In-situ Deposition of Silver Nanoparticles of Different Colors on Cotton for Imparting Antibacterial and Antifungal Properties

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ABSTRACT

This paper reports the preparation characterization of silver nanoparticle suspensions and silver nanoparticles attached cotton fibers and a study of their antimicrobial properties. Silver nanoparticle sols with several different colors were prepared and their antimicrobial activities were studied. The sol with the smallest particle size (17 nm), which was dark green in color, has the highest inhibition zone for several clinically important bacteria \textit{[Staphylococcus aureus (ATCC 6538), Methicillin resistant Staphylococcus aureus (MRSA) (ATCC25923) and E. coli]}. Silver nitrate solution exhibited the least inhibition zone. The wavelength at the maximum absorption ($\lambda_{max}$) of the UV-visible spectrum increased with increasing particle size and the diameter of the inhibition zone for bacteria decreased. Silver nanoparticles, when entrapped in cotton, imparts various colors to cotton and killing effects to above mentioned bacteria and fungi (air-born \textit{Aspergillus niger}). Effects of standard washing cycles performed for the silver nanoparticle-modified cotton on the thickness of inhibition zone are also reported. The dark green coloured cotton which contains the smallest silver nano-particles had the widest inhibition zone for bacteria and fungi used in this study when compared with those of the cotton pieces modified by larger silver nanoparticles. Considering the matching colour of the military uniforms and tent cloths and the antimicrobial activities exhibited by the dark green coloured cotton, it is, therefore, possible to use this material in military wear and tents. Described also are the effect of standard washing cycles on the thickness of the inhibition zone of the silver nanoparticle-modified cotton. The dark green colored cotton piece has the widest inhibition zone for the above bacteria and fungi. This material is, therefore, very attractive for use in military wear and tents.

INTRODUCTION

Among the many different nano-materials used to impart antimicrobial activities in textile materials, nanoparticles of silver, titanium dioxide and zinc oxide stand out to be the most common materials. Silver nanoparticles are special as they can impart antimicrobial properties as well as characteristic colors such as brown, cream, yellow, dark green or purple according to the size and shape of the Ag particles deposited on the textile. Use of silver as an antimicrobial agent has a long history. Recently, silver nano-particle incorpor-
rated textile materials have attracted a great deal of potential technological attention in the medical field applications as they can be used in wound dressings and in hospital clothing.

Many researchers have investigated the effects of Ag nanoparticles on microbes. Some of them found that, silver species such as Ag atoms, Ag(I) and Ag(II) ions can interact with thiol groups in proteins to result in the inactivation of respiratory enzymes. They have also shown that Ag species could lead to the production of reactive oxygen species (ROS) for further attacks (Banerjee et al., 2010; Li et al., 2008). Another study revealed that Ag species could prevent DNA replication hence affecting the structure and permeability of the cell membrane (Chen and Schluesener, 2008). Silver species are also photoactive in the presence of UV radiation, leading to enhanced inactivation of bacteria and viruses (Zhao et al., 2007; Divya et al., 2009).

Several methods have been used for the incorporation of silver nanoparticles on textile materials. Ultra-sound irradiation of silver nanoparticles in the colloidal solution was used to deposit silver particles onto the surfaces of nylon, polyester and cotton fabrics. This has resulted in a modified textile materials, which produced uniform coatings of 80 nm sized silver nanoparticles. An excellent antibacterial effect was found quantitatively on E. coli and S. aureus (Perelshtein et al., 2008). Reduction of silver ions, with hydrazine and glucose as reducing agents, has resulted in a modified silk fabric having 10 nm to 35 nm sized silver nanoparticles. The antimicrobial test on gram positive bacterium S. aureus gave a positive result (Gulrajani et al., 2008). Preparation of silver nanoparticles by reducing silver ions with sodium citrate is used to make silver nanoparticles incorporated into silica and polyethylene terephthalate (PET) (Sileikaite et al., 2006). They have obtained 100 nm sized nanoparticles attached onto silica and PET substrates. Antimicrobial activity of silver-based polymer prepared by reducing silver nitrate with sodium citrate has been reported (Monteiroa et al., 2009).

The objectives of the current study are to prepare silver nanoparticle suspensions of different sizes, to investigate their antimicrobial properties, and to attach the silver nanoparticles to cotton for the study of antibacterial and antifungal properties in order to develop a material most suitable for use in military wear and tents.

MATERIALS AND METHODS

Materials Used

Raw white cotton pieces were purchased from Sri Lanka and silver nitrate, sodium hydroxide, Muller Hinton agar, Nutrient agar, Nutrient broth, Brain Heart and infusion broth were purchased from Aldrich Chemical Co. Ltd and citric acid from the British Drug Houses Ltd, England. Clinically important bacteria were obtained from the Microbiology laboratories of the Faculties of Dental Science and Veterinary Medicine and Animal Science of University of Peradeniya, Sri Lanka. The fungal species used was grown in situ.

To prepare silver nanoparticle-attached cotton fabrics, bleached raw cotton sample pieces were inserted separately into three-neck flasks containing 100 ml of 1 mmol dm$^{-3}$ silver nitrate in deionized water. The solutions were heated up to the required temperatures in the range from 75°C to 100°C and stirred for 30 min while adding 10 ml of 0.36 mol dm$^{-3}$ of previously prepared aqueous solution of trisodium citrate using a dropping funnel. The color of the resulting silver nanoparticles depend on the reaction temperature and the reaction time at the desired temperature. A yellowish-brown cotton fabric samples were obtained after 30 min of reaction at 75°C. Others were prepared at different temper-
atures from 70° C to 95° C but keeping the same reaction period. UV-visible spectroscopic (JASCO V-570) investigations were carried out for all the colloidal solutions prepared in order to determine the Surface Plasmon Resonance of silver nanoparticle sols with different particle sizes. The solutions were used to study the antibacterial activity of silver nanoparticles. The characterizations of the resulting modified textile samples were carried out by XRF (Fischer XAN-FD) and SEM (ZEISS Supra TM 35VP, Germany) techniques. Antimicrobial efficiencies of modified textile materials were tested according to the SN 195920 (Agar diffusion) method. Gram positive bacteria used were *Staphylococcus aureus* (ATCC 6538) and Methicillin resistant *Staphylococcus aureus* (ATCC25923). Gram negative *E. coli* were also used to test antibacterial activity in modified textile materials (Rajendran et al., 2011). Antifungal activities were studied for air-born *Aspergillus niger*.

**Antibacterial Activity Assessment**

Antibacterial activities of nanosilver-adsorbed cotton were determined by both qualitative and quantitative methods. For the qualitative analysis, single bacterial colony of *E. coli*, *S. aureus* and MRSA were inoculated separately into brain heart infusion broth. For the quantitative analysis only *E. coli* and *S. aureus* were used. The bacterial solutions were incubated in water bath shaker (OLS 200) for 8 h at 37°C. The antibacterial activity of different sized silver nanoparticles attached fabrics were quantified according to the method of AATCC 100-1999 (Fan, 2005). The unmodified and modified fabrics were cut into 2 x 2 cm pieces. The fabric samples were disinfected by autoclaving for 10 min at 121°C. The experimental flow chart is shown in figure 1 (Li et al., 2006). The unmodified fabric was kept in the sample number 2 container while the seven modified samples in container numbers 3, 4, 5, 6, 7, 8, and 9 respectively. 0.5 ml of bacterial solution was added into each of the 2 to 9 containers. An amount of 9 ml of saline water and 0.5 ml of plane brain heart infusion were added into container 1. A container number 1 here acts as a negative control. Nine milliliters of saline water was added to each of 3 to 9 sample tubes. The sample containers were incubated at 37°C for 24 h. Subsequently, 0.1 ml from each of the samples was taken from all the sample containers and separately spread on to Muller Hinton Agar (MHA) plates. All the plates were incubated at 37°C for 24 h. However, *S. aureus* containing testing samples was incubated for 48 h at the same conditions.

The numbers of viable bacterial colonies were counted and the reductions of bacterial percentage were calculated using the following equation. Percentage Reduction Rates of Bacterial Colonies = (A – B) x 100/A; where A is the number of bacterial colonies in untreated textile containing tube and B is the number of bacterial colonies in tubes containing modified textile materials.

**Antibacterial Activity Determination**

Modified textiles possessing antimicrobial activities and unmodified textiles materials were placed on separate places of MHA thin agar plates in aseptic conditions. Another thin layer of MHA was poured on to the textiles so that textile materials are sandwiched between the MHA layers. For the lower layer, 10 ml of MHA were poured into sterilized Petri plates in aseptic conditions. For the upper layer 10 ml of MHA was poured on to the textiles. Bacterial concentration was determined by measuring the optical density (OD) at 600 nm. The OD value 0.3 corresponds to the bacteria concentration of $1 \times 10^8$ CFU/ml (Kimberly et al., 2006). An amount of 100 µl of the bacterial solution was inoculated on to Muller Hinton Agar plates and evenly...
spread. The inoculated agar plates were incubated at 37°C for 24 h.

The antibacterial properties of silver nanoparticles of different average particle sizes in solution were studied by preparing wells in the Agar plate and pouring the colloidal solutions into these wells. The similar procedure outlined above was carried out to determine the inhibition zones against the three types of bacteria used in this work.

**Antifungal Activities Determination**

Modified and unmodified textile samples were placed on the culture medium (Suborn Dextrose Agar) and a thin SDA gel layer was poured on the textile samples. The parallel streaks were drawn on the top gel layer using the loop touched with airborne *Aspergillus niger*. The samples were incubated at 37˚C for 72 hours and at the end of 72 hours the fungal growth on streaks was visualized on textile samples.

**RESULTS AND DISCUSSION**

Figure 2 shows the optical images of cotton fabrics modified with silver nanoparticles of different dimensions. As can be seen from these images, the cotton pieces become coloured due to the adsorption of silver nanoparticles. The colour of the cotton pieces depends on the colour of the silver nanoparticle sol, which depends on the surface plasmon resonance of silver nanoparticles. The wavelength at maximum absorption ($\lambda_{\text{max}}$) of surface Plasmon resonance of silver nanoparticles depends on the particle size and hence depending on the particle size, silver nanoparticle-modified cotton with different colours (yellow, brown, green and dark green) were obtained.

**Antibacterial Activities of Silver Nanoparticle**

Figure 3 shows the inhibition zones of *S. aureus* with the nanosilver colloids of different particle sizes. Table 1 depicts the sizes of the inhibition zones for the bacteria used in this study together with the respective particle size of the silver nanoparticles and the colour of the silver nanoparticle sols. It is clear from the data in Table 1 that for the smallest particle size, the largest inhibition zone is obtained. This could be explained by considering the possibility of smaller nanoparticles to penetrate through the cell walls to damage DNA and enzymes (Banerjee et al., 2010; Li et al., 2008). Since 17 nm silver nanoparticles in suspension gives the best performances, further studies were carried out for 17 nm particle attached bleached cotton pieces. They were characterized using XRF and SEM studies prior to the qualitative and quantitative analysis of antimicrobial activities.

**Table 1:** Particle size, $\lambda_{\text{max}}$ and the extent of inhibition zones for *S. aureus* of different Ag nanoparticle suspensions together with the inhibition zone of AgNO<sub>3</sub>.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Particles size/nm</th>
<th>$\lambda_{\text{max}}$/nm</th>
<th>Inhibition zone/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>4.2</td>
</tr>
<tr>
<td>Brown</td>
<td>70</td>
<td>429</td>
<td>10.2</td>
</tr>
<tr>
<td>Pale Brown</td>
<td>51</td>
<td>425</td>
<td>12.8</td>
</tr>
<tr>
<td>Pale Green</td>
<td>30</td>
<td>415</td>
<td>14.3</td>
</tr>
<tr>
<td>Dark Green</td>
<td>17</td>
<td>410</td>
<td>18.1</td>
</tr>
</tbody>
</table>

**XRF Characterization**

The XRF studies have shown that the number of counts, which is proportional to the amount of Ag atoms on the surface of the modified cloth, reduces with washing cycles performed according to the standard washing procedure (UNI EN ISO 26330). The percentage reduction of silver atoms is only 15% after 10 washing cycles. This is a very small reduction for 10 washing cycles as 85% of the activity from the unwashed...
Figure 1: A flow chart showing the steps involved in the quantification of viable bacterial colonies.

Figure 2: (i) UV-Visible spectra of different colored nano silver colloids, a: Yellow, b: Brown, c: Green and (ii) The colors of cotton pieces modified with silver nanoparticles with different particle sizes, (a) yellow, (b) dark brown (c) green and (d) dark green.

An unwashed sample can be expected after 10 washing cycles (wide infra Figure 5d). Twenty washing cycles has reduced 34 % from the original counts and the activity is almost diminished after 30 washing cycles (90 % reduction in the counts).
SEM Images of Cotton

The SEM image of figure 4(a) shows the fibers of bare cotton and 4(b) shows cotton fiber containing silver nanoparticles of dark green color. It is interesting to note the presence of several small particles typically less than 20 nm as well as some aggregated particles with a wide distribution of particle sizes up to about 100 nm. These data agree very well with the UV-Visible absorption spectral data of the dark green silver nanoparticle suspension. The surface plasmon resonance spectrum is broad and the $\lambda_{\text{max}}$ showing a hypsochromic shift indicating a majority of small particles with a few but a wide range of aggregated particles are present in the solution. The SEM image gives the similar result for the dark green silver nanoparticle attached cotton.

Figure 3: Antibacterial activity of suspensions a: AgNO$_3$, b: 17 nm Ag nanoparticle suspension, c: 30 nm Ag nanoparticle suspension d: 51 nm Ag nanoparticle suspension and e: 70 nm Ag nanoparticle suspension against Staphylococcus aureus on Muller Hinton Agar plate (MHA).

Antibacterial Activities of Silver Modified Cotton

The antibacterial performance of dark green silver (17 nm) modified cotton against S. aureus is shown in figure 5(a) where 5a(i) demonstrates the absence of antibacterial activity for the blank sample of unmodified cotton and 5a(ii) shows the absence of antibacterial activity for the peroxide-bleached unmodified cotton.

Figure 4: (a) The SEM of bare cotton and (b) The SEM of cotton modified with silver nanoparticles

Figure 5a (iii) shows the presence of antibacterial activity of 17 nm silver nanoparticle-modified cotton on an MHA culture plate. A clear inhibition zone of 18.1 mm indicates the diffusion length of silver particles and their inhibition effects for S. aureus. Figure 5(b) shows the similar effects for E. coli and Figure 5(c) gives the respective data for MRSA. The cotton used to test antibacterial activities for E. coli was washed 10 times using the standard washing procedure and its antibacterial activities are shown in figure 5(d). The ten washings have reduced the antibacterial activity only by 15%. Similar tests were done for cotton textiles modified with other sizes of nanosilver particles. The antibacterial effect is largest for the smallest particle size used.
Antibacterial Activity - Viable Cell Counting Method

Table 2 gives the viable cell counts for two different bacteria (S. aureus and E. coli) as a result of 24 h exposure of the bacterial solutions to the dark green colored 17 nm silver nanoparticle-attached bleached cotton samples with reference to the control of unmodified bleached cotton. The percentage reduction of the viable cell counts exceed 90% in both cases demonstrating the excellent killing effect of dark green silver nanoparticle-attached bleached cotton in a period of 24 hours. Treatment with more than 24 h gives even higher reduction of the viable cell counts. The percentage reduction of viable cell counts within 24 h is between 90-95 for E. coli and it is between 96 to 98 for S. aureus. This is a clear indication of the permeability of silver nanoparticles through the cell walls and cell membranes of different bacteria. The viable cell reduction rate is higher for gram positive bacteria than that for gram negative bacteria.

Antifungal Activities of Nanosilver Modified Cotton

The antifungal activities of cotton modified with nanosilver particles of 17 nm were carried out using parallel streak method. The fungal growth was inhibited on the modified textile but fungi were well grown on the unmodified cotton control sample. Figure 6 shows the antifungal activities of nanosilver on Aspergillus niger used in this work. The antifungal activities of dark green colored nanosilver-modified cotton are of unique importance for tents, socks and clothing of military forces.

CONCLUSIONS

The citrate reduction of silver nitrate aqueous solution at different temperatures in the range from 70°C to 90°C results in silver nanoparticle sols with different particle sizes and hence different colours (yellow, brown, green, dark green). The UV-visible spectra of the sols clearly show the surface plasmon resonance and the $\lambda_{\text{max}}$
Table 2: The viable cell reduction percentage due to 17 nm silver nanoparticle-coated bleached cotton after 24 hours of incubation in each of the two bacterial solutions.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No of colony forming units in the suspension containing unmodified textiles</th>
<th>No of colony forming units in the suspension containing 17 nm Ag particle-modified textiles</th>
<th>Viable cell reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>87</td>
<td>8</td>
<td>90.8</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>5</td>
<td>94.5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>94</td>
<td>3</td>
<td>96.8</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>2</td>
<td>97.6</td>
</tr>
</tbody>
</table>

depends directly on the average particle size, lower the average particle size the lower is the $\lambda_{\text{max}}$. The width of the inhibition zones for bacteria used in this study depends inversely on the particle size of the silver nanoparticle sols. Silver nanoparticles can be adsorbed on to cotton fabrics when they are placed in the preparative solution and the same trend of inhibition zones can be obtained. Only 15% reduction in silver nanoparticles on the surface of cotton fabrics was obtained when they are subjected to 10 washing cycles. The dark green coloured cotton fabric containing 17 nm silver nanoparticles may find applications as military wear and tents.

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REFERENCES


