Electron beam/$\gamma$-ray irradiation synthesis of gold nanoparticles and investigation of antioxidant activity

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Abstract
Colloidal solutions of 1 mM gold nanoparticles (AuNPs) were synthesized by $\gamma$-ray Co-60 and electron beam irradiation using 1% water soluble chitosan (WSC) with different molecular weight (Mw) as stabilizer. The AuNPs size measured from TEM images was of 7.1 and 15.1 nm for electron beam and $\gamma$-ray Co-60, respectively. The AuNPs sizes of 9.8, 15.1 and 22.4 nm stabilized by different WSC Mw of $155 \times 10^3$, $78 \times 10^3$ and $29 \times 10^3$ g mol$^{-1}$, respectively, were also synthesized by $\gamma$-ray Co-60 irradiation. Antioxidant activity of AuNPs with different size from 7.1 to 20.0 nm was investigated using free radical 2,2$'\,$-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS$^+$). Results indicated that the smaller size of AuNPs exhibited higher antioxidant activity. In particular, the antioxidant efficiency was of nearly 100, 75, 65, 52 and 30% for 7.1, 9.8, 15.1, 20.0 nm AuNPs and WSC 0.1%, respectively, at the same reaction time of 270 min. Thus, due to the compatibility of WSC and the unique property of AuNPs, the pure colloidal AuNPs/WSC solutions synthesized by irradiation method can be potentially applied in biomedicine, cosmetics and in other fields as well.

Keywords: gold nanoparticle, $\gamma$-ray, electron beam, antioxidant
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1. Introduction
Gold nanoparticles (AuNPs) with different size and shape have unique optical [1], antioxidant [2, 3] and catalytic properties [4]. A number of methods have been reported for the synthesis of AuNPs, such as chemical [3, 5–7], photochemical [8], sonochemical [9] and radiolytic [10–12] reduction. In these methods, ionizing radiation has been considered as an effective method due to several advantages, as described in our previous papers [12, 13]; especially, mass production can be carried out and satisfies the requirements of clean production.

Synthesis of AuNPs of desired size and shape has enormous importance in the field of scientific research and practical application because small changes in the size or shape of nanoparticles can have a great effect on a variety of physical properties of the material. Change in size caused the AuNPs to have different applications. Therefore, some approaches were used to adjust AuNPs size such as change of concentration of Au$^{3+}$, stabilizer or molecular weight of stabilizer, reduction rate and reducing agent, etc.

AuNPs used in biological applications such as DNA sensor [14] drug delivery [15], in cosmetics (www.utilisegol.com), in cancer diagnostics and therapy [16] should be biocompatible and nontoxic. Thus, besides the ‘green’ synthesis method, natural polymers such as chitosan [2, 6, 17, 18], alginate [11], hyaluronan [12], gum arabic [19], protein [20], soluble starch [21], heparin [22] have been used as stabilizers. Because of the biocompatibility, biodegradability and nontoxicity, many works have used chitosan as a stabilizer agent for AuNPs. However, usually chitosan is only soluble in acid but insoluble in water, which makes chitosan unsuitable for applications in biomedicine and/or in cosmetics.

In this work, colloidal 1 mM AuNPs solutions were synthesized by $\gamma$-ray and electron beam (EB) irradiation using 1% water soluble chitosan (WSC) with different molecular
weight (Mw) as stabilizer. The effect of dose rate (γ-ray and EB) and Mw of WSC on the size of AuNPs was studied. The antioxidant activity of AuNPs with different sizes was also investigated using free radical 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulphonic) (ABTS•+). [23, 24].

2. Experimental

2.1. Materials

HAuCl₄.3H₂O and pure water of reagent grade were obtained from Merck, Germany. WSC with degree of deacetylation about 50% and different Mw of 155 × 10³, 78 × 10³ and 29 × 10³ g mol⁻¹ was prepared by reacetylation of chitosan with acetic anhydride according to the process of [25] with some modifications [26].

2.2. Synthesis and characterization of AuNPs

To investigate the effect reduction rate, 2 ml of 10 mM HAuCl₄ was added to 10 ml of 2% (w/v) aqueous WSC solution (Mw = 78 × 10³ g mol⁻¹), then the mixture was filled with water to 20 ml for preparing a solution of 1 mM Au³⁺/1% WSC. Irradiation was carried out on a γ-ray Co-60 irradiator SVST Co-60/B (dose rate ∼1.1 kGy h⁻¹) and on an electron beam accelerator UERL-10-15S2, 10 MeV, 1.5 mA (dose rate ∼5 kGy s⁻¹) at VINAGAMMA Center, Ho Chi Minh City with dose of 7, 14 and 21 kGy.

To investigate the effect of Mw of WSC, 2 ml of 10 mM HAuCl₄ was added to 10 ml of 2% (w/v) aqueous WSC with Mw of 155 × 10³, 78 × 10³ and 29 × 10³ g mol⁻¹. Then the mixtures were filled with water to the final volume of 20 ml for preparing a solution of 1 mM Au³⁺/1% WSC. Irradiation was carried out on the above-mentioned Co-60 irradiator with a dose of 7 kGy. The UV–vis spectra of AuNPs solutions, diluted by water to 0.1 mM calculated as Au³⁺ concentration were recorded using spectrophotometer UV-2401PC, Shimadzu, Japan. The size of AuNPs was characterized by transmission electron microscope (TEM) model JEM 1010, JEOL, Japan operating at 80 kV.

2.3. Antioxidant activity of AuNPs

To investigate the antioxidant activity of AuNPs/WSC, 2,2′-azino-bis (3-ethylbenzo-thiazole-6-sulphonic acid) (ABTS) was dissolved in water with concentration of 7.4 mM. 2 ml of 7.4 mM ABTS was mixed with 2 ml of 2.6 mM K₂S₂O₈ for creation of free radical cation ABTS•+. The ABTS•+ solution was kept in the dark for 16 h at 23 °C. ABTS•+ solution was diluted by water with volume ratio of 1:18 to obtain optical density of about ±0.1 at the wavelength of 734 nm. For studying antioxidant activity, 0.6 ml water (control sample) and 0.6 ml of AuNPs solution with different sizes of 7.1, 9.8, 15.1 and 20.0 nm were added into cuvettes containing 1 ml of diluted ABTS•+ solution. The optical density (OD) of samples was measured over time on a UV–vis spectrophotometer at λ = 734 nm. The antioxidant efficiency (AE) of free radicals capture was calculated as follows [23, 24]

\[ AE(\%) = 100 \times \frac{(OD_{AC} - OD_{AS})}{OD_{AC}}, \]

where ODₐc is the optical density of the control solution (ABTS•+/H₂O) and ODₐs is the optical density of the studied solutions (ABTS•+/AuNPs and ABTS•+/WSC).

3. Results and discussion

3.1. Synthesis and characterization of AuNPs

Reduction rate plays an important role in the preparation of AuNPs either by ionizing radiation or chemical reduction method [12, 27]. For ionizing radiation method, the size, OD and λmax of AuNPs will depend on dose rate, i.e. on reduction rate.

AuNPs were synthesized from solution 1 mM Au³⁺/1% WSC by γ-ray and EB at doses of 7, 14 and 21 kGy. The results of UV–vis spectra in figure 1 and TEM images in figures 2(A, a) and figures 3(D, d) indicated that the characteristics of AuNPs were also affected by dose rate. In particular, when dose rate increased from 1.1 kGy h⁻¹ (γ-ray) to 5 kGy s⁻¹ (EB), the AuNPs size decreased from ∼15 to 7 nm and λmax shifted from 526 to 517 nm, respectively. The reason for this phenomenon is due to the competition between the adsorption of Au³⁺ onto the nascent Au clusters and the reduction reaction of Au³⁺ → Au²⁺ → Au¹⁺ → Au⁰ to form new Au clusters [12]. At high dose rate, the reducing reaction is predominant; therefore, many new clusters are formed to create smaller AuNPs. In contrast, at low dose rate, the adsorption of Au³⁺ onto clusters is predominant, therefore AuNPs will be larger. Hien et al also obtained similar results in the study of synthesis of AuNPs by γ-ray Co-60 using hyaluronan as stabilizer [12]. The AuNPs size decreased from

![Figure 1. The UV–vis spectra of AuNPs prepared by γ-ray Co-60 and EB at a dose of 7 kGy.](image-url)
Figure 2. TEM images and size distributed histogram of AuNPs synthesized by EB at dose of 7 kGy (A, a), 14 kGy (B, b) and 21 kGy (C, c).

Figure 3. TEM images and size distributed histogram of AuNPs synthesized by γ-ray at dose of 7 kGy (D, d), 14 kGy (E, e), 21 kGy (F, f).
9.5 to 5 nm and $\lambda_{\text{max}}$ shifted from 533 to 517 nm when dose rate increased from 0.5 to 5 kGy h$^{-1}$.

Jana et al. also recognized that the size of the AuNPs decreased with the increase in the rate of chemical reduction [27].

Results in table 1 and figure 2 showed that no significant change in particle size was observed for AuNPs prepared by EB. Meanwhile, the size was changed from 15 to 20 nm when dose increased from 7 to 21 kGy, respectively, for AuNPs prepared by $\gamma$-ray (see table 1 and figure 3). The reason for this phenomenon can be due to WSC in low concentration, which was seriously degraded at high dose together with low dose rate of $\gamma$-ray leading to forming larger AuNPs [28]. In contrast, Naghavi et al. [29] reported that the size of silver nanoparticles is decreased along with higher dose. However, they used polyvinylpyrrolidone (PVP) as stabilizer. PVP belongs to radiation crosslinkable polymer group [30], so PVP did not degrade at high dose; therefore, PVP protected silver nanoparticles well. Further study on the effect of stabilizer and dose on AuNPs size should be carried out.

The effect of WSC Mw on characteristics of AuNPs was manifested in table 2. The obtained results showed that the higher the Mw, the shorter the $\lambda_{\text{max}}$ and the smaller the size of AuNPs attained. The reason may be due to the cumbersomeness of high WSC Mw, which could anti-agglomerate Au clusters to prevent forming big AuNPs better than low WSC Mw. Phu et al. [13] obtained similar results in the study of the effect of chitosan Mw on radiation synthesis of silver nanoparticles.

In order to elucidate the stabilization effect of WSC for AuNPs, a schematic diagram of WSC capped AuNPs was proposed as in figure 4. Polysaccharides such as chitosan, hyaluronan, alginate with oxygen-rich structure in hydroxyl and ether groups, which can be tightly bound with metal clusters and nanoparticles via steric and/or electrostatic stabilization mechanism [12].

### 3.2. Antioxidant activity of AuNPs

Antioxidant activity of AuNPs with size from 7.1 to 20.0 nm at the same concentration of 20 mg L$^{-1}$ was investigated by free radical cation ABTS$^+$ scavenging method [23, 24].

The results in figure 5 showed that the antioxidant efficiency increased with the increase of the reaction time and with the decrease of AuNPs size. In addition, the antioxidant activity of WSC was also tested. The results indicated that the antioxidant efficiency of WSC at concentration of 0.1% was of the smallest value (30%) compared with that of AuNPs of 7.1 nm (100%), 9.8 nm (75%), 15.1 nm (65%) and 20.0 nm (52%) at the same reaction time of 270 min. Thus, WSC

| Table 1. OD, $\lambda_{\text{max}}$ and size (d) of AuNPs synthesized by $\gamma$-ray and EB at dose 7, 14 and 21 kGy. |
|---|---|---|---|
| Methods | Dose (kGy) | OD | $\lambda_{\text{max}}$ (nm) | d (nm) |
| EB | 7 | 0.39 | 517 | 7.1 ± 0.8 |
| | 14 | 0.39 | 516 | 6.8 ± 0.7 |
| | 21 | 0.39 | 516 | 7.0 ± 0.8 |
| $\gamma$-ray | 7 | 0.36 | 526 | 15.1 ± 1.8 |
| | 14 | 0.34 | 528 | 17.6 ± 2.2 |
| | 21 | 0.32 | 530 | 20.0 ± 3.2 |

| Table 2. OD, $\lambda_{\text{max}}$ and d of AuNPs/WSC with different Mw synthesized by $\gamma$-ray at dose 7 kGy. |
|---|---|---|---|
| Mw × 10$^3$ (g mol$^{-1}$) | OD | $\lambda_{\text{max}}$ (nm) | d (nm) |
| 155 | 0.38 | 522 | 9.8 ± 1.1 |
| 78 | 0.35 | 526 | 15.1 ± 1.8 |
| 29 | 0.32 | 532 | 22.4 ± 2.0 |

Figure 4. Schematic diagram of WSC capped AuNPs synthesized by ionizing irradiation.

Figure 5. Antioxidant efficiency as a function of time and AuNPs with different sizes.

Figure 6. Reaction scheme for the formation of AuNPs.
possesses certain antioxidant efficiency to contribute to the total antioxidant capacity of AuNPs/WSC composition.

The obtained results on antioxidant activity of AuNPs with various sizes were in good agreement with the results of Yakimovich et al who investigated the antioxidant effect (capture •OH radicals) of chitosan stabilized AuNPs by electron spin resonance (ESR) spectroscopy technique [3]. The authors claimed that the interaction of the AuNPs with the free radicals depended on the size, specific surface, and concentration. Furthermore, Yakimovich et al also mentioned that the AuNPs with larger size have lower specific surface area that will manifest lower antioxidant activity compared to the AuNPs with smaller size [3]. Esumi et al [2] also investigated the antioxidant effect of AuNPs/chitosan and they concluded that AuNPs stabilized by chitosan from 0.01 to 0.1% showed high rate constant of free radical scavenging, but with higher concentration of chitosan, the antioxidant activity of AuNPs/chitosan was decreased. This was because AuNPs were covered by chitosan with high concentration that restricted the interaction of AuNPs with free radicals, and subsequently the antioxidant activity of AuNPs was somewhat inhibited. Results of our previous work also proved that AuNPs of 9.8 nm exhibited the highest antioxidant activity among AuNPs sizes from 9.8 to 53 nm [26]. Besides the antioxidant activity, Pokharkar et al reported that LD50 (a dose required to kill half of the population) value of AuNPs/chitosan was found to be greater than 2000 mg kg\(^{-1}\) body weight of rats by oral administration [31]. They concluded that AuNPs/chitosan produced no toxicity in rats that can be exploited for potential therapeutic applications. Recently, Luan et al [32] reported the studied results of biodistribution of AuNPs after intravenous administration in mice. They concluded that AuNPs of 20 nm were not toxic for mice by intravenous injection at dose of 33.3 mg kg\(^{-1}\) of body weight. And more importantly, spherical AuNPs of different sizes (5–70 nm) are not inherently toxic to human skin cells [33]. Thus, AuNPs/WSC holds great promise for biomedical and cosmetics applications.

4. Conclusion

AuNPs were favourably synthesized by γ-ray Co-60 and/or EB using WSC as stabilizer. The size of AuNPs synthesized by EB was smaller compared to that by γ-ray Co-60. The Mw of WSC stabilizer affected the size of AuNPs. AuNPs with small size (7.1 nm) exhibited higher antioxidant activity compared to that of large size (20 nm). Thus, due to the compatibility of WSC and the unique attribute of AuNPs, the pure colloidal AuNPs/WSC solution as-prepared by ionizing radiation method can be potentially applied in biomedicine, cosmetics and in other fields as well.

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