Research Article

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Fabrication of *N*-halamine polyurethane films with excellent antibacterial properties

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Abstract: An *N*-halamine precursor, namely, 2-amino-5-(2-hydroxyethyl)-6-methylpyrimidin-4-one (AHM), was used as a chain extender in the preparation of a series of *N*-halamine polyurethane (PU) films, in order to also instill antibacterial properties. The mechanical properties, thermodynamic performance, and antimicrobial performance of the functionalized PU films were systematically studied. The results showed that the addition of AHM could improve the thermodynamic and mechanical properties of the developed PU films. Conducting tests in the presence of *Escherichia coli* and *Staphylococcus aureus* as the model microorganisms revealed that prior to chlorination the antibacterial properties of the chlorinated PU-AHM-Cl films improved significantly relative to the analogous films. The excellent antibacterial properties and the overall superior performance of the PU-AHM-Cl films allow their potential application in microbiological protection materials and related fields.

Keywords: polyurethane, *N*-halamine, chlorination, antibacterial, surface property

1 Introduction

In contrast to the low stability, low acid resistance, and low mechanical strength of some natural antibacterial polymers, polyurethane (PU) has advantageous physical and chemical properties. This polymer is also readily available, inexpensive, and highly biocompatible, thus

making PU an extremely versatile polymer that can be utilized for producing synthetic leather, furniture, or biomaterials, or for other purposes in health care and related fields (1–4). However, similar to most other polymers, PU materials are commonly contaminated with microorganisms, leading to cross-contamination, cross-infection, or transmission of bacterial diseases (5–9). Therefore, the ongoing challenge is to develop an effective strategy for optimizing the antibacterial properties of PU materials (10). In general, the majority of reported antibacterial PU polymers are synthesized by either (i) physical mixing of antibacterial substances (e.g., metal ions, reactive oxygen species, antibiotics, quaternary ammonium salts) into PU or (ii) surface modification, i.e., by binding antimicrobial agents onto the surface of PU (11–18).

Among the numerous potential antibacterial substances, *N*-halamine compounds are highly desirable agents for fabricating antibacterial materials owing to their inherent economic advantages, long-term stability, high durability, regenerability, and nontoxicity to humans and the environment (19–23). *N*-halamine compounds kill microorganisms by releasing strongly oxidizing free halogen cations that interfere with cellular enzyme activity and metabolic processes (24). Interestingly, once the oxidative halogens are consumed, *N*-halamine compounds are easily recharged by soaking them in bleach. Based on their unique antibacterial performance, *N*-halamine compounds have been used extensively in the medical and health fields, specifically for water treatment, food safety, and textile materials (25–27).

Various preparation techniques, including physical blending, surface coating, chemical grafting, and copolymerization, have been used to introduce *N*-halamine compounds into the target materials (28–33). Compared with physical methods, chemical modification results in higher durability, long-term stability, and regenerability because the antibacterial groups are tightly bound to the base (34). Recently, surface grafting of *N*-halamine compounds on PU has been shown to induce antibacterial properties. For example, an *N*-halamine precursor, 5,5-dimethylhydantoin (DMH), was covalently linked to the surface of PU using hexamethylene diisocyanate as the

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Figure 1: Synthetic scheme describing the preparation of (a) AHM and (b) PU-AHM.

coupling agent, and the new *N*-halamine-based PU material exhibited effective, durable, and rechargeable antimicrobial activity (35). Similarly, Tan *et al.* successfully grafted an *N*-halamine precursor (2,2,5,5-tetramethylimidazolidine-4-one (TMIO) hydantoin) onto the surface of microporous PU films (36) and found that the as-prepared PU films provided an effective hygienic protection barrier after chlorination. Kemao Xiu *et al.* diffused a polymerizable *N*-halamine precursor, 3-(4'-vinylbenzyl)-5, 5-dimethylhydantoin (VBDMH), in PU to prepare PU/PVBDMH (PUV) semi-interpenetrating polymer networks (semi-IPNs). They reported that the PVBDMH-based *N*-halamines were primarily on the surface of the semi-IPNs rather than in the bulk. The antimicrobial efficiency of the PUV *N*-halamine semi-IPNs resulted in complete extermination of the testing bacteria after 30 min of contact (37). However, most approaches described in the literature are not economical, and the products exhibit non-durable, unstable, and inefficient activities, among other problems. Some chemical surface modifications even reduce the mechanical and thermodynamic properties of the polymer. Therefore, it is challenging but necessary to develop a simple strategy for preparing antimicrobial PU polymers with excellent mechanical and thermodynamic properties.

Herein, we introduce a simple procedure for preparing an antimicrobial PU polymer by integrating a

pyrimidine amino acid-containing *N*-halamine compound (2-amino-5-(2-hydroxyethyl)-6-methylpyrimidin-4-one (AHM)), which simultaneously functions as a chain extender and an antibacterial agent. Following chlorination, the antimicrobial activities of these PU-AHM-Cl films were tested against *Escherichia coli* and *Staphylococcus aureus* to evaluate their antibacterial properties. The mechanical properties and thermodynamic performances of the as-prepared PU-AHM and PU-AHM-Cl films were also studied.

2 Materials and methods

2.1 Materials

Guanidine carbonate (98%) and α -acetyl- γ -butyrolactone (99%) were purchased from Shanghai Macklin Reagent Co., Ltd. Household bleach (5% sodium hypochlorite according to the label), triethylamine (TEA; analytical grade), hydrochloric acid (HCl; 35%), dibutyltin dilaurate (DBTDL; 98%), and 1,4-butanediol (BDO) were purchased from Sinopharm Chemical Reagent Co., Ltd. Isophorone diisocyanate (IPDI, 99%) and poly propylene glycol (PPG;

$M_n = 2,000$ g/mol, chemically pure) were provided by Bayer (China) Co., Ltd. *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC29213) were purchased from the Institute of Microbiology, Chinese Academy of Sciences. All other reagents were of analytical grade.

2.2 Synthesis of 2-amino-5-(2-hydroxyethyl)-6-methylpyrimidin-4-one (AHM)

The synthetic route for producing AHM, according to a previously reported method, is shown in Figure 1a (38). In a typical procedure, guanidine carbonate (18 mmol, 11.3 g), α -acetyl- γ -butyrolactone (18 mmol, 2.0 mL), TEA (5.2 mL), and absolute ethyl alcohol (29 mL) were added sequentially into a three-necked flask, and the mixture was stirred for 1 h at 30°C. Then the reaction was allowed to proceed for 24 h at 78°C with mechanical stirring, after which, a light-yellow suspension was obtained. Following suction filtration, the obtained solid material was rinsed several times with ethyl alcohol and dried in a vacuum oven at 50°C for 10 h. Finally, the white target compound, AHM, was obtained, characterized, and used for subsequent experiments. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ : 10.93 (s, 1H), 6.35 (s, 2H), 4.53 (s, 1H), 3.36 (s, 2H), 2.44 (s, 2H), and 2.06 (s, 3H). The $^1\text{H-NMR}$ spectrum of AHM is provided in Figure S1.

2.3 Synthesis of PU-AHM

The synthesis of PU-AHM is shown in Figure 1b. After adding 20.0 g PPG-2000 to a 250-mL three-necked flask, the flask was dehydrated under vacuum for 2 h at 110°C. It was then allowed to cool naturally to 50°C, and a solution (prepared by dissolving 4.5 g IPDI in 10 mL dimethylformamide [DMF]) was added dropwise into the flask under an N_2 atmosphere. After adding two drops of DBTDL (about 0.16 mmol) into the flask, the reaction proceeded at 78°C for 2.5 h. Then a mixture containing BDO and AHM in various proportions was added to the flask at 50°C; the amount of AHM dissolved in 20 mL DMF was 0, 1.0, 1.5, 2.0, or 2.5 mmol, and the total number of moles of (BDO + AHM) was 10 mmol. This reaction was carried out for 4 h at 78°C. After cooling to room temperature, the solution was dropped into a Teflon groove and dried under vacuum for 12 h. Finally, a series of PU-AHM films with varying proportions of AHM (expressed as PU,

PU-AHM1.0, PU-AHM1.5, PU-AHM2.0, and PU-AHM2.5, according to the concentrations stated above, respectively) were prepared. All films were repeatedly extracted with acetone to remove the unreacted components. The $^1\text{H-NMR}$ spectrum of PU-AHM is presented in Figure S2. The gel permeation chromatography (GPC) analysis of the PU and PU-AHM films is shown in Figure S3 and Table S1.

2.4 Chlorination of PU-AHM films

To transform the AHM moieties into *N*-halamines, the as-prepared PU-AHM films with varying AHM contents were immersed in 5% bleach solution (the pH was adjusted to 7 using 6 mol/L HCl) at room temperature for 30 min under ultrasonication (Figure 1b). Afterwards, the films were washed copiously with deionized water to remove the free chlorine, and the water used for rinsing was tested with potassium iodide/starch to ensure thorough washing. The obtained PU-AHM-Cl films were dried in a vacuum oven for 4 h at 50°C and stored in a dehydrator for 8 h. The active chlorine content in the PU-AHM-Cl films was determined via iodometric titration.

2.5 Characterization and instrumentation

Fourier-transform infrared spectroscopy (FTIR; NEXUS-870, Nicolet) was used to characterize the surface of the films in the frequency range 4,000–600 cm^{-1} . The $^1\text{H-NMR}$ spectra were obtained using a Bruker 400 MHz spectrometer. The tensile strength and elongation at break of the films were measured with a tensile testing instrument (Instron 5967; Instron, USA) by applying a speed of 100 mm/min at $25 \pm 1^\circ\text{C}$. The area specification of the samples was 4×25 mm, and at least five replicates of each sample were evaluated to calculate an average value. The thermal stabilities of the films were determined using a thermogravimetric analyzer (Pyris-1; Perkin Elmer, USA). The samples were heated at a rate of 10°C/min, from 30°C up to 800°C, under a nitrogen atmosphere (flowing at 20 mL/min). The elemental compositions of and chemical bonding variations in the films were determined using X-ray photoelectron spectroscopy (XPS; Axis Ultra DLD/ESCA, Kratos, UK). The molecular weight and molecular weight distributions of the polymers were determined with a Waters 1515 GPC, equipped with a refractive index detector. The permeation chromatographs

were calibrated with standard polystyrene samples. DMF was used as the eluent at a flow rate of 0.5 mL/min at 35°C.

2.6 Active chlorine content analysis by titration

The active chlorine contents of the chlorinated films were determined via iodine titration (23). Specifically, about 0.05 g of the chlorinated PU or PU-AHM film was cut into small pieces and dispersed in deionized (DI) water. Then 5 mL of 0.5 mol/L sulfuric acid solution and 0.40 g of KI were added successively with continuous mixing. The generated I_2 was titrated with a sodium thiosulfate solution, using a starch solution as an indicator. Testing of each specimen was repeated three times, and the mean values were calculated. The weight percentage of oxidative chlorine content in each film was determined using the following equation:

$$Cl^+ (\%) = \frac{35.45 \times N \times V}{2 \times W} \quad (1)$$

where N is the normality of the titrant, V is the volume of the consumed titrant, and W is the weight of the film (g).

2.7 Antibacterial testing

The leaching characteristics of the films were characterized using the ring diffusion test. All laboratory equipment were sterilized with high-temperature and ultraviolet treatments. The nutrient agar for culturing bacteria was tiled in a petri dish, and these tiled agar plates were inoculated with 0.10 mL of diluted suspensions of model microorganisms. The population of the diluted suspensions was about $(5-10) \times 10^5$ cfu/mL (cfu = colony-forming unit). Then the test film wafers measuring 10 mm in diameter were added onto the surface of the inoculated agar plates. All of these agar plates were incubated for 24 h at 37°C, at which point, the diameters of the inhibition zones were measured. Each system was tested and repeated three times in order to calculate the mean values.

The antibacterial properties of the films were evaluated in accordance with the American Association of Textile Chemists and Colorists (AATCC) test 100-2004 (39). To investigate the antimicrobial functions of the chlorinated PU-Cl and PU-AHM-Cl specimens, *E. coli* and *S. aureus* were used as the model microorganisms. In a typical experiment, 0.05 mL of a diluted suspension

of model microorganisms at about $(5-10) \times 10^5$ cfu population was added onto the surface of the film (the size of each specimen was about 2×2 cm). In order to enable sufficient contact, a sterilized PE film was placed on the surface of the inoculated film. After 5 min of contact time, the film was placed into 20 mL of a saline solution as an eluent. To quench the biological interaction, an appropriate amount of 0.02 mol/L sodium thiosulfate was added into the eluent and shaken to mix. Then 0.01 mL of the mixture was evenly placed across the surface of the nutrient agar plate. After 24 h of incubation at 37°C, the viable bacterial colonies on the plates were counted, and the reduction was calculated. The formula for calculating the inhibition rate based on the number of bacteria is:

$$W = (A - B)/A \times 100\% \quad (2)$$

where W is the bacteriostatic rate, and A and B are the number of colonies observed in the control and composite film samples, respectively. Each specimen was tested three times in order to calculate the mean values.

2.8 Statistical analysis

Data management and analysis were performed using the SPSS software (v.17.0, SPSS Inc., Chicago, IL). The results are reported as the mean $\pm$ standard deviation (SD), and the statistical significance was set at the customary level of $p < 0.05$.

3 Results and discussion

3.1 Chemical composition and surface structure of the as-prepared films

The successful preparation of antibacterial PU-AHM films using AHM as the chain extender was confirmed by FTIR (Figure 2). The wide absorption peaks between 2,960 and 2,866 cm^{-1} observed in the FTIR spectra were assigned to the $-CH_2$ and $-CH_3$ stretching vibrations, and the peak at 3,334 cm^{-1} was confirmed as the N-H stretching frequency of the carbamate. After the chain-extending reaction, the characteristic peak of $-NCO$ (at 2,280–2,240 cm^{-1}) did not appear, indicating that the isocyanate ($-NCO$) groups from IPDI were fully consumed in the reaction. Compared with PU (Figure 2a), which contained only BDO as a chain extender, the spectrum of PU-AHM2.0 (Figure 2b) contained the characteristic peaks associated

Figure 2: FTIR spectra: (a) PU film, (b) PU-AHM2.0 film, and (c) PU-AHM2.0-Cl film.

with C=N (1550 cm^{-1}) and C=O (1637 cm^{-1}) on the pyrimidine ring of AHM. These peaks appeared following the addition of AHM as the chain extender (40), which verified the successful polymerization reaction between AHM and PU.

Interestingly, the characteristic peak of C–N (observed in the spectrum of PU-AHM2.0; Figure 2b), located at 1550 cm^{-1} , remained in the PU-AHM2.0-Cl after chlorination, and the stretching frequency of the carbonyl band shifted to 1641 cm^{-1} . The shift of this characteristic carbonyl band to a higher wave number was in good agreement with the reported literature (41) and was attributed to the change in the chemical environment (i.e., increased positive charge on a nearby atom). Overall, the FTIR spectra confirmed the success of the chain-extending reaction during the preparation of PU-AHM films, using AHM as the chain extender; after chlorination, the N–H bonds were converted into N–Cl bonds.

3.2 Characterization of the chlorinated polymer films

The prepared PU-AHM films were chlorinated via immersion in 5% bleach solution, and the surface chemical composition of each PU-AHM sample before and after chlorination was investigated using XPS, as shown in Figures 3 and 4. It is clear from Figure 3a and b that the main distinction between PU-AHM2.0 and PU-AHM2.0-Cl was the new peak at about 200 eV in the PU-AHM2.0-Cl spectrum. This new peak was considered as Cl_{2p} , which was attributed to the generation of N–Cl bonds. Furthermore,

Figure 3: XPS spectra: (a) PU-AHM2.0 film and (b) PU-AHM2.0-Cl film.

the high-resolution XPS spectrum of the PU-AHM2.0 film in Figure 4a has an N_{1s} peak at about 400 eV, which was assigned to the amide nitrogen atom (N–H). However, as shown in Figure 4b, the peak of N_{1s} shifted to 401 eV after chlorination, which was consistent with the formation of *N*-halamine nitrogen atoms (N–Cl). This change in binding energy (between N–H and N–Cl) can be explained based on the higher electronegativity of Cl relative to H (42).

3.3 Mechanical properties

Figure 5 shows the tensile strength and elongation at break of the PU-AHM films with different proportions of AHM, before and after chlorination. Without addition of any AHM as a chain extender, the tensile strength and elongation at break of the PU film were 1.2 MPa and 660%, respectively. However, upon incorporating AHM as the chain extender, the mechanical properties of the PU-AHM polymers greatly enhanced. In particular, there was a significant increase in the tensile strength of the film produced with 1.0 mmol of AHM as the chain extender in the reaction. In general, the tensile strength of the PU-AHM films increased further as the proportion of AHM increased, such that the tensile strength of PU-AHM2.5 reached 15.3 MPa. This significant improvement can be interpreted in terms of the chemical reaction. Specifically, the amine moiety of the *N*-halamine precursor reacted with isocyanate to form a carbamide group, which was more polar than the urethane group. In addition, employing AHM as the chain extender leads to the formation of more hydrogen bonds within the polymer,

Figure 4: XPS high-resolution nitrogen (N 1s) spectra: (a) PU-AHM2.0 film and (b) PU-AHM2.0-Cl film.

Figure 5: Tensile strength and elongation at break of PU-AHM and PU-AHM-Cl films as a function of their AHM content.

thus enhancing the intermolecular interactions. These hydrogen bonds promoted the formation of hard segment domains, which played a key role in improving the strength, hardness, and modulus performance of the PU films. Furthermore, introduction of the AHM compounds increased the cross-linking density of the PU polymer, which also strengthened the PU films. Therefore, the tensile strength of the prepared PU-AHM polymers markedly improved because of the addition of AHM as a chain extender. However, the stretching ability of the films was limited because of the formation of the cross-linked network of hydrogen bonds (43). As a result, the elongation at break of the PU-AHM films before and after chlorination gradually reduced as the AHM content increased. However, as shown in Figure 5, the differences in the tensile strength and elongation at break of PU-AHM and PU-AHM-Cl films were small, which indicated that

chlorination had only a minor effect on the mechanical properties of the polymers. This is likely because chlorination occurs primarily on the surface of the PU-AHM polymers, so the majority of the bulk PU-AHM structure undergoes negligible changes following chlorination.

3.4 Thermal properties

The thermal stability of the PU and PU-AHM films were confirmed by thermogravimetric analysis (TGA), and the results are presented in Figure 6a and Table 1. In general, the TGA curves of the PU-AHM films clearly shifted to the right, relative to the PU film, indicating that the thermostability of the PU-AHM films was increased because of the addition of AHM as the chain extender. The TGA data compiled in Table 1 show that the 10% and 50% thermal weight loss temperatures of the PU film were 295.7°C and 352.8°C, respectively. However, after adding AHM (rather than just BDO) as the chain extender, the 10% and 50% thermal weight loss temperatures of the PU-AHM films increased. For example, considering the PU-AHM2.5 film, the $T_{10\%}$ and $T_{50\%}$ increased by about 44°C and 36°C, respectively, as compared with the PU film. This change is likely because the introduction of AHM as a chain extender effectively enhanced the cross-linking density of the PU-AHM films by forming more hydrogen bonds (44,45).

To investigate the impact of chlorination on the thermostability of the PU-AHM films, the TGA results for PU-AHM2.0 before and after chlorination were compared (Figure 6b). Based on the thermal weight loss curves of PU-AHM2.0 and PU-AHM2.0-Cl, it is clear that only a very small difference exists between these two data sets, indicating that chlorination did not have an appreciable

Figure 6: TGA curves comparing: (a) PU and PU-AHM with various AHM content and (b) PU-AHM2.0 and PU-AHM2.0-Cl.

effect on the thermal stability of the PU-AHM film. These comparative analytical results further illustrated that chlorination occurred mainly on the surface of the prepared PU-AHM films, without inducing major structural damage.

3.5 Active chlorine content of the prepared films

The active chlorine contents in the PU and PU-AHM films after treatment with bleach solution are provided in Table 2. According to the titration analysis, the chlorinated PU films had only $(0.03 \pm 0.01)\%$ of active chlorine. In contrast, the chlorinated PU-AHM1.0-Cl had a total active chlorine density of $(0.13 \pm 0.01)\%$. Additionally, the active chlorine content increased with greater AHM concentration (from 1.0 to 2.5 mmol), such that the PU-AHM2.5-Cl film had an active chlorine atom density that reached $(0.19 \pm 0.01)\%$. The orders-of-magnitude change in active chlorine density among the prepared films was attributed to chlorination of increasing quantities of N–H groups of AHM that were exposed at the surface of the PU-AHM films. In addition, this facilitated and accelerated the release rate of free oxidative chlorine ions from the N–Cl moieties on the antibacterial *N*-halamine agent, AHM (46).

Table 2: Determination of active chlorine content in the tested films

Sample	Cl ⁺ concentration (wt%)
PU	0
PU-AHM	0
PU-Cl	0.03 ± 0.01
PU-AHM1.0-Cl	0.13 ± 0.01
PU-AHM1.5-Cl	0.16 ± 0.01
PU-AHM2.0-Cl	0.18 ± 0.01
PU-AHM2.5-Cl	0.19 ± 0.01

The storage stability of chlorinated PU-AHM-Cl films was also evaluated. After storing the films under routine conditions for 2 months (i.e., samples were each placed in a sealed container at room temperature), the chlorine content of PU-AHM2.0-Cl was determined to be $(0.17 \pm 0.01)\%$. Therefore, the PU-AHM-Cl films demonstrated sufficient storage stability, which is an important aspect when considering practical applications (37,47).

3.6 Assessment of antibacterial properties

The antibacterial activities of the unchlorinated and chlorinated PU and PU-AHM films against *E. coli* and *S. aureus* were investigated using the ring-diffusing test. In general, the different diffusion abilities of the antibacterial

Table 1: TGA data for the PU films with different AHM content

Sample	PU	PU-AHM1.0	PU-AHM1.5	PU-AHM2.0	PU-AHM2.5
$T_{10\%}$ (°C)	295.7	312.6	318.9	330.2	339.8
$T_{50\%}$ (°C)	352.8	368.8	372.2	382.4	388.9

Table 3: Inhibition zone diameter and antibacterial results for films with different AHM content after contact with bacteria for 5 min

Sample	Inhibition zone diameter (mm)		Bacterial reduction (%)	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
PU	10.0	10.0	0	0
PU-AHM	10.0	10.0	0	0
PU-Cl	11.8 ± 0.1	11.2 ± 0.1	29.17	26.65
PU-AHM1.0-Cl	14.2 ± 0.1	12.1 ± 0.1	78.32	72.41
PU-AHM1.5-Cl	16.0 ± 0.1	13.3 ± 0.2	100.00	100.00
PU-AHM2.0-Cl	19.2 ± 0.2	15.6 ± 0.3	100.00	100.00
PU-AHM2.5-Cl	22.7 ± 0.3	19.4 ± 0.2	100.00	100.00

substances on the agar plate led to the formation of differently sized transparent circles due to the inhibition of the surrounding microorganisms' growth. The size of this inhibition zone was used to determine the activity of the antibacterial films. As shown in Table 3, the unchlorinated PU and PU-AHM films did not exhibit any antibacterial effects (no inhibition zone was observed). However, the chlorinated PU-Cl and the series of PU-AHM-Cl films demonstrated effective antimicrobial activity. The activity of the PU-Cl was attributed to the fact that the halogenation reaction replaced the N-H moieties of the urethane linkages. Interestingly, the enhancement in the antimicrobial properties of the PU-AHM-Cl films paralleled the increasing proportion of AHM. For example, the diameter of the *S. aureus* and *E. coli* inhibition zones generated by PU-AHM2.5-Cl was about 22.7 and 19.4 mm, respectively. This inhibition zone study confirmed that the oxidative chlorine in the PU-AHM-Cl film could be released, thus allowing them to diffuse into the agar and inhibit the

growth of the surrounding microorganisms. A larger inhibition zone indicated superior antimicrobial activity and a more rapid release rate in terms of the active chlorine. Therefore, it is clear that the chlorinated PU-AHM-Cl films demonstrated remarkable antimicrobial properties. This activity originated from the strong oxidative nature of the halogen atoms in the N-Cl bonds of the *N*-halamine components, which are known to demonstrate antibacterial activity against pathogens.

The biocidal efficiencies of the PU, PU-Cl, and the various PU-AHM and PU-AHM-Cl films are summarized in Table 3 and Figure 7 (determined according to the AATCC test method 100-2004). After 5 min of contact and 24 h of incubation, the unchlorinated PU and PU-AHM films did not cause as expected appreciable bacterial reduction. However, the chlorinated PU-Cl and especially the PU-AHM-Cl samples showed clear antibacterial activity against both *E. coli* and *S. aureus*. For example, the PU-AHM1.0-Cl film deactivated about 78.32% of *S. aureus* and 72.42% of *E. coli* after 5 min of contact, and the PU-AHM1.5-Cl sample deactivated almost all *S. aureus* and *E. coli* after 5 min. This bactericidal testing further confirmed that the chlorinated PU-AHM-Cl films had powerful antibacterial properties, allowing them to effectively (i) prevent the growth of microorganisms by releasing free oxidative chlorines and (ii) deactivate bacteria via direct contact in the presence of the *N*-halamine monomers, as reported previously (48,49).

4 Conclusions

In this study, an *N*-halamine precursor (AHM) was successfully used as a chain extender for the preparation of PU-AHM polymers, which demonstrated antimicrobial

Figure 7: Testing the antibacterial activity of the PU films, the PU-AHM films, the chlorinated PU films, and the chlorinated PU-AHM-Cl films (containing various relative amounts of AHM) in contact with *S. aureus* and *E. coli* bacteria for 5 min.

activity following a chlorination process. Based on the presented mechanical and thermodynamic analyses, the PU-AHM films exhibited superior performance compared with the PU films because the addition of AHM increased the cross-linking density within the PU-AHM polymers by forming more hydrogen bonds. In addition, the bactericidal and ring-diffusing tests revealed that as the amount of the incorporated AHM increased, the diameter of the induced inhibition zone became larger. In fact, when the amount of AHM reached 1.5 mmol, the model microorganisms completely devitalized after 5 min of contact with the PU-AHM film. Overall, this work reported the synthesis, characterization, and activity of a series of new PU-AHM polymers with excellent antibacterial activity and thermal stability. These materials could be applied as microbiological protection agents in protective clothing or in other specialized fields.

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