FABRICATION OF POLYVINYL ALCOHOL NANOFIBRES WITH SILVER NITRATE AND CHITOSAN FOR WOUND DRESSING

A PROJECT REPORT

Submitted by

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BONAFIDE CERTIFICATE

Certified that this project report **"FABRICATION OF POLYVINYL ALCOHOL NANOFIBERS WITH SILVER NITRATE AND CHITOSAN FOR WOUND DRESSING"** is the bonafide work of **B.SURESH KUMAR, P.TAMILARASU ARUL PRASHANNA, S.VAITHILINGAM and S.VASANTHRAJ** who carried out the project work under my supervision.

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ABSTRACT:

Electrospinning is a technique that is used to produce nanofibres and is gaining wide popularity among researchers. Polyvinyl alcohol (PVA) offers a combination of cost efficient, non toxic and performance. In this work, the optimum concentration of PVA required to produce good fibers is determined. PVA is taken as the base polymer and is blended with Silver nitrate (AgNO3) in the concentration PVA/AgNO3 – 10/0.1 wt %. Chitosan(Cs), a chemical that has excellent bio compatibility, easily available is made into a solution with 90 % aqueous acetic acid. The Chitosan solution is blended with PVA solution in two different proportions, PVA/Cs – 70/30 and 50/50. The two blends are electrospun and are characterized by FTIR analysis and SEM morphology. The PVA/AgNO3 solution when electrospun, produced fibers that were light yellow in color, hence those fibers were analysed using EDAX. The PVA/Cs and PVA/AgNO3 fibers are subjected to Anti bacterial, Anti Allergic and Anti Inflammatory test for applications in wound dressing.

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INTRODUCTION

1.1Nanotechnology and nanofibers:

Nanotechnology is predicted as the second industrial evolution in the world.

Nanotechnology is an emerging interdisciplinary area that is exposed to have wide ranging implications in all fields of science and technology such as material science, mechanics, electronics, optics, medical textiles, plastics, energy, aerospace etc. Nanophasic and nanostructured materials are also attracting a great deal of attention of the textile and polymer researchers and industrialists because of their potential applications for achieving specific processes and properties, especially for functional and high performance textile applications.

Nanofibers are traditionally defined as nanostructures with a diameter below 1000 nm and a length-to-width ratio typically greater than50.

The origins of nanofibers can be traced back to the end of the 19th century, when the physical phenomena occurring during the electrospinning process were first observed. Since then, however, nanofibers have been produced in limited quantities, typically in a laboratory setting, largely due to very low process throughput.

Over the years, several types of nanofibers have been developed: polymeric, carbon, ceramic, glass, metallic, and composite .

During the last 6 years, the number of research activities resulting in patent applications and issued patents has increased rapidly, by almost a factor of 15, leading to the development of mass-production fabrication methods, innovative

compositions, and a large variety of applications spanning many industrial sectors.

The growing interest in the utilization of these nanostructures primarily stems from their unique physical, mechanical, and electrical properties associated with their very high surface area. These properties make nanofibers suitable for the creation of numerous technologically advanced products within many fields of application. With development activities related to nanofiber technology intensifying rapidly, one can reasonably project that these nanostructures will achieve widespread commercialization within the next 5 to 10 years.

There is also a need to evaluate the current status and future trends of the nanofiber industry from a global standpoint. As use of this technology expands, information on regional production, sales, and type of suppliers, becomes more valuable.

A survey conducted by BCC showed the increase in global market for nanofibers:

- The global market for nanofibers increased from $43.2 million in 2006 to an estimated $48.0 million by the end of 2007. It should reach $176 million in 2012 and grow to $825 million by 2017, compound annual growth rates (CAGR) of 30% and 36%, respectively.

- The mechanical/chemical sector is projected to account for 73.5% of total revenues in 2007. Other key sectors are energy and electronics.

- Nanofibers are generating great interest in certain industry segments, where alternative materials are characterized by limited performance or much

higher unit prices (a good example is the utilization of carbon nanofibers as an alternative to carbon nanotubes for electron emitters in flat panel displays).

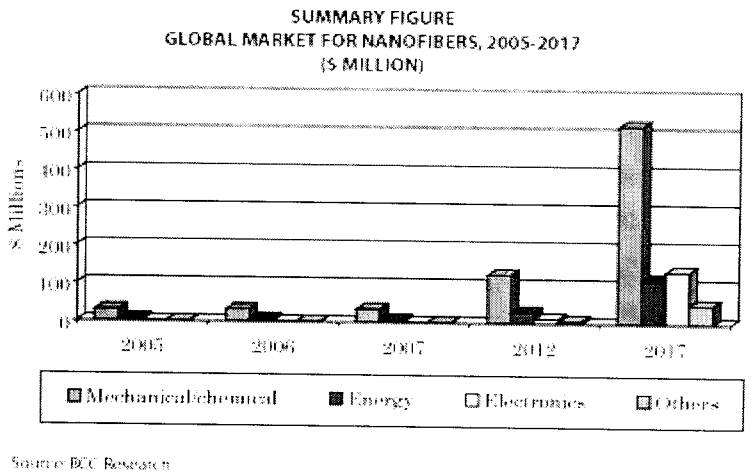


Fig 1.1. Global Market for Nanofibers

1.2 Nanotechnology in Textile:

The nanotechnology research in the textile area mainly centres on creating unique properties in everyday fabric such as self-cleaning, water and oil repellence, stain proof, anti bacterial, UV protective, antistatic, improved moisture regain and comfort in synthetic based textiles but without compromising the original hand, breathability and durability of the fabric. It also shows promising applications in developing advanced textile materials such as composite nanofibers and other nanomaterial incorporated textiles for applications in medical, defence, aerospace and other technical textile applications such as filtration, protective clothing besides a range of smart and intelligent textiles.

The impact of nanotechnology in the textile finishing area has brought up innovative finishes as well as new application technique. Particular attention has been paid in making chemical finishing more controllable and more thorough.

Ideally, discrete molecules or nanoparticles of finishes can be brought individually to designated sites on textile materials in a specific orientation and trajectory through thermodynamic, electrostatic or other technical approaches.

Nanofibers are an exciting new class of material used for several value added applications such as medical textiles, filtration, barrier, wipes, personal care, composite, garments, insulation, and energy storage. Special properties of nanofibers make them suitable for a wide range of applications from medical to consumer products and industrial to high-tech applications for aerospace, capacitors, transistors, drug delivery systems, battery separators, energy storage, fuel cells, and information technology .

Nano-fibers also have a great scope in the medical textiles in tissue engineering, scaffold engineering, wound dressing, drug delivery and wound healing applications.

CHAPTER-2

LITERATURE REVIEW

2.1 Polymer Nanofibers - an Overview of Applications and Current Research into Processing Techniques

Polymer fibers are ubiquitous in many spheres of human life. Clothing, apparels, cosmetics, cigarette filters, air conditioning filters, fishing nets, composites, surgical masks, extracorporeal devices, vascular grafts, heart valves, are to name a few examples. Fibers used in these applications are typically in 5 to 50 micrometer diameter range, and made from a variety of polymers of both synthetic and natural origin. According to one estimate, the world fiber consumption is reaching 60 million tons per annum.

Both, the surface area to weight ratio and surface area to volume ratio of fibers increase significantly. Increased surface area means a feasibility to enhance the functionality of fibers. This would lead to a highly sensitive filter made of polymer nanofibers that is able to selectively capture viruses or bacteria and able to neutralize or kill them.

Researchers around the world are searching for ways to produce polymer nanofibers. There are more than 70 research groups worldwide investigating polymer nanofibers produced by electrospinning method. Year 2003 alone saw publication of over 200 articles in this emerging field. Figure (see below) illustrates the broad domains of polymer nanofibers that are being actively researched on. About 50% of the papers are focused on the electrospinning process development and characterization of fibers. Others are focused on innovative use of polymer nanofibers for a variety of applications in medicine, biotechnology, and engineering.

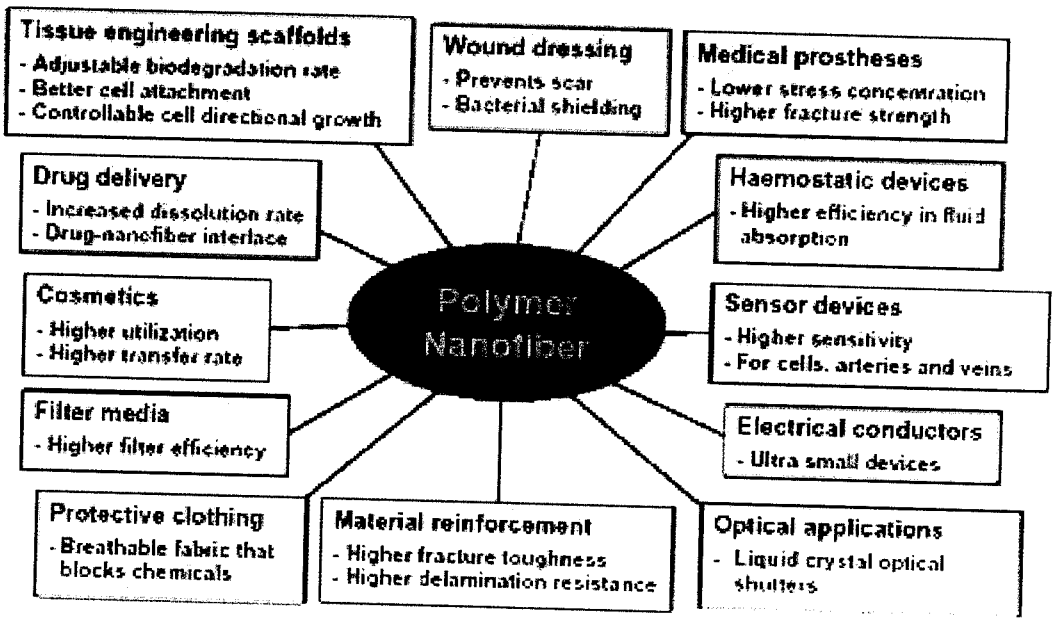


Fig **2.1** Potential applications of polymer nanofibers.

2.2 Electrospinning: A fascinating fiber fabrication technique:

With the emergence of nanotechnology, researchers become more interested in studying the unique properties of nanoscale materials. Electrospinning, an electrostatic fiber fabrication technique has evinced more interest and attention in recent years due to its versatility and potential for applications in diverse fields. The notable applications include in tissue engineering, biosensors, filtration, wound dressings, drug delivery, and enzyme immobilization. The nanoscale fibers are generated by the application of strong electric field on polymer solution or melt. The non-wovens nanofibrous mats produced by this technique mimics extracellular matrix components much closely as compared to the conventional techniques. The sub-micron range spun fibers produced by this process, offer various advantages like high surface area to volume ratio, tunable porosity and the ability to manipulate nanofiber composition in order to get desired properties and function. Over the years, more than 200 polymers have been electropun for various applications and the number is still increasing

gradually with time. With these in perspectives, we aim to present in this review, an overview of the electrospinning technique with its promising advantages and potential applications. We have discussed the electrospinning theory, spinnable polymers, parameters (solution and processing), which significantly affect the fiber morphology, solvent properties and melt electrospinning (alternative to solution electrospinning).

2.3 Molecular weight dependent structural regimes during the electrospinning of PVA

The cumulative effects of molecular weight and concentration on the structural transitions in the electrospun polymer have been studied. Experiments have been conducted with water as the solvent for molecular weights of polyvinylalcohol (PVA) ranging from 9500 g/mol to 155,000 g/mol. The structural regimes for beads, beaded fibers, complete fibers and flat ribbons have been mapped. The development of a stable fiber structure generally corresponds to the onset of significant molecular entanglements.

The molecular weight of the polymer has a significant role in establishing the structure in the electrospun polymer. The molecular weight dependent concentration regimes for beads, beaded fibers and fibers have been mapped. At a constant concentration, the structure changes from beads, to beaded fibers, to complete fibers and to flat ribbons as the molecular weight is increased.

Lower molecular weight polymers require a higher concentration of the polymers to produce good, round fibres, whereas higher molecular weight require a much lower concentration.

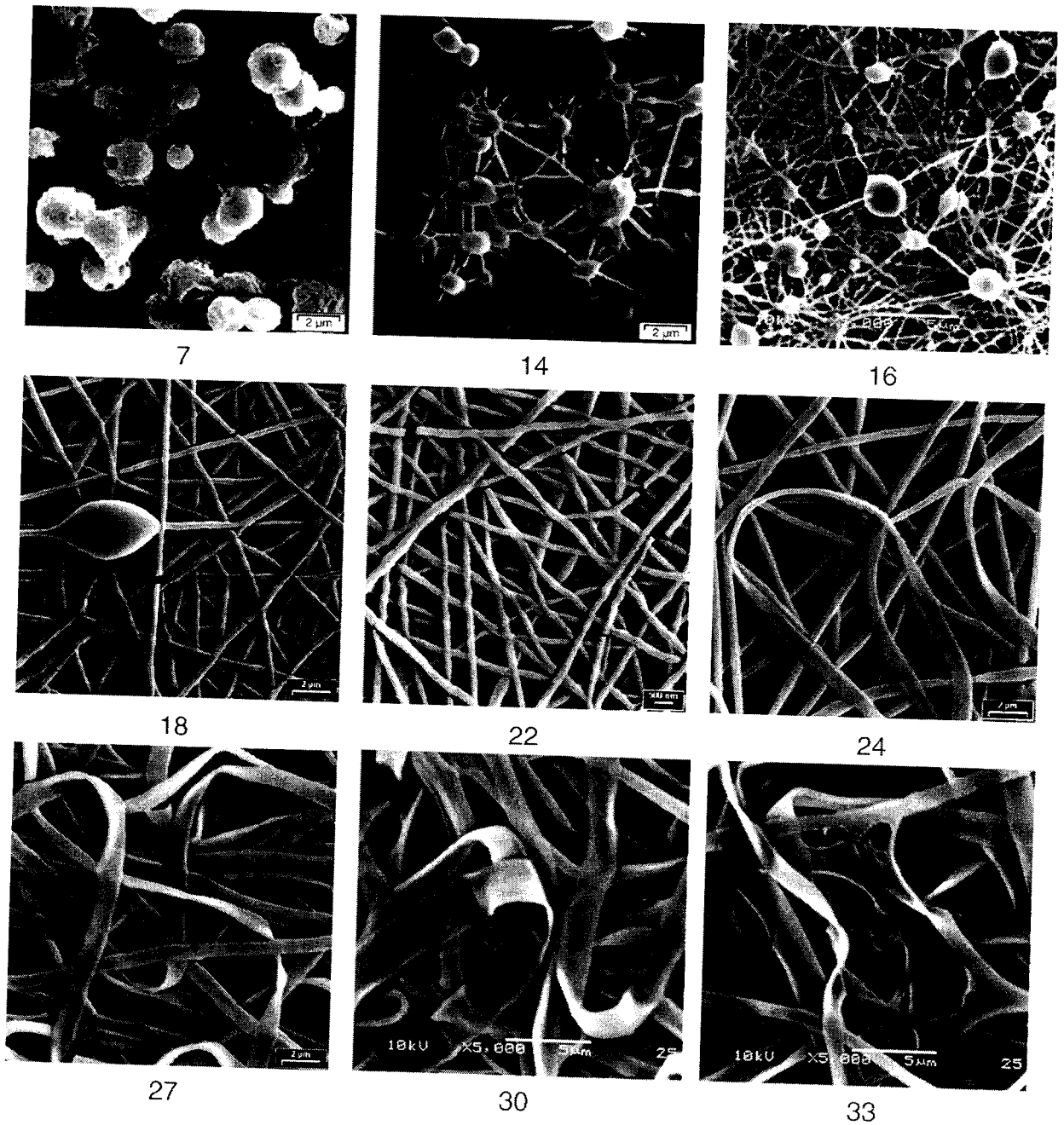


Fig 2.2 SEM image of PVA nanofibers at different concentrations.

This picture shows SEM photos of the nanofibers electrospun with PVA of molecular weight 18,000 g/mol, at different concentrations (wt%). The optimum concentration for the molecular weight was found out to be 22 wt%.

2.4 Preparation of Antimicrobial Poly(vinyl alcohol) Nanofibers Containing Silver Nanoparticles:

2.4.1 Introduction:

Polyvinyl alcohol (PVA) nanofibers containing Ag nanoparticles were prepared by electrospinning PVA/silver nitrate (AgNO3) aqueous solutions, followed by short heat treatment, and their antimicrobial activity was investigated for wound dressing applications. Since PVA is a water soluble and biocompatible polymer, it is one of the best materials for the preparation of wound dressing nanofibers. After heat treatment at 155 8C for 3 min, the PVA/AgNO3 nanofibers became insoluble, while the Ag+ ions therein were reduced so as to produce a large number of Ag nanoparticles situated preferentially on their surface. The residual Ag+ ions were reduced by subsequent UV irradiation for 3 h. The average diameter of the Ag nanoparticles after the heat treatment was 5.9 nm and this value increased slightly to 6.3 nm after UV irradiation. It was found that most of the Ag+ ions were reduced by the simple heat treatment. The PVA nanofibers containing Ag nanoparticles showed very strong antimicrobial activity.

2.4.2 Experimental:

PVA (98–99% hydrolyzed granules; typical Mw,85,000–124,000) and AgNO3(99.998%) were purchased from Aldrich. To prepare the PVA nanofibers containing the Ag nanoparticles, a PVA/AgNO3 (10/0.1 wt %) aqueous solution was electrospun. The pH value of the PVA/AgNO3 solution was adjusted to 4 with an aqueous solution of HNO3, to prevent the reduction of the Ag+ ions. The electrospinning setup used in this study consisted of a hypodermic syringe, a plastic tip (ID ¼ 0.95 mm), a graphite electrode(black lead), an aluminum collecting drum, and high voltage supply .The syringe pump

connected to the hypodermic syringe controlled the flow rate. The PVA/AgNO3 aqueous solution was electrospun at a positive voltage of 20 kV, a tip-to-collector distance of 11.5 cm, and a solution flow rate of 0.66 mL/h.

2.4.3 Reduction of Ag+ Ions:

To stabilize the PVA/AgNO3 nanofiber webs and reduce the Agþ ions there in, the nanofiber webs were placed between the hot plates of a hot press. The plates were set at 155° C and placed about 1 cm apart. A ultra-violet (UV) lamp (UV-A, 315–380 nm, 10 W) was used for the photoreduction treatment.

2.4.4 Generation and Characterization of the Ag Nanoparticles in the PVA Nanofibers:

When the PVA/AgNO3 nanofiber web was heat treated at 155 8C for 3 min, its color changed from white to light yellow, as shown in below figure. This indicates that the Ag+ ions in the PVA/ AgNO3 nanofibers were reduced and aggregated into Ag nanoparticles during the heat treatment. The size of the PVA/AgNO3 nanofiber webs was slightly decreased after the heat treatment.

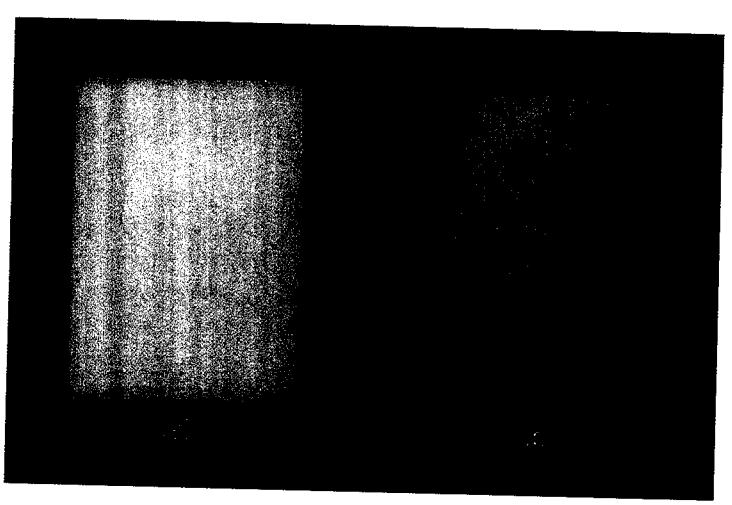


Fig 2.3 PVA/AgNO3 nanofiber webs (a) before and (b) after heat treatment at 155 °C for 3 min.

2.5 Silver nanoparticles as a new generation of antimicrobials:

Silver has been in use since time immemorial in the form of metallic silver, silver nitrate, silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. But due to the emergence of several antibiotics the use of these silver compounds has been declined remarkably. Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize, which drastically changes the chemical, physical and optical properties of metals. Metallic silver in the form of silver nanoparticles has made a remarkable comeback as a potential antimicrobial agent. The use of silver nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics. Hence, silver nanoparticles have emerged up with diverse medical applications ranging from silver based dressings, silver coated medicinal devices, such as nanogels, nanolotions, etc.

2.5.1 Mechanism of action of silver nanoparticles

The silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death.

The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity

2.5.2 Mechanism of action of silver ions/AgNO3

The mechanism for the antimicrobial action of silver ions is not properly understood however, the effect of silver ions on bacteria can be observed by the structural and morphological changes. It is suggested that when DNA molecules are in relaxed state the replication of DNA can be effectively conducted. But when he DNA is in condensed form it loses its replication ability hence, when the silver ions penetrate inside the bacterial cell the DNA molecule turns into condensed form and loses its replication ability leading to cell death. Also, it has been reported that heavy metals react with proteins by getting attached with the thiol group and the proteins get inactivated.

2.6 Spinnability and Defect Formation of Chitosan/Poly Vinyl Alcohol Electrospun Nanofibers:

This work is focused on the influence of poly vinyl alcohol (PVA) on the formation of chitosan (CS) nanofiber. PVA with different molecular weights, 72,000 and 145,000, and CS with a molecular weight of 180,000 were used. Blend solution was prepared from chitosan and poly vinyl alcohol in water by varying %PVA for a given % of CS. The amount of PVA was varied from 1 to 11 % wt/vol and CS was varied 1 to 3 % wt/vol. It was found that the content of PVA had to be not less than 2% wt/vol to initiate the fiber formation for all chitosan contents. Spinnability was good when using chitosan contents of 1 and 1.5 % wt/vol. PVA with larger molecules seemed to help stabilize the

spinnability when increasing the polymer concentration in the appropriate spinnable range and could prevent a sharp increase of nanofiber diameter for the whole range of polymer concentrations. In addition, chitosan/PVA nanofibers seemed to have fewer defects than PVA nanofibers

2.6.1 Experimental

Water soluble chitosan (degree of deacetylation of 94%) which has a molecular weight of 180,000 was synthesized in our laboratory. Poly vinyl alcohol with different molecular weights of 72,000 and 145,000 were obtained from Merck and were used as received

2.6.2 Preparation of the aqueous solution of Chitosan/Poly(vinyl alcohol)

The maximum solubility of chitosan in water was 3% wt/vol. For a given concentration of chitosan which was 1, 1.5, 2, or 3 %wt/vol., PVA was added at various amounts ranging from 1 to 11 % wt/vol. First, PVA had to be dissolved in 12 ml hot water (about 80-90 °C). The solution was stirred rigorously until complete dissolution was obtained. Then, the specified amount of chitosan was added and continuously mixed until the solution was homogeneous.

2.6.3 Nanofiber formation by electrospinning

2 ml solution was poured into a syringe held by a stall. The positive electrode from the positive power supply was connected to a syringe metal tip, while the other was connected to a plate collector wrapped by a piece of aluminum foil being used as ground. The applied voltage was fixed at 18kV and the distance between the tip and the collector was set at 9 cm. The spinning time was 5 min for all samples.

2.6.4 Morphology characterization

The deposited foil was cut into small piece and attached to a brass stub with carbon tape. All samples were then coated with a gold sputtering device before being investigated under the scanning electron microscope (SEM).

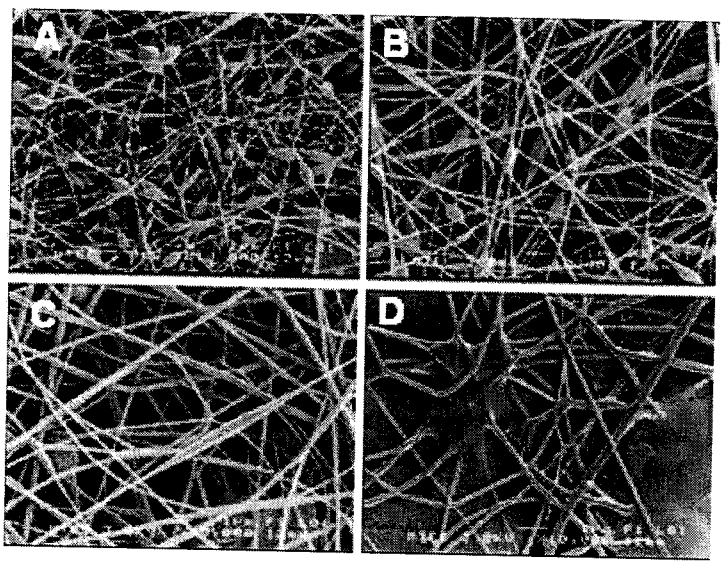


Fig2.4 Morphologies of nanofibers produced from spinning solutions which contain 1.5% wt/vol chitosan and various poly(vinyl alcohol) contents: (A) 3%, (B) 5%, (C) 7%, (D) 8%. PVA has Mw = 145,000.

2.7 Chitin and chitosan polymers: Chemistry, solubility and fiber formation:

Chitin and chitosan (CS) are biopolymers having immense structural possibilities for chemical and mechanical modifications to gencerate novel properties, functions and applications especially in biomedical area. Despite its huge availability, the utilization of chitin has been restricted by its intractability

and insolubility. The fact that chitin is as an effective material for sutures essentially because of its biocompatibility, biodegradability and non-toxicity together with its antimicrobial activity and low immunogenicity, points to immense potential for future development. Despite its huge annual production and easy availability, chitin still remains an under utilized resource primarily because of its intractable molecular structure [10,16]. The non-solubility of chitin in almost all common solvents has been a stumbling block in its appropriate utilization.

Chitin fibers stand apart from all the other biodegradable natural fibers in many inherent properties suchas biocompatibility, non-toxicity, biodegradability, low immunogenicity, nontoxicity, etc. These properties in combination with good mechanical properties make them good candidate materials for sutures that form the largest groups of material implants used in human body. It was reported that the chitin suture was absorbed in about 4 months in rat muscles. Chitin's micro-fibrillar structure indicated its potential as fibre- and film-former, but as chitin was found to be insoluble in common organic solvents, the N-deacetylated derivative of chitin, CS, was developed.

Table 2.1 General properties of chitin and chitosan(CS):

Property	**Chitin**	**Chitosan**
Molecular wt	(1–1.03)×106 to 2.5×106	105 to 5×103
Degree of deacetylation	~10%	60–90
Viscosity of 1% soln. in 1% acetic acid, cps	-	200–2000
Moisture Content	-	6–7
Solubility	DMAc – LiCl /TCA–MC	Dilute acids TCA–MC

2.8 Study of Wound-Healing Properties of Chitosan:

Of the presently existing broad assortment of polymercoatings for wounds and burns, resorbable coatings maximally meet all biomedical requirements and can be useful both at early and later stages of treating wounds and burns. Consequently, the development of resorbable, adhesive polymer coatings with various times of biodegradation is an urgent direction in the creation of effective applications on wounds and burns. The natural polysaccharide chitosan has a broad spectrum of action. It derivatives regulate proliferation of fibroblasts and stimulate normal regeneration of skin. The analgesic and antimicrobial action is due to the unique ability of chitosan to interact nonspecifically with pain receptors and the cell wall of microorganisms.

One of the causes of the effective influence of chitosan on healing wounds is the stimulating effect on the immune system since it can be regarded as an analogue of liposaccharides of cells walls of microorganisms, performing the role of activators of macrophages. A substantial problem of postburn areas is scars forming at places of skin regeneration. The use of chitin and its derivatives allows a considerable reduction of hyperproliferation of granulation tissue. It is known that chitin derivatives have structural characteristics similar to glucosamines of the skin and can serve as a substrate for the growth of keratinocytes and fibroblasts.

2.9 Electrospinning of chitosan dissolved in concentrated acetic acid solution

2.9.1. Experimental

Chitosan nanofibers were electrospun from aqueous chitosan solution using concentrated acetic acid solution as a solvent. A uniform nanofibrous mat of average fiber diameter of 130nm was obtained from the following optimum condition: 7% chitosan solution in aqueous 90% acetic acid solution was successfully electrospun in the electric field of 4 kV/cm. The aqueous acetic acid concentration higher than 30% was prerequisite for chitosan nanofiber formation, because more concentrated acetic acid in water progressively decreased surface tension of the chitosan solution and concomitantly increased charge density of jet without significant effect on solution viscosity. However, acetic acid solution more than 90% did not dissolve enough chitosan to make spinnable viscous concentration. Only chitosan of a molecular weight of 106,000 g/mol produced bead-free chitosan nanofibers, while low- or high-molecular-weight chitosans of 30,000 and 398,000 g/mol did not. Average fiber diameters and size distribution decreased with increasing electric field and more bead defects appeared at 5 kV/cm or more.

2.9.2 Electrospinning

High surface charge densities enhance a whipping mode rather than axisymmetric mode, where bending of the jet produces highly stretched polymeric fiber with simultaneous rapid evaporation of the solvent. Important parameters in electrospining are not only polymer and solution properties such as molecular weight, viscosity, conductivity and surface tension, but also electrospinning conditions such as applied electric voltage, tip-to collector distance, feeding rate, etc. .

2.9.3 Chitosan merits:

Nanofibers have amazing characteristics such as very large surface area-to-volume ratio and high porosity with very small pore size. Therefore, nanofibers can be promising materials for many biomedical applications such as tissue templates, medical prostheses, artificial organ, wound dressing, drug delivery, pharmaceutical composition . It seems more difficult to produce biomacromolecular nanofibers due to their limited solubility to most organic solvents, ionic character in dissolved state and three-dimensional networks of strong hydrogen bonds. Chitosan is an N-deacetylated product of chitin, the second -most abundant natural polysaccharide next to cellulose, which is embedded in a protein matrix of a crustacean shell or a squid pen. Chitosan has many useful properties such as biocompatibility, biodegradability, antimicrobial activity, wound healing property, antitumor effect, etc. Electrospinning of chitosan solution has been reported. While both produced blend nanofiber of chitosan/poly(ethylenoxide) or poly(vinyl alcohol) from the chitosan solution mixed with PEO or PVA, the latter also reported that electrospinning of homogeneous chitosan nanofiber using trifluoroacetic acid/dichloromethane solvent, which requires additional extraction of the organic solvents. And it was also mentioned by the latter that chitosan solution dissolved in 0.2M acetic acid and its solvent mixtures with various volatile organic solvents or aprotic solvents cannot produce chitosan nanofiber via electrospinning. However, we electrospun homogeneous nanofibers of pure chitosan dissolved in strongly concentrated aqueous acetic acid solution without addition of other solvents, which have not been reported yet. It is considered that the surface tension depression produced by increasing acetic acid concentration in water is the most important solution factors in the electrospinning of chitosan.

CHAPTER 3

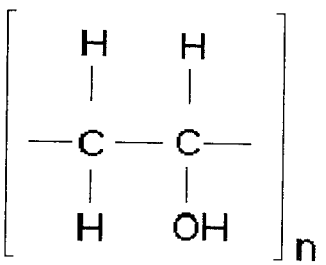
MATERIALS AND METHODS

3.1 MATERIALS:

3.1.1. Poly Vinyl Alcohol:

Manufactured by NICE Chemicals

Chemical formula of PVA:



Specifications:

- Molecular weight – 1,15,000 g/mol approx
- Ash =1% max
- Degree of hydrolysis = 98%. min
- Viscosity of 4% aqueous solution at 20°C = 25-32 CPs

Characteristics:

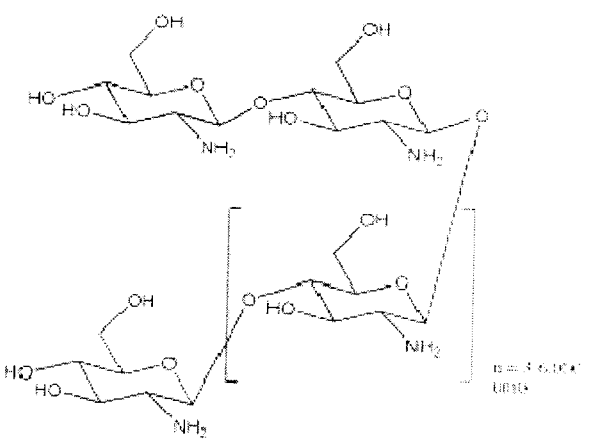
- Polyvinyl alcohol (PVOH, PVA, or PVAL), a synthetic polymer is an odourless and tasteless, translucent, white or cream coloured granular powder.
- It is soluble in water and a 5% solution of polyvinyl alcohol exhibits a pH in the range of 5.0 to 6.5.

- Polyvinyl alcohol has a melting point of 180 to 190°C.
- PVA is nontoxic.
- It has high tensile strength and flexibility.
- Polyvinyl alcohol, has excellent film forming, emulsifying, and adhesive properties. It is also resistant to oil, grease and solvent.

3.1.2 Chitosan (Cs), from crab shells:

Manufactured by SIGMA-ALDRICH

Chemical Formula:



Specifications:

- Average molecular weight - 5,000 g/mol
- Total impurities: ≤1% insoluble matter
- Ign. residue : ≤2% (as SO_4)
- Loss on drying: ≤ 10%
- Viscosity: <200 mPas, 1 % in acetic acid(20 °C)

- IUPAC Name = 2-Amino-2-deoxy-(1→4)-β-Dglucopyranan.

Characteristics:

- Chitosan has excellent biocompatibility and admirable biodegradability with ecological safety and low toxicity.
- Versatile biological activities such as antimicrobial activity and low immunogenicity.
- Chitosan is soluble in most of the acids.
- Chitosan is hypoallergenic, and has natural anti-bacterial properties.
- It is essentially a natural derivative of cellulose with unique properties.
- Chitosan is inexpensive, biodegradable, and nontoxic for mammals.

3.1.3 Silver Nitrate (AgNO3):

Manufactured by Nice Chemicals.

Specifications:

Molecular weight – 169.08 g/mol

3.2 Methodology:

3.2.1Electrospinning:

Solution based electrospinning is a unique method of producing continuous polymer fibres. It has received great deal of attention in recent times due to its versatility in the spinning of wide variety of polymeric fibres and its consistency in producing polymeric fibres with fibre diameter on submicrometer or nanometre scale. The as-spun fibres, often in the form of a

non-woven mat, have an extremely high surface area to mass ratio and highly porous structure.

The electrospinning apparatus consists of a nozzle (syringe) that contains the polymer solution, collector (aluminium foil) and these two are connected to a voltage source. The syringe is connected to a syringe pump, which pumps out the solution according to the flow rate. Both the emitter and the collector are placed at some distance apart which is generally the spinning distance. The syringes used for electrospinning are normal disposable syringes.

3.2.2 Parameters affecting electrospinning and fibre morphology:

Operating parameters:

- Applied electrical field.
- Flow rate of polymer solution.
- The distance between the nozzle and the collector (spinning distance).
- The diameter of the spinneret.

Material parameters :

- Polymer concentration
- Solution viscosity
- Surface tension.

3.2.3 Solvent evaporation:

One of the most fascinating features of electrospinning is the ability of the spun jet to oust small solvent molecules during extremely short periods of

time when the jet travels from the electrode to the collector. In other words, the elongated and narrowed whipping jet operates as a significant operative drier even at room temperature. The principle of the phenomenon leading to the evaporation of more then 80% of the solution from the jet during a fraction of second has its explanation in the realm of thermo-dynamics.

3.3 Production:

3.3.1 Solvent preparation:

Formula considered:

To determine amount of solute (polymer), when concentration is considered in weight (wt) % is:

Amount of solute = Desired amount of solution

X

(Given concentration/100)

Note:

Solvent = solution – solute

3.3.1.1 Polyvinyl alcohol:

- Poly vinyl alcohol, being a water soluble polymer was dissolved in distilled water.
- The concentrations considered for preparing the solution were 9.5 wt % and 10.7 wt %.

- The solutions were dissolved by stirring and simultaneously heating it up at 65° C for three hours.
- The optimum concentration will be determined depending on the spinnability of the fibres.

3.3.1.2 Chitosan:

Chitosan was dissolved using various solvents.

The solvents used were:

- Distilled water.
- Acetic acid and water in 90:10 ml ratio.
- Formic acid and Ethanol in 80:20 ml ratio .
- Trifluoro acetic acid and Dimethyl formamide in 80:20 ml ratio.

All solutions were stirred and simultaneously heated up at 50° C for a period of four hours.

After heating, stirring was continued for 5 hours.

From the above we infer:

- Acetic acid and water was found to be dissolved with Chitosan and was found to be soluble with suitable viscosity which was optimum condition requirement for electrospinning.
- Formic acid and Ethanol dissolved with Chitosan was found to be soluble but the viscosity was found to be less which did not apt for the electrospinning.

Hence, acetic acid and water was the suitable solvent for Chitosan.

3.3.1.3 Silver nitrate:

- PVA/Agno3 was taken in the ratio 10/0.1 wt% in same distilled water.

For example:

1.5 grams of PVA (10 wt% of 15 grams of solution)

0.015 grams of PVA (0.1wt% of 15 grams solution)

are mixed with 13.485 grams of distilled water to produce a solution of weight 15 grams.

- Few drops of concentrated Nitric acid(Hno3) was added in the solution in order to maintain the pH level.
- Stirring and mild heating at 40°C was applied for one hour. Stirring was continued for three hours.

3.3.2. Electrospinning:

The solution is transferred to a disposable 2 ml syringe of needle size 0.55 x 25 mm. The collector used is an aluminium foil.

Generally there are two types of collectors:

- Static collectors
- Dynamic collectors

Static collectors:

Static collectors are generally stationary aluminium foils, slits of conducting material fixed to the base of the apparatus by means of a tape.

Dynamic collectors:

Dynamic collectors are drums wound with aluminium foil that rotate on its own axis. The speed of the drum can be varied.

Before electrospinning, check whether:

- The syringe has to be fitted into the holder properly.
- There is flow of liquid from the needle tip.
- The collector is placed in the spinning chamber and connected to the electrode.
- Enter the flow rate value in the Automated system.
- Apply high voltage and run the machine.

3.3.2.1.Polyvinyl alcohol:

The two solutions of 9.5 wt % and 10.7 wt % were taken for electrospinning at different parameters. The solutions are taken in syringes and electrospun. The concentration that has good spinnability (i.e) without needle clotting, no drops on the collector, beads free fibres is taken as the optimum concentration.

Table 3.1. Spinning conditions of polyvinyl alcohol

S.no	Polymer	Concentration *(wt %)*	Operating Parameters			Collector
			Applied voltage *(Kilo volts)*	Spinning distance *(cms)*	Flow rate *(ml/hour)*	
1.	PVA	9.5 %	25	20	0.25	Rotating drum
2.	PVA	9.5 %	20	17	0.25	Rotating drum
3.	PVA	10.7 %	20	12	0.2	Rotating drum
4.	PVA	10.7 %	22	7	0.35	Rotating drum
5.	PVA	10.7 %	20	12	0.45	Sheet collector
6.	PVA	10.7 %	20	12	0.45	Rotating drum
7.	PVA	10.7 %	20	12	0.5	Rotating drum
8.	PVA	10.7 %	20	12	0.55	Sheet collector

From Table 3.1. it is clear that PVA was experimented more with concentration 10.7 % and less with 9.5 % because the former had better spinnability, very less formation of drops on the collector and the needle did not get blocked. The flow rate was varied from 0.2 ml/hr to 0.55 ml/hr.

3.3.2.2.PVA/Silver nitrate:

The PVA/AgNO3 solution when dissolved is transferred to the syringe and is electrospun. PVA/AgNO3 showed good spinnability similar to the PVA of concentration 10.7 %. The slightly brown colored solution is transferred to the syringe and is electrospun.

Table 3.2. Spinning conditions of PVA/AgNO3

S.no	**Polymer**	**Concentration** *(wt %)*	**Operating Parameters**			**Collector**
			Applied voltage *(Kilo volts)*	**Spinning distance** *(cms)*	**Flow rate** *(ml/hour)*	
1.	PVA/AgNO3	10/0.1 %	20	12	0.3	Sheet collector
2.	PVA/AgNO3	10/0.1 %	20	12	0.35	Sheet Collector

From Table 3.2. we can see that only sheet collectors are used to collect the fibres so as to check whether there are drops incident on the collector surface.

3.3.2.3.PVA/Chitosan:

The chitosan-acetic acid solution is considered for blending as it is more easily soluble and has better viscosity when compared to the other solvents.

PVA and chitosan are blended at two different ratios:

- 50:50 (PVA:Cs)
- 70:30 (PVA:Cs)

Both these blends are then stirred again until they become a homogenous mixture. The solution is then electrospun.

Table 3.3. Spinning conditions of PVA/Chitosan

S.no	Polymer	Blend ratio	Operating Parameters			Collector
			Applied voltage (*Kilo volts*)	Spinning distance (*cms*)	Flow rate (*ml/hour*)	
1.	PVA/Cs	70/30	22	22	0.15	Sheet collector
2.	PVA/Cs	50/50	25	22	0.05	Sheet collector
3.	PVA/Cs	50/50	25	20	0.3	Sheet collector
4.	PVA/Cs	50/50	20	23	0.1	Sheet collector
5.	PVA/Cs	70/30	20	16	0.2	Sheet collector
6.	PVA/Cs	70/30	20	18	0.1	Sheet collector

From Table 3.3. we can see that sheet collectors are used to collect the fibres so as to check whether there are drops incident on the collector surface. The flow rate used here was pretty low, since the viscosity of chitosan used was very low.

3.4. Characterisation of nanofibers:

3.4.1. SEM analysis:

Nano fibres can be characterised by SEM photography. The SEM photography reveals the orientation of fibres, fiber diameter, presence of beads. The SEM photography is the first step to characterise nanofibers. SEM photography was taken in a Hitachi Model S-3200 Scanning Electron Microscope at a magnification of 10,000 X to 60,000 X at 15 KV.

3.4.2.FTIR analysis:

Shimadzu Fourier Transform Infrared Spectroscopy (FTIR) 8400S in ATR mode was used to assess the presence of chemical groups present in the fiber to that present in the solution form. The finger prints were obtained with wave numbers in the range of 4000 to 400 cm^{-1}, resolution of 4cm^{-1} and final 20 scans to reduce noise effects in measurements.

3.4.3.Anti-bacterial test:

Anti-bacterial test is performed by the method AATCC-147, which is a qualitative analysis done by using two bacterias, one gram positive and one gram negative bacteria. These microbes are made to grow on sterile petri dishes for 24 hours after incubation and their anti bacterial function is studied.

3.4.4. Anti Allergic test : Patch Test

Anti- allergic test is carried out to check whether the polymers and other chemical substances used were bio-compatible with the skin and the person subjected to wound dressing does not pick up any allergy due to the product.

3.4.5. Anti inflammatory test:

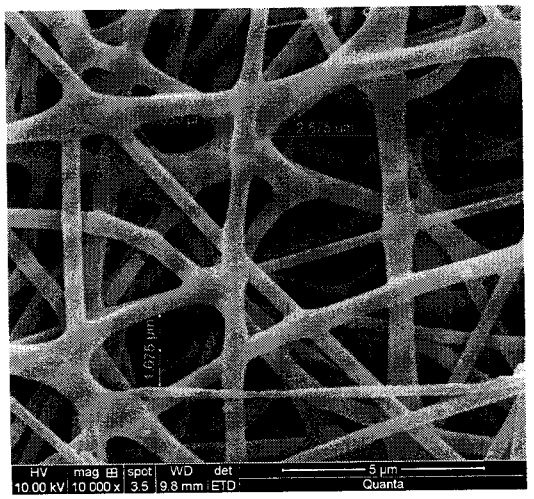
The normal anti inflammatory test is a long process and consists of five stages. The type of testing done for the nanofibre sample is that the microbes that are prone to develop in a patient's post operative wound's pus is isolated at the PSG Hospital, Coimbatore and these microbes are made to grow on sterile petri dishes for 24 hours. Any anti bacterial effect exhibited by the samples is related to the anti inflammatory effect of the corresponding sample.

CHAPTER 4

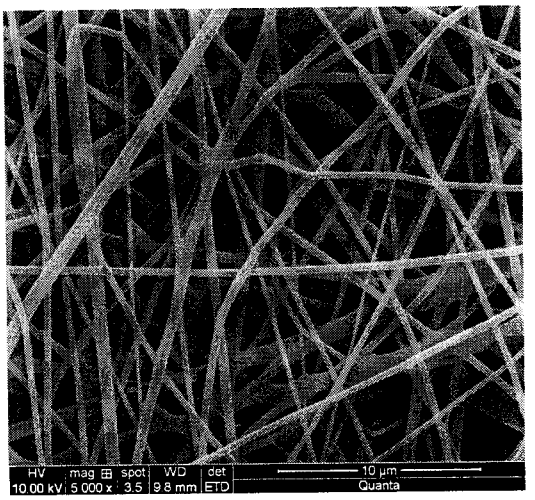
RESULT AND DISCUSSION

4.1 SURFACE MORPHOLOGY ANALYSIS

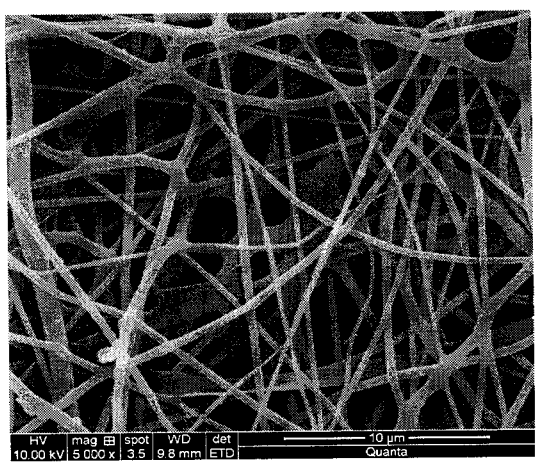
The Surface morphology of the produced PVA nanofibers are characterized by the SEM photos. Scanning Electron Microscopy(SEM) is one of the prime techniques to identify the presence of nanofibers. PVA fibers were used as the main component of the work and SEM micrographs show that PVA nanofibers have come out well as nanofibers. The SEM photos shows the orientation, diameter of the nanofibers and the fiber density present in the sample. In certain cases the nanofibers are coated with a conducting metal in order to transmit the electrons. Present below are a series of SEM micrographs of the PVA nanofibers, PVA/AgNO3 blend nanofibers and PVA/Chitosan blend nanofibers. Scanning Electron Microscope images were taken at a magnification of 10,000 X to 60,000 X at 15 KV.



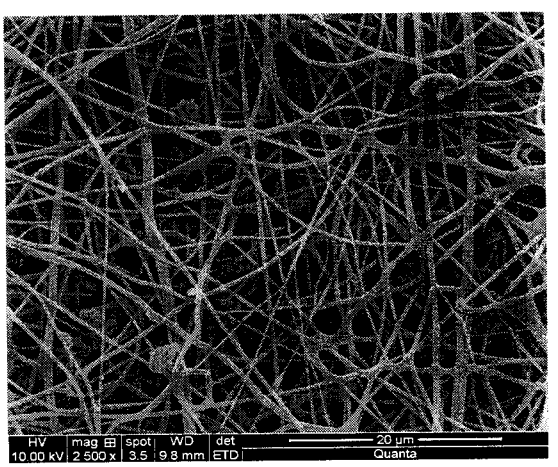
a) magnification- 10,000X



b) magnification- 5,000X



c) Magnification-5,000 X

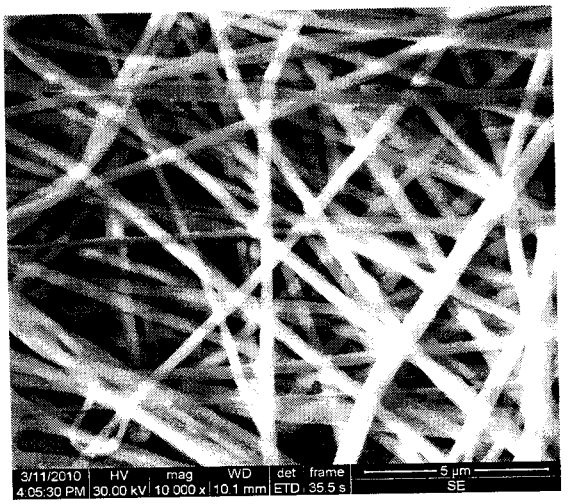


d) Magnification-2,500

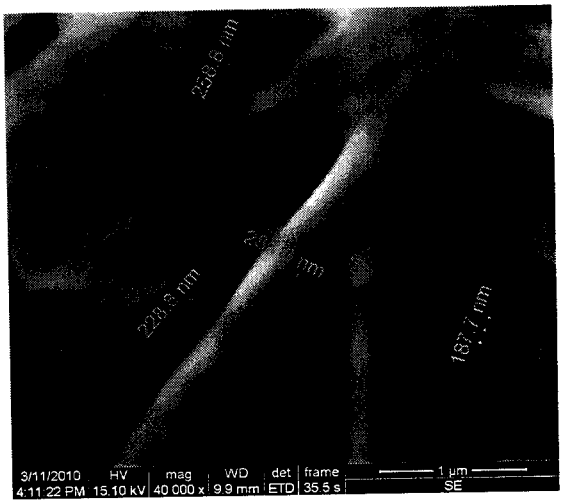
(Concentration 9.5%, Distance between the Emitter and Collector-17 cm. Spinning time-1.5 Hrs Voltage =20kv Flow-rate=0.25ml/hr.)

Fig. 4.1 SEM Micrograph of PVA Nanofibre of Concentration 9.5%

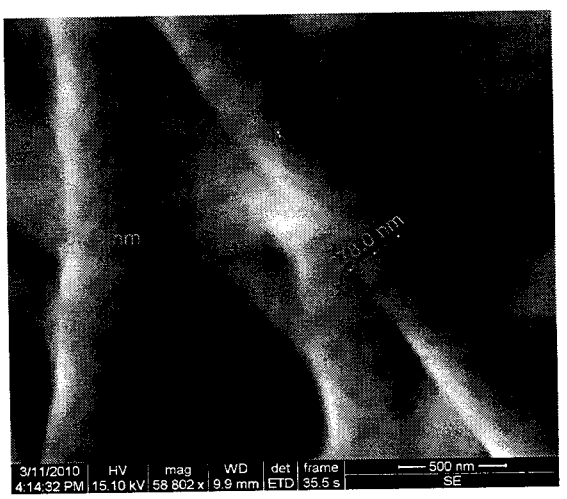
The SEM images of PVA 9.5 % concentration shows good fiber density, but also formation of beads along with the fibers (Fig 4.1 d). Also the size of the fibers is pretty big as it is clearly evident from the magnification power used.



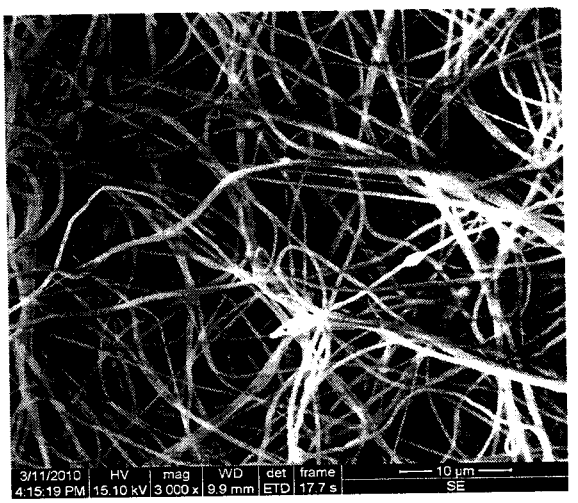
a) Magnification-10,000 X



b) Magnification-40,000 X



c) Magnification-58,802 X

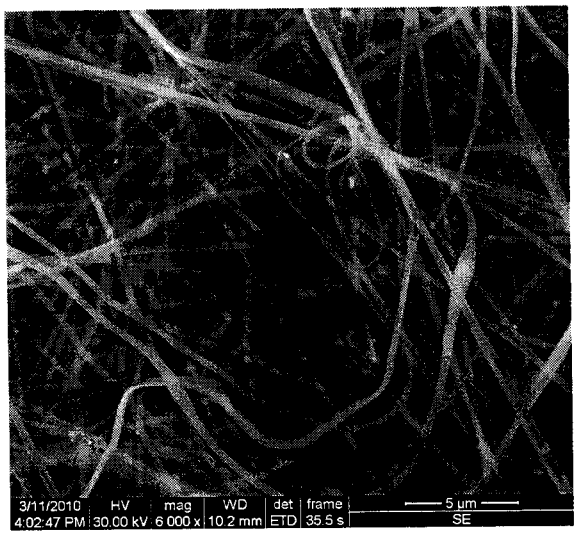


d) Magnification-3,000 X

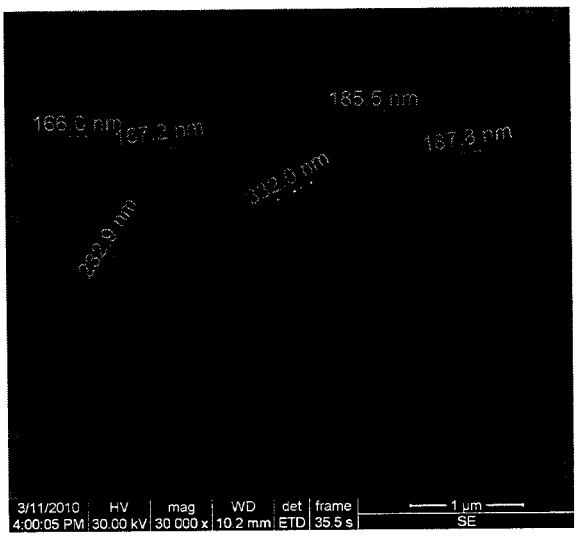
(Concentration 9.5%, Distance between the Emitter and Collector-12 cm.
Spinning time-1.5 Hrs, Voltage =20KV, Flow-rate=0.5ml/hr.)

Fig. 4.2 SEM Micrograph of PVA Nanofibre of Concentration 10.7 %

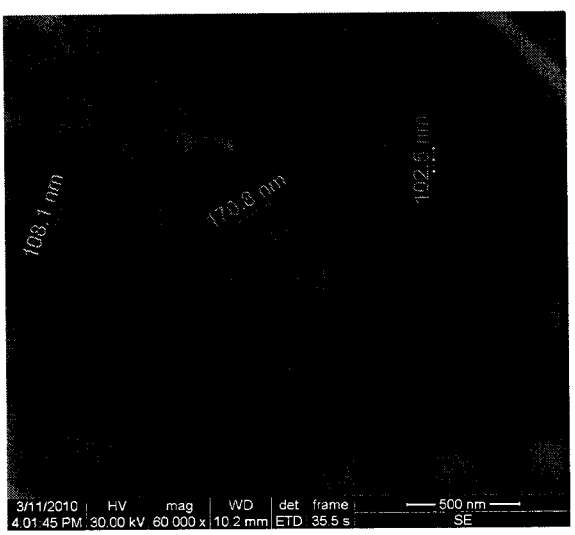
The images of 10.7 % exhibits better fiber density, bead free fibers and hence the flow rate was varied to increase production.



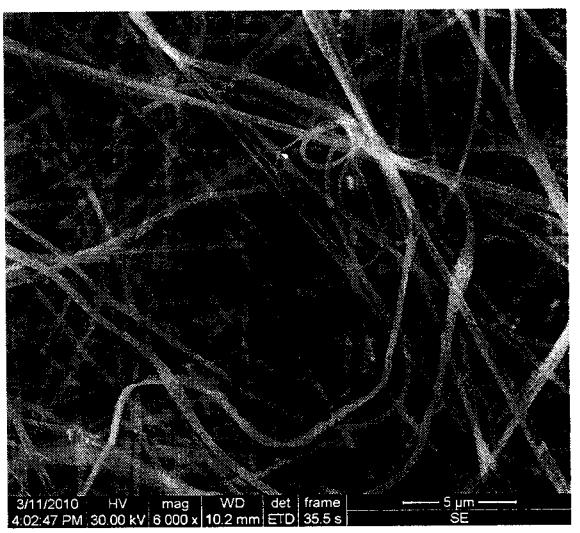
a) Magnification 6,000X



b) Magnification 30,000X



c) Magnification-6,000X



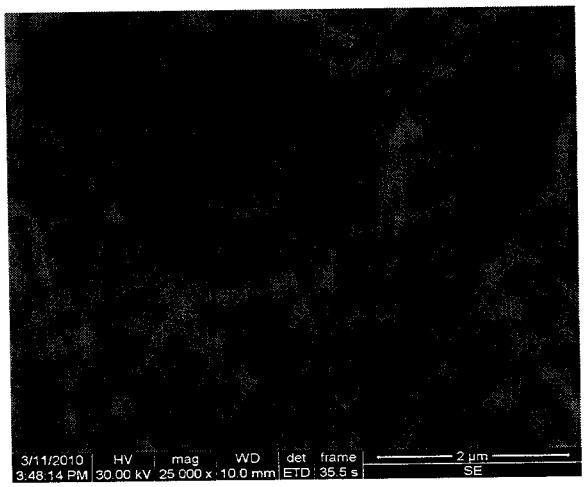
d) Magnification 30,000X

(Concentration-10.7 %, Distance between the Emitter and Collector-12 cm. Spinning time-1.5 Hrs, Voltage = 20kv, Flow-rate=0.55 ml/hr.)

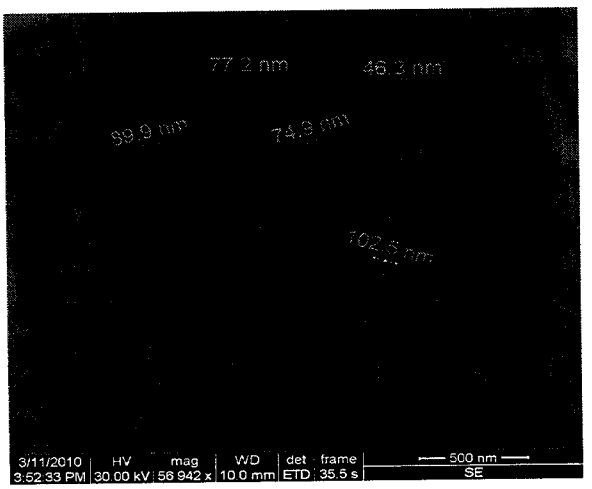
Fig 4.3 SEM micrograph of PVA of varying Flow-rate.

Change in flow rate did not bring much change to the fiber density and the fiber diameter.

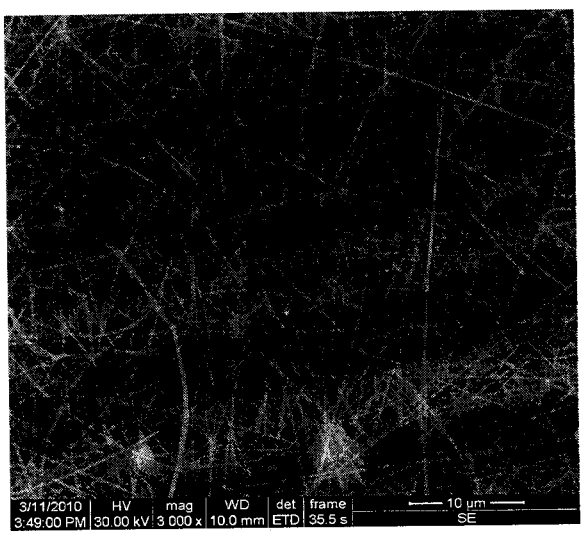
When PVA was blended with AgNO3, the fibres produced were of better orientation and lesser diameter. The lesser diameter can be attributed to the presence of Ag + nanoparticles in the fibers.



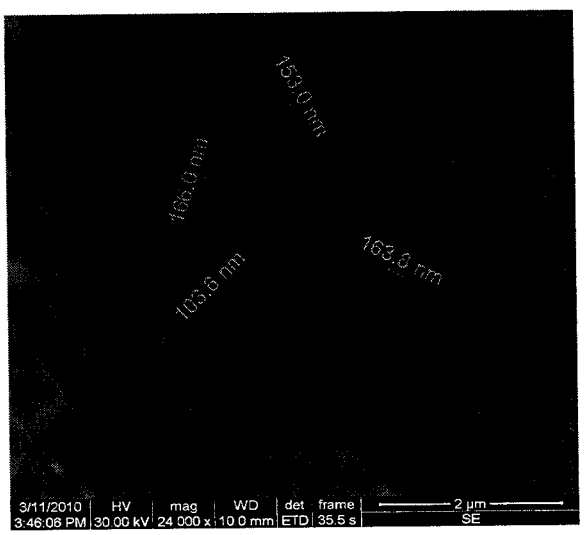
a) Magnification-25,000X



b) Magnification-57,000X



c) Magnification – 3,000 X



d) Magnification – 24,000 X

(Concentration-10.7 %, Distance between the Emitter and Collector-12 cm. Spinning time-1.5 Hrs, Voltage = 20kv, Flow-rate=0.55 ml/hr.)

Fig 4.4 SEM micrograph of PVA/AgNO3.

The produced PVA/AgNO3 nanofibres was yellow in color, which might be an indication for presence of Ag+ ions (nanoparticles) since the nanofibre turns yellow only after heating up to 155°C for 3 minutes which indicates the presence of Ag+ ions. Hence SEM analysis with EDAX was taken in order to see the components present in the PVA/AgNo3.

4.1.1.EDAX Analysis:

EDAX analysis shows the presence of elements that are present. EDAX analysis doesn't show the presence of compounds.

Processing option : All elements analyzed
Number of iterations = 4

Standard :
C CaCO3 1-Jun-1999 12:00 AM
O SiO2 1-Jun-1999 12:00 AM
Na Albite 1-Jun-1999 12:00 AM
Cl KCl 1-Jun-1999 12:00 AM
Cu Cu 1-Jun-1999 12:00 AM
Zn Zn 1-Jun-1999 12:00 AM
Ag Ag 1-Jun-1999 12:00 AM

Table 4.1. EDAX result

Element	Weight%	Atomic%
C K	100.86	80.67
O K	30.33	18.21
Na K	0.77	0.32
Cl K	0.42	0.11
Cu K	2.17	0.33
Zn K	1.22	0.18
Ag L	2.06	0.18
Totals	137.82	

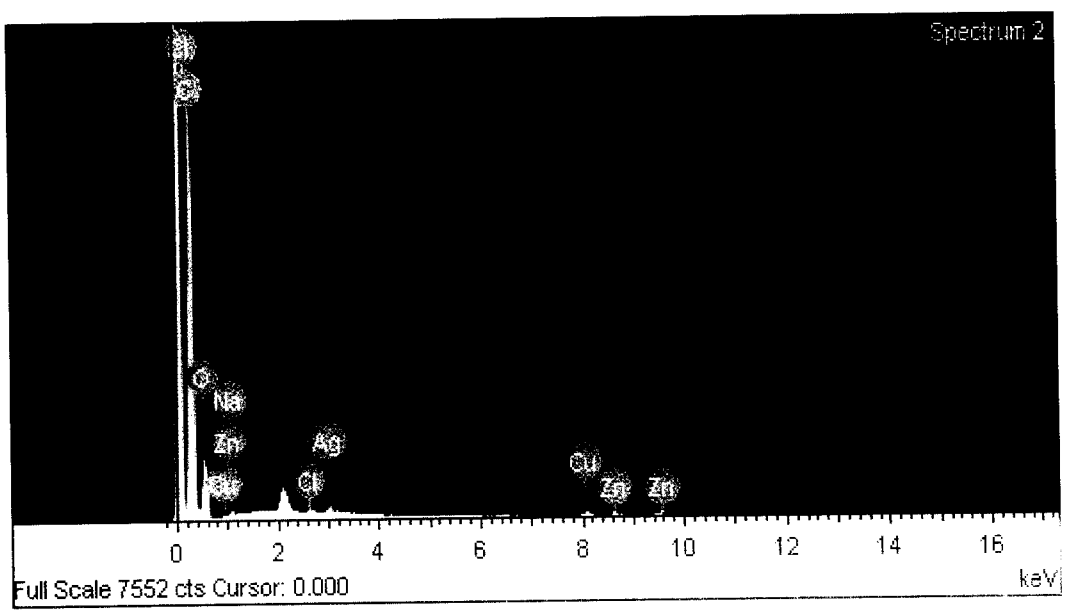
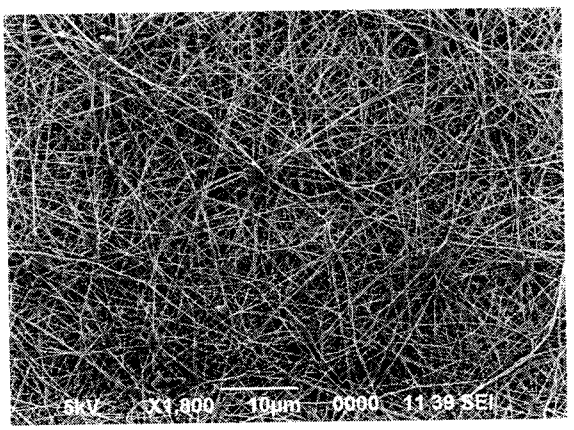


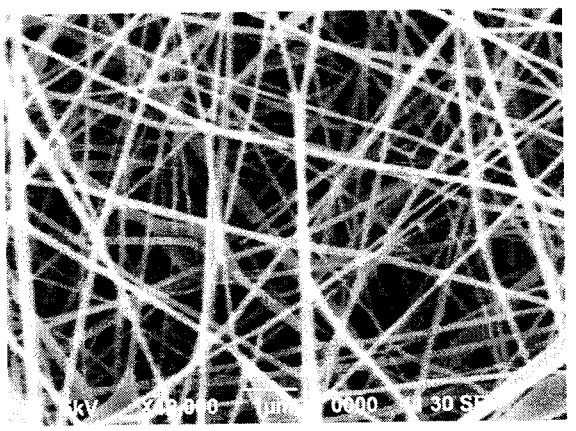
Fig 4.5 EDAX graph of PVA/AgNO3.

Silver is present as free radical in the fibres and it is being present as nano particles which is evident from the size of the fibres.

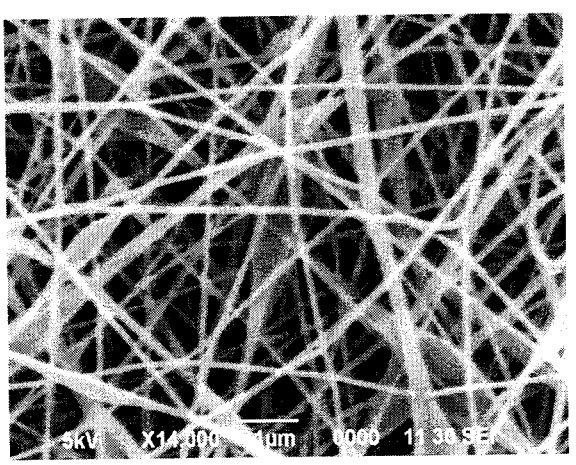
PVA/Chitosan solution was Electro-spun. The nanofibres formed were of less beads and of lesser diameter. The formation of beads is due to the low viscous solution of chitosan.



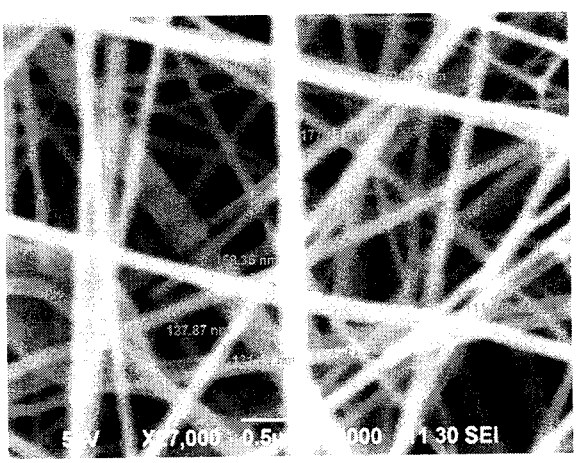
a)Magnification – 1,800 X



b) Magnification – 12,000 X



c) Magnification – 14,000 X



d) Magnification – 27,000 X

Material- PVA/, Distance between the Emitter and Collector-22 cm.

Spinning time-1.5 Hrs,Voltage =25kv, Flow-rate=0.1ml/hr

Fig 4.6 SEM micrograph of PVA/Chitosan.

4.2 FTIR SPECTRA ANALYSIS:

FTIR spectra analysis is done to determine whether there are any changes in chemical nature of the samples due to electrospinning.

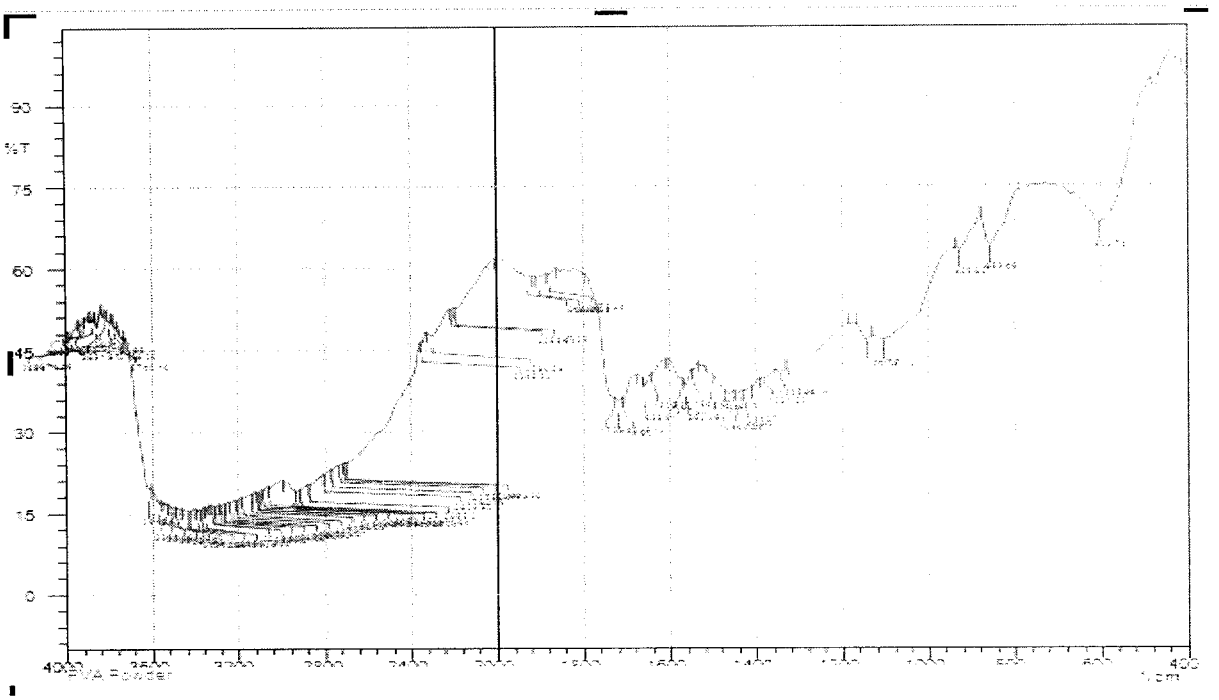


Fig 4.7 FTIR Spectra of PVA Powder

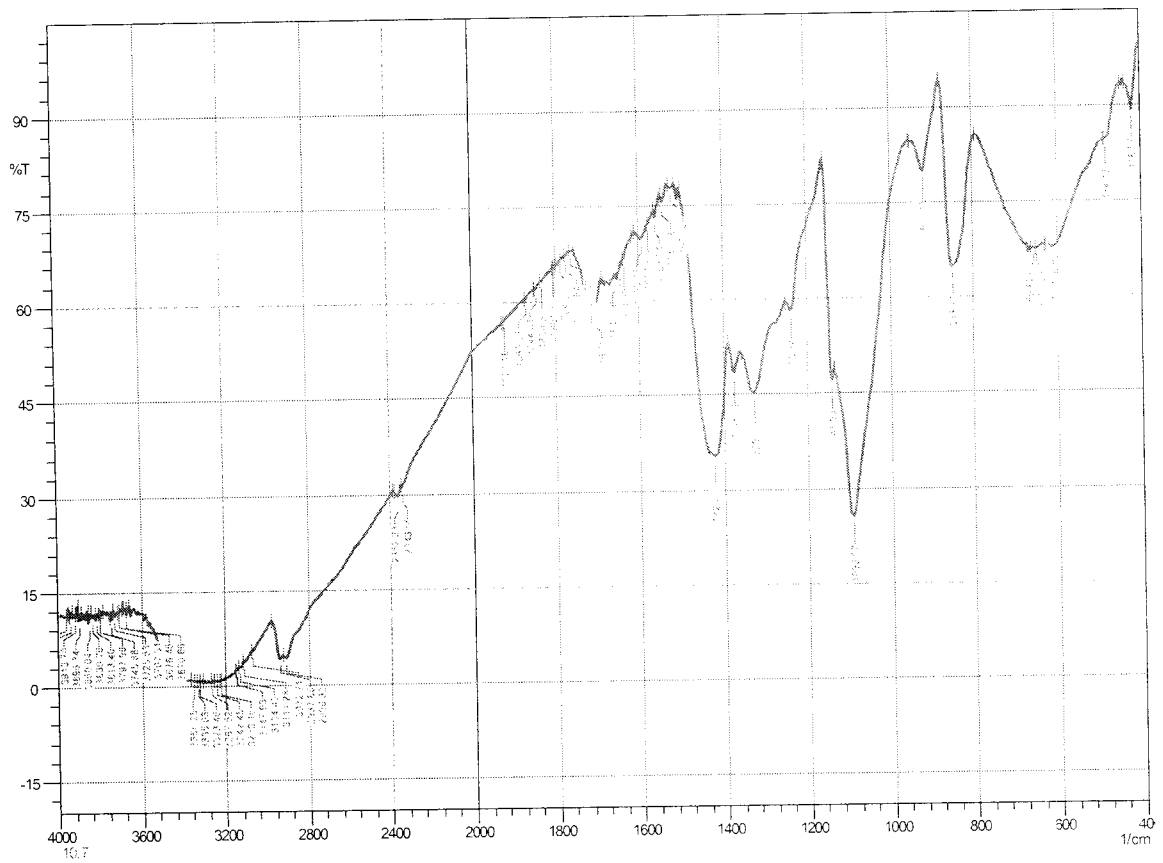


Fig 4.8 FTIR Spectra of PVA Nanofiber of concentration 10.7%

The FTIR graph of PVA powder and PVA fiber contains the corresponding peaks that are characteristic of methylene group and OH peaks.

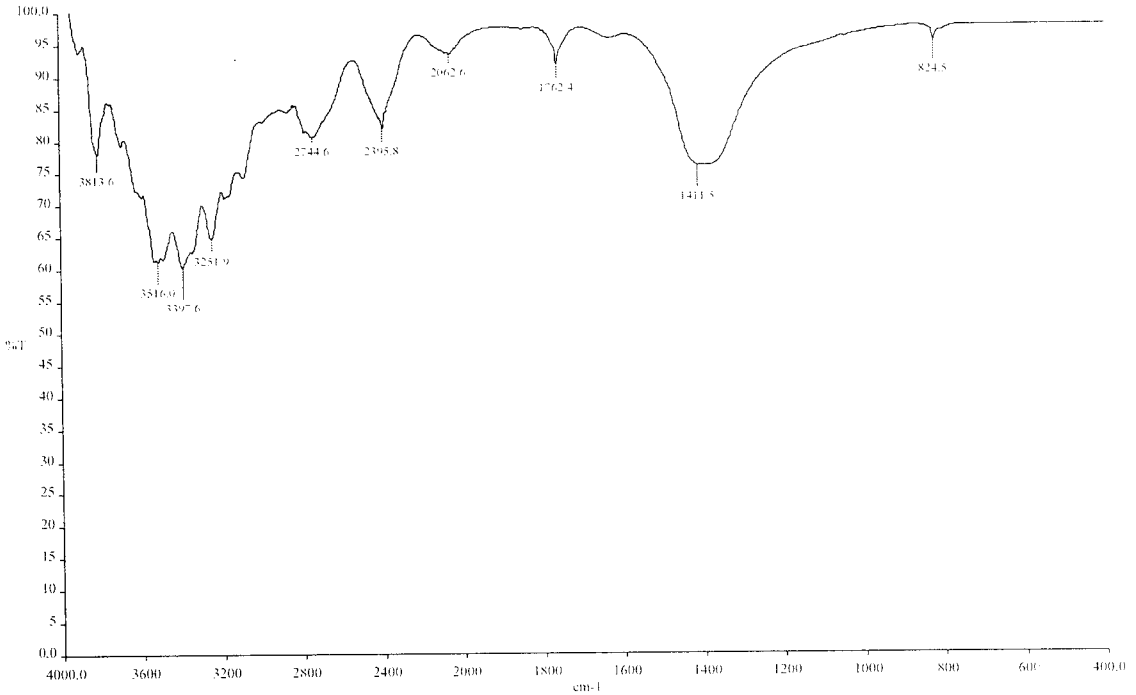


Fig4.9 FTIR Spectra of Silver Nitrate Powder

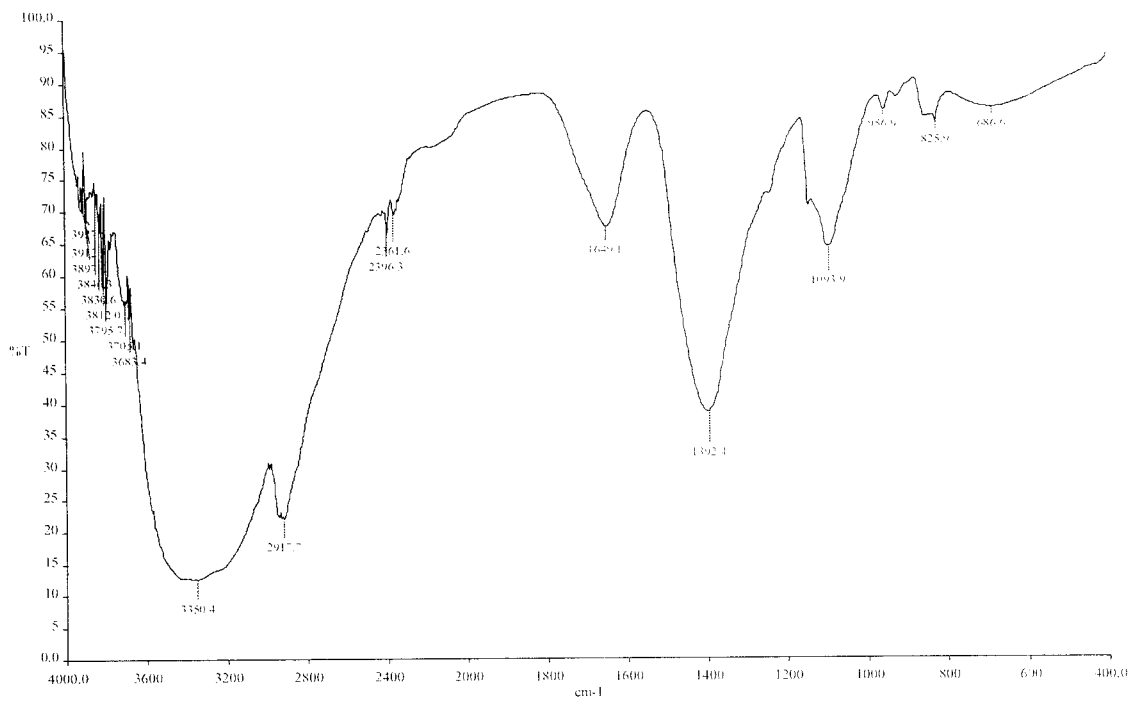


Fig 4.10 FTIR Spectra of PVA/AgNo3 Nanofibre

Sharp peaks present shows the homogenous blending of PVA and AgNO3.

Peaks in the starting region shows presence of OH groups (fig 4.10)

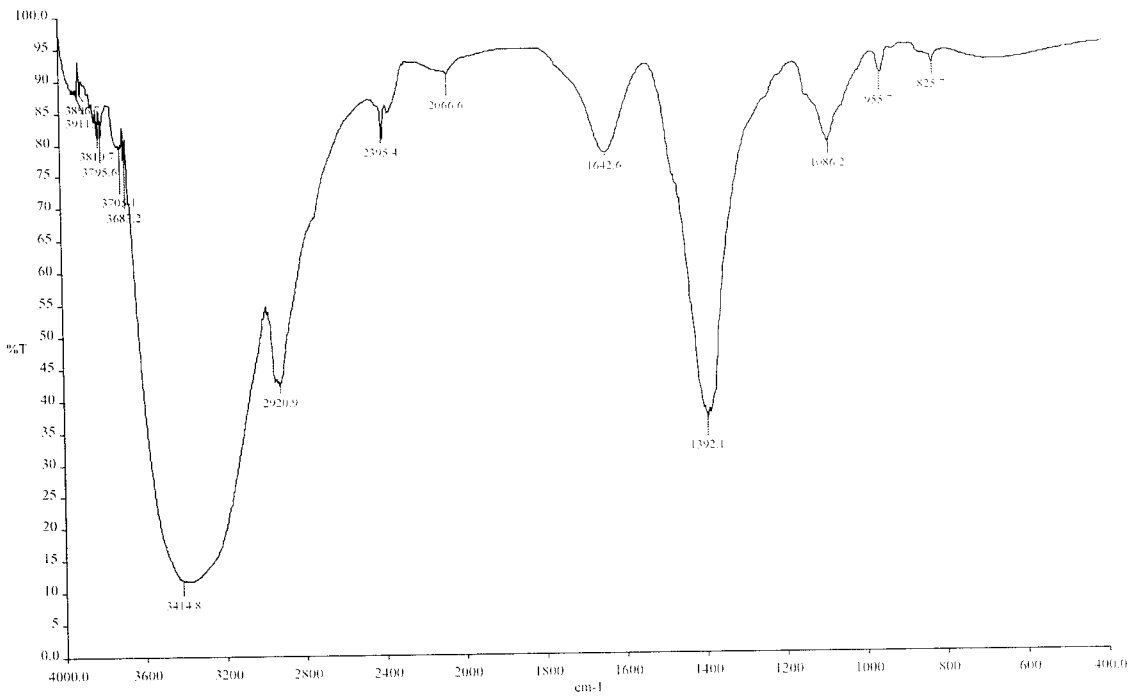


Fig 4.11 FTIR Spectra of PVA/Chitosan Nanofibre

Sharp peaks present shows the homogenous blending of PVA and Chitosan.

Peaks in the starting region shows presence of OH groups (fig 4.11)

4.3 Antibacterial analysis by AATCC-147

Sterile bacteriostasis agar was dispensed in sterile petri dishes. Broth cultures (24 hours) of the test organisms were used as an inoculum. Using sterile cotton swab the test organisms (*Escherichia coli & Staphylococcus aureus*) were swabbed over the surface of the agar plate. The fabric samples (21mm dia) were placed over the agar surface. The plates were incubated at 37°C for 18 to 24 hours. After incubation the plates were examined for the

interruption of growth over the inoculum. The size of the clear zone was used to evaluate the inhibitory effect of the test sample.

Sample 1: PVA/AgNO3 nanofiber

Sample 2: PVA/Chitosan nanofiber.

Result:

Table 4.2 Anti Bacterial Test Result

S.No	Samples	Antibacterial activity (Zone of Bacteriostasis – mm)	
		Escherichia coli	Staphylococcus aureus
1.	Sample 1	16	18
2.	Sample 2	0	0

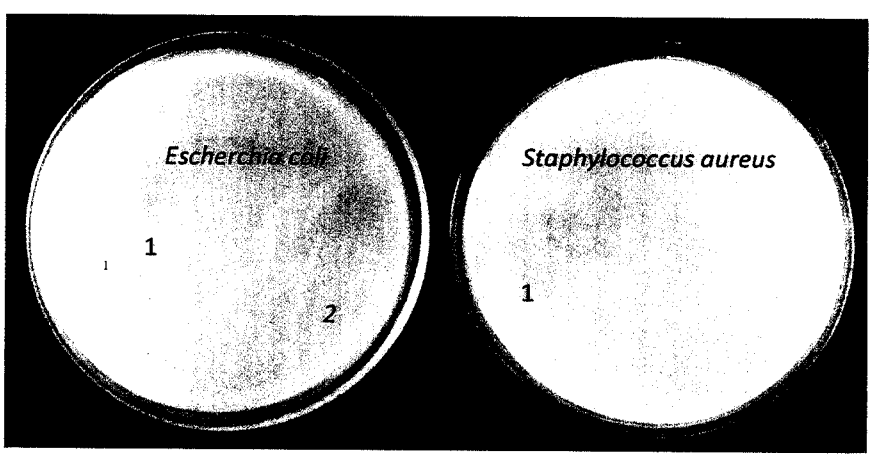


Fig 4.12 Sterile petri dishes for testing anti microbial activity.

Interpretation:

The zone of bacteriostasis in millimeter shows the area of anti bacterial activity of that sample. Sample 1, PVA/AgNO3 fiber showed good anti bacterial activity, whereas sample 2, PVA/Chitosan nanofiber showed no anti bacterial activity.

4.4.ANTIALLERGIC TEST : Patch Test

Subjects tested **random volunteers** representing three males and three females were used for the present study.

Principle:

The fabrics were patched on the normal skin and observed for the specified period of time for the development of the symptoms leading to contact dermatitic allergy.

Procedure:

1. Non hairy part of the skin of the subjects were selected
2. The surface of the skin was cleaned with cotton swabs dipped in clean water
3. The patches of the fabric sample were made and plastered on the surface of the cleaned skin.
4. The site of patching was observed for any immediate allergic response

5. For the observations were made up to 24 hours for the symptoms such as reddishness, rashes, irritations, etc.,
6. The time of observation may be extended for another 24 hours to confirm the effect.

Sample 1: PVA/AgNO3 nanofiber

Sample2: PVA/ Chitosan nanofiber

Patch test

Table 4.3 Anti allergic Test Result

Subjects	Sample 1	Sample 2
Subject 01 (Male / 21 yr)	-	-
Subject 02 (Male / 30 yr)	-	-
Subject 03 (Male / 35 yr)	-	-
Subject 04 (Female / 22 yr)	-	-
Subject 05 (Female / 25 yr)	-	-
Subject 06 (Female / 29 yr)	-	-

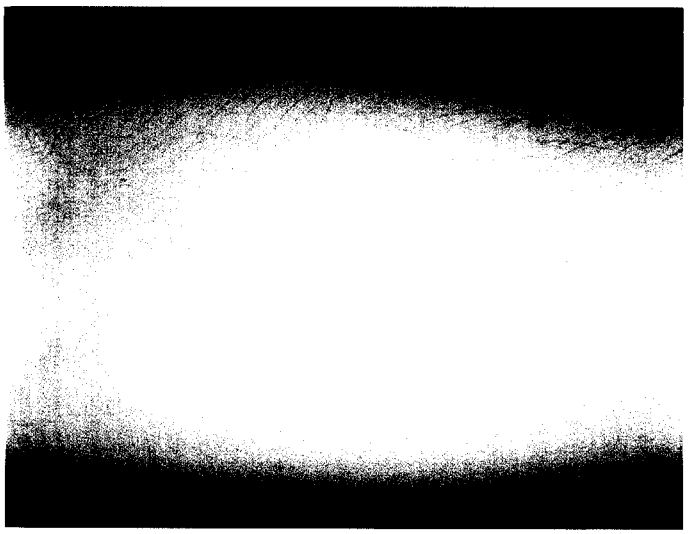


Fig 4.13 Area of skin before patching.

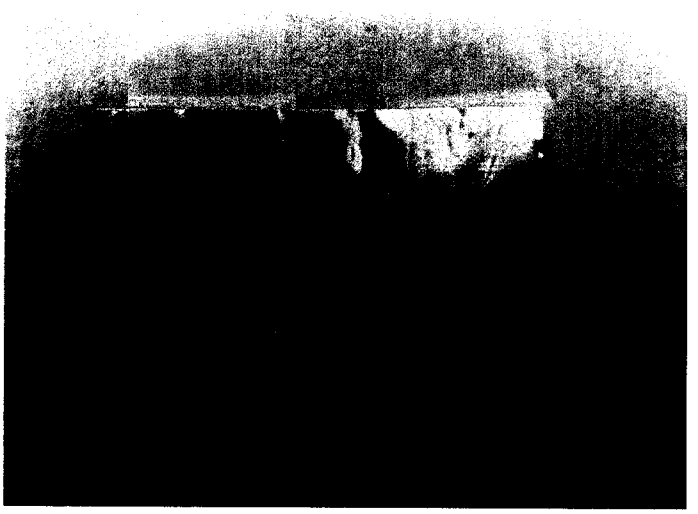


Fig 4.14 Sample 1 patched onto skin.

Fig 4.15 Sample 2 patched onto skin.

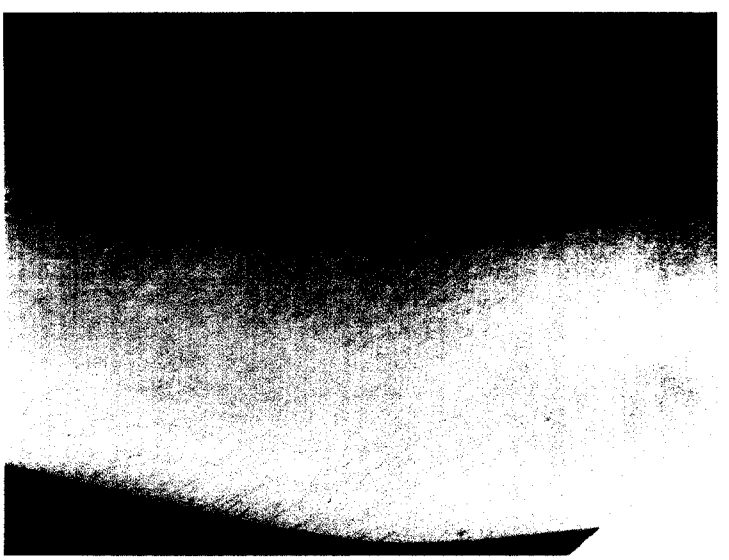


Fig 4.16 Area of skin after removing Sample 1.

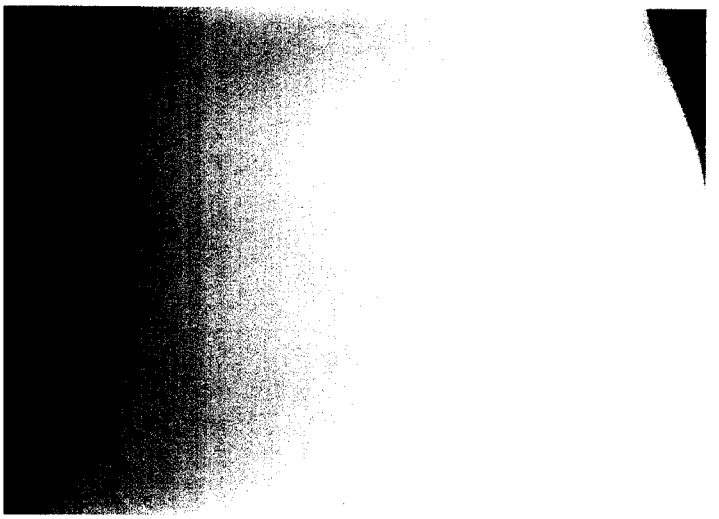


Fig 4.17 Area of skin after removing Sample 2.

Interpretation:

No allergic reaction found for both samples.

4.5.Anti inflammatory test (In House Method)

Source of the bacterial isolates the bacterial organisms such as *Staphylococcus aureus, Streptococcus pyogenes , Klebsiella pneumoniae, pseudomonas aeruginosa* were isolated and identified from the pus samples of post operative wound infected patients.

The anti-inflammatory response was studied based on the capacity to suppress the bacterial growth there by reducing the sequence of events leading to the development of inflammation. The anti-bacterial effect was studied using AATCC147 as described earlier. The results are presented in the following table.

Sample 1: PVA/AgNO3 nanofiber

Sample 2: PVA/Chitosan nanofiber.

Anti inflammatory test (In House Method)

Table 4.4 Anti Inflammatory Test Result

Samples	**Bacteria producing inflammation**	**Antibacterial activity (Zone of bacteriostasis – mm)**	**Anti inflammatory reaction**
Sample 1	**Staphylococcus aureus**	18	++
	Streptococcus pyogenes	17	++
	Klebsiella pneumoniae	15	++
	Pseudomonas aeruginosa	17	++
Sample 2	**Staphylococcus aureus**	0	--
	Streptococcus pyogenes	0	--
	Klebsiella pneumoniae	0	--
	Pseudomonas aeruginosa	0	--

Intepretation:

The results of the tests are presented in the following table demonstrating that the sample 1 had anti inflammatory response and sample 2 did not exhibit this.

CHAPTER – 5

CONCLUSION

From the study we conclude that:

- PVA exhibits good spinnability at a concentration of 10.7 %.
- The PVA/AgNO3 fibers produced turned to yellow without heating, which indicates presence of Ag+ ions as nanoparticles.
- The solution during electrospinning evaporates 80 % which is shown by the FTIR spectra.
- The SEM photos shows that PVA nanofibers with average diameter 260nm.
- PVA/AgNO3 fibers have an average diameter of 120 nm.
- PVA/Cs fibers have an average diameter of 160 nm.
- When subjected to medical tests, PVA/AgNO3 showed better anti microbial, anti inflammatory characteristics than PVA/Cs blend, thus making PVA/AgNO3 suitable for wound dressing.

CHAPTER - 6

SCOPE FOR THE FUTURE

- Identifying other bio-compatible, non toxic polymers.
- Polycaprolactone, Cellulose acetate, Polyethylene oxide are few of the other polymers that are capable of producing nanofibers.
- Cell cultures and bacterial cultures can be developed to see the performance of the nanofibres for implantable dressings.
- Commercialisation of electrospinning could be a big break for industrial uses as electrospinning is still used only for researches.
- Face masks that can be used for plastic surgery can also be fabricated.
- Nanostructured materials are also attracting a great deal of attention in Textile and Polymer researchers and industrialists because of their potential applications for achieving specific processes and properties.

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Figure 7. Release profiles of Agþ ions from (a) thePVA/AgNO3 nanofibers after the heat treatment and (b) the PVA/AgNO3 nanofibers after the heat treatment and subsequent UV irradiation. 2474 HONG ET AL.

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