Optimization of antimicrobial finishing on cotton muslin fabric by treatment with PEG, chitosan and cetrimide

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In the present work, combinations of different concentrations of binary mixture of chitosan + PEG, chitosan + cetrimide and PEG + cetrimide have been applied on bleached cotton muslin fabric by pad-dry-cure process in the presence of citric acid + sodium hydrogen phosphate catalyst, and the consequent percentage strength retention after treatment, percentage strength retention after soil burial test and average bacterial reduction % are evaluated with an aim to optimize the concentrations of chitosan, polyethylene glycol and cetrimide to achieve a balance between antimicrobial properties and tenacity retention. Optimization results show that the application of 2% chitosan + 2% PEG (50:50) with citric acid and SHP as mixed catalyst system gives shows very good antimicrobial/rot resistant properties without much loss of tenacity.

Keywords: Antimicrobial finishing, Cotton muslin fabric, Chitosan, Epoxy-propyl trimethyl-ammonium chloride, Rot resistance, Tenacity

1 Introduction

One of the major problems of cotton fabric is its microbial susceptibility which needs to be addressed properly. Cotton muslin fabric is more vulnerable to the same, as it has considerably lower tenacity and durability because of light weight (low GSM) and lower cover factor 1,2 than normal cotton fabrics. Hence, there is an essential need of identifying some eco-friendly & economical antimicrobial and rot resistant finish formulations for such delicate cotton fabric that cause minimum tensile strength reduction during treatment use and pose resistance (or less prone) towards microbial attack during storage. There are many reports on antimicrobial finish /rot resistance finish on cotton and ligno cellulose fabrics 3-10. A few reports have mentioned about the use of chitosan, citric acid and polyethylene glycol (PEG)3-10 for improving antimicrobial properties of cotton. But none of them has used properly optimized chemical proportions in mixture to obtain balanced results between deterioration in tensile strength and effectiveness of antimicrobial activity. Only one recent preliminary report of such work has been published but using the above-said three chemicals on muslin cotton delicate fabric without optimization of the formulations. However, as the cotton muslin fabric is a finest and delicate khadi product, there is need of proper optimization of these three chemicals whether used separately or in combinations for imparting desired balance in between the bacterial reduction % as a measure of antimicrobial activity and tenacity retention after soil burial test as a measure of rot resistance. From the experience of earlier preliminary work ¹, the dosages of each such chemicals (PEG, CA and SHP) has been decided. In the present work, different proportions /concentrations of binary mixtures of (i) chitosan + PEG, (ii) chitosan + cetrimide + (iii) PEG + cetrimide have been applied on bleached cotton muslin fabric in presence of CA + SHP mixed catalyst under pre-determined conditions treatment by pad–dry–cure process. The statistical optimization of the chemical concentrations (quantitatively) as critical input variables has also been studied with an aim to achieve a balance between antimicrobial properties (bacterial reduction %) and tenacity retention after soil burial test using Box- Behnken statistical experimental design 11.

Efficacies of these chemicals/ formulations are also compared with the performance of a commercially available and widely used chemical epoxy-propyl trimethyl-ammonium chloride (EPTAC), a specific quaternary ammonium compound.
2 Materials and Methods

2.1 Materials

Cow-dung bleached plain woven fine cotton muslin fabric made out of very fine 2.7 tex (400 Nm) un-sized handspun cotton yarn with 38 g/m² aerial density, 150 PPI (pick density) and 122 EPI (end density) was used in the present study.

PEG 200 obtained from E Merck (India), chitosan (100 mesh powder) obtained from Mahtani Chitosan Pvt. Ltd. and cetrimide obtained from Loba Chem, India were used as antimicrobial agents for cotton. Citric acid (CA) and sodium di-hydrogen phosphate (SHP) obtained from E Merck India were used as catalyst to achieve a durable and wash fast effect on the cotton muslin fabric. Sodium hydroxide (NaOH) was obtained from Navdeep Chemicals Pvt Ltd, Mumbai for the present study.

2.2 Treatment Methods

2.2.1 Bleaching

Cow-dung bleaching 12-14, which is a conventional mild bleaching process specially meant for such delicate “cotton muslin fabric” and “ayurvastra”, was carried out by treating the fabric in filtered cow dung solution (pH 7-8) for one and half hour followed by steaming at 100°C for 30 minutes, then washing and drying at ambient conditions. This process was standardized earlier by controlling pH, temperature and time at MGIRI, Wardha. This is eco-friendly biopretreatment process.

2.2.2 Antimicrobial/Rot Resistance Treatment

Bleached cotton muslin fabric was initially treated with 2% (20 gpL) aqueous solution (with 100% expression) of each of cetrimide, chitosan and PEG and a mixture of two or three such agents in different proportions. For the treatment, pad-dry-cure method was used in absence and presence of the mixed catalyst system such as citric acid + sodium di-hydrogen phosphate. The pH was maintained at 5 - 5.5 by addition of soda ash and acetic acid as and when required with additional use of sodium acetate and acetic acid buffer solution. Being insoluble in water, chitosan was dissolved in the aqueous solution of 2% acetic acid at 60°C and pH 5-5.5 by constant stirring for 60 min.

The above treated bleached cotton muslin fabric was padded in a lab padding mangle with 1.5 kg/cm² nip pressure to maintain 100% wet pick up for 1-4 % (10gpL-40 gpL) application of each of these three chemicals in their binary combination, followed by drying at 100°C for 10 min, curing at 140°C for 4 min followed by rinsing, washing (to remove the unreacted chemicals) and drying in air at ambient conditions. The amount of optimum catalyst and best curing conditions were pre-decided. Hundred per cent wet pick up was maintained using special type of pneumatic controlled RAPID–China Make lab padding mangle.

As EPTAC is usually available with different trade names and it is not sure to get the right compound for comparison of antimicrobial and rot resistance performance properties, another set of cotton muslin fabric was additionally padded with a mixture of 60g/L 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTAC) and 24 g/L hydrated NaOH (after keeping the mixture for 10 min in room temperature) to produce in situ EPTAC15. This compound dried and cured in the same manner as described above. Unless otherwise indicated, all such cured fabrics were thoroughly washed and dried in air at ambient conditions.

2.3 Test Methods

2.3.1 Rot Resistance

Rot resistant performance, i.e. resistance to microbial attack of treated and untreated cotton muslin fabric samples was assessed by determining the % retention of tensile strength after subjecting the fabric to a standard soil burial test for 21 days as per IS 1623:1992 standard test16.

2.3.2 Tensile Strength and Tenacity Retention

Tensile strength of selected fabric samples was measured by the raveled strip method as per ASTM D 5034 standards using Digi strength tester (make Paramount Instruments, India) with a traverse speed of 300 mm/min. The final gauge length (sample size) of the fabric sample was 100×150 mm under the two jaws for the tensile strength test of treated and untreated cotton muslin fabrics. The test results reported are an average of 10 tests for each sample. Fabric tenacity was calculated as follows:

$\text{Tenacity (cN / tex) = } \frac{\text{Breaking load (cN)}}{\text{Fabric wt (gm / mt)}} \times \frac{\text{Fabric width (mm)}}{100}$

The retention% of the tensile strength or tenacity for both untreated and treated cotton muslin fabric was calculated using following relationship:

$\% \text{ Retention} = \frac{\text{Treated fabric tenacity}}{\text{Untreated fabric tenacity}} \times 100$
2.3.3 Antimicrobial Properties

Antimicrobial properties of these treated and untreated samples were studied as per AATCC 147-2011 and AATCC 100-2012 standards in the incubation condition of 37°C for 24 h (ref. 18).

2.4 Statistical Optimization

As per Box-Behnken statistical experimental design, for the three input variables namely chitosan conc. (X1), PEG (X2) and cetrimide conc. (X3) at 3 levels, total 15 experimental run, are needed (Table 1). The corresponding values of the two resultant response variables are average bacterial reduction % (Y1) and tenacity retention % (Y2) (Table 2). This is guided by the following quadratic equation as per Box-Behnken statistical optimization response equation for each response variable

\[ Y_i = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{i=1}^{k} b_{ij} x_i x_j \]  

(1)

where \( b_0, b_i \) and \( b_{ii} \) are the corresponding regression coefficients.

3 Results and Discussion

3.1 Rot Resistance and Antimicrobial Properties

As a preliminary work, rot resistance property of treated cotton muslin fabrics with and without catalyst under fixed and comparable conditions of treatment was measured by evaluation of fabric tenacity before and after soil burial test. The extent of fabric damage by microorganism is indicated by percentage loss in strength or in terms of percentage retention of tenacity after soil burial test. Relevant results of preliminary study are shown in Table 3. The fabric treated with mixture of polyethylene glycol 200 + chitosan with citric acid + sodium hydrogen phosphate as mixed catalyst system has been found to perform well in terms of tenacity retention after treatment, tenacity retention after soil burial test and average bacterial reduction % as compared to all other combinations. Therefore, treatment of cotton muslin with PEG + chitosan in combination (1:2, 1:1, 2:1 and 2:3) has shown much less deterioration of strength after soil burial testing (80-92% retention). Untreated cotton muslin fabric has shown higher degradation after 21 days soil burial test and retains only 53.2% tensile strength as shown in Table 3, whereas the rot resistance property imparted on PEG + chitosan treated cotton is observed, as the attached PEG imparts thermal adaptivity and moisture adaptability besides the special surfactant nature with slippery surface of poly-ol array of treated cotton fabric and also by polycationic nature and some degree of cross-linking with chitosan, thus preventing cellulose degradable enzymes to act upon. The ability of chitosan to immobilize microorganism derives from its poly cationic character. Its protonated amino group blocks the protein sequences of microorganism, thus inhibiting further proliferation.

Moreover, in presence of citric acid+SHP catalyst system, the carboxylate group of citric acid and OH group of chitosan form ester, and also carboxylate group of citric acid may react with amino group of chitosan forming salt linkages, thus converting relatively hard and stiff chitosan to more ductile, mobile and easy flowing and less crystalline chitosan micro molecules, favoring higher spread of chitosan film with its polycationic character on surface of treated cotton. Cationic character of cetrimide as a quarternary ammonium compound like EPTAC is assured to have effective antimicrobial/rot resistant effect on cotton.

Moreover, some degree of cross-linking of chitosan-PEG adduct, having -OH end group of polyol in one end and -COOH end group of chitosan at the other end, with cellulose of cotton can not be excluded. This cross-linking additionally enhances the antimicrobial property of cotton treated with

<table>
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<th>Level</th>
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<tr>
<td>Chitosan (X1)</td>
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<tr>
<td>PEG (X2)</td>
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<tr>
<td>Cetrimide (X3)</td>
<td>0</td>
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Table 2 — Experimental run for critical process variables

<table>
<thead>
<tr>
<th>Sample code</th>
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<tr>
<td>S1</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>4</td>
<td>2</td>
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Table 1 — Three levels of critical process variables
chitosan+PEG combination, particularly showing good results for 2% PEG-200 + 2% chitosan combination.

For varying proportions of chitosan and PEG blends (1:1, 1:1.5, 1:2 & 2:1), as the PEG content is increased from 1% to 2%, the rot resistance and other textile related properties mainly tenacity retention is better balanced with measurable improvement in rot resistance properties. However, in case of PEG from 2% upto 4% it does not proportionately improve all these properties, rather impairs some of them. This may be due to the fact that excessive higher amount of PEG causes higher amount of hydrogen bonding interaction with chitosan, partially restrains the movement of chitosan molecule and its immobility is increased, causing the above impairing effect. The addition of more PEG with respect to chitosan creates more elongated porous structure which ultimately results in a weak membrane of chitosan–PEG adduct, showing some drop in tenacity values. The application of 2% each PEG-200 and cetrimide or 2% each chitosan and cetrimide also show comparable rot resistance performance, particularly for the former under same conditions of treatment.

It is observed that 2%PEG and 2% chitosan is most suitable for ensuring overall balance amongst the rot resistance and other related properties of cotton muslin fabrics in presence of 1/5th of CA + SHP mixed catalyst. The findings are then compared with a commercially available quarternery ammonium compound EPTAC.

Thus, an increase of chitosan from 1% to 2% is found to be advantageous, but its increase above 2%, keeping PEG % same, impairs the overall balance in the property parameters of the fabric. For understanding the individual and mutual interactions of these mixture, the selective mixture of PEG-200, chitosan and cetrimide needs to be optimized statistically, which is the reported hereunder.

### 3.2 Statistical Optimization

#### 3.2.1 Use of Box and Behenken Experimental Design

To derive maximum and significant interaction using three critical process variables at three different levels against two resultant response variables, Box and Behenken experimental design technique has been used. Table 4 shows the actual response of the three process variables (chitosan, PEG and cetrimide) and their effect on two average bacterial reduction% and tenacity retention% of cotton muslin fabric. To establish the relationships between these variables, corresponding regression analysis has been carried out and the regression coefficients are used in quadratic equation for the determination of predicted values of all the 15 experimental run. The significance of the effect of input process variables are tested by F-ratios. These quadratic equations are also used to obtain possible treatment combinations for each experimental assessment and the respective responses using computer software (Minitab 17.3.1.0). The contour diagrams were plotted to study the effect of input process variables on the response variables. It is observed that each regression coefficient always shows a value (Tables 4 and 5), either positive or negative and so has an effect on the three experimental process variables. For a process variable to have a significant effect on resultant property, its coefficient must be greater than twice its standard error coefficient. However, the non significant coefficient should not be dismissed altogether and might have some interactive effect.
Thus, the effect of three process variables (dosage of chitosan, PEG and cetrimide) under prefixed dosage of CA + SHP mixed catalyst system is shown in Table 4. As per the preliminary study, the dosages of the three chemicals were varied between 2% and 4% only.

### 3.2.2 Results of Tenacity Retention

Data in Tables 5 and 6 indicate that on one side concentrations of chitosan and PEG and on the other side concentration of cetrimide have reasonable effect on rot resistant property in terms of % tenacity retention after soil burial test as depicted in the ANOVA data. Table 6 shows that both $b_0$ and $b_1$ have positive values, thus indicating a direct effect of concentrations of chitosan on tenacity retention value. Positive regression values of $b_1$ and $b_2$, also show a direct effect of interacting Tenacity retention value with increase in chitosan as well as PEG. However, again $b_{1.1}$, $b_{2.2}$ and $b_{2.3}$ also show positive regression coefficient which indicates that the increase in square term of both chitosan and PEG individually or PEG and cetrimide have positive effect on tenacity retention value i.e. interactions are positive in nature.

The negative square term for cetrimide and mixtures of chitosan + PEG or chitosan + cetrimide ($b_{3.3}$ and $b_{1.2}$, $b_{1.3}$ respectively) indicates that the increase in concentration of these variable have a negative effect, i.e. tenacity retention value starts decreasing. So, by analysis of Box and Behnken experimental design, a quadratic equation [Eq(1)] is obtained to predict the tenacity retention value along with values of $b_0$, $b_1$, $b_2$, $b_3$, $b_{1.1}$, $b_{1.2}$, $b_{1.3}$, $b_{2.3}$ putting $X1$ as chitosan, $X2$ as PEG and $X3$ as cetrimide, the regression equation for determining tenacity retention% is as follows:

$$
\text{Tenacity retention} \% = b_0 + b_1(X1) + b_2(X2) + b_3(X3) + b_{1.1}(X1^2) + b_{1.2}(X2^2) + b_{3.3}(X3^2) + b_{1.2}(X1.X2) + b_{1.3}(X1.X3) + b_{2.3}(X2.X3) \quad \text{(2)}
$$

### 3.2.3 Analysis of Box-Behnken on Tenacity Retention

Following parameters are used:

- **Factors**: 3
- **Base runs**: 15
- **Base blocks**: 1
- **Center points**: 3
- **Replicates**: 1
- **Total runs**: 15
- **Total blocks**: 1
Regression equation in uncoded units after putting the values of $b_0$, $b_1$, $b_2$, $b_{11}$, $b_{12}$, $b_{13}$ and $b_{23}$ are as follows:

Tenacity retention (%) = 92.20 + 2.88 $(X_1)$ + 1.68 $(X_2)$ - 0.68 $(X_3)$ + 2.66 $(X_1^2)$ + 0.96 $(X_2^2)$ - 2.44 $(X_3^2)$- 2.18 $(X_1X_2)$ - 1.68 $(X_1X_3)$ + 0.22 $(X_2X_3)$  

Each response value obtained by using this equation is thus predictable and obtained by contour curves as shown in Figs 1(a) - (c) for tenacity retention after soil burial test of 21 days for varying dosage of chitosan, PEG and cetrimide.

A comparison of contour curves indicates that all possible interactive effects of dosage of chitosan and PEG on tenacity retention values (after soil burial test for rot resistance) are positive, whereas the increase in dosage of cetrimide indicates a negative impact on the same.

3.2.4 Analysis of Box-Behnken Design on Bacterial reduction

Tables 7 and 8 show the ANOVA analysis using the regression coefficients and their positive or negative effect on bacteria reduction% for both single-way and two-way interactions. It is observed from the regression coefficient values that chitosan, PEG and cetrimide individually have a positive effect, i.e. with the increase in dosage of these three chemicals, the bacteria reduction percentage increases. Two-way interactions of chitosan +PEG also show a positive effect (Table 8), i.e. an increase in bacteria reduction%. But considering two-way interactions of chitosan + cetrimide or cetrimide + PEG, it shows a reduction in bacteria reduction%. Square term for PEG, chitosan and cetrimide individually shows negative effect on bacteria reduction%, indicating that individually each has to be applied within certain limits to impart an optimized antimicrobial effect but the excess of these has detrimental effect on % bacterial reduction (Table 8).

The general regression equation [Eq. (1)] is also followed in this case, putting chitosan as $X_1$, PEG as $X_2$ and cetrimide as $X_3$, the regression equation for average bacterial reduction% is as follows:

Average bacteria reduction% = $b_0+b_1 (X_1)+b_2 (X_2)$ + $b_3 (X_3) +b_{1,1} (X_1^2) + b_{1,2} (X_2^2) + b_{3,3} (X_3^2)$ + $b_{1,2} (X_1X_2)+ b_{1,3} (X_1X_3)$ + $b_{2,3} (X_2X_3)$  

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<table>
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<tr>
<th>Coded coefficients</th>
<th>Process variables</th>
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<th>SE coefficient</th>
<th>T-value</th>
<th>P-value</th>
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<td>Constant</td>
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<td>2.46</td>
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<td>1.51</td>
<td>1.91</td>
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<tr>
<td>PEG</td>
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<td>1.51</td>
<td>1.11</td>
<td>0.317</td>
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<td>Cetrimide</td>
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<td>-0.68</td>
<td>1.51</td>
<td>-0.45</td>
<td>0.673</td>
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<tr>
<td>Chitosan × Chitosan</td>
<td>$b_{1,1}$</td>
<td>2.66</td>
<td>2.22</td>
<td>1.20</td>
<td>0.284</td>
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<td>PEG × PEG</td>
<td>$b_{2,2}$</td>
<td>0.96</td>
<td>2.22</td>
<td>0.43</td>
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</tr>
<tr>
<td>Cetrimide × Cetrimide</td>
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<td>-1.10</td>
<td>0.322</td>
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<td>Chitosan × PEG</td>
<td>$b_{1,2}$</td>
<td>-2.18</td>
<td>2.13</td>
<td>-1.02</td>
<td>0.354</td>
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<tr>
<td>Chitosan × Cetrimide</td>
<td>$b_{1,3}$</td>
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<td>2.13</td>
<td>-0.79</td>
<td>0.467</td>
</tr>
<tr>
<td>PEG × Cetrimide</td>
<td>$b_{2,3}$</td>
<td>0.22</td>
<td>2.13</td>
<td>0.11</td>
<td>0.920</td>
</tr>
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</table>

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Fig. 1 — Contour curves showing interactive effect of (a) PEG + chitosan, (b) PEG + cetrimide and (c) cetrimide + chitosan on tenacity retention
Now after putting the values of $b_0, b_1, b_2, b_3, b_{11}, b_{12}, b_{13}, b_{23}$ in Eq. (4), the following equation is obtained for determining the average bacteria reduction%:

$$
\text{Average bacteria reduction(\%)} = 90.567 + 0.775 (X_1) + 0.275 (X_2) + 0.250 (X_3) - 0.433 (X_1.2) - 1.083 (X_2.2) - 0.583 (X_3.2) + 0.350 (X_1.3) - 0.100 (X_1.3) + 0.050 (X_2.3) \quad \ldots (5)
$$

The contour diagrams for all the variables vs tenacity retention, obtained by Minitab software, are shown in Figs 2 (a)-(c).

A comparison of contour curves [Figs 2(a)-(c)] for different responses indicates that the increase in both chitosan and PEG has a positive effect on average bacterial reduction% but increase in concentration of cetrimide has a negative effect.

Table 9 shows the antimicrobial properties of three types of fabrics against two common microbes present in the air and moisture. It is observed that the chitosan and PEG mixtures show the optimum protection of the fabric against the microbes with minimum loss of tenacity value. After 24 h of incubation, the reduction in microbes (bacteria) is depicted in Fig 3, tested as per quantitative AATCC100 standard. The corresponding values of average reduction% in bacteria after 21 days of soil burial are depicted in Table 3.

As shown from Table 9, the effect of chitosan alone or in mixture with PEG shows absence of growth under specimen and zone of inhibition together, indicating a bacteriostatic antimicrobial activity on the fabric, whereas PEG alone or in mixture with chitosan or cetrimide shows growth of microbes.
combination with cetrimide shows a diffusible antimicrobial activity. In the latter case, zone of inhibition more than 1 mm shows good antimicrobial activity.

4 Conclusion

4.1 The effect of three input variables (concentrations of chitosan, PEG and cetrimide) on two resultant output variables (Average bacterial reduction%, and tenacity retention%) after soil burial test can effectively be interpreted by the obtained quadratic equations with known constant value and can be explained by corresponding standard error, regression coefficients and also from contour curve diagram.

Table 9 — Observation of presence of bacteria after 24 hours of incubation as per AATCC 147-2011 method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staphylococcus aureus AATCC 6538</th>
<th>Klebsiella pneumonia AATCC 4352</th>
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</thead>
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<tr>
<td></td>
<td>Growth under specimen</td>
<td>Zone of inhibition</td>
</tr>
<tr>
<td>Untreated fabric</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Treated with 2% chitosan</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Treated with 2% PEG</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Treated with 2% PEG + 2% chitosan</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Treated with 2% PEG + 2% cetrimide</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>

Fig. 3 — Presence of two microbes Staphylococcus aureus and Klebsiella pneumoniae after 24 h of incubation for (a) untreated fabric and (b) fabric treated with 2% chitosan + 2% PEG

4.2 The Box–Behnken experimental design of the input parameters thus indicate that binary mixture of chitosan + PEG in certain % would increase the average bacteria reduction% with a bit compromise on tenacity retention% values, but use of excess of the same has a negative effect as well. As the antimicrobial properties play a vital role in obtaining maximum storage life of cotton muslin fabric in long run, particularly for medical textile application, antimicrobial property improvement with a little bit compromise on tenacity retention values can be monitored and achieved by the response surface for statistical optimization of the said process using the regression equation generated.
4.3 It was finally revealed that 2% PEG + 2% chitosan mixture render optimum retention of tenacity percentage after soil burial test of 21 days and best average bacteria reduction% values after incubation for 24 h. Thus, this optimum treatment to cotton muslin fabric can be more useful for its application in medical textiles like surgical gown/dresses of nurse and medical bed linen etc.

References
2. Textile Terms and Definition (The Textile Institute), 7th edition, 1975