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Antimicrobial activity of cotton and silk fabric with herbal extract by micro encapsulation

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ABSTRACT

Objective: To explore the antimicrobial activity of microcapsules encapsulated with mixture of herbs like neem, tulsi and turmeric, and its application on cellulosic fabric in the form of microcapsules. **Methods:** The microcapsules were prepared from the mixture of herbs by plain diffusion method, a natural encapsulation technique with yeast and applied on cotton and silk fabric by pad-dry-cure method. The microcapsules were fixed on cotton and silk fabric using the binder UF Silpure FBR-5(PA)B at 120 ℃. The antimicrobial activities of the finished fabric were assessed by using three types of bacteria including *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas*. **Results:** The results of antimicrobial activity from the tests including parallel streak method and disc diffusion method showed that activity of the mixture of herbs was very effective among the three types of bacteria selected, and the antimicrobial activity of prepared microcapsule against *Pseudomonas* was very good. **Conclusions:** The herbal microcapsule treated fabric could be applied in the field of medicine. The scanning electron microscope photographs ensure the fixing of the microcapsules firmly in the yarn structure of plain woven cotton and silk fabric.

1. Introduction

Micro encapsulation may be defined as the micro packaging technique where in active core materials are encapsulated in a polymer shell of limited permeability[1]. The objective of this technology is either to protect the active core material from the external environment till required or to affect the controlled release of the active core on active desired delay until the right stimulus is encountered. This process is initially developed for the carbonless copy industry. However, it has now attracted the attention of wide range of industries including pharmaceutical, agriculture, chemical, food processing, cosmetics and also textiles[2,3].

Antimicrobial fabrics gained significant importance in the recent years due to its wide acceptance as surgical apparels, baby clothing, undergarments etc[4]. In this present investigation, herbal plant extracts are used as antimicrobial finishing agents. The control of bacteria, fungi and dust mites can be achieved using normal textile finishing process to create a value added product with a strong appeal for consumers[5,6].

In a liquid mixture, molecules can either be in solution, or suspension, or they will precipitate (settle out and usually to the bottom or top). Molecules (in our case, photochemical) that have been extracted by a solvent from the plant material will precipitate out if the solvent is removed. We remove solvent so to prevent precipitation, and encapsulate (surround) the solutes with other molecules to isolate them from the remaining solvent deficient liquid in a sense[7]. The micro sphere is called a micelle and they range from1 to 100 microns in size. When newly made regular tinctures or fluid extracts are saturated and suffer precipitation on storage due to agglomerization of solutes in the presence of solvents like a mixture of alcohol and water[8], and solvents are removed through evaporation, the phytochemcials get precipitated which can be filtered off if the final product is a liquid. The mixture can still be at "saturation" meaning it holding all the molecules in solution that it can. However, part of the herb has been lost, filtered or decanted away. It is no longer "holistic" at that point. If all the liquid is evaporated, then every thing left is a precipitate and the extract is known as a concentrate. It may, however be un-absorbable by the body at this point. So as to have an holistic approach we made microcapsules of herbal extracts and fixed in the cotton and silk fabrics using ultra-fresh (UF) silpure[9,10].

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2. Materials and methods

The core material was defined as the specified material to be coated and it can be either solid or liquid in nature. The core material of herbal extract mixture were from leaves of neem (*Azadirachta indica*), leaves of turmeric (*Ocimum sanctum*) and roots of tulsi (*Curcuma longa*), which were in semisolid form.

Fresh yeast was used as the wall material, with good capsulating capacity compared with other natural products. UF silpure FBR -5 (PA) was taken as the binder with good adherence of the microcapsules to materials.

Woven fabric of cotton and silk were initially soured and bleached. Plain weave cotton (60 s count, EPI of 120, PPI of 92) and silk (60 s count, EPI of 124, PPI of 94) were taken for the study. The microcapsules were applied by pad-dry -cure method.

2.1. Preparation of herbal extract

1 g of each core material (Neem, tulsi and turmeric) was taken separately and distilled water was added to the float level. 1% of chloroform was added to each of the core material in a distilled bath and allowed to dissolve for 24 hours.The extracts were filtered and kept in the water bath, heated until a semisolid form was obtained. The extracts were kept for drying in the sunlight till the standard form was obtained which would take 3 to 5 days. The standard form of core material obtained including neem in semi liquid residue, tulsi in semisolid residue and turmeric in dry coarse powder.

2.2. The effect of cell viability on encapsulation of herbal drug in yeast cells

To assess the effect of cell viability on the encapsulation process, cells were pre-treated prior to the encapsulation process. 50 g of the suspension of fresh yeast was treated overnight with 2 g of sodium azide which was a respiratory inhibitor used to prevent the cell from performing energy dependent processes. The solution was kept for an overnight. These cells were taken and sterilized in autoclave at 120 ℃ for 20 minutes prior to encapsulation. Sterilization by autoclaving is a thermally destructive process denaturing any carrier protein molecules likely to be involved in facilitated diffusional process, which may be responsible for encapsulation process.

2.3. Preparation of microcapsules

45 g of he herbal extract (drug), 180 mL of yeast and 540 mL of distilled water were taken at standard volumetric ratio of 1∶4∶8. The concoction was agitated in a gallenkamp rotary incubator at 100 rpm of 40 ℃ for 4 hours. These cells were centrifuged for 10 minutes then wash was given for five cycles with distilled water and freeze dried for 2-4 days.

2.4. Application of microcapsules on cotton and silk material

The microcapsules were fixed in cotton and silk fabric with a binder by pad-dry method. The microcapsules were diluted in water at the concentration of 6 gm/L along with 1.5 mL of binder (ultra fresh-silpure). The microcapsules were applied on fabric by pad-dry cure method with material at liquor ratio of 1∶10 and pH of 5.5-6. The curing was carried out at 120 ℃ for 2 minutes.

2.5. Parallel streak method (AATCC 143 1993)

Dispensed sterilized nutrient (or appropriate medium) agar, which was cooled to (47±2) ℃ by pouring (15±2) mL into each standard flat-bottomed Petri dish (15 mm×100 mm). Set agar firmly before inoculating.

Prepared inoculum by transferring (1.0±0.1) mL of a 24 hours broth culture into (9.0±0.1) mL of sterile distilled water contained in a test tube or small flask. Mixed well using appropriate agitation. Used a 4 mm inoculating loop, loaded one loopful of the diluted inoculum agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart covering the central area of a standard Petri dish without refilling the loop. Did not to break the surface of the agar while making the streaks. Gently pressed the test specimen transversal across the five inoculum streaks to ensure intimate contact with the agar surface. This would be accomplished more easily by pressing the specimen to the agar surface with a biological section lifter or with a spatula, which had been sterilized by flaming and then air, cooled immediately before using. If the specimen curled, prevented from intimate contract with the inoculated surface, placed sterile glass slides on the ends of the specimen to hold it in place. Incubated at (35± 2) ℃ for 18 -24 hours.

Examined the incubated plates for interruption of growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge. The average width of a zone of inhibition along a streak on either side of the test specimen was calculated using the following equation.

$W = (T-D)/2$

W = width of clear zone of inhibition in mm; T= total diameter of test specimen and clear zone in mm; D= diameter of the test specimen in mm.

2.6. Disc diffusion methods

Cups were cut in the medium using sterile cork borer about 10 mm in diameter, and the agar disc were removed by vaccum device or a splayed-out steel pen nib. Cylinders of plain steel, glazed porcelain, pyrex glass or sterilisable plastic with external diameter of about 8 mm and a height of about 10 mm. These were usually warmed so that they sank slightly to a constant depth when placed on the agar. Filter paper of cellulose discs was used to absorb a fixed volume of solution. Standard ceramic insulation beads (fish spine beads) were used which could attract a fixed volume as touched on the surface of the solution. The surface of the agar would be dry.

The test and standard solutions were placed in the container randomli in order to prevent the bias that could be caused by a regular order of plating. The volume was critical for the cup method but not significant when cylinders were used provided they were at least two-thirds full. Did not seal the tops of the cylinders with the lid of the plate. The plates were left at room temperature for 2 hours to allow diffusion of the antibiotic to get ahead of growth of organisms. Then they were incubated at the appropriate temperature, usually for about 16 hours. After incubation, inhibition of the growth could be seen as a clear zone around each container. The diameter of this was proportional to

the log of concentration of the antibiotic. As soon as each diameter was measured, an optical system was used to projecte the image of the plate on to a large grid. 2 diameters at the right angles were used as a check on ellipticity of the zone.

The results could be processed in two ways:

(1)A graph was plotted in log concentration of standard against zone diameter and the results for the test preparation were plotted on the same graph. If these two liners were parallel, the relative potencies of the standard and test were represented by the horizontal distance between the lines.

(2)Parallelism between the lines could be confirmed mathematically and the potency of the test was obtained by calculation.

2.7.Air permeability tester

Prior to test, these samples were conditioned to moisture equilibrium in the standard atmosphere of (65±2)% relative humidity and (27±2) ℃ from dry side as laid down in Is:6359-1971. The test was carried out in the standard atmosphere.

Took the conditioned specimen and mounted a portion between the clamp and circular orifice with sufficient tension to eliminate wrinkles to see if the fabric was distorted in its own place. Started the suction fan or other means to force the air through the fabric and adjusted the rate of flow air till pressure drop of 1 cm water head across. Noted the rate of flow of air in cm^3/s. Repeated the test at different places. Carried out at least 5 tests.

Calculated the rate of flow of air per cm^2 of fabric in cm^3/s by the following formula:

R = r/a

R = rate of flow of air/cm^3 of fabric in cm^2; r=mean rate of flow of air in cm^3/s and a=area cm^2 of fabric under test in cm^2 .

The prepared microcapsules were applied on the cotton and silk fabric by pad-dry cure method and the presence of microcapsules were examined at the magnification level of 1 000× and 2 000× for cotton and silk fabrics, respectively under Scanning Electron Microscope (SEM). The morphological analysis of the treated fabric and microcapsule was given in the photographs using the optical microscope and QUANTA SEM.

3. Results

3.1. Antimicrobial activity of microcapsules

Figure 1 showed the disc diffusion results of mixture of microcapsules containing neem, tulsi and turmeric against *Staphylococcus*, *Escherichia coli* and *Pseudomonas*. It was found that the microcapsule from the Table 1 shows higher zone of inhibition against the *Pseudomonas* was 12.5 mm compared to the *Staphylococcus* and *Escherichia coli*.

3.2.Antimicrobial activity of fabrics treated with microcapsules

It was found that the *Pseudomonas* showed higher zone of inhibition against *Staphylococcus* and *Escherichia coli*.

3.3. Air permeability test

The comparison of air permeability between control and the treated fabric showed that the air permeability was less in the treated fabric than in the control fabric in case of cotton. This may be due to the less cover factor of cotton than silk which showed no change in air permeability after the treatment (10.32% reduction). The air permeability of silk fabric was the same for both the treated fabric nand the control fabric in case of silk (Table 2&3).

3.4. Evaluation of microcapsule treated fabric under SEM

Bifocal microscopy of the microcapsules containing the herbal extract was shown in Figure 2, which showed the surface characteristics and the microcapsules with the minimal size, without any magnification and also burst characteristics.

Figure 3&4 showed the SEM photograph of the treated samples. From the SEM photos it was inferred that the microcapsules were entrapped between the yarn structures in a very strong condition.

Table 1
Disc diffusion method.

Types bacteria	Capsule size (mg)	D (mm)	T (mm)	W = (T-D)/2 (mm)
Pseudomonas	5	3	13	5
	10	4	20	8
	15	5	30	12.5
Staphylococcus	5	3	11	4
	10	4	18	7
	15	5	27	11
Escherichia coli	5	3	9	3
	10	4	12	4
	15	5	15	5

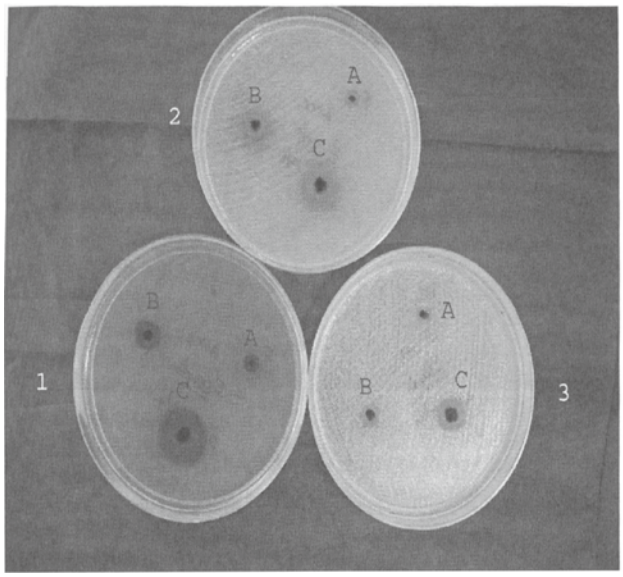

Figure 1. Antimicrobial activity of microcapsules.

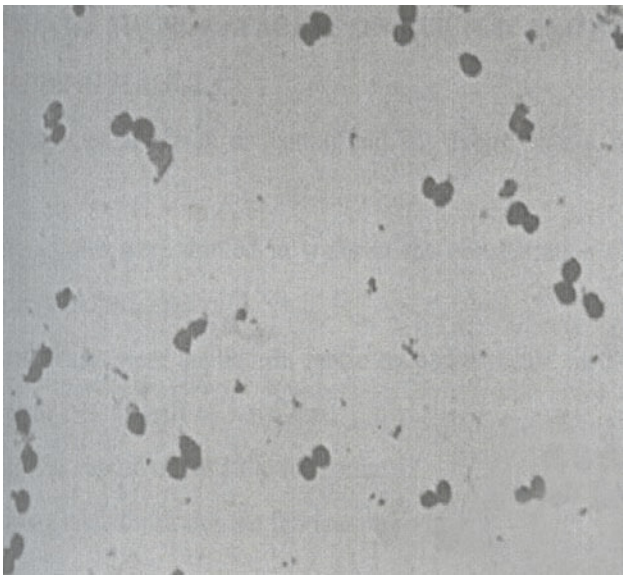

Figure 2. Microcapsules.

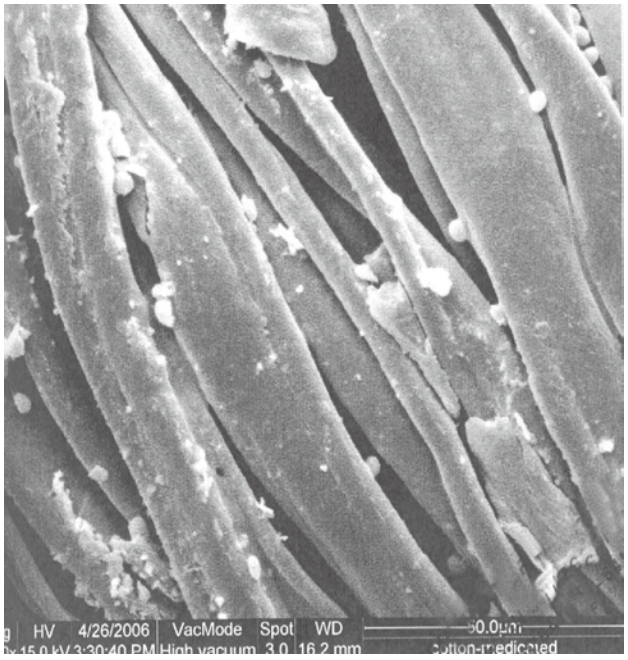

Figure 3. SEM photo of treated cotton.

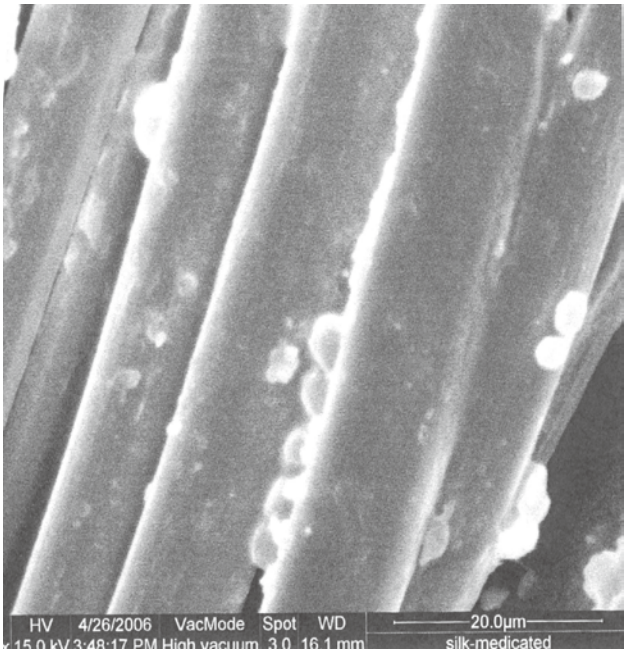

Figure 4. SEM photo of treated silk.

Table 2
Air permeability of cotton fabric.

S.NO.	1000 (lph)		100 (lph)		10(lph)		Total		Air permeability($cm^3/cm^2/sec$)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	2500	2050	475	390	58	35	3033	2475	210.62	171.87
2	2500	2000	475	220	55	37	3030	2257	210.41	156.73
3	1500	1500	175	225	40	38	1715	2313	119.09	160.62
4	1400	1500	150	250	38	35	1588	1785	110.27	123.95
5	2400		350	210	40	38	2790	1748	193.75	121.38
				Average					163.82	146.91

Table 3
Air permeability of silk fabric.

S.NO.	1000 (lph)		100 (lph)		10(lph)		Total		Air permeability($cm^3/cm^2/s$)	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	800	980	175	110	19	21	994	1111	69.03	77.15
2	1000	850	150	100	19	20	1169	970	81.18	67.36
3	1050	950	150	125	18	19	1218	1094	84.58	75.97
4	1000	1100	150	150	18	19	1168	1269	81.11	88.12
5	950	1050	150	155	19	19	1119	1224	77.70	85.00
				Average					78.72	78.72

4. Discussion

A microcapsule of herbs mixture like neem, turmeric and tulsi has been developed by natural encapsulation technique using yeast.Neem was used in the form of semi-liquid residue, tulsi in the form of semi-solid residue, turmeric in the form of dry coarse powder, and fresh yeast was used as the wall material with very good encapsulating capacity compared to other natural products. UF silpure, FBR-5 (PA) was taken as the binder with good adherence of the microcapsule to the finish of the material.

The treated fabric shows effective antimicrobial activity. The micro capsule impregnated in the textile containing neem, tulsi and turmeric is against *Staphylococcus*, *Escherisia coli* and *Pseudomonas*. It is found that the microcapsules shows higher zone of inhibition against the *Pseudomonas* compared to *Staphylococcus* and *Escherisia coli*.

The antimicrobial activity of treated fabric confirms that it can be applied for medicinal fabric. The zone of inhibition of *Pseudomonas* is higher and the air permeability test is less in treated fabric, then in controlled fabric in case of cotton. The comparison of air permeability in control and the treated fabric in silk is analyzed. And due to this, it can be applied for medicinal fabric. Since the amount of permeability of air can be controlled through the fabric and the medicinal properties impregnated on the fabric helps to control various forms of micro organisms when they attack the medicinal fabric.

The presence of microcapsule in the finished sample is visualized by a SEM and photographs are taken at various magnification level. The SEM does not show any bursting characteristics of the micro capsule. The surface morphology is very clear with no leakage of the material form which is impregnated microcapsule on the fabric.

Conflict of interest statement

We declare that we have no conflict of interest.

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