GREEN SYNTHESIS OF SILVER NANOPARTICLES USING CYCLODEXTRIN GLYCOSYLTRANSFERASE PRODUCED BY *ALKALIPHILIC BACILLUS*

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Introduction

Cyclodextrin glycosyltransferase (CGTase) is an extracellular enzyme commonly produced by alkaliphilic Bacilli. In this article, we report a one-step synthesis of silver nanoparticles (Ag-NPs) through a green-method using CGTase as both reducing and stabilizing agents. Structural, morphological and optical properties of the Ag-NPs were characterized using X-ray diffraction (XRD), transmission electron microscopy (TEM) and UV-Vis spectroscopy. The formations of Ag-NPs with an absorbance band centered at 450nm were confirmed. A TEM image displayed that the nanoparticles are spherical in shape with size ranging from 10 to 25nm. The XRD pattern showed that the nanoparticles were crystalline in nature. The bio-formed nanoparticles are expected to have prominent-uses in pharmaceutical and biomedical areas.

Researchers in the field of nanotechnology are turning to 'Nature' to provide inspiration, for stimulating and innovative approaches of nano-production. Synthesizing new metallic nanoparticles based on the notion of green nanotechnology is obtaining momentum. Green nanotechnology mixes the principles of green engineering and green chemistry to produce safe and eco-friendly nanoparticles, which do not use toxic substances in their synthesis procedure [1]. The synthesis of nanoparticles of noble metals, such as Ag-NPs, is of excessive interest because of their unique characteristics. Manipulation of their shape and size creates unique properties that have potential applications in biomedical uses such as antibacterial, anti-HIV activity, controlling plant pathogens and as a biosensor and catalyst [2-5].

Current chemical and physical techniques for the production of Ag-NPs use hazardous substances, for example; hydrazine, dimethyl formamide (DMF) and sodium borohydride, as reducing agents, and may also need to use costly instruments. These approaches produce Ag-NP efficiently; nevertheless downstream processing to distinct the nanoparticles from the toxic compounds is high cost and time-consuming. The existence of even a slight trace of toxic compounds makes these Ag-NPs incompatible for pharmaceutical and biomedical applications.

Since 2000, the production of inorganic nanoparticles using bacteria [6], fungus [7] and plant extracts such as rose [8], for nanoparticle synthesis are under potent investigations [9]. The investigations can be a feasible substitute for the current physicochemical processes of producing nanoparticles [10]. Hence, in the present study, the CGTase was used for the production of the Ag-NPs. The CGTase is an extracellular, inducible enzyme produced by microbial cells and the alkaliphilic *Bacilli* strains are the best producers of the CGTase. In this paper, we report a simple, fast and cost-effective process to produce Ag-NPs that are stable with extended shelf life.

Methodology

Materials

$AgNO_3$ (99.98%), which was applied as a silver precursor, was purchased from Merck (Darmstadt, Germany). The CGTase was produced using the previous method [11]. In the experiments, all reagents were of analytical grade and all the solutions were made using deionized water.

Synthesis of Ag-NPs

A volume of 25 mL of the CGTase was added to 0.1 mM $AgNO_3$ aqueous solution under gentle stirring at 30˚C for 1 h, and then it was kept at room temperature for another 2 h. The resulting solid product was collected by centrifugation at 8,000 rpm for 15 min and carefully washed with distilled water and dried at 45˚C overnight.

Instrument

The crystalline structure of the sample was examined by XRD analysis, which recorded by a diffractometer (XPERTPRO) at room temperature at a voltage of 40 kV /and current of 30 mA. The morphology and size of the sample were determined by HITACHI H-700 TEM. The pure sample was analyzed for its UV–visible spectrum using a UV–vis spectrophotometer (Lambda 25-Perkin Elmer) in the range of 200 to 800nm.

Results

Preliminarily, the synthesis of Ag-NPs was confirmed through visual assessment. The reaction solution turned to dark brown color from brownish-yellow color within 30 min specified the formation of Ag-NPs. The appearance of dark brown color may be owing to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$ [12]. UV–Vis spectrum of reaction solution showed strong absorption peak with centering at 450 nm (Figure 1) specified the formation of Ag-NPs. This absorption is near to that seen for silver nanoparticles formed by different methods [13].

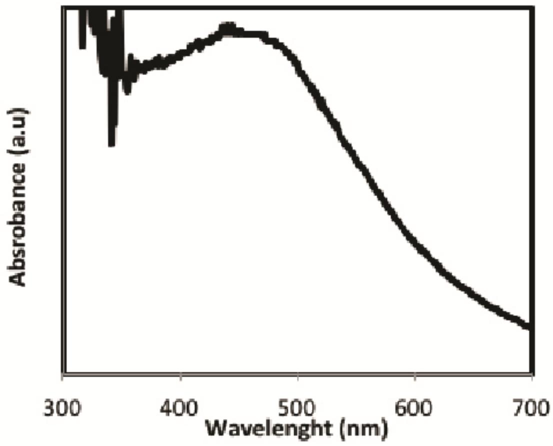

Figure 1. UV absorption spectrum of bioformed Ag-NPs.

The XRD pattern (Figure 2A) displays that the particles are crystalline. The lattice planes (111), (200), (220), and (311) were identified with the corresponding Bragg's angles of 37.95°,45.84°, 64.07°, and 76.43°, respectively, which confirm the face-centered cubic structure of the formed Ag-NPs. The TEM image Figure 2B shows the Ag-NPs formed were well dispersed with a spherical structures and a particle size ranging from 10 to 25 *n*m.

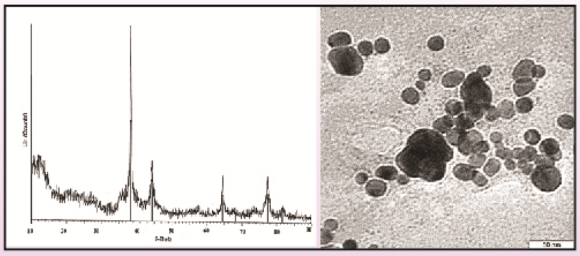

Figure 2. XRD pattern (A) and TEM image (B) of biosynthesized Ag-NPs.

Conclusions

This article highlights on the development of a simple, eco-friendly and economic, biological procedure to synthesize Ag-NPs. The biosynthesized silver nanoparticles have spherical shapes, and the particle size ranges from 10 to 25 *n*m. The biosynthesized silver nanoparticles are expected to have important applications in pharmaceutical and biomedical fields.

References

[1] Castro, L., Blazquez, M.L., Munoz, J.A., Gonzalez, F., Garcia-Balboa, C., Ballester, A., Biosynthesis of gold nanowires using sugar beet pulp. *Process Biochem.*, 2011, 46(5), 1076-1082.

[2] Elechiguerra, J.L., Burt, J.L., Morones, J.R., Camacho-Bragado, A., Gao, X., Lara, H.H., Yocaman, M., Interaction of silver nanoparticles with HIV-1. *J. Nanobiotechnol.*, 2005, doi:10.1186/1477-3155-3-6.

[3] Shiraishi, Y., Toshima, N., Oxidation of ethylene catalyzed by colloidal dispersions of polym(sodium acrylate)-protected silver nanoclusters. *Colloids Surf*. A., 2000, 169, 59–66.

[4] Jain, P., Pradeep, T., Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnol. Bioeng.*, 2005, 90, 59–63.

[5] Krishnaraj, C., Jagan, E.G., Ramachandran, R., Abirami, S.M., Mohan, N., Kalaichelvan, P.T., Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Plant growth metabolism. *Process Biochem.*, 2012, 47(4), 651–658.

[6] Kalishwaralal, K., Deepak, V., Ram Kumar Pandian, S., Gurunathan, S., Biological synthesis of gold nanocubes from *Bacillus licheniformis. Bioresour. Technol.*, 2009, 100(21), 5356–5358.

[7] Du, L., Xian, L., Feng, J.-X., Rapid extra-/intracellular biosynthesis of gold nanoparticles by the fungus *Penicillium sp. J. Nanopart. Res.*, 2011, 13(3), 921–930.

[8] Ghoreishi, S.M., Behpour, M., Khayatkashani, M., Green synthesis of silver and gold nanoparticles using *Rosa damascena* and its primary application in electrochemistry. *Physica*. E., 2011, 44(1), 97–104.

[9] Nadagouda, M.N., Hoag, G., Collins, J., Varma, R.S., Green synthesis of Au nanostructures at room temperature using biodegradable plant surfactants. *Cryst. Growth Des.*, 2009, 9(11), 4979–4983.

[10] Karn, B., The road to green nanotechnology. *J. Ind. Ecol.*, 2008, 12(3), 263–266.

[11] Azin, A., Rosfarizan, M., Raha, A.R., Rosli, M.I., Farideh, N., Joo Shun, T., Sahar, A. Cyclodextrin glycosyltransferase biosynthesis improvement by recombinant *Lactococcus lactis* NZ:NSP:CGT: medium formulation and culture condition optimization. *Biotechnol. Biotechnol. Equip.*, 2015, 29(3), 555-563.

[12] Mulvaney, P., Surface plasmon spectroscopy of nanosized metal particles. *Langmuir,* 1996, 12, 788–800.

[13] Lee, Ch.J., Karim, M.R., Vasudevan, T., Kim, H.J., Raushan, K., Jung, M.J., Kim, D.Y., Lee, M.S., A comparison method of silver nanoparticles prepared by the gamma irradiation and in situ reduction methods. *Bull. Korea Chem.* Soc. 2010, 31, 1993–1996.

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