

Fabrication of monodisperse silica–polymer core–shell nanoparticles with excellent antimicrobial efficacy†

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Monodisperse nanoparticles with antimicrobial polymer shells were fabricated using a seeded copolymerization; they exhibited excellent antibacterial activities against gram-positive bacteria as well as gram-negative bacteria.

Versatile antimicrobial organic materials, including quaternary ammonium salts,¹ phosphonium salts,² and *N*-halamine compounds,³ have been studied for a wide range of applications such as disinfection of hygienic areas, water purification, and food packaging.⁴ In particular, *N*-halamine compounds containing one or more nitrogen–halogen covalent bonds are of great importance due to their inherent advantages such as long-term stability and high durability and regenerability.³ The antimicrobial mechanism of *N*-halamines involves the direct transfer of positive halogen from the *N*-halamine to bacterial cells. Since the halogen atom is one electron short of a full outer shell, it is highly reactive to gain an electron for a full electron shell. Therefore, the halogen has a strong tendency to participate in ionic reactions or to combine with another element, thereby leading to destruction or inhibition of metabolic processes in microorganisms.⁵ Considerable research efforts have been devoted to synthesizing various *N*-halamine polymers.³ In general, the antibacterial performances of *N*-halamine-based polymers strongly depended on the surface area and contact time. In this regard, it is expected that antibacterial nanostructures with high surface areas will provide enhanced efficacy compared with their bulk counterparts.

Recently, core–shell nanoparticles have received considerable attention due to their promising applications in the fields of materials science, optics, and biophysics.⁶ Major research efforts have been devoted to the development of diverse core–shell nanoparticles in order to provide the combined characteristics of the core and the shell. Typically, polymer shells on inorganic nanoparticles prevent particle–particle aggregation and offer excellent compatibility in a polymer matrix.⁷ Furthermore, functional shells such as conducting polymers, emissive polymers, and polyelectrolytes endow the core nanoparticles with versatile electrical, optical, and structural properties.⁸ Hence, the fabrication route to well-defined core–shell functional nanoparticles makes it possible to obtain desirable properties compared with either pristine core or shell component.

Although improvement of the performance of antibacterial agents has been recognized to be important, most studies have depended on the use of microspheres as supports to enhance the activated surface.⁹ There has been limited information concerning biocidal polymer nanoparticles. Herein, we report the facile fabrication of silica–poly(3-allyl-5,5-dimethylhydantoin-*co*-methyl methacrylate) (poly(ADMH-*co*-MMA)) core–shell nanoparticles as a biocidal polymeric agent using a seeded polymerization. Colloidal silica nanoparticles of three different diameters (7, 12, and 22 nm) were used for the core material and were encapsulated with poly(ADMH-*co*-MMA) to improve the antibacterial performance against *Escherichia coli* (*E. coli*) as a typical gram-negative bacterium and *Staphylococcus aureus* (*S. aureus*) as a gram-positive bacterium.

The overall synthetic procedure for the fabrication of silica–poly(ADMH-*co*-MMA) core–shell nanospheres is illustrated in Fig. 1. The silica nanoparticles were dispersed in distilled water. ADMH was dissolved in methyl methacrylate (MMA) monomer, and the mixture was added dropwise into the colloidal silica solution. The ADMH–MMA mixture is adsorbed on the surface of silica nanoparticles due to their hydrophobic properties. The solution was continuously stirred to prevent particle–particle aggregation, and then ceric ammonium nitrate (CAN) was added into the solution for the redox-initiated radical polymerization of ADMH with MMA.¹⁰ In general, allylic monomers undergo so-called “autoinhibition” during radical polymerization, which is ascribed to the high resonance stability of the allylic radicals generated.¹¹ Therefore, the allylic structure of ADMH prevents chain propagation reactions due to its autoinhibition mechanism during polymerization, inevitably resulting in low molecular-

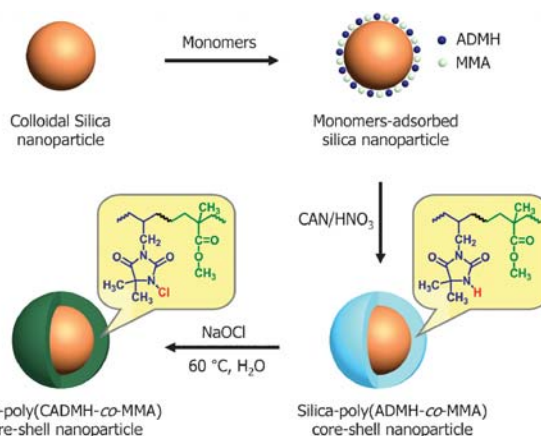


Fig. 1 Schematic illustration of the fabrication of silica–poly(CADMH-*co*-MMA) core–shell nanoparticles.

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weight products. On the other hand, methyl methacrylate and methacrylonitrile monomers are favorable to radical polymerization because ester and nitrile substituents stabilize the radicals and simultaneously enhance the reactivity of the monomers toward chain propagation reaction. In this regard, ADMH was copolymerized with methyl methacrylate to form a stable polymer shell on the silica core. After the polymerization, silica-poly(ADMH-co-MMA) was precipitated from the solution and the resulting product was dried at 30 °C. Halogenated derivatives of 3-allyl-5,5-dimethylhydantoin (ADMH) belong to the class of cyclic *N*-halamines, which can provide great stability in a wide range of temperatures and pH values.³ The hydantoin moieties of the core-shell nanoparticles were transformed into *N*-halamine structures (1-chloro-3-alkyl-5,5-dimethylhydantoin: CADMH) by immersion in sodium hypochlorite solution. Since the *N*-halamine structure has no α -hydrogen next to the halamine bond, the release of hydrochloric acid as a result of an elimination reaction of α -hydrogen and chlorine on the *N*-halamine (N-Cl) bond is prevented.

Fig. 2 presents field-emission scanning electron microscopy (FE-SEM) images of the resulting core-shell nanoparticles and histograms showing particle size distribution. Spherical nanoparticles with fairly uniform diameters were exclusively observed, and there was no serious particle-particle aggregation. The nanoparticles had increased diameters (11, 16, and 26 nm) compared with those (7, 12, and 22 nm) of the silica core (see the histograms). Transmission electron microscopy (TEM) observation also provided typical core-shell images, as shown in the inset images. These results mean that the silica core was successfully encapsulated with a thin polymer and the sizes of the core-shell nanoparticles were controlled by the diameter of the silica core.

To further ascertain the encapsulation of poly(CADMH-co-MMA) on the silica core, zeta-potential analyses of the nanoparticles were also performed at pH 7. The zeta-potential of silica nanoparticles showed a negative value (−50.3 mV) due to the negatively charged −OH groups on the silica surface. In the case of silica-poly(CADMH-co-MMA) core-shell nanoparticles, the zeta-potential value was −5.3 mV, indicating that negatively charged sites on the silica surface were protected by the coated poly(CADMH-co-MMA) shell.

The biocidal efficacy of silica-poly(CADMH-co-MMA) core-shell nanoparticles was examined for *E. coli* as a typical gram-negative bacterium. The antibacterial activity was evaluated from the viewpoint of copolymer concentration using the spread

plate method. This method involves plating *E. coli* cells on nutrient agar incubated at 37 °C for 24 h, followed by counting the number of viable colonies. As shown in Fig. 3a, the antimicrobial activity increased as the concentration of the silica-poly(CADMH-co-MMA) core-shell nanoparticles increased. It was found that silica-poly(CADMH-co-MMA) core-shell nanoparticles of ca. 10 mg mL^{−1} could completely inactivate the bacteria for 30 min. The antimicrobial activity experiments were also carried out in aqueous suspensions containing 2 × 10⁹ colony forming unit (CFU) mL^{−1} of *S. aureus*, a gram-positive bacterium (Fig. 3b). Silica-poly(CADMH-co-MMA) core-shell nanoparticles had higher biocidal efficacy against *S. aureus* than *E. coli*. Typically, 11 nm-diameter core-shell nanoparticles presented a total kill of *S. aureus* at a concentration of 3 mg mL^{−1}. On the other hand, the lowest concentration for a total kill of *E. coli* was 5 mg mL^{−1}. This result is likely attributable to the different cell structures of gram-positive and gram-negative bacteria. The lipid bilayer cell wall of gram-positive bacteria is mostly covered by a porous peptidoglycan layer, which does not exclude most antibacterial agents. The cell wall of gram-negative bacteria also contains ca. 20% peptidoglycan. However, gram-negative bacteria are surrounded by two membranes, and the outer membrane acts as an efficient permeability barrier because it includes lipopolysaccharides and porins.^{9a} For this reason, it is considered that gram-positive bacteria would be more vulnerable than gram-negative bacteria against antibacterial agents. The bacterial concentration (2 × 10⁹ CFU mL^{−1}) used for evaluating biocidal activities was three to four orders of magnitude higher compared to the concentrations used in previous studies.³ Nevertheless, the antibacterial performance of the core-shell nanoparticles was considerably higher than that of other existing antibacterial agents.^{3,9} Accordingly, it is evident that silica-poly(CADMH-co-MMA) core-shell nanoparticles are highly effective against various bacterial species. It is also important to note that the biocidal efficacy was strongly dependent on the size of the core-shell nanoparticles. As the nanoparticle size decreases, the surface area becomes larger. The BET surface areas of 11 nm, 16 nm, and 26 nm diameter core-shell nanoparticles were measured to be 284, 190, and 125 m² g^{−1}, respectively. The feeding amount of ADMH was approximately 3 mol% relative to the amount of MMA. Based on the BET data, the maximum values of effective *N*-halamine sites on the nanoparticle surface are calculated to be 54.8, 36.5, and 24.9 μ mol g^{−1} for 11 nm, 16 nm, and 26 nm diameter core-shell nanoparticles, respectively. Therefore, the

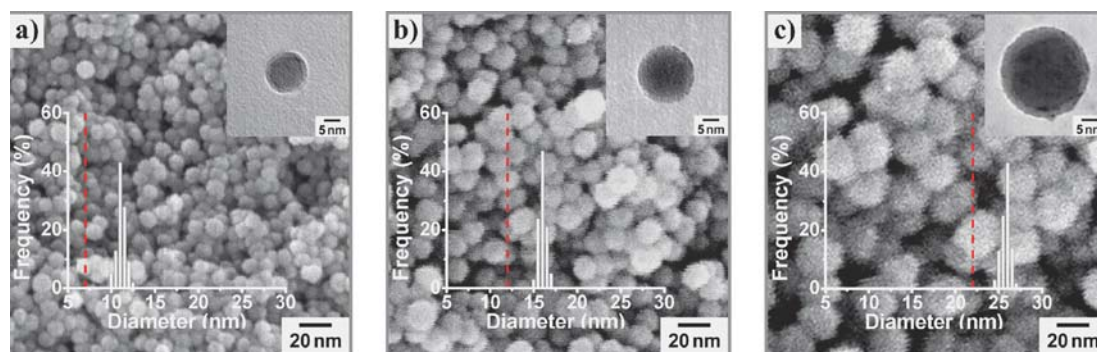


Fig. 2 FE-SEM images and size distribution histograms of core-shell nanoparticles with different diameters (insets: TEM images of a single core-shell nanoparticle): (a) 11 nm, (b) 16 nm, (c) 26 nm. In the histograms, the dotted line indicates the diameter of the silica core used.

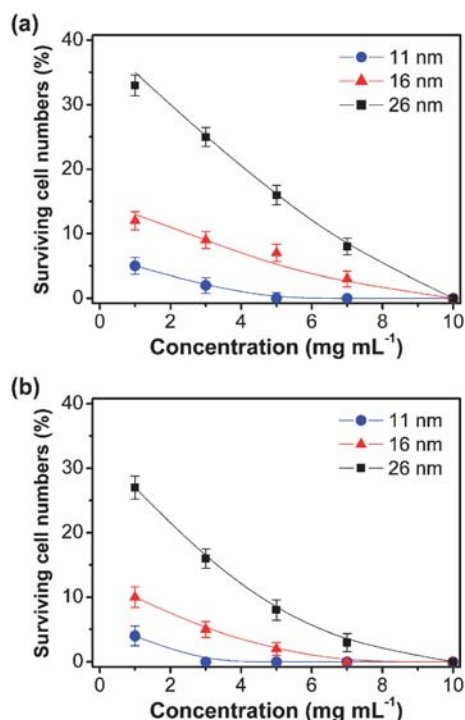


Fig. 3 Antimicrobial activities of silica-poly(CADMH-co-MMA) core-shell nanoparticles with different diameters as a function of particle concentration (contact time 30 min): (a) *E. coli* and (b) *S. aureus*.

nanoparticles with smaller diameters were capable of providing more *N*-halamine functional sites on the surface of core-shell nanoparticles and this *N*-halamine increment would be responsible for the enhanced efficiency of biocidal activity.

When a suspension of bacteria is exposed to antimicrobial agents, the contact time is one of the major factors in killing the microorganisms. Table 1 summarizes the antibacterial activity of silica-poly(CADMH-co-MMA) core-shell nanoparticles against *E. coli* as a function of different contact times. As a control, the bulk powder of poly(CADMH-co-MMA) was prepared by the same polymerization method without colloidal silica nanoparticles. The bulk counterpart was able to kill all the bacteria after 120 min exposure. However, 11 nm-diameter silica-poly(CADMH-co-MMA) core-shell nanoparticles were able to cause complete inactivation of 97% bacteria within a contact time of 1 min. Photographs of the culture plates visualize the survival rate of *E. coli* upon contact with the core-shell nanoparticles of different diameters (Fig. S2, ESI†). *E. coli* colonies are observed as small white dots. At the same contact time (30 min), the population of white dots distinctly increased in the order of 11 nm < 16 nm < 26 nm < bulk. As mentioned previously, the high surface area of the nanoparticles could be responsible for the enhanced antibacterial activity of silica-poly(CADMH-co-MMA) core-shell nanoparticles.

In conclusion, silica-poly(CADMH-co-MMA) core-shell nanoparticles of three different diameters were fabricated by a seeded polymerization. The silica-poly(CADMH-co-MMA) core-shell nanoparticles had uniform copolymer shells with a thickness of about 2 nm. The core-shell nanoparticles showed ca. 4 times faster biocidal activity than the bulk powder.

Table 1 Antibacterial activities of silica-poly(CADMH-co-MMA) core-shell nanoparticles (5 mg mL⁻¹) against *E. coli*^{a,b,c}

Contact time/min	Number of viable colonies (%)			
	11 nm	16 nm	26 nm	Bulk
1	3	57	84	100
30	NS	7	13	100
60	NS	NS	NS	73
120	NS	NS	NS	NS

^a Antimicrobial properties were tested according to the spread plate method, which involved plating *E. coli* cells on nutrient agar incubated for 24 h at 37 °C and counting the number of viable colonies. ^b The original *E. coli* concentration was 2 × 10⁹ CFU mL⁻¹. ^c NS: none surviving.

In addition, the biocidal efficacy of silica-poly(CADMH-co-MMA) nanoparticles increased with decreasing diameter of the core-shell nanoparticles. Namely, the smaller nanoparticles intrinsically had more active sites to contact bacteria and thus provided enhanced antibacterial activities. Importantly, this synthetic methodology for silica-polymer core-shell nanosystems might be expanded to allow the fabrication of novel metallic and inorganic polymer core-shell nanomaterials.

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