

Nanofiber synthesis of almond resin polymer blended with starch to make antimicrobial fiber sheet with polyhexanide as drug

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ABSTRACT

Cellulose nanofiber is produced and then blended with starch to be used in treating wounds in diabetic patients. Since it is of plant origin, it prevents the toxic effects to the body like itching, swelling etc. Cellulose is extracted from a common source almond gum which is easily available and has greater amount of cellulose in it. Cellulose is blended with starch, which is also a plant origin compound and has greater effect in wound healing property. Starch has a natural wound healing property which is why it is blended with cellulose. PVA is a synthetic polymer which is used in the nanofiber synthesis along with starch and cellulose. PVA maintains the tensile strength and helps to maintain the surface to volume ratio. It also helps in the proper drug distribution throughout the fiber. The method used to produce nanofiber is electrospinning technique. And it provides the proper texture for the nanofiber and also the proper distribution. The voltage is being kept at 12KV. The flow rate is kept at 0.1ml/min and it is adjusted depending upon the nanofiber which is being produced. High voltage and potential difference is used in the production of nanofiber. The concentration of the components in nanofiber and PVA is optimized for the effective production of nanofiber. The drug used in the nanofiber is polyhexanide which is effective against gram positive and gram-negative bacteria. Concentration of the drug is also optimized for its effective activity. Finally, the components are optimized, and the fiber is produced by electro spinning method. The fiber produced is then checked for its antimicrobial activity in three different bacterial strains in MHA agar and then given for SEM analysis which confirms the diameter of the fiber.

Keywords: Cellulose, Starch, Poly vinyl Alcohol (PVA), Drug distribution, Polyhexanide, Nanofiber, Electrospinning Technique, Polymer.

INTRODUCTION

Nanoscience and nanotechnology are at the forefront of modern research. The fastest growing economy in this area requires experts who have an outstanding knowledge of nanoscience in combination with the skills to apply this knowledge in new products. A multidisciplinary scientific education is crucial to provide industry and research institutes with top quality experts who have a generic background in the different sub disciplines such as electronics, physics. The word Nanoscience refers to the study, manipulation and engineering of matter, particles and structures on the nanometer scale (one millionth of a millimeter, the scale of atoms and molecules). Important properties of materials, such as the electrical, optical, thermal and mechanical properties, are determined by the way molecules and atoms assemble on the nanoscale into larger structures.

Nanotechnology is science and engineering at the scale of atoms and molecules. It is the manipulation and use of materials and devices so tiny that nothing can be built any smaller. The term

'nanotechnology' was used first by the Japanese scientists Norio Taniguchi (1912-1999) in 1974 on production technology that creates objects and features on the order of a nanometer. The American engineer K.Eric Drexler (1995) is credited with the development of molecular nanotechnology, leading to nanosystems machinery manufacturing. The invention of scanning tunneling microscope in the 1980s by IBM Zurich scientists and then the atomic force microscope allowed scientists to see materials at an unprecedented atomic level. The concepts of nanotechnology though considered being modern science, has history is the 9th century. Nanoparticles of gold and silver were used by the artisans of Mesopotamia to generate glittering effects of pots. The therapeutics use of gold can be traced back to Chinese medical history in 2500BC since ancient time colloidal gold and silver under the name Swarna Bhasma and Roupya Bhasma is still used in the Indian ayurvedic and Unani medicine for rejuvenation, revitalization and for treating various diseases.

The importance of nanoparticles to improve human health, electronic, magnetic and optoelectronic, biomedical, pharmaceutical,

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cosmetic, energy, environmental, catalytic and materials applications. Nanoparticles can be further used to immobilize microbial cells that can degrade or bio-recover specific chemicals and also be used as biocatalysts for reductive dechlorination (gupta and silver 1998). The use of nanoparticles already established for some medical application like wound infection, wound dressing and treatment of preclinical stages. Nanomaterials show very high efficiency in destroying cancer cells and are already undergoing clinical trials. The results are so promising that nanomaterials might become an alternative to traditional cancer therapy, mostly due to the fact that they allow cancer cells to be targeted specifically and enable detailed imaging of tissues, making planning further therapy much easier. Nanotechnology has the potential to bring major advances in medicines. Nanobots could be sent into a patient arteries to clear away blockages. Surgeries could become much faster and more accurate. Injuries could be repaired cell-by-cell. It may even become possible to heal genetic conditions by fixing the damaged genes. Nanotechnology could also be used to refine drug production, tailoring drugs at a molecular level to make them more effective and reduce side effects. Nanotechnology offers many potential benefits to medical research by making pharmaceuticals more efficacious and by decreasing their adverse side-effects.

MATERIALS AND METHODS

Production of cellulose:

Almond gum is used to extract cellulose for making cellulose nanofiber. The almond gum was washed with distilled water to remove dirt, dust and water soluble impurities. Almond gum was treated with 4% sodium hydroxide solution at 80°C for 2 hours, under mechanical stirring which removed the residual additives, such partially solubilized pectin, lignin, and hemicelluloses impurities. After each treatment, the obtained reaction mass was filtered and washed with distilled water, until the filtrate became neutral. After this alkali treatment, the residues was decolorized with 3% sodium chlorite to bleach and leach the residues. This process was accomplishment at 80°C, for 2 hours. The resultant residue was washed continuously in distilled water, until the complete remove of sodium chlorite. After this, the residue was hydrolyzed using 10% sulphuric acid at 80°C, for 2 hours, using mechanical stirring. After the acid hydrolysis, the reaction mass was cooled with ice cubes to quench the hydrolysis, washed with distilled water, and centrifuge for 20 minutes, 8000rpm. The suspension and pellet was collected separately. Extraction of celluloses was carried out by mixing the almond gum with 0.25M sodium hydroxide with ratio of 1:25 (W / V). Then the mixture was stirred at 400rpm and heated at constant temperature of 55°C for 2 hours. Then after stirring the mixture was filtered, the pH solution was adjusted to pH 5-6 by adding 6M hydrochloric acid. Then the mixture was kept at 4.0°C for 24 hours. Then the mixture was centrifuged at 3500 rpm for 15 minutes. Then the liquid fraction was treated with ethanol and 10% acetic acid solution was adding 1:1 ratio and 1:2 ratios. Then the liquid fraction to precipitate this precipitated sample was centrifuged at 3000rpm for 10 minutes. After centrifugation the pellet was collected and dried for 24 hours.

Phenol-sulfuric acid method:

Phenol-sulfuric acid method is the most reliable method among all the quantitative assays for carbohydrate estimation that is cellulose confirmation. In hot acidic medium, glucose is dehydrated to

hydroxyethyl furfural. This forms a yellow-brown-colored product with phenol and has absorption maximum at 490 nm UV spectroscopy. This obeys Beer-Lambert's law which states that when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of absorbing solution is proportional to incident radiation as well as the concentration of solution. The values obtained of absorbance were helpful to plot a graph which is very crucial in estimating carbohydrate content. The values should be positive, above zero to plot a graph. After the plotting of a graph, the total amount of carbohydrate in the sample from glucose standard graph was calculated. The standard curve of absorbance was plotted at 490 nm on "Y" axis representing absorbance at 490 nm versus concentration of glucose in µg/ml on X axis.

Electrospinning method:

The mixture containing cellulose, starch, PVA and polyhexanide is being loaded in a 10 ml syringe and then the loaded in the electrospinning setup. Electrospinning is a simple and comprehensive process for generating an ultrafine fibre from varieties of materials which include polymer, composite and ceramic. The electrospinning setup consists of three major components namely, high voltage power supply, syringe with metal needle and a conductive collector. It is, in fact, very sophisticated, but a simple, processing mechanism of producing nanofiber. The electrospinning process can be classified into several techniques like vibration electrospinning, magneto-electrospinning, siro-electrospinning and bubble electrospinning. As the charge liquid jet moves from the syringe tip to the collector, the mode of current flow changes from ohmic to convective flow as the charge moves instead to the fibre surface.

A slow acceleration is a characteristic of the ohmic flow, since the geometry of the Taylor cone is controlled by the ratio of the surface tension to electrostatic repulsion. After successfully addressing the ohmic flow, the jet travels at a rapid acceleration, which includes the transition zone from liquid to dry solid. In the end, the jet penetrates the collector. The name 'Taylor Cone' simply represents the conical shape formed at the needle tip see. The electrospinning apparatus is really a simple idea, carrying only three main components: a high voltage power supply, a polymer solution reservoir (e.g., a syringe, with a small diameter needle) with or without a flow control pump, and a metal collecting screen. A high voltage power supply with adjustable control can well provide up to 50-kV DC output and, depending on the number of electrospinning jets, the multiple outputs that function independently, are necessitated. The polymeric solution is kept in a reservoir and connected to a power supply to establish a charged polymer jet. Charging the polymer solution could be done either with a syringe with a metal needle or a capillary with a metal tip in the polymer solution. If the syringe is not placed horizontally, polymer flow can be driven by gravity. However, to remove the experimental variables, a syringe pump is engaged to control the precise flow rate. The fibre collecting screen is expected to be conductive and it can either be a stationary plate or a rotating platform or substrate. The plate can produce non-woven fibers, whereas a rotating platform can produce both nonwoven and aligned fibers. Presently, two standard electrospinning setups are available namely the vertical and horizontal, with three new electrospinning setups with different angles for the study of the effect of the gravity. As a result of the increasing interest in this

technology, many research groups have developed sophisticated mechanisms by which more complex nanofibers structures, can be fabricated in a more controlled and efficient manner. For instance, motor-controlled multiple jets and fibre-collecting targets provide avenue for producing a single nanofibrous scaffold consisting of multiple layers, with each layer obtained from a different polymer type. Furthermore, this technology can be used to manufacture polymer composite scaffolds where the fibers of each layer represent a combination of various polymer types. Presently, two standard electrospinning setups are available namely the vertical and horizontal, with three new electrospinning setups with different angles for the study of the effect of the gravity. As a result of the increasing interest in this technology, many research groups have developed sophisticated mechanisms by which more complex nanofibers structures, can be fabricated in a more controlled and efficient manner. For instance, motor-controlled multiple jets and fibre-collecting targets provide avenue for producing a single nanofibrous scaffold consisting of multiple layers, with each layer obtained from a different polymer type. Furthermore, this technology can be used to manufacture polymer composite scaffolds where the fibers of represent a combination of various polymer types. In this way cellulose nanofibers were produced and tested for antimicrobial activity of the cellulose nanofiber.

RESULTS AND DISCUSSIONS

Extraction of cellulose:

Cellulose was extracted from almond gum using chemical method. Almond gum was made into powder and then dissolved in 100 ml distilled water. Then cellulose is precipitated using isopropyl alcohol. Then cellulose is dried overnight in Hot air oven. Then the cellulose is taken and kept in a container. The dried cellulose is taken for UV test to determine its confirmation.

Confirmation of cellulose:

The confirmation of cellulose was done by a method called Phenol Sulphuric Acid method by using UV spectroscopy. The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. The phenol-sulfuric acid method is a simple and rapid colorimetric method to determine total carbohydrates in a sample. The method detects virtually all classes of carbohydrates, including mono-, di-, oligo-, and polysaccharides. These compounds then react with phenol to produce a yellow-gold color.

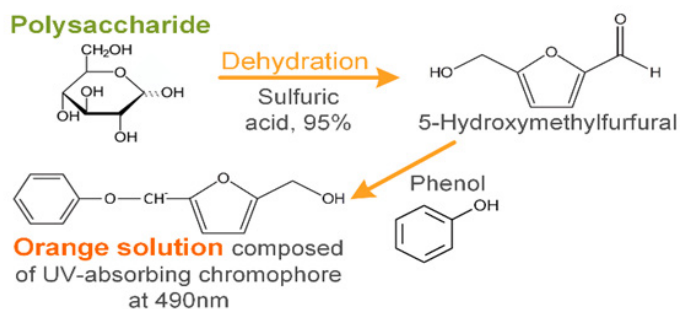


Figure 1: Reaction to find the absorbance of cellulose at 490nm

Among many colorimetric methods for carbohydrate analysis, the phenol-sulfuric acid method is the easiest and most reliable method. It has been used for measuring neutral sugars in oligosaccharides, proteoglycans, glycoproteins, and glycolipids. This method is used widely because of its sensitivity and simplicity.

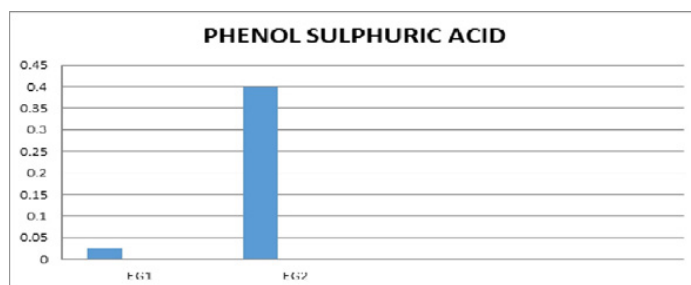


Figure 2: Graphical study of phenol Sulphuric acid assay

Another method for cellulose confirmation is Fourier Transform Infrared (FTIR). FTIR works essentially by applying infrared radiation (IR) to samples of materials, FTIR analysis measures a sample's absorbance of infrared light at various wavelengths to determine the material's molecular composition and structure. In FTIR analyses, Infrared light from the light source passes through a Michelson interferometer along the optical path. The Michelson interferometer comprises a beam splitter, moving mirror, and fixed mirror. FTIR analysis measures the range of wavelengths in the infrared region that are absorbed by a material. A simple device called an interferometer is used to identify samples by producing an optical signal with all the IR frequencies encoded into it. The signal can be measured quickly. The absorption bands are observed in wave number regions of 3300 - 4000 cm^{-1} .

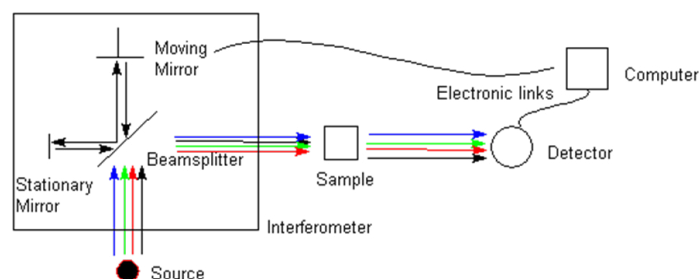


Figure 3: FTIR principle

The optimization was done in the ratio of 1:1. The components added to the cellulose materials are Polyvinyl Alcohol, Starch and Polyhexanide as the drug. These were added and optimized in the ratio of 1:1. The mixture was taken to produce nanofibers by using electrospinning instrument.

Electrospinning is a method to produce ultrafine (in nanometers) fibers by charging and ejecting a polymer melt or solution through a spinneret under a high-voltage electric field and to solidify or coagulate it to form a filament. The voltage applied was 18 KV. The flow rate was 1mL/hr. The temperature was 50 °C. The produced nanofiber was taken for the Antimicrobial assay.

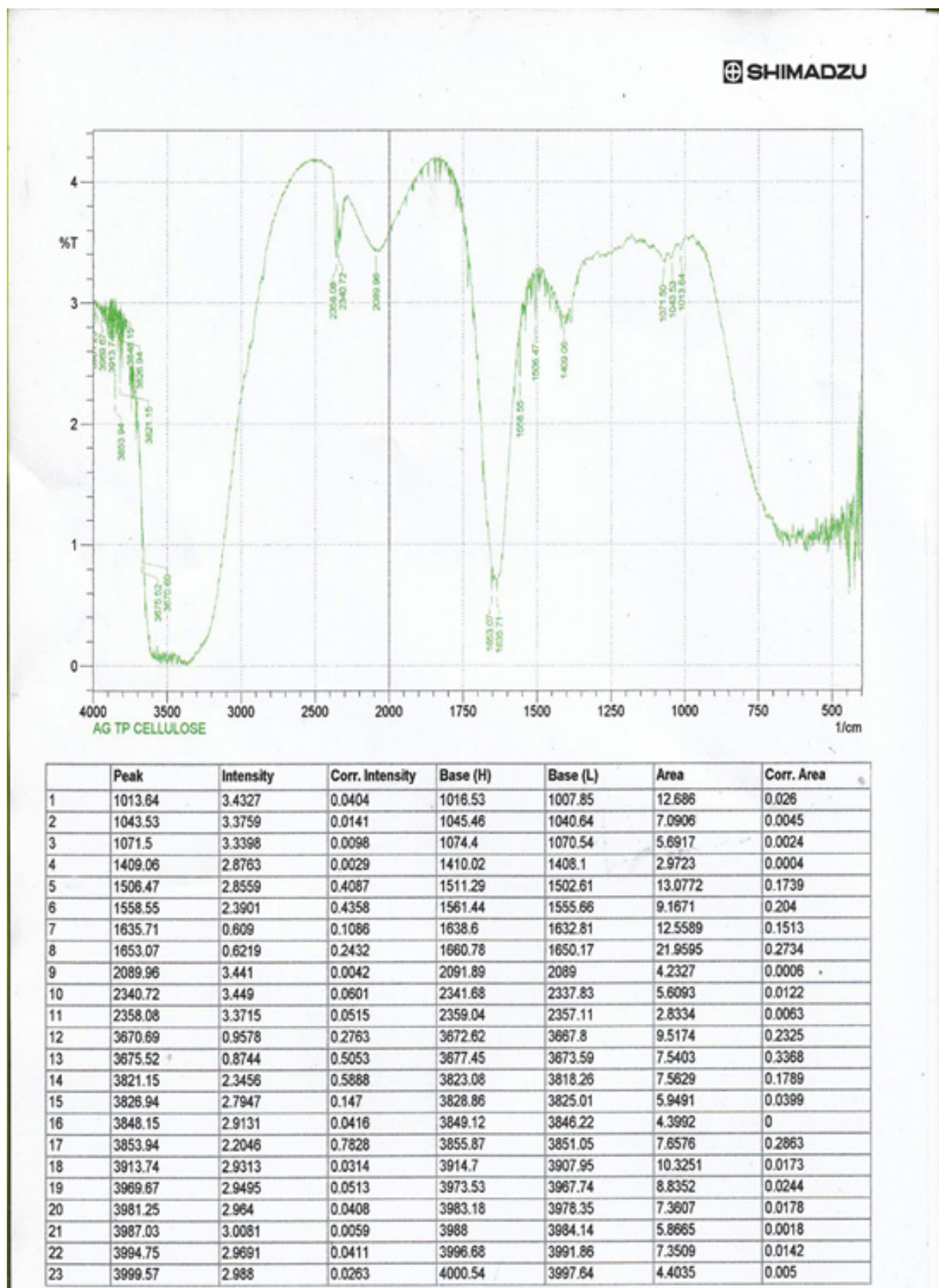


Figure 4: FTIR report

Antimicrobial assay:

Antimicrobial activity can be defined as a collective term for all active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial colonies, and may destroy microorganisms. Antibacterial activity is the most important characteristic of medical textiles, to provide adequate protection against microorganisms,

biological fluids, and aerosols, as well as disease transmission. Antimicrobial assay is being carried out in MHA agar which consist of Tryptone - 17.5g/100ml, Beef extract - 2.2g/100ml, Starch-1.7g/100ml, Agar-2g/100ml. And autoclave the nutrient agar and petriplate. After autoclave the nutrient agar was cool it then mixed with 1ml of staphylococcus culture, E.coli culture and staptococcus culture poured in petriplate. After pouring the agar was solidified.

After solidification the gel was cut the well and 500µl of sample was added and incubated for 37°C for 24 hours. The zone is being calculated by using a scale and then checked for its antimicrobial property. The Zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The zone of inhibition can be used to measure the susceptibility of the bacteria towards the antibiotic.

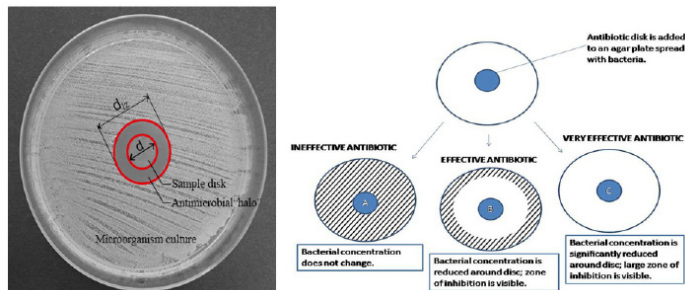


Figure 5: Antimicrobial Study and its analysis

The microorganisms used for antimicrobial activity includes *Klebsiella pneumoniae*, *Pseudomonas*, *Streptococcus pneumoniae*.

Klebsiella pneumoniae is a gram-negative bacterium that normally lives inside human intestines, where it doesn't cause disease. But if *K. pneumoniae* gets into other areas of the body, it can lead to a range of illnesses, including pneumonia, bloodstream infections, meningitis, and urinary tract infections. *Klebsiella* bacteria are mostly spread through person-to-person contact. Less commonly, they are spread by contamination in the environment. As with other healthcare-associated infections, the bacteria can be spread in a health care setting via the contaminated hands of health care workers.

Zone diameter: 0.95 cm

Pseudomonas species are Gram-negative, aerobic bacilli measuring 0.5 to 0.8, µm by 1.5 to 3.0 µm. Motility is by a single polar flagellum. Species are distinguished by biochemical and DNA hybridization tests.

Zone diameter: 1 cm

Streptococcus pneumoniae are lancet-shaped, gram-positive, facultative anaerobic bacteria with 100 known serotypes. Most *S. pneumoniae* serotypes can cause disease, but only a minority of serotypes produce the majority of pneumococcal infections. Pneumococci are common inhabitants of the respiratory tract.

Zone Diameter: 1.05 cm

Anti-inflammatory test:

The produced cellulose nanofiber was taken for the anti-inflammatory. Test for anti-inflammatory activity was done by using HRBC method. Human red blood cell membrane stabilization (HRBC method) has been used as a method in estimating the anti-inflammatory property. The present study aimed to authenticate that traditional information by both in vitro and in vivo anti-inflammatory screening. HRBC method was used for the estimation of anti-inflammatory activity in vitro. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were

separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged at 3 000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

$$\text{Percentage protection} = 100 - (\text{OD sample} / \text{OD control}) \times 100$$

Scanning electron microscope:

The Scanning electron microscope (SEM) is used to measure the produced nanofiber diameter. Scanning electron microscope (SEM) is one of the most widely used techniques used in characterization of nanomaterials and nanostructures. The signals that derive from electron-sample interactions reveal information about the sample including surface morphology (texture), chemical composition of the sample. Advances in scanning electron microscopy (SEM) enable the high-resolution imaging of single nanoparticles with sizes well below 10 nm.

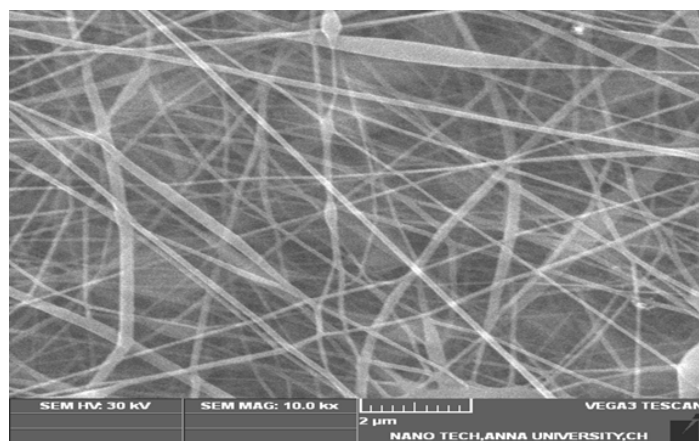


Figure 6: SEM image of the fiber

The diameter of the nanofiber is found to be 0.151. The nanofiber was found out to have pores so that it can intake oxygen for healing purpose and for the decaying of the nanofiber, so that it doesn't cause harmful effects.

CONCLUSION

Cellulose Nanofiber has effectively been used in the treatment of topical wounds and is of greater ability. Since it is of plant origin, it prevents the toxic effects to the body like itching, swelling etc. Cellulose is extracted from a common source almond gum which is easily available and has greater amount of cellulose in it. Cellulose is blended with starch, which is also a plant origin compound and has greater effect in wound healing property. Starch has a natural wound healing property which is why it is blended with cellulose. PVA is a synthetic polymer which is used in the nanofiber synthesis along with starch and cellulose. PVA maintains the tensile strength and helps to maintain the surface to volume ratio. It also helps in the proper drug distribution throughout the fiber. The method used for the production of nanofiber is electrospinning technique. And it provides the proper texture for the nanofiber and also the proper distribution. The voltage is being kept at 12KV. The flow rate is kept at 0.1ml/min and it is adjusted depending upon the nanofiber which is being produced. High voltage and potential difference is used in the production of nanofiber. The concentration of the components in nanofiber and PVA is optimized for the effective production

of nanofiber. The drug used in the nanofiber is polyhexanide which is effective against gram positive and gram negative bacteria. Concentration of the drug is also optimized for its effective activity. Finally the components are optimized and the fiber is produced by electro spinning method. The fiber produced is then checked for its antimicrobial activity in three different bacterial strains in MHA agar and then given for SEM analysis which confirms the surface to volume ratio and the tensile strength.

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