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INTRODUCTION

Gold nanoparticles (AuNPs) are metallic nanoparticles that have attracted much attention due to the unique optical properties, being easily visualized and detected by spectroscopic techniques [1]. AuNPs are considered as non-toxic and are attractive to be applied in many applications. The particle size and surface modification of AuNPs can be controlled by chemical composition [2]. The particle size of nanoparticles is thought to one of stability indices of the nanoparticles. Little or no change in particle size is possibly implied the stability of the nanoparticles especially after time storage. The measurement of nanoparticle size, however, is likely to depend upon the methodology and interpretation of the data. Recently, some recent mentioned that the different techniques employed led to the variation in particle sizes of the nanoparticles [3]. In this study, the citrate-stabilized gold nanoparticles (AuCt) and polyethyleneimine-stabilized gold nanoparticles (AuPEI) were synthesized and determined for their nanoparticle sizes and size distribution using several methods namely, UV-vis spectroscopy, transmission electron microscopy and dynamic light scattering. The variation in size with different time intervals of either AuCt or AuPEI was also evaluated.

MATERIALS AND METHODS

Materials For nanoparticle synthesis, hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4.3H_2O$), trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) and polyethyleneimine (PEI,-$(CH_2CH_2NH)_n$-, MW = 750 KDa) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Glassware were cleansed with aqua regia ($HCl:HNO_3$ = 3:1, Carlo Erba, Milan, Italy) for the purpose of circumventing gold contamination, rinsed with ultrapure water and oven dried prior to use.

Synthesis of gold nanoparticles The chemical reduction method was used for synthesis of 200 ppm (1,015 μM) AuCt and AuPEI. The thirty-five microliters of 30% (w/w) hydrogen tetrachloroaurate trihydrate were added to 49.5 mL ultrapure water. The sample was then heated up to 90°C in a water bath with stirring. Then, 0.5 mL of 0.4 M trisodium citrate dihydrate was added in to the gold solution and the mixture was continued heating until the color became dark red. The molar ratio of Au atom to trisodium citrate used in the synthesis was 1:8. In addition, AuPEI at the same concentration of AuCt was synthesized using polyethyleneimine as a polymeric stabilizer. The same procedure as mentioned above was used for synthesis AuPEI except for 0.5 mL of 0.36 M PEI solution was used instead of the citrate solution. Notably, the molar ratio of Au atom to PEI was 1 to 0.072. The synthesized AuCt and AuPEI were stored in a light-protected container at room temperature until use.

Size measurement of gold nanoparticles The appearance and color of freshly prepared AuNPs were visually observed prior to size measurement. The measurement of either AuCt or AuPEI was performed using i) UV-vis spectroscopy ii) transmission electron microscopy and iii) dynamic light scattering.

UV-vis spectroscopy The optical properties of AuNPs are conquered by collective oscillations of electrons (plasma oscillations) that are in resonance with the incident electromagnetic radiation [4]. The surface plasmon resonance (SPR) band of AuNPs was characterized by using UV-visible spectrophotometer model Evolution 600 (Thermo Scientific, UK). The UV quartz cuvette with a path length of 10 nm was used and the ultrapure water was a reference standard for measurement.

The analysis of nanoparticle size was done according to the following equation [3]:

$$d = 3 + (7.5 * 10^{-5}) X^4 \quad \text{for} \quad X < 23 \qquad (1)$$

$$d = \frac{(\sqrt{X\text{-}17} - 1)}{0.06} \quad \text{for} \quad X \geq 23 \qquad (2)$$

where d is the diameter of the nanoparticle (nm), when $5 \leq d \leq 100$, and X is the maximum absorbance wavelength (λ_{max}) – 500.

Transmission electron microscopy (TEM) Ten microliters of sample solution was dropped on the carbon-coated grid (200 mesh) and dried in the air at room temperature prior to observation under TEM model JEM-2100 (Jeol, Japan). The size distribution of AuCt and AuPEI was calculated using SemAfore *program version 5.21.*

Dynamic light scattering The hydrodynamic sizes of AuCt and AuPEI were also measured by using dynamic light scattering (DLS) method or referred as photon correlation spectroscopy (PCS) technique. The scattered intensity of the Brownian motion of nanoparticles is recorded and calculated using Stokes-Einstein equation to obtain hydrodynamic diameter (d_h) [5]:

$$d_h = \frac{kT}{3 \cdot \P \cdot \eta \cdot D} \qquad (3)$$

where k is the Boltzmann's constant (1.38065 x 10^{-23} $J \cdot K^{-1}$), T is the temperature (K), η is the viscosity of the solvent ($nm^{-2} \cdot s$) and D is the diffusion coefficient ($nm^2 \cdot s^{-1}$).

Then, the hydrodynamic diameter of nanoparticle was calculated by the cumulant method using the software provided with the instrument. The width of size distribution was also reported as polydispersity index (PdI). All PCS measurements were performed at 25°C using Zetasizer Nano ZS (Zen3600, Malvern, UK) equipped with He-Ne laser operating at a wavelength of 633 nm and a photon detector. The measurement was performed at a scattering angle of 173°. Prior to size measurement, AuCt and AuPEI were diluted with ultrapure water to eliminate multiple scattering effects. The data was represented as a mean particle diameter of three measurements.

Stability study of gold nanoparticles AuNPs were kept at room temperature with light protection. AuNPs were determined for instability after storage for 1, 4 and 12 weeks. The change in appearance and color of AuNPs were visually observed. The particle sizes of AuNPs were evaluated by DLS method as described previously.

RESULTS

Particle size analysis of gold nanoparticles The SPR characteristic of AuNPs was presented as the UV absorbance spectra. The maximum absorption wavelengths (λ_{max}) were found to be 522.5 ± 0.5 nm for AuCt and 521 nm for AuPEI (Figures 1A and 2A). For AuCt, the particle size was calculated according to equation-1 for 'X' value of 22 (λ = 522 nm) and equation-2 for 'X' value of 23 (λ = 523 nm). Thus, the sizes of AuCt were in a range of 20.57 to 24.16 nm. Since the 'X' values of AuPEI were less than 23 (λ = 521 nm), the particle size of AuPEI was then calculated using equation-1. The AuPEI contained particle sizes of 17.59 nm.

The morphology and size of the AuNPs were detected by a TEM technique. The TEM result presented the spherical shape of either AuCt or AuPEI (Figures 1B and 2B). The average size and size distribution of AuNPs from TEM result were further analysed. The mean particle size and of AuCt was 10.76 ± 1.26 nm (n=200) and that of AuPEI was 7.71 ± 0.53 nm (n=279). In order to obtain the hydrodynamic size of AuNPs, the DLS technique was performed. The hydrodynamic sizes of AuCt and AuPEI were 18.37 ± 0.34 nm (n=3) and 18.59 ± 0.59 nm (n=3), respectively. The polydispersity index which was similar to

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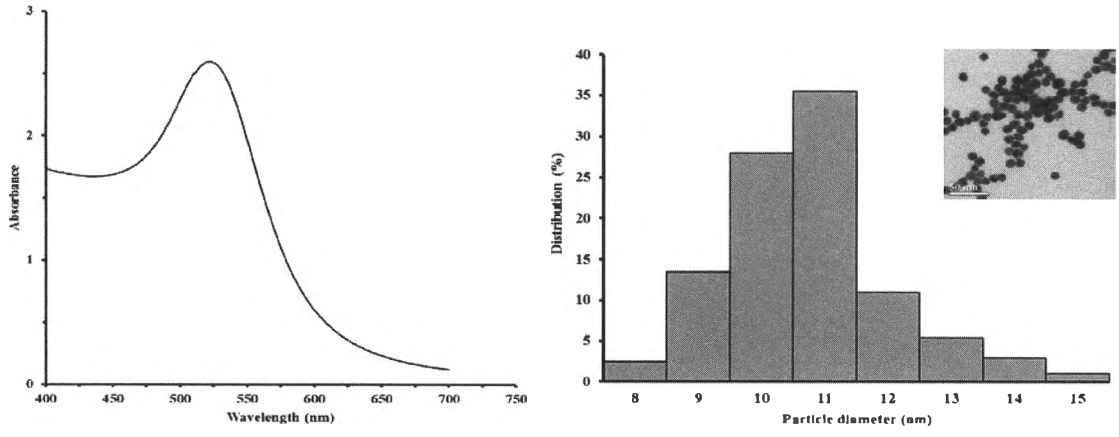
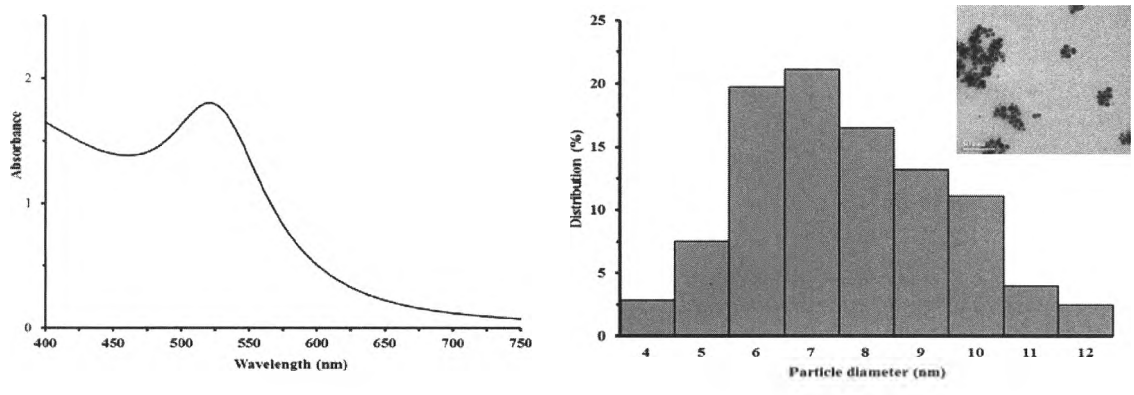
the distribution of size was 0.39 for AuCt and 0.27 for AuPEI. In comparison, the results of particle size measurement of AuCt and AuPEI by several methods are tabulated in Table 1.

Stability study of gold nanoparticles After being kept for several time periods, AuNPS (AuCt and AuPEI) presented rather the same physical appearance as their corresponding freshly prepared samples. Likewise, the UV-vis absorption spectra of AuNPs after storage were in similar pattern to those of freshly prepared samples (data not shown). The variation in size and size distribution of week-old AuNPs was evaluated by DLS technique. The PCS results are illustrated in Figure 3.



(A) (B)

Figure 1 UV-visible absorption spectra (A) and TEM image (inset; scale bar of 50 nm) with corresponding size distribution histograms (B) of AuCt.



(A) (B)

Figure 2 UV-visible absorption spectra (A) and TEM image (inset; scale bar of 50 nm) with corresponding size distribution histograms (B) of AuPEI.

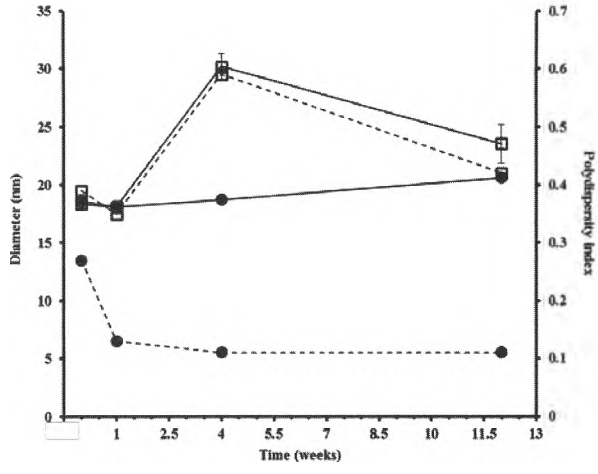


Figure 3 Variation in hydrodynamic diameter (nm) (mean ± S.D., n=3) of and polydispersity index of AuCt (□) and AuPEI (●) with different time storage. Solid line and dash line represent particle diameter and polydispersity index, in orderly.

Table 1 Particle size analysis of AuCt and AuPEI

AuNPs	Particle size (nm) measured by different methods		
	i) UV spectroscopy	ii) TEM	iii) DLS
AuCt	20.57 ; λ = 522 nm	10.76 ± 1.26	18.37 ± 0.34
	24.16 ; λ = 523 nm		
AuPEI	17.59 ; λ = 521 nm	7.71 ± 0.53	18.59 ± 0.59

DISCUSSION AND CONCLUSION

In this study, gold nanoparticles stabilized by either citrate or polyethyleneimine were characterized for particle sizes using the commonly used methods namely, UV-vis spectroscopy, transmission electron microscopy and dynamic light scattering or so-called photon correlation spectroscopy. The results of particle sizes obtained from UV-vis spectroscopy and DLS were considerably in the same range for AuCt and fairly similar for AuPEI. Surprisingly, the particle sizes of AuCt and AuPEI became smaller (nearly 50% or more) when determined by the TEM analysis. Considerably, the result was not unexpected since the stabilizers of AuNPs, citrate and polyethyleneimine were hydrophilic in nature. The hydrophilic stabilizer can then form the layer surrounding the particles and interact with the water molecules in the dispersion medium [6,7]. Conversely, the TEM technique allows only dehydrated form of a tested sample to be detected, hence the interaction with the water molecules or interparticle interaction were unable to be occurred. However, although the smaller sizes detected with the TEM results, this technique was the only one that indicated the morphology of the AuNPs. In this study, the results were in the same trend when the size measurement was performed by UV-vis spectroscopy and DLS. It was mentioned however that the size analysis of UV-vis spectroscopy and DLS techniques was dependent on the assumption of spherical shape and monodisperse non-interacting particles [8]. Notably, the DLS method provides better advantages than UV-vis spectroscopy in term of the polydispersity index (PdI). Owing to less change in particle sizes and lower PdI values of AuPEI after time storage, AuPEI seemed to be more stable and narrower in size distribution than AuCt.

In conclusion, the size measurement of AuNPs should be concurrently performed using at least two techniques. The TEM technique is inevitable when the assumption on size morphology or distribution is required for analysis of particle sizes.

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