Synthesis of water soluble chitosan stabilized gold nanoparticles and determination of uric acid

Thi Lanh Le¹, Quang Khieu Dinh², Thai Hoa Tran², Hai Phong Nguyen², Thi Le Hien Hoang² and Quoc Hien Nguyen³

¹ Quang Nam Junior of Economy-Technology, 431 Hung Vuong Street, Tam Ky City, Vietnam
² College of Sciences, Hue University, 77 Nguyen Hue Street, Hue City, Vietnam
³ Research and Development Center for Radiation Technology, Viet Nam Atomic Energy Institute, 202A Street 11, Linh Xuan Ward, Thu Duc District, Ho Chi Minh City, Vietnam

E-mail: trthaihoa@yahoo.com and hien7240238@yahoo.com

Received 12 April 2014
Accepted for publication 14 April 2014
Published 7 May 2014

Abstract
Gold nanoparticles (Au-NPs) have been successfully synthesized by utilizing water soluble chitosan as reducing and stabilizing agent. The colloidal Au-NPs were characterized by UV-Vis spectroscopy and transmission electron microscopy (TEM). The results showed that the colloidal Au-NPs had a plasmon absorption band with maximum wavelength in the range of 520–526 nm and the diameters were about 8–15 nm. In addition, a new Au-NPs-modified electrode was fabricated by self-assembling Au-NPs to the surface of the L-cysteine-modified glassy carbon electrode (Au-NPs/L-Cys/GCE). The Au-NPs-modified electrode showed an excellent character for electro-catalytic oxidization of uric acid (UA) in 0.1 mol L⁻¹ phosphate buffer solution (pH 3.2). Using differential pulse anodic stripping voltammetry (DP-ASV), a high selectivity for determination of UA has been explored for the Au-NPs-modified electrode. DP-ASV peak currents of UA increased linearly with their concentration at the range of 2.0 × 10⁻⁶ to 4.0 × 10⁻⁵ mol L⁻¹ with the detection limit of 2.7 × 10⁻⁶ mol L⁻¹ for UA. The proposed method was applied for the detection of UA in human urine and serum samples with satisfactory results.

Keywords: gold nanoparticles, water-soluble chitosan, uric acid, electrochemical analytic
Classification numbers: 2.07, 4.02

1. Introduction
Synthesis of gold nanoparticles (Au-NPs) has received much attention due to their efficient applications in many fields include catalysis [1], electrochemistry [2], cancer diagnosis and therapy [3]. A number of different methods have been reported for the synthesis of Au-NPs. Most common is chemical reduction [1–4] and irradiation method [5, 6].

Recently, some works have introduced a green method using natural polymers to synthesize Au-NPs for the purpose of minimizing or eliminating pollution to the environment. Raveendran et al [7] used β-D-glucose as reducing and soluble starch as stabilizing agents for synthesizing gold and silver nanoparticles. Among natural polymers, chitosan is used widely because of its biocompatibility, biodegradability, nontoxicity, and adsorption properties [8, 9]. However, chitosan is insoluble in water and can only be dissolved in acid. Therefore, the applications of Au-NPs solution is restricted.

Uric acid (2,6,8-trihydroxypurine, UA) is the primary end product of purine metabolism. UA concentration of normal people is about 240–520 μM in blood serum and 1.4–4.4 mM in urine [10]. Abnormal levels of UA are symptoms of several diseases, including gout, hyperuricemia and Lesch Nyan disease [11]. Therefore, the great importance of detecting UA content in biological samples such as blood serum and urine has been well recognized.

Up to now, many analytical methods such as photometry [11], liquid chromatography [12], enzymatic [13], electrochemical analytic [14]... have been developed for the detection of UA. However, photometry method is rarely exact
because of ascorbic acid (AA) effect [14]. Enzymatic method has high selectivity but instrumentation are rather expensive. Electrochemical analytic is of much interest because it is highly selective, cheaper and a more rapide method [14].

Recently, many works have developed a modified electrode to determine UA in a biological sample. Metal nanoparticles have received great attention due to their wide applications in the fields of chemical modified electrodes. As is well-known, Au-NPs can enhance the conductivity, facilitate the electron transfer and improve the detection limit of electrode [2, 15, 16].

In this work we use water soluble chitosan (denoted as WSC) instead of chitosan to synthesize Au-NPs and create a modified electrode on background glassy carbon electrode (GCE) with L-cysteine (L-Cys) and Au-NPs, and apply it to the determination of UA content in urine and blood serum sample.

2. Experimental

2.1. Chemicals

Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄ · 3H₂O), L-cystein and uric acid (UA) (Merck) were used in this paper. Water soluble chitosan with molecular weight of 1.8 × 10⁴ g mol⁻¹ and deacetylation degree of about 52% was prepared according to the method of Duy et al [6]. KH₂PO₄ and K₂HPO₄ were obtained from Kanto, Japan. Phosphate buffered saline (PBS) (0.1 M) at various pH were prepared using 1 M K₂HPO₄, 1 M KH₂PO₄. Double-distilled water was used throughout the experiments.

2.2. Apparatus

Absorption spectra were recorded on a JASCOV630 UV-Vis spectrophotometer, Shimadzu, Tokyo, Japan. Transmission electron microscopy (TEM) measurements were performed on a JEOL-JEM 1010, Japan. The size and size distribution were calculated statistically from TEM images. X-ray diffraction (XRD) was recorded on a D8 Advanced, Brucker, Germany. Infrared spectroscopy (IR) was recorded on a IR-prestige-21, Shimadzu, Japan. All electrochemical experiments were performed with 693 VA Processor Electrochemical analyser, 694 VA-stand electrode and 797 VA Computrace, Metrohm, Switzerland. The conventional three-electrode system included: a modified glassy carbon electrode (GCE) as working electrode, a platinum wire as auxiliary electrode and a silver (Ag/AgCl with 3 M KCl) electrode as a reference electrode. pH was recorded by 340 WTW pH meter, Germany.

2.3. Method

2.3.1. Preparation of gold nanoparticles. In a typical experiment, 1.25 mL of 10 mM HAuCl₄ was added to 12.5 mL of 1% (w⁻¹) aqueous WSC solution, the mixture was filled with distilled water to the final volume of 25 ml for obtaining solution of 0.5 mM Au³⁺/0.5% WSC, stirring and heating the mixture at 85 °C. After the color of the suspension changed gradually from yellow to purple, the mixture was kept at 85 °C for 8 h. Then, Au-NPs were separated from the solution by centrifugation at 9000 rpm for 30 min. The supernatant was decanted to collect Au-NPs at the bottom and re-dispersed in double-distilled water. Then, Au-NPs solution were acidified using 0.1 M HCl to remove WSC, centrifuged at 9000 rpm for 30 min and finally re-dispersed in double-distilled water to collect Au-NPs solution.

2.3.2. Preparation of working electrode. First, the glassy carbon electrode (GCE) was successively polished to a mirror using 0.05 μm alumina slurry. Next, the electrode was dipped in 2 M KOH solution in 10 min. Then, the electrode was washed with ultrasonic in 2 M H₂SO₄ solution in 15 min. and washed thoroughly by double-distilled water. After that, GCE was cleaned by circle voltammetry (CV) between 0.0 V to 1.0 V at scanning rate ν = 100 mV s⁻¹ in 0.5 M PBS buffer pH 7. Then, GCE was washed thoroughly by double-distilled water and soaked in Au-NPs solution for 12 h at 4 °C and dried at room temperature.

3. Results and discussion

3.1. Characterization of gold nanoparticles

Gold nanoribbons (Au-NPs) were synthesized by chemical reduction using the water soluble chitosan (WSC). It is worth noting that the acetylated chitosan is a polymeric molecule and involves acetylated groups, thus it not only functions as an aqueous stabilizer, but also as a reducing agent for synthesizing (Au-NPs). Notably, we carried out the synthesis of (Au-NPs) in the acetylated chitosan dispersion without using any additional reducing agent.

The results of UV-Vis absorption spectra, in figure 1(a), indicated that WSC and Au³⁺ solution have no absorption peak in visible region. However, solution of 0.25 mM of Au³⁺ has maximum absorption wavelength (λmax) at 288 nm. Duy et al also reported mostly the same λmax results of Au³⁺ solution, particularly at 286 nm when the authors synthesized Au-NPs by γ-irradiation method [6]. After reacting, the color of Au³⁺/WSC solution turned from yellow to red with newly appearing λmax at 522, which is a surface plasmon resonance characteristic of Au-NPs [1–9], and the disappearance of the peak of Au³⁺ solution at 290 nm indicated that all Au³⁺ ions have been completely reduced by WSC. According to the literature, the distinct absorption peak from the surface plasmon absorption of the spherical Au-NPs is in the range of 510–530 nm [1–9]. The TEM images and corresponding histogram of size distribution (figures 1(b) and (c)) of Au-NPs...
showed nearly mono-dispersion and uniformity of spherical Au-NPs with the average size of 8–10 nm.

The FT-IR spectra of WSC before and after capping Au-NPs (WSC/Au) are shown in figure 2(a). The results indicated that unlike the WSC, new IR bands at 1740 cm⁻¹ were observed in the stabilized Au-NPs which are thought to result from the formation of carboxyl groups in the acetylated chitosan stabilizer. We suggest that in acidic media of the Au-NPs synthesis, the acetylated chitosan was partially degraded to a hemi-acetalic structure with a few exposed −CHO groups. These −CHO groups can reduce Au³⁺ to Au⁰ and then convert to −COOH groups. This analysis demonstrates the
coating of the acetylated chitosan on the Au-NPs surface through reduction of the aldehyde groups of the stabilizer. XRD patterns (figure 2(b)) of the acetylated chitosan-stabilized Au-NPs show four broadened diffraction peaks of (111), (200), (220), (311) lattice planes, characteristic of a face-centered-cubic [fcc] phase and crystallinity of the gold nanostructure [17]. This crystalline phase is in contrast to an amorphous structure of the acetylated chitosan stabilizer.

We speculate that the surface of the Au seeds generated by reduction of –CHO hemi groups state in negative charge from the adsorption of AuCl₄⁻ species. The negatively charge surfaced Au-NPs seeds can interact with polycation of the WSC to form a primary protected layer. These positively charge surfaced Au-NPs colloids can be further adsorbed with the excess stabilizers to yield multilayer-coated Au-NPs with positively charge surfaces outward of water. This proposed mechanism indicates that the multilayer coating of the chitosan stabilizers is crucial for controlling the particle size, while their positive charge surfaces give rise to a repulsive force between particles resulting in the well-stabilized aqueous colloid suspension (figure 3).

3.2. Detection of uric acid

The prepared water soluble chitosan-stabilized Au-NPs probes were then used to design electrodes to detect uric acid by differential pulse anodic stripping voltammetry (DPA-SV) method. Based on the water solubility of the synthesized...
materials, we bound the chitosan-stabilized Au-NPs on the surface of glassy carbon electrode (GCE) by using L-cystein cross-linker. The selective capping of thio groups and amine groups of the L-cystein molecules to the Au-NPs surface and the GCE surface, respectively, resulted in the tight attachment of the mono-disperse Au-NPs on the GCE (figure 4(a)) [18].

Cyclic voltammetric stripping (CVS) profiles (figures 4(b) and (c)) of the GCE before and after surface functionalization have only a stable anodic signal without cathodic peak, suggesting that irreversible electrochemical reactions of uric acid occurred on the electrodes. In comparison, the GCE−Cys−Au-NPs electrode exhibits a stable anodic peak with intensity of 12-fold higher than that of the GCE electrode.

The peak current of UA exhibited linear correlation to their concentration in the range from $2.0 \times 10^{-6}$ to $4.0 \times 10^{-5}$ mol L$^{-1}$ ($R^2 = 0.998$) (figure 5). The limit of detection (LOD) was found to be $2.7 \times 10^{-6}$ mol L$^{-1}$. The proposed method was applied for the detection of UA in three of human urine samples (U1, U4, U5) and three of serum samples (using the method of standard addition with satisfactory results). The result in table 1 showed UA concentration in all human urine samples was normal degree ($1.4 - 4.4$ mM). Table 2 showed UA concentration in human serum was greater than normal ($240 - 520$ μM) [10].

### 4. Conclusion

In this work, we have demonstrated an effective method to synthesize colloidal Au-NPs using water soluble chitosan both as a stabilizing and a reducing agent. The obtained colloidal Au-NPs solutions are stable and have plasmon properties at $\lambda_{\text{max}} \sim 520$ nm. The sizes of Au-NPs are about 8–15 nm. We can use the prepared Au-NPs to analyze uric acid by rational design of Au-based nanoelectrodes via surface functionalization by DP-ASV method. Our analyzed results affirmed that DP-ASV method using GCE/Lys/Au-NPs electrode was realizable to detect UA content with low limit of detection of 2.7 μM.

### Acknowledgements

This work was supported by National Foundation Science and Technology Development (NAFOSTED) of Vietnam (No. 104.03-2012.54).

### References

[1] Campbell C T et al 2011 Faraday Discuss. 152 227
[2] Kannan P and John S A 2009 Anal. Biochem. 386 65
[3] Huang X et al 2007 Nanomedicine 2 681
[4] Nghiem T H L et al 2012 Adv. Nat. Sci.: Nanosci. Nanotechnol. 3 015002 (5pp)
[5] Hien N Q et al 2012 Carbohydr. Polym. 89 537
[6] Duy N N et al 2013 Coll. Surf. A: Physicochem. Eng. Aspects 436 633
[7] Raveendran P, Fu J and Wallen S L 2006 Green Chem. 8 34
[8] Sun C et al 2008 Carbohydr. Res. 343 2595
[9] Wei D and Qian W 2008 Coll. Surf. B: Biointerfaces 62 126
[10] Vietnam Ministry of Health 2012 Routine Tests Applied in Clinical Practice (Hanoi, Vietnam: Medicinal Publisher)
[11] Khajehsharifi H and Pourbasheer E 2013 J. Iran. Chem. Soc. 8 1113
[12] Vlassa M, Coman V and Dragomir C 2009 Archiva Zootech. 12 59
[13] Stanknov M, Djurdjevi P and Stankov D 2003 J. Serb. Chem. Soc. 68 691
[14] Fang B et al 2011 Microchim. Acta 173 27
[15] Wang C et al 2012 Anal. Chim. Acta 741 15
[16] Wang Y 2007 Microchim. Acta 2011 172 419
[17] Wei D et al 2007 Carbohydr. Res. 342 2494
[18] Hua G et al 2008 Electrochim. Acta 52 6610