Journal of Molecular Liquids 213 (2016) 75–81

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Synthesis, antibacterial, cytotoxicity and sensing properties of starch-capped silver nanoparticles

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ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form 21 October 2015
Accepted 4 November 2015
Available online xxxx

Keywords:
Silver nanoparticles
Starch
Antibacterial
Toxicity
H_2O_2

ABSTRACT

In this study, we report the antibacterial, cytotoxicity and sensing properties of dextrose reduced starch-capped silver nanoparticles (Ag-NPs) synthesized via a completely green method. The synthesis involved the use of water, starch, and dextrose as the solvent, the stabilizing and the reducing agents respectively. Silver nitrate was used as the silver precursor without the use of any accelerator. The as-synthesised Ag-NPs were characterized with UV–vis absorption spectroscopy, Fourier transform infra-red spectroscopy (FTIR), Raman spectroscopy, X-Ray diffraction analysis (XRD) and high resolution transmission electron microscopy (HR-TEM). All the as-synthesised Ag-NPs showed good antibacterial activities against *Escherichia coli* and two strains of *Pseudomonas aeruginosa*, which are antibiotic sensitive and resistant bacteria. The study also indicated that the time of reaction did not have any significant effect on the antibacterial activity of the Ag-NPs synthesized despite the different particle sizes of the as-synthesised Ag-NPs. The cytotoxicity evaluation on human THP-1 monocyte cell line indicated that the as-synthesised Ag-NPs are less toxic than $AgNO_3$ at lower concentrations (2 μg/ml). Furthermore, the as-synthesised Ag-NPs were found to be very useful for colorimetric detection of hydrogen peroxide (H_2O_2) at lower concentration up to 10^{-10} M with a linear regression coefficient value of 0.8822.

1. Introduction

Metal particles in the nanosize regime possess unique features with extensive applications in diverse fields [1–3]. These have been attributed to their reduced dimension in relation to the excitonic radius of the bulk material and high surface/volume ratios. Studies have also shown that the incorporation of metal nanoparticles in polymer matrices improve their stability [4,5] which lend them to potential applications in nanoelectronic devices, sensors, molecular optical devices and optoelectronic applications [6–10]. Among various metal nanoparticles, silver nanoparticles (Ag-NPs) have received considerable attention due to its outstanding plasmonic activity, broad spectrum of antibacterial activities, chemical stability, good thermal, electrical conductivity and catalytic properties [11]. Silver nanoparticles can be synthesized using various methods such as chemical reduction [12], electrical [13] γ-radiation [14], photochemical [15] and laser ablation [16]. Among these various methods, the most common and cost effective method for large scale synthesis and easy processing is the chemical reduction method. Chemical reduction method involves the use of reducing agents such as sodium borohydride, hydroxylamine hydrochloride, trisodium citrates, dimethylformamide with or without accelerators such as NH_3 and NaOH, especially for the production of smaller-sized (1–10 nm) materials [17–20]. However, these reducing agents, as well as the accelerators, are usually associated with some levels of environmental and biological toxicity. Thus, developing an environmentally benign synthetic route has become an important aspect of fundamental research. It is envisaged that these approaches will not only maximize safety and efficiency, but it will also reduce the hazard to health and to the environment. In the green synthesis of silver nanoparticles, utilization of (i) nontoxic chemicals as reducing agent, (ii) environmentally benign solvents, and (iii) renewable materials as stabilizing or passivating

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http://dx.doi.org/10.1016/j.molliq.2015.11.010
0167-7322/

agent are very important steps to be considered. A completely green synthesis of Ag-NPs was first reported by Raveendran et.al., in which water, starch and β-D-glucose were used as the solvent, capping agent and reducing agent respectively [21]. Thereafter, there have been several reports on the green synthesis of Ag-NPs [22–25]. However, only a few of these reports can be regarded as completely green synthesis of Ag-NPs. The reason for this is that most of these acclaimed green syntheses still use small quantities of NH_3, $NaBH_4$, NaOH or citrate, as an accelerator, reducing agent, complexing agent or as size directing agent. Such reagents have been associated with environmental toxicity and biological hazards. A completely green synthesis should be devoid of such chemicals. Most of the reported work on the completely green synthesis of Ag-NPs involved the use of various biopolymers such as gelatin, chitin, cellulose, polyvinyl pyrrolidone (PVP) and polyvinyl alcohol (PVA) as reducing and stabilizing agents [26–31]. Our group has previously also reported a completely green synthesis of Ag-NPs using cellulose and gelatin as capping agents and different types of sugars and plant extract as reducing agent [27,32–34]. The results showed that the particle size as well as the mechanism of the reaction depend on the biopolymer used as capping agent. It is envisaged that the synthesis of Ag-NPs via a completely green method will increase their antibacterial activity and most importantly reduced their cytotoxicity. Thus research on the antibacterial activities and cytotoxicity of Ag-NPs synthesized via completely green method to justify this belief has become an area of interest.

Hydrogen peroxide, a reactive oxygen specie, has been reported as a potential pollutant in both aquatic environment and biological systems. It is a product of the reactions catalysed by various oxidase enzymes in biological systems. When H_2O_2 is present in excess, it contributes to DNA damage in living systems [35]. Therefore its detection, especially at trace ppm level concentration, has become essential for some industrial processes. The several methods reported [36,37] for the detection of H_2O_2 offer excellent sensitivity however, these methods are not cost effective, and they involve time-consuming procedures which are skill dependent. As a meet to these challenges, the use of NPs as colorimetric sensors has been proven to be a promising approach for simple and cost-effective protocols with high sensitivity for tracking environmental pollutants in biological systems. The methods involving the use of Ag-nanocomposite for the detection of H_2O_2 [24,28], also have some drawbacks such as: the use of toxic reagent like NH_3 and $NaBH_4$ as reducing agent for the preparation of the Ag-nanocomposite, longer detection time and limited detection limit. Therefore in this work, the antibacterial, cytotoxicity and sensing properties of starch-capped Ag-NPs synthesised via a completely green method were investigated for the first time. The antibacterial activities of the Ag-NPs produced at different reaction times were tested against *Escherichia coli* and two strains of *Pseudomonas aeruginosa*, which are antibiotic sensitive and multidrug resistant bacteria. The cytotoxicity evaluation of the as-synthesized Ag-NPs was carried out against human THP-1 cells. Furthermore, the synthesized nanoparticles were used for colorimetric sensing of H_2O_2.

2. Experimental procedure

2.1. Materials

All chemicals were of analytical grade and they were used as purchased without any further purification. $AgNO_3$ was purchased from Alba CHEME; while starch, dextrose and H_2O_2 were purchased from Merck. All glasswares used in the experiment were cleaned and washed thoroughly with double distilled water and dried with acetone before use. A cultivating medium, Mueller–Hinton broth, used in the antibacterial assays was supplied by HIMEDIA CHENNAI. *E.coli ATCC 10536*, and two *P. aeruginosa b*acterial strains (*PA 27853*) and (*PA.MDR*) isolated from human clinical material were used. Human THP-1 monocyte cell line was obtained from American Type Culture Collection (ATCC, TIB-202TM, Manassas, VA, USA).

2.2. Synthesis of dextrose reduced starch-capped Ag-NPs

The synthesis is simple and straightforward. In a typical reaction, 1.0 g of starch was added to 95 mL of distilled water in a round bottom flask and heated to a temperature of 40 °C to obtain a clear solution. 0.07 M (10 mL) dextrose solution was added to the starch solution followed by the addition of aqueous $AgNO_3$ (5 mL, 1 M) under continuous stirring. The reaction temperature was elevated to 70 °C and allowed to react for several hours. Aliquots were taken at different time intervals to monitor the growth of the particles.

2.3. Characterisation

UV–vis absorption measurements were carried out using a SHIMDTH UV 2401PC spectrophotometer in the 300–700 nm wavelength range. FTIR spectra were recorded with Nicolet-Nexus 670. The Raman spectra were recorded with Horiba Jobin Vyon LabRAM HR with a laser wavelength of 633 nm. A JEOL JEM-3010 electron microscope operating at 200 kV was used for the TEM and HRTEM measurements. XRD measurements were performed on a Bruker D8 Advance diffractometer operating in the reflection mode with Cu-Kα radiation (40 kV, 20 mA) and diffracted beam monochromator. The samples for the XRD measurements were prepared by casting the Ag-NPs solution on a glass substrate and subsequently air-dried under ambient conditions.

2.4. Antimicrobial and bactericidal assays

2.4.1. Evaluation of antibacterial activity of nanoparticles

Antibacterial activity was evaluated using disc diffusion method. 18 h Mueller–Hinton broth (MHB) cultures of one clinical isolates of *E. coli* and two strains of *P. aeruginosa* were evaluated in this study. 10 mg of the compound was dissolved in 1 mL sterile milliQ water. 10 μL of the Ag-NPs solution was put on a filter paper disc and dried at 30 °C in an incubator. A stock solution of $AgNO_3$ was made with the same concentration for the purpose of comparison. Strict aseptic conditions were maintained throughout the procedure. Bacterial cultures were swabbed on Mueller–Hinton agar (MHA) plate and the surface of the media was allowed to dry for 30 min. This was followed by pressing the nanoparticle impregnated discs gently on the agar surface. After incubation at 37 °C overnight, formation of inhibition zones were observed and diameter of zones were measured.

2.4.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MHB broth culture (18 h) of clinical isolates of *E. coli* and *P. aeruginosa* isolates were selected for the evaluation of MIC. The assay was performed in a 96-well microtitre plate. Inoculum density of the test organisms were adjusted to that of 0.5 Mc Farland standards (10 μL. 1×10^8 CFU/mL). The broth was dispensed into the wells of the microtitre plate followed by addition of the Ag-NPs solution and inoculum. Serial dilutions were performed by the addition of various quantities of Ag-NPs solution into the microtitre wells with MHB to reach concentrations of 5–90 μg/mL. Microtitre wells containing only fresh medium/broth served as a negative control and those containing bacterial isolates only served as a positive control. Total volume of the assay system in each well was kept at 200 μL. Plates were incubated at 37 °C for 18 h and read at 600 nm in a plate reader (BIORAD 680). MIC was recorded as the lowest concentration at which growth was observed. The measurements were carried out in triplicates. The content of the wells showing no visible growth as well as the positive and negative controls were subcultured on nutrient agar plates by streaking and re-incubation at 37 °C overnight. On the incubation of the plates, the dilution from the minimum concentration from those streaks showing no growth was considered as MBC. The highest dilution showing at least 99% inhibition was taken as MBC.