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ORIGINAL ARTICLE

Coleus aromaticus leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity

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Abstract The utilization of various plant resources for the biosynthesis of metallic nanoparticles is called green nanotechnology, and it does not utilize any harmful chemical protocols. The present study reports the plant-mediated synthesis of silver nanoparticles using the plant leaf extract of *Coleus aromaticus*, which acts as a reducing and capping agent. The silver nanoparticles were characterized by ultraviolet visible spectroscopy, X-ray diffraction, scanning electron microscopy, energy-dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy, and the size of the silver nanoparticles is 44 nm. The bactericidal activity of the silver nanoparticles was carried out by disc diffusion method that showed high toxicity against *Bacillus subtilis* and *Klebsiella planticola*. Biosynthesis of silver nanoparticles by using plant resources is an eco-friendly, reliable process and suitable for large-scale production. Moreover, it is easy to handle and a rapid process when compared to chemical, physical, and microbe-mediated synthesis process.

Keywords Biological synthesis · Silver nanoparticle · Electron microscopy · *Coleus aromaticus* · UV–vis spectroscopy

Introduction

Nanotechnology is likely to prominently manipulate science, economy and day-to-day life in this twenty-first century (Krumov et al. 2009) and their potential effects are used widespread in both in vivo and in vitro biomedical applications and research (Singh et al. 2008). Nanoparticles are the basic essential elements in the wall of nanotechnology and it exhibits fabulous advanced characteristic features based on their properties such as size, morphology and other size dependent properties (Smith et al. 2006). These unique features of nanoparticles may lead to play a crucial role in biomedicine, energy science, optics and other health care applications (Fayaz et al. 2010). Among nanoparticles, silver nanoparticles have potential applications in the arena of life sciences especially in food chemistry (Li et al. 2009), forensic science (Cantu 2008), agriculture (Park et al. 2006) and cosmetics (Kokura et al. 2010).

The physical (Xu et al. 2008) and chemical processes (Wang et al. 2005) are the classical general methods used for the fabrication of nanoparticles, but these methods are not environmentally benign (Dubey et al. 2010) and due to the presence of some toxic metals in the synthesis process that may create some dicey effects in biomedical applications (Bar et al. 2009a). These snags in the nanoparticle synthesis are overcome by microbe-mediated and plant-mediated biological process and this bio-route attracts a considerable interest because of its eco-friendliness and biocompatibility (Krumov et al. 2009). Several works has been reported on biosynthesis of silver nanoparticles by using microorganisms such as bacteria (Shahverdi et al. 2007), either intracellularly (Kalishwaralal et al. 2008a) and extracellularly (Kalishwaralal et al. 2008b) fungus (Basavaraja et al. 2008; Balaji et al. 2009) and algae (Vivek et al. 2011). Recently, plant assisted synthesis of nanoparticles have captured a considerable attractive interest in the arena of modern Nanoscience and technology due to its flexibility and eco-friendly nature. Some of the plant materials such as leaves

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(Satyavani et al. 2011a; Cruz et al. 2010; Elumalai et al. 2010; Huang et al. 2007; Daizy 2011), seeds (Bar et al. 2009b), fruits (Dubey et al. 2010; Jain et al. 2009), latex (Bar et al. 2009a) and barks (Sathishkumar et al. 2009) are involved in the metal reduction process.

Coleus aromaticus Lour. known as Indian Borage belongs to the Lamiaceae family, and it is a commonly available medicinal plant, which is used for the biofabrication of silver nanoparticles. It is a tender, fleshy, highly aromatic pubescent herb with distinctive smelling medicinal plant contains many phytochemicals such as carvacrol (monoterpenoid), caryophyllene (bicyclic sesquiterpene) and patchoulene and flavonoids like quercetin, apigenin, luteolin, salvigenin, and genkwanin (Ram and Mehrotra 1970). It is used for the treatment of malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic and convulsions (Nadkarni 1996). In this present study, the leaves of *C. aromaticus* was used for biosynthesis of silver nanoparticles, biosynthesized silver nanoparticles are characterized by UV–vis spectroscopy, X-ray diffraction (XRD), EDAX, and the capping agent for the silver nanoparticles synthesis was confirmed by Fourier transform infrared spectroscopy (FTIR). Furthermore, the bactericidal effect of silver nanoparticles was also analyzed by disc diffusion method.

Materials and methods

Plant materials and chemicals

Medicinal plant *C. aromaticus* plant was cultivated at in vivo condition at SPK Centre for Environmental Sciences, Alwarkurichi, Tamilnadu, India. Silver nitrate and all analytical grade chemicals were purchased from Himedia Chemicals Ltd., Mumbai.

Preparation of leaf extract

About 10 g of fresh leaves (Fig. 1) was collected and thoroughly washed the leaves with tween 20 and the detergent SDS for 3–4 times. The leaves were chopped into fine pieces and boiled it at 60 °C for 5 min. The extract was filtered and stored at 4°C and then used for nanoparticle synthesis.

Synthesis of silver nanoparticles

The silver nanoparticles were prepared by treating 90 ml of 1 mM silver nitrate with 10 ml of plant extract solution and incubate it at room temperature for 10 min and observed the brown colour formation, indicating the production of silver nanoparticles (Fig. 2).

Fig. 1 Picture of *Coleus aromaticus* leaves

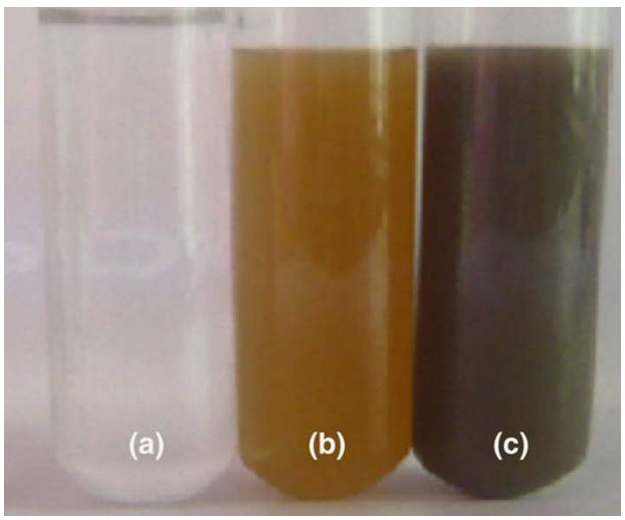

Fig. 2 Formation of silver nanoparticles and it is identification by the colour change. **a** Silver nitrate solution, **b** plant extract without silver nitrate, **c** plant extract with silver nitrate solution

Characterization of silver nanoparticles

The silver nanoparticle was characterized by UV-spectra at the wavelength ranges from 340 to 740 nm (Perkin Elmer Lambda double beam UV-Spectrophotometer). The purified silver nanoparticle was obtained by centrifuge the solution at 15,000 rpm for 2–3 times. The solution was filtered and the pellet was dried in the hot air oven. The dried powder of silver nanoparticles was further analyzed by XRD (Philips PW 1830), EDAX (Philips XL-30). Morphology and size of the silver nanoparticles was characterized by Scanning electron microscopy (SEM) (Philip model CM 200). The FTIR was obtained on a SHIMADZU instrument with the sample as KBR pellet in the wave number region of 500–4,000 cm^{-1}.

Bactericidal activity of silver nanoparticles

The bactericidal effect of the silver nanoparticle was analysed by disc diffusion method. The two bacterial cultures *Bacillus subtilis* (3053) and *Klebsiella planticola* (2277) were purchased from MTCC, India. These two bacterial cultures were grown in Muller Hinton agar (Himedia, Mumbai) petriplates with different concentration of silver nanoparticles containing disc such as 10, 20, 30, 40 and 50 μL at 37 °C for 24 h. After incubation, the antibacterial effect was measured by the formation of zone in the petriplates. The bactericidal activity of biosynthesized silver nanoparticles was also compared with leaf extract and chemically synthesized silver nanoparticles. The standard error was calculated using three replicates of experiments.

Results and discussion

The formation of nanoparticles was primarily characterized by UV–visible spectroscopy analysis. The formation of dark brown colour clearly indicates the production of silver nanoparticles and it confirmed by the surface plasmon resonance (Shankar et al. 2004) and the formation of dark brown colour because of the oscillation of free electron in the silver nanoparticles (Mulvaney. 1996). Figure 3 shows the UV–visible spectra of silver nanoparticles was measured at different time intervals from 0–24 h. Figure 3 exhibits the UV–vis spectra ranges from 340–740 nm and the strong SPR bands was observed at 460 nm. This SPR band indicates the presence of spherical silver nanoparticles in the solution. The broadening of peaks indicates the nanoparticles are monodispersed. The production of silver nanoparticles was initiated from 2–10 min onwards and vigorously it increases up to 24 h. After 24 h there was no colour was occurred indicate the completion of the nanoparticles synthesis process. The similar result was reported that silver nanoparticles were formed at 24 h by using the *Lippia citriodora* leaf extract (Cruz et al. 2010). Previously, microorganisms such as bacteria and fungi play an important role in the silver nanoparticle synthesis, but some demerits were found in those processes that is the time required for the synthesis of nanoparticle is from 24 to 120 h. In this investigation, using the *C. aromaticus* leaf the silver nanoparticle was synthesized and the nanoparticle formation was initiated at 10 min and it gets the saturation period is 24 h.

Crystalline size and structure of the silver nanoparticles were carried out by XRD. The biosynthesized silver nanostructure by employing *C. aromaticus* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Fig. 4). The four distinct diffraction peaks of the 2θ values of 38.1°, 44.1°,

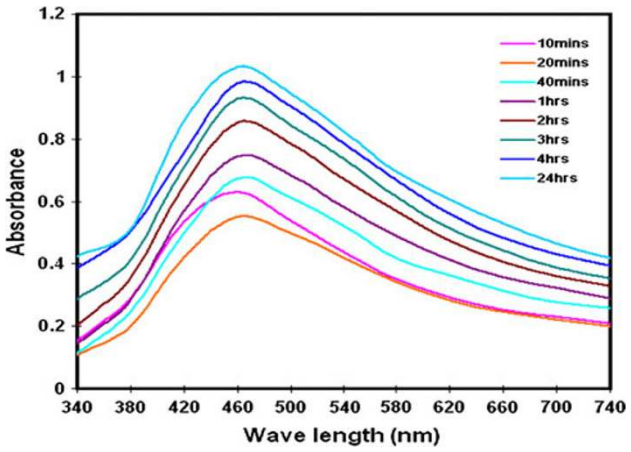

Fig. 3 UV–vis spectra recorded as a function of reaction time of an aqueous solution of 1 mM of $AgNO_3$ with the 10 ml of leaf extract. The reaction time is indicated next to the respective curves

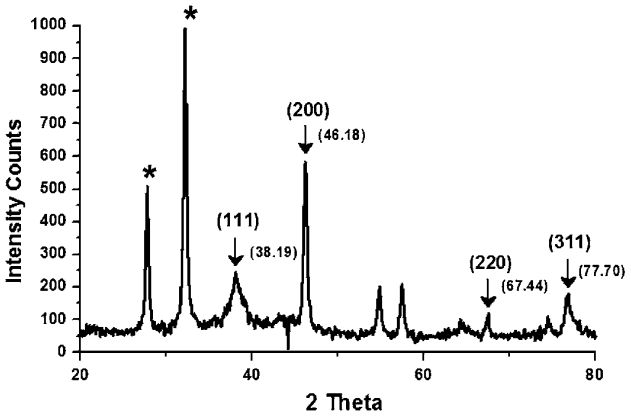

Fig. 4 XRD pattern of silver nanoparticle

27.8° and 78.1° can be assigned the plane of (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively indicates the silver nanoparticles are fcc and crystalline in nature (JCPDS file no. 84-0713 and 04-0783). The broadening of Bragg's peaks indicates the formation of nanoparticles. The mean size of silver nanoparticles was calculated using the Debye–Scherrer's equation

$$D = 0.94\,\lambda/\beta\,\mathrm{Cos}\,\theta$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. It is found that the calculated average size is 36.1 nm from FWHM of peaks. A few unassigned peaks (marked with stars) were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles. Similar results in silver nanoparticles synthesized using edible mushroom extract (Daizy. 2009) and also this similar result was reported by using geranium leaves (Shankar et al. 2003).

The surface morphology and size of the silver nanoparticles was analyzed by Scanning Electron Microscope. SEM image had shown individual silver nanoparticles as well as number of aggregates (Fig. 5). It illustrates the particles are predominantly spherical in shape and aggregates into larger particles with no well-defined morphology. This aggregation may be due to the presence of secondary metabolites in the leaf extracts. The SEM image shows the size of the silver nanoparticles ranging from 40–50 nm. Similar result of the silver nanoparticles size was reported by using *Aloe vera* extract (Chandran et al. 2006) and by using *Euphorbia hirta* leaves (Elumalai et al. 2010).

An EDAX study was used to confirm the formation of silver nanoparticles. The EDAX (Fig. 6) recorded from silver nanoparticles showed strong signal of silver from 3 keV with weak signals from O. Weak signals of O due to X-ray emission from carbohydrates/proteins/enzymes present with in the leaves of *C. aromaticus*. Throughout the scanning range of binding energies, there is no peak to detect the impurity. This result indicated that the product was composed of high purity Ag nanoparticles.

FTIR measurements were carried out to identify the potential functional groups of the biomolecules in the *C. aromaticus* leaf broth responsible for the reduction of the silver ions. These functional molecules are associated with silver nanoparticles. Figure 7 had shown the FTIR spectra shows that the band at 3,843, 2,640 cm^{-1} corresponds to NH group of amines. The weak band at 3,337.65 cm^{-1} shows characteristics of O–H stretching of secondary alcohols. The band at 1,636.40 cm^{-1} corresponds to C=O stretching of alcohols (Satyavani et al. 2011a) amide I band and nitro groups. The band at 1,383.65 cm^{-1} corresponds to C–N stretching vibrations of aromatic amines (Satyavani et al. 2011b). The absorbance

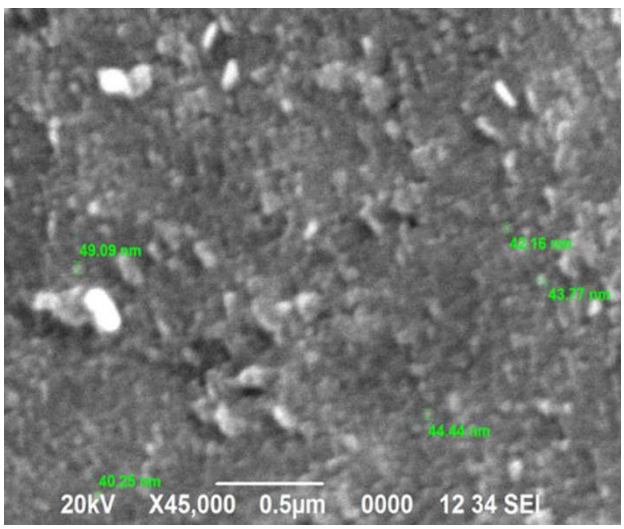

Fig. 5 SEM images of silver nanoparticle

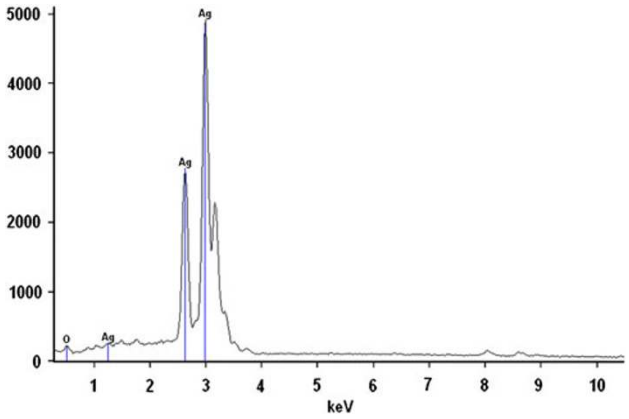

Fig. 6 EDAX pattern of silver nanoparticle

peak at 1,058 cm^{-1} was disappeared in the capped silver nanoparticles indicates to C–N stretching vibrations of aliphatic amines or alcohols or phenols representing the presence of polyphenols may be flavonoids. In this study of FT–IR spectrum confirmed the presence of aromatic amine, amide (I) groups, phenolic groups and secondary alcohols may act as reducing agents for the synthesis of silver nanoparticles.

Bactericidal activity of silver nanoparticles

The synthesized silver nanoparticle by green route has more highly toxic against *B. subtilis* and *K. planticola* were investigated by disc diffusion method. Table 1 showed a clear inhibition zone whereas the standard antibiotics like chloramphenicol and various concentrations of silver nanoparticles. The concentration of silver nanoparticles was varied from 10–50 µL. The inhibition of zone increased while increasing the concentration of silver nanoparticles.

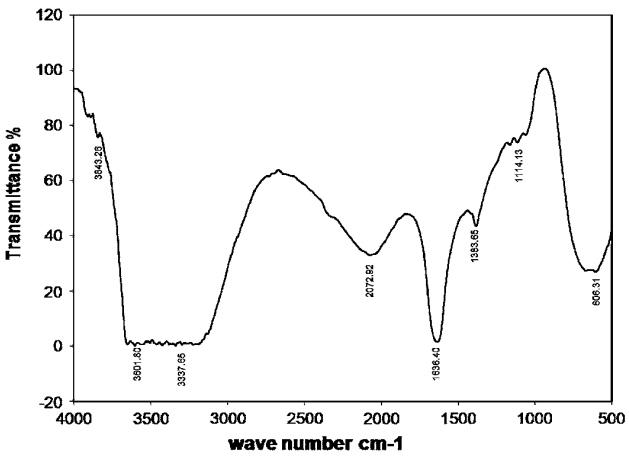

Fig. 7 FT–IR spectrum of leaf extract associated with silver nanoparticles

Table 1 Shows inhibition zone of silver nanoparticles at various concentration (diameter in mm)

Concentration of silver nanoparticles	Zone of inhibition (mm in diameter)	
	Bacillus subtilis	*Klebsiella planticola*
10 µl	8.67 ± 0.33	7.00 ± 0.578
20 µl	10.33 ± 0.33	8.33 ± 0.334
30 µl	10.33 ± 1.203	9.00 ± 0.578
40 µl	10.67 ± 0.668	10.00 ± 0.335
50 µl	12.33 ± 1.203	11.00 ± 0.335
Chloroamphenicol (Control)	24.67 ± 0.334	23.33 ± 0.883

± Standard deviation

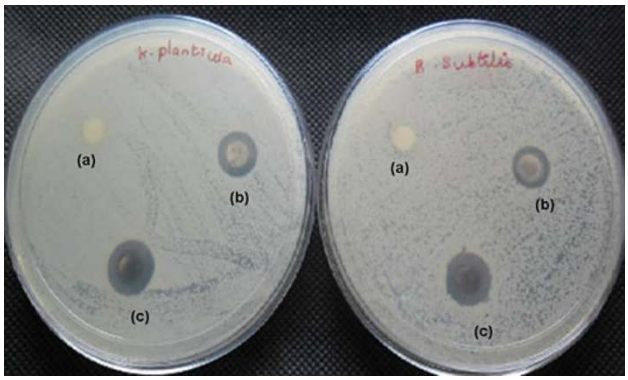

Fig. 8 Comparative study of bactericidal activity of (**a**) leaf extract of *C. aromaticus*, (**b**) chemically synthesized silver nanoparticles and (**c**) biosynthesized silver nanoparticles against *Bacillus subtilis* and *Klebsiella planticola*

The zone of inhibition in diameter was tabulated in Table1. Silver nanoparticles inhibit the growth of Gram positive bacteria around the disc of 12.33 ± 1.203 mm in diameter in the concentration of 50 µL. In the Gram negative bacteria the zone inhibition was 11.00 ± 0.335 mm, observed at 50 µL concentration. Our study coincided with the report of Mahitha et al. (2011); they have examined the antibacterial effect of silver nanoparticles against the gram positive (*Staphylococcus aureus* and *B. subtilis*) and gram negative bacterium (*E.coli* and *K. pneumonia*).

Figure 8 shows high zone inhibition of *C. aromaticus* leaf mediated biosynthesized silver nanoparticles than the leaf extract of *C. aromaticus* and chemically synthesized silver nanoparticles. Biosynthesized silver nanoparticles exhibit more zone inhibition in *B. subtilis* and *K. planticola* was 14.17 ± 0.602 and 12.83 ± 0.442, respectively, than leaf extract and chemically synthesized silver nanoparticles (Table 2).

The mechanism of inhibitory action of silver nanoparticles on microorganisms is partially known. Silver nanoparticles are have positive charge, it will attach with the negative charged microorganisms by the electrostatic attraction in the cell wall membrane (Dibrov et al. 2002) and silver nanoparticles are associated with thiol groups of cell wall resulted in the generation of reactive oxygen species and disrupting the cell (Lara et al. 2010). The silver nanoparticles closely associated with cell wall of bacteria by forming 'pits' finally it affects the permeability, and cause cell death (Sondi et al. 2004). The silver nanoparticles were small in size so it easily enters into the bacterial cell and affect the intracellular processes such as DNA, RNA and protein synthesis. Silver nanoparticles were binding with bacteria depends on the surface area for the interaction. Smaller particles affect the larger surface area of the bacteria thus it has more bactericidal activity than the larger sized nanoparticles (Shrivastava et al. 2007).

Previously, scientists used plant materials such as leaves (Satyavani et al. 2011a, b), fruits (Dubey et al. 2010), seeds (Bar et al. 2009b), latex (Bar et al. 2009a) and barks (Sathishkumar et al. 2009) for synthesis of silver nanoparticles. When compare to others plant materials, leaf mediated synthesis gains more advantageous like simple and rapid synthesis of silver nanoparticles (Daizy 2011). Herein, our plant extract synthesized silver nanoparticles within 10 min, and it was confirmed by the colour changes from yellow to brown. The earlier UV–vis spectroscopy reports demonstrated that the silver nanoparticles require ~10 or more than 10 min to form in the reaction mixture (Krishnaraj et al. 2010). Whereas, the silver nanoparticles could be synthesized 2–10 min by the *C. aromaticus* leaf extract. The oxidation/reduction property of flavonoids (apigenin, quercetin, myricetin, isorhamnetin and kaempferol) (Rasineni et al. 2008) present in the leaf extract might play an important role in the synthesis of silver nanoparticles. These flavonoids may act as both reducing and stabilizing agents in the synthesis process. The synthesis of silver nanoparticles using *C. aromaticus* is facile and fine compatible method for large-scale production.

Table 2 Shows comparative of bactericidal activity

Concentration of silver nanoparticles	Zone of inhibition (mm in diameter)	
	Bacillus subtilis	*Klebsiella planticola*
Biosynthesized silver nanoparticles	14.17 ± 0.602	12.83 ± 0.442
Chemically synthesized silver nanoparticles	11.67 ± 0.334	10.67 ± 0.334
Pure *C. aromaticus* leaf extract	6.17 ± 0.167	6.5 ± 0.289

± Standard deviation

Conclusion

The eco-friendly green mediated synthesis of silver nanoparticles using *C. aromaticus* leaf extract was attained with successfully and very rapidly. Silver nanoparticles formation was achieved with in 10 min, which was demonstrated by UV–vis spectroscopy in the absorbance peak at 460 nm. The synthesized nanoparticles were confirmed by XRD and also average size of the nanoparticles was 36.1 nm calculated by Debye–Scherrer's equation. The EDAX results showed the significant presence of silver. The silver nanoparticles size was in the range of 40–50 nm established by SEM. Capping agent/stabilizing agent play the major role in the reduction of silver ion, which was characterized by FTIR. Green synthesized silver nanoparticles had the bactericidal activity against *B. subtilis* and *K. planticola* was successfully demonstrated by disc diffusion method with zone inhibition on the agar plate. Therefore, this green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials.

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