Green synthesis of silver and gold nanoparticles by using *Citrus reticulata* juice

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Nanoparticles are generally prepared by a variety of chemical methods which are not environmentally friendly. Silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) were successfully synthesized**, respectively** from $AgNO_3$ and $HAuCl_4$ through a simple eco-friendly route using the juice of *Citrus reticulata* fruit as reducing as well as capping agent. AgNPs and AuNPs were carried out at 25, 55, 25 and 50 ^oC respectively. Nanoparticles were **characterized** with the help of UV–Vis, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Energy Dispersive X-ray Analysis (EDAX), and Scanning electron microscopy (SEM).

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Keywords: Citrus reticulata; AgNPs; AuNPs; Green synthesis

1. Introduction

Nowadays precious metal recovery technologies use harmful chemicals that may represent a risk to the environment and public health. This is the reason why it is necessary to develop clean, non-toxic and environmentally friendly procedures to recover precious metals. In the last time, the use of bio-organisms in synthesis and assembly of nanoparticles has received increasing attention. There are already eco-friendly “green” methods for the synthesis of noble metal nanoparticles using bacteria, yeast, fungi and plant extracts.

Several plants have been successfully used for efficient and rapid extracellular synthesis of AgNPs and AuNPs. Leaf extracts of *Saururus chinensis* [1] *Teraxacum officinale* [2], *Cymbopogon flexuosus* [3], *Cinnamomum camphora* [4], *Azadirachta indica* [5], *Aloe vera* [6], *Tamarindus indica* [7] fruit extract of *Emblica officinalis* [8], *Pseudocydonia sinensis* [9] flower extract of *Carthamus tinctorius* [10], *Lonicera japonica* [11], *Dioscorea batatas rhizome* [12] have shown potential in reducing Au (III) ions to form gold nanoparticles, Au (0) or silver nitrate to form silver nanoparticles, Ag (0).

Fruits from citrus species have been widely used as a source of traditional medicine. These fruits include anti-allergic agents, anti-oxidants and anti-immune-modulating agents [13]. Citrus fruits contain a range of key nutrients such as vitamin C and A, folate, dietary fibers and minerals and also are a rich source of citric and ascorbic acids as well as of many other phytochemicals including flavonoids, carotenoids, such as beta-cryptoxanthin, limonoids, including obacunone 17 beta-D-glucopyranoside, nomilinic acid 17 beta-D-glucopyranoside, limonin, nomilin, coumarins, monoterpenes, triterpenes, phenolic acids, and a limonoid glucoside mixture.

2. Materials and methods

Fresh *Citrus reticulata* fruits were purchased from a local super-market in Gyeongju, South Korea. $AgNO_3$ was purchased from MERCK. $HAuCl_4$ purchased from Sigma-Aldrich Chemical Pvt. Ltd, South Korea.

2.1. Preparation of AgNPs and AuNPs using *Citrus reticulata* juice

Citrus reticulata fruits were carefully washed with deionized water. Fruits were squeezed to extract the juice which was later strained through a fine pore nylon mesh. The juice obtained was centrifuged at 10, 956.4 g for 5 min to remove any undesired impurities. For reduction of silver and gold ions, 5 mL of *Citrus reticulata* juice was dropwise added into 95 mL of 1 mM $AgNO_3$ and $HAuCl_4$ solutions.

2.2 UV-Vis spectra analysis

The reduction of pure Ag^+, $AuCl_4^-$ ions were monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (Cary 4000 UV-Vis spectrophotometer).

2.3 SEM and EDAX analysis

After reaction completion, the biomass spontaneously precipitated, in 1 hour, at the bottom of conical flasks. After the precipitation, the suspension was sampled for SEM-EDAX observations. SEM samples of the aqueous suspension of nanoparticles were fabricated by dropping the suspension onto clean electric glass and allowing water to completely evaporate. Samples were coated by carbon and SEM analyses were performed on a Hitachi s-3500N.

2.4 XRD analysis

The AgNPs and AuNPs solutions were purified by repeated centrifugation at 2, 739.1 g for 20 min followed by redispersion of the pellet of AgNPs and AuNPs into 10 mL of deionized water. After freeze drying of the purified nanoparticles, their structure and composition were analyzed by XRD (Rigaku RINT 2100 series). The dried mixture of AgNPs and AuNPs were collected for the determination of the formation of AgNPs and AuNPs by an X'Pert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ- 2 θ configuration.

2.5 FTIR analysis

To remove any free biomass residue or compound that are not the capping ligands of the nanoparticles, the residual solution of 100 mL was centrifuged at at 2, 739.1 g for 10 min and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifugation and redisperion processes were repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR (Bruker model, TENSOR 37).

2.6 Particle size measurement

Particle sizing experiments were carried out by means of laser diffractometry, using Zeta sizer nano series (Malvern).

3. Results and discussion

3.1 UV-Vis study

Reduction of silver and gold ions into Ag and Au particles during exposure to the plant extracts could be followed by color modification. AgNPs and AuNPs exhibit respectively, wine red color (at 25 °C), reddish brown (at 55 °C), purple (at 25 °C) and Indian red (50 °C) color in aqueous solution due to the surface plasmon resonance (SPR) phenomenon (Fig.1).

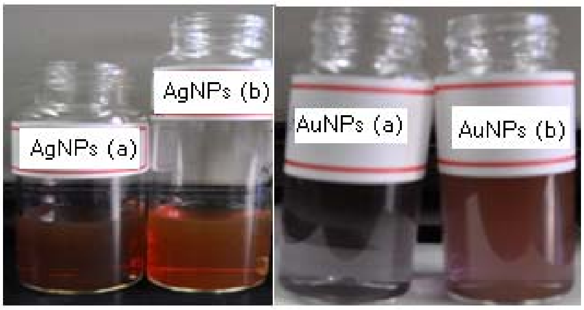
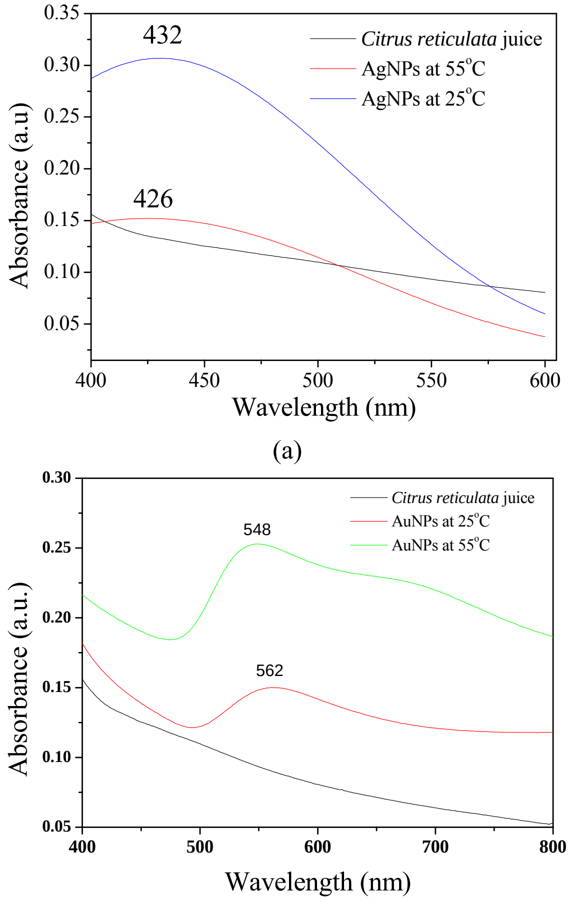


Fig. 1 Color change of juice extracts of Citrus reticulate. Left: (a) AgNPs at 55 °C (b) AgNPs at 25 °C Right: (a) AuNPs at 50 °C (b) AuNPs at 25 °C.

UV–Vis spectroscopy is an important technique to ascertain the formation and stability of metal NPs in aqueous solution.

Metal NPs such as silver and gold have free electrons, which give rise to SPR absorption band [14]. The characteristic SPR resonance band of biogenic AgNPs occurs at 432 and 426 nm for reaction carried out at 25 °C and 55 °C, respectively (Fig 2a), AuNPs occurs 562 and 548 nm at 25 °C and 50 °C (Fig 2 b). This may be due to the temperature increment. The reactants are consumed rapidly eventually leading to the formation of smaller nanoparticles [15, 16]. At low temperature, the rate of growth controls the size of the particles, while at higher temperature, a number of nuclei are formed, small size particles being observed.



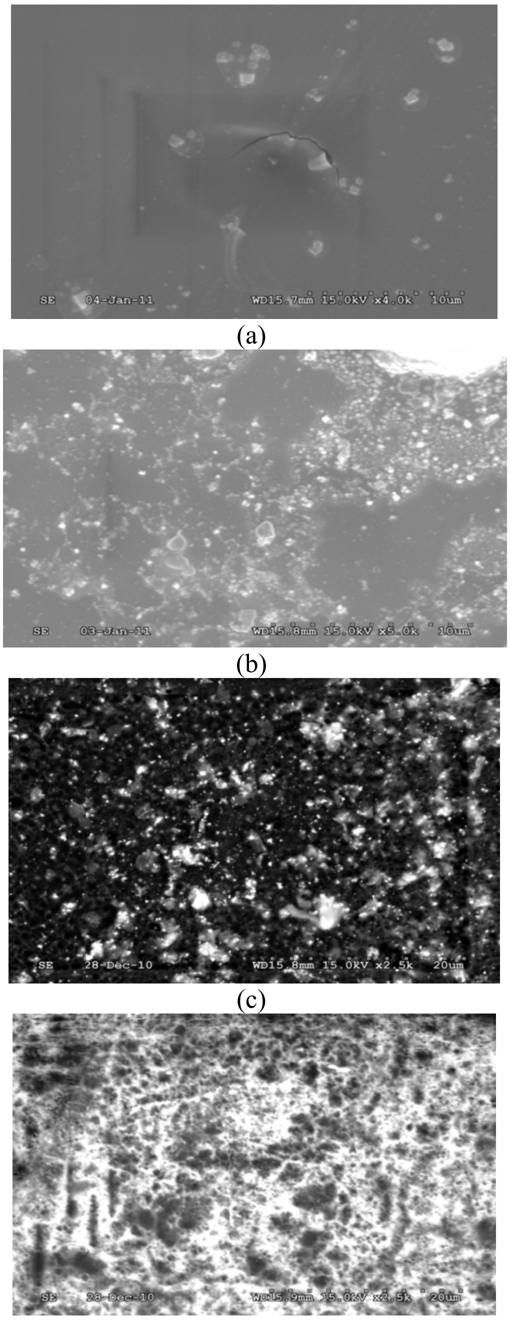
(a)

(b)

Fig. 2 UV-Vis absorption spectra (a) AgNPs (b) AuNPs.

3.2 SEM and EDAX studies

Fig. 3 (a-d) shows, the SEM images of the synthesized AgNPs and AuNPs. The morphology of AgNPs is cubic, round in shapes and well-dispersed, at 25 °C cubic in shapes and mono-dispersed, at 55 °C, AuNPs are more clearly seen, the particles shapes being spherical, octahedral, pyramidal, cubic and mono-dispersed at 25 °C, while at 50 °C they are irregular and well-dispersed.



(a)

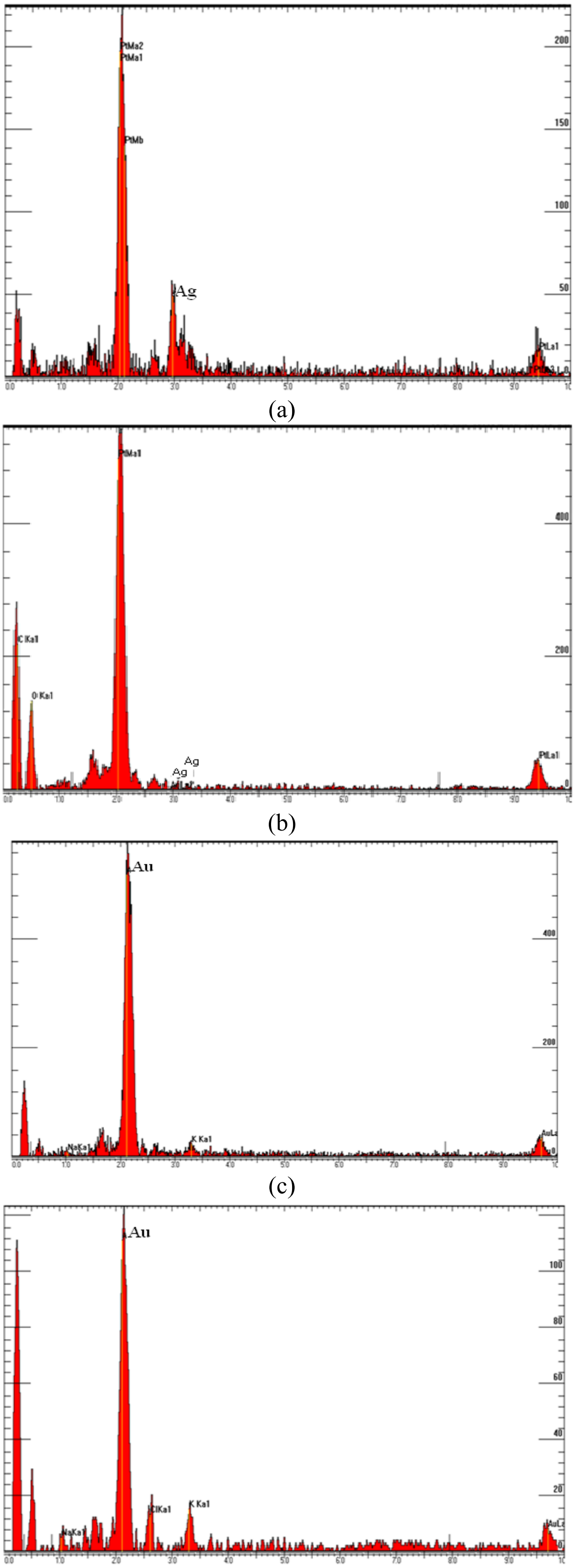
(b)

(c)

(d)

Fig. 3 SEM images at different magnifications of the green synthesized of AgNPs (a) AgNPs at 25 ^{o}C b) AgNPs at 55 ^{o}C (c) AuNPs at 25 ^{o}C (d) AuNPs at 50 ^{o}C.

Fig. 4 (a-d) shows the EDAX spectrum of AgNPs synthesized at 25 °C and 55 °C, and of AuNPs at 25 ^{o}C and 50 ^{o}C. Strong signals from the silver and gold atoms in the nanoparticles were observed, and also signals from O and C atoms (AgNPs); Na and K (AuNPs) were also recorded. The C and K, signals were likely due to X-ray emission from carbohydrates/proteins/enzymes present in the cell wall of the biomass. The presence of the elemental silver and gold can be observed in the graph obtained from EDAX analysis, which also supports the XRD results. This indicates the reduction of silver and gold ions into elements of silver and gold.



(a)

(b)

(c)

(d)

Fig. 4 EDAX spectrum of AgNPs and AuNPs (a) AgNPs at 25 ^{o}C (b) AgNPs at 55 ^{o}C (c) AuNPs at 25 ^{o}C (d) AuNPs at 50 ^{o}C.

3.3 XRD, FTIR and Particle size

The XRD pattern of the AgNPs and AuNPs is shown in the figure 5 (a-d). Various Bragg reflections clearly indicate the presence of (111) lattice planes and on this basis, they can be indexed as face-centered-cubic (FCC) structure of silver and gold. Hence, from the XRD pattern it is clear that AgNPs and AuNPs are formed using *Citrus reticulata.* In addition to the Bragg peaks representative of FCC AgNPs and AuNPs, additional unassigned peaks are also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the nanoparticles.

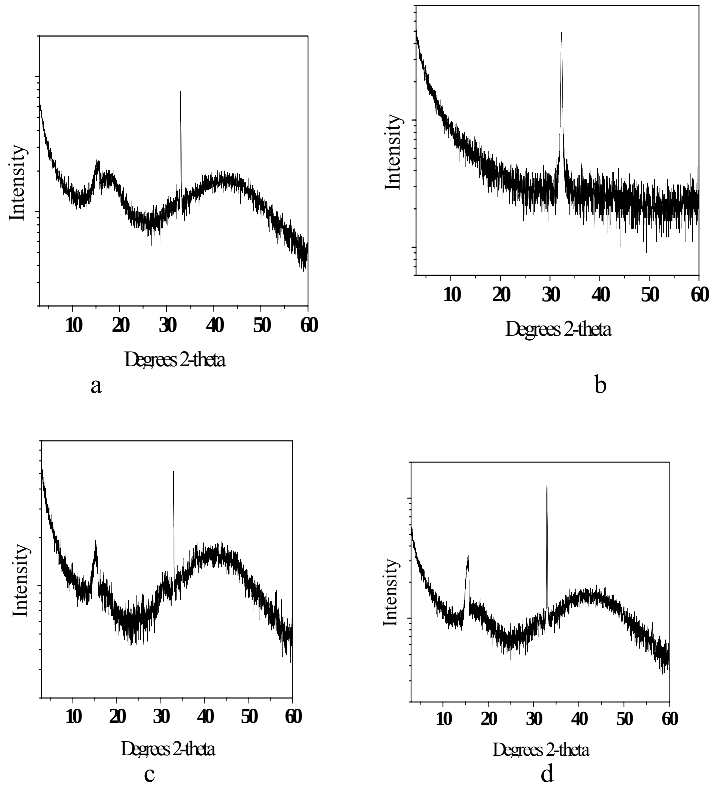


Fig. 5 XRD spectrum of (a) AgNPs at 25 °C, (b) AgNPs at 55 °C, (c) AuNPs at 25 °C and (d) AuNPs at 50 °C.

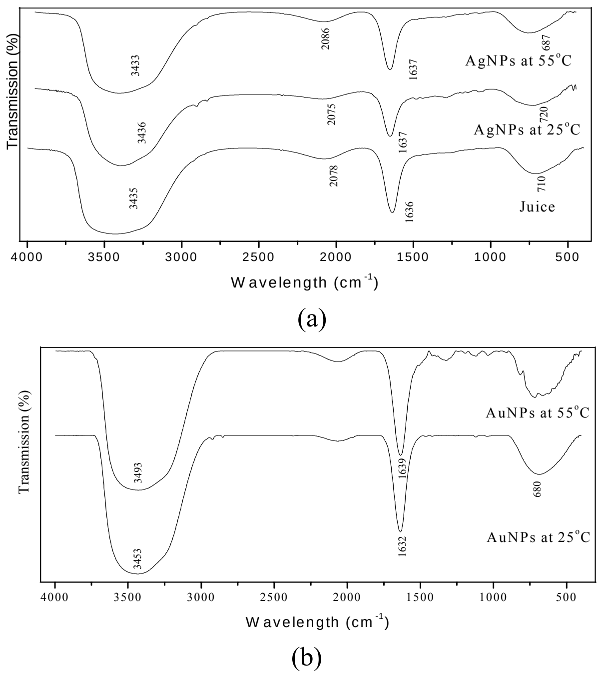
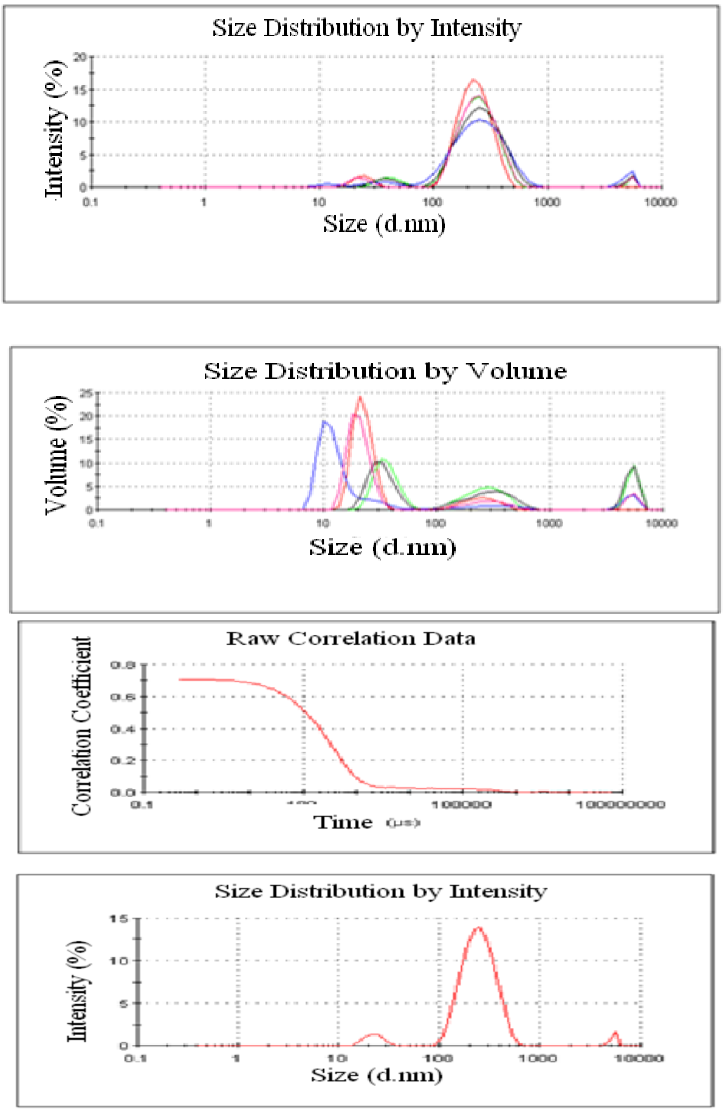


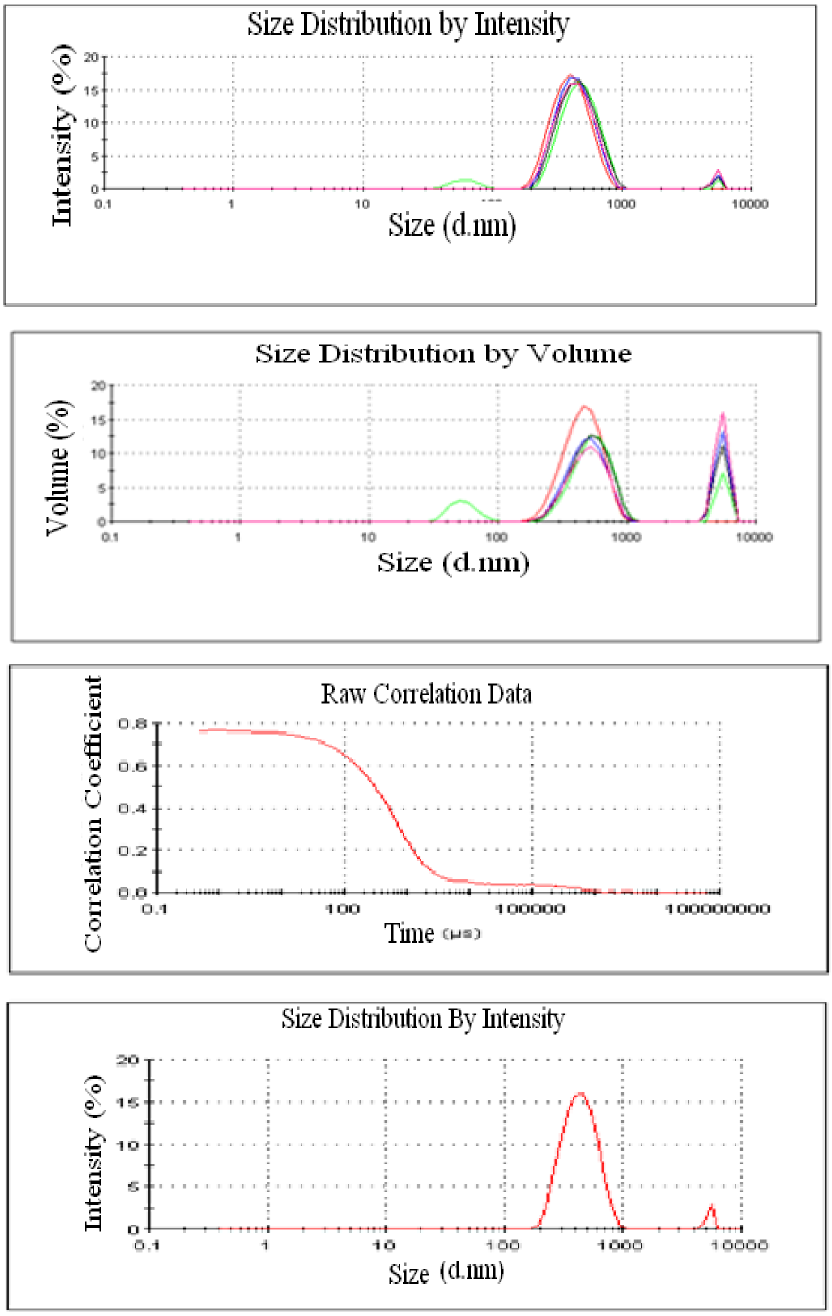
Fig.6. FT-IR spectrum of Citrus reticulata juice, AgNPs (a) and AuNPs (b).

FTIR results reveal that absorption bands (Fig. 6 a and b) at 3, 436, 2, 075, 1, 637 and 720 cm^{-1} (AgNPs at 25 °C), 3, 433, 2, 086, 1, 637 and 687 cm^{-1} (AgNPs at 55 °C); 3, 453, 1, 632 and 680 cm^{-1} (AuNPs at 25 °C), 3, 493 and 1, 639cm^{-1} (AuNPs at 50 °C), respectively. The vibrational bands situated in the region range, 680 to 3, 453cm^{-1}, are corresponding to the bonds such as amino (N-H), alkane (C=C), alcohols (O-H). The most wide spectrum absorption was observed at 3, 453 and 1, 639 cm^{-1}.

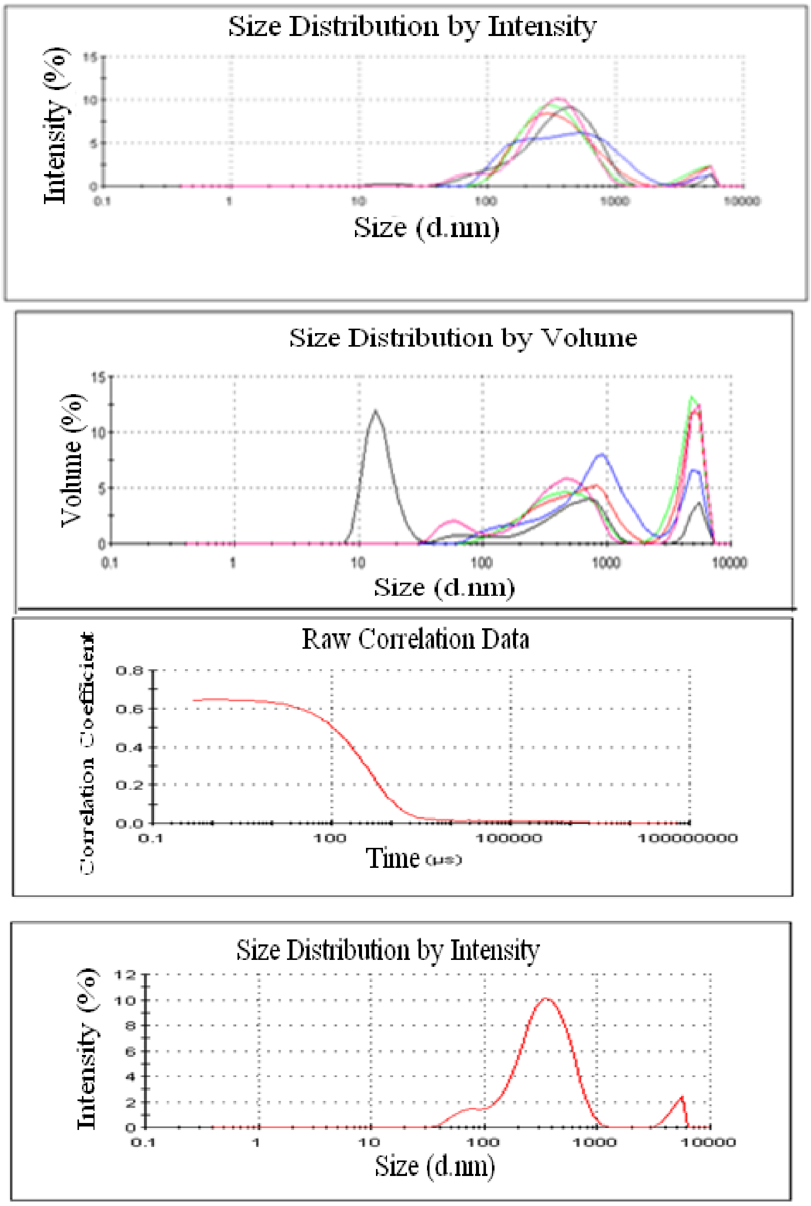
Particle size determination of the formulated AgNPs and AuNPs were shown under different categories like size distribution of volume and intensity (Fig. 7a-d). The size distribution by volume gives a bell shaped pattern which indicates the wide range size distribution of nanoparticles in the sample. The volume (%) of the samples were found to be in the range of 0.1 to 10, 000 (AgNPs and AuNPs). The formed AgNPs and AuNPs are well distributed with respect to volume and intensity, an indication of the formation of well built AgNPs and AuNPs and of their mono and poly disparity, respectively.



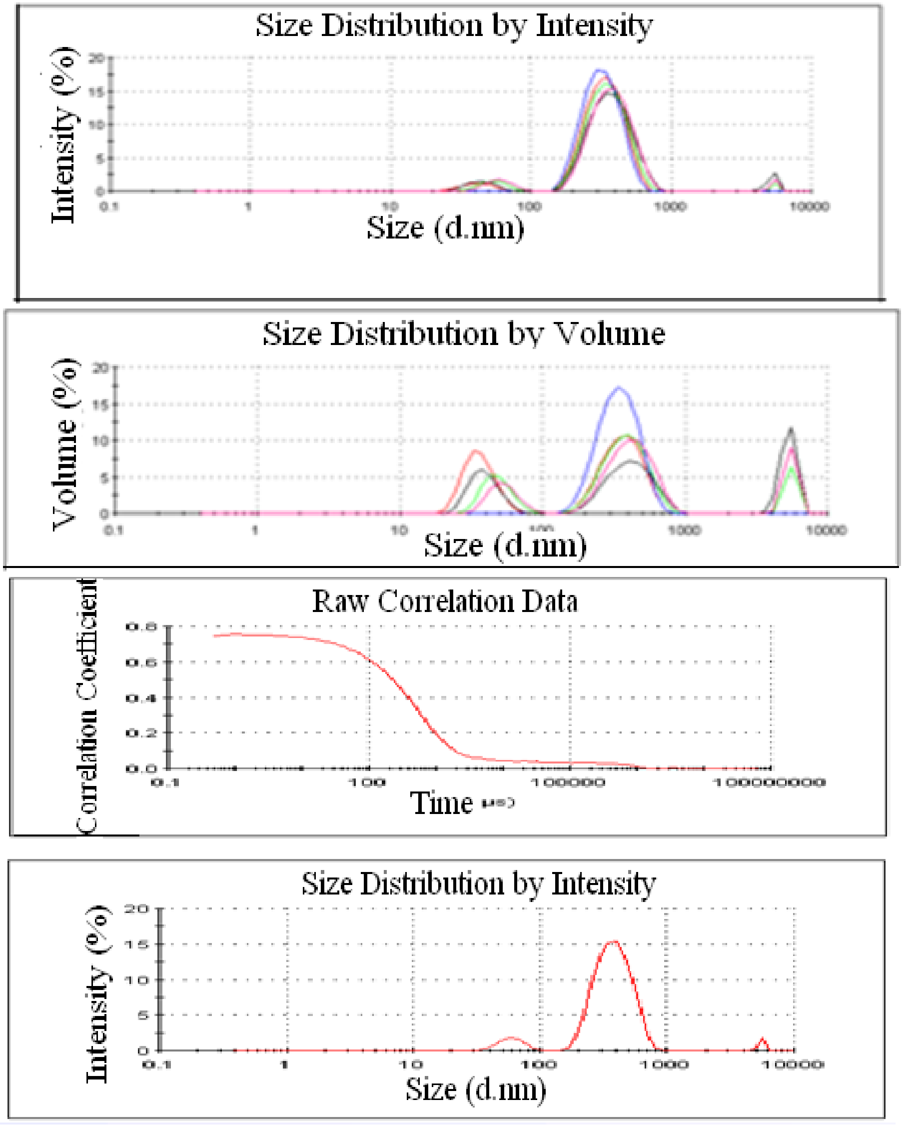
(a)


Size Distribution by Intensity
Intensity (%)
20
15
10
5
0
0.1
1
10
1000
10000
Size (d.nm)
Size Distribution by Volume
Volume (%)
20
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)
Raw Correlation Data
Correlation Coefficient
0.8
0.6
0.4
0.2
0.0
0.1
100
100000
100000000
Time (µs)
Size Distribution By Intensity
Intensity (%)
20
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)

(b)


Size Distribution by Intensity
Intensity (%)
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)
Size Distribution by Volume
Volume (%)
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)
Raw Correlation Data
Correlation Coefficient
0.8
0.6
0.4
0.2
0.0
0.1
100
100000
100000000
Time (µs)
Size Distribution by Intensity
Intensity (%)
12
10
8
6
4
2
0
0.1
1
10
100
1000
10000
Size (d.nm)

(c)



(d)

Fig. 7 Particle size distribution by intensity and volume (a) AgNPs at 25 ^oC (b) AgNPs at 55 ^oC (c) AuNPs at 25 ^oC (d) AuNPs at 50 ^oC).

4. Conclusion

Here we have reported and characterized the synthesis of AgNPs and AuNPs using *Citrus reticulata* fruit juice in the aqueous solution as a reducing and capping agent. The structural characterizations of the samples were performed using UV-Vis spectrometry, FTIR, SEM and XRD analysis. This green chemistry approach of the synthesis of AgNPs and AuNPs have many advantages, being more economic and an effective alternative to the chemical synthesis of AgNPs and AuNPs.

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