Development of Colorimetric Analytical Methods to Monitor Quaternary Amine Grafted Surfaces

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ABSTRACT: Quick and simple colorimetric analytical techniques were developed to monitor functionalization of surfaces with quaternary amine bearing polymers as complementary qualitative tests to the traditionally used antimicrobial test protocols. First, an established antimicrobial quaternary ammonium salt (QAS) was chosen as a probe compound and an acrylol functional group was introduced (acrylol-QAS) to facilitate its polymerization with acrylic monomers. Surface graft polymerization was carried out with methyl methacrylate (MMA) and acrylol-QAS on cotton fabric surface. The success of graft polymerization, durability after repetitive aqueous extraction, and the effect of crosslinking agents on the durability and extent of polymerization were tested with a fluorescein spot and a bromophenol solution test. The results of these colorimetric analytical methods were in perfect agreement with those of antimicrobial tests, XPS, and SEM analysis. J Appl Polym Sci 118: 2397–2407, 2010

Key words: coatings; crosslinking; graft copolymers; surfaces

INTRODUCTION AND BACKGROUND

Development of durable antimicrobial surfaces has been at the forefront of research on functional textiles.[1,2] Numerous antimicrobial agents such as quaternary ammonium salts (QAS), *N*-halamines, phosphonium salts, silver ions, and natural bioactive agents have been utilized to impart antimicrobial properties to surfaces.[3–7] Generally, functional moieties are applied to surfaces, particularly to textiles, by three different methods. The first method is the dispersion/blending of active agents with desired properties in textile finishing chemicals or coating formulations followed by pad-dry-cure, exhaustion, foaming, or any other fabric application method.[8–10] The resulting and lasting impact is difficult to predict with this technique, though, since the active agent may be entrapped in the depths of the coating matrix and/or the activity may not be sustainable due to perpetual leaching off of the surface with repetitive washing.

The second widely used method is to chemically anchor active agent to the textile surface such as cotton, polyester, and nylon via a crosslinker.[1,11] With this approach, a crosslinker between the surface and the functional group is required such as acryl, epoxy, sulfonate, amino, hydroxyl, or carboxyl groups to chemically attach functional moieties to fibers.[12] However, a high degree of surface functionalization and immobilization is required to achieve the desired antimicrobial properties. The presence of textile finishing chemicals on the surface can also be problematic since they can interfere or inhibit the reaction between surface and the active agent. In addition, monomeric antimicrobial agents due to their low molecular weight suffer from many disadvantages such as toxicity to the environment upon leaching off of the surface and ineffective antimicrobial activity.

The third method used to engineer functional surfaces is to introduce active agents as chemically tethered in high molecular weight polymers.[2,11,13–36] Antimicrobial monomers could be readily copolymerized with most acrylic, substituted-acrylic, vinyl and siloxane prepolymers, generating so called high molecular weight "biocidal polymers."[13] Enhanced antibacterial activity, reduced toxicity, longer lifetime, and durability have been reported with biocidal polymers bearing antimicrobial agents as comonomers.[2,11,14–36] Biocidal polymers can cover the entire surface area in a uniform manner while having pendant active groups intact and available on the surface. For instance, Klibanov and coworkers have developed high molecular weight hydrophobic biocidal polymers on glass surface by first immobilizing PVP, poly(4-vinylpyridine) to a NH_2-glass slide followed by *N*-alkylation.[15] These slides were highly efficient against *S. aureus* (94 ± 4% kill), and

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even deadlier to *S. epidermidies* (>99%), as well as common Gram-negative pathogens *E. coli* and *P. aeruginosa*. There was no leaching off of the active agent or development of antibacterial resistance. In another study, Sun et al. have copolymerized cyclic *N*-halamines with acrylonitrile, vinyl acetate, and methyl methacrylate (MMA) obtaining high antibacterial activities.[37,38] It is imperative to maintain structural integrity of the polymer while achieving optimum antimicrobial activity. Sun et al. found that 5 wt % of the hydantoin comonomers were adequate to obtain desired antibacterial properties without deterioration of the original properties of the biocidal polymers. Worley and coworkers successfully synthesized siloxane biocidal polymers and applied as antimicrobial coatings.[29–31] Wijas et al. reported that polysiloxane biocidal polymer containing QAS had a good antimicrobial activity whereas QAS monomer has no activity when bonded to the surface alone.[35] It is also possible to increase the concentration of antimicrobial groups in the polymer by simply using polyhedral oligomeric silsesquioxane (POSS) which can take up to eight functional groups per molecule while maintaining structural integrity of the polymer.[39] The high density of antimicrobial groups combined with simplicity of siloxane chemistry translates to high antimicrobial surfaces with extended durability.

A good illustration of these three methods explained above to generate antimicrobial surfaces is a series of studies by Hong and Sun.[40–42] First, they physically blended benzophenone, a photoinduced antimicrobial agent, as a functional additive with polymers.[40] Second, they chemically anchored benzophenone molecules via hydroxyl functional groups onto cotton surface.[41] Third, they polymerized benzophenone with prepolymers to generate biocidal poly (styrene-*co*-vinylbenzophenone) polymer.[42] Although effective antimicrobial activity was reported in all three studies, they did not provide any comparative data as to which method is effective in terms of antimicrobial activity and durability.

The method of antimicrobial activity evaluation is critical in assessing the effectiveness of surface treatments. Screening studies of biocidal activity generally fall into two categories: the agar diffusion test and suspension test. Agar diffusion (AATCC 147) is rather a qualitative test which involves placing a treated surface directly onto an agar plate onto which the tester organisms have been freshly spread or streaked. After the incubation, if biocidal activity is present zones of inhibition (ZOI) of bacterial growth around the substrate will be generated in proportion to the concentration and diffusibility of biocide.

A suspension test is used to determine the minimum amount of biocide, minimal inhibitory concentration (MIC), required to kill the microorganisms tested. It is done by adding serial twofold dilutions of the biocidal material to a series of freshly inoculated culture broths and observing where growth is inhibited. Quantitative testing of biocidal surfaces, however, is a more complicated process because it involves application of microorganisms to surfaces and recovery for enumeration by swabbing after periods of time. The accuracy of this approach thus depends on the efficiency of microorganism recovery and the similarity of incubation conditions to those intended for the actual coating application. For textile surfaces, swabbing is too inefficient since internal contaminated fibers cannot be reached. Results might differ by two orders of magnitude with this technique. In addition, both mentioned techniques can be very time consuming.

Therefore, there is a need for a quick, accurate, and easy method to screen functionalized surfaces. Colorimetric analytical methods have been widely used to monitor variety of analytes in the literature.[43,44] For instance, fluorescein salt dye staining technique was successfully used by Huang et al. to show the surface modification with QAS groups.[43] In solution, bromophenol blue is used to detect QAS by monitoring the shift of λ_{max} from 613 nm to 592 nm as a result of complex formation between chromophore and the analyte.[44] In this work, two colorimetric analytical methods have been developed to monitor surface functionalization of textiles with biocidal polymers containing QAS groups. Fluorescein spot and bromophenol solution tests were employed in parallel to monitor extent of functionalization of textile surfaces with QAS containing biocidal polymers. Results of both techniques are in good agreement with those of lengthy and cumbersome standard antimicrobial test methods and elemental analysis. These two easy and quick colorimetric analytical techniques can be used when a large amount of samples needs to be screened in a short period of time.

MATERIALS AND METHODS

Bleached desized cotton (#400) was purchased from TestFabrics, (West Pittson, PA). Trimethylolpropane trimethacrylate esters (SR350®) and acrylate esters (CN309®) were kindly supplied by Sartomer Company. Cylink® NMA (48 wt % *N*-methylolacrylamide in aqueous solution) was kindly supplied by Cytek Industries. Toluene (reaction solvent), methanol (dissolving/collecting polymers), and hexane (washing polymers) were purchased from VWR. Fluorescein, bromophenol blue, potassium persulfate, acrylic acid, methyl methacrylate (MMA), acrylol chloride, 4-dimethylamino pyridine (DMAP), and benzoyl peroxide (BPO) (reagent grade 97%) were purchased

from Sigma Aldrich. Triethylamine was obtained from EMD Chemicals. All chemicals were used as received. QAS biocide and ensuing QAS biocidal polymers were prepared at Luna Innovations laboratories, Blacksburg, VA.

Characterization

Fourier transform infrared (FTIR) spectroscopy was performed with a Shimadzu 600 FTIR spectrometer and measurements were carried out either by casting thin films of soluble samples and by evaporating the methanol solvent, or by preparing and scanning insoluble powders in the presence of potassium bromide.

UV–Vis absorption spectra were measured with a Shimadzu UV–2450 UV–vis spectrophotometer in a wavelength range of 300–600 nm with a 1-cm quartz cell.

Scanning electron microscopy (SEM) evaluation of the polymer grafted cotton samples was carried out using JEOL JSM 6060 LV scanning electron microscope (Virginia Tech, Blacksburg), where samples were coated with a 15 nm layer of gold before observation.

X-ray photoelectron spectroscopy (XPS or ESCA) analyses were carried out on a PHI-TFA XPS spectrometer (Physical Electronics) at Virginia Tech facility. The analyzed area was 0.4 mm in diameter and about 3–5 nm in depth. Sample surfaces were excited by X-ray radiation from a monochromatic Al source at a photon energy of 1486.6 eV. C 1s, F 1s, O 1s, N 1s and Si 2p spectra were acquired with an energy resolution of about 1.0 eV with an analyzer pass energy of 58 eV. Quantification of surface composition was performed from XPS peak intensities measured on three different spots of the sample, taking into account the relative sensitivity factors provided by the instrument manufacturer.

EXPERIMENTAL

Functionalization of quaternary ammonium salt (QAS) with acryloyl group

Antimicrobial agents that contain reactive functional groups such as hydroxyl, carboxyl, or amino groups can be covalently linked to a wide variety of polymerazible monomers. Most widely used biocidal polymers are acrylic types of antimicrobial agents.[45] The QAS selected for this study was *N*-acrylolylated with acryloyl chloride to introduce functional acryloyl group to the biocide for its further polymerization with mono- and bi-functional monomers.

Selected QAS biocide (200 g, 0.5 mol) was dissolved in 1.5 L of dry chloroform in flame-dried glassware under nitrogen purge. Triethylamine (99.1 mL, 0.7 mol) as well as catalytic amount of DMAP was added to the mixture and the reaction was then cooled to $\sim 0^{\circ}C$ in an ice water/NaCl bath. Acryloyl chloride (53.4 mL, 0.7 mol) was dissolved in 50 mL of dry chloroform in an addition funnel and then added drop wise to the reaction flask over a period of 3 h. The reaction was allowed to warm to room temperature over an 8–10 h period with stirring. After complete addition of the acryloyl chloride the reaction mixture was yellow in color. The reaction mixture was again cooled to $0^{\circ}C$ and a second equivalent of acryloyl chloride was added drop wise to the mixture over a period of two hours. A miniscale work-up was performed the next morning to check for reaction completion. Approximately 4 mL of the reaction mixture was diluted with 10 mL of chloroform, washed with brine, then washed with water, and dried over magnesium sulfate. Starting material was still present by proton NMR. As a result the reaction mixture was once again cooled to $0^{\circ}C$ in an ice water bath and 23.6 mmol of acryloyl chloride in 25 mL chloroform was added to the mixture drop wise over a period of 3 h and allowed to warm to room temperature overnight. The next morning the reaction mixture was yellowish-orange in color. The reaction was then further diluted with chloroform. The mixture was then washed with water ($3 \times 1L$), and then with brine ($3 \times 1L$). The solution was then dried over magnesium sulfate and collected. The material was then concentrated first on a rotary evaporator and then under nitrogen purge. No starting material was present by NMR. Acrylol-QAS is water soluble.

Polymerization of Acrylol-QAS with Methyl Methacrylate (MMA)

The biocidal polymer Acrylol-QAS-*co*-MMA contains 50 wt % acrylol-QAS. Acrylol-QAS (3.0 g) was dissolved in 250 mL toluene in a 1L round-bottom flask. MMA (3.0 g) and 0.18 g benzoyl peroxide as initiator ($\sim$3 wt % of total copolymers) were added and solution was mixed with a stir bar. The solution was then degassed for 45 min with nitrogen gas. The reaction was then heated to $70^{\circ}C$ and maintained at that temperature for 4 h under constant stirring and inert atmosphere. At the end of the reaction, solvent was removed by rotary evaporator followed by hexane wash. The polymer was finally dried overnight in vacuum oven at 60–$65^{\circ}C$.

Polymerization of Acrylol-QAS with MMA in the presence of cross-linkers

Two crosslinking agents were tested in combination with SR350® (trimethylolpropane trimethacrylate)

from Sartomer Company: Cylink® NMA (*N*-methylolacrylamide) monomer from Cytec Industries and CN309® (proprietary acrylate esters) from Sartomer Company. Syntheses were carried out the same way as described above by addition of each crosslinker to the reaction medium. 5 wt % Sartomer SR350® was added to each formulation along with 5 wt % of either Cylink® NMA or CN309® to increase durability.

Surface graft polymerization

Cotton fabrics were grafted with copolymers of Acrylol-QAS and MMA with and without crosslinkers of CN309® and Cylink® NMA. Graft polymerization reactions were carried out in the presence of 3 × 3 inch cotton swatches (3 pieces) by following the same procedure explained above. At the end of reaction, cotton swatches were vacuum dried to remove the solvent entirely for further analysis. Polymers in the reaction flask were collected by evaporation and hexane wash.

Fluorescein spot test

This colorimetric analytical test is designed to answer following questions: (i) is QAS biocidal polymer effectively grafted on the cotton surface and (ii) is the grafted QAS biocidal polymer durable after repetitive washing.

Fluorescein salt dye is known to selectively bind to quaternary ammonium groups.[46] Fluorescein salt dye staining technique was successfully used by Huang et al. to show the surface modification with QAS groups.[43] It clearly showed in accordance with ATR-FTIR measurements an increase in color intensity as the surface concentration of QAS group increases.[43]

Fluorescein salt dye has an absorption maximum at 501 nm. Fluorescein (1 wt %) sodium salt was prepared in aqueous solution and filtered to remove impurities before use. The resulting solution was bright yellow. For the colorimetric analytical detection of QAS presence, 1 mL of fluorescein solution was applied to the surface of 1 × 1 inch cotton swatches, grafted and ungrafted as control. Very distinct yellow circles were observed. Dye molecules are expected to form a strong complex with the QAS present on the surface and hence will resist any attempt to extract them into solution. After application of fluorescein dye solution, each fabric was placed in 40 mL EPA vial containing 10 mL deionized water. The vials were vigorously shaken to remove any uncomplexed fluorescein molecules. The solution was analyzed with UV–vis spectrophotometer for the presence of eluted fluorescein molecules after removal of cotton swatches.

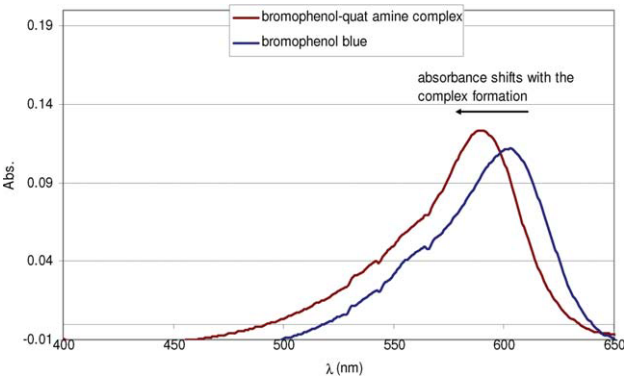


Figure 1 Bromophenol absorption maximum shifts to shorter wavelength upon forming a complex with quaternary amine groups (QAS). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Bromophenol blue solution test

Bromophenol blue solution colorimetric analytical test is designed to monitor the durability of surface grafted QAS biocidal polymer upon repetitive washing.

Bromophenol blue is a dye which forms a complex with QAS and experience a shift of λ_{max} from 613 nm to 592 nm as a result of complex formation (Fig. 1), which is discernible to naked eye.[44] An aqueous solution of 0.5 wt % bromophenol blue was prepared and the solution was buffered with Na_2CO_3 (pH ~7.0) to avoid absorbance changes due to pH fluctuations; although no pH variation was observed with the addition of extracts from the grafted cotton washings to the bromophenol solutions.

Cotton swatches were subjected to repetitive extraction with 10 mL deionized water in 40-mL EPA vials as described previously. A 5 mL aliquot of extracts was then added to 10 mL of 0.5 wt % buffered bromophenol blue solution. UV–vis measurements were recorded between wavelength regions of 400–650 nm. Extraction repeated 5, 10, and 20 times (5, 10, and 20×).

MIC determination to evaluate biocidal activity

MICs of QAS biocide alone, Acrylol-QAS biocide, Acrylol-QAS-*co*-MMA (Sample 1), Acrylol-QAS-*co*-MMA with crosslinker CN309® (Sample 2), and Acrylol-QAS-*co*-MMA with crosslinker Cylink® NMA (Sample 3) were determined. Test organisms, *Staphylococcus aureus* 12,598, *Acinetobacter baumannii* 49,466, and *Escherichia coli* 0157:H7, were inoculated into 5 mL of Luria-Bertani (LB) broth and allowed to grow at 37°C overnight. The following day, the cultures were adjusted to an optical density of 0.5 at 600 nm using fresh LB broth. In addition, the unknown biocides were dissolved in phosphate buffered saline at a concentration of 1 mg/mL. Each biocide was serially diluted twofold in LB broth

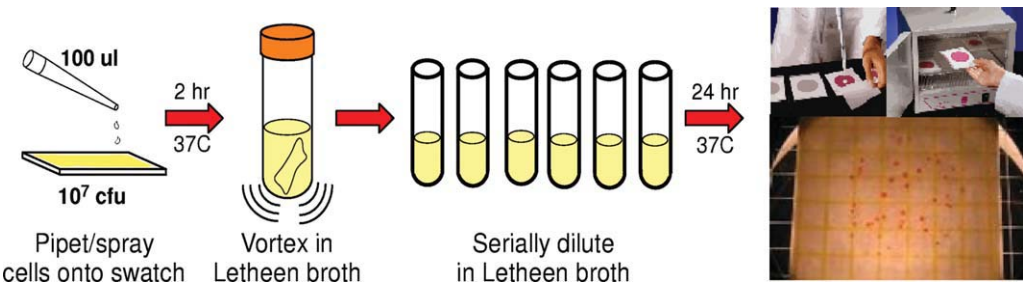


Figure 2 AATCC method 100 for evaluating biocidal textiles. Swatches are contaminated with bacteria, incubated for varying times and vortexed in broth. Then, the resuspended cells are serially diluted and plated onto aerobic count PetriFilms. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

beginning with 200 μg/mL and ending with 6 ng/mL. A 20 μL solution of test organism (OD 600 = 0.5) was added to each dilution of biocide as well as a broth control tube and incubated at 37°C for 24–48 hours. Growth in the tubes was measured at 600 nm by UV–vis spectrophotometer and the MIC was determined by the lowest concentration of biocide that showed no growth of organism.

Plate count enumeration test [AATCC 100 method (2004)]

Samples from three different places on each treated and untreated cotton swatches were aseptically cut (~1.5 × 1.5 cm squares) and placed in sterile 60 mm polystyrene petri dishes. Overnight LB broth cultures of *S. aureus* 12,598, *A. baumannii* 49,466, and *E. coli* 0157:H7 were adjusted to ~10^8 cfu/mL and diluted 1/10 in sterile PBS buffer (~10^7 cfu/mL). Triplicate swatches of treated and untreated cotton control samples then were spotted with 100 μL (~10^6 cfu) of each of the diluted tester organisms. As a nonincubated control, one of each of the test and control swatches was immediately enumerated for the presence of viable bacteria as described later. The remaining swatches, including a second set of controls, were incubated at ambient temperature and relative humidity in a covered petri plate for 2 h. Following the 2 h incubation, the control and bacteria-treated swatches were placed into 5 mL Letheen broth in a sterile 50 mL tube and vortex for 1 minute. The rinsed swatches were then serially diluted 10^{-1} to 10^{-5} in Letheen broth and 1 mL of the rinse, and each dilution were "plated" directly onto PetriFilms in duplicate. PetriFilms (3M Corporation) for aerobic counts are reliable and ready made plating system containing standard nutrients in a cold water soluble gelling agent and a tetrazolium indicator dye which facilitates colony counting. The dilutions and the PetriFilms then were incubated at 37°C for 24 h after which the colonies were counted and cfu/mL were calculated (Fig. 2). In addition, the growth in the dilutions is used for confirmation of the quantitative colony enumeration, i.e., the last tube to grow organisms is the end point dilution (EPD) and indicates relative log reduction in viable organisms.

Parallel-streak test [AATCC 147 method (2004)]

An overnight culture of each *S. aureus* , *E. coli, and A. baumannii* was diluted 1/10 in PBS buffer and

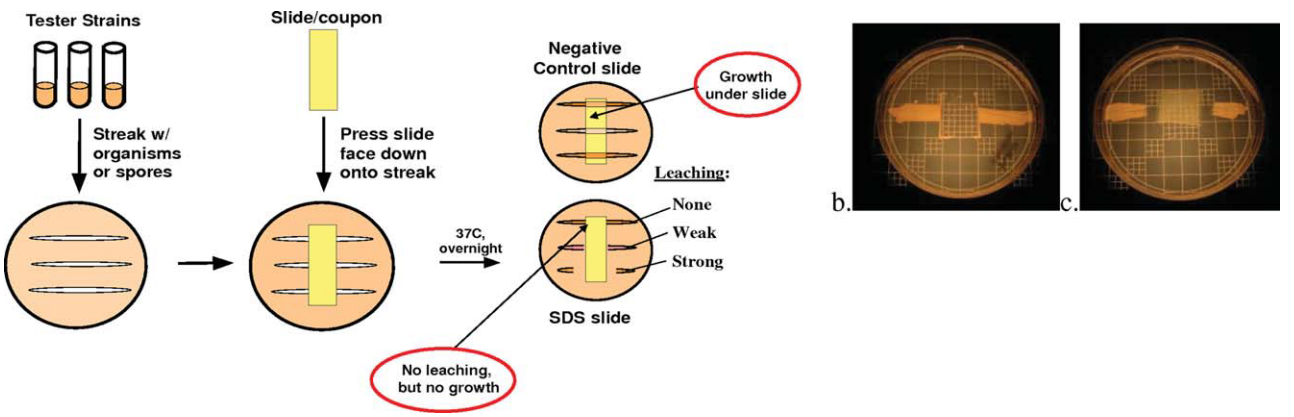


Figure 3 Modified AATCC method #147 for evaluating biocidal textiles. (a) biocidal textiles are placed directly across streaked tester strains and incubated (b) control slide (no biocide), growth occurs up to and under the slide indicating no activity (c) biocide treated textile, no growth near slide indicating considerable leaching. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Minimum Inhibitory Concentrations (MIC) of Biocides Synthesized

	S. aureus	*E. coli*	*A. baumannii*
QAS biocide	781 ng/mL	25 μg/mL	12.5 μg/mL
Acryloyl-QAS biocide	781 ng/mL	25 μg/mL	6.25 μg/mL
Acrylol-QAS-*co*-MMA (Sample 1)	1.56 mg/mL	100 μg/mL	25 mg/mL
Acrylol-QAS-*co*-MMA with crosslinker CN309® (Sample 2)	9.76 ng/mL	50 μg/mL	781 ng/mL
Acrylol-QAS-*co*-MMA with crosslinker Cylink® NMA (Sample 3)	1.56 mg/mL	25 mg/mL	6.25 mg/mL

streaked onto LB agar plates. Duplicate ~1.5 cm^2 treated and untreated cotton swatches were pressed evenly onto the streaks and the plates were incubated at 37°C for 24 h. Following incubation the plates were examined for zone of inhibition and growth underneath the swatches (Fig. 3). The size of the zone of inhibition from the edge of the cotton swatches indicates the degree of leaching and relative biocidal activity of the textile. Growth up to but not underneath the swatch indicates nonleaching bacteriostatic activity (prevention of growth), whereas growth underneath indicates no activity.

RESULTS AND DISCUSSION

MIC results

MICs, the lowest concentration of the synthesized biocides that will inhibit visible growth of a microorganism after overnight incubation, are shown in Table I. As it can be seen from the MIC data, QAS biocide did not lose its biocidal activity upon functionalization with acrylol group. According to the data, the biocidal activity increased with the addition of crosslinkers. Most importantly, although copolymerization of acryloyl-QAS with MMA (Sample 1) loses its effectiveness against *S. aereus* (twofold), *A. baumannii* (twofold), and *E. coli* (fourfold), addition of crosslinkers (Samples 2 and 3) seems to bring it back to its original form. In the case of *A. baumannii*, Acrylol-QAS-*co*-MMA crosslinked with Cylink® NMA (Sample 3) showed even higher activity (twofold) than that of QAS biocide itself. MIC control experiments were performed with MMA, MMA reacted with SR350®, MMA with crosslinker CN309® and Cylink® NMA, and no biocidal activity was observed.

Plate count results

Plate count tests reveal that all 3 samples were highly effective at killing selected test organisms (Table II). To test the success of graft polymerization on the cotton surface and the durability of graft after water extraction, samples were subjected to extraction with deionized water and air dried. Microbial tests show that the samples with crosslinkers continue to be effective whereas Sample 1 has no bioactivity after one aqueous extraction.

Fluorescein spot test results

To test the hypothesis that the presence of fluorescein dye in the extract of treated samples would either signal absence of QAS on the cotton or leaching off of the substrate, fluorescein qualitative spot test was performed as explained in the experimental section. As seen in Figure 4, Sample 1 extract has a

TABLE II
Samples 2 and 3 Retain Antimicrobial Activity Upon Aqueous Extraction Suggesting Durable Grafting While Sample 1 Is Not Durable

	S. aureus	*E. coli*	*A. baumannii*
	Log kill	Log kill	Log kill
No coating and no washes (untreated cotton)	0	0	0
Acrylol-QAS-*co*-MMA (Sample 1)	6	4	6
1× H_2O extracted Sample 1	0	0	0
Acrylol-QAS-*co*-MMA with crosslinker CN309® (Sample 2)	6	4	6
1×H_2O extracted Sample 2	5	2	4
Acrylol-QAS-*co*-MMA with crosslinker Cylink® NMA (Sample 3)	7	4	6
1×H_2O extracted Sample 3	7	4	6

Bacteria concentration 10^6–10^7 CFU/mL, contact time 2 h. One log reduction means 90% kill, two log reduction means 99% kill, and six log reduction is 99.9999% kill.

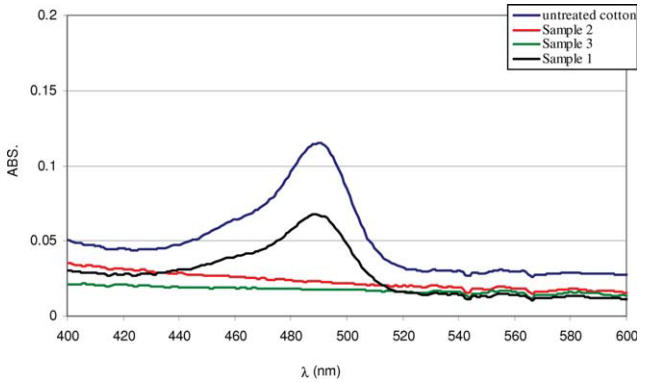


Figure 4 Untreated cotton has no QAS to retain fluorescein and strong absorption band around 501 nm indicates that fluorescein is not retained. Fluorescein in Sample 1 extract might be an indication of either dissolution of QAS or its absence on the surface. Sample 2 and 3 suggests successful and durable grafting with no observable fluorescein presence (All samples 1× extracted). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

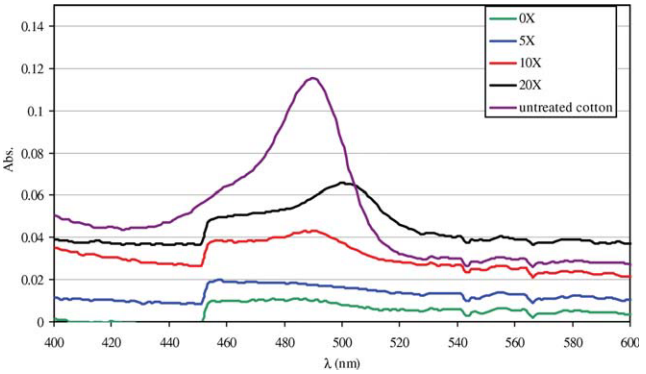


Figure 5 Sample 2 graft starts to dissolve after cycle 5 of aqueous extraction. Crosslinker CN309® imparts a considerable durability compared to Sample 1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

strong absorption band around 501 nm while Sample 2 and 3 extracts have no absorptions upon one extraction. Fluorescein experiments reveal that Sample 1 either does not have QAS permanently grafted on the surface or upon aqueous extraction it is leaching off of the surface. Samples 2 and 3 seem to retain the fluorescein salt on the cotton substrate after one aqueous extraction. These results correlate well with the biocidal activity data obtained from plate count experiments, showing that Sample 1 lost its biocidal activity upon aqueous extraction, whereas Samples 2 and 3 retained theirs, the latter having better activity (Table II). They also validate the use of fluorescein spot test as a quick and easy motoring method of QAS-treated surfaces. Untreated cotton swatch was also tested as control, showing it does not retain fluorescein salt which exhibited strong fluorescein absorption band around 501 nm in the extract.

Upon 1× aqueous extraction, Sample 2 showed lesser biocidal activity compared to Sample 3 (Table II). Samples 2 and 3 were subjected to further extraction and the extracts of 5, 10 and 20 cycles (5, 10, and 20×) were used for fluorescein spot test analysis. Figure 5 shows the results of the fluorescein spot test of Sample 2 (Acrylol-QAS-*co*-MMA crosslinked with CN309®). Between cycle 5 and 10, fluorescein absorption peak appears in the extract. The fact that unwashed Sample 2 has no fluorescein absorption indicates the presence of QAS on the cotton surface, and the appearance of the peak can be attributed to the slight dissolution of the graft from the surface after 5×. Cycle 20 has a discernable absorption peak, indication the QAS graft continues to dissolve from the surface.

Figure 6 shows the fluorescein spot test for Sample 3 (Acrylol-QAS-*co*-MMA crosslinked with Cylink® NMA). No leaching of the graft was observed up to 20× aqueous extraction. Compared to Sample 2 (Fig. 5), Sample 3 showed considerably higher durability. The only difference between Sample 2 and Sample 3 is the nature of the crosslinker; Sample 2 has a crosslinker with methacrylate functional group whereas Sample 3 has a crosslinker with hydroxyl and amide functional groups. The reason for the weak durability of methacrylate polymers compared to acrylamides might be due to the hydrolytic cleavage of the ester bond, leading to the eventual loss of the surface grafted polymer film. While the vinyl functionality of Sample 3 taking part in polymerization, hydroxymethyl groups might be reacting with hydroxyl groups of the cotton surface, generating more durable grafted polymer with biocidal activities. The interchain crosslinking in addition to the covalent bonding with the surface provides extra durability to the biocidal polymer grafting.

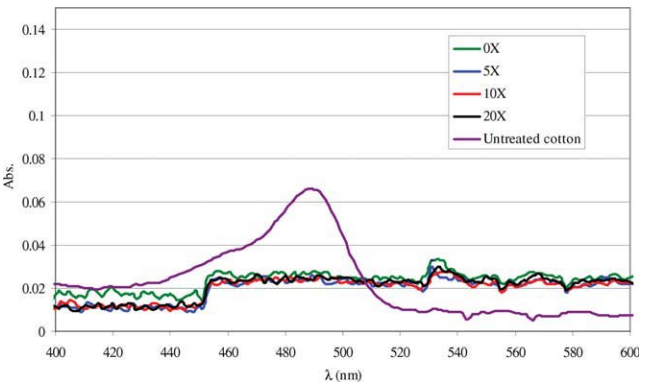


Figure 6 Sample 3 shows excellent durability up to 20 cycle of aqueous extraction which can be seen from the lack of any fluorescein absorption peak in the extracts. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III
Sample 3 Shows Biocidal Activity After 20X, Suggesting a Good Durability of the Biocidal Polymer Graft. Sample 2 Loses its Biocidal Activity After 20×

	S. aureus	*E. coli*	*A. baumannii*
	Log kill	Log kill	Log kill
No coating and no washes (untreated cotton)	0	0	0
Acrylol-QAS-*co*-MMA with crosslinker CN309® (Sample 2)	6	4	6
20× extracted Sample 2	0	0	0
Acrylol-QAS-*co*-MMA with crosslinker Cylink® NMA (Sample 3)	7	4	6
20× extracted Sample 3	6	3	6

After 20 cycles of extraction, residual antimicrobial activity of Sample 2 and Sample 3 was tested by plate count enumeration test (Table III). Table III corroborates well with the fluorescein spot test results. More durable Sample 3 still showed biocidal activity after 20 repetitive aqueous extractions. Sample 2, however, displays no residual biocidal activity after 20× extraction.

Bromophenol solution test results

Once the nature of durability is established, the second step would be to test if the grafted polymer is dissolving from the surface with repetitive water extraction. Bromophenol solution test is designed so that any QAS leaching off of the substrate can be qualitatively detected by its complex formation with bromophenol in the solution. This QAS:bromophenol complex results in an discernible shift of bromophenol λ_{max} to a shorter wavelength (Fig. 1).

Aliquots (5 mL) of Sample 2 extracts (5, 10, and 20×) were added to 10 mL of buffered bromophenol solution as explained in the experimental section. Figure 7 shows bromophenol solution analysis of Sample 2. No QAS in the extract was observed up to 5× cycle. Repetitive extractions beyond 5× result in leaching off the QAS containing graft of the substrate, indicated by the shift of bromophenol absorption peak. This result is in good agreement with both that of the fluorescein spot test and biocidal activity measured by plate count test.

Sample 3 bromophenol solution tests are shown in Figure 8. It is evident from the figure that there is no QAS in the solution, i.e., λ_{max} did not shift with subsequent washes up to 20×. Bromophenol solution test combined with fluorescein spot test shows that the graft polymerization of Acryloyl-QAS-*co*-MMA with crosslinker Cylink® NMA (Sample 3) is durable with no detectable QAS leaching off of the substrate up to 20 cycle of water extraction. This result is supported by the results of fluorescein spot test and plate count enumeration test as well.

XPS analysis results

Atomic percentages (atom %) for untreated cotton, and Samples 1, 2, and 3 are shown in Table IV. As expected, untreated cotton has no nitrogen atom within experimental error. Sample 1 has 5.40% higher concentration of C atom and 19.5% lower concentration of O atom compared to untreated

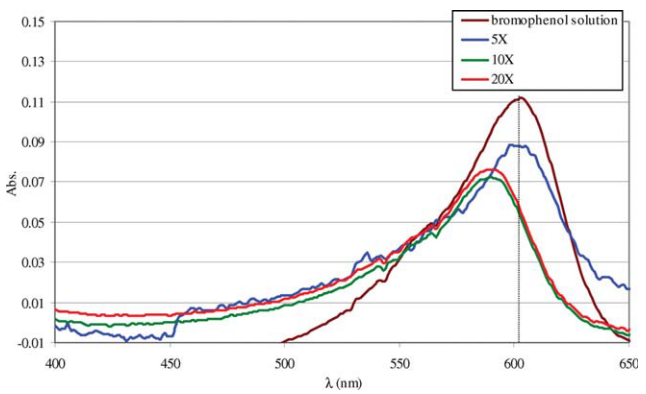


Figure 7 Sample 2 aqueous extracts start showing a λ_{max} shift after 5×, suggesting dissolution of QAS containing biocidal polymer from the cotton fabric. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

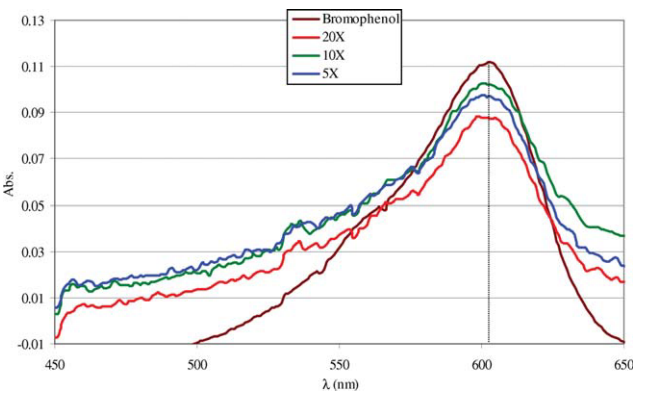


Figure 8 Sample 3 reveals no λ_{max} shift, an indication that the biocidal polymer graft is still intact after 20×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE IV
X-Ray Photoelectron Spectroscopy Showing the Atomic Concentration of Samples Treated (Experimental Error: ±0.1, Standard Deviation: ±0.0)

Atomic concentration table (%)			
	C1s	N1s	O1s
(untreated cotton)	63.0	0.10	36.8
Acrylol-QAS-*co*-MMA (Sample 1)	66.4	0.59	29.6
Acrylol-QAS-*co*-MMA with crosslinker CN309® (Sample 2)	74.0	1.46	23.5
Acrylol-QAS-*co*-MMA with crosslinker Cylink® NMA (Sample 3)	81.8	1.97	15.7

cotton. In addition, nitrogen atoms were observed on Sample 1 surface. This result is expected since Acrylol-QAS-*co*-MMA grafted cotton (Sample 1) has more carbon atom coming from the grafted polymer, less oxygen atom since the covalent bonding decreases the hydroxyl surface groups, and some nitrogen atoms (0.59%) coming from the QAS groups tethered in the graft.

Sample 2 has 17.4% higher concentration of C atom and 36.0% lower concentration of O atom compared with untreated cotton. Sample 2 is Acrylol-QAS-*co*-MMA with crosslinker SR350®, a high molecular weight polymer, grafted on cotton fabric. There is also high concentration of N atoms detected on Sample 2 (1.46%), suggesting high amount of QAS copolymerization. Clearly, use of crosslinking agent both increased the amount of QAS incorporated into the polymer and graft yield on the surface. In fact, Sample 2's concentration of N atoms (1.46%) is 2.5 times higher than Sample 1 (0.59%).

Sample 3 has a 29.9% higher concentration of C atom and 57.2% lower concentration of O atom compared to untreated cotton. Sample 3 has also the highest amount of nitrogen atom concentration among the samples tested (1.97%). Sample 3 has a bifunctional crosslinking agent, Cylink® NMA, with vinyl and hydroxymethyl groups which can result in higher molecular weight polymers and better grafting on the surface. These XPS results compare favorably to the results of fluorescein spot test, bromophenol solution test, and biocidal activity of samples tested.

SEM

Field emission SEM measurements showed that at 100× magnification, all samples after 5× aqueous

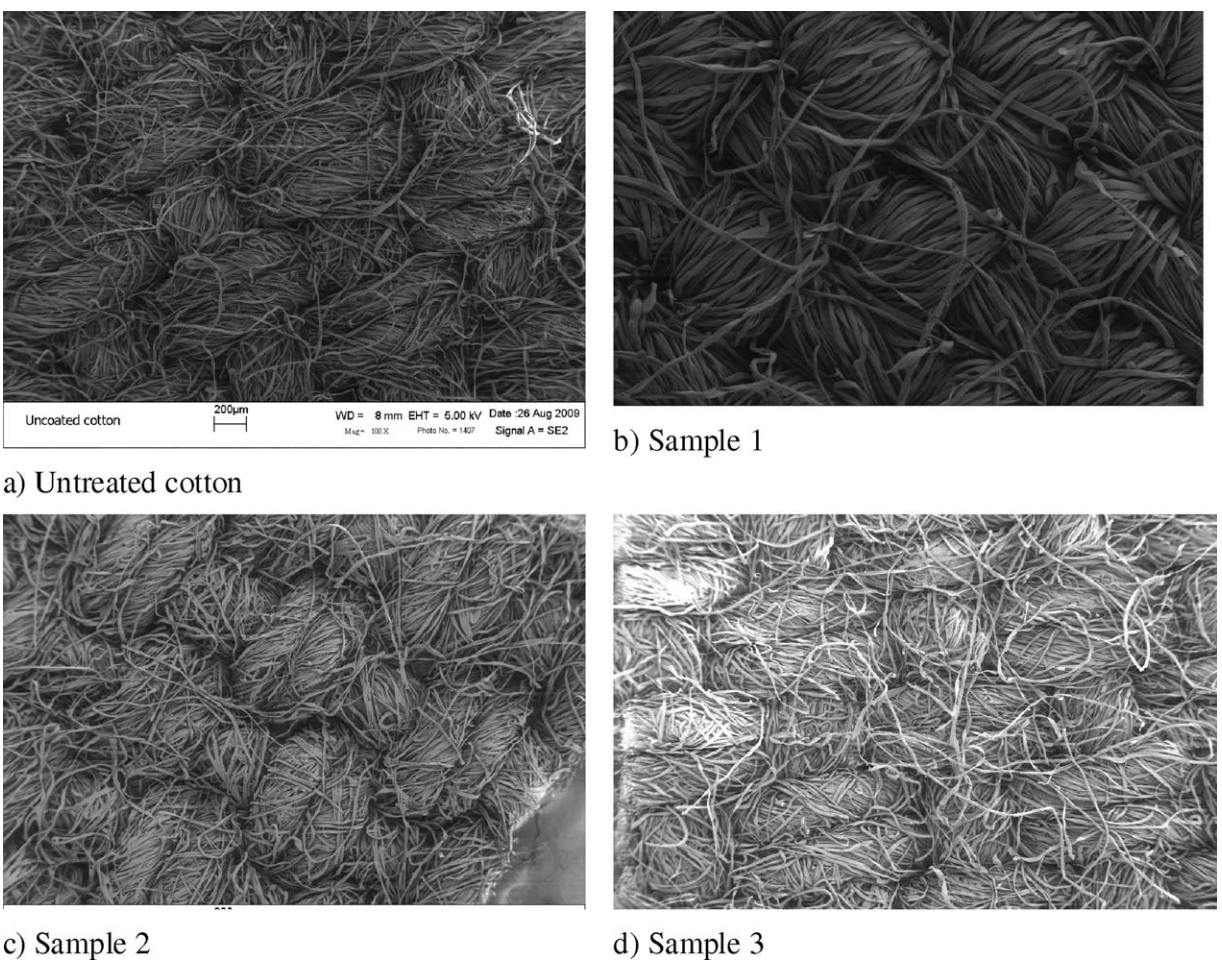
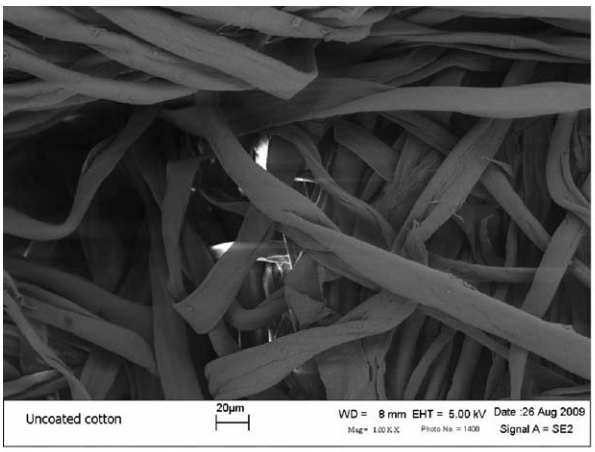
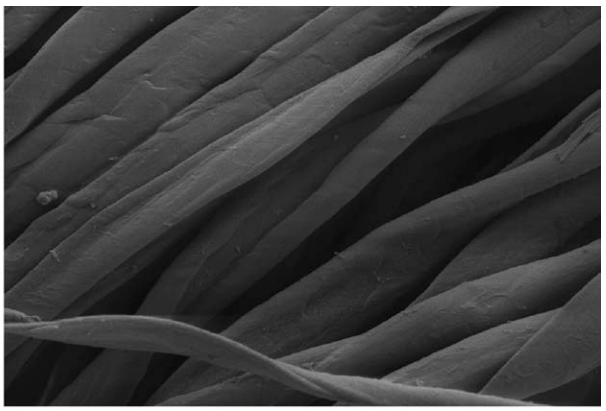


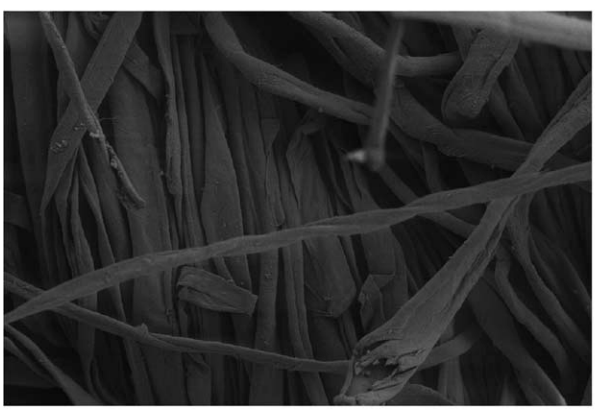
Figure 9 100× magnification of treated and untreated cotton samples.



a) Untreated cotton



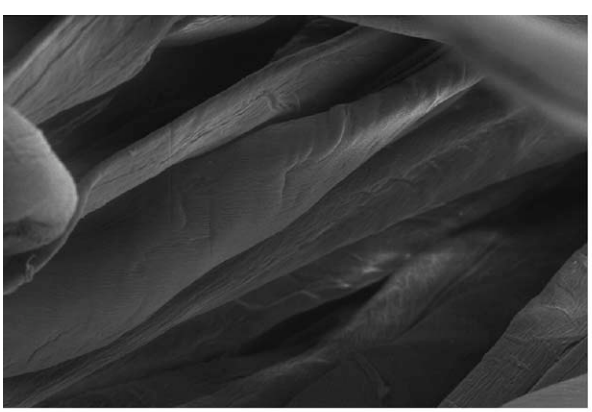
b) Sample 1



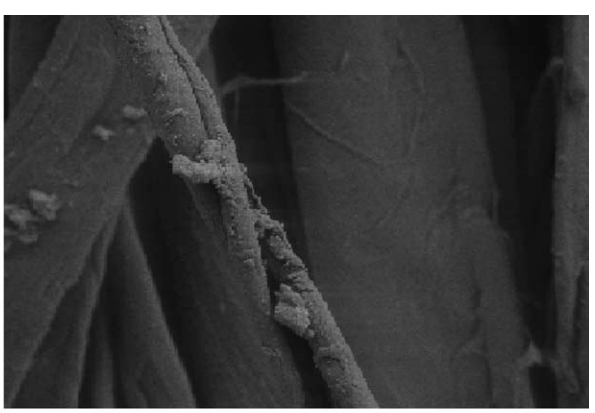
c) Sample 2

d) Sample 3

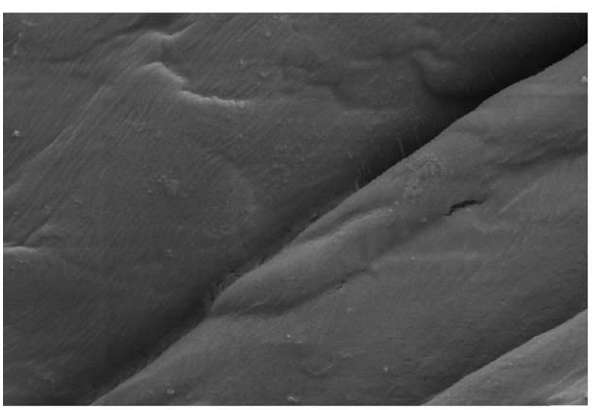
Figure 10 1000× magnification of treated and untreated cotton samples.



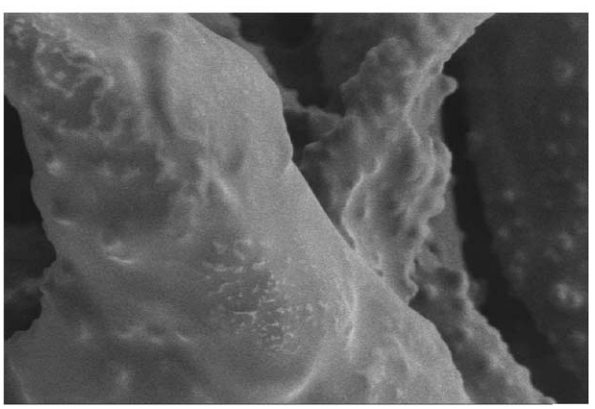
a) Untreated Cotton



b) Sample 1



c) Sample 2



d) Sample 3

Figure 11 5000× magnification of treated and untreated cotton samples.

extraction from various treatments have similar appearances (Fig. 9). Higher magnification, however, reveals the presence of polymer in Sample 3, indicating high graft polymerization (Fig. 10). A thick, sticky polymer can be easily seen in Sample 3 between fiber bundles. Figure 11 also shows the successful coating of the fibers itself in Sample 3, enveloping each fiber. Sample 2 has a fiber coating as well, although binding between fiber bundles are not observed. Sample 1 shows no indication of polymer presence, which is also supported by its lack of biocidal activity after aqueous extractions. This observation is in complete agreement with the hypothesis that fluorescein spot test and bromophenol solution test, used in tandem, can provide quick and reliable information as to the extent of graft polymerization of quaternary amine containing polymers and their durability on fabric surfaces.

CONCLUSION

The purpose of this study is to develop quick and easy colorimetric analytical techniques to monitor grafting of textile surfaces with QAS containing biocidal polymers. Fluorescein spot test can be employed to verify the presence of QAS on the surface and durability after repetitive washing. Bromophenol solution test can be used to monitor QAS in the solution, suggesting a leaching off the biocidal polymer of the treated surface. These two tests can be used in lieu of more cumbersome, time consuming and hence expensive standard protocols when a qualitative screening of samples are required.

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