

# Supporting Information

## UV-irradiation Induced Templated/In-Situ Formation of Ultrafine Silver/Polymer Hybrid Nanoparticles as Antibacterial

Mengjun Chen, Yining Zhao, Wantai Yang, Meizhen Yin\*

State Key Laboratory of Chemical Resource Engineering, Key Laboratory of Carbon Fiber and Functional Polymers, Beijing University of Chemical Technology, 100029 Beijing, China. E-mail: yinmz@mail.buct.edu.cn

### Abbreviation

Ag	Silver
Ag <sup>+</sup>	Silver ion
Ag(0)	Silver elementary substance
AOT(Aerosol OT)	Sodium bis(2-ethylhexyl) sulfosuccinate
APS	Ammonium peroxodisulfate
CFU	Colony forming units
DMAA	<i>N,N</i> -dimethylacrylamide
EDS	Energy disperse spectrum
HR-TEM	High resolution-transmission electron microscope
IME	Inverse microemulsion
IR	Infra-red
MBA	<i>N,N'</i> -methylenebisacrylamide
NP	Nanoparticle
PBS	Phosphate buffer solution
TMEDA	<i>N,N,N',N'</i> -tetramethylethylenediamine
UV	Ultraviolet

## Experimental Section

### Materials

DMAA (Alfa Aesar, 99.5%), AOT (Alfa Aesar, 96%), TMEDA (Beijing Chemical Reagents Company, 99.0%), AgNO<sub>3</sub> (Beijing Chemical Works, 99.8%) and hexane (Beijing Modern Eastern Finechemical, 95%) were used without further purification. MBA (Sinopharm Chemical Reagent Co., Ltd, 98%) and APS (Sinopharm Chemical Reagent Co., Ltd, 98%) were recrystallized from acetone and water in sequence.

### Characterization

**IR** spectra were recorded on a Nicolet Nexus 670 IR spectrometer with KBr pellets at a scan resolution of 4 cm<sup>-1</sup>. High resolution transmission electron microscopy (**HRTEM**) images and energy disperse spectrum (**HRTEM/EDS**) figures were obtained with a JEOL JEM-3010 high resolution transmission electron microscope operating at an acceleration voltage of 200 kV. Optical characterizations were carried out by **fluorescence microscope** with Olympus IX-81 microscope and **UV-Vis** spectroscopy with a GBC Centra 20 spectrophotometer, using a 0.1 cm path length quartz cuvette. The **fluorescence spectroscopic** studies were performed on a fluorescence spectrophotometer (Horiba Jobin Yvon FluoroMax-4 NIR, NJ, USA) at room temperature (25 °C).

### Synthesis of ultrafine polymeric PDMAA nanoparticles

**General Procedure:** The IME polymerization of DMAA emulsified by sodium bis(2-ethylhexyl) sulfosuccinate (AOT) was carried out in *n*-hexane by using *N,N'*-methylenebisacrylamide (MBA) as the cross-linking agent, with redox initiated system of ammonium peroxydisulfate (APS)/ *N,N,N',N'*-tetramethyl-ethylenediamine (TMEDA).

AOT (5.6 g, 12.6 mmol) was dissolved in *n*-hexane (20 mL). To the solution DMAA (557 mg, 5.6 mmol) and MBA (45 mg, 0.3 mmol) were added, which formed an anhydrous inverse microemulsion system. When extra water was added into the system, an aqueous solution of the monomer was used in inverse microemulsion. After well dispersed by ultrasound, the mixture was then stirred at 25°C for 30 min under N<sub>2</sub> atmosphere. Subsequently, APS (100 mg/mL, 10 μL) and TMEDA (10 μL) were added to initiate the polymerization. The polymerization was carried out at 35°C for 12 hr. Finally extra solvent (*n*-hexane) was added to accelerate the precipitation of the product particles. The white solid product was collected

by centrifugation, rinsed with *n*-hexane for several times and dried under vacuum. By altering the water content in the system, PDMAA NPs ranging in size from 2.6 nm to 10 nm can be obtained.

### **Synthesis of Ag/PDMAA hybrids with polymeric PDMAA nanoparticles as templates**

Obtained template PDMAA nanoparticles (8 mg) were dispersed in 10 mL ethanol, while 200  $\mu$ L aqueous AgNO<sub>3</sub> (0.04 mol/L) was dropped into the dispersion. The system was stirred for 30 min under N<sub>2</sub> atmosphere and then was exposed under UV-light irradiation (25 w/m<sup>2</sup>) for varied time.

### **In-situ formation of Ag/PDMAA hybrids via inverse microemulsion polymerization**

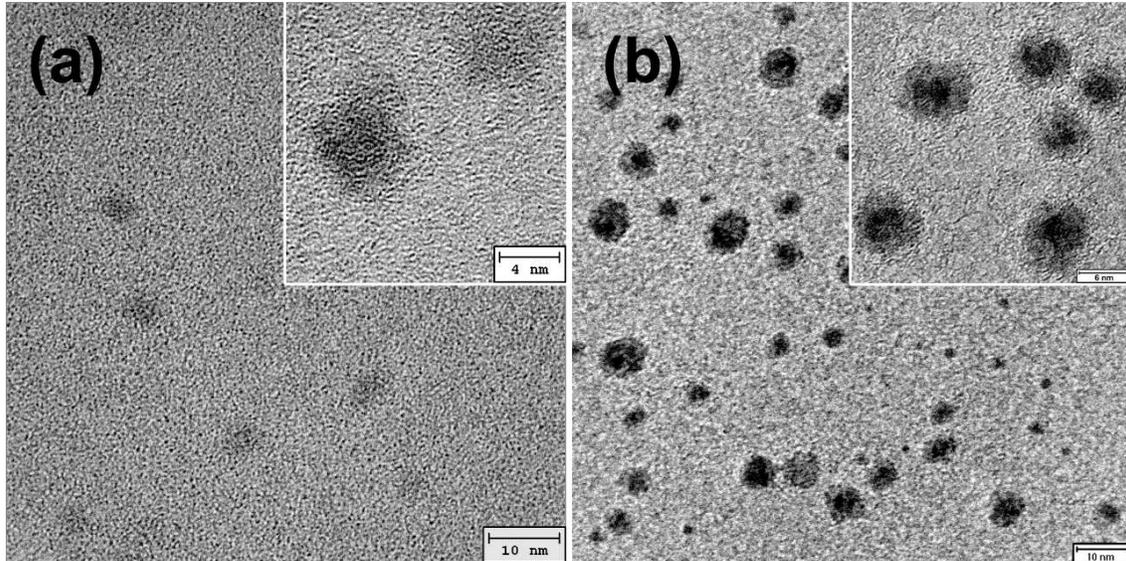
AOT (5.6 g, 12.6 mmol) was dissolved in *n*-hexane (20 mL). To the solution DMAA (557 mg, 5.6 mmol), MBA (45 mg, 0.3 mmol), varied amount of aqueous AgNO<sub>3</sub> (0.04 mol/L) were added to adjust the *w* (n(water)/n(surfactant)) of the system. After well dispersed by ultrasound, the mixture was then stirred at 25°C for 30 min under N<sub>2</sub> atmosphere. Subsequently, APS (100 mg/mL, 10  $\mu$ L) and TMEDA (10  $\mu$ L) were added to initiate the polymerization. The polymerization was carried out at 35°C for 12 hr. Finally extra solvent (*n*-hexane) was added to accelerate the precipitation of the product particles. The brown solid product was collected by centrifugation, rinsed with *n*-hexane for several times and dried under vacuum.

### **Antibacterial activity of Ag/PDMAA hybrids**

The dynamic shake flask method has been used to assess the antibacterial ability of the Ag/PDMAA hybrid NPs. A colony of Gram negative *E. coli* bacteria was cultivated in Luria-Bertani broth (containing 10 g/L peptone, 10 g/L sodium chloride, and 5 g/L yeast extract) at 37 °C, shaking at 160 rpm for 24 hr. The bacteria were diluted with 0.1 mol/L phosphate buffer solution (PBS, pH=7) to the desired concentration. The as-prepared Ag/PDMAA hybrids (10 mg/mL in PBS, pH=7) were immersed in the bacterial suspension with the volume ratio of 2% and then shaken at 37°C for 2 hr. A certain amount of suspension was taken, diluted appropriately, and plated on L-agar plates for 48 hr incubation. Theoretically, each surviving bacterium develops into a distinct colony after incubation and the number of viable bacterium was then determined as colony forming units (CFU), thus providing a direct

measure of bacterial viability.

All the above-mentioned materials and apparatus were treated with high-temperature sterilization at 121°C for 30 hr to ensure the aseptic condition.



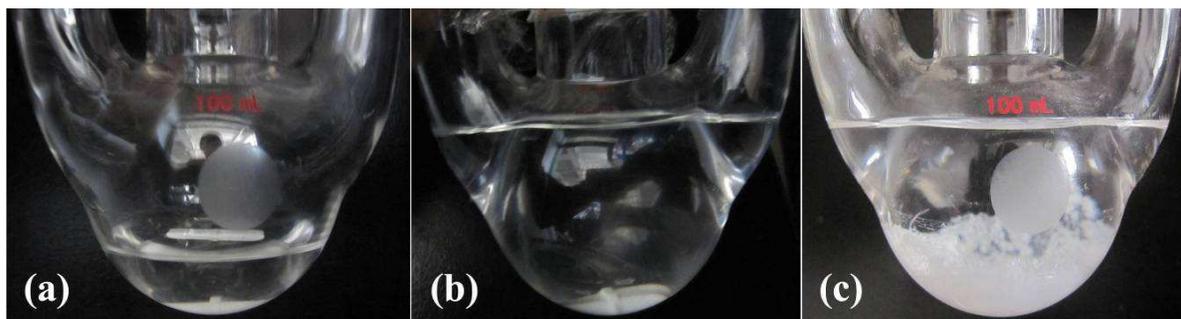
**Fig. S1** (a) HRTEM images of PDMAA NPs produced via IME polymerization, with sizes of 6.4 nm, scale bar is 10 nm. (b) HRTEM images of Ag/PDMAA NPs obtained through template method by applying template PDMAA NPs with sizes of 6.4 nm.

### Auto-precipitation phenomenon of in-situ formation method

We have reported the auto-precipitation of PDMAA NPs in IME system, and the mechanism was well interpreted in the previous communication<sup>1</sup>.

“We assumed that, the produced hydrophilic polymeric nanoparticles aggregated with each other after polymerization due to the flocculation performance of PDMAA, and the aggregation led to the rearrangement and departure of the surfactants surrounding the polymeric nanoparticles. When the aggregates reached a certain size, they couldn't be stabilized by surfactants anymore and then precipitated from the nonpolar phase (n-hexane).”

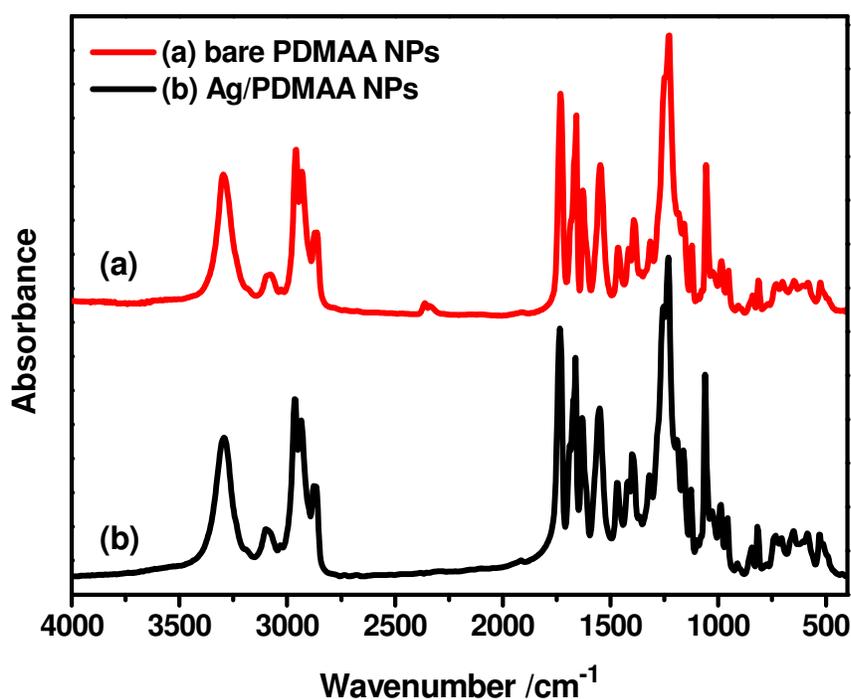
As mentioned above, the auto-precipitation of such system was caused by the flocculation property of the monomer (DMAA). Even with the content of Ag in the reaction system, the auto-precipitation of Ag/PDMAA nanoparticles could be observed (*Fig. S2*).



**Fig. S2** Digital photographs of auto-precipitation phenomenon during in-situ formation of Ag/PDMAA hybrids. (a) IME system after polymerization of 12 hr. (b) Extra *n*-hexane was added into the system right after polymerization of 12 hr. (c) After a certain time, flocculent precipitate of Ag/PDMAA hybrids separated out spontaneously from the polymerization system.

IR spectrum of PDMAA nanoparticles (*Fig. S3(a)*) showed an absorption peak at  $\sim 3300\text{ cm}^{-1}$ , which should be shown only in secondary amine, corresponded to the bending vibration of N-H in MBA. The unique characteristic absorption peak of tertiary amine was observed as a doublet at  $1660\text{-}1620\text{ cm}^{-1}$  that was assigned to stretching vibrations of C=O, which was usually known as amide I band. Amide II band at  $1548\text{ cm}^{-1}$  was observed to be dependent upon the effect of coupling between bending vibration of N-H and stretching vibrations of C-N. Its intense overtone band appeared at  $3080\text{ cm}^{-1}$ . The strong bands near  $1228\text{ cm}^{-1}$  corresponds to the stretching vibrations of C-N.

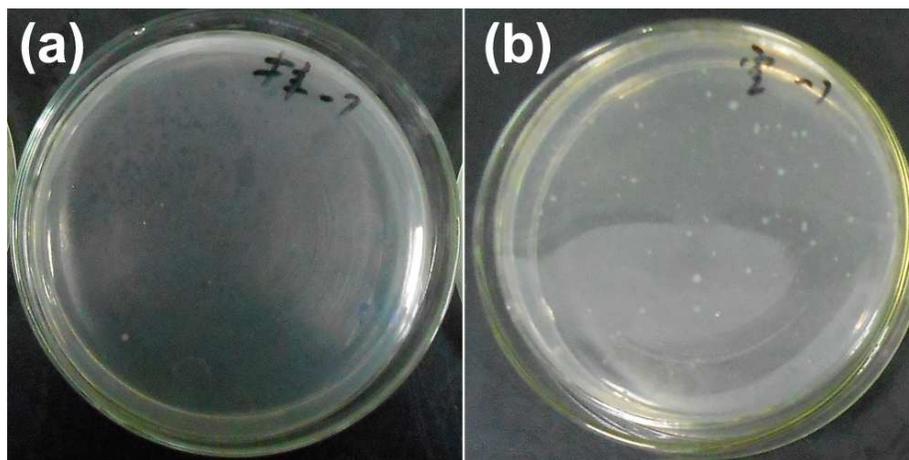
In *Fig. S3(b)*, IR spectrum of Ag/PDMAA hybrids prepared via in-situ formation method showed the same peaks as that of bare PDMAA NPs, which indicating the successful polymerization of DMAA in this procedure.



**Fig. S3** IR spectrum of (a) bare PDMAA NPs prepared under  $w=5$  by adding deionized water; (b) Ag/PDMAA NPs prepared under  $w=5$  by adding aqueous  $\text{AgNO}_3$  (0.04 mol/L). The sample powder (1-2 mg) was mixed with dry KBr (200 mg) and then compacted into a tablet for IR measurement.

Antibacterium of Ag/Hybrid NPs obtained from template method was assayed by contacting with viable Gram negative bacteria *E. coli* in suspension. Obviously, by treated with 2 % of the produced Ag/PDMAA hybrids (obtained from template method) at a concentration of 10 mg/mL, nearly no bacterial colony was observed on the plate incubated with bacteria suspension, while the control plate was covered with significant bacterial colonies ( $3.6 \times 10^5$  CFU).

The result indicated that Ag/PDMAA NPs prepared from template method also perform well as antibacterial.



**Fig. S4** Photographs of L-agar plates onto which *E. coli* suspension of PBS (pH=7) contained (a) Ag/PDMAA hybrids obtained from template method (hybrids content:  $10 \text{ mg/mL} \times 2\% = 0.2 \text{ mg/mL}$ ) and (b) no hybrids were deposited and incubated for 48 hr.

### Reference

1. Chen, M.; Yang, W.; Yin, M., Size-controllable synthesis and functionalization of ultrafine polymeric nanoparticles. *Small* **2013**, 9 (16), 2715-2719.