



Antibacterial functionalization of reactive-cellulosic prints via inclusion of bioactive Neem oil/ β CD complex

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ABSTRACT

In the present research enhancing the antibacterial activity of cellulosic fabrics printed with reactive dyes was achieved through combined reactive printing and β CD loading in one step followed by subsequent treatment with Neem oil, as an eco-friendly antimicrobial agent. Retention of Neem oil with its main compound azadirachtin within the hydrophobic cavities of β CD moieties-attached the reactive cellulosic prints, via formation of host–guest inclusion complexes, to impart antibacterial functionality against G+ve (*Staphylococcus aureus*) and G–ve (*Escherichia coli*) bacteria was carried out. The experimental results reveal that post-treatment with Neem oil results in a remarkable improvement in the antibacterial activity of the treated reactive prints along with darker depth of shade and without adversely affecting the UV-blocking properties of the final products. Mode of interactions, surface morphology as well as washing durability of antibacterial and anti-UV functions were also investigated.

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1. Introduction

In recent years, development and manufacturing of high added value textile products with multifunctional properties like antimicrobial, UV-protecting, insect repellent, self cleaning and anti-radiation, taking in consideration fashion, comfort, ecological and economic demands, have been become extremely important (Growri et al., 2010; Ibrahim, Refaie, & Ahmed, 2010; Schindler & Hauser, 2004; Simonic & Tomsic, 2010). As a consequence, a wide range of finishing additives suitable for textile applications has been used to bring new functional properties to the final textile products. The extent of improvement in the demanded functional properties depends on the type of substrate, chemical structure and functionality of the nominated finishing agent, method of application and durability to wash (Bajaj, 2002; Holme, 2007; Ibrahim, Refaie et al., 2010).

Antimicrobial finishing of textile materials protects both the users from pathogenic or odor-generation microorganism and the textiles from damage and/or undesirable aesthetic changes (Gao & Cranston, 2008; Purwar & Joshi, 2004; Simonic & Tomsic,

2010). Antimicrobial agents can be classified according to their effectiveness, mode of action and washing resistance (Simonic & Tomsic, 2010). Antimicrobial finishes can be divided into biocides that kill bacteria and fungi, and biostats that inhibit the growth of microorganism (Gao & Cranston, 2008; Ibrahim, El-Gamal, Gouda, & Mahrous, 2010; Simonic & Tomsic, 2010). The major classes of antimicrobial agents for textile include quaternary ammonium compounds (Gao & Cranston, 2008; Marini, Bondi, Iseppi, Toselli, & Pilati, 2007; Zhao, Sun, & Song, 2003), N-halamines (Ibrahim, Aly & Gouda, 2008; Ibrahim, Fahmy, Rehim, Sharaf, & Abo-Shosha, 2010; Ibrahim, 2008; Ren, Kocer, Worley, Broughton, & Huang, 2009; Simonic & Tomsic, 2010), chitosan (Lim & Hudson, 2009; Öktem, 2003; Shanmugasundaram, 2006), Polybiguanides (Kawabata & Taylor, 2007; Simonic & Tomsic, 2010), triclosan (Orhan, Kut, & Gunesoglu, 2009), nanosized inorganic particles (Growri et al., 2010; Gorensek & Pecelj, 2007; Ibrahim, Eid, Hashem, Refie, & El-Hossamy, 2010; Ibrahim, Refaie et al., 2010), and bioactive plant based products (Gupta, Khare, & Laha, 2004; Holm, 2005; Ibrahim, El-Gamal, et al., 2010; Simonic & Tomsic, 2010). On the other hand, the application of herbal oil, e.g. Neem oil, onto textile materials to impart antimicrobial activities as well as to get medicinal fabrics has very recently received growing interest (Joshi, Ali, & Rajendran, 2007; Sayed & Jawale, 2006; Vaideki, Jayakumar, Thilagavathi, & Rajendran, 2007).

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In the current study, our attention was focused both on upgrading the antibacterial efficiency of cellulosic reactive prints via incorporation of MCT- β CD in the printing formulations followed by subsequent treatment of the obtained prints with Neem oil, and on evaluating the impact of this after-treatment on the UV-protecting properties.

2. Experimental

2.1. Materials

Mill-scoured and bleached plain weave cotton (130 g/m²) and viscose (110 g/m²) fabrics were used throughout this work.

Commercial grade reactive dyes used in this study are Reactive Red 120, Reactive Red 141, Reactive Blue 160, Reactive Red 195 and Reactive Red 198. They are kindly supplied by Oh Yon Ind. Co, Ltd., China.

Cavaso[®] W7MC, monochlorotriazine- β -cyclodextrin MCT- β CD [average molecular weight \approx 1560, degree of substitution 0.3–0.6 anhydroglucose unit, Wacker, Germany], Neem seed oil [Ozone Biotech Division, Shivanshu Sintered Product Pvt. Ltd., India], as well as Cecalgin[®] HV/KL-600 [medium viscosity sodium alginate-Ceca Kolloid-Chemie, Paris] were of commercial grade. Other chemicals such as polyethylene glycol, sodium bicarbonate (NaHCO₃), urea and methyl alcohol were of laboratory reagent grade.

2.2. Methods

2.2.1. Reactive printing

Fabric samples were printed with the following printing formulation:

Constituent	g/kg paste
Reactive dye	20
Stock thickening (10%)	700
MCT- β CD	20
Na-bicarbonate	20
Urea	50
PEG-600	20
Water	170
Total	1000

using the flat screen technique

After printing, the fabric samples were then dried at 100 °C for 3 min and steam fixed at 110 °C for (5–15 min) using Ariolt[®] CSL-Steamer-Italy, rinsed thoroughly in water and washed according to the dyestuff manufacturer recommendations to remove unreacted and any soluble byproducts followed by drying at 80 °C/5 min.

2.2.2. Post-treatment with Neem oil

The printed fabric samples were immersed in the methanolic solution (20 g/L) of the Neem oil with LR of 1/20 for 30 min at room temperature followed by roll squeezing to a 80% wet pick up and drying at 80 °C/15 min.

2.3. Testing

Percentage added-on (wt. add-on%) was calculated from the following equation:

$$\text{Add-on (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

where W_1 and W_2 are the weight of the printed and printed \rightarrow post-treated fabric samples, respectively.

The depth of the obtained prints, expressed as K/S , was measured at the wavelength of maximum absorbance using an automatic filter spectrophotometer and calculated by the Kubelka–Munk equation (Ibrahim, Mahrous, El-Gamal, Gouda, & Husseiny, 2010):

$$\frac{K}{S} = \frac{(1 - R)^2}{2R}$$

where K is the absorption coefficient, S is the scattering coefficient, and R is the reflectance of the printed samples. The higher the K/S value is, the darker the depth of the reactive prints.

Color fastness to washing, perspiration and rubbing were evaluated according to AATCC Test Methods: (61-1972), (15-1973) and (8-1972), respectively.

UV-protection factor (UPF) was determined according to the Australian/New Zealand Standard (AS/NZS 4399-1996).

Anti-bacterial activity assessment against Gram-positive bacteria (G+ve, *Staphylococcus aureus*) and Gram-negative bacteria (G–ve, *Escherichia coli*) was evaluated according to AATCC Test Method (147-1988) and expressed as zone of growth inhibition (mm).

SEM analysis was made to compare surface morphology of the untreated and printed fabric samples without reactive dye using Scanning Electron Microscope JEOL-JZA-840A, after the samples were plated with gold.

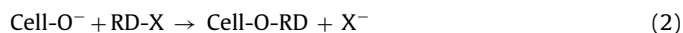
Durability to washing was evaluated according to AATCC test method 124.

3. Results and discussion

Since the main task of the present work was to render reactive cellulosic prints antibacterial and anti-UV blocking properties without adversely affecting their printing properties, attempts have been made to include MCT- β CD in reactive printing formulations to get reactive prints having the ability to host a guest bioactive agent, Neem oil, in their internal cavities thereby imparting antibacterial properties to the post-treated reactive prints. Variables studied include: type of cellulosic substrate, type and concentration of additive, steaming time, type of reactive dye as well as Neem oil concentration. The results obtained along with the appropriate discussion follow.

3.1. NaHCO₃ concentration

Fig. 1 shows that, the K/S values of the obtained reactive prints reaches a maximum at 20 g/kg and then decrease for both the cellulosic substrates. The data so obtained reflect the positive impact of the using NaHCO₃ at proper concentration on enhancing the swellability of the cellulose structure, accessibility of its reactive sites, OH groups, and inducing ionization of the cellulose-hydroxyl groups thereby increasing the extent of dye fixation via formation of covalent bond as follows:



where Cell-OH: cellulose substrate, RD-X: reactive dye

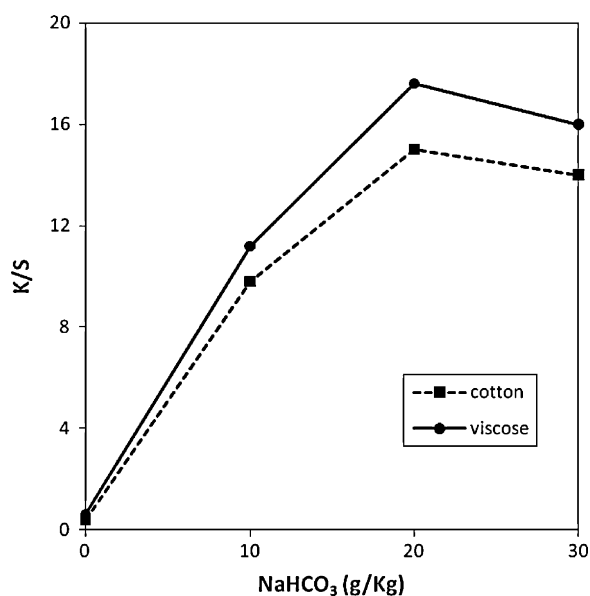
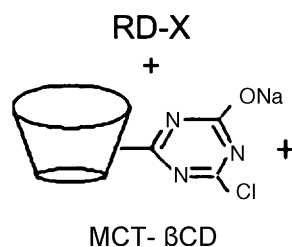
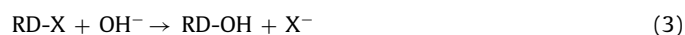


Fig. 1. Effect of NaHCO_3 concentration on depth of the obtained reactive prints. Reactive Red 198 (20 g/kg); stock-thickening agent –10% (700 g/kg); R- β CD (20 g/kg); urea (50 g/kg); PEG 600 (20 g/kg). Drying at 85 °C/5 min; steaming at 110 °C/10 min.

Further increase in NaHCO_3 concentration, beyond 20 g/kg, is accompanied by a decrease in the depth of the obtained prints, expressed as K/S values, most probably due to partial hydrolysis of the used reactive dye thereby decreasing the dye-fiber chemical interaction as follows (Ibrahim & El-Sayed, 1993):



Additionally, the extent of increase or decrease in the depth of the obtained reactive prints is determined by type of the cellulosic substrate, viscose > cotton, reflects the difference between them in surface area, amorphous to crystalline regions, reactivity as well as number, location and availability of active sites, i.e. hydroxyl groups (Ibrahim, Abo-Shosha, Allam, El-Zairy, & El-Zairy, 2006).

On the other hand, side-interactions between the used reactive dye and the added MCT- β CD, under the given printing conditions, cannot be ruled out, thereby leading to washable adducts, and decreasing the extent of dye-fiber interaction and fixation, thus giving lower K/S values as follows (Ibrahim, E-Zairy, & Eid, 2010):

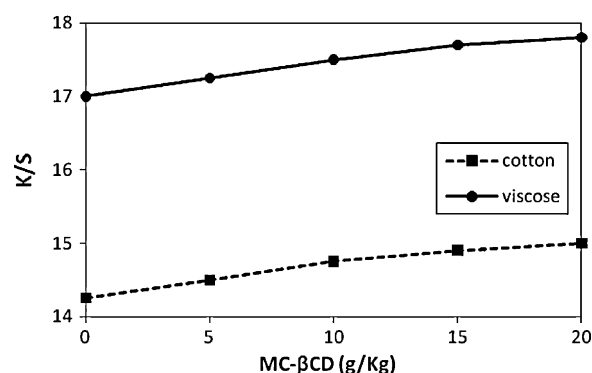
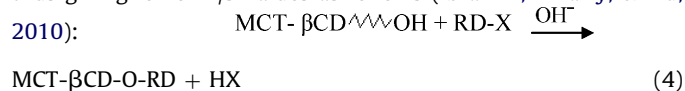
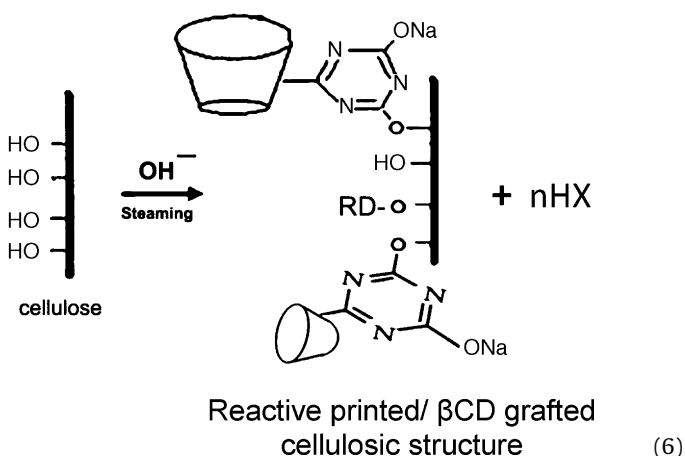


Fig. 2. Effect of R- β CD concentration on depth of the obtained reactive prints. Reactive Red 198 (20 g/kg); stock-thickening agent –10% (700 g/kg); NaHCO_3 (20 g/kg); urea (50 g/kg); PEG 600 (20 g/kg). Drying at 85 °C/5 min; steaming at 110 °C/10 min.



3.2. MCT- β CD concentration

Fig. 2 shows that increasing MCT- β CD concentration in the printing formulation up to 20 g/kg is accompanied by an improvement in the K/S values of the obtained reactive prints irrespective of the used substrate. This implies that combined reactive printing and β CD-loading with its internal hydrophobic cavities and hydrophilic outside in one step has practically a positive impact on dye building up and accommodation onto/within the cellulose structure through modification of the cellulose structure along with imparting beneficial physicochemical properties via formation of an inclusion complexes with guest molecules for attaining new functional properties as follows (Ibrahim, E-Zairy, & Eid, 2010; Nostro, Fratoni, Ridi, & Baglioni, 2003; Romi, Nostro, Bocci, Ridi, & Baglioni, 2005):



Needless to say, the extent of variation in the K/S values of the obtained reactive prints is determined by the nature of the cellulosic substrate as discussed earlier and extend of its modification.

On the other hand, Fig. 3 shows the SEM micrographs of untreated and MCT- β CD-treated cotton and viscose fabric samples with a magnification of 2500. SEM micrographs confirm the grafting and loading of β CD onto the treated fabric samples.

3.3. Urea concentration

Fig. 4 shows the effect of including urea in the printing formulation on the depth of the obtained prints. The K/S values reached a maximum at 50 g/kg, regardless of the used substrate, reflecting the positive impact of urea on enhancing the swellability of the cellu-

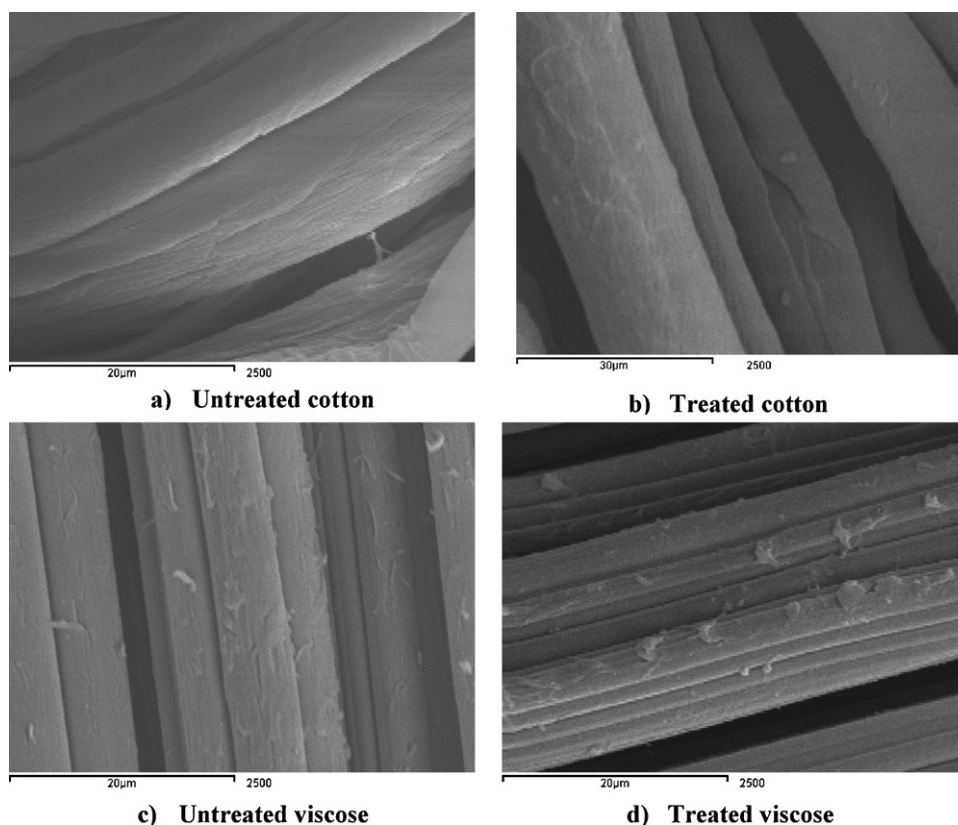


Fig. 3. SEM of untreated and treated fabric samples.

losic structure, improving disaggregation of dye molecules and dye solubility, retarding the evaporation of water during drying step, increasing the extent of water condensation on the surface of the printed fabric samples as well as facilitating the release and transfer from the thickener film into the modified cellulose structure, in turn enhancing the extent of dye-fiber interaction and opportunity for covalent bonding (Eq. (2)) (Ibrahim et al., 2006; Ibrahim, El-Zairy, & Abo-Shosha, 1994).

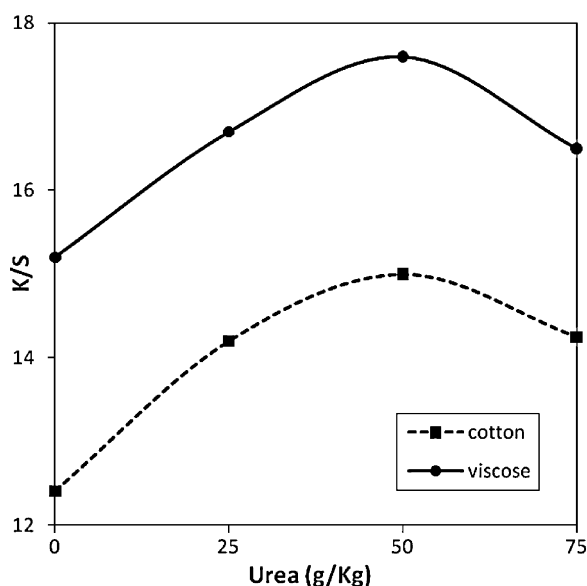


Fig. 4. Effect of urea concentration on depth of the obtained reactive prints. Reactive Red 198 (20 g/kg); stock-thickening agent –10% (700 g/kg); R-βCD (20 g/kg); NaHCO₃ (20 g/kg); PEG 600 (20 g/kg). Drying at 85 °C/5 min; steaming at 110 °C/10 min.

At higher urea concentration, i.e. beyond 50 g/kg, lower depth of shades are obtained. This is due to a side interactions with the Na-alginate thickener thereby changing its rheological properties and/or the used reactive dye along with facilitating undue penetration of the dye molecules within the highly swelled cellulose structure, in turn decreasing the extent of dye fixation, i.e. lower values of the obtained reactive prints (Ibrahim, Abo-Shosha, Allam, El-Zairy, El-Zairy, 2006; Ibrahim et al., 1994).

3.4. PEG-600 concentration

The effect of including PEG-600, as a hygroscopic agent, in the printing formulation on the extent of reactive printing is shown in Fig. 5. For a given set of printing conditions, it is clear that increasing PEG-600 concentration up to 20 g/kg results in a significant increase in the K/S values. This can be ascribed to its positive impact on: swellability of both the modified cellulosic structure and thickener film, promoting dye solubility, disaggregation of dye molecules, transferring dye molecules to the fabric surface and enhancing water condensation on the print during the steam fixation step. The net effect of the abovementioned factors is a significant improvement in the extent of dye fixation and accommodation, i.e. darker depth of shades (Ibrahim et al., 1994; Ibrahim, El-Zairy, El-Zairy, & Khalil, 2010). Beyond 20 g/kg, undue penetration of dye molecules within the fabric structure takes place thereby resulting in a decrease in the K/S values of the obtained reactive prints.

3.5. Steam fixation time

The data presented in Fig. 6 shows a gradual increase in the K/S values of the obtained reactive prints by prolonging the steaming time up to 15 min at 110 °C regardless of the used cellulosic sub-

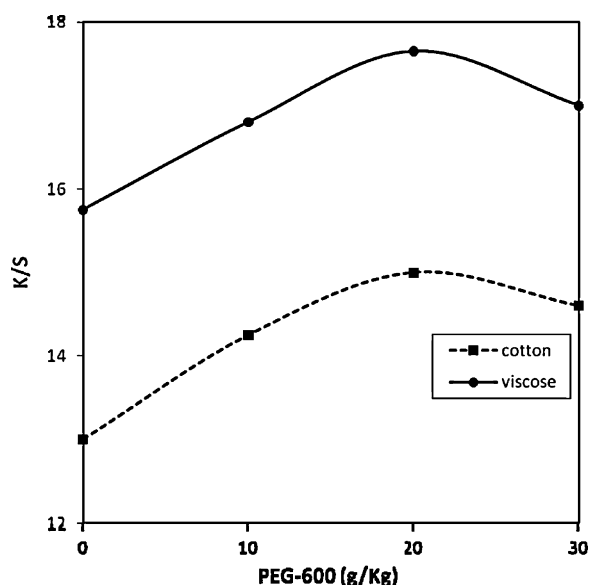


Fig. 5. Effect of PEG-600 concentration on depth of the obtained reactive prints. Reactive Red 198 (20 g/kg); stock-thickening agent –10% (700 g/kg); R-βCD (20 g/kg); NaHCO₃ (20 g/kg); urea (50 g/kg). Drying at 85 °C/5 min; steaming at 110 °C/10 min.

strate. This gradual increase in the depth of the obtained prints reflects the positive impact of proper steam fixation time on: enhancing the extent of modification of the cellulose structure with βCD-moieties, swelling both the modified cellulose structure and the thickener film components, facilitating the release of dye molecules from the thickener film onto/within the modified cellulose structure and enabling the interaction among the dye molecules, MCT-β-CD and the active sites of the modified cellulose structure and formation of covalent bonds (Ibrahim et al., 2006; Ibrahim et al., 1994).

As previously explained herein, proper steam fixation can generate the simultaneous and competitive reactions of the reactive dye fixation and β-CD grafting onto/into the cellulose structure.

3.6. Printing properties

Data in Table 1 indicate that the depth and fastness properties, using different reactive dyes and keeping other parameters fixed, of the obtained reactive prints is governed by: (i) the type substrate, i.e. viscose > cotton, (ii) the kind of the reactive dye, i.e. molecu-

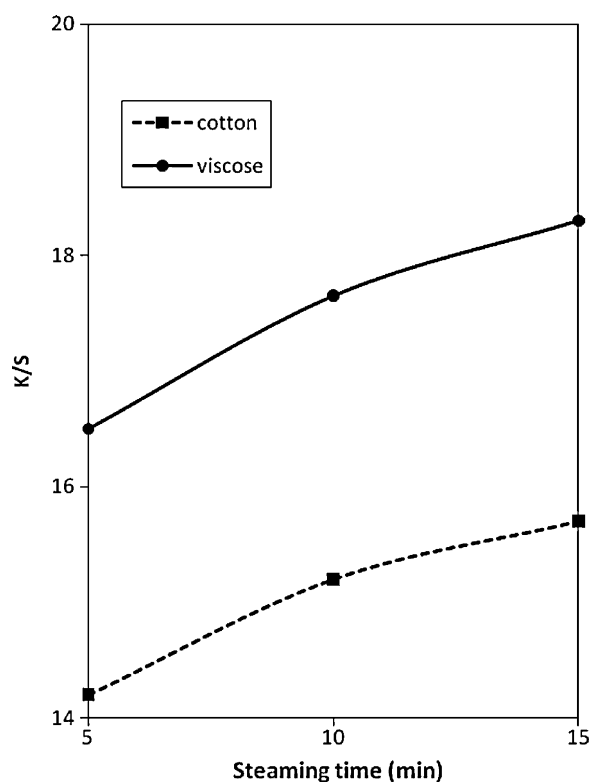


Fig. 6. Effect of steaming time on depth of the obtained reactive prints. Reactive Red 198 (20 g/kg); stock-thickening agent –10% (700 g/kg); R-βCD (20 g/kg); NaHCO₃ (20 g/kg); urea (50 g/kg); PEG 600 (20 g/kg). Drying at 85 °C/5 min.

lar size, chemical composition, chromophoric system, functionality (mono or bi: homo or hetero), mode of interaction (substitution and/or addition), (iii) compatibility with other printing paste components, (iv) stability at the used printing and steam fixation conditions (Ibrahim et al., 2006; Ibrahim et al., 1994; Ibrahim & El-Sayed, 1993), and (v) extent of simultaneous printing and β-CD grafting.

On the other hand, the wash, perspiration and rubbing fastness properties of the obtained reactive prints were good to excellent (4 to 5).

3.7. Functional properties

As far as the changes in the weight, K/S, UPF and antibacterial properties of the reactive printed/β-CD-loaded cellulosic

Table 1
Printing properties using different reactive dyes.

Reactive dye	Substrate	K/S	WF		PF				RF	
			Alt.	C	Acidic		Alkaline		Dry	Wet
					Alt.	St.	Alt.	St.		
C.I. Reactive Red 120 λ _{max} = 525	Cotton	14.01	5	4–5	5	5	5	5	5	4–5
	Viscose	15.28	4–5	4–5	4–5	4–5	5	5	4–5	4
C.I. Reactive Red 141 λ _{max} = 565	Cotton	10.86	4–5	4–5	5	5	5	5	5	5
	Viscose	12.46	4–5	4–5	4–5	5	5	3–4	4–5	4–5
C.I. Reactive Blue 160 λ _{max} = 630	Cotton	10.69	5	5	4–5	4–5	4–5	4	4–5	4
	Viscose	12.28	4–5	4–5	4–5	4–5	4–5	4	4–5	4–5
C.I. Reactive Red 195 λ _{max} = 560	Cotton	10.82	5	5	4–5	5	5	5	5	5
	Viscose	13.88	4–5	4–5	4–5	4–5	4–5	4–5	4–5	4
C.I. Reactive Red 198 λ _{max} = 530	Cotton	15.80	5	5	4–5	4–5	5	5	5	4–5
	Viscose	18.35	5	5	4–5	4–5	5	5	4–5	4

Printing paste: reactive dye (20 g/kg); stock-thickening agent –10% (700 g/kg); NaHCO₃ (20 g/kg); R-βCD (20 g/kg); urea (50 g/kg); PEG 600 (20 g/kg). Drying at 85 °C/5 min; steaming at 110 °C/15 min. K/S: color strength; WF: washing fastness; PF: perspiration fastness; RF: rubbing fastness; Alt: alteration; St.: staining on cotton.

Table 2

Effect of Neem-treatment on some performance and functional properties of reactive prints.

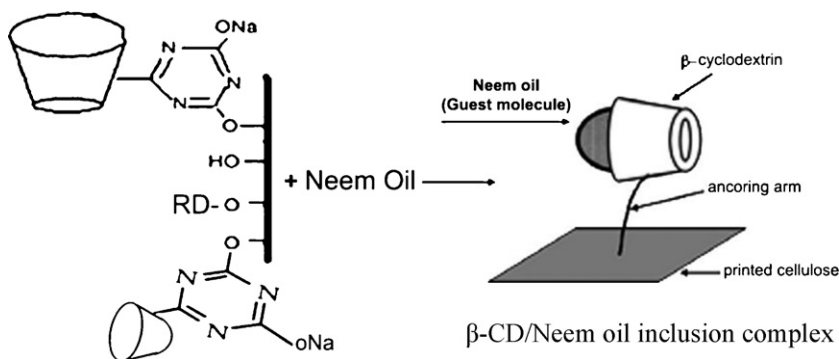
Reactive dye	Substrate	Incr. in weight (%)	K/S		UPF		Antimicrobial properties (zone mm)			
			U	T	U	T	G +ve		G –ve	
							U	T	U	T
C.I. Reactive Red 120 $\lambda_{\max} = 525$	Cotton	2.95	14.01	14.58	115	104 (excell) ^a	5.2 (1.8)	13.0 (11.0)	4 (1.3)	10.0 (8.2) ^b
	Viscose	3.45	15.28	15.76	42	35 (v. good)	6.2 (2.3)	14.2 (11.3)	4.5 (1.6)	11.0 (9.1)
C.I. Reactive Red 141 $\lambda_{\max} = 565$	Cotton	3.27	10.86	11.16	101	97 (excell)	2.5 (0.5)	11.5 (9.2)	1.0 (0.3)	10.2 (8.5)
	Viscose	3.72	12.46	12.82	35	26 (v. good)	4.1 (1.4)	13.1 (10.9)	3.2 (0.8)	10.5 (9.0)
C.I. Reactive Blue 160 $\lambda_{\max} = 630$	Cotton	3.44	10.69	11.00	58	53 (excell)	4.0 (1.3)	8.3 (7.0)	3.5 (1.1)	7.2 (5.5)
	Viscose	4.15	12.28	12.80	30	22 (v. good)	4.9 (1.6)	10.3 (8.5)	4.2 (1.4)	8.4 (7.3)
C.I. Reactive Red 195 $\lambda_{\max} = 560$	Cotton	3.18	10.82	11.40	111	92 (excell)	5.0 (1.7)	11.0 (9.0)	4.3 (1.5)	10.0 (8.6)
	Viscose	3.85	13.88	14.15	41	32 (v. good)	6.0 (2.0)	13.2 (10.8)	4.5 (1.6)	12.0 (10.4)
C.I. Reactive Red 198 $\lambda_{\max} = 530$	Cotton	2.29	15.80	16.25	83	75 (excell)	3.1 (1.0)	9.3 (7.8)	2.0 (0.6)	8.2 (7.0)
	Viscose	2.59	18.35	18.89	50	44 (excell)	4.5 (1.5)	10.5 (9.0)	4.0 (1.2)	8.5 (7.5)

Printing paste: reactive dye (20 g/kg); stock-thickening agent –10% (700 g/kg); NaHCO₃ (20 g/kg); R-βCD (20 g/kg); urea (50 g/kg); PEG 600 (20 g/kg). Drying at 85 °C/5 min; steaming at 110 °C/15 min. Neem oil concentration (20 g/L). UPF: UV Protection Factor; ZI: zone of inhibition; U: untreated; T: Neem-treated.

^a UV-protection grade.

^b Durability of antibacterial effect after 15 washing cycles.

substrates as a function of subsequent inclusion of Neem oil, as an eco-friendly natural bioactive agent, into the hydrophobic central cavities of the grafted β-CD, Table 2 reveals the following: (i) post-treatment with Neem oil-alcoholic solution (20 g/L) is accompanied by an increase in both the wt. add on (%), and the depth of the obtained prints along with a slight decrease in UPF values regardless of the used substrate and reactive dye, (ii) formation of inclusion complex (Eq. (7)) onto/within the attached-β-CD hydrophobic cavities results in a significant enhancement in the antibacterial activity against G+ve bacteria (*S. aureus*) and G–ve bacteria (*E. coli*) reflecting the biological activity/antibacterial efficacy of Neem oil-functional ingredients especially azadirachtin (C₃₅H₄₄O₁₆) (Biswas, Chattopadhyay, Banerjee, & Bandyopadhyay, 2002; Joshi et al., 2007; Vaideki et al., 2007), (iii) the increase in the



(7)

%Add-on, K/S as well as antibacterial activity is governed by the type of modified substrate and its ability to pick up and retain the used and active ingredient and follows the decreasing order: viscose > cotton keeping other parameters constant, (iv) the UPF values of printed-post-treated fabric samples follows the descending order: cotton > viscose reflecting the differences in fabric construction, i.e. porosity, thickeners, mass, as well as moisture content, which in turn affected the extent of absorption or reflection of the harmful UV-B radiation (Ibrahim, Eid, et al., 2010; Ibrahim, Refaie, et al., 2010), (v) the antibacterial activity against G+ve bacteria was better than that against G–ve bacteria, keeping other parameters fixed most probably due to the differences in biological response exhibited by the two species towards the bioactivity of the Neem oil components as well as in their cell wall structure and outer membrane (Ibrahim, Aly, & Gouda, 2008; Ibrahim, El-Gamal, et al., 2010; Shanmugasundaram, 2006), (vi) the impact of substrate

type, nature of the reactive dye as well as the extent of modification of the printed substrate via incorporation of the βCD moieties onto/within the cellulose structure on formation of the host-guest inclusion complexes, which in turn affected the antibacterial functionality, cannot be phased out, (viii) the variation in UPF values of the printed fabric samples reflects the differences among them in fabric construction as well as the ability of the printed substrate to absorb harmful UV radiation (Ibrahim, Mahrous, et al., 2010), (viii) retention of the antibacterial activity of Neem-treated reactive prints even after 15 washing cycles reflects the positive role of the hydrophobic cavities of grafted β-CD in fixing and incorporating the bioactive agent onto/into the cellulose structure, and (ix) the antibacterial activity of the untreated reactive prints most probably

attributed to the ability and bioactivity of the used anionic dyes to attack microorganisms (Ibrahim, Mahrous, et al., 2010).

4. Conclusions

Cotton and viscose cellulosic fabrics were printed with reactive dyes in the presence of MCT-βCD under alkaline conditions so that the free hosting βCD hydrophobic cavities would be available for the direct formation of host guest inclusion complexes with an-eco-friendly bioactive ingredients, azadirachtin, via subsequent treatment with methanolic solution of Neem oil. Inclusion of the used bioactive agent onto/within the reactive prints results in a significant improvement in their antibacterial activity against *E. coli* and *S. aureus*, regardless of the used reactive dye and cellulosic substrate. The post-treated reactive prints showed a darker depth of

shades most probably due to a slight yellowness of Neem treated reactive prints along with practically a slight or no effect on their UPF values. The durability of the imparted antibacterial activity was confirmed after 15 home laundering cycles.

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