CHAPTER IV
MATERIALS AND METHODS

4.1 Introduction

The details about the materials taken and chemicals used were given in this chapter. The chemical treatments such as chitosan modifications process, pretreatments of cotton fabric by conventional and enzymatic process, chemical modifications of cellulose and crosslinking of modified chitosan with cotton fabric were discussed.

The instrumental techniques used for characterization of all the prepared samples were also reported in the subsequent sections.

4.2 Materials

4.2.1 Chitosan

Chitosan was purchased from M/s South India Sea Foods, Kochi, Kerala, India. It was extracted from crustacean exoskeletons, had an average molecular weight of 180 kDa and was 90% deacetylated. The chitosan is designated as CS.

4.2.2 Fabric

Plain weave grey cotton fabric with Ends/in.:140, Picks/in.:80, Count: 40s, Weight/sq cm – 0.015g (kindly supplied by Lakshmi mills, Coimbatore) was used throughout the study.
4.2.3 Chemicals

The chemical treatment given to the fabric, chemicals used and its grade are given in Table 4.1. Enzymes were kindly supplied by Resil limited. Deionized water was used throughout the study.

Table 4.1: Chemical treatments and chemicals

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chemicals used</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali desizing</td>
<td>HCl</td>
<td>LR</td>
</tr>
<tr>
<td>Alkali scouring</td>
<td>NaOH, Na₂CO₃, Wetting agent</td>
<td>LR</td>
</tr>
<tr>
<td>Enzyme desizing</td>
<td>Resil Ezysize ultima</td>
<td>LR</td>
</tr>
<tr>
<td>Bioscouring</td>
<td>Resil Ezycare Bio-prep, Resiscour LFSCL, soda ash</td>
<td>LR</td>
</tr>
<tr>
<td>Acrylonitrile treatment</td>
<td>Acrylonitrile</td>
<td>LR</td>
</tr>
<tr>
<td>Solvent</td>
<td>Acetone</td>
<td>LR</td>
</tr>
<tr>
<td>Neutralization</td>
<td>Acetic acid</td>
<td>LR</td>
</tr>
<tr>
<td>Composite preparation</td>
<td>Acetic acid, NaOH, Zn(NO₃)₂.6H₂O</td>
<td>LR</td>
</tr>
<tr>
<td>Washing durability</td>
<td>Ariel detergent washing powder</td>
<td>LR</td>
</tr>
</tbody>
</table>
4.3 Pretreatment of cotton fabric

4.3.1 Acid desizing

The grey cotton fabric was wetted with water and then steeped in 0.5% HCl with material to liquor ratio 1:30. The treatment was allowed to proceed for 1 hour at 50 °C. The fabric was washed with water and then neutralized with sodium carbonate. The fabric was washed again and air dried at room temperature.

4.3.2 Alkali scouring

The desized cotton fabric was treated with NaOH (4%) and Na₂CO₃ (2%) at 80-85 °C for 2.30 hours. The fabric was washed with water and then neutralized with acetic acid. The fabric was washed again and air dried at room temperature. This sample is designated as AS.

4.3.3 Enzyme desizing

The grey cotton fabric was desized with bath containing 1% Resil Ezysize ultima at 6.5 pH. This process was continued with occasional stirring for 30 minutes. Then the fabric was taken out and washed with hot water followed by cold water.

4.3.4 Bioscouring

The enzyme desized cotton fabric was treated with enzyme in HTHP beaker dyeing machine using Resil Ezycare Bio-prep enzyme (0.5 g/L) with
liquor ratio of 1:100. The pH was maintained at pH 9.0 using Resiscour LFSCL (Wetting agent) (0.2 g/L) and soda ash (0.75 g/L). The temperature was raised slowly to 50 °C and maintained for 1 hour. After enzymatic treatment, the fabrics were washed twice in hot distilled water (80 °C) for 30 sec each to deactivate the enzyme and then air-dried for 24 hours. Then it was thoroughly washed with cold water and air-dried. This sample is designated as BS.

4.4. Chemical modifications

4.4.1 Modification using acrylonitrile

The bioscoured cotton fabric was kept immersed in aqueous sodium hydroxide (18% w/w) solution for about 30 min at room temperature, squeezed well, and then treated with acrylonitrile (1:10) for about 30 min at room temperature. Then the fabric was squeezed well, washed, neutralized with acetic acid, washed again and air-dried. This sample is designated as AN.

4.4.2 Modification using acrylonitrile and acetone

The bioscoured cotton fabric was kept immersed in aqueous sodium hydroxide (18% w/w) solution for about 30 min at room temperature, squeezed well, and then treated with reaction mixture of acrylonitrile (1:6) and acetone (1:4) for about 30 min at room temperature. Then the fabric was squeezed well,
washed, neutralized with acetic acid, washed again and air-dried. This sample is designated as AA.

4.5 Crosslinking of chitosan on cotton fabric

4.5.1 Coating of chitosan on fabric

The chitosan solution was prepared by stirring a dispersion of chitosan (1.5g owb) in 20% (v/v) aqueous acetic acid solution (100 mL) for 1 hour at 60 ºC. The bioscoured cotton fabric was immersed in the chitosan solution with constant stirring for 2 hours at 60 ºC. The fabric was squeezed to 100% wet pick-up and cured at 80 ºC for 5 min. The dried fabric samples were divided into two parts.

4.5.2 Crosslinking of chitosan using acrylonitrile

With one part of cured fabric, the acrylonitrile treatment was carried out as explained in the section 4.4.1 and this sample is designated as AN-CS.

4.5.3 Crosslinking of chitosan using acrylonitrile and acetone

With second part of the cured fabric, the crosslinking process was carried out with addition of acetone as was mentioned in the section 4.4.2 and this sample is designated as AA-CS.

4.6 Raw materials for composite preparation

In this work, three different compositions of Zinc (II) nitrate and the sodium hydroxide are selected basis on the following equation.
The Zinc (II) nitrate and the sodium hydroxide formed two compounds, Zinc (II) hydroxide and sodium nitrate. The Zinc (II) hydroxide proved to be insoluble due to it containing hydroxide. The hydroxide is insoluble, therefore making the compound form a precipitate. The sodium nitrate is soluble; therefore it broke down into Na⁺ and NO₃⁻ ions. The standard equation was a double displacement reaction Ax+By → Ay+Bx.

Molecular Equation

\[ Zn(NO_3)_2(aq) + 2NaOH(aq) \rightarrow Zn(OH)_2(s) + 2NaNO_3(aq) \]

Ionic Equation

\[ Zn^{2+}(aq) + (NO_3)^2(aq) + 2Na^+(aq) + 2OH^-(aq) \rightarrow Zn(OH)_2(s) + 2Na^+(aq) + 2NO_3^-(aq) \]

Net Ionic Equation

\[ Zn^{2+}(aq) + 2OH^-(aq) \rightarrow Zn(OH)_2(s) \]

4.6.1 Preparation of chitosan–ZnO composite

The commercial chitosan flakes (1 g) was taken and dissolved with 5 mL of acetic acid (0.1 M). Then 0.1 M Zn(NO₃)₂.6H₂O (100 mL) was added slowly under constant stirring using magnetic stirrer and the temperature was raised to 80 ºC. After 45 min 100 mL of 0.4 M NaOH (1:4) was added dropwise to the viscous solution; a white precipitate was formed, which was then allowed to settle for 24 hours. The supernatant solution was discarded and precipitate was rinsed with deionized water several times; then it was filtered through suction pump and oven dried at 80 ºC for 1 hour (Figure 4.1). This sample was designated as CZS-4.
Figure 4.1 Preparation process of Chitosan–ZnO composite
The above process was repeated by taking commercial chitosan (C), zinc nitrate (Z) and sodium hydroxide (S) with ratio of 1:8 and 1:16 and the samples were designated as CZS-8 and CZS-16 respectively. For control sample preparation, CZS-4 procedure was followed without adding of chitosan.

4.7 Crosslinking of composites

4.7.1 Coating of composite

Coating of modified chitosan (CZS-4) on cotton fabric was done as explained in the section 4.5.1 and dried fabric samples were divided into two parts.

4.7.2 Crosslinking of composite using acrylonitrile

With first part of the dried fabric, the acrylonitrile treatment was carried out as explained in the section 4.4.1 and this sample is designated as AN-CSZ-4.

4.7.3 Crosslinking of composite using acrylonitrile and acetone

With second part of the dried fabric, the crosslinking process was carried out with addition of acetone as was mentioned in the section 4.4.2 and this sample was designated AA-CZS-4.
4.8 Characterizations and testing

4.8.1 Elemental analysis

The elemental analysis of the samples carried out by using Perkin Elmer 800 atomic absorption spectrometry. Ion sorption studies of the composites were carried out by stirring 0.1 g of modified chitosan powder in 100 mL beaker containing 98 mL water and 2 mL dilute HNO₃ for 30 min. Metal ion uptake is expressed as follows [214]:

$$\text{Percent uptake (P_u)} = \frac{\text{Amount of metal ions sorbed}}{\text{Total amount of metal ions present}} \times 100$$

4.8.2 FTIR spectroscopy

FTIR is most useful for identifying chemicals that are either organic or inorganic. It can be utilized to quantitate some components of an unknown mixture. It can be applied to the analysis of solids, liquids, and gasses. In the present work, Fourier transform infrared (FT-IR) spectra were recorded on Nicolet 5700 instrument (Nicolet Instrument, Thermo company, Madison, USA) using KBr pellet in the range between 4,000-400 cm⁻¹ at room temperature. The samples were thoroughly ground in a smooth agate mortar to form powered sample. 2 mg of this powdered sample was intimately mixed with 350 mg of pure, dry, powdered spectroscopic grade potassium bromide. The mixture of sample and potassium bromide was pressed with special dies under a pressure of 10,000 to 15,000 psi for 10 minutes in the hydraulic pressure and then released
to get a transparent disk. The disk was loaded on to the FTIR spectral instrument to perform spectral analysis over a range of 400-4000 cm and analyzed.

### 4.8.3 UV-Vis spectroscopy

It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I₀). The ratio I / I₀ is called the transmittance, and is usually expressed as a percentage (%T). The absorbance, A, is based on the transmittance:

\[
A = - \log(\%T / 100%)
\]

The UV-vis absorption spectra of the samples were recorded using UV-visible spectrophotometer (2401 PC model; Shimadzu, Kyoto, Japan), the wavelength of incident ray was selected in the range of 200-700 nm.

### 4.8.4 Photoluminescence spectrophotometer

Varian Cary Eclipse Photoluminescence spectrophotometer employing 15W Xe flash lamp was used for the photoluminescence studies of composites. The absorbance was measured in the range of 200-800 nm at room temperature.

### 4.8.5 X-ray diffractometer

The X-ray powder diffraction studies were carried out using X’Pert PRO diffractometer system with Cu Kα radiation (λ=0.15418 nm) with the scanning rate of 0.01°/step and with 2θ ranging from 5° to 80°.
The degree of crystallinity ($X_c\%$) and the size of the crystallites ($L_{hkl}$) were obtained using following equations [215-216] respectively.

\[ X_c\% = \frac{I_c}{(I_c + I_a)} \times 100 \]

where $X_c$ is the degree of crystallinity, and $I_c$ and $I_a$ represent the integrated intensity of crystalline and amorphous regions, respectively.

\[ L_{hkl} = \frac{K\lambda}{\beta \cos \theta} \]

where $L_{hkl}$ is the size of crystallites from the normal direction of hkl plane; factor $k$ is the scherrer constant (0.94); $\lambda$ is the wavelength of the X-ray used; $\beta$ represent half widths of the peak and $2\theta$ is the Bragg angle.

Crystallinity Index (CI) was calculated using the following equation [217-218]:

\[ CI = 100 \left(\frac{(I_{002} - I_{am})}{I_{002}}\right) \]

where $I_{002}$ is the intensity of the principal Cellulose I peak at $2\theta = 22.7^\circ$ and $I_{am}$ is the intensity attributed to amorphous cellulose given at $2\theta = 18^\circ$.

### 4.8.6 SEM and EDAX

The SEM (Scanning electron microscopy) morphology and EDAX (Energy-dispersive x-ray analysis) of the samples were studied using Hitachi S-3600N VP scanning electron microscope equipped with EDAX elemental composition analyzer at an acceleration voltage of 15 kV. A gold coating of thickness upto 200 µm was deposited on samples in vacuum before observing and photographing and the samples were observed at different magnifications.
4.8.7 Thermogravimetric analysis

Thermogravimetric studies were performed on a SDT Q600 TG-DTA analyzer under nitrogen atmosphere at a heating rate of 10 °C/min. TG/DTG is a simple analytical technique that measures the weight loss (or weight gain)/derivative weight of a material as a function of temperature. As materials are heated, they can loose weight from a simple process such as drying, or from chemical reactions that liberate gases. Some materials can gain weight by reacting with the atmosphere in the testing environment. Since weight loss and gain are disruptive processes to the sample material or batch, knowledge of the magnitude and temperature range of those reactions are necessary in order to design adequate thermal ramps and holds during those critical reaction periods. Thermogravimetric curves are characteristics for a given compound or systems because of the unique sequence of physicochemical reactions, which occur over definite temperature ranges and at rates that are a function of the molecular structure.

4.8.8 Antibacterial activity

The antibacterial activity of the samples was tested qualitatively by an inhibition zone method [219]. In this method, two Gram-positive bacteria S. aureus, S. pyogenes and two Gram-negative bacteria E. coli, K. aerogenes were selected as the experimental bacteria. For qualitative measurement of the antibacterial activity, the samples are put together to form a circular zone, and the antimicrobial activity was tested using modified agar diffusion assay (disc
test). The plates were examined for possible clear zone after incubation at 30 °C for 2 days. The presence of any clear zone around samples on the plates was recorded as an inhibition against the microbial species.

4.8.9 Washing durability

The washing fastness of the fabric was determined after repetitive washing in an AATCC Atlas Launder-O-Meter Standard Instrument, which is widely used for evaluating laundry results on a laboratory scale. One wash in a Launder-O-Meter (ISO-105-CO1:1989(E) Standard Method) provides an accelerated washing treatment corresponding to five home washings. The finished fabric samples were washed repetitively up to 10 times; the duration of the washing cycles was 30min and was carried out in a solution of Ariel detergent powder with a concentration of 5gpl, previously heated to 40 °C, to give a liquid ratio of 50:1.

After washing, the samples were rinsed in cold distilled water, held under a cold tap water for 10 min and squeezed and dried at room temperature. The quality of the fabric coatings was assessed after the first and tenth washing cycles.
### 4.9 List of samples and its designation

The prepared samples were designated and listed in Table 4.2.

**Table 4.2: Sample designation**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the product</th>
<th>Section</th>
<th>Page No.</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkali scoured fabric</td>
<td>4.3.2</td>
<td>96</td>
<td>AS</td>
</tr>
<tr>
<td>2</td>
<td>Bioscoured fabric</td>
<td>4.3.4</td>
<td>96</td>
<td>BS</td>
</tr>
<tr>
<td>3</td>
<td>Modified fabric using acrylonitrile</td>
<td>4.4.1</td>
<td>97</td>
<td>AN</td>
</tr>
<tr>
<td>4</td>
<td>Modified fabric using acrylonitrile and acetone</td>
<td>4.4.2</td>
<td>97</td>
<td>AA</td>
</tr>
<tr>
<td>5</td>
<td>Commercial Chitosan</td>
<td>4.2.1</td>
<td>94</td>
<td>CS</td>
</tr>
<tr>
<td>6</td>
<td>Chitosan crosslinked using acrylonitrile</td>
<td>4.5.2</td>
<td>98</td>
<td>AN-CS</td>
</tr>
<tr>
<td>7</td>
<td>Chitosan crosslinked using acrylonitrile and acetone</td>
<td>4.5.3</td>
<td>98</td>
<td>AA-CS</td>
</tr>
<tr>
<td>8</td>
<td>Chitosan-ZnO composite prepared using 1:4 ratio</td>
<td>4.6.1</td>
<td>99</td>
<td>CZS-4</td>
</tr>
<tr>
<td>9</td>
<td>Chitosan-ZnO composite prepared using 1:8 ratio</td>
<td>4.6.1</td>
<td>99</td>
<td>CZS-8</td>
</tr>
<tr>
<td>10</td>
<td>Chitosan-ZnO composite prepared using 1:16 ratio</td>
<td>4.6.1</td>
<td>99</td>
<td>CZS-16</td>
</tr>
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<td>11</td>
<td>CZS-4 crosslinked using acrylonitrile</td>
<td>4.7.2</td>
<td>101</td>
<td>AN-CZS-4</td>
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<tr>
<td>12</td>
<td>CZS-4 crosslinked using acrylonitrile and acetone</td>
<td>4.7.3</td>
<td>101</td>
<td>AA-CZS-4</td>
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