Synthesis and Characterization of Neem Chitosan Nanocomposites for Development of Antimicrobial Cotton Textiles

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ABSTRACT
Health and hygiene are the primary requirements for human beings to live comfortably and work with maximum efficiency. The present study focuses on the development of herbal chitosan nanocomposite finishes for protective clothing. The medicinal plant (*Azadirachta indica*) was selected, and bioactive compounds were extracted. The neem chitosan nanocomposites were prepared using multiple emulsion/ solvent evaporation method. The neem chitosan nanocomposites were finished on to 100% cotton fabrics using pad dry cure method. The antibacterial activity of the fabrics were assessed using standard AATCC 100 and 147 test methods. The neem chitosan nanocomposites treated fabrics showed an increased antimicrobial activity than the other fabric treatments (neem chitosan composite, neem, and chitosan). The scanning electron microscopic results showed that the nanocomposites were essentially spherical in the size range of 50-100nm.

Keywords: nanocomposite, *Azadirachta indica*, antibacterial activity, chitosan

INTRODUCTION

The textile and clothing industry normally seen as “traditional industry” is an important part of the European and Asian manufacturing industry. Because of the increased competition, the industries have to move towards more innovative, high quality products in order to differentiate themselves and compete with other competitors. In the development of fabrics, functional aspects such as anti-bacterial and UV protection are playing an increased important role [1,2]. To protect the mankind and to avoid cross contamination, a special finish like antimicrobial finish has become necessary. As consumers have become more aware of hygiene and potentially harmful effects of microbes, the demand for antimicrobial finished clothing is increasing [2-7]. Though a number of commercial antimicrobial agents have been introduced in the market, their compliance to the regulations imposed by international bodes like EPU is still unclear [8]. The herbal antimicrobial finishes overcome the disadvantages of chemical finishes. They will not cause damage to the fabrics, are eco-friendly, non-toxic, non allergic and since naturally occurring herbs are used, the cost factor is also feasible [9-12].

Chitosan, a natural polysaccharide, is a derivative of chitin, which is commonly found in shells and exoskeletons of some crustacean and is the second most abundant bio-polymer with unique structural and physiological characteristics [2, 13, 14]. They have a unique combination of properties such as biocompatibility, biodegradability and antibacterial activity which makes it an ideal polymer for industrial applications including textiles, agriculture, food science, pharmaceuticals and biomedical [2,15,16]. Nanoencapsulation, is a new technique rapidly emerging and widely used in Pharmaceutical, chemical, cosmetics, food processing and in recent years to textile finishing [1]. A potential application of nanoencapsulation with regards to finishing is the slow controlled release of the active ingredient (antimicrobial agent) to achieve the desired delay until the right stimulus is obtained [17]. Composites are formed by the combination of two or more materials that have quite different properties. These different materials work together to give the unique properties of the composite which is the materials’ individual properties. Hence, in the present study the cotton fabrics finished with neem chitosan composite were compared with those fabrics finished with neem chitosan nanocomposites by placing the neem treated and the chitosan treated fabrics as the basic material of comparison. The antimicrobial activity of the nanocomposite finished cotton fabrics were assessed against bacteria that normally exist in textiles like *E.coli* and *S. aureus* using standard AATCC 100 and 147 test methods. This study would bring out the best antimicrobial finish of the above combinations used.

COLLECTION, PROCESSING AND EXTRACTION OF NEEM

The medicinal herb selected for study was *Azadirachta indica* which was collected in and around Coimbatore. The collected leaves were shade dried at room temperature to reduce the moisture content. The 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 filterate was further used for the treatment of cotton fabrics [18].

PREPARATION OF CHITOSAN SOLUTION

Chitosan was procured from India Sea Foods, Cochin. About 1g of the procured chitosan was dissolved in 1% acetic acid (1ml of acetic acid in 99ml of distilled water).

PREPARATION OF NEEM CHITOSAN COMPOSITE

About 15ml of the Neem extract was added drop wise to the prepared chitosan solution under constant stirring.

PREPARATION OF NEEM CHITOSAN NANOCOMPOSITE

Neem Chitosan nanocomposites were prepared using Multiple Emulsion/ Solvent Evaporation procedure. 3% Chitosan solution was added with 5% (w/v) Tween 80 and placed in rotary shaker for 5 minutes. Neem extract was added to the emulsion and kept in the rotary shaker for 5 minutes. Simultaneously, 5% Span 80 was prepared with palm oil and stirred for 10 minutes. Both the solutions were mixed in 9:1 ratio and stirred well for 5 minutes. To this 0.01g of TPP (Tri poly phosphate) was added and kept in the rotary shaker for 5 minutes. The entire suspension was then incubated for ½ hour in a water bath at 50ºC and then cooled. Nanocomposites were segregated from the palm oil by repeated washing with petroleum ether. The pellets were dissolved in Phosphate buffer [17].

FABRIC TREATMENT

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. The prepared solutions (neem extract, chitosan solution, neem chitosan composite and neem chitosan nanocomposite) 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 above said solutions 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 1 kgf/cm^2 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 [19].

ANTIMICROBIAL ASSESSMENT OF FINISHED COTTON FABRICS

Test organisms: *Staphylococcus aureus*, *Escherichia coli*

Parallel Streak Method (AATCC Test Method 147-1988) [20]

Test specimens (non sterile) were cut into pieces (25mm x 50mm). A 50mm length permits the specimen to lay across 5 parallel inoculums streaks each of diminishing width from both 8mm to 4mm wide. 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 $37^{o}C$ for 18-24 hours.

Evaluation

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 Eq. (1).

$$\text{Zone of inhibition (mm)} = (T-I)/2 \qquad (1)$$

Where:

T - width of zone of inhibition,
I - width of specimen.
The antibacterial activity of the fabrics treated were interpreted using multivariable ANOVA analysis.

<u>Quantitative Bacterial Reduction Test (AATCC test method 100-2004) [21]</u>

About 1.0 ml of the test inocula (*Staphylococcus aureus* and *Escherichia coli)* were loaded on the swatches (treated and untreated) of 4.8±0.1cm diameter. They were then transferred to the respectively labeled sterile AATCC Bacteriostasis broth. After an incubation of 24 hours, serial dilutions were made up to 10^{-7} for all the samples. 0.1ml sample from each dilution were spread plated on to the sterile AATCC Bacteriostasis agar plates and incubated at 37 ^{o}C for 24 hours. The percentage reduction of bacteria by the treatment can be calculated by the formula Eq. (2).

$$R = 100(B-A)/B \qquad (2)$$

Where:
R= % reduction and A and B are the number of bacteria recovered from the inoculated treated and untreated swatches respectively.

WASH DURABILITY TEST

Washing was carried out as per test no:1 of IS: 687-1979 by using a neutral soap (5gpl) at $40^{0}C \pm 2^{0}C$ for 30 minutes, keeping the materialistic liquor ratio at 1:50, following by rinsing, washing and drying. After drying, the test samples were assessed for antimicrobial activity using AATCC 100 procedure up to 30 laundering cycles [22].

SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopic analysis was done to study the surface morphology of the fabrics. The surface morphology of the Neem chitosan nanocomposite treated fabric was studied using SEM (JEOL/EO JSM 6390). Metal coating was used as the conducting material to analyze the sample.

RESULTS AND DISCUSSION

<u>Antibacterial Efficiency</u>

Test Method–I: Qualitative assessment of the antibacterial activity of the treated cotton fabrics were carried out by Parallel Streak Method (AATCC 147). The zone of inhibitions obtained in the different treatments was observed and tabulated in *Table I* and *II.* The maximum inhibition of 14mm for *E.coli* and 20mm for *S.aureus* was found in the fabric treated with Neem chitosan nanocomposite. Such an increased zone of inhibition could be due to the combination of neem and chitosan in the form of nanocomposites (both being good antimicrobial agents by themselves). Satyavati *et al.*, [23] and Sairam, [24] had performed many clinical studies and have proved the antibacterial properties of the neem leaves. The use of chitosan as an antimicrobial agent in cotton fabrics was evident from the results of Zhang *et al*., [25]. Hence on the combination of both neem and chitosan in the form of nanocomposites, an increased zone of inhibition when compared to neem and chitosan treated fabrics were obtained.

Table III shows the values derived from multivariable ANOVA analysis using Microsoft excel 2007 version. Freom the tabulated values, there is a significant difference found between neem extract and chitosan solution at Factual> Fcritical (82.409 > 9.2766) at 95% confidence level and also noticed significant difference in all the different combinations (neem, chitosan, neem chitosan composite, neemchitosan nanocomposite). It may be due to the better activity of neem extract and chitosan nanocomposite against *E.coli* and *S.aureus* on the cotton fabrics.

Test Method-II: Quantitative Bacterial Reduction of cotton fabrics was carried out by AATCC 100 method in which the percentage in reduction of the test bacteria (*S. aureus* and *E.coli*) was confirmed. The results were calculated and tabulated in *Table IV*. Due to the combined activities of the two antimicrobial agents in the form of nanocomposites, the fabrics treated with herbal nanocomposite showed an increased bacterial reduction percentage when compared to other antimicrobial agents used in the study. It is evident that the herbal nanocomposites showed 100% reduction against *S. aureus* and 93% reduction against *E.coli*. Whereas the fabrics finished with the bulk neem chitosan composite reduced 98% of *S.aureus* and 89.1% of *E.coli*. This difference can be attributed to the fabric finishing with nanoparticles in the former, which paves the way for the better surface properties of the antimicrobial agent resulting in enhanced activity. While the neem and chitosan treated fabrics reduced 92% and 87% of *S.aureus* and 86.9% and 83.5% of *E.coli* respectively. The antibacterial activity of bulk *Azadirachta indica* and bulk chitosan were studied by Chopra *et al.*, [26] and Chung *et al*.,[27] respectively which proves the potent antibacterial property of the neem leaf extract and chitosan.

TABLE I. Comparison of Antibacterial (*E.coli*) Activity of Treated Cotton fabrics-Parallel Streak method (AATCC 147).

Antibacterial agent used	Antimicrobial Activity (Zone of inhibition in mm)			
	Trials			
	1	2	3	Ave
Neem extract	9.8	10.3	9.9	10
Chitosan solution	5	5	5	5
Neem chitosan composite	12.4	12.3	12.8	12.5
Neem chitosan nanocomposite	14	14.1	13.9	14

TABLE II. Comparison of Antibacterial (*S. aureus*) Activity of Treated Cotton fabrics-Parallel Streak method (AATCC 147).

Antibacterial agent used	Antimicrobial Activity (Zone of inhibition in mm)			
	Trials			Ave
	1	2	3	
Neem extract	15.9	15.9	16 .2	16
Chitosan solution	12.7	13.1	13 .2	13
Neem chitosan composite	18.8	19.2	19	19
Neem chitosan nanocomposite	20	20	20	20

TABLE III. Multivariable ANOVA Analysis.

Source of variance	SS	df	MSS	Fact	P value	Fcri t
Between neem extract and chitosan	77.7	2	25.9	82.4	0.002	9.27
Chitosan and neem chitosan composite	87.8	2	65.2	102.3	0.001	9.27
Neem chitosan composite and neem chitosan nanocomposite	67.8	2	90.2	61.4	0.001	9.27
Neem extract and neem chitosan nanocomposite	92.9	2	88.9	81.4	0.004	9.27

Note: SS-Sum of Square, df-Degree of freedom, MSS-Mean sum of square

TABLE IV: Comparison of Quantitative Bacterial Reduction of the Treated fabrics. (AATCC 100)

Antibacterial agent used	Bacterial reduction (%)	
	E.coli	*S. aureus*
Neem extract	86.9	92
Chitosan solution	83.5	87
Neem chitosan composite	89.1	98
Neem chitosan nanocomposite	93	100

Wash Durability Testing

The percentage reduction of the antibacterial activity after 30 laundering cycles were determined using Wash Durability Testing. The fabric samples treated with all the antimicrobials used in the study were subjected to laundering procedures. The fabrics were then tested for the percentage in reduction of the microorganisms using AATCC 100. The results were calculated and tabulated in *Table V.*

TABLE V. Wash Durability of the Treated Fabrics.

Fabric Treatments	No. of Laundering cycles	Bacterial reduction (%)	
		E.coli	*S.aureus*
Neem extract	5	86	91.5
	10	85.2	90
	15	83.3	88.7
	20	80	86.2
	25	76	83.4
	30	71	79
Chitosan solution	5	82.3	86.5
	10	80.9	84
	15	78.6	82.5
	20	75	80.2
	25	72.1	77.6
	30	69	73
Neem chitosan composite	5	88	97.2
	10	87.6	96
	15	87	94
	20	85	92.9
	25	82	91
	30	76	85
Neem chitosan nano composite	5	92.5	100
	10	92	100
	15	92	100
	20	92	100
	25	89	98.5
	30	86	97

From the tabulated results, it can be seen that the bacterial reduction of 100% exhibited by herbal nanocomposites against *S.aureus* have sustained for 20 laundry washes. After this, there occurs a decrease in the reduction percentage during the consecutive washing. This retention of the activity by the finished fabrics can be attributed to the uniform coating of the nanoparticles and the sustained release of the active principle from the nanocomposites. Similar results were obtained by Wen-Li Du *et al* [10] and Thilagavathi *et al.*, [3] who worked on micro and nanocapsules. With respect to the other antimicrobial finished fabrics subjected to wash durability, the bacterial reduction percentage has been found to decrease constantly owing to the fact that these antimicrobials do not have a sustained release unlike the nanocomposites. This meager retention in the reduction percentage exhibited by these fabrics during the initial washes could be attributed to the usage of citric acid as the fixative agent as opined by Thilagavathi *et al.*,[3].

SEM Analysis

The surface morphology of all the antimicrobials finished cotton fabrics were studied using SEM (JEOL/EO JSM 6390). Here the metal coating was used as the conducting material to analyze the sample. Herbal nanocomposite treated fabrics were subjected to SEM analysis with a particle magnification of 100,000X, 25kV.

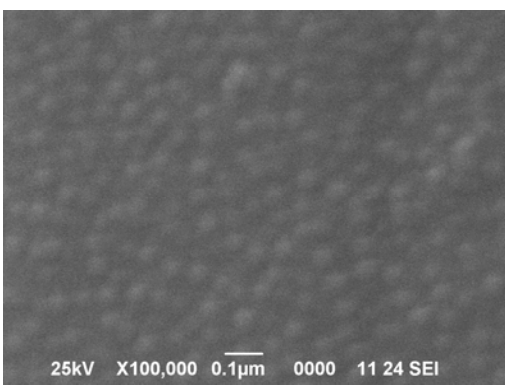

FIGURE 1. SEM micrograph of the neem chitosan nanocomposite finished fabric.

Figure 1 shows the uniform coating of neem chitosan nanocomposites on the fibers of the cotton fabric with a particle size ranging 30nm.This accounts for the enhanced antibacterial activity of these nanocomposites when compared to other antimicrobial finished fabric which is evident from the results of the AATCC Standard procedures. The surface properties of the nano particle allow for the sustained and slow release of it from the fabric on washing.

CONCLUSIONS AND PROSPECTIVE FUTURE RESEARCH

The antimicrobial activities of the nanocomposites treated cotton fabrics were found to be higher than that of the fabrics treated with neem and chitosan separately which was substantiated using AATCC 147 and AATCC 100 tests. Neem chitosan nanocomposite treated fabric exhibited a better bacterial reduction until 30 washes. This study, on herbal nanoparticles will pave way for more research in the areas of polymer material, size, wall thickness, core material, time of emulsification, type of solvent, cross linking and kinetics of the release of the nanocomposites.

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