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Completely green synthesis of dextrose reduced silver nanoparticles, its antimicrobial and sensing properties

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ABSTRACT

We herein report the green synthesis of highly monodispersed, water soluble, stable and smaller sized dextrose reduced gelatin capped-silver nanoparticles (Ag-NPs) via an eco-friendly, completely green method. The synthesis involves the use of silver nitrate, gelatin, dextrose and water as the silver precursor, stabilizing agent, reducing agent and solvent respectively. By varying the reaction time, the temporal evolution of the growth, optical, antimicrobial and sensing properties of the as-synthesised Ag-NPs were investigated. The nanoparticles were characterized using UV–vis absorption spectroscopy, Fourier transform infra-red spectroscopy (FT-IR), X-ray diffraction (XRD), transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HR-TEM). The absorption maxima of the as-synthesized materials at different reaction time showed characteristic silver surface plasmon resonance (SPR) peak. The as-synthesised Ag-NPs show better antibacterial efficacy than the antibiotics; ciproflaxin and imipenem against *Pseudomonas aeruginosa* with minimum inhibition concentration (MIC) of 6 μg/mL, and better efficacy than imipenem against *Escherichia coli* with MIC of 10 μg/mL. The minimum bactericidal concentration (MBC) of the as-synthesised Ag-NPs is 12.5 μg/mL. The sensitivity of the dextrose reduced gelatin-capped Ag-NPs towards hydrogen peroxide indicated that the sensor has a very good sensitivity and a linear response over wide concentration range of 10^{-1}–10^{-6} M H_2O_2.

1. Introduction

Noble metals in quantum size regime have generated a lot of interest among researchers from various disciplines over the last decades (Hermanson, Lumsdon, Williams, Kaler & Velev, 2001; Jennifer, Bettye, Maddux & James, 2007; Kim, Eunye, Kim, Park, & Park, 2007; Zhenhua et al., 2003). This is due to the unique and attractive, optical and electronic properties of metal nanoparticles (NPs) such as silver (Ag), gold (Au), platinum (Pt) etc., which are significantly different from those of bulk materials. These properties are being influenced by several parameters, most importantly their size and shape. Among these materials, interest in Ag-NPs is very high due to their outstanding plasmonic activity, bacterial inhibitory and bactericidal effects compared with the other metal nanoparticles. The design of Ag-NPs especially using the bottom-up technique has been widely investigated for various applications and researchers are continuously developing newer methods for the synthesis of highly monodispersed and stable nanoparticles. Conventionally, stable metal nanomaterials are synthesized using either chemical or physical methods. In chemical methods, reducing agents like borohydrides, hydroxylamine hydrochloride, trisodium citrates, dimethylformamide etc are usually used (Chreigton, Blatchford & Albrecht, 1979; Lee & Meisel, 1982). The two main problems normally associated with the chemical synthetic route are the aggregation of the nanoparticles formed and the toxicity of the reagents used. As part of developing eco-friendly method, in order to address these concerns, new synthetic

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routes based on green chemistry principles are being developed (Bozanic, Trandafilovic, Luyt, & Djokovic, 2010; Raveendran, Fu & Wallen, 2006).

There has been an upsurge of interest in implementing green chemistry principles into the synthesis of silver nanoparticles in order to maximize safety and efficiency, and minimise the environmental and societal impact of these materials. In the green synthesis of silver nanoparticles, three important factors to be considered are: (i) use of green solvents, (ii) use of an eco-friendly benign reducing agent, and (iii) use of a nontoxic material as a stabilizer. One of the green methods for preparing silver nanoparticles is the polysaccharide method. In this method, water is normally used as an eco-friendly benign solvent and polysaccharides as capping agents. Raveendran et al. (2003) reported the first completely green synthesis of Ag nanoparticles using water, starch and β-D-glucose as the solvent, capping agent and reducing agent respectively (Raveendran, Fu & Wallens, 2003). The use of starch makes it possible to avoid the use of relatively toxic organic solvents. Based on the modification of this method, synthesis of Ag-NPs have been reported using different sugars as reducing agent (Panacek et al., 2006) and biopolymers such as starch (Batabyal, Basu, Das & Sanyal, 2007) gelatin (Darroudi, Ahmad, Abdullah & Ibrahim,2011), polyvinylpyrolindone (PVP) (Filippo, Serra & Manno, 2009)and so on as passivating agent with or without accelerating agent such as NaOH (Darroudi et al., 2011a,b; Filippo, Serra, Buccolieri & Manno,2010; Stevanovic, Kovacevic, Petkovic, Filipic & Uskokovic, 2011). In a new development, our group also reported the synthesis of small highly stable and monodispersed Ag-NPs using maltose, a disaccharides sugar as reducing agent while gelatin and starch were used as passivating agent without the use of any accelerating agent (Oluwafemi et al., 2013a; Oluwafemi et al., 2013b). In another development, Eid and Azzay reported the synthesis of anisotropic Ag-NPs using dextrose, trisodium citrate and NaOH as reducing agent, capping agent and accelerating agent respectively (Eid & Assay, 2012). In their report, different sizes of robust hollow flower like nanostructures were produced by changing the concentration of the $AgNO_3$, dextrose, NaOH and trisodium citrate. However, such capping and accelerating agent may be associated with environmental toxicity or biological hazards. In this work, we reported, the synthesis of highly monodispersed, water soluble, stable and smaller sized gelatin capped-silver nanoparticles (Ag-NPs) via a completely green method by using dextrose as reducing agent without any accelerating agent. The antibacterial property of the as-synthesised dextrose reduced, gelatin-capped Ag-NPs at different stages of growth were tested for the first time against *Escherichia coli* and *Pseudomonas aeruginosa*, which are multidrug resistant bacteria and were compared with two antibacterial drugs; imipenem and ciprofloxacin using disc diffusion method. In addition, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the as-synthesised Ag-NPs were also evaluated. Furthermore, the sensing property of the Ag-NPs against H_2O_2, one of the reactive oxygen species (ROS) that possess a serious threat to biological system was also investigated.

2. Experimental procedure

2.1. Materials

All the chemicals were of analytical grade and used as purchased without any further purification. $AgNO_3$ was purchased from Alba scheme, while gelatin, dextrose and H_2O_2 were from Merck. All glasswares used in the experiment were cleaned and washed thoroughly with double distilled water and dried before use. A cultivating medium, Mueller–Hinton broth, used in the antibacterial assays was supplied by HIMEDIA Chennai. *E. Coli* ATCC 10536, and *P. aregumosa* bacterial strains isolated from human clinical material were used.

2.2. Synthesis of dextrose reduced gelatin coated Ag-NPs

In a typical synthesis, 1.0 g of gelatin was added to 95 mL of distilled water in a round bottom flask and heated to 40 °C to get a clear solution. 5 mL of $AgNO_3$ solution (1 M) was added to the gelatine solution with continuous stirring to obtain Ag^+/gelatin solution. This was followed by the addition of 10 mL dextrose solution (0.07 M) under continuous stirring. The reaction was maintained at 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. Characterization

A SHIMDTH UV 2401PC spectrophotometer was used for the absorption measurement in the 300–700 nm wavelength range. FT-IR spectra were recorded with Nicolet-Nexus 670. A JEOL JEM-3010 electron microscope operating at 200 kV was used for the TEM and HRTEM measurements. XRD measurements were performed on the 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 silver nanoparticle solution on glass substrate and subsequently air-drying 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. Mueller–Hinton broth (MHB) cultures (18 h) of two clinical isolates of *E. coli* and *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 added on filter paper disc and dried at 30 °C in an incubator. A stock solution of $AgNO_3$ was made with the same concentration and checked for the purpose of comparison. Strict aseptic conditions were maintained throughout the procedure. Bacterial cultures were swabbed on Mueller–Hinton agar (MHA) plate and surface of the media was allowed to dry for 30 min, then the nanoparticles incorporated discs were pressed gently on the agar surface at specified distance. Ciprofloxacin (5 μg) and imipenem (10 μg) discs were also pressed separately on the agar surface for the purpose of comparison. After incubation at 37 °C for overnight, formation of inhibition zone was checked and diameter of zone was 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 was selected for the evaluation of MIC. The assay was performed in 96-well microtitre plates. Inoculum density of the test organisms was adjusted to that of 0.5 Mc Farland standards (10 μL, $1 \times 10^{8-}$ CFU/mL). Broth was dispensed into the wells of microtitre plate followed by addition of the Ag-NPs solution and inoculum. Serial dilutions were performed by addition of various quantities of Ag-NPs solution to the microtitre wells with MHB to reach concentrations of 10– 100 μg/mL. The antibiotic imipenem and ciprofloxacin was also serially diluted with broth at a concentration ranging from 8–128 μg/mL. Microtitre wells containing fresh medium served as a negative control and that containing bacterial growth 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