

# An Effective Antimicrobial Treatment for Wool Using Polyhexamethylene Biguanide as the Biocide, Part 1: Biocide Uptake and Antimicrobial Activity

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**ABSTRACT:** Antimicrobial treatments of textiles have received substantial attention in recent years, both in academic research and in commercial productions. Such treatments prevent the odor formation and fabric deterioration that arise from the growth of microbes on the textiles during their use or storage. So far, antimicrobial treatments have mainly focused on cotton and synthetic fibers, and a viable treatment for wool is lacking. In this report, we demonstrate a novel and effective antimicrobial finishing for wool. We found that pretreatment with peroxymonosulfate and sulfite, a treatment previously developed for shrink resistance, uniquely enabled wool

to exhaust up to 5% (on the weight of the fabric) of the biocide polyhexamethylene biguanide (PHMB) under mild conditions. In contrast, untreated or chlorinated wool had little affinity for this biocide. The exhaustion was facilitated by higher temperatures but was not affected by pH over a broad range. Wool fabrics finished with PHMB exhibited strong antimicrobial abilities that could deactivate the bacterium *Escherichia coli* within a few minutes of contact. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 117: 3075–3082, 2010

**Key words:** fibers; biofibers; biopolymers

## INTRODUCTION

The growth of microbes, in particular bacteria, on textiles leads to a number of undesirable outcomes, including an unpleasant odor, the discoloration of the fabric, and a reduction in fabric strength. All of these can be prevented or diminished by the antimicrobial finishing of the textile. The intensive research on antimicrobial textiles has been reviewed in a number of recent articles.<sup>1–3</sup> Such intensive research, coupled with consumers' demand for hygienic clothing and active wear, has seen the production of large quantities of commercial antimicrobial textiles. It is also estimated that the production of antimicrobial textiles increased by more than 15% per year in Western Europe between 2001 and 2005; this makes it one of the fastest growing sectors in the textile market.<sup>4</sup>

Commercial antimicrobial textiles use the biocides silver (or its salts), quaternary ammonium compounds (QACs), polyhexamethylene biguanide (PHMB), and triclosan, and several other biocides are in the development stage.<sup>1–3</sup> Of these chemicals, PHMB is polymeric and potent with broad spectral activity against bacteria but with low toxicity to

higher organisms. It has a long history of safe use as a disinfectant in the food industry and in the sanitization of swimming pools.<sup>5</sup> PHMB can be directly exhausted onto cotton<sup>6,7</sup> or applied in a pad-dry-cure process.<sup>8</sup> Because of its cationic nature, PHMB attachment to cotton is believed to be predominantly through ionic and hydrogen bonding.<sup>9</sup> The carboxyl groups on cotton fabrics that have originated from chemical finishing are involved in some of these interactions.<sup>10</sup> Commercial antimicrobial cotton fabrics using PHMB are available under the trade name Purista.<sup>11</sup> PHMB does not exhaust well onto synthetic fibers. Nevertheless, Purista was recently extended to include some synthetic fibers with an unspecified process.<sup>12</sup>

Research activities on antimicrobial treatments have been largely confined to cotton and synthetic fibers (e.g., polypropylene, polyester, nylon). In contrast, reports on the antimicrobial treatment of wool have been only sporadic, in part because of the difficulty in the finishing. In these few studies, wool has been exhausted with QAC,<sup>13,14</sup> covalently linked to thiol-containing QAC,<sup>15</sup> adsorbed with metallic salts ( $\text{Ag}^+$  and  $\text{Cu}^{2+}$ ),<sup>16,17</sup> or coated with chitosan.<sup>18–20</sup> However, because of the toxicity of the process, the adverse effect of the treatment on the fabric handle or the poor durability of the finishing, none of these processes can be adopted for the commercial production of antimicrobial wool fabrics. Very recently, Nanohorizon, Inc., announced a silver-based

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treatment for wool but provided little information about the finishing or its effectiveness.<sup>21</sup> As such, an effective antimicrobial treatment for wool is largely lacking.

In this study, we found that pretreatment with peroxymonosulfate (PMS) and sodium sulfite, a process developed for wool shrink resistance, enabled the otherwise unreceptive wool to exhaust large quantities of the biocide PHMB. This novel treatment imparted strong antimicrobial functionality to the wool.

## EXPERIMENTAL

### Materials

Unless specified otherwise, the wool fabric used in this study had a mass of 190 g/m<sup>2</sup>. Fabric construction was 2 × 1 twill with untreated and unchlorinated Australian Merino wool. PMS (trade name Oxone) was purchased from Dupont (Sydney, Australia). Sodium sulfite, yeast extract, and tryptone were purchased from Sigma (Sydney, Australia). PHMB in a 20% (w/v) aqueous solution was purchased from Arch Chemicals (Singapore).

### Methods

#### PMS/sulfite pretreatment

Wool fabrics (typically, 150 × 150 mm<sup>2</sup>, ~ 4.5 g) were pretreated before they were used for PHMB exhaustion. The pretreatment, adopted from a wool pretreatment for shrink resistance,<sup>22</sup> consisted of two steps. The fabrics were first treated with a PMS solution (2 g/L) containing 1 mL/L of the nonionic surfactant Triton X-100 at room temperature for 15 min, rinsed in water, and squeezed to remove excess water. Subsequently, the fabrics were treated with sodium sulfite (10 g/L, pH adjusted to 8.2–8.5 with 2M sulfuric acid) at room temperature for 15 min, again rinsed in water, and squeezed to remove excess water. The liquor-to-wool ratio for both the PMS and sulfite treatments was 40 : 1 (vol/wt). All fabrics, including the untreated fabrics, were dried in an oven at 80°C for 45 min and stored at room temperature before use. This drying process did not alter the wool's affinity for PHMB.

#### Determination of the PHMB exhaustion by absorbance

The PHMB stock solution was adjusted to pH 7–7.5 with NaOH. Unless stated otherwise, the untreated or PMS/sulfite-pretreated wool fabric (0.40 g) was incubated with 20 mL of solution containing 1.6 mg/mL PHMB and 1 mL/L Triton X-100 with continuous shaking at 22°C (room temperature) or

40°C (in a water bath) for up to 60 min. The amount of PHMB was therefore 8% owf (on weight of the fabric), and the liquor-to-wool ratio was 50 : 1 (vol/wt). At designated time points, 50 µL of the solution was withdrawn and diluted to 5 mL with deionized water for an absorbance reading at 236 nm. The exhaustion of PHMB was calculated from the initial absorbance at time 0 and the absorbance at a designated time.

#### Determination of the PHMB uptake by mass gain

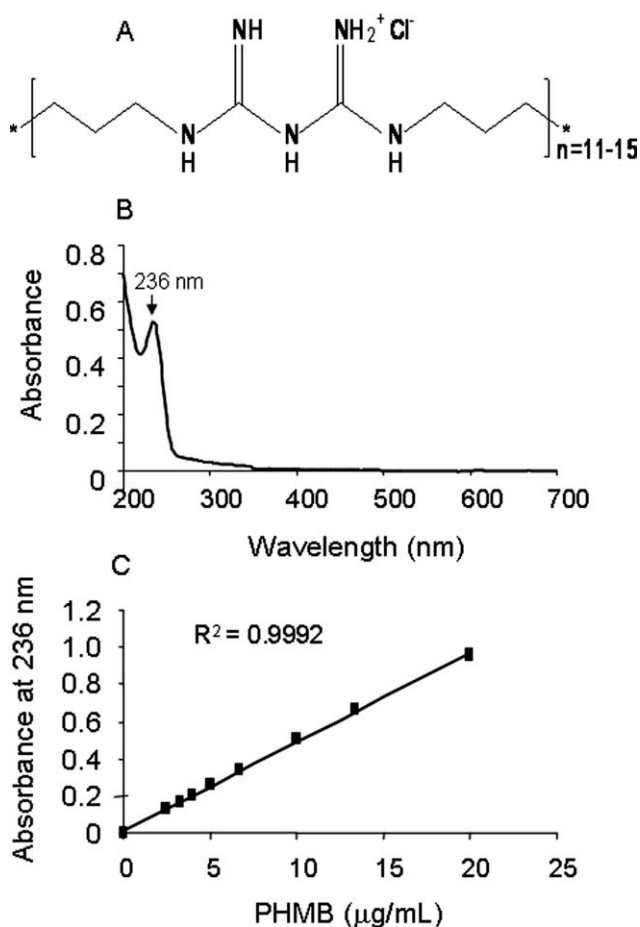
To determine the actual amount of PHMB uptake, the PMS/sulfite-pretreated fabrics (150 × 150 mm<sup>2</sup>, ~ 4.5 g) were conditioned at 20°C and 65% relative humidity for 2 days and weighed to an accuracy of ±1 mg. These fabrics were then used for PHMB exhaustion in various amounts at 40°C for 60 min. The absorbance of the solutions at 236 nm at the beginning and at the end of exhaustion was also determined to calculate the amount of PHMB exhaustion for comparison with weight gain. These fabrics were then rinsed in deionized water for 1 h with three changes of water and gentle shaking to remove loosely bound PHMB. The weight of the fabrics was determined again after conditioning, as described previously. The actual PHMB uptake was calculated from the weight difference before and after PHMB exhaustion.

#### Antimicrobial assays

Quantitative antimicrobial assays on the textiles were performed as per AATCC Test Method 100–1999 with the bacterial species *Escherichia coli* (ATCC 4352, Gram negative) and *Staphylococcus aureus* (ATCC 6538, Gram positive) cultured in lysogeny broth (5 g/L yeast extract, 10 g/L tryptone, and 10 g/L NaCl, with the pH adjusted to 7.2 with NaOH). In these assays, 0.25 mL of overnight culture (diluted to 4 × 10<sup>7</sup> cells/mL with lysogeny broth) was applied to four layers of fabrics (0.4 g in total). The inoculum was fully adsorbed without any excess liquid remaining. The fabrics were placed in sealed jars (250 mL volume) and incubated for 5 h at 37°C. The bacteria were eluted with 100 mL of sterile water by vigorous shaking. The total number of live *E. coli* cells was counted by serial dilution and plating on nutrient agar plates. The percentage of bacterial reduction was calculated with the following equation:

$$\text{Bacterial reduction (\%)} = 100(C - A)/C$$

where *A* is the number of colonies from the test fabrics after 5 h of incubation and *C* is the number of colonies from a PMS/sulfite-pretreated fabric (no



**Figure 1** (A) Structures of PHMB, (B) spectrum of PHMB absorption, and (C) linear relationship between its concentration and absorbance at 236 nm.

PHMB finishing) at time zero. If  $A$  is greater than  $C$ , there is no reduction.

## RESULTS

### Monitoring PHMB exhaustion by UV absorbance

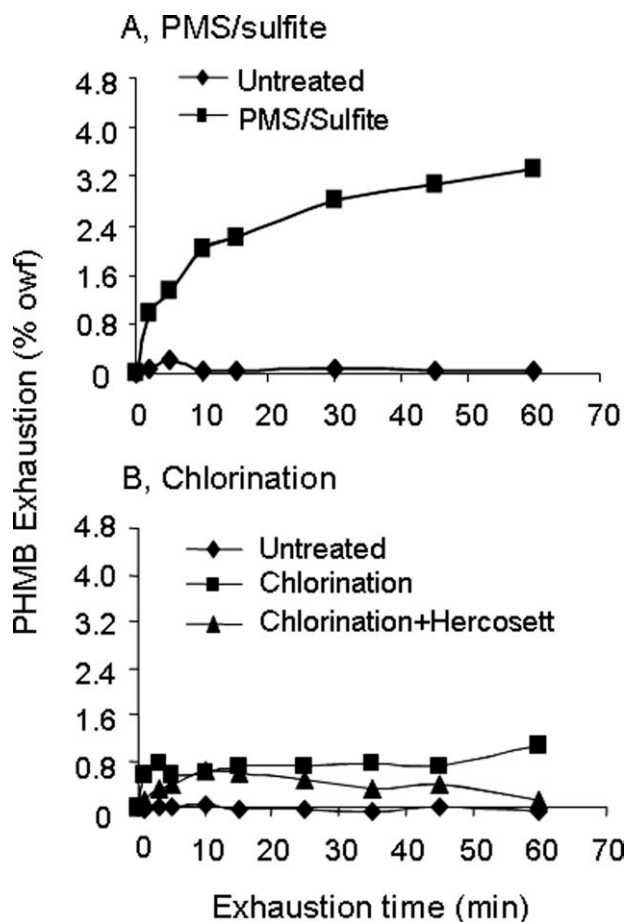
PHMB [Fig. 1(A)] was reported to absorb UV light,<sup>10</sup> probably because of the C=N double bonds in the structure. We confirmed that PHMB absorbed UV with a single sharp peak at wavelength of 236 nm [Fig. 1(B)]. The absorbance was found to observe the Beer-Lambert law; that is, it was proportional to the concentration of PHMB [ $R^2 = 0.9992$ ; Fig. 1(C)]. The absorbance coefficient appeared to be very high, 0.52 at 10  $\mu\text{g}/\text{mL}$  in water. This property, thus, provided a simple and sensitive means to quantify PHMB's exhaustion onto fabrics from solutions. Because of the strong absorbance, the exhaustion solutions (e.g., 1.6 mg/mL PHMB) used in this study had to be diluted 100-fold in water before the absorbance could be read. This large dilution also served to eliminate interference in the absorbance from the surfactant Triton X-100 used in the exhaustion solution and any small

amount of proteins that may have leached out of the wool during the exhaustion process.

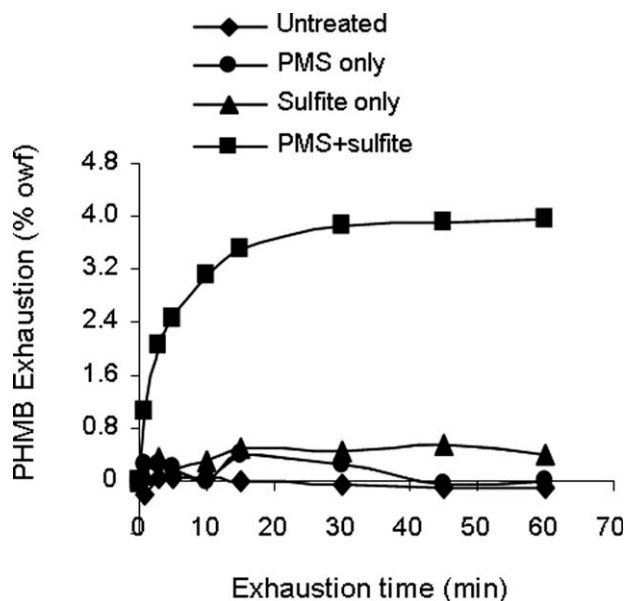
### PHMB exhaustion on the PMS/sulfite-pretreated wool

Untreated or PMS/sulfite-pretreated wool fabrics were used for PHMB exhaustion at 8% owf PHMB in the bath and a liquor-to-wool ratio of 50 : 1. Figure 2(A) shows that, with the absorbance reading method described previously, the PMS/sulfite-pretreated wool fabric was able to exhaust 3.4% owf PHMB or 42.5% of the PHMB present in the exhaustion bath in 1 h at 22°C. The untreated wool fabrics exhausted a negligible amount of PHMB.

We also tested whether chlorinated wool was able to exhaust PHMB. For this purpose, matched fabrics made from untreated wool, chlorinated wool, or chlorinated wool with Hercosett coating were used. Figure 2(B) shows that the untreated wool, again, did not take up PHMB. The chlorinated wool fabrics exhausted approximately 0.8% owf PHMB. Further coating with Hercosett reduced the uptake to



**Figure 2** Exhaustion of PHMB by (A) PMS/sulfite-pretreated and (B) chlorinated wool fabrics at 22°C. The total amount of PHMB in the exhaustion baths was 8% owf for both experiments.



**Figure 3** Effect of PMS oxidation, sulfite reduction, and their combination on PHMB exhaustion at 40°C. The total amount of PHMB was 8% owf in the exhaustion bath.

approximately 0.4% owf. We suspected that there might have been some wax on the yarns of these fabrics arising from the spinning process. This wax may have prevented the wool fiber from directly contacting and, therefore, taking up, PHMB. However, washing the fabrics with Triton X-100 (0.2% v/v) for 15 min at 55°C followed by rinsing in warm water, a process to that was expected to remove the wax (if any), did not improve the PHMB exhaustion on these fabrics (data not shown). These results indicate that wool after chlorination, in contrast to the PMS/sulfite treatment, did not exhaust any significant amount of PHMB.

#### Both PMS and sulfite in the pretreatment were essential for PHMB uptake

The PMS/sulfite pretreatment comprised an oxidative and a reductive step. We examined which step was critical for PHMB exhaustion. For this purpose, fabrics were pretreated with PMS only, sulfite only, or a combination of the two. Figure 3 shows that the untreated and PMS-treated fabrics exhausted a negligible amount of PHMB, whereas sulfite-treated fabrics exhausted approximately 0.5% owf PHMB over 1 h at 40°C. On the other hand, the PMS/sulfite-pretreated fabrics exhausted 4.0% under these conditions. It was, thus, clear that both PMS and sulfite were needed in the pretreatment for efficient PHMB exhaustion.

#### Effect of the temperature and pH on PHMB exhaustion

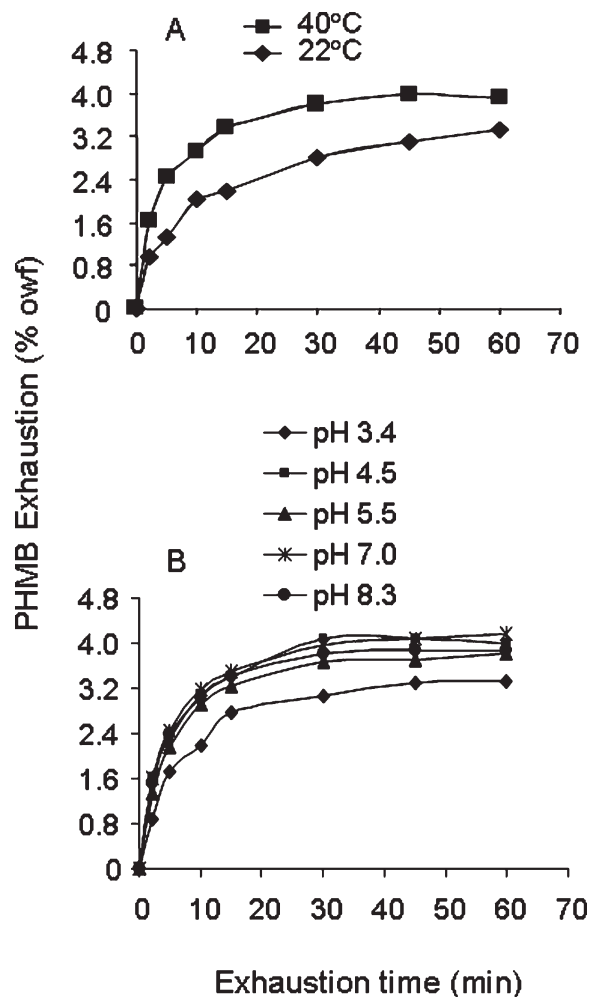
Temperature and pH typically have an effect on the exhaustion of chemicals onto textiles. Figure 4(A)

shows that the exhaustion was faster and more efficient at 40°C than at 22°C. The exhaustion at 40°C largely plateaued after 30 min and reached 4.0% owf over a 1-h period. On the other hand, the exhaustion at 22°C continued to rise after the first 30 min and only reached 3.4% owf after 1 h.

The effect of pH on PHMB exhaustion was studied by careful adjustment of the pH to the desired levels with concentrated NaOH. The pH was not maintained with a buffer during the exhaustion. Variation in the range of pH of 4.5–8.3 had little effect on PHMB exhaustion; however, a further decrease in the pH to 3.4 decreased the exhaustion from approximately 4.0 to 3.2% owf [Fig. 4(B)].

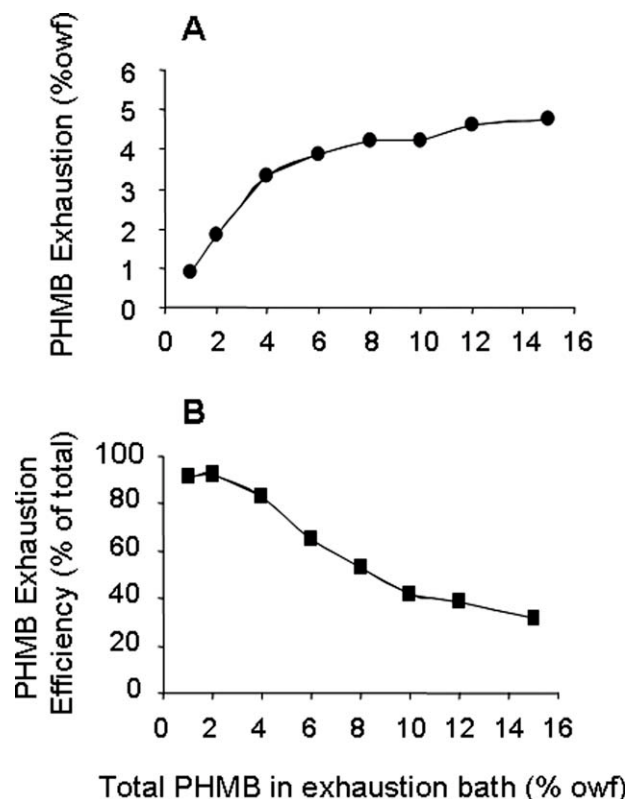
#### Saturation of PHMB exhaustion

As demonstrated previously, the PMS/sulfite-pretreated wool fabrics were capable of taking up 4.0% owf PHMB at a liquor ratio of 50 : 1 at 40°C when a



**Figure 4** Effects of (A) temperature and (B) pH on PHMB exhaustion for PMS/sulfite-pretreated wool fabrics at 40°C. The total amount of PHMB was 8% owf in the exhaustion bath.





**Figure 5** The amount (A) and efficiency (B) of PHMB exhaustion by PMS/sulfite-pretreated wool under saturation conditions at 40°C.

total of 8% owf PHMB was present in the bath. It was likely that the fabrics were still unsaturated with PHMB under those conditions. To examine the maximum amount of PHMB that the pretreated fabrics could take up, individual pretreated fabrics were incubated with various amounts of PHMB in the exhaustion bath at 40°C for 1 h (1–16% owf, liquor ratio = 50 : 1). The exhaustion initially increased almost linearly with increasing PHMB amount in the bath but started to level off above 6% owf PHMB in the bath [Fig. 5(A)]. As such, increasing PHMB in the exhaustion bath from 8 to 15% owf only increased the actual exhaustion from 4.2 to 4.8% owf [Fig. 5(A)]. The percentage uptake depended on the total amount of PHMB in the bath. The fabrics almost depleted the chemical when 2% owf or less PHMB was available but could take up only half of the 8% owf PHMB in the bath [Fig. 5(B)].

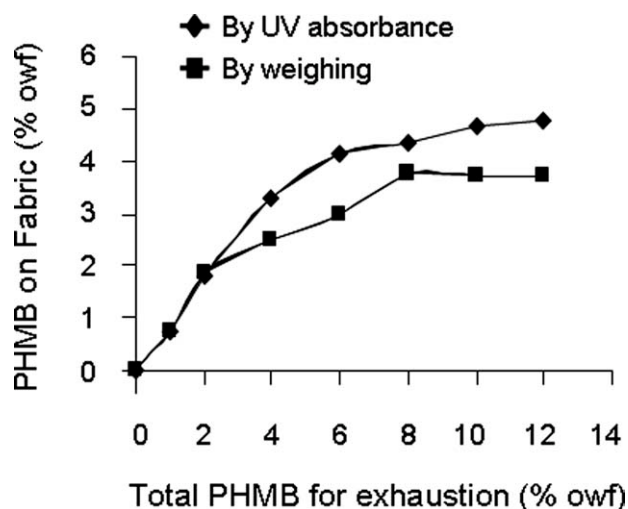
#### Determination of PHMB on the fabrics by mass gain

In the experiments described previously, the amount of PHMB exhaustion was conveniently determined by its absorbance at 236 nm. We also sought to directly determine the uptake by fabric mass gain. This would serve to further validate the spectrophotometric method. In this experiment, in addition to

monitoring the exhaustion by absorbance, we determined the amount of PHMB exhausted on the fabrics by conditioning and weighing the fabrics before and after the exhaustion. We noted that, in two independent experiments, each with two fabrics, shaking fabrics in 0.1% Triton X-100 at 40°C for 60 min (i.e., exhaustion without PHMB) resulted in a loss of 0.55% of their mass, presumably due to the leaching of a small amount of protein from the wool. We corrected for this mass loss in all mass determinations. Figure 6 shows that, under limiting amounts of PHMB (1 or 2% owf PHMB in the bath), the mass increased almost linearly with higher amounts of PHMB in the bath. These results agreed very well with those obtained by the absorbance method. Under higher PHMB concentrations, the uptake determined by mass gain was slightly less than that calculated from absorbance. The reason for this small difference is discussed later.

#### Antimicrobial effect of the PHMB-finished wool fabrics

The antimicrobial activity of the PHMB-finished fabrics was assessed quantitatively against Gram-negative *E. coli* and Gram-positive *S. aureus*. Untreated or PMS/sulfite-pretreated wool fabrics were exhausted with 1, 2, 4, and 8% owf PHMB in the bath at 22°C. The untreated fabrics did not exhaust a significant amount of PHMB in any of these concentrations and, consequently, did not have antimicrobial activity against these two species (Table I). The finishing of the PMS/sulfite-pretreated fabrics with 1, 2, 4, and 8% owf PHMB in the bath led to the exhaustion of the biocide at 0.82, 1.38, 2.70, and 3.25% owf, respectively. Fabrics containing 0.82% owf PHMB did not exhibit antimicrobial activity against the two



**Figure 6** Comparison of PHMB uptake of PMS/sulfite-pretreated fabrics by absorbance reading at 236 nm and by mass gain.

TABLE I  
Quantitative Antimicrobial Assays of PHMB-Finished Wool Fabrics with *E. coli* and *S. aureus*

Fabric pretreatment	Total PHMB for exhaustion (% owf)	PHMB exhaustion (% owf)	Antimicrobial activity against <i>E. coli</i> (% reduction)	Antimicrobial activity against <i>S. aureus</i> (% reduction)
None	0	0	NR	NR
None	1	0.12	NR	NR
None	2	0.08	NR	NR
None	4	0.11	NR	NR
None	8	0.18	NR	NR
PMS/sulfite	0	0	NR	NR
PMS/sulfite	1	0.82	NR	NR
PMS/sulfite	2	1.39	NR	29.6
PMS/sulfite	4	2.70	91.5	99.9
PMS/sulfite	8	3.25	99.9	99.9

Untreated or PMS/sulfite-pretreated fabrics were exhausted with various amounts of PHMB in a bath at 22°C for 1 h. NR = no reduction.

species, whereas those containing 1.38% owf PHMB had some bacterial reduction against *S. aureus* but not against *E. coli*. Fabrics containing 2.70% owf reduced *E. coli* growth by 91.5% and *S. aureus* by 99.9% (Table I). When PHMB was further increased to 3.25% owf, the fabrics were able to reduce both bacterial species by 99.9%. These results indicate that PHMB-treated wool was more effective against *S. aureus* than against *E. coli*. This was consistent with the toxicological information that PHMB has a lower minimal inhibitory concentration against *S. aureus* (0.5 ppm) than *E. coli* (1 ppm; technical information from Arch Chemicals). They also indicate that the PHMB on the fabrics required for effective bacterial control should be around 3% owf or more, an amount comparable to the 2–4% owf required for a durable cotton treatment.<sup>23</sup>

#### Kinetics of the PHMB-finished wool fabrics in deactivating bacteria

The length of contact time required to kill bacteria is a consideration. Preferably, the bacteria should be deactivated quickly when they come into contact with the treated fabrics. The pretreated fabrics finished with PHMB (8% owf in the bath at 40°C for 1 h), which contained approximately 4% PHMB owf (see Figs. 3 and 4), were inoculated with *E. coli* and incubated in sealed jars at 37°C for the indicated length of time (up to 5 h). Live cells were then eluted and counted by serial dilution and plating on nutrient agar plates. The PHMB-finished fabrics worked very rapidly, killing 98.2% of the cells within 5 min of contact and nearly 100% after 20 min of contact (Fig. 7 and its inset graph).

#### DISCUSSION

In this report, we have demonstrated that the PMS/sulfite pretreatment, which was initially developed

as a wool shrink-resistance treatment,<sup>22</sup> uniquely enabled wool to exhaust large amounts of the biocide PHMB. Such PHMB-finished wool fabrics had strong and fast antimicrobial abilities, deactivating more than 99% of bacteria within several minutes of contact. To the best of our knowledge, this represents the first report that PHMB can be successfully applied to wool.

PMS/sulfite-pretreated wool fabrics maximally took up approximately 5% owf PHMB in an exhaustion process that was facilitated by higher temperatures (e.g., 40°C) but was generally not affected by pH. We measured the uptake routinely and spectrophotometrically by following the disappearance of PHMB from the exhaustion solution, and this was confirmed by direct mass gain in the fabrics after the exhaustion. At low bath concentrations of PHMB ( $\leq 2\%$  owf), the results from these two methods agreed very well. However, at higher PHMB levels, the amount of PHMB on the fabric determined by mass gain was slightly lower than that calculated

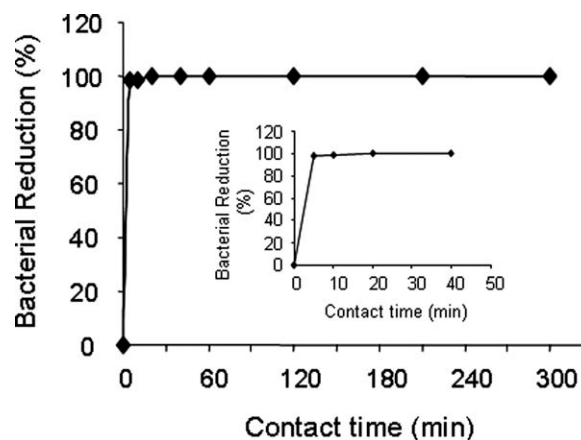


Figure 7 Bacterial deactivation after bacteria were in contact with PHMB-finished wool for various times. The inset graph is an expansion of the x axis in the first 40 min of contact.

from absorbance changes of the solution (Fig. 6). One plausible explanation for this small difference may be as follow. When a large excess of PHMB was present in the bath, the PHMB molecules competed for the wool surface for adsorption, which led to a small portion of the PHMB molecules being loosely attached to the wool. These loosely bound molecules were labile and could be lost in the rinse step before weight gain determination. In any case, both methods indicated that PHMB was efficiently exhausted onto the pretreated wool.

We observed that, unlike the highly effective PMS/sulfite pretreatment, PMS oxidation alone and the chlorination process did not generate wool surfaces receptive to PHMB exhaustion (Figs. 2 and 3). Similar differences in the interaction between wool and other cationic substances have been observed in the literature. For instance, colloidal cationic polymer particles could adsorb onto PMS/sulfite-treated wool but not PMS-treated wool,<sup>24</sup> and the cationic polymer Hercosett could be coated on chlorinated but not PMS-treated wool in shrink-resistance treatment.<sup>25</sup> The underlying mechanism for this difference has attracted considerable interest and is presumably related to the surface chemistry, such as the lipid layer and the oxidation of cystine.<sup>24,26</sup> As far as PHMB adsorption is concerned, the removal of the lipid layer appeared not to be critical because chlorination, which removes most of the lipid layer, only slightly improved PHMB exhaustion (Fig. 2). On the other hand, Bunte salts may play an important role in wool's ability to exhaust PHMB. Denning et al.<sup>22</sup> reported that Bunte salts were not produced by either PMS and chlorine oxidation of wool surface. However, after a reduction step with sulfite, PMS-treated wool and, to a much lesser extent, chlorine oxidized wool contained large quantities of Bunte salts.<sup>22</sup> There exists a clear positive correlation between Bunte salts and PHMB exhaustion.

The interaction between PHMB and the PMS/sulfite-treated wool surface is a matter of interest. Because of its cationic nature, PHMB attachment on cotton has been found to be predominantly due to ionic interaction with the carboxylic acid at low concentrations and hydrogen bonding at higher concentrations.<sup>9,10</sup> The dyeing of cotton fabrics with reactive dyes, which introduces additional anionic sulfonic groups in the fabric, further increases the uptake of PHMB.<sup>27</sup> It is known that both PMS and chlorine oxidization of the wool surface produce large quantities of cysteic acid,<sup>22</sup> which has the ability to form ionic bonds with cationic substances. If ionic bonding was also the major force in the PHMB exhaustion on wool, one would expect that wool after such oxidations would have a high affinity for PHMB at the neutral pH used in this study. However, this was not the case (Fig. 3). This low affinity

of PMS- or chlorine-treated wool for PHMB seemed to eliminate ionic interaction as the major interaction force. If Bunte salts were indeed responsible for PHMB exhaustion on the PMS/sulfite-treated wool, their interaction with PHMB remains to be determined.

The finishing of the PMS/sulfite-pretreated wool fabrics with PHMB conferred them strong antimicrobial activity. This was expected, as the fabrics exhausted large quantities of PHMB, whose biocidal action is well documented. There was a minimal amount of PHMB on the wool that was required for effective antimicrobial activity. An amount of less than 1.5% owf had little effect on the growth of the Gram-negative bacteria *E. coli*, whereas more than 2.5% owf deactivated more than 90% of the cells (Table I). This latter amount was similar to the 2–4% owf required and recommended for cotton antimicrobial finishing.<sup>23</sup> This appears to be a relatively large amount when one considers that PHMB is a potent biocide with a minimal inhibitory concentration of 0.05–10 ppm against a range of bacterial species.<sup>3</sup> It is possible that PHMB loses some of its activity or becomes less readily available for interaction with bacterial cells after immobilization on a textile surface.

An apparent application of PHMB-treated wool is in antimicrobial socks. Traditionally, wool is one of the best fibers for socks because of the comfort it offers and its ability to absorb large amount of moisture. The warm and moist environment in socks during wearing is very conducive to bacterial growth and odor formation, and there is, therefore, a genuine need for antimicrobial finishing. Indeed, it has been estimated that over 20% of all antimicrobial fibers or fabrics are used for socks.<sup>3</sup> PHMB-finished wool offers the potential in the manufacturing of premium antimicrobial woolen socks.

## CONCLUSIONS

We discovered a novel and effective antimicrobial treatment, in which a combinational use of PMS oxidation and sulfite reduction in the pretreatment produced receptive wool for the exhaustion of the biocide PHMB, and the pretreatment could not be replaced by chlorination. Further work in the characterization of the fabrics, including washing durability studies, compatibility with dyeing, and effects on the fabric physical properties, will be reported in part II of this study.

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