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Microwave assisted greener synthesis of silver nanoparticles using Karanjin and their antifungal activity

Nadur L. Naveena, Rudra Pratap, T. R. Ravikumar Naik*, S. A. Shivashankar

Centre for Nano Science and Engineering, Indian Institute of Science, Bangalore, India

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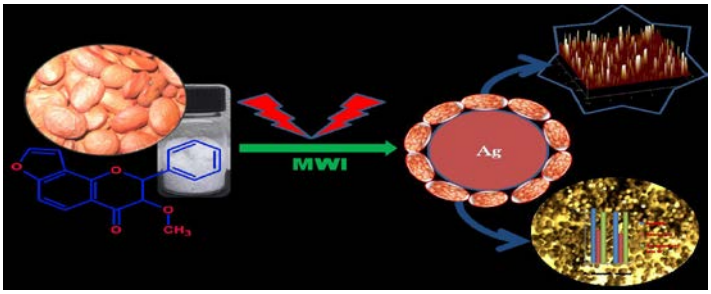
ABSTRACT



In recent days, the synthesis of nanoparticles using biomaterials is growing very rapidly due to their non toxicity and eco friendliness. In the present study we demonstrated the role of individual organic compounds present in the plant extracts in the synthesis of silver nanoparticles (Ag NPs) using Karanjin, a natural flavonoid extracted from seeds of *Pongamia pinnata* L. assisted by microwave technique. The plasmon resonance absorbance peak was observed at 424 nm in UV–visible spectroscopy. The TEM results showed presence of spherical shape with ~20 nm in size. The FTIR spectrum indicates there is no organic molecule present in the powder material and it confirms the formation of Ag NPs by microwave method. Further, confirmed by X-ray diffraction (XRD) and Energy Dispersive Spectroscopy (EDS) analysis. AFM images explained the topography of the Ag NPs. The obtained nanoparticles were stable and it confirms the activity of *Karanjin* as a reducing and capping agent in synthesis of Ag NPs. The synthesized Ag NPs demonstrated good antifungal activity against *Aspergillus flavus* and *A. niger*.

Keywords: Silver nanoparticles, Microwave synthesis, Karanjin, Flavonoids, Pongamia pinnata

INTRODUCTION

Nanotechnology is one of the fastest growing areas of manufacturing in the world today and there is an increasingly frantic search for new nanomaterials and methods to make them. One of the fields in which nanotechnology finds extensive applications is nano-medicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. A number of nanoparticles based therapeutics has been approved clinically for infections, vaccines, and renal diseases.[1] Further, nanoparticles are in high demand due to their effectiveness and various properties like, optical, electronic, magnetic and catalytic; and are being widely used in different fields.[2] In order to meet the increasing demand, metallic nanoparticles are synthesized mainly by various chemical and physical methods such as reduction of metal salts, sonochemical decomposition,[3] metal evaporation,[4] ion sputtering, chemical reduction, sol gel, thermal decomposition in organic solvents and chemical and photo reduction in reverse micelles.[5,6]

Silver nanoparticles are one of the most commercialized nano materials[7] due to their peculiar properties like, smaller size, high surface area, easy suspension in liquids and access to cells and cell organelles.[8] Its production in recent times has reached 500 tons per year and their application has spread in various fields such as high

*Corresponding Author: Dr.T. R. Ravikumar Naik
Address: Centre for Nano Science and Engineering, Indian Institute of Science, Bangalore, India
Tel: +91 9900792675
Email: *E-mail: naikravi7@gmail.com; nlnaveena@gmail.com

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sensitivity bio-molecular detection, catalysis, biosensors, medicine, optoelectronics, nano-electronics, surface-enhanced Raman spectroscopic (SERS) studies,[9] nano-bio technology,[10] pest management[8] and anti-angiogenesis activities.[11]

Most silver nanoparticles are synthesized by either physical or chemical methods using toxic solvents as reducing agents which affect the human health and environment.[12] Therefore microwave irradiation method (here after MWI) which is an environmentally friendly technique has been employed to synthesize various metal nano materials.[13] This method is rapid, gives higher yield and has other advantages compared to conventional methods.[9]

Recently, Ag NPs have been synthesized by using plant extracts as reducing agents instead of chemicals ($NaBH_4$ etc..) via MWI and other green synthesis or biosynthesis schemes.[14] Use of extracts of neem, menthol, aloevera, clove, edible mushroom, coffee, tea and other potential plants has been reported.[15] Secondary metabolites such as flavonoids, terpenoids and polyphenol compounds present in plants acts as reducing agents and support the antimicrobial activity of Ag NPs.[16] However, there is a less information on the role of individual secondary metabolite in the reaction,[17] and studies in this direction are very much needed to unravel the success behind the biosynthesis of nanoparticles. In this paper, karanjin a flavonoid extracted from seeds of *Pongamia pinnata* L. was used as a reducing agent to produce silver nanoparticles through MWI.

Pongamia pinnata L. (family: Leguminosae) is a tree commonly found in India and neighboring countries[18] and each part of this tree has been used in Ayurveda and Siddha medicine. Its therapeutic values include remedies against various human ailments such as ulcerogenic, inflammatory, analgesic, antiquity, viral, bacterial and fungal infections.[19] Flavonoids, terpenes and steroids can be found in its various organs such as leaves, bark, flowers and seeds.[20]

To the best of our knowledge, the synthesis of Ag NPs using naturally extracted karanjin as a reducing agent by microwave method has not been reported earlier. During the synthesis reaction, reduction of Ag^+ to Ag^0 and stable Ag nanoparticles has been achieved.

EXPERIMENTAL

Materials

All chemicals required for the study were purchased from M/s Sigma-Aldrich, and Merck, USA and M/s S.D. Fine Chem. Pvt. Ltd, Mumbai, India. *Pongamia pinnata* seeds were collected from the forest area of Indian Institute of Science Campus, Bangalore, India

Extraction

Seeds (2 kg) were dried under shade, powdered and used for extraction using Soxhlet apparatus with methanol. After evaporation of methanol, the obtained semi solid compound was fractionated by column chromatography over silica gel (60mm) and confirmed the compounds by spectroscopic techniques (LC-MS, NMR). Further, this extracted compound (karanjin) was used for synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

About 10 mL of 1 mM solution of silver nitrate was prepared in a beaker using double distilled water. Then 1mL of karanjin was added and the reaction mixture was sonicated (3 minutes) and exposed to microwave irradiation for two minutes at 850W. The resultant reaction mixture was cooled to room temperature and centrifuged at 15,000 rpm for 10 min at room temperature. The obtained pellets were re-suspended in de-ionized water and centrifuged for 5 min at 15,000 rpm. The resulting powder was dried in hot-air oven at 55°C for 24hr and used for characterization.

Characterization

Bio-reduction of silver nitrate in a solution with karanjin was monitored by UV- visible spectroscopy (Schimadzu UV- Visible spectrophotometer, model UV-1800). X-Ray Diffraction (XRD) – analysis was done with Rigaku X-ray difractometer at a scanning speed of 0.15°/min and 20-90° (2θ-degree). Transmission electron microscope (TEM) (FEI Technai™ using an accelerating voltage of 300 kV field emission and methanol as a solvent), and Energy-dispersive spectroscopy (EDS) (Bruker, Germany). Fourier transform infrared spectroscopy (FT-IR) studies were carried out using a Thermofisher Scientific FTIR spectrophotometer (Nicolet 6700 FT-IR). The samples were prepared using KBr pellet method and analyzed to check the presence of bio-functional moieties of karanjin and the surface chemistry of the reduced silver ion. The FTIR spectrums were collected by using XT-KBr beam splitter and DTGS KBr detectors at a spatial resolution of 4 cm^{-1} in the transmission mode, between 3500–400 cm^{-1} respectively. Atomic force microscopic (AFM) measurements were recorded in tapping mode using an AFM instrument (Bruker, Germany) to understand the surface information.

Antifungal activity

Antifungal activity of karanjin mediated biosynthesized AgNPs was tested against saprophytic and pathogenic fungi, *Aspergillus flavus* and *A. niger* by the standard agar well diffusion method. The fungal strains were grown in a broth media containing potato dextrose for 72 hr and used for the study.[21] To examine the antifungal activity of AgNPs, Potato dextrose agar (PDA) media was prepared and poured on sterilized petriplates and allowed to solidify. After solidification, microorganisms were inoculated with the help of spreader. Then, suspension of AgNPs were prepared (1ml of 100 ppm) with deionized water and sterilized whatman No.1 filter paper discs (6.0 mm diameter) were impregnated in 30 µl of suspension and placed on agar plates. The negative (distilled water) and positive (amphotericin B) controls, along with karanjin were included for the antifungal activity assay. The petriplates were then incubated at 25 °C for 72 h in an incubator. The zone of inhibition (mm) around the disc was observed and recorded including disc diameter.

RESULTS AND DISCUSSION

When karanjin was added to aqueous solution of silver salt ($AgNO_3$), the change in yellowish white colour of the reaction mixture to brown and then to black was observed. This change in colour of the solution is due to the formation of silver nanoparticles from reduction of silver salt. The complete

reduction of Ag^{+} to Ag^{0} is due to the presence of reducing agent karanjin. It was reported that compounds like caffeine and theophylline act as reducing agents when *Acalypha indica* leaf extract was used.[22] while terpenoids and flavanones in neem and other plant extracts[15] reduce silver salt to silver nanoparticles.

The complete colour change (black) was observed after 2 minutes of irradiation in domestic microwave at higher power (850W) and, when the reaction mixture was stabilized there was no further change in colour. This indicates that silver salt present in the reaction mixture has been reduced completely. However, this colour change took about 30 minutes when extracts from leaves or other plant parts were used and the reaction mixture was incubated at room temperature.[14]

UV-vis Spectroscopy

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations.[23] As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from colorless to yellowish brown due to reduction of silver ion which indicates the initiation of forming silver nanoparticles (Fig. 1.).

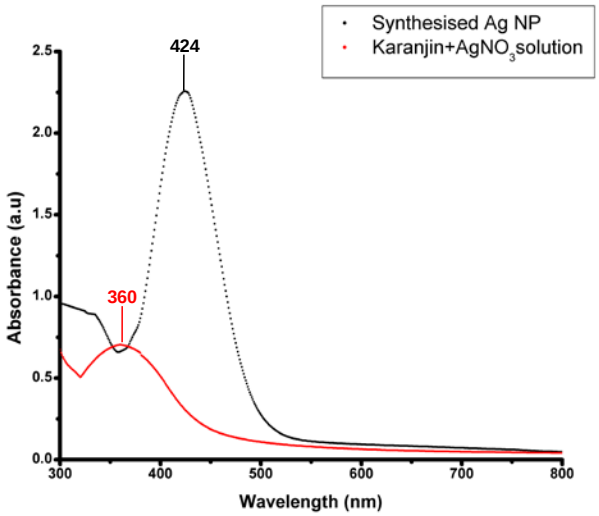

Figure 1. UV spectroscopy of karanjin mediated silver nanoparticles.

XRD Analysis

The XRD pattern of Karanjin mediated synthesized AgNPs is shown in Fig. 2. There were five major peaks that appeared at 38.12°, 44.30°, 64.45°, 77.41° and 81.55°. These peaks correspond to the (111), (200), (220), (311) and (222) planes of face centered-cubic (fcc) geometry of silver nanoparticles, which is in agreement with the standard data file (JCPDS file No. 42-0783).[11] We also observed several small peaks which may correspond to crystallized karanjin. However, these are weaker than Ag peaks, which clearly indicate the presence of Ag as the core material. Overall the XRD spectra clearly indicate that the synthesized material is in crystalline nature.

The synthesized nanoparticles were analyzed for its size in TEM. It was calculated as approximately 20 nm in size. Further, the observed silver nanoparticles were mostly in spherical and near spherical shapes and are poly-disperse. The image of TEM and HRTEM of the synthesized silver nanoparticles is shown in the Figure 3.

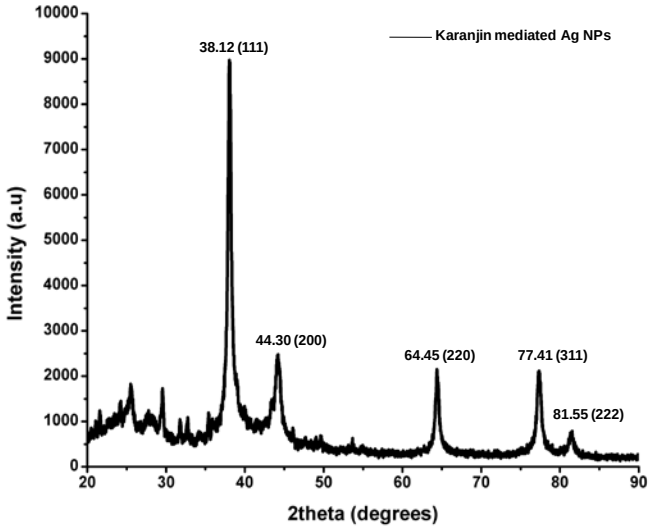

Figure 2. XRD Pattern of karanjin mediated silver nanoparticles synthesized by microwave heating.

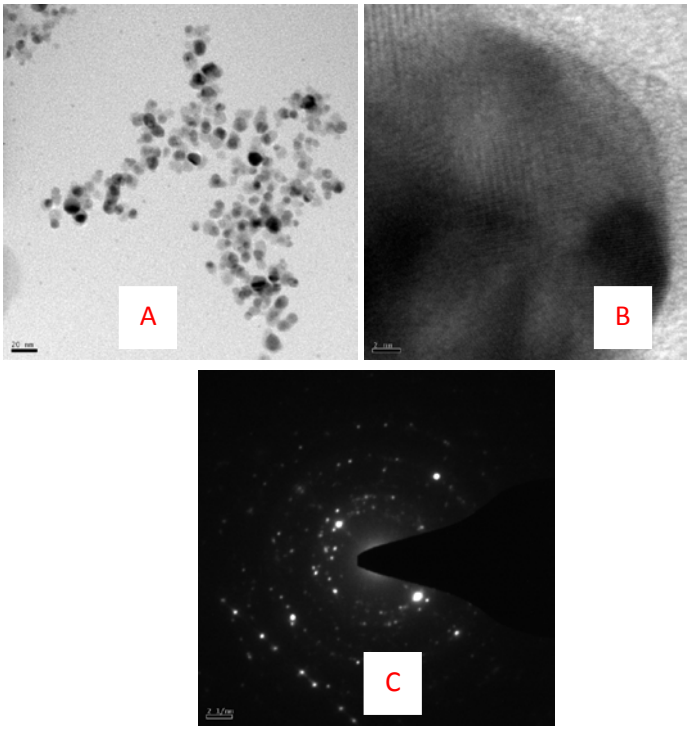

Figure 3. A) TEM B) HR TEM images of the synthesized karanjin mediated silver nanoparticles and C) Electron diffraction pattern recorded from the particles shown in figure 2 with lattice planes of fcc silver.

Energy Dispersive Spectroscopy (EDS) analysis

This analysis was carried out to confirm the formation of metallic silver nanoparticles in the reaction mixture. The EDS analysis of stable silver nanoparticles synthesized with karanjin is shown in Fig. 4. The intense signal of the Ag atoms is observed at

3 keV, and also weak signals of C are seen, this confirms the presence of elemental silver.[11,16]

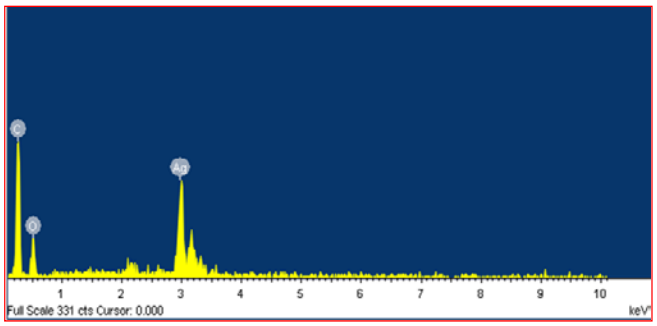

Figure 4. Spot profile EDS spectrum image of synthesized silver nanoparticles.

Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR spectra of synthesized Ag NPs (Fig. 5) were obtained between the wave length ranges of 3500–400 cm^{-1} respectively. The major IR bands recorded at, 3122, 2837, 2357, 1610, 1587, 1518, 1410, 1488, 1269, 1218, 1130, 1065 and 820 cm^{-1}. It is confirmed that the various functional groups in karanjin acts as reducing and capping agent in synthesis of stabilized Ag NPs. The band at 1160 cm^{-1} and 1065 cm^{-1} can be assigned to the ether linkages or – C-O- functional groups of the products of flavones, terpinoids and polysaccharides.[26] The band at 1410 cm^{-1}, corresponding to -NO_3 stretching which comes from silver nitrate.[27,28] For C–C stretching vibration an intense band is calculated at 1539 cm^{-1} which is found to be in good agreement with the experimental one, that is, 1526 cm^{-1} In aromatic compounds C-H stretching was observed at 2837 cm^{-1} region.

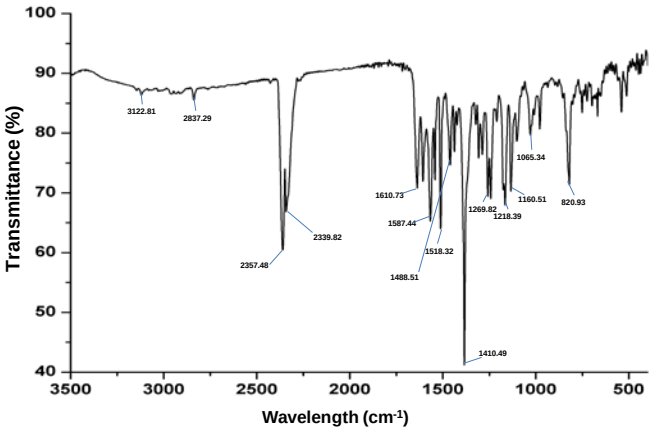

Figure 5. FTIR spectra of synthesized AgNPs.

Mode of action of karanjin in the formation of silver nanoparticles

Karanjin is a furonoflavonoid, having furan ring with carbonyl and alkoxy groups acts as reducing as well as capping agent during the synthesis of Ag NPs. The possible mechanism of reduction and capping is depicted in Fig.6. When mixing karanjin and $AgNO_3$ solution Ag^+ ions react with carbonyl and alkoxy groups, converted into Ag nanoparticles. However, when we use a crude extract containing various phyto-chemicals then the mechanism of action is slightly elongated and was reported by many workers.[24,25]

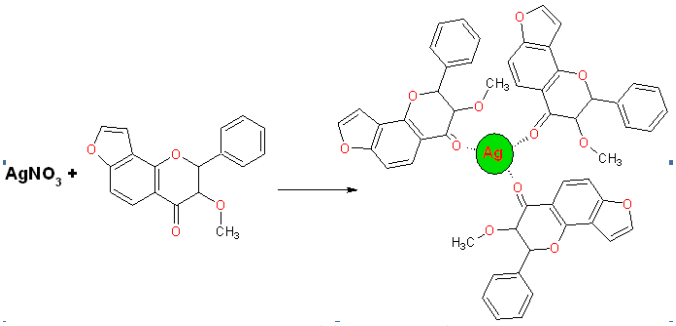

Figure 6. Possible mechanism of formation of silver nanoparticles using karanjin

Atomic force microscopic (AFM) analysis

The 3D and 2D topographic images of bio-synthesized AgNPs are given in Figure 7. The tapping mode AFM image clearly shows the formation of nanoparticles with different heights of the material. The topography of AFM reveals the AgNPs produced were in high number and in small to medium sizes.

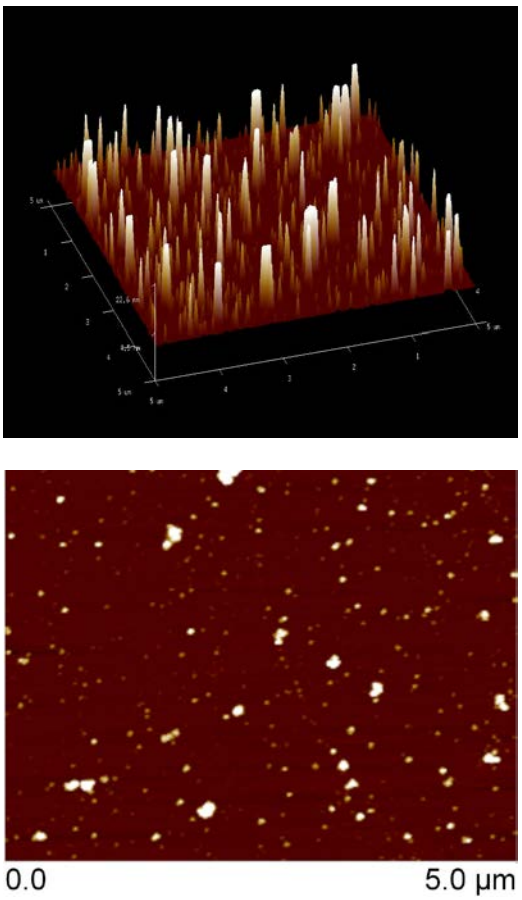

Figure 7. 3D and 2D image of surface/topography of biosynthesized Ag NPs.

Antifungal Activity

The antifungal activity of biosynthesized AgNPs and karanjin was evaluated against saprophytic and pathogenic fungi,

Aspergillus flavus and *A. niger* and results are given in table 1. The karanjin mediated synthesized silver nanoparticles showed the maximum zone of inhibition against *A. flavus* (15 mm) and *A. niger* (14 mm) compared to karanjin.[29] On the other hand, the negative control (distilled water) did not exhibit any zone of inhibition. The positive control showed antifungal activity against both the fungi.

Table 1. Activity of biosynthesized silver nanoparticles on fungi

Components*	Zone of inhibition (mm)	
	A. flavus	*A. niger*
AgNPs	15	14
Karanjin	9	8
Amphotericin B	14	14
DI Water	NA	NA

*=30µl; NA-No activity

CONCLUSION

Karanjin, a flavonoid present in *Pongamia pinnata* plants has various properties. It acts as a reducing and capping agent in the reaction of microwave assisted synthesis of silver nanoparticles. The synthesized silver nanoparticles were stable with enhanced properties and also good antifungal agents. This method satisfies all the conditions of a green chemical process and proves to be an eco-friendly, simple, rapid, energy efficient and cost effective. The obtained nanoparticles can be used in medicine and other fields as they are free from toxic substances.

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AUTHORS BIOGRAPHIES

Dr. Nadur L Naveena was born in Nadur, Karnataka, India. He completed his Ph.D in Agricultural Entomology from University of Agricultural Sciences, Bangalore in 2013. He has worked on management of insects in food grains for his doctoral studies. Later he worked as a Research Associate at University of Agricultural and Horticultural Sciences, Shimoga, India and in 2015 he joined Centre for Nanoscience and Engineering, Indian Institute of Science as Post Doctoral Fellow. He has more than 10 peer reviewed research articles in reputed journals. His research area of interest is in application of nanotechnology for the management of insect pests in Agriculture in particularly food grains and crop pests.

Prof. Rudra Pratap: Rudra Pratap (Ph.D., Cornell University, 1993, M.S., University of Arizona, 1987, B.Tech., IIT Kharagpur, 1985) is a professor at the Centre for Nano Science and Engineering (CeNSE), IISc Bangalore. He is also an associate faculty of Mechanical Engineering, IISc, where he served full time from 1996 until2010 when he moved to CeNSE as the Founding Chairperson. For the last 20 years, he has worked in the area of Micro-electro-mechanical systems (MEMS) and dynamics of micro and nano-scale systems. His research interests include MEMS and NEMS, vibroacoustics, bioacoustics, mechano-biology, and computational mechanics. He is an Associate Editor of IEEE/ASME Journal of MEMS and Journal of ISSS. He is an elected Fellow of the National Academy of Engineering and National Academy of Science.

Dr. T. R. Ravikumar Naik received his PhD in Industrial Chemistry from Kuvempu University, INDIA, in 2008. From 2008-2011, he worked as Dr. DSK Postdoctoral Fellow, Dept of Organic Chemistry, Indian Institute of Science (IISc), Bengaluru He was awarded with TWAS-USM Postdoctoral fellowship, Malaysia in 2012. Later in 2012, Senior facility technologist at CeNSE, (IISc) from 2012 to 2017. He joined the full-time faculty member of the Chemistry department, Veerashaiva College, VSK University, Ballari, INDIA. He has more than 40 peer reviewed research articles in reputed journals. His current research interests are in the development of chemical precursors for the synthesis of nanomaterials and nanostructures for biological applications including nanostructured thin films, for different applications.

Prof. S.A. Shivashankar received his PhD in Chemical Physics from Purdue University, USA, in 1980. From 1980-1900, he was a Research Staff Member at the IBM Watson Research Center, New York. He joined the faculty of the Materials Research Centre, Indian Institute of Science (IISc), Bengaluru, India, in 1980 and became a member of the “founding faculty” of the Centre for Nano Science and Engineering (CeNSE) when it was established at IISc 2010. Since retiring from formal service in 2012, he has been serving at CeNSE in a full-time Emeritus capacity. His current research interests are in the development of chemical precursors for the synthesis of nanomaterials and nanostructures, including nanostructured thin films, for different applications.