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Green synthesis of silver nanoparticles from aqueous *Aegle marmelos* leaf extract

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ABSTRACT

Synthesis of nanoparticles by green route is an emerging technique drawing more attention recently because of several advantages over the convention chemical routes. The present study reports one-pot synthesis and in situ stabilization of silver nanoparticles using *Aegle marmelos* leaf extract. Nanoparticles of almost uniform spherical size (~60 nm) were synthesized within ~25 min reaction time at room temperature. The size of particles depends on the ratio of $AgNO_3$ and leaf extract. The crystallinity, size, and shape of the nanoparticles were characterized by X-ray diffraction, dynamic light scattering, and scanning electron microscopy respectively. The size stability was attained by the capping effect of polyphenolic tannin compound, procatacheuate in the extract. The capped polyphenols can be removed from the particle surface by simple NaOH/methanol wash. The involvement of phenolic compounds in metal ion reduction and capping were supported by UV–visible spectroscopy, infrared spectroscopy, high performance liquid chromatography, and zeta potential measurements.

1. Introduction

Silver nanoparticles (SNPs) are very important among the most widely used metal nanoparticles. These possess many potential applications such as anti-microbial agents, water purifiers, air purifiers and ingredient for inks used in inkjets printers [1–3]. Among many of these interesting properties, antimicrobial property is very important in several applications and known since ancient times [4]. Considering the importance of SNPs, various research groups have explored many possible routes to synthesize and stabilize nanoparticles for different applications. Among all available methods, wet synthesis methods can be broadly classified into (i) chemical and (ii) biological or other green routes. In the later methods, natural compounds from plants and microorganisms have been extensively used to reduce silver ions to metallic nanoparticles [5–13].

In the recent years nanoparticles synthesis using plant sources are gaining more interest, specifically the use of various parts of the plants such as leaf [12–17], tuber [18], bark [19] and buds [20,21]. Reported studies related to biological syntheses of SNPs especially using medicinal plants have been promising [3,13,15,19,22–25]. The methods using plant extracts involve phytochemicals such as terpenoids [25], flavonoids [20], phenol derivatives [26], plant enzymes (hydrogenases, reductases, quinones) and their derivatives, di-hydric phenols [23] and so on act as reductants in the presence of metal salt. Duran et al. reviewed the mechanism of formation of SNPs by different plant compounds range from proteins to phytophenols [27]. This review suggested the formation of SNPs by polyols is because of the oxidation of these water soluble compounds to their intermediate products with apparent hydrogen participation of their hydroxyl groups. Additionally, some polyphenols present in the plant extracts act as natural capping agents too to control the particle size in situ. For example, Satyavani et al. reported the formation of small sized (~31 nm) polyphenol capped SNPs by leaf extract of *Citrullus colocynthis* [22]. The activity of polyphenols is also specific to different sources of the same plant, as Kumar et al. reported larger size (~92 nm) SNPs with higher synthesis rate from the seed extract than other extracts of *Syzygium cumini* plant [24]. The above evidences state the dependency of source, structure and type of phenolic phytochemical on different sized nanoparticles formation.

Moreover, synthesis of nanoparticles using plant extracts is more advantageous over microbial route such as simple and user friendly process, economical and less reaction time etc. [28]. *Aegle marmelos* is one of the useful medicinal trees popularly known from pre-historic time for its nutritional, environmental, and commercial importance [29]. The leaves contain broadly alkaloids, phenylpropanoids, terpenoids and other polyphenols which were well recognized for their healing power toward wide variety of bacterial and fungal infections [30,31]. To the best of our knowledge till to date there is no study available on *A. marmelos* leaves for the reduction of metal salts to get nanoparticles. In this study, we report an inexpensive one-pot synthesis of SNPs by green route at room temperature, stabilized in situ using *A. marmelos* leaf extract. Additionally, kinetics of the particle

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formation, identification of the responsible compounds involved in metal salt reduction and understanding of the in situ particle stabilization mechanism are focused in this study.

2. Experimental

2.1. Preparation of leaf extract (LE) and nanoparticle synthesis

Fresh leaves of *A. marmelos* were picked from the tree, thoroughly washed thrice with deionized water, dried in a hot air oven at 40–45 °C for 48 h to ~6% final moisture content (analyzed by Sartorious MA-150 moisture analyzer), and then crushed into powder using a grinder. An intense brown color LE was obtained by mixing 1 g of leaf powder in 20 mL ultrapure water (i.e. 5%, w/v) and then heated at 100 °C for ~10 min in a hot water bath. The residue was separated by filtration through Whatman 42 filter paper and subsequently centrifuged at 20,000 rpm (Sigma, Model No. 3-30K, Germany) to remove very fine suspended particles.

SNPs were prepared from aqueous $AgNO_3$ solutions (0.1–1 mM) as a precursor purchased from Merck India. The variation in absorbance with respect to time because of SNPs was observed by UV-vis. spectrophotometer (UV-3600 Shimadzu). The residue of the reaction mixture was separated by centrifugation and then washed twice with water and finally dried. To know the residual $AgNO_3$ content, supernatants (after centrifugation) were treated with NaCl solution (0.1 M) to see the formation of precipitate if any.

2.2. Detection of phenolic compounds

To know the presence of phenolic compounds in *A. marmelos* leaf extracts; the ferric ion reducing test was done with the addition of $FeCl_3$ solution (30 mM) to the aqueous leaf extracts before and after the addition of $AgNO_3$ and the color change of the extracts was identified. Hydrolysable tannins give blue-black color and condensed tannins brownish-green while forming ferrous compound [32].

2.3. Test for bound phenols

Synthesized SNPs were separated out from the LE by centrifugation and washed thrice with distilled water, followed by treated serially with ethanol and diethyl ether. Further, the dried particles were suspended in NaOH (0.5 M) and waited for overnight at room temperature. The sample then centrifuged to get the residue and the supernatant was analyzed by UV–visible spectrophotometer [33].

2.4. Characterization of nanoparticles

The zeta (ζ) potentials and the particle size was measured by dynamic light scattering (DLS) technique using a Malvern zeta size analyzer, (Nano ZS, UK). The crystallite size and the particle confirmation were done by XRD using Philips (PW1830 HT) X-ray diffractometer, in the range of 20–90° (2θ) at 0.05°/min scanning rate. The accelerating voltage and applied current used was 35 kV and 30 mA respectively. The particle size and shape was confirmed using Field Emission gun based Scanning Electron Microscope (FESEM, Carl Zeiss, Neon 40), as well as scanning electron microscope (JEOL T-330) equipped with EDX attachment. The FT-IR analysis of LE and the nanoparticles were carried out using Perkin Elmer, Model No. S2000, USA. The LE before and after addition of $AgNO_3$ was also characterized by HPLC (Jasco, Japan, MD2015*plus*) with C_{18} reversed phase (RP) column with diode array detector. Mobile phase (isopropanol 20%; glacial acetic acid 1%; water 79%) of HPLC grade was used to detect phenolics [34]. Thermogravimetric analysis (TGA) was also studied using water washed SNPs under atmospheric condition (Shimadzu, DTG-60H, Japan). All the size and zeta potential measurements were done in triplicate and the average values reported.

3. Results and discussion

3.1. Concentration of leaf extract (LE) on particle size

Similar to chemical route, during the particle formation using LE; it is also very important to know the approximate stoichiometry ratio of extract to $AgNO_3$ for completion of the reaction. To test that the obtained 5% LE was diluted to 4, 10, 14, 17, 20, and 50 times and allowed to react with 1 mM $AgNO_3$ for overnight. A color change from brownish yellow to light yellow was observed in each test tube depending on the dilution. These samples were then centrifuged and the supernatants were collected to test the presence of unreacted $AgNO_3$ by the addition of NaCl. The visible turbid appearance was observed because of the formation of AgCl in the last two tubes of 20 and 50 times dilutions which indicate the presence of unreacted $AgNO_3$ (Supporting information, Fig. S1). UV absorption measurements before and after addition of NaCl also confirm no change in absorption pattern up to 17 times dilution (Fig. S1). While upon further dilution, a change in spectrum pattern was visible because of the formation AgCl in the solution. For convenience the identified critical concentration (17 times dilution) will be denoted further as 0.3% and other strengths in terms of % LE.

3.2. Particle size and distribution

The particle size was checked by lowering the strengths of the reactants within the critical proportion of 1/0.3 ($AgNO_3$ (mM)/LE (%)). The size variation and the distribution for each composition are shown respectively in Table TS1 and Fig. S2 (Supporting information). The DLS measurements show that there is no significant variation in average size and distribution pattern of nanoparticles within the critical concentration. Further with an increase in $AgNO_3$ concentration ($AgNO_3$/LE = 2/0.3, 3/0.3 and 4/0.3) beyond the critical concentration, resulted in the formation of $\sim 123 \pm 8$, 141 ± 4, and 164 ± 12 nm sized SNPs (Table TS1). The increase in size may attribute to the insufficient capping action by the active compound present in LE which was not enough to prevent agglomeration. Additionally, it could also be because of less number of nuclei formation in the presence of excess $AgNO_3$ resulted in increased particle size with a decrease in reaction rate.

3.3. Kinetics of particle formation

The kinetics of particle formation was also studied spectrophotometrically to know the equilibrium time for the particle formation. The UV–visible absorption spectra during the reduction reaction at different time intervals are shown in Fig. 1a. The spectrum shows the maximum absorbance (λ_{max}) at 422 nm which increased gradually because of the formation of SNPs and finally saturated after 24 min. This indicates that the reduction reaction might have completed within 25 min with an intense color change (Fig. 1a insert). Fig. 1b shows the change in absorbance as a function of time at a constant wavelength of 422 nm. It can be observed that the sharp change in absorbance is within 10 min, after that the increase is slow and beyond ~25 min reaches to a plateau level. This may specify chemical reaction as well as nucleation process completed within 10 min and there after growth continued till 25 min.