ELECTROSPUN KERATIN/PA6 COMPOSITE NANOFIBROUS MAT FOR AIR AND WATER FILTRATION

Chetan Mahajan¹, Shishir Tyagi²

Wool Research Association, P.O. Sandoz Baug, Kolshet Road, Thane - 400 607, Maharashtra, India.

Abstract: In this article, we have prepared the Keratin/PA6 nanofibers by electrospinning technique and physicochemical, and filtration property of Keratin/PA6 nanofibers was studied. Keratin/PA6 nanofibers composite were made with different proportions of keratin concerning PA6, i.e., 1:0, 1:1, 1:2, 2:1. Keratin/PA6 nanofibers were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetric (DSC) and Field emission scanning electron microscopy (FESEM). FTIR showed that Keratin gets compatible with PA6. XRD patterns illustrated keratin was in crystalline nature and helps to increase the crystalline quality of PA6 nanofibers. FESEM images showed the uniform formation of Keratin/PA6 nanofibers. The Keratin/PA6 nanofibers diameter was found to be around 76 nm in size with tiny pore size. Nanofibers filtration efficiency against a particulate matter was obtained more than 99.42% and the unique nature of removal to heavy metals in water.

Keywords: Keratin/PA6 nanofibers, air-water filtration

I. INTRODUCTION

In recent years, research on the electrospinning of biocompatible polymeric materials has greatly increased due to the abundance of potential biomedical applications for nanofibrous materials. Electrospining is a technique that utilizes a high voltage to create an electrically charged jet of polymer that is drawn toward a grounded collection plate. The resulting fibers have diameters in the nano- to micro-scale range and are randomly arranged to form a non-woven fibrous mat. The enhanced physical configuration (i.e., small pore size, high porosity, three-dimensional features, and high surface area-to-volume ratio) of nanostructured nonwovens promotes cell adhesion and growth, which has led to the development of electrospun membranes for such uses as bandages for wound healing and scaffolds for tissue engineering. Recently, the electrospinning 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. Much work has been done to characterize the intermolecular interactions between the keratin and “additive” macromolecule in order to correlate the properties of the blend solution to the properties of the electrospun fibers.

Fibroin regenerated from silk has also been used to improve the processability of keratin for electrospinning applications (Zoccola et al., 2008). Characterizations of the rheological behavior of keratin/fibroin solutions revealed macromolecule interactions that promoted the formation of network structures with maximum synergy at a 50/50 (w/w) blend ratio. At this ratio, the synergistic effects on the protein interactions resulted in the formation of smaller-diameter, finer nanofibers as compared to fibers formed using solutions of unequal ratios of keratin/fibroin.

Wrzesniewska-Toski et al. (2007) also employed wet-spinning techniques to create novel fibrous keratin-based materials that have potential application as hygienic fabrics. Keratin and bio-modified cellulose were combined and used to create fibers that were characterized as having better sorption properties, higher hygroscopicity and a smaller wetting angle than cellulose-only fibers. Although introduction of keratin into cellulose fibers decreased the mechanical properties, a level was achieved that still enabled their application for manufacturing composite fibrous materials. In addition, the cellulose-keratin fibers had better biodegradation than cellulose fibers.

In this present study focused on extraction of keratin from wool waste and prepare the keratin based PA6 nanofibers by electrospinning techniques and evaluate the applications of nanofibers for air and water filtration.
II. MATERIALS AND METHOD

Materials:
Deccani waste wool was used for keratin extraction. Petroleum Ether, Urea, 2-Mercaptoethanol, sodium dodecyl sulfate (SDS), Formic acid 85%, Acetic acid were purchased from SD Fine Chemicals Limited (Mumbai, Maharashtra), Cellulose Dialysis Tubing (molecular weight cut off 12000-14000Da) obtained from Himedia laboratories (Mumbai, Maharashtra), PA-6 available at WRA laboratory.

2.1 Extraction of Keratin from Wool Waste:
Keratin protein was extracted from wool waste fibre according to the method reported previously (Yamauchi et al., 1996). Briefly, wool fibres were soaked in petroleum ether for 4 hours to de-waxing it. Then the protein was extracted by incubating 10 grams of wool with a mixture of 0.2 M SDS, 8 M urea and 2 mercapto ethanol at 60 °C for 5 hours. The solution was then filtered to remove the un-dissolved wool fibre and thoroughly dialyzed against distilled water using cellulose tubing to remove the chemicals and then the aqueous keratin solution was obtained. The keratin solution was poured into a Petri dish and placed it in oven for 2 days and to get Keratin powder.

2.2 Preparation of PA6-Keratin nanofibers
The solutions of PA-6 and PA-6/Keratin at 1:0, 1:1, 2:1, and 1:2 concentrations of the polymers in formic acid (85%) were electrospun at a 15-cm working distance to ensure that the nanofibers were dried. The applied voltages were between 15-20 kV. 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 2 ml/hr of the solution through the capillary, and the electrospinning process started. The process was stopped after about 1hr. During the electrospining process, Environmental conditions were kept in check; in particular, the temperature was ranged from 20 to 25°C, and the relative humidity was in the range of 35–55%.

III. CHARACTERIZATION:

3.1 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.

3.2 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.

3.3 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.

3.4 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 CuK\(\alpha\) radiation (\(\lambda = 1.5406 \text{ Å}\)) at 40 kV and 40 mA. Scan rate was 50/min between the angles 5-80°.

3.5 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.
3.6 Air permeability:

Determination air permeability of keratin/PA6 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.

3.7 Heavy metal absorption:

Heavy-metal adsorption from aqueous solutions by keratin/PA6 nanofibrous membranes was performed in dynamic conditions. Practically, accurately weight bio-film nanofibers mat 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.

Where the removal efficiency was evaluated using the following equation

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

Adsorption capacity of the 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. De-ionized water was used in all experiments and all chemicals used were of analytical grade.

3.8 Filtration efficiency 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. RESULTS AND DISCUSSION

4.1.1 UV Visible spectroscopy:

![UV Visible spectra of extracted keratin](image)

Figure 1. UV visible spectra of extracted keratin

UV-Vis absorption measurement is a very simple method to explore the structural change. Keratin solution shows $\lambda_{max}$ at 273 nm. The UV-Vis absorption of keratin is shown in fig 1. Keratin absorbs mainly in the far UV, but has an absorption tail out as far 380nm. The wide peak in the region 250-280 nm. The main chromospheres absorbing in the UV region are aromatic amino acids, tryptophan, tyrosine and phenylalanine, which are present in the keratin chain.

4.1.2 FTIR:

![FTIR Spectra of Keratin](image)

Figure 2. FTIR Spectra of Keratin

Fig 2. Showed characteristic absorption band for peptide bonds (–CONH–) are seen. The vibrations in the peptide bonds can be attributed to Amide A, amide I, II, III. The amide A band, which falls at 3327.12 cm$^{-1}$ is due to the stretching vibration of N–H bonds. The amide I band due to C–O stretching vibration occurs in the range of 1700–1600 cm$^{-1}$. Sharp peak was observed at 1656.8 cm$^{-1}$. The amide II, which falls at 1544.47 cm$^{-1}$ is related to N–H bending and C–H stretching vibration. The amide III band (1220–1300 cm$^{-1}$) was observed at 1245.18 cm$^{-1}$ as a sharp peak. The carbohydrate moieties band (1000–1100 cm$^{-1}$) was observed at 1083.10 cm$^{-1}$ as a sharp peak.
4.1.3 DSC analysis of keratin

DSC has most popular thermal technique, which measures the heat absorbed or liberated during various transitions in the sample due to temperature treatment. The DSC thermogram 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. Appreciably high thermal stability of the biomaterial would be an advantage for applications where thermal stability is important.

![DSC analysis of Keratin](image1.png)

Figure 3 DSC analysis of Keratin

4.1.4 X Ray diffraction of Keratin:

An x-ray diffraction spectrum of extracted keratin was shown in fig4. In XRD spectra peak arise at 2θ, 21.2° are index for the β-sheet crystalline structure of keratin. XRD spectra clearly indicate the extracted keratin was in highly crystalline state.

![X-ray diffraction of Keratin](image2.png)

Figure 4. X -ray diffraction of Keratin

4.2. Evaluation of PA6/Keratin bio-film:

4.2.1 FTIR analysis:

The FTIR spectra of PA6/ keratin blend films with different proportions are shown in fig.5. The bands corresponding to the crystalline α form (1030, 960, 930 and 830 cm⁻¹) and the bands corresponding to amide V and amide VI in the α structure (690 and 580 cm⁻¹ respectively) increased in the cast films, where these bands were still present with reduced intensity. Modification of the lattice vibrations intensity, corresponding to vibrations of the CH2 groups (1475, 1465, 1440 and 1420 cm⁻¹) confirmed the rearrangement of the polyamide chains both for cast films and electrospun film. Spectra of pure polyamide 6 and protein from
wool showed overlapping bands at about 3300, 3080, 1630 (Amide I) and 1530 cm$^{-1}$ (Amide II). Only in the region between 1000 and 800 cm$^{-1}$ absorptions in polyamide 6 spectrum were present.

4.2.2 XRD analysis:

Keratin and PA6 keratin blend was shown in fig 6, the crystalline structures PA6 were assessed by using XRD. The diffraction peaks located at about 20 - 19.5$^\circ$ and 24$^\circ$ correspond to (200) and (002,202) reflections of the $\alpha$-form crystals of the PA6. X-ray diffraction spectra of the blends films XRD spectra peak arise at $2\theta$, 19.5$^\circ$ are index for the b-sheet crystalline structure of keratin. XRD spectra clearly indicate the extracted keratin merge with PA6 and improve the crystallization nature.
4.2.3 DSC analysis:

A pure polyamide 6 DSC peak was showed in fig 7. A melting temperature peak (Tm) at 261°C. The height of the highest temperature peaks decreased with increasing the protein content. The highest melting point may be associated to the α-form of polyamide 6. In the blends, the lower crystallinity of polyamide 6 could be due to the inability of the polymer chains to be fully incorporated into the growing crystalline lamella, because of the presence of wool proteins. Moreover, the low temperature melting protein may simply reflect changes in the crystallite.

4.2.4 Heavy metal adsorption (%) Efficiency:

Metal like Cd, Ni Pb and Fe were removed with the help of keratin/PA6 nanofibers with different weight ratio. Metal salt of known concentration containing solution pass through Nanofibrinous mat and evaluate the amount of the heavy metal adsorb on the surface of the mat. PA6 nanofibers shows small amount of heavy metal adsorption but in case of keratin/PA6 and nanofibers shows very good adsorption ability than neat PA6 nanofibers shown in table 1. The dramatically improved 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. Keratin in the form of the nanofibers gives larger binding site adsorbs the large amount of metal ions.

![DSC analysis of PA6/keratin films](image)

**Table 1. Heavy metal adsorption efficiency of PA6 and Keratin/PA6 based nanofibers**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Cd</th>
<th>Ni</th>
<th>Pb</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA 6 nanofibers coated non woven fabric (PA6-1)</td>
<td>2.4</td>
<td>1.9</td>
<td>2.14</td>
<td>8.19</td>
</tr>
<tr>
<td>PA6/keratin nanofibers coated non woven fabric(PA6-2)</td>
<td>66.19</td>
<td>64.25</td>
<td>71.8</td>
<td>94.3</td>
</tr>
<tr>
<td>PA6/keratin nanofibers coated non woven fabric(PA6-3)</td>
<td>66.17</td>
<td>63.12</td>
<td>69.98</td>
<td>91.22</td>
</tr>
<tr>
<td>PA6/keratin nanofibers coated non woven fabric(PA6-4)</td>
<td>71.2</td>
<td>68.77</td>
<td>71.79</td>
<td>95.33</td>
</tr>
</tbody>
</table>

4.2.5 Particulate matter filtration efficiency:

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.

The particulate matter (aerogel) passed through face mask 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 density 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. Evaluation of filtration efficiency (%) of nanofibers based face mask

<table>
<thead>
<tr>
<th>Face mask</th>
<th>Inlet Count (C1)</th>
<th>Outlet count (C2)</th>
<th>Filtration efficiency (%)</th>
<th>Pressure drop(ΔH) mmwg</th>
<th>Air flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PA6-1)</td>
<td>121746</td>
<td>3320</td>
<td>97.27</td>
<td>19</td>
<td>95L/m</td>
</tr>
<tr>
<td>(PA6-2)</td>
<td>146571</td>
<td>1956</td>
<td>98.66</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>(PA6-3)</td>
<td>141487</td>
<td>3263</td>
<td>97.66</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>(PA6-4)</td>
<td>144798</td>
<td>834</td>
<td>99.42</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

4.2.6 Surface Morphology Keratin/PA6 Nanofibers:

Keratin based nanofibers are prepared by using electrospinning techniques, details procedure explained in methods. The surface morphology of keratin based nanofibers evaluated by scanning electron microscopy shown in fig. 8. Nanofibers are prepared with mixture of Keratin with PA6. Surface morphology of keratin based PA6 nanofibers shown in fig 8. Nanofibers are uniformly formed with the diameter 52nm-74 nm. The Nanofibers make non woven fibrous mat with continues structure and uniform. Surface morphology of Nanofibers clearly shown the nanofibers form the porous structure which helps in filtration.
Figure 8. SEM images of PA6/keratin nanofibers

V. CONCLUSION:

Keratin extracted from deccani wool waste and blended with PA6 at different concentration. The electrospinning process appeared stable and successful for keratin/PA6 blend solutions. All the nanofibers were found to be smooth and bead-free with favorable compatibility between keratin and PA6. Formation of hydrogen bonds between the two components was confirmed by FTIR spectroscopy. The crystalline nature of nanofibers improved with the incorporation of keratin, but the orientation of nanofibers was favorable to crystallization. The filtration efficiency keratin/PA6 nanofibers were shown 99.42 filtration efficiency and excellent heavy metal adsorption from water. Overall, the present work demonstrated the possibility to incorporate Keratin into 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


