TEXTILE TECHNOLOGY

Using the Reactive Dye Method to Covalently Attach Antibacterial Compounds to Cotton

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ABSTRACT

Fabric quality and durability are a concern with fibers that contain natural antibacterial properties or are treated to provide antibacterial properties. The textile industry has developed antibacterial fabric to address the public's desire for improved sanitation and personal protection against disease transmission. The approach has been to attach biocidal or some bacteriostatic groups to the fabric surface. In this study, well described antibacterial drugs were attached to cotton fabric with the goal that if this could be accomplished easily, treated fabric could act as barriers against specific diseases or wound infections. Trimethoprim and sulfamethoxazole were modified to act as reactive dyes and were covalently bonded to the surface of cotton in order to impart antibacterial properties. Some of the treated fabric was subjected to multiple washings to determine durability. The treated fabrics were then assayed for antibacterial properties. The preliminary results suggest that the antibacterial compound trimethoprim is tightly bound to the cotton fabric and imparts to the fabric antibacterial properties, which are durable through multiple washes. The results show that both trimethoprim and sulfamethoxazole impart antibacterial properties to cotton fabric. These results indicate that other compounds may be used to attach specific antibacterial compounds to fabric to create specific usage, designer, or tailored fabrics to meet specialized needs.

Antibacterial finishing of textiles first appeared in 1941 in response to a need to protect the apparel of military personnel from hot and humid environmental conditions in the South Pacific, which were ideal for the growth of organisms on natural fiber substrates.

More recently, an awareness of general sanitation, contact disease transmission, and personal protection have led to the development of antibacterial fibers to protect wearers against the spread of bacteria and diseases rather than to protect the quality and durability of the textile material. Most of these approaches entail the attachment of a biocidal or bacteriostatic agent to the fabric surface. The mechanisms used to attach these agents to the fabric include the layer deposition of silver nanoparticles onto fabric structures (Dubas et al., 2006), graft polymerization of N-halamide monomers onto cellulosic substrates (Lui and Sun, 2006), placement of quaternary ammonium salts onto cotton fabrics using a covalently bound adduct (Son et al., 2006), covalent attachment of a chloromelamine derivative (Sun et al., 2005), and the attachment of chitosan to cotton fabric via cross-linking agents (El-talawy et al., 2005; Ye et al., 2006).

The approach in this study was to modify two well described antibacterial drugs, previously or currently in use for treating diseases, for direct attachment to cotton fabric. Assuming this can be performed easily and cost effectively, the potential exists to attach specific antibacterial drugs in situations where treated fabric could act as a barrier against specific diseases or wound infections (Parikh et al., 2005). Such a situation would be advantageous, because the history and action of most antibacterial drugs have been well researched and established, making its regulatory acceptance less of a burden. Also, many more antibacterial compounds or drugs have come on the scene in the last 50 years. Some of these antibacterial drugs have become so cheap and readily available that they are routinely added to animal feed as supplements to promote rapid weight gain of the animal. Since these drugs are antibacterial, by covalently attaching them to fabric the chance of imparting desired antibacterial properties to the fabric is expected to be high, which would provide the possibility of creating designer or tailored anti-microbial fabric or yarn for specific medical, as well general usage. The approach in this research also has the potential for obtaining bacteriostatic fabric that does not degrade upon washing, as occurs in some

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of the other described methods of attachment. In addition, treating for bacteriostatic properties by a reactive dye mechanism will potentially prevent an alteration in the hand and moisture migration properties of the treated fabric, which is one drawback of a polymer graft mechanism of attachment.

This study looked at the feasibility of utilizing two common antibacterial drugs and chemically converting them in order to obtain a reactive dye-type molecule, which could be applied to cotton fabric with the goal of imparting the antibacterial properties of the antibiotic compounds to the fabric. The two compounds used were trimethoprim and sulfamethoxazole, which both possess bacteriostatic properties effective against a wide range of bacteria. The drugs are often used together as part of a synergistic combination for the treatment and prevention of urinary tract infections, diarrhea, respiratory infections, and prevention and treatment of infections by *Pneumocystis* (Beers and Berkow, 1999). The drugs interfere with folic acid synthesis in bacteria and eventually with bacterial DNA synthesis. These two compounds were reacted with cyanuric chloride, and the resultant product was then cross-linked to cotton fabric using an exhaust methodology. Treated fabrics were then assayed for antibacterial properties.

MATERIALS AND METHODS

Test fabric. The fabric from a commercial producer was supplied by Cotton Inc. (Cary, NC). The fabric was a white, 100% cotton, tight-weave denim-like fabric, weighing approximately 271.3 g/m^2, which had been commercially scoured and bleached. The fabric was cut into large squares, approximately 12.8 cm x 12.8 cm, before being treated. After treatment, the large squares were ironed to remove wrinkles, cut into 18.1 cm^2 squares, 4.25 cm on a side, and sterilized in an autoclave using a dry cycle prior to the antibacterial assay.

Synthesis of a reactive trimethoprim and sulfamethoxazole to covalently bond with the cotton fabric. Synthesis of 2,4-bis (2,4-dichloro-6-amino-s-triazino)-5-(3,4,5-trimethoxybenzyl)pyrimidine was accomplished by suspending 5.80 g (0.02 mole) trimethoprim (2,4-diamino-5-3,4,5-trimethoxybenzyl pyrimidine) (Sigma Chemical Co.; St. Louis, MO) in 20 ml deionized water in an ice bath at 5 °C. To this suspension, 7.36 g (0.04 mole) cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) (Aldrich Chemical Co.; St. Louis, MO) was added. The suspension was

maintained at 5 °C during the drop-wise addition of 40 ml 1.0 N NaOH (0.04 mole).

Synthesis of 4- (2,4-dichloro-6-amino-s-triazino)-N-(5-methyl-3-isoxazolyl)benzenesulfonamide was accomplished by suspending 7.59 g (0.03 mole) sulfamethoxazole (4-amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide (Sigma Chemical Co.; St. Louis, MO) in 20 ml deionized water in an ice bath at 5 °C. To this suspension, 5.52 g (0.03 mole) cyanuric chloride was added. The suspension was maintained at 5 °C during the drop-wise addition of 30 ml 1 N NaOH (0.03 mole).

Bonding the reactive antibiotic to cotton fabric. An exhaust dyeing method was used to bind the reactive antibiotic to the cotton fabric. The dye bath was prepared by adding 0.5 ml of Triton-X-100 (octylphenol ethoxylates; Dow Chemical Co.; Midland, MI), 75 g of sodium sulfate, and 6.5 g of the reactive antibiotic, or 3.25 g of each of the two reactive antibiotics to 1.2 L of deionized water. Three, 20-g squares of the cotton fabric were submerged in the dyebath heated to 60 °C. After 30 min of incubation, 12 g NaOH that had been dissolved in 100 ml of deionized water was added. The temperature was then raised to 80 °C, and the fabrics heated for another 30 min. The fabric was then rinsed in deionized water and heated for 10 min at 80 °C in deionized water, then rinsed and kept in a convection oven at 105 °C until dried.

Assay for antibacterial properties. The assay used for measuring antibacterial properties was based on the 'AATCC Test Method 100-1999, Antibacterial Finishes on Textile Materials: Assessment of' (Anonymous, 1999a), which has been described previously (Chun et al., 2006). Prior use of the assay found that the population densities after incubation often remained the same or increased even in the controls, so the population densities of the zero time of incubation for the controls and treatments were not determined. Test and control swatches were inoculated with challenge bacteria and after a period of incubation, the bacteria were eluted from the swatches with known volumes of extraction solution. Then the numbers of viable bacteria present in the extraction solutions were determined and the population densities compared.

Two bacterial species, Gram-positive *Staphylococcus aureus* (ATCC No. 6538), and Gram-negative *Klebsiella* (ATCC No. 4352) were used throughout the experiment. Stock cultures were maintained on slants of Difco Brain Heart Infusion Agar (Difco Laboratories; Detroit, MI). The stock cultures were

transferred once every 3 to 4 wk by incubating a freshly inoculated slant at 37 ± 2 °C for 2 d before storing at 5 ± 1 °C.

For each assay, the challenge bacteria were incubated in either a trypticase soy broth (TSB; Becton Dickinson and Co.; Cockeysville, MD) or on trypticase soy agar slants (TSA; Becton Dickinson and Co.) at 37 ± 2 °C for 1 to 3 d before being used to inoculate broth (TSB) cultures for testing. The inoculated broth cultures were incubated in a shake incubator (37 ± 2 °C and 300 rpm) for 24 h. At the end of incubation, the broth cultures were placed in an ice bath until chilled.

To get a standardized density of bacteria for inoculation, the chilled cultures were diluted with TSB to a pre-determined turbidity that provided approximately 2×10^9 CFU/ml or 2×10^8 CFU/ml. The turbidity was measured at 500 nm on a Beckman DU-7 Spectrophotometer (Beckman Instruments, Inc.; Irvine CA). Chilled TSB from the same batch as the cultures were grown in was used to zero the instrument. Then the diluted broth cultures were serially diluted with chilled diluent without Tween-80 or gelatin (Chun and Perkins, 1996) for a final approximate bacterial density of 1 to 2×10^5 CFU/ml. Based on prior experience, 0.5 ml of the initial broth culture was added early in the dilution series to compensate for the observed loss from the expected population starting density to the actual starting density used in the assays. The bacterial suspensions were kept in an ice bath. A magnetic stir bar and stirring plate was used to keep the bacteria suspended during inoculation.

For each replicate sample, 1.0 ± 0.1 ml of inoculum was dispersed as droplets over the 3 swatches using a Rainin EDP-Plus Electronic Pipette (RAININ Instrument Co., Inc.; Woburn, MA). The swatches were inoculated in pre-sterilized 237 ml (half pint) canning jars. The band and lid of the canning jar were screwed on the jar to prevent evaporation. After all the samples were inoculated, the jars were incubated at 37 ± 2 °C for 24 h before being assayed for bacterial population density.

The bacterial population density was determined by extracting the bacteria from the fabric by adding 100 mL of diluent to each jar and shaking the jars on a tabletop shaker for 1 min. Then aliquots were removed and plated directly into Petri dishes or further diluted before being plated. The pour plate method was used to determine the bacterial density (Chun and Perkins, 1996). No antibiotics were used,

and plates were incubated at 37 ± 2°C for at least 24 h before the plates were counted.

Experimental design and statistical analysis. Four main fabric treatments were (A) a control fabric that was not chemically altered; (B) fabric that had trimethoprim covalently bonded to it; (C) fabric that had sulfamethoxazole covalently bonded to it; and (D) fabric that had both trimethoprim and sulfamethoxazole covalently bonded to it. Before assaying the 4 treatments, a trial run using just the control and fabric treated with trimethoprim was conducted to see if the antibiotic would covalently bond to the fabric, and if the attachment of the antibiotic would persist through multiple washings. The fabric was washed by Cotton Inc. (Cary, NC), based on the AATCC Test Method 124-1996 (Anonymous, 1999b) laundering procedure, which used a normal/cotton sturdy cycle, 1.81 kg (4 lb) load, warm water temperature, and AATCC detergent without optical brightener. The treated and control samples were washed 3 and 10 times, respectively. For the trial run, only results with *Klebsiella pneumoniae* will be presented.

In the antibacterial assays, the bacterial inoculum was dispersed over 3 swatches per replicate sample as droplets. Replicate tests were done and the observations were combined and used for statistical analysis. A $\log_{10}(CFU +1)$, where CFU = microbial population as colony forming units, transformation was used for the analysis dealing with the microbial data. Data were analyzed with SAS (release 8.00; SAS Institute Inc., Cary, NC) for Duncan mean comparisons when the anlysis of variance analysis yielded significant F-values to indicate a high degree of difference of the variable. Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA) was used to randomize treatment assignments, to enter and store data, to sort data and prepare for SAS analysis, to transform data, to summarize and tabulate results, to obtain simple treatment statistics (means, standard deviations, regressions, *t*-test comparison, etc.), and to perform other spreadsheet functions.

RESULTS AND DISCUSSION

Initial testing determined whether reactive antibiotics would covalently bond to the cotton fabric and impart antibiotic properties to the fabric. A pilot test was done with trimethoprim using both the *Staphylococcus aureus* and *Klebsiella pneumoniae* as challenge bacteria. With *S. aureus*, bacterial populations of the control swatches averaged 6.291

[$\log_{10}$(CFU + 1)], and the swatches treated with trimethoprim averaged 5.101 [$\log_{10}$(CFU + 1)]. With *K. pneumoniae*, the control swatches averaged 7.743 [$\log_{10}$(CFU + 1)], and the swatches treated with trimethoprim averaged 4.333 [$\log_{10}$(CFU + 1)]. The difference between the means of the control and the trimethoprim-treated swatches were highly significant using *t*-test analysis for *S. aureus* and *K. pneumoniae*, $P = 0.011$ *and* $P < 0.001$, respectively. This indicated that the trimethoprim was bound to the cotton fabric, and the antibacterial activity of the compound was not affected.

The large swatches of treated and untreated cotton fabric were washed 3 and 10 times at Cotton Inc. to determine if the antibiotic binding to the fabric would be durable through normal washing. After washing, these large swatches were cut into smaller swatches, sterilized, and then assayed for antibacterial properties. Both *S. aureus* and *K. pneumoniae* were used, but only the results for *K. pneumoniae* will be reported. Populations of *S. aureus* between the unwashed control and the trimethoprim treated swatches were 5.758 and 4.202 [$\log_{10}$(CFU + 1)], respectively, and were significantly different. The results from the washed portions of the assay, even after being repeated, were unexplainable and anomalous. The observations from two tests using *K. pneumoniae* were combined for analysis. The bacterial density for the untreated fabric was 7.2, 6.8, and 6.7 $\log_{10}$ (CFU + 1) for the unwashed, washed 3 times, and washed 10 times, respectively. These averages were not significantly different from one another, which indicated that washing alone did not affect the bacterial density. The bacterial density of the treated fabric was 3.4, 3.3, and 3.6 $\log_{10}$ (CFU + 1) for the unwashed, washed 3 times, and washed 10 times, respectively. Both unwashed and washed treated fabrics had significantly lower bacterial density than the untreated fabric, and the averages were not significantly different among the three treated fabrics. These results indicate that the antibacterial property imparted by binding trimethoprim to cotton was durable and retained at least through 10 washes.

In a second experiment, fabric was treated with trimethoprim, sulfamethoxazole, and a 1:1 mixture of trimethoprim and sulfamethoxazole each at half the strength. The observations from three separate antibacterial assays were combined for analysis (Table 1). The results indicate that both trimethoprim and sulfamethoxazole individually or together depressed the bacterial density of *K. pneumonia* and

S. aureus significantly after 24-hr incubation. Sulfamethoxazole was less effective than trimethoprim alone or when both trimethoprim and sulfamethoxazole were attached to the fabric. But the fabric treated with trimethopriman and trimethoprim and sulfamethoxazole were not significantly different. Although fabric treated with sulfamethoxazole was bacteriostatic compared with the untreated control for *K. pneumoniae*, sulfamethoxazole was not significantly different than trimethoprim alone. The total amount of trimethoprim and sulfamethoxazole in the 1:1 mixture treatment, however, was half the amount used to evaluate the compounds individually. There is a possibility that the two compounds may have had a synergistic effect to account for the low bacterial density compared with trimethoprim alone or the amount of the trimethoprim applied alone was in excess to what is needed to effectively lower the bacterial density to this level. Of course, this also suggests that the reactive trimethoprim may have been preferentially attached to the cotton fabric and that even at half the dose could account for the lower bacterial density when combined sulfamethoxazole. To resolve this, further research must be carried out comparing different concentrations of the antibiotics, both alone and together, in influencing bacterial densities, and the potential influence of competition between dyes for the same hydroxyl groups on cotton fabric. Since trimethoprim and sulfamethoxazole were both easily prepared to act as reactive dyes and that many, if not most, of other commonly known antibiotic compounds have the same or similar reactive sites, many other antibiotics may be used in a similar manner, and future research should be

Table 1. Bacterial density after 24-hr incubation on cotton fabric treated with trimethoprim, sulfamethoxazole, or both compounds

Treatment	Density [$\log_{10}$(CFU + 1)][z]	
	Klebsiella pneumoniae	*Staphylococcus aureus*
Control	6.830 a	6.084 a
Treated with trimethoprim	2.793 bc	3.794 c
Treated with sulfamethoxazole	3.618 b	4.407 b
Treated with trimethoprim and sulfamethoxazole	2.147 bc	3.773 c

[z] **Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$)**

expanded to include testing a wide spectrum of antibiotic compounds. This creates an opportunity to design or tailor antimicrobial fabric or yarn using this reactive dye method to attach antibacterial compounds to cotton. For example, one area where this approach may prove to be of value would be to attach scarce antibacterial drugs to dressings to act as barriers to specific drug resistant bacteria to help prevent or reduce infection and its spread.

In summary, trimethoprim and sulfamethoxazole could be prepared as reactive dyes that can covalently bind to cotton fabric. The treated cotton fabric displayed antibacterial properties that persisted through 10 launderings. Antibiotic concentration should be investigated to determine efficacious rates. The ease of application may extend to the use of other antibiotic drugs to provide value to cotton fabric where tailored or designer antibacterial fabric is desired.

DISCLAIMER

Mention of a trademark, warranty, proprietary product or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply approval or recommendations of the product to the exclusion of others that may also be suitable.

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