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Fluorinated and Un-fluorinated N-halamines as Antimicrobial and Biofilm-controlling Additives for Polymers

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Abstract

The objective of this study was to evaluate the effects of fluorination on the antimicrobial and biofilm-controlling activities of N-halamine-based additives for polymers. A fluorinated N-halamine, 1-chloro-3-(1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (Cl-FODMH), and its un-fluorinated counterpart, 1-chloro-3-octyl-5,5-dimethylhydantoin (Cl-ODMH), were synthesized and characterized with FT-IR, ¹H-NMR, and DSC studies. Polyurethane (PU) films containing Cl-ODMH and Cl-FODMH as antimicrobial additives were fabricated through solvent casting. With the same additive contents (1wt%-5 wt%), PU films with Cl-FODMH showed higher contact angle values. AFM, SEM and DSC results revealed that while Cl-ODMH distributed evenly within PU, Cl-FODMH aggregated and formed macro-domains in PU. Antimicrobial studies showed that PU films with Cl-ODMH had higher antimicrobial and biofilm-controlling potency against Gram-positive and Gram-negative bacteria than PU samples with Cl-FODMH. These results demonstrated the importance of distribution of additives in polymers on antimicrobial performances, shedding lights on future antimicrobial material design strategies.

1. Introduction

A biofilm can be defined as a microbial community enclosed in a self-produced polymeric matrix and bathed in fluid.^{1,2} Microorganisms readily colonize conventional polymeric materials and form biofilms in a wide range of industrial, environmental, institutional and medical/hygienic applications, which can cause serious problems including transferring infectious agents, reducing heat transfer in industrial cooling towers, corroding pipes, and blocking filters. Consequently, considerable efforts have been devoted to the development of antimicrobial and biofilm-controlling polymers. A number of polymers with anti-biofilm effects have been reported, and some of these studies have achieved encouraging results.³⁻¹⁵

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The research interests in this lab are to use N-halamine-based polymers for antimicrobial and biofilm-controlling applications. An N-halamine is a compound containing one or more nitrogen-halogen covalent bonds.¹¹ Upon contact, N-halamines can transfer positive halogens to appropriate receptors in microbial cells (either directly or indirectly),¹⁴ resulting in the expiration of the microorganisms.¹¹ N-halamines have similar antimicrobial potency as chlorine bleach, one of the most widely used disinfectants, but they are much more stable, less corrosive, and have a much less tendency to generate halogenated hydrocarbons, making them attractive candidates for the antimicrobial treatments of various polymeric materials.^{5,7,10,15,16}

Our previous studies used N-halamine compounds as antimicrobial additives for polymers to achieve antimicrobial and biofilm-controlling activities.^{3,10,17} To provide further information about the structure-property relationships of this class of additives, in this study, we evaluated the influences of fluorination of N-halamines on their antimicrobial performances in polymers. It has been recognized that fluorinated surfaces can have lower levels of biofilm formation and/or easy of biofilm removal.^{2,18-20} The effects of combining N-halamine structures with fluorinated moieties on biofilm-controlling functions are currently unknown. We therefore synthesized a fluorinated N-halamine, 1-chloro-3-1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (Cl-FODMH), and compared its performance with the un-fluorinated counterpart, 1-chloro-3-octyl-5,5-dimethylhydantoin (Cl-ODMH), in polyurethane (PU) as antimicrobial additives. We found that while Cl-ODMH distributed evenly within PU, Cl-FODMH aggregated in PU with rougher surfaces, which led to lower antimicrobial and biofilm-controlling functions. These results demonstrated that the distribution of antimicrobial additives within the polymer matrix plays a paramount role in the antimicrobial and biofilm-controlling effects of the resulting polymers.

2. Experimental section

2.1. Materials

The polyether-based thermoplastic PU was supplied by A-dec (Newberg, OR).

Trichloroisocyanuric acid (TCCA), 5,5-dimethylhydantoin (DMH), 1-bromooctane (BO), and 1H,1H,2H,2H-perfluorooctyl iodide (IFO) were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were analytical grade and used as received. The bacteria, *Staphylococcus epidermidis* (*S. epidermidis* ATCC 35984, Gram-positive) and *Acinetobacter calcoaceticus* (*A. calcoeticus* ATCC 31926, Gram-negative) were obtained from American Type Culture Collection (ATCC, Manassas, VA).

2.2. Instruments

FT-IR spectra of the samples were recorded on a Nicolet iS10 Mid-IR spectrometer. ¹H-NMR studies were performed using a 500 MHz spectrometer (Bruker, Switzerland). Melting points of the samples were measured using a TA DSC-20. Scanning electron microscope (SEM) observation was performed on a JEOL JSM 7401 FE-SEM. Contact angle was measured on a VCA optima surface analysis system (AST, MA) using water as the testing liquid. Atomic force microscopy (AFM) studies were conducted on a PSIA XE-150 (PSIA, CA).

2.3. Synthesis of 1-chloro-3-octyl-5,5-dimethylhydantoin (Cl-ODMH)

Cl-ODMH was synthesized following a procedure we reported previously.¹⁰ In a typical run, 3.20 g DMH were dissolved in 30 mL methanol in the presence of 1.68 g potassium hydroxide. The mixture was kept at 50 °C for 30 min. After evaporation of the solvent, the potassium salt of DMH was dried in a vacuum oven at 60 °C for three days. The anhydrous salt was then dispersed in 100 mL N,N-dimethylformamide (DMF) at 95 °C for 10 min under constant stirring, after which 4.83 g BO were added into the mixtures. The reaction was continued for 4 h at 95 °C. At the end of the reaction, the formed KBr was filtered off. After the removal of DMF by distillation under reduced pressure, the residual substance was recrystallized from ethanol. 3-octyl-5,5-dimethylhydantoin (ODMH) was obtained as white powders. Yield: 3.90 g (65.0%).

In the synthesis of Cl-ODMH, 0.5g ODMH was dissolved in 30 mL chloroform, to which 30 mL of 10% (v/v) Clorox® regular chlorine bleach [the final concentration of sodium hypochlorite was 0.6% (w/v)] was added. The mixture was stirred vigorously at ambient temperature for 30 min. The chloroform layer was washed with saturated sodium chloride aqueous solution, and dried with anhydrous sodium sulfate. After evaporation of chloroform, Cl-ODMH was obtained as a clear, viscous liquid. Yield: 0.35 g (61.2%).

2.4. Synthesis of 1-chloro-3-1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (Cl-FODMH)

3-1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (FODMH) was synthesized using similar procedures as in the preparation of ODMH. Briefly, 3.20 g DMH were dissolved in 30 mL methanol in the presence of 1.68 g potassium hydroxide. The mixture was kept at 50 °C for 30 min. After evaporation of the solvent, the potassium salt of DMH was dried in a vacuum oven at 60 °C for three days. The anhydrous salt was then dispersed in 100 mL N,N-dimethylformamide (DMF) at 95 °C for 10 min under constant stirring, after which 11.85 g IFO were added into the mixtures. The reaction was continued for 4 h at 95 °C. At the end of the reaction, the formed KI was filtered off. After the removal of DMF by distillation under reduced pressure, the residual substance was recrystallized from ethanol. 3-1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (FODMH) was obtained as white powders with a yield of 60.7% (7.22 g).

In the preparation of Cl-FODMH, 0.5 g FODMH and 0.74g trichloroisocyanuric acid (TCCA) were dissolved in acetone.¹⁰ Here, TCCA was employed because our screening tests showed that using chlorine bleach as the chlorination agent could not chlorinate FODMH successfully due to the very hydrophobic nature of FODMH. The solution was vigorously stirred for 30 min at ambient temperature. At the end of the reaction, acetone was evaporated, hexane was added to the mixtures, and the insoluble solids were filtered off. After removing hexane from the filtrate by evaporation, Cl-FODMH was obtained as white powders with a yield of 59.2%.

2.5. Preparation of polyurethane (PU) films containing Cl-ODMH or Cl-FODMH

A known amount of Cl-ODMH or Cl-FODMH (1%-5% of the weight of PU) was added into 10 wt% PU solution in THF with constant stirring until a clear solution was formed. The solution was poured onto glass slides. After evaporation of most of the solvent in a fume

hood at ambient temperature for 3 days, the samples were further dried in a vacuum oven at ambient temperature for 2 days. PU films were obtained with a thickness of $100 \pm 10 \mu\text{m}$. Pure PU films were prepared similarly to serve as controls.

2.6. Antimicrobial activities of PU films containing CI-ODMH or CI-FODMH

Staphylococcus epidermidis (*S. epidermidis* ATCC 35984, Gram-positive) and *Acinetobacter calcoaceticus* (*A. calcoaceticus* ATCC 31926, Gram-negative) were selected as model microorganisms because of their biofilm-formation capabilities. The bacteria were cultured in nutrient broth solution overnight, harvested by centrifuge, washed twice with sterile PBS, and then resuspended in sterile PBS to densities of 10^8 - 10^9 CFU/mL.

In the static antimicrobial tests, 2 μL of *S. epidermidis* or *A. calcoaceticus* PBS suspensions were placed onto the surface of a PU circular film (0.6 cm of diameter) containing a certain amount of CI-ODMH or CI-FODMH. The film was “sandwiched” with another identical film. Caution was taken to ensure that no bacteria suspension leaked out of the film “sandwich”. Under our test conditions with 2 μL of the bacteria suspension, this could be readily achieved. After a certain period of contact time (e.g., 15 min), the whole “sandwich” was transferred into 1.0 mL sterile sodium thiosulfate aqueous solution (0.03 wt%).^{3,10,17} The resulting solution was vortexed for 1 min to separate the two films and sonicated for 5 min to remove the adherent bacteria into the solution. Our screening studies with pure PU films have shown that such a sonication/quenching treatment did not affect the growth of the bacteria. An aliquot of the solution was serially diluted, and 100 μL of each dilution were plated onto nutrient agar plates. Bacterial colonies on the plates were counted after incubation at 37 °C for 48 h. The same procedure was applied to pure PU films to serve as controls. Each test was repeated three times.

2.7. Anti-biofilm function of PU films containing CI-ODMH or CI-FODMH

An overnight culture of *S. epidermidis* or *A. calcoaceticus* was centrifuged, washed with PBS, and diluted with sterile PBS to densities of 10^8 - 10^9 CFU/mL. CI-ODMH- or CI-FODMH- containing PU films (0.6 cm of diameter) were immersed individually in 1.0 mL of the microbial suspension, which was shaken gently at 37 °C for 60 min to permit bacterial adhesion.^{2,10,15} The films were taken out of the suspension and washed gently with 10 mL non-flowing PBS to remove the loosely attached bacteria. The washed films were transferred into 10 mL nutrient broth solutions. The whole system was put in a water bath and gently shaken at 37 °C. After 72 h of incubation, the films were rinsed gently with 0.1 M sodium cacodylate buffer (SCB) to remove loosely attached bacteria. Some of the films were used to determine the level of adherent bacteria, as described above, and the rest of the films were fixed with 3 % glutaraldehyde in SCB at 4 °C for 24 h. After washing gently with SCB, the films were dehydrated with 30%, 50%, 75%, 90%, and 100% of ethanol (10 min at each concentration),² mounted onto sample holders, sputter coated with gold-palladium, and observed under SEM to check for the presence of adherent bacteria/biofilms. The same procedure was also applied to pure PU films to serve as controls.

3. Results and discussion

3.1. Synthesis and characterization of Cl-ODMH and Cl-FODMH

The chemical structures of Cl-ODMH and Cl-FODMH were shown in Fig. 1, which were prepared by alkylation of 5,5-dimethylhydantoin (DMH) to produce ODMH or FODMH, followed by chlorination to transfer the amide N-H groups into N-Cl groups. The structures of the samples were characterized with FT-IR studies. As shown in Fig. 2, in the spectrum of DMH, the broad band centered around 3280 cm^{-1} was caused by N-H stretching vibrations, and the 1770 , 1732 and 1713 cm^{-1} bands were related to the carbonyl groups of the imide and amide groups. After reacting with 1-bromooctane (BO), strong bands in the region of 2854 cm^{-1} - 2956 cm^{-1} were observed, which were caused by the newly introduced alkyl chains. In addition, the carbonyl bands of the imide and amide groups shifted to 1782 cm^{-1} and 1707 cm^{-1} , respectively. Upon chlorination treatment, the N-H bond in ODMH was transformed into N-Cl bond. Consequently, in the spectrum of Cl-ODMH, the N-H stretching vibration band around 3280 cm^{-1} disappeared, and two weak bands at 758 and 735 cm^{-1} could be detected, which were associated with the N-Cl groups.¹⁰ Moreover, the transformation of N-H bonds to N-Cl groups broke the N-H---O=C hydrogen bonding in ODMH. Thus, the C=O bands shifted from 1782 and 1707 cm^{-1} in ODMH to 1794 and 1728 cm^{-1} in Cl-ODMH, respectively. Similar phenomena were observed in the “DMH-FODMH-Cl-FODMH” transformation.

The chemical structures of the samples were also characterized with ^1H NMR analysis. As shown in Fig. 3, in the spectrum of DMH, the methyl protons showed signals at 1.60 and 1.50 ppm , the imide proton showed a peak at 7.89 ppm , and the amide proton displayed a peak at 5.64 ppm . After reacting with BO, the imide proton signal disappeared, the amide proton signal shifted to 5.35 ppm , and new signals corresponding to the alkyl protons appeared in the spectrum of ODMH. After chlorination, the amide proton peak at 5.35 ppm disappeared in the spectrum of Cl-ODMH, suggesting that the N-H group was transformed into N-halamines. Similar results were obtained in the preparation of Cl-FODMH (Fig. 3).

To provide further information about the samples, Fig. 4 presented the DSC curves of DMH, ODMH, FODMH, Cl-ODMH and Cl-FODMH. DMH had a melting point of $180.0\text{ }^\circ\text{C}$. After reacting with BO or IFO, because the imide hydrogen was replaced by long alkyl chains, the melting points of ODMH and FODMH decreased to $34.8\text{ }^\circ\text{C}$ and $106.7\text{ }^\circ\text{C}$, respectively. After chlorination, the amide N-H groups were transformed into N-Cl groups. Thus, the melting points of Cl-ODMH (a viscous liquid at ambient temperature) and Cl-FODMH dramatically decreased to $-73.2\text{ }^\circ\text{C}$ and $40.5\text{ }^\circ\text{C}$, respectively. All these findings strongly suggested that Cl-ODMH and Cl-FODMH were successfully synthesized.

3.2. Distribution of Cl-ODMH and Cl-FODMH in polyurethane (PU)

Cl-ODMH and Cl-FODMH were used as antimicrobial additives in PU, one of the most versatile polymers in industrial, environmental, institutional and medical applications. Shown in Table I were the contact angle data of the samples. PU had a contact angle of $106.18 \pm 1.65\text{ }^\circ$. Adding up to 5% of Cl-ODMH into PU did not noticeably affect the contact angles of the samples. In the case of Cl-FODMH, however, with the increase of Cl-FODMH

content, the contact angles rapidly increased, suggesting the presence of Cl-FODMH on the film surfaces.

The surface topologies of the samples were characterized with AFM, and the results were displayed in Fig. 5. Pure PU had a smooth surface with the roughness (R_z) of $1.5 \pm 0.3 \mu\text{m}$. With 1% - 5% of Cl-ODMH as additives, the surface roughness was relatively unchanged. In the case of Cl-FODMH, however, with the increase of Cl-FODMH content, the surface roughness of the samples significantly increased. Similar trends were observed in SEM studies (Fig. 6). While PU samples containing 1%-5% of Cl-ODMH had relatively smooth surfaces (Fig. 6 B-D), the presence of 3% or 5% Cl-FODMH in PU led to much rougher surfaces (Fig. 6 F-G) with domains of surface-aggregated additives.

To provide further information about the distribution of Cl-ODMH and Cl-FODMH in PU, Fig. 7 showed the DSC curves of the samples. With 1%-5% of Cl-ODMH in PU, no melting peaks around -73.2°C could be observed, suggesting that Cl-ODMH had good compatibility with PU and did not crystallize in PU. On the other hand, in the study of Cl-FODMH, with 3% or 5% of the additive, melting peaks at 42.4°C and 43.1°C were observed in the DSC curves, which corresponded to the melting transition of Cl-FODMH (Fig. 4). These findings confirmed that the flaky aggregations observed on PU surfaces (Fig. 5 and Fig. 6) were indeed crystalline domains of Cl-FODMH formed on PU, suggesting “surface blooming”²¹ of Cl-FODMH at higher than 3% of the additive content.

Since Cl-ODMH and Cl-FODMH have the same DMH-based N-halamine structures, the low compatibility of Cl-FODMH with PU must be caused by the fluorinated alkyl chain. That is, the fluorine -containing alkyl chain led to low solubility of Cl-FODMH in PU. At concentrations that are higher than its solubility, Cl-FODMH could migrate to the PU film surface and aggregate to form its own domains, leading to rougher surfaces, particularly at higher than 3% of Cl-FODMH contents.

3.3. Performances of Cl-ODMH and Cl-FODMH as antimicrobial additives in PU

The antimicrobial efficacies of PU films containing Cl-ODMH and Cl-FODMH were evaluated against Gram-negative bacteria *A. calcoeticus* and Gram-positive bacteria *S. epidermidis*. Pure PU films were used as controls. All the additive-containing PU films demonstrated antimicrobial effects against the Gram-positive and Gram-negative bacteria, but to different extents, as shown in Table II. In the testing of *A. calcoeticus*, $(1.81 \pm 0.17) \times 10^5$ CFU/cm² of the bacteria were isolated from the pure PU films after 15 min of contact. With the increase of Cl-ODMH content in the films, this level rapidly decreased. At higher than 3% Cl-ODMH content, no recoverable bacteria could be isolated, suggesting powerful antimicrobial effects of the samples. In the case of Cl-FODMH, however, the antimicrobial efficacy was much lower. Even with 5% of Cl-FODMH, $(2.58 \pm 1.14) \times 10^2$ CFU/cm² of *A. calcoeticus* were recovered from the film. Similar trend was observed in the evaluation of *S. epidermidis*, as shown in Table II.

The lower antimicrobial efficacy of Cl-FODMH than that of Cl-ODMH in PU films could be related to the low compatibility of Cl-FODMH with PU (see Fig. 5-7). Because of the structural differences between the fluorine-containing Cl-FODMH and PU, Cl-FODMH had

low solubility in the polymer. Thus, at higher than 3% additive content, Cl-FODMH aggregated and formed its own domains on PU surfaces (“surface blooming” of additives²¹). As a result, not all the added Cl-FODMH could make contact with the bacteria to provide antimicrobial effects, leading to lower antimicrobial potency. Another factor could be due to the high hydrophobicity of Cl-FODMH (see Table I), which limited its contact with the bacteria in the aqueous medium.

In biofilm-controlling studies, PU films containing different amounts of Cl-ODMH and Cl-FODMH were exposed to 10^8 - 10^9 CFU/mL of the bacteria for 60 min to allow initial adhesion, followed by incubation in the corresponding broth solutions for 72 h to facilitate microbial colonization and biofilm formation. Fig. 8 presented the representative SEM results of pure PU films (the control) and PU films containing 5% of Cl-ODMH and Cl-FODMH after biofilm-controlling studies. Here, because all the samples were treated with 30%, 50%, 75%, 90%, and 100% of ethanol (10 min at each concentration) to dehydrate the bacteria before SEM observations,² and ethanol could dissolve Cl-ODMH and Cl-FODMH, no aggregated Cl-FODMH domains were observed on the sample surfaces. After biofilm testing, a large amount of adherent *A. calcoeticus* (Fig. 8 A) or *S. aureus* (Fig. 8 D) could be detected on the surface of the pure PU film. The bacteria cells aggregated together and formed several layers, suggesting formation of biofilms. PU films containing 5% of Cl-ODMH or Cl-FODMH showed cleaner surfaces (Fig. 8 B-C and E-F) with much lower bacterial adherent levels.

The levels of adherent bacteria on the sample surfaces were quantified. As shown in Table III, in the testing of *A. calcoeticus*, $(1.65 \pm 0.78) \times 10^5$ CFU/cm² of adherent bacteria were recovered from pure PU. With the increase of Cl-ODMH content in the samples, the recoverable level gradually decreased. With 5% of Cl-ODMH, the recoverable level was more than 100 times lower than that from the pure PU. In the presence of Cl-FODMH, the recoverable level of bacteria was lower than that from the pure PU, but higher than that from PU samples containing Cl-ODMH. Similar trend was observed in the testing with *S. epidermidis*, as shown in Table III. The biofilm-controlling function must be due to the antimicrobial effects of Cl-ODMH or Cl-FODMH: when bacteria came into contact with the films, some of them were inactivated during and/or after adherence/colonization, resulting in lower recoverable level. The films with Cl-FODMH had lower biofilm-controlling effects than those with Cl-ODMH. This could be related to two factors: (i) PU films with Cl-FODMH had lower antimicrobial effects (Table II), and/or (ii) PU films with Cl-FODMH had rougher surfaces, which could promote bacterial adhesion/biofilm formation.^{1,2}

4. Conclusion

We synthesized 1-chloro-3-1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (Cl-FODMH), a fluorinated N-halamine, and 1-chloro-3-octyl-5,5-dimethylhydantoin (Cl-ODMH), an un-fluorinated N-halamine, to determine the effects of fluorination on the antimicrobial and biofilm-controlling activities of N-halamine-based additives for polymers. While Cl-ODMH distributed evenly within polyurethane (PU), one of the most versatile polymers for a wide range of applications, Cl-FODMH aggregated and formed macro-domains on PU surfaces. As a result, PU films containing Cl-ODMH provided more potent

antimicrobial and biofilm-controlling effects against Gram-positive and Gram-negative bacteria than PU films containing Cl-FODMH. These results suggested that the distribution of additives within the polymer matrix plays a vital role in antimicrobial performances, providing valuable guidance in designing new antimicrobial polymeric materials for various industrial, institutional, environmental, hygienic, and/or biomedical applications.

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REFERENCES

1. Stoodley LH, Costerton JW, Stoodley P. *Nature Rev.* 2004; 2:95–108.
2. An, YH.; Friedman, RJ., editors. *Handbook of bacterial adhesion principles, methods, and applications.* Humana press; New York: 2000.
3. Chen ZB, Sun YY. *Macromolecules.* 2005; 38:8116–8119.
4. Sun YY, Chen ZB, Braun M. *Ind. Eng. Chem. Res.* 2005; 44:7916–7920.
5. Sun YY, Sun G. *Macromolecules.* 2002; 35:8909–8912.
6. Ren X, Zhu C, Kou L, Worley SD, Kocer HB, Broughton RM, Huang TS. *J. Bioact. Compat. Polym.* 2010; 25:392–405.
7. Sun X, Zhang L, Cao Z, Deng Y, Liu L, Fong H, Sun Y. *ACS Appl. Mater. Interface.* 2010; 2:952–956.
8. Ahearn DG, Grace DT, Jennings MJ, Borazjani RN, Boles KJ, Rose LJ, Simmons RB, Ahanotu EN. *Curr. Microbiol.* 2000; 41:120–125. [PubMed: 10856378]
9. Sun YY, Sun G. *Ind. Eng. Chem. Res.* 2004; 43:5015–5020.
10. Chen ZB, Sun YY. *Ind. Eng. Chem. Res.* 2006; 45:2634–2640. [PubMed: 18714370]
11. Worley SD, Williams DE. *Crit. Rev. Env. Contr.* 1988; 18:133–175.
12. Dagostin VS, Golçalves DL, Pacheco CB, Almeida WB, Thomé IP, Pich CT, Paula MMS, Silva L, Angioletto E, Fiori MA. *Mater. Sci. Eng. C.* 2010; 30:705–708.
13. Fiori MA, Paula MMS, Bernardin AM, Riella HG, Angioletto E. *Mater. Sci. Eng. C.* 2009; 29:1569–1573.
14. Wiese A, Munstermann M, Gutschmann T, Lindner B, Kawahara K, Zahringer U, Seydel UJ. *Membr. Biol.* 1998; 162:127–138.
15. Chen Z, Luo J, Sun Y. *Biomaterials.* 2007; 28:1597–1609. [PubMed: 17184837]
16. Sun YY, Sun GJ. *Appl. Polym. Sci.* 2003; 88:1032–1039.
17. Sun X, Cao Z, Sun YY. *Ind. Eng. Chem. Res.* 2009; 48:607–612.
18. Hyde FW, Alberg M, Smith K. *J Ind Microbiol Biotechnol.* 1997; 19:142–149. [PubMed: 9366095]
19. Chen M, Yu Q, Sun H. *Int. J. Mol. Sci.* 2013; 14:18488–18501. [PubMed: 24018891]
20. Privett BJ, Youn J, Hong SA, Lee J, Han J, Shin JH, Schoenfisch MH. *Langmuir.* 2011; 27:9597–9601. [PubMed: 21718023]
21. Zweifel, H. *Stabilization of polymeric materials.* Springer; Verlag: 1998.

Highlights

- Fluorinated and un-fluorinated N-halamines were used as antimicrobial additives.
- Fluorination led to poor compatibility of the N-halamine with polyurethane.
- Fluorination decreased antimicrobial and biofilm-controlling activity.
- We show the structure-property relationship of N-halamine antimicrobial additives.

Fig. 1.

Chemical structures of 5,5-dimethylhydantoin (DMH), 3-octyl-5,5-dimethylhydantoin (ODMH), 3-1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (FODMH), 1-chloro-3-octyl-5,5-dimethylhydantoin (Cl-ODMH), and 1-chloro-3-1H,1H,2H,2H-perfluorooctyl-5,5-dimethylhydantoin (Cl-FODMH).

Fig. 2.
FT-IR spectra of DMH, ODMH, FODMH, CI-FODMH, and CI-ODMH

Fig. 3.

Proton NMR spectra of DMH, 1-bromooctane (BO), ODMH, Cl-ODMH, 1H,1H,2H,2H-perfluorooctyl iodide (IFO), FODMH, and Cl-FODMH, in CDCl₃

Fig. 4.
DSC curves of DMH, FODMH, Cl-FODMH, ODMH and Cl-ODMH

Pure PU

1% CI-ODMH PU

1% CI-FODMH PU

3% CI-ODMH PU

3% CI-FODMH PU

Samples	R_z (μm) (n=3)
Pure PU	1.5 ± 0.3
1% CI-ODMH PU	1.4 ± 0.1
3% CI-ODMH PU	1.3 ± 0.2
5% CI-ODMH PU	1.2 ± 0.3
1% CI-FODMH PU	2.1 ± 0.2
3% CI-FODMH PU	3.4 ± 0.8
5% CI-FODMH PU	4.4 ± 1.0

Fig. 5. AFM images and the roughness R_Z (ten points average roughness, reported as the arithmetic average of the five highest peaks and five lowest valleys) of PU samples containing different amounts of Cl-ODMH or Cl-FODMH

Fig. 6.

SEM images of (A) pure PU film, (B) PU film containing 1% of Cl-ODMH, (C) PU film containing 3% of Cl-ODMH, (D) PU film containing 5% of Cl-ODMH, (E) PU film containing 1% of Cl-FODMH, (F) PU film containing 3% of Cl-FODMH, and (G) PU film containing 5% of Cl-FODMH

Fig. 7.
DSC curves of PU films containing different amounts of Cl-ODMH or Cl-FODMH

Fig. 8. SEM images of *A. Calcoaceticus* grown on (A) pure PU films, (B) PU films containing 5% of Cl-ODMH, and (C) PU films containing 5% of Cl-FODMH, and *S. epidermidis* grown on (D) pure films, (E) PU films containing 5% of Cl-ODMH, and (F) PU films containing 5% of Cl-FODMH

Table I

Water contact angles (degree) of PU films containing different amounts of Cl-ODMH or Cl-FODMH

Additive contents (%)	PU with Cl-ODMH	PU with Cl-FODMH
0	106.2 ± 1.7	106.2 ± 1.7
1	104.9 ± 0.3	106.0 ± 0.4
3	108.3 ± 0.5	120.0 ± 0.8
5	110.1 ± 0.2	139.1 ± 0.6

Table II

Level of recoverable bacteria from PU samples containing different amounts of Cl-ODMH or Cl-FODMH in the direct contact antimicrobial tests^{*}

Sample	Level of recoverable <i>A. calcoaeticus</i> from the film (CFU/cm ²) ^{**}			Level of recoverable <i>S. epidermidis</i> from the film (CFU/cm ²) ^{***}		
	Additive content in the film (%)			Additive content in the film (%)		
	1	3	5	1	3	5
PU-Cl-ODMH	$(2.4 \pm 1.7) \times 10^4$	0	0	$(2.91 \pm 1.17) \times 10^4$	0	0
PU-Cl-FODMH	$(1.65 \pm 0.38) \times 10^4$	$(8.1 \pm 6.9) \times 10^2$	$(2.58 \pm 1.14) \times 10^2$	$(4.78 \pm 1.5) \times 10^4$	$(2.04 \pm 0.50) \times 10^3$	0

^{*} The contact time was 15 min.

^{**} From pure PU film (0% additive content), $(1.81 \pm 0.17) \times 10^5$ CFU/cm² of *A. calcoaeticus* were recovered.

^{***} From pure PU film (0% additive content), $(2.0 \pm 0.23) \times 10^5$ CFU/cm² of *S. epidermidis* were recovered.

Table III

Level of recoverable bacteria from PU samples containing different amounts of Cl-ODMH or Cl-FODMH in the biofilm-controlling studies *

Sample	Level of recoverable <i>A. calcoaeticus</i> from the film (CFU/cm ²) **			Level of recoverable <i>S. epidermidis</i> from the film (CFU/cm ²) ***		
	Additive content in the film (%)			Additive content in the film (%)		
	1	3	5	1	3	5
PU-Cl-ODMH	$(1.0 \pm 0.17) \times 10^4$	$(4.01 \pm 0.16) \times 10^3$	$(1.16 \pm 0.35) \times 10^3$	$(8.91 \pm 0.67) \times 10^4$	$(1.37 \pm 0.058) \times 10^4$	$(7.70 \pm 0.35) \times 10^2$
PU-Cl-FODMH	$(1.26 \pm 0.69) \times 10^4$	$(1.30 \pm 0.86) \times 10^4$	$(2.69 \pm 0.32) \times 10^4$	$(2.25 \pm 0.83) \times 10^5$	$(4.34 \pm 1.51) \times 10^4$	$(1.98 \pm 0.35) \times 10^4$

* The bacteria contacted the films in PBS for 60 min; the films were then incubated in broth solutions at 37 °C for 72 h.

** From pure PU film (0% additive content), $(1.65 \pm 0.78) \times 10^5$ CFU/cm² of *A. calcoaeticus* were recovered.

*** From pure PU film (0% additive content), $(1.21 \pm 0.32) \times 10^6$ CFU/cm² of *S. epidermidis* were recovered.