Indian Journal of Fibre & Textile Research
Vol. 45, June 2020, pp. 183-189

# Novel method for biosynthesis of silver nanoparticles using melanin and its application on wool to impart antimicrobial activity

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*Received 6 June 2018; revised received and accepted 28 May 2019*

A novel method for biosynthesis of silver nanoparticles using a polyphenolic pigment (melanin) has been proposed to impart antimicrobial properties to wool fabric. A diphenolic catechol has been combined with potato juice to produce melanin, which is used as a simultaneous reducing and dispersing agent for the synthesis of silver nanoparticles. The process variables used for the synthesis of melanin have been optimized to produce melanin with maximum reduction potential. The synthesized silver nanoparticles are then characterized and quantified. The optimum conditions used for the synthesis of melanin are found to be 0.1% catechol, 25% potato juice, 6.5 *p*H, 95˚C temperature and 45 min treatment time. Spherical shaped silver nanoparticles with average sizes of 35 nm and 50 nm are obtained and then applied on wool fabrics by exhaustion method to observe antimicrobial effect. It is observed that the treated wool has excellent antimicrobial activity against both the *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria.

**Keywords**: Antimicrobial activity, Melanin, Potato juice, Silver nanoparticles, Wool

## 1 Introduction

Wool fibre is a superior natural textile material due to its resilience and comfort characteristics. However, wool fibres provide an excellent environment for the growth of microorganisms because of their ability to retain moisture and richness of protein source[1]. Retention of microbes leads to generation of unpleasant odor, stains and discoloration in the fabric and/or reduction of mechanical strength, thus making it imperative to protect wool against microbial growth. To overcome such problems, many antimicrobial agents have been employed to impart antimicrobial property to wool fabrics. Among these agents, silver has been widely used due to its broad spectrum of antimicrobial property and low toxicity to mammalian cells[2]. At nanoscale, silver shows good antibacterial property because of its large surface area to volume ratio that can provide a better contact condition with bacterial cell membranes[3]. Recently, the major research interests in textile industry have been focused on the synthesis & application of silver nanoparticles to different textile fibres for providing antimicrobial effects. There are several research works in which wool fabrics have been loaded with silver nanoparticles to provide antimicrobial properties[4].

Melanin is a ubiquitous pigment widely distributed in nature. Melanin is the main pigment responsible for the various pigmentations found in animal and human skin, hair, and eyes. Melanins have been studied extensively for their various biological features, including photosensitization, metal ions chelation, anti-oxidizing and free radical scavenging behaviour, electrical conductivity, photo-protection, and redox properties[5]. There is also an interest in producing melanin and using it as a reducing and stabilizing agent for synthesis of nanoparticles[6, 7]. It is worthwhile to note that in the biological methods of synthesis of silver nanoparticles using melanin, melanin was produced by the microbial route only. The processes using plant extracts are simpler and readily scalable when compared with relatively expensive methods based on microbial processes used for the synthesis of nanoparticles[8].

Potato (*Solanum tuberosom*), an extremely important plant crop, is rich in several vitamins, minerals, fats, sugars proteins, phenolic compounds and polyphenol oxidase (PPO) enzymes[1-9]. Potatoes when cut and exposed to air becomes brown in color, due to the PPO enzyme catalysed browning reactions. These enzymes catalyse two different reactions in the presence of molecular oxygen, namely the hydroxylation of monophenols (monophenolase activity) and the oxidation of *o*-diphenols to

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*o*-quinones. This reaction is followed by non-enzymatic polymerisation of quinones giving rise to melanins pigments of high molecular mass and dark colour[10].

Potato starch processing industry produces a large amount of potato juice as waste effluent[11]. For producing a ton of potato starch, approximately 3.5 tons of potato juice is produced as a byproduct[12]. In our previous studies, the fresh potato juice was combined with diphenolic catechol (a known substrate for PPO) to develop series of brown shades on wool fabric[13,14]. In the current study, freshly extracted juice of potato tuber is combined with catechol to produce melanin. The process parameters used for synthesis of melanin, on its ability to mediate the synthesis of silver nanoparticles, were studied. The synthesised silver nanoparticles were characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM), dynamic light scattering (DLS) and inductively coupled plasma mass spectrometry (ICP-MS). The silver nanoparticles were applied to merino wool fabric under variable process conditions to determine the *p*H and temperature required for maximum exhaustion. The treated wool fabrics were characterized by scanning electron microscopy (SEM). The antimicrobial activity of treated wool against both the Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) was also assessed. Till date, to the best of our knowledge, the synthesis of melanin from plant sources for mediating the synthesis of silver nanoparticles remains unexplored and its application on wool fabrics to impart antimicrobial activity is not reported.

## 2 Materials and Methods

### 2.1 Materials

Fresh potato tubers (*Solanum tuberosom)* of LSG packed variety were procured from the local market. Potatoes were washed, padded dry, grated and squeezed to extract the juice. One hundred fifty grams of potatoes yield about 100 mL of potato juice (PJ). The juice was allowed to stand for 20 min at 37°C (room temperature) in a beaker to allow the starch to settle down. The clear juice at the top was decanted and then used within half an hour of preparation to prevent any enzymatic oxidation. Before use, the juice sample was characterized in terms of enzyme activity and total phenolic content. Enzyme activity of polyphenol oxidases enzyme (PPO) was estimated spectrophotometrically as recommended by Sigma Aldrich[15]. Catechol (1,2 dihydroxy benzene) was procured from Sigma Aldrich Pvt Ltd. Deionized water was used for all experiments. Merino wool fabric (matt weave, 30 µm fibre diameter, 86.3 g/m$^2$ areal density, 23.5/18.02 tex warp/weft count and 22/22 ends/picks per cm) was used. Ready for dyeing (RFD) fabric was obtained from Shingora Textiles, Ludhiana, Punjab and used without further preparation.

### 2.2 Synthesis of Reductant

Freshly extracted juice of potatoes was centrifuged twice with 8000 rpm at 25˚C for 20 min. The juice was decanted to ensure complete removal of starch, as any residual starch could also have a reducing effect. The supernatant was filtered using Whatman filter paper 2. Filtered potato juice (25%) was combined with catechol (0.1%) and incubated in a shaking water bath at 95°C for 60 min. Further, the parameters were varied, one at a time, keeping the other parameter constant (Table 1). The *p*H of bath was maintained with acetic acid and sodium hydroxide.

### 2.3 Synthesis of Silver Nanoparticles

The optimum combination of parameters that would yield melanin with maximum reducing power was identified by testing the efficiency of each solution to mediate the reduction of $Ag^+$ to $Ag^0$. Each of the melanin solutions synthesized was added at the rate of 20 mL (5 mg/mL) to 5mL of silver nitrate solution (2mM) in a beaker and incubated in a shaking water bath (150 rpm, 100˚C, 5 min).

### 2.4 Characterization of Silver Nanoparticles

#### *2.4.1 UV–visible Spectroscopy*

The formation of silver nanoparticles was monitored using UV-Vis spectrophotometer (Model D-2750, Shimadzu, Singapore). Solutions were diluted and UV -vis spectra scan was recorded from 300 nm to 700 nm.

#### *2.4.2 Transmission Electron Microscopy*

The particle size and morphology of the synthesised silver nanoparticles were examined using (FEI Tecnai $TF_{20}$) transmission electron microscope

Table 1 — Process parameters varied for synthesis of melanin and for silver nanoparticle exhaustion on wool fabric

| Parameter | Values | |
|---|---|---|
| | Synthesis | Exhaustion |
| *p*H | 3, 4.5, 6.5, 8, 9, 10 | 3, 4, 6.3, 8, 10 |
| Temperature, ˚C | 60, 80, 95 | 40, 50, 60, 70, 80 |

(TEM), equipped with FEG source operating at an accelerated voltage of 200 kV. Samples were prepared by dropping 20µL of silver nanoparticles on a grid of copper mesh and drying it at 37°C (room temperature).

#### *2.4.3 ICP-MS Study*

The concentration of silver nanoparticles was determined by means of an Inductively Coupled Plasma (ICP) (Agilent Technologies, 7900) source equipped with a mass spectrometer (MS).

### 2.5 Application of Silver Nanoparticles on Wool

Silver nanoparticles produced in the study were applied as such on to wool fabric by exhaust method. Effect of bath parameters, namely *p*H and temperature on adsorption of nanoparticles by wool was studied as per plan given in Table 1. Samples were immersed in beakers containing silver nanoparticles (MLR 1:50) and kept in a shaking water bath at 150 rpm. The *p*H of the bath was adjusted with the help of acetic acid and sodium hydroxide. Treated fabrics were dried at 50˚C without rinsing and finally tested for antimicrobial activity.

### 2.6 Characterisation of Wool Fabric Treated with Silver Nanoparticles

#### *2.6.1 Colour*

Attachment of silver brings about a change in the colour of fabric, and hence colour value (*K/S*) can be used as a measure of concentration of silver nanoparticles adsorbed by the fabric. The *K/S* value of treated wool fabrics was recorded using Gretag Macbeth Colour-Eye 7000A spectrophotometer with $D_{65}$ illuminant and 10˚ observer.

#### *2.6.2 Morphology*

Surface morphology of wool fibre treated with silver nanoparticles was studied using scanning electron microscope (ZEISS EVO50SEM) at a resolution of ~3 nm using an SE detector, 20 kV power. Samples were given a gold coating of 100Å before testing.

#### *2.6.3 Antimicrobial Activity*

The antimicrobial activity of samples treated with silver nano particles was evaluated by modified colony counting method (AATCC 100) against both *S.aureus* and *E.coli* bacteria.

## 3 Results and Discussion

In this study, the melanin pigment has been synthesised by oxidation of catechol with potato juice, and used as a reductant for *in vitro* synthesis of silver nanoparticles. Enzyme activity and total phenolic content of potatoes as gallic acid equivalent (GAE) from the calibration curve of gallic acid is found to be 47147.88 units/mg and 85.34 mg/g GAE[16] respectively.

### 3.1 Parameters Affecting Reducing Ability of Melanin

A series of reductant (melanin) solutions is prepared by varying the *p*H and temperature. Prepared solutions are used to reduce the silver nitrate solution. The reducing ability of various melanin solutions is assessed by the appearance of the characteristic surface plasmon resonance (SPR) bands.

#### *3.1.1 Effect of pH of Treatment Bath*

The original colour of the reductant solution is dark brown. When silver nitrate is added and the bath heated to 95˚C for 5 min, the solutions change their visual appearance and each bath acquires a distinct colour [Fig. 1(a)]. The bath turns light brown in colour at *p*H 3, 4.5, 10 and 12, yellow at *p*H 8 and green at *p*H 6.5. However, formation of a silver

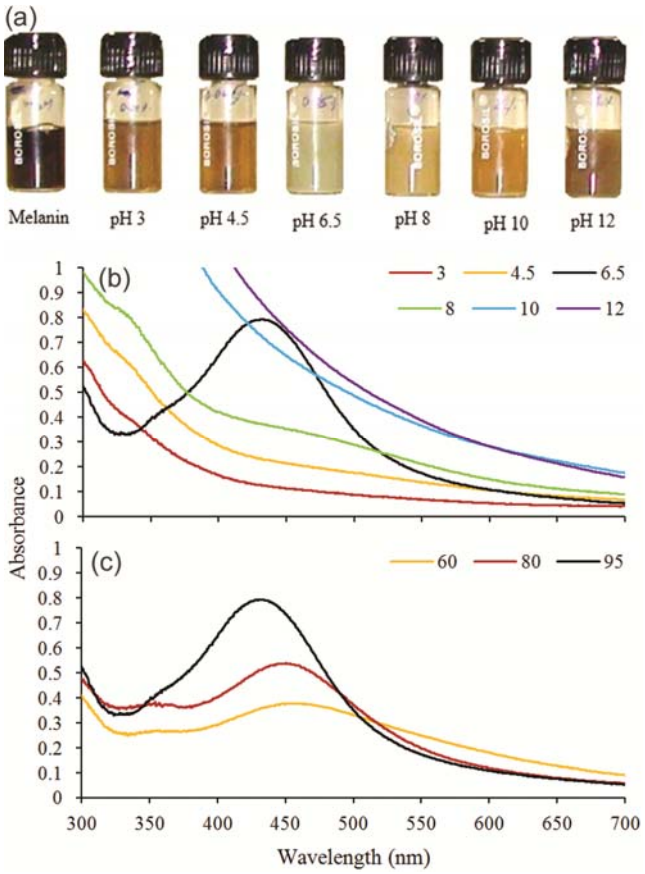


Fig. 1 — (a) Silver nitrate solutions reduced with melanin synthesized at different *p*H, (b) UV vis spectra of silver nitrate solutions reduced with melanin synthesized at varying *p*H (0.1% catechol, 25% PJ, 95˚C), and (c) effect of temperature on formation of silver nano particles (0.1% catechol, 25% PJ, *p*H 6.5)

hydrosol colloid could only be observed in the bath at *p*H 6.5.

The presence of nanoparticles in the bath is further confirmed by UV- visible spectra. The sample at *p*H 6.5 shows a sharp peak at 430 nm corresponding to the surface plasmon excitation vibrations of silver nanoparticles [Fig. 1(b)]. Samples treated at all other *p*H show only a diffused spectrum in the visible region, indicating the absence of silver nanoparticles. It can be concluded that *p*H of the reaction bath plays a key role in the synthesis of melanin, which, in turn, affects the reduction of silver into nanoparticles. This could be because *p*H of the bath affects the degree of polymerization of the synthesized melanin[16,17]. The polyelectrolyte nature of melanins is dependent on the ionization state of groups such as carboxylic, phenolic and amine, and also on the ionic strength of the environment. In acidic conditions, melanin has a tendency to aggregate, while a net negative charge at higher *p*H leads to its dissociation into smaller oligomers. At *p*H 6.5 which is near to its isoelectric point, optimum polymerization of melanin occurs. The melanin formed at this *p*H effectively reduces silver nitrate into silver nanoparticles. The surface plasmon resonance (SPR) band observed at 430nm at 6.5 *p*H indicates the presence of silver nanoparticles of the size 10nm. For all subsequent experiments, *p*H of the bath is maintained at 6.5.

#### *3.1.2 Effect of Temperature*

The SPR bands recorded at various temperatures is shown in Fig. 1(c). The intensity as well as sharpness of peak increase with increase in temperature. At $60^0$C, the peak is observed at 450 nm with absorption intensity of 0.375, which increases to 0.538 at $80^0$C. When the temperature is increased to $95^0$C, the peak shift towards lower wavelength (430nm) with a sharp increase in intensity to 0.791. Maximum peak intensity as well as peak sharpness is observed in reactions carried out at 95˚C. The reason for the maximum peak intensity and sharpness at 95°C is attributed to the higher melanin formation that has facilitated the synthesis of silver nanoparticles. The experiments show that the reducing power of melanin depends on the conditions that yield melanin with maximum synthesis of silver nanoparticles, such as 0.1% catechol, 25% potato juice, 6.5 *p*H, 95˚C temperature and 45 min treatment time.

### 3.2 Reduction of Silver Nitrate using Melanin

The reduction mechanism of silver metal ions ($Ag^+$) for formation of metallic silver ($Ag^0$) using melanin is proposed in Fig. 2. Melanin acts as an electron exchanger, either oxidizing or reducing the silver nitrate. The phenolic (-OH) groups, present in catechol after reaction with potato juice containing PPO enzymes, convert into quinone groups in melanin. These quinone groups generate reducing equivalents ($-2e^+/-2H^+$) that are used for the transformation of silver metal ions into silver nanoparticles[6].

### 3.3 Characterisation of Silver Nanoparticles

From the TEM images (Fig. 3), the diameter of nanoparticles is found to lie in the narrow range of 45 nm $\pm$ 5nm. No aggregation is observed and the nanoparticles exhibit an isotropic nature in terms of being nearly spherical in shape and uniform in size. Results indicate that melanin reduces the silver nitrate solution effectively, while acting both as a reducing as well as a dispersing agent. The yield of silver is high, the size of nanoparticles is small and uniform, and there is no aggregation.

### 3.4 Application of Silver Nanoparticles on Wool

Silver nanoparticles synthesized in the study are applied on to wool fabric using exhaust method. Effect of bath parameters such as bath *p*H and temperature on adsorption of nanoparticles by wool fabric has been studied. To establish the significance due to bath *p*H during the application of silver nanoparticles, statistical analysis is carried out using the unpaired Student's *t*-test. A value of $p<0.05$ is considered to be statistically significant. Bath exhaustion is studied spectrophotometrically by recording the *K/S* (λ-360 nm) of wool samples which have been coloured due to the SPR of silver nanoparticles. The colour of samples ranges from light green to brown.

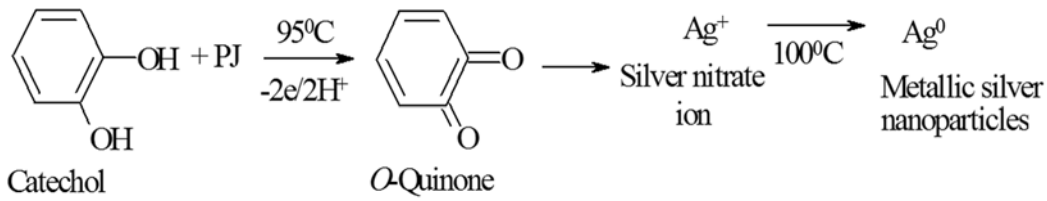


Fig. 2 — Schematic diagram showing synthesis of silver nanoparticles by melanin

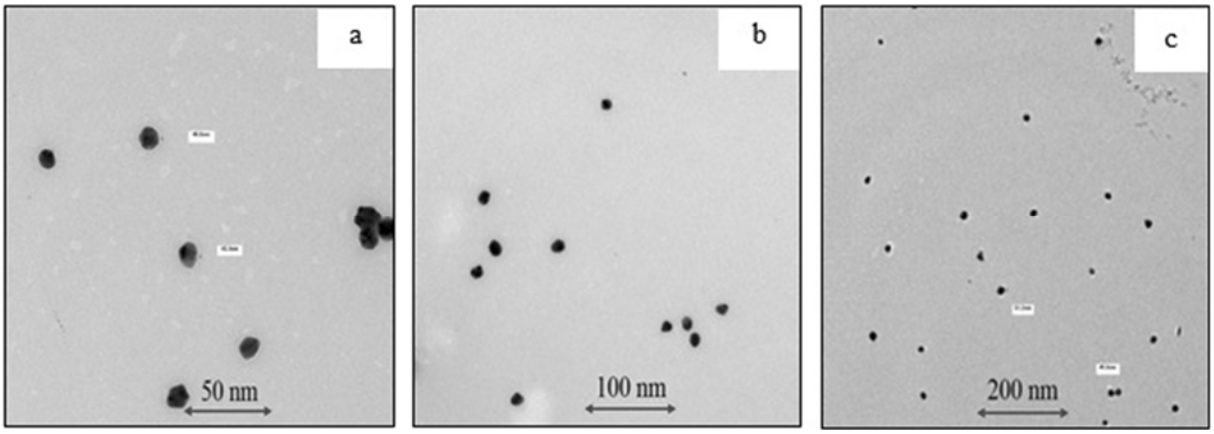


Fig. 3 — TEM images of silver nanoparticles recorded at different magnifications (a) × 50,000, (b) × 2,50,000 and (c) ×520,000

#### *3.4.1 Effect of Bath pH*

*K/S* of wool samples treated with silver nanoparticles at varying *p*H is shown in Table 2. With increase in *p*H of bath from acidic (*p*H 3) to alkaline (*p*H 10), a progressive decrease in *K/S* value is observed, as it reduces from 3.28 to 1.36. Most amino groups in wool are protonated at *p*H 3, giving it a net positive charge. This leads to high absorption and binding of anionic silver nanoparticles to the positively charged groups of wool. At higher *p*H values (>4.5), wool fibre acquires a negative charge, which acts as a deterrent to the adsorption of silver nanoparticles[4].

Experiments at each *p*H are repeated six times and the *t*-test for significance ($\alpha = 0.05$) is performed between *K/S* values at *p*H 3 and at other *p*H values to observe whether the *K/S* value at *p*H 3 is statistically significant. The calculated standard deviation and standard error of difference are presented in Table 2. It is also found that all the calculated *p* values are lesser than 0.0001, indicating that *K/S* value at *p*H 3 is extremely statistically different. Therefore, it can safely be inferred that at *p*H 3, there is a significant difference in the silver nanoparticle uptake.

#### *3.4.2 Temperature*

*K/S* value of wool treated with silver hydrosol at different temperatures is shown in Fig. 4. *K/S* increases from 2.53 at 40˚C to 3.95 at 95˚C. This can be attributed to the higher kinetic energy of the system with increasing temperature. Increase in *K/S* beyond 60˚C is found marginal.

### 3.5 Characterisation of Wool Fabric Treated with Silver Nanoparticles

Based on the findings above, the following conditions are taken as optimum for maximizing the exhaustion of silver nanoparticles on wool fabric: *p*H 3, 60˚C for 30 min. The silver nanoparticles of concentrations 150 ppm and 200 ppm are applied on wool by exhaust method using these optimum conditions. At 150 ppm of silver hydrosol concentration, the concentration of nanosilver loaded on the wool fabric is found to be 40 ppm with an exhaustion percentage of 26.66%. Upon increase in the

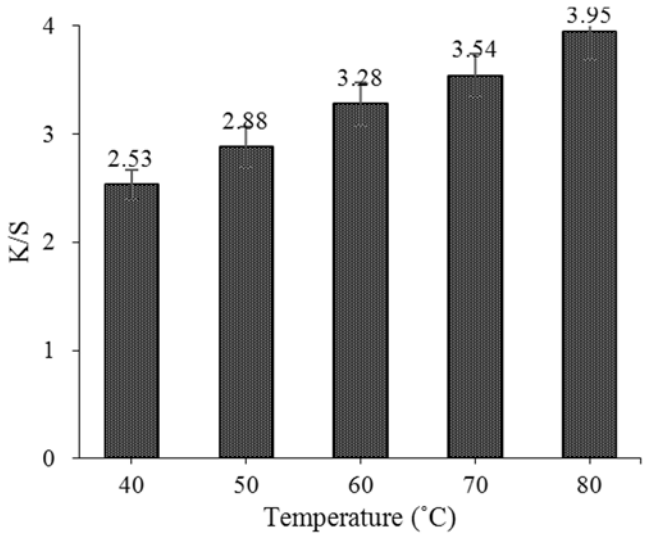


Fig. 4 — *K/S* of wool treated with silver hydrosol at different temperature (*p*H 3)

Table 2 — *K/S* of wool fabrics treated [60˚C, 30 min] with silver hydrosol at various *p*H

| *p*H | *K/S* value | S.D | S.E |
|---|---|---|---|
| 3 | 3.28 | 0.20 | 0.102 |
| 4 | 2.03 | 0.15 | 0.087 |
| 6.3 | 1.44 | 0.07 | 0.088 |
| 8 | 1.42 | 0.08 | 0.090 |
| 10 | 1.36 | 0.09 | - |

S.D. – Standard deviation, S.E – Standard error.

concentration of silver hydrosol to 200 ppm, the concentration of nanosilver loaded on the wool fabric increases to 70 ppm with exhaustion percentage of 35%. The untreated wool fabric changes its color from white to dark green (150 ppm) and brown (200 ppm) after treatment with silver nanoparticles (Not shown). Treated fabrics are subjected to SEM analysis.

#### *3.5.1 SEM Analysis*

SEM images of untreated wool fibres and those treated with silver nanoparticles are shown in Fig. 5. In fibres treated with 150 ppm silver [Fig. 5 (b)], deposition of silver nanoparticles on the scales can be clearly seen. The distribution of nanoparticles is denser and more uniform when the concentration of silver is increased to 200nm [Fig. 5 (c)].

### 3.6 Antimicrobial Activity of Wool Treated with Silver Hydrosol

Wool samples treated with different concentrations of silver nanoparticles are tested for antimicrobial activity against *S.aureus* & *E.coli* bacteria. Results are reported in Table 3. Treated fabrics show 99.5% antimicrobial activity at 150 ppm and 100% activity at 200 ppm. Representative photographs of antimicrobial test results are shown in Fig. 6.

Table 3 — Antimicrobial activity of wool treated with silver nanoparticles

| Conc. of nanosilver ppm | Antimicrobial activity, % | |
|---|---|---|
| | *S.aureus* | *E.coli* |
| 0 | 0 | 0 |
| 150 | 99.5 | 99.5 |
| 200 | 100 | 100 |

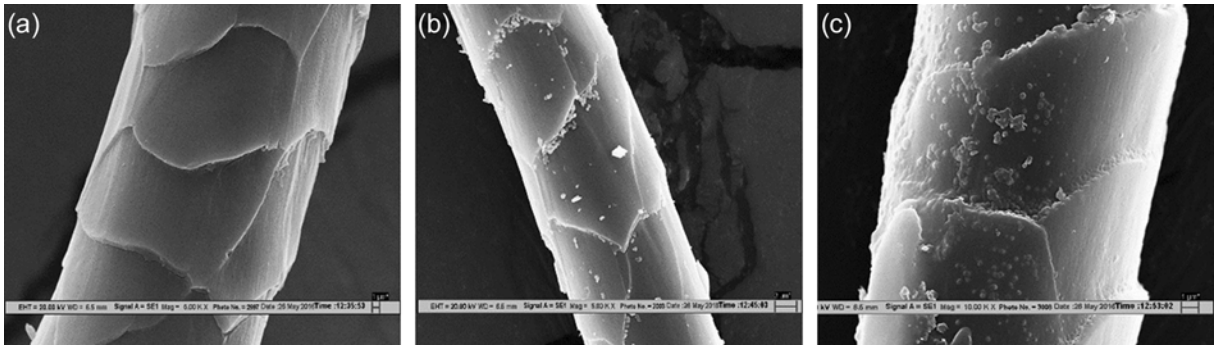


Fig. 5 — SEM images of (a) untreated, and treated with (b) 150 ppm and (c) 200 ppm nanosilver

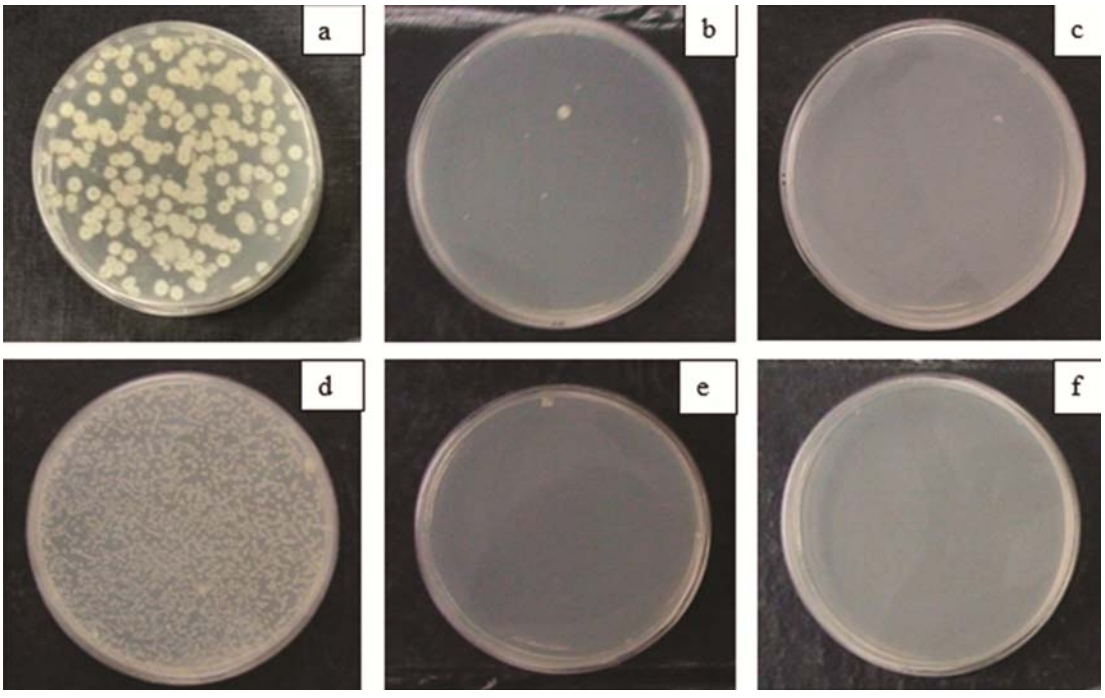


Fig. 6 — Antimicrobial testing of nanosilver treated wool against *S. aureus* (a) control (b) 150 ppm and (c) 200ppm; and against *E.coli* (d) untreated, (e) 150ppm and (f) 200ppm

Untreated fabrics show a high number of bacterial colonies, while few colonies are observed for the sample treated with 150 ppm silver and no colonies are seen for sample treated with 200 ppm of silver nanoparticles.

## 4 Conclusion

A simple process, using melanin pigment synthesized by oxidative polymerization of catechol by fresh potato juice, have been used effectively for *in vitro* synthesis of silver nanoparticles on wool fabric. Process conditions used in the synthesis of melanin influence its ability to reduce silver nitrate to nanosilver. Optimum *p*H and temperature lead to synthesis of melanin with high reduction potential as evidenced from the increase in intensity and sharpness of surface plasmon resonance bands in the 430-450 nm range. Melanin with maximum reduction potential is obtained when 0.1% catechol and 25% potato juice are reacted at *p*H 6.5 at $95^0$C for 45 min. Spherical shaped monodispersed silver nanoparticles with average particle size of 45 ± 5 nm is obtained. Rapid synthesis of $Ag^0$ is done in this single step process without use of a capping agent. The synthesized silver nanoparticles could be applied successfully to merino wool fabric by exhaust method (*p*H 3, 60˚C, 30 min) to impart high antimicrobial activity. This study opens up a novel ecofriendly approach to biosynthesize silver nanoparticles using melanin and functionalization of wool fabrics with antimicrobial effects which can be utilized for sports and medical applications.

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