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FABRICATION OF ANTIMICROBIAL TEXTILES USING ZINC OXIDE NANO PARTICLES

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ABSTRACT: The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, textiles, environmental protection, and biotechnology is an area of constant interest. lately, an awareness of general sanitation, contact disease transmission, and personal protection has led to the development of antimicrobial textiles. The development of antimicrobial cotton fabrics using Zinc oxide nanoparticles has been investigated in this present work. The ZnO nanoparticles were prepared by wet chemical method and were directly applied on to the 100% cotton woven fabric using pad-dry-cure method. The antibacterial activity of the finished fabrics was assessed qualitatively by agar diffusion and parallel streak method, quantitatively by percentage reduction test. The topographical analysis of the treated fabric and untreated fabric were studied and compared. The results show that the finished fabric demonstrated significant antibacterial activity against in both qualitative and quantitative tests. The SEM analysis discovered the embedding of ZnO nanoparticles in treated fabrics. The wash durability study of the treated fabric was also carried out and found to withstand up to 25 wash cycles.

I. INTRODUCTION

Nanoscale science and technology have emerged over the past decade as the forefront of science and technologies. The intersecting fields of study that create this domain of science and engineering perfectly typify the rapid, multidisciplinary advancement of contemporary science and technology. . Inorganic materials such as metal and metal oxides have attracted lots of attention over the past decade due to their ability to withstand harsh process conditions. Of the inorganic materials, metal oxides such as TiO harsh process conditions but also generally regarded as safe materials to human beings and animals. The use of nanoparticles of silver and zinc oxide has been seen as a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles. The intrinsic properties of a metal nanoparticle are mainly determined by size, shape, composition, crystallinity and morphology (Dickson and Lyon 2000). In view of the
textile industry's innovative history, it is no wonder that nanotechnology has found its way into this sector so quickly. Nanotechnology is forecasted as the second industrial evolution in the world. The novel properties and low material consumption amount has attracted global interest across disciplines and industries. The textile sector is no exception. As stated by the "European Technological Platform for Textiles and Fashion", the textile industry to thrive must improve and reduce the costs of the processes, offer innovative products for traditional markets, develop new products for new markets. Nanotechnology can have an important role to achieve these goals and, in effect, all over the world public and private research institutions and private enterprises are actively engaged in nanotechnology research aimed at applications in the textiles sector. The competition is growing and technological innovation is crucial to keep pace with it. Health concerns along with customer satisfaction have made functionally finished textiles a fast-paced and fast growing industry.
With growth in world population and the spread of disease, the number of antibiotic resistant microorganisms is rising along with the occurrence of infections from these microorganisms. With this increase in health awareness, many people focused their attention on educating and protecting themselves against harmful pathogens. It soon became more important for antimicrobially finished textiles to protect the wearer from bacteria than it was to simply protect the garment from fiber degradation . The need for antimicrobial textiles goes hand-in-hand with the rise in resistant strains of microorganisms. Functional textiles include everything from antimicrobial finished textiles, to durable, or permanent press finished garments, to textiles with self-cleaning properties, and also textiles with nanotechnology. With the above back ground information the present

study was carried out with the main objective of evolving a simple method for the synthesis of ZnO nanoparticles, design a method to finish ZnO nanoparticles onto cotton fabrics to confer antimicrobial function and finally evaluate the finished fabrics in terms of antibacterial, wash durability and topographical function.

II.MATERIALS AND METHODS

2.1 Nanoparticle preparation

The zinc oxide nanoparticles were prepared by wet chemical method using zinc nitrate and sodium hydroxide as precursors and soluble starch as stabilizing agent. Different concentrations of soluble starch (0.1%, 0.5% and 1.0%) were dissolved in 500 ml of distilled water by using microwave oven. Zinc nitrate, 14.874 g (0.1 M) was added in the above solution. Then the solution was kept under constant stirring using magnetic stirrer to completely dissolve the zinc nitrate.. After complete dissolution of zinc nitrate, 0.2 M of sodium hydroxide solution (20 ml was used in our study) was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 Hours after complete addition of sodium hydroxide. After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was then discarded carefully. The remaining solution was centrifuged at 10, 000 X g for 10 mins and the supernatant was discarded. Thus obtained nanoparticles were washed three times using distilled water. Washing was carried out to remove the byproducts and the excessive starch that were bound with the nanoparticles. After washing, the nanoparticles were dried at 80 degree C for overnight. During drying, complete conversion of zinc hydroxide into zinc oxide takes place.

2.2 Application onto fabrics

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. ZnO nanoparticles were applied on cotton using pad-dry-cure method. The cotton fabric cut to the size of 30 ×30 cm was immersed in the solution containing ZnO (2%) and acrylic binder (1%) for 5 min and then it was passed through a padding mangle. 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. The fabric was then immersed for 5 min in 2 g/l of sodium lauryl sulfate to remove unbound nanoparticles. Then the fabric was rinsed at least 10 times to completely take out all the soap solution. The fabric thus washed was air-dried. Simultaneously, bulk-ZnO coating was carried out for comparison.

2.3 Assessment of antibacterial activity

2.3.1 Qualitative tests

2.3.1.1 Agar diffusion method

Bacteriostasis agar was dispensed in sterile petriplates.24 hours broth cultures of the test organisms (E.coli and S.aureus) were used as inoculums. Using sterile cotton swab the test organisms were swabbed over the surface of the agar plates. The test fabrics (fabrics treated ZnO nanoparticles) & Control (fabrics treated with ZnO bulk) was gently pressed in the center of the mat culture. The plates were incubated at 37°C for 18-24 hours.

2.3.1.2 Parallel streak method:

Sterile bacteriostasis agar was dispensed in petriplates. 24 hours broth cultures of the test organisms E.coli and S.aureus) were
used as inoculums. Using 2 mm inoculation loop, 1 loop full of culture was loaded and transferred to the surface of the agar plate by making 7.5cm long parallel streaks 1cm apart in the center of the plate, without refilling the loop. The test specimen (fabrics treated ZnO nanoparticles & Control i.e. fabrics treated with ZnO bulk) was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with the agar surface. The plates were incubated at 37°C for 18-24 hours.

2.3.2 Quantitative tests

2.3.2.1 Percentage reduction test

Specimens of the test material were shaken in a known concentration of bacterial suspension and the reduction in bacterial activity in standard time was measured. The efficiency of the antimicrobial treatment is determined by comparing the reduction in bacterial concentration of the treated sample with that of control sample expressed as a percentage reduction in standard time. The evaluation of modified Hohenstein test was made on

the basis of the percentage reduction of bacteria by the sample. Percentage reduction was calculated using the following formula.

Where *R* is percentage reduction, *A* is the number of bacteria in the broth inoculated with treated test fabric sample immediately after inoculation i.e., at zero contact time and *B* is the number of bacteria recovered from the broth inoculated with treated test fabric sample after the desired contact period (18 hours).

2.4 Topographical analysis by SEM:

The topographical analysis of the test fabrics (finished with ZnO nanoparticles) and the fabrics finished with ZnO bulk were studied comparatively based on the Scanning Electron Microscopic analysis.

2.5 Wash durability of the finished fabric :

The wash durability testing of the finished fabrics was carried out using a neutral soap at 40º C (+/- 2º C) for 30 minutes, keeping the material : liquour 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 methods as described earlier .

Zinc Oxide (ZnO) Nanoparticle Preparation Commercially obtained ZnO nanoparticles (Himedia) with a size approximately <100 nm were procured. Stock suspensions with concentrations of 1 mg/mL were prepared by suspending in distilled water and sonication is carried out for about 5 minutes to allow better dispersion of nanoparticles and obtain homogenised suspension. Preparation of Staphylococcus aureus Culture Mannitol salt agar (MSA) is a selective medium used for the isolation of pathogenic staphylococci. On MSA, pathogenic Staphylococcus aureus produces small colonies surrounded by yellow zones. The reason for this change in colour is that S. aureus ferments the mannitol, producing an acid, which, in turn, changes the indicator from red to yellow. The growth of other types of bacteria is inhibited. S. aureus was grown in MSA medium at room temperature. The lysogeny broth was prepared in six different conical flasks. The flasks were treated with different concentrations of Zinc Oxide nanoparticles (20 µg, 40 µg, 60 µg,80 µg 100µg). Each of the conical flasks was inoculated with S. aureus strain and kept it in shaker for incubation at room temperature at 150 rpm. Antibacterial Test - The Agar Diffusion Test or Bauer-Kirby Test Staphylococcus aureus is inoculated over the dried surface of Muller-Hinton agar plate by streaking using L rod or swab with 12 hours incubated organism over the entire sterile agar surface. This procedure was repeated two more times, and the plate was rotated to ensure an even distribution of inoculum. The appropriate number of wells is made on the surface of agar plate and load with different concentrations of ZnO nanoparticles with 20µg/20µL to 100µg/100µL. After 16-18 hours incubation, each plate was examined and measured for the diameters of the zones of complete inhibition including the diameter of the wells. Colony-Forming Unit The serial dilutions of the bacterial suspensions were required. The plates are divided into numbered sectors. The inoculum suspension is deposited as drops of 0.02ml from a height of 2.5cm on to the medium where it spreads over an area of 1.5 – 2.0cm diameter. Each of the 6 plates receives one drop of each dilution in separate numbered sectors. The plates are incubated for 18 – 24 hours and observed for growth. Sectors where more than 20 colonies are present without any confluence are utilized to make the viable counts. Viable count per 0.02ml for a dilution is obtained by taking the average of counts for that dilution in all the six plates. Growth rate of Staphylococcus aureus Freshly grown bacterial inoculum is incubated in the presence of 20,40,60,80, 100µl/ml of Zinc Oxide nanoparticles are added in different flasks to observe the bacterial cell growth pattern at room temperature and 150 rpm. Total solution used in each flask is 50 mL of broth and the growth rate is indexed by measuring optical density (OD) at 600nm. The readings obtained were plotted and comparative studies are performed for different concentrations of ZnO nanoparticles. Estimation of Protein The Staphylococcus aureus culture samples have been taken in six different conical flasks. These samples were treated with different concentrations of zinc oxide nanoparticles ranging from 0, 20, 40, 60, 80 and 100 µg/mL. The cultures were then kept for incubation overnight at room temperature at 150 rpm in shaker. The samples are then taken after 24 hours for protein estimation by Lowry's method12. However for estimation of protein by Sodium dodecyl Sulphate Poly-Acryl amide Gel Electrophoresis (SDS-PAGE - 12%) samples have to be prepared separately Staphylococcus aureus strain, grown without agitation for 24 h at 37°C in lysogeny broth. Cells from 20 ml of culture were harvested by centrifugation at 10000 rpm, washed twice with phosphate- buffered saline without Mg2+ and Ca2+, and recentrifuged. The cells were suspended in 10 ml of ice-cold acetone (analytical grade), allowed to stand on ice for 5 min, and collected by centrifugation 10000 rpm. Samples treated with acetone for 5 to 30 min or the use of more acetone did not change the efficiency of protein extraction. The proteins extracted by incubating with 1.0 ml of 1% sodium dodecyl sulphate

III RELATED WORK

Antibacterial activity of ZnO Nanoparticles against S. aureus As the concentration of nanoparticles increases, the zone of inhibition also increases. This maybe due to the destructive effect of ZnO nanoparticles with the cells and increased production of active oxygen such as H2O2, leads to the cell death. ZnO nanoparticles, after its adherence to the surface of the cell membrane, results in disturbance in its respiration as it interact with enzymes of the respiration chains of bacteria. These results were in accordance with the result obtained by others9.

Colony Forming Units (CFU) in S. aureus when treated with ZnO Nanoparticles

CFU has reduced significantly with increasing ZnO nanoparticle loadings. Thus the present observation indicated that the growth rate of S. aureus is much affected by the ZnO nanoparticles. The growth rate of the bacteria is affected due to the interaction of the nanoparticles in the cells. Nanoparticles have larger surface area available for interactions which enhances the bactericidal effect than the large sized particles and hence they impart cytotoxicity to the micro organisms.Our results was in accordance with13 They reported the enhancing effect of ZnO nanoparticles on antibacterial activity13.

Growth rate of Staphylococcus aureus

At a lower concentration of nanoparticle i.e. at 20 µg/mL and 40 µg/mL the optical absorption is lesser than that at higher concentration. This has been attributed to the
reduced growth of the bacterial cells. Higher optical density means greater growth. Hence the concentrations of 20 µg/mL and 40 µg/mL of ZnO nanoparticles have been found to be more effective bactericides than higher concentrations when compared to the control this was due to the higher optical absorption. This suggests that the effect of ZnO nanoparticles is not entirely dependent on increasing concentration of nanoparticles. There is an optimum concentration which has higher effect compared to those concentrations that are more or less than the optimum.

Estimation of Protein ZnO nanoparticles, which have a good bacteriostatic effect causes membrane disorganisation in Gram Positive organism. The surface modification of ZnO nanoparticles causes an increase in membrane permeability and the cellular internalization of these nanoparticles. This causes changes in the level of Proteins14. The level of protein decreased as the concentration of the nanoparticle increases. This may be due to Zinc oxide nanoparticle which is toxic and reactive towards proteins. A possible explanation is that the antibacterial effect of ZnO is based on the abrasive surface texture of ZnO. ZnO nanoparticles have been found to be abrasive due to surface defects6 and they bind to protein molecules and as a result cellular metabolism is inhibited causing death of micro organism. It is believed that nanoparticles after penetration into bacteria inactivate their enzymes, generate Hydrogen peroxide and cause bacterial cell death15.

IV CONCLUSION

In prokaryotic systems, cell death due to interactions between reactive oxygen species (ROS) and proteins, DNA, or membrane structures can be induced by oxidative stress. There occurs a concern in toxicity and safety issues regarding the expanding growth of nanotechnology and nano biotechnology, and related industrial products. Because of their wide range of practical applications including their use in sunscreens and cosmetics, and these recent indications of their toxic nature, nano scale metal oxides such as ZnO is a current focus of the Nanotechnology Safety Initiative under National Institute of Environmental Health and Safety.
Experimental observations have explained significantly the antibacterial behaviour of Zinc Oxide (ZnO) nanoparticles. Previous studies with silver (Ag) nanoparticles, which is widely used as a biocide, showed that it is effective only with Gram negative bacteria. In the present study, it is well observed that the zinc oxide nanoparticles can be used as an effective biocide for Gram positive bacteria Staphylococcus aureus. From the results obtained in our study it is well understood that the proteins are the important biological molecules which are fundamental to the proper functioning of cells in the micro organisms.

The result of this paper raises other areas to be concentrated for further research to answer a number of questions before a concrete conclusion could be drawn. Innovative applications of nanotechnology using engineered catalysts exploiting unique properties of nanomaterials are capable of making an impact include the minimization of industrial wastes, sensors to detect toxic molecules in the biosphere. Improved nanostructured photocatalysts are useful for degrading toxic contaminants into benign frag- ments

utilizing solar energy. The concentration of toxic materials and infectious microorganism in the natural resources of drinking water is constantly increasing causing severe environmental pollution.

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