Blue orange light emission from biogenic synthesized silver nanoparticles using Trichoderma viride

Mohammed Fayaz\textsuperscript{a},\textsuperscript{a}, C.S. Tiwary\textsuperscript{b}, P.T. Kalaichelvan\textsuperscript{c}, R. Venkatesan\textsuperscript{c}

\textsuperscript{a} CSIR in Botany, Universit of Madras, Guindy Campus, Chennai 600025, India
\textsuperscript{b} Department of Physics, National Institute of Technology, Durgapur, West Bengal, India
\textsuperscript{c} National Institute of Ocean Technology, Ministry of Earth Sciences, Chennai, India

\textbf{ABSTRACT}

Recent advances in nanomaterial have produced a new class of fluorescence labels by conjugating noble metal with biomolecules. The nanometer size metal conjugates are water soluble, biocompatible and provide important advantage over the florescence dyes. In this regard we synthesized silver nanoparticles at the size of 2–4 nm using biological route and studied fluorescence property of these nanoparticles. We observe that these silver (Ag\textsuperscript{+}) ions when exposed to filtrate of Trichoderma viride are reduced in solution, thereby leading to the formation of an extremely stable silver hydrosol. These silver nanoparticles were characterized by means of UV-vis spectrophotometer, FTR, HRTEM, EDX, XRD and fluorescence spectroscopy. The nanoparticles exhibit maximum absorbance at 405 nm in UV-vis spectrum. The presence of proteins was identified by FTR. The HRTEM micrograph revealed the formation of monodispersed spherical nanoparticles and the presence of elemental silver was confirmed by EDX analysis and XRD. These monodispersed silver nanoparticles showed emission in the range of 320–520 nm wavelength.

1. Introduction

In recent years the nanoparticles of II–VI group are very much important due to its emission properties. These nanoparticles (CdS, ZnS, CdSe, etc.) are used for wide range of application in the field of cathode ray tube, flat-panel display, sensor, laser devices, etc. [1–5]. But for biological application these particles are not very successfully applicable. On the other hand, the intense light emission properties of noble metals (gold, silver, etc.) nanoparticles have caught a lot of attention. These nanoparticles are extensively used for biological labeling [6], easy to prepare and have a good chemical and thermal stability [7].

Silver (Ag) nanoparticles (noble metal) have potential application in electronics, optoelectronics [8], in heterogeneous catalysis [9], and surfaces of heat exchange, gas sensors and as conductive inks [10]. But a limited amount of attention has been given to the luminescence from Ag nanoparticles due to its very low efficiency. The absence of band gap makes luminescence exceedingly improbable for these nanoparticles [11]. It was reported that Ag nanoparticles emits light in rare gas matrix at cryogenic temperature under photo activation or electro activation and this photoluminescence was attributed to sp to sp like transition analogy to inter-band transition in bulk silver [12]. The optical properties got enhanced due to lanthanide addition [13]. But for biological application apart from luminescence synthesis method is also equally important. There are many methods for synthesis of Ag nanoparticles are reported, including both chemical and biological methods. In recent times, scientist has endeavored microorganisms as possible eco-friendly nano-factories, for synthesis of silver nanoparticles. The Bacterium Pseudomonas stutzeri AG255 isolated from silver mine, when placed in a concentrated aqueous solution of AgNO\textsubscript{3}, played a major role in the reduction of the Ag\textsuperscript{+} ions and it makes silver nanoparticles of well-defined size and distinct topography within the periplasmic space of the bacteria [14].

While intracellular synthesis in principle may accomplish a better control over the size and shape distributions of the nanoparticles, product harvesting, and recovery are more cumbersome and expensive. The extracellular synthesis by comparison is more adaptable to the synthesis of a wider range of nanoparticles systems [15]. However, optical properties of the biologically synthesized silver nanoparticles have been rarely reported.

In our current investigation, non-pathogenic fast growing fungus Trichoderma viride, which habituated in dead organic materials, was used for extracellular biosynthesis of silver nanoparticles. The biologically synthesized silver nanoparticles were characterized using UV–vis spectrophotometer (Cary 300 Conc UV–vis spectrophotometer) and fluorescence spectroscopy (PerkinElmer). The size and morphology were characterized using HRTEM (high

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\* Corresponding author. Tel.: +91 (088446658); fax: +91 44 23252494.
E-mail address: nanofayaz@gmail.com (M. Fayaz).

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resolution transmission electron microscope, Technai 300) with EDX (energy dispersive spectroscopy), XRD (X-ray diffraction, Pan Analytical), and Fourier transformation infrared spectroscopy (PerkinElmer). The PL emission is found to be 320–520 nm with blue color emission along with very narrow range of particle distribution which is stabilized by protein molecules.

2. Experimental details

The fungus T. viride was obtained from Culture Collection Center, CAS in Botany, University of Madras, India and maintained in Potato Dextrose Agar slant at 27 °C.

To prepare the biomass for biosynthesis studies the fungi were grown aerobically in liquid broth containing (g/l): KH₂PO₄ – 7; K₂HPO₄ – 2; MgSO₄·7H₂O – 0.1; (NH₄)₂SO₄ – 1; yeast extract – 0.6; glucose – 10. The culture flask was incubated in an orbital shaker at 27 °C and agitated at 150 rpm and the biomass was harvested, after 72 h of growth by sieving through plastic sieve followed by extensive washing with sterile double distilled water to remove any medium components from the biomass.

Typically 20 g (wet weight) brought in contact with 100 ml sterile double distilled water for 48 h at 27 °C in an Erlen Meyer flask and agitated as described earlier, after incubation the cell filtrate was obtained by passing it through Whatman filter paper No. 1. In 100 ml cell filtrate, carefully weighed quantity of silver nitrate was added to the Erlen Meyer flask to yield an overall Ag⁺ ions concentration of 10⁻⁵ M in cell filtrate solution and the reaction is carried out in dark condition at 40 °C. The synthesized nanoparticles were dissolved in water and used for measuring optical properties. A thin film is coated on glass plate for measuring the XRD. The water dispersed nanoparticles are seen in HRTEM using carbon coated grid.

3. Results and discussion

The optical properties of silver nanoparticles are related to the excitation of plasma resonance or inter-band transition, particularly on the size effect. The UV-vis absorbance spectrum of colloidal silver can be calculated from the wavelength dependence of optical constant of the particle relative to the surrounding medium using ‘MIE’ theory. According to ‘MIE’ theory silver nanoparticles less than diameter 52 nm (electron mean free path of silver) result in a broadening of the plasma absorbance bands, meanwhile the height of the absorbance peak also decreases [16].

Fig. 1 shows the UV-vis spectrum obtained from biologically synthesized silver nano solution. It is observed from the spectra that the silver surface plasmon band occurs at 405 nm in addition to prominent band at around 260 nm. These peaks are characteristic plasmon band for silver nanoparticles [17]. At the initial stage of the reaction, a characteristic absorbance band centered at approximately 405 nm, as reaction proceeds the intensity of the band significantly increased and the full-width at half-maximum (FWHM) of the peak position changed slightly. As there is an increase in the intensity of the absorbance, the concentration of silver particle increases with reaction time. Furthermore, there is no significantly different wavelength shift in the absorption spectra at different reaction time interval. The absorbance band at lower wavelength with a good symmetry indicate that the mean diameter of silver nanoparticles is very small with a uniform size distribution.

In addition to a prominent band at above 260 nm, it is attributed to electronic excitation in tryptophan and tyrosine residues in protein which indicates the presence of protein molecule in colloidal solution. It is interesting to note from our study that NADPH dependent reduce enzymes, not only hereby Pusarium oxysporum as suggested by other [18], but also involves in the reduction of Ag⁺ to Ag. In case of fungus T. viride under experimental condition the possible mechanism suggests that the reduction of Ag⁺ to Ag is mainly due to conjugate between the electron shutter with NADPH dependent reduce participation [18].

FTIR spectrum of silver nanoparticles is shown in Fig. 2. This spectrum shows the presence of bands at 1650, 1540, 1423 and 1060 cm⁻¹. The bands at 1650 correspond to primary amine NH band, similarly, 1540 and 1060 corresponds to secondary amine NH band and primary amine CN stretch vibrations of the proteins, respectively [19]. The positions of these bands were close to that reported for native proteins [20]. The FTIR result indicates that the secondary structures of proteins were not affected as a consequence of reaction with Ag⁺ ions or binding with silver nanoparticles. The band at 1425 cm⁻¹ is assigned to methylene scissoring vibration from the protein in the solution.

XRD pattern taken using Cu Ko target in the range 20–90 of the Ag nanoparticles is shown in Fig. 3. The peaks matches with JCPDF Card No-008-0720. There is no new peak because protein is found in this range. The peak listing is shown in Table 1. The

<table>
<thead>
<tr>
<th>d-spacing (Å)</th>
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<th>(hkl)</th>
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<tr>
<td>2.354</td>
<td>2.354</td>
<td>(111)</td>
</tr>
<tr>
<td>2.0386</td>
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<tr>
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<td>1.4115</td>
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<td>1.2393</td>
<td>(311)</td>
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<td>1.177</td>
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<td>(222)</td>
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obtained pattern is for fcc cubic crystal structure. The peak plane matches with the card. The crystalline size is calculated from the full-width at half-maximum (FWHM) of the diffraction peaks using the Debye–Sherrer formula

\[ D = \frac{0.9 \lambda}{\beta \cos \theta} \]  

where \( D \) is the mean grain size, \( \lambda \) is the X-ray wavelength for Cu target, \( \beta \) is the FWHM of diffraction peak and \( \theta \) is the diffraction angle. In order to measure the size of nanoparticles accurately each peak is Gaussian fitted and also the instrumental broadening is subtracted using Si standard sample broadening. The size of nanoparticles from all the peaks is in the range of 2–4 nm. To observe the effect of protein molecule on crystal structure the lattice parameter is calculated using \( d \) spacing of each peak. The calculated lattice parameter is found to be 4.084 Å. It confirms that biologically synthesized silver nanoparticles lattice is unaffected by secreted protein molecules by T. viride.

The HRTEM technique was used to visualize the size and shape of the silver nanoparticles formed. Fig. 4a shows the typical bright field HRTEM micrograph of the synthesized silver nanoparticles. The morphology of nanoparticles is spherical which is observed in micrograph. The HRTEM micrograph suggests that the size of particles is around 2–4 nm. In EDX analysis, Fig. 4b inset shows the peak in silver region confirming the presence of elemental silver. The peak is observed approximately at 3 KeV, which is typical for the absorption of metallic silver nanocrystalline [21] due to surface plasmon resonance. From this we confirmed the presence of nanocrystalline elemental silver.

The photoluminescence (PL) of silver metal and that of noble metal is generally attributed to electronic transition between the upper d band and conduction sp band [22]. Luminescence of silver can be induced by irradiating the metal surface or film with electron [23], photon [24] or laser beam [25], with reported emission peak positions distributed over a wide range of 320–520 nm. However, most of the reports are silver metal, little has been reported about luminescence from silver nanoparticles.

The PL shown in Fig. 5 shows emission in a range of 320–520 nm. The three peaks at 364 nm, 410 nm and 460 nm are observed in the PL emission. The peak at 410 nm is so weak that can be neglected in whole photoluminescence spectra; meanwhile another two strong bands staying apart at both side of 410 nm were observed. The strong emission band that approximately occurred at 364 nm should be assigned to Ag–Ag interaction. Xu et al. have shown visible luminescence at 448 nm on excitation of 309 nm, which is due to metal–ligand charge transfer absorption [26].

Theoretical work demonstrates that photoluminescence of silver metals can be viewed as excitation of electrons from occupied d bands into states above the Fermi Level. Subsequently electron–phonon and hole–phonon scattering process leads to energy loss and finally photoluminescence recombination of an electron from an occupied Sp band with the hole [22,23]. This mechanism of the emission is shown in Fig. 5, where the band structure for a typical noble metal is represented by a sample model which includes s–p conduction bond and two sets of d band in K-space. Excitation occurs from states in upper d bands to level and above the Fermi energy. Because of the small photon momentum the interband transition are assumed to be direct. The emission arises from direct recombination of conduction band electron below the Fermi energy with holes in the d band that have scattered to momentum state less than the Fermi momentum \( k_F \) [27].
4. Conclusion

Silver nanoparticles were synthesized by a novel route using T. viride. The UV–vis studies showed Plasma Resonance behaviors. The size of monodisperse silver nanoparticles is 2–4 nm which is confirmed by HRTEM and XRD. The FTIR studies show the presence of protein molecules. The PL most interestingly shows an emission in the range of 320–520 nm, which fall in blue orange region. The biocompatible silver nanoparticles that are synthesized by the biological method in future can be used for biosensor and bioimaging application.

References