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Characterization and antibacterial properties of N-halamine-derivatized cross-linked polymethacrylamide nanoparticles

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ABSTRACT

N-halamine-derivatized cross-linked polymethacrylamide nanoparticles with sizes ranging between 18 ± 2.0 and 460 ± 60 nm were prepared via surfactant-free dispersion co-polymerization of methacrylamide (MAA) and the cross-linking monomer N,N-methylenebisacrylamide (MBAA) in an aqueous continuous phase, followed by a chlorination process using sodium hypochlorite. The effect of various polymerization parameters (monomer concentration, initiator type and concentration, polymerization duration, polymerization temperature, and the weight ratio [MBAA]/[MAA]) on the size and size distribution of the produced cross-linked P(MAA–MBAA) nanoparticles was elucidated. The effect of various chlorination parameters (hypochlorite concentration, chlorination period and temperature) on the bound oxidative chlorine atom (Cl) content of the P(MAA–MBAA) nanoparticles was also investigated. The bactericidal activity of these chloramine-derivatized nanoparticles was tested against two common bacterial pathogens (*Escherichia coli* and *Staphylococcus aureus*), and they were found to be highly potent. Furthermore, these nanoparticles also exerted their antimicrobial activity against multi-drug resistant (MDR) bacteria, further demonstrating their efficacy.

1. Introduction

Various organic antimicrobial agents, such as quaternary ammonium salts [1–5], phosphonium salts [6–8], and N-halamine compounds [9–14], have been extensively investigated over the past 20 years. The motivation for the development of organic antimicrobial agents is the demand for antibacterial coatings that can prevent the bacterial colonization of surfaces (i.e., biofouling) for industrial applications (e.g., water treatment) and medical applications (e.g., medical devices). Compared with halogens, which are inorganic, N-halamines are more stable and less corrosive [15], and their numerous sought-after qualities (i.e., effectiveness at killing toward a broad spectrum of microorganisms, long-term stability, the possibility of recycling, low cost, and safety for humans and the environment) make N-halamines particularly attractive. [9] The dissociation constant of N-halamine compounds in water is relatively low and varies based on chemical structure in the order amine < amide < imide, as presented in Table 1 [16]. However, in the presence of microorganisms, oxidative Cl transfer from the N-halamine bond to the microorganism is significantly favored over hydrolysis (see Fig. 1). Amine-halamine is the most stable of all halamine bonds but has a slower bactericidal rate than amide-halamine. In contrast, imide-halamine has a rapid bactericidal rate because it is the least stable of all halamine bonds and can rapidly release active Cl into the medium [16]. Both the functional group and the substituent type and concentration may affect the dissociation rate: e.g., a Cl atom bound to secondary amide will dissociate more rapidly than a Cl atom bound to the primary amide. [17] Compounds containing amide-halamine bonds are considered the most practical for industrial applications because they exhibit a moderate rate of transfer of the oxidative Cl from the N-halamine to bacteria in aqueous solution and thus provide reasonably rapid bactericidal activity. Although the hydrolysis constant of amide-halamines is in the range of 10^{-8} [16], the Cl transfer to the bacteria is the more favorable process [18–21].

N-halamine polymers can be divided into cyclic and acyclic N-halamines. Cyclic N-halamine polymers such as hydantoins, oxazolidinones and imidazolidinones have been extensively investigated as antibacterial particles and coatings [10,11,18,22–27]. However, acyclic N-halamine polymers have primarily been

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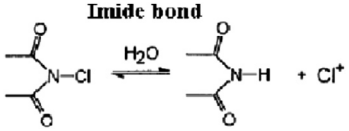
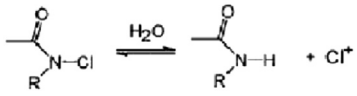
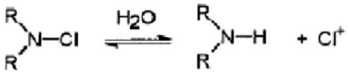
0142-9612/

Table 1
Dissociation constants of Cl-amine compounds in water.

Dissociation reaction	Dissociation constant
Imide bond 	8.5×10^{-4}–1.6×10^{-2}
Amide bond 	2.6×10^{-8}
Amine bond 	$<10^{-12}$

investigated for use as antibacterial coatings [1,9,11,28]. Cyclic N-halamines are expected to be more stable than acyclic N-halamines and to withstand hydrolysis of the N-halogen bond. This stability is a result of their chemical structures, in which the electron-donating alkyl group is substituted on the heterocyclic ring adjacent to the oxidative N-halamine moieties, thus hindering the release of the oxidative halogen into the aqueous solution [29]. The N-halogen bonds of acyclic N-halamine polymers are more sensitive to hydrolysis because of their open structure, but this sensitivity can be overcome to some extent by cross-linking the acyclic N-halamine polymers. Such cross-linking may decrease the hydrolysis rate because of the "tight polymer package" structure. Substitution of the N-halogen of the acyclic form also affects the stability of this bond. A previous study demonstrated that the N-halogen bonds of secondary amide groups (e.g., N-tert-butylacrylamide) are more resistant to hydrolysis than those of primary amide groups (e.g., acrylamide), but halogenation of the secondary amide groups is more difficult because of increased steric hindrance [30,31]. To benefit from the reactivity of the primary amide and the stability of the secondary amide, a polymer containing both primary and secondary amides may be advantageous. The current manuscript describes the formation of N-halamine-derivatized cross-linked polymethacrylamide nanoparticles via aqueous surfactant free copolymerization of methacrylamide (MAA) (a monomer containing a primary amide group) and N,N′-methylenebisacrylamide (MBAA) (a cross-linking monomer containing secondary amide groups), followed by a chlorination process using household bleach. The effect of various polymerization parameters (monomer concentration, initiator type and concentration, polymerization duration, polymerization temperature, and the weight ratio [MBAA]/[MAA]) on the size and size distribution of the produced P(MAA–MBAA) cross-linked nanoparticles was investigated, and the antibacterial activity of the P(MAA–MBAA)-Cl nanoparticles was tested against both Gram-positive and Gram-negative bacteria.

2. Materials and methods

2.1. Materials

All chemicals were of analytical-grade and used with no further purification. MAA, MBAA sodium hypochlorite (4%), potassium persulfate (PPS), and 2,2′-Azobisisobutyronitrile (AIBN) were purchased from Sigma–Aldrich (Rehovot, Israel); sodium iodide was purchased from Strem Chemicals (Newburyport, MA, USA); acetic acid was purchased from Fisher Scientific (Loughborough, UK); sodium thiosulfate (0.01 N) was purchased from Acros Organics (Geel, Belgium); and water was purified by passing deionized water through an Elgastat Spectrum reverse osmosis system (Elga LTD, High Wycombe, UK).

2.2. Instruments

Attenuated total reflectance (ATR) analysis was performed with Bruker Platinum ATR QuickSnap TM sampling modules A220/D-01. The samples were analyzed over 100 scans at a 4 cm^{-1} resolution. The hydrodynamic diameter and size distribution of the particles dispersed in water were measured at room temperature with a particle analyzer, model NANOPHOX (Sympatec GmbH, Germany). The size and size distribution of the dried particles were measured with a cryogenic transmission electron microscope (cryo-TEM). For this purpose, a small droplet of an aqueous dispersion of the nanoparticles was placed on a perforated carbon film supported on a TEM copper grid held by tweezers. The drop was blotted with a piece of filter paper, resulting in the formation of thin films of 100–300 nm thickness within the micropores of the carbon-coated lace-like polymer layer supported on the grid. The specimen was subsequently plunged into a reservoir of liquid ethane cooled by liquid nitrogen to ensure its vitrification (rapid freezing) and to prevent ice crystal formation. The vitrified specimen was transferred under liquid nitrogen and mounted on a cryogenic sample holder cooled to −170 °C. All samples were observed under low-dose conditions. Vitrified samples were examined in an FEI T12 G2 Cryo-TEM operating at 120 kV and equipped with an Oxford CT-3500 cryo-holder system. Images were recorded with a Gatan US1000 high-resolution cooled CCD camera and processed with DigitalMicrograph version 3.3.1 software. The ramp-shaped optical density gradients in the background were digitally corrected. The thermal behavior of the P(MAA–MBAA) and P(MAA–MBAA)-Cl nanoparticles was measured by thermogravimetric analysis (TGA) (TGA/DSC 1 STAR^e System, Mettler Toledo, Switzerland). This analysis was performed with approximately 10 mg of dried sample under a nitrogen atmosphere (200 mL/min) at a heating rate of 10 °C/min.

2.3. Preparation of the cross-linked P(MAA–MBAA) nanoparticles

P(MAA–MBAA) nanoparticles of hydrodynamic sizes ranging from 18 ± 2 to 460 ± 60 nm were formed by surfactant-free dispersion copolymerization of the monomers MAA and MBAA in water as a continuous phase. In brief, P(MAA–MBAA) nanoparticles of 27 ± 3 nm hydrodynamic diameter were formed by dissolution of 4.4 g of MAA, 3.6 g of MBAA (2% w/v total monomers), and 240 mg of PPS in 400 mL of distilled water. The 1 L round-bottom flask containing this solution was stirred with a mechanical stirrer (200 rpm) at 100 °C for 1 h. The MAA and MBAA residues were subsequently removed from the nanoparticle aqueous dispersion by extensive dialysis against water. The dried P(MAA–MBAA) nanoparticles were obtained by lyophilization.

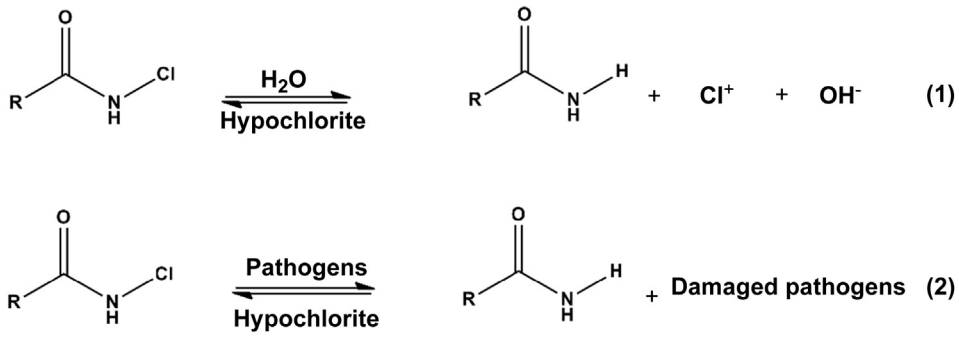


Fig. 1. Oxidative Cl transfer scheme from an amide-Cl to water and microorganisms.