2007 Beltwide Cotton Conferences, New Orleans, Louisiana, January 9-12, 2007

USING THE REACTIVE DYE METHOD TO ATTACH ANTIBACTERIAL COMPOUNDS TO COTTON

David T.W. Chun
Gary R. Gamble
USDA-ARS, Cotton Quality Research Station
Clemson, SC

Introduction

Fibers and fabric with natural antibacterial properties or treatments to provide antibacterial properties were the emphasis where the quality and durability of the textile material was the main concern. Now, greater concern centers on antibacterial fabric to protect the wearers against spread of disease by providing personal protection against disease transmission. But adding antimicrobial properties to textiles is an old practice which may be as simply involved as adding finishes or incorporating a polyester fiber impregnated with silver. Our goal was to determine if we could easily attach known antibacterial compounds to the surface of cotton fabric and impart antibacterial properties to the cotton fabric. A version of the reactive dye method was used to activate the antibacterial compound and then covalently bind it to cotton fabric. For these initial studies, trimethoprim and sulfamethoxazole were chosen. Trimethoprim and sulfamethoxazole are generally used together in the prophylaxis and treatment of urinary tract infections, and are commonly described as bacteriostatic antibiotics (Anonymous, 2006). After attaching the antibacterial agent to cotton fabric, the durability and antibacterial activity of the treated fabrics was evaluated and the results will be presented.

Materials and Methods

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); and it has been described previously (Chun et al., 2006). Test and control swatches are inoculated with test bacteria and after a period of incubation, the bacteria are eluted from the swatches with known volumes of extraction solution. Then the numbers of viable bacteria present in the extraction solutions are determined and the populations' densities compared. For all of the tests, the bacterial inoculum was dispersed over 3 swatches per replicate sample as droplets. Three swatches were used instead of 4 since the fabric was highly absorbent and 3 replicate samples were used for each treatment.

Test Fabric.

The fabric was supplied by Cotton Inc. (Cary, NC) from a commercial producer. The fabric was a white 100% cotton tight-weave denim-like fabric which appears made from upland cotton, weighing approximately 271.3 g/m², which had been commercially scoured and bleached. The fabric is cut into large squares, approximately 12.8 cm x 12.8 cm, before being treated. After treatment, the large squares are ironed to remove wrinkles, cut into swatches of 18.1 cm^2 squares, 4.25 cm on a side, and sterilized in an autoclave using a drying cycle prior to the antibacterial assay.

Synthesis of a reactive Trimethoprim (2,4-diamino-5-3,4,5-trimethoxybenzyl pyrimidine) and sulfamethoxazole (4-amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide) 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 done by first suspending 5.80 g (0.02 mole) trimethoprim (from Sigma Chemical Co., St. Louis, MO) in 20 ml deionized water in an ice bath at 5°C. To this suspension was added 7.36 g (0.04 mole) cyanuric chloride (2,4,6-trichloro-1,3,5-triazine; from Aldrich Chemical Co., St. Louis, MO). The suspension was maintained at 5°C during the dropwise addition of 40-ml 1 N NaOH (0.04 mole).

Synthesis of 4- (2,4-dichloro-6-amino-s-triazino) -N-(5-methyl-3-isoxazolyl)benzenesulfonamide was done by first suspending 7.59 g (0.03 mole) sulfamethoxazole (from Sigma Chemical Co., St. Louis, MO) in 20 ml deionized

water in an ice bath at 5°C. To this suspension was added 5.52 g (0.03 mole) cyanuric chloride. The suspension was maintained at 5°C during the dropwise 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. First, a dyebath was prepared by the addition to 1.2 L of deionized water, 0.5 ml of Triton-X, 75 g of sodium sulfate and of 6.5 g of the reactive antibiotic, or 3.25 g of each of the two reactive antibiotics. Three 20-g squares of the cotton fabric were submerged in the dyebath and the dyebath heated to 60°C. After 30 minutes of incubation, 12 g NaOH which had been dissolved in 100 ml of deionized water was added. The temperature was then raised to 80°C and the fabrics incubated for another 30 minutes. After this time, the fabric was rinsed in deionized water and then incubated for 10 minutes at 80°C in deionized water, then rinsed and kept in a convection oven at 105°C until dried.

Treatments.

Four main fabric treatments were considered: (A) a control fabric which had not been chemically altered; (B) fabric which had trimethoprim covalently bonded to it; (C) fabric which had sulfamethoxazole covalently bonded to it; and (D) fabric which had both trimethoprim and sulfamethoxazole covalently bonded to it. Before assaying these 4 treatments, a trial run using just the control and trimethoprim treated fabric was conducted to see if covalently bonding the antibiotic would take and if the attachment of the antibiotic would persist through multiple washings. The fabric washing was done by Cotton Inc. which was based on the AATCC Test Method 124-1996 (Anonymous, 1999b) laundering procedure: 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. For this trial run, only the *Klebsiella pneumoniae* challenge results will be presented.

Results and Discussion

The synthesis of antibacterial compounds with reactive sites which could covalently bond to the surface of cotton seems straight forward. But before allocating too much time and resources into this project, a preliminary trial using just the trimethoprim treated fabric was done to justify pursuing the use of this method to impart the fabric with antibacterial properties. In this test, with the *Staphylococcus aureus* challenge, the control swatches averaged 6.291 (Log_{10}[(CFU/ml + 1]) while the trimethoprim-swatches averaged 5.101 (Log_{10}[(CFU/ml + 1]); with the *Klebsiella pneumoniae* challenge, the control swatches averaged 7.743 (Log_{10}[(CFU/ml + 1]) while the trimethoprim-swatches averaged 4.333(Log_{10}[(CFU/ml + 1]). The differences between the means of the control and the trimethoprim-treated swatches were highly significant using t-test analysis for the *Staphylococcus aureus* and *Klebsiella pneumoniae* challenge, $P = 0.011$ and $P < 0.001$, respectively.

Now knowing that the antibacterial compound could be attached to cotton surfaces, a washing test was performed on this first batch of treated fabric to determine it's retention through normal washing. The large swatches of treated and untreated cotton fabric were sent out to be washed 3 and 10 times. When these large swatches were returned, they were cut into smaller swatches and sterilized before being used in the antibacterial assay. Both the *Staphylococcus aureus* and *Klebsiella pneumoniae* challenge were used, but only the *Klebsiella pneumoniae* challenge results will be reported. The *Staphylococcus aureus* challenge results showed that the difference between the unwashed control and the trimethoprim treated swatches were significantly different as before, 5.758 and 4.202 (Log_{10}[(CFU/ml + 1]), respectively; but the results from the washed portions of the assay, even after being repeated, were unexplainable and anomalous. The observations from two tests using the *Klebsiella pneumoniae* challenge (Table 1) were combined and analyzed. The results indicate that the antibacterial property imparted by binding trimethoprim to cotton was retained at least through 10 washes. The 3 controls all showed the same high bacterial density of about 7.0 (Log_{10}[(CFU/ml + 1]). The treated fabric, both unwashed and washed, were significantly lower with an average density of about 3.5 (Log_{10}[(CFU/ml + 1]); and the average were not significantly different whether the treated fabric was unwashed or washed 3 or 10 times.

Table 1. Bacterial density, $Log_{10}(CFU/ml + 1)$, after 24-hr incubation on cotton fabric treated with trimethoprim after 0, 3 and 10 washes.

Treatment	*Klebsiella pneumoniae* density, $Log_{10}(CFU/ml + 1)$[1]
Control, Unwashed	7.265[A]
Control, Washed 3 Times	6.847[A]
Control, Washed 10 Times	6.774[A]
Treated with Trimethoprim, Unwashed	3.442[B]
Treated with Trimethoprim, Washed 3 Times	3.310[B]
Treated with Trimethoprim, Washed 10 Times	3.645[B]

[1] Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

The observations from three separate antibacterial assays on fabric which had been treated with trimethoprim, sulfamethoxazole, or both, were combined and analyzed (Table 2). The results indicate that both trimethoprim and sulfamethoxazole individually or together will depress the bacterial density significantly after the 24-hr incubation in both the *Klebsiella* and *Staphylococcus* challenge. Binding sulfamethoxazole to fabric produced a weaker effect than trimethoprim alone or when both trimethoprim and sulfamethoxazole were attached to the fabric. But the trimethoprim treated fabric and the trimethoprim and sulfamethoxazole treated fabric were not significantly different; that is, while sulfamethoxazole treated fabric was bacteriostatic compared to the controls, when used in combination with trimethoprim, its added effect to the effect of trimethoprim alone was not significantly different than using just trimethoprim alone. However, the total amount of trimethoprim and sulfamethoxazole in the 1:1 mixture treatment was half the amount used in the tests of the compounds tested individually. This argues for the possibility that the two compounds may have had a synergistic effect to account for the low bacterial density comparable to trimethoprim used alone or the amount of the individually applied antibiotic was in excess to what is needed to effectively lower the bacterial density to this level. Of course, the possibility also exists that the reactive trimethoprim may have been preferentially attached to the cotton fabric and that at half the dose, alone could account for the lower bacterial density. To resolve this, further work must be carried out comparing different concentrations of the antibiotics in influencing bacterial densities. Regardless, the opportunity to create designer or tailored antimicrobial fabric appears possible using this reactive dye method to attach antibacterial compounds to cotton.

Table 2. Bacterial density, Log_{10}(CFU/ml + 1), after 24-hr incubation on cotton fabric treated with either trimethoprim or sulfamethoxazole or both.

Treatment	*Klebsiella pneumoniae* density, Log_{10}(CFU/ml + 1)[1]
Control	6.830[A]
Treated with Trimethoprim	2.793[BC]
Treated with Sulfamethoxazole	3.618[B]
Treated with Trimethoprim and Sulfamethoxazole	2.147 [BC]
Treatment	***Staphylococcus aureus* density, Log_{10}(CFU/ml + 1)[1]**
Control	6.084[A]
Treated with Trimethoprim	3.794[C]
Treated with Sulfamethoxazole	4.407[B]
Treated with Trimethoprim and Sulfamethoxazole	3.773[C]

[1] Mean separation within column by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

In summary, trimethoprim and sulfamethoxazole could be made to behave as reactive dyes which can covalently bind to cotton fabric. When doing so, the cotton fabric is imparted with antibacterial properties which appear to persist through multiple laundering. As currently applied, the effect of antibiotic concentration should be investigated to determine areas of efficacy. However this ease of application may 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.

Acknowledgements

We gratefully acknowledge Cotton Inc. for providing the cotton fabric used in the study and especially Norma M. Keyes and Vikki B. Martin for their help in washing the test fabric.

References

Anonymous. 1999a. AATCC Test Method 100-1999, Antibacterial Finishes on Textile Materials: Assessment of. Pages 147-149, In: AATCC Technical Manual. Vol. 75, 2000 Technical Manual of the American Association of Textile Chemists and Colorists, 1999, AATCC, Triangle Park, NC.

Anonymous. 1999b. AATCC Test Method 124-1996, Appearance of Fabrics after Repeated Home Laundering. Pages 205-208, In: AATCC Technical Manual. Vol. 75, 2000 Technical Manual of the American Association of Textile Chemists and Colorists, 1999, AATCC, Triangle Park, NC.

Anonymous. 2006. Trimethoprim – Wikipedia, the free encyclopedia. http://en.wikipedia.org/wiki/Trimethoprim. March 28, 2006.

Chun, D.T.W., , Foulk, J.A., and McAllister III, D.D. 2006. Modification, Use of, and Description of AATCC Test Method 100-1999 to Test For Antibacterial Properties OF Denim Made from Flax Fabric. pgs, 2050-2050h, *In:* Proc. Beltwide Cotton Conf., San Antonio, TX. 3 – 6 Jan. 2006. Natl. Cotton Counc. Am., Memphis, TN. http://www.cotton.org/beltwide/proceedings/2006/pdfs/2050-2050.pdf