Preparation and Evaluation of Antimicrobial Hyperbranched Emulsifiers for Waterborne Coatings

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ABSTRACT: Nosocomial infections are a major problem in medical health care. To solve this problem, a series of antimicrobial waterborne paints were prepared by using antimicrobial hyperbranched (HB) emulsifiers. The HB-emulsifiers were prepared by polymerizing AB₂ monomers obtained in a one-step reaction of bis(hexamethylene)triamine and carbonyl biscaprolactam. The blocked isocyanate end groups (B groups) of the HB-polymer were utilized to introduce tertiary amino groups through the reaction with compounds comprising either a hydroxyl or a primary amino group and a tertiary amino group. Quaternization of the tertiary amines with 6 different alkyl bromides resulted in 12 amphiphilic cationic species. The 12 emulsifiers showed the successful inhibition and killing of 8 bacterial and 2 fungal strains. The killing efficacy increased with increasing alkyl chain length. The octyl-functionalized compound was chosen for suspension polymerizations because of the good compromise between killing and emulsifying properties. With this emulsifier, aqueous poly(methacrylate) suspensions were prepared, which were stable and had excellent killing properties.

INTRODUCTION

Nosocomial infections are a global health care problem in hospitals and nursing homes because of the high susceptibility of elderly and immune-compromised people.¹−⁸ Intensive house cleaning is obviously extremely important and effective to remove all pathogens.⁵ Individuals who are susceptible can become seriously ill or can even die. Infections are not only very inconvenient for patients but also costly. According to the WHO, 4.5 million people in Europe alone are infected every year, creating a financial burden of billions of dollars.⁶−⁸ All kind of touchable surfaces in hospital, such as door handles, grips, furniture, walls, and floors, can become contaminated and can transmit microorganisms. Without proper environmental cleaning, bacteria can survive on inanimate surfaces for up to several months.⁹ This is underlined by reports that show that patients have an increased risk of being infected by pathogens such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), and Acinetobacter baumannii if a hospital room was previously occupied by a patient who was infected with these pathogens.¹⁰−¹²

Besides the standard labor-intensive decontamination procedures, there is growing interest in antimicrobial surfaces that kill bacteria on contact. Applying antimicrobial coatings to walls, furniture, and equipment may offer the solution of reducing or even preventing the transfer of bacteria. Although coatings are primarily intended for decoration and protective purposes, additional functionalities can be added. For instance, leachable biocides are commonly added to paints to reduce the growth of microorganisms, which can start during storage. However, after being applied, these biocides are deplete in due time and lose their activity while contaminating the environment. Another undesired feature of paints is the use of organic solvents because they cause health and milieu problems as well. Waterborne coatings are therefore the state of the art, but with water as the medium, the use of emulsifying agents is indispensable for preparing stable dispersions because coating resins are always hydrophobic in nature.

Emulsifiers are composed of a hydrophilic and a hydrophobic part, of which the hydrophilic part can be anionic, nonionic, or cationic. Quaternary ammonium compounds (QUATs) are well-known cationic species having both surfactant and biocidal properties. Low-molecular-weight cationic surfactants, such as cetyltrimethylammonium chloride, are well-known potent biocides but have poor emulsifying properties. As a result, only low monomer concentrations can
be used, along with high biocide concentrations.\textsuperscript{13–15} The incorporation of quaternary ammonium comonomers in coating resins, such as in polyacrylates and polyurethanes, has been described often, giving biocidal and emulsifying properties.\textsuperscript{16–20} However, in these cases the coating properties will be influenced as well because the cationic moieties are randomly distributed throughout the whole coating resin. In contrast, only a very few attempts have been reported for preparing antibacterial polymeric emulsifiers that will be located only at the periphery of the paint droplets, without influencing the coating properties. Such amphilphilic AB-block cationic copolymers, prepared via living polymerization techniques,\textsuperscript{21–24} are potential biocides and emulsifiers for waterborne paint, but AB-block-copolymers are composed of only one hydrophobic and one hydrophilic part, which limits the number of structural options. In contrast, hyperbranched block copolymers offer a much larger pallet of desired structural compositions.

There are several technologies for preparing hyperbranched polymers, but not all of them are suitable for fulfilling the desired requirements.\textsuperscript{25} Dendrimers have a perfect hyperbranched structure and are very suitable for drug delivery, but not as emulsifiers because of their fixed shape.\textsuperscript{26,27} Hyperbranched polymers can also be prepared from $A_2$ and $B_1$ monomers, which are absolutely available and cheap, but are not suitable because of their ill-defined structure.\textsuperscript{28} In contrast, $A_2B_1$ monomers are very well suited for making hyperbranched polymer emulsifiers with well-defined structures, but so far the preparation of $A_2B_1$ monomers has been too laborious to be applicable.\textsuperscript{29,30}

Here we report the preparation and evaluation of amphiphilic hyperbranched antimicrobial emulsifiers based on $A_2B_1$ monomers prepared by a one-step synthesis route, starting from commercially available compounds. Through postpolymer modifications of the hydrophobic hyperbranched polymers by cationic species, emulsifiers were obtained with a hydrophobic core and a hydrophilic cationic shell. As the degree of polymerization increased, the hydrophobic part and the number of reactive end groups increased as well. The B groups, at the end of each polymer chain, were provided with quaternary ammonium moieties to create hydrophilicity and antimicrobial properties. The antimicrobial properties of 12 emulsifiers were evaluated with 10 microorganisms. The emulsifiers were able to kill bacteria and fungi and allow the preparation of stable aqueous antimicrobial suspensions of poly(methyl methacrylate).

\section*{MATERIALS AND METHODS}

Carbonyl biscaprolactam (CBC, >99\%) was obtained from Actu-all (Oss, The Netherlands). Bis(hexamethylene)triamine (BHTA, high purity), $N,N$-dimethylethenediamine (DMEN, $\geq$98\%), $N,N$-di-methylethenediamine, 1-bromoethane, 1-bromobutane, 1-bromo-hexane, 1-bromooctane, and 1-bromododecane (99\%) were purchased from Sigma-Aldrich. Poly(vinyl alcohol) (PVA, 88\% hydrolyzed, average $M_w = 22,000$ Da) was purchased from Acros Organics. Benzoyl peroxide (Luperox A75, 75\%, remainder water) was obtained from Merck. Surfactants Tego\textsuperscript{\textregistered} wet 245 and 250 were purchased from Evonik Tego Chemie. DMF (anhydrous) and toluene (anhydrous) were obtained from Sigma-Aldrich. All of the chemicals were used as received and without purification.

\textit{Synthesis of $A_2B_1$ Monomers (1).} To a three-necked flask equipped with a reflux condenser, a nitrogen inlet, and a connector to a vacuum pump, $A_2B_1$ monomers (1, 18 g) were added. After three evacuation cycles while flushing with nitrogen to remove the oxygen, the mixture was dissolved in 60 mL of DMF and stirred for 1 h under a nitrogen atmosphere at 145 °C. After most of the DMF was removed under reduced pressure (80 °C and ca. 80 mbar), the mixture was dissolved in 40 mL of CHCl\textsubscript{3} and washed with saturated aqueous sodium chloride ($5 \times 100$ mL) to remove impurities and residual DMF. The organic layer was collected and dried with anhydrous sodium sulfate, the salt was filtered off, and all of the solvent was removed under reduced pressure. A transparent yellow resin was obtained.

$^{1}$H NMR (400 MHz, CDCl\textsubscript{3}): $\delta = 1.27$–1.60 (m, CH\textsubscript{2}), 1.63–1.82 ((12H, m, CH\textsubscript{2} ring), 2.57 (4H, m, CH\textsubscript{2}NHCH\textsubscript{3}), 2.68 (4H, t, CH\textsubscript{2}CON), 3.26 (4H, m, CH\textsubscript{2}NH CO), 3.96 (4H, t, CH\textsubscript{2}NCO), 9.23 (2H, t, NHCO). Yield 92\%.

\textit{Modification of HPB with $N,N$-Dimethylethenediamine (DMEN, 3).} To a three-necked flask equipped with a reflux condenser, a nitrogen inlet, and a connector to a vacuum pump was added the HPB (2) (18 g, with on average 6 caprolactam groups/molecule). After three cycles of evacuation, while flushing with nitrogen to remove the oxygen, $N,N$-dimethylethenediamine (DMEN, 15.56 mL, 0.142 mol) dissolved in DMF (180 mL) was injected into the vessel and stirred at 125 °C for 48 h under a nitrogen atmosphere. Then the mixture was concentrated under reduced pressure to remove most of the DMF (80 °C and ca. 80 mbar), dissolved in 40 mL of CHCl\textsubscript{3} and washed with saturated aqueous sodium chloride ($5 \times 100$ mL) to remove the excess of DMEN, $\epsilon$-caprolactam, and residual DMF. The organic layer was dried with anhydrous sodium sulfate. After the salt was filtered off and all of the solvent was removed under reduced pressure, a slightly colored resin was obtained and was denoted as the HPB-NH\textsubscript{2} system.

$^{1}$H NMR (400 MHz, DMSO): $\delta = 1.14$–1.50 (m, CH\textsubscript{2}), 2.11 (s, CH\textsubscript{2}), 2.21 (t, CH\textsubscript{2}N(CH\textsubscript{2}))$), 2.95, 3.05, and 3.18 (CH\textsubscript{2}NCO, CH\textsubscript{2}NHCO), 5.60 to 6.02 (NHCONH, NHCONH).

\textit{Modification of HPB with 2-(Dimethylamino)-1-ethanol (DMAE, 4).} To a three-necked flask equipped with a reflux condenser, a nitrogen inlet, and a connector to a vacuum pump was added HPB resin (2.25 g, with 6 caprolactam groups/molecule). After three cycles of evacuation and flushing with nitrogen to remove the oxygen, 2- (dimethylamino)-1-ethanol (DMAE, 27 mmol, 2.40 g) and tin(II)-2- ethylhexanoxide (catalyst, 1–5 wt \%) were injected into the vessel with DMF (30 mL) and stirred at 125 °C for 48 h under a nitrogen atmosphere. Then the mixture was concentrated under reduced pressure to half its original volume, dissolved in 30 mL of chloroform (CHCl\textsubscript{3}) and washed with saturated aqueous sodium chloride ($5 \times 100$ mL) to remove excess DMAE, impurities, and residue DMF. The organic layer was collected, followed by drying with sodium sulfate, filtering off the salt, and removing all of the solvent under reduced pressure. Finally, wine red resin was obtained and was denoted as the HPB-OH system.
with a rehydration stock on blood agar plates under aerobic conditions for 24 h.

Suspensions. Next, 500 μL of bacterial suspension (24 h at 37 °C under aerobic conditions) was added to a 250 mL glass reactor equipped with a mechanical stirrer and a PTFE bearing to prevent solvent evaporation. The temperature was set to 80 °C. To a solution of amine-functionalized HBPs (0.1 g) were added 1-bromooctane (14.0 mL, 67.2 mmol), and the resulting mixture was stirred overnight in a three-necked glass reactor with a mechanical stirrer and a PTFE bearing to prevent solvent evaporation. The temperature was set to 80 °C.

Preparation of QUAT-Functionalized HBPs (Emulsifier, 5). A control suspension polymer was prepared using poly(vinyl alcohol) (PVA, 88% hydrolyzed, average Mw = 22 000 Da), and 0.1 wt % (0.1 g) PVA was used to yield a suspension under the same experimental conditions as for the C8 emulsifier.

Bacterial Strains and Growth Conditions for Bacterial Suspensions. Bacterial strains were cultured from frozen dimethyl sulfoxide stocks on blood agar plates under aerobic conditions for 24 h at 37 °C. Subsequently, a preculture of 10 mL of liquid growth medium was used for inoculation for 24 h at 37 °C under aerobic conditions. Next, 500 μL of the preculture was used to inoculate 10 mL of the main culture for 18 h at 37 °C under aerobic conditions. Next, 500 μL of the preculture was used to inoculate 10 mL of the main culture for 18 h at 37 °C under aerobic conditions. Next, 500 μL of the preculture was used to inoculate 10 mL of the main culture for 18 h at 37 °C under aerobic conditions. Next, 500 μL of the preculture was used to inoculate 10 mL of the main culture for 18 h at 37 °C under aerobic conditions. Next, 500 μL of the preculture was used to inoculate 10 mL of the main culture for 18 h at 37 °C under aerobic conditions.

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RESULTS AND DISCUSSION

Figure 1. Preparation of AB2 monomers from triamines and carbonyl biscaprolactam (CBC, where R1 and R2 are alkyl chains).

Figure 2. Schematic representation of the polymerization of AB2 monomers followed by a functionalization step (F = functional group, X = O or NH).

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1H NMR (400 MHz, DMSO): δ = 1.12 to 1.55 (m, CH3), 1.64 (m, OCH2CH2CH3), 2.11 (s, CH3), 2.22 (t, CH2N(CH3)), 2.95, 3.07, and 3.18 (CH2NCO, CH2NHCOO), 3.93 (t, COOCH2), 5.66 to 6.25 (t, NHCON), 7.05 (t, NHCOO).

Preparation of QUAT-Functionalized HBPs (Emulsifier, 5). To a solution of amine-functionalized HBPs (3 or 4), (20 g, ~10 mmol, ±6 amine groups/HA molecule) in dry DMF (120 mL) was added 1-bromooctane (14.0 mL, 67.2 mmol), and the resulting mixture was stirred overnight in a three-necked glass flask provided with a reflux condenser at 70 °C. Next, the solution was cooled to room temperature and poured into diethyl ether. The organic suspension was extracted with water (100 mL) to dissolve the precipitate and washed with diethyl ether (8 × 150 mL) to remove the DMF, excess alkylating agent, and other impurities. After the water was removed by freeze-drying, slightly colored resin was obtained, which was denoted as HBP-NH2-C8.

1H NMR (400 MHz, DMSO): δ = 0.86 (t, (CH2)2CH3), 1.14–1.50 (m, CH2), 2.11 (s, CH3), 2.21 (t, CH2N(CH3)), 2.95, 3.05, and 3.18 (CH2NCO, CH2NHCOO), 5.60 to 6.02 (NHCON), 6.02 (NHCONH).

Suspension Polymerization (7). MMA (25 wt %, 25 g, 26.7 mL), benzoyl peroxide (1 wt %, 1 g), double distilled water (73.3 mL), and C8-emulsifier (4) (0.1 wt %, 0.1 g) were added to a 250 mL glass reactor equipped with a mechanical stirrer and a PTFE bearing to prevent solvent evaporation. The temperature was set to 80 °C and the reaction was stirred for 6 h to yield a homogeneous suspension.

A control suspension polymer was prepared using poly(vinyl alcohol) (PVA, 88% hydrolyzed, average Mw = 22 000 Da), and 0.1 wt % (0.1 g) PVA was used to yield a suspension under the same experimental conditions as for the Cu emulsifier.

Bacterial Strains and Growth Conditions for Bacterial Suspensions. Bacterial strains were cultured from frozen dimethyl sulfoxide stocks on blood agar plates under aerobic conditions for 24 h at 37 °C. Subsequently, a preculture of 10 mL of liquid growth medium was used for inoculation for 24 h at 37 °C under aerobic conditions. Next, 500 μL of the preculture was used to inoculate 10 mL of the main culture for 18 h at 37 °C under aerobic conditions. Next, 500 μL of the preculture was used to inoculate 10 mL of the main culture for 18 h at 37 °C under aerobic conditions.

Staphylococcus epidermidis ATCC 12228, Staphylococcus aureus ATCC 12600, Staphylococcus epidermidis ATCC 1457, Staphylococcus epidermidis ATCC 35984 (MRSE), Staphylococcus aureus ATCC BAA-1696 (MRSA), Acinetobacter baumannii 1, Klebsiella pneumoniae 1, Escherichia coli ATCC 25922, Candida albicans GB 1/2, and Candida parapsilosis were all cultured with Tryptone soya broth growth medium and BD Bacto agar ref. 314010 (Oxoid, ref. CMO129, Basingstoke, U.K.).

In case of bacterial aggregation, the main cultures were sonicated for 10 s at 30 W (Vibra Cell model 375, Sonics and Materials Inc., Danbury, CT, USA) to suspend bacterial clumps. Subsequently, the bacterial concentration was determined using the Bürker Türk counting chamber.

Estimation of the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal and Fungicidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC). A sterile 96-well plate (Falcon Flat Bottom 353072, Tyne & Wear, U.K.) was used to mix 200 μL of TSB growth medium containing the bacterial suspension to a final concentration of 107 bacteria/mL with emulsifier concentrations ranging from 0 to 2560 μg/mL. Gentamicin at 10 μg/mL was used as a positive control. The 96-well plate was incubated for 24 h at 37 °C under aerobic conditions. Bacterial growth was examined visually for each well by assessing changes in the turbidity of the suspension after 24 h. The MIC was defined as the well with the lowest emulsifier concentration for which no growth was observed.

Next, 10 μL of bacterial suspension from the wells that did not show any visual signs of growth was used to inoculate TSB agar plates of the corresponding growth medium. The agar plates were incubated for 24 h at 37 °C under aerobic conditions. The MBC/MFC was defined as the agar plate inoculated with medium (bacteria and emulsifier) with the lowest emulsifier concentration for which no growth was observed. All experiments were performed in triplicate with separate bacterial cultures.
group (A group), were prepared from triamines comprising two primary amino groups and one secondary amino group. It was found that all triamines with various R spacer groups (C2, C3, C4, and C6) yielded AB2 monomers in high yields. The polymerization of these AB2 monomers starts above 100 °C via the reaction of the secondary amine (A group) with the blocked isocyanates (B group), yielding the corresponding hyperbranched polyurea (HBP). If R1 and R2 are short alkyl chains (i.e., with fewer than six CH2 units), then cyclization takes place, besides polymerization, yielding chain stoppers, limiting the attainable molecular weights. From six CH2 units on, no cyclization takes place unless highly dilute conditions are used. When the AB2 monomers, obtained from bis-(hexamethylene) triamine and CBC, are heated, high-molecular-weight hyperbranched polyurea were obtained with blocked isocyanates at the end of each polymer branch, enabling subsequent functionalization reactions (Figure 2).

It is noteworthy to mention that, because of the quantitative yield of the synthesis of AB2 monomers, the reaction steps depicted in Figures 1 and 2 can be conducted in a one-pot procedure without intermediate purification steps, making this system even more simple and therefore economically feasible. In Figure 3, a 1H NMR spectrum of a hyperbranched polymer is given. Peak 7 (at 9.3 ppm) is characteristic of the NH of the blocked isocyanate group. The large chemical shift to 9.3 ppm is due to the strong hydrogen bond between the NH and CO groups of caprolactam. This strong interaction has a stabilization effect and, as a result, prevents the reaction between amino groups and these blocked isocyanate groups below 100 °C. The peak at 6.2 ppm represents the NH of the urea moiety, indicating the formation of polyurea. The number of monomer units (N) in the hyperbranched polymers (and thus the molecular weight) can be obtained from the 1H NMR spectra by N = 2P2/(16P4 − 2P2), where P2 and P4 (Figure 3) are areas of the corresponding peaks or from N = P2/(P2 − 16P4). According to these calculations, the number-average molecular weight (Mn) after a polymerization time of 1 h at 145 °C was about 1500 Da, which was the desired value for emulsifiers.

Low-molecular-weight amphiphilic quaternary ammonium surfactants (QUATs) are well-known potent biocides and have been used for decades in disinfectants and cosmetic products.37 It is also well established that the biocidal behavior of these QUATs depends not only on the positive charge but also on the presence and the length of the alkyl chain.38 The aim of this study was to functionalize a hydrophobic hyperbranched polymer (core) with hydrophilic quaternary ammonium moieties at the end of each polymer branch (shell) in order to make biocidal emulsifiers. Amphiphilic compounds (surfactants) can be used as detergents, wetting agents, emulsifiers, foaming agents, and dispersants, depending on their structure. Low-molecular-weight surfactants can be excellent detergents but are not well suited as emulsifiers. Moreover, they are leachable and will finally end up in the environment. Although QUATs are less toxic to human cells than to bacteria, they still can be harmful. Therefore, the leaching of potentially toxic substances must be avoided.39 It is reasonable to expect that the physical interaction of polymeric biocidal emulsifiers with coating resins is much stronger and therefore less leachable. Well-designed hyperbranched polymeric emulsifiers may even perform better in that respect because of the greater freedom to control their structural features. To prepare hyperbranched polymeric emulsifiers provided with QUATs (F in Figure 2), the caprolactam blocking group was substituted by compounds comprising one hydroxyl or one primary amino group and a tertiary amino group. Suitable compounds were N,N-dimethylamino ethanol and N,N-dimethylethlenediamine, which reacted on heating above 125 °C with the blocked isocyanates of the HBPs, thereby introducing a tertiary amino group. The reaction was
monitored with $^1$H NMR by the disappearance of the NH peak of the blocked isocyanate at 9.3 ppm and the appearance of peaks at about 5.75 ppm for the new urea moieties (Figure 4).

$N\,N$-Dimethylethylenediamine reacted considerably faster than $N\,N$-dimethylamino ethanol because the amino group is much more nucleophilic than the hydroxyl group. The reaction rate of the latter can be considerably increased by titanium hexanoate, which is FDA-approved.

It is well known that the antimicrobial properties of QUATs depend on the length of the alkyl group attached to the positively charged nitrogen atom, but for new compounds, the optimal chain length for the biocidal behavior has to be established experimentally. Therefore, various alkyl bromides (CH$_3$(CH$_2$)$_n$Br, in which $n = 1, 3, 5, 7, 9, and 11$) were used to prepare a series of QUATs (Figure 5). The success of the alkylation step was demonstrated by $^1$H NMR, in which the CH$_3$ endgroups of the alkyl chains were clearly visible at 0.9 ppm (Figure 4 inset).

To get a first indication of the surfactant properties, critical micelle concentrations (CMCs) in water were determined (Table 1). The CMC was measured by the change in the UV emission spectrum. At the CMC of an aqueous solution of Nile red with increasing concentration of the surfactant, the UV spectrum changed substantially. It can be seen that all compounds formed micelles. The CMC decreased as the length of the alkyl chain increased, which was to be expected because the hydrophobicity increased. Importantly, the CMCs of the compounds starting from C6 were of the same order of magnitude as the CMCs of commercial coating emulsifiers (Tego* wet KL 245 and 500), which makes it reasonable to expect that these compounds could have emulsifying properties in paints as well.

Besides the surfactant properties, the biocidal behavior was of course of eminent importance. The antimicrobial properties were established by determining the minimum inhibitory concentration (MIC), minimum biocidal concentration (MBC), and minimum fungicidal concentration (MFC) of

![Figure 4](image1.png) 1H NMR spectra of hyperbranched polyurea after reaction with $N\,N$-dimethylethylenediamine and after alkylation with octyl bromide (HBP-C8NH$_2$-N$^+$, inset).

![Figure 5](image2.png) Hyperbranched emulsifiers prepared from $N\,N$-dimethylethylenediamine (left) and $N\,N$-dimethylamino ethanol (right) in which R is CH$_3$(CH$_2$)$_n$ and $n = 1, 3, 5, 7, 9, and 11$.

| alkyl chain length | emulsifier HBP-NH$_2$-N$^+$R (mg/mL) | emulsifier HBP-OH-N$^+$R (mg/mL) |
|-------------------|-----------------------------------|----------------------------------|
| C2                | 4.7                               | 6.8                              |
| C4                | 3.7                               | 4.9                              |
| C6                | 1.5                               | 3.7                              |
| C8                | 1.2                               | 2.3                              |
| C10               | 0.4                               | 0.5                              |
| C12               | 0.1                               | 0.4                              |
| Tego* wet KL 245  | 0.4                               |                                   |
| Tego* wet KL 500  | 1.5                               |                                   |

Table 1. Critical Micelle Concentration of 12 Emulsifiers and 2 Commercial Products
eight bacterial strains and two fungi (Table 2). The biocidal activity was strongly dependent on the alkyl chain length and increased with increasing length of the alkyl moiety. The inhibition of compounds with short alkyl chains was more effective for Gram-negative strains (A. baumannii, K. pneumoniae, and E. coli) and yeast or fungal strains (C. albicans). A hexyl chain is the minimal length needed to obtain reasonable antimicrobial behavior. Inhibitory properties also existed for methicillin-resistant bacteria such as S. epidermidis ATCC 12228 and S. aureus ATCC BAA-1696 (MRSA). Bacterial or fungidical concentrations are generally 2–4 times higher than the inhibitory concentrations, although for some strains (A. baumannii and K. pneumoniae) the concentrations were equal to the inhibitory concentrations. The emulsifiers obtained from N,N-dimethylethlenediamine perform slightly better than those obtained from N,N-dimethylamino ethanol. The need for a tin catalyst with N,N-dimethylamino ethanol and the somewhat better performance of N,N-dimethylethlenediamine makes the latter somewhat more preferred. It is noteworthy that Gentamicin, a well-known antibiotic agent, performs much worse than these new emulsifiers with long alkyl chains (≥C6).

Although the longest alkyl chains exhibit better biocidal behavior, they might reduce the emulsifying properties due to the influence of the hydrophobic moiety, which is too dominant. The octyl chain had a good balance between the biocidal activity and emulsifying properties (Table 1), and this compound was therefore chosen to be an emulsifier for suspension polymerization. Polycrylates are well-known, often-used materials in waterborne paints.40 Methyl methacrylate (MMA) was therefore selected as a representative monomer for preparing an aqueous suspension (25 wt %) in the presence of 0.10 wt % of the octyl emulsifier (HBP-NH2-N+C8) and 0.1 wt % benzoyl peroxide and was polymerized at 80 °C for 6 h. The MIC values of the suspension were evaluated with S. epidermidis ATCC 12228 (Table 3).

The MIC calculation was based on the amount of C8 emulsifier in the suspension. Inhibition took place at 4 μg/mL of emulsifier, meaning that the PMMA concentration that was 250 times higher (25/0.1) was 250 × 4 μg/mL = 1 g/L, thus far below the 25 wt %. This result demonstrates that an aqueous PMMA suspension, prepared with the C8-emulsifier, displays

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Biocidal Concentration (MBC) of Eight Bacterial Strains and the Minimum Fungicidal Concentration (MFC) of Two Fungi in μg/mL.44

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|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| emulsifier      | S. epidermidis ATCC 12228 | S. aureus ATCC 12600 | S. epidermidis ATCC 1457 | S. epidermidis ATCC 35984 | S. aureus ATCC BAA-1696 |
| HBP-NH2-N+C2    | >2560           | >2560           | >2560           | >2560           | >2560           |
| HBP-NH2-N+C4    | >1280           | >2560           | >2560           | >2560           | >2560           |
| HBP-NH2-N+C6    | 40              | 320             | 160             | 320             | 40              |
| HBP-NH2-N+C8    | 5               | 40              | 10              | 40              | 5               |
| HBP-NH2-N+C10   | 5               | 20              | 10              | 20              | 5               |
| HBP-NH2-N+C12   | 5               | 20              | 10              | 20              | 5               |
| HBP-OH-N+C2     | >2560           | >2560           | >2560           | >2560           | >2560           |
| HBP-OH-N+C4     | 1280            | 2560            | 2560            | 2560            | 1280            |
| HBP-OH-N+C6     | 40              | 320             | 80              | 320             | 40              |
| HBP-OH-N+C8     | 5               | 20              | 5               | 40              | 5               |
| HBP-OH-N+C10    | 5               | 5               | 5               | 10              | 5               |
| HBP-OH-N+C12    | 5               | 5               | 5               | 5               | 5               |

10 μg/mL gentamicin yes (3) yes (3) no (2) yes (3) yes (3) yes (3) yes (3) yes (3)

1 = no inhibition, 2 = not bactericidal, 3 = inhibition, 4 = bactericidal, and 5 = not fungicidal.

Table 3. Minimum Inhibitory Concentration (MIC) of the PMMA Suspension with the HBP-NH2-N+C8 Emulsifier and with Poly(vinyl alcohol) (PVA) (S. epidermidis ATCC 12228 × 10^5 bac/mL)

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|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| emulsifier      | polymerization temperature (°C) | MMA (wt %) | emulsifiers (wt %) | MIC of C8 emulsifier (μg/mL) |
| HBP-NH2-N+C8 PVA | 80              | 25              | 0.10             | 4 ± 8          |
high biocidal activity with respect to S. epidermidis ATCC 12228. Importantly, the 25 wt % PMMA suspension was stable for at least 1 month. To exclude that the biocidal activity was caused by residual monomer (MMA) or other impurities, such as the decomposition products of benzoyl peroxide, suspension polymerizations were performed under identical conditions with poly(vinyl alcohol) (PVA) as the emulsifier. It can be seen that these suspensions did not show any biocidal activity (MIC > 640 μg/mL).

**CONCLUSIONS**

A series of amphiphilic hyperbranched antimicrobial emulsifiers have been prepared. The hyperbranched polyurea were obtained by the polymerization of AB₃ monomers prepared by a one-step reaction of bis(hexamethylene)triamine with carbonyl biscaprolactam. The corresponding hyperbranched polyurea comprised a secondary amino group in the focal point and a number of blocked isocyanates (BIs) at the end of each polymer branch. The number of BIs depends on the molecular weight, which in turn depends on the polymerization time, temperature, and monomer concentration. The nucleophilic polymer branch. The number of BIs depends on the molecular weight, which in turn depends on the polymerization time, temperature, and monomer concentration. The nucleophilic substitution of caprolactam of the BIs was successfully accomplished by compounds comprising one hydroxyl or a primary amino group and a tertiary amino group. In this way, the hyperbranched polymers were furnished with tertiary amino groups at the end of each polymer branch. To study the influence of the length of the alkyl moiety on the antimicrobial properties, the tertiary amine groups were alkylated with six alkyl bromides with increasing chain length (C₂ to C₁₂). The properties, the tertiary amine groups were alkylated with six alkyl bromides with increasing chain length (C₂ to C₁₂). The MIC, MBC, and MFC were measured for eight Gram-positive and were killed to a similar extent. Starting from the hexyl group, good biocidal properties were found. The C₈-modified polymer was chosen for the suspension polymerizations in order to have good balance between the antimicrobial and emulsification properties. The suspension polymerization of MMA (methyl methacrylate) in the presence of the C₈ emulsifier resulted in a stable suspension with good antimicrobial properties.

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**Notes**

The authors declare no competing financial interest.

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