Materials Science and Engineering C 76 (2017) 73–80

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Synthesis, characterization and antibacterial study on the chitosan-functionalized Ag nanoparticles

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ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 23 February 2017
Accepted 25 February 2017
Available online 28 February 2017

Keywords:
Hydrothermal synthesis
Chitosan
Ag nanoparticles
Nanotriangle
Antibacterial activity

ABSTRACT

This study provided a facile, one-step hydrothermal method to synthesize stable Ag colloid in aqueous solution by utilizing chitosan as both reductant and stabilizer. The formation of chitosan-functionalized Ag nanoparticles was verified by UV–Vis, FTIR, TEM, AFM and XRD measurements. FTIR results revealed that the primary amine groups and amide groups of chitosan have specific interactions with the surface of Ag nanoparticles. The average diameter of the Ag nanoparticles is 10.0 ± 5.4 nm as determined by TEM. Ag nanoparticles are highly crystalline as revealed by HR-TEM and XRD measurements. The size and shape of Ag nanoparticles are also found to depend on the pH condition in the synthesis. Ag nanoparticles were the main products at pH 5.0 whereas large Ag nanotriangle and truncated triangular nanoplate were dominant at pH 4.0 in the synthesis. Due to its monodispersity and good stability, the chitosan-functionalized Ag colloid synthesized at pH 5.0 was further tested for its antibacterial activities against gram-positive bacteria, gram-negative bacteria and fungus. The results of zone of inhibition, inhibition ratio and SEM characterization revealed that chitosan-functionalized Ag nanoparticles have great bactericidal efficiency against both bacteria and fungus.

1. Introduction

Synthesis of different Ag nanomaterials has been an active research area in recent years, because Ag nanomaterials have potential applications in catalysis [1], biodiagnostics [2], bactericide [3], biological labeling, imaging, and sensing [4–6]. It has been revealed that the physicochemical properties of Ag nanomaterials depended on their size, shape and surface capping agent [7,8]. Functionalized Ag nanomaterials with different polymer shells are of great interest owing to their good stability, superior electro-optical properties and biological applications. A number of polymers such as poly(vinylpyrrolidone) (PVP) [7], hyperbranched polyethylenimine (PEI) [9], amine-terminated hyperbranched poly(amidoamine) (HPAMAM-NH_2) [10], poly(propyleneimine) dendrimer (PPI) [11] have been explored as shells or capping agents of Ag nanomaterials. In order for the biological applications, the polymer shell of Ag nanomaterials with good biocompatibility or biopolymer is preferential to be used.

Chitosan, a linear polysaccharide composed of random repeating units of β-(1,4)-linked 2-amino-2-deoxy-D-glucan and 2-acetamido-2-deoxy-D-glucan, is mainly obtained from the partial *N*-deacetylation of chitin under alkaline conditions [12]. Chitin is the second most abundant natural polymer after cellulose and estimated to be produced annually almost as much as 10 billion tons. As an abundant natural cationic biopolymer with outstanding biological and chemical properties such as biodegradability, biocompatibility, bioactivity [13], chitosan has been used in various research fields such as in gene and drug delivery [14], for constructing biosensors [15], antibacterial film [16] and modification of nanoparticle surfaces [17].

Functionalized Ag nanomaterials with chitosan have received considerable attention due to their potential applications in biological labeling, imaging, sensing and antibacterial field [4]. So far, there are a few studies on the synthesis of chitosan-modified Au and Ag nanomaterials and nanocomposites [4,18–22]. However, the shape, morphology and crystalline of the obtained Ag nanomaterials in these studies were varied significantly with the synthetic methods. For instance, chitosan-modified popcorn-like Au–Ag nanoparticles (CSPNPs) with diameter of 50 nm to 120 nm can be synthesized by ascorbic acid reduction method [4]. Under quiescent conditions, a common heating process at 45–95 °C can produce Ag and Au nanoparticles on the basis of chitosan

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http://dx.doi.org/10.1016/j.msec.2017.02.154
0928-4931/

as a mediator agent [19]. However, the obtained Ag nanoparticle tended to aggregate especially the relatively high concentration of silver salts was used for the synthesis [19]. Chitosan-functionalized Ag nanomaterials with high crystalline and good stability are still demanded for further exploiting their applications.

Hydrothermal synthesis refers to the preparation by chemical reactions of substances in a sealed heated solution above ambient temperature and pressure [23]. Hydrothermal process can produce high temperature and high pressure, which has been proved to be efficient to promote some redox reactions. Hydrothermal synthesis has been successful used for the preparation of different nanomaterials such as metal oxides, quantum dots, complex oxide ceramics, and magnetic materials [23–25]. Recently, our group has successfully synthesized the Ag nanoparticles modified by amino acid and hyperbranched polyethylenimine (PEI) by hydrothermal synthesis method [9,26]. These studies inspired the further application of hydrothermal methods in the Ag nanomaterial synthesis. In this study, we explored hydrothermal synthesis of chitosan functionalized Ag nanomaterials and their antibacterial activities.

2. Materials and methods

2.1. Materials

Chitosan (CAS number: 9012-76-4, average molecular weight 310 kDa) used in the present work was purchased from Sigma-Aldrich Co. with a degree of deacetylation of 75–85%. 0.7% (m/v) chitosan solution was prepared by dissolving the pure chitosan in 1% (v/v) acetic acid solution. Other chemical reagents including ammonia water (28%), acetic acid (AR), $AgNO_3$ (GA) were used without further purification. *Escherichia coli* (ATCC8739), *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (ATCC 10231) were purchased from Heilongjiang Provincial Academy of Sciences Institute of Applied Microbiology (Harbin, China). They were kept on an agar slant nutrient medium at 4 °C before use. Ultra pure water was used throughout this study and its resistivity was >18 MΩ cm.

2.2. Synthesis of chitosan-functionalized Ag nanomaterials

In a typical experiment, 10 ml of 0.7% of chitosan solution was added into a beaker, then 25 ml pure water was added and the solution pH was adjusted by ammonia water to 5.0. 1 ml of 1% $AgNO_3$ solution was then added and pH of the mixed solution was further adjusted by ammonia water and HNO_3 to 5.0. Finally, appropriate volume pure water was added to obtain a 40 ml solution and then kept in dark >40 min. The final solution was transferred into a Teflon-lined autoclave of 50 ml, and then sealed and maintained at 140 °C for 4 h. A yellow solution was obtained, which indicated that chitosan-functionalized Ag nanoparticles were produced. As for obtaining Ag nanotriangle and truncated triangular nanoplate, the same procedure was performed as described above except that the solution pH was adjusted to 4.0 in the synthesis.

2.3. Characterization of Ag nanoparticles

2.3.1. UV–Vis absorption spectroscopy

UV–Vis absorption spectra of Ag colloid solution were measured by UV-5500 spectrophotometer (Metash). 4 ml of dilute colloid solution was added into a quartz cuvette for the measurement.

2.3.2. Fourier transform infrared spectroscopy

FTIR spectra were measured by IR Affinity-1(Shimadzu) using KBr pellet method. The sample was prepared by mixing the precipitate of the Ag nanoparticles (derived from the centrifugation of the Ag colloidal solution) with a small amount of solid KBr. FTIR spectra of pure chitosan was also measured for comparison.

2.3.3. Transmission electron microscopy

The produced Ag nanomaterials were imaged using JEM-2100 transmission electron microscope (JEOL Ltd.) at 200 kV. The sample for TEM characterization was prepared by placing 5 μl of the as-synthesized Ag colloid solution on a carbon coated copper grid and dried at room temperature.

2.3.4. X-ray diffraction

Powder X-ray diffraction patterns were record using D/MAX 2200VPC (40 kV/40 mA). The sample for XRD determination was derived from centrifugation of the Ag colloidal solution.

2.3.5. Atomic force microscopy

50 μl of the as-synthesized Ag colloid solution were dropped onto a freshly cleaved HOPG surface and then rinsed by a copious of pure water. The AFM samples were dried under a flow of pure Ar gas and stored in a desiccator prior to AFM imaging. A PicoPlus II AFM system from Molecular Imaging Inc. was used. AFM images were obtained at 512 × 512 resolution.

2.4. Antibacterial tests

Antibacterial activity of Ag nanomaterials has been investigated against *E. coli* as the model gram-negative bacteria, *S. aureus* as the model gram-positive bacteria and *C. albicans* as the model fungus using a standard disc diffusion method. *E. coli* and *S. aureus* were refreshed from the nutrient agar to nutrient broth at 37 °C, and *C. albicans* in YPD broth at 28 °C. Ag nanoparticles and control samples impregnated antibiotic discs were prepared by loading the filter papers (6 mm diameter). 10^8 cfu/ml bacterial suspensions including *E. coli*, *S. aureus* and *C. albicans* were inoculated on the agar plates under sterile condition. Chitosan-Ag nanoparticles and control discs were gently placed onto the top of agar. After 24 h of incubation at 37 °C for *E. coli* and *S. aureus*, and 28 °C for *C. albicans*, zone of growth inhibition around each sample was captured by digital camera. Ag colloid by $NaBH_4$ reducing method [27] (the Ag colloid solution was prepared by the reduction of $AgNO_3$ using sodium borohydride in an ice bath), $AgNO_3$ solution, chitosan solution and penicillin were used as the control samples.

Quantitative antibacterial experiments were carried out by determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Two-fold (1:2) dilutions of Ag colloid in concentration ranging from 158.8 μg/ml to 0.31 μg/ml were prepared by using sterile distilled water and then transferred into 96-well microtiter plates. Freshly prepared microbial suspensions

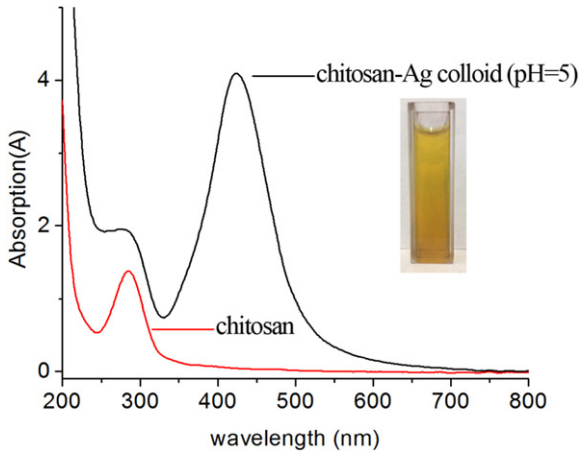

Fig. 1. UV–Vis absorption spectrum of the obtained Ag colloid at pH 5.0 in a typical experiment. UV–Vis absorption spectrum of pure chitosan without $AgNO_3$ after a hydrothermal process was also shown as a reference.