www.amlett.com, DOI: 10.5185/amlett.2010.9155 Published online by the VBRI press in 2010

Biogenic synthesis of silver nanocubes and nanorods using sundried *Stevia rebaudiana* leaves

Ratnika Varshney[a]*, Seema Bhadauria[a], Mulayam S.Gaur[b]

[a]*Microbiology and Nanotechnology Research Lab, Department of Botany, Raja Balwant Singh College, Khandari, Agra 282004, India*

[b]*Department of Physics, Hindustan College of Science & Technology, Farah, Mathura, India*

*Corresponding author. Tel: (+91) 902 7224510; Fax: (+91) 562 2881414; E-mail: ratnika_bt@rediffmail.com

Received: 13 Sept 2010, Revised: 8 Oct 2010 and Accepted: 11 Oct 2010

ABSTRACT

This work reports a novel biological method for the synthesis of rod shaped silver nanoparticles by exploiting sundried *Stevia rebaudiana* leaves at ambient conditions. On treatment of aqueous solutions of silver with leaf powder, not only the rod shaped silver nanoparticles ranging from 80-200 nm diameter and 400-800 nm height, but also cubes ranging from 55 to 80 nm in size, could be rapidly fabricated. The rate of reduction is much faster than those observed in earlier studies, highlighting the possibility that biological methodologies will achieve rates of synthesis comparable to those of chemical methods. The approach also appears to be a cost-efficient alternative to conventional methods, so it would be suitable for developing a biological process for large scale production. Instead of the boiled leaf broth used in previous studies, sundried leaf biomass could be preserved as an excellent bio-reductant, conveniently available any time for biosynthesis of the nanoparticles. Copyright © 2010 VBRI press.

Keywords: *Stevia rebaudiana*; biosynthesis; nanorods; sundried biomass, silver nanoparticles.

Ratnika Varshney is presently working as a Senior Research Fellow in project funded under the Nano Science & Technology Initiative scheme of Department of Science & Technology, New Delhi, India. She did her M.Sc. in biotechnology from Jiwaji University, Gwalior, India. Her areas of research interests are biosynthesis of nanomaterials of silver, copper and bioremediation of industrial waste waters.

Seema Bhadauria is presently working as a Reader in Microbiology, Department of Botany, R.B.S.College, Agra. She did her Ph.D. from Agra College, Agra. She has published more than 100 research papers in the field of Biodeterioration and Bioremediation, Soil Testing, Vermicomposting, Bioethanol production and synthesis of nanoparticles and their use in bioremediation of industrial waste waters. She has completed five sponsored projects as a principal investigator funded by Department of Science & Technology and Department of Biotechnology, Govt. of India and has two ongoing sponsored projects. She has one patent on soil testing kit to her credit. She is guiding ten PhD students. in the field of Microbiology, Biotechnology.

M.S. Gaur received his M.Sc., M. Phil, and PhD degrees in Physics from the Rani Durgawati University, Jabalpur (MP), India. He has started his career as a Lecturer in 1996. He has published more than 30 research papers in the field of multidisciplinary sciences particularly electroactive properties of polymer/polymer nanocomposites and synthesis of nanoparticles for biosensor applications. He has completed two sponsored projects as a principal investigator and currently has two ongoing sponsored projects. He is guiding six PhD students. Presently, he is a Professor and Head, Department of Physics, Hindustan College of Science & Technology, Farah, Mathura (UP), India. His research interests are synthesis and characterization of nanoparticles, optoactive and electroactive properties of polymer nanocomposites.

Introduction

Nanotechnology is the fastest growing area of manufacturing in the world today and there is an increasingly frantic search for new nanomaterials and methods to make them. There have been impressive developments in the field of nanotechnology in the recent past, with numerous methodologies formulated to synthesize nanoparticles of particular shape and size depending on specific requirements. It has been well known that living cells are the best examples of machines that operate at the nano level and perform a number of jobs ranging from generation of energy to extraction of targeted materials at very high efficiency [**1**].

Recognizing the importance of developing eco-friendly nanoparticles synthesis methods, more and more researchers have turned to biological micro-organisms for inspiration. Many biological organisms, both unicellular and multicellular, are known to produce inorganic materials either intra- or extra-cellularly [**2, 3**] often of nanoscale dimensions and of exquisite morphology and hierarchical assembly. The accumulation of inorganic particles in microbes has been reported by Zumberg et al. [**4**] (gold in Precambrian algal blooms), Hosea and coworkers [**5**] (gold in algal cells), Beveridge and coworkers [**6**] (gold in bacteria), Aiking and coworkers [**7**] (CdS in bacteria), Reese and coworkers [**8**] (CdS in yeast), Temple and Leroux [**9**] (ZnS in sulfate-reducing bacteria) and Blakemore et al. [**10**] (magnetite in bacteria). Some well-known examples of bio-organisms synthesizing inorganic materials include magnetotactic bacteria (which synthesize magnetite nanoparticles) [**11**] and actinomycetes like extremophilic actinomycete, *Thermomonospora sp*. [**12**] and alkalotolerant actinomycete *Rhodococcus sp* [**13**].

Beveridge and co-workers have demonstrated that gold particles of nanoscale dimensions may be readily precipitated within bacterial cells by incubation of the cells with Au^{3+} ions [**14**]. Klaus-Joerger and co-workers have shown that the bacterium *Pseudomonas stutzeri* AG259 isolated from silver mine, when placed in a concentrated aqueous solution of $AgNO_3$, resulted in the reduction of the Ag^+ ions and formation of silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria [**15**]. Nair and Pradeep have synthesized nanocrystals of gold, silver and their alloys by reaction of the corresponding metal ions within cells of lactic acid bacteria present in buttermilk [**16**]. Sastry et al. have shown that eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical compositions and sizes. A number of different genera of fungi have been investigated in this effort and it has been shown that fungi are extremely good candidates in the synthesis of gold [**17, 18**] and silver [**19-21**]. One area of untapped potential is the use of plants to fabricate nanoparticles. Very recently, the synthesis of nanoparticles of variable morphology using leaves of different plants, sprouts, roots and stems of live alfalfa plants have been demonstrated include bacteria for silver [**15, 22, 23**], gold [**14, 16, 24, 25**], CdS [**26-28**], ZnS (sphalerite) [**29**], magnetite [**30, 31**] and iron sulphide [**32-41**].

The biosynthetic method employing plant extracts has received some attention as a simple and viable alternative to chemical procedures and physical methods synthesizing metal nanoparticles only in recent years [**42**]. Jose-Yacaman et al firstly reported the formation of gold and silver nanoparticles by living plants [**37, 43**]. It has been reported that the Au (III) ions are reduced in the solid media to Au (0) by the plant and then the atoms are absorbed into the plant where the nucleation and growth of gold nanoparticles takes place. This method can be very efficient in decontaminating soil polluted with heavy metal ions.

Sastry et al. aforementioned attained the biosynthesis of metal nanoparticles by plant leaf extracts and their potential applications. They studied bioreduction of chloroaurate ions or silver ions by a broth of geranium leaf [**40**] or Neem leaf [**44**]. Further, they had explored the formation mechanism of gold nanotriangles by lemongrass extracts. The nanotriangles seemed to grow by a process involving rapid reduction, assembly and room-temperature sintering of 'liquid-like' spherical gold nanoparticles [**39**]. Very recently, they have demonstrated synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extracts [**45**]. It was explained that only biomolecules of molecular weights less than 3 kDa caused reduction of chloroaurate ions, leading to the formation of gold nanotriangles. Nevertheless, the bioreduction of silver ions proceeded merely in the presence of ammonia. Most of the above research on the synthesis of silver or gold nanoparticles utilizing plant extracts employed broths resulting from boiling fresh plant leaves.

Here, novel sundried *Stevia rebaudiana* leaf, previously unexploited for bioreduction and different from such boiling procedures, was used to synthesize rod shaped silver nanoparticles in aqueous solutions at ambient conditions, without any additive protecting nanoparticles from aggregating, template shaping nanoparticles or accelerants like ammonia. To our knowledge, it is the first report of synthesis of silver nanorods using dried leaves powder of *Stevia*, a zero calorie sweetener.

Experimental

The biomass used for the reduction was prepared by crushing the dried *Stevia rebaudiana* leaves and then screening the leaf powder by a 20 mesh sieve. Silver nitrate ($AgNO_3$) was purchased from Hi-media Company and was used as received. For the synthesis of silver nanoparticles, carefully weighted 0.5 g dried powder of *Stevia rebaudiana* leaf, was added to 50 mL of 1 mM aqueous $AgNO_3$ solution at room temperature. The bioreduction of Ag^+ 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 silver nanoparticles produced were carried out as a function of bioreduction time at room temperature on ELICO UV spectrophotometers at a resolution of 1 nm. X-Ray diffraction (XRD) measurements of the bioreduced silver nitrate solution drop-coated onto glass substrates were done for the determination of the formation of Ag 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*a* radiation. The biomass after reaction

spontaneously precipitated at the bottom of the conical flasks in 1 h. After the precipitation, the suspension above the precipitate was centrifuged at 5000 rpm, 4°C sampled for Scanning Electron Microscopy-Energy Dispersive X-ray Analysis (SEM-EDX). Samples of the aqueous suspension of silver nanoparticles were fabricated by dropping the suspension onto clean glass slide and allowing water to completely evaporate and then coated with carbon. SEM analyses were performed on a Zeiss EVO 300 Electron Microscope with Bruker EDX after carbon coating of the samples. After bioreduction, the biomass residue was obtained by centrifuging the residual solution at 5000 rpm for 10min. Then the biomass residue was completely dried at 60 °C. The dried biomass before bioreduction and the residue of *S. rebaudiana* leaf after bioreduction were analysed by Nicolet Avatar 660 (Nicolet, USA) Fourier Transform Infrared spectrophotometer (FT-IR).

Results and discussion

Biological systems, masters of ambient condition chemistry, synthesized nanomaterials that are hierarchically organized from the nano- to the macroscale. In this work, a zero calorie sweetener plant *Stevia rebaudiana* leaves were used for the synthesis of silver nanoparticles. Reduction of the aqueous silver nitrate ions during exposure to the *Stevia* leaves may be easily followed by UV-vis spectroscopy. It is well known that silver nanoparticles exhibit lovely red colors, this color arises due to the excitation of surface plasmon vibrations in the silver nanoparticles. The biomass incubated with deionized water (positive control) retained its original color i.e. green, while the silver nitrate treated biomass turned dark red (as shown in **Fig. 1a**) after 6 h due to the formation of silver Nanoparticles extracellularly. This shows that it was a quite fast process. This color is primarily due to the surface plasmon resonance of silver nanoparticles. In case of negative control (silver nitrate solution alone), no change in color was observed and the silver nanoparticles analysed by UV-Vis spectra and SEM were stable after 180 days (as shown in **Fig. 1b**).

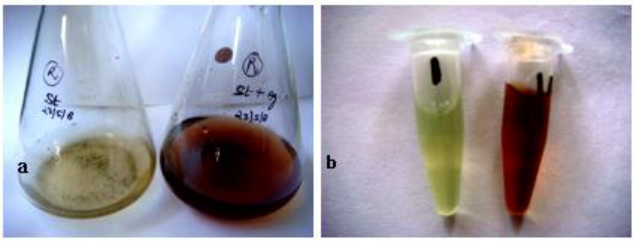

Fig. 1. (a) Flasks containing the Stevia leave biomass and incubated with an aqueous solution of $AgNO_3$ solution after 6 h and (b) Tubes containing control and silver nanoparticles after 6 months.

It is generally recognized that UV–vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions [**38**]. **Fig. 2a** shows the UV-Vis absorption spectra of the silver nitrate solution challenged with the *Stevia* biomass after 6 h of reaction. The evolution of the absorbance spectra emanating from silver nanoparticles over time manifests increasingly sharp absorbance with increasing time of reaction at around 420 nm attributed to the surface plasmon resonance band (SPR) of the nanocrystalline silver particles. While no absorption band was observed in both controls (positive and negative) not shown in the graph. After 6 h of incubation, no change in intensity at 420 nm was observed indicating the complete reduction of silver ions (**Fig 2a**).

It may be noted that the intensity of the plasmon peak initially increases with number of days of incubation due to increasing concentration of the silver nanoparticles (since the intensity of the plasmon peak is proportional to the concentration of silver nanoparticles produced) and then it saturates upon completion of the reaction (**Fig. 2b**).

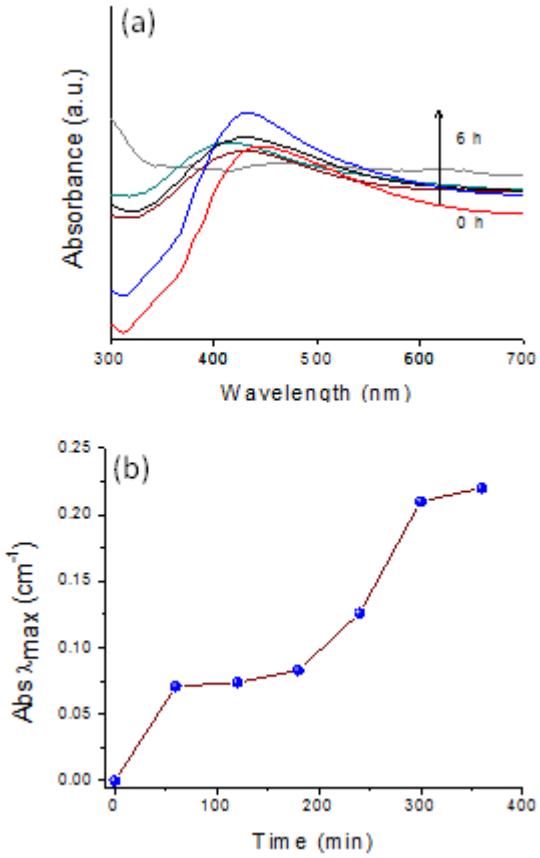

Fig. 2. (a) UV-Vis spectra recorded as a function of time of reaction of 1 mM $AgNO_3$ aqueous solution. (b) Plot of maximum absorbance vs time of reaction for the silver nanoparticles.

The plasmon bands are broad with an absorption tail in the longer wavelengths, which could be in principle due to the size distribution of the particles [**46**]. Since the varying intensity of the plasmon resonance depends on the cluster size [**39**]. After the reaction the silver nanoparticles solution was filtered from the biomass and aged for 6 months. Then the stability of the synthesized nanoparticles was studied by measuring its UV-Vis spectra at 420 nm over a period of 6 months at room temperature. A little change in intensity was observed. The results indicate that the solution was stable for at least six months with only little aggregation of particles in the solution.

Fig. 3 shows the XRD pattern recorded from a drop-cast film of the silver nanoparticles in sample. A number of strong Bragg reflections can be seen which correspond to the (111), (200), (220) reflections of *fcc* silver. The XRD

results thus show that the silver nanoparticles formed are crystalline.

Additionally, the diffractions at around $2\theta = 22°$ resulting from the biomass or the biomass residue are also notable. No spurious diffractions due to crystallographic impurities were found. The size of the Ag nanoparticles estimated from the Debye–Scherrer formula is ca. 42 nm.

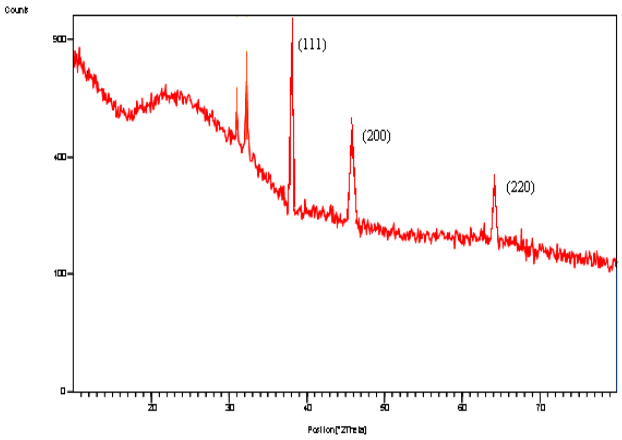

Fig. 3. XRD patterns recorded from drop-coated films of silver nanoparticles synthesized using Stevia leaf powder on glass substrates.

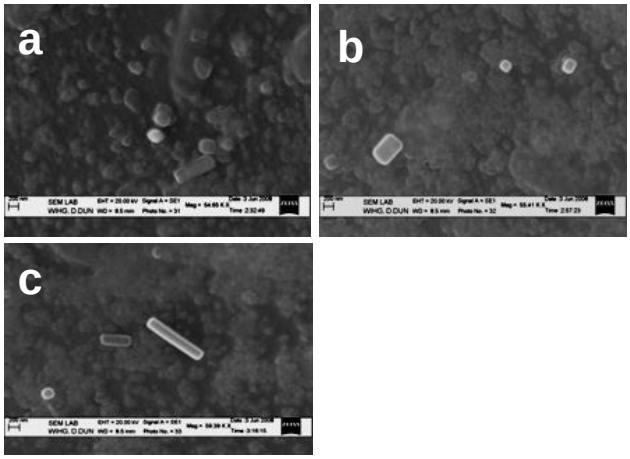

Fig. 4. SEM images from a to c illustrating the formation of Silver Nanorods by exposing 0.5 g of Stevia Biomass to 50 ml of 1 mM aqueous $AgNO_3$ at 26 ° C. Scale bars: (200 nm).

Fig. 4 shows representative SEM images recorded from drop-coated films of the silver nanoparticles, synthesized by treating silver nitrate solution with *Stevia* leaves for 6 h. In Fig. 4a, cubic silver nanoparticles ranging from 55 to 80 nm in size and particles growing into rods ranged in size from 80-200 nm diameters and 400-800 nm height can be seen. Silver nanoparticles are quite polydisperse in nature. In **Fig. 4b** and **c**, the morphology of the rod shaped silver nanoparticles is clearly seen. While triangular nanoparticles of silver and gold have been observed in earlier studies, there have been no reports on biogenic silver nanorods [**22, 47**]. The results indicate that some rectangular silver nanocubes in the size of 50-300 nm are also found.

The rod shaped nanoparticles were verified to contain Ag using EDX analysis in **Fig. 5** and confirmed as elemental Ag (0) using XRD as shown in **Fig. 3**. Strong signals from the silver atoms in the nanoparticles are observed, while signals from C, O, K, Al, Ca, Mg and Na atoms were also recorded. These signals are likely to be due to X-ray emission from proteins/enzymes present in the cell wall of the biomass.

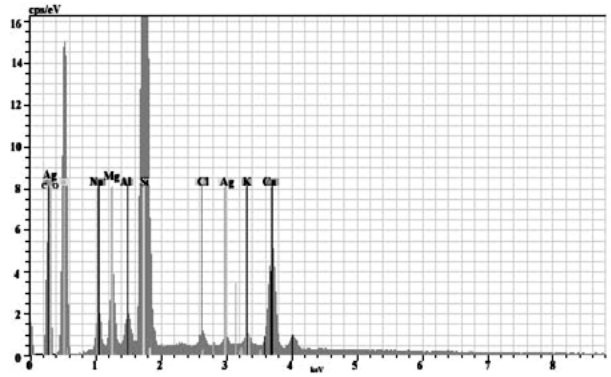

Fig. 5. EDAX (energy dispersive analysis of X-rays) spectrum recorded in the spot-profile mode from one of the densely populated silver nanorods regions.

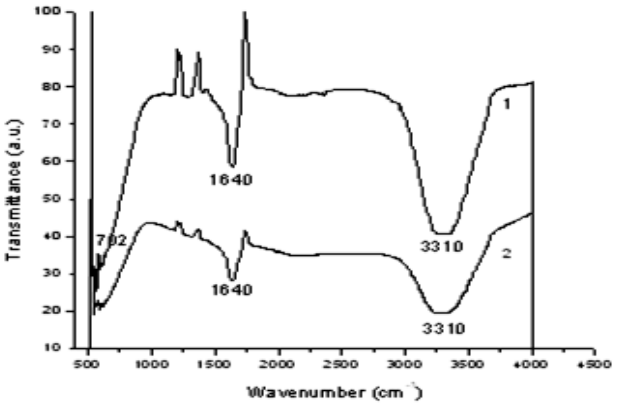

Fig. 6. Typical FT-IR absorption spectra of the leaf biomass before bioreduction (1) and after bioreduction of silver ions (2).

FT-IR spectroscopic studies were carried out to investigate the plausible mechanism behind the formation of these silver nanoparticles and offer information regarding the chemical change of the functional groups involved in bioreduction. Study on extraction of pharmaceutical components from the *S. rebaudiana* leaves showed that alkaloids, tannins, sterols, tri-terpenes, cardiac glycosides, saponins, reducing compounds, anthraquinones and cyanogenetic glycosides exist in such a leaf [**48**]. **Fig. 6** shows the spectra of cell-free extracts of leaves analysed before and after bioreduction.

The broad band in the region 3100-3400 cm^{-1} is due to O–H stretching vibrational frequencies and the presence of bands in these regions in the spectra strongly indicates the presence of organic molecules with alcohol functional groups [**49**]. It may be observed that the intensity of the band decreases significantly in (b), indicating a decrease in the concentration of the peptide linkages in the solution.

The spectra also exhibit an intense band at ~1640 cm^{-1} assigned to the N–H stretching band in the free amino groups of biomacromolecules. The intensity of this band also decreases in (b) due to the decrease in the concentration of peptide linkages in the solution in conformity with our previous observation. Moreover, both the spectra exhibit a low frequency band at ~760 cm^{-1} attributed to the bending vibration in the S–H moiety bonded to the CH_2 group [**50, 51**]. It may be observed that the intensity of the band at ~760 cm^{-1} decreases drastically due to possible changes in the chemical environment and hence symmetry around the S–H bond.

Medium to weak and broad peaks observed between 1200-1300 cm^{-1} in the spectra, are possibly molecules bind to the surface of the Ag nanoparticle through the carboxylate groups. These originate from the O–H bending vibrational modes from alcohol functional groups or from C–O stretching and O–H bending vibrations from COOH groups. Possibly the molecules capping Ag nanoparticles are the oxidized products of Steviosides (e.g. steviol glycosides, rebaudiosides etc.) [**52**] or reducing agents those are usually present as a large fraction in the plant extracts. The oxidation of reducible alcoholic or aldehydic group to carboxylic group possibly leads to the reduction of the Ag^+ ions to Ag^0 state and formation of nanoparticles stabilized by the carboxylic functionality.

There appear to be no peaks in the amide I and amide II regions characteristic of proteins/enzymes which have been found to be responsible for the reduction of metal ions when using microorganisms such as fungi for synthesis of metal nanoparticles.

The peaks observed for the silver nanoparticles at 1640 cm^{-1} (C=C groups or from aromatic rings), suggest the presence of the steviosides or reducing agents adsorbed on the surface of metal nanoparticles. It is also possible that the terpenoids may play a role in reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids.

Previously, we have observed from FT-IR studies that there is a decrease in the concentration of the amide linkages in the aqueous solution after the formation of silver nanoparticles. This suggests that some of the peptide linkages from amino acids first undergo hydrolysis to produce free carboxylate ions and free amino groups which then possibly act as the capping agents to the silver nanoparticles.

Conclusion

In conclusion, it has been demonstrated that the *Stevia rebaudiana* leaves powder is capable of producing cubic and rod shaped silver nanoparticles extracellularly at room temperature conditions without using any additive protecting nanoparticles from aggregating, template shaping nanoparticles or accelerants like ammonia and these nanoparticles are quite stable in solution. The glycosides components were mainly responsible for the reduction of silver ions and the stabilization of the nanoparticles. And this is a cost efficient, eco-friendly and simple process. This report will also lead to the development of a rational biomaterials-based synthesis procedure for other metal nanomaterials such as gold and platinum with the *Stevia* leaves powder and there is no need to use high pressure, energy, temperature and toxic chemicals and downstream processing and handling of the nanoparticle suspension would be much easier. Sundried biomass has the advantage over broth, previously used for reduction, as fresh leaves are seasonal so they would not be readily available for the bioreduction all the time. It is quite difficult to control some parameters accurately such as the optimum boiling time while handling the broth.

Acknowledgement

The authors gratefully acknowledge the financial support given to this research from the Department of Science & Technology, New Delhi, India under the NSTI (SR/S5/NM-22) Scheme. We are also grateful to the Director-NPL, Dr. N. K. Saini, Dr. N. K. Juyal and Dr. Samay Singh, WIHG, Dehradun for providing XRD and SEM-EDX facility. We acknowledge Dr. Renu Pasricha, National Physical Laboratory, New Delhi, India for valuable discussions and encouragement.

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