A low cost technique for synthesis of gold nanoparticles using microwave heating and its application in signal amplification for detecting *Escherichia Coli* O157:H7 bacteria

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Abstract

In the present work a low cost technique for preparation of gold nanoparticles (AuNPs) using microwave heating was developed. The effect of different elements (precursor reagents, irradiation time, and microwave radiation power) on the final morphology of AuNPs obtained through the microwave assisted technique has been investigated. The characterization of the samples has been carried out by transmission electron microscopy, UV–vis absorption spectroscopy, Fourier transform infrared spectroscopy, and powder x-ray diffraction. The results showed that to some extent the above-mentioned characterizations influenced the size of synthetized nanoparticles and application of microwave heating has many advantages such as low cost, rapid preparation and highly uniform particles. As an application in quartz crystal microbalance (QCM) immunosensor, AuNPs are conjugated with the *Escherichia coli* (*E.coli*) O157:H7 antibodies for signal amplification to detect *E.coli* O157:H7 bacteria residual in QCM system.

Keywords: gold nanoparticles, particle size and distribution, microwave synthesis, biosensing, amplification, QCM immunosensor

Classification numbers: 2.04, 4.02, 6.09

1. Introduction

For the last decade many different methods for preparation of gold nanoparticles (AuNPs) such as chemical [1], microwave irradiation [2], and γ-irradiation methods [3] were investigated. The famous strategy for synthesis of AuNPs is to produce nanostructures of small and uniform size distribution. One of the traditional method for preparation of AuNPs, the Turkevich method [4], is the most common synthesis route of AuNPs because of its simplicity. The method is based on a reduction of chloroauroic acid (H[AuCl₄]·4H₂O) with sodium citrate (Na₃Ct) as reducing agent. In this method, Na₃Ct is added into heating solution of H[AuCl₄] on a heating hot-plate and then colloidal AuNPs are formed after ten minutes. However, a problem in this method is that conductive heating with using a heating hot-plate is not efficient to achieve fast and uniform heat transfer to the reactants. Hence, the size and shape of AuNPs in this method are affected, and the size distribution is not uniform.

Microwave-assisted method of AuNPs synthesis is gaining popularity because of its high rate of reaction, high efficiency...
in energy transfer, simplicity, uniformity of products, and environmental friendly nature. In this process material is directly heated by radiation instead of indirect heating by thermal sources leading to higher temperature homogeneity in the reaction mixture. In this process of heating, microwave radiation interacts with the polar molecules possessing dipole moment and makes them reorient through rotation. A large number of molecules try to orient together resulting in collision and production of heat. Thus microwave heating is energy conversion method in which electromagnetic radiation is converted into heat energy rather than heat transfer by convection in conventional heating. The result is that the reactants could enhance reactions. Therefore, the synthesis of AuNPs by microwave heating has the advantage over conventional methods [5–7].

The objective of the present work is to synthesize AuNPs by microwave irradiation. The products prepared by the microwave and the conventional heating methods are compared. Effects of different elements (precursor reagents, irradiation time, and microwave radiation power) on gold particles size and structural property, morphology and absorption spectroscopy of the AuNPs are also discussed. Further, application of AuNPs conjugated with the E. coli O157:H7 antibodies for signal amplification to detect E. coli O157:H7 bacteria residual in quartz crystal microbalance (QCM) system is shown.

## 2. Experimental

### 2.1. Material and reagents

Sodium citrate (Na$_3$Ct) and chloroauric acid (HAuCl$_4$.3H$_2$O) were purchased from Merk and Sigma-Aldrich Company. De-ionized water was used throughout experiments. Affinity purified antibodies E. coli O157:H7 were purchased from Abcam Company, England. Protein A-soluble, 16-mercaptopentadecanoic acid (MHDA), 1-ethyl-3- (3-dimethyaminopropyl) carbodimide (EDC), ester N-hydroxysuccinimide (NHS), phosphate buffered saline (PBS) pH 7.4, bovine serum albumin (BSA) were supplied from Sigma Aldrich (United States). Ethanol, NaOH, acetone HCl, H$_2$SO$_4$ (98%), H$_2$O$_2$ were purchased from Merck Company (Germany) and were used without treatment. All chemical materials were GR grade.

### 2.2. Instruments

The preparation of AuNPs was carried out by a microwave system (Electrolux, EMM1908W, max power: 700 W). The UV−vis absorption behaviors for AuNPs were recorded by

![Figure 1. The model for synthesis of gold nanoparticles by (a) conventional heating (b) microwave techniques.](image)

| Samples | Na$_3$C$_6$H$_5$O$_7$ (%) | Microwave heating (W) | Time (min) |
|---------|-------------------------|------------------------|------------|
| Comparison of microwave and conventional heating of 140 °C | 0.1 | 210 | 5 |
| Effect of concentration sodium citrate | 0.1 | — | 5 |
| 0.2 | 210 | 5 |
| 0.3 | 210 | 5 |
| 0.4 | 210 | 5 |
| 0.5 | 210 | 5 |
| 1 | 210 | 5 |
| 2 | 210 | 5 |
| Effect of irradiation time | 0.3 | 210 | 5 |
| 0.5 | 210 | 5 |
| Effect of power | 0.3 | 210 | 5 |
| 0.3 | 50 | 5 |
| 0.3 | 380 | 1 |
using UV–vis spectrophotometer (Dynamca, HALO RB-10). Transmission electron microscopy (JEOL JEM-1400) and x-ray diffraction (XRD) by using a Brucker D5005 diffractometer were employed to characterize the AuNPs. The particle size was carried by 2D grain analysis after digitizing the photo image. The testing of E. coli O157:H7 was carried out by 5 MHz QCM stanford research systems company.

Figure 2. UV–vis absorption spectra of synthesized colloidal AuNPs with microworave (curve a) and conventional heating (curve b).

Figure 3. TEM image of synthesized gold nanoparticles prepared by (a) microwave, (b) conventional heating, and (c) corresponding histograms for both cases.

Figure 4. FTIR spectra of colloidal AuNPs prepared by microwave heating.
2.3. Preparation of AuNPs by microwave and thermal heating methods

For preparation of AuNPs by using microwave and conventional heating techniques (figure 1), the effect of different variables (precursor reagents, irradiation time, and microwave radiation power) on synthesized AuNPs are shown in table 1. For microwave heating, 1 ml chloroauric acid solution and 1 ml sodium citrate solution were dissolved in 18 ml H2O and the solution was placed into the microwave oven chamber (EMM1908W, Electrolux Co) to react for 10 min at 210 W. Besides, 1 ml chloroauric acid, 1 ml sodium citrate solution and 18 ml H2O were put into 100 ml vial equipped with a magnetic stirring bar. Then the vial was put into a 1000 °C hot-plate (model HJ-3, China) to react for 20 min. The temperature of the solution was controlled by using thermometer.

2.4. Method of applying AuNPs in amplification with 5 MHz QCM system for detecting E. coli O157:H7

First, the method for fabrication of QCM based immunosensor was described in [8]. This method gave good results in the range of 10^2–10^7 CFU ml⁻¹ E. coli O157:H7. The QCM sensors were pretreated with 1 M NaOH for 20 min, 1 M HCl for 5 min in ultrasonic bath and piranha etch solution (H₂O₂: H₂SO₄ = 2:3) for 1 min, in sequence, to obtain a clean and highly hydrophobic Au surface. After each pretreatment, the QCM sensors were rinsed with ethanol and water successively and dried in a stream of nitrogen. The pretreated QCM sensors were immersed in an ethanol solution of 200 μl MHDA for 24 h to forming self-assembled monolayers with...

Figure 5. XRD spectra of colloidal AuNPs prepared by microwave heating.

Figure 6. UV–vis spectra of gold nanoparticles produced by microwave heating with effect of different concentrations of sodium citrate.
one side of the crystal exposed to the solution. After rinsing with ethanol and water, the MHDA-modified crystals were treated with 75 mM EDC and 15 mM NHS for 2 h to convert the terminal carboxylic group to an active NHS ester. After rinsing with water and drying, 100 μl of 0.1 mg ml$^{-1}$ anti-

E. coli O157:H7 antibodies were added onto one side of the QCM sensor, spread over the entire Au electrode for 2 h at 37°C. The excess antibodies were removed by rinsing with PBS. This crystal was treated continuously with BSA-PBS solution for 1 h to block the untreated and nonspecific sites. After rinsing with PBS and water, the QCM sensors were dried in nitrogen, and finally the sensors were ready.

Second, method for binding AuNPs to E. coli O157:H7 bacteria to amplify signal in QCM system was shown in [9]. Following the capture of bacteria by the immobilized first antibody, the second antibody (BSA-coated antibody of E. coli O157:H7-AuNPs) was circulated through the QCM system for testing E. coli O157:H7 in the limit of 10 CFU ml$^{-1}$ E. coli O157:H7 at 1 h. The system was rinsed using PBS between steps to provide a frequency baseline for subsequent comparison.

3. Results and discussion

3.1. Characterization of AuNPs

3.1.1. Comparison of optical characterizations of AuNPs prepared by microwave and thermal heating methods. Figure 2 shows a comparison of the UV-visible absorption of god nanoparticles produced by microwave radiation and thermal heating, where the concentration of precursor reagents, irradiation time, and microwave radiation power were the same. After the reaction, both the solutions were diluted the same extent for UV test. It can be seen that the absorbance of AuNPs produced by microwave radiation was higher than that of thermal heating. Following the Beer–Lambert law, it was known that a UV–vis absorbance is directly proportional to the path length and concentration of the suspension. From the figure, we can conclude that the suspension produced by microwave heating has a higher concentration of AuNPs.

A representative TEM images and corresponding size distribution histogram of AuNPs synthesized by microwave
radiation and thermal heating under the same reaction conditions is shown in Figure 3. It can be seen that spherical particles were more abundant than particles of other shapes. Besides, AuNPs produced by microwave heating have a narrow size distribution because the heating by microwave radiation is more uniform. It is found that keeping uniform temperature distribution was an important factor in narrow distribution of nanoparticles [10]. In addition, microwave radiation can penetrate the reaction solution with different wavelengths for heating the whole solution quickly and uniformly. On the other hand, since the thermal conductivity of the reaction vehicle and the solution are small, and in the case of the thermal heating the heat must transfer from outside...
into the solution, the thermal heating is less efficient compared to the microwave heating. Moreover, the microwave heating method is suitable for synthesis of narrowly dispersed AuNPs. The FTIR spectra of AuNPs and surfactants are shown in figure 4.

Figure 11. TEM images and histogram of synthesized gold nanoparticles with effect of irradiation time at power of 50 W ((a) and (c)) [9] and of 380 W (b) and (d).

Figure 12. UV–vis spectra of AuNPs solution before and after 1 month.

Figure 13. Frequency shift of the QCM immunosensor as a function of time for *E. coli* O157:H7 suspension of different concentrations: (a) $10^1$, (b) $10^2$, (c) $10^3$, (d) $10^4$, (e) $10^5$, (f) $10^6$ and (g) $10^7$ CFU ml$^{-1}$. Measurement reference is phosphate buffered saline (PBS).
In addition, XRD analysis was employed to identify the crystallinity of the as-synthesized AuNPs. The result shows that the recorded XRD pattern (figure 5) exhibits peaks at 38.19°, 44.37°, 77.58° in the 2θ range of 20°–80° which are assignable to (111), (200), (220), and (311) planes of gold with face-centered-cubic crystalline structure. Secondly, lattice constants determined from XRD pattern are 4.077, 2.693, 4.079, and 4.078 Å in good agreement with the standard diffraction pattern of cubic gold metal (pattern 4-784). Similar trend was also observed by Alzoubi et al [11] in their research when they synthesized AuNPs by chemical reduction method with sodium citrate and chloroauric acid solution.

3.1.2. Effects of the concentration of sodium citrate, irradiation time and microwave radiation power. Figure 6 shows the UV–vis of absorption spectra of AuNPs produced by microwave heating with effect of the concentrations of sodium citrate (0.2%, 0.3%, 0.4%, 0.5%, 1.0%, 2.0%). The results of maximum absorbance wavelength (λ_max) with effect of the concentration of sodium citrate (0.3%, 0.4%, 0.5%) for AuNPs are ~518~542 nm with size 14~18 nm as shown in figures 7(a)–(c). However, with the increase of the concentration of sodium citrate (1%–2%) in microwave radiation method, maximum absorbance wavelength about 524–535 nm and the average diameter of AuNPs decrease and the size distribution becomes narrower are shown in figures 7(d) and (e). This result is similar to that of Seol et al [12] by using the reduction of chloroauric acid (HAuCl₄) reacted with sodium citrate (Na₃Ct) in de-ionized water with high microwave power.

The UV-visible spectra of AuNPs produced under different irradiation times by microwave heating are shown in figure 8. With a longer irradiation time, a red shift of the absorption peak on the UV–vis spectrum was observed. It can be seen that with the increase of the irradiation time (from 2 to 5 min), the maximum absorbance wavelength increased and the size distribution of AuNPs (about 18–22 nm) became broader (figure 9). This suggests that the longer irradiation
time can also promote the particle aggregation to form larger particles, leading to a red shift of the absorption peak.

The UV–vis spectra of AuNPs produced under different microwave radiation power by microwave heating are shown in figure 10. The high reaction radiation power resulted in a red shift of the absorption peak, indicating that the size of AuNPs increased with the increase of microwave radiation power when all the other reaction conditions were identical. This size increase was further confirmed by the TEM results and the size analysis data (figure 11). This result suggests that the growth of AuNPs was enhanced with the increase of microwave radiation power.

**3.1.3. The stability of AuNPs synthesized by microwave irradiation.** Although the stability of AuNPs can be estimated by observing the color of colloid solution which did not change with time, we have carefully investigated this stability by applying the UV–vis spectroscopy. Figure 12 shows the UV–vis spectra of AuNPs at two different time moments: when the AuNPs were just fabricated and 1 month later. Both spectra are almost the same. The size of AuNPs was also maintained and their aggregation did not take place.

**3.2. AuNPs-based signal amplification for detecting E. coli O157:H7**

In QCM immunosensor the principle of the detection of E. coli O157:H7 is based on the dependence of the resonance frequency of QCM on the mass change due to the presence of E. coli O157:H7 at the crystal surface, and AuNPs amplify the resonant frequency, might be due to the plasmonic enhancement effect [13–15].

Figure 13 shows that in the entire working range of $10^5$–$10^7$ CFU ml$^{-1}$ of E. coli O157:H7, the higher E. coli O157:H7 concentration, the greater the sensor responses. Without the amplification due to the conjugation of E. coli O157:H7 with AuNPs, in the solution of $10^2$–$10^5$ CFU ml$^{-1}$ of E. coli O157:H7 the sensor response ranged from $-15$ to $-140$ Hz. However, with the E. coli O157:H7 concentration of $10^7$ CFU ml$^{-1}$, the temporal response curves could not be distinguished from the baseline of negative control (about 5 Hz).

The AuNPs were conjugated with E. coli O157:H7 captured by the sensor for signal amplification, as shown in figure 14(a). The method of conjugating AuNPs with E. coli O157:H7 and applying for detecting E. coli O157:H7 was described in [9]. The result showed that the signal was amplified and the frequency change was about 35 Hz as shown in figure 14(b).

**4. Conclusion**

In this paper, AuNPs were prepared by a reduction method where microwave frequency was used as a heating source with different elements such as concentration of sodium citrate, irradiation time, and microwave radiation power. Compared to a conventional heating method, microwave heating provided a much faster reaction, resulting in a higher concentration of AuNPs with the same conditions. Besides, the size of gold particles decreases and the size distribution becomes narrower with increasing the concentration of sodium citrate. Also, a longer reaction time and higher microwave radiation power increased the size of AuNPs. It can be concluded that microwave heating had a strong effect on the yield of the AuNPs. By using microwave heating, the higher yield and narrower distribution of AuNPs were achieved and time for stability for AuNP was about 1 month. In addition, we have fabricated AuNPs conjugated with antibody E. coli O157:H7 captured by the probe for signal amplification of testing E. coli O157:H7 at $10^9$ CFU ml$^{-1}$ with QCM 5 MHz system.

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