CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter contains information about the materials and methods used for this study. The overall experimental plan is given in Figure 3.1.

The first unit describes methods related to the handle improvement process for wool using acid protease and the comparative study with commonly used alkaline protease treatment.

The second unit describes methods related to the evaluation of microbial resistance character of bleached and enzyme treated wool materials and development of combined bleaching and antimicrobial finishing process.

The third unit describes methods related to synthesis of silver nanoparticles, characterization and application on wool materials and the effect of bleaching and enzyme pretreatments on antimicrobial efficacy of wool.

The fourth unit consists of two parts. The first part describes the methods related to the development of natural dyeing process for wool using leaf extracts. The second part describes methods related to the combined dyeing and antimicrobial finishing of wool pretreated with hydrogen peroxide and protease enzyme in acid and alkaline conditions using developed natural dyeing process.
Handle Improvement of Wool

Acid Peroxide Bleaching
  Acid Protease

Alkaline Peroxide Bleaching
  Acid Protease
  Alkaline Protease

Testing of Handle using KES Method

Comparative Study of Enzyme Treatments for Handle

Testing of Antimicrobial Efficacy of Bleached and Enzyme Treated Fabrics

Antimicrobial Finishing to Bleached and Enzyme Treated Fabrics

Application of Ag Nanoparticles
  Synthesis and Characterization of Ag Nanoparticles

Application of Natural Dyes
  Extraction of Dyes from Plant Leaves Containing High % of Tannin

Testing the Antimicrobial Efficacy of Treated Materials and Comparative study

Figure 3.1 Schematic diagram of experimental plan
3.2 HANDLE IMPROVEMENT OF WOOL USING ACID AND ALKALINE PROTEASE ENZYMES

3.2.1 Materials

A pure woolen fabric with 350 g/m² weight made of fine wool was used for this study. The detailed specifications of the wool fabric were given in Appendix 1. An alkaline protease enzyme (Perizym AFW) supplied by M/S Textilchemie DR. Petry GMBH, Germany and an acid protease enzyme (Palkoacid HP) supplied by Maps Enzyme (India) Ltd, India were used as such. A nonionic detergent Sandoclean PCJ (Clariant India Ltd) was used as wetting and scouring agent. All the other chemicals were of analytical grade purchased from M/S SD Fine Chemicals, India.

3.2.2 Enzyme Treatment Methods

The wool fabrics were treated with acid and alkaline protease enzyme by three methods as outlined in Figure 3.2. For all the three methods, scoured wool fabrics (using 3% non ionic detergent in pH–7 at 50°C for 30 min) were used. An optimization study was conducted for method A & B in order to find out the suitable enzyme concentration, pH and time of treatment on wool fabric.

3.2.2.1 Method A

In this method, the bleaching and subsequent enzyme treatments were carried out completely in acidic conditions. The scoured wool fabric was bleached in acidic pH using the following recipe (Karunditu et al 1994).

Fabric weight - x g
Wetting agent - 1%
Hydrogen Peroxide - 3%
Citric acid - 3 % (owm)
Sodium acetate - 2 % (owm)
pH - 5.5
Temperature - 100°C
Time - 90min

The bleached fabric was then treated with acid protease using the following recipe

Fabric weight - x g
Wetting agent - 0.5%
Enzyme - x % (owm)
pH - 4.5
Temperature - 40°C
Time - 60min

**Figure 3.2 Schematic diagram of three enzyme treatments**
An optimization study was conducted to determine the suitable enzyme concentration, pH and time of enzyme treatment using Box and Behnken technique with three variables in three levels as per Table 3.1. The enzyme-treated fabric by this method using optimized process parameters was given a code name ACB-ACP.

Table 3.1  Optimization of acid protease enzyme process using Box and Behnken experiment design

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Independent Variable</th>
<th>Coded levels</th>
<th>Dependant variable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1 0 +1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Concentration</td>
<td>1 2 3</td>
<td>Y1, Weight loss (%)</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>4.5 5.5 6.5</td>
<td>Y2, Strength loss (%)</td>
</tr>
<tr>
<td>3</td>
<td>Time</td>
<td>30 60 90</td>
<td>Y3, Subjective softness rating</td>
</tr>
</tbody>
</table>

3.2.2.2  Method B

In this method, the bleaching and enzyme treatments were carried out completely in alkaline condition. The wool fabric was first bleached with alkaline hydrogen peroxide using the following recipe.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabric weight</td>
<td>x g</td>
</tr>
<tr>
<td>Wetting agent</td>
<td>1%</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>3%</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>3% (owm)</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td>2% (owm)</td>
</tr>
<tr>
<td>pH</td>
<td>9</td>
</tr>
<tr>
<td>Temperature</td>
<td>70°C</td>
</tr>
<tr>
<td>Time</td>
<td>90min</td>
</tr>
</tbody>
</table>
The bleached fabric was then treated with alkaline protease enzyme using following recipe

- Fabric weight - x g
- Wetting agent - 0.5%
- Enzyme - x % (owm)
- pH - 9 (using buffer solution)
- Temperature - 70°C
- Time - 60min

An optimization study was conducted to determine suitable enzyme concentration and time of treatment using full factorial experimental design with two variables at three levels. The enzyme concentration (1%, 2% and 3%) and time of enzyme treatment (30, 45 and 60 min) were the variables. The enzyme treated fabric by this method using optimized process parameters was given a code name ALB-ALP.

**3.2.2.3 Method C**

In this method, the scoured wool fabric was bleached in alkaline condition using alkaline peroxide followed by acid protease enzyme treatment in acidic condition as per the recipes outlined in earlier sections. The enzyme treated fabric by this method was given a code name ALB-ACP.

**3.2.3 Testing Methods**

The weight loss of the fabric was determined using oven dried weights of the fabric before and after treatments using weighing balance. The strength loss of the fabric was determined in similar way using tensile strength tester. The subjective softness of the treated fabrics was determined by five judges with rating from 1 to 5. The rating ‘5’ is very good and the
rating ‘1’ is very poor. The average value of the ratings was taken for this study. The judges selected for this study were post-graduate students of textile technology having knowledge in handle-related properties of textile materials. The coefficient of variation (CV %) of the subjective ratings of the judges was determined by dividing the standard deviation of the ratings with the mean value. An ANOVA test was also carried out to study the variations in the ratings of the judges for the treated fabrics.

The alkali solubility of the materials was determined using ASTM D1283. The whiteness (Stephenson Method) and the yellowness indices (ASTM E313) were determined using Premier Colorscan SS5100A Spectrophotometer. The shrinkage was determined as per the standard procedure (AATCC 143, 2001c). The chemical changes on the wool were determined with a FT-IR spectrometer using KBr pelleting method. Spectra were collected at 4cm\(^{-1}\) resolution with an average of 300 scans. A semi-quantitative analysis was carried out measuring band intensities in the second-order derivative absorbance spectrum after normalizing on amide III band at 1232 cm\(^{-1}\). The absorbance ratio between the each selected frequency (1040, 1075 and 1124 cm\(^{-1}\) corresponding to cysteic acid, cystine monoxide and cystine dioxide) versus the amide III band was calculated and compared with the corresponding absorbance ratio of untreated scoured wool (Jovancic et al 2001).

3.2.3.1 Testing of handle of the treated fabrics

The handle of the treated fabrics was determined using Kawabata Evaluation System for fabric (KES-F). The coefficient constants of men’s suiting fabric were used for determining primary and total hand values (Kan and Yuen 2006).
The evaluation system consists of four modules to evaluate the tensile, shear, bending, compression and surface properties of fabrics by determining sixteen low stress mechanical properties as shown in Table 3.2.

**Table 3.2 Low stress mechanical properties from KES-F**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tensile</td>
<td>Linearity of Load/extension curve</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>LT</td>
<td>Tensile energy</td>
<td>N/m</td>
</tr>
<tr>
<td>2</td>
<td>WT</td>
<td>Tensile resilience</td>
<td>%</td>
</tr>
<tr>
<td>3</td>
<td>RT</td>
<td>Tensile resilience</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Bending</td>
<td>Bending rigidity</td>
<td>$10^{-4}$Nm</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>Hysteresis of bending moment</td>
<td>$10^{-2}$N</td>
</tr>
<tr>
<td>5</td>
<td>2HB</td>
<td>Hysteresis of bending moment</td>
<td>$10^{-2}$N</td>
</tr>
<tr>
<td></td>
<td>Shearing</td>
<td>Shear stiffness</td>
<td>N/m Deg.</td>
</tr>
<tr>
<td>6</td>
<td>G</td>
<td>Hysteresis of shear force at 0.5° of shear angle</td>
<td>N/m</td>
</tr>
<tr>
<td>7</td>
<td>2HG</td>
<td>Hysteresis of shear force at 5° of shear angle</td>
<td>N/m</td>
</tr>
<tr>
<td>8</td>
<td>2HG5</td>
<td>Hysteresis of shear force at 5° of shear angle</td>
<td>N/m</td>
</tr>
<tr>
<td></td>
<td>Compression</td>
<td>Linearity of compression/thickness curve</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>LC</td>
<td>Compressional energy</td>
<td>N/m</td>
</tr>
<tr>
<td>10</td>
<td>WC</td>
<td>Compressional resilience</td>
<td>%</td>
</tr>
<tr>
<td>11</td>
<td>RC</td>
<td>Coefficient of friction</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>MMD</td>
<td>Mean deviation of friction</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>SMD</td>
<td>Geometrical roughness</td>
<td>$\mu$m</td>
</tr>
<tr>
<td>14</td>
<td>T</td>
<td>Fabric thickness</td>
<td>mm</td>
</tr>
<tr>
<td>15</td>
<td>W</td>
<td>Fabric weight/unit area</td>
<td>$10 \text{ g/m}^2$</td>
</tr>
</tbody>
</table>

- Average of the values in warp and weft directions is applied
Sample of 20 cm × 20 cm dimension in triplicates was taken for this study. The KES-FB1 instrument was used to measure extensibility at 500 gf/cm (EMT), tensile energy in deformation (J/m$^2$) (WT), resilience to deformation (RT)-the ratio between recovering energy and tensile energy and linearity in extension (LT). The KES-FB1 instrument was also used to measure shear stiffness (N/m*degree) (G), hysteresis of shear stiffness at 0.5° deformation (N/m) (2HG) and hysteresis of shear stiffness at 5° deformation (N/m) (2HG5).

The KES-FB2 instrument was used to determine bending rigidity per unit length (μN*m$^2$/m) (B) and hysteresis of bending moment per unit length (mN*m/m) (2HB). The KES-FB3 instrument was used to determine thickness T of the fabric subjected to a pressure of 0.5 cN/cm$^2$, deformation energy (J/m$^2$) (WC) and resilience to compression (RC). This last property was the ratio between elastic recovery energy and deformation energy. The ratio between deformation energy (WC) and the deformation energy of a perfectly elastic body subjected to the same conditions of compressions (LC).

The KES-FB4 instrument was used to determine the surface characteristics such as mean coefficient of friction (MIU), standard deviation of MIU (MMD) and the geometrical roughness (SMD) (μm).

Except compression property, the other mechanical properties were evaluated both in warp and weft direction and the average value was taken as the final reading.

The above sixteen parameters obtained from KES-F system combined with the calculation model developed by Prof. Niwa and Kawabata to obtain an objective evaluation of the primary hand of the fabrics as mentioned in following equation (Kawabata 1980, Kawabata and Niwa 1989).
\[ Y_k = C_o + \sum C_{ki} X_i \]  

where \( Y_k \) = \( k^{th} \) hand value such that, \( k=1 \) is stiffness (Koshi), \( k=2 \) is smoothness (Numerii) and \( k=3 \) is fullness (Fukurami) for winter/autumn suiting fabrics

\( C_o \) and \( C_i \) = Constants calculated by Kawabata for each primary hand values

\( X_i \) = deviation of the value from the mean parameter \( x_i \) measured from the mean of \( n_i \) of the universe, express in the form of a standard deviation \( \sigma_i \)

\[ x_i = (X_i - M_i) / \sigma_i \]

Having calculated the primary hand values in terms of smoothness, stiffness and fullness for winter suiting fabrics, Kawabata et al (1989) then worked out the equation of total hand value (THV) which units all the primary hand value into a single value as mentioned in Equation (3.2).

\[ THV = C_o + \sum Z_k \]  

\[ Z_k = \frac{C_{k1}(Y_k + M_{k1})}{\sigma_{k1}} + \frac{C_{k2}(Y_k^2 - M_{k2})}{\sigma_{k2}} \]

where \( Z_k \) = Contribution of the \( k^{th} \) primary hand value to total hand value

\( M_{k1} \) and \( \sigma_{k1} \) = Population means and standard deviation of \( Y_k \)

\( M_{k2} \) and \( \sigma_{k2} \) = Population means and standard deviation of \( Y_k^2 \)

\( C_{k1} \) and \( C_{k2} \) = Constant coefficients

\( C_o \) = Constants calculated by Kawabata for each Total hand values
3.3 MICROBIAL RESISTANCE OF BLEACHED AND ENZYME TREATED WOOL MATERIALS

3.3.1 Materials

The acid peroxide bleached, alkaline peroxide bleached, acid protease treated and alkaline protease treated wool fabrics as per the previous unit were taken for this study.

3.3.2 Citric Acid Treatment for Wool

In this process, 3% citric acid was applied separately on the alkaline peroxide bleached fabric at 70°C for 60 minutes. This process was done to study the possibility of incorporating citric acid after bleaching treatment on wool.

3.3.3 Testing of Microbial Resistance

The microbial resistance of the treated wool fabrics was determined using qualitative Agar Diffusion Method [SN 195920-1992 (Swiss Norm)] and quantitative AATCC 100 (2001a) method respectively as per the literature (Thilagavathi et al 2005) against *Staphylococcus aureus* and *Escherichia coli*.

3.3.3.1 Agar diffusion method

This method was used to find out the antimicrobial efficacy of the treated fabrics qualitatively according to the Swiss standard- SN195920. For this test, AATCC bacteriostatic agar medium was used as a growth medium for evaluation. The test specimens (non-sterile) were taken and they were cut into pieces according to convenient size with round shape and untreated fabric sample cut in the same size was used as a control.
The bacteriostatic agar was sterilized in an autoclave. 15-20 ml of the sterilized agar was then added on a petri dish and allowed to harden for 30 minute. The required bacterial culture (24 hours incubated) of the test organism was then evenly spread on the agar using a cotton swab. In this experiment, a gram-positive *Staphylococcus aureus* and a gram-negative *Escherichia coli* were taken as representative bacteria for evaluation. The treated and control fabrics were placed on the solid agar inoculated with bacterial culture. The petri dish was then incubated at 37° C for 18-24 hours.

At the end of the incubation time, the test dish was observed. The evaluation was made based on absence or presence of an effect of bacteria in the contact zone under the specimen and the possible formation of a zone of inhibition around the test specimen. The area of inhibition zone in mm was a measure of antimicrobial effectiveness of the treated sample.

### 3.3.3.2 AATCC 100 method

The AATCC 100 method was used to quantitatively determine the bacterial reduction percentage of treated fabrics.

#### 3.3.3.2.1 Principle

Swatches of test and control textile materials were qualitatively tested for antibacterial activity using agar diffusion and parallel streak method. Those showing activity were evaluated quantitatively by this method. Test and control swatches were inoculated with the test organisms. After incubation, the bacteria were eluted from the swatches by shaking in known amounts of neutralizing solution. The number of bacteria present in this liquid was determined and the percentage reduction by the treated specimen was calculated.
3.3.3.2 Test organisms

*Staphylococcus aureus* was used as a representative gram-positive organism and *Escherichia coli* was used as a representative gram-negative organism.

3.3.3.2.3 Culture medium and test specimens

AATCC bacteriostasis broth/nutrient agar was used as a growth medium for evaluation. The composition was

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10 g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Heating to boiling was done to dispense ingredients; pH was adjusted to 6.8 with 1N NaOH solution if necessary. 15 ml of the broth was dispensed in conventional bacteriological culture tubes, plugged and sterilized at 103 K Pa (15 psi) for 15 minutes.

Circular swatches of 4.8 ± 0.1 cm in diameter were cut from the test fabric. The swatches were stacked in a 250 ml wide mouth glass jar with screw cap. Swatch of the same fibre type and fabric construction as test sample but containing no antimicrobial finish was used as the control.

1.0± 0.1 ml of an appropriate dilution of 24 hour broth culture of the test organism was applied so that recovery from the untreated control fabric swatches or treated test fabric swatches at ‘0’ contact time (placed as soon as possible after inoculation) showed a count of appropriate number of organisms. The dilutions of the test organisms were made in nutrient broth.
3.3.3.2.4 Procedure

The 24-hour culture of the test organism was shaken and allowed to stand for 15-20 minute before preparing the inoculum. The swatches were placed in a sterile Petri dish and using a micro liter pipette inoculation was done making sure that there is even distribution of the inoculum. The swatches were then transferred aseptically to the jar. The jar tops were screwed on tightly to prevent evaporation. As soon as possible after inoculation (‘0’ contact time) 100 ml of neutralizing solution (sterile distilled water) was added to each of the jars containing the inoculated untreated control swatches, the inoculated treated test swatches and the uninoculated treated swatches.

The jars were shaken vigorously for one minute. The serial dilutions were made with water and plated (in duplicate) on nutrient agar. Dilutions of $10^0$, $10^1$, $10^2$ were usually suitable. Additional jars containing inoculated untreated control swatches and jars containing inoculated treated test swatches were incubated at $37 \pm 2^\circ C$ ($99 \pm 3F$) for 18-24 hours. Similar jars were incubated over other periods (1-6 hours) to provide information about the bactericidal activity of the treatment over such periods. After incubation, 100 ml of neutralizing solution was added to jars containing untreated control swatches and to jars containing treated test swatches. The jars were vigorously shaken for 1 minute. Serial dilutions were made and plated (in duplicate) on nutrient agar. Dilutions of $10^0$, $10^1$, $10^2$ were usually suitable for treated test fabric. Several different dilutions were required for untreated control fabrics depending on the incubation period. All the plates were incubated for 48 hours at $37 \pm 2^\circ C$ ($99 \pm 3F$).
3.3.2.5 Evaluation

The bacterial counts were reported as the number of bacteria per sample (swatches in jar) not as the number of bacteria per ml of neutralizing solution. ‘0’ counts at $10^0$ dilution was reported as “less than 100”. The percent reduction of bacteria by the specimen treatments were calculated using any one of the following formulas

$$100 \frac{(B-A)}{B} = R$$  \hspace{1cm} (3.3)

where $R$ = % reduction

$A$ = the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period.

$B$ = the number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at ‘0’ contact time).

$$100 \frac{(C-A)}{C} = R$$ \hspace{1cm} (3.4)

where $C$ = the number of bacteria recovered from the inoculated untreated control specimen swatches in the jar immediately after inoculation (at ‘0’ contact time)

If ‘B’ and ‘C’ are not similar, the larger number should be used.

In this study, the equation (3.4) was mostly used to determine percent reduction of bacteria.
3.3.4 Nuclear Magnetic Resonance Spectra

The solid state NMR spectrometer (BRUKER DSX-300 solid state NMR spectrometer) using $^{13}$C nuclei was used to study the bond formation between wool and citric acid under oxidizing conditions in the bleached and enzyme treated fabrics and also on the alkaline peroxide sample post treated with 3% citric acid.

3.3.5 Effect of Citric Acid on the Whiteness and Handle of Acid Peroxide Bleached Wool

3.3.5.1 Bleaching

The scoured wool fabric was bleached with hydrogen peroxide in acidic conditions using citric acid as per the following recipe:

- Fabric weight: $x$ g
- Wetting agent: 1%
- Hydrogen peroxide: 3%
- Citric acid: 3%, 4% and 5% (owm)
- Sodium acetate: 2% (owm)
- pH: 4.5-5
- Temperature: 100°C
- Time: 90min

The control wool fabric was bleached with alkaline hydrogen peroxide using the following recipe:

- Fabric weight: $x$ g
- Wetting agent: 1%
- Hydrogen peroxide: 3%
- Sodium carbonate: 3% (owm)
Trisodium phosphate - 2 % (owm)

pH - 9
Temperature - 70°C
Time - 90min

3.3.5.2 Testing methods

The antimicrobial efficacy of the bleached fabrics was determined using AATCC 100 method. The whiteness index and yellowness index of the treated materials were assessed by ASTM E313 method with 2° observer using Premier Colourscan SS5100A Spectrophotometer. The handle of the materials was determined using Kawabata Evaluation Method (KES-F) with the coefficient constants of men’s suiting fabric for determining primary hand values namely smoothness, stiffness and fullness (Kan and Yuen 2006).

3.4 SYNTHESIS OF SILVER NANOPOWDER AND ITS APPLICATION ON WOOL FOR ANTIMICROBIAL FINISHING

3.4.1 Chemicals and Fabrics

Analytical grade silver nitrate and trisodium citrate purchased from M/s SD Fine chemicals, India and polyvinyl pyrrolidone (PVP) purchased from M/s. Himedia, India were used as such. The alkaline hydrogen peroxide bleached, plain woven wool fabric having 350 g/sq.m weight was used for this study.

3.4.2 Preparation of Silver Nanoparticles

A sono-chemical method was used for synthesizing silver nanoparticles. All solutions were prepared in deionized water. In a typical recipe, $1 \times 10^{-3}$ M of silver nitrate solution containing 2% PVP was sonicated
in a 20 kHz, 130 watts ultra sonicator (Model VX 130 of M/s Sonics, USA) for 3 h. The sonicated solution was then immediately heated to boil followed by drop-by-drop addition of 1% trisodium citrate until the colour of the solution turns yellow. The appearance of yellow colour in the solution was taken as the preliminary indication of the formation of silver nanoparticles.

3.4.3 Spray Drying

The synthesized silver nanoparticle solution was converted to powder using a laboratory spray dryer (Model – LU222 Advanced of M/s. Lab Ultima, India) operated in the co-current mode. The liquid feed rate was 2 ml/min through 0.7 mm diameter nozzle at 300 kPa (3 bar) air pressure. The atomization was done in the aspirator with the vacuum of 35 mm water column. Spray drying was performed at an inlet air temperature (T\textsubscript{inlet}) of 135°C, corresponding to an outlet air temperature (T\textsubscript{outlet}) of 90°C. The spray drying solution contained 2% w/v of total dissolved solids. The powder was collected from I and II cyclones of the spray dryer and stored in airtight container for further applications.

3.4.4 Characterization

UV-Visible spectrum was used to qualitatively confirm the presence of the silver in nanometer size in the prepared nanopowder using Shimadzu UV-1601 spectrometer. Silver in nanometer size has the ability to produce absorption peak in the wavelength region of 400-440 nm. Atomic absorption spectrum was used to find out concentration of silver in the synthesized powder using Varian Model Spectraa 220 spectrometer. TEM analysis was carried out to determine the size of the silver nanoparticles in the nanopowder using Philips CM 200 model machine by drop coating method. The nano powder was dissolved in water and drop-coated on the copper grids
for TEM analysis. The elemental analysis on the individual nanoparticle was done using EDAX facility attached with the TEM itself.

3.4.5 Application on Wool

The wool fabric was treated with the aqueous solution of silver nanopowder by exhaustion method. The material was treated for 45 min at boiling condition followed by washing with normal water. The durability of the finished samples was determined after 5, 10 and 20 washes as per AATCC 124 (2001b) method using IFB washing machine.

3.4.6 Assessment of Microbial Resistance

The antimicrobial efficacy of the treated wool fabrics along with their washed samples was determined using qualitative agar diffusion method [SN 195920-1992 (Swiss Norm)] and quantitative AATCC 100 method respectively as per the procedure given earlier against *Staphylococcus aureus* and *Escherichia coli*.

3.5 EFFECT OF BLEACHING AND ENZYMES PRETREATMENTS ON THE ANTIMICROBIAL EFFICACY OF WOOL TREATED WITH NANO SILVER

The developed nano silver based antimicrobial finishing process was used to give antimicrobial finishing for differently bleached and enzyme treated wool fabrics in acid and alkaline condition. All the four types of fabrics, namely, acid peroxide bleached (ACB), alkaline peroxide bleached (ALB), acid protease treated (ACP) and alkaline protease treated (ALP) were taken for this study. The materials were treated with aqueous solution of silver nanopowder by exhaustion method. The treated fabrics were then assessed for their antimicrobial efficacy by qualitative agar diffusion method.
and quantitative AATCC 100 method respectively as per the literature (Thilagavathi et al 2005) against Staphylococcus aureus and Escherichia coli.

The silver content of the all the treated wool fabrics was determined using atomic absorption spectra (AAS) using Varian Model Spectraa 220 spectrometer.

3.6 DEVELOPMENT OF NATURAL DYEING PROCESS FOR WOOL USING LEAF EXTRACTS CONTAINING TANNIN

3.6.1 Materials

Scoured 100% woollen yarn (4NM) from fine wool of 20-22μ diameter was used for all the dyeing experiments. The five types of leaves, namely, silver oak [Grevillea Robusta (SOL)], flame of forest [Spathodea campanulata (FOF)], tanner’s senna [Cassia auriculata (AL)], wattle [Acacia decurrens (WL)] and serviceberry [Amelauchier arborea (SL)], were collected from Western Ghats of India and were used as dye sources. Aluminum sulphate [Al₂(SO₄)₃], stannous chloride [SnCl₂] and ferrous sulphate [FeSO₄] of analytical grade were used as mordants for dyeing wool.

3.6.2 Extraction, Dyeing and Mordanting

Leaf materials with the moisture content of 40-50% were used for extraction of dye. Aqueous extract of leaves were prepared by boiling 250g of the leaf material with 5 litres of water for 1 hour. The extracts were filtered and stored in containers with the small addition of acetic acid to prevent any contamination with microorganisms. All the dyeing experiments were carried out by exhaustion method in a water bath, keeping material to liquor ratio to 1:50. The well-scoured woollen yarn (5g) in the form of hank was introduced into dye bath at room temperature and temperature was increased to 85°C
with gentle stirring. The dyeing was then continued for one hour. Throughout the dyeing, a pH level of 7 was maintained. The simultaneous mordanting with different salts was carried out in the same bath after exhaustion of the dye. The temperature of the bath was allowed to cool below 50°C and the mordant solution of 3% concentration on the weight of the fibre (owm) was added to the bath. The temperature was then raised to 85°C and dyeing continued for another one hour. After mordanting, the samples were taken out of the bath and thoroughly washed with water, followed by washing with detergent.

3.6.3 Testing Methods

The light and washing fastness of the dyed samples were done as per the standard methods, i.e. ISO 105 (CO3) and BS 1006 (BO2), respectively. The spectral values of the dyed samples like Integrated K/S, ΔL, Δa, Δb and ΔE (CIE 2000) values were determined using a Minolta 508 spectrophotometer with Macbath Match View software in D65 day light. K/S value is linearly related to the concentration of dyes in the material. The colour difference (ΔE) was calculated according to Equation (3.5).

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

(3.5)

where, ΔL, Δa, Δb are the difference between sample and standard. If, ΔL < 0, the sample is darker than the standard; ΔL > 0, the sample is lighter than the standard; Δa < 0, the sample is greener than the standard; Δa > 0, the sample is redder than the standard; Δb < 0, the sample is bluer than the standard; Δb > 0, the sample is more yellow than the standard.
COMBINED DYING AND ANTIMICROBIAL FINISHING OF WOOL USING NATURAL DYES AND THE EFFECT OF BLEACHING AND ENZYME PRETREATMENTS

3.7.1 Materials

Scoured 100% woollen yarn (4NM) from fine wool of 20-22μ diameter was used as an initial material for this experiment. The four types of leaves, namely, silver oak [Grevillea Robusta (SOL)], flame of forest [Spathodea campanulata (FOF)], tanner’s senna [Cassia auriculata (AL)] and wattle [Acacia decurrens (WL)], were collected from Western Ghats of India and were used as dye sources. Aluminum sulphate [Al₂(SO₄)₃], stannous chloride [SnCl₂] and ferrous sulphate [FeSO₄] of analytical grade were used as mordants for dyeing wool.

An alkaline protease enzyme (Perizym AFW) supplied by M/S Textilchemie Dr. Petry GMBH, Germany and an acid protease enzyme (Palkoacid HP) supplied by Maps Enzyme (India) Ltd., India were used as such. A nonionic detergent Sandoclean PCJ (Clariant India Ltd.) was used as wetting and scouring agent. All the other chemicals are of analytical grade purchased from M/s. SD Fine Chemicals, India.

The schematic diagram of the different processes used in this study is shown in Figure 3.3. The scoured wool yarns (using 3% non-ionic detergent at 50°C for 30 min in neutral pH) were treated with hydrogen peroxide and protease enzymes under acidic and alkaline condition followed by natural dyeing process.
In this method, the bleaching and subsequent enzyme treatment were carried out completely in acidic conditions. The scoured wool yarn was bleached in acidic pH using the following recipe:

- Fabric weight - x g
- Wetting agent - 1%
- Hydrogen Peroxide - 3%
- Citric acid - 3% (owm)
- Sodium acetate - 2% (owm)
- pH - 5.5
- Temperature - 100°C
- Time - 90min

Figure 3.3  Schematic diagram of dyeing and antimicrobial finishing process

3.7.2  Acid Protease Treatment
The bleached yarn was then treated with acid protease using the following recipe:

- Fabric weight: x g
- Wetting agent: 0.5%
- Enzyme: 2% (owm)
- pH: 4.5
- Temperature: 40°C
- Time: 60min

### 3.7.3 Alkaline Protease Treatment

In this method, the bleaching and enzyme treatments were carried out completely in alkaline condition. The wool yarn was first bleached with alkaline hydrogen peroxide using the following recipe:

- Fabric weight: x g
- Wetting agent: 1%
- Hydrogen Peroxide: 3%
- Sodium carbonate: 3% (owm)
- Trisodium phosphate: 2% (owm)
- pH: 9
- Temperature: 70°C
- Time: 90min

The bleached yarn was then treated with alkaline protease enzyme using following recipe:

- Fabric weight: x g
- Wetting agent: 0.5%
- Enzyme: 2% (owm)
3.7.4 Extraction of Colouring Materials and Conversion to Powder

All the plant leaves were dried in shadow to the moisture content of 10-20% and then ground into powder form. 40g of each crude powder was then soaked in 200ml of water for 24 hours followed by boiling in water bath for two hrs for extraction of water-soluble colouring mater. The crude extract was then filtered and spray dried using Labultima LU 222 spray dryer into powder. The powdered natural dye was then used for dyeing experiments.

3.7.5 Dyeing and Mordanting

All the dyeing experiments were carried out by exhaustion method in a water bath, keeping material to liquor ratio to 1:50. The bleached and the enzyme treated woollen yarns (5g) in the form of hank was introduced into dye bath containing 5% dye (owm) at room temperature and temperature was increased to 85°C with gentle stirring. The dyeing was then continued for one hour. The mordanting with different salts was carried out in the same bath after exhaustion of the dye. The temperature of the bath was allowed to cool below 50°C and the mordant solution of 3% concentration on the weight of the material (owm) was added to the bath. The temperature was then raised to 85°C and dyeing continued for another one hour. After mordanting, the samples were taken out of the bath and thoroughly washed with water, followed by washing with detergent.
3.7.6 Testing Methods

The light and washing fastness of the dyed samples were done as per the standard methods, i.e. ISO 105 (CO3) and BS 1006 (BO2), respectively. The CIE 2000 (ΔL, Δa, Δb and ΔE) spectral values of the dyed samples were determined using a Minolta 508 spectrophotometer with Macbeth Match View software in D65 day light and compared with control sample (standard).

3.7.7 Testing of Antimicrobial Efficacy

The microbial resistance of the treated wool fabrics along with their different washed samples was determined using qualitative Agar Diffusion Method (SN 195920) as per the procedure given earlier against *Staphylococcus aureus* and *Escherichia coli*.

3.8 COMPARATIVE STUDY OF THE DEVELOPED ANTIMICROBIAL FINISHING TREATMENTS

A comparative study among the three different antimicrobial finishing processes developed for wool in this research was made in terms of antimicrobial efficacy, ease of application and suitable materials for finishing.