

Characterization of Copper Nanoparticles Synthesized by a Novel Microbiological Method

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The exploitation of various biomaterials for the biosynthesis of nanoparticles is considered as green technology as it does not involve any harmful chemicals. The present study reports the synthesis of copper nanoparticles which involves non-pathogenic bacterial strain Pseudomonas stutzeri, isolated from soil. These copper nanoparticles are further characterized for size and shape distributions by ultraviolet-visible spectroscopy, x-ray diffraction, and high resolution transmission electron microscopy techniques. The results showed that the particles are spherical and quite stable in nature and shows surface plasmon resonance clearly featured in the optical spectra in visible region.

INTRODUCTION

Low-dimensional nanoscale materials are currently of great interest due to their unique electronic, optical, and mechanical properties.¹⁻⁴ Among the coinage metals, Ag and Au have been studied the most because of their stability and their intense absorption band in the visible region, often called surface plasmon absorption. Cu, in turn, is the least studied out of the coinage metals due to its high instability for oxidation, and most of the time it forms complexes with water molecules in aqueous media.^{5,6} Particularly in transition metal oxides, Cu-based nanoparticles and nanowires have been synthesized by a variety of methods, and because of a reduction in dimensionality, their ferromagnetic polarizations are quite different from those observed in transition metals.⁷⁻⁹ Cu nanoparticles were widely used as alternative catalysts,¹⁰ such as selective hydrogenation and methanol synthesis reactions, which make them suitable for application in the field of catalysis. Nano-sized Cu particles are widely used

in fields of lubricants, polymers/plastic, metallic coating and ink. Cu sulfide has potential applications in solar cells, IR detectors and lubrication.¹¹

There are several reports available of physical and chemical synthesis of Cu and Cu based nanomaterials. Chuncheng Hao et al. have reported the preparation of Cu nanoparticles encapsulated in graphitic carbon shells using modified arc plasma method.¹² Surfactant-assisted electrochemical proce-

dures have been shown to be a powerful tool for preparing stable nanoparticles composed of a wide range of metals and compounds.¹³⁻¹⁸ The most varied and versatile preparation route appears to be the so-called "sacrificial anode" electrolysis that is usually carried out in the presence of cationic surfactants such as tetra-alkyl-ammonium salts. In a seminal paper based on the combined use of transmission electron and scanning tunneling microscopies, the group of M. Reetz demonstrated that these metal nanoparticles possess a core-shell structure in which the metallic core is stabilized by a monolayer of tetra alkyl-ammonium species, and the thickness of this shell is linearly correlated with the length of the alkyl chains.¹⁹ In the last decade, the electrolytic production of nanoparticles and nanocomposites containing platinum group elements has been extensively studied, due to the strong catalytic properties of these products.^{13,20-25} The electro-synthesis of Cu and Ag colloids, on the other hand, has been the subject of only a few reports.^{14,26-28} But no reports are available on the biological synthesis of Cu nanomaterials. It is, therefore, important to develop synthetic strategies that are simple, cost-effective, environment friendly, easily scalable and at the same time with parameters to control size and shape of the materials. Hence the development of a novel method for the preparation of Cu nanoparticles is inevitable. With these ideas in mind, an attempt has been made in this investigation to synthesize Cu nanoparticles by a novel biological method using non-pathogenic bacterial strain *Pseudomonas stutzeri* isolated from soil and characterize them for their properties by methods of absorption optical spectroscopy and transmission electron microscopy (HRTEM).

How would you...

...describe the overall significance of this paper?

This paper reports a bacterial strain for the synthesis of copper nanoparticles. This is a novel method as it doesn't involve any harmful and environmentally toxic chemicals used previously in conventional chemical reduction methods for the preparation of copper nanoparticles.

...describe this work to a materials science and engineering professional with no experience in your technical specialty?

Copper nanoparticles synthesized with this method are spherical and well dispersed. They have organic coating on the surface which is reducing and not only acts as capping agent but also prevents them from oxidation, which seems to be a major problem with the previous methods involved in the copper nanoparticles synthesis.

...describe this work to a layperson?

This method reports use of a non-pathogenic bacterial strain and spherical nanoparticles of copper are synthesized which are very useful in the field of catalysis. It is a simple, rapid, cost efficient and green method which is free from using any toxic reducing chemicals. This provides a platform for new metallic nanoparticles synthesis.

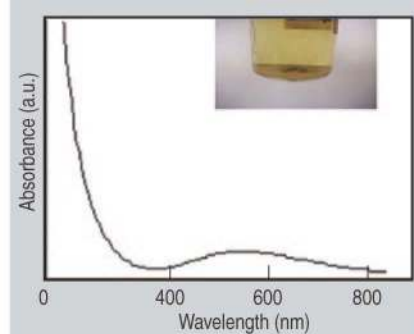


Figure 1. UV-Visible spectra recorded from solution of Cu nanoparticles; inset shows the appearance of yellow color indicating the synthesis of nanoparticles.

The size and shape distributions of Cu nanoparticles are determined from the data of TEM and optical spectroscopy. X-ray diffraction (XRD) analysis was performed to identify the crystalline nature of Cu nanoparticles.

See the sidebar for experimental procedures.

RESULTS AND DISCUSSION

Nanosized Cu together with other noble metals such as Au and Ag are the most studied metallic nanoparticles as the surface plasmon resonances are clearly featured in the optical spectra, and are located in visible region.²⁹ Cu nanoparticles were first indicated by the appearance of a yellow color solution (Figure 1 inset). This is due to Plasmon resonance, with a significant contribution from inter-band transition which produces yellow hydrosol having I_{\max} at 570 nm (Figure 2). The yellow color of the colloidal solution remains stable after one month of synthesis. The position and shape of the plasmon absorption peaks are dependent on the particle morphology, dielectric functions of the metal and the surrounding medium as well as surface-adsorbed species.²⁹ The presence of the single surface-plasmon peaks implied that the formed nanoparticles were nearly spherical attributed to the coherent oscillation of the conduction electrons caused by oscillating electric field when they are irradiated by light.³⁰

According to Mie theory, spectra of the particles with radii between about 2 and 10 nm are independent of the particle size. For particles with dimensions beyond 10 nm the absorption peak broadens and shifts to longer wavelength. Analogous considerations

can be made for the absorption spectra of Cu nanoparticles. In this case, the broad and low absorption peak can be related to the small particle size due to a reduced mean free path for conduction electrons. At very small sizes, limitation of the mean free path by the particle boundary broadens and decreases peak absorption.³¹ The spectra of nanoparticles obtained by biological synthesis gives the better band than the traditional chemical methods, proves the less share of impurity, and also, probably, the less dispersion in the size of particles.

The XRD pattern of the sample is shown in Figure 2. Diffraction peaks can be indexed to those of pure face-centered cubic (f.c.c.) Cu (JCPDS, File No. 04-0836), corresponding to the (111), (200), and (220) planes. The crystallite size can be found by applying Sherrer's equation and the average crystallite size is found to be 11 nm. The observation of diffraction peaks for the Cu nanoparticles indicates that these are crystalline in nature.

Previous studies found that Cu nanoparticles without the coating oxidize gradually.³² To confirm the stability of nanoparticles, we performed diffraction analysis of Cu nanoparticles as prepared and after one month. The graph in

Figure 2a illustrates the crystallized particles immediately after the preparation consisted of Cu and in Figure 2b, crystallized particles of Cu after one month illustrates an insignificant change in intensity. Since it is clear from Figure 2 that Cu nanoparticles have not been oxidized in over one month, it was demonstrated that the organic coating on the metal nanoparticles is effective in preventing them from oxidation.

To confirm the spectra analysis findings and identify the existence of Cu nanoparticles structure, HRTEM analysis was performed for the sample. Typical HRTEM images and SAED pattern of the Cu nanoparticles synthesized are presented in Figure 3. In the HRTEM images, the dark spots and light features corresponds to Cu nanoparticles and carbon matrix, respectively. HRTEM study shows that particles produced were almost spherical and 8–15 nm in size range (Figure 3a). In Figure 3b, a thin coating layer can be observed on all particles and the thickness is a few nanometers. This indicates that the bacterial surface acts both as reducing as well as capping agent. The micrograph also demonstrates that as-synthesized Cu nanoparticles are well-dispersed with no conspicuous agglomeration and

EXPERIMENTAL PROCEDURES

Bacterial strains were enumerated from soil around the sewage outfall of the small scale electroplating industry from Dhakran, Agra by Serial dilution-agar plating method (Nigam 1965). $1 \times 10^{-3} \text{ dm}^3$ inoculum was transferred into $50 \times 10^{-3} \text{ dm}^3$ medium containing (g/l) Peptone, 5; Beef extract, 3; Sodium chloride, 5; Agar, 15; pH 7.2 in Erlenmeyer flasks. Cells were grown at 37°C for 24 h and then harvested by centrifugation (8,000 r min⁻¹, 10 min. at room temperature). The cell pellet was resuspended and centrifuged three times in deionized water. Copper sulfate was purchased from Hi-media, and was used as received. In a typical synthesis for nanoparticles using *Pseudomonas stutzeri*, the carefully weighted 0.1 g biomass was added to 100 ml of 1 mM aqueous CuSO₄ solution, in conical flasks of 250 ml content. The flasks were thereafter incubated in incubator-shaker at 150 rpm at room temperature.

To confirm the synthesis of nanoparticles, characterization was done by UV-Vis spectroscopy, x-ray diffraction, high-resolution transmission electron microscopy and selected area electron diffraction techniques. The bioreduction of Copper ions in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml deionized water and subsequently measuring ultra violet visible (UV-vis) spectra of the resulting diluents. UV-vis spectroscopy analyses of copper nanoparticles produced were carried out on ELICO UV spectrophotometers at a resolution of 1 nm. X-ray diffraction measurements of the bioreduced solution, drop-coated onto glass substrate, were done by an X'Pert Pro x-ray diffractometer instrument operating at a voltage of 45 kV and a current of 40 mA with Cu K α radiation. Samples for HR-TEM analysis were prepared on carbon-coated copper grids. The films on the copper grids were allowed to stand for 2 min following which the extra solution was removed using a blotting paper and the grid was allowed to dry prior to measurement and analysis was carried out on Tecnai G² F30 S-Twin (FEI; Super Twin lens with Cs = 1.2 mm) instrument operated at an accelerating voltage at 300 kV (field emission gun), having a point resolution of 0.2 nm and lattice resolution of 0.14 nm.

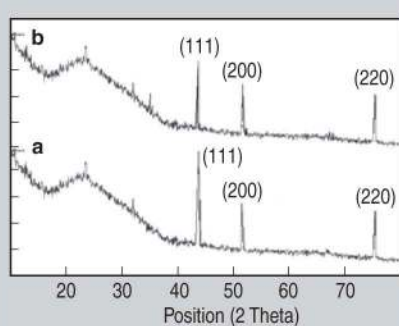


Figure 2. XRD patterns recorded from drop-coated films of Cu nanoparticles on glass substrates (a) after one h; (b) after one month.

stable even up to one month; since the x-ray diffraction studies confirmed.

Figure 3c depicts the high magnification view of nanoparticles which shows lattice fringes on the surface, which is in good agreement with the inter-planar spacings of Cu (111). Inset of Figure 3c shows the selected area electron diffraction (SAED) pattern obtained from the Cu nanoparticles. SAED depicts the Scherrer ring patterns, characteristic of the face centered cubic (fcc) Cu, indicating that the structures seen in the HRTEM images are nanocrystalline in nature. From phase identification by transmission electron microscopy and x-ray diffraction analysis, it was found that the Cu nanoparticles have been passivated and have not been oxidized in over one month.

CONCLUSION

We have prepared spherical Cu nanoparticles in nanoregime by a novel biological synthesis technique which is simple and environmentally benign. It is an easy, fast, and cost effective technique and doesn't involve any harmful and environmentally toxic chemicals used previously in conventional chemical reduction methods. Aqueous solutions of Cu nanoparticles with very good stability have been synthesized. It has been also discussed that the biomolecules present in the biomass not only reduce the metal ions and but also stabilize the metal nanoparticles by preventing them from being oxidized after the preparation. To the best of our knowledge, this is the first report of Cu nanoparticles synthesis using any bacterial strain.

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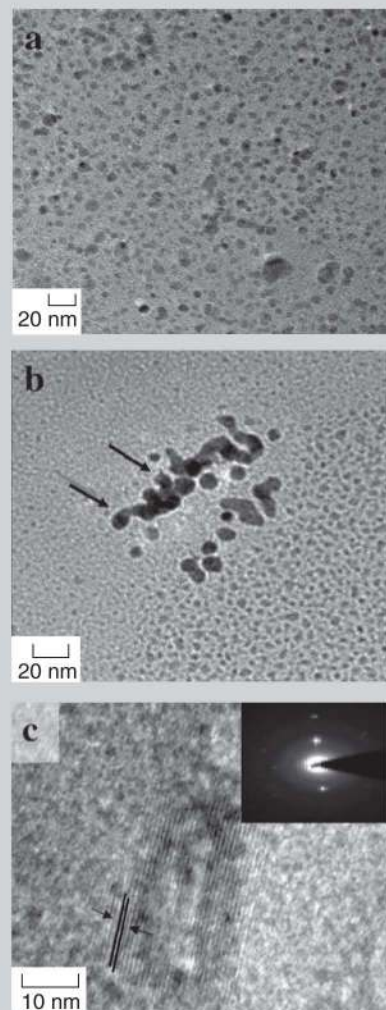


Figure 3. HRTEM images of Cu nanoparticles formed by bacteria *Pseudomonas stutzeri* shows: (a) spherical Cu nanoparticles; (b) a thin bacterial layer around nanoparticles which acts as capping agent; (c) close-up view of nanoparticles showing lattice fringes; inset shows selected electron diffraction pattern.

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