Green synthesis of silver nanoparticles by using carambola fruit extract and their antibacterial activity

S J Mane Gavade¹, G H Nikam¹, R S Dhabbe¹, S R Sabale¹, B V Tamhankar¹ and G N Mulik²

¹Department of Chemistry, Jaysingpur College, Jaysingpur-416101 (M S), India
²Department of Chemistry, Balwant College, Vita (M S), India

E-mail: baputamhankar@gmail.com

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Abstract
In this study well defined silver nanoparticles were synthesized by using carambola fruit extract. After exposing the silver ions to the fruit extract, the rapid reduction of silver ions led to the formation of stable AgNPs in solution due to the reducing and stabilizing properties of carambola fruit juice. The synthesized NPs were analyzed by ultraviolet-visible spectroscopy and x-ray diffraction pattern. The as-synthesized AgNPs were phase pure and well crystalline with a face-centered cubic structure. The AgNPs were characterized by TEM to determine their size and morphology. The antimicrobial activity of the synthesized AgNPs was investigated against Escherichia coli and Pseudomonas aeruginosa by agar well diffusion method. This newly developed method is eco-friendly and could prove a better substitute for the current physical and chemical methods for the synthesis of AgNPs.

Keywords: green synthesis, AgNPs, carambola fruit extract, characterization, antibacterial

Classification numbers: 2.04, 4.02

1. Introduction

Metal nanoparticles (NPs) have a high specific surface area and a small fraction of surface atoms, and have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, optics, electronics, antibacterial, antimicrobial and biomaterial production [1–3]. The methods reported for the synthesis of metal NPs by using plants are rapid, cost effective and have an eco-friendly nature. There are several chemical methods for the synthesis of silver nanoparticles (AgNPs) [4–6] that may pose a risk to the environment due to the presence of toxic chemicals. Thus, there is a need to develop environmentally benign methods for synthesis that are free from toxic chemicals. Biosynthesis of nanoparticles employing microorganisms or plants can potentially eliminate this problem by making the nanoparticles more biocompatible.

Sometimes the synthesis of nanoparticles using various plants and their extracts can be advantageous over other biological processes which involve tedious methods of maintaining microbial culture media [7]. Many experiments have already been carried out such as synthesis of various metal nanoparticles using fungi such as fusarium oxysporum, penicillium sp. and using some bacteria such as Bacillus subtilis, Idiomarina sp. etc [8–12]. The advantages of using plant material for the synthesis of nanoparticles is that it is easily available, safe to handle and possesses a broad variety of metabolites that may aid in reduction. Plant mediated synthesis of nanoparticles is formed due to the presence of biomolecules such as proteins, amino acids, vitamins, polysaccharides, polyphenols, terpenoids and organic acids such as citrates etc present in the plants as their phytochemicals [13]. Apart from mediating the synthesis, these molecules also stabilize the nanoparticles formed with different sizes and shapes. Various studies indicated that biomolecules not only play a role in reducing the ions to nanosize, but also play an important role in the capping of nanoparticles [14, 15].

A convenient and rapid process for the formation of AgNPs with natural reducing and capping agents of daucus carota (L.) was reported [16]. Many researchers reported the
2.2. Preparation of carambola fruit extract

The fruit was washed with distilled water and cut into small pieces. The aqueous fruit extract was prepared by boiling 5 g of fruit pieces with 100 ml distilled water for 15 min. The extract was cooled to room temperature and filtered through filter paper. The filtrate was collected as stock solution and used for further experiment of synthesis of AgNPs.

2.3. Synthesis of AgNPs using carambola fruit extract

A stock solution of 4 mmol L$^{-1}$ AgNO$_3$ was prepared and kept against sunlight. In a typical procedure, 10 ml of fruit extract (5%) was taken and 25 ml AgNO$_3$ stock solution was added to it with constant stirring at 40 °C. The colorless fruit extract solution changes to reddish brown slowly indicating the formation of AgNPs. Schema 1 shows a schematic illustration of the synthesis of AgNPs.

To investigate the optimum factors responsible for AgNPs synthesis, the experiment was carried out at different reaction conditions such as temperature and fruit extract concentration. The effect of these parameters on the synthesis of AgNPs was monitored by UV–vis spectrophotometer.

2.4. Characterization techniques

The synthesized AgNPs were characterized by using various analytical techniques. The reduction of the pure Ag$^+$ ions was monitored by measuring the absorbance of the reaction medium with UV–Vis–NIR spectrophotometer (Shimadzu, Model UV-3600). The x-ray diffraction pattern of synthesized AgNPs was recorded on a Philips automated x-ray diffractometer (Model PW-3710) equipped with a crystal monochromator employing Cu-K$_\alpha$ radiation of wavelength 1.5406 Å in 2θ range from 20° to 80°. Transmission electron microscopy analysis of AgNPs was performed with JEM-2100 (Jeol), operated at 200 kV. Biosynthesized AgNPs were analyzed for their antimicrobial activity by agar well diffusion method.

3. Results and discussion

3.1. UV–vis spectral analysis of AgNPs

The formation and stability of AgNPs in an aqueous colloidal solution was investigated by using UV–vis-NIR spectral analysis. As expected, AgNPs turned yellowish brown in the aqueous solution, which has been attributed to the excitation of surface plasmon resonance in AgNPs. Figure 1 shows time dependent UV absorption spectra exposed from the reaction of reduction of silver ions which have dispersed nanoparticles with broadening peak in the absorbance band at the wavelength of 448 nm. Moreover, it has been noticed that the absorption peak width gradually became narrower with time, which suggests the narrow size distribution of newly formed AgNPs.

It is already reported that the Averrhoa carambola leaf extract and fruit extract contains a lot of biomolecules, such as proteins, enzymes, polysaccharides, amino acids and vitamins possessing antioxidant and antimicrobial activities [23]. It acts as a reducing as well as a capping agent for the preparation of AgNPs. The polysaccharide (C$_x$H$_{2y}$O$_z$) and ascorbic acid (C$_6$H$_8$O$_6$) present in carambola fruit extract are act as reducing agent [24]. It is clear that with increase in OH$^-$ ions concentration, the forward reaction is favorable resulting spontaneous increase in AgNPs. In general, the overall reaction can be represented as in schema 2.
3.1.1. Effect of temperature. Temperature is one of the important physical parameter affecting on the synthesis of AgNPs. Figure 2 shows UV–vis-NIR a spectrum of the AgNPs prepared at different temperature (25 °C, 30 °C, 40 °C and 60 °C). It was observed that the absorbance increases with increasing temperature. Increased reaction temperature led to a rapid reduction rate of the Ag⁺ ions and the subsequent homogeneous nucleation of silver nuclei allowing for the formation of AgNPs with small size. The broadening of the peak observed at low temperature shows formation of large sized nanoparticles while the peak became narrower at higher temperature, which indicates that the formed nanoparticles were small in size and a higher rate of reduction of silver ions had occurred.

3.1.2. Effect of concentration. The effects of fruit extract concentration on the synthesis of AgNP’s were examined by using UV–vis spectroscopy. Figure 3 shows the absorption spectra of AgNPs prepared using different concentrations of fruit extract. All experiments were carried out by varying the concentration of carambola fruit extract, keeping other conditions constant (using 4 mmol L⁻¹ AgNO₃) at 40 °C. Generally, a broad peak at a higher wavelength indicates an increase in particle size, whereas a narrow line at a shorter wavelength represents smaller particle size [25]. In this case, as the concentration of the fruit extract varied from 1% to 5%, the sharpness of surface plasmon resonance (SPR) band peak increased with a simultaneous increase in the absorption coefficient.

3.2. XRD studies of AgNPs

Analysis through x-ray diffraction (XRD) was carried out to confirm the crystalline nature of the AgNPs. The XRD pattern

Schema 2. Reaction of AgNPs with polysaccharide (C₆(H₂O)₇) and ascorbic acid (C₆H₈O₆) in carambola fruit extract.
The crystalline size of AgNPs was calculated from the highest intensity peak using Scherer’s equation \[ D = \frac{K\lambda}{\beta \cos \theta}, \]
where \( D \) is the crystallite size of AgNPs, \( \lambda \) is the wavelength of the x-ray source (0.1541 nm) used in XRD, \( \beta \) is the full width at half maximum of the diffraction peak, \( K \) is the Scherer constant with a value from 0.9 to 1 and \( \theta \) is the Bragg angle. The average crystallite size for as-synthesized AgNPs was found to be 18 nm.

3.3. TEM studies of AgNPs

The shape and size distribution of colloidal particles were characterized by transmission electron microscopy (TEM). TEM grids were prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp. Figure 5 shows the typical bright field TEM micrograph of the synthesized AgNPs. It was observed that most of the AgNPs were spherical in shape. A few agglomerated AgNPs were also observed in some places, thereby indicating possible sedimentation at a later time. It was evident that there was variation in particle sizes and the average size estimated was 20 nm and the particles size ranged from 10 nm to 40 nm. The selected area electron diffraction (SAED) pattern was obtained by directing the electron beam perpendicular to one of the spheres. The SAED pattern recorded from one of the nanoparticles confirmed the crystalline nature of AgNPs. The hexagonal symmetry of diffraction spots pattern is shown in figure 6 which confirmed the spherical particles with well crystalline nature. The high resolution transmission electron microscopy (HR-TEM) image of single AgNPs is presented in figure 7 showing the spherical morphology of nanoparticles.
3.4. Antimicrobial activity of AgNPs

AgNPs are extensively used in the pharmaceutical industry and have inhibitory activities on various microorganisms. Biosynthesized AgNPs were analyzed for their antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* by agar well diffusion method. In this method petriplates containing 20 ml Muller Hinton medium were seeded with 24 h culture of bacterial strains. Wells were cut and AgNPs were added. The plates were then incubated at 37 °C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993) and chloramphenicol disc was used as a positive control [28]. The images for antibacterial activity of biosynthesized AgNP’s against *Escherichia coli* and *Pseudomonas aeruginosa* are shown in figures 8(a) and (b). The table 1 shows summarized results of antibacterial activity.

The mechanism of antibacterial activity of AgNPs on microorganisms is partially known. AgNPs may attach to the surface of the bacterial cell membrane via interacting with sulfur-containing proteins [29, 30], disturbing permeability and respiration functions of the cell, resulting in cell death. This explanation was supported by the TEM results obtained in this work. It is also possible that AgNPs not only interact with the surface of the membrane, but can also penetrate inside the bacteria [31]. The action of AgNPs on the bacteria was also due to the interaction with thiol group compounds found in the respiratory enzymes of bacterial cells thus inhibiting the respiration process in bacteria [32, 33]. Moreover, AgNPs entered into bacteria cells and condensed DNA as a result preventing DNA from replication and cells from reproduction [34].

3.5. Comparison between different AgNPs synthesis method

The presently investigated method for the synthesis of AgNPs was compared with other biosynthesis methods with respect to various criterions like synthesis conditions, results of bactericidal activity etc. The inhibition zone against *E. coli* was 26 mm which is more than other reported methods as

| Test organisms         | Zone of inhibition (diameter in mm) |
|------------------------|-------------------------------------|
|                        | Carambola fruit extract | AgNPs100 ppm | AgNPs 50 ppm |
| *Escherichia coli*     | 2 | 26 | 14 |
| *Pseudomonas aeruginosa* | 0 | 8 | 6 |

Table 2. Different fruit extract used for AgNPs synthesis.

| No | Name of plant and fruit     | Quantity | Time | Temperature (°C) | Particle size (nm) | Inhibition zone against *E. coli* (mm) | Reference |
|----|-----------------------------|----------|------|------------------|--------------------|----------------------------------------|----------|
| 1  | Bamboo leaves               | 20%      | 10 min | 65               | 13.5               | 9                                      | [35]     |
| 2  | *Vitis vinifera* fruit      | 20%      | 24 h   | rt               | 18–20              | 18                                     | [36]     |
| 3  | Pomegranate fruit           | 10%      | 5 h    | rt               | 30                 | 2.2                                    | [37]     |
| 4  | *Paederia foetida* leaf     | 20%      | 6 h    | rt               | 24                 | 16                                     | [38]     |
| 5  | *Moringa oleifera* leaf    | 20%      | 10 min | 65               | 13.5               | 9                                      | [39]     |
| 6  | Lemon                       | 10 ml    | 1 h    | 40               | 75                 | 3                                      | [40]     |
| 7  | Camara fruit                | 10 ml    | 1 h    | rt               | 12.77              | 22                                     | [41]     |
| 8  | Carob leaf                  | 5 ml     | 2 min  | rt               | 5–40               | 8.12                                   | [42]     |

rt: room temperature.
shown in table 2. For synthesis of plant extract 5% plant extract was sufficient for completion of AgNPs in current study whereas in other reported methods 10% to 20% plant extract is needed [35–39]. In the reported method synthesis starts within 5 min and completed after 1 h whereas in some previous methods more than 5 h are required [36, 37, 39]. In some other reported method synthesis completed within 2 min to 1 h but inhibition zone against E. coli is less than current study [40–42].

4. Conclusion

In this study rapid and green synthesis of AgNP’s using carambola fruit extract is reported. The formation of AgNP was demonstrated by UV-vis-NIR spectroscopy in the absorbance peak at 448 nm. The synthesized NPs were confirmed by XRD and also average size of NPs was 18 nm calculated by Debye–Scherrer’s equation. The particle size of AgNP was in the range 10–40 nm, established by TEM. Green synthesized AgNPs with the bactericidal activity against Escherichia coli and Pseudomonas aeruginosa was successfully demonstrated by agar well diffusion method. Therefore, this green chemistry approach towards the synthesis of AgNPs has many advantages such as the process can be easily scaled up and has economic viability. Application of such eco-friendly nanoparticles in bactericidal activity makes this method potentially exciting for the large scale synthesis of nanoparticles.

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