Advanced Powder Technology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Advanced Powder Technology

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

Original Research Paper

A facile method to prepare size-tunable silver nanoparticles and its antibacterial mechanism

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ARTICLE INFO

Article history:
Received 14 August 2017
Received in revised form 20 November 2017
Accepted 21 November 2017
Available online xxxx

Keywords:
Size-tunable silver nanoparticles
Preparation
Structure characterization
Antibacterial mechanism

ABSTRACT

The antibacterial effect of silver nanoparticles (denoted as Ag NPs) is closely related to size. This could partly explain why size controllable synthesis of Ag NPs for bactericidal application is drawing much attention. Thus, we establish a facile and mild route to prepare size-tunable Ag NPs with highly uniform morphologies and narrow size distributions. The as-prepared Ag NPs with averaged sizes of 2, 12 and 32 nm were characterized by transmission electron microscopy (TEM), ultraviolet–visible absorption spectroscopy (UV–vis), X-ray powder diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The antimicrobial effect of the as-prepared Ag NPs with different particles size was assessed by broth dilution and disk diffusion as well as measurement of optical density (OD_{600}). Moreover, their antibacterial mechanism was discussed in relation to morphology observation of microorganism by scanning electron microscopy (SEM) and to concentration detection of Ag^+ by stripping voltammetry. It was found that the parameters such as reactant molar ratio, reaction time, dropping speed, and most of all, pH of the reactant solutions, have significant influences on size-regulation of Ag NPs. The as-prepared Ag NPs exhibit excellent antibacterial properties, and their antimicrobial activities increase with decreasing particles size. Besides, two kinds of mechanisms, i.e., contact action and release of Ag^+, are responsible for the antimicrobial effect of Ag NPs.

1. Introduction

The ability to control the size of nanoparticles (NPs) is critical to their applications in engineering [1–7]; and metal NPs, particularly those of noble metals (e.g., Ag and Cu) with strong antibacterial effect are of significance for reducing and/or eliminating bacteria-related infections [8–13]. This is because metal NPs with a high surface-to-volume ratio often exhibit peculiar and greatly improved physical, chemical and biological properties as compared with the macro-sized counterparts [14–19]. Some researchers have found that the antimicrobial activity of metal NPs is highly dependent on size, which is why they have made enormous efforts to achieve size-controllable preparation of metal NPs [14,16,20]. For example, our group and other two groups found that the antibacterial properties of Ag NPs are strongly dependent on size and size distribution and often vary with varying synthetic methods as well as reducing agents and stabilizers [21–23]. Therefore, it is a significant challenge to achieve size-tunable synthesis of Ag NPs with large surface area and surface activity as well as poor stability and strong aggregation tendency [8,10,24,25].

Raza et al. [10] prepared Ag NPs with tri-sodium citrate and sodium borohydride as the reducing agents. However, the as-prepared spherical Ag NPs have a wide range of diameter (15–90 nm), and the particles size cannot be controlled by adjusting stirring condition. Li et al. [24] obtained Ag NPs with different sizes (5, 15 and 55 nm) by altering reductant, temperature, reactant concentration and solution pH value; the as-obtained Ag NPs also exhibit a relatively wide size distribution, and those with an average diameter of 55 nm are easy to aggregate. We found in our previous work that poly acrylic acid (PAA) modified Ag NPs exhibit antimicrobial activities; and the particles size of Ag/PAA NPs can be tuned within a very narrow range (8–10 nm) by changing the molar ratio of reactants PAA and $AgNO_3$ [8]. To date, however, it still remains disputable how silver exerts its antibacterial activity to kill bacteria [26,27]. Some researchers speculate that three kinds of hypotheses, including contact action, generation of reactive oxygen species (ROS), and release of Ag^+, could account for the antibacterial effect of Ag NPs. For example, Morones et al. [28] suggest that Ag NPs can be attached to the surface of the cell

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https://doi.org/10.1016/j.apt.2017.11.028
0921-8831/

membrane of bacteria to disturb the functions of the cell membrane and penetrate through the bacteria to cause the leakage of cytoplasm, thereby killing the bacteria. Wang et al. [29] attribute the antibacterial effect of Ag NPs to the increase in the concentration of ROS. They suggest that ROS leads to bacteria death by inducing intracellular oxidation, membrane potential variation, and cellular contents release [29,30]. Namely, Ag NPs can absorb energy at levels higher than the band gap energy; as a result, the electrons are excited to the conductive band, leaving a hole (h^+) in the valance band. The electron in the conductive band can react with molecular oxygen to produce superoxide (O_2^-), while the h^+ can obtain electrons from water molecules to produce a hydroxyl species ($OH^\cdot$) [31]. These oxides and hydroxides act as precursors to generate other ROS with damage to proteins and DNA [32,33]. Different from the abovementioned, Kumar et al. [34] propose that the antimicrobial activity of silver is dependent on Ag^+ which is strongly bonded to electron donor groups in biological molecules containing sulphur, oxygen or nitrogen. In fact, silver can interact with H^+ to release Ag^+ upon exposure to oxygen [30,33,35]. This could support the Ag^+ release hypothesis in that DNA loses its replication ability and cellular proteins become inactivated upon Ag^+ encounter. Besides, some researchers suggest that Ag^+ can attack the functional groups in proteins and cause denaturation [35,36].

In the present research, we establish a simple route to prepare a series of size-tunable water-soluble Ag NPs with highly uniform morphologies and narrow size distributions. Using low-cost and eco-friendly sodium borohydride ($NaBH_4$) and sodium citrate as the reducing agent and modifying agent, respectively, we successfully obtained a series of Ag NPs whose size can be precisely tuned to be 2, 12, and 32 nm by adjusting the pH value of the reactant solution. This paper reports the preparation of the Ag NPs and their structure characterization based on X-ray powder diffraction (XRD), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and ultraviolet–visible absorption spectroscopy (UV–vis); and it also deals with the antibacterial mechanism of the as-prepared Ag NPs against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) by broth dilution and disk diffusion methods as well as optical density (OD_{600}) measurement and stripping voltammetry detection of Ag^+ concentration in bacterium suspension.

2. Experimental section

2.1. Materials

Silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), acetone (C_3H_6O), sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), nitric acid ($HNO_3$), polyvinyl alcohol (PVA), potash nitrate (KNO_3) and $NaBH_4$ were purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Ammonia solution ($NH_3{\cdot}H_2O$, 25–28%), glutaraldehyde and absolute ethanol were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). All the reagents are of analytical grade, and they were used as-received without further purification. Biochemical reagents nutrient agar and broth medium were purchased from Beijing Aoboxing Biotechnology Corporation (Beijing, China). *E. coli* (ATCC 23282) and *S. aureus* (ATCC 35696) bacterial strains were purchased from China General Microbiological Collection Center. Distilled water was used as the solvent or for rinsing.

2.2. Synthesis of Ag NPs

Ag NPs were synthesized by reducing $AgNO_3$ with $NaBH_4$ in aqueous phase. Briefly, 40 mL of $Na_3C_6H_5O_7{\cdot}2H_2O$ solution (1.25 mmol/L) and 1.0 mL of $AgNO_3$ solution (10 mmol/L) were formulated and injected into a three-neck flask under stirring at ambient temperature. Then 1.2 mL of the fresh solution of $NaBH_4$ (10 mmol/L) was added dropwise to the flask and stirred for 3 min to allow the pH value of the solution to approach 7. Upon completion of stirring, the resultant solution was aged at room temperature for 2 h, followed by vacuum-rotary evaporation and acetone addition (8 mL) to afford Ag NPs precipitate. The as-obtained sediment was separated by centrifugation at 8500 r/min for 10 min, followed by fully washing with acetone and absolute ethanol as well as vacuum drying at ambient temperature to provide Ag NPs. The as-prepared Ag NPs were kept in dark place. For fabricating Ag NPs with different sizes, a proper amount of $NH_3{\cdot}H_2O$ solution (1.45 mol/L) and NaOH solution (0.1 mol/L) was immediately added into the reactant solution to adjust the pH value to be 7–11 right after the addition of $NaBH_4$. The Ag NPs obtained at a reactant solution pH of 11, 9 and 7 are denoted as sample-1, sample-2 and sample-3), respectively.

2.3. Structure characterization

TEM images were recorded with a transmission electron microscope (JEOL JEM-2100, Japan) at an acceleration voltage of 200 kV. XRD patterns were collected with an X-ray diffractometer (BRUKER D8-ADVANCE, Germany; Cu Kα radiation (λ = 1.5418 Å), voltage: 40 kV, current: 40 mA). UV–vis absorption spectra were recorded with an UV–vis absorption spectrophotometer (Lambda35, US) in a wavelength range of 300–800 nm. FTIR spectra were measured with a Fourier transform infrared spectrometer (NICOLET AVATAR 360, US) in a wavenumber range of 4000–400 cm^{-1}. SEM images were taken with a field emission scanning electron microscope (FEI Nova NanoSEM 450, US; voltage: 5 kV or 10 kV, spot size: 3.0 nm, working distance: 5 mm).

2.4. Antibacterial test

Gram-negative bacteria of *E. coli* and Gram-positive bacteria of *S. aureus* were selected as the indicators to evaluate the antibacterial activity of Ag NPs. Prior to antibacterial tests, the glassware, suction nozzles and culture medium were sterilized in an autoclave at 0.1 MPa and 121 °C for 20 min. The minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by twofold serial broth dilution method to assess the antimicrobial activities of the as-prepared Ag NPs with different sizes. MIC is defined as the lowest concentration of the tested samples at which the visible growth of the bacteria, detected by turbid-metric method after incubation, is inhibited. MBC is defined as the minimum concentration of the tested samples at which 99.9% of the bacteria should be killed after a defined period of incubation. As to MIC tests with Ag NPs free bacterial broth suspension as the control, the serial broth solutions containing different concentrations (1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 μg/mL) of Ag NPs were separately mixed with 20 μL of bacterial suspension containing 10^8–10^9 colony-forming units (CFU)/mL. The resultant mixed solutions were incubated at 37 °C for 22 h in an ATS-03M2R shaking bath (Shanghai Kanxin Instrument Co., Ltd.; Shanghai, China) operating at 120 r/min. For the measurements of MBC, the bacterial suspensions containing different concentrations (higher than that for MIC test) of the tested samples were coated onto nutrient agar plates and incubated in a DHP-9082 constant temperature incubator (Zhengzhou Nanbei Instrument & Equipment Co., Ltd.; Zhengzhou, China) at 37 °C for 24 h. The number of the survival colonies was counted at pre-set incubation intervals. When the number of the survival colonies on some tested nutrient agar plate was determined to be zero or

Please cite this article in press as: Y. Wu et al., A facile method to prepare size-tunable silver nanoparticles and its antibacterial mechanism, Advanced Powder Technology (2017), https://doi.org/10.1016/j.apt.2017.11.028