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Ultra-efficient photocatalytic deprivation of methylene blue and biological activities of biogenic silver nanoparticles

Arif Ullah Khan [a,*], Qipeng Yuan [a,*], Yun Wei [a], Zia ul Haq Khan [b], Kamran Tahir [a], Shahab Ullah Khan [c], Aftab Ahmad [a], Shafiullah Khan [d], Sadia Nazir [a], Faheem Ullah Khan [a]

[a] State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, PR China
[b] Department of Chemistry, University of Science and Technology Bannu, KP, Pakistan
[c] Department of Physics, University of Peshawar, 25000 KP, Pakistan
[d] Institute of Chemical Sciences, Gomal University D.I.Khan, KP, Pakistan

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ABSTRACT

Phytosynthesis of metal nanoparticles is considered as a safe, cost-effective, and green approach. In this study, silver nanoparticles (AgNPs) were successfully synthesized using the aqueous extract of Lychee (*Litchi chinensis*) fruit peel and an aqueous solution of silver nitrate ($AgNO_3$). The synthesized nanoparticles were characterized by several analytical techniques i.e. UV–Vis Spectroscopy, XRD (X-ray diffraction spectroscopy), EDX (electron dispersive X-ray), SAED (selected area electron diffraction), HRTEM (high-resolution transmission electron microscopy), and FTIR (Fourier transform infrared spectroscopy). HRTEM and XRD results indicated that the prepared AgNPs are spherical in shape, well dispersed and face centered cubic crystalline. AgNPs showed potent antibacterial properties against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The minimum inhibitory concentration (MIC) values were 125 μg against *E. coli* and 62.5 μg against both *S. aureus* and *B. subtilis*. AgNPs induce efficient cell constituent release from bacterial cells, which indicates the deterioration of cytoplasmic membrane. Moreover, antioxidant studies on the as-synthesized nanoparticles reveal efficient scavenging of the stable or harmful DPPH free radical. The cytotoxicity assay confirmed that biosynthesized AgNPs are nontoxic to normal healthy RBCs. AgNPs exhibited consistent release of Ag^+ determined by ICP-AES analysis. AgNPs exhibited extraordinary photocatalytic degradation (99.24%) of methylene blue. On the other hand, commercial silver nanoparticles have moderate biological activities against the tested bacterial strains and negligible photocatalytic degradation of methylene blue. The significant biological and photocatalytic activities of the biosynthesized silver nanoparticles are attributed to their small size, spherical morphology and high dispersion.

1. Introduction

Metal nanoparticles have attracted significant interest in the past two decades. These nano-scale materials acquire unique physiochemical characteristics, which are different than their bulk states [1–5]. Nanoparticles exhibit a high surface to volume ratio with decreased nanosize. The specific surface area plays an important role in catalytic activity and other biological properties. Platinum, gold and silver nanoparticles are broadly applied to human contact areas such as soaps, shampoos, detergents, cosmetic products, shoes and toothpaste as well as medical and pharmaceutical applications [6].

A number of physical and chemical methods have been in practice for the synthesis of noble metal nanoparticles. Chemical method is considered to be an important method among the different approaches employed for the synthesis of nanoparticles [7,8]. However, the toxicity of the chemicals limits their applications in nanoparticle synthesis, especially if the synthesized nanoparticles have intervention in the biological system. Therefore, it is a growing demand to develop green or eco-friendly protocols for nanoparticle synthesis. The synthesis of nanoparticles has been reported using biological methods like microorganisms [9–11] and enzymes [12] which are suggested as possible green or eco-friendly alternatives to chemical and physical methods. However, the concern of time and cost effectiveness led to the use of whole plant or a plant extract in nanoparticle synthesis. Nowadays various plants species such as *Citrus sinensis* var. Kozan yerly fruit [13], *Cirsium arvense* [14], *Nerium oleander* [15] and *Sargentodoxa cuneata* [16] have been used in the synthesis of AgNPs.

The use of whole plants or plant extracts for nanoparticle synthesis is favorable and has advantages over other biological methods such as the microbial protocol that accounts for long laborious and less cost-effectiveness [17]. Many industries including the production of cosmetics, food, paint, paper, plastics and textiles use synthetic dyes which cause serious environmental pollution and problems due to their intense color and are refractory to degradation. Many conventional

* Corresponding authors.
E-mail addresses: khanbuct@gmail.com (A.U. Khan), yuanqp@mail.buct.edu.cn (Q. Yuan).

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treatment methods including adsorption, ultra- filtration, coagulation, reverse osmosis etc. are ineffective for decolorization and mineralization of these pollutants due to their complex aromatic structure and high stability. Therefore, a new treatment technique is required either for the eradication and degradation of these persistent pollutants or for their conversion to a harmless product. Nanocatalysis is a fast emergent area of research in which metal nanoparticles are used as catalysts for a wide range of chemical reactions [18–20]. In this study, AgNPs were synthesized using Lychee (*Litchi chinensis*) fruit peel aqueous extract on the time scale (≤150 min). The biosynthesized Ag nanoparticles have been evaluated for their biological activities and photocatalytic degradation of methylene blue. The shorter processing time and production of highly dispersed, spherical and small size (4–8 nm) nanoparticles with efficient photocatalytic and antibacterial activity make our study prominent than other biogenic AgNPs.

2. Materials and Methods

2.1. Materials

Silver nitrate ($AgNO_3$), methylene blue, nutrient agar, nutrient broth, Triton X-100, methanol, DPPH and vitamin C were purchased from Beijing Chemical Works, Beijing, China. Commercial AgNPs (PVP stabilized) were purchased from Sigma Aldrich, USA. All the chemicals were used without further purification.

2.2. Preparation of Litchi Fruit Peel Aqueous Extract

Lychee (*L. chinensis*) fruit was purchased from the local market (Beijing, China) and peeled out. The peels were shade dried for one month and made to powder by grinding. 15 g of powdered peel was soaked in 120 ml water and heated at 60 °C for 2 h. The obtained aqueous extract was filtered through Whatman no. 1 filter paper. The filtrate was centrifuged at 5000 rpm for 10 min at 4 °C to remove any suspended plant debris. The clear supernatant (100 ml) obtained was used as a reducing, capping and stabilizing agent for the synthesis of AgNPs.

2.3. Synthesis of AgNPs

To synthesize AgNPs, 10 ml aqueous extract of Lychee (*L. chinensis*) fruit peel was added to 25 ml of 6 mM aqueous solution of $AgNO_3$ (sigma Aldrich) in 100 ml beaker. The reaction mixture was stirred in the dark (to minimize the photoactivation of silver nitrate) for complete mixing at room temperature. The colloidal solution of AgNPs was centrifuged at 10,000 rpm for 15 min followed by re-dispersion of the pellet in double distilled water. The resulted pellet was freeze-dried under vacuum (VirTis freeze mobile 6ES) and stored at 4 °C for further analysis.

2.4. Characterization

The biosynthesis of AgNPs was checked regularly by scanning the aliquot samples in the wavelength range of 350–800 nm (Shimadzu UV–Vis 2450 spectrophotometer) at a resolution of 1 nm. The X-ray diffraction (XRD) measurements were carried out on a Rigaku Miniflex X-ray diffractometer at a scanning rate of 20 min^{-1} with an operating voltage of 30 kV and a current of 15 mA with CuKα radiation (1.5405 A°) monochromatic filter in the range of 20–70°. A Hitachi EDX elemental microanalysis system and JEOL-3010 high-resolution transmission electron microscope were used to study the elemental composition, morphology and size of the nanoparticles respectively. FTIR study was performed (ABB MB3000 spectrophotometer) to detect the biomolecules that are involved in the reduction and capping of AgNPs.

Silver ion concentration was determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) analysis.

2.5. Antibacterial Activity

2.5.1. Bacterial Strains

Escherichia coli, *Staphylococcus aureus*, and *Bacillus subtilis* were used in the antimicrobial assay. These bacterial strains were obtained from China General Microbiological Culture Collection Centre (Beijing, China). The selected bacteria were maintained on agar slants at 4 °C in the College of Life Science and Technology Beijing University of Chemical Technology, Beijing for antimicrobial tests.

2.5.2. Screening for Antibacterial Activity

The antibacterial activity was carried out by agar well diffusion method [21]. All the bacterial strains were grown in nutrient broth (Oxoid) and incubated at 37 °C for 24 h till turbidity became equivalent to McFarland 0.5 turbidity standard. The inocula of *B. subtilis*, *S. aureus* and *E. coli* were streaked onto the condensed Muller Hinton Agar (Oxoid) in Petri plates by a sterilized cotton swab in order to make sure a uniform thick lawn of growth following incubation. Wells of 8 mm in diameter were formed with the help of sterilized cork borer onto nutrient agar plates. 50 μl (4 mg/ml in sterile double distilled water) of biosynthesized AgNPs and commercial AgNPs (standard) was put into the wells and the plates were allowed to stand for 2 h at room temperature. Finally, the plates were incubated at 37 °C for 20–24 h and the resulting diameters of zones of inhibition were measured. The experiments were performed in triplicates and the data were expressed as means ± SD.

2.5.3. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of Ag nanoparticles was determined by agar dilution method [21,22]. The sterilized Muller Hinton Agar (oxoid) was allowed to cool at 50 °C and about 19 ml of this was added to sterilized test tubes which contained 1 ml of different concentrations of AgNPs. This mixture was gently mixed and poured into pre-labeled sterile Petri dishes. Petri dishes having only growth media were prepared in the same way so as to serve for comparison with Petri plates containing Ag nanoparticles. The concentrations of AgNPs used in this assay were ranged in 31.25, 62.5, 125, 250, 500, 1000 and 2000 μg/ml. The suspensions of the respective microorganisms having density adjusted to 0.5 McFarland turbidity standards were inoculated onto the series of agar plates using a standard loop. The plates were then incubated at 37 °C for 24 h. The lowest concentration which inhibited the growth of the respective microorganism was taken as MIC. All tests were carried out in triplicate.

2.5.4. Assessment of the CellConstituent Release

The release of cell constituents into the media was assessed as previously described [23] with a little modification. In brief, working cultures of the three tested bacteria were collected by centrifugation (10 min, at 5000 rpm) after being grown for 24 h in nutrient broth for bacterial cells from 50 ml relevant suspension, washed 3 times with 0.1 M PBS (pH 7.0) and the pellet was re-suspended in 50 ml of 0.1 M PBS. The cell suspensions were incubated at 37 °C under agitation for 2 h in the presence of AgNPs at three different concentrations (2 × MIC, MIC and control). 3 ml of sample suspensions was collected and centrifuged for 2 min at 12,000 rpm and afterward, 2 ml of the supernatant was processed to measure UV absorption at 260 nm to determine the concentration of the cell constituent released.

2.5.5. Hemolytic Activity Assay

The in vitro hemolytic property of AgNPs was assessed by measuring the hemoglobin released from the red blood cells (RBCs) with the treatment of silver nanoparticles. The blood was collected from male Wistar albino mouse in a sterile lithium heparin vacutainers. The tube was centrifuged at 1500 rpm for 10 min. The supernatant and buffy coat were carefully removed and the pellet was washed three times with phosphate-buffered saline (PBS) at pH 7.4. AgNPs in different