

Green synthesis of silver nanoparticles using *Citrus Unshiu* peel extract

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ABSTRACT

In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO₃ solution through the extract simple and cheap route using peel extract of *Citrus Unshiu* as reducing and capping agent. Formation of nanoparticles were characterized using UV-Vis spectrophotometer, Energy Dispersive X-ray (EDX), Scanning Electron Microscopic (SEM), and FT-IR spectroscopy. SEM analysis showed the silver nanoparticles ranges from 40.18 to 48.73 nm.

Keywords: *Citrus Unshiu*, Silver nanoparticles, Bio-synthesis

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. New applications of nanoparticles and nanomaterials are emerging rapidly [1-3]. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics [4], antimicrobials and therapeutics [5, 6], catalysis [7] and micro-electronics [8]. Also, silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [9, 10]. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds [11].

Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions [12], chemical and photochemical reactions in reverse micelles [13], thermal decomposition of silver compounds [14], radiation assisted [15], electrochemical [16], sonochemical [17], microwave assisted process [18], and recently via green chemistry route [19-21].

The use of environmentally benign materials like plants extract [22], as reducing and capping agent for fabrication of nanocrystalline silver particles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Thus, green syntheses of nanoparticles could be an alternative to chemical and physical methods due to it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals [23].

Citrus Unshiu peel contains a wide range of active ingredients and research is still underway in finding uses for them as they are rich in 1-limonene, γ -terpinene, β -linalool and β -myrcene[24]. Here in, we report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous peel extract of *Citrus Unshiu*.

MATERIALS AND METHODS

Plant material and preparation of the extract

Citrus Unshiu peel was collected from Ramsar, Province of Mazandaran, Iran, in December 2014. The water extract solution of *Citrus Unshiu* Peel was prepared by boiling of 5 g of air dried finely cut peel tissue of *Citrus Unshiu* in 100 ml deionized water for 10 min. The mixture then filtered using Wattman No.1 filter paper and filtered solution was kept in 4°C for further use.

Synthesis of Silver Nanoparticles

1 mM aqueous solution of silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Citrus Unshiu* extract was added into 90 ml of aqueous solution of 1mM Silver nitrate for reduction Ag^+ ions to silver nanoparticles and kept at room temperature for 5 hours.

UV-Vis Spectra analysis

The reduction of Ag^+ ions to silver NPs was monitored by measuring the UV-Vis spectrum of the reaction medium at different time after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done using Varian Carry 300UV-Vis spectrophotometer from 380 to 550 nm.

FT-IR spectral analysis

FT-IR was used for determination of the active functional groups in reducing procedure of Ag^+ ions. To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution, after reaction was centrifuged at 13000 rpm for 20 min and the resulting suspension was re-dispersed in 1 ml sterile deionized water. The centrifuging and re-dispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally the dried samples were palletized with KBr and analyzed by a Nicolet Nexus FT-IR spectrophotometer.

Particle size

The silver nanoparticle solution thus obtained was centrifuged at 13000 rpm for 20 min such as preparation procedure for FT-IR, the achieved pellet used for SEM, and EDX analyses. Scanning Electron Microscopic (SEM) analysis was done using Leo 440i SEM instrument. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. The element analysis of the silver nanoparticles was performed using EDX. The freeze-dried silver nanoparticles were mounted on specimen stubs with double-sided taps, and analyzed by EDX 3000 instrument.

RESULTS AND DISCUSSION

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [25]. As the *Citrus Unshiu* extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to brown due to reduction of silver ion, which indicated formation of silver nanoparticles.

It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. Figure 1 shows the UV-Vis spectra recorded from the reaction medium as a function of time. As the time is increased, the absorbance value at the absorption peak of 450 nm also increases. However, the percentage increase in absorbance after 1 hr is very less. The UV-Vis data also revealed that formation of AgNPs occurred rapidly within the first 1 hr only and the AgNPs in solution remained stable even after 5 h of completion of reaction. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 450 nm, broadening of peak indicated that the particles are polydispersed.

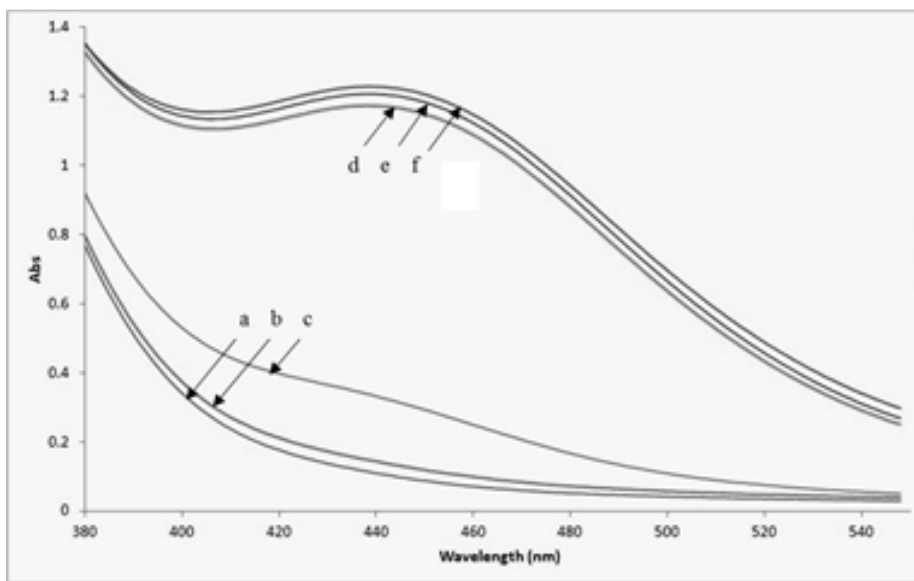


Fig. 1: UV-Vis curves of AgNPs production using *Citrus Unshiu* peel water extract as reduction agent for different incubation times(a) 1 min., (b) 5 min., (c) 15 min., (d) 1 hr., (e) 3hr, (f) 5 hr

FT-IR analysis was used for the characterization of the extract and the resulting nanoparticles. FT-IR absorption spectra of water soluble extract before and after reduction of Ag^+ ions are shown in Figure 2. FT-IR analysis confirmed that the polyols are mainly responsible for the reduction of Ag^+ ions, whereby they themselves get oxidized to carbonyl derivatives leading to a broad peak at $3200-3500\text{ cm}^{-1}$ ($C=O$) and a sharp peak at 1730 cm^{-1} (OH). Further elucidation of FT-IR spectra of silver nanoparticles presents one peaks of absorption band at 868 cm^{-1} representing presence of $Ag-O$ bond.

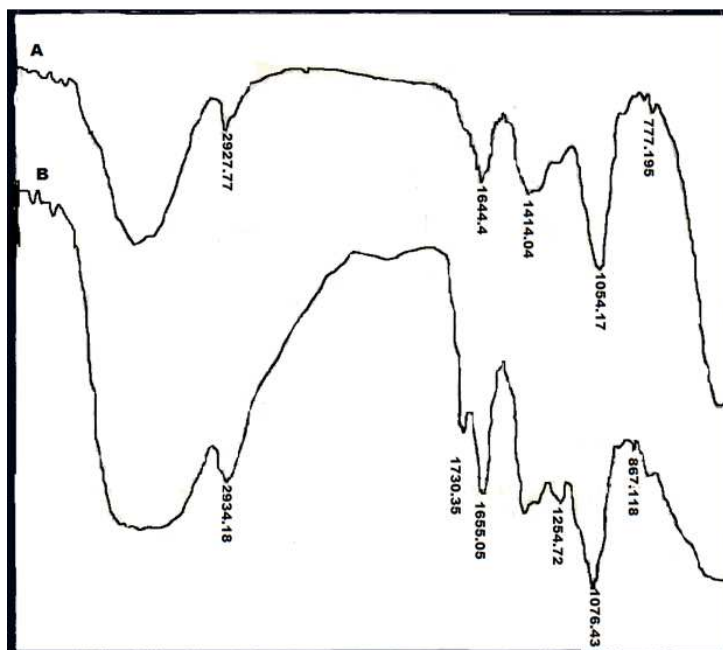


Fig. 2: FT-IR spectra (A) *Citrus reticulata* extract (B) Ag nanoparticles + extract

EDX spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles (Figure 3). The vertical axis displays the number of x-ray counts whilst the horizontal axis displays energy in KeV. Silver

nanoparticles generally show typical absorption peak approximately at 3 KeV due to surface plasmon resonance and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified.

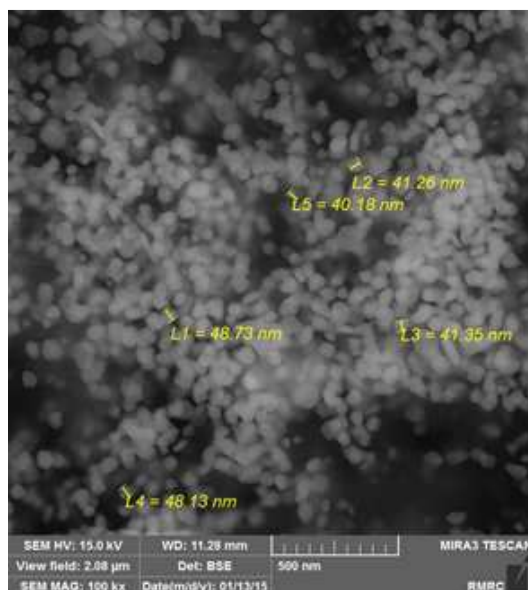


Fig. 3: EDX of green synthesized of Ag nanoparticles

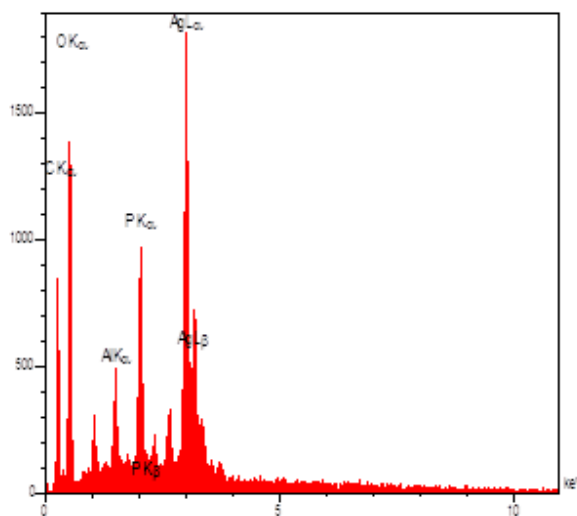


Fig. 4: SEM image of green synthesized AgNP

The SEM analysis showed the particle size between 40.18-48.73nm as well the cubic structure of the nanoparticles(Figure4). The present study showed a simple, rapid and economical route to synthesized Silver nanoparticles.

CONCLUSION

In the present study we found that fruits can be also good source for synthesis of silver nanoparticles. This green chemistry approach toward the other chemical and physical synthesis of silver nanoparticles has many advantages such as, cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. In addition, the produced AgNPs by peel extract are suitable for pharmaceutical and biological applications due to they have not any chemical contaminations.

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