# FABRICATION OF CHITOSAN BASED NANOFIBERS BY ELECTROSPINNING AND THEIR CHARACTERISATION

*A Thesis submitted in partial fulfilment of the requirements for the degree of*

**Bachelor of Technology**

**in**

**Biotechnology**

**By**

**FARHA NAAZ (109BT0649)**

*Under the guidance of*

**Prof. K. Pramanik**

Department of Biotechnology & Medical Engineering
National Institute of Technology
Rourkela 769008

# National Institute of Technology
# Rourkela

## CERTIFICATE

This is to certify that the thesis entitled, **“ FABRICATION OF CHITOSAN BASED NANOFIBERS BY ELECTROSPINNING AND THEIR CHARACTERISATION”** submitted by **Farha Naaz (109BT0649)** in partial fulfilment of the requirements for the award of **Bachelor of Technology Degree** in **Biotechnology** at National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Place: Rourkela
Date:

Supervisor
Prof. K. Pramanik
Department of Biotechnology and Medical Engineering
National Institute of Technology,Rourkela-769008

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Place:

Date:

Farha Naaz

109BT0649

B.Tech

Biotechnology

NIT Rourkela

# ABSTRACT

Electrospinning is used to produce fibers in the nanometer range by stretching a polymeric jet using electric field of high magnitude. Electrospinning leads to the formation of continuous fibers ranging from 0.01 to 10 μm . The ultra-fine fibers produced by electro spinning are expected to have two main properties, a very high surface to volume ratio, and a relatively defect free structure at the molecular level . The development of nanofibers by electrospinning process has led to potential applications in filtration, military protective clothing, and biological applications such as tissue engineering scaffolds, drug delivery devices , artificial organ components etc. The present study is an attempt to fabricate composite nanofibers that can be used as tissue engineering scaffolds. The approach involves the blending of two different polymers both being biocompatible and biodegradable but one is natural and other is synthetic along with a surfactant. Composites in the form of nanofibers were formed via electrospinning technique. Different ratios of Chitosan:PEO(Polyethylene glycol:DATB(Dodecyltrimethylammonium bromide) blends were prepared and successfully electrospunned so that the nanofibers obtained could mimic the natural ECM(Extra Cellular Matrix). It was found that usage of DTAB in the blend yielded fibers in the range of 50-250 nm which could be suitable for tissue engineering.. The prepared composite scaffolds were characterised using several techniques such as SEM(Scanning Electron Microscopy), FTIR(Fourier Transform Infrared Spectroscopy, XRD(X-ray Diffraction) . Also solubility and biodegradability tests were carried out for the prepared scaffolds. It was found that at feed rate 0.5ml/hr and voltage 25kV ,Chitosan:PEO ratios of 70/30 and 80/20 with DTAB concentration of 15mM yielded better nanofibers as compared to higher DTAB concentrations.

Keywords: electrospinning, biocompatible, biodegradable, electrospinning, SEM, FTIR,XRD

# TABLE OF CONTENTS

**Contents**

# List of Figures

# List of Tables

# Chapter 1

## -Introduction

# Introduction

Tissue/organ transplantation has proved to be one of the greatest medical achievements of all time. Accidents and diseases sometimes result in loss of tissues and organ failures that result in devastating and life threatening situation. Presently autograft and allograft transplantation are major approaches used to repair/replace damaged or lost tissue and organs. Allografts trigger an immune response in the host body and carry the risk of disease transfer whereas autografts avoid eliciting immune response in the patient after re-implantation yet are associated with limitations such as donor site morbidity and limited availability. The lack of sufficient amount of donor tissues and organs led to the development of a new technology called tissue engineering.

Tissue engineering is the use of combination of cells, engineering and materials methods, and suitable biochemical and physico-chemical factors so as to improve or replace biological functions. It provides long-term solutions, which are much safer than other options (auto/allografts) and are cost-effective; the presence of residual foreign material is eliminated as well. It provides a novel way to renew physiological function by seeding cells onto scaffolds fabricated from natural or artificial materials, alongwith the growth factors and other signalling molecules so as to modulate cell proliferation and differentiation.

With the development of the electrospinning process, electrospun nanofibrous scaffolds with large surface area-to-volume ratio, high porosity, and good mechanical properties and morphology similar to the extracellular matrix (ECM) are used in tissue engineering. For electrospun nanofibrous scaffolds to be used in biomedical applications , its physical and biological properties such as hydrophilicity , mechanical modulus and strength, biocompatibility, biodegradability , and specific cell interactions are grealy determined by materials' chemical compositions. Hence by selecting combination of proper components and adjusting the component ratio, properties of electrospun scaffolds can be modified to obtain desired new functions.

Polymeric nanofibers are produced using several different techniques, such as self-assembly, phase separation, and electrospinning. Electrospinning is the only technique that allows dimensional control, use of various polymers , fiber formation in different orientations, and production in huge quantities as compared to other methods. This technique is based on the application of electric potentials with high magnitude (5-50 kV) and low current (0.5-1 μA) in which a jet of fluidic polymeric material is accelerated and stretched, producing fibers with small diameters.

Essentially a scaffold needs to maintain its stability and promote cell growth and proliferation, but gradually degrade along with the construction of new tissue and finally to be replaced completely by the new tissue .

Chitosan is N-deacetylated derivative of chitin. Due to its biocompatibility , biodegradability, antimicrobial and non-toxic properties , chitosan has been extensively studied for various applications such as drug delivery controlled systems, for covering wounds and for other biomedical devices. Fiber formation from chitosan is difficult because of its limited solubility and poly cationic nature in solution. Chitosan has been electrospun in several solvent systems across a broad range of concentrations; however, no ultrafine fibers were obtained even at the concentrations in which the chitosan chains were extensively entangled[8].

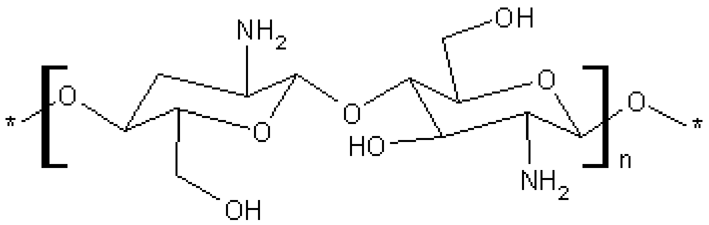


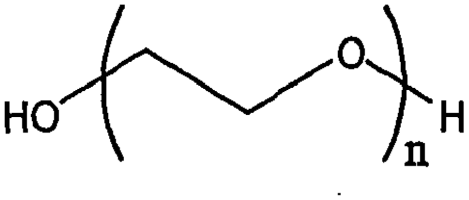
**Fig 1**:- Molecular structure of chitosan

With chitosan solutions, even moderate concentrations become too viscous to overcome the electric field and cannot be successfully electrospun. Also, chitosan is a cationic biopolymer which affects the rheology of the solutions. With a typical laboratory electrospinning setup, the range of the viscosity of the solution must be maintained for nanofibers to form successfully. Above the upper threshold, the solution becomes too viscous and fiber formation is hindered because the electric field is not strong enough to overcome the viscosity of the solution. Below the lower limit, the polymer chains are not entangled so fiber formation is not possible and polymer beads often are created. Therefore, several research groups have blended chitosan with other polymers in an attempt to improve the electrospinability of the solutions.

### 1.1 Electrospinning of blends of chitosan and synthetic polymers

It is commonly observed while electrospinning from polymers that the formation of beads takes place due to to an inadequate stretch of the filaments during the whipping of the jet because of a low charge density. To overcome this defect, salts , ionic surfactants or ionic polyelectrolytes can be added into the polymer solution to improve the net charge density that enhances the whipping instability. The jet is stretched under stronger charge repulsion and at a higher speed, resulting in an depletion of the bead structure. Since chitosan is a linear cationic polymer, it is observed that chitosan can act like other ionic additives and reduce fiber diameter and thus producing bead free fibers. Natural polymers have weak mechanical properties so they are blended with synthetic polymers so as to enhance their mechanical properties and durability. Recently, the electrospun composite nano -fibers have been developed using chitosan and synthetic polymers such as poly(vinyl alcohol) (PVA), poly(ethylene terpthalate) (PET), poly(ethylene oxide)(PEO), polycaprolactone (PCL), poly(lactic acid) (PLA), nylon-6 and others. These composite nanofibers are more advantageous over the electrospun nanofibers from pure chitosan, because the mechanical, biocompatible, antibacterial and other properties of the nanofibers are drastically enhanced by the addition of these polymers.

For our study we shall focus on Polyethylene oxide(HO-CH2-(CH2-O-CH2-)n-CH2-OH) as the co-polymer. PEO can be dissolved in both organic solvents and aqueous solutions, including pure water. It is non-toxic and can be eliminated by renal and hepatic pathways, making it suitable for many biomedical applications. It is used because of its excellent electrospinning characteristics, its ability to form ultrafine fibers, its linear structure with flexible chains, its biocompatibility, its solubility , and its capability to form hydrogen bonds with other macromolecules.



<u>Fig 2</u>:- Molecular structure of PEO

In a chitosan–PEO blend, PEO acts as a plasticizer facilitating orientation and flow of chitosan by uncoiling and wrapping around chitosan chains [9].

Besides using PEO we shall also use D-TAB surfactant in the polymer blend. Surfactants are used in a wide array of applications because of their potential to lower surface or interfacial tension of the medium in which they are dissolved [10]. Each molecule contains both a hydrophilic and a hydrophobic part. An ionic surfactant, which has an ionic hydrophilic head, also improve the electrical conductivity of the solution, promoting bending instability during the electrospinning process, thereby facilitating thinner fiber formation with a higher degree of orientation [11]. Further, surfactants may also self-assemble to form colloidal aggregates above a critical concentration, the so-called critical micellar concentration or cmc [12]. These micellar solutions are able to serve as solubilization vesicles to improve solubility and protect and deliver lypophilic functional ingredients. Incorporation of micelles into nanofibers could thus offer a novel means to further functionalize biopolymer nanofibers and their blends. Finally, polymer–surfactant interactions may modulate the molecular structure and interactions of polymer molecules thereby altering rheological and interfacial properties of polymer dispersions [11], which are critical factors in the successful preparation of nanofibers by electrospinning. For example, addition of small amounts of nonionic surfactant was found to improve both the onset voltage and the reproducibility of electrospinning [13]. In nonionic polymer solutions, non-ionic surfactants did not stop bead formation but greatly reduced it, while cationic surfactants prevented beaded fibers and lead to fibers with smaller mean diameters [11].

## 1.2 Electrospinning process

Electrospinning is a fiber forming processes by the application of electric potential where electrostatic forces are employed to control the production of fibers. It is closely related to the more established technology of electrospraying, where the droplets are formed. Electrospinning readily leads to the formation of continuous fibers ranging from 0.01 to 10 μm. The development of nanofibers by electrospinning process has led to to potential applications in filtration, protective clothing, and biological applications such as tissue engineering scaffolds, and drug delivery devices. A typical electrospinning setup consists of a capillary through which the liquid to be electrospun is forced; a high voltage source with positive or negative polarity, which injects charge into the liquid; and a grounded collector .A syringe pump, gravitational forces, or pressurized gas are typically used to force the liquid through a small-diameter capillary forming a pendant drop at the tip. An electrode from the high voltage source is then immersed in the liquid or can be directly attached to the capillary if a metal needle is used. The voltage source is then turned on and charge is injected into the polymer solution. Increasing the electric field strength causes the repulsive interactions between like charges in the liquid and the attractive forces between the oppositely charged liquid and collector to begin to exert tensile forces on the liquid, elongating the pendant drop at the tip of the capillary. As the electric field strength is increased further a point will be reached at which the electrostatic forces balance out the surface tension of the liquid leading to the development of the Taylor cone. If the applied voltage is increased beyond this point a fiber jet will be ejected from the apex of the cone and be accelerated toward the grounded collector. While the fiber jet is accelerated through the atmosphere toward the collector it undergoes a chaotic bending instability, thereby increasing the transit time and the path length to the collector and aiding in the fiber thinning and solvent evaporation processes. The solid polymer fibers are then deposited onto a grounded collector.

### 1.3 History Of Electrospinning

The first patent for electrospinning setup was issued to Formhals in the year 1934 (US patent 1-975-504). In the past several decades, this technique has been used to create fibers from a wide range of polymers including biopolymers, engineering plastics, conducting polymers, and polymer blends.

In the late 16th century William Gilbert describe the behavior of magnetic and electrostatic phenomena. He observed that when a suitably electrically charged piece of amber was brought near a droplet of water it would form a cone shape and small droplets would be ejected from the tip of the cone: this is the first recorded observation of electro spraying.

The process of electrospinning was patented by J.F. Cooley in May 1900 and February 1902 (U.S. Patent 692,631) and by W.J. Morton in July 1902 (U.S. Patent 0,705,691 .

Between 1964 and 1969 Sir G. I. Taylor proposed that the fluid droplets make a cone when it ejected from the tip of the syringe under the effect of an electric field; this characterized droplet shape is n known as the Taylor cone.

In the 1990s Reneker and Rutledge proposed that many organic polymers can be electrospun into nanofibers by electrospinnig. After that, the number of publications about electrospinning increased exponentially.

Since 1995 Reznik et al. describes the extensive work on the shape of the Taylor cone and the subsequent ejection of a fluid jet.

## 1.4 The electrospinning process may be broken down into several operational components:

(i) Charging of the fluid,

(ii) Formation of the cone-jet,

(iii) Thinning of the jet in the presence of an electric field,

(iv) Instability of the jet, and

(v) Collection of the jet (or its solidified fibers) on an appropriate target.

### Charging of the polymer fluid

Polymer solution is filled inside the syringe which is charged to a very high potential, usually around 10-30 kV, by means of a high potential electrode known as induction charging. An electrical double layer is formed where ion or ion pairs are generated as charge carriers based on the polarity of the solution. For non-conducting fluid , charges are introduced by applying an electrostatic field.

### Taylor cone Formation

Similar charges in the electrical double layer repel each other based on the applied voltage and act against the surface tension of the fluid so that it deforms into a cone shaped structure known as Taylor cone. The Taylor cone, beyond a particular aoltage becomes unstable and the emission of jet takes place from the tip of the cone. [4]

### Formation of a thin jet

The charged fluid gets accelerated in the presence of an electric field and forms a thin stream of fluid. This region is usually linear and thin.

### Instability of the jet stream

The jet tends to come towards the collector . The fluid is accelerated in the presence of an electric field and due to one or more bending instabilities follows a spiral and distorted path before getting deposited on the collector plate[5]. This region of instability is known as whipping region.

### Collection of fibers on the collector plate

The collector plate is usually held at a lower potential where the charged fluids gets deposited. The morphology of fibers collected may vary in accordance to the type of collector such as static collector, rotating drum collector, moving belt collector etc.

Table 1.-**PARAMETERS AFFECTING THE ELECTROSPINNING PROCESS**

| S.no | Solution Properties | Processing Parameters | Ambient Parameters |
|---|---|---|---|
| 1 | Surface tension | Feed Rate | Temperature |
| 2 | Molecular wt.and viscosity | Diameter of needle | Humidity |
| 3 | Effect of Solvent | Effect of Collector Plate | Atmospheric Pressure |
| 4 | Solution conductivity | Distance between tip and collector | |
| 5 | Volatility of solvent | | |

## 1.5 Objectives

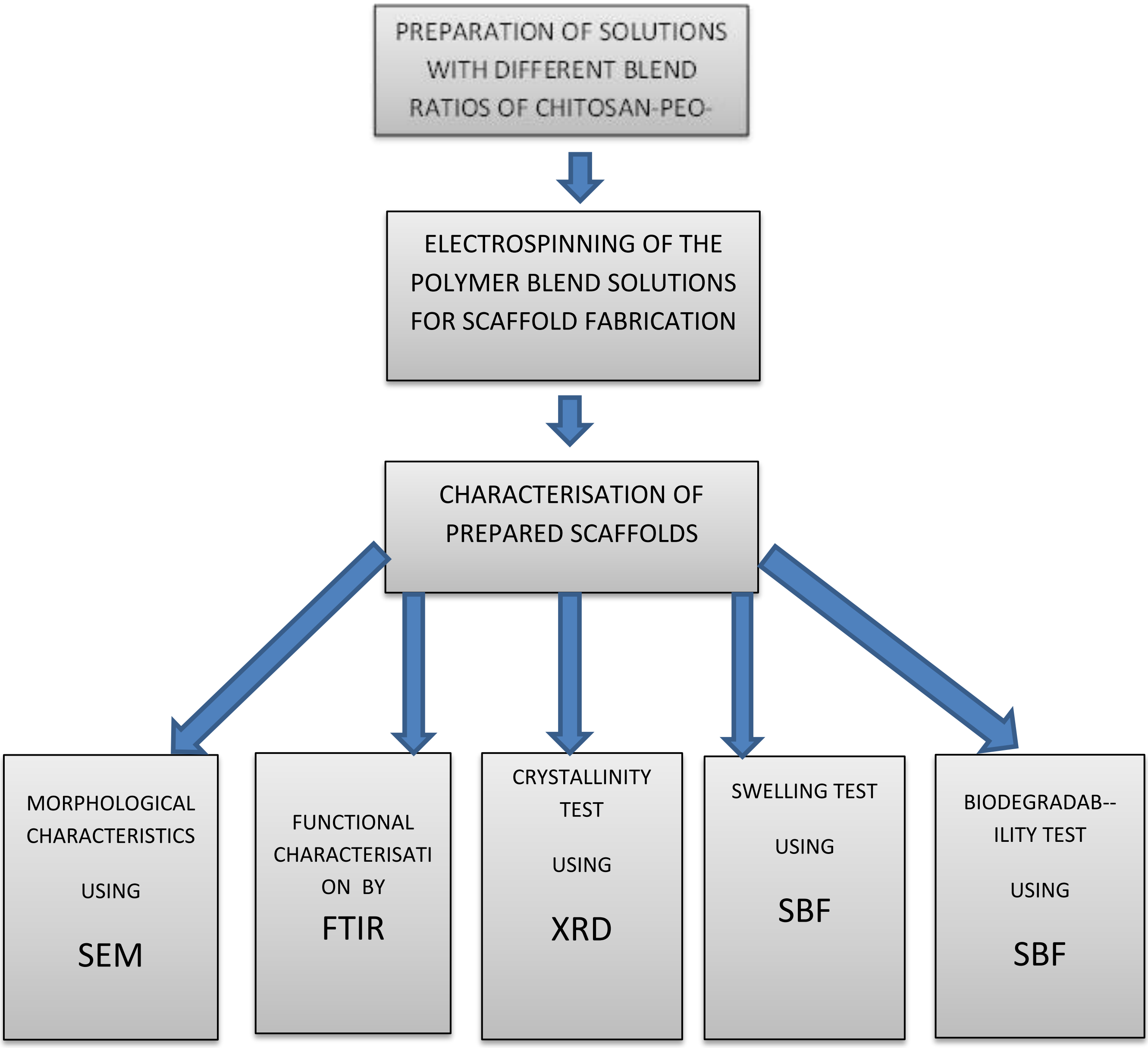
**The objectives of the project are as follows-**

1) Preparation of chitosan based electrospun nanofibrous mat .
2) Study the effect of key process parameters on the yield of nanofibers.
3) Characterisation of the prepared scaffolds by several techniques such as SEM, FTIR and XRD.
4) Biodegradability and swelling studies using SBF(Simulated Body Fluid).

## 1.6 Scope of the study

The scope of this present work involves fabrication of nanofibrous polymer blend scaffolds. The blend of Chitosan-PEO have been prepared using acetic acid and distilled water. As chitosan , biocompatible and biodegradable, itself is not electrospinnable hence a need of a copolymer arises which will increase the mechanical strength of the nanofibers . PEO is non-toxic and can be eliminated by renal and hepatic pathways, making it suitable for many biomedical applications. It is used due to its excellent electrospinning characteristics, its ability to form ultrafine fibers, its linear structure with flexible chains, its biocompatibility, its solubility in aqueous media, and its capability to form hydrogen bonds with other macromolecules. In a chitosan–PEO blend, PEO acts as a plasticizer facilitating orientation and flow of chitosan by uncoiling and wrapping around chitosan chains. DTAB used in the blend also possess several beneficial effects such as they lower surface or interfacial tension of the medium in which they are dissolved. They also decrease the viscosity and increase the conductivity of the solution. Also DTAB is pharmaceutically approved .The micellar solutions are able to serve as solubilization vesicles to improve solubility and protect and deliver lypophilic functional ingredients. incorporation of micelles into nanofibers could thus offer a novel means to further functionalize biopolymer nanofibers and their blends. The morphological, structural and functional characterisations were done by SEM, XRD, FTIR. Biodegradability and swelling tests were also performed.

# METHODOLOGY



# Chapter 2

## -Literature review

# LITERATURE REVIEW

1) For tissue-engineering scaffold applications, fibers with diameters in the range from several micrometers down to less than 100 nm have a very high surface area to mass ratio, and can be electrospun into 3-D scaffolds with very high porosity. In this way, biomimetic matrices can be fabricated by electrospinning which facilitate cell attachment, support cell growth, and regulate cell differentiation. In electrospinning, the diameter of the nanofiber can be controlled by adjusting parameters including polymer concentration, flow rate of the polymer solution, solvent conductivity and temperature,and so on.

2) Polyethylene oxide (PEO) is also a biocompatible polymer that has been used as cartilage tissue repair and wound dressing . Many nanofibers of the blends of PEO and chitosan have also been fabricated by electrospinning. For examples, Klossner et al. [1] fabricated defect-free nanofibers with average diameters ranging from 62±9 to 129±16 nm by electrospinning of blended solutions of chitosan and PEO in acetic acid. Their study showed that as total polymer concentration (chitosan + PEO) increased, the number of beads decreased, and as chitosan concentration increased, fiber diameter decreased.

3) Chitosan/PEO solutions phase would separate over time, so blended solutions were able to be electrospun easily within 24 h of initially being blended. The addition of NaCl stabilized these solutions and increased the time the blended solutions could be stored before electrospinning.

4) Bhattarai et al. [2] prepared nanofibers with an average fiber diameter controllable from a few microns down to ~40 nm and a narrow size distribution by electrospinning solutions containing chitosan, PEO and Triton X-100™. It was found that the matrix with a chitosan/PEO ratio of 90/10 retained excellent integrity of the fibrous structure in water.

5) Hybrids of chitosan/PEO could be electrospun in the presence of micellar surfactant solutions[3]. The presence of surfactants resulted in the formation of needle-like, smooth or beaded fibers. It was revealed that nanofibers consisted of both polymers and surfactants with concentration of the constituents differing from that in polymer solutions.

6) In another article nanofibers were fabricated by electrospinning a mixture of cationic chitosan and neutral polyethylene oxide (PEO) at a ratio of 3:1 in aqueous acetic acid . In this blend, PEO acts as a plasticizer facilitating orientation and flow of chitosan by uncoiling and wrapping around chitosan chains .

7) In order to further modify the quality and morphology of the nanofibers, Zeng et al. added several surfactants to the electrospinning solutions. The addition of ionic surfactants to polyionic polymers as chitosan alters its solution properties such as viscosity, conductivity and surface tension. In turn, these changes in solution properties alter the Taylor cone formation, jet expulsion and jet bending/whipping, influencing the type, structure and dimensions of the nanostructures formed. Among different surfactants cationic dodecyl trimethyl ammonium bromide (DTAB) is a positively charged surfactant whose head groups should be electrostatically repelled from the cationic groups on the chitosan backbone. Nevertheless, DTAB may still bind to polymers through hydrophobic interactions between its nonpolar tail and any non-polar groups on the polymer chain. Binding occurs and there is an increased electrostatic repulsion between chitosan/DTAB complexes thus decreasing entanglement and viscosity. Also it was reported that fibers obtained were in the range of 50-130 nm .

8) Reports on chitosan-PEO blends with Tween-20 ,Triton X and other surfactants are also available(Ziani et al.[6])

9) Ultrafine fibers could be generated by controlling the addition of PEO in 2:1 or 1:1 mass ratios of Chitosan to PEO from 4–6 wt% Chitosan/PEO solutions ( Duan et al.[7]). It was also shown that addition of PEO brings about additive effects in enhancing the formation of a fibrous structure. With the PEO/chitosan mass ratio of 2:1 or 1:1, fine fibers with two diameter distributions (the diameter ranged from 80 nm to 180 nm) were obtained from solutions of 4–6 wt.% chitosan/PEO concentrations. They found that thick and thin fibers were formed mainly by PEO and chitosan, respectively.

10) As acetic acid concentration increased from 50% to 90%, surface tension of all solutions without added surfactants decreased from approximately 38 to 30 mN/m (Fig. 3). The presence of any polymer had little influence on the surface tension, i.e. the surface tension of solutions containing no polymer, 1.2–1.6% chitosan or 0.4–1.6% PEO varied betweenw37 and 38 mN/m in 50% acetic acid and decreased to 30–32 mN/m in 90% acetic acid. Upon addition of surfactants, surprisingly little change in surface tension was observed, with exception of addition of SDS in 50% acetic acid, where surface tension significantly decreased to below 30 mN/m. In 90% acetic acid, addition of SDS again had little influence on surface tension[Kriegel et al.[8]).

11) With addition of PEO to chitosan, a well defined Taylor cone was formed, a jet was obtained and a deposition indicative of nanofiber formation was observed at the collector plate surface. FESEM images showed interesting nanofibrous structures with nanofibers having average diameters ranging from 10 to 250 nm[Kriegel et al.[8]).

# Chapter 3

## -Material and Method

## 3.1 Materials required-

1)Chitosan(85% deacetylated)(MERK, India)

2)PEO(polyethylene oxide)(Aldrich,India) – 6000kDa

3)DTAB(Dodecyltrimethylammonium bromide)(HiMedia, India)

4)Glacial Acetic acid

## 3.2 Steps involved in the preparation of the solutions

1) Preparation of 2% chitosan solution- 2g of chitosan was weighed in an electronic weight balance and then mixed in 100ml solution of glacial acetic acid and distilled water(50ml of 50% acetic acid +50ml distilled water) and kept for stirring for around 10-11 hours until an uniform and clear solution was obtained.

2) Preparation of 2% PEO solution - 1g of PEO was weighed and mixed in 100 ml solution of acetic acid and distilled water and kept for stirring for 8-9 hours until a clear and homogenous solution was obtained.

3) Preparation of blend – Chitosan and PEO solutions were added in beaker in different ratios (90/10 , 80/20 , 70/30, 60/40) with varying concentrations of DTAB and kept under stirring for 8-9 hours so that proper mixing and internal binding in the solution takes place.

## 3.3 Electrospinning of the prepared solutions

Different blended solutions were taken for electrospinning. The solutions were then loaded into a plastic syringe(5ml) and this was placed inside the electrospinning machine Espin Nano. A blunt-ended 20-gauge stainless steel needle was used as the nozzle. The emitting electrode from a Gamma High Voltage Research ES30P power supply capable of generating DC voltages up to 30 kV was attached to the needle. The grounding electrode from the same power supply was attached to a rectangular piece of aluminium foil which was used as collector plate and was placed approximately 12 cm below the tip of the needle. Upon application of a high voltage ranging between 20-25kV across the needle and the collector plate(grounded), a fluid jet was initiated from the nozzle. As the jet accelerated towards the collector, the solvent gradually evaporated, leaving only ultrathin fibers on the collector. Obtained fibers were left exposed to air for complete drying so that they can be stored .

### 3.4 Following parameters were studied in the experiment-

- Determination of electrospinnability of the fibers considering various process parameters
- Determination of morphology and size of fibers obtained using SEM
- Determination of interaction between Chitosan and peo using FTIR
- Determination of crytallinity of the obtained fibers using XRD
- Determination of swelling and biodegradability of fibers

Table 2:- **PROCESS PARAMETERS FOLLOWED FOR NANOFIBER FORMATION**

| Voltage applied(kV) | Flow rate(ml/hr) | Tip-collector distance(cm) |
|---|---|---|
| 15 | 0.5 | 15 |
| 20 | 0.5 | 15 |
| 22 | 0.5 | 15 |
| 25 | 0.5 | 15 |
| 25 | 1 | 15 |
| 25 | 0.5 | 15 |
| 25 | 0.3 | 15 |
| 25 | 0.3 | 12 |

Fig 3:- Espin-nano ( electrospinning machine) used for electrospinning of solutions

Fig 4:- Thin fibers being obtained on aluminium foil by the application of high voltage

### 3.5 SEM STUDY

Fiber morphology , texture and dimensions of the fibers were studied using scanning electron microscope with an accelerating voltage of 20 kv . Obtained images were analysed using ImageJ software for the calculation of the average diameter of the nanofibers. A scanning electron microscope (SEM) is a kind of electron microscope which produces images of a sample by scanning it with a focused beam of electrons. The electrons from the machine interact with electrons in the sample, producing various signals that are detected and thus we get information regarding the sample's surface topography and composition. For SEM analysis, a small piece of sample is taken which is given a platinum coating and then it is placed on the SEM multi-holder. It was then inserted into the chamber and on application of high accelerating voltage , SEM images were obtained.

### 3.6 XRD ANALYSIS

To establish the crytallinity of the sample this study was done.X-ray crystallography is a method used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of X-rays to diffract into many specific directions and thus these diffracted rays gives an idea about the crystalline structure of the molecule. In this technique, the sample was kept in the sample holder and inserted in the machine which thereafter produced a graph with characteristic peaks for different samples thus showing their crystalline nature.

### 3.7 FTIR ANALYSIS

The chemical bonds in the sample which are under constant vibrational and bending motions absorb infrared radiations. FTIR measures how well a sample absorbs light at each wavelength corresponding to their unique chemical bondand thus give a unique set of infrared absorption spectra. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. It is an effective analytical tool for detecting functional groups and characterization covalent bonding information. The bonds such as N-H, C-O ,C=O ,C-O-C are analysed whose peaks are unique for different samples. In this technique scaffold was first taken and mixed with small amount of KBr. It was then powdered and formed into a pellet which was inserted in the machine. The intensity of the samples is measured using infrared detectors. And finally we get the FTIR graphs.

## 3.8 SWELLING PROPERTY

Samples with different Chitosan:PEO ratios were taken for the swelling studies. In this method , weight of the samples were measured using electronic weighing balance and it was named as dry weight . Almost same weights of the samples were taken. They were then immersed in SBF and left for 1hour . the samples were then dried using tissue paper and then the weight was again taken which was termed as wet weight. Similarl readings were taken for consecutive 1 hour durations. The next day one reading was taken for 24 hours and finally another reading after 2 days for 48 hours.

Calculation for swelling is done as %age water uptake –

$$\% \text{ water uptake} = [(W_s - W_d)/ W_s] * 100$$

Ws- wet weight of the sample

Wd- dry weight of the sample

## 3.9 BIODEGRADABILITY STUDY

Same procedure was followed as that of the swelling test . It just differs in the formula used.

$$\% \text{ masss remaining} = [(Wi - Wf)/Wi] * 100$$

Wi – initial weight

Wf – final weight

# Chapter 4

## -Result and Discussion

## FOLLOWING RESULTS WERE OBTAINED

**4.1.** Electrospinning of the Chitosan-PEO-DTAB blend was carried outunder different process parameters (Table 2 ) such as voltage applied, flow rate and tip collector distance and it was found that fibers were obtained at 25kV with polymeric flow rate of 0.3ml/hr and tip to collector distance of 12cm .

**4.2.** Electrospinning of the Chitosan-PEO-DTAB blend solutions were performed and the formation of fibers was observed. Two kinds of study were done- one by varying the Chitosan:PEO(keeping the DTAB concentration constant) ratio and the other by varying the DTAB concentration(keeping the Chitosan:PEO ratio constant). Following tables(3,4,5,6) show the results.

Table 3:- **EFFECT OF VARYING THE RATIO OF CHITOSAN:PEO KEEPING THE CONCENTRATION OF DTAB CONSTANT(36mM) ON THE YIELD OF NANOFIBERS**

| Chitosan:PEO ratio | DTAB CONCENTRATION (mM) | OBSERVATIONS |
|---|---|---|
| 90/10 | 36 | Fibers obtained |
| 80/20 | 36 | Fibers obtained |
| 70/30 | 36 | Fibers obtained |
| 60/40 | 36 | Fibers obtained |

Table 4:- **EFFECT OF VARYING THE RATIO OF CHITOSAN:PEO KEEPING THE CONCENTRATION OF DTAB CONSTANT(30mM) ON THE YIELD OF NANOFIBERS**

| Chitosan:PEO ratio | DTAB CONCENTRATION (mM) | OBSERVATIONS |
|---|---|---|
| 90/10 | 30 | Fibers not obtained |
| 80/20 | 30 | Fibers obtained |
| 70/30 | 30 | Fibers obtained but not good |
| 60/40 | 30 | Fibers not obtained |

Table 5:- **EFFECT OF VARYING THE RATIO OF CHITOSAN:PEO KEEPING THE CONCENTRATION OF DTAB CONSTANT(20mM) ON THE YIELD OF NANOFIBERS**

| Chitosan:PEO ratio | DTAB CONCENTRATION (mM) | OBSERVATIONS |
|---|---|---|
| 90/10 | 20 | Fibers not obtained |
| 80/20 | 20 | Fibers obtained |
| 70/30 | 20 | Fibers obtained |
| 60/40 | 20 | Fibers not obtained |

Table 6:- **EFFECT OF VARYING THE RATIO OF CHITOSAN:PEO KEEPING THE CONCENTRATION OF DTAB CONSTANT(15mM) ON THE YIELD OF NANOFIBERS**

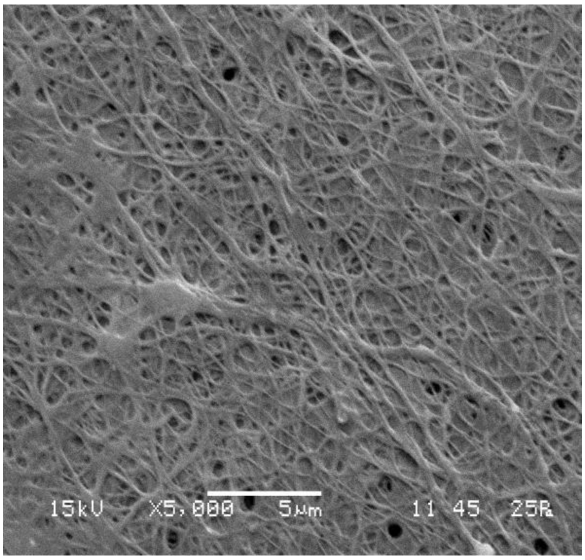
| Chitosan:PEO ratio | DTAB CONCENTRATION (mM) | OBSERVATIONS |
|---|---|---|
| 90/10 | 15 | Fibers not obtained |
| 80/20 | 15 | Fibers obtained |
| 70/30 | 15 | Fibers obtained |
| 60/40 | 15 | Fibers not obtained |

## 4.3. SEM STUDY

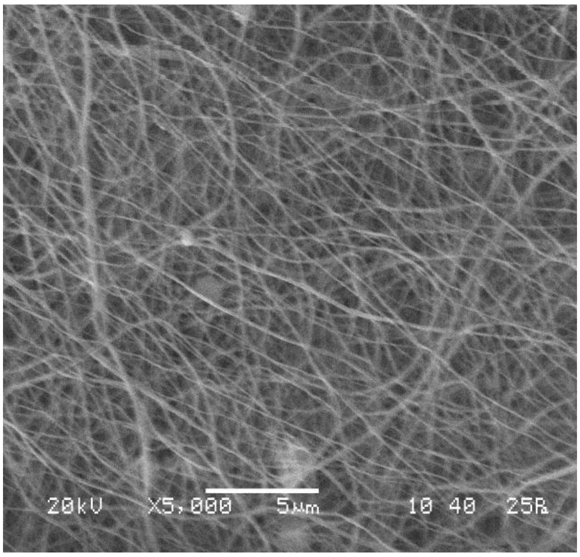
Following are the SEM results for the fibers obtained . Images of certain compositions could not be taken as they didn’t give fibers with good morphology. It is observed that at higher DTAB concentrations , proper fibers are not obtained unlike that in the lower concentartions of DTAB. Chitosan:PEO ratio of 70/30 didn’t give good fibers. Also images for ratios 90/10 and 80/20 were not obtained as the resultant fibers were not good.

It is clearly visible that low concentrations of DTAB yield good fibers and that too in the ratios of Chitosan:PEO of 70/30 and 80/20. For ratio 60/40- DTAB(36mM) fibers obtained were in the range of 50-150nm as calculated from ImageJ software . For ratio 70/30- DTAB(20mM) fibers obtained were in the range of 50-200nm as calculated from ImageJ software . And for ratios 70/30- DTAB(15mM) and 80/20 – DTAB(15mM) , fibers were found to be in the range of 50-250 nm. The results are shown below -

(A)



(B)



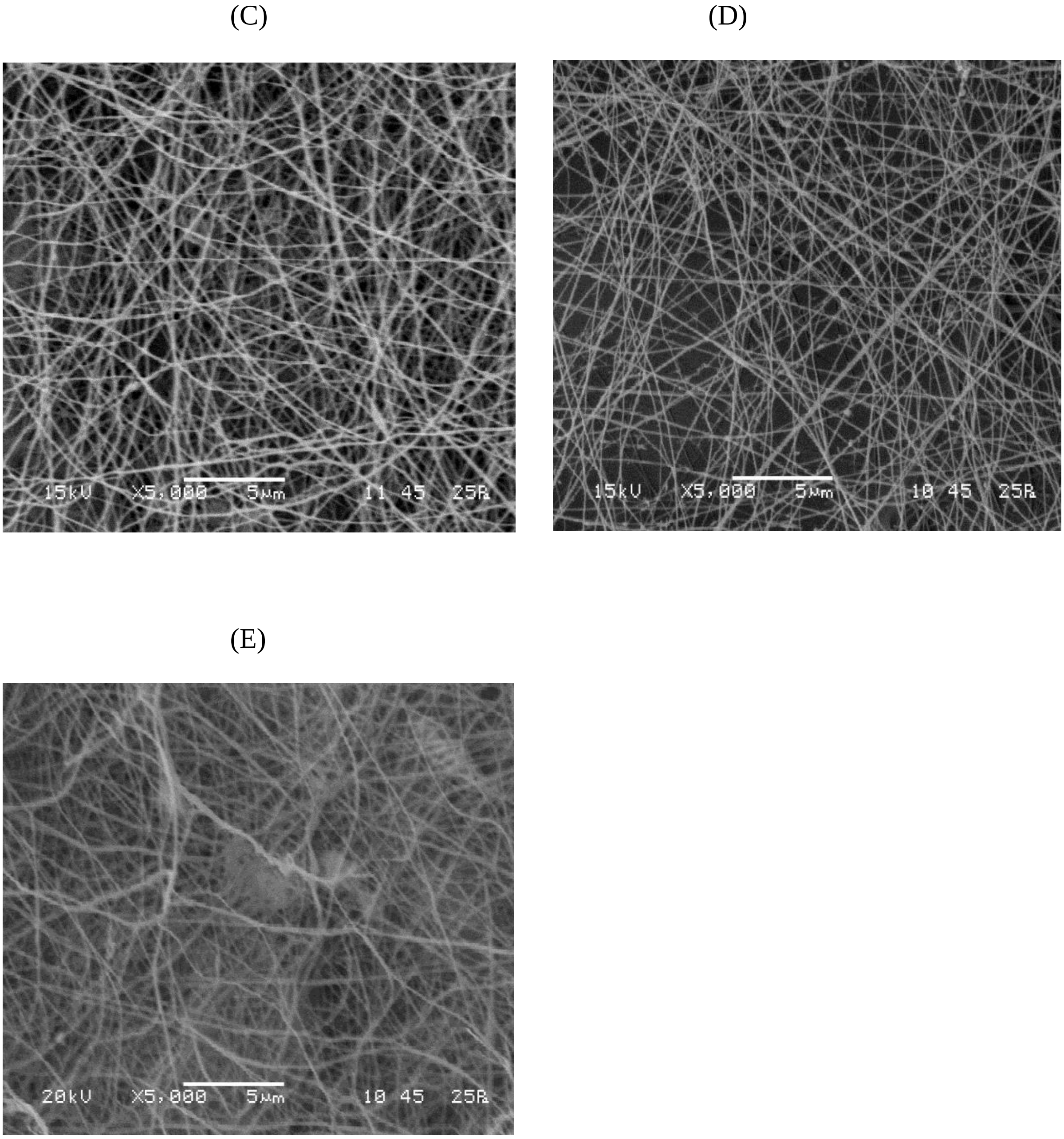

(C) (D) (E)

Fig 5:- Scanning Electron Microsgraph of Chitosan:PEO in different ratios with varying DTAB concentration for under magnification of 5000X.

(A) 70/30 – DTAB(36mM) (B) 60/40 – DTAB(36mM) (C)70/30 – DTAB(20Mm)

(D) 80/20- DTAB(15mM) (E) 70/30 – DTAB(15mM)

Also it is clear that more and bigger beads are obtained at 15mM DTAB concentration as compared to that of 20Mm DTAB concentration. Also finer fibers are obtained with Chitosan:PEO ratio of 70/30 – DTAB(20mM) ranging between 50-200nm. It is observed that with increasing DTAB concentrations fiber diameter gradually decreases. But yet fiber morphology is better in case of fibers with low DTAB concentrations as evident from the following figures.

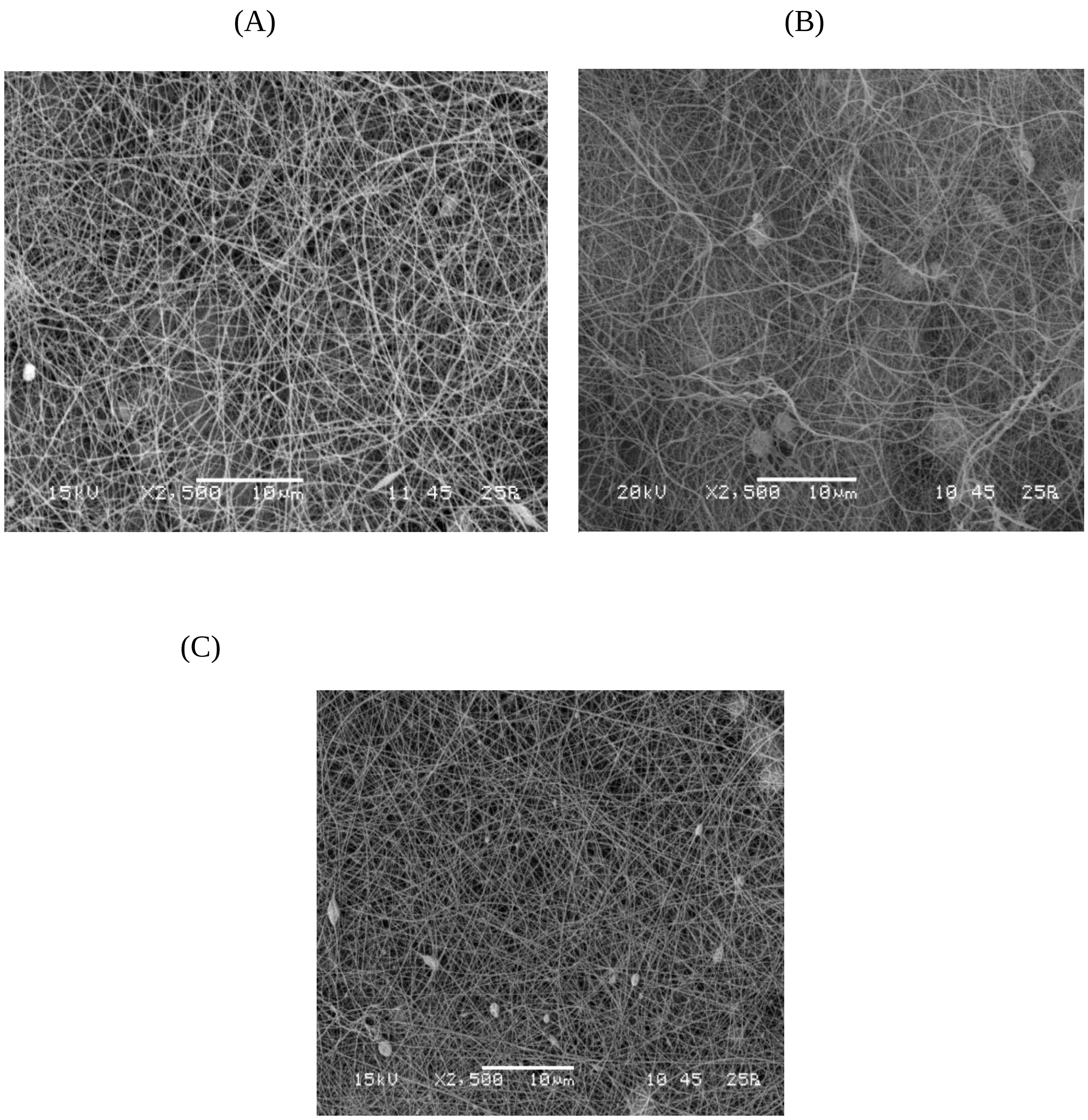

(A) (B) (C)

Fig 6:- Scanning Electron Microsgraph of Chitosan:PEO in different ratios with varying DTAB concentration for under magnification of 2500X.

(A) 70/30 – DTAB(20mM) (B) 70/30 – DTAB(15mM) (C)80/20 – DTAB(15Mm)

## 4.4.FTIR STUDY

Chitosan gives peak at 1653( N-H bond) , at 2874(C-H bond), at 1070(C-O-C bond) , 1653 and 2364. Also PEO gives peak at 2891(C-H bond), 1101(C-O-C bond) , 961&841(C-$H_2$ bond) , 1968 and 1474. It is observed that in the composite [chitosan-PEO-DAB] , the 1071 peak of chitosan is intact. Also 1653 peak of chitosan has shifted a little to 1600. Peak of 2364 is quite visible in all the three i.e. chitosan , PEO and composite. FTIR spectra thus confirms the interaction of chitosan and PEO as shown in the following figure.

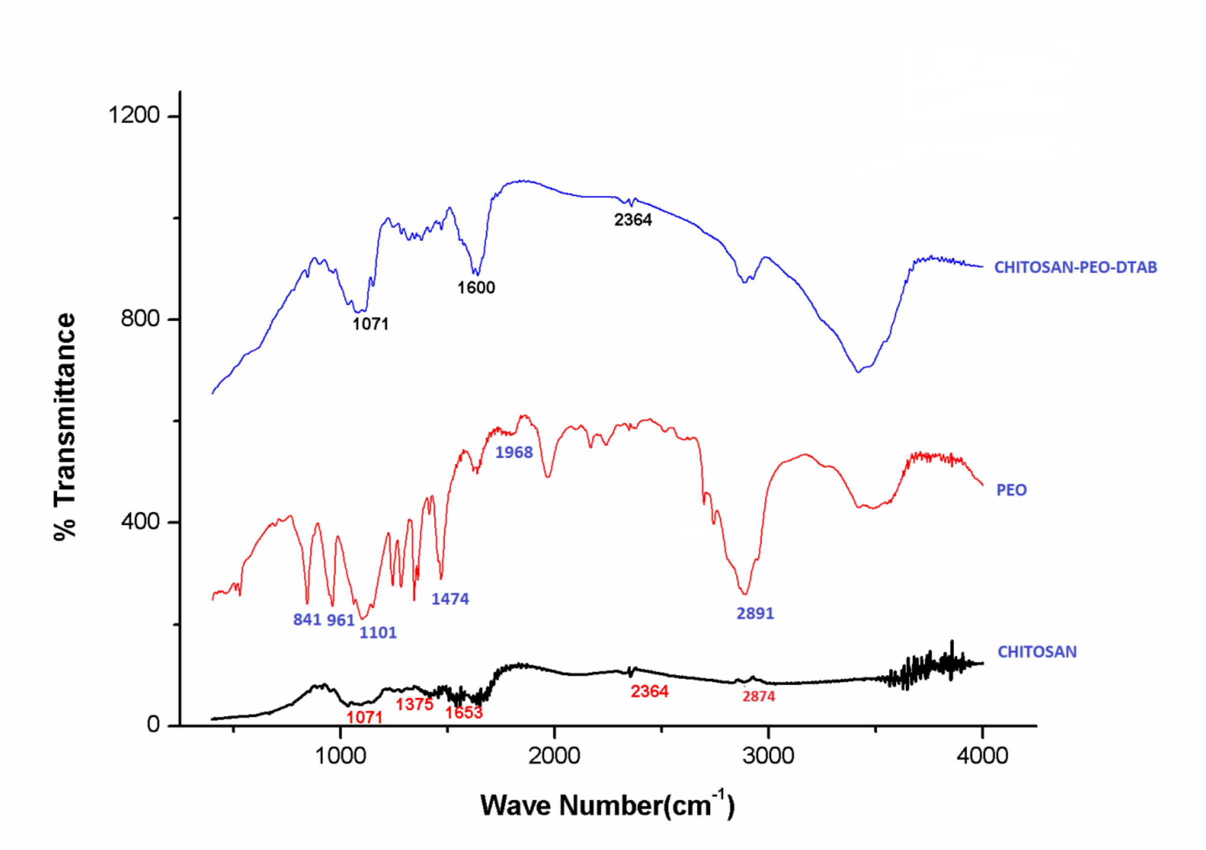


Fig 7:- Comparative result of FTIR spectra of chitosan , PEO and composite nanofiber.

### 4.5. XRD ANALYSIS

It was observed that the scaffold formed possessed crystalline nature. It is evident from the XRD plot that the composite contains the peak of both chitosan and PEO.

The intensity of the peak for PEO in the composite has decreased(below 200 arbitary unit) from that of the original( around 3000 arbitrary unit). Value for chitosan almost remains the same in the composite. The comparative results thus clearly shows the presence of crystalline nature in the composite[CPD:- chitosan-PEO-DTAB].

Following results were obtained with XRD.

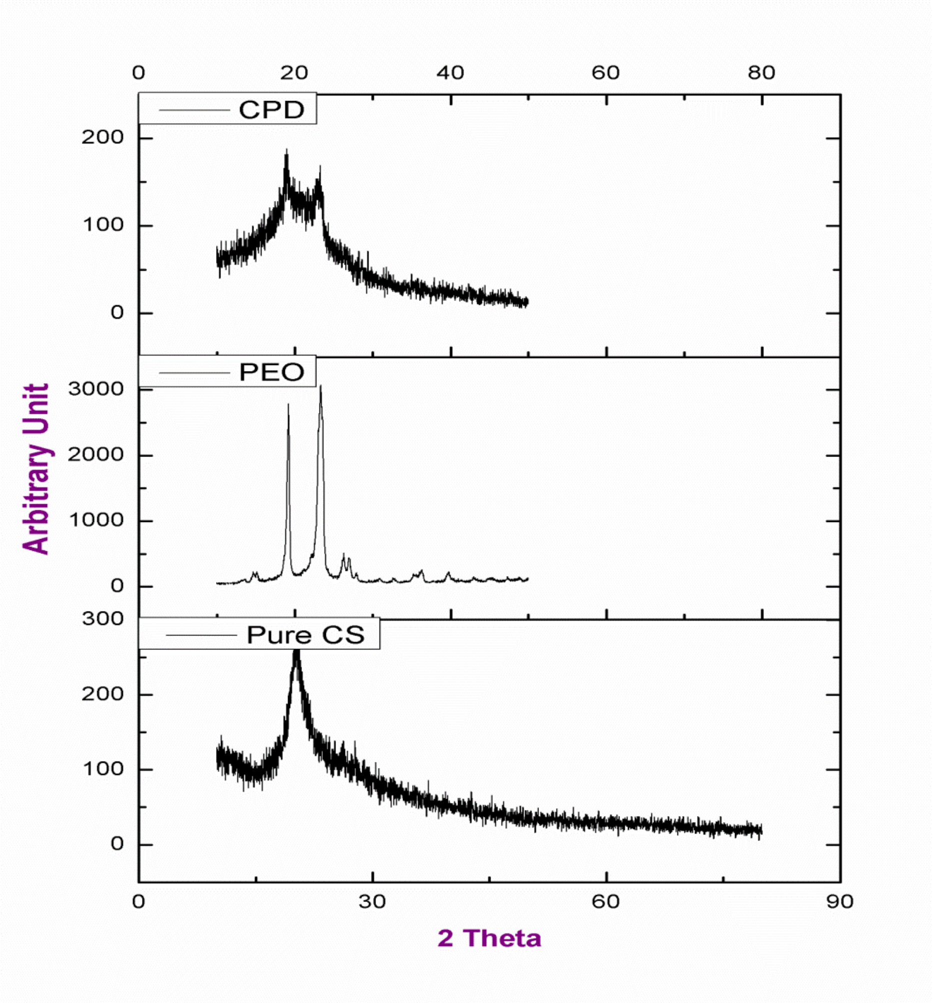


Fig 8:- XRD plot of chitosan , PEO and composite nanofiber(CPD)

### 4.6.BIODEGRADABILITY STUDY

It was observed that for chitosan:PEO of 60/40 %age mass remaining decreased faster as compared to the other samples which shows that sample 60/40 will degrade faster i.e. it will sustain inside the body for shorter period of time as compared to other samples.

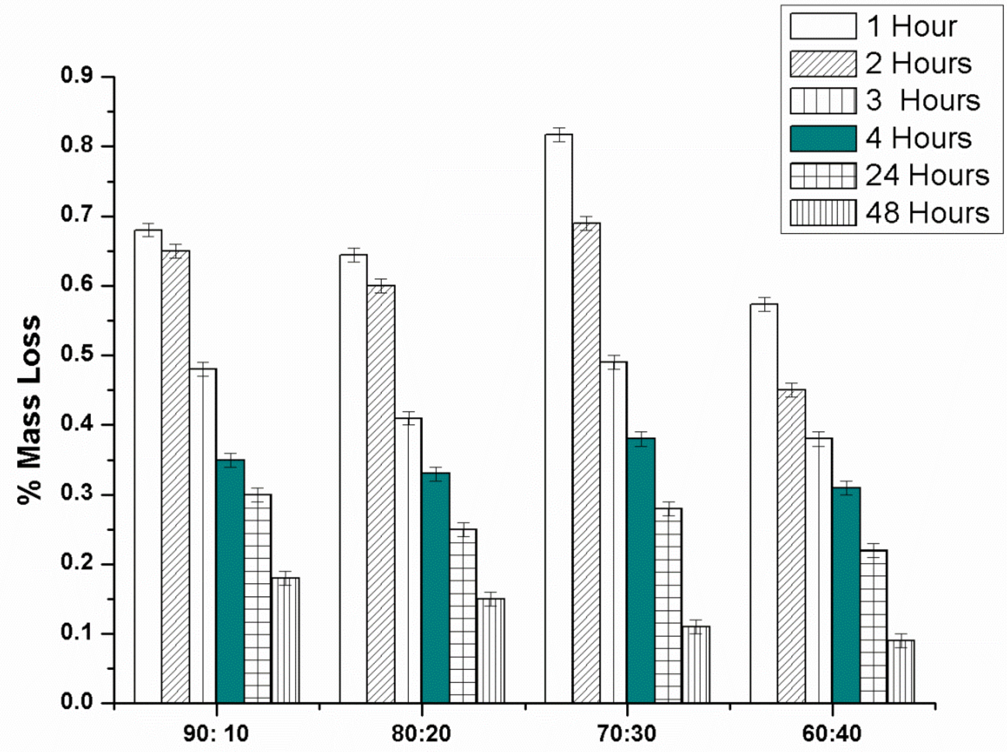


Fig 9:- Graph for biodegradability of the scaffolds of different chitosan-PEO ratios

### 4.6 SWELLING STUDY

Water uptake capacity of the sample 60/40 was overall found to be higher as compared to other samples and that of the sample 90/10 was found to be the least overall leaving samples 80/20 and 70/30 in between.

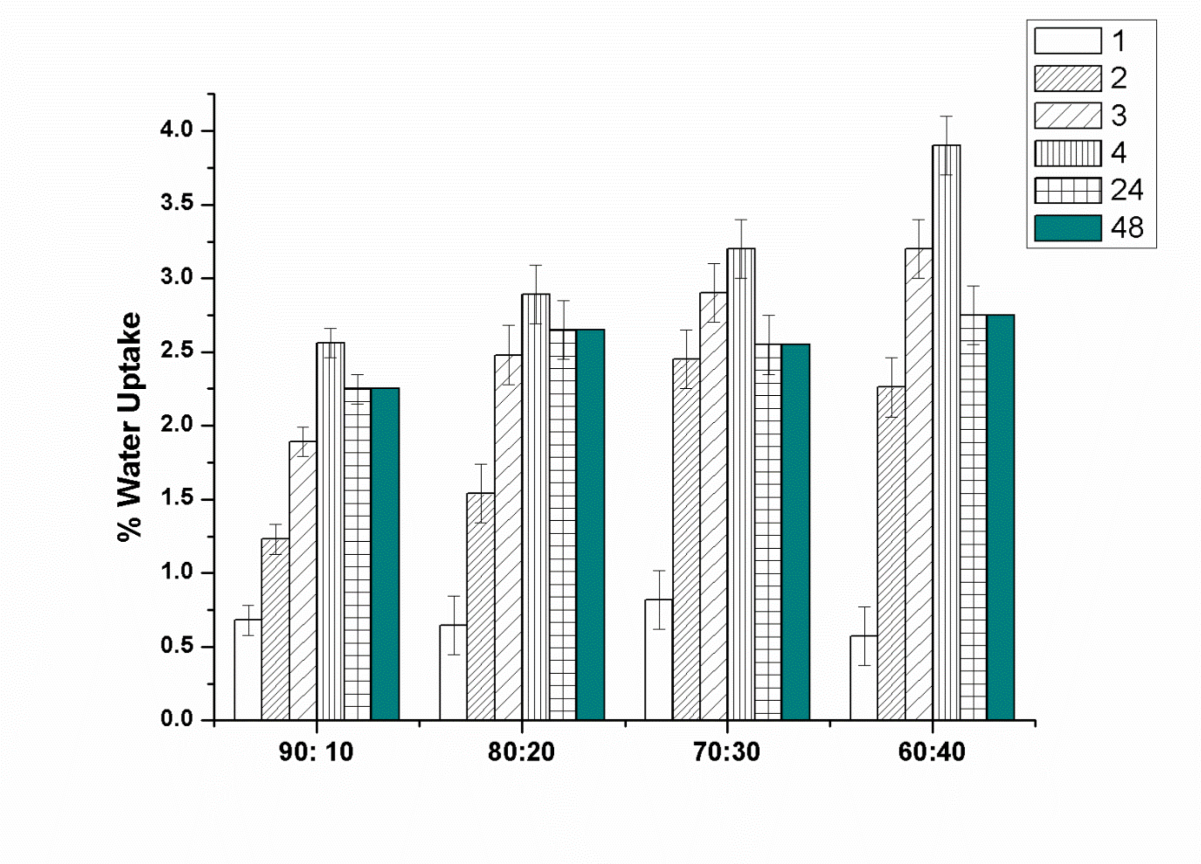


Fig 10:- Graph for %age water uptake of the scaffolds of different chitosan-PEO ratios

# Chapter 5

## -Conclusions

The present study dealt with the preparations of nanofibers from blends of Chitosan:PEO:DTAB varying in ratios and concentrations. Also various characterisation techniques such as SEM , FTIR, XRD were used for morphological and chemical studies. Besides that biodegradability and swelling tests were also done. Effect of varying the concentration of DTAB keeping the ratio constant was also observed in the present study.

To sum up, the conclusions are as follows-

1) Composites in the form of nanofibers were successfully fabricated by electrospinning of chitosan with PEO with varying ratios in solvent acetic acid:water and in presence of DTAB surfactant.

2) It was found that not all compositions of chitosan:PEO:DTAB yielded fibers. Fibers with low DTAB concenrations and chitosan:PEO ratios of 80/20 and 70/30 yielded good fibers in the range of 50-250 nm.

3) Fibers were obtained at 0.3ml/hr with a tip-collector distance of 12cm and applied voltage of 25kV.

4) Samples with chitosan:PEO ratios in between i.e. 80/20 and 70/30 had their values of biodegradability and swelling tests in between. Also these ratios had good yield of fibers as evident from SEM results at low DTAB concentrations. Thus we conclude that fibers should be processed with ratios 80/20 and 70/30 or somewhere between ratios 90/10 and 60/40 and that too at low DTAB concentration so that it is not much harmful to the cells .

# Chapter 6

## -Future Work and suggestions

## FUTURE WORK AND SUGGESTIONS:-

1)Different other ratios of chitosan:PEO can be tried with low DTAB concentrations.

2) Use of DTAB can be further explored as an active carrier of drugs in the micelles for the seeded cells.

3) Further low concentration of acetic acid can be used so that the cells are least harmed by the residual acid(left even after electrospinning and other treatments) present in the scaffold.

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