Antimicrobial Treatment of Nylon

JIAN LIN,[1] CATHERINE WINKELMAN,[1] S. D. WORLEY,[1] R. M. BROUGHTON,[2] J. F. WILLIAMS[3]

[1] Department of Chemistry, Auburn University, Auburn, Alabama 36849

[2] Department of Textile Engineering, Auburn University, Auburn, Alabama 36849

[3] Halosource Corporation, Seattle, Washington 98121

Received 2 September 2000; accepted 16 October 2000

ABSTRACT: The preparation of an antimicrobial nylon material and its properties are discussed. Biocidal cyclic *N*-chloramine moieties were covalently bonded to Nylon 66. These moieties, which included hydantoins, oxazolidinones, and imidazolidinones, were stable during at least 3 months of dry storage, and their antimicrobial activities, once lost by reaction with reducing sodium thiosulfate, could be regenerated by exposure to free chlorine. Biocidal swatch tests showed that the nylon fabrics containing *N*-chlorinated hydantoin functional groups provided a 7.2 log reduction of *Staphylococcus aureus* and a 7.1 log reduction of *Escherichia coli* at a contact time of only 10 min. Antimicrobial nylon should find a variety of important uses such as in clothing, carpets, sutures, brushes, and so forth. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 943–947, 2001

Key words: antimicrobial; nylon; *N*-halamine; chlorination; fabric

INTRODUCTION

Some microorganisms are highly undesirable because they are a cause of odors, skin irritation, and illness. The odor on clothing arises primarily as a result of bacteria and fungi that grow in the perspiration and on skin cells that are in the clothing. Bacteria and fungi are deposited on carpets through the normal traffic of people and animals, food and beverages spilled on the carpet, and animal and infant excreta. Frequent, long-lasting local infections may be brought about by nylon surgical sutures incorporated into tissues and soaked with liquids that are potential culture media for bacteria.[1] Therefore, it would be useful if products made of nylon could have antimicrobial activity.

Recently, in response to the demand for a safe antimicrobial and deodorizing treatment, chemical methods have been proposed that use nontoxic halamines, such as *N*-halohydantoins, as antimicrobial components.[2–4] These types of moieties have successfully been incorporated into fabrics produced from cellulose.[5–7] In the present work, three types of cyclic chloramine moieties were chemically bound to the surfaces of Nylon 66 fabrics and fibers using the chemistry shown in **Scheme 1**.

EXPERIMENTAL

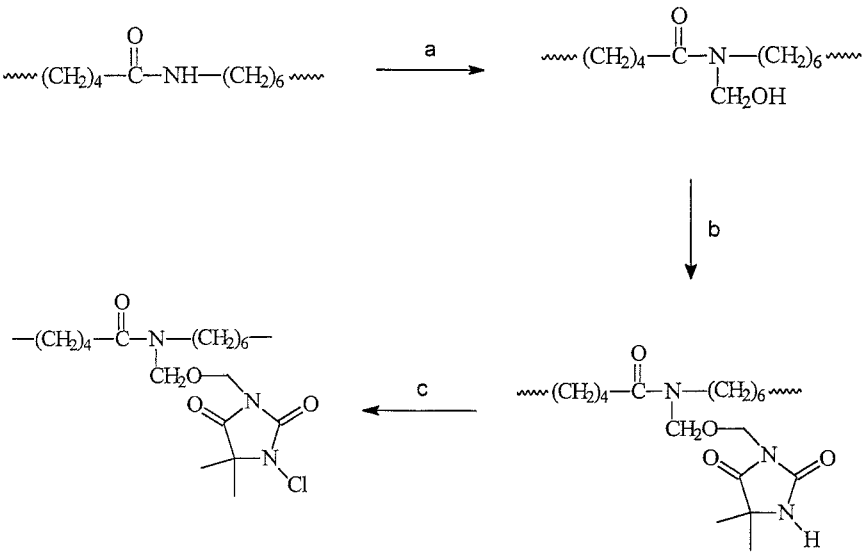
Treatment Process

An example of the treatment process used in this study follows. A 2.0-g sample of Nylon 66 fabric (or

Correspondence to: S. Worley.

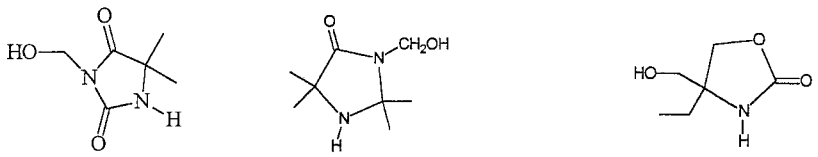
Contract grant sponsor: U.S. Department of Commerce; contract grant number: 99-27-07400.

Journal of Applied Polymer Science, Vol. 81, 943–947 (2001)



a = formaldehyde

b =



hydroxymethylhydantoin (as shown in above scheme) hydroxymethylimidazolidinone hydroxymethyloxazolidinone

c = free chlorine

Scheme 1 Reaction scheme used in producing the antimicrobial nylon samples.

fibers) was soaked in 200 mL of 10% concentration of formaldehyde solution under basic conditions (0.5*N* NaOH) at 80°C for 2 h. The fabric or fibers were washed with distilled water until a neutral pH was obtained. Then the fabric or fibers now containing a hydroxymethyl functional group at the amide nitrogen of Nylon 66 were placed in a treatment bath containing 10 g of 4-hydroxymethyl-4-ethyl-2-oxazolidinone (prepared as described in Eknoian et al.[4]), 0.5 g of $MgCl_2$, 0.2 g Triton X-100 wetting agent, and 200 mL of distilled water held at pH 3.5 and 80°C for 30 min. The treated Nylon 66 now containing the oxazolidinone functional group on the surface of the material was then cured in an oven at 130°C for 15 min. After curing, it was washed with detergent at 50°C for 30 min. The treated material was then soaked in dilute bleach (0.75% active chlorine) at ambient temperature for 3 h before antimicrobial testing. Nylon 66 materials were also treated with 3-hydroxymethyl-2,2,5,5-tetramethylimidazolidin-4-one and both 3- and 1-hydroxymethyl-5,5-dimethylhydantoin using analogous procedures (see **Scheme 1**).

Titration Analyses of Chlorine on the Fabric

The dry fabrics were stored in closed plastic bags at room temperature for a period up to 3 months. Over a selected time interval, a piece of the fabric was removed from the stock bag and cut into strips. The strips were dipped in 100 mL of distilled water in a beaker, and about 1 mg of potassium iodide was then added. The mixture was held for 5 h to ensure complete reaction of potas-

sium iodide with the combined chlorine moiety, after which the solution containing the fabric was analyzed by the standard iodometric titration method. The equation used to calculate the concentration of chlorine (in mg) on the surface of one side of fabric is

$$W_{Cl} = (V \times N \times 35.45)/(S \times 4)$$

where W_{Cl} is the mg of chlorine on one side of 1.00 cm^2 of fabric, V is the volume of titrant (mL), N is the normality of sodium thiosulfate titrant, and S is the sample surface area (in cm^2).

Each analysis was run in triplicate, and the three titration results were always within 5% precision.

Antibacterial Tests

The antibacterial efficacies of the biocidal Nylon 66 fibers and fabric swatches were evaluated as follows. The test and control (unchlorinated) fiber samples were tested quantitatively for antibacterial activity using a column bacteria test. In this test, the sample was placed in a sterile glass buret or pipet (i.e., the column). The empty bed volume of the sample was measured to calculate the contact time of the inoculum with the sample. This was done by measuring the volume of water that exactly filled the region of the column containing the fibers. The sample was tested to ensure that no free, unbound chlorine was present. This was achieved by repeatedly washing the sample in the column with chlorine demand–free water, and testing the resultant wash water with chlorine indicator strips, until the strips indicated a concentration of less than 0.2 mg/L free chlorine in the effluent. A known volume of inoculum containing about 9.2×10^7 CFU/mL of *Staphylococcus aureus* in pH 7 buffer, typically 1.0 mL, was passed through the column and collected, during which time the flow rate was recorded. A 25.0-μL aliquot of the collected solution was quenched with an equal volume of 0.02N sodium thiosulfate, and then a 25.0-μL sample of this mixture was plated onto a nutrient agar plate. The remaining bacterial solution was then passed through the column once again, and again it was sampled and plated onto agar. This procedure was repeated typically for a total of six passes of the 1.0-mL inoculum. The resultant aliquots were then incubated for a period of 48 h. The bacteria colonies were counted at 24 and 48 h, providing information with regard to the contact time required to produce an efficient antibacterial activity. An identical column containing unchlorinated fibers was used as a control.

Table I Titration Results for Chlorine on the Surface of Nylon Fabric Treated with a Hyroxymethylhydantoin Monomer

Storage Time (days)	Cl on Surface (mg/cm^2)	% Chlorine Retention
1	0.0119	100
3	0.0107	89.6
7	0.0104	87.0
14	0.0101	84.4
28	0.0096	80.5
61	0.0091	76.6
94	0.0081	67.5

For the fabric swatches, antibacterial tests were conducted using American Association of Textile Chemists and Colorists (AATCC) Method 100. In the method, sized and shaped treated swatches were placed in sterile petri dishes. A known volume of inoculum containing bacteria [about 10^7 or 10^8 CFU/mL (*S. aureus* 1.1×10^7–5.0×10^8 and *E. coli* 2.0×10^7)] in pH 7 buffer solution was used. Complete absorption of the bacterial solution was required with no free solution being available. Swatches of identical fabric, but containing no biocidal finish, acted as controls. After inoculation, each swatch was transferred into a sterile wide-mouth glass vessel containing 0.02N sodium thiosulfate to quench disinfectant action. The vessel and contents were shaken, and an aliquot of the resulting mixture was removed. A set of serial dilutions were performed using pH 7 buffer. A 25.0-μL aliquot of each dilution was then plated on nutrient agar and incubated for a period of 24–48 h. Bacterial counting was performed after 24 and 48 h of incubation.

RESULTS AND DISCUSSION

Stability of Chlorine on the Fabric

The results of the stability tests for nylon treated with the hydroxymethylhydantoin monomer and chlorinated are tabulated in Table I. It was observed that the retention of chlorine on the surface of the fabric was about 70% after 3 months of storage. The analogous experiments with the other two monomers were not performed. How-

Table II Bactericidal Performance of Nylon 66 Swatches Treated with Chlorinated Hydroxymethylhydantoin

Bacterium	Contact Time (min)	Log Reduction[a]
S. aureus	10	7.2
E. coli	10	7.1
S. aureus	30	7.2
E. coli	30	7.1

[a] The control swatches showed a reduction of only about 1 log.

ever, it was observed here that *N*-chlorooxazolidinones and *N*-chloroimidazolidinones are inherently more stable to loss of chlorine than are *N*-chlorohydantoins, so it can be anticipated that nylon treated with the other two monomers will retain its chlorine for periods exceeding 3 months.

Antibacterial Efficacies

The Nylon 66 fibers that were treated with the hydroxymethylhydantoin and subsequently chlorinated provided an 8 log reduction (complete inactivation) of *S. aureus* within 16.8 s, whereas the unchlorinated control fibers gave no reduction of the bacteria, even at a contact time of 71 s. Thus, an authentic inactivation of the bacteria occurred rather than just filtration. The results of the swatch testing are shown in Tables II and III.

It is clear from the results in the two tables that the treated Nylon 66 fibers and swatches were bactericidal, with the hydantoin treatment being the most effective, at least in terms of efficacy at the contact times studied for the freshly prepared samples. However, it should be noted that, for *N*-halamine moieties, the biocidal efficacy, as assessed by contact time necessary to achieve a given log inactivation, is inversely related to the stability of the N—Cl or N—Br covalent bond. In other words, compounds or materials containing cyclic *N*-halamine moieties that require longer contact times for antimicrobial activity will be more stable to loss of halogen and possess enhanced long-term biocidal activity.

Table IV Regeneration of Antibacterial Activity Against *S. aureus*

Chlorination[a]	Challenge Time (min)	Log Reduction
First	60	8.1
Second	60	8.1

[a] After the first bacterial challenge the swatch was dechlorinated using sodium thiosulfate as a reducing agent, and then rechlorinated, followed by a second bacterial challenge.

An experiment was also performed to determine whether the antibacterial activity could be regenerated after its loss. Fabric samples were treated with the hydroxymethylhydantoin compound and chlorinated. Test and unchlorinated control fabric samples were tested quantitatively for antibacterial activity against *S. aureus* (1.23×10^8 CFU) using the swatch bacteria test. The two samples were then exposed to 100 mL of 0.02*N* sodium thiosulfate for 1 min. A second chlorination was performed on the previously chlorinated sample, and the swatch test was performed a second time for both the chlorinated and unchlorinated samples, the results of which are shown in Table IV. It is clear that antibacterial activity was restored to the previously chlorinated sample by a second chlorination.

The nitrogen–chlorine bond is very stable in these treated Nylon 66 materials. The mechanism

Table III Bactericidal Performance of Nylon 66 Swatches Treated with Three Different *N*-Halamines Against *S. aureus*

Treatment Monomer[a]	Chlorination	Challenge (logs)	Contact Time (min)	Reduction (logs)
HMHY	Yes	8.7	60	8.7
HMHY	No	8.7	60	1.3
HMOX	Yes	8.6	60	6.1
HMOX	No	8.6	60	0.6
HMIM	Yes	8.6	60	5.5
HMIM	No	8.6	60	0.8

[a] HMHY = hydroxymethylhydantoin; HMOX = hydroxymethyloxazolidinone; HMIM = hydroxymethylimidazolidinone.

of action must be a direct contact of the bacterial cell with the bound chlorine atom, resulting in transfer of the chlorine and subsequent cell inactivation. The bound chlorine atom is subject to loss mechanisms upon chemical interaction with reducing agents, but as noted earlier, it can be replenished by subsequent exposure to a source of free chlorine such as bleach.

Work in these laboratories is currently being directed toward the biocidal treatment of commercial nylon articles using techniques similar to those discussed earlier as well as other important fiber, textile, and industrial materials.

CONCLUSIONS

This study demonstrated that Nylon 66 fibers can be rendered antimicrobial by chemically bonding a heterocyclic *N*-halamine functional group to the Nylon 66 molecule at the amide nitrogen using formaldehyde as a linking agent. The treated materials were shown to be bactericidal against the bacteria *S. aureus* and *E. coli*. Upon loss of the antimicrobial chlorine atom, activity can be restored by exposure to a source of free chlorine such as bleach. It is anticipated that this technology will be useful in rendering antimicrobial such commercial products as clothing, carpets, surgical sutures, and brush bristles.

This work was supported in part by U.S. Department of Commerce Grant 99-27-07400 to the National Textile Center.

REFERENCES

1. Buchenska, J. J Appl Polym Sci 1996, 61, 567.
2. Sun, G.; Wheatley, W. B.; Worley, S. D. Ind Eng Chem Res 1994, 33, 168.
3. Worley, S. D.; Sun, G. Trends Polym Sci 1996, 4, 364.
4. Eknoian, M. W.; Putman, J. H.; Worley, S. D. Ind Eng Chem Res 1998, 37, 2873.
5. Bickert, J. R.; Xu, X.; Sun, G.; Williams, J. F. Presented at the International Conference on Safe and Protective Fabrics '98, Baltimore, MD, 1998, 1.
6. Sun, G.; Xu, X. Tex Chem Col 1998, 6, 26.
7. Sun, G.; Xu, X. Tex Chem Col 1999, 31, 21.