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Antimicrobial activity of monochlorotriazinyl-β-cyclodextrin/chlorohexidin diacetate finished cotton fabrics

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ABSTRACT

Linear electron beam radiation was used as an initiator for the grafting of glycidyl methacrylate/monochlorotriazinyl-β-cyclodextrin mixture (GMA/MCT-β-CD) onto cotton fabric. The obtained grafted fabric (Cell-g-GMA/MCT-β-CD) was loaded with chlorohexidin diacetate and subjected to several washing cycles. Grafted cotton fabrics (before/after loading) and untreated cotton were characterized for antimicrobial activity against different bacteria and fungi by using the Agar-Diffusion Method. Grafted fabrics loaded with the antimicrobial agent were found to show very good antimicrobial activity in comparison with control and grafted fabrics which are not loaded with antimicrobial agent. The results reported in this study demonstrate also that the GMA/MCT-β-CD grafted fabrics loaded with antimicrobial agent retain good durability toward antimicrobial activity after five washings. This is due to the cavities present in cyclodextrin moieties which are used as a host for the antimicrobial agent, resulting in long lasting antimicrobial efficiency.

1. Introduction

Antimicrobial finishing, by definition, inhibits the growth of or kills microorganisms. It has long been recognized that microorganisms can grow on textile substrates. These microorganisms can cause fiber degradation by feeding on unreacted monomers and/or chemicals found in the fibers such as oils, dyes, finishes, and coatings. Natural fibers such as cotton are more susceptible than synthetics because their porous hydrophilic structures that retain water, oxygen and nutrients which provide a perfect environment for growth of microorganisms (Nobmann, Smith, Dunne, Henehan, & Bourke, 2009).

Microbial infestation has unpleasant consequences such as unpleasant odors, mold and mildew stains, discoloration and loss of functional properties (e.g. tensile strength and elasticity). Microbes can disrupt textile manufacturing processes like dyeing, printing and finishing operations through the reduction of viscosity, fermentation, and mold formation. Microbial infestation cannot be removed by the most frequent washing with the exception of washing at boiling temperature, which is not suitable for some textiles (Lin, Gong, Wang, Li, & Wang, 2011).

In general, antimicrobial properties of textile materials can be achieved by incorporating functional agents onto fabrics, by either chemical or physical finishing (Shin, Yoo, & Min, 1999; Sun & Xu, 1998) or by modification of cotton by biopolymers (El-Shafei, Fouda, Knittel, & Schollmeyer, 2008; Fouda, El Shafei, Sharaf, & Hebeish, 2009). The durability of antimicrobial finishing can be grouped into two categories, temporary and durable (Payne & Kudner, 1996; Tang et al., 2011). Temporary finishing of textiles is easy to achieve, but is also easily washed off (Hong & Sun, 2011; Pinto, McGahan, Steiner, & Priefer, 2011). Durable antimicrobial finishing is generally achieved by the slow-release method, in which the treated fabrics slowly release the antimicrobial agent to inactivate microorganisms (Abdel-Halim, Fouda, Hamdy, Abdel-mohdy, & El-sawy, 2010; Abou-Zeid et al., 2011; Yao, Li, Danny, Gohel, & Chung, 2011).

Cyclodextrins were first isolated in 1891 by Villiers as degradation products of starch. Cyclodextrins can be obtained by enzymatic degradation of starch. In this process compounds with six to twelve

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glucopyranose units per ring are produced. Depending on the enzyme and how the reaction is controlled, the main product is α, β or γ-cyclodextrin (6, 7 and 8 glucopyranose units, respectively). β-Cyclodextrin is the most commercially produced type of the three natural cyclodextrins because of the ease of production, availability, cavity diameter and price. It is the most widely used type of cyclodextrins and represents at least 95% of all produced and consumed cyclodextrins (Szejtli, 1994).

Cyclodextrins can form inclusion complexes with numerous different guest molecules (Fouda, Knittel, Hipler, Elsner, & Schollmeyer, 2006). This would account for the changes in characteristics of the guest compounds occurring after the encapsulation process is achieved (Szejtli, 1982). The importance of cyclodextrin inclusion complexes in pharmaceutical, food and cosmetics industries or in agriculture has increased considerably during the last years. Fabrics containing cyclodextrins fixed on their surfaces are considered to be models for medical, technical and geo-textiles.

Monochlorotriazinyl-β-cyclodextrin (MCT-β-CD) is the first reactive cyclodextrin derivative manufactured on an industrial scale (Abdel-Mohdy, El-Aerf, & Aly, 2005; Parrish, 1987). It has a monochlorotriazinyl group as a reactive anchor. This derivative is able to fix onto cotton cellulose following a reactive dye reaction mechanism (Wei, Yang, & Hong, 2011).

The aim of this study is to investigate the incorporation of the antimicrobial agent; chlorohexidin diacetate into cyclodextrin cavities, covalently bonded to cotton fabrics to give the fabric durable antimicrobial activity.

2. Experimental

2.1. Materials

Duck cotton fabric was supplied by Misr Company for Spinning and Weaving, Mehalla El–Kobra, Egypt. The fabric was further laboratory purified by scouring at 100 °C for 60 min in an aqueous solution containing (2 g/l) sodium carbonate and 2 g/l non-ionic wetting agent using a material to liquor ratio of 1:20, then thoroughly washed with water and dried at ambient temperature.

Monochlorotriazinyl-β-cyclodextrin (MCT-β-CD) was supplied by Wacker Chemie GmbH, München, Germany. Glycidyl methacrylate (GMA) was supplied by Fluka Chemie AG, Buchs, Switzerland. Chlorohexidin diacetate was supplied by Synopharm GmbH & Co. Kg, Barsbüttel, Germany. All other chemicals were laboratory grade reagents.

2.2. Radiation grafting onto cotton fabric

GMA or GMA/MCT-β-CD mixtures were grafted onto cotton fabric using linear electron beam irradiation technique. The grafting reaction was conducted at the National Centre of Radiation Research and Technology, Nars City, Cairo, Egypt. The samples were irradiated with linear electron beam accelerator (energy 1.5 Mev, power 37.5 Kw, beam current 25 mA and scan width variable up to 90 cm).

Samples of pre-scoured cotton fabric were completely immersed in finishing bath containing calculated concentrations of GMA or GMA/MCT-β-CD mixture for 1 h, and then squeezed to a wet pick up of ca 100%. The fabric samples were then irradiated with the said linear electron beam accelerator to initiate the grafting reaction. The grafted samples were then thoroughly washed with the proper solvent to remove the non-reacted matters and ungrafted polymers. The grafted samples were finally dried at ambient conditions and the graft yield was determined gravimetrically.

2.3. Reaction of MCT-β-CD with grafted cotton samples (retreatment process)

A set of cotton fabrics previously radiation-grafted with GMA or GMA/MCT-β-CD mixture was retreated with solution containing 2% (ows) MCT-β-CD, 1 M NaCl and 1% (w/v) NaOH, using material to liquor ratio of 1:20. The system was kept under continuous shaking at 80 °C for 1 h. The treated samples were washed thoroughly with distilled water and then dried at ambient conditions (Gawish, Matthews, Wafa, Breidt, & Bourham, 2007).

2.4. Incorporation of chlorohexidin diacetate into grafted cotton fabrics

Treated and control cotton fabrics were further treated by dipping the fabrics at 25 °C for 2 h in ethanolic solution containing 2% (w/v) chlorohexidin diacetate (antimicrobial agent), using material to liquor ratio of 1:10. The samples were then roll-squeezed to wet pick-up of 100% and then washed to remove the adsorbed antimicrobial agent from the fabric surface.

Several methods are available for the determination of antimicrobial agents in the treated fabrics. Most of the methods developed so far for the analysis of commercially available antimicrobial agents formulations are gas chromatography (GC) and liquid chromatography (HPLC) (Scalia et al., 2006; Wang & Cai, 2008). In the present study a fast, selective and sensitive method of chlorohexidin diacetate estimation in treated cotton fabrics was employed. The analysis was carried out using gas chromatography (GC) with electron capture for both detection and quantification of the chlorohexidin diacetate. Analysis of chlorohexidin diacetate extract was conducted with a Hewlett–Packard Model 5890 A GC, equipped with column HP-1(25 cm × 0.2 mm × 0.2 μm film thickness) and flame ionization detector (FID). The injector and detector were operated at 250 °C and 280 °C, respectively. The oven temperature was programmed from 200 °C to 250 °C at 5 °C/min and held for 1 min.

2.5. Evaluation of treated fabrics

2.5.1. Determination of graft yield

Graft yield was determined from the gain in weight of cotton fabric due to graft polymerization after removing the ungrafted polymers with the proper solvent according to the following equation.

$$\%\ \text{Graft yield} = \frac{W_2 - W_1}{W_1} \times 100$$

where (W_2) is the weight of dry fabric sample after grafting and (W_1) is the weight of dry fabric sample before grafting.

2.5.2. Quantification of MCT-β-CD fixed onto cotton fabrics

The amount of MCT-β-CD fixed onto the cotton fabrics was estimated in terms of nitrogen content values according to standard Kjeldhl method (Vogel, 1995a,b).

2.5.3. Determination of remaining epoxide

α-Epoxides are group of cyclic ethers in which the oxygen atom forms a three-membered ring with two adjacent carbon atoms. Because of the strained three-membered ring, α-epoxides are the most reactive of the oxides and are far more reactive than ordinary ethers. Thus they react with hydrogen chloride to form the corresponding chlorohydrins. This reaction is the base for the determination of α-epoxy groups. This is termed the acidimetric method (Vogel, 1995a,b).

2.5.4. Quantitative analysis of antimicrobial agents

Chlorohexidin diacetate was extracted from the fabric and portion of the resulting extract was analyzed by a gas chromatograph (GC) Hewlett–Packard Model 5890 A GC, The GC analysis was carried out at Mycotoxin Central Laboratory, National Research Centre.

2.5.5. Fabric washing

Treated cotton fabrics were washed in an aqueous solution containing 2 g/l sodium carbonate and 5 g/l non-ionic wetting agent at 60 °C for 15 min using material to liquor ratio of 1:20. The washing process was carried out to examine the ability of fabric to retain its antimicrobial activity after washing.

2.5.6. Estimation of fabric's antimicrobial activity

The antimicrobial activities of both treated and control fabrics were measured according to the Diffusion Disk Method (Grayer & Harbone, 1994; Irob, Young, & Apderson, 1996; Muanza, Kim, Euler, & Williams, 1994) at Micro Analytical Centre, Faculty of science, Cairo University. Two kinds of fungus – *Candida albicans* and *Aspergillus flavus* and two kinds of bacteria: *Escherichia coli* and *Staphylococcus aureus*, were selected to investigate the antimicrobial activity. In order to investigate the durability of antimicrobial performance, the antimicrobial activity of the fabrics was also tested after washing.

The ability of fabric to exhibit antimicrobial activity after washing is expressed as percent retention.

$$\% \text{ Retention} = \frac{\text{Antimicrobial activity after washing}}{\text{Antimicrobial activity before washing}} \times 100$$

2.5.7. Infrared spectroscopy

Cotton fabrics were evaluated using infrared spectroscopy. The infrared analysis of the treated cotton fabrics were carried out at the Infrared Laboratory, Central Services Laboratory, National Research Centre, using JAS Co 1 Japan, FTIR 6100, Fourier Transion Infrared Spectrophotometer.

2.5.8. Scanning electron microscopy (SEM)

SEM analysis of control and treated fabrics was performed at Scanning Electron Microscope Division; Central Services Laboratory, National Research Centre, using a JOEL (JXA–840 A); Electron Probe Micro-Analyzer, Edward, England, 150 A. Sputter Coater. Samples were coated with gold according to the method described in the operation manual provided by the manufacturer. Photos were collected at range from 1000× to 2000×.

3. Results and discussions

3.1. Infrared spectroscopy

Cotton fabrics were subjected to grafting and the resulting preparation was loaded with chlorohexidin diacetate (antimicrobial agent). This approach represents a way to the development of new textiles of antimicrobial activities.

Structures of the grafted fabrics before and after incorporation of the antimicrobial agent were tested by comparative inspection of their IR-spectra particularly in the fingerprint region (1500–650 cm^{-1}).

The cotton fabric grafted with glycidyl methacrylate (GMA) was prepared by treating the fabric with GMA. The main functional groups attributable to cellulose and GMA are apparent in the spectrum (Fig. 1a). These include the absorption bands at 3414 (OH), 2906 (CH, aliphatic), 1725 (C=O, ester), 1164 (C–O, stretching) and at 1114 cm^{-1} (–C–O–C, epoxide). The absorption band present

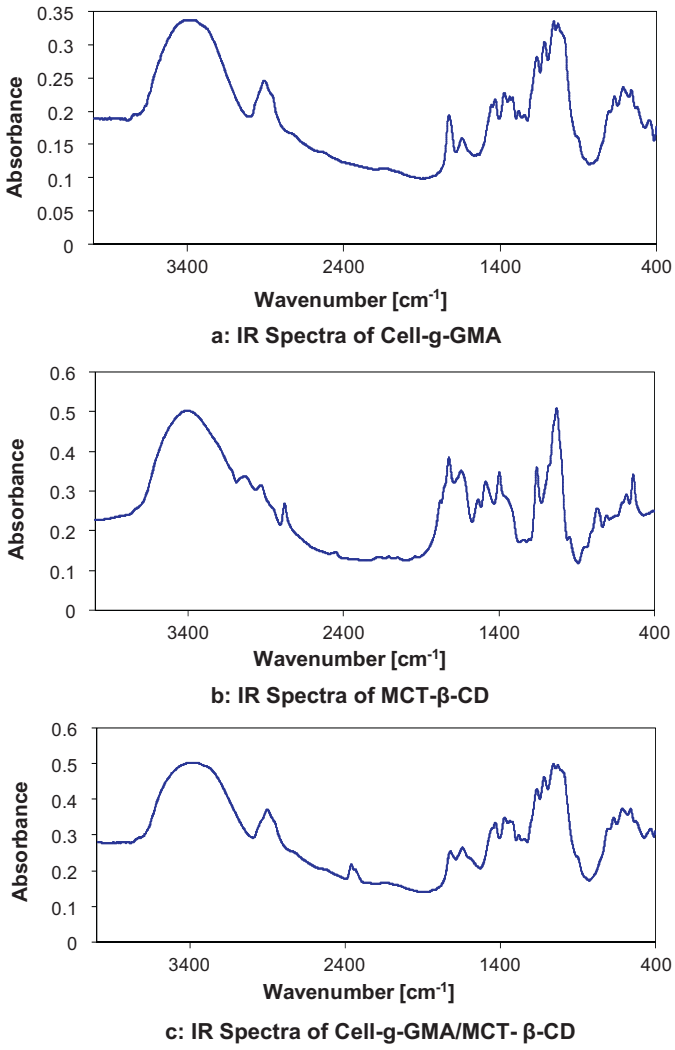

Fig. 1. IR spectra of the copolymer components before loading with antimicrobial agent.

at 1641 cm^{-1} cannot be attributed to the CH=C– group absorption in GMA since it is also recorded in the spectrum of cellulose itself. This indicates that this group is implicated in the grafting process.

The cotton fabric grafted with GMA was further treated with monochlorotrizinyl-β-cyclodextrin (MCT-β-CD). The IR-spectra of MCT-β-CD reagent alone (Fig. 1b) disclosed the presence of strong absorption bands at 3410 (OH), 2932, 2778 (CH), 1643 (C=N) and at 1027 cm^{-1} (Cl–C=N).

The IR-spectra of Cell-g-GMA/MCT-β-CD (Fig. 1c) is neither super imposable with the IR-spectra of Cell-g-GMA nor with the IR-spectra of the reagent MCT-β-CD. The characteristic bands in the IR-spectra of Cell-g-GMA/MCT-β-CD were recorded at 3412 (OH), 2903 (CH), 1723 (C=O, ester), 1643 (C=N) and at 1115 cm^{-1} (–C–O–C–). The strong Cl–C=N– absorption band present at 1027 cm^{-1} in the IR-spectra of MCT-β-CD was absent in the IR-spectra of Cell-g-GMA/MCT-β-CD. This indicates that the chlorine atom in MCT-β-CD is involved in the grafting process which leads to Cell-g-GMA/MCT-β-CD.

Fig. 2 represents the IR-spectra of Cell-g-GMA/MCT-β-CD loaded with chlorohexidin diacetate while Fig. 3 represents the IR-spectra of chlorohexidin diacetate alone. The IR-spectra of chlorohexidin diacetate revealed the presence of absorption bands at 3332 (NH), 3100 (CH, aromatic), 2934 (CH, aliphatic), 1643

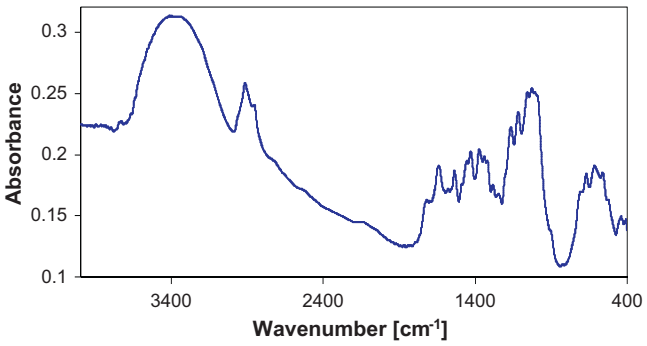

Fig. 2. IR Spectra of Cell-g-GMA/MCT-β-CD loaded with chlorohexidindiacetate.

(C=O), 1532 (C=C, aromatic), 1162 (C–O, stretching) and 1084 cm^{-1} (Cl–Ar).

Careful comparison of the IR-spectra of samples Cell-g-GMA/MCT-β-CD (Fig. 1c) and Cell-g-GMA/MCT-β-CD loaded with chlorohexidin diacetate (Fig. 2), particularly in the fingerprint region indicated that they are not identical. The absorption band at 1550 cm^{-1} corresponding to (C=C, aromatic) is present in the IR spectra of both chlorohexidin diacetate (Fig. 3) and Cell-g-GMA/MCT-β-CD loaded with chlorohexidin diacetate (Fig. 2) while it is absent in the IR spectrum of Cell-g-GMA/MCT-β-CD before loading with the antimicrobial agent (Fig. 1c). However, the intensity of the peak at 1550 cm^{-1} in the IR spectra of Cell-g-GMA/MCT-β-CD loaded with chlorohexidin diacetate (Fig. 2) is smaller than its intensity in the IR spectra of chlorohexidin diacetate alone (Fig. 3). This may indicate some sort of chemical reaction between the antimicrobial agent and the grafted cellulose and not just incorporation of chlorohexidin diacetate in the grafted cellulose prepared.

3.2. Scanning electron microscopy (SEM)

SEM was conducted to view the effect of grafting on the cotton fabrics morphology. Fig. 4a shows the SEM micrograph of a sample of untreated cotton fabric, where the surface is smooth and free from any additions. Fig. 4b shows a sample of cotton fabric grafted with GMA, in which it is clear that the surface has layer of addition and particulates on fibers. Fig. 4c illustrates an SEM micrograph of a cotton fabric grafted with GMA and MCT-β-CD, in which the surface morphology is different from that of GMA only, some flakes of MCT-β-CD are apparent on the surface. The particulate and speckled matter on the cotton fibers in the resulting micrographs confirm the grafting of GMA and additional MCT-β-CD compound onto cotton fabric.

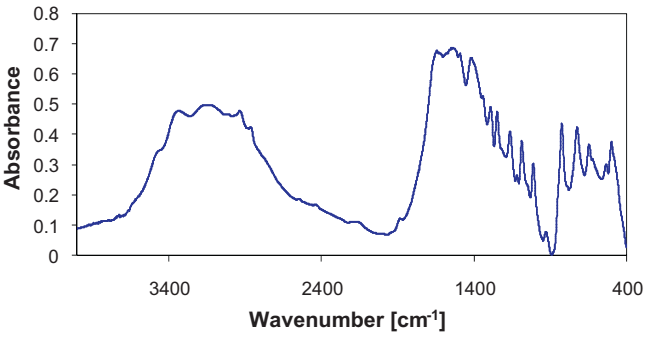

Fig. 3. IR Spectra of chlorohexidindiacetate.

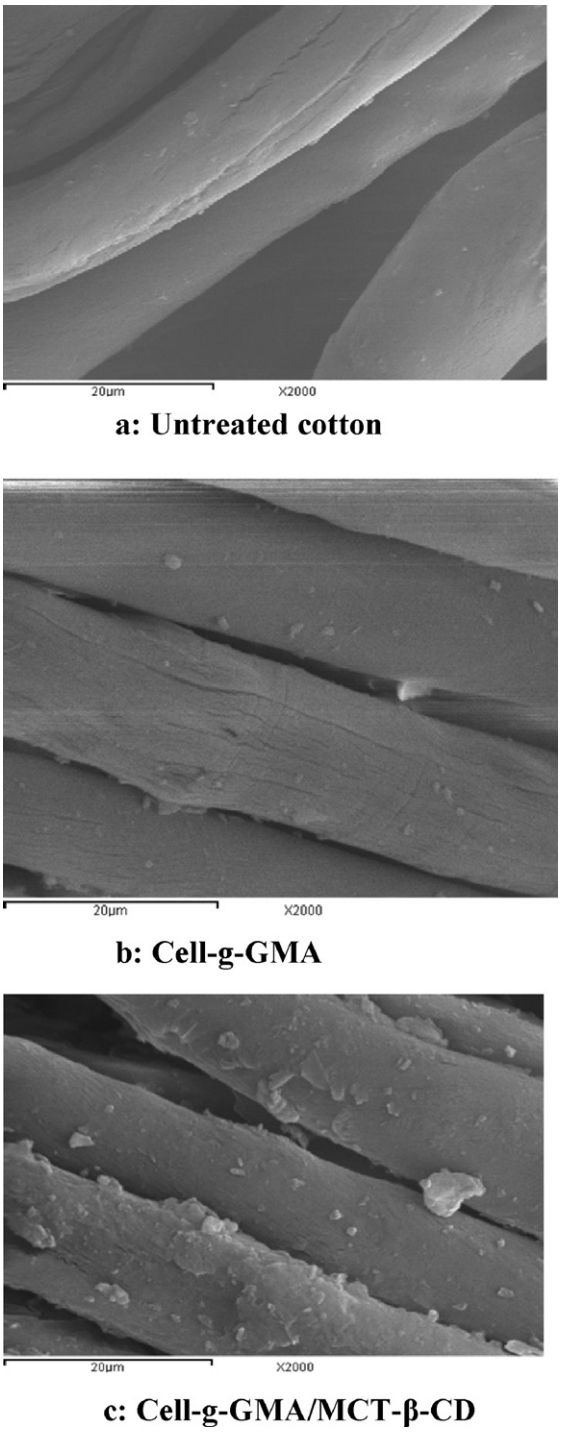

Fig. 4. Scanning electron micrographs of differently treated cotton fabrics.

3.3. Antimicrobial assessment

The antimicrobial fabrics were prepared by means of the methods outlined above. The antimicrobial activities of differently treated cotton fabrics were tested using diffusion disk method, and the results are shown in Table 1. Besides testing the antimicrobial activity of fabrics loaded with the antimicrobial agent, the antimicrobial activities of untreated cotton fabric (control) and cotton fabrics grafted with GMA or GMA/MCT-β-CD were also tested.

Results in Table 1 show that untreated cotton fabric and cotton fabrics grafted with GMA or GMA/MCT-β-CD do not show any

Table 1
Antimicrobial activities of differently treated cotton fabrics.

Extent of treatment	Incorporated antimicrobial agent (mg/m^2)	Inhibition zone diameter (mm/1 cm sample)			
		Escherichia coli (G$^-$)	*Staphylococcus aureus* (G$^+$)	*Candida albicans* (fungus)	*Aspergillus flavus* (fungus)
A	0.0	0.0	0.0	0.0	0.0
B	0.0	0.0	0.0	0.0	0.0
C	0.0	0.0	0.0	0.0	0.0
D	19.5	16	17	18	14
E	24.3	28	24	26	23

Antimicrobial agent, chlorohexidin diacetate; antimicrobial test, diffusion disk method; test carried out after one wash. A: untreated fabric (control), B: fabric grafted with only GMA, C: Fabric grafted with GMA/MCT-β-CD, D: fabric grafted with GMA/MCT-β-CD and then loaded with antimicrobial agent, E: above grafted fabric was regrafted with MCT-β-CD and then loaded with antimicrobial agent.

Table 2
Antimicrobial activities of cotton fabrics with different amount of fixed MCT-β-CD.

Finished fabric		Inhibition zone diameter (mm/1 cm sample)			
Fixed MCT-β-CD (%)	Incorporated antimicrobial agent (mg/m^2)	*Escherichia coli* (G$^-$)	*Staphylococcus aurous* (G$^+$)	*Candida albicans* (fungus)	*Aspergillus flavus* (fungus)
4.28	20.6	24	25	25	26
6.25	26.9	21	21	22	23
10.27	31.7	24	26	24	24
10.42	32.0	25	25	26	24
11.31	33.6	24	26	24	25

Fabric sample: cell-g-GMA/MCT-β-CD loaded with chlorohexidin diacetate as an antimicrobial agent.

Table 3
Effect of repeated washing on the antimicrobial activity of Cell-g-GMA/MCT-β-CD loaded with chlorohexidindiacetate as an antimicrobial agent.

Number of washes	Remaining antimicrobial agent (mg/m^2)	Inhibition zone diameter (mm/1 cm sample)			
		Escherichia coli (G$^-$)	*Staphylococcus aureus* (G$^+$)	*Candida albicans* (fungus)	*Aspergills flavus* (fungus)
0	38.2	33	35	33	33
1	24.3	28	24	26	23
5	19.4	21	20	18	14

Washing conditions: 2 g/l sodium carbonate, 5 g/l non-ionic wetting agent, *M*/*L* ratio 1:20, washing at 60 °C for 15 min.

antimicrobial activity. This means that neither cotton itself nor GMA and MCT-β-CD moieties have any antimicrobial activities by themselves. When cotton fabric grafted with GMA/MCT-β-CD is loaded with the antimicrobial agent, the latter is incorporated to the cavities of MCT-β-CD moiety and the fabric shows good antimicrobial activity. When cotton fabric grafted with GMA/MCT-β-CD is regrafted with MCT-β-CD and loaded with the antimicrobial agent, the fabric shows improvement in its antimicrobial activity. This is because the regrafting process increases the number of hosting cavities and accordingly the amount of incorporated antimicrobial agent increases which leads to better antimicrobial activity.

Table 2 shows the antimicrobial activities of cotton fabrics having different amounts of fixed MCT-β-CD. It is clear from the results that increasing the amount of fixed MCT-β-CD is accompanied by an increase in the amount of incorporated antimicrobial agent through the treatment and accordingly the antimicrobial activity increases. This is logic because the guest molecules depositing property of the fabric is dependent on the amount of monochlorotriazinyl-β-cyclodextrin anchored to the fabric.

Table 3 and Fig. 5 show the effect of repeated washing on the durability of antimicrobial activity of Cell-g-GMA/MCT-β-CD loaded with antimicrobial agent. The durability of antimicrobial activity was expressed in terms of percent retention of antimicrobial activity (inhibition zone of different types of bacteria and fungi) after repeated washings. Although the antimicrobial activities of the said fabrics decrease by repeated washing, but the fabrics still retaining good deal of their antimicrobial properties. This good retention of antimicrobial activity after repeated washing means that the antimicrobial agent is not only just physically adsorbed on the fabric surface but the cavities present in cyclodextrin moieties play an important role in hosting and keeping the antimicrobial agent inside them, resulting in long lasting antimicrobial activity of the treated fabrics (Wang & Cai, 2008).

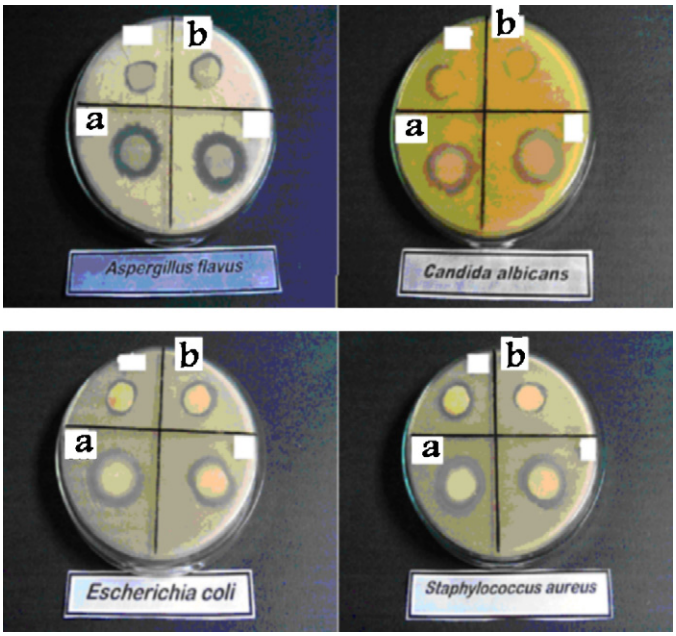

Fig. 5. Inhibition zone of antimicrobial finished cotton fabrics before and after washing.

4. Conclusions

GMA/MCT-β-CD was grafted onto cotton fabric by irradiation technique using linear electron beam for initiation. The so obtained grafted cotton fabric was loaded with commercially available antimicrobial agent (chlorohexidin diacetate). Grafted cotton fabric loaded with the antimicrobial agent, in addition to control and cotton fabrics grafted with GMA or GMA/MCT-β-CD (not loaded with any antimicrobial agent) were tested toward their antimicrobial activity. The fabrics loaded with the antimicrobial agent were found to show very good antimicrobial activity in contrary to control and grafted fabrics which are not loaded with antimicrobial agent. On the other hand, it was found that as the amount of MCT-β-CD, fixed on the fabric increases, the amount of incorporated antimicrobial agent and accordingly the antimicrobial activity of the fabric increases. The results reported in this study demonstrate that the GMA/MCT-β-CD grafted fabrics loaded with antimicrobial agent retain good deal of their antimicrobial activity after five washings. This good retention of antimicrobial activity after repeated washing is due to the cavities present in cyclodextrin moieties which are used to host and keep the antimicrobial agent, resulting in long lasting antimicrobial activity.

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