



Nanoherbal coating of cotton fabric to enhance antimicrobial durability

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ABSTRACT

The present investigation emphasizes on screening of herbs with potent antimicrobial activity, preparation of nanoparticles of their extracts, treatment of cotton fabrics with herbal nanoparticles, assessment of their antimicrobial efficacy and finally assessment of wash durability. Based on the antimicrobial activity and availability Neem was selected and the herbal Nanoparticles were prepared using Neem extract. Results confirmed that the neem Nanoparticles treated fabrics showed maximum antimicrobial activity than the plain Neem extract treated fabrics against *S. aureus* and *E. coli* in both qualitative and quantitative tests. The antimicrobial activity of the Neem Nanoparticles treated fabrics was retained upto 25 washes where as the plain neem extract treated fabrics retained the activity only up to 10 washes. These results indicate that the application of nanotechnology is ideal for improving the antimicrobial activity and wash durability of herbal-based antimicrobial textiles.

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Introduction

Nanotechnology is defined as the study and use of structures between 1 nanometer and 100 nanometers in size. To give an idea of how small that is, it would take eight hundred 100 nanometer particles side by side to match the width of a human hair. Making composite fabric with nano-sized particles or fibers allows improvement of fabric properties without a significant increase in weight, thickness, or stiffness as might have been the case with previously-used techniques. Therefore, there is an increasing use of nanotechnology application in various areas of R&D and industry. This included coating of textiles to impart better performance properties such soil release, dye affinity water absorbency and wash durability as described below.

The cotton garments, by virtue of their characteristics and proximity to human body, provide an excellent medium for the adherence, transfer and propagation of infection causing microbial species, especially in cases where work wears are worn, in places such as hospitals, clinics, food industry, hygiene industry or babies and elders wears who need extra care. The presence and growth of these microorganisms can cause health problems, odors and finally fabric deterioration (Deepti, 2007) (Sarkar R. K, Purushottam. De., Chauhan, P.D. 2010). As microbes often attack the additives applied to textiles, discoloration and loss of textile's functional properties such as elasticity (brittleness) or tensile strength can also occur. In order to overcome the deleterious effect of microorganisms on textile materials a special type of finishing called antimicrobial finishing is required (Sathianarayanan *et al.*, 2010). In the last few decades, with the increase in new antimicrobial finishing technologies and the growing awareness about cleaner surroundings and healthy lifestyle, a range of textile products based on synthetic antimicrobial agents such as triclosan, metal and their salts, organometallics, phenols and quaternary ammonium compounds, have been developed and quite a few are also available commercially (Joshi *et al.*, 2009). Although the synthetic antimicrobial agents are very

effective against a range of microbes and give a durable effect on textiles, they are a cause of concern due to the associated side-effects, action on non-target microorganisms and water pollution. Hence, there is a great demand for antimicrobial textiles based on eco-friendly natural agents which not only help to reduce effectively the ill effects associated due to microbial growth on textile material but also comply with the statutory requirements imposed by regulating agencies (Joshi *et al.*, 2009). Among natural biocides used in antimicrobial finishing in textiles plant extracts and essential oils play an ever growing role as the extracts and essential oils from different plants are widely known for bactericidal and fungicidal properties (Kozlowski and Walentowska, 2008) (Simoncie B., Tomsic B 2010). One major problem associated with the herbal antimicrobial finishing is that they are not durable. There are many approaches tried to increase the durability and one such way is the use of nanotechnology as manipulation of nanoscale phenomena to create synergies between natural fibers and nanomaterials could offer a solution for this apparent dichotomy by creating functionality while preserving appearance and comfort (Vigneshwaran *et al.*, 2009). The present investigation aims at developing antibacterial finish for cotton fabrics from herbal nanoparticles. Some selective species of plants were identified and screened for their antibacterial activities. Their extracts were then used for nanoparticle preparation and the nanoparticles were characterized topographically. The fabrics were finished by pad-dry- cure method. An extensive study was conducted to assess the antibacterial activity of the finished fabrics. The fabrics were subjected to wash durability testing to find the durability of the finish and the findings are discussed in this paper.

Materials and Methods

The following commercially available materials were used as received. Bovine Serum Albumin (Himedia Laboratories pvt. Limited, Mumbai), 25% Glutaraldehyde (S.D.Fine Chemicals, Mumbai) 99% Absolute Alcohol (Changshu Yangyuan

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Chemical, China), Mannitol (Qualigen fine Chemicals, Mumbai), Phosphate Buffer (Prepared from NaH_2PO_4 and were Na_2HPO_4 obtained from Qualigen fine Chemicals, Mumbai).

Collection, Processing and Extraction of medicinal herb

The medicinal herb selected for this study was *Azadirachta indica* (commonly called as neem) and it was collected from in and around Coimbatore. The collected leaves were shade dried at room temperature to reduce the moisture content. The dried leaves were then powdered and sieved. 20 grams of the ground herbal powder was suspended in 100ml methanol and incubated overnight. The supernatant was filtered twice using Whatman No.1 filterpaper and the filtrate was further used for preparation of nanoparticles.

Preparation of the Nanoparticles

The herbal extract loaded albumin Nanoparticles were prepared by coacervation process followed by crosslinking with glutaraldehyde according to Meridio *et al* (2001). The *Azadirachta indica* (neem) extract was incubated with required amount of protein solution (2% w/v) for one hour at room temperature and the pH was adjusted to 5.5 by 1M HCl using digital pH meter (Orion meter model 720). Then ethanol was added to the solution in 2:1 ratio (v/v) at the rate of 1ml/min.⁸ The coacervate so formed was hardened with 25% glutaraldehyde (1.56 $\mu\text{g}/\text{mg}$ of protein) for 2 hours to allow cross-linking of protein. Organic solvents were then removed under reduced pressure by Rotary Vacuum evaporation and the resulting nanoparticles were purified by centrifugation (Remi cooling centrifuge) at 4°C. Pellets were then suspended in phosphate buffer (pH 7.4; 0.1 M) and each sample finally was lyophilized with mannitol (2% w/v) at -48°C and 28×10^{-3} M Bar pressure for 24 hours (Freeze Dry System/Free zone, 4.5 Labconco Model 117). Albumin Nanoparticles controls were also prepared by the same procedure without herbal extracts loading.

Characterization of nanoparticles by SEM

Lyophilised Nanoparticles were suspended in phosphate buffer (pH 7.4) and the topographical characterization of the Nanoparticles were done using SEM (JEOL-JSAL 6360).

Fabric Specification and Coating of cotton fabrics with nanoparticles

A fine-medium weight 100% cotton woven fabric (plain weave, 75×30 g/m²; ends 75/inch; picks 60/inch) was used for the application purpose. Herbal Nanoparticles and plain herbal extract were applied on cotton using pad-dry-cure method. The cotton fabric cut to the size of 30 X 30 cm was immersed in the solution containing Nanoparticles (2%) and citric acid binder (1%) for 5 min and then it was passed through a padding mangle (R. B .Electronic and Engineering, Mumbai), running at a speed of 15 m/min with a pressure of 2 kgf/cm² to remove excess solution. A 100% wet pick-up was maintained for all of the treatments. After padding, the fabric was air-dried and then cured for 3 min at 140°C and immersed for 5 min in 2 g/l of sodium lauryl sulfate to remove unbound nanoparticles and rinsed to remove the soap solution followed by air-drying. The fabric without any treatment was used as control.

Antimicrobial assessment of finished cotton fabrics

The test organisms used in the study were *Staphylococcus aureus* (representative of gram positive bacteria) and *Escherichia coli* (representative of gram negative bacteria) as per AATCC standards. The qualitative antibacterial assessment was done using Parallel streak method (AATCC test method 147) and the quantitative antibacterial assessment was done by Percentage Reduction Test (AATCC 100)

Parallel streak method (AATCC 147)

Test specimens (non sterile) were cut into pieces (25mm x 50mm). Sterile AATCC bacteriostasis agar plates were prepared. Using sterile 4mm inoculating loop, one loop full of culture was loaded and transferred to the surface of the agar plate by making five parallel inoculum streaks spaced 10mm covering the central area of the petridish without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with agar surface. The plates were incubated at 37°C for 18-24 hours. The inoculated plates were examined for the interruption of growth along the streaks of inoculum beneath the fabric and for a clear zone of inhibition beyond the fabric edge. The average width of the zone of inhibition around the test specimen calculated in mm using the following formula

$$\text{Zone of inhibition (mm)} = (T-I)/2$$

T-width of zone of inhibition

I-width of specimen

Percentage Reduction Test (AATCC 100)

The non-sterile test specimens were taken, and they were cut into pieces according to standard size (20 mm radius) with round shape recommended by AATCC and sterilized under UV. Test specimens were soaked inside the test tubes containing sterile AATCC bacteriostasis broth and a loop full of test organisms was inoculated. Initial cell concentration was calculated by using viable count method. The test samples were incubated at 37°C for 18 hrs. After 18 hrs of incubation, final concentration of cell in control and the test samples were calculated using viable count method. The plates were incubated at 37°C for 18-24 hours. After incubation period the number of colonies present in each dilution plates was counted. The percentage in bacterial reduction was calculated using the following formula

$$\% \text{ Bacterial Reduction} = [(A-B)/A] \times 100$$

A = initial no. of cells; B = final no. of cells

Wash Durability Testing

The wash durability testing of the finished fabrics was carried using a neutral soap at 40°C ($\pm 2^\circ\text{C}$) for 30 minutes, keeping the M:L ratio at 1: 50, followed by rinsing washing and drying. After drying the test fabrics and the control were assessed for antimicrobial activity by the method as described earlier (Sec. 2.5.2)

Results and discussion

Characterization of nanoparticles:

Nanoparticles of the neem extract was prepared by coacervation method using Bovine serum albumin (BSA) protein. The topographical characterization of the nanoparticles done using SEM showed that the nanoparticles were roughly spherical and the size of the nanoparticles were in the range of 50 – 70nm .The SEM image of the nanoparticles were shown in the Fig (1)

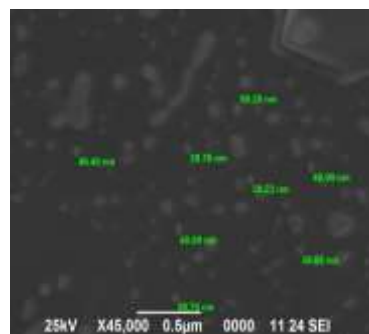


Fig. (1) SEM image of nanoparticles

Parallel streak method (AATCC 147)

The antibacterial activity of the nanoparticles finished cotton fabric was assessed by parallel streak method. The test results presented in figure 2 showed that the bactericidal activity of the nanoparticle treated fabrics was found to be higher when compared to the plain extract treated fabrics and the untreated fabrics (control) has no bactericidal activity. In terms of zone of inhibition maximum zone was found with nanoparticle treated fabric against *S.aureus* with a zone of 27 mm. These results are similar to the earlier reports of Thilagavathi *et al.*, 2004 on antibacterial activity of neem treated fabrics. They found that antibacterial activity of neem treated samples is stronger for *S.aureus* than *E.coli*.

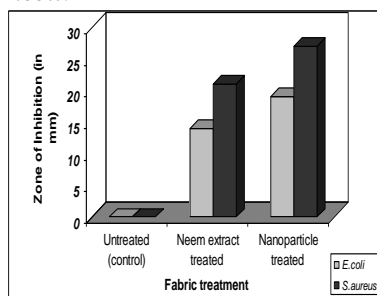


Fig.2 : Antibacterial Assesment -AATCC 147

Percentage Reduction Test (AATCC 100)

The quantitative assessment of the antibacterial activity by percentage reduction test was shown in Table-1. The results showed that the maximum reduction percentage was observed with the fabrics treated with the neem extract nanoparticles with a percentage reduction of 100% and 87.48% for the test organisms *S.aureus* and *E.coli* respectively. A study by Vaideki *et al.*, (2007) confirmed that the percentage reduction for *S.aureus* was 86% in case of neem extract treated fabrics.

As the untreated fabric (control) has no bactericidal activity, the final number of surviving cells will be much higher than the initial cell. Hence percentage reduction cannot be calculated.

Wash durability test:

Table 2 shows the wash durability studies carried out on treated fabrics.

It was found that directly applied herbal extract samples does not retain the antibacterial activity after 10 washes. This is because that the extracts were coated only on the surface without any firm bonding. The fabrics treated with the nanoparticles of the neem extract retained the antimicrobial activity effectively upto 25 wash cycles. This may due to the entanglement of the nanoparticles onto the fibres very strongly. It was reported by Thilagavathi and Krishna Bala (2007) that the fabrics treated with neem extract alone do not show much activity after 10 washes.

Conclusion

Nanoparticles of the *Azadirachta indica* extracts were prepared by co-acervation method using bovine serum albumin cross-linked with glutaraldehyde. The antimicrobial activities of the nanoparticles treated cotton fabrics were found to be higher than that of the neem extract alone treated fabrics and untreated fabrics and the results were confirmed by standard AATCC tests. Since the leaves are abundantly available in many of the countries, the scope of implementation and commercialization of herbal extract nanoparticles to impart antibacterial finish in textile is high. It is also considered as eco-friendly treatment since it uses only herbs but only chemical residues are to be avoided by thorough rinsing and neutralization. The rinsing

agent such as acetic acid could be recommended specially it is used by the hygiene textile industry.

The reduction in antibacterial activity is clear for the case of neem extract for both types of bacteria after three washes the bacterial reduction is totally zero, while in the case of Nano neem extract it was up to 25 washes where there is still bacterial reduction. This can be attributed to the low surface tension of the Nano solution in general and the contact between the fabric and the nano extract is increased as explained below.

Contact angle, θ , is a quantitative measure of the wetting of a solid by a liquid. It is defined geometrically as the angle formed by a liquid at the three phase boundary where a liquid, gas and solid intersect as shown below:

low values of contact angle (θ) indicates that the liquid spreads, or wets well, while a high contact angle indicates poor wetting. If the angle θ is less than 90 degrees the liquid is said to wet the solid. If it is greater than 90 degrees it is said to be non-wetting. A zero contact angle represents complete wetting.

The measurement of a single static contact angle to characterize the interaction is no longer thought to be adequate. For any given solid/ liquid interaction there exists a range of contact angles which may be found. The value of static contact angles are found to depend on the recent history of the interaction. When the drop has recently expanded the angle is said to represent the 'advanced' contact angle. When the drop has recently contracted the angle is said to represent the 'receded' contact angle. These angles fall within a range with advanced angles approaching a maximum value and receded angles approaching a minimum value.

Measurements of surface tension yield data, which directly reflect thermodynamic characteristics of the liquid tested. Measurement of contact angles yield data, which reflect the thermodynamics of a liquid/solid interaction. If you wish to characterize the wetting behavior of a particular liquid/solid pair you only need to report the contact angle. It is possible to characterize the wettability of your solid in a more general way. To characterize the thermodynamics of the solid surface itself more elaborate analysis is required. Various methods are used but the same basic principle applies for each. The solid is tested against a series of liquids and contact angles are measured.

Calculations based on these measurements produce a parameter (critical surface tension or surface free energy), which quantifies the characteristics of the solid and mediates the properties of the solid substrate. The critical surface tension or the surface free energy obtained in this way can be regarded as the "surface tension" of the solid substrate where is in this case is the cotton fabric, which is a characteristic property of cotton in the same way as the surface tension is for the extract. There may be more complications to be considered in this interaction between the cotton fabric and the extract when attachment of the extract molecule when normal or nano, the nano size of the extract droplets has rather more surface area that the normal extract which more surface energy and less surface tension. This enables the nano extract to cling to the fibre surface.

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Table-1: Antibacterial Assesment –AATCC 100

Fabric Treatment	Antibacterial activity (Bacterial reduction, %)	
	Test organisms	
	S.aureus	E.coli
Neem extract treated	98.73	86.84
Nanoparticles of neem extract treated	100	91.48

Table-2 Wash durability of treated samples

No. of washes	Antibacterial Activity			
	Neem extract treated fabric		Nanoparticles of neem extract treated Fabric	
	% Bacterial reduction			
	S.aureus	E.coli	S.aureus	E.coli
0	98	79	100	87
5	68	62	86	81
10	49	41	74	69
15	0	0	66	61
20	0	0	59	52
25	0	0	45	40
30	0	0	0	0