lower electric field.

2.5.2.1.2) Conductivity/Surface charge Density

This factor influences the electrospinning process and fiber morphology. Most of the polymers are conductive in nature and jet formation is accompanied by charged ions present in the polymeric solution. Type of polymer, solvent used, ionisable

salts availability determined the solution conductivity. The fibers diameter decreases with the increase in the conductivity where as the effect of the low conductivity is the insufficient elongation of a jet by electrical forces which produce the uniform fiber and some beads may also be present in it.

2.5.2.2) Processing Parameters

2.5.2.2.1) Applied voltage

This is the main parameter on which whole electrospinning technique is based. There is some threshold value of the voltage, after reaching to this value only charges on the solution is developed with electric field and initiation of fibers starts in electrospinning. The optimization of the voltage is essential in the process of spinning. Researchers have proved that the initiating drop shapes changes with the spinning conditions. Larger diameter fibers are obtained when the voltage is higher is reported by some researchers and some have reported that if the applied voltage is high then there will be narrowing of the fibers diameter due to increase in the electric field strength. The effect can be understand as, when the high voltage is applied the fiber diameter reduction takes place because of the greater columbic forces in the jet due to which the stretching of the solution takes place and the solvent evaporation also occurs. But higher voltage is avoided in the electrospinning technique because it leads to formation of the beads.

2.5.2.2.2) Rate of flow

The flow rate needs to be optimized in the electrospinning process because it affects the jet velocity and material transfer rate. Various experiments result shows that the solution flow rate should be less so that solvent gets enough time to evaporate. Evaporation of solution is necessary otherwise it will form beads. Higher flow rate will form bead as the solvent will not get enough time for

evaporation. Proper drying of the fibers is necessary before reaching to the collector plate so flow rate should be less.

2.5.2.2.3) Type of Collector

The type of collector to be used plays an important role in electrospinning. For the collection of Nanofibers to drum act as conductive substrate. Usually aluminum foil is used for collecting purpose but one problem associated with this type of collection is transferring the collected fibers. If there is requirement of the aligned fibers then other types of collectors are available namely conductive papers, conductive cloth, wire mesh, rotating rod or wheel. Comparison between the wire screen with aluminum and without aluminum can be made in the same conductive area and it was recognized that pure wire screen is better than other because of the ease of the transfer of the fibers to some other substrates. The fibers alignment is affected by the collector and its speed of rotation. The fibers are deposited on the rotating drum in a random manner because of the instability of the highly charged jet.

2.5.2.2.4) Tip to Collector distance

The diameter and fiber morphology also depends on the tip to collector distance. The distance between these two should be such that the solvent gets enough time to evaporate so that fibers collected on the drum is in dried state. If the distance is too far or too close then beads may be observed. When the distance between the tip and collector is close the shape of fiber is slightly flatter but when the distance is increased the fibers shape becomes round. Thus the optimization of the distance is very necessary so that proper evaporation of solvents takes place.

2.5.2.2.5) Electrospinning solvents

The solvent is the important and basic requirement of the electrospinning as the very first step is to make the polymeric solution by dissolving polymers into the solvents. There are certain properties of the solvents like it should be volatile enough to evaporate, vapor pressure, boiling point, etc. So the choice of solvents for electrospinning of polymer is very important parameter. Polymer solvent system intermolecular interaction is either attractive or repulsive totally depends on the type of solvents.

2.6) Past Problem

Important role of bone is the proper functioning of the human body, proper movement. It also helps in the internal regulation so that stable working condition of the body is maintained [40]. Bone plays a crucial role in providing the sufficient amount of Ca^{2+} and PO_4^{2-} for the cells and also maintains the calcium level in blood vessel. With the increase in age the mechanical strength of the bone reduces and the ability of the osteoblasts to repair and produce new bone decreases so the new bone formation do not takes place easily. So the reason for suffering more and more bone injuries in old age prevails [41].

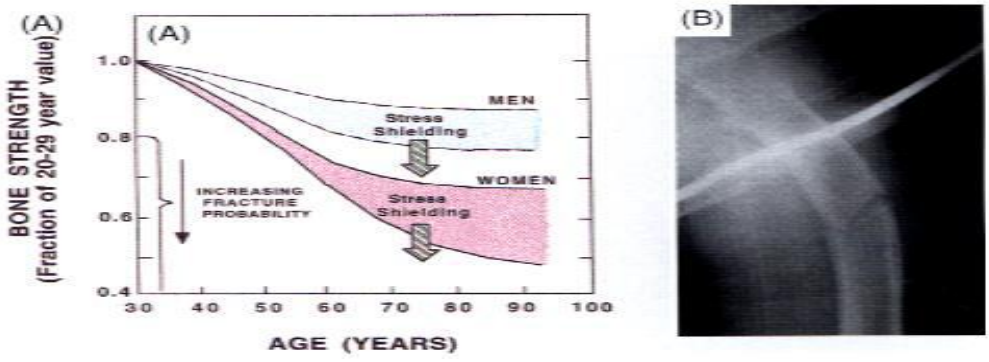


Figure 7 A) Effect of age on the strength of bone (B) Fractured femoral (thigh) bone caused by osteoporosis [41]

Impact of osteoarthritis on health persists all over the world. It is mostly common in knee joint due to the reason that bone protective layer wears out early in this portion as in this area shear and impact forces are dominant. The problem of bone

wear can be solved by tissue engineering which helps in restoration of tissues. This field reduces the need of transplantation of new organ as it will leads to the development of new drugs that abolish the need for bone replacement. When surgery for this problem is done then it will need the proper time for recovery and process of operation will be followed. The time consumption will be high which can be reduced by scaffolds preparation which imitates the structure of ECM chemically as well as physically.

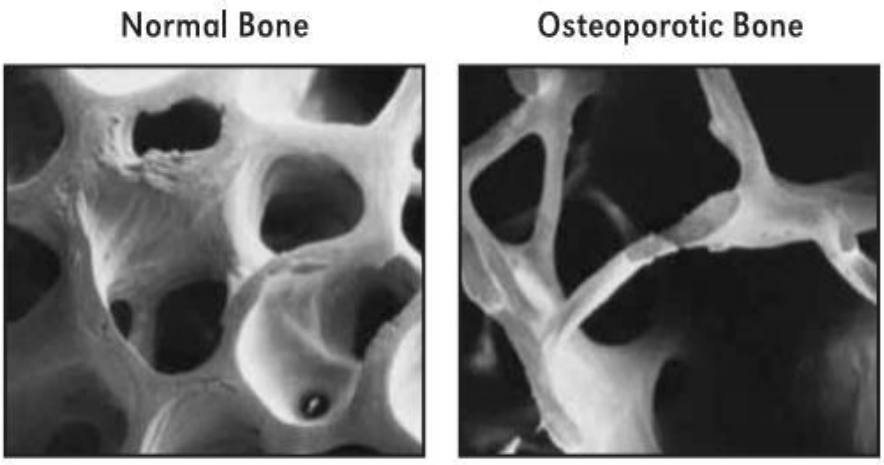


Figure 8 SEM images from biopies of a normal and an osteoporotic bone [41].

The ECM is an agglomerate of molecules secreted by cells that together form a structure that provides strength, resistance to tension, cell movement, integrity and support to surrounding cells. The main components of the ECM are collagen proteoglycans, laminins and fibronectin. There are certain properties which must be present in materials like non-toxicity, non inflammatory, good mechanical strength, biocompatible, biodegradable.

CHAPTER 3

EXPERIMENTAL

3.1) Materials

3.1.(a) For Gelatin Pectin fibers

The reagents used for the preparation of Nanofibers are Gelatin is taken 25 weight percent, purchased from Loba Chemie Pvt Ltd, pectin 0.08 gm from Central Drug House (P) Ltd Vardhan House Daryaganj New Delhi. These two materials are dissolved in the formic acid (2.5 ml) and milli Q water (17.5 ml) solution of 20ml. Formic acid is procured from Central Drug House (P) Ltd. This is the materials used for making the gelatin pectin fibers.

3.1.(b) For Gelatin-Pectin-Bioactive glass fibers

The Bioactive Glass fibers are prepared by using the bioglass precursors namely 9.29 gm Tetraethyl orthosilicate (TEOS) from Sigma Aldrich Inc USA, 1 gm Triethyl Phosphate (TEP) from Sigma Aldrich Inc USA, 6.26 gm Sodium acetate from Sigma Aldrich Inc USA, 4.21 gm Calcium Acetate from Sigma Aldrich Inc USA. These are the materials used for the preparation of bioactive glass Nanofibers.

3.1.(c) For Gelatin-Pectin-Bioactive glass drug loaded fibers

The next step is the loading of drug in the fibers. The drug used is precursor of vitamin D i.e. 7- dehyrocholestrol purchased from Sigma Aldrich Inc USA. The quantity of the precursor for loading is 0.5 mg/ml. The vitamin D precursor is not water soluble so for this purpose N,N-Dimthyl Formamide from High Purity Laboratory Chemicals Pvt Ltd Mumbai and Ethanol from Changshu Yangyuan Chemical china are used.

3.1.(d) For Buffer solution

Milli Q water 160 ml is to be used, 1.6 gm sodium chloride from Fisher Scientific, 0.04 gm of Potassium Chloride from Fisher Scientific, 0.288 gm of di-Potassium Hydrogen Orthophosphate from Fisher Scientific, 0.48 gm of Potassium dihydrogen Phosphate from Fisher Scientific are used to make PBS buffer of 7.4 pH in order to perform the release behavior of drug loaded fibers.

3.2.) Methods

The very first step to start the process is to prepare the gelatin pectin fibers because pectin does not have capability to form fibers due to the polyelectrolyte nature of the pectin. So gelatin is added as it has ability to form the fibers. The gelatin face

the problem in dissolving in water at room temperature because it forms gel so formic acid is used and pectin and gelatin are dissolved in the mixture of water and formic acid. This mixture is then tested for electrospinning to conform that fiber forms and it is in nm range.

The next step will be to use the bioactive glass precursor in the solution to make fibers of this solution. There is particular step to make bioglass solution. Bioactive glass needs a template for its formation for which gelatin pectin will act as template. So BAG will be added to the above prepared solution of gelatin and pectin in a defined order drop by drop. In the very first step TEOS is added and after 15 min TEP is added, after next 15 min sodium acetate is used and finally after next 15 min calcium acetate is added.

This addition of chemicals is done during the strong stirring of the solution o the magnetic stirrer. After the strong stirring this solution must be sonicated in order to remove bubbles and any particles. Sonication is performed after stirring but before electrospinning. The parameters of ESP for this solution are optimized.

Now after the above mentioned method it has been proved that BAG can makes fibers when gelatin and pectin is used as template. The next step will be addition of drug into the solution. Before adding the drug into the solution its must be prepared separately (1 ml) in DMF as it is easily soluble in it and DMF can be used as solvent for electrospinning. So vitamin D precursor is dissolved in it and the mixed it in the above prepared solution of BAG gelatin pectin and stir it properly.

After making each solution individually the ESP is also performed individually one by one. Because the very first step is to check for pectin fibers and then to check for BAG fibers and then finally drug loaded sample is formed. The electrospinning parameters are optimized individually for each solution. The three important parameters are applied voltage, flow rate, distance between tip and collector. So following are the optimized parameters.

For Gelatin Pectin fibers

Applied voltage – 18 KV

Flow Rate – 1 ml/hr

Tip to collector distance – 13 cm

For Gelatin Pectin BAG fibers

Applied voltage – 19.5 KV

Flow Rate – 1 ml/hr

Tip to collector distance – 13 cm

For Gelatin Pectin BAG drug loaded fibers

Applied voltage – 19.5 KV

Flow Rate – 0.8 ml/hr

Tip to collector distance – 13 cm

The parameters for each fiber are optimized and finally drug has been loaded. The next step is to perform the release test for which PBS of 7.4pH is required. The PBS solution is made in this manner first take milli Q water and then adds sodium chloride, Potassium Chloride, di-Potassium Hydrogen Orthophosphate, Potassium dihydrogen Phosphate in the same manner it is mentioned. And then check for pH of 7.4. Since the vitamin D is fat soluble so ethanol is used along with PBS for dissolution of vitamin D precursor. Ethanol and PBS are used in the ratio of 1:2 of pH 7.4 and this solution will be used for release test.

3.3.) Instrumentation Techniques

3.3.1.) Scanning electron microscopy

Scanning electron microscope (Hitachi S-3700N SEM, Germany) at voltage of 15 kV is used for determining the surface morphology of the fibers. In SEM analysis gold coating is provided on the sample before analyzing the samples.

3.3.2.) Universal Testing Machine

The UTM is a machine which is used to determine the behavior of materials mechanically. The maximum strength at fracture and young's modulus can be determined by this testing. INSTRON 3369 15kN load cell is used for the testing purpose. The fibers are deposited on the surface of aluminum foil which is cut in the rectangular shape 2.5 cm width and then measure the thickness with digital vernier caliper. The strain rate for the test is 1 mm/min. The final result is shown on the screen when the fracture of material takes place.

3.3.3) Fourier Transform Infrared Spectroscopy

FTIR of fiber was carried out with scientific Nicolet 380 spectrophotometer, USA using transmittance mode. Scanning was carried out using KBr pellet in frequency range of $500cm^{-1}$ to $4000cm^{-1}$. KBr pellet was prepared by 1 part of and 20 part of KBr.

3.3.4.) Thermogravimetric Analysis

Thermogravimetric analyses (TGAs) of the TFF and CMTFF were performed with a thermogravi-metric analyzer (PerkinElmer Pyris-6). The experiment was carried out under a nitrogen atmosphere by the heating of the sample (5 mg) in an aluminum crucible at 10 ^{0}C/min from 30 to 650 ^{0}C. Thermo gravimetric analyses (TGAs) of fibers were performed with a thermo gravimetric analyzer (PerkinElmer Pyris-6). The experiment was carried out under a nitrogen atmosphere by the heating of the sample (5 mg) in an aluminum crucible at 10^0C/min from 30 to 650 ^{0}C. Thermogravimetric analyzer was used for the analysis of thermal stability decomposition of the fibers made up of different materials. Nitrogen gas is purged in inside atmosphere by the heating of the sample in an aluminum crucible at 10^0C/min from 40^0 to 900^0C.

3.3.5.) UV-Vis Spectrophotometer

The release behavior of drug is determined by the UV-Vis spectra (Agilent Technologies Carry 300 UV-Vis). The result of release is recorded at the wavelength of 285 nm. The fiber is dipped in the solution of PBS and ethanol (2:1) for different time duration up to 8 hours.

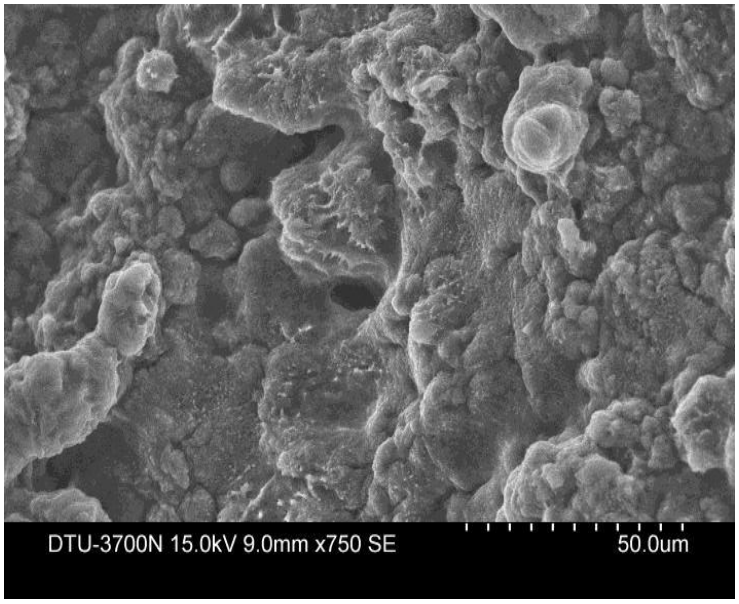
CHAPTER 4

RESULTS AND DISCUSSIONS

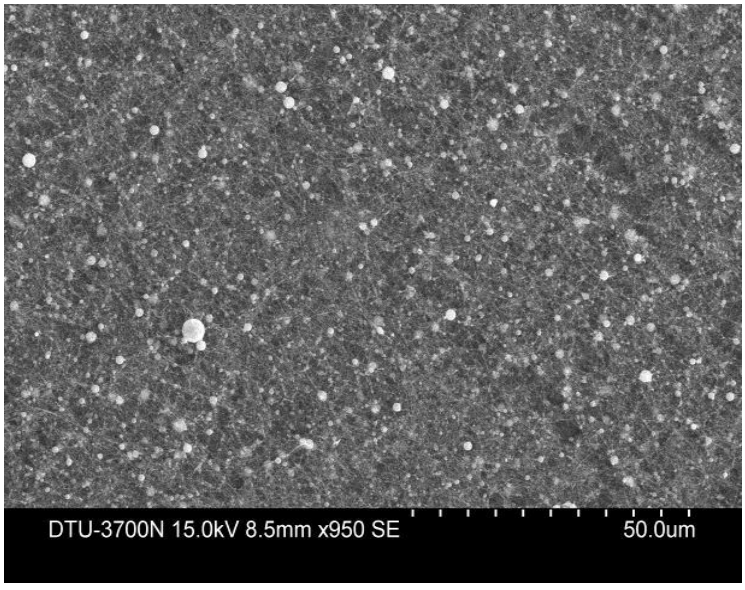
4.1) Scanning Electron Microscopy

The morphology of the fibers is clearly analyzed from SEM images. First step is to identify whether pure pectin forms fibers or not. The SEM image fig 4.1.1(a) clearly shows no fiber formation of pure pectin with water as solvent, as electro spraying takes place while electrospinning. So for the fiber formation of pectin there is requirement of carrier polymer which help pectin in making fibers as pectin nature is polyelectrolyte. So gelatin is used as carrier polymer along with pectin. The image fig 4.1.1(b) shows that at 15 wt% of gelatin forms beads with some fibers. So the percent of gelatin is increased to 20 wt% in pectin which forms fibers along with presence of beads shows in fig 4.1.1(c). So for proper formation of

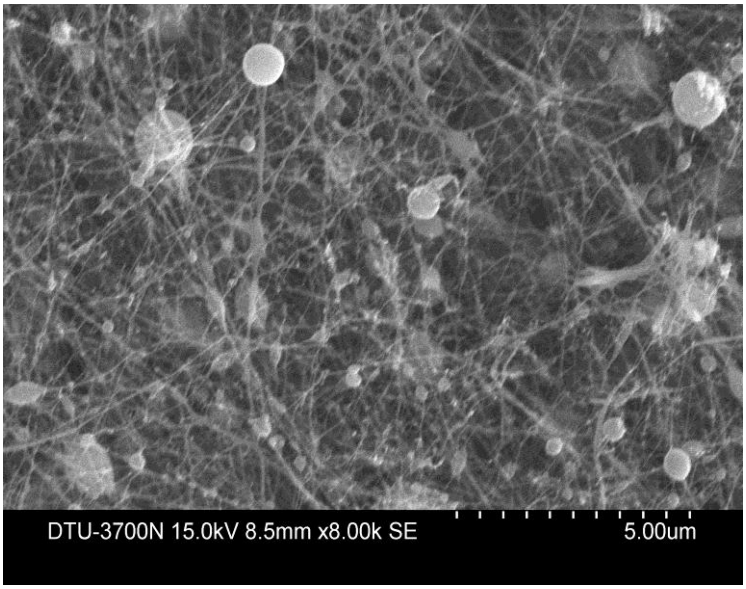
beads the optimum percentage of gelatin is 25wt% in fig 4.1.1(d). This amount of gelatin leads to the formation of very fine fibers in nano meters range. The SEM shows the clear picture of the fibers without any bead formation.



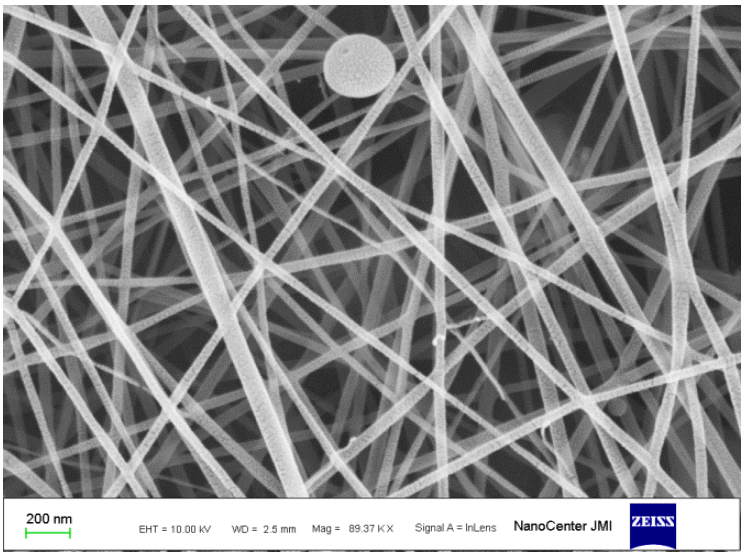
(a)



(b)



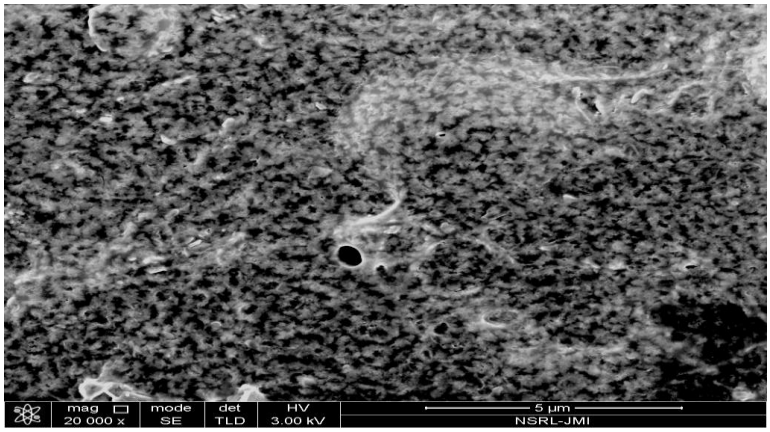
(c)



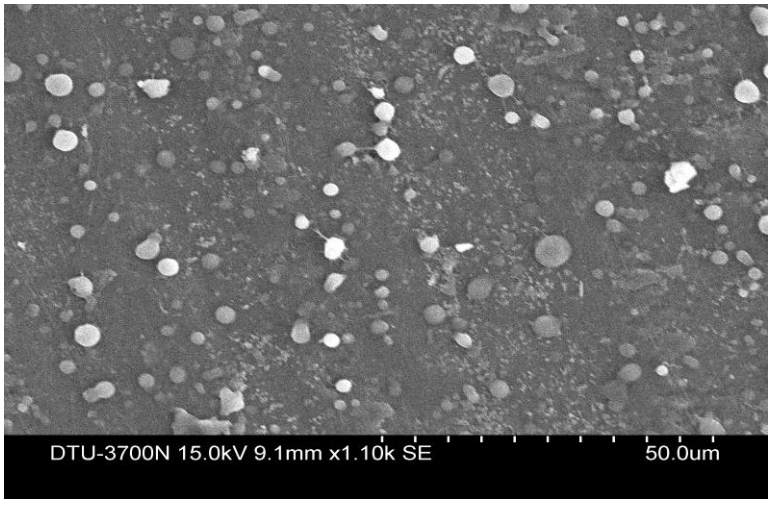
(d)

Figure 9(a) Pure Pectin (b) 15wt% gelatin (c) 20wt% gelatin (d) 25wt% gelatin, in pectin

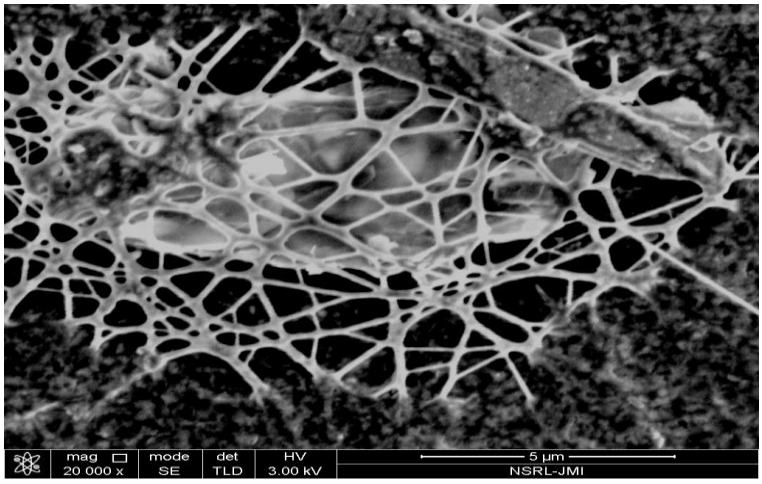
BAG in TRIZMA buffer shows no fiber formation when electrospun is shown in fig 4.2(a) Now gelatin pectin BAG nano fibers SEM image fig 4.2 (b) shows no formation of fibers when BAG in TRIZMA buffer is added into the gelatin pectin solution and mixed properly.


mag
20 000 x
mode
SE
det
TLD
HV
3.00 kV
5 µm
NSRL-JMI

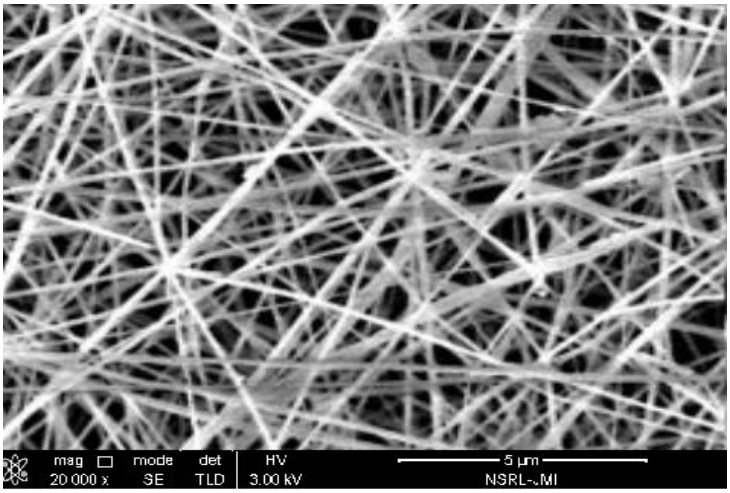
(a)


DTU-3700N 15.0kV 9.1mm x1.10k SE
50.0um

(b)


mag
20 000 x
mode
SE
det
TLD
HV
3.00 kV
5 µm
NSRL-JMI

(c)



(d)

Figure 10 (a) BAG in TRIZMA buffer (b) BAG when mixed with GP solution (c) GP mixed with BAG (d) drug loaded fibers

So it is decided to make the BAG spinnable and for this reason only the optimization of the parameters of gelatin pectin is necessary and gelatin pectin solution is used as template for BAG. The image shows that the formation of fibers fig 4.2(c). The next step is the loading of drug 7 dehydrocholestrol into the solution of the gelatin pectin and BAG. The SEM image of the sample shows the proper formation of the Nanofibers of BAG. This is due to the reason that the concentration of the BAG is slightly reduced in the solution which support the proper formation the fibers.

4.2.) FTIR

The results of FTIR show the presence of various functional groups in the fibers. The presence of caboxylic acid group, methy ester group and alcohol, etc shows the peaks in the spectrum at various wavenumber. The polymeric solution is simply blend no reaction is ocuuring in it as no new functional groups are introduced in the gelatin-pectin fibers spectrum other than groups of gelatin and pectin. There is very

little variation in the peaks of the gelatin, pectin, gelatin-pectin fibers as the functional groups are similar but gelatin contains amide I and amide II and carbonyl groups, carboxyl groups.

From the FTIR data it can be concluded that the peaks of the gelatin-pectin fiber is slightly broader than the gelatin fiber and pectin fiber peaks, this may be due to the presence of slight moisture in it fig 4.2.1 (a). The structural changes in the fibers are very difficult to find out as the spectra are almost similar. Now the peaks of gelatin shows the characteristic absorption bands for –COO groups asymmetric stretching vibration and symmetric stretching vibration (1449.9cm^{-1}) are detected in the spectrum. Amide I band peaks for C=O stretching vibrations is present at 1636.9 cm^{-1} and amide II peaks for NH bending (1234.4cm^{-1}), for CN stretching (1081cm^{-1}) can be seen. The presence of high wavenumber peaks conforms the presence of –OH stretching (3431.3cm^{-1}) and –CH stretching (2927.1cm^{-1}) is present in the gelatin FTIR spectra.

The peaks for pectin fibers are analysed from the spectrum shown in fig.4.2.1 (a). The stretching vibration peaks for –OH (3416.6cm^{-1}) and –CH (2936.8cm^{-1}) is present in pectin as well. The peaks at 1747.4 cm^{-1} is due to the presence of carboxymethyl group (-$COOCH_3$) of the pectin and 1634.9cm^{-1} is for –C=O stretching vibration peaks. In the pectin there is presence of –CH2 and –OH bending vibration at 1444.2 cm^{-1} and 1335 cm^{-1} respectively. The aliphatic cyclic secondary alcohol peak for –CH-OH is shown at 1151.91 cm^{-1} and for –CH-O-CH stretching at 1016 cm^{-1} is shown in fig. the blend of gelatin-pectin fibers shows no considerable differences in terms of peaks which proves no reactions takes place between gelatin and pectin. From the FTIR spectra as shown in the figure 4.2.1 (a) it can be concluded that the peaks for this is slightly broader than the other two peaks for –OH stretching vibration peak at 3403.3 cm^{-1}. This may be due the presence of strong intermolecular interaction or due to the presence of hydrogen bonding [24].The peaks for C-O stretch of secondary alcoholic group are 1081cm^{-1}, 1239 cm^{-1}.

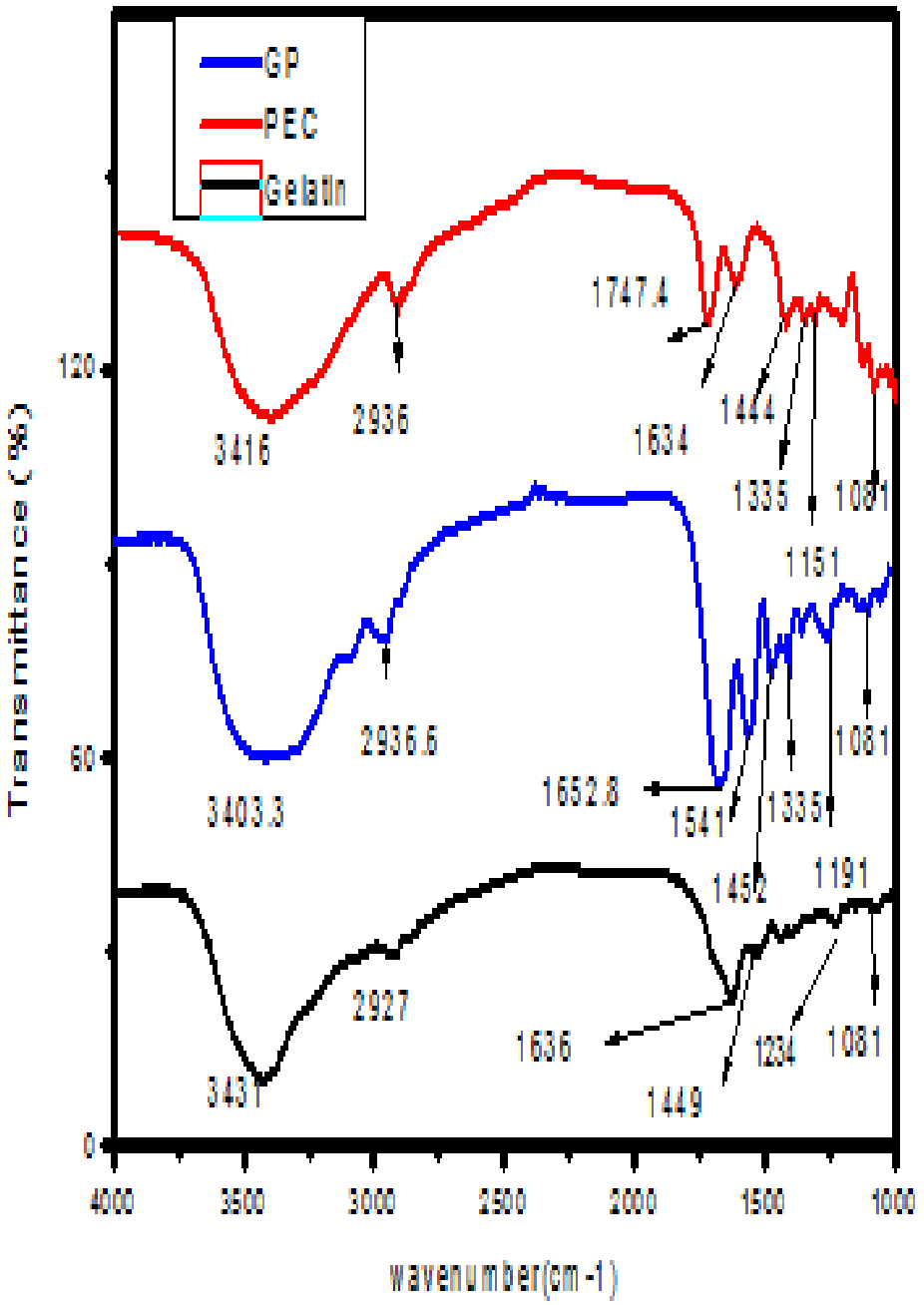
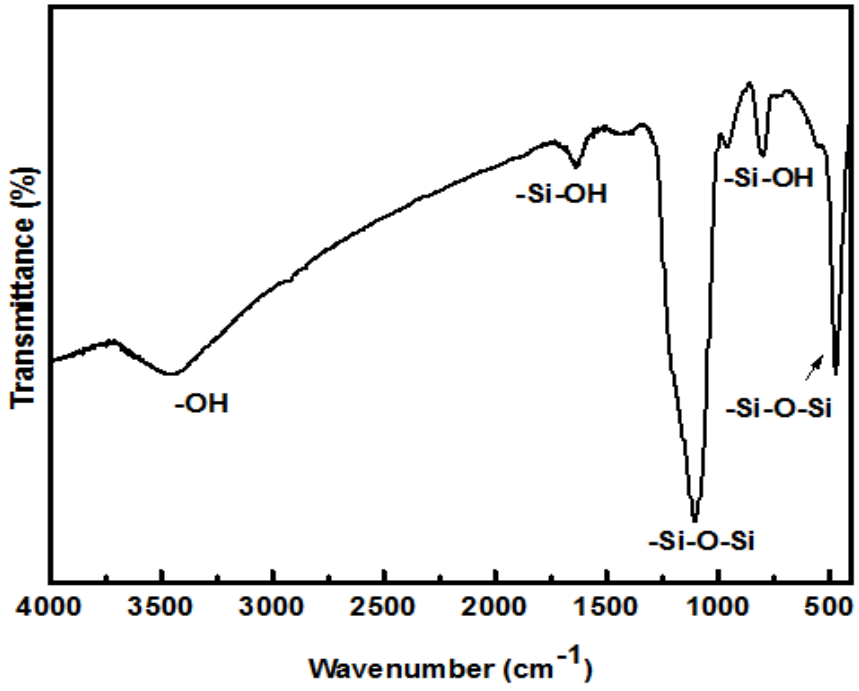


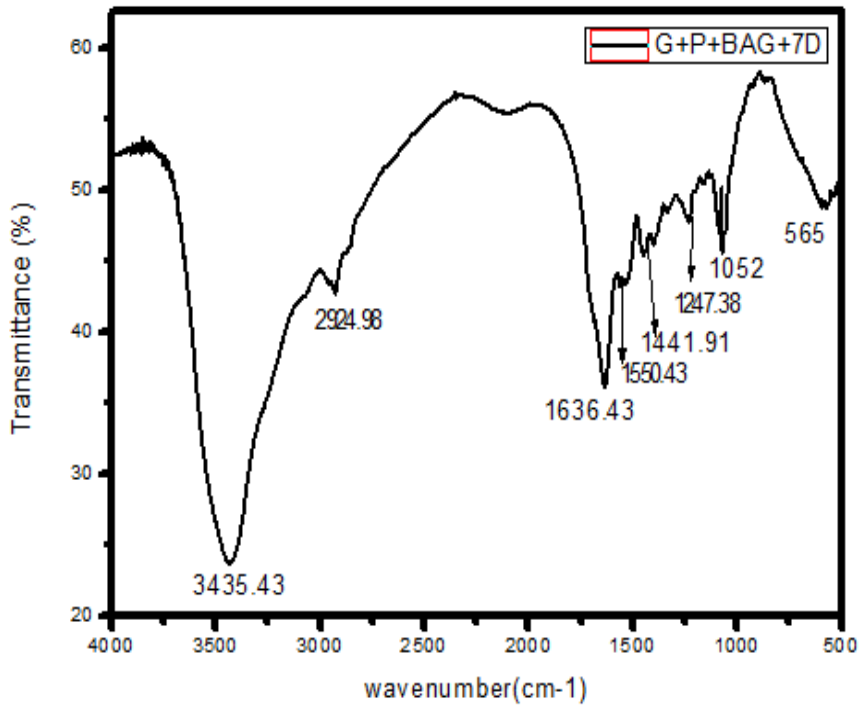
Figure 11(a) FTIR spectra of Gelatin, Pectin, Gelatin-Pectin

The same peaks at 1652 cm^{-1} is also present in the gelatin-pectin fibers for –C=O stretching vibration peaks. –CH stretching peaks is present in the blend fibers at 2936.6 cm^{-1}.

The various peaks in Fig. 4.2.3 shows the FTIR spectra of BAG and drug loaded BAG. The peak Si-O-Si is present in the BAG at Si-O-Si due to stretching vibrations and bending vibration at 1134 cm^{-1} and at 537 cm^{-1}. Si-OH peaks are also present in the spectrum which is at 850 cm^{-1}. And –OH peak is also present in it due to stretching vibrations. The fiber of gelatin pectin BAG and 7 dehydrocholestrol FTIR is shown in fig 4.2.3 (b) which represents the peaks at 3435 cm^{-1} because of presence of –OH stretching. This –OH group is present in 7 dehydrocholestrol as well as in gelatin, pectin, BAG. –CH stretching peak is present at 2924 cm^{-1} and 1636 peak is due to –C=O peaks. –COO groups asymmetric stretching vibration and symmetric stretching vibration peak at 1449.9cm^{-1}. Si-O-Si due to stretching vibrations and bending vibration at 1052 cm^{-1} and at 565 cm^{-1} is present in the FTIR spectrum. So the fibers of all four shows similar peaks like the individual one. Formation of new functional group is not seen in the spectrum.



(a)



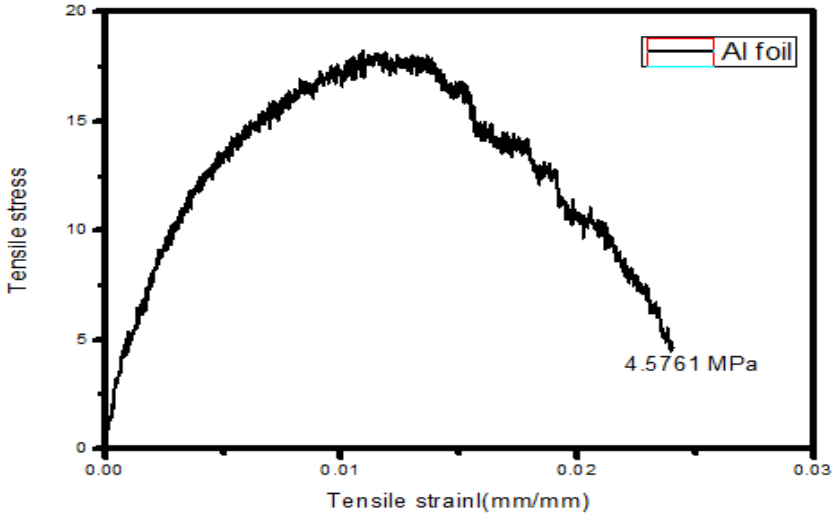
(b)

Figure 12 FTIR spectra of (a) BAG (b) drug loaded fibers

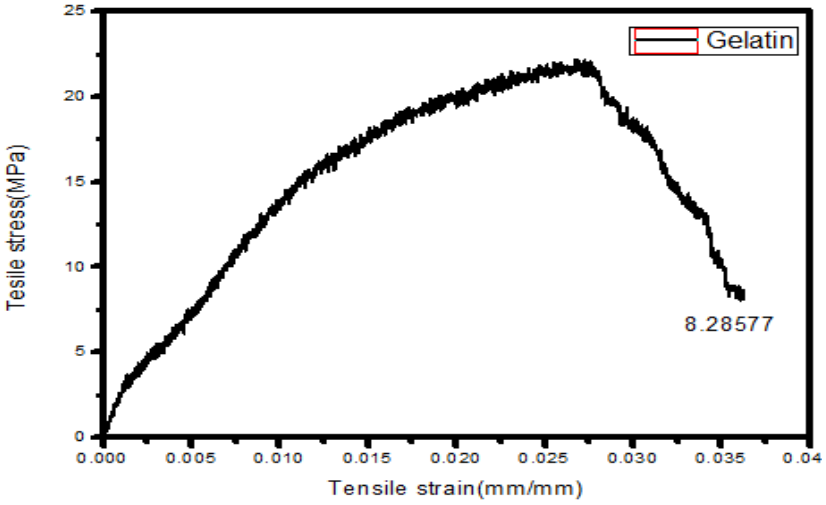
4.3) Tensile Strength Results

The mechanical properties like tensile strength. Young's modulus can be determined by UTM in which a strain rate (1 mm/min) is provided and length, thickness, width is entered. The figure 4.3.1 (a) shows the tensile strength of aluminum foil (4.5761 MPa) only so that comparison with gelatin fiber on aluminum foil can be done. The next step is to find out the gelatin fiber strength fig 4.3.1 (b) which is 8.28577 MPa which is higher than Al foil strength and its young's modulus value also increases from 1361.47418 MPa to 2117.696 MPa. The addition of pectin (fig 4.3.1.(c)) into the solution of gelatin results in slight increase of strength as well as increase in its young's modulus with value 10.9882 MPa and 2658.41306 MPa. BAG addition (fig 4.3.1.(d)) into the fiber lead to considerable increase in the strength (27.8776 MPa) but modulus (2457.8142 MPa)

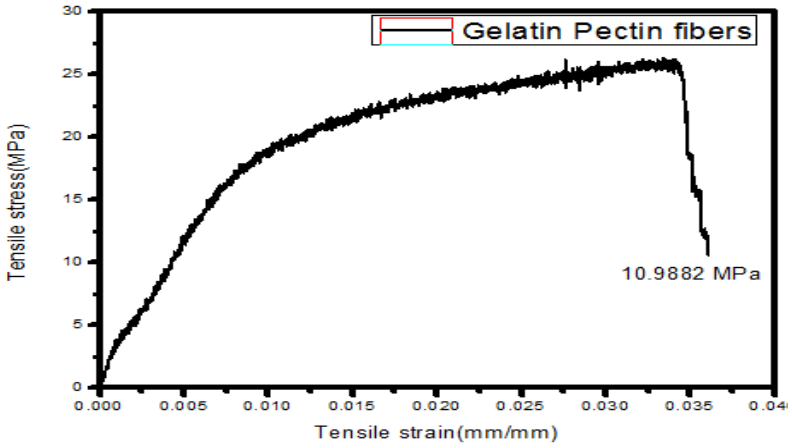
value decreases. The decrease in modulus is favorable for bone healing phenomenon as modulus high value means high stiffness, but in bone healing phenomenon some amount of flexibility is desirable. The addition of vitamin D precursor 7 dehydoxycholestrol does not cause any significant increase in the strength and modulus, both the value decreases slightly, 26.71 MPa and 2411.1043 MPa respectively.



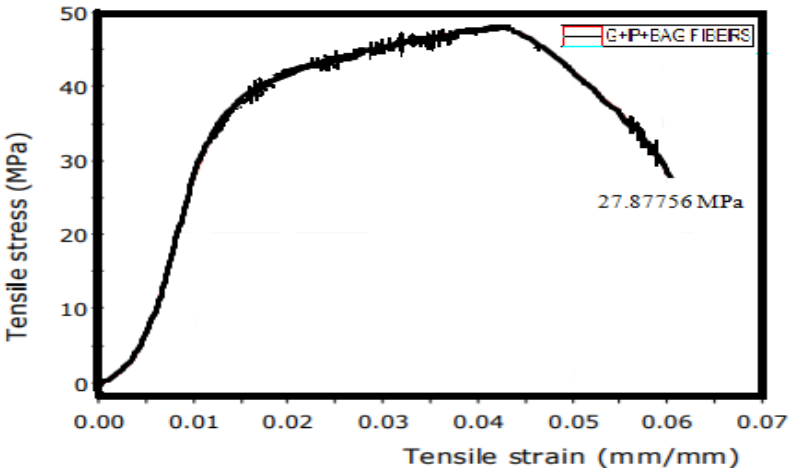
(a)



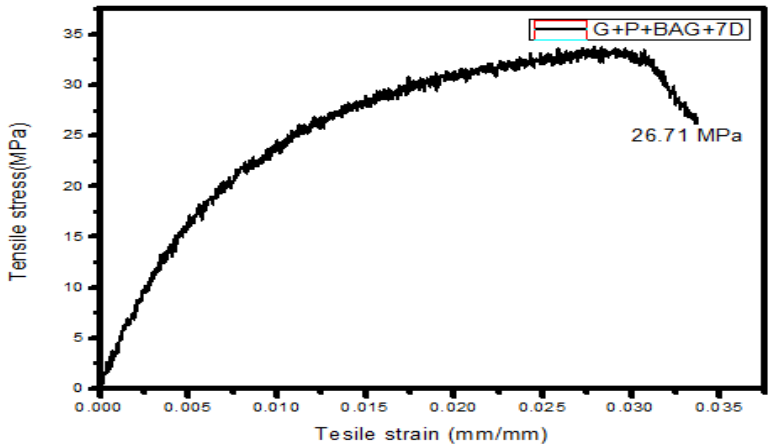
(b)


Gelatin Pectin fibers
Tensile stress(MPa)
Tensile strain(mm/mm)
10.9882 MPa

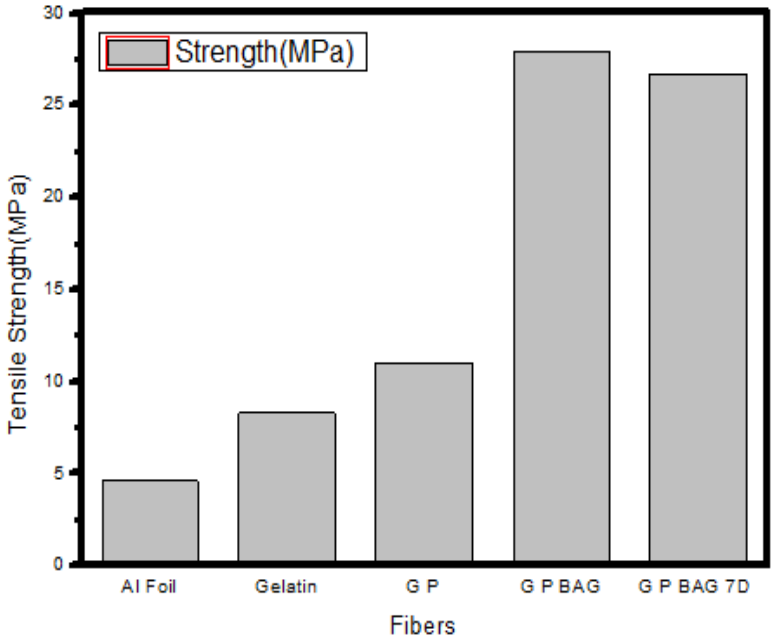
(c)


G+P+BAG FIBERS
Tensile stress (MPa)
Tensile strain (mm/mm)
27.87756 MPa

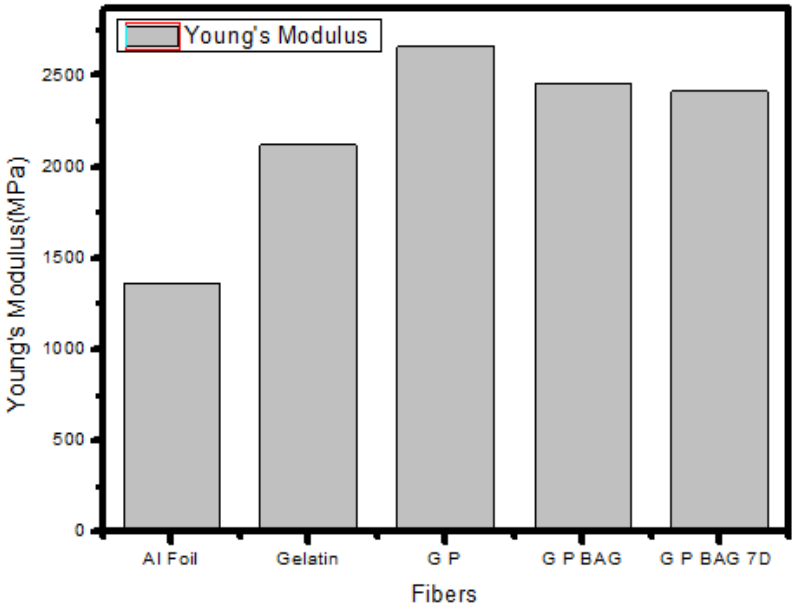
(d)


G+P+BAG+7D
Tensile stress(MPa)
Tesile strain (mm/mm)
26.71 MPa

(e)



(f)

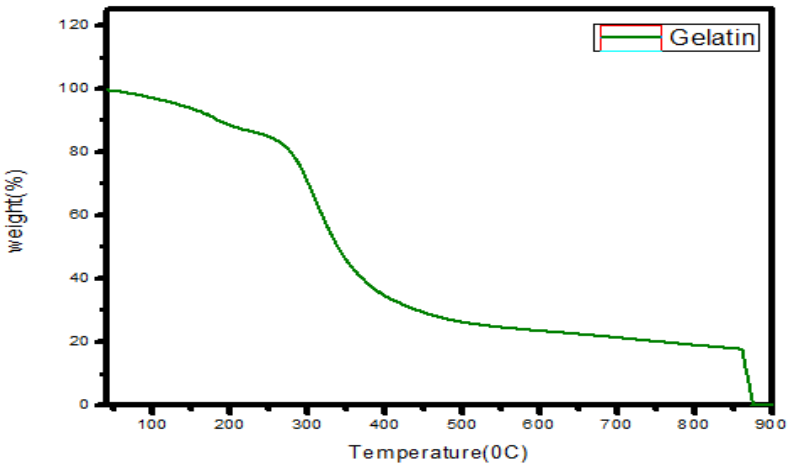


(g)

Figure 13 strength of (a) Al foil (b) gelatin (c) gelatin-pectin (d) gelatin-pectin-BAG (e) gelatin-pectin-BAG-drugloaded fibers (f) strength variation (g) modulus variation

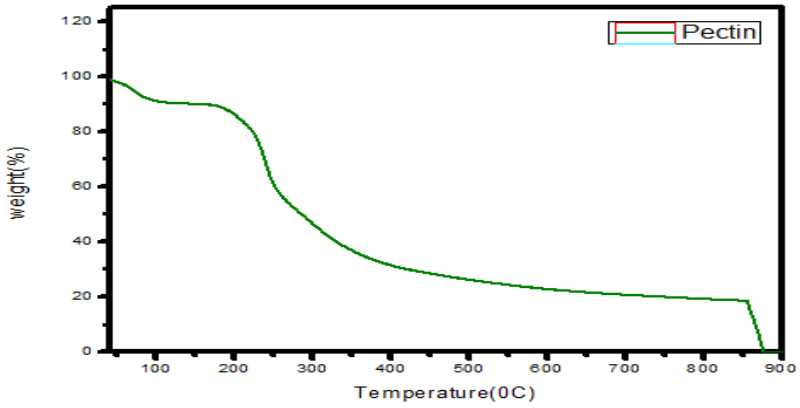
4.4.) TGA Analysis

TGA method is widely used to identify the range of stability and its decomposition temperature. The gelatin fibers results fig 4.4.1.(a) shows that there is first drop which signifies the loss of moisture in the sample from 40^0C to 171.53^0C and then decomposition starts from 171.2^0C to 526.24^0C and then thermal stability is attained before complete decomposition.



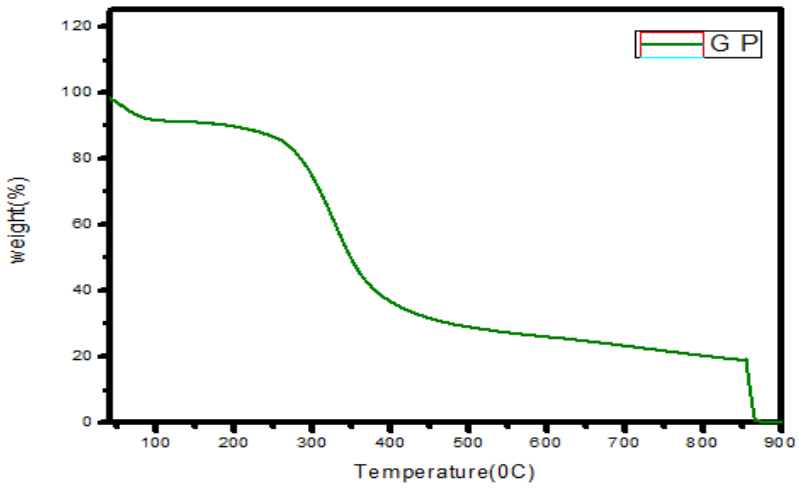
(a)

The pectin powder TGA (fig 4.4.1.(b)) shows the water loss from 40^0C to 89.23^0C and then thermal decomposition is there from 160.75^0C to 543.13 ^{0}C and the weight loss associated with the pectin powder is 64.88%, finally it also attain stability range before complete decomposition.

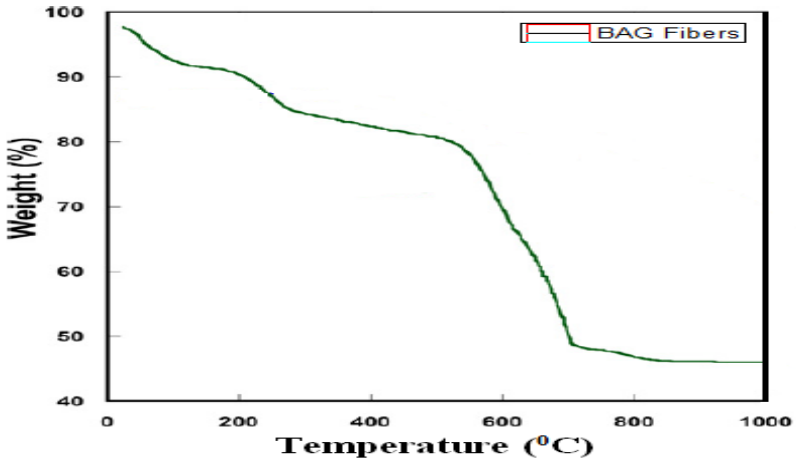


(b)

The TGA of gelatin pectin fibers in fig 4.4.1 (c) shows little variation water loss takes place from 40^0C to 88.92^0C then thermal stability occurs from 88.92^0C to 188.21^0C. The decomposition of gelatin pectin fibers starts from 188.21^0C to 574.34^0C and the corresponding weight loss is 62.835^0C. So gelatin pectin fibers stability range is increased from gelatin fibers as well as from pectin powder and weight loss percent also decreases.



(c)



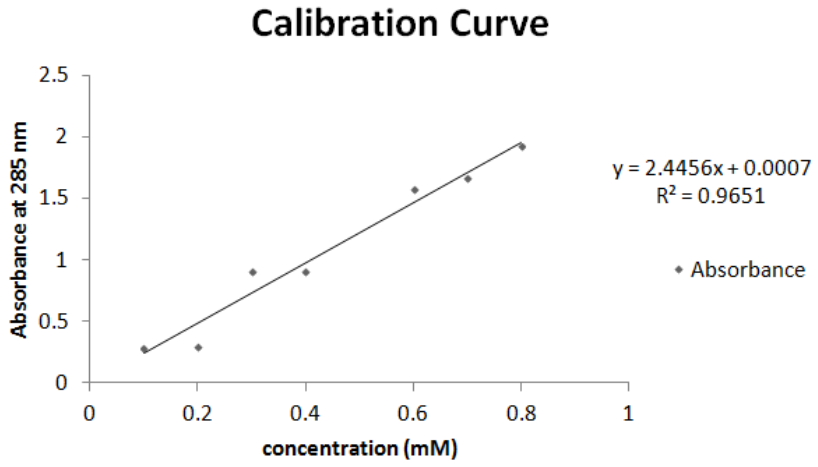
(d)

Figure 14 TGA plot of (a) gelatin (b) pectin powder (c) gelatin pectin (d) BAG-gelatin-pectin fibers

In the analysis of TGA of BAG gelatin pectin fiber shown in fig.4.4.1 (d) the moisture content of fibers which is absorbed is removed by evaporation and the loss is approximately 10%. One more loss is shown in the spectrum from 200^0C to 230^0C which is 12.2% and this amount of loss is due to the precursor condensation i.e. loss of water from fibers. This curve shows the highest amount of loss of weight which is approximately 29.78%. So from TGA curve best thermal stability range is obtained. The addition of BAG results in reduction of the loss of weight and increase in thermally stable temperature.

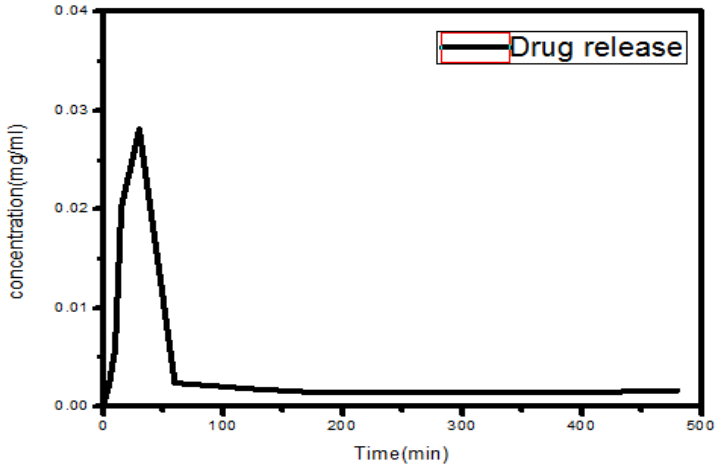
4.5) Drug Release Behavior

The drug release characteristic is shown in the fig 4.5.1 (a), in which the release behavior of drug can be seen. In the drug release from biomaterials the two most important phases are “burst” release and next stage is “sustain” release, these two phases have their own advantages in the field of bone healing [42]. But literature says that first phase release behavior plays very important role in this field and avoid the side effects of excessive drug concentration [43]. In our case, BAG shows the first stage of burst release in first 30 min in which ~63% release occurs and then there is decrease in the rate of release of drug and finally the sutained release is obtained.



(a)

Such type of release behavior favor the bone healing treatment as initial high amount of drug releases provides immediate relief followed by prolonged release to promote gradual healing. It is well-known that BG materials induce apatite formation at the surface in the physiological environment, and thus the surface morphology of BG fibers significantly varies. Such phenomenon has been considered as a main impetus for the sustained protein release.



(b)

Figure 15 Calibration curve at 285 nm (b) Drug release from BAG nanofibers

CHAPTER 5

CONCLUSION AND FUTURE WORK

Nano fibers of Gelatin-Pectin –BAG used for bone healing in which drug is loaded shows favorable results for this purpose. Sem images of the fibers conforms the formation of the nano meter range fiber. Then FTIR of the sample are recorded which shows that no reaction is taking place in between the polymeric materials as no new function group is seen in the blend fibers. Tensile strength of the materials increases with use of BAG in the gelatin pectin solution upto 27.87756 MPa but modulus of the material decreases from 2658.41306 MPa to 2457.8142 MPa which good for bone healing as this process need some flexibility for its growth as very high stiffness value materials will not support the bone healing phenomena efficiently. With the loading of drug no increase in strength is seen, its value remains almost similar i.e. 26.71 MPa and modulus value is almost similar to favorable value. The increase in strength signifies that it is good choice for bone healing. The TGA analysis shows that when BAG is used with pectin and gelatin

fibers its thermal stability range increases. The drug release behavior is also favorable for this purpose as there is burst release in the starting and then sustained release can be seen which helps in initial relief in the fractured part and then there will be bone healing with time in a sustained manner. Sunlight exposure shows that the intensity of peak firstly increases up to 15 min at 1593 cm^{-1}.

This work can be further used in the field of biomedical sciences for wound healing, tissue engineering, etc. This BAG based nanofibers strength can be further increased by using some other kind of natural polymer. The impact on tensile strength by using various concentration of the 7- dehydrocholestrol can be seen. It may possible that increase in concentration enhance the mechanical properties of BAG nanofibers which are desirable in bone healing.

REFERENCES

1. Seeram Ramakrishna, Kazutoshi Fujihara, Wee-Eong Teo, Thomas Yong, Zuwei Ma, and Ramakrishna Ramaseshan, Electrospun fibers: Solving global issues, ISSN: 1369 7021, 2006.
2. Kyung-Tae Noh, Hye-Young Lee, Ueon-Sang Shin, Hae-Won Kim Composite nanofiber of bioactive glass nanofiller incorporated poly (lactic acid) for bone regeneration, Materials Letters 64 (2010) 802–805.
3. Cann MC, Roberts K (1996) Plant cell wall architecture: the role of pectins and pectinases. In: Visser J, Voragen AGJ (eds) Pectins and pectinases. Elsevier science, Amsterdam, Netherlands, pp 91–107.
4. Rockwell, P.L., Kiechel, M.A., Atchison, J.S., Toth, L.J. and Schauer, C.L. (2014) Various-Sourced Pectin and Polyethylene.Oxide Electrospun Fibers. Carbohydrate Polymers, 107, 110-118.
5. Fishman, M.L., Chau, H.K., Hoagland, P. and Ayyad, K. (2000) Characterization of Pectin, Flash-Extracted from Orange Albedo by Microwave Heating, Under Pressure. Carbohydrate Research, 323, 126-138.
6. Liu, L.S., Fishman, M.L., Kost, J. and Hicks, K.B. (2003) Pectin Based Systems for Colon Specific Drug Delivery via Oral Route. Biomaterials, 24, 3333-3343.
7. Fishman ML, Coffin DR, Ly TV (1996) J of Appl Poly Sci 61:71.
8. Alborzi, S., Lim, L. T., & Kakuda, Y. (2010). Electrospinning of sodium alginate–pectin ultrafine fibers. Journal of Food Science, 75(1), C100–C107. Canteri-Schemin, M. H., Fertonani, H. C. R., Waszczynskyj, N., & Wosiacki, G. (2005). Extraction of pectin from apple pomace. Brazilian Archives of Biology and Technology, 48(2), 259–266.
9. Ramachandran GN (1967) Ramakrishnan C. In: Ramachandran GN, Reddi AH (eds) Biochemistry of Collagen. Plenum Press, New York.
10. Brodsky B, Ramshaw JAM (1997) Matrix Biol 15:545.
11. Kawai K, Suzuki S, Tabata Y, Ikada Y, Nishimura Y (2000) Biomaterials 21:489.

12. Zhao F, Yin Y, Lu WW, Leong JC, Zhang W, Zhang J, Zhang M, Yao K (2002) Biomaterials 23:3227.
13. Ito A, Mase A, Takizawa Y, Shinkai M, Honda H, Hata KI, Ueda M, Kobayashi T (2003) J of Bio & Bioeng 95:196.
14. Di Silvio L, Guruv N, Kayser MV, Braden M, Downes S (1994) Biomaterials 15:931.
15. Luís Jorge Marques de Carvalho, Characterization of bioactive glass electrospun fibers and influence of RGD binding motifs on stem cell activity, Master of science thesis, 2014.
16. Yangyang Li, Binbin Li, Gang Xu, Zeeshan Ahmad, Zhaohui Ren, Yan Dong, Xiang Li, Wenjian Weng, Gaorong Han, A feasible approach toward bioactive glass nanofibers with tunableprotein release kinetics for bone scaffolds, Colloids and Surfaces B: Biointerfaces 122 (2014) 785–791.
17. Reginna E. Scarbergregg, Optimization and characterization of bioactive glass nanofibers and nanocomposites, PhD thesis, 2011.
18. Youliang Hong, Xuesi Chen, Xiabin Jing, Hongsong Fan, Zhongwei Gu, and Xingdong Zhang (2010): Fabrication and Drug Delivery of Ultrathin Mesoporous Bioactive Glass Hollow Fibers .Advanced Functional Material 20, 1503–1510.
19. Hamidreza Pirayesh and John A. Nychka, Sol–Gel Synthesis of Bioactive Glass-Ceramic 45S5 and its in vitro Dissolution and Mineralization Behavior, J. Am. Ceram. Soc., 96 [5] 1643–1650 (2013).
20. L. L. Hench, "feature 1705," vol. 28, 1998.
21. H. Services, "Bone Health and Osteoporosis A Report of the Surgeon General."
22. L. G. Griffith and G. Naughton, "Tissue engineering-current challenges and expanding opportunities.," Science, vol. 295, no. 5557, pp. 1009–14, Feb. 2002.
23. Wedad Z. Mostafa , Rehab A. Hegazy, Vitamin D and the skin: Focus on a complex relationship: A review, Journal of Advanced Research (2015) 6, 793–804.
24. Kyung-Tae Noh, Hye-Young Lee, Ueon-Sang Shin, Hae-Won Kim, Composite nanofiber of bioactive glass nanofiller incorporated poly(lactic acid) for bone regeneration, Materials Letters 64 (2010) 802–805.

25. Changfeng Chen, Carl P. Tripp, A comparison of the behavior of cholesterol, 7-dehydrocholesterol and ergosterol in phospholipid membranes, Biochimica et Biophysica Acta 1818 (2012) 1673–1681.

26. Vanchinathan V, Lim HW. A dermatologist's perspective on vitamin D. Mayo Clin Proc 2012;87:372–80.
27. Kochupillai N. The physiology of vitamin D: current concepts. Indian J Med Res 2008;127:256–62.
28. Gilchrest BA. Sun exposure and vitamin D sufficiency. Am J Clin Nutr 2008;88:570S–7S.
29. CIE. Action spectrum for the production of previtamin D3 in human skin. Technical report 174; 2006.
30. Krause R, Bohring M, Hopfenmu¨ ller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. Lancet 1998; 352:709–10.
31. Stamp TC, Haddad JG, Twigg CA. Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. Lancet 1977; 1:1341–3.
32. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesize vitamin D3. Lancet 1982; 1:74–6.
33. Committee of Revision. Drug information for the health care professional. Rockville, MD: United States Pharmacopeial Convention Inc.; 1997.
34. Institute of Medicine. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Vitamin D. Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press; 1997. p. 250–87.
35. Medicines Commission. British Pharmacopoeia 1980. London, United Kingdom: Her Majesty's Stationery Office; 1980.
36. Greiner, A. and Wendorff, J.H. (2007) Electrospinning: A Fascinating Method for the Preparation of Ultrathin Fibres. Angewandte Chemie-International Edition, 46, 5670-5703.

37. Stijnman, A.C., Bodnar, I. and Tromp, R.H. (2011) Electrospinning of Food-Grade Polysaccharides. Food Hydrocolloids, 25, 1393-1398.
38. Rockwell, P.L., Kiechel, M.A., Atchison, J.S., Toth, L.J. and Schauer, C.L. (2014) Various-Sourced Pectin and Polyethylene Oxide Electrospun Fibers. Carbohydrate Polymers, 107, 110-118.
39. Viness Pillay, Clare Dott, Yahya E. Choonara, Charu Tyagi, Lomas Tomar, Pradeep Kumar, Lisa C. du Toit, and Valence M. K. Ndesendo, A Review of the Effect of Processing Variables on the Fabrication of Electrospun Nanofibers for Drug Delivery Applications, Journal of Nanomaterials Volume 2013, Article ID 789289, 22 pages.
40. F. Rosso, A. Giordano, M. Barbarisi, and A. Barbarisi, "From cell-ECM interactions to tissue engineering.," J. Cell. Physiol., vol. 199, no. 2, pp. 174–80, May 2004.
41. L. L. Hench, "Founders Award , Society for Biomaterials 24th Annual Meeting , San Diego , CA ,April 22 – 26 , 1998 Bioactive materials : The potential for tissue regeneration," 1998.
42. Shih-Feng Chou, Kim A. Woodrow, Relationships between mechanical properties and drug release from electrospun fibers of PCL and PLGA blends, Journal of the mechanical behavior of biomedical materials 65 (2017) 724–733.
43. Muhidinov, Z.K., Kasimova, G.F., Bobokalonov, D.T., Khalikov, D.K., Teshaev, K.I., Khalikova, M.D., et al. (2010) Pectin/Zein Microspheres as a Sustained Drug Delivery System. Chemical Pharmaceutical Journal, 44, 20-24.
44. Bodo Lehmann, Thurid Genehr, Peter Knuschke, Jens Pietzsch,* and Michael Meurer, UVB-Induced Conversion of 7-Dehydrocholesterol to 1a,25-Dihydroxyvitamin D3 in an In Vitro Human Skin Equivalent Model, J Invest Dermatol 117:1179-1185, 2001.