CHAPTER 4

ANTIBACTERIAL STUDIES OF FABRIC COATED WITH CaP-Ag

4.1 INTRODUCTION

Silver in various forms has been used for centuries to prevent certain infections. Ancient Greeks practiced disinfecting the stored liquids by using silver coins.\(^1\) Silver nitrate solution was used in late 1800’s for preventing ocular infection in neomates and was also observed to be effective against typhoid and anthrax.\(^2\)

With the discovery of organic drugs, the use of silver in preventing infections decreased. Thirty years after World War II, silver once again began to receive clinical importance.\(^3\) Among the various antimicrobial agents available, the use of silver in wound treatment on skin is high, as silver is considered to be relatively safe.\(^4\)\(^-\)\(^8\) Silver ions have been described as oligodynamic as even their minute concentrations show bactericidal effects.\(^9\)\(^-\)\(^11\)

Silver ions and silver based compounds show strong biocidal effects on many species of bacteria and are considered to be non-toxic to the mammalian cell.\(^12\)\(^-\)\(^14\) The ability to develop resistance to silver by the bacteria is relatively low.\(^9\)

Both gram positive and gram negative bacteria are susceptible to attack by the silver ions.\(^2\)\(^,\)\(^3\)\(^,\)\(^9\)\(^,\)\(^15\) \textit{Staphylococcus aureus} (\textit{S. aureus}) causing diseases like endocarditis, osteomilitis, foreign body related infections, etc.\(^11\) was found to be affected by silver in appropriate concentrations.\(^16\)
Recently, using hydrofibre technology, commercial products impregnated with silver are available like, foam, film, gauze, dressing with hydrocolloid, etc.\(^9\)

Physically silver bonded to cellulose latex, polyethylene, polypropylene, etc., have been used largely in the field of medical services.\(^{17,18,19}\) It is also used in wood preservation, antibiotic bandages and water purification.\(^{20-22}\) Antibacterial water filter containing silver nano particles coated on polyurethane foams was found to be effective in its performance as no bacteria was detected in the output water, with a bacterial load of \(1 \times 10^{-5}\) to \(1 \times 10^6\) CFU/mL in the input water.\(^{23}\)

### 4.1.1 Literature on phosphate glass containing silver

Phosphate based glasses with silver incorporated in it are soluble materials and are effective in delivering silver ions in a controlled manner.\(^{21}\) Control of urinary tract infections by silver incorporated phosphate glass was observed in patients who need long term indwelling catheritis.\(^{19}\) Glass composition with high antibacterial activity was achieved by introducing a small amount of antibacterial component (\(\text{Ag}_2\text{O}\)) and this glass was used to synthesize antibacterial polymer.\(^{24}\)

### 4.1.2 Mechanism of action by silver

Feng \textit{et al},\(^{26}\) studied the antibacterial effects of silver ion in both \textit{Escherichia coli} (\textit{E. coli}, gram negative) and \textit{Staphyloccocus aureus} (gram positive) and suggested that the bactericidal activity was due to
inability of DNA to replicate and inactivation of proteins after coming into contact with silver ions.\textsuperscript{25-26} Soluble forms of silver ions or silver metal may inhibit the electron transport and thereby cause conformational changes on the cell membrane apart from interfering in DNA replication.\textsuperscript{13}

The mechanism of antibacterial action of silver ions was related to their interaction with thiol groups in enzymes, proteins and also involves other cellular components.\textsuperscript{27} Fuhrman \textit{et al},\textsuperscript{28} reported the release of potassium ions from microbial cytoplasmic membrane which are associated with many important enzymes and are target sites for silver ion activity.\textsuperscript{28-30} Detailed discussion on specificity of silver ion for thiol groups were made by Russel and Hugo.\textsuperscript{27} Didrov \textit{et al},\textsuperscript{31} reported the proton leakage through the bacterial membrane occurred at low concentration of silver ions resulting in cell death.

### 4.2 PROCEDURE FOR ANTIBACTERIAL ANALYSIS

The classical microbial test methods to evaluate the substrate-bound antimicrobial for regular quality control and screening tests have the inherent difficulties which include ensuring contact of inoculum to treated surface, less flexibility of retrieval at different contact times, difficulties related to sensitivity and reproducibility. In order to overcome the above difficulties, ASTM-E-2149 procedure was designed to evaluate the resistance of non-leaching antimicrobial active specimen to growth of microbes under dynamic contact conditions. Thus, for evaluating the antibacterial property exhibited by Ag-CaP and silver-coated fabric, ASTM-E-2149 standard procedure was followed.
4.2.1 Summary of the test-method

The antibacterial activity of a substrate-bound antimicrobial is dependent upon direct contact of microbes with the active surface containing chemical agent (in the present case, phosphate glass containing silver). The antibacterial activity of treated specimen was determined by shaking samples of surface-bound materials in a concentrated bacterial suspension for a contact time of one hour. The suspension was serially diluted both before and after contact and cultured. The number of viable organisms in the suspension was determined and the reduction percent was calculated based on the initial counts or on retrievals from appropriate untreated controls.

4.2.2 Preparation of Reagents

Buffer Solution: 34 g of potassium dihydrogen phosphate was transferred into 1000 mL beaker containing 500 mL of distilled water. The pH was adjusted to 7.2 using a dilute solution of NaOH and further diluted to 1000 mL. For working buffer solution (0.3 mM KH$_2$PO$_4$), 1 mL of stock buffer solution was transferred, with a sterile pipette to flask containing 800 mL of distilled water. It was capped and sterilized.

4.2.3 Test Specimen and Test Organism

Dimensions of the fabric specimens were 5 cm x 5 cm (weighing about 1 - 1.5 g) for both treated and untreated controls. ATCC type culture was selected for the antibacterial studies. Test organisms selected for the above studies were *E. coli* (gram negative, ATCC No:
25922) and *S. aureus* (gram positive, ATCC No. 6568 P). The above strains of bacteria were obtained from NCIM, NCL, Pune. The purity of the cultures were maintained and checked periodically by streak plate technique and observed for a single species characteristic type of colonies.

### 4.2.4 Details of Instrumentation

**Apparatus:** Sterilizer, incubator, laminar flow chamber, wrist-shaker, water bath, glassware, contact flask, 250 mL Erlenmeyer flask, autoclave, dilution vessels and micropipette.

**Media:** Nutrient broth or media (HIMEDIA)

![Figure 4.1: Pictorial representation of bacterial culture grown on agar slant.](image-url)
Bacterial inoculums: The working bacterial dilution was prepared by growing a fresh 18 hour shake culture of the test organism in a sterilized nutrient broth for each series of samples.

The culture was diluted with sterilized buffer solution to obtain a final concentration of $1.5 \times 10^8$ to $3.0 \times 10^8$ CFU/mL.

### 4.2.5 Procedure for determining antibacterial activity

One treated piece and un-treated piece of each specimen of identical composition were required for each series of specimen tested.

Sterilized 250 mL screw-cap Erlenmeyer flasks were taken each for treated and untreated specimen. This was in addition to one ‘innoculum only’ sample. 50 mL of working solution of bacterial inoculum (prepared as explained above) was added into each of the flask.

The flasks were capped and subjected to shaking using wrist-shaker for 1 min. Each flask was considered to be a part of zero contact time subgroup. For determining the bacterial concentration of solution at ‘0’ contact time, serial dilutions and standard ‘plate count’ techniques were adopted. Duplications have been performed at ‘0’ contact time.

Immediately after preparation of ‘0’ contact time sub samples, treated specimen was introduced in one of the flasks and untreated specimen was introduced into another. The flasks were re-capped and agitated using wrist-action shaker for 1 hour. One mL from each flask were transferred to test-tubes, which was followed by serial dilutions
and standard plate count methods for determining the bacterial concentration after 1h of shaking. Duplications similar to ‘0’ contact time sub group were performed. All the petri dishes from both the subgroups were allowed to incubate for 24 – 48 hours.

The colonies formed in each of the respective petridishes were counted. Average of petridish counts were worked out and then converted to ‘Colony Forming Units per millilitre’ (CFU/ mL) respectively. The percentage reduction of the organism resulting from the specimen was calculated using the formula, given below. Results were reported as percentage reduction of CFU/ mL.

\[
\text{Reduction, } \% \text{ (CFU)} = \frac{(B - A)}{B} \times 100
\]

Where,

\[A = \text{ CFU/ mL (or mean } \log_{10} \text{ density of bacteria) for the flask containing the treated substrate after the specified contact time i.e. One hour}\]

\[B = \text{ CFU/ mL for the flask at ‘0’ contact time (before the addition of treated substrate).}\]

Since, CFU/ mL for the flask containing the ‘inocculum only’ control after one hour and CFU/ mL for the flask where the untreated specimen was introduced and shaken for one hour was within 15%, the antibacterial activity (\% reduction) was calculated using the formula shown above. Hence, further computations as per ASTM standards were found to be non-applicable.
Antibacterial studies using ASTM-E-2149 procedure

**Figure 4.2: Schematic representation of test-procedure -ASTM-E-2149**

- **‘INOCULUM ONLY’**
  - 50 mL working solution of bacterial inoculum
  - 1 mL transferred, serially diluted, plate counted, & CFU/mL at '0' contact time was determined
  - 1 min shaking
  - Shaking continued for 60 min.
  - 1 mL transferred, serially diluted, plate counted, & CFU/mL at '60' min. was determined

- **TREATED SPECIMEN**
  - 1 mL transferred, serially diluted, plate counted, & CFU/mL at '0' contact time was determined
  - 1 min shaking
  - Treated cloth introduced & Shaking continued for 60 min.
  - 1 mL transferred, serially diluted, plate counted, & CFU/mL at '60' min. was determined
Figure 4.3: Pictures of the instruments/equipments used for antibacterial analysis.
4.3 COATING TECHNIQUES

4.3.1 Dip-Coating Technique

The glass beads were dissolved in distilled water to form glass solution with known concentration of silver. Pure cotton fabric of 5 cm X 5 cm dimension was immersed in 25 mL of the above solution for 5 minutes. It was then removed using sterilized forceps and dried under shade in dust free atmosphere. The silver coated fabric was sealed and labelled.

Figure 4.4: Dip-coating technique.
4.3.2 Steam-Pressing technique

The deposition of silver can also be effected by steam-pressing technique. In this technique, the silver glass solution was introduced in chamber provided to produce steam in hot-pressing machine. The fabric of dimension 5 cm X 5 cm was steam-pressed with the machine. The silver dissolved in the solution was transferred on the surface of the fabric along with steam drops. The silver-coated fabric was sealed and labelled.

![Steam-pressing coating technique](image)

Figure 4.5: Steam-pressing coating technique.

4.3.3 Spin-Washing technique

Another method for coating silver over the fabric was carried out by dipping the fabric of 5 cm X 5 cm dimension in 1 litre beaker containing 250 mL distilled water. The glass beads were sealed in nylon knitted pouch and suspended in the beaker. The contents were stirred at 50 rpm for 20 min. The silver released from the glass matrix
gets deposited on to the surface of the fabric. The fabric was removed
with sterile forceps and dried in dust free environment. The silver
coated fabric was sealed and labelled.

Figure 4.6: Spin-washing coating technique.

4.4 ANTIBACTERIAL ANALYSIS

When the Ag-CaP glass degrades in water, phosphate units containing
silver dissolves out to form glass solution. This moiety containing silver ions,
gets deposited on the surface of the fabric and imparts antibacterial property.
4.4.1 Antibacterial analysis of Dip-coated fabric

Glass solutions containing different concentrations of silver were used for coating the fabric by dip-coating method. The dried fabric was evaluated for its antibacterial activity against two strains of bacteria, viz., gram positive bacteria (*S. aureus*) and gram negative bacteria (*E. Coli*).

Table 4.1 A: Antibacterial analysis of dip-coated fabric

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Concentration of Ag in solution, ppm</th>
<th>REDUCTION % (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram Positive organism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>1.</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>2.</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>3.</td>
<td>10</td>
<td>82</td>
</tr>
<tr>
<td>4.</td>
<td>50</td>
<td>96</td>
</tr>
</tbody>
</table>

It was observed that acceptable antibacterial activity (>75%) was found with fabric coated with glass solution containing 10 ppm of silver content. Almost 100% antibacterial activity was observed with fabric coated with glass solution containing 50 ppm of silver content. Therefore, duplications / trials were carried out over fabrics coated with glass solution containing 10 ppm of silver content and the results are given in the Table 4.1-B.
The graph shows that with an increase in silver content in glass solution, the antibacterial activity increases (i.e., increase in reduction percentage of CFU/mL).

**Table 4.1-B: Duplications of antibacterial analysis of dip-coated fabric.**

<table>
<thead>
<tr>
<th>S.</th>
<th>Experiment trial</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Con. of silver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>E. coli</td>
<td>S. aureus</td>
<td>E. coli</td>
<td>S. aureus</td>
</tr>
<tr>
<td>1.</td>
<td>10 ppm</td>
<td>78</td>
<td>89</td>
<td>81</td>
</tr>
</tbody>
</table>

**Antibacterial Analysis - Dip coating**

![Antibacterial activity of dip-coated fabric with different concentrations of silver.](image)

**Figure 4.7:** Antibacterial activity of dip-coated fabric with different concentrations of silver.
Studies by Dunn K et al.\textsuperscript{2} showed that both gram positive and gram negative bacteria are susceptible to attack by ionic silver. Sabeel P. Valappil et al.\textsuperscript{16} studied the effect of increasing silver content in phosphate glass on \textit{Staphylococcus} biofilms.

### 4.4.2 Antibacterial analysis of steam-pressed fabric

Silver glass solutions with varying silver concentration in the range of 1 to 50 ppm were used for coating over the fabric by steam-pressing technique. Antibacterial activity of the steam-coated fabric was evaluated for gram positive and gram negative bacteria and the results are presented in Table 4.2-A.

**Table 4.2-A: Antibacterial analysis of fabric coated by steam pressing technique.**

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Concentration of Ag in solution, ppm</th>
<th>Gram Positive organism</th>
<th>Gram Negative organism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>\textit{Staphylococcus aureus} ATCC NO: 6568 P</td>
<td>\textit{Escherichia coli} ATCC NO: 25922</td>
</tr>
<tr>
<td>1.</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>5</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>10</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>4.</td>
<td>50</td>
<td>57</td>
<td>52</td>
</tr>
</tbody>
</table>

Table 4.2-A shows that the fabric coated with glass solution containing 1 ppm of silver showed zero activity. When the silver
content was increased to 10 ppm, the antibacterial activity increased to around 25%. Upon further increasing the silver content to 50 ppm, there was no significant increase in such activity. Results of the duplications of the antibacterial activity are presented in Table 4.2-B. The antibacterial activity increases with the increase in concentration of silver (Fig. 4.8).

### Table 4.2-B: Duplication of antibacterial analysis of steam-pressed fabric.

<table>
<thead>
<tr>
<th>S</th>
<th>Experiment trial</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Con. of silver</td>
<td>S. aureus E. coli</td>
<td>S. aureus E. coli</td>
<td>S. aureus E. coli</td>
</tr>
<tr>
<td>1</td>
<td>10 ppm</td>
<td>25</td>
<td>27</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 4.8: Antibacterial activity of Steam-press coated fabric with different concentrations of silver.
4.4.3 Antibacterial analysis of spin-washed fabric

Different quantities of CaP-Si-6 glass sample were suspended during the coating silver on the fabric. When the fabric was analyzed for antibacterial activity, nearly 100% CFU/mL reduction was observed with 50 grams of CaP-Si-6 beads. This corresponds to nearly 12 ppm (based on calculations made using solubility data of the sample). Trials or duplications were performed with fabrics coated with glass solution prepared by dissolving 50gms of glass beads.

The antibacterial activity analysis was duplicated with 50 grams of CaP-Si-6, in order to confirm the results and are presented in Table 4.3-B.

Table 4.3-A: Antibacterial analysis of fabric coated by spin-washing technique.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Weight of CaP-Si-6 sample, grams</th>
<th>REDUCTION % (CFU/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram Positive organism - Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATCC NO: 6568 P</td>
</tr>
<tr>
<td>1.</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>2.</td>
<td>30</td>
<td>85</td>
</tr>
<tr>
<td>3.</td>
<td>50</td>
<td>96</td>
</tr>
</tbody>
</table>
Table 4.3-B: Duplication of antibacterial analysis of spin-washed fabric.

<table>
<thead>
<tr>
<th>S</th>
<th>Experiment trial</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td>50 grams</td>
<td>96</td>
<td>99</td>
</tr>
</tbody>
</table>

Weight CaP-Si-6 ↓

Figure 4.9: Antibacterial activity of spin-wash coated fabric by varying quantity of CaP-Si-6.

Graphical representation of the antibacterial activity is presented in Fig.4.9. An increase in quantity of the glass used, increases the
antibacterial activity due to increase in concentration of silver released during the spin-washing process.

### 4.4.4 Comparison of antibacterial activity – different coating techniques

The fabric coated with ~10 ppm of silver was selected for the comparative studies. The results are presented in Table 4.4.

**Table 4.4: Antibacterial analysis of silver-coated fabrics using different coating techniques.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Coating Technique</th>
<th>Concentration of silver, ppm</th>
<th>REDUCTION % (CFU/ ml)</th>
<th>Gram Positive organism - Staphylococcus aureus ATCC NO: 6568 P</th>
<th>Gram Negative organism - Escherichia coli ATCC NO: 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dip-coating</td>
<td>10</td>
<td>82</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Steam-Pressing</td>
<td>10</td>
<td>26</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Spin-washing</td>
<td>12 (corresponding to 50g)</td>
<td>96</td>
<td>99</td>
<td></td>
</tr>
</tbody>
</table>

From the above table, enhanced antibacterial activity was observed with fabric coated by dip-coating and spin-washing techniques. This may be due to the fact that the silver present in the solution and that released from the glass gets coated on the fabric.
The low activity observed in steam-pressed fabric may be due to transfer of less quantity of silver from the solution to the fabric through steam droplets.

In order to compare the efficiency between 2 coating techniques, viz., dip-coating and spin-washing, the respective fabrics were subjected to wash-cycle studies.

4.4.5 Retention of antibacterial activity after washing – cycle studies

The retention of antibacterial activity of silver coated fabric was tested by subjecting it to repeated wash-cycle studies. The fresh silver-coated fabrics from the above studies (dip-coated fabric – 10 ppm; steam-press coated fabric – 10 ppm; spin-wash coated fabric – 12 ppm) were subjected to conventional domestic washing procedure, dried under shade in dust free environment and analyzed for antibacterial activity. The results are reported as Cycle-I. The same fabric from Cycle-I (without coating silver for the second time), were again subjected to conventional washing procedure and analyzed for antibacterial activities and are reported as Cycle-II). The dip-coated fabric showed less CFU reduction in Cycle-I (Fig. 4.11-B), which dropped to nearly zero activity in Cycle-II. This reveals poor adherence of silver over the dip coated-fabric. The steam-pressed fabric showed zero activity in Cycle-I, itself. On the other hand, the spin-wash coated fabric retained its antibacterial activity to an appreciable extent upto Cycle-II.
Figure 4.10-A: Antibacterial activity against *S. aureus* exhibited by silver-coated fabric using spin-washing technique using 50 g CaP-Si-06.
Figure 4.10-B: Antibacterial activity against *E. coli* exhibited by silver-coated fabric using spin-washing technique using 50 g CaP-Si-06.
Figure 4.11: Antibacterial activity studies exhibited by silver-coated fabric in Cycles using (A) Steam-pressing (B) Dip-coating and (C) Spin-washing techniques.

The uptake of silver by the fabric from glass solution containing 10 ppm of silver, using 3 washing techniques was analyzed and the results are presented in Table 4.5.

It is evident from the table that amount of silver deposited on the fabric by dip-coating and spin-washing technique was nearly same. This is reflected in their respective antibacterial activities (Table 4.4). As expected the intake of silver onto the fabric using steam pressing technique was very low.
Table 4.5: Concentration of silver deposited on the fabric using different coating techniques

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Coating technique</th>
<th>Concentration of silver in glass solution, ppm</th>
<th>Uptake of silver by the fabric (silver deposited), ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dip-coating</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>2.</td>
<td>Steam-Pressing</td>
<td>10</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>3.</td>
<td>Spin-washing</td>
<td>12</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Eventhough, the quantity of silver deposited on the dip-coated and spin-washed fabric was nearly the same, the adherence nature of silver in spin-washed fabric was proved to be better through wash-cycle studies.

4.5 CONCLUSIONS

Silver released from Ag-CaP glasses in aqueous solution was coated on the fabric by three conventional techniques, viz., dip-coating, steam-pressing and spin-washing. The antibacterial activities for the silver coated fabric using above techniques were evaluated using ASTM-E-2149 procedure for *S. aureus* and *E. coli* cultures. After a detailed investigation, it was concluded that fabric coated by spin-washing technique showed nearly 100% antibacterial activity with relatively less silver content. Wash cycle studies were performed over the above fabrics coated with silver using the above 3 techniques. The spin-washed fabrics retained high antibacterial activity up to washing cycle-II, while fabrics coated using other methods showed nearly zero activity during cycle-II and even before. Thus, the concentration of silver, composition of glass and coating technique of silver on the fabrics were optimized to get longer and enhanced antibacterial activity.
References


