

Biocompatible antimicrobial cotton fibres for healthcare industries: a biogenic approach for synthesis of bio-organic-coated silver nanoparticles

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Abstract: Cotton fibres coated with biogenically fabricated silver nanoparticles (SNPs) are most sought material because of their enhanced activity and biocompatibility. After successful synthesis of SNPs on cotton fibres using leaf extract of *Vitex negundo* Linn, the fibres were studied using diffuse reflectance spectroscopy, scanning electron microscopy, nanoparticle tracking analysis, energy dispersive X-ray, and inductively coupled plasma atomic emission spectrometry. The characterisation revealed uniformly distributed spherical agglomerates of SNPs having individual particle size around 50 nm with the deposition load of 423 µg of silver per gram of cotton. Antimicrobial assay of cotton-SNPs fibres showed effective performance against pathogenic bacteria and fungi. The method is biogenic, environmentally benign, rapid, and cost-effective, producing highly biocompatible antimicrobial coating required for the healthcare industry.

1 Introduction

Design and manufacturing of nanomaterial-coated textiles is highly explored area in the field of nanoscience and nanotechnology. The nanomaterial-modified textile fibres find widespread applications in UV-radiation protection, chemical warfare agent destruction, water-repellent, fire retardants, skin care products, wound-dressing materials, and hospital clothing [1, 2]. The achievement of required structures with retention of desirable features of core fibre such as softness, comfort, and aesthetics are given priority during development of nanoparticle-impregnated textile fibre. The nanoparticle impregnation can be achieved by variety of physical methods like ultrasonication [3], UV-treatment, plasma deposition [4], and laser deposition. Biogenic antimicrobial polymers are in great demand. Among them, silver nanoparticles (SNPs) are of utmost importance. Owing to its broad-spectrum antimicrobial nature, they are highly explored as antimicrobial coatings on textile fibres [5–8]. The fabrication of SNP-impregnated textile fibres has been attempted by various methods like reducing the metal ion on the polymer using external reducing agent [9, 10] or seeding of the polymer substrate with pre-synthesised silver colloids [7, 11].

Antimicrobial finishing of textile fibres is assuming greater importance in order to minimise acquired infections in hospitals, effective wound-healing in burns etc. [12]. Variety of nanoscale antimicrobials such as copper [13, 14], zinc oxide [15], triclosan, TiO₂, chitosan [16, 17], and alginate-silver [18, 19] have been reported recently. These nanoscale materials offer certain advantages such as higher surface area to volume ratio, greater adherence, and long-lasting effects when compared with classic antimicrobials such as quaternary ammonium salts which are active only for short duration.

Among nanoscale antimicrobials, SNPs are considered as highly attractive and promising candidates due to its long-lasting activity against multiple micro-organisms like bacteria, fungi, and viruses [20]. Owing to this, SNPs are widely being used in burn treatment, medicinal clothing, wound-dressing, and water disinfection.

A majority of the methods for producing SNP-impregnated textile fibre requires chemical reduction in liquid medium, aid of surfactants, and templates [8, 21]. Several studies pertaining to the

production of SNP-impregnated wool, cotton [22], polyester, polyamide, and silk [23–25] have been reported recently. The use of stronger binding agents reduces the activity against pathogens and imposes environmental problems associated with production of SNPs over the core fibres.

Consequently, biogenic fabrication of SNPs over textile fibre is one of the most important topics in nanoscience research today. Biogenic synthesis offers high stability, reduced toxicity [26], and biocompatibility. In this direction, in situ fabrication of SNPs over textile fabric using biogenic reducing agents such as fungi and plant extract has been reported recently [5, 10, 27]. Plant extract mediated synthesis can be considered advantageous over the other biological methods as it eliminates lengthy process of growing and maintaining cultures. Moreover, it can be extended for large-scale synthesis. Biogenic synthesis of metal nanoparticles is green approach because water is commonly used as an environmentally benign solvent, replacing toxic organic solvents, and biomolecules have been reported to serve as both reducing and stabilising agents [28, 29]. *Vitex negundo* occurs wild in most parts of India near moist places. To our opinion, it possess high amount of total phenolic compounds and flavonoids. Additionally, their leaves are the most potent for medicinal use [30]. Hence, in situ fabrication of SNPs over textile fibres using *V. negundo* is an environmentally benign and convenient method.

Herein, we report the in situ synthesis of SNPs on the cotton fibres at ambient conditions with emphasis on their physicochemical properties, biocompatibility, and antimicrobial action.

2 Materials and methods

Leaves of *V. negundo* Linn were collected from Mahim Nature Park, Mumbai, and cotton balls were collected from the National Bureau of Plant Genetic Resources (NBPGR) Akola, Maharashtra State, India. Silver nitrate (AgNO₃), streptomycin antibiotic disc, Dulbecco's modified Eagle's medium, foetal bovine serum, Muller-Hinton medium, and agar were procured from HiMedia, India.




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2.1 Preparation of leaf extract

For the preparation of extract, leaves of *V. negundo* were washed with deionised water to remove dust particles and thereafter sundried to remove residual moisture. The 0.5 g of fine powder of dried leaves was dispersed in 100 ml of deionised water thoroughly and separated with Whatman filter paper to obtain clear solution which was used as the reducing agent for the synthesis of SNPs.

2.2 In situ growth of SNPs on cotton fibres

The 10 g of clean cotton fibres was separated with the help of comb and forceps from the fresh cotton ball and mixed thoroughly using a glass rod into the leaf broth for 5 min, avoiding air bubbles getting trapped into the cavities of the fibre. The cotton was blot dried and allowed to dry completely by exposing them to sunlight in covered Petri dish. For the synthesis of SNPs, 1 mM AgNO₃ solution (100 ml) was poured onto the fibres and allowed to soak for a while and later exposed to the sunlight for 5 min. The colourless cotton fibres turned reddish brown within 5 min [31] which specified the formation of SNPs. Fibres were then washed with deionised water for four times to ensure the removal of unreacted silver ions. Similarly, the cotton fibres were treated with AgNO₃ solution without any reducing agent, which served as a negative control. The cotton-SNPs fibres were analysed using diffused reflectance measurement, nanoparticle tracking analysis (NTA), field emission gun-scanning electron microscope (FEG-SEM), energy dispersive analysis of X-rays (EDAX), and inductively coupled plasma atomic emission spectroscopy (ICP-AES). The SNP-impregnated cotton fibres were also tested for antimicrobial activity and cellular toxicity.

2.3 Diffuse reflectance spectroscopy measurements

The reflectance spectral analysis was performed on UV-visible spectrophotometer using reflectance measurement attachment (Model-Shimadzu UV 1800). Bare cotton was used for base line correction, the cotton-SNPs fibres were scanned in the range of 190–1100 nm for the reflectance.

2.4 Scanning electron microscopy and energy dispersive X-ray analysis

Scanning electron micrographs were obtained using JEOL FEG-SEM 6030 operated at a vacuum of the order of 10–5 Torr and 20 kV. The dried cotton-SNPs fibres were placed above the double-sided carbon tape stuck over 1×1 mm of brass stubs. Excess sample were brushed off before analysis and then studied for morphology. The elemental composition was determined by spot analysis using the energy dispersive X-ray (EDX).

2.5 NTA analysis

To determine the size distribution and particle concentration adsorbed on the cotton fibres, the nanoparticles were desorbed from the cotton fibres by subjecting them to ultrasonication. One milligram of cotton was taken in 2 ml of deionised water in a small beaker and was sonicated for 5 min using probe sonicator (Sonics Vibro cell V SX130, USA) at an amplitude of 50% with a pulse rate of 10:2. The resulting dispersion of SNPs in the water was analysed using nanoparticle tracking analyser (LM-20, Nanosight, UK).

2.6 ICP-AES analysis

To precisely determine the amount of silver deposited on the cotton fibres, ICP-AES (SPECTRO Analytical Instruments GmbH, Germany) analysis was performed. For ICP-AES sample preparation, 1 mg of cotton-SNPs fibres was dissolved in 1 ml of HNO₃ and left for overnight period. To the dissolved cotton fibres, 11 ml of water was added and 4 ml of solution was analysed.

2.7 Antimicrobial testing

The antimicrobial activity of cotton-SNPs fibres was studied against gram-negative *Escherichia coli* JM-103 (ATCC-39403) and *Klebsiella pneumoniae* (clinical isolates); gram-positive *Staphylococcus aureus* (ATCC-25923) and diploid fungus *Candida albicans* using the disc diffusion method [32]. The 18 h revived culture of the above-mentioned microorganisms was prepared in Mueller-Hinton (MH) broth [33].

To each of the agar plates, 50 µl of the inoculum from the overnight grown bacterial and fungal culture (inoculum having turbidity standard 0.5 McFarland standard equal to 1–2 × 10⁸ CFU/ml) was poured separately in the sterile Petri dishes and spread uniformly with the help of a spreader in the laminar air flow chamber. After spreading of the test organism, three cotton discs (6 mm diameter) viz. cotton-SNPs fibres, cotton-AgNO₃ fibres, and bare cotton and standard antibiotic disc of streptomycin (positive control) were placed with the help of sterile forceps onto the surface of inoculated MH agar plate. Each disc was pressed down to ensure complete contact with the agar surface such that they must be distributed evenly and are no closer than 24 mm from centre to centre. The plates were then incubated at 37°C. After the overnight incubation, each plate was examined for the appearance of inhibition zones. The resulting zone of inhibition around each disc was calculated and their efficacy was comparatively studied.

2.8 Biocompatibility test

The biocompatibility of cotton-SNPs fibres was performed over mouse normal fibroblast cell line (L929) acquired from National Center for Cell Science (NCCS), Pune, India. A total of 10⁴ cells were seeded in 96-well plate and left for 12 h to adhere. Later, 1 mg of cotton fibres was dispersed in 300 µl of media. It was added in triplicates to 96-well plate. The plate was incubated at 37°C and 5% CO₂ for 24 h. Supernatant was taken out and wells were washed thrice using phosphate buffer saline, thereafter fresh media containing 5% of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye were added. After 4 h of incubation, 200 µl of dimethyl sulphoxide was added and absorbance was recorded at 570 and 690 nm using microplate reader (Tecan Infinite M200).

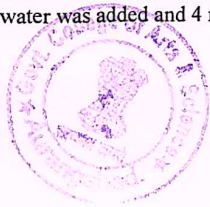
3 Results

A simple and biogenic method has been stated without any requirement of external binder or stabiliser. Cotton fibres treated with leaf extract and aqueous silver ion solution showed the colour change from colourless to brown under the exposure of sunlight for 5 min. SNPs exhibit a brownish colour due to surface plasmon resonance of electrons with the frequency of electromagnetic waves. These colour changes were quantified by diffused reflectance measurements on the dried sample in UV-Vis range and are shown in Fig. 1b. The presence of nanoscale silver metal is marked by maxima around 500 nm in the reflectance spectra.

The morphology of nano-sized SNPs fabricated on cotton fibres was observed using scanning electron microscopy (SEM) (Fig. 2). The SEM revealed that the impregnated SNPs were uniformly distributed over the surface of cotton fibres. However, the surface morphology of the SNPs was not clearly visible. The exact size of the SNPs was also not assessed precisely, but large cluster formation was observed in the size range of 180–250 nm.

The size of the SNPs was further ascertained by NTA and it revealed the mean size of 45 nm with the standard deviation of ±32 nm. Fig. 3 shows size distribution as a function of particle concentration. The spot EDX analysis of the SNP-impregnated cotton fibres (Fig. 4) divulges the presence of the main elements – silver, carbon, oxygen, and chlorine. Carbon and oxygen may be assigned to the bio-organic compounds present in the shell (orbit) of the SNPs. The details of the elemental composition and their atomic percentage are tabulated in Table 1. The load of silver was found to be 423 µg/g of cotton as determined using ICP-AES.

To examine the antimicrobial efficacy of the cotton-SNPs fibres, the activity of the cotton-SNPs fibres was studied against *E.*



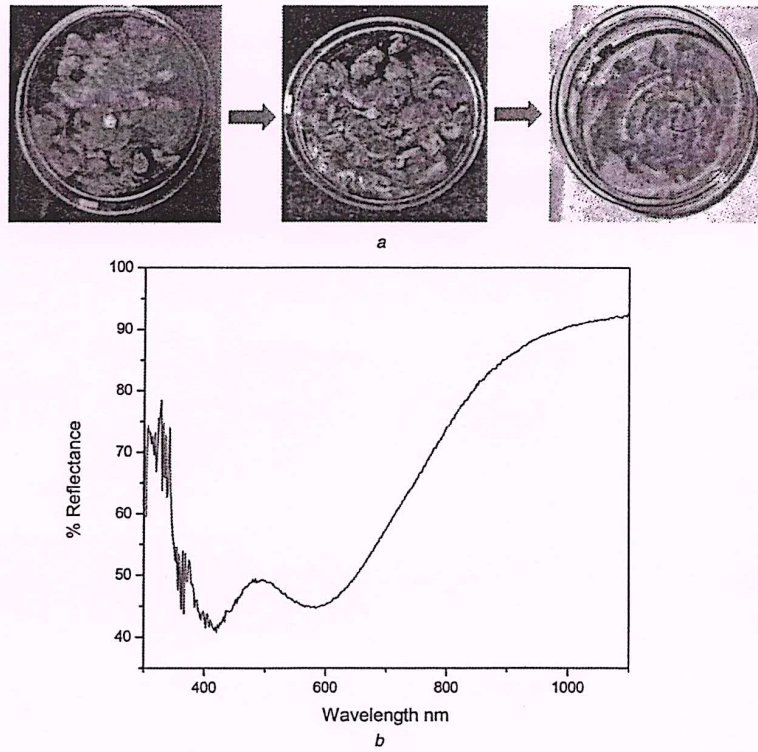


Fig. 1 Visual Observation and spectroscopic study of silver nanoparticle impregnated cotton fibres
 (a) Cotton fibres in leaf broth, in leaf broth + silver nitrate, and after reduction of silver nitrate to SNPs respectively from left to right, (b) Diffuse reflectance spectra of cotton-SNPs fibres

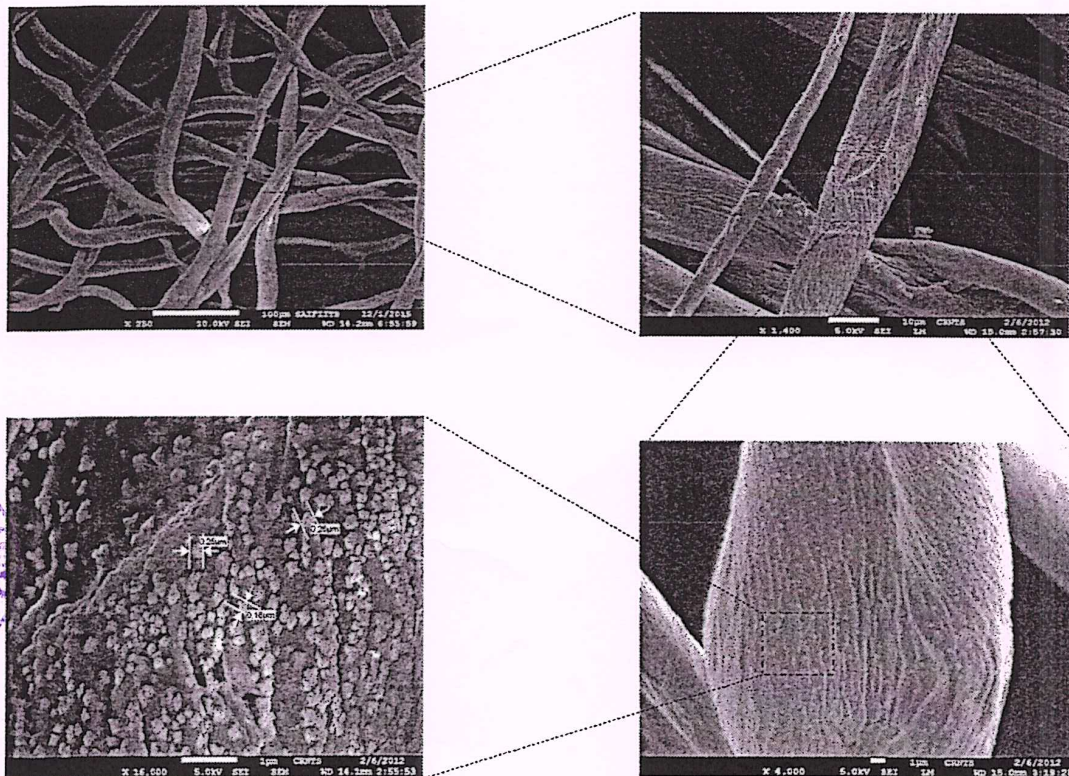


Fig. 2 SEM images of cotton-SNPs fibres at different magnifications showing uniform adherence of SNPs over cotton fibres

coli JM-103 (ATCC-39403), *K. pneumonia*, *S. aureus* (ATCC-25923), and *C. albicans* and proved to be effective (Table 2). Except for *E. coli*, the zone of inhibition for cotton-SNPs

fibres is higher against all the studied microbes compared with cotton-AgNO₃ fibres.

The cotton-SNPs fibres were also tested for cellular toxicity. It was found that L929 cells treated with cotton and cotton-SNPs

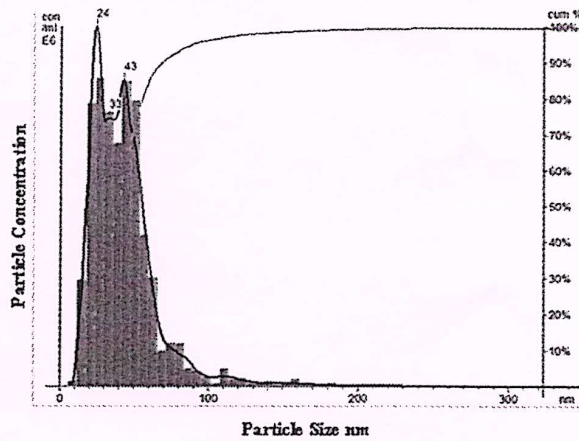


Fig. 3 Size distribution curve of desorbed nanoparticles from cotton-SNPs fibres as a function of particle concentration

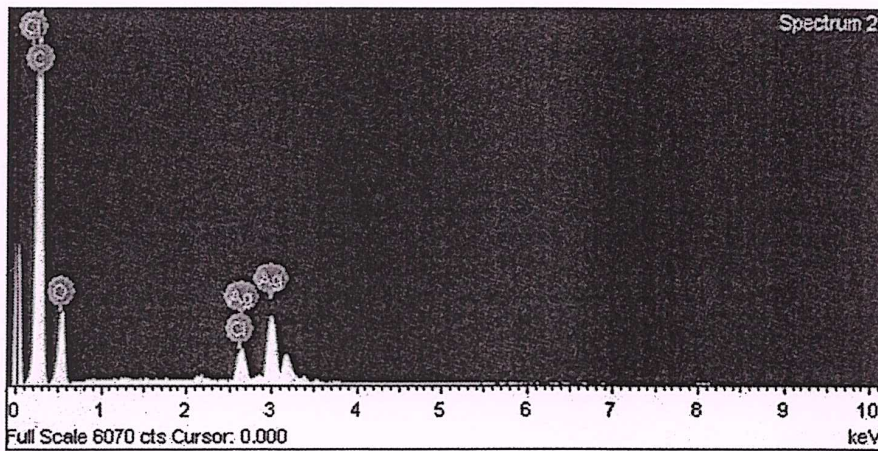


Fig. 4 EDAX spectrum of cotton-SNPs fibres showing the presence of silver

Table 1 Elemental composition of cotton-SNPs fibres in EDAX

Element	Weight %	Atomic %
C K	54.13	71.13
O K	25.32	24.97
Cl K	2.97	1.32
Ag L	17.58	2.57
total	100	100

Table 2 Antibacterial efficacy study of cotton-SNPs fibres and corresponding controls

Microbial strains	Antibacterial efficacy zone of inhibition in mm			
	Cotton-SNPs fibres (NP)	Bare cotton (C)	Antibiotic disc streptomycin (Ab)	Cotton-AgNO ₃ fibres (Ag)
<i>Escherichia coli</i> (ATCC 8739)	7.72	nil	12.49	6.71
<i>Klebsiella pneumoniae</i> (clinical isolate)	3.70	nil	9.85	2.52
<i>Staphylococcus aureus</i> (ATCC 6538P)	4.47	nil	14.98	2.12
<i>Candida albicans</i> (clinical isolate)	8.37	nil	9.28	2.11

showed >80% viability which is close to negative control (cells without any treatment) (Fig. 5). Quantitative results were also reflected in qualitative analysis of cells as morphology of cells treated with cotton and cotton-SNPs were found to be similar to negative control cells without any remarkable changes.

4 Discussion

The applications of biocidal and biostatic metal nanoparticles over polymer substrates offers promising alternative to minimise acquired infections in hospitals which occurs mainly through surface contacts. These polymers mainly include synthetic polymers such as polypropylene, polyamide etc. However, the cellular toxicity of this material impedes the effective wound-healing process. Therefore, biogenic antimicrobial polymers are in demand. Owing to its broad-spectrum antimicrobial potential, SNPs are highly explored as antimicrobial coatings on textile fibres. In our study, we described a simple and green method of in situ fabrication of SNPs over cotton fibres using leaf broth of *V. negundo* Linn as reducing source. The plant is widely available in the region in ample amount. Moreover, the present protocol requires only 0.5 g of their dry mass. Hence, the use of this plant will not have significant effects on the flora and fauna of the region where it grows, thereby proving it to be environmentally benign protocol. The characterisation studies of SNP-impregnated cotton fibres were assessed to confirm the morphology, particle size, elemental composition, and toxicity of the material prepared. Brownish colour change of cotton fibres and quantification by UV-Vis studies confirmed the presence of SNPs. The presence of nanoscale silver metal is marked by maxima around 500 nm in the reflectance spectra. This response obtained can be accounted in terms of two-flux theory of Kubelka and Munk [34, 35]. The



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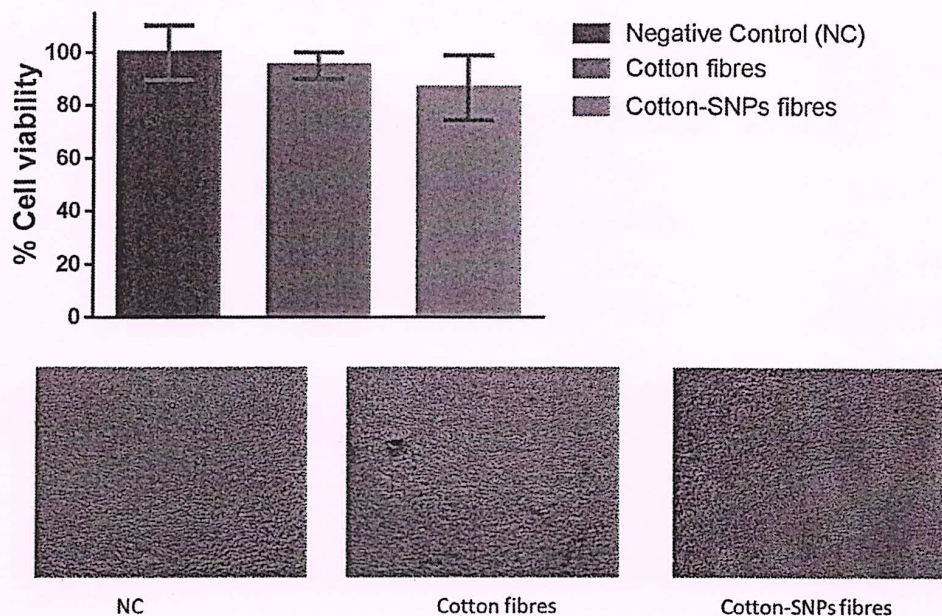


Fig. 5 Quantitative and qualitative analysis of cotton-SNPs fibres biocompatibility over mouse normal fibroblast L929 cells

response of electromagnetic radiation towards densely packed particle assembly, e.g. paints, and coatings, can be explained in terms of *many-flux theories*. Among these, two-flux model of Kubelka and Munk is quite popular due to its simplicity and ability to give more accurate results. Based on two-flux model of Kubelka and Munk, the computed reflectance spectra for spherical SNPs with 50 nm diameter exhibit maxima at 510–550 nm range [34]. A similar result obtained in our case ratifies the size of the SNPs. It is well established that intense yellowish brown colour of SNPs arise due to surface plasmon resonance of electrons with the frequency of electromagnetic waves [36]. However, SEM images show clusters in the range of microns, which can be attributed to the agglomeration of SNPs due to high surface energy.

As an antimicrobial agent, the size of SNPs is also very important. NTA analysis gives accurate estimates of size, size distribution, and concentration of bio-reduced nanoparticles. In NTA, the sizing of the nanoparticles in dispersion is done on the basis of laser light microscopy and CCD camera, the Brownian motion of particles is observed and sphere equivalent hydrodynamic radius of the particles is calculated from the movement of particle via the Stoke–Einstein equation [37]. The observed particle size in NTA analysis matches with the diffuse reflectance measurement. From the results obtained, we can depict the important features of the synthesised SNPs being of desirable size, uniformly distributed, strongly adhered over surface of the cotton fibres, the second major attention of this work is the design of biogenic, biocompatible, and antimicrobial cotton for wound-dressing and healthcare purpose. The antimicrobial studies revealed the potential application of the SNP-impregnated on cotton fibres. AgNO_3 , a water-soluble salt, has been in use as an antimicrobial agent from many decades [38]. However, in many cases, it has been reported that, when compare with silver salts, SNPs have better efficacy against most of the microorganisms [6, 39, 40]. Dissolved silver ions are responsible for showing their antibacterial activity. However, it is speculated that SNPs are about three times lethal when compared with silver ions [41]. This is because they offer slower release of silver ions. Hence, there is pressing need to compare the antibacterial activity of SNPs and silver nitrate. The mechanism underlying the antibacterial efficacy of SNPs is not fully established. However, literature review divulges that the electrostatic attraction between negatively charged cell membrane of microorganism and positively charged nanoparticles plays a decisive role in the antimicrobial activity [42–45]. The higher efficacy of SNPs is attributed to the sustained release of silver ions over a period of time rather than having higher dosage of silver ions for short duration [46, 47]. The SNPs

serve as a reserve of the Ag^+ ions which when comes in contact with the extracellular fluid of the bacteria will release the Ag^+ ions. It is this combine effect of the Ag^+ ions and the properties of the SNPs that act as strong bactericidal effect.

The environmental fate of nanoparticles is also important from application point of view. At present though regulatory studies are ongoing, in the majority of cases, SNPs thought to be non-toxic. This could be due to the formation of silver sulphide upon release to the environment [48]. Apart from environmental threats, the toxicity of the product during their usage is also important. The cellular toxicity study indicates that the cotton-SNPs fibres produced in the present study are safe and biocompatible. High biocompatibility of cotton-SNPs could be attributed to biological method of in situ synthesis. The present greener method of fabrication of these fibres is attractive due to rapid, low cost, versatility, and easy process ability that could be easily processed on large scale. To the best of our knowledge, this is the first report on in situ biogenic reduction in silver ions over natural cellulosic polymer like cotton at ambient conditions. The major advantages of these fibres are its biocompatibility, reliability, non-toxic effect, ecofriendlyness, and cheap. These cotton-SNPs fibres can be widely used for topical applications, wound-dressings, healthcare purpose, and as an antimicrobial textile.

5 Conclusion

The present method of fabrication of cotton-SNPs is totally green, rapid, cost-effective, and easy to obtain antibacterial cotton for topical application. Compared with the conventional methods for SNP-impregnation, the present approach of biogenic and in situ reduction of silver ions on the cellulosic fibre has advantage of being biocompatible, authentic, and reliable. Since it is free from toxic additives, there will not be any deleterious effect. The cellular toxicity studies indicate that the cotton-SNPs fibres produced biogenically are safe and biocompatible and can be used as antimicrobial textiles. Undoubtedly, the method is very rapid and cost-effective that could be easily extended on the industrial scales.

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