Surface Self-Concentrating Amphiphilic Quaternary Ammonium Biocides as Coating Additives

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ABSTRACT A variety of amphiphilic quaternary dimethylammonium compounds bearing *n*-alkyl and oxyethylene groups have been designed and synthesized as antimicrobial additives for use in self-decontaminating surfaces. The effectiveness of these additives stems from a unique ability to self-concentrate at the air−polymer interface without the incorporation of exotic perfluorinated or polymeric functionalities. X-ray photoelectron spectroscopy analysis reveals surface enrichment as high as 18-fold, providing a 7-log reduction of both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The migration to the surface is a consequence of the hydrophobicity of the additive within the hydrophilic polyurethane resin, over which an unprecedented level of control can be exerted by altering the lengths of the *n*-alkyl and oxyethylene groups. Thus, for the first time, specific surface and bulk coating concentrations can be achieved as desired using a single class of antimicrobial additives.

KEYWORDS: additives • antimicrobial • biocide • coating • quaternary ammonium • self-cleaning • surface-concentrating

The rational design of effective self-decontaminating surfaces has been the goal of a number of research groups (1−5), and a variety of methods for the surface modification of polymeric materials have been utilized toward the synthesis of antimicrobial coatings for use in hospitals and in defense of biological weapons (6). The use of biocidal additives in paints and coatings imparts antimicrobial properties without the need for complex or costly postmanufacturing steps associated with surface-modifying post-treatments (7). For this purpose, the self-concentration of additives at the surface is advantageous not only to preserve the physical properties of the bulk material but also to increase the antimicrobial activity. Furthermore, a surface-concentrated additive is more efficient than an additive that is evenly distributed throughout the coating because much of the additive in the latter case will be ineffective within the bulk material. One challenge in the development of such an additive is to impart the ability to surface-concentrate without leaching from the surface because leaching invokes environmental concerns (8). Moreover, while the release of biocidal agents can be an effective method of surface self-decontamination (9), the activity will diminish as the agents are depleted. On the other hand, coatings subject to weathering and abrasion would require biocidal additives that are well-dispersed throughout the material, so that the biocidal properties are not lost upon damage of the surface.

Quaternary ammonium biocides are used in a variety of commercial applications ranging from cosmetic preservatives to hospital disinfectants and sanitizers (10). These species have many advantages over other biocide classes (e.g., phenols and aldehydes) including broad-spectrum antimicrobial activity, effectiveness over a wide pH range, low human toxicity, low vapor pressure, amphiphilic solubility, and lack of a detectable odor. Polymer surface modifiers (PSMs) have recently been used to concentrate quaternary ammonium groups and other biocidal moieties at the surface−air interface (11−13), driven primarily by low solubility and/or low surface energy (14). One such case is a class of PSMs comprised of copolymers containing fluoroalkyl groups adjacent to biocidal moieties (12, 13). The fluoropolymer segments were found to migrate through the bulk polyurethane and served to "chaperone" the chloroamide or quaternary ammonium biocidal components to the surface.

It was envisioned that homologous quaternary dimethylammonium compounds bearing hydrophobic *n*-alkyl groups and hydrophilic oxyethylene chains, both of variable length, would provide a series of novel biocides that allow adjustment in hydrophilicity and hydrophobicity as desired. Herein, we report a new class of discrete amphiphilic quaternary ammonium compounds that are highly effective antimicrobial additives in polyurethane films and do not exhibit any evidence for leaching biocidal agents. This work represents the first example of a biocidal additive with the ability to self-concentrate at the surface of a polymer film without exotic polymer functionalities. We further detail simple structural modifications that provide an unprecedented level of control over the additive's tendency to surface-concentrate (Figure 1), such that the properties of the coating can be fine-tuned to achieve maximum surface reactivity or to maintain biocidal activity upon damage or wearing.

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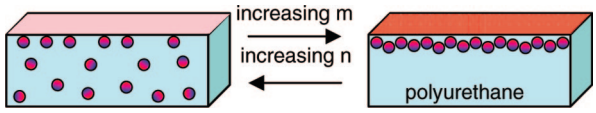

FIGURE 1. Graphic representation of the effect of altering the lengths of the *n*-alkyl (red) or oxyethylene (blue) groups on the surface concentration.

Scheme 1. Synthetic Scheme for the Preparation of Amphiphilic Quaternary Ammonium Antimicrobials

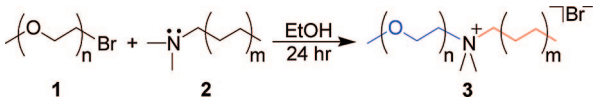

Table 1. Antimicrobial Activity and XPS Results for 3a−h

					MIC[a] (mmol/L)		log kill[b]		XPS[c]	
entry	product	*m*	*n*	yield (%)	*S. aureus* (G+)	*E. coli* (G−)	*S. aureus* (G+)	*E. coli* (G−)	% N obsd	% N calcd
1	**3a**	2	1	83	9.3	9.3	5	3	0.1	0.05
2	**3b**	2	2	82	16.0	8.0	6	3	0.3	0.05
3	**3c**	2	3	96	14.0	7.0	3	1	0.1	0.05
4	**3d**	2	4	53	6.2	6.2	3	1	0.1	0.04
5	**3e**	3	1	79	0.7	2.0	7	6	0.5	0.05
6	**3f**	3	2	87	0.9	7.3	7	7	0.9	0.05
7	**3g**	3	3	94	1.3	6.5	5	4	0.3	0.04
8	**3h**	3	4	85	1.9	3.0	4	4	0.2	0.04

[a] Minimum inhibitory concentration. [b] Log reduction starting with 107 CFU/cm^2 on a coating of Hydrothane containing 1% biocide. [c] % N obsd is the weight percent of biocidal nitrogen in the surface, excluding hydrogen, as observed by XPS. [d] % N calcd is the calculated number expected if the additive was evenly distributed throughout the coating, with no surface concentration.

As depicted in Scheme 1, these amphiphilic biocides were prepared by reacting monodispersed methoxy-terminated oxyethylene bromides (**1**) with tertiary amines (**2**) in ethanol to afford the desired ammonium bromide compounds (**3**) in good yields (Table 1).

All compounds were subjected to minimum inhibitory concentration (MIC) studies for effectiveness comparisons against the bacteria *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), as shown in Table 1. Biocides possessing *n*-octyl alkyl groups (**3e−h**) were generally more effective antimicrobials than the corresponding analogues possessing *n*-hexyl groups (**3a−d**), particularly against *S. aureus*. Furthermore, the *n*-hexyl series was slightly more effective against *E. coli* than *S. aureus*, while the *n*-octyl series was significantly more effective against *S. aureus*. It is possible that the slightly longer and more hydrophilic *n*-octyl group is more effective than *n*-hexyl at disrupting the cell wall of Gram-positive bacteria, whereas the more complex Gram-negative cell wall is not as vulnerable to this functionality.

Variations in the oxyethylene chain length were also found to affect the activity. In the case of the *n*-octyl series, an increase in the oxyethylene chain length corresponded to a gradual decrease of the antimicrobial activity against *S. aureus*. However, the compounds bearing oxyethylene chains of 1 or 4 repeat units (**3e** or **3h**) were significantly more

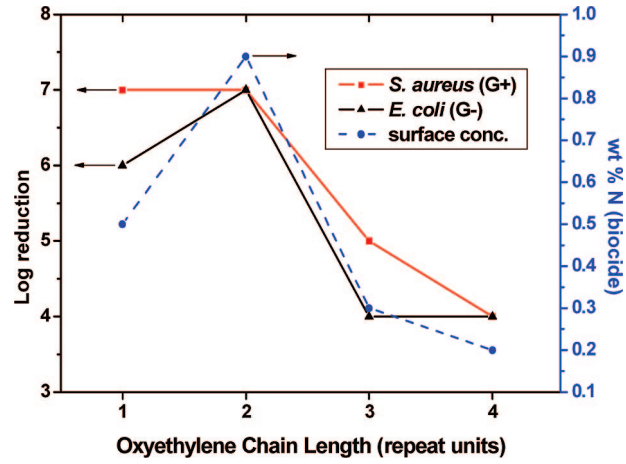

FIGURE 2. Effect of increasing the oxyethylene chain length on log reduction of *S. aureus*/*E. coli* and the surface concentration of *n*-octyl bearing quaternary ammoniums.

effective against *E. coli* than those possessing chains of 2 or 3 units (**3f** or **3g**). In contrast, the *n*-hexyl derivatives bearing oxyethylene chains of 1 or 4 units showed increased activity against *S. aureus* (relative to those with chains of 2 or 3 units), while increasing the length of the oxyethylene chain corresponded to an increase in the antimicrobial activity against *E. coli*. It was further noted that increasing the length of the oxyethylene chain assisted in solubilization, correlating to the predicted increase in hydrophilicity; however, it is likely that the amphiphilic nature of these molecules results in aggregation into micelles in aqueous solution. This could be hindering the antimicrobial activity through obstruction of the biocidal functional groups, contributing to the apparent lack of congruity with respect to the observed trends in the effects of altering the oxyethylene chain length.

The effectiveness of **3a−h** as antimicrobial additives in coatings was conducted using Hydrothane polyurethane resin, selected for its hydrophilicity and lack of pigments, fillers, and any other additives that would hinder analysis. Films were cast at 1% (w/w) biocide loading and tested against the same bacteria as were employed in the MIC studies. Satisfactorily, all samples were found to retain their antimicrobial activity in the polyurethane, with log reduction as high as 7 (Table 1). Once again, the *n*-octyl derivatives were consistently more active than their *n*-hexyl counterparts, although contrary to the solution results, both series were found to be more effective against *S. aureus* than *E. coli* bacteria. Furthermore, while altering the length of the oxyethylene chain did not consistently affect the antimicrobial activity in solution, a clear trend emerges from the surface testing. In both the *n*-octyl and *n*-hexyl series, compounds bearing oxyethylene chains of 1 repeat unit are nearly (or equally) as effective as those with chains of 2 units, whereas a sharp decline in the activity is displayed by compounds with chains of 3 or 4 units.

In order to shed light on this trend, X-ray photoelectron spectroscopy (XPS) analysis was conducted on each film (Table 1). A direct correlation between the antimicrobial activity and the biocide surface concentration was immediately evident. Figure 2 shows the effect of increasing

the oxyethylene chain length of the *n*-octyl series on log reduction of bacteria and the observed surface concentration. The two most effective additives (**3e** and **3f**) were found to have the highest surface concentrations, with a 10- and 18-fold increase of the expected values, respectively. Furthermore, the trend in the effect of the oxyethylene chain length on the antimicrobial activity ($n = 2 \geq 1 \gg 3 \geq 4$) is mirrored in the surface concentration data. From these observations, we have concluded that the long hydrophobic alkyl chains drive the surface concentration of the amphiphilic quaternary ammonium compounds via segregation from the hydrophilic polyurethane matrix. An increase in the length of the oxyethylene chain serves to decrease the hydrophobicity of the compound, which, in turn, lowers the energy gained by segregation and inhibits migration to the surface. That these effects are more pronounced in the *n*-octyl series provides additional evidence that the surface concentration is a consequence of the balance between the hydrophobicity of the *n*-alkyl group, the hydrophilicity of the oxyethylene chain, and, presumably, the hydrophilicity of the polymer matrix. Thus, the molecule can be tailored to achieve a desired surface concentration in a given polymeric system by simply altering the lengths of the *n*-alkyl and oxyethylene groups, as shown in Figure 1.

The leaching of a biocidal agent from a surface is a concern for any additive, particularly low molecular weight antimicrobials. Fortunately, films containing **3f** (chosen because of the high surface concentration) did not exhibit evidence of leaching when subjected to zone of inhibition studies, tested both as prepared and after immersion in water for 7 days. Also, samples did not show a decrease in the antimicrobial activity, and the water was not found to inhibit bacterial growth or contain **3f** within the limits of high-performace liquid chromatography detection. This suggests that the additives are mobile and able to surface-concentrate in the uncured polyurethane but become "locked" in place once the resin has cured. Thus, diffusion of the antimicrobial agents out of the film is prevented despite the inherent water solubility.

In conclusion, a novel class of quaternary ammonium compounds that self-segregate to the polymer−air interface were designed and synthesized and were proven to be effective antimicrobials against both Gram-positive and Gram-negative bacteria. To our knowledge, this is the first report of nonpolymeric biocidal additives capable of self-concentrating at the surface of a polymeric coating. Altering the lengths of the alkyl groups and oxyethylene chains provided a unique method of controlling the surface concentration of these molecules within polyurethane films, as demonstrated by XPS analysis, affording an unprecedented ability to "dial-in" antibacterial properties to a polymer surface as desired for a specific application. Methods of refining control over the surface concentration and improving the antimicrobial activity through further alterations of the *n*-alkyl groups and other functionalities, as well as the extension of this work into other resin systems, are currently under investigation.

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Supporting Information Available: Synthetic procedures, biological evaluation procedures, and analytical details. This material is available free of charge via the Internet at http://pubs.acs.org.

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