



Green Synthesis and Characterization of Silver Nanoparticles Using Red Apple (*Malus domestica*) Fruit Extract at Room Temperature

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Received 31 Dec 2013, Revised 23 Feb 2014, Accepted 23 Feb 2014

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Abstract

The present work reports on simple and effective ecofriendly approach for the synthesis of silver nanoparticles (AgNPs) from silver nitrate using *Malus domestica* (red apple) fruit. The fruit extract act as both reducing and capping agents. The synthesized AgNPs were characterized using various instrumental techniques including ultraviolet-visible spectroscopy (UV-Vis), Fourier transformed infrared spectroscopy (FTIR), x-ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive spectroscopy (EDS) and dynamic light scattering (DLS). Surface Plasmon Resonance (SPR) for AgNPs was observed at 422 nm. The synthesized AgNPs were found to be flower-like in shape with average diameter of 145 nm. The zeta potential of AgNPs was found to be -65.0 mV. This large negative zeta potential value indicates repulsion among AgNPs and their dispersion stability.

Keywords: Red apple, Silver nanoparticles, Biosynthesis, Zeta potential, Characterization, Green Chemistry

1. Introduction

Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which has grown very rapidly all over the world for the past decade. Silver nanoparticles (AgNPs) are very important among the most widely used metal nanoparticles. They can be synthesized using different methods including physical and chemical methods [1, 2], electrochemical reduction [3], photochemical reduction [4] and heat evaporation [5]. Most of the methods reported in the literature are extremely expensive and also involve the use of toxic, hazardous chemicals such as stabilizers which may pose potential environmental and biological risks. Because of the increasing environmental concerns by chemical synthesis routes, an environmentally sustainable synthesis process has led to biomimetic approaches, which refers to applying biological principles in materials formation [6].

The use of plant extracts to synthesize nanoparticles is receiving attention in recent times because of its simplicity. Also, the processes are readily scalable and may be less expensive. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. A number of plant extract mediated synthesis of AgNPs have been reported in the literature. For instance the use of *Ficus benghalensis* [7], *Rosa rugosa* [8], *Arbutus Unedo* [9], *Stevia rebaudiana* [10] leaves extracts, *Euphorbia milii* latex [11], *Iresine herbstii* leaf extract [12], *Brucea javanica* (L) rind extract [13], *Ixora coccinea* leaf extract [14], *Aegle marmelos* leaf extract [15], *Artemisia nilagirica* leaf extract [16], leaf extract of *Vitex negundo* L.[17], *Cocos nucifera* coir extract [18], *Hibiscus cannabinus* leaf extract [19], *Sesbania grandiflora* leaf extract [20], *Dillenia indica* fruit extract [21], *Ocimum sanctum* leaf extract [22] to mention but a few has been reported. Mittal *et al.* [23] and Iravini [24] in their recent reviews have highlighted various plant extracts that have been used to synthesize AgNPs.

Apples are obtained from the medium-sized tree belonging to the *Rosaceae* family. The apple tree is originated in the mineral-rich mountain ranges of Kazakhstan, and now being cultivated in many parts of the world. Apple fruit features oval or pear shape; and the outer skin has different colors depending upon the cultivar type. Internally, the juicy pulp is off-white to cream in color and has a mix of mild sweet and tart taste. Its seeds are inedible because of their bitter taste. Apples are rich in antioxidant, phytonutrients, flavonoids and polyphenolics. Some of the important flavonoids in apples are quercetin, epicatechin, and procyanidin B₂. Additionally, they are also good in tartaric acid that gives tart flavor to them [25].

Although there are some reports on the synthesis of AgNPs from fruits, to the best of our knowledge till date there is no report available on *Malus domestica* fruits extract for the reduction of metal salts to get nanoparticles. As part of our contributions to the growing interest of bio-mediated synthesis of SNPs, we report for the first time an inexpensive one-pot synthesis of AgNPs by green route at room temperature, stabilized in situ using *Malus domestica* fruit extract.

2. Experimental

2.1. Preparation of *Malus domestica* extracts

Fresh fruits of *Malus domestica* were bought from King Fahd University of Petroleum and Minerals (KFUPM) commercial shopping centre. They were thoroughly washed thrice with double distilled water, sliced into pieces, dried in a hot air oven at 70 °C for 24 h and then crushed into powder using a grinder (Joya, 16-002). An intense brown colour *Malus domestica* extract was obtained after mixing 10 g of the fruit powder in 200 mL double distilled water and then boiling it for 10 min in a hot plate. The residue was separated by filtration through Whatman No. 1 filter paper. The filtrate obtained was stored in a refrigerator for further use.

2.2 Synthesis of AgNPs

Silver nitrate (AgNO₃) (BDH, Poole, England) of analytical reagent grade was used as a precursor for the synthesis of AgNPs. Silver nanoparticles were prepared by adding 5 mL of the extract to 50 mL of aqueous silver nitrate solution (0.1 M) at room temperature. The mixture was hand shaken and kept in the laboratory at room temperature. The experiment was continuously observed via color change with naked eye as well as UV–vis spectrophotometer. The effects of different parameters such as (i) concentration of silver nitrate, (ii) concentration of *Malus domestica* extract and (iii) reaction time on nanoparticle formation were examined. Five millilitres of the extract was added to different concentrations (0.001, 0.01 and 0.1 M) of silver nitrate in order to assess the influence of AgNO₃ concentration on the synthesized nanoparticles whereas different concentrations of the extract (1 – 5 mL) were added to a fixed concentration of AgNO₃ (0.1 M) in order to determine the effect of extract concentration on the synthesized nanoparticles.

2.3 Characterization of AgNPs

2.3.1 Uv-visible measurements

Spectral analysis for the development of nanoparticles at different reaction conditions were observed using JASCO 670- UV–Vis spectrophotometer from 300 to 700 nm at a resolution of 1 nm at room temperature. AgNPs gave sharp peak in the range of visible region of the electromagnetic spectrum.

2.3.2 SEM-EDS analysis

Scanning Electron Microscope (SEM) JSM- 5800 LV, JEOL was used to determine the shape of developed nanoparticles. Samples were prepared by depositing a drop of colloidal solution on an aluminium grid sample holder and drying at room temperature. Elemental composition of the sample was analyzed with energy dispersive analysis of X-ray spectroscopy (EDS) coupled to the Scanning Electron Microscope.

2.3.3 XRD analysis

Resulting solution of the developed nanoparticles of silver was centrifuged at 10,000 rpm for 30 min. The solid residues of AgNPs were washed twice with double distilled water and then redissolved in absolute ethanol and evaporated to dryness at 80 °C to obtain powder AgNPs used for X-ray powder diffraction measurements. The

powder X-ray diffraction (XRD) patterns were recorded on Rigaku Mini-flex II system using nickel filtered Cu K α radiation $\lambda = 1.5406 \text{ \AA}$ at 40 kV and 30 mA.

2.3.4. Particle size and Zeta potential measurements

Particle size was determined by dynamic light scattering technique using Microtrac S3500 and zeta potential of AgNPs was measured using Zetatrac (Microtrac, USA).

2.3.5. FTIR analysis

Fourier transformed infrared (FTIR) spectrum of the sample was recorded by Fourier transform infrared (Nicolet 6700 FT-IR, Thermo Scientific) spectrophotometer. The FTIR spectrum ranged from 4000 to 450 cm^{-1} at a resolution of 4 cm^{-1} by making a KBr pellet with AgNPs.

3. Results and discussion

3.1. UV-vis analysis

The synthesis of well-dispersed, single, structurally flower like silver nanoparticles was accomplished via one-pot reaction involving the reduction of silver salt using aqueous extract of red apple fruit. UV-vis absorption spectrum of the solution showed the surface Plasmon resonance derived from the silver nanoparticles at around 409 - 448 nm. Reaction parameters viz. concentration of silver salt, aqueous extract and reaction time were optimized for maximum yield of silver nanoparticles. Figure 1 shows the UV-vis spectra of the nanoparticles obtained at different concentrations of the silver salt (0.001, 0.01 and 0.1 M) after 168 h reaction time. Absorption peak obtained with 0.001 M silver salt is broad indicating the presence of nanoparticles of different sizes. The digital photograph showing the colour evolution (Fig. 1b) due to AgNPs formation clearly shows the dependent of silver salt concentration on AgNPs formation. The colour changes from yellowish brown through reddish brown to dark brown with increase in concentration of the silver salt from 0.001 M through 0.01 M to 0.1 M could be visibly observed. It is also observed from Figure 1a that the absorption peaks increases with increase in the concentration of the silver salt. Best result was obtained with 0.1 M AgNO_3 solution hence this concentration was chosen to evaluate other reaction parameters.

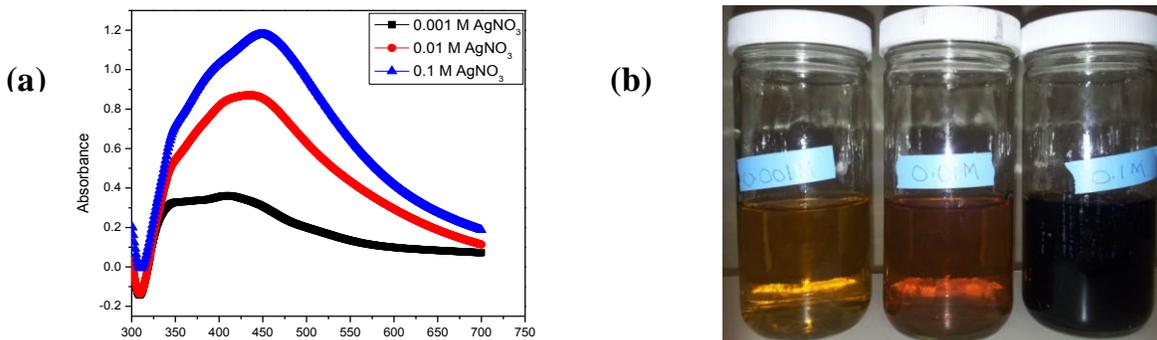


Figure 1: (a) UV-visible spectrum of AgNPs synthesized using aqueous AFE with different concentrations of AgNO_3 after 168 h (b) Digital photograph showing the colour change of (a) 0.001 M, (b) 0.01 M and (c) 0.1 M AgNO_3 when aqueous AFE was added after 168 h reaction time.

Figure 2 shows the UV-vis spectrum of AgNPs synthesized using aqueous AFE as a function of reaction times (1, 6 and 24 h). A broad peak could be seen after 1 h reaction time and sharp peaks are observable after 6 and 24 h reaction times. Also the Surface Plasmon Resonance (SPR) shifted to higher wavelength with increase in reaction time. Experiments were also undertaken to determine the influence of the amount of AFE on AgNPs formation. The red apple fruit extract quantity was varied from 1 to 5 mL in 50 mL of 0.1 M silver nitrate solution. It is interesting to note that the formation of well dispersed uniformly shaped silver nanoparticles was influenced significantly by the aliquot ratio of the extract to the silver precursor quantity.

The higher the concentration of the extract being added, the higher was the possibility of the green reduction mechanism of the silver nitrate occurring, which would lead to the formation of well defined and stable silver nanoparticles. The result shown in Figure 3 reveals a consistent increase in the absorbance peak with increase in the amount of extract. However, the shapes of the AgNPs were not altered as could be seen in Figure 4.

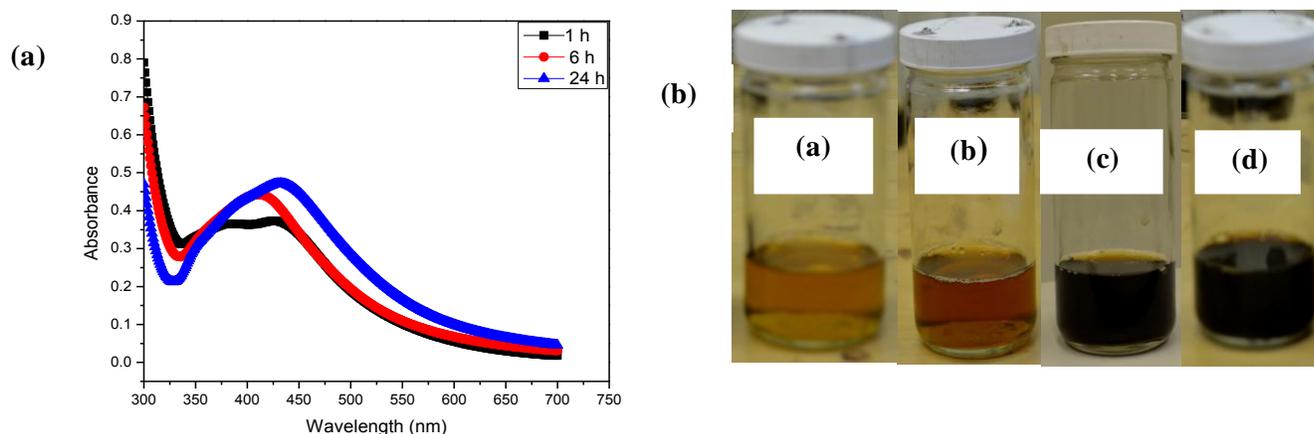


Figure 2: (a) UV-visible spectrum of AgNPs synthesized using aqueous AFE at 1 h, 6 h and 24 h reaction time; (b) Digital photograph showing the colour change of AgNO₃ on addition of aqueous AFE at (a) 0 h, (b) 1 h, (c) 6 h and (d) 24 h reaction time

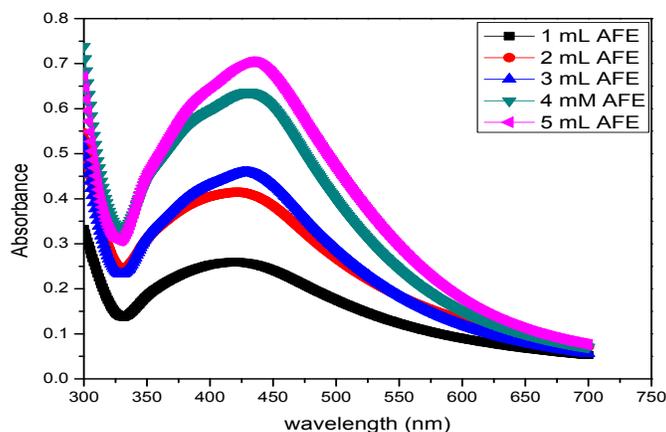


Figure 3: UV-visible spectrum of AgNPs synthesized using different concentrations of aqueous AFE after 24 h reaction time.

3.2. SEM and Energy dispersive X-ray analysis

Figure 4 depicts the SEM images obtained from drop coated films of AgNPs synthesized from (a) 1, (b) 3 and (c) 5 mL aqueous *M. domestica* extract. The SEM images showed flower-like shaped structures. EDS analysis gives qualitative as well as quantitative status of elements that may be involved in formation of nanoparticles. Figure 5 shows elemental profile of synthesized nanoparticles using red apple fruit extracts and confirms the formation of silver nanoparticles. Figure 5 also shows higher counts at 3 keV due to silver nanoparticles. Generally metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance [19]. The elemental analysis of the silver nanoparticles shown in the figure revealed highest proportion of silver followed by Al, and O. The O peaks are from the biomolecules bound to the surface of the silver nanoparticles and Al peaks are due to sample grid holder which is made from Al alloy. It has been reported that

nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from the plant leaf broth and are, thus, stable in solution several months after synthesis [20].

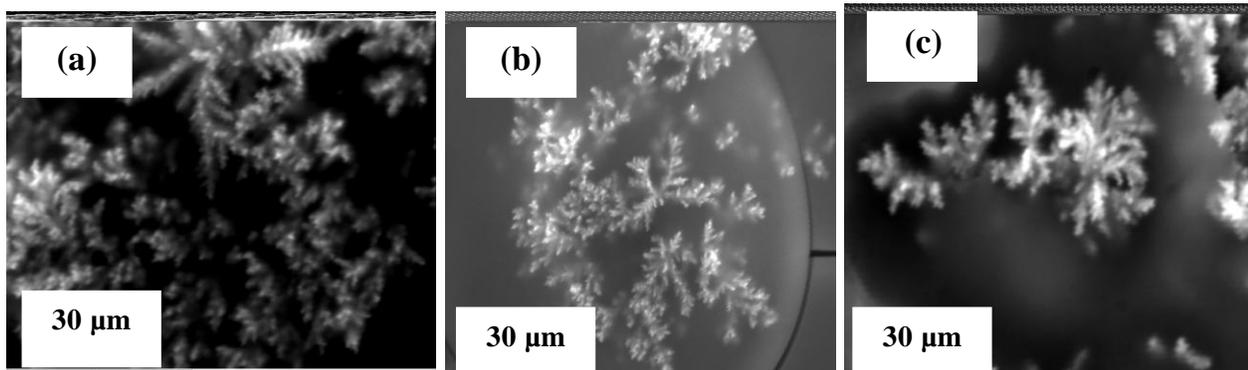


Figure 4: SEM images of synthesized AgNPs using different concentrations of aqueous AFE (a) 1 mL, (b) 3 mL and (c) 5 mL after 24 h reaction time

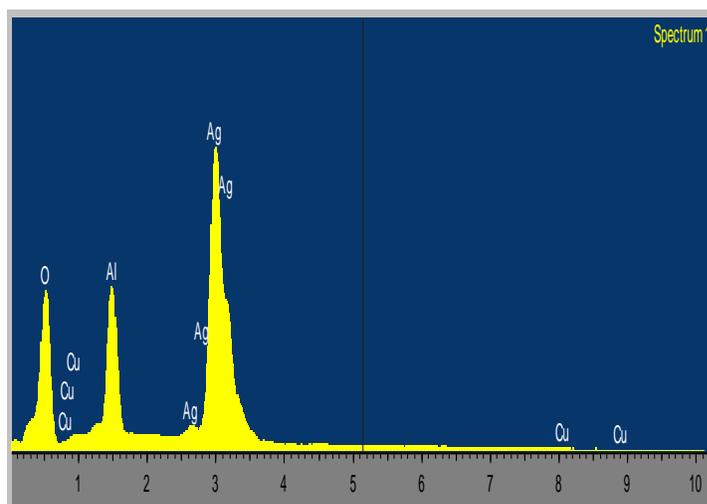


Figure 5: EDX images of synthesized AgNPs

3.2. Particle size and Zeta potential measurements

The particle size of the synthesized silver nanoparticles was determined using dynamic light scattering measurement technique. Dynamic light scattering (DLS) is a technique for characterizing the size of colloidal dispersions which utilizes the illumination of a suspension of particles or molecules undergoing Brownian motion by a laser beam. The time-dependent fluctuations in the intensity of scattered light that occur are analyzed using an autocorrelator which determines the autocorrelation function of the signal [26]. The size distribution of the synthesized AgNPs is depicted in Figure 6. From the figure, it is observed that the particles obtained are polydisperse mixtures in the range 50 to 300 nm. The average size of the synthesized silver nanoparticles using red apple fruit extract is around 150 nm. Sizes and shapes of metal nanoparticles are influenced by a number of factors including pH, precursor concentration, reductant concentration, time of incubation, temperature as well as method of preparation. The zeta potential of the synthesized AgNPs was determined in water as dispersant. The zeta potential was found to be -65.07 mV. The high value confirms the repulsion among the particles and thereby increases in stability of the formulation [22]. The zeta potential value could be positive or negative; the negative potential value shown by AgNPs could be due to the possible capping of the bio-organic components present in the extract [27, 28].

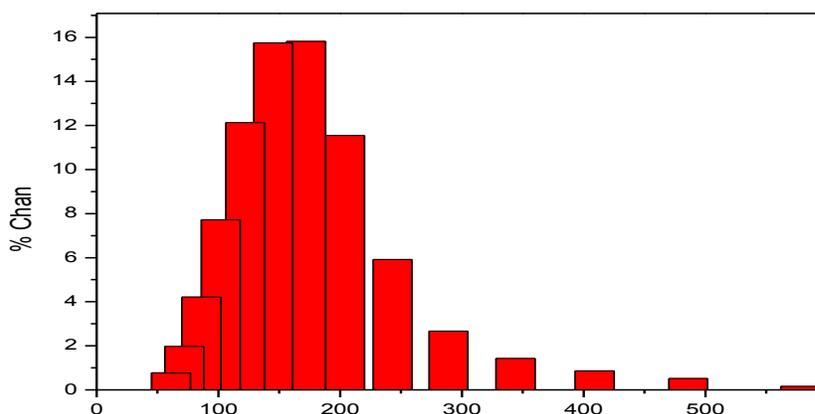


Figure 6: Particle size distribution of silver nanoparticles from dynamic light scattering measurements

3.4. XRD analysis

The crystalline nature of AgNPs was confirmed by the analysis of XRD pattern as shown in Figure 7. The XRD spectrum showed four distinct diffraction peaks at 38.28° , 44.33° , 64.33° , and 77.53° corresponding to lattice plane value indexed at (111), (200), (220) and (311) planes of face centered cubic (FCC) silver with a lattice parameter of $a = 4.08 \text{ \AA}$ which were in good agreement with reference of FCC structure from joint committee of powder diffraction standard (JCPDS) Card No-087-0720.

3.5. FTIR Spectroscopy

FTIR spectroscopy is useful in probing the chemical composition of the surface of the silver nanoparticles and the local molecular environment of the capping agents on the nanoparticles.

The FTIR spectrum of silver nanopowder is shown in Figure 8. Three main bands could be observed from the figure. The broad band appearing at 3450 cm^{-1} is assigned for O–H stretching vibration indicating the presence of hydroxyl groups in the reducing agent [20]. The strong intense peaks at 1379 and 1625 cm^{-1} corresponds to C–N stretch vibrations as well as to the amide I bands of proteins in the apple fruit extract. The result of this FTIR spectroscopic study confirmed that the red apple fruit extract has the ability to perform dual functions of reduction and stabilization of silver nanoparticles.

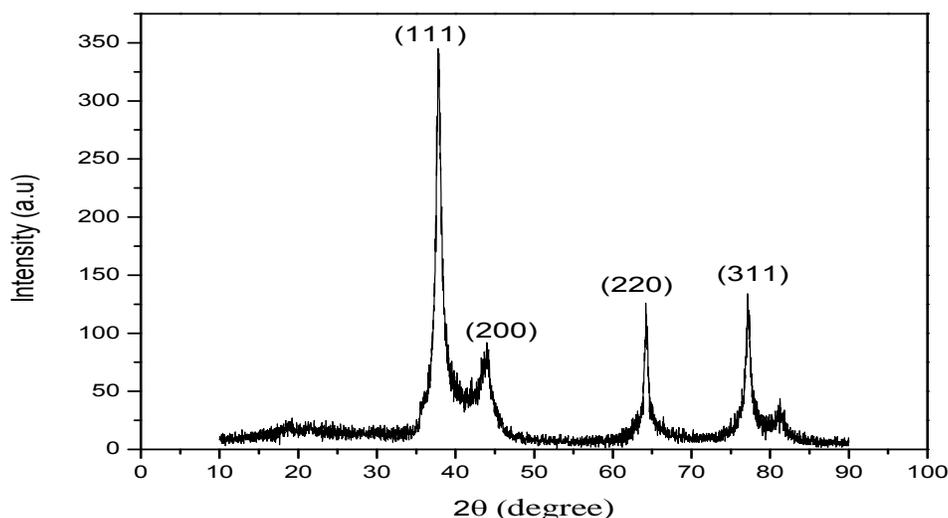


Figure 7: XRD patterns of Cu nanoparticles synthesized by treating 5 mL aqueous AFE with 0.1 M aqueous AgNO_3 solution.

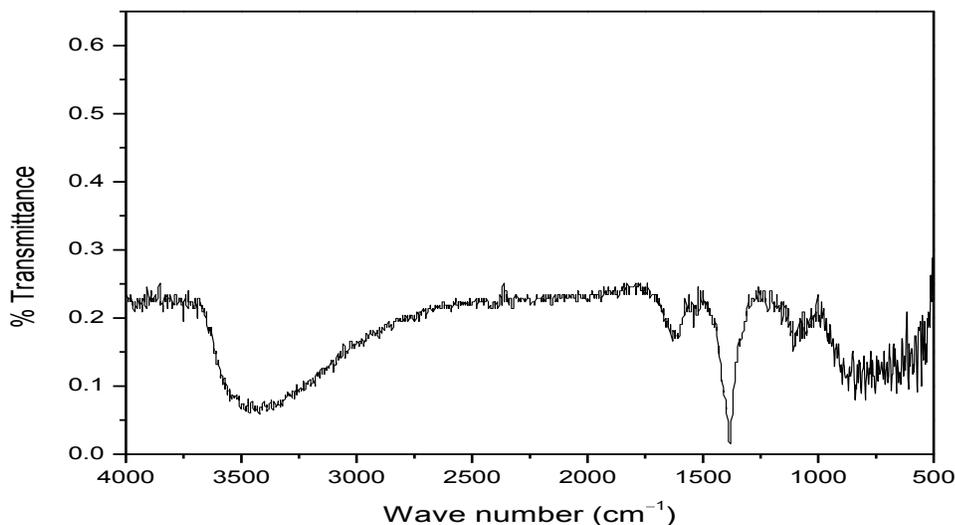


Figure 8: FTIR of silver nanoparticles synthesized by treating 5 mL aqueous AFE with 0.1 M aqueous AgNO₃ solution.

Conclusions

The present study shows that aqueous extract of red apple fruit can be used efficiently for the synthesis of silver nanoparticles having a flower-like shape. The synthesized silver nanoparticles were found to be stable at room temperature as revealed by the negative value of zeta potential due to the presence of natural products, such as flavanoids, proteins and reducing sugars present in the extract. Powder diffraction study showed the face-centered cubic lattice nature of AgNPs.

Acknowledgments-The authors are pleased to acknowledge King Fahd University of Petroleum and Minerals (KFUPM) for providing the facilities for the research. Also the Director, Centre of Research Excellence in Nanotechnology (CENT) is gratefully acknowledged for his kind permission to use their facilities for the characterization.

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