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Preparation of NanoSilver coated Cotton Fabric and its bactericidal evaluation

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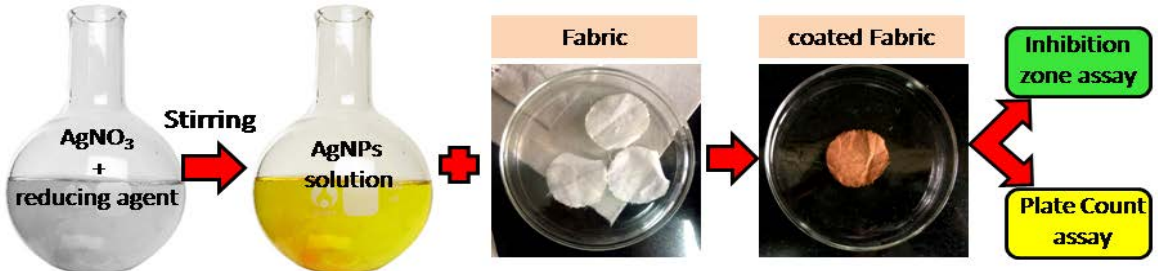
ABSTRACT



We have developed silver nanoparticle coated cotton fabric and evaluated its anti-bacterial efficacy. Silver nanoparticles were synthesized by chemical reduction of silver nitrate using hydrazine hydrate as reducing agent and polyvinyl alcohol as stabilizing agent. It is coated over cotton cloth by ultrasonication method. The anti-microbial efficacy has been studied by inhibition zone assay and agar plate count assay against gram negative bacteria. The longevity of anti-bacterial property of clothes has also been determined by checking its bactericidal effect after washing the cloth several times by Ezee brand liquid soap.

Keywords: Nano silver, Chemical reduction, Coating, Cotton fabric, Bactericidal

INTRODUCTION

Anti bacterial fabric is prepared by incorporating anti bacterial agent either at the surface or within the fibres.[1] Natural fabric like cotton and wool etc have large surface area and these are hygroscopic therefore they provide optimum environment for bacterial growth and may pose health risks. The growth of microorganisms on the fabric causes decrease in mechanical strength, development of bad odour, production of stain etc.[2] It is very important to keep a check on harmful bacteria (microbes) as they lead to adverse health effects. Microorganisms adhere on the surface followed by growth under suitable environmental conditions to form so called biofilms, which are notoriously difficult to remove.[3] A trend in hygiene textile has evolved with busy lifestyle of consumers and therefore seeking low maintenance for their textile products.[4] Antimicrobial finishes on fabrics are developed to protect consumers against disease or odour causing microorganisms as the human skin and fabrics are in direct contact all the day.[5] As the human skin is a shelter for microorganisms like bacteria, (*Staphylococcus aureus, S. typhi* and *Escherichia coli)*, viruses and fungi which are considered major disease causing micro organisms. Several reports are available in literature which describes the manufacturing of anti bacterial textile fabric by loading/coating various anti bacterial agents like quaternary ammonium compounds, chitosan, metal nano particle and plant extract etc.[6-7] Among these nano silver (AgNp) have gained much interest and is widely used as an anti bacterial agent for preparation of anti bacterial fabric.[8-11]

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The current paper describes the formation of nano silver by chemical reduction of silver nitrate using hydrazine hydrate. Formation of nano silver has been confirmed by Surface Plasmon Spectroscopy (SPS). Nano silver was coated on cotton cloth using 10 and 50 ppm nano silver solution by ultra-sonication. Antibacterial activity of the cotton cloth has been checked by inhibition zone assay and agar plate count assay.

EXPERIMENTAL DETAIL

Synthesis of AgNp using hydrazine hydrate and PVA

Hydrazine hydrate ($N_2H_4.H_2O$) is the simplest diamine molecule that can essentially reduce the metal salts. For preparation of 50 ppm nano silver solution, 50 mL of silver nitrate ($AgNO_3$) (10 mM) solution was mixed with 4 mL of PVA (polyvinyl alcohol) (10 % w/w) under magnetic stirring. To this, freshly prepared solution of hydrazine hydrate (0.1 M) was added drop by drop under vigorous stirring. The addition was done until solution acquire yellow colour which indicates the formation of silver nano particles.[12] Once the solution acquired yellow colour it was allowed to stand for 30 min under continuous stirring. Completion of reaction was checked by adding saturated solution of sodium chloride to the reaction mixture. Similarly 10 ppm nano silver solution was also prepared. The 10 and 50 ppm nano silver solution coated fabric was designated as Ag-10 and Ag-50 respectively. The concentration of silver nitrate has also been confirmed by argentometric titrations using Vohlard's method.[13]

Coating of nano silver onto cotton fabric

Cotton cloth was cut into small circular disc shape and washed thoroughly several times with distilled water, and air dried in the laboratory. Cotton cloth was dipped into the 1M KOH solution for 30 seconds and was dried. In a beaker 20 mL nanosilver solution was taken and dried cotton cloth was dipped into the solution and ultrasonicated for 30 minutes at 60°C.[14-15] After 30 minutes cotton cloth was taken out from the beaker and was dried between the folds of filter papers, and further drying was done in hot air oven maintained at 60°C. After drying, cloth was stored in airtight container and its antibacterial activity was measured.

Anti bacterial Activity

The antibacterial activity of silver nanoparticles coated cotton fabrics was tested against *E.coli* by disc diffusion assay and plate count agar method.[16] Disc diffusion assay is the modification of Kirby-Bauer disc diffusion assay which is a standard method used to check anti bacterial activity.[17] 100 µL of three hour old culture of *E. coli* was spread uniformly over the agar plate using sterile 'L' rod to get uniform distribution of bacteria. The control cotton fabric, Ag-10 and Ag-50 fabric were placed on the agar surface. Positive control was also maintained by keeping the antibiotic disc coated with Piperacilin on the agar plate to ensure that the tested organisms were susceptible to a common antibiotic and were not resistant strains.

The plates were incubated for 24 hours at 37°C. The results were recorded by measuring the diameter of inhibition zone. Plate count agar method test includes both ASTM E 2149-01 (American Society for Testing and Materials) and AATCC Test Method 100-1999 (American Association of Textile Chemists and Colourists). This test is used to get a quantitative value on the antimicrobial activity. One day old *E.coli* culture (100 µL) was inoculated in to 10 mL LB broth at incubated at 37°C for 3 hours to achieve 1.5–3.0 x 10^5 CFU/mL (colony forming unit). Three test tubes having 10 mL LB broth was inoculated with 100 µL of 3 hours old culture. Control fabric, Ag-10 and Ag-50 fabric was added in these three different test tubes and kept in shaker cum incubator at 37°C at 190 rpm for 1 hour. 100 µL of the sample was taken from each tube and serially diluted with 0.9% saline and 1 mL of it was plated on LB agar petriplates. The inoculated plates were incubated at 37°C for 18-24 hours at inverted position. After that the number of colonies were counted. The absorption at 610 nm was also taken for all the control and test groups after 24 hours of incubation. The anti bacterial activity is expressed as percentage inhibition in the growth of microorganisms in the experimental group compared to the control group. It is calculated using the following formula,

Percentage reduction (CFU/mL) = B-A/B x 100

A – number of cells or OD at 610 nm in the experimental group

B – number of cells or OD at 610 nm in the control group

RESULTS AND DISCUSSION

Many chemical reduction methods have been used to synthesize silver nanoparticles from silver salts.[18-19] In fact, the preparation of nano size metallic silver particles via chemical reduction of silver salts involves the reduction of ionic metal salt in a suitable medium in the presence of stabilizing and reducing agents.[20] For the synthesis of nano silver we have used hydrazine hydrate as reducing agent and PVA as stabilizing agent. During the addition of the reducing agent to the aqueous $AgNO_3$ solution, light yellow colour slowly appeared in the mixture, indicating the formation of AgNPs.[21] The UV-Vis spectrum is a good method to confirm the formation and growth of AgNPs. The maximum absorption peak observed at 420 nm confirms the formation of AgNPs which is shown in figure 1 (a).[22] To study the effect of time on the concentration of nanosilver, samples were carefully withdrawn at different time intervals and absorption was measured. Figure 1 c depicts the enhancement in the intensity of colour of the reaction mixture with time. It was observed that only one sharp symmetric absorption peak at 424 nm was observed which steadily increases in intensity as a function of time without any shift in the peak wavelength.[23] These results indicate that concentration of nano silver particles increases with time. The completion of reaction was checked by adding saturated solution of NaCl to the reaction mixture. Absence of any turbidity or white precipitate of silver chloride indicates the completion of reduction of silver nitrate into nano silver. The golden-yellow solution of nano silver was found to be stable over a long period of time there by indicating that the nano particles have no tendency to agglomerate.[24] The stability of the synthesized Ag nanoparticles were studied by measuring the absorption over a period of 4 months. Figure 1(b) shows the UV spectra of nano silver solution after 120 days from its synthesis. No significant change in the absorbance was observed, which shows that aggregation of nano particles has not occurred which may be because of effective

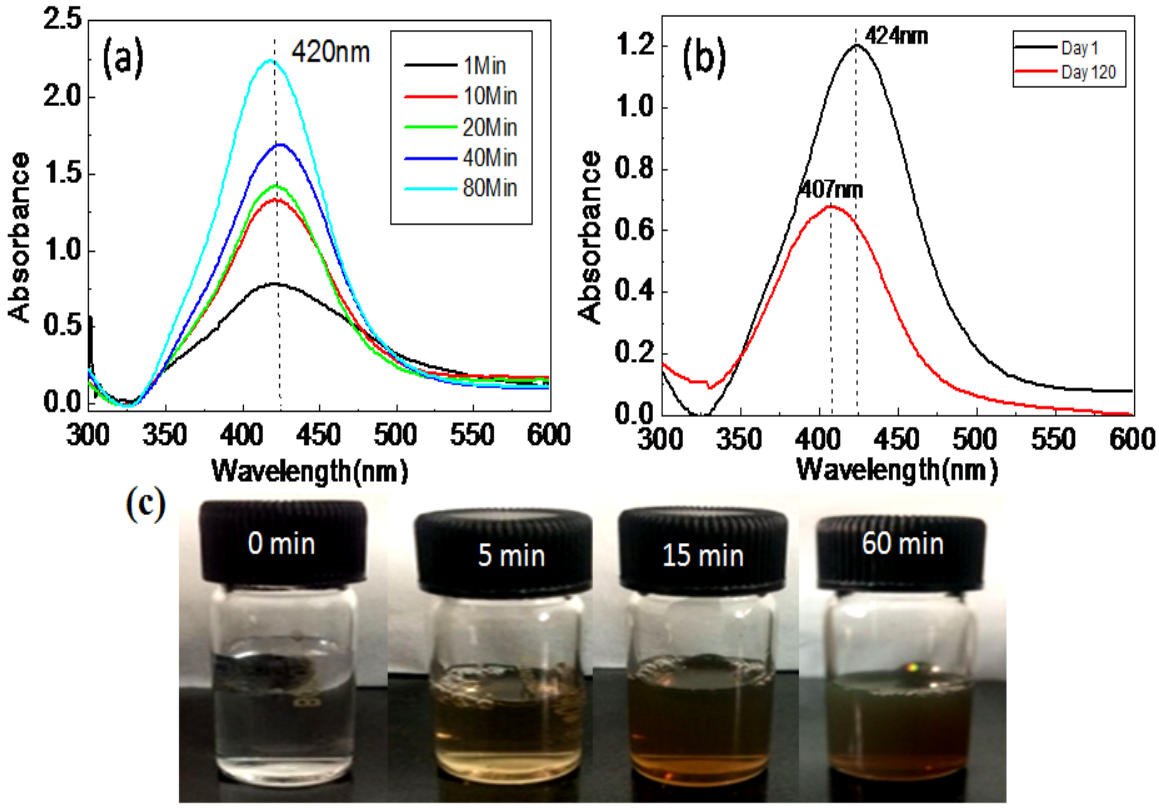

Figure 1 (a) UV-visible absorption of nano silver solution at various time intervals (b) UV-visible absorption of nano silver to demonstrate stability of AgNp (c) Digital photographs of nano silver solution drawn at different time interval

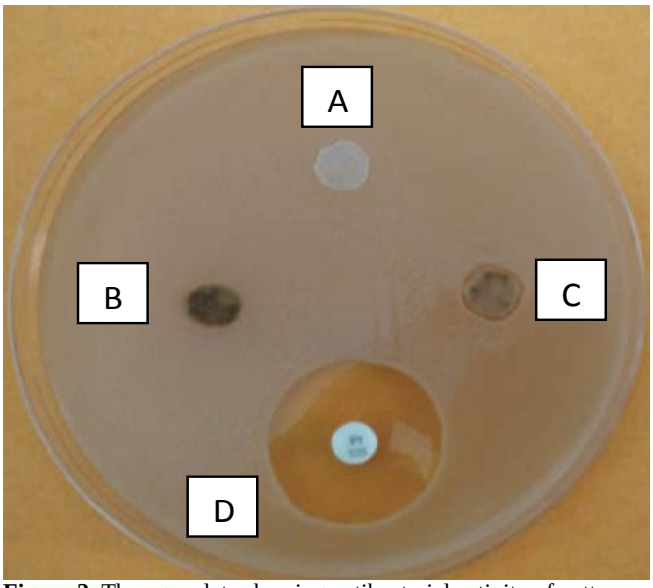

Figure 2. The agar plate showing antibacterial activity of cotton fabrics Where A= control, B= Ag-50, C = Ag-10 and D = antibiotic disc

capping by PVA which may restricts the mobility of silver ions during the reaction and avoid its agglomeration.[25-26] The nano silver was coated on the fabric using ultra sonication.[27] Before coating, activation of cotton surface was performed by alkali treatment. By alkali treatment (KOH) hydroxyl group present on cellulose get converted into O^-K^+ and get exchanged with Ag^+.[28]

The antibacterial activity of the cotton fabrics coated with AgNps was tested against *E. coli*. By placing the coated fabrics on the agar, AgNp diffuses in the agar and inhibit bacterial growth which is measured as inhibition zone formed around the fabrics.[29] It was very evident that the fabric coated with Ag-50 had a clear zone of 1 mm (Figure 2). Very less inhibitory effect was noted for Ag-10 and no inhibitory effect was observed for control group.[30-31] The results indicates that Ag-50 fabric is better anti bacterial than Ag-10 fabric. The clear zone formed around the antibiotics disc is mainly due to the presence of high concentration of antibiotic present in the disc. In the quantitative assay, the bacterial growth was inhibited by the fabric coated with Ag-50 as observed in the petriplates where the number of bacterial colonies was minimal which is shown in figure 3. The quantitative assay has been performed with Ag-10 and Ag-50 after 5 wash cycles. The coated fabric was washed with ezee liquid and rinsed with water followed by drying. The washing steps were repeated five times and after five wash antibacterial activiy was again determined. There is slight decrease in antibacterial activity after washing for both the fabrics. Figure 3 demonstrates the reduction in growth of bacteria before and after wash. The percentage reduction of growth of bacteria was calculated by measuring absorption at 610 nm. The results are summarized in table 1. Figure 4 depicts the digital photographs of the samples from which absorption was measured. The highest percentage of inhibition (94.6%) was observed for Ag-50. The anti bacterial activity of Ag-50 fabric was retained even after 5 wash.

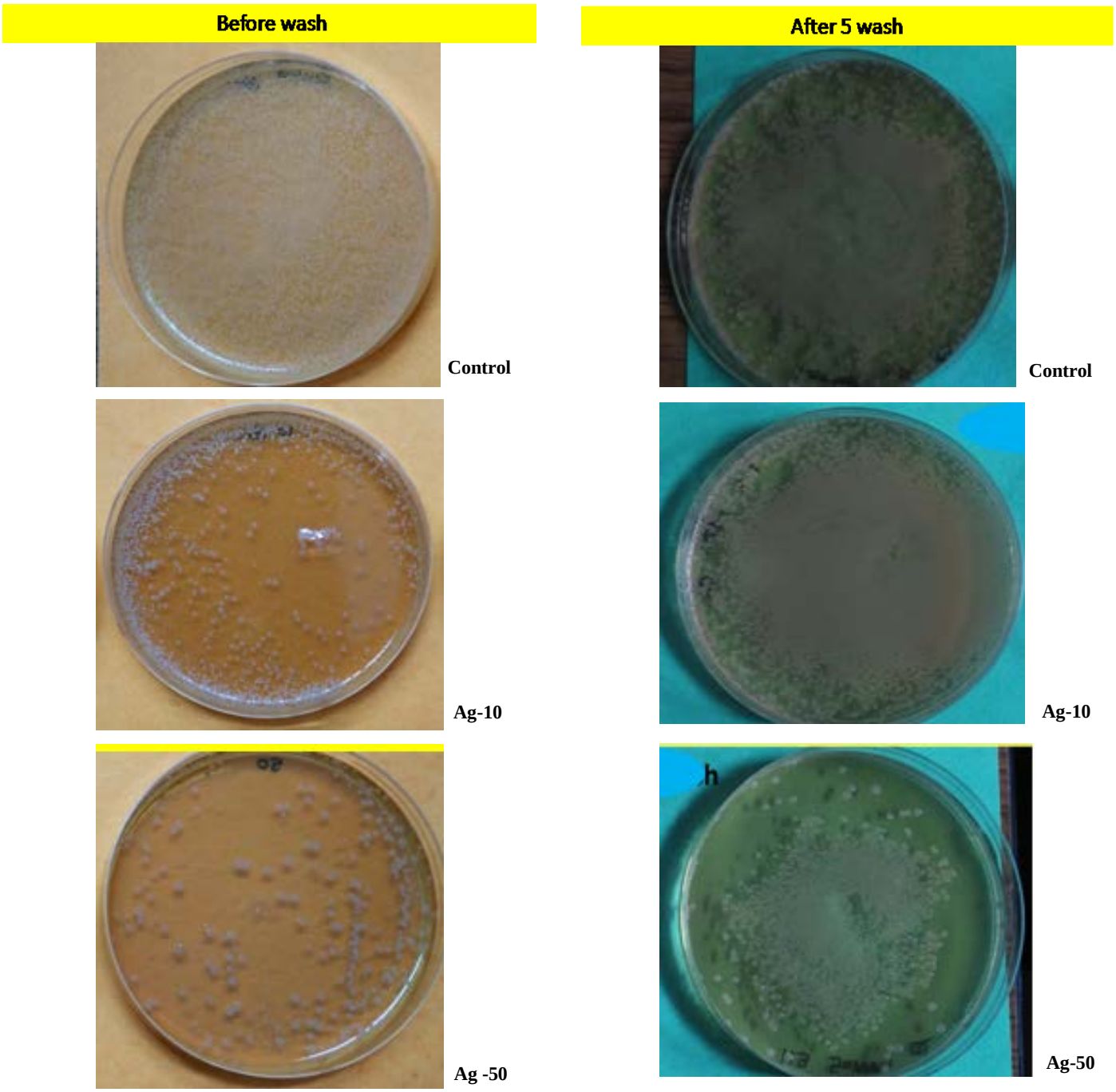

Figure 3: Petriplates showing reduction in the growth of bacteria before wash and after 5 wash

The fabric coated with Ag-10 shows less bactericidal activity with the percentage inhibition of 10.9% before wash and 6.7% after 5 wash. The observed inhibition of bacterial growth may be due to inhibition of cell wall synthesis and protein synthesis in bacteria by AgNp.[32-33] So based on these results, we can conclude that the fabric coated with Ag-50 showed an excellent antibacterial activity against *E.coli.* The long-lasting bactericidal activity was observed in this fabric even after 5 wash. The results observed by zone inhibition method (qualitative method) are consistent with the results obtained by plate count method (quantitative method). Therefore, it is a safe and eco-friendly method to impart anti bacterial activity to cotton fabric.

Figure 4: Test tubes showing reduction in the growth of bacteria before wash and after 5 wash

Table 1.Reduction in growth of bacteria (based on OD at 610nm)

Sample	OD at 610 nm		% age inhibition	
	Before Wash	After 5 wash	Before wash	After 5 Wash
Control	0.55	0.60	--	--
Ag-10	0.49	0.56	10.9	6.70
Ag-50	0.03	0.04	94.56	93.3

CONCLUSIONS

Nano silver was synthesized by silver nitrate using hydrazine hydrate as reducing agent and effect of time on completion of reaction was demonstrated using Surface Plasmon spectroscopy. The nano silver was coated on cotton fabric using ultra sonication and its anti bacterial activity was investigated. The coated cotton fabric showed excellent killing effect even after washing the fabric several times. The coated fabrics can have potential applications in wound dressing, sport wear and as medicinal bandages. In nutshell, a simple and cost effective method for the preparation of nano silver coated antibacterial cotton fabric was described.

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