Preparation of gold nanoparticles by microwave heating and application of spectroscopy to study conjugate of gold nanoparticles with antibody \textit{E. coli} O157:H7

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Abstract
Gold nanoparticles (AuNPs) of 15–20 nm size range have attracted attention for producing smart sensing devices as diagnostic tools in biomedical sciences. Citrate capped AuNPs are negatively charged, which can be exploited for electrostatic interactions with some positively charged biomolecules like antibodies. In this paper we describe a method for the low cost synthesis of gold nanoparticles using sodium citrate (Na\textsubscript{3}Cit) reduction in chloroauric acid (HAuCl\textsubscript{4.3}H\textsubscript{2}O) by microwave heating (diameter about 13–15 nm). Gold nanoparticles were functionalized with surface activation by 3-mercaptopropionic acid for attaching antibody. These nanoparticles were then reacted with anti-\textit{E. coli} O157:H7, using N-hydroxy succinimide (NHS) and carbodiimide hydrochloride (EDC) coupling chemistry. The product was characterized with UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy and zeta potential. In addition, the binding of antibody-gold nanoparticles conjugates to \textit{E. coli} O157:H7 was demonstrated using transmission electron microscopy (TEM).

Keywords: gold nanoparticles, anti-\textit{E. coli} O157:H7, conjugate, biosensor

Classification numbers: 4.02, 6.09

1. Introduction
In recent years, the attention to nanoscience and nanotechnology has increased because of the unique physical, chemical, and biological properties displayed by nanoscale materials compared with bulk material [1]. Of special interest are their small size and large specific surface area. For these reasons, metal nanoparticles ranging from noble to transition elements have found uses in many applications in different fields such as delivery [2], sensors [3], catalysis [4], bioelectronics and photonics [5]. Gold nanoparticles are one of the most applicable and usable metal nanoparticles that were synthesized by simple and ‘green’ methods such as microwave heating [6]. Gold nanoparticles are useful in areas such
as catalyst activity and optoelectronic. However, due to its conjugation property with antibodies, nano gold mainly has biomedical applications including biomolecular detection, diagnostic, photothermal therapy, and drug delivery application [7]. For instance, gold nanoparticles can be used to enhance electrical signals for obtaining detection limit information on many biological species of interest, which were previously unattainable using piezoelectric biosensor settings [8].

*Escherichia coli* (*E. coli*) O157:H7, which can be either food- or water-borne, is a gram-negative non-spore forming rod and a representative microorganism of the enteric bacteria. This bacterium causes serious illnesses such as bloody diarrhea, bloody feces, anemia, kidney failure [9, 10]. It has been estimated that *E. coli* O157:H7 causes up to 8 million deaths worldwide every year from diarrheal diseases [11]. Traditional methods for detecting of *E. coli* O157:H7 include plating and culturing, enumeration methods and biochemical testing [12]. However, these technics are lengthy and laborious. Besides, polymerase chain reaction (PCR), which is based on the amplification of DNA from a bacteria cell in ~1 h, has limited usefulness in examining environmental samples [13].

In recent years, the use of nanoparticles for detecting biomolecules received great interest in the field of biosensors due to their exquisite sensitivity in chemical and biological sensing [14, 15]. Many kinds of nanoparticles, including metal nanoparticles, oxide nanoparticles, and semiconductor nanoparticles were used in biosensors. For example, some groups have combined ligandmagnetic nanoparticles (LMNPs) enrichment with a fluorescent silica nanoparticles (FSiNPs) based two-color flow cytometry assay for the detection of *E. coli* O157:H7 [16]. Besides, luminescent CdSe/ZnS nanocrystals was applied to detect low levels of *E. coli* O157:H7 [17].

Among the nanomaterials used as components in biosensors, gold nanoparticles received the greatest interest because their optical properties are not affected when attaching biomolecules, such as antibodies [14, 18]. Moreover, gold nanoparticles have a sufficiently high surface-to-volume ratio and surface energy for stable immobilization of a large amount of biomolecules retaining their bioactivity. In addition, they allow fast and direct electron transfer between a wide range of electroactive species [18, 19].

Here, we report the synthesis and optical characterization of gold nanoparticles by microwave heating. The goal of the study was to characterize gold nanoparticles conjugation with antibodies directed at *E. coli* O157:H7. It could be useful for the detection of this bacterium. The gold nanoparticles (about 12–14 nm diameter) were prepared using a colloidal synthetic method by a reduction of chloroauric acid (HAuCl₄·4H₂O) with sodium citrate (Na₃Ct) in the presence of a mixture of 3-mercaptopropionic. The anti *E. coli* O157:H7 was anchored to the carboxyl acid via N-hydroxyl succinimide (NHS) carboxylimide hydrochloride (EDC) coupling. Finally, the binding of the antibody-gold nanoparticles conjugates with *E. coli* O157:H7 was investigated using transmission electron microscopy (TEM).

2. Experimental

2.1. Material and reagents

Chloroauric acid (HAuCl₄·3H₂O) was purchased from Merk Company (Germany). Sodium citrate (Na₃Ct), N-hydroxyl succinimide 98% (NHS), carboxylimide hydrochloride (EDC), 3-mercaptopropionic acid 99%, bovine serum albumin (BSA), phosphate buffered saline (PBS) and ethanol 99% were obtained from Sigma-Aldrich Company (USA). Affinity purified antibodies *E. coli* O157:H7 were purchased from Abcam Company (England). De-ionic water was used throughout experiments. All chemical materials were G R grade.

Target bacteria *E. coli* O157:H7 were supplied from the Pasteur Institute of Ho Chi Minh City, Vietnam. All strains were morphologically and physiologically checked, maintained on nutrients agar slant and kept in refrigerator at 4 °C.

2.2. Method for preparation of gold nanoparticles by microwave heating

Gold nanoparticles were prepared by the Turkevich method [20]. For preparation of gold nanoparticles by microwave heating technique, 1 ml HAuCl₄ solution (5 mM) and 1 ml Na₃Ct solution (25 mM) 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. The model for microwave heating equipment is shown in figure 1. The UV–vis absorption behaviors of gold nanoparticles were recorded using UV–vis spectrophotometer (model CARY 100 CONC, Varian). Transmission electron microscopy (JEOL JEM—1010-Japan) and zeta potential (zetasizer nano ZS) were employed to characterize the gold nanoparticles. The particle size was measured by 2D grain analysis after digitizing the photo image.
2.3. Method for thiol surfactants-coated gold nanoparticles and conjugation of antibody E. coli O157:H7 on gold nanoparticles

Antibody E. coli O157:H7 was conjugated to gold nanoparticles using a modified version of a method reported by Pasqua et al [21]. The nano gold colloidal, which was prepared by microwave heating, was centrifuged during 30 min at 4500 rpm under approximately 4 °C temperature. 100 μl of 3-mercaptopropionic was mixed with gold nanoparticles at room temperature during 24 h. Then 1 ml gold nanoparticles was added to a solution containing N-hydroxy succinimide 98% (NHS), carbodiimide hydrochloride (EDC) in 27 μl 0.01 M PBS (pH 7.4) and 1 mg anti-E. coli O157:H7. The suspension was stirred with 100 μl BSA at room temperature during 24 h. The material was collected via centrifugation, washed once with 0.01 PBS and ethanol. This material was then characterized using UV–vis spectrophotometer (model CARY 100 CONC, Varian-Japan), FTIR spectroscopy (model TENSOR 37, Burker), transmission electron microscopy (JEOL JEM—1010-Japan) and zeta potential (etasizer nano ZS).

2.4. Method for binding of antibody-gold nanoparticles to E. coli O157:H7 bacteria

The antibody E. coli O157:H7 conjugated with gold nanoparticles were suspended in a 0.01 M PBS (pH 7.4) solution containing E. coli O157:H7 at 1:1 ratio. After 2 h, the bacteria in the solution were fixed by adding 3 ml of 7% formaldehyde. Then a drop of the resulting solution was placed on a formvar/carbon-coated copper grid. After drying in air, TEM images of the sample were collected using transmission electron microscopy (JEOL JEM—1010-Japan).

3. Results and discussion

3.1. Characterization of gold nanoparticles

Figure 2(a) shows UV–vis absorption spectrum of gold nanoparticles produced by microwave heating. The surface plasmon resonance (SPR) peak of gold nanoparticles was found at the wavelength of 521 nm and indicated the formation of gold nanoparticles. The obtained result was similar with the one published by Seol [22] showing an absorption peak at 521 nm on a size of 12.04 ± 1.35 nm. Previous reports on the citrate reduction method illustrating that formation of gold nanoparticles was obtained by three successive steps, including nucleation, aggregation, and spherical shaping after reduction [23, 24].

Figure 2(b) shows the morphology of gold nanoparticles in the colloidal solution. It is obvious that gold nanoparticles are mainly spherical, uniform in size and the average size is 14 ± 3 nm (figure 2(c)). It was found that keeping uniform temperature distribution significantly narrows the distribution of nanoparticles, as reported by Fuelong et al [25]. Besides, it has been known that microwave radiations can penetrate the reaction solution with different wavelength to heat the whole solution quickly and uniformly.

Figure 3 shows the zeta potential measurement of the prepared gold nanoparticles by citrate method with microwave heating. The result revealed that the zeta potential of
Gold nanoparticles is $-23.9 \text{ mV}$. It confirmed the formation of negative charges on the surface of gold nanoparticles. Negative charges resulted from citrate that is used both as a reducing and a stabilizing agent of gold nanoparticles. It also protected gold nanoparticles from aggregation and precipitation over time [24].

3.2. Analysis of thiol surfactants-coated gold nanoparticles

From the data of the UV–vis absorption spectrum shown in figure 4, curve (a) it was found that the gold nanoparticles have a peak of absorbance at wavelength of 521 nm. After
addition the prepared surfactant (3-mercaptopropionic) to the gold nanoparticles, the above peaks disappeared and another peak appeared at (wavelength of 260 nm), which was related to thiol group absorbance as shown in figure 4 curve (b). This result shows that the prepared surfactant attached the surface of the gold nanoparticles, which is in accordance with results previously published by Azzam et al [26].

The FTIR spectroscopy results shown in figure 5 for the individual surfactants and their nanostructures with gold nanoparticles show the following peaks: O-H stretch around 3318.51 cm\(^{-1}\), stretch C = O around 1636.45 cm\(^{-1}\) and stretch C-OH around 1087.67 cm\(^{-1}\). After addition of the 3-mercaptopropionic to gold nanoparticles, the peak at 1274.10 cm\(^{-1}\), showing the stretching of S-H disappeared and another peak appeared at 1087.67 cm\(^{-1}\) which was due to the stretch C-OH as shown in curve (a).

Besides, figure 6 shows the TEM image of the nanostructures of the prepared thiol surfactant (3-mercaptopropionic) with the gold nanoparticles. It was noticed from figure 2(b) and figure 6 that the nano size of thiol surfactant-coated gold nanoparticles decreases with the nanostructure of the individual gold nanoparticles. In addition, the result in figure 6 shows the large space between the gold nanoparticles when attached to the prepared thiol surfactants. This effect of the prepared thiol surfactants on the nano size of the gold nanoparticles led to the stabilization of the nano size of the gold nanoparticles due to the formation of nano shells between these surfactants and the gold nanoparticles. This observation is similar to the results previously published by Azzam et al [26].

In addition, gold nanoparticles of the prepared thiol surfactant (3-mercaptopropionic) were diluted in 0.6 mg ml\(^{-1}\) and transferred into fold capillary cells for zeta potential. The sample was analyzed using zetasizer nano ZS. The observed zeta potentials (mV) are presented in figure 7. The zeta potential (mV) of thiol-gold nanoparticles was \(-14.8\) mV while the one of gold nanoparticles was \(-23.9\) mV as shown in figure 3. Gold nanoparticles have a higher negative charge in comparison with thiol-gold nanoparticles. Hence, these results show that thiol groups attached the surface of the gold nanoparticles.

### 3.3. Analysis of the conjugation of antibodies on gold nanoparticles and binding of E. coli O157:H7 bacteria using antibody-gold nanoparticles

Figure 8 shows the UV–vis spectra of antibody-gold nanoparticle conjugates after addition of the anti-\(E.\ coli\) O157:H7 antibodies in nano gold colloidal solution. The peak at 260 nm which is shown in curve (b) was due to anti-\(E.\ coli\) O157:H7 antibodies and the peak at 521 nm which is due to gold nanoparticles disappeared, as shown in curve (a). Similar results were also found by Zhao et al [27].

The FTIR spectra of MPA/gold nanoparticles shows an intense peak at 1087.67 cm\(^{-1}\) (curve (a) in figure 5) that is caused by the C-OH stretching of carboxylic group present in the MPA. However, after immobilization of antibodies on gold nanoparticles with \(E.\ coli\) O157:H7 bacteria. The characteristic peak of C-OH stretching of carboxylic group decreased, as shown in curve (c) of figure 5. Besides, the zeta potential (mV) of gold nanoparticles conjugated with anti \(E.\ coli\) O157:H7 was \(-2\) mV (figure 9) while that of thiol-gold nanoparticles was \(-14.8\) mV as shown in figure 7. It is notable that gold nanoparticles conjugated with anti-\(E.\ coli\) O157:H7 have a lower negative charge in comparison with thiol-gold nanoparticles. Therefore, it indicates successful binding of antibodies on gold nanoparticles with \(E.\ coli\) O157:H7 bacteria.

**Figure 9.** Zeta potential of gold nanoparticles conjugated with anti-\(E.\ coli\) O157:H7.

**Figure 10.** TEM images of (a) \(E.\ coli\) O157:H7 and (b) gold antibody \(E.\ coli\) O157:H7 conjugates.
In addition, the binding of gold nanoparticles anti-*E. coli* O157:H7 antibodies conjugates with *E. coli* O157:H7 was studied by TEM. Figure 10(a) shows a single isolate *E. coli* O157:H7 with the expected rod-like shape and lengths and widths between 1500 and 500 nm. Addition of the Au-antibody conjugate to a suspension containing *E. coli*, resulted in the binding of the conjugate to the surface of the cell through antibody-antigen recognition as shown in figure 10(b). A similar trend was also observed by Pasqua *et al.* [21] in their research when they showed the synthesis and characterization of gold nanoparticles conjugated with the antibody anti-*E. coli* O157:H7.

4. Conclusion

Gold nanoparticles are successfully prepared using sodium citrate as a reduction agent of gold salt as well as a stabilizer by microwave heating of particles with sizes of about 13–14 nm. In addition, we successfully synthesized thiol group functionalized-gold nanoparticles containing N-hydroxy succinimide, carbondimide hydrochloride and 3-mercaptohexadecanoic acid conjugated with anti-*E. coli* O157:H7 antibodies for the detection of waterborne *E. coli* O157:H7 pathogen. Observations made by UV–vis, FTIR spectroscopy, zeta potential and TEM suggest that the synthesized thiol surfactant was attached to the gold nanoparticles and the antibody-conjugated gold nanoparticles were shown to successfully bind with *E. coli* O157:H7.

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References

[1] Behari J 2010 *Indian J. Exp. Biol.* **48** 1008
[2] Xu Z P *et al.* 2006 *Chem. Eng. Sci.* **61** 1027
[3] Doria G *et al.* 2012 *Sensors* **12** 1657
[4] Bigall N C *et al.* 2008 *Angew. Chem. Int. Edit.* **47** 7876
[5] Pérez-López B and Merkoç A 2011 *Anal. Bioanal. Chem.* **399** 1577
[6] Thanh N V *et al.* 2014 *Commun. Phys.* **24** 153 (in Vietnamese)
[7] Tiwari P M, Vig K, Dennis V A and Singh S R 2011 *Nanomaterial* **1** 31
[8] Cao X, Ye Y and Liu S 2011 *Anal. Biochem.* **417** 1
[9] Bunchnan R L and Dolye M P 1997 *Food Technol.* **51** 69
[10] Griffin P M and Tauxe R V 1991 *Epidemiol. Rev.* **13** 60
[11] Velusamuy V, Arshak K, Korostynska O, Oliwa K and Adley C 2010 *Biotechnol. Adv.* **28** 232
[12] Deisingh A K and Thompson M 2004 *J. Appl. Microbiol.* **96** 419
[13] Oda M, Morita M, Unno H and Tanji Y 2004 * Appl. Environ. Microbiol.* **70** 527
[14] Tansil N C and Gao Z 2006 *NanoToday* **1** 28
[15] Zhao X *et al.* 2004 A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles *Proc. Natl. Acad. Sci. USA* **101** 15027–32
[16] He X, Zhou L, He D, Wang K and Caao J 2011 *Analyst* **136** 4185
[17] Liu Y, Brandon R, Cate M, Peng X, Stony R and Johnson M 2007 *Anal. Chem.* **79** 8796
[18] Wang J, Polsky R and Xu D 2001 *Langmuir* **17** 5739
[19] Wang J, Xu D and Polsky R 2002 *J. Am. Chem. Soc.* **124** 4208
[20] Turkevich J, Stevenson P C and Hillier J 1951 *Discuss. Faraday Soc.* **11** 55
[21] Pasqua A J *et al.* 2009 *Mater. Lett.* **63** 1876
[22] Seol S K, Kim D, Junga S and Hwu X 2011 *Mater. Chem. Phys.* **131** 331
[23] Ojea-Jimenez I and Campanera J M 2012 *J. Phys. Chem. C* **116** 23682
[24] Pong B-K, Elim H I, Chong J-X, Ji W, Trout B L and Lee J-Y 2007 *J. Phys. Chem. C* **111** 6281
[25] Fueleg D N, Launikonis A and Sasse W H F 1984 *J. Chem. Soc. Faraday Trans. 1* **80** 571
[26] Azzam E M S, Badawi A M, Alawady A R E and Soliman A 2009 *J. Disper. Sci. Technol.* **30** 540
[27] Zhao X *et al.* 2010 *African J. Microbio. Research* **4** 663