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High-efficacy antibacterial polymeric micro/nano particles with N-halamine functional groups

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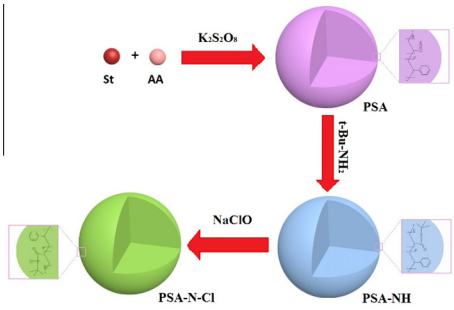
HIGHLIGHTS

- Design and synthesis of novel high-efficacy antibacterial polymeric micro/nano particles.
- The characterization of micro/nano particles.
- The good storage stability of antibacterial micro/nano particles.
- Low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values.

GRAPHICAL ABSTRACT



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ABSTRACT

Novel polymeric micro/nano particles (MNPs) containing amide functional groups, poly (styrene-co-N-(t-Bu)-acrylamide) (PSA-NH) MNPs, have been synthesized by a copolymerization of styrene and acrylic acid and then an amination with tert-butylamine. The as-synthesized PSA-NH MNPs were converted to antibacterial polymeric MNPs containing N-halamine groups, poly (styrene-co-N-(t-Bu)-N-chlorine-acryl amide) (PSA-N-Cl) MNPs, by a facile chlorination in diluted NaOCl solution. The as-prepared PSA-N-Cl MNPs were characterized by field emission scanning electron microscopy (FE-SEM), Brunauer–Emmet–Teller (BET) analysis, X-ray photoelectron spectra (XPS), and Fourier Transform infrared spectroscopy (FTIR). Antibacterial tests showed that the as-prepared PSA-N-Cl MNPs possess powerful antibacterial activity against both *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Minimum inhibitory concentration (MIC) values of PSA-N-Cl MNPs against *E. coli* and *S. aureus* were 0.20 μg/mL, whereas minimum bactericidal concentration (MBC) values of them against *E. coli* and *S. aureus* were 5.00 and 7.00 μg/mL, respectively. The storage stability test indicated that PSA-N-Cl MNPs are very stable under common storage conditions.

1. Introduction

Over the recent decades, microbial threats on human health and safety have become a serious public concern. In response to the wide spreading of infectious diseases, antibacterial materials that can effectively inhibit the growth of microorganisms have attracted significant research interests. Antibacterial compounds have been underlined by their broad applications in the fields of biomedicine, food packaging, and sterilization of hygienic areas [1]. These antibacterial compounds include quaternary ammonium salts [2–4], quaternary phosphonium salts [5], metal ions [6], N-halamines [7,8] etc. N-halamines containing one or more nitrogen-halogen covalent bonds are of great importance due to their inherent advantages such as excellent antibacterial efficacies, sta-

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bilities in aqueous solution and in dry storage [9], lack of corrosive surfaces [10], safety to humans [11], and relatively low expense [12,13]. Stable N-halamines are effective oxidizing agents which can oxidize the molecules on cell surfaces which are vital for cell survival [9]. Therefore, N-halamine chemistry has proved to be important in the development of effective antibacterial compounds [14,15].

The antibacterial mechanism of N-halamine materials involves the direct transfer of positive halogen from the N-halamine to bacterial cells, and the oxidative halogen has a strong tendency to participate in ionic reactions or combine with another element, thereby leading to destruction or inhibition of metabolic processes in microorganisms [16]. Therefore, antibacterial performances of N-halamine materials as contact biocides strongly depend on their activated surface areas. N-halamine materials with larger surface areas can provide more N-halamine functional sites to contact the bacteria. Nanometer-sized materials have shown remarkable potentials because those materials have large surface areas [17]. Therefore, for enhancement of antibacterial capacity, fabrication of N-halamine materials with nanostructure to enlarge activated surface area is advisable [18]. Recently, some antibacterial silica nanoparticles (NPs) grafted with N-halamine functional groups have been developed [1,19,20]. However, the contents of oxidative chlorines in those nanoparticles were comparatively low, which limit their applications.

In this paper, novel poly(styrene-co-N-(t-Bu)-N-chlorine-acryl amide) (PSA-N-Cl) MNPs were synthesized. The three-step synthetic route was shown in Fig. 1. Commercially available styrene and acrylic acid were copolymerized to form poly(styrene-co-acrylate acid) (PSA) MNPs which reacted with tert-butylamine (t-Bu-NH_2) to form the porous poly(styrene-co-N-(t-Bu)-acryl amide) (PSA-NH) MNPs. Finally, The PSA-NH was transferred to PSA-N-Cl structure by exposure to a dilute sodium hypochlorite solution. Successful preparation of PSA-N-Cl was evidenced by different techniques like FTIR and XPS. The antibacterial tests showed that the as-prepared PSA-N-Cl MNPs have excellent antibacterial activities against both Gram-positive and Gram-negative bacteria. MIC values of PSA-N-Cl MNPs against *Escherichia coli* and *Staphylococcus aureus* were 0.20 μg/mL, whereas MBC values of them against *E. coli* and *S. aureus* were 5.00 and 7.00 μg/mL, respectively, which are extremely lower than those of literature-reported N-halamine NPs [1,19]. The as-prepared high-efficacy antibacterial MNPs will have potential applications in many areas such as disinfection of hygienic areas, water purification, antibacterial paint, and food packaging.

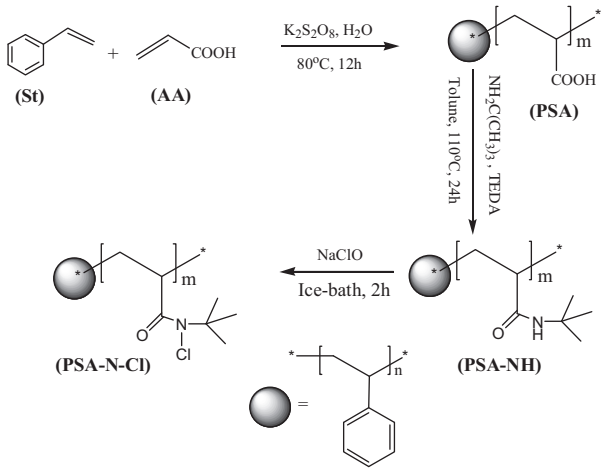

Fig. 1. The synthetic route for PSA-N-Cl MNPs.

2. Experimental section

2.1. Materials

Styrene (St), acrylic acid (AA), 1,4-diazabicyclo [2.2.2] octane (TEDA), tert-butylamine, potassium peroxydisulfate ($K_2S_2O_8$), were provided by Shanghai Aladdin reagent Co., Ltd. Toluene, hydrochloric acid (HCl), and concentrated sulfuric acid (H_2SO_4) were offered by Jiangsu Qiangsheng Chemical Co., Ltd. sodium hypochlorite (NaOCl) solution was obtained from Shanghai Chlor-Alkali Chemical Co., Ltd. 0.1 N Sodium thiosulfate aqueous solution ($Na_2S_2O_3$), monopotassium phosphate(KH_2PO_4), and disodium hydrogen phosphate ($Na_2HPO_4{\cdot}12H_2O$) were available from Shanghai Lingfeng Chemical Reagent Co., Ltd. Beef extract was purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. The bacteria employed were *S. aureus* ATCC 6538P (*S. aureus*) and *E. coli* O157:H7 ATCC 11229 (*E. coli*) (Shanghai Institute of Materia Medica of the Chinese Academy of Sciences).

2.2. Synthesis of poly(styrene-co-acrylate acid) (PSA) MNPs

0.72 g of AA was added to 43.2 mL of deionized water. After the added AA was completely dissolved, 1.44 g of styrene were added. The mixture was vigorously stirred for about 30 min at room temperature and then 0.032 g of $K_2S_2O_8$ was added. The reaction mixture was vigorously stirred at 80 °C for 12 h under nitrogen atmosphere [21]. After the reaction was over, the formed PSA MNPs were collected by centrifugation and then washed 3 times with distilled water. The wet PSA MNPs were dried under vacuum at 60 °C for 2 h.

2.3. Synthesis of poly (styrene-co-N-(t-Bu)-acryl amide) (PSA-NH) MNPs

1.00 g of PSA MNPs, 4 mL of t-Bu-NH_2 and 0.03 g of TEDA were added into 15 mL of toluene in a pressure reactor. The reaction mixture was stirred at 110 °C for 24 h [22,23]. The formed PSA-NH MNPs were collected by centrifugation and then washed 3 times with distilled water. The wet PSA-NH MNPs were dried under vacuum at 60 °C for 2 h.

2.4. Synthesis of poly (styrene-co-N-(t-Bu)-N-chlorine-acryl amide) (PSA-N-Cl) MNPs

About 0.40 g of PSA-NH MNPs was dispersed into 15 mL distilled water and then 20 g of 1.00% sodium hypochlorite solution were added dropwise with pH adjusted to 7.5. The mixture was stirred for 2 h in ice bath. The formed PSA-N-Cl MNPs were collected by centrifugation and then washed 4 times with distilled water. The wet PSA-N-Cl MNPs were dried under vacuum at 45 °C for 2 h.

2.5. Analytical titration procedure

For the determination of oxidative chlorine (Cl^+) content of PSA-N-Cl MNPs, a standard iodometric/thiosulfate titration procedure was employed [24]. For example, about 0.02 g of PSA-N-Cl MNPs was added in 80 mL of a 0.025 N H_2SO_4 solution. After addition of 0.10 g of KI and 0.20 mL of 0.5% of starch water solution as an indicator, the solution was titrated with 0.01 N of sodium thiosulfate until the blue color disappeared at the end point. The Cl^+ weight percent of PSA-N-Cl MNPs could then be determined using the following equations [25]:

$$Cl^+\% = \frac{N \times V \times 35.45}{2 \times W} \times 100\%$$