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Polymeric *N*-Halamine Latex Emulsions for Use in Antimicrobial Paints

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ABSTRACT A new *N*-halamine monomer, *N*-chloro-2,2,6,6-tetramethyl-4-piperidinyl methacrylate (Cl-TMPM), was synthesized and used to prepare water-based polymeric *N*-halamines by emulsion polymerization. The chemical structures of the samples were characterized with Fourier transform IR, ^{13}C NMR, UV/vis, and differential scanning calorimetry analyses. Upon the addition of a small amount of the polymeric *N*-halamine latex emulsions into commercial water-based latex paints as antimicrobial additives, the new paints provided potent antimicrobial activities against *Staphylococcus aureus* (*S. aureus*; Gram-positive bacteria), methicillin-resistant *S. aureus* (MRSA; drug-resistant Gram-positive bacteria), vancomycin-resistant enterococcus (VRE; drug-resistant Gram-positive bacteria), *Escherichia coli* (*E. coli*; Gram-negative bacteria), *Candida tropicalis* (*C. tropicalis*; fungi), MS2 virus (15597-B; virus), and *Stachybotrys chartarum* spore (*S. chartarum*; mold), and they successfully prevented biofilm formation and development. The antimicrobial functions of the new paints were long-lasting for more than 1 year under normal in-use conditions, easily monitorable by a simple potassium iodine/starch test, and readily rechargeable if the functions were accidentally lost as a result of challenging conditions such as heavy soil, flooding, etc.

KEYWORDS: polymeric *N*-halamine • antimicrobial • antibacterial • antifungal • antiviral • antimold • rechargeable • paint

INTRODUCTION

Because of the fast growing need to control surface microbial contamination in residential, commercial, institutional, industrial, and hygienic applications, antimicrobial paints, paints that can inhabit/inactivate microorganisms upon contact, are experiencing tremendous growth (1, 2). One of the primary applications of antimicrobial paints is to control mold. When any building material or furnishing is damp for more than 48 h, mold may grow. Water damage due to roof or plumbing leakage, floods, and poor drainage of rainwater runoff or landscape irrigation can significantly promote mold growth. Cleaning can temporarily remove mold, but if the dampness/humidity is not properly controlled, mold regrowth will occur. Mold can physically destroy the building materials on which it grows, it is unsightly and may produce offensive odors, and mold can sensitize and produce allergic responses in allergic individuals. For individuals with impaired immune systems, indoor mold exposure can also cause serious fungal infections (3, 4).

Another increasingly important application of antimicrobial paints is to help to control the wide spreading of healthcare-associated infections (HAIs) and community-acquired infections (5–8). HAIs, which are increasingly associated with multi-drug-resistant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE), cause an estimated 88 000 deaths and $4.5 billion in excess healthcare cost in the United Sates (9, 10). Lately, multi-drug-resistant species have also spread out of healthcare facilities, and MRSA infections have been reported in public sites, posing a growing risk for the general public (11). Environmental sources contaminated with these microorganisms play a very important role in cross-contamination and cross-infection, and they are responsible for about 20% of the documented outbreaks of HAIs (12). Although painting "high-risk" surfaces with antimicrobial paints will not completely eliminate the transmission of such infections, it has the potential to significantly reduce it.

While a number of antimicrobial paints are commercially available, none of them can provide biocidal functions against bacteria (including the drug-resistant species), mold, fungi, and viruses simultaneously. The narrow inhibiting spectrum reduces the effectiveness and limits the applications of the current antimicrobial paints. To solve this problem, we developed polymeric *N*-halamine latex emulsions for use in antimicrobial paints (13). An *N*-halamine is a compound containing one or more nitrogen–halogen covalent bonds that are formed by the chlorination of imide, amide, or amine groups. *N*-Halamines have biocidal efficacy against bacteria, mold, fungi, and viruses similar to that of hypochlorite bleach, one of the most effective disinfectants, but they are much more stable and noncorrosive, with no documented cases of microbial resistance, and have a much lower tendency to generate halogenated hydrocarbons. Therefore, *N*-halamines have found wide applications as food and water disinfectants (14, 15). Most recently, these compounds have also been incorporated into various polymeric materials to achieve biocidal functions (16–20).

In the current study, we developed a new polymerizable *N*-halamine monomer, *N*-chloro-2,2,6,6-tetramethyl-4-piperidinyl methacrylate (Cl-TMPM). Cl-TMPM could be readily polymerized using a semicontinuous emulsion polymerization tech-

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nique, forming stable, water-based, latexlike emulsions. The polymeric *N*-halamine latex emulsions could be directly added into commercial water-based latex paints as antimicrobial additives, providing potent antimicrobial activities against bacteria (including the drug-resistant species), mold and other fungi species, and viruses. The antimicrobial functions were durable for longer than 1 year under normal in-use conditions and could be easily monitored by a potassium iodine/starch test; if challenging conditions (e.g., heavy soil, flooding, etc.) consumed more chlorines and reduced the antimicrobial functions, the lost functions could be readily regenerated by another chlorination treatment. These properties point to the great potential of the new polymeric *N*-halamine latex emulsions for use in the antimicrobial surfacing of a wide range of related residential, commercial, institutional, industrial, and hygienic applications to reduce the risk of microbial contamination.

EXPERIMENTAL SECTION

Materials. Ammonium persulfate [$(NH_4)_2S_2O_8$], 2,2,6,6-tetramethyl-4-piperidyl methacrylate (TMPM), dichloroisocyanurate sodium (DCCANa), and dioctyl sulfosuccinate sodium (DSS) were purchased from Sigma-Aldrich and used as received. The microorganisms, *Staphylococcus aureus* (*S. aureus*; ATCC 6538), *Escherichia coli* (*E. coli*; ATCC 15597), methicillin-resistant *S. aureus* (MRSA; ATCC BAA-811), vancomycin-resistant *E. faecium* (VRE; ATCC 700221), *Candida tropicalis* (*C. tropicalis*; ATCC 62690), *Stachybotrys chartarum* (*S. chartarum*; ATCC 34915), and MS2 virus (ATCC 15597-B1) were obtained from American Type Culture Collection (ATCC).

Instruments. Fourier transform IR (FT-IR) spectra were recorded on a Thermo Nicolet 6700 FT-IR spectrometer (Woburn, MA). ^{13}C NMR studies were carried out using a Varian Unity-200 spectrometer (Palo Alto, CA) at ambient temperature in $CDCl_3$. UV spectra of the samples in chloroform were obtained on a Beckman DU 520 UV/vis spectrophotometer. Thermal properties of the samples were characterized using DSC-Q200 (TA Instruments, New Castle, DE) at a heating rate of 10 °C/min under a N_2 atmosphere. Gel permeation chromatography (GPC) studies were performed in tetrahydrofuran on a GPC system equipped with a Waters 515 HPLC pump. The dual detection system consisted of a Waters 2414 RI detector and a multi-wave-length Waters 486 UV detector. The instrument was calibrated using polystyrene standards.

Synthesis of *N*-Chloro-2,2,6,6-tetramethyl-4-piperidinylmethacrylate (Cl-TMPM). Cl-TMPM was prepared by the chlorination of TMPM with DCCANa. In a typical run, a solution of DCCNa (12.1 g, 0.06 mol) in water (50 mL) was added to a solution of TMPM (11.25 g, 0.05 mol) in chloroform (50 mL). The mixture was vigorously stirred at room temperature for 1 h. After filtration, the chloroform layer was separated and dried with magnesium sulfate for 24 h. Magnesium sulfate was filtrated off, and chloroform was evaporated. The residual was recrystallized from water/ethanol at 0 °C. Cl-TMPM was obtained as a white powder (12.6 g, yield 96.3%; mp 15 °C by differential scanning calorimetry, DSC) and changed to a colorless oil upon storage at room temperature.

Preparation of the Polymeric *N*-Halamine Emulsion by Seed Emulsion Polymerization. The polymeric *N*-halamine latex emulsion was prepared by a semicontinuous emulsion polymerization technique as reported previously (21, 22). DSS and TX-100 were used as emulsifiers. A stable monomer preemulsion was prepared by stirring a mixture of 20% Cl-TMPM, 1% DDS, and 1% TX-100 in water for 30 min and then sonicating for 10 min. In the first stage of the polymerization, a dispersion of seed particles was prepared by batch emulsion polymerization. In a typical run, the monomer preemulsion (1.25 g), water (20 mL), DSS (0.025 g), and TX-100 (0.025 g) were added into a 250 mL three-necked flask equipped with a mechanical stirrer, a nitrogen inlet, a reflux condenser, and a liquid inlet system. The flask was immersed into a water bath at 70 °C. The whole system was thoroughly purged with N_2 during the reaction. An initiator solution [0.1 g of $(NH_4)_2S_2O_8$ in 5 mL of water] was added into the reactor. The mixture was stirred for about 30 min until a light-blue emulsion appeared.

In the second stage, the monomer preemulsion was continuously dropped into the dispersion of the seed particles at a rate of 0.1 mL/min for 3 h. After the addition was completed, the system was further maintained at 70 °C for 0.5 h under constant stirring. The resultant latex emulsion was cooled to room temperature for future use.

To determine the active chlorine contents of the samples, the emulsions were cast into paint films on poly(tetrafluoroethylene) and dried for 1 week at room temperature. Around 0.05 g of the dried paint film was dispersed in 20 mL of *N*,*N*-dimethylformamide and 20 mL of water containing 1.0 wt % acetic acid. A total of 1 g of potassium iodide was added, and the mixture was stirred for 1 h at room temperature under a N_2 atmosphere. The released iodine was titrated with a 0.01 mol/L sodium thiosulfate aqueous solution. Blank titrations were performed under the same conditions to serve as controls. The percentage of the chlorine content was calculated according to the following equation:

$$\% \ Cl = \frac{35.5}{2} \frac{(V_{Cl} - V_0) \times 10^{-3} \times 0.01}{W_{Cl}} \qquad (1)$$

where V_{Cl} and V_0 are the volumes (mL) of sodium thiosulfate solutions consumed in the titration of the polymeric *N*-halamine film and the control, respectively, and W_{Cl}(g) is the weight of the dry film. Each test was repeated three times, and the average was recorded (23).

Preparation of Polymeric *N*-Halamine-Containing Antimicrobial Paints. The polymeric *N*-halamine latex emulsions could be directly added into commercial water-based latex paints to provide antimicrobial functions without any phase separation/coagulation. In the current study, a white latex paint (Color Place latex semigloss house white paint, Wal-Mart Stores, Inc., Bentonville, AR) and a blue latex paint (Auditions satin paint, Valspar Corp., Min-

Table 1. Microorganisms Tested in This Study and the Media Used for Their Growth and Incubation

	bacteria		drug-resistant bacteria		yeast	virus	mold
	S. aureus 6538[a]	*E. coli* 15597[b]	MRSR BAA-811[a]	VRE 700221[a]	*C. tropicalis* 62690	MS2 15597-B1	*S. chartarum* 34915 spore
broth	tryptic[c] soy broth	LB broth[d]	tryptic soy broth	tryptic soy broth	YM broth[d]	EC Medium broth[c]	N/A
agar	tryptic[d] soy agar	LB agar[c]	tryptic soy agar	tryptic soy agar	YPD agar[d]	LB agar[c]	cornmeal agar[d]

[a] Gram-positive bacteria. [b] Gram-negative bacteria. [c] Purchased from Difco Laboratories (Detroit, MI). [d] Purchased from Fisher Scientific (Fair Lawn, NJ).

neapolis, MN) were used as representative commercial paints. The new paints containing different amounts of polymeric *N*-halamines were painted onto polystyrene sheets and dried for 7 days at room temperature to prepare paint films.

Antibacterial, Antifungal, and Antiviral Functions of the New Polymeric *N*-Halamine-Containing Paints. All microbial tests were performed in a Biosafety Level 2 hood. The guidelines provided by the U.S. Department of Health and Human Services were followed (24), and appropriate protective equipment including gowns and gloves and recommended decontamination protocols were used to ensure lab safety. In the antibacterial study, *S. aureus* (ATCC 6538) and *E. coli* (ATCC 15597) were used as typical examples of nonresistant Gram-positive and Gram-negative bacteria, respectively. MRSA (ATCC BAA-811) and VRE (ATCC 700221) were selected to represent drug-resistant strains because these species have caused serious HAIs and community-acquired infections (5, 6, 8). *Candida tropicalis* (*C. tropicalis* 62690) was employed to challenge the antifungal activities of the samples, and *E. coli* bacteriophage MS2 15597-B1 virus was used to represent viral species.

To prepare the bacteria or yeast suspensions, *S. aureus* 6538, *E. coli* 15597, MRSA BAA-811, and VRE 700221 were grown in the corresponding broth solutions (see Table 1) at 37 °C for 24 h, and *C. tropicalis* 62690 was grown in YM broth at 26 °C for 36 h. Cells were harvested by centrifuge, washed twice with sterile phosphate-buffered saline (PBS), and then resuspended in sterile PBS to 10^8-10^9 colony-forming unit (CFU)/mL. In the preparation of the viral suspensions, the freeze-dried bacteriophage MS2 virus was dispersed into DifcoTM EC Medium broth containing 10^8-10^9 CFU/mL of 24-h-old *E. coli* 15597 as the host. The viral suspension was diluted with EC Medium broth to 10^8-10^9 plaque-forming unit (PFU)/mL.

In the current study, to simulate possible scenarios in real applications, both waterborne and airborne microbial challenging conditions were evaluated.

Waterborne Tests. A modified AATCC (American Association of Textile Chemists and Colorists) Test Method 100-1999 was used to evaluate the antimicrobial efficacies of the polymeric *N*-halamine-containing paint films. In this test, 200 μL of a bacterial, yeast, or viral suspension was placed onto the surface of a polymeric *N*-halamine-containing paint film (ca. 2 × 2 cm); the film was then "sandwiched" using another identical film to ensure full contact. After different periods of contact time, the entire "sandwich" was transferred into 10 mL of a sterilized sodium thiosulfate ($Na_2S_2O_3$) aqueous solution (0.03 wt %). The mixtures were vigorously vortexed for 1 min and sonicated for 5 min to separate the films, quench the active chlorines, and detach adherent cells from the film surfaces into the solution. The resultant solutions were serially diluted, and 100 μL of each diluent was placed onto the corresponding agar plates (see Table 1). In the testing of the MS2 virus, the diluent was placed onto a Luria–Bertant (LB) agar plate overlaid with LB soft agar containing 24-h-old *E. coli* 15597 as the host, as suggested by ATCC. The same procedure was also applied to the original commercial paint films to serve as controls. Viable microbial colonies (for bacteria and yeast) or lysis (for the MS2 virus) on the corresponding agar plates were visually counted after incubation at 37 °C for 24 h (in the testing of the bacterial and viral species) or at 26 °C for 36 h (in the testing of *C. tropicalis* 62690). Each test was repeated three times, and the longest minimum contact time of the three tests for a total kill of the microbes (the weakest antimicrobial efficacy observed) was reported. This test was designed to simulate possible microbial challenges in real applications when microorganisms were suspended in water.

Airborne Tests. The antimicrobial activity of the polymeric *N*-halamine-containing paint films under airborne conditions was evaluated according to a method reported previously (25, 26). This method was designed to evaluate the antimicrobial activity of the paint against microorganisms that were in air or from coughing/sneezing of infected humans/animals. In the current study, *S. aureus* 6538, *E. coli* 15597, MRSA BAA-811, VRE 700221, and *C. tropicalis* 62690 were grown and harvested as described above. For each bacteria or yeast strain, 200 μL of a microbial suspension (10^8-10^9 CFU/mL) was sprayed onto a paint film (4 × 4 cm) in a biosafety hood using a commercial sprayer (25). After a certain period of contact time (10–60 min), the film was transferred into 10 mL of a sterilized sodium thiosulfate solution (0.03%). After vortexing and sonication, the solution was serially diluted, and 100 μL of each diluent was placed onto the corresponding agar plates (see Table 1). Viable microbial colonies on the agar plates were visually counted after incubation at 37 °C for 24 h (for the bacteria) or at 26 °C for 36 h (for the yeast), as described above. Each test was repeated three times, and the longest minimum contact time of the three tests for a total kill of the microbes (the weakest antimicrobial efficacy observed) was reported. The original commercial paint films were evaluated under the same conditions as those of the controls.

Antimold Function. In this study, the antimold efficacy of the new polymeric *N*-halamine-containing paints was tested with spores derived from *S. chartarum* (ATCC

34915). *S. chartarum* is a toxin-producing species that is commonly found in buildings with significant water damage, and it is responsible for mold growth (27, 28). *S. chartarum* was cultured on cornmeal agar plates at 37 °C until a profusion of conidia was present. Once this was achieved, the culture plate was washed using 10 mL of a sterile PBS and 0.1% Tween 80 solution to separate the conidia from the spore. The spore concentration was determined through serial dilution, plating, and enumeration, and the final concentration for the antimold test was adjusted to 10^8-10^9 CFU/mL with sterile PBS (29).

In each test, 200 μL of the mold solution was inoculated onto the surface of a polymeric *N*-halamine-containing paint film (ca. 4 × 4 cm). The film was placed in a sterile Petri dish containing 1 mL of sterile water. The dish was closed and placed into a static microbial test chamber (ca. 32 × 39 × 51 cm) constructed following ASTM D6329-98 (2008). The chamber was sealed, and the internal condition was maintained at 100% relative humidity (RH) and room temperature. Growth of *S. chartarum* on the films was inspected weekly within a 3-month test period, and mold growth at each inspection was recorded by measuring the covering ratios of visible mold on the film surfaces (29). Triplicate sample films were processed for each paint formulation (the original commercial paint and the new paints containing different amounts of polymeric *N*-halamines).

Biofilm-Controlling Function. The ability of the polymeric *N*-halamine-containing paint film to prevent biofilm formation was evaluated using scanning electron microscopy analysis. In this study, *S. aureus* 6538 was grown and harvested as described above. A polymeric *N*-halamine-containing paint film (ca. 1 × 1 cm) was immersed in 10 mL of sterile PBS containing 10^8-10^9 CFU/mL of the bacteria. The mixture was gently shaken at 37 °C for 30 min. The film was taken out of the bacteria solution and gently washed three times with 10 mL of sterile PBS to remove loosely attached bacteria. The film was immersed into tryptic soy broth and incubated at 37 °C for 3 days. After incubation, the film was rinsed gently with 0.1 M sodium cacodylate buffer (SCB) and fixed with 3% glutaraldehyde in SCB at 4 °C for 24 h. After being gently washed with SCB, the samples were dehydrated through an alcohol gradient method and dried in a critical point drier (23). Thereafter, the samples were mounted onto sample holders, sputter-coated with gold–palladium, and observed under a Hitachi S-3200N scanning electron microscope. The same procedure was also applied to the original commercial paint films to serve as controls.

Zone of Inhibition Study. In this test, the surface of a tryptic soy agar plate and LB agar plate were overlaid with 1 mL of 10^8-10^9 CFU/mL of *S. aureus* 6538 and *E. coli* 15597, respectively. The plates were then allowed to stand at 37 °C for 2 h. Each polymeric *N*-halamine-containing paint film (1 × 1 cm) was placed onto the surface of each of the bacteria-containing agar plates. The film was gently pressed with sterile forceps to ensure full contact between the film and the agar. The same procedure was also applied to the original commercial paint film to serve as controls. After incubation at 37 °C for 24 h, the inhibition zone around the films was measured. Afterward, the films were removed sterilely from the agar plates and washed gently with nonflowing sterile PBS (3 × 10 mL) to remove loosely attached bacteria. The resultant films were vortexed for 1 min and sonicated for 5 min in 10 mL of PBS to detach adherent bacteria. The solution was serially diluted, and 100 μL of each dilution was plated onto the corresponding agar plates (see Table 1). Recoverable microbial colonies were counted after incubation at 37 °C for 24 h.

Stability Study. To investigate the stability of the chorines in the *N*-halamines, a series of polymeric *N*-halamine-containing paint films (ca. 2 × 2 cm) were immersed in 10 mL of deionized water under constant shaking (50 rpm) at room temperature. After a certain period of time, 1 mL of solution was taken out of the immersing water and tested with a Beckman DU 520 UV/vis spectrophotometer in the range of 190–400 nm to determine whether TMPM- or Cl-TMPM-containing compounds were released from the paint film into the solution (characteristic absorption peaks of pure TMPM, 254 nm, and Cl-TMPM, 285 nm). Afterward, the water sample was iodometrically titrated to determine the level of active chlorines in the soaking solutions.

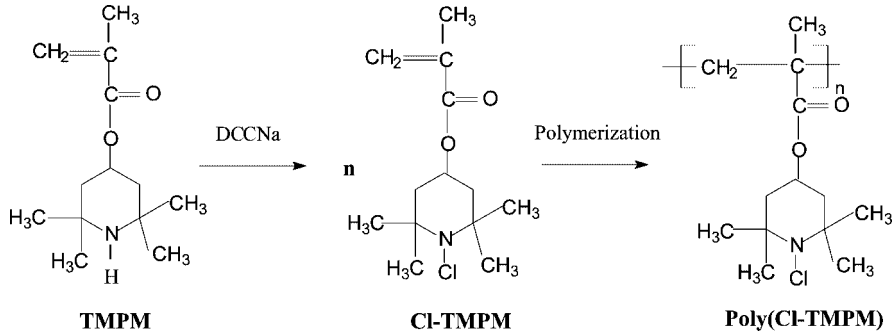
Durability and Rechargeability of the Antimicrobial Function. The polymeric *N*-halamine-containing paint films were tested for retention of antimicrobial functions under storage. Paint films with known chloride contents were stored under normal lab conditions (25 °C, 30–90% RH). The chloride contents and the antibacterial and antifungal functions were tested periodically over a 12-month storage period.

To test rechargeability, the polymeric *N*-halamine-containing paint films were first treated with a 0.1 M sodium thiosulfate aqueous solution at room temperature for 24 h to quench the bound chloride and then wiped using a cellulosic cleaning cloth with 1 wt % of a DCCNa aqueous solution for 30 s. The films were left to air-dry overnight, washed with distilled water to remove the remaining DCCNa, and air dried. After different cycles of this "quenching–recharging" treatment, the chloride contents and antibacterial and antifungal functions of the resultant films were reevaluated.

RESULTS AND DISCUSSION

Synthesis of Cl-TMPM and Poly(Cl-TMPM). To date, all of the reported polymeric *N*-halamines have been synthesized by a "posthalogenation" approach. In this approach, *N*-halamine precursor structures were first incorporated into the target polymers, and then the resultant polymers were halogenated to transform the *N*-halamine precursors into *N*-halamines (16, 23, 26). In the current study, however, we developed a new "prechlorination" approach in the preparation of polymeric *N*-halamines, as shown in Scheme 1. In this approach, an *N*-halamine monomer, Cl-TMPM, was synthesized through chlorination of TMPM. While TMPM is a solid at room temperature (mp 62 °C), Cl-TMPM has a melting point of 15 °C (by DSC), and it is a clear liquid at room temperature. The liquid nature of

Scheme 1. Preparation of Cl-TMPM Monomer and Poly(Cl-TMPM) Emulsion through Emulsion Polymerization



Cl-TMPM made it much easier to disperse Cl-TMPM evenly into water in the presence of conventional emulsifiers to form stable emulsions, which was difficult to do when TMPM was used. Polymerization of Cl-TMPM transformed the monomer into poly(Cl-TMPM) (M_w = 5572 Da and polydispersity = 1.94 by GPC), which was a stable water-based emulsion and could be directly added into commercial latex paints to provide antimicrobial functions. To our knowledge, this prechlorination approach was the first time that an *N*-halamine monomer (not an *N*-halamine precursor as reported in earlier studies 16, 22, 26) was used to form polymers, and no posthalogenation treatment was needed in order to provide the resulting polymers with antimicrobial functions. Because of the simplicity in the preparation of the monomer and polymer emulsions and the ease in use of the final products, it is highly possible that the prechlorination approach will be adopted widely in the preparation of other polymeric *N*-halamines to control microbial contamination in a broad range of related applications.

FT-IR analysis was used to follow the reactions. Figure 1 shows the IR spectra of TMPM, Cl-TMPM, and poly(Cl-TMPM). In the spectrum of TMPM, the 3312 and 3340 cm^{-1} peaks were attributable to N−H stretching vibrations. The peak at 1635 cm^{-1} could be related to the C−C double bonds, and the 1700 cm^{-1} band was caused by the ester carbonyl, in good agreement with the literature data (30, 31).

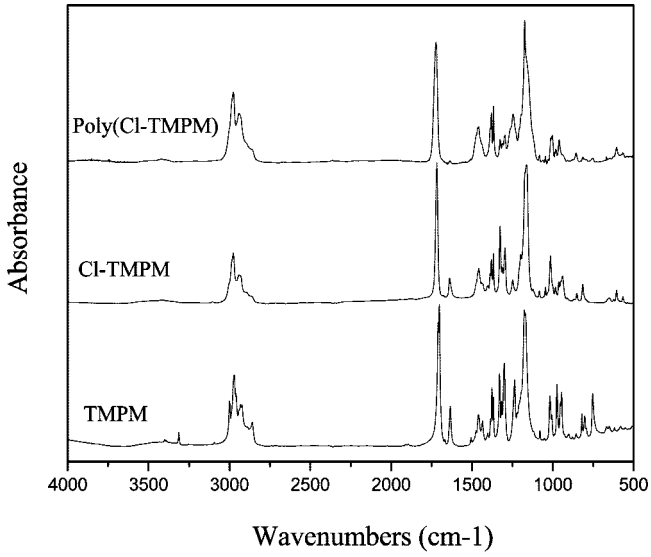


FIGURE 1. FT-IR spectra of TMPM, Cl-TMPM, and poly(Cl-TMPM).

Upon chlorination, the N−H structure was transferred into N−Cl. Thus, the N−H stretching vibrations disappeared in the spectrum of Cl-TMPM. Furthermore, the ester carbonyl band shifted from 1700 to 1716 cm^{-1}, which could be caused by the breakage of the "C═O---H−N" hydrogen bonds. After polymerization, Cl-TMPM was transformed into poly(Cl-TMPM). As a result, the double-bond band around 1635 cm^{-1} disappeared in the spectrum of poly(Cl-TMPM), and the ester carbonyl band further shifted from 1716 to 1721 cm^{-1}.

The FT-IR results were confirmed by ^{13}C NMR studies, as shown in Figure 2. In the spectrum of TMPM, the peaks at 136.8 ppm (C^2) and 125.0 ppm (C^3) were caused by the carbons of the double bonds, and the signal at 51.5 ppm was related to the two neighboring carbons (C^5) of the N−H group. After chlorination, the 51.5 ppm peak shifted to 62.9 ppm in the spectrum of Cl-TMPM. This change was attributed to the replacement of the N−H structure with a N−Cl group because the latter has a stronger electron-withdrawing effect than the N−H group (32). After polymerization, the two double-bond carbon peaks disappeared in the spectrum of poly(Cl-TMPM), confirming the formation of polymers.

The FT-IR and NMR results agreed well with UV studies. As shown in Figure 3, TMPM showed an adsorption peak around 254 nm. After chlorination, a strong adsorption peak around 282 nm could be observed in the spectrum of Cl-TMPM. UV absorptions of *N*-halamines have been well established (33−36), and this peak could be caused by the disruption/dissociating of the N−Cl bond and/or the transition from a bonding to an antibonding orbital, indicating that after chlorination the N−H groups in TMPM were transformed into N−Cl structures. In the spectrum of poly(Cl-TMPM), the N−Cl peak could still be observed, suggesting that the N−Cl structure survived in the emulsion polymerization process. This finding was further strengthened by iodimetric titration, which showed that while Cl-TMPM had 13.68% of active chlorine, after polymerization, the resulting poly(Cl-TMPM) had 13.07% of active chlorine, retaining 95.5% of the theoretical value.

To provide further information about the reactions, the samples were characterized by DSC studies, and the results are presented in Figure 4. TMPM showed a melting point at 62 °C. After chlorination, the N−H bond was transformed into the N−Cl bond, and because of the lack of hydrogen

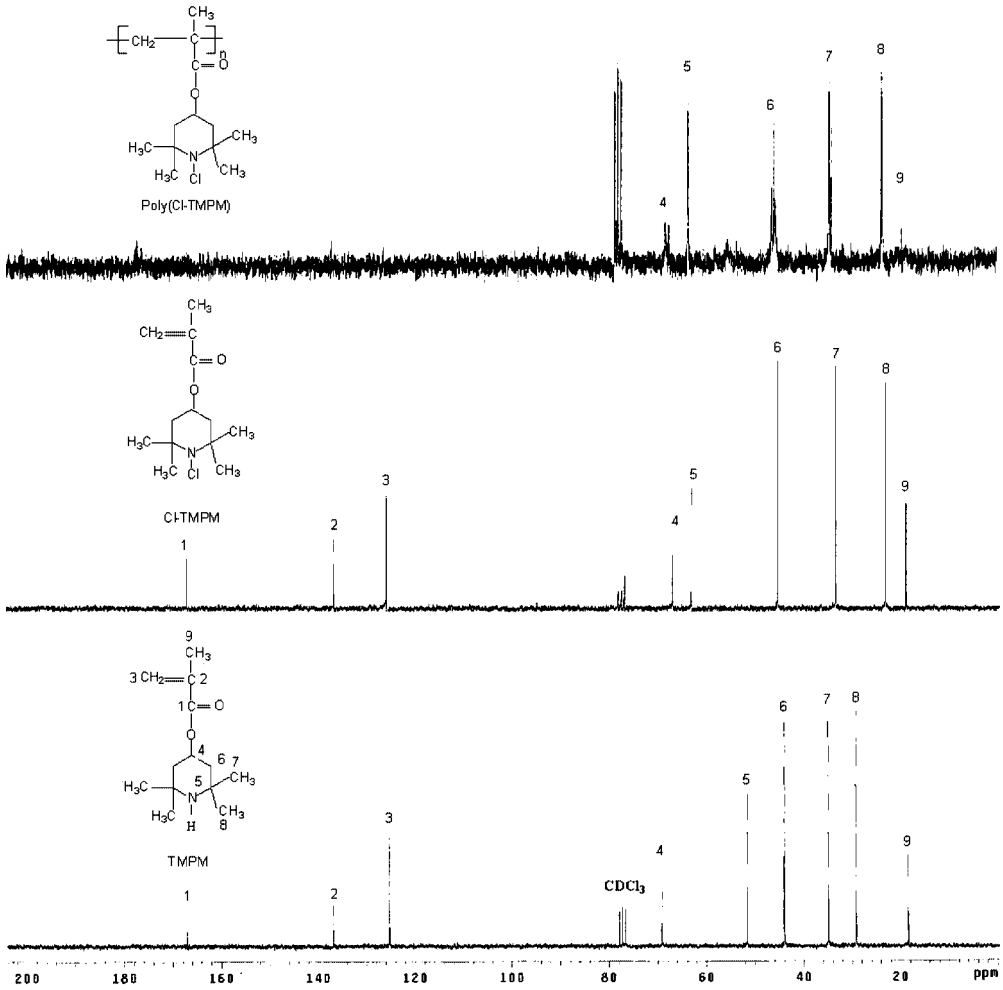


FIGURE 2. ^{13}C NMR spectra of TMPM, Cl-TMPM, and poly(Cl-TMPM).

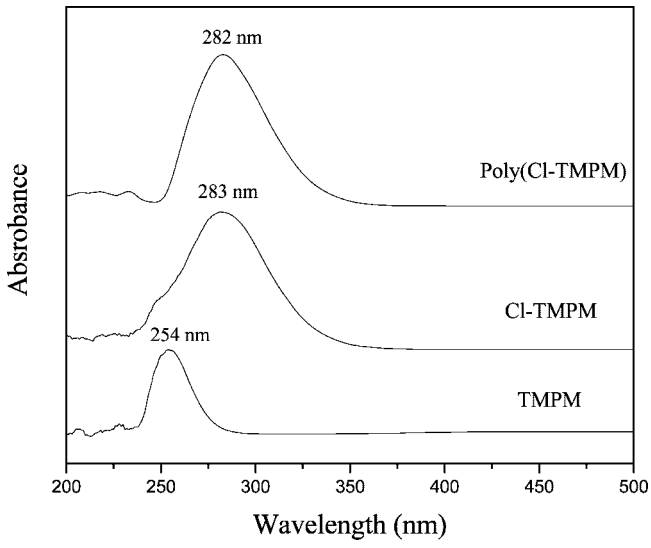


FIGURE 3. UV/vis spectra of TMPM, Cl-TMPM, and poly(Cl-TMPM) in chloroform.

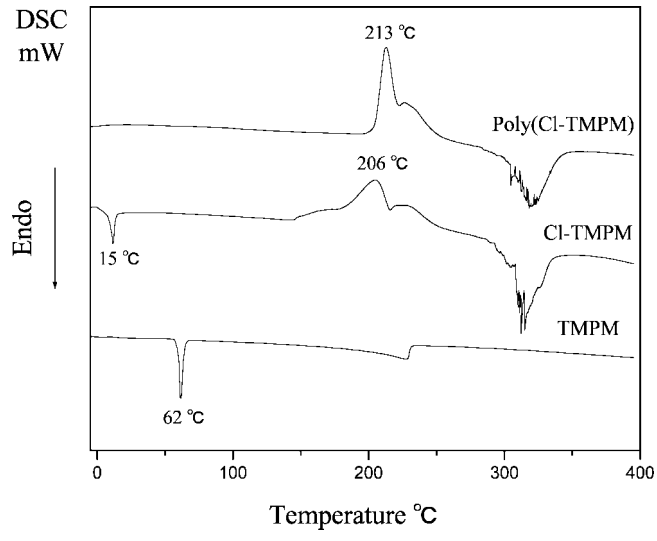


FIGURE 4. DSC curves of TMPM, Cl-TMPM, and poly(Cl-TMPM).

bonding, the melting point of Cl-TMPM decreased to 15 °C. The broad exothermal peak at 206 °C must be caused by the thermal decomposition of the N—Cl structure. After polymerization, the melting point at 15 °C disappeared, and the N—Cl decomposition temperature slightly increased to 213 °C in the DSC curve of poly(Cl-TMPM). All of these findings strongly suggested that Cl-TMPM and poly(Cl-TMPM) latex emulsions have been successfully synthesized following the procedure as illustrated in Scheme 1.

Antimicrobial Functions of the Poly(Cl-TMPM)-Containing Paints. The poly(Cl-TMPM) emulsion itself could be used as a paintlike coating to provide potent antimicrobial functions. Nevertheless, the major focus of this

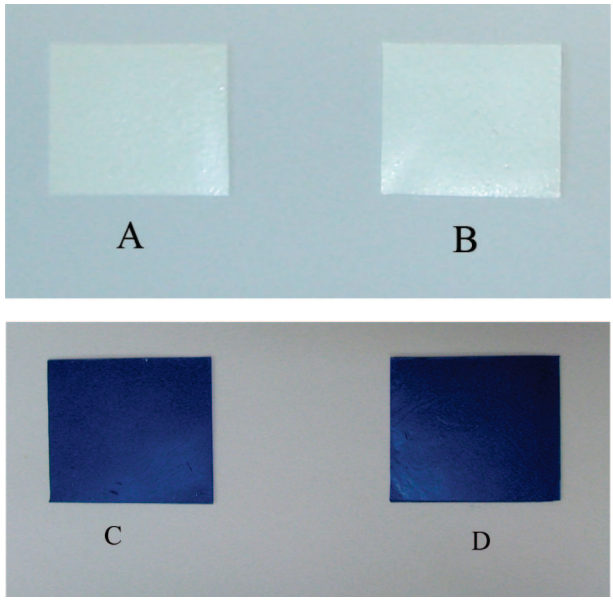


FIGURE 5. Paint films of (A) Color Place exterior latex semigloss house paint, white paint, (B) Color Place exterior latex semigloss house paint, white paint containing 20 wt % of poly(Cl-TMPM), (C) Auditions satin paint, blue paint, and (D) Auditions satin paint, blue paint containing 20 wt % of poly(Cl-TMPM).

study was to use poly(Cl-TMPM) emulsion as an additive in commercial water-based latex paints (which are gaining increasing importance in the paint industry because of their "greener" nature than solvent-based paints) to transform the conventional paints into antimicrobial paints. It was encouraging to find that poly(Cl-TMPM) emulsions could be freely mixed with most commercial water-based paints at any ratios without coagulation and/or phase separation. The covering capacity and appearance of the new paints were not negatively affected by the presence of poly(Cl-TMPM). As an example, Figure 5 shows the same polystyrene plastic films painted with a commercial white paint and blue paint and with the new paints containing 20 wt % (by solid content) of poly(Cl-TMPM), respectively.

The antibacterial, antifungal, and antiviral efficacies of the poly(Cl-TMPM)-containing paints were evaluated under both waterborne and airborne test conditions. The original commercial paints were used as controls, which did not show any antimicrobial effects. The poly(Cl-TMPM)-containing paints, however, demonstrated encouraging antimicrobial efficacy, as summarized in Table 2. In waterborne tests, poly(Cl-TMPM) contents showed a significant influence on the antimicrobial potency. For example, with 1 wt % of poly(Cl-TMPM), it took the paints 120 and 60 min to provide a total kill of 10^8–10^9 CFU/mL of *S. aureus* 6538 (Gram-positive bacteria) and *E. coli* 15597 (Gram-negative bacteria), respectively. When the poly(Cl-TMPM) content was increased to 5 wt %, the contact time for a total kill of the same species dramatically decreased to 10 and 5 min, respectively.

It was a striking finding that the poly(Cl-TMPM)-containing paints provided potent antibacterial activity against drug-resistant species including MRSA BAA-811 and VRE 700221 (see Table 2), which are major concerns in healthcare settings and a wide range of related community facilities, causing serious healthcare-related infections and community acquired infections (9, 10). These results pointed to the great potential of the new poly(Cl-TMPM)-containing paints for use in antimicrobial surfacing in related facilities to help reduce the risk of such infections.

The antifungal function of the new paints was evaluated with *C. tropicalis* 62690, and at 5 wt % of the poly(Cl-TMPM) content, the new paints provided a total kill of 10^8–10^9 CFU/mL of the yeast in 30 min in waterborne tests. Higher poly(Cl-TMPM) contents led to even faster antifungal action. The virus (*E. coli* bacteriophage MS2), which has been widely used as a surrogate of enteric viral pathogens, was relatively difficult to kill. With 5% of poly(Cl-TMPM), it took 240 min for the new paint films to offer a total kill of 10^8–10^9 PFU/mL of the virus in the waterborne test. When the content of poly(Cl-TMPM) was increased to 10 and 20 wt %, the contact time for a total kill of the virus decreased to 120 and 60 min, respectively. Similar results on the resistance of the MS2 virus to other polymeric *N*-halamines have also been reported (33).

The airborne antimicrobial efficacies of the poly(Cl-TMPM)-containing paint films were challenged with *S. aureus* 6538, *E. coli* 15597, MRSA BAA-811, VRE 700221, and *C. tropicalis* 62690. To simulate the deposition of airborne microorganisms, a common route of spreading infectious agents generated, for example, by talking, sneezing, coughing, or just breathing, a small commercial sprayer was used to spray the test organisms onto the poly(Cl-TMPM)-containing paint films (25, 26). Shown in Table 2 are typical results. It was found that at the same poly(Cl-TMPM) content the contact time for a total kill of the same species was slightly longer under the airborne conditions than in the waterborne conditions. This could be caused by the antimicrobial mechanism of *N*-halamines. It has been suggested that *N*-halamines provided antimicrobial effects by donating chlorines to microbial cells, leading to expiration of the microorganisms (14–17). Under airborne conditions, less water/moisture was involved when the microbial aerosols made contact with the paints; thus, a longer contact time was needed for a total kill. Nevertheless, even under the airborne conditions, the new paints could still provide a total kill of 10^8–10^9 CFU/mL of the bacteria (including the drug-resistant species) and yeast in 30–60 min at 5 wt % of the poly(Cl-TMPM) content. When the poly(Cl-TMPM) content was increased to 10 wt %, the contact time for a total kill of the bacteria or the yeast was further reduced to 10–30 min.

In addition to antibacterial (including the drug-resistant species), antifungal, and antiviral functions, the new poly(Cl-TMPM)-containing paints demonstrated potent antimold function. As shown in Table 3, after 1 month of growth, about 30% of the original paint surface was already covered by mold. When the growing time was extended to 3 months, 100% of the original paint surface was covered by mold. On the new paints containing 5 or 10 wt % of poly(Cl-TMPM), however, no mold growth could be detected during the 3-month testing period. Because the general public is increasingly concerned about mold growth and indoor mold exposure, the antimold effects of the poly(Cl-TMPM)-con-

Table 2. Effect of Poly(Cl-TMPM) Contents in the New Paints on the Minimum Contact Time for a Total Kill of the Bacterial, Yeast, and Viral Species

antimicrobial test method	content (%)	*S. aureus* (min)	*E. coli* (min)	MRSA (min)	VRE (min)	*C. tropicalis* (min)	MS2 (min)
waterborne	1	120	60	60	120	120	
waterborne	2	60	30	30	60	60	
waterborne	5	10	5	10	30	30	240
waterborne	10	5	5	5	10	30	120
waterborne	20	2	2	2	5	10	60
airborne	5	30	30	30	30	60	
airborne	10	30	10	10	10	30	

S. aureus, *E. coli*, MRSA, VRE, and *C. tropicalis* concentrations were 108−109 CFU/mL, and the MS2 virus density was 108−109 PFU/mL; the new paints contained 1−20 wt % poly(Cl-TMPM). Each test was repeated three times, and the longest minimum contact time of the three tests for a total kill of the microbes (the weakest antimicrobial efficacy observed) was reported.

Table 3. Surface Mold Covering Ratios on Paints Containing Different Amounts of Poly(Cl-TMPM) over Time

	surface mold covering ratio (%)		
time (month)	0 wt % poly(Cl-TMPM)	5 wt % poly(Cl-TMPM)	10 wt % poly(Cl-TMPM)
0.5	<10	0	0
1	30	0	0
2	60	0	0
3	100	0	0

taining paints would further strengthen the potential for the new paints to be used in real applications.

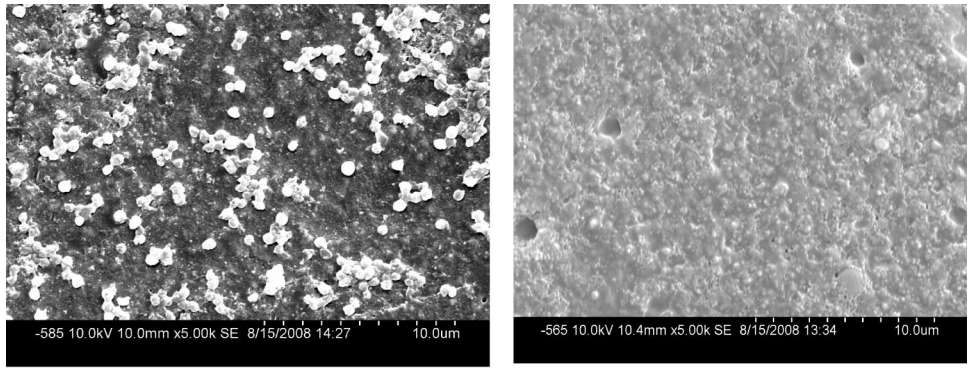
Biofilm-Controlling Function of the Poly(Cl-TMPM)-Containing Paints. The formation and development of biofilms could cause serious industrial, environmental, and institutional problems (37, 38). Because the poly(Cl-TMPM)-containing paints were able to effectively kill microbes, it was highly possible that they could prevent the formation and development of biofilms. To provide detailed information about the biofilm-controlling effect, the original paint film and the new paint film containing 10 wt % of poly(Cl-TMPM) were contacted with *S. aureus* 6538 for 30 min to allow initial adhesion, and the samples were then immersed in tryptic soy broth to facilitate the formation and development of bacterial biofilms (33). As shown in Figure 6, after 3 days of incubation, a large amount of bacteria adhered onto the surface of the original commercial paint film, forming microcolonies and developing into biofilms (Figure 6A). On the other hand, the poly(Cl-TMPM)-containing paint film showed a much clearer surface (Figure 6B): no adherent bacteria could be observed, and no biofilms were formed, suggesting potent biofilm-controlling activity.

Zone of Inhibition and Stability Studies. To provide a deeper understanding of the antimicrobial action of the poly(Cl-TMPM)-containing paints, zone of inhibition studies of the samples were performed. As shown in Table 4, the original commercial paint did not provide any inhibition zones against *S. aureus* 6538 or *E. coli* 15597. However, the new paints containing 5 wt % of poly(Cl-TMPM) generated a zone of 1.9 ± 0.1 mm against *S. aureus* 6538 and a zone of 2.2 ± 0.1 mm against *E. coli* 15597 ($n = 3$). Further increasing the poly(Cl-TMPM) content to 10 wt % did not significantly increase the zone sizes against the Gram-positive or Gram-negative bacteria.

After zone of inhibition tests, the paint film samples were washed and sonicated to recover surface adherent bacteria. As shown in Table 4, from the original commercial paint film, as high as 4.7×10^6 ($\pm 1.7 \times 10^5$) CFU/cm^2 of *S. aureus* 6538 or 1.9×10^6 ($\pm 1.6 \times 10^5$) CFU/cm^2 of *E. coli* 15597 could be recovered ($n = 3$). From the paint films containing 5 wt % of poly(Cl-TMPM), the recoverable level of *S. aureus* 6538 decreased to 10^3 CFU/cm^2 and the recoverable level of *E. coli* 15597 dropped to 10^2 CFU/cm^2. When the poly(Cl-TMPM) content was increased to 10 wt %, the levels of the recoverable bacteria further decreased to the range of 10^1 CFU/cm^2.

These results suggested that during the tests at least some of the antimicrobial agents diffused away from the poly(Cl-TMPM)-containing paint films to kill the bacteria. To determine what is responsible for this action, a series of the new paint films containing 10 wt % of poly(Cl-TMPM) (2 × 2 cm) were immersed in 10 mL of deionized water under constant shaking at room temperature, and an UV/vis spectrophotometer was used to test the immersing solutions. Within the test period of 72 h, the soaking solution was very clear, and no suspension/precipitation was observed. In the range of 190−400 nm, no UV absorption could be detected, suggesting that almost no detectable Cl-TMPM-containing compounds were released into the water system.

Thus, the inhibition zones could be created by positive chlorines generated by the dissociation of the amine N−Cl bonds (33). To confirm this, a quantitative evaluation of the positive chlorine contents in the immersing solutions was conducted by iodometric titration. Figure 7 presented the positive chlorine content in the solution as a function of the releasing time. It was found that in the initial stage (1−4 h) the positive chlorine content gradually increased; after that, the increasing trend became much slower, and when the equilibrium of the dissociation of the N−Cl bond was achieved, the chlorine content in the solution was kept constant at around 0.094 μg/mL (0.094 ppm). This value is much lower than the current EPA Maximum Residual Disinfectant Level (MRDL) in drinking water of 4 ppm. In other words, although the new paints contained 10 wt % of poly(Cl-TMPM) (1.307% of covalently bound chlorines), only 0.094 μg/mL of positive chlorine would be released from the



A. The original commercial paint, after 3 days of incubation in tryptic soy broth

B. Polymeric N-halamine-containing paint, after 3 days of incubation in tryptic soy broth

FIGURE 6. Biofilm-controlling function of the samples against *S. aureus* [the polymeric *N*-halamine-containing paint contained 10 wt % of poly(Cl-TMPM)].

Table 4. Inhibition Zones of Paint Films and Levels of Recoverable Bacteria

	inhibition zone (mm)		bacteria recovered (CFU/cm^2)	
poly(Cl-TMPM) content (wt %)	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
0	0	0	4.7×10^6 ($\pm 1.7 \times 10^5$)	1.9×10^6 ($\pm 1.6 \times 10^5$)
5	1.9×0.1	2.2 ± 0.1	1.5×10^3 ($\pm 2.8 \times 10^2$)	7.2×10^2 ($\pm 6.4 \times 10^1$)
10	2.3 ± 0.2	2.4 ± 0.1	5.0×10^1 ($\pm 3.7 \times 10^0$)	1.6×10^1 ($\pm 7.2 \times 10^0$)

paint films under equilibrium conditions if no microbial challenges were presented. Considering the extremely low dissociation constant of hindered amine *N*-halamines ($<10^{12}$) (39), these were reasonable findings, and these also explained the fact that the poly(Cl-TMPM) emulsions and the resulting paint films did not have any detectable chlorine smells under normal storage conditions.

On the other hand, in the presence of microbial challenges (as seen in the zone of inhibition study and antimicrobial tests), the dissociated chlorines could be quickly consumed by the surrounding microorganisms. This disturbed the *N*-halamine dissociation equilibrium, resulting in more chlorine to be continuously released to maintain the equilibrium. Thus, an inhibition zone and relatively rapid antimicrobial action could be observed (see Tables 2 and 4). After all of the microbial challenges were cleared, however, the *N*-halamine dissociation equilibrium could be easily achieved and maintained; thus, a very small amount of dissociated chlorines would be presented (0.094 ppm under our testing conditions), and this would lead to exceptional chlorine storage stability, as can be seen in the sections below.

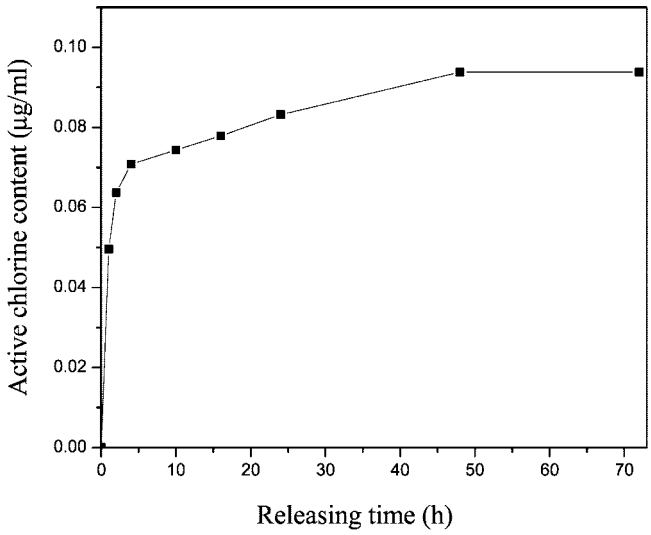


FIGURE 7. Positive chlorine content in the releasing solution (the polymeric *N*-halamine-containing paint had 10 wt % of poly(Cl-TMPM), and the total active chlorine content was 1.307%).

Durability, Monitorability, and Rechargeability of the Antimicrobial Effects. The nonleaching nature of the Cl-TMPM-containing components in the paints and the extremely low level of dissociation of the amine N—Cl bonds led to excellent durability of the new poly(Cl-TMPM)-containing paints. Under normal lab conditions (25 °C, 30—90% RH), the paint samples have been stored for more than 12 months without any significant changes of the active chlorine contents in the paints as well as the antimicrobial efficacies against the bacteria and yeast species, pointing to long antimicrobial durations in real applications.

On the other hand, challenging conditions (e.g., heavy soil, flooding, etc.) in real applications might consume more chlorine and thus shorten the antimicrobial duration. Nevertheless, the antimicrobial function of the new poly(Cl-TMPM)-containing paints could be easily monitored with a simple potassium iodine/starch test by contacting the paint surface with potassium iodine/starch test strips on an unobvious spot. As shown in Figure 8 as a demonstration, poly(Cl-TMPM) in the new paints would react with potassium iodine to produce iodine, and this would generate a dark-blue color with starch almost instantly. This simple test could be performed even by the end users in real applications, and if potassium iodine test showed that the antimicrobial func-

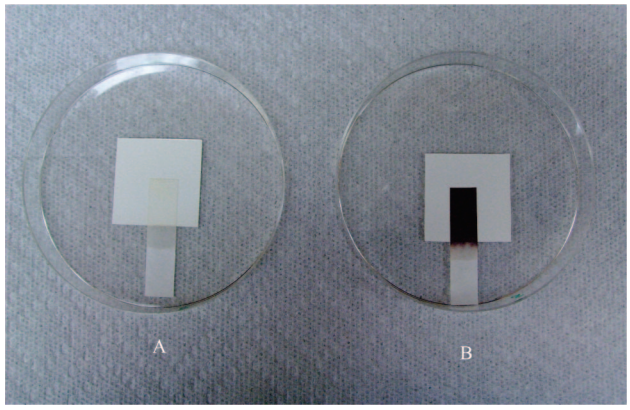


FIGURE 8. Potassium iodine/starch test after 30 s of contact with (A) a pure commercial paint film and (B) a paint film containing 5 wt % of poly(Cl-TMPM).

tion was lost, the lost chlorines could be recharged by another chlorination treatment.

To preliminarily evaluate rechargeability, a series of new paint films containing 5 wt % of poly(Cl-TMPM) were first treated with 0.3% sodium thiosulfate to quench the active chlorine and then rebleached with 1% of DCCNa at room temperature (see the Experimental Section for details). After 10 cycles of the quenching–rebleaching treatments, the chlorine contents and antimicrobial activities of the new paints were essentially unchanged, indicating that the antimicrobial function was fully rechargeable.

CONCLUSIONS

In this study, a new "prehalogenation" approach for the preparation of polymeric *N*-halamines was developed through emulsion polymerization of a new *N*-halamine monomer, Cl-TMPM. The major advantages of the new approach were that Cl-TMPM was a liquid at room temperature, which could be dispersed evenly into water in the presence of conventional emulsifiers to form stable emulsions, the resulting monomer emulsions could be readily polymerized to form poly(Cl-TMPM) latex emulsions, and the new poly(Cl-TMPM) emulsions could be directly used for antimicrobial applications without the "exposure to a halogen source" step that was critical in the conventional "posthalogenation" polymeric *N*-halamine preparation approach (16, 23, 26). The chemical structures of the samples were confirmed by FT-IR, ^{13}C-NMR, UV/vis, iodometric titration, and DSC analyses. The poly(Cl-TMPM) latex emulsions could be directly mixed with commercial water-based latex paints at any ratios without coagulation and/or phase separation. The covering capacity and appearance of the paints were not negatively affected by the presence of the poly(Cl-TMPM) latex emulsions. The new poly(Cl-TMPM)-containing paints provided potent antimicrobial effects against bacteria (including multi-drug-resistant species), fungi, and viruses, completely inhibited mold growth, and successfully prevented bacteria biofilm formation on the paint surfaces. The antimicrobial activity was long-lasting and could be easily monitored by a potassium iodine/starch test, and if the activity was accidentally lost, it could be regenerated by another halogenation treatment. As in the development of any new technologies, more studies are needed to determine the acceptance of the prechlorination approach and the antimicrobial efficacy and safety of the new polymeric *N*-halamine-based latex paints in real applications.

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