Biosynthesis of spherical and highly stable gold nanoparticles using Ferulago Angulata aqueous extract: dual role of extract

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Abstract

A biocompatible method for synthesizing of highly disperses gold nanoparticles using Ferulago Angulata leaf extract has been developed. It has been shown that leaf extract acts as reducing and coating agent. Various spectroscopic and electron microscopic techniques were employed for the structural characterization of the prepared nanoparticles. The biosynthesized particles were identified as elemental gold with spherical morphology, narrow size distribution (ranged 9.2–17.5 nm) with high stability. Also, the effect of initial ratio of precursors, temperature and time of reaction on the size and morphology of the nanoparticles was studied in more detail. It was observed that varying these parameters provides an accessible remote control on the size and morphology of nanoparticles. The uniqueness of this procedure lies in its cleanliness using no extra surfactant, reducing agent or any capping agent.

1. Introduction

In modern nanotechnology, there is a growing need to develop environmentally friendly methods for a clean synthesis of metallic nanoparticles [1–13]. Gold nanoparticles (AuNPs), with a variety of tunable properties, have attracted considerable interest in a range of applications, e.g. catalyst, sensing, photonics, diagnostics, and therapeutics [14–20]. The physical and chemical properties of AuNPs can be tuned by tailoring their size, shape and dispersity. The control of size and morphology of metal nanoparticles play a key role in manufacturing advanced materials on a large scale. Therefore, controlled procedures are necessary to unambiguously correlate the structural properties of these materials [21]. Of those parameters, morphology is often not feasible enough to be tailored deliberately. As a result, there is a growing demand to develop simple and environmentally safe methods that favor the synthesis of shape-controlled metal nanoparticles. Basically, parameters such as initial concentration of precursors, nature of reducing and stabilizing agents, seeds, solvent, pH, incubation time and etc are the main factors affecting the nucleation and growth steps and eventually the shape of formed AuNPs [22].

Vast numbers of chemical [23, 24], physical [25] and biological strategies [1, 26–29] have been developed to synthesize AuNPs of different shapes. Among them, biological processes are preferred for environmental and economic concerns. Physical and chemical methods require expensive high technology, high temperature, high pressure, toxic environment, and are neither suitable for mass production nor energy-efficient. In recent years, the biosynthetic methods employing plant extract is gaining attention due to its simplicity and environmental friendly process. Moreover, the AuNPs produced by extracts are stable and the rate of synthesis is high [1].

The genus Ferulago comprises of some thirty-five species. Seven of which are found in Iran. Limited studies have been done on this genus and its essential oil and its components were determined in Turkey and Greek. The Ferulago Angulata (F.A.) is an endemic plant of Iran, which belongs to west part of Iran and is an important medicinal plant and contains variety of components with different therapeutically effects [30, 31]. Traditionally, this plant was added to different production such as diary and oil ghee to prevent from decay and giving a
pleasant taste to them. We previously reported that extract of F.A. mainly contains two types of flavonoids: quercetin and rutin, which are known as antioxidant compounds [32]. We hypothesized that these antioxidants containing various phenolic units will provide suitable reduction power for the bio-reduction of Au$^{3+}$ ions into elemental Au$^0$ atoms in a clean and safe manner. Accordingly, the possible dual activation role of aqueous extract of F.A. as an bio-reducing and capping agent in generation of stable AuNPs, without using extra chemical reducing or capping agents was screened in detail. The obtained results confirmed that this clean and tuneable approach could be considered as a facile alternative to the conventional methods of producing gold and other noble metal nanoparticles.

2. Experimental

2.1. Materials and methods

Hydrogen tetrachloroaurate ($\text{HAuCl}_4.3\text{H}_2\text{O}$) was purchased from Merck Co. Double distilled water (Milli-Q, Billerica, MA) was used to prepare the aqueous solution of metal salt. The plant material was gathered in Oramanat area of Kermanshah province (west of Iran).

2.2. Preparation of extract

After harvesting in order to get extract from flowers and other parts, the plant was dried and grounded. Then, the powder ($60 \text{ g}$) was extracted with deionized water ($500 \text{ ml}$) by Soxhlet apparatus for $24 \text{ h}$). The crude extract was filtered and concentrated in a rotary evaporator and then the residue was dried in oven for $1 \text{ d}$ at $50^\circ \text{C}$ leading to a brown powder with a pleasant odor.

2.3. Biosynthesis of AuNPs

To synthesize AuNPs, to a vigorously stirred $50 \text{ ml}$ of $0.3 \text{ mM}$ aqueous $\text{HAuCl}_4.3\text{H}_2\text{O}$ solution, $10 \text{ ml}$ of solution of F.A. extract ($1\% \text{ w/v}$) was added and the mixture was stirred for few minutes at room temperature. Reduction of Au$^{3+}$ with the help of extract was evident from an immediate color change and completed within $5 \text{ min}$ with a stable red color leading to the formation of 1-AuNPs. After completion of the reaction (monitored by no more change in color), the solution was centrifuged and nanoparticles were collected and re-dispersed in water. The centrifugation process was repeated for several times so the other impurities may get washed out. At the final step, AuNPs were dispersed in water and stored for several months ($\text{more than 6 months}$) without any considerable aggregation. To obtain other AuNPs with different sizes ($2\text{-AuNPs, }\ldots, 16\text{-AuNPs}$) we followed the same procedure unless the parameters like initial ratio of Au$^{3+}$/F.A., reaction time and temperature were varied as explained in next sections.

2.4. Tests for flavonoids

2.4.1. Shinoda test

To $1 \text{ ml}$ of fresh aqueous extract of F.A., $5 \text{ ml}$ of $95\%$ ethanol and few drops of conc. HCl were added. Then, $0.5 \text{ g}$ of magnesium turnings was added. Observance of an immediate pink color indicated the presence of flavonoids.

2.4.2. Lead acetate test

To $1 \text{ ml}$ of fresh aqueous extract of F.A., $1.5 \text{ ml}$ of a $0.2 \text{ mM}$ lead acetate solution was added. Immediate formation of yellow precipitates showed the presence of flavonoids.

2.4.3. Sodium hydroxide tests

Upon addition of sodium hydroxide to a $1 \text{ ml}$ aqueous solution of F.A., yellow color was observed which was immediately decolorized after addition of few drops of acid.

3. Results and discussion

3.1. Colorimetric standard assays for evaluation of the reductant potential of F.A. extract

Extraction of water soluble components from F.A. plant was performed using Soxhlet apparatus and a brown powder with a pleasant odor was collected. Then, colorimetric assays (Shinoda [33, 34], lead acetate [35], and sodium hydroxide [36]) were carried out to evaluate the antioxidant potential of the extract (figure 1). Shinoda tests is the general and well-known tests for the recognition of flavonoids and involves a reductive transformation of colorless flavones and flavonols into deeply colored products among which are anthocyanidin [33, 34]. Moreover, lead acetate test [35] caused the formation of a yellow precipitate which approved the presence of flavonoids in extract possessing several hydroxyl groups making them able to form insoluble complex with Pb$^{2+}$ ions (figure 1).
3.2. Synthesis of AuNPs

The bio-assisted formation of AuNPs was followed as a function of initial ratio of Au\(^{3+}/\)F.A., reaction time and temperature by UV–visible spectroscopy in the range of 400–800 nm. 1 ml of AuNPs colloids was diluted to 3 ml in the UV–visible experiments. After washing several times with deionized water to remove the free entities and re-dispersing and centrifuging at 12000 rpm for 15 min, the colloids were dried in a vacuum desiccator and subjected to XRD analysis. The crystalline structure of AuNPs was determined by XRD (Bruker D8Advance) with CuK\(\alpha\) radiation (\(\lambda = 1.5406 \text{ Å}\)) in the 2\(\theta\) range of 30°–80° operated at voltage of 40 kV and a current of 30 mA. The FTIR analysis of AuNPs was carried out by Shimadzu 8300 spectrometer in the range 400–4000 cm\(^{-1}\). The same sample preparation for XRD analysis was used for FTIR analysis.

For scanning electron microscope (SEM) studies, samples were cast on glass slides and dried at room temperature. Then, these glass slides were fixed on copper supports and probed with the SEM (JEOL JSM 6701 F, Japan) that operates at 0–20 kV. The SEM is equipped with an energy-dispersive spectroscopy (EDX) unit for further composition analyses of the produced AuNPs. The morphology and size of the biosynthesized nanoparticles were measured with high resolution transmission electron microscopy using JEM-2100 that operates at 200 kV. To run TEM measurements, samples were prepared by placing a drop of aqueous solution of nanoparticles on carbon-coated copper grids and dried at room temperature.

3.3. Effect of the ratio of precursors (Au\(^{3+}/\)F.A. extract)

The bio-synthesis of AuNPs with water extract of F.A. began with the optimization of reaction conditions. As mentioned earlier, in the chemical reductive routes to noble metal nanoparticles, the mean size of the metallic core can be finely adjusted by choice of metal ion/reducing agent/capping agent ratio, the temperature and rate at which the reduction is conducted. In this regard, at a constant concentration of extract of F.A. (1% w/v), various concentrations of Au\(^{3+}\) (0.1, 0.3, 0.5 and 0.9 mM) (table 1, entries 2–5) or at a constant concentration of Au\(^{3+}\) (0.25 mM) (table 1, entries 6–13) were used in aqueous solution at room temperature and the formation of AuNPs was evidenced with the change in color of the solution. At the first combination set of starting materials (F.A. 1% w/v and Au\(^{3+}\) 0.1 mM) (table 1, entry 2), stirring the solution for 60 min caused no change in initial yellow color indicating that the Au\(^{3+}\) ions were not reduced under this condition. This might be due to the insufficient concentration of Au\(^{3+}\) ions which could be completely embedded within the huge network of biomolecules of extract. In contrast, in another set of experiment, it was observed that, as the concentration of Au\(^{3+}\) increased up to 0.3 mM (table 1, entry 3), stirring the solution for 5 min led to a naked-eye detectable color change from yellow into red. Figure 2 displays the optical image and absorption spectrum (curve (b)) of the colloidal (1-AuNPs) suspension synthesized using 0.3 mM extract of F.A. In addition, 0.1 mM aqueous solution of HAuCl\(_4\) shows a strong absorption peak appeared at ~300 nm (figure 2, curve (a)) corresponding to the ligand-to-metal charge-transfer transition of the [AuCl\(_4\)]\(^-\) ions [36]. Disappearance of this peak and the appearance of an intense
new absorption at 540 nm in curve (b), in addition to the significant change in color, all are in a good agreement with the successful reduction of Au$^{3+}$ ions to elemental Au$^0$ nuclei nucleuses followed by an immediate growth process.

The optical properties of AuNPs are dominated by collective oscillation of electrons at surfaces, known as surface plasmon resonance (SPR) and dependence of SPR of spherical AuNPs on their size, shape and surrounding medium are widely investigated \[37\]. The UV–vis spectra of AuNPs can be analyzed using the Mie theory mainly focusing on the position of the SPR maximum to estimate the size of spherical AuNPs \[38, 39\]. In particular, Haiss and his co-workers has reported worthwhile studies and developed a very feasible and simple calibration procedure which would allow the size of spherical AuNPs in aqueous solutions from UV–vis spectra to be determined without performing calculations \[38\]. Accordingly, the estimated size of 1-AuNPs using above-mentioned calibration curve was simply obtained to be 13.21 nm (table 1, entry 3). On the other hand, it is well-known that there is a nucleation growth-stabilizing

Table 1. Experimental condition, optical data and size of synthesized AuNPs using various ratio of precursors.

| No. | Sample | (Au$^{3+}$) (mM) | (Extract) (%w/v) | Time (min) | Temp. (°C) | $\lambda_{\text{max}}$ (nm)$^a$ | Exp.$^b$ | Calc.$^c$ |
|-----|--------|----------------|----------------|----------|----------|----------------|--------|--------|
| 1   | —      | 0.10           | 0.00           | —        | —        | 305            | —      | —      |
| 2   | —      | 0.10           | 1.00           | 60       | 25       | 12.75 ± 1.10   | 13.21  | —      |
| 3   | 1-AuNPs | 0.30           | 1.00           | 5        | 25       | 540            | 13.85 ± 1.75 | 13.90  |
| 4   | 2-AuNPs | 0.50           | 1.00           | 5        | 25       | 546            | 17.52 ± 1.14 | 17.82  |
| 5   | 3-AuNPs | 0.90           | 1.00           | 5        | 25       | 567            | 16.93  | —      |
| 6   | 4-AuNPs | 0.25           | 0.10           | 5        | 25       | 569            | 15.02 ± 1.10 | 15.34  |
| 7   | 5-AuNPs | 0.25           | 0.20           | 5        | 25       | 557            | 13.81  | —      |
| 8   | 6-AuNPs | 0.25           | 0.30           | 5        | 25       | 547            | —      | —      |
| 9   | 7-AuNPs | 0.25           | 0.40           | 5        | 25       | 542            | 13.65  | —      |
| 10  | 8-AuNPs | 0.25           | 0.75           | 5        | 25       | 535            | 13.30  | —      |
| 11  | 9-AuNPs | 0.25           | 1.00           | 5        | 25       | 533            | 13.06 ± 1.75 | 13.12  |
| 12  | 10-AuNPs | 0.25         | 1.25           | 5        | 25       | 532            | 11.60 ± 1.59 | 11.70  |
| 13  | 11-AuNPs | 0.25         | 2.50           | 5        | 25       | —              | —      | —      |
| 14  | 12-AuNPs | 0.4           | 1.00           | 10       | 4        | —              | —      | —      |
| 15  | 13-AuNPs | 0.4           | 1.00           | 120      | 4        | 532            | 9.20 ± 1.25 | 10.01  |
| 16  | 14-AuNPs | 0.4           | 1.00           | 10       | 25       | 532            | 12.75  | —      |
| 17  | 15-AuNPs | 0.4           | 1.00           | 120      | 25       | 538            | 13.73 ± 1.04 | 13.91  |
| 18  | 16-AuNPs | 0.4           | 1.00           | 10       | 55       | 534            | 13.30 ± 1.23 | 14.20  |
| 19  | 17-AuNPs | 0.4           | 1.00           | 120      | 55       | 547            | 14.92 ± 1.75 | 15.32  |

*a* SPR.  
*b* According to TEM images.  
*c* Obtained from calibration curve31.  
*d* Spectrum showed no detectable SPR to measure.

Figure 2. The optical images and UV–vis spectra of HAuCl$_4$ aqueous solution (a) and AuNPs synthesized using extract of F.A. (1% w/v) at different concentration of Au$^{3+}$ aqueous solution: (b) 0.3 (1-AuNPs), (c) 0.5 (2-AuNPs), (d) 0.9 (3-AuNPs) mM.
sequence in synthesizing of AuNPs [40, 41], which controls the size of nanoparticles and this sequence is affected by various experimental parameters, mainly by the ratio of concentrations of Au$^{3+}$/stabilizing/reducing agents. In the present study, further increase in the Au$^{3+}$ concentration (table 1, entries 4 and 5) resulted in deeper color change from initial yellow into deep-red and purple indicating the formation of larger nanoparticles (figures 2, 2-AuNPs and 3-AuNPs) with a clear red shift in their SPRs and broadened spectra.

At a constant concentration of Au$^{3+}$ (0.25 mM), varying the concentration of F.A. extract (table 1, entries 6–13) also resulted in the formation of a series of AuNPs with various colors and corresponding SPRs with different position and intensity. For instance, the UV–visible spectrum of the aqueous solution of Au$^{3+}$ (0.25 mM) exposed to a 0.1% w/v aqueous solution of F.A. extract for 5 min at room temperature (table 1, entry 6) showed a very broadened absorption spectrum with $\lambda_{max}$ at 569 nm (figure 3, curve (a)) which is obviously a proof of formation of nearly large nanoparticles [14, 37]. Employing the calibration procedure [38], the estimated size of these nanoparticles (4-AuNPs) was determined to be 16.93 nm. On the other hand, upon exposure of starting yellow Au$^{3+}$ solution to 0.2, 0.3, 0.4, 0.75, 1.00 or 1.25% w/v aqueous solutions of F.A. extract under stirred conditions for 5 min, the solution showed remarkable change in solution color and position and intensity of SPR (table 1, entries 7–12). As seen, contemporary with the increase in F.A. extract concentration, there was a remarkable blue shift in SPR of the corresponding absorption spectra (figure 3, curves (a)–(g)) changing from 569 nm in a broadened spectrum of 4-AuNPs to 529 nm in a nearly weak spectrum of 10-AuNPs. In addition, notably absent from SPR in figure 3, curve (h) was any hint of the formation of very small nanoparticles ($<5$ nm) [37]. This clear dependance of plasmon frequency on the ratio of Au$^{3+}$/F.A.

Dependence of the position of the main SPR bands of the synthesized AuNPs on the ratio of Au$^{3+}$/F.A. extract and the blue shift observed in figure 3 were depicted by plotting $\lambda_{max}$ of SPR against F.A. extract concentration. As shown in figure 4, the SPR peak for AuNPs concomitantly undergoes a blue shift with increase in the concentration of F.A extract suggesting a possible control on the size of nanoparticles. Such an observation and changes in the absorption response can be easily justified in terms of the size of the particles.

From a mechanistic point of view, it is concluded that the reaction mechanism is consistent with a nucleation-growth-passivation process [40, 41] and the higher concentration of F.A. causes a huge delivery of reductant and quickly nucleates cores into very small particles. On the other hand, these initially generated small nanoparticles can be quickly passivized with various polyols and polyphenols components found in F.A. extract [30–32]. This, in turn, prevents further growth of initially formed cores yielding small nanoparticles with the uniform core size. Although the extract of F.A. contains a variety of phytochemicals like flavonoids, polyphenols, alkaloids, terpenoids, proteins, carbohydrates and etc however the major components are rutin, quercetin (scheme 1). It is proposed that the antioxidant behavior of flavonoids (Fl–OH) is probably the main reducing agent of Au$^{3+}$ ions to Au$^0$. Due to the lower redox potentials of flavonoids ($E^0$ = from +0.23 to +0.75 V), they are thermodynamically able to reduce Au$^{3+}$ ions with redox potential $E^0 = +1.002$ V and during a redox reaction flavonoids can simply provide electrons to reduce the Au$^{3+}$ ions to Au$^0$. In the present study, the proposed mechanism for the reduction of Au$^{3+}$ ions to AuNPs by virtue of the oxidation of catechol moieties of rutin and quercetin (found in leaf extract) to their corresponding quinone are schematically depicted in scheme 1.
3.4. XRD assay

In order to verify the result of the UV–vis spectral analysis, the sample of the Au\(^{3+}\) ions exposed to the extract (0.3 mM Au\(^{3+}\) to 1% w/v extract; 1-AuNPs) was examined by XRD. As seen in figure 5, the powder XRD pattern

**Figure 4.** Plot of \(\lambda_{\text{max}}\) (SPR) values against concentration of F.A. extract showing blue shift with increase in the concentration of extract.

**Scheme 1.** The structures of flavonoids found in F.A. and oxidation half reaction of flavonoids (Fl–OH) provide the electrons needed for reducing of Au\(^{3+}\) to AuNPs.

3.4. XRD assay

In order to verify the result of the UV–vis spectral analysis, the sample of the Au\(^{3+}\) ions exposed to the extract (0.3 mM Au\(^{3+}\) to 1% w/v extract; 1-AuNPs) was examined by XRD. As seen in figure 5, the powder XRD pattern
of 1-AuNPs showed that the obtained nanoparticles were crystalline in nature and four distinct diffraction peaks appeared at 38.07°, 44.21°, 64.41° and 79.38° which correspond to crystal facets of (111), (200), (220) and (311) planes, respectively with the majority of particles showing (111) plane having face centered cubic (fcc) structure. This was in a good agreement with reference JCPDS Card No. 089-3697. All of these Bragg reflection peaks are broadened indicating the formation of AuNPs and the peak corresponding to the (111) plane is more intense than the other planes suggesting that it is the predominant orientation. X-ray diffraction is also an excellent indirect and feasible method for accurate measurement of the diameter of nanoparticles providing more reliable information from the statistical point of view. The mean crystallite diameter of the biosynthesized 1-AuNPs was calculated using the Scherrer’s equation by determining the width of the (111) Bragg reflection and was estimated to be 12.03 nm which is nearly in consistent with the TEM measurement (table 1, entry 3).

3.5. Field emission scanning and transmission electron microscopies

Figure 6(a) shows representative SEM image of 1-AuNPs synthesized by using an aqueous solution of F.A. extract wherein, the majority of the produced AuNPs are highly monodispersed, uniform in diameter and have spherical morphology. It is also worth mentioning that there is a cloudy or nearly opaque layer seen in SEM image. This presumably belongs to the organic capping layer. The elemental analysis of resultant 1-AuNPs was performed by EDX probe and the spectrum spot profile was made from the densely populated region of nanoparticles on the slide surface (shown as 001 in SEM image, figure 6(a)). The presence of Au was confirmed by EDX spectrum as the strong signal of Au peak was observed approximately at 2.2 keV (figure 6(b)). Along with this, the recorded signals of C and O atoms are presumably due to x-ray emission from the capping organic material in F.A. extract.
The TEM images showed the accurate size and morphology of the synthesized nanoparticles. The nanoparticles were all spherical in shape and quite uniform in size (figures 7(a)–(f)). In addition, the high resolution TEM image of AuNPs (figure 8) confirmed their spherical shape and revealed that they are crystalline, and the lattice spacing of ~0.232 nm related to the (111) plane, matching the d-spacing in the (111) plane of Au with a fcc structure. This clear observation of interplanar spacing supports that the AuNPs synthesized utilizing F.A. extract are highly crystallized with an inter-planar spacing of approximately 0.232 nm.

It was also observed that the nanoparticles were uniformly spread within an organic matrix (For instance, figure 7(c)) which presumably could act as the capping or passivating agent. This also may well explain the fact that the nanoparticles showed a very good dispersion inside the bio-reduced aqueous solution, even in the macroscopic scale and were highly stable in the solution showing no aggregation even after several months. In order to evaluate the effect of pH varying on the stability of bio-synthesized AuNPs, the pH of the colloidal solutions was adjusted between 2 and 12 by drop wise addition of 1 M HCl or NaOH solutions. It was found that in acidic pH the starting red solution retained its initial color for several days, however, upon addition of a few
drops of 1 M NaOH solution, the color of starting solution immediately turned to deep-blue. We believe that phytochemicals like polyphenols, alkaloids, terpenoids, proteins, carbohydrates and etc which act as stabilizing molecules at the surface of AuNPs and prevent them from aggregation, can be simply deprotonated in alkaline solution. As a result, the small initial AuNPs intend to agglomerate and make larger particles in alkaline solution.

It is also worth mentioning that almost the same results were observed when the effect of NaCl salt on the stability of bio-synthesized AuNPs were studied and an aggregation of starting AuNPs was observed with addition of 300–350 μl of 0.1 5 M NaCl solution. All together, these studies confirm the stability of bio-synthesized AuNPs in neutral and acidic solution and suggest an aggregation of them in alkaline solution.

From these observations, it can be concluded that these small AuNPs are encapsulated by the organic network of the extract and have less average velocity and are not allowed to easily move and aggregate. In other words, immediately after nucleation step, the ultra-small nanoparticles fall into the growth step but since they are all bound to the organic network, their further growth is prohibited because of their very slow movement. As a result, this core–shell structure prevents the nanoparticles from any possible aggregation and stabilizes them as small spherical monodispersed particles. The presence of biomolecules on the shell at the surface of synthesized nanoparticle was also monitored by dynamic light scattering (DLS) and FT-IR experiments. For instance, the sample of 1-AuNPs (0.3 mM Au3+ to 1% w/v extract) was examined by DLS studies. As seen in figure S-7 (Supporting information), the hydrodynamic diameter of nanoparticles in this sample is around ∼30 nm although the theoretical calculation and TEM analyses showed it about 12–13 nm (table 1, entry 3). This is presumably due to the presence of an organic layer (shell) surrounding the nanoparticles (cores) and acting as capping and passivizing agent. The presence of biomolecules at the surface of synthesized nanoparticle was also monitored by TEM analysis (figure 7) and FT-IR (figure S-1) experiments. Additionally, in FT-IR spectra, it was found that almost the same characteristic peaks were appeared in the spectra of F.A. extract and 1-AuNPs. (Figure S-1, supporting information).

3.6. Effects of temperature and reaction time

The effect of temperature and time of reaction on particle size were also investigated in detail. Three different temperatures (4 °C, 25 °C and 55 °C) and various stirring time (3–120 min) were selected. Also, the concentration of Au3+ solution was set at 0.4 mM and 1% w/v for F.A. extract. The UV–vis absorption spectra of the Au colloid solutions obtained at 4 °C (figure S-2, supporting information) showed no SPR at this low temperature unless the reaction mixture kept stirring at least for 60 min After 120 min, the resultant nanoparticles (13-AuNPs, table 1, entry 15) showed a measurable SPR at 525 nm. In contrast, at 25 °C and 55 °C the corresponding SPRs of the resultant nanoparticles (14-AuNPs 16-AuNPs, table 1, entries 16 and 18) appeared at 532 and 534 after 10 min and in each temperature, SPR was concomitantly red-shifted with an increase in the reaction temperature and time (figure S-3, 4, 5, 6, supporting information), providing a control on the size of nanoparticles. In addition, it was observed that the higher the temperature was, the larger nanoparticles were (figure 9). This could be due to the decrease of supersaturation ratio as shown in equation (1):

\[
SSR = \frac{Q - S}{S},
\]

where Q is concentration of solute and S is equilibrium solubility [45]. Increasing in temperature usually increases the solubility of solute and according to equation (1), decreases the SSR. On the other hand, according...
**Figure 9.** Correlation diagram between time and diameter of the synthesized nanoparticle at Au$^{3+}$ ion concentration of 0.4 mM and 1% w/v of F.A. extract at 25 and 55 °C.

**Figure 10.** TEM images of gold nanoparticles synthesized at various temperature and reaction time using 0.4 mM of Au$^{3+}$ aqueous solution and 1% w/v of F.A. extract. (a) 4 °C/120 min; (b) 25 °C/120 min; (c) 55 °C/10 min; (d) 55 °C/120 min.
to relationship between SSR and precipitation size, any decrease in SSR favors a considerable decrease in nucleation process as well as the number of nucleuses being formed as central core. As a result, in competition with nucleation process, the growth process plays the key role and occurs efficiently on those initially formed cores and hence, bigger particles form. This assumption was clearly proven with TEM investigation and TEM images of 13-AuNPs (figure 10(a)) confirmed the formation of smaller but polydispersed spherical particles while 15-AuNPs, 16-AuNPs and 17-AuNPs all were found to be larger and highly monodispersed spherical particles. (Figures 10(b)–(d).)

4. Conclusion

The biosynthesis of a series of spherical AuNPs with remarkable monodispersity through an environmental friendly and rapid route using water extract of F. A. has been developed. The uniqueness of this approach lies in its cleanliness using no extra surfactant, reductant and capping agent. The polyhydroxyl and polyphenols found in F.A. extract were assumed to play a key dual reducing and passivizing role. The structural elucidation of the prepared nanoparticles employed several high throughput techniques indicated that the particles are as elemental gold with spherical morphology, narrow size distribution and high stability. Additionally, it was demonstrated that the size of AuNPs can be altered by controlling the initial ratio of precursors, temperature and time of reaction. From a technological point of view, this clean protocol with its several advantages like cost-effectiveness, instrumental feasibility and compatibility for medical and pharmaceutical applications, as well as large scale commercial production may found potential applications in the field of biological synthesis of noble metal nanoparticles.

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