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Dyeing and antimicrobial characteristics of chitosan treated wool fabrics with henna dye

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ABSTRACT

Chitosan, a naturally available biopolymer which is now increasingly being used as a functional finish on textile substrates to impart antimicrobial characteristics and increase dye uptake of fabrics was applied on wool fabrics. Henna a natural dye with proven bactericidal properties was applied on wool fabrics along with chitosan to impart antimicrobial characteristics. The effect of chitosan application on the dyeing properties of wool fabrics was studied by measuring the *K*/*S* values of the treated substrates at various concentrations of chitosan and the dye. The antimicrobial properties of chitosan and natural dyes both when applied independently and collectively on fabrics were assessed. The results proved that the chitosan treated wool fabrics showed increase dye uptake of fabrics. The treated fabrics were found to be antimicrobial and the chitosan treatment enhances the antimicrobial characteristics of the dyes. Fastness properties of the applied finish to washing, rubbing and perspiration have also been discussed.

1. Introduction

The population explosion and the environmental pollution in the recent years have forced researchers to find new health and hygiene related products for the well being of mankind. Textiles find immense applications in day to day life and there has been a growing need to develop finishes for textile materials that can offer improved protection to the users from microbes (bacteria, mould or fungi), which cause numerous problems. The large surface areas and ability to retain moisture make textile fabrics more prone to bacterial growth. Clothing and other textile materials can act as carriers for microorganisms such as pathogenic or odour-generating bacteria and moulds and when in contact with the human body, they offer an ideal environment for microbial growth, providing oxygen, water and warmth, as well as nutrients from spillages and body exudates (Gupta, Khare, & Laha, 2004). Hence there is a pressing need to develop textiles that are resistant to microbes as the textile substrates find various applications such as masks, hospital covers, surgical gowns apart from the conventional apparel usage.

Antimicrobial textiles are classified as those textile and fibrous materials subjected to various finishing techniques to afford protection for both the user of textile materials (against bacteria, yeast, dermatophytic fungi and other related microorganisms for aesthetic, hygienic or medical purposes) and the textile itself (biodeterioration caused by mould, mildew and rot producing fungi) without negatively affecting the other important characteristics of the textiles (Clemo, 2004; Nagwekar & Irwin, 1984; Ramachandran, Rajendrakumar, & Rajendran, 2004; Saravanan, 2005; Sarkar, Purushottam, & Chauhan, 2001). Commercially available biopolymer, chitosan (Fig. 1) has many chemical attributes to make it an interesting candidate for antimicrobial applications. The treatment of chitosan to textiles is considered as multi-functional finish as the chemical attributes not only contribute to the antimicrobial properties but also result in enhancement of colour strength thus generating much interest towards chitosan (Bandyopadhyay, Seth, & Moni, 2001; Sang-Hoon & Samuel, 2003; Seungsin, Jeong-Sook, & Gilsoo, 1999). Apart from increasing the colour strength of fabrics with synthetic dyes (Julia, Pascual, & Erra, 2000; Sang-Hoon & Samuel, 2004), they also established the application as an auxiliary in printing of textiles. It has been reported that the printed samples have comparable colour fastness to that of commercial printed samples but for its stiffness of the fabrics (Bahmani, East, & Holme, 2000).

Natural products (plant, animal or mineral origin) have been known for a long time for dyeing as well as medicinal properties.

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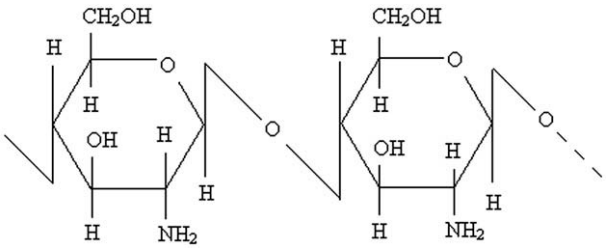

Fig. 1. Chemical structure of chitosan.

Many of the plants used for dye extraction are classified as medicinal and thus can be useful antimicrobial agents (Gupta, Jain, & Panwar, 2005; Hans & Yang, 2005). However, henna (*Lawsonia inermis*) is one such natural dye and it contains Lawsone a red–orange pigment (the molecule of which is also known as hennotannic acid is the chief constituent of henna leaves). Chemically, the molecule of Lawsone is 2-hydroxy-1,4-naphthoquinone structure of which is shown in Fig. 2. Industrial classifications also describe Lawsone as Natural Orange 6 and CI 75480, it acts as a substantive dye for keratin and imparts orange colour (Gulranjani, Gupta, Varsha, & Manoj, 1992). The aqueous extract of henna leaf is found to inhibit the growth of both Gram-positive and Gram-negative bacteria. In addition, henna leaf extracts are capable of inhibiting the growth of microorganisms that are involved in causing burn wound infections. Use of henna in management of burns may offset the complications that arise in the use of conventional wound dressings such as silver nitrate, which imparts stains, and is time consuming apart from being able to cause hyponatraemia or hypokalaemia (Malekzadeh, 1968; Muhammad & Muhammad, 2005; Habbal, Al-Jabri, El-Hag, Al-Mahroogi, & Al-Hasmi, 2005; Riffel et al., 2002). It has been proven through some preliminary studies that chitosan improves the dye uptake of fibers. However, the combined antimicrobial effect of chitosan and natural dyes has not been explored. Hence the aim of the present study is to investigate the combined effect of chitosan and henna on dyeing of wool fabrics and its antimicrobial properties.

2. Materials and methods

2.1. Materials

Commercial scoured wool fabrics with plain weave (26^S Ne × 23^S Ne) were purchased and used for antimicrobial finishing. Chitosan with degree of deacetylation of 0.82 viscosity 300 cps obtained from Central Institute of Fisheries Technology as a gift sample was used as such for coating of the fabrics. Commercially available henna (leaves) powder was used as such for dyeing. Acetic acid was used for preparation of chitosan solution, ethanol and distilled water were used for carrying out dyeing.

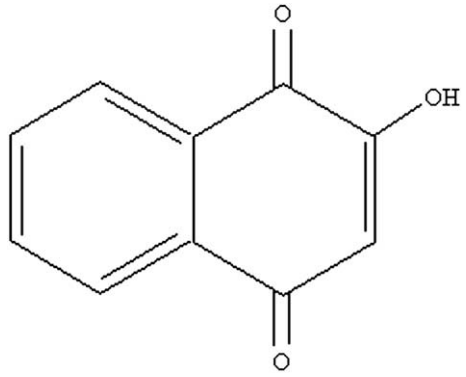

Fig. 2. Chemical structure of Lawsone.

2.2. Coating with chitosan

Chitosan solutions of concentrations 0.5%, 1.0% and 1.5% (w/v) were prepared in 2.0% (v/v) aqueous acetic acid by stirring the dispersion for 1 h at 60 °C. The scoured fabrics were than immersed in chitosan solutions of different concentrations for 24 h at room temperature. The fabrics were then padded and cured in the curing chamber at 120 °C for 5 min.

2.3. Dyeing with natural dyes

The henna powder was immersed in water–ethanol mixture (90:10 v/v) for 24 h and extracts were obtained. The dyeing was carried out using the extracts in water–ethanol mixture (90:10 v/v) for 90 min at 60 °C with material-to-liquor ratio 1:50. The dyeing was carried out for three different shades % viz., 5%, 10%, 15% (owf) for the dyes. The samples were then washed and dried.

2.4. Determination of colour strength and related parameters

Reflectance values of the treated samples were measured using UV–Vis spectrophotometer (U-3210, Hitachi, Japan) at λ_{max} and K/S value of the fabrics were determined using the Kubalka–Munk equation given below

$$\frac{K}{S} = \frac{(1 - R_{\lambda_{max}})^2}{2R_{\lambda_{max}}}$$

where K is the coefficient of absorption; S is the coefficient of scattering; $R_{\lambda_{max}}$ is the reflectance value of the fabric at peak wavelength.

The colour difference and relative colour strength between chitosan coated dyed samples and uncoated dyed samples were also obtained using following relationships.

$$\text{Relative colour strength } (\%) = \frac{\text{K/S of treated sample}}{\text{K/S of untreated sample}} \times 100$$

$$\Delta E = \sqrt{(\Delta l)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where $\Delta L = L_{coated} - L_{uncoated}$; $\Delta a = a_{coated} - a_{uncoated}$; $\Delta b = b_{coated} - b_{uncoated}$'$L$' describes lightness; '$a$' measures redness or greenness and 'b' measures yellowness or blueness.

2.5. Determination of fastness properties

The treated samples were washed as per the conditions specified in the test method IS 3361:1988 to determine the change in colour and staining of adjacent fabrics after washing. Rubbing fastness tests were carried out according to the test method IS 766:1988. The treated materials were evaluated for their perspiration fastness using the test method IS 971:1983.

2.6. Antimicrobial activity of treated fabrics

ASTM E 2149-01 was used to analyze the antimicrobial activity of the treated wool fabrics. The organisms taken for this study were *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). To evaluate the antimicrobial activities of the treated fabrics, the reduction in colony number between the treated and untreated fabrics after incubation was determined. The percentage reduction was calculated with the following equation

Table 1
Spectrophotometer characterization of henna dyed samples

Dye concentration (% owf)	Chitosan concentration (% w/v)	*K*/*S*	Relative colour strength (%)	L^*	a^*	b^*	ΔE
3	Untreated	0.3439	100	57.37	16.82	33.10	–
	0.5	0.4707	137	56.44	13.77	32.56	3.23
	1.0	0.5341	155	55.24	11.93	32.43	5.37
	1.5	0.6218	180	53.63	12.10	31.33	6.27
9	Untreated	0.5726	100	49.97	18.90	36.25	–
	0.5	0.7688	134	48.65	14.98	36.01	4.13
	1.0	0.8735	153	46.98	14.33	31.44	7.27
	1.5	0.8901	156	46.96	13.85	32.32	7.06
15	Untreated	0.8167	100	45.94	17.98	33.59	–
	0.5	0.8525	104	45.74	16.95	34.76	1.57
	1.0	0.8845	108	45.88	15.96	34.90	2.40
	1.5	0.9406	115	44.68	16.62	33.72	1.85

$$\text{Reduction rate } (\%) = \frac{(B-A)}{B} \times 100$$

where '*B* and *A*' are the numbers of bacteria recovered from the untreated and the treated wool fabrics, respectively, after inoculation and incubation.

2.7. Statistics

All quantitative results were obtained from triplicate samples. Data were expressed as means ± SD. Statistical analysis was carried out using the unpaired Student's *t*-test. A value of $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Colour strength and related parameters

Chitosan has been proved to increase the rate of dye uptake and dye exhaustion of wool fabrics in the case of acid and reactive dyes (Sang-Hoon & Samuel, 2003; Stephen & Yulin, 1994). However, the effect of dye uptake of chitosan on fabrics when natural dyes are used has not been investigated so far and therefore it has been taken up in the present study. *K*/*S* value of a dyed material is directly proportional to the amount of dye present in the material. The *K*/*S* values and the relative colour strength of henna dyed samples are given in Table 1. From the tables it can be seen that the *K*/*S* values of chitosan coated dyed fabrics are higher than that of uncoated dyed fabrics. As the chitosan concentration increases, the dye uptake also increases which is reflected in the values. This enhancement in (*K*/*S*) values of chitosan treated wool fabrics is associated with the introduction of chitosan primary amino groups into the fiber structure. The SEM micrographs of the chitosan treated fabrics and untreated wool fabrics are shown in Fig. 3. The photographs reveal the deposition of chitosan on the treated fabrics. Table 1 also shows that the relative colour strength of the samples increases with the increase in chitosan concentration for each shade % of the dye. This result further affirms that chitosan increases the amount of dye uptake in the treated wool fabrics.

To evaluate the colour parameter and the colour difference, CIE Lab system is used, where $L*$ refers to lightness–darkness values from 100 to 0 representing white to black, $a*$ values run from negative (green) to positive (red) and $b*$ values run from negative (blue) to positive (yellow) and the total colour difference is given by ΔE. Lower $L*$ values indicate that the sample becomes darker to that of control sample. It can be seen from Table 2 that the $L*$ values decrease with the increase in chitosan concentration indicating that the sample becomes darker compared to that of the control sample. The colour difference (ΔE) values are also given in Table 1 and from the tables it can be clearly seen that there is a significant colour difference between the samples treated with different chitosan concentrations though dyed to the same shade %.

3.2. Determination of fastness properties

Textiles are subjected to frequent washing, rubbing and perspiration during their usage. Hence durability of the finish applied on the textile material to these conditions is extremely important and hence has been assessed and is given in Table 1. Wash fastness ratings for staining of adjacent fabrics in case of henna dyed samples are very good (4–5) and those for change in colour are also good

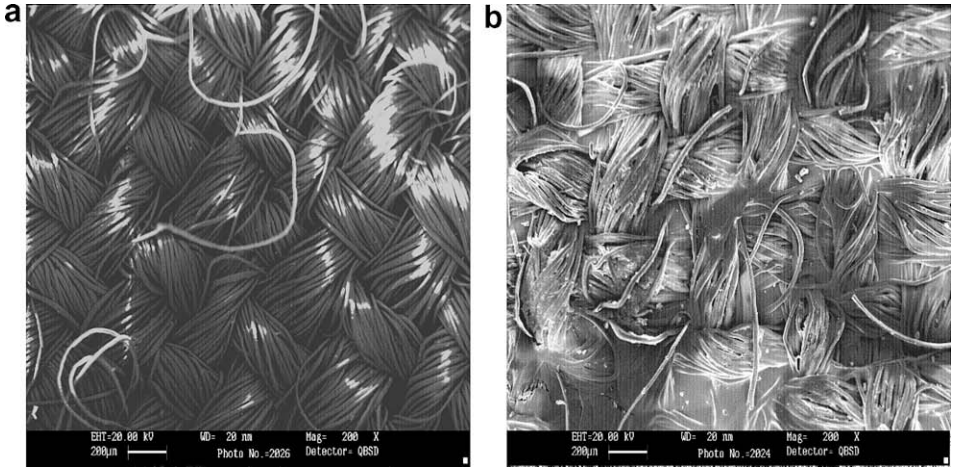

Fig. 3. SEM photographs of the surface of untreated and chitosan treated cotton fabrics. (a) Untreated wool fabric (b) 1.5% chitosan treated wool fabric.

Table 2
Fastness rating of henna dyed samples

Dye concentration (% owf)	Chitosan concentration (% w/v)	Washing fastness			Rubbing fastness				Perspiration fastness			
		Assessment of change in colour	Assessment of staining		Assessment of staining		Assessment of change in colour		Assessment of staining			
									Acidic		Alkaline	
			With cotton	With wool	Dry	Wet	Acidic	Alkaline	With cotton	With wool	With cotton	With wool
3	Untreated	2–3	4–5	4–5	4–5	4	2–3	2–3	4–5	3	4–5	3
	0.5	2–3	4–5	4–5	4–5	3–4	2–3	2–3	4–5	3	4–5	3
	1.0	2–3	4–5	4–5	4–5	4	2–3	2–3	4–5	3	4–5	3
	1.5	2–3	4–5	4–5	4–5	4	2–3	2–3	4–5	3	4–5	3
9	Untreated	2–3	4–5	4–5	4–5	4–5	2–3	2–3	4–5	3	4–5	3
	0.5	2–3	4–5	4–5	4	4	2–3	2–3	4–5	3	4–5	3
	1.0	3–4	4–5	4–5	4	4	2–3	2–3	4–5	3	4–5	3
	1.5	3–4	4–5	4–5	3–4	3–4	2–3	2–3	4–5	3	4–5	3
15	Untreated	2–3	4–5	4–5	4–5	4–5	2–3	2–3	4–5	3	4–5	3
	0.5	2–3	4–5	4–5	4–5	4	2–3	2–3	4–5	3	4–5	3
	1.0	3–4	4–5	4–5	4–5	4–5	2–3	2–3	4–5	3	4–5	3
	1.5	3–4	4–5	4–5	4	3–4	2–3	2–3	4–5	3	4–5	3

(3–4). It should be noted that the improvement in change in colour is observed at higher dye and chitosan concentration. Rubbing fastness of the samples assessed in terms of dry and wet rubbing indicates very good fastness to rubbing for dry (4–5) and wet (3–4). Perspiration fastness properties (acidic and alkaline) of the samples in terms of ratings for staining of adjacent fabrics and change in colour are also given in Table 1 for henna dyed samples. In case of henna dyed samples, the ratings for staining of adjacent fabrics are very good (4–5) with wool as adjacent fabric are also good (3) with wool as adjacent fabric, while ratings for change in colour are fair (2–3) in case of both acidic and alkaline solutions as shown in Table 2. The lower ratings for change in colour at both acidic and alkaline conditions indicate that the sensitivity of the chitosan dyed samples related to pH. This may be due to the degradation of the dyes at acidic and basic conditions.

3.3. Antimicrobial activity of treated fabrics

The antimicrobial activities of fabrics have been tested for the various possible treatment combinations viz., treatment with chitosan alone; dyeing with the natural dyes without chitosan treatment and treatment with chitosan followed by dyeing with natural dyes. The antimicrobial activities of the treated fabrics have been determined using the ASTM test method E2149-01. The microorganisms chosen for the study were *S. aureus*, a pathogenic Gram-positive bacterium, which is the most frequently evaluated species, as it is the major cause of cross-infection in hospitals as well as in commercial and home laundry practices. It causes skin and tissue infections, septicemia, endocarditis and meningitis (Bhat, Nagasampagi, & Sivakumar, 2005) and *E. coli* a Gram-negative bacterium, which is a popular test organism and is resistant to common antimicrobial agents. It causes urinary tract and wound infections, common in gastrointestinal tract and accounts for 25% of hospital infections (Bhat et al., 2005).

The microbial reduction % values for chitosan treated samples against both *E. coli and S. aureus* are presented in Figs. 4 and 5, these figures show that the microbial reduction is better against both bacteria. The most accepted mechanism for microbial inhibition by chitosan is the interaction of the positively charged chitosan with the negatively charged residues at the cell surface of many fungi and bacteria, which causes extensive cell surface alterations and alters cell permeability. This causes the leakage of intracellular substances such as electrolytes, UV-absorbing material, proteins, amino acids, glucose and lactate dehydrogenase. As a result, chitosan inhibits the normal metabolism of microorganisms and finally leads to the death of these cells (Sang-Hoon & Samuel,

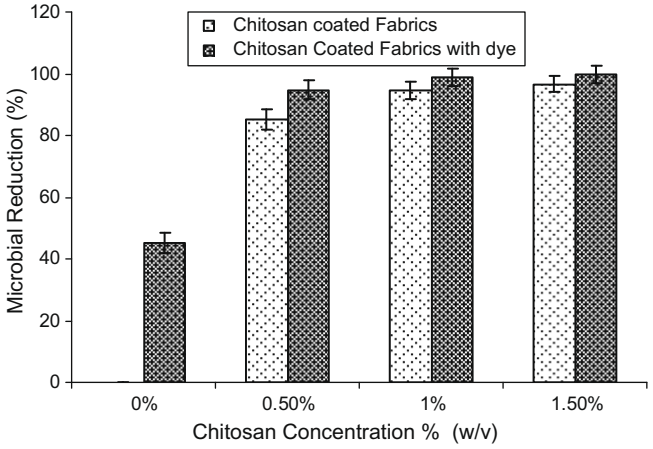

Fig. 4. Microbial reduction % values of fabrics against *E. coli* bacteria.

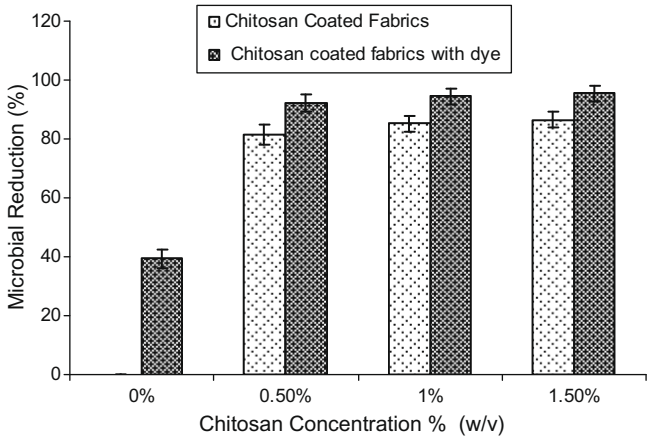

Fig. 5. Microbial reduction % values of fabrics against *S. aureus* bacteria.

2003). *S. aureus* being a Gram-positive bacterium has a thicker cell wall and hence is more resistant to chitosan than *E. coli*.

Fabrics with highest dye conc. i.e. 15% owf were chosen to be tested for their antimicrobial activities. The antimicrobial activities of the treated samples in terms of their reduction % values against *S. aureus* and *E. coli* for henna dyed samples are given in Figs. 4 and 5. From the figure, it is clear that even fabrics dyed with henna alone without chitosan application do exhibit significant antimicrobial activity. This is due to the inherent antimicrobial character-

istics of these dyes. As mentioned before, henna has an orange-coloured pigment called Lawsone (2-hydroxy-1,4-naphthoquinone), which is responsible for both its dyeing and antimicrobial characteristics. Quinones are aromatic rings with two ketone substitutions which are illustrated in Fig. 2. They are ubiquitous in nature and characteristically highly reactive. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function and hence they find numerous applications as antimicrobial agents. Portable targets in the microbial cell are surface exposed adhesions, cell wall polypeptides and membrane bound enzymes. Quinones may also render substrates unavailable to the microorganism and have been proven to inhibit cell growth (Habbal et al., 2005).

However, the reduction % values exhibited by chitosan treated and subsequently dyed fabrics are higher than those values reported in the earlier case proving that chitosan treatment enhances the antimicrobial activity of the dyed fabrics in case of henna dyes applied to fabrics. The combined antimicrobial effect of chitosan and natural dyes is very good and can be used to develop clothes for protecting against common infections and moreover, can be effectively used in home textiles as they are major propagators of common infections.

4. Conclusions

Chitosan, which is a very useful non-toxic biopolymer, can be used as an effective antimicrobial finish and can also be used to increase the dye uptake of the fabrics. The chitosan application to wool fabrics before dyeing has twofold effects: One it improves the dye uptake of wool fabrics; the other is that it significantly improves the antimicrobial activity of the dyes. The fastness properties of these fabrics are good against washing, light and perspiration. Thus, a non-toxic, eco-friendly, multi-functional finish has been developed for wool fabrics.

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