International Journal of Innovative Research in Advanced Engineering (IJIRAE) ISSN: 2349-2163
Issue 02, Volume 6 (February 2019) www.ijirae.com

PREPARATION AND EVALUATION OF WOOL KERATIN BASED CHITOSAN NANOFIBERS FOR AIR AND WATER FILTRATION

Chetan Mahajan
Wool Research Association, PO. Sandoz Baug, Kolshet Road,
Thane (W) 400607, Maharashtra, India
crmahajan4985@gmail.com

Shishir Tyagi
Wool Research Association, PO. Sandoz Baug, Kolshet Road
Thane (W) 400607, Maharashtra, India
zamiiict@gmail.com

Manuscript History
Number: **IJIRAE/RS/Vol.06/Issue02/FBAE10083**
Received: 03, February 2019
Final Correction: 10, February 2019
Final Accepted: 15, February 2019
Published: **February 2019**
Citation: Mahajan & Tyagi (2019). PREPARATION AND EVALUATION OF WOOL KERATIN BASED CHITOSAN NANOFIBERS FOR AIR AND WATER FILTRATION. IJIRAE::International Journal of Innovative Research in Advanced Engineering, Volume VI, 37-44.**doi://10.26562/IJIRAE.2019.FBAE10083**
Editor: Dr.A.Arul L.S, Chief Editor, IJIRAE, AM Publications, India

Abstract— In this article, we have extracted keratin from deccani wool waste and prepared the wool keratin based Chitosan nanofibers by electrospinning technique. The prepared nanofibers mat were prepared with different weight percent ratio like 1wt.%, 3wt.% and 5wt.% with respect to polymer i.e Chitosan. The physicochemical and filtration properties of wool keratin based Chitosan nanofibers were studied. Wool keratin based Chitosan nanofibers were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC) and scanning electron microscopy (FESEM). The filtration efficiency of keratin Chitosan nanofibers were investigated through DOP test and heavy metal removal capacity of evaluated through Atomic absorption spectroscopy. FTIR results were showed that Keratin gets compatible with Chitosan. XRD patterns revealed keratin was in crystalline nature and increase the crystalline nature of Chitosan nanofibers. FESEM images showed that uniform nanofibers generation with average fiber diameter 80nm. Nanofibers filtration efficiency against a particulate matter in air was obtained more than 99.53% and excellent property of removal of heavy metal.

Keywords — Wool keratin; Chitosan; nanofibers; Air filtration; Water filtration;

I. INTRODUCTION

Keratin is the major structural protein of wool fiber; also this is the key structural material making up the outer layer of human skin. Wool fiber in its native state belongs to a group of proteins called alpha-keratins, which is the crystalline portion of the protein having a particular X-ray diffraction pattern associated with the alpha -helical structure for proteins. Keratins are defined as natural, cellular systems of fibrous proteins cross-linked by cysteine sulphur. Keratin based biomaterials are extensively used in biomedical applications because of the several key properties of keratins that contribute to the overall physical, chemical and biological behaviour of these biomaterials. The extracted keratin proteins have an intrinsic ability to self-assemble and polymerize into porous, fibrous scaffolds.

This phenomenon of self-assembly is evident in the highly conserved superstructure of the wool fiber and when processed correctly, is responsible for the reproducible architecture, dimensionality and porosity of keratin-based materials. The keratin is biomaterials and extracted from wool and human hair. [1-3]. The synthetic and natural origin polymers has been used to create electrospun fibers for different applications [4]. Recently, research on the electrospun nanofibers of biocompatible polymeric materials has very much enlarged due to versatile application. Electrospining is a technique based on electrostatic charges that utilizes a high voltage charge helps to create jet of polymer and travels towards collecting plate due to charge difference. The resulting fibers have diameters in the rages from nano- to micro-scale and form a non-woven fibrous mat. The non-woven fibrous mat enhanced physical properties due to small pore size, high porosity, three-dimensional features, and high surface area-to-volume ratio. Recently, the electrospining process has also been extended to include regenerated keratin extracted from hair and wool fibers. Due to the intrinsically poor mechanical characteristics of pure keratin, however, many researchers have resorted to the addition of synthetic or natural polymers in order to increase the processability of keratin for fiber formation [5-13]. This present focuses on the preparation of keratin embedded Chitosan nanofibers for air and water filtration.

II. MATERIALS AND METHODS

A. Materials: Waste wool supplied by Raymond ltd, Petroleum Ether, Urea, 2-Mercaptoethanol, sodium dodecyl sulfate (SDS), Formic acid 85 %, Acetic acid was procured from SD Fine Chemicals Limited (Mumbai, Maharashtra), Cellulose Dialysis Tubing (molecular weight cut off 12000-14000Da) and chitosan purchased from Himedia laboratories (Mumbai, Maharashtra).

B. Keratin extraction: Extraction of Keratin from waste as per method reported previously (Yamauchi et al., 1996). The wool fibres were flooded in petroleum ether for 4 hours to de-waxing and removal of impurities like dust etc. The keratin was extracted with a mixture of 0.2 M SDS, 8 M urea and 2 mercapto ethanol at 70°C for 5 hours. Wool fibers were dissolved in the mixture solution and form the dark red colour. The keratin solution was then filtered to remove the un-dissolved wool fibre and thoroughly dialyzed against distilled water using cellulose dialysis tubing to remove the chemicals. The pure form of aqueous keratin solution was obtained, poured the solution into a petri dish and placed it in oven for 2 days and to get hydrolysed keratin powder.

C. Preparation of Keratin Chitosan nanofibers: Chitosan solution and keratin/Chitosan composites were prepared in 2% acetic acid. The keratin/Chitosan composites were prepared with different weight % ratio with respect to chitosan (C1) like 1wt.%(C2), 3wt.% (C3)and 5wt.% (C4). The keratin/Chitosan composites were electrospun at a 15-cm working distance between collecting plate and tip of syringe. The applied voltages were between 25-30 kV and about 2 mL of the polymer solution was placed in the syringe. The tip was positively charged by the generator, when a steady voltage was reached between the tip and collector, the delivery pump switched on and fed the fixed flow 0.5 ml/hr of the solution through the capillary, and the electrospinning process started. The process was stopped after about 4hr. During the electrospinning process the temperature was ranged from 20 to 25°C, and the relative humidity was in the range of 30–45%.

III. CHARACTERIZATION

A.UV Visible spectroscopy: UV Visible spectroscopy of powdered keratin was obtained by making the solution in water and analyzing UV Visible spectra with help of Specord 201 Analytik Jena Germany.

B.Fourier Transform Infra Red (FTIR) Analysis: Fourier transform infra red (FTIR) spectra of lyophilized reduced keratin was obtained by using the ATR technique (Attenuated Total Reflection). An infrared spectrum of keratin was recorded from 5000 to 500 cm-1 using Alpha Bruker spectrophotometer Germany

C. Differential Scanning Calorimeter (DSC) Analysis: Differential scanning calorimeter (DSC) analysis of keratin after conditioning the samples at 24°C, 65% R.H was performed from 30°C to 500°C, at 10°C/min using instruments DSC 3 Mettelar Toledo, Switzerland,. The instrument was calibrated by an indium standard and the calorimeter cell was flushed with 40 ml/min liquid nitrogen.

D. X-Ray diffraction analysis: Crystalline nature and structure was judged by Bruker, D8 ADVANCE (Bruker Corporation, Tokyo, Japan) X-ray powder diffractometer (XRD) using monochromatic CuKα radiation (λ = 1.5406 Å) at 40 kV and 40 mA. Scan rate was 50/min between the angles 5-80°.

E. Scanning electron microscopy: Surface morphology was observed using field emission scanning electron microscope (FE-SEM), HITACHI S-4800, operated at 5 to 15 Kv. Suspension was drop casted onto carbon tape and dried at room temperature. Prior to analysis sample was coated with gold to avoid degradation or burning due to high power.

F. Air permeability: This test method covers the measurement of the air permeability--the rate of air flow passing perpendicularly through a known area under a prescribed air pressure differential between the two surfaces of a material of textile fabrics and is applicable to most fabrics including woven fabrics, Non woven fabric, air bag fabrics, blankets, napped fabrics, knitted fabrics, layered fabrics, and pile fabrics. Nanofibrous mat comes under None woven fabric, therefore it's important to evaluate air permeability of nanofibrous mat.

Determination air permeability of keratin based nanofibrous mat under designated pressure analyzed standard ASTM D 0737 by applying pressure 125pa and test area 38cm2 using Air permeability tester MO211A SDL ATLAS Hong kong.

G.Heavy metal absorption: Heavy-metal adsorption from aqueous solutions by keratin based bio films and nanofibrous membranes was performed in dynamic conditions. Practically, accurately weight bio-film nanofibers mat and, was placed in the solution containing heavy metals for required period of time. All the adsorption experiments were carried out at a temperature of 25°C. The concentration of the metal ions after the adsorption was determined with an atomic absorption spectroscopy 906AA GBC Scientific Australia.

$$\text{Removal Efficiency } (\%) = \frac{C_0 - C_t}{C_0} \times 100$$

where The removal efficiency was evaluated using the following equation adsorption capacity of the biofilms and nanofibers membrane at C0 and Ct (mg/L) are the initial heavy-metal ion concentration and final heavy-metal ion concentration respectively, Heavy metal stock solutions of 1000 ppm were prepared using their chloride and nitrate salts, from which solutions of lower concentrations were obtained by dilution. Batch adsorption tests were conducted in conical flasks with the desired adsorbent to adsorbate ratio. The suspensions were agitated by shaking in a water bath shaker at 100 revolutions per minute (rpm). The conditioning temperature was adjusted and maintained at the desired level. Deionized water was used in all experiments and all chemicals used were of analytical grade.

H. Filtration efficiency (DOP) Test: Filtration efficiency with pressure drop evaluated by PALAS2010 instrument were 0.3µ-4µ particle size dust/aero-gel passed through nanofibers coated membrane and evaluate % retention of solid particles and pressure drop. DOP/PAO testing is a very quick process that tests the integrity of the HEPA (High Efficiency Particulate Air) filter using DOP or PAO solutions in their operational conditions. These solutions generate a gas type smoke and then generate gas particles that will be greater than 0.3 microns. The test will certify the HEPA filter is fully functioning and there is no leakage or damage.

IV. RESULT AND DISCUSSION

A. UV Visible spectroscopy:

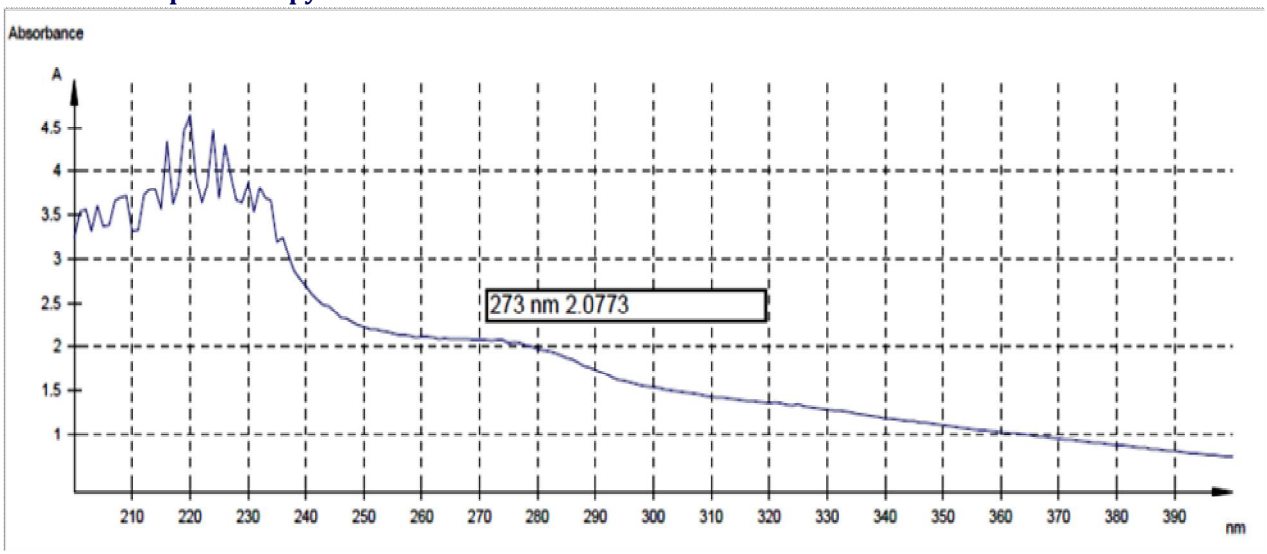

Fig. 1.UV visible spectra of extracted keratin

UV-Vis absorption measurement of extracted keratin solution (fig 1) shows λ_{max} at 273 nm with the absorbance 2.0779. Keratin was absorbs mainly in the far UV, but has an absorption tail out as far 380nm. The main chromospheres absorbing in the UV region are aromatic amino acids, tryptophan, tyrosine and phenylalanine, which are present in the keratin chain. [11]

B. FTIR analysis:

FTIR analysis of keratin showed fig 2. the peptide bonds (–CONH–) were seen in the vibrational spectra. The vibrations in the peptide bonds can be attributed to Amide A group which is characteristic bond in keratin i.e amide I, II, III. The amide A band, which falls at 3327.12 cm^{-1} represents stretching vibration of N–H bonds .The amide I band due to C––O stretching vibration occurs in the range of 1700–1600 cm^{-1}. 1544.47 cm^{-1} represent amide II was related to N–H bending and C–H stretching vibration. The amide III band observed in between 1220–1300 cm^{-1} was highlighted at 1245.18 cm-1 as a sharp peak. The carbohydrate moieties band was observed at 1083.10 cm^{-1}. [10-12]

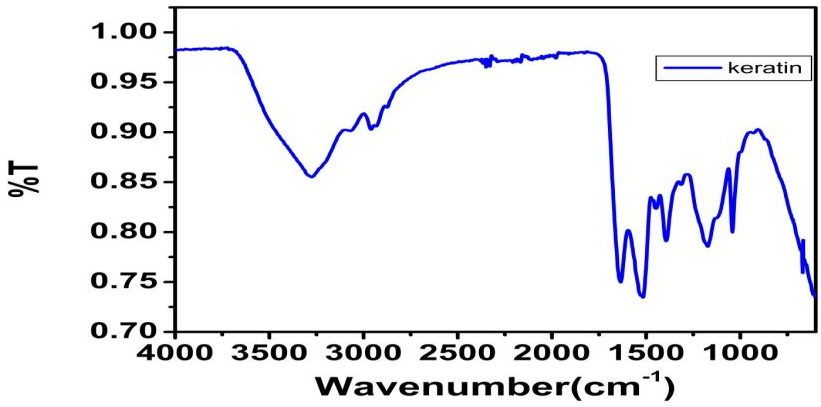

Fig. 2.FTIR Spectra of Keratin

C. DSC analysis of keratin

The DSC thermo gram of keratin is shown in Fig 3. The denaturation temperature observed around 56°C is due to water evaporation. The endothermic peak observed above 148°C is reported for α helix denaturation in keratin. The second endothermic peak for the hoof keratin is observed around 224°C.

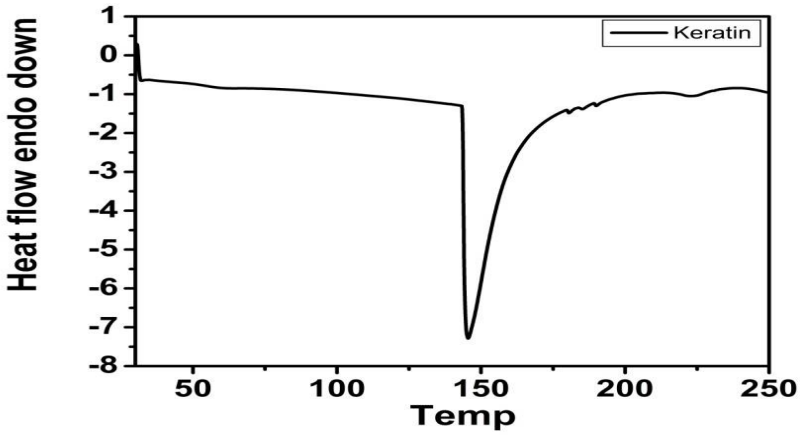

Fig. 3 DSC analysis of Keratin

D. X Ray diffraction of Keratin:

An x-ray diffraction spectrum of extracted keratin was shown in fig 4. In XRD spectra peak arise at 2Θ, 21.2° are index for the b-sheet crystalline structure of keratin. XRD spectra clearly indicate the extracted keratin was in highly crystalline state. [11]

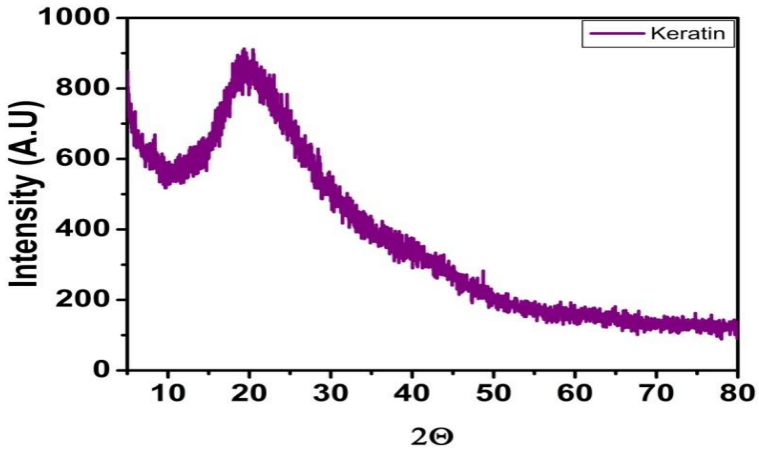

Fig. 4. X -ray diffraction of Keratin

Evaluation of Keratin/Chitosan composite nanofibers:

F. FTIR analysis:

The FT-IR spectra of prepared Chitosan showed significant absorption bands to identify the characteristic functional groups which were recorded in the middle infrared (4000 cm^{-1} to 500 cm^{-1}). The infrared spectra for chitosan biopolymers are shown in Fig. 5. The stretching vibrations of -OH bond of the prepared chitosan was found at 3478.68cm^{-1} and that for C–H were observed at 2924.13 cm^{-1}. The absorption peaks at 1656.88 cm^{-1} , 1571.05 cm^{-1} , 1422.53 cm^{-1} , 1378.16 cm^{-1} were associated with the presence of the C=O stretching of the amide I band, bending vibrations of the N–H (N-acetylated residues, amide II band), C-H bending, OH –1 bending respectively. The peak at 1157.31 cm was assigned for anti- symmetric stretching of bridge, 1075.33cm^{-1} and 1025.18 cm^{-1} were anticipated to the skeletal vibration involving C–O stretching. Spectra of pure polyamide 6 and protein from wool showed overlapping bands at about 3478cm^{-1} (Amide I) and 1571 cm^{-1} (Amide II) . Only in the region between 1000 cm^{-1} and 800 cm^{-1} absorptions in Chitosan spectrum were present

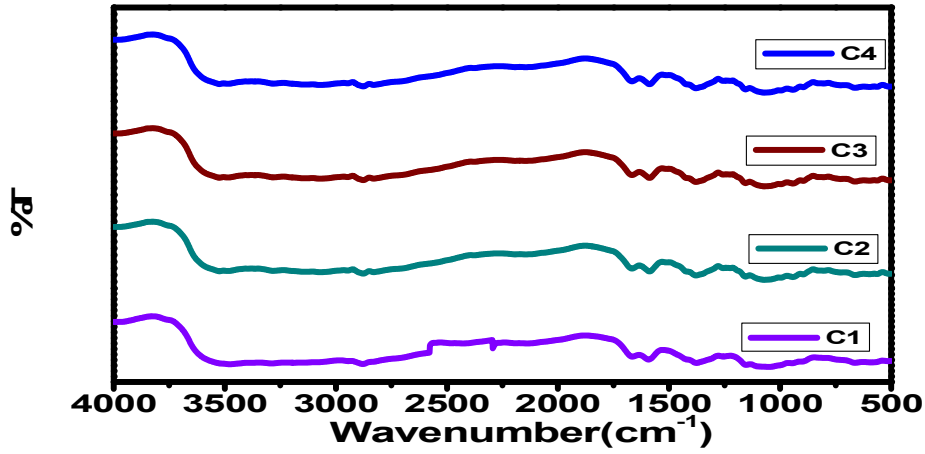

Fig. 5. FTIR spectra of keratin/chitosan composite nanofibers

F. XRD analysis:

X-ray diffraction spectra of keratin and keratin/Chitosan composite nanofibers was shown in fig.6. In XRD spectra peak has been rises at 2θ, 21.2° are index for the b-sheet crystalline structure of keratin. XRD spectra clearly indicated the extracted keratin was in highly crystalline state. In case of keratin/ Chitosan nanofibers intensity of peak at 2θ, 21.2° increased due to addition of keratin.

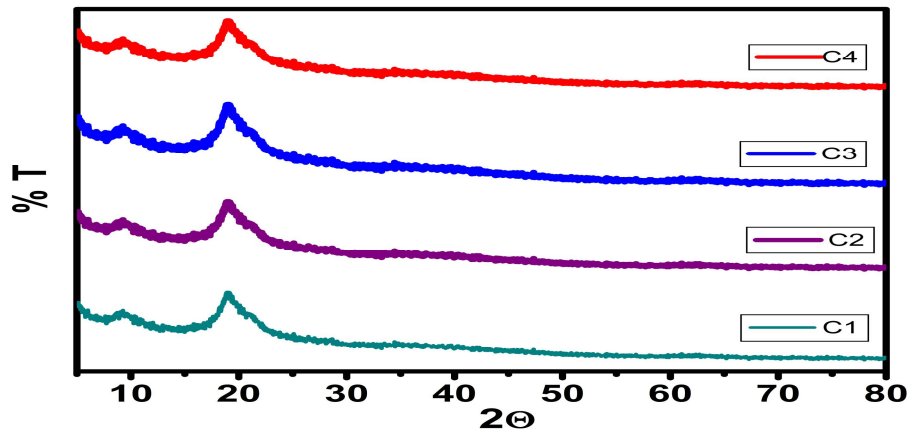

Fig. 1. XRD analysis of Keratin /Chitosan composite nanofibers

G. DSC analysis:

Fig 7 illustrated the DSC curves of keratin/Chitosan composite nanofibers, the endothermic peak at approximately 78.59 °C can be attributed to the evaporation of bound water, representing the energy required to vaporize bound water present in the films, followed by another endothermic peak observed at about 238°C related to the crystalline melting (Tm) of the keratin/Chitosan composite nanofibers.

Chitosan has interferes with the keratin self-assembling, causing the protein chains to organize in a thermally more stable than neat chitosan. Degradation temperature for keratin/Chitosan composite nanofibers was found to be 339.10°C .

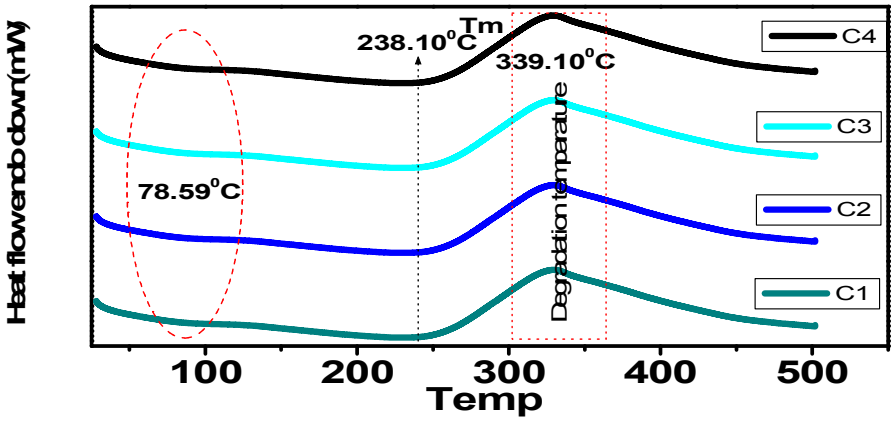

Fig. 7. DSC analysis of keratin/Chitosan composite nanofibers

H. Heavy metal adsorption (%) Efficiency in water:

Metal like Cd, Ni Pb and Fe were removed with the help of keratin/chitosan nanofibers with different weight ratio. Metal salt of Cd, Ni Pb and Fe in known concentration containing solution was passed through Nanofibrous mat and evaluated the amount of the heavy metal adsorbed on the surface of the Nanofibrous layer. Chitosan nanofibers shows good amount of heavy metal adsorption but in case of keratin/Chitosan composite nanofibers shows excellent adsorption ability than neat chitosan nanofibers shown in table 1. The spectacularly enhanced in adsorption efficiency due to special characteristic nature of keratin and nanofibers. Keratin is functionally active protein which has functional moieties helps to bind the metal ions to them, therefore keratin showed additive effect for removal of heavy metals with Chitosan nanofibers.

Table 1. Heavy metal adsorption efficiency of Chitosan and Keratin/chitosan composite nanofibers

Membrane	Heavy metal removal efficiency (%)			
	Cd	Ni	Pb	Fe
Chitosan nanofibers coated non woven fabric (C1)	24.9	28.74	22.1	31.65
Keratin/chitosan composite nanofibers non woven fabric(C2)	88.9	86.4	89.46	97.11
Keratin/chitosan composite nanofibers coated non woven fabric (C3)	89.2	87.1	88.46	96.56
Keratin/chitosan composite nanofibers coated non woven fabric (C4)	90.1	91.47	89.58	98.97

I. Particulate matter filtration efficiency: Filtration efficiency with pressure drop was evaluated by DOP test (Dispersed oil particulate). The 0.3µ-4µ particle size dust/aero-gel passed through Chitosan and Keratin/chitosan composite nanofibers coated membrane and evaluate % retention of solid particles and pressure drop. The particulate matter (aerogel) passed through nanofibers at 95 L/m and evaluates the filtration efficiency. Obtained result shows excellent filtration efficiency 97-98% due to the presence of nanofibers. Nanofibers are play an important role in filtration, nanofibrous mat contains very small pores upto 100nm which helps to restrict particulate matter which will more than 100nm. The densities of nano pores are numerous due to that air can easily pass through membrane but particulate matter get restricted. Maximum density of nano pores helps to maintain pressure drop.

Table 2. Evalution of filtration efficiency (%) Chitosan and Keratin/chitosan composite nanofibers

Membrane			(%) Filtration efficiency	Pressure drop(ΔH) mmwg	Air flow
	Inlet Count (C1)	Outlet count (C2)			
(C1)	141476	3458	97.55	20	95L/m
(C2)	149548	2248	98.49	24	
(C3)	114597	1146	98.99	24.6	
(C4)	179892	892	99.53	25.1	

J. Surface Morphology Chitosan and Keratin/ Chitosan Nanofibers:

The surface morphology of keratin/Chitosan nanofibers were evaluated by scanning electron microscopy shown in fig. 8. Nanofibers are prepared with mixture of Keratin with Chitosan.

Nanofibers are uniformly formed with the diameter 68nm-79 nm. The Nanofibers make non woven fibrous structure which was uniform in nature. The surface morphology of nanofibers has been undoubtedly shown that the nanofibers mat forms the mesh like structure which helps in filtration air as well as water.

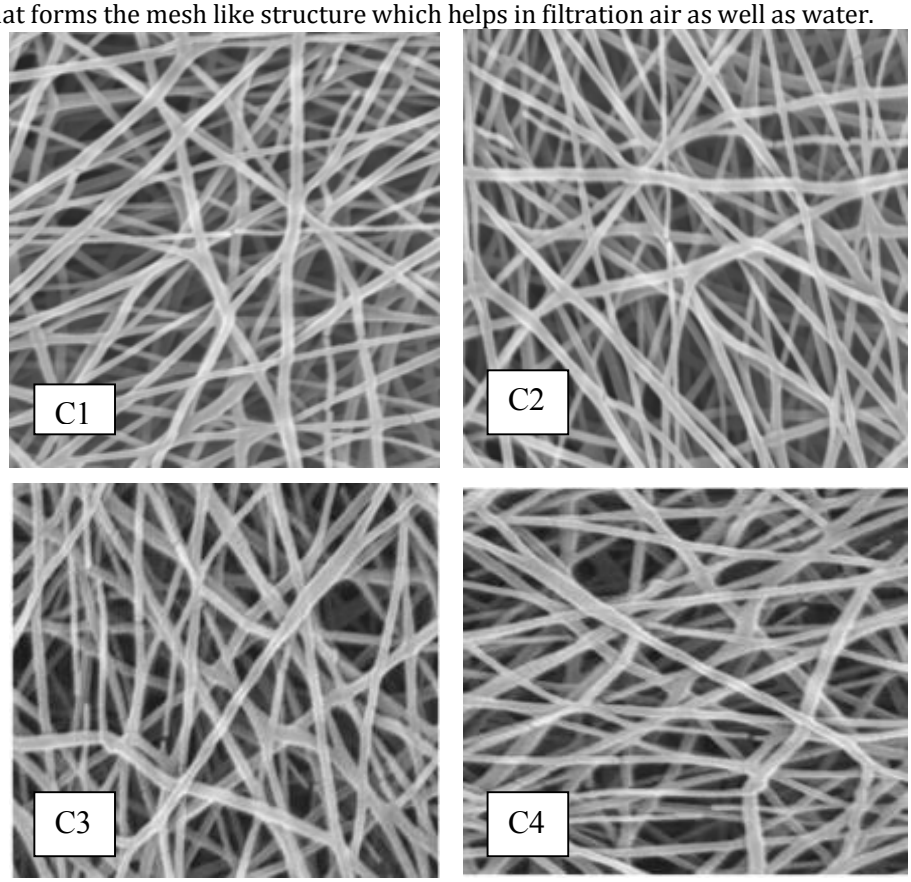

Fig.8. SEM analysis of Chitosan and keratin/Chitosan composite nanofibers

V. CONCLUSION

Keratin extracted from deccani wool waste and blended with chitosan at different concentration. The electrospinning process appeared stable and successful for keratin/chitosan composite solutions. All the nanofibers were found to be smooth and bead-free with favourable compatibility between keratin and Chitosan. The filtration efficiency keratin/chitosan nanofibers were shown 99.53% filtration efficiency and excellent heavy metal adsorption from water for Cd, Ni,Pb and Fe metals . Overall, the present work demonstrated the possibility to incorporate Keratin into Chitosan nanofibers that have the potential to be applied in the filtration for air as well as water.

ACKNOWLEDGMENT

Authors thank to Ministry of Textile, Govt of India for financial support.

REFERENCES

1. Katoh, K. Shibayama, M. Tanabe, T. Yamauchi, K. Preparation and properties of keratinpoly (vinyl alcohol) blend fiber. J. Appl. Polym. Sci. 91, 756–762, 2004,
2. Katoh, K. Tanabe, T. Yamauchi, K. Novel approach to fabricate keratin sponge scaffolds with controlled pore size and porosity. Biomaterials 25, 4255–4262, 2004,
3. Katoh, K. Shibayama, M. Tanabe, T.; Yamauchi, K. Preparation and physicochemical properties of compression-molded keratin films. Biomaterials 25, 2265–2272, 2004
4. Edwards, A., Jarvis, D., Hopkins, T., Pixley, S., & Bhattarai, N. Poly(-caprolactone)/keratin-based composite nanofibers for biomedical applications. J Biomed Mater Res B. 103, 21-30, 2015.
5. Aluigi, A. Varesano, A.Montarsolo, A.Vineis, C. Ferrero, F. Mazzuchetti, G.; Tonin, C. Electrospinning of keratin/poly(ethylene oxide) blend nanofibers. J. Appl. Polym. Sci. 104, 863–870,2007
6. Adekogbe, I. Ghanem, A.Fabrication and characterization of DTBP- crosslinked Chitosan scaffolds for skin tissue engineering, Biomaterials, 26, 7241-7250, 2005.
7. Aluigi, A.Varesano, A.Montarsolo, A. Vineis, C. Ferrero, F.Mazzuchetti, G.; Tonin, C. Electrospinning of keratin/poly(ethylene oxide) blend nanofibers. J. Appl. Polym. Sci., 104, 863–870. 2007.

8. Dror, Y. Ziv, T. Makarov, V. Wolf, H, Admon, A.; Zussman, E. Nanofibers made of globular proteins. Biomacromolecules , 9, 2749–2754, 2008.
9. Gillespie, J. M.; Lennox, F. G. Keratin derivatives extracted from wool with alkaline thioglycollate solutions. Australian Journal of biological. Sciences, 8, 97. 1955.
10. Rouse, J. G.; Dyke, M. E. V. A Review of Keratin-Based Biomaterials for Biomedical Applications. Materials, 3, 999-1014, 2010.
11. Yamauchi, K. Yamauchi, A. Kusunoki, T.; Kohda, A. Konishi, Y. Preparation of stable aqueous solution of keratins, and physiochemical and biodegradational properties of films. J. Biomed. Mater. Res., 31, 439–444, 1996.
12. Yamauchi, K. Maniwa, M. Mori, T. Cultivation of fibroblast cells on keratin-coated substrata. J. Biomat. Sci.-Polym. E., 9, 259–270, 1998.
13. Yamauchi, K. Hojo, H. Yamamoto, Y.Tanabe, T. Enhanced cell adhesion on RGDS-carrying keratin film. Mat. Sci. Eng. C-Bio. S., 23, 467–472, 2003.

