

Extinction coefficient of gold nanoparticles with different sizes and different capping ligands

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Abstract

Extinction coefficients of gold nanoparticles with core size ranging from ~4 to 40 nm were determined by high resolution transmission electron microscopy analysis and UV–vis absorption spectroscopic measurement. Three different types of gold nanoparticles were prepared and studied: citrate-stabilized nanoparticles in five different sizes; oleylamide-protected gold nanoparticles with a core diameter of 8 nm, and a decanethiol-protected nanoparticle with a diameter of around 4 nm. A linear relationship between the logarithms of extinction coefficients and core diameters of gold particles was found independent of the capping ligands on the particle surface and the solvents used to dissolve the nanoparticles. This linear relation may be used as a calibration curve to determine the concentration or average size of an unknown nanoparticle or nanoparticle–biomolecule conjugate sample.

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1. Introduction

Gold nanoparticles are among one of the most extensively studied nanomaterials. Since the first report of gold colloids more than 100 years ago by Faraday [1], numerous studies have been reported on the synthesis, property study and application development of gold clusters, colloids, and nanoparticles [2–6]. Gold nanoparticles exhibit many unique and interesting physical and optical properties such as surface plasmon resonance (SPR), surface enhanced Raman scattering (SERS), nonlinear optical properties (NLO), and quantized charging effect [7–9]. The surface plasmon resonance of gold nanoparticles is an optical property that holds promising potentials in biosensing, molecular imaging, and photothermal treatment of diseases. Mirkin et al. demonstrated the use of gold nanoparticles as an optical probe to detect DNA molecules based on the color change of individually scattered nanoparticles and the complementary DNA-hybridized nanoparticle aggregates [10]. Recently the photothermal conversion property of gold nanoparticles and nanoshells has attracted a keen interest from the scientific community [6,9,11–13]. Gold nanoparticles or nanoshells that are

irradiated by a laser beam at a wavelength around the SPR band can efficiently convert the photon energy to thermal energy, which leads to the destruction of biological cells such as tumor and bacteria [12,13].

The analysis of surface plasmon resonance absorption band can also provide valuable information on the size, structure and aggregation properties of gold nanoparticles. We herein present in our study of using UV–vis absorption spectroscopy and transmission electron microscopy to determine the extinction coefficients of gold nanoparticles with different sizes and different capping ligand monolayer. The extinction coefficient is an important parameter that can be used to calculate the nanoparticle concentration or estimate the nanoparticle size. Although this may appear to be a trivial issue, the accurate calculation or estimation of the molar concentration of nanoparticles is actually a challenge. The main reason is that nanoparticles are not monodispersed. For some particles synthesized from one batch to another, the size and size dispersity may vary significantly. Recently, El-Sayed et al. reported a theoretical calculation of extinction coefficient of gold nanoparticles and its dependence on the nanoparticle size [14]. Experimental studies reported by the same group earlier on a citrate-stabilized gold nanoparticles were in accordance with these theoretical calculations [15]. In our study, we examined three different types of gold nanoparticles, citrate-stabilized gold nanoparticles with size ranging from

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approximately 5–35 nm; oleylamide-protected gold nanoparticles with an average diameter around 8 nm; and a decanethiol-stabilized gold nanoparticle with an average core diameter of 4 nm. Our analysis revealed that despite the different capping ligands and the solvents used to dissolve gold nanoparticles, the logarithms of extinction coefficient and nanoparticle core diameter follow a linear relationship. This correlation can be used as an effective rule-of-thumb to estimate nanoparticle concentration or core size.

2. Experimental

2.1. Chemicals and materials

Citrate stabilized gold nanoparticles (5, 10 and 20 nm, named as C_i AuNPs-1, C_i AuNPs-2 and C_i AuNPs-3, respectively) were purchased from Aldrich. Oleylamine ($C_{19}H_{37}NH_2$; tech., 70%), decanethiol, tetraoctylammonium bromide, sodium borohydride, trisodium citrate and all solvents (ACS Reagents) were purchased from Aldrich (Milwaukee, WI). Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4 \cdot xH_2O$) was purchased from Strem Chemicals (Newburyport, MA).

2.2. Synthesis of gold nanoparticles

1-Decanethiol protected gold nanoparticles (D_t AuNPs) were synthesized according to a modified Brust–Schiffrin reaction [16]. Briefly, $HAuCl_4 \cdot xH_2O$ (310 mg, 1 equiv.) was dissolved in 25 mL nanopure water and phase transferred to 80 mL toluene using 1.5 g of tetraoctylammonium bromide. Following the addition of 1-decanethiol (17 μ L, 1/11 equiv.) and aqueous solution of $NaBH_4$ (0.38 g, 10 equiv., in 25 mL nanopure water) into the organic phase, the reaction solution was stirred at room temperature for 3 h. Oleylamide-protected gold nanoparticles (O_A AuNPs) were synthesized by following a reported method [17]. Typically, 35 mg of $HAuCl_4 \cdot xH_2O$ was dissolved in 80 mL nanopure water and heated up to 80 °C before addition of 200 μ L oleylamine by a syringe. The reaction was allowed to continue for 3 h. After the reaction mixture was cooled down to room temperature, 240 mL methanol was added to the solution and nanoparticles were collected by precipitation and washing a few times with a copious amount of methanol. Trisodium citrate-protected gold nanoparticles with size larger than 20 nm (C_i AuNPs-4 and C_i AuNPs-5) were synthesized according to Turkevich method [18].

2.3. High resolution transmission electron microscopy (HRTEM)

TEM grids were treated with polylysine solution first before depositing nanosphere water solutions onto grids. The HRTEM images of each sample were obtained using a FEI TECNAI F30 transmission electron microscope. The accelerating voltage used in the measurement was 300 keV. The 400 mesh carbon/formvar-coated copper grids for citrate-stabilized nanoparticles were first treated with a poly-L-lysine (M.W. 93800, Sigma) solution (0.0381 g/mL in 1:3 H_2O :MeOH mixture solvent). TEM

sample grids were then prepared by extracting 5 μ L sample solution, casting onto polylysine treated grids and vacuum dried. Average gold core diameter (D), size distributions and standard deviations were calculated for each nanoparticle sample by averaging 200 particles from the TEM images using ImageJ software (developed at the National Institutes of Health).

2.4. UV–vis spectroscopy

All UV–vis spectra were recorded using a Cary 300 Bio double-beam UV–vis spectrophotometer at a 300 nm/min scanning rate from 300 to 700 nm. For each sample, UV–vis spectra at five different concentrations were recorded by directly diluting the as-prepared nanoparticle solution with appropriate solvents to the expected relative concentrations. For citrate-stabilized nanoparticle samples (C_i AuNPs-1 to C_i AuNPs-5), solutions with relative concentrations of 1.0, 0.8, 0.6, 0.4, and 0.2 to the initial concentrations were prepared using Nanopure water as solvent. For D_t AuNPs, relative concentrations of 0.088, 0.070, 0.053, 0.035 and 0.018 to the initial concentration of the as-prepared sample were obtained by diluting the sample with toluene. For O_A AuNPs sample, THF was used as the solvent to dilute the as-prepared sample to achieve relative concentrations of 0.064, 0.124, 0.244, 0.515 and 1.040 to the initial concentration. For calculating the extinction coefficient, the maximum absorbance of the SPR band was recorded for each sample.

3. Results and discussion

3.1. Calculation of average number of gold atoms per nanoparticle

The average number of gold atoms per nanoparticle may be calculated from high resolution TEM analysis. The HRTEM images of four nanoparticle product synthesized in this study are shown in Fig. 1. Using ImageJ software, the average core diameters of the particles (D , nm) were measured and summarized in Table 1. Assuming a spherical shape and a uniform fcc structure [19], the average number of gold atoms (N) for each type of nanosphere was calculated by Eq. (1), where ρ is the density for fcc gold (19.3 g/cm³) [20,21] and M stands for atomic weight of gold (197 g/mol):

$$N = \frac{\pi \rho D^3}{6 M} = 30.89602D^3 \quad (1)$$

3.2. Determination of molar concentrations of nanoparticle solution

The molar concentration of the nanosphere solutions were calculated by dividing the total number of gold atoms (N_{total} , equivalent to the initial amount of gold salt added to the reaction solution) over the average number of gold atoms per nanosphere (N) according to Eq. (2), where V is the volume of the reaction solution in liter and N_A is the Avogadro's constant. It is assumed that the reduction from gold(III) to gold atoms was 100% complete. The concentrations of each diluted solution may

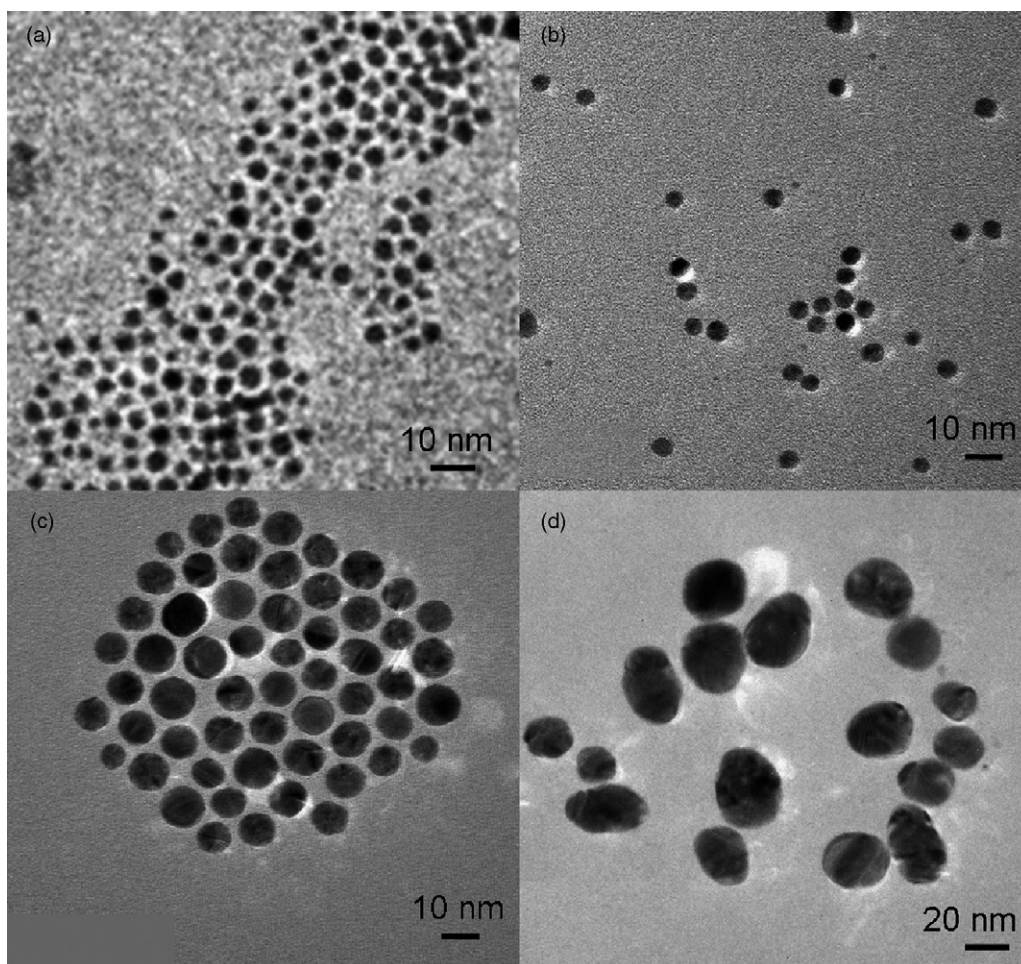


Fig. 1. High resolution transmission electron microscopy images of (a) 3.76 nm 1-decanethiol-capped D_t AuNPs; (b) 4.61 nm citrate-stabilized C_i AuNPs; (c) 8.76 nm oleylamide-protected O_A AuNPs; (d) 25.67 nm citrate-stabilized C_i AuNPs.

be calculated from this initial concentration according to their relative concentration as stated in Section 2:

$$C = \frac{N_{\text{Total}}}{NVN_A} \quad (2)$$

3.3. Extinction coefficient

The extinction coefficient of each nanoparticle sample was determined according to Lambert–Beer law, Eq. (3). The as-prepared or purchased gold nanoparticle solution was diluted into solutions into different concentrations as stated in Section

2. The absorption spectrum of each solution was measured. The absorbance at 506 nm was recorded for each sample and plotted versus the molar concentration of the solution. The extinction coefficient can be obtained from the slope of the linear region of the absorbance–concentration curve. Other samples were analyzed in the similar way:

$$A = \epsilon bC \quad (3)$$

Fig. 2a–d is an illustration of the UV–vis absorption spectra and the linear fitting curve of absorbance–concentration plots for decanethiol and oleylamide-protected gold nanoparticles. Both

Table 1

The extinction coefficients (ϵ) of gold nanoparticles with different core sizes and capping ligands determined in this study

Sample name	Surface ligand	Solvent matrix	Solvent dielectric constant (25 °C)	Initial concentration (mol/L)	Core size, D (nm)	ϵ ($M^{-1} \text{ cm}^{-1}$)
D_t AuNPs	Decanethiol	Toluene	2.4	9.45×10^{-6}	3.76 ± 0.65	$(3.61 \pm 0.08) \times 10^6$
C_i AuNPs-1	Citrate	Water	78.4	9.42×10^{-8}	4.61 ± 0.48	$(8.56 \pm 0.09) \times 10^6$
C_i AuNPs-2	Citrate	Water	78.4	1.49×10^{-8}	8.55 ± 0.79	$(5.14 \pm 0.07) \times 10^7$
O_A AuNPs	Oleylamine	THF	7.58	1.30×10^{-7}	8.76 ± 1.11	$(8.79 \pm 0.03) \times 10^7$
C_i AuNPs-3	Citrate	Water	78.4	1.07×10^{-9}	20.60 ± 1.62	$(8.78 \pm 0.06) \times 10^8$
C_i AuNPs-4	Citrate	Water	78.4	4.92×10^{-10}	25.67 ± 5.62	$(2.93 \pm 0.02) \times 10^9$
C_i AuNPs-5	Citrate	Water	78.4	2.22×10^{-10}	34.46 ± 4.34	$(6.06 \pm 0.03) \times 10^9$

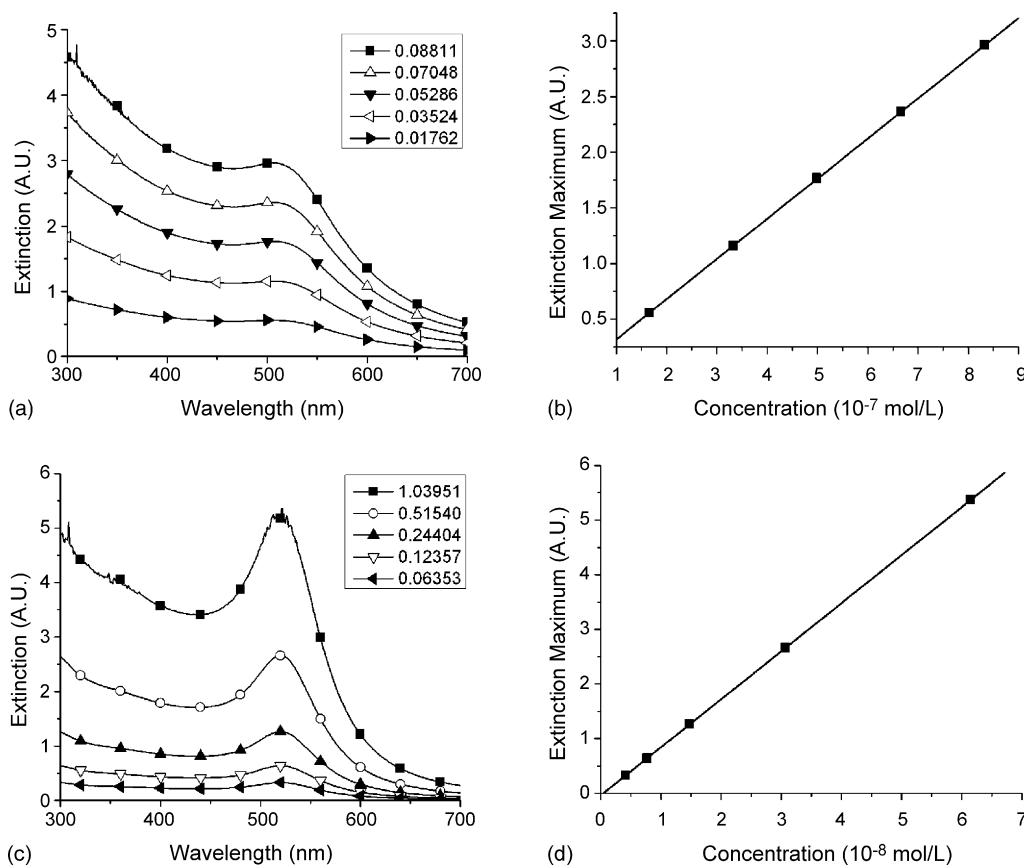


Fig. 2. Extinction spectra of (a) 1-decanethiol and (c) oleylamide-protected gold nanoparticles at different relative concentrations; (b) and (d) are plots of experimental data of maximum extinction at the surface plasmon resonance band vs. corresponding concentration and the linear fitting curves for decanethiol and oleylamide-protected gold nanoparticles, respectively.

curves are linear with a correlation coefficient very close to 1.0. A good linear fitting of the experimental data was found for all other nanoparticle samples. The types of nanoparticles analyzed and the extinction coefficients calculated from the linear fitting curve were summarized in Table 1. From this table, one can notice immediately that the extinction coefficients of gold nanoparticles are orders of magnitude higher than organic dye molecules. The measured extinction coefficients of citrate-stabilized gold nanoparticles are approximately in line with what has been reported by El-Sayed and coworkers [14]. For example, the extinction coefficient values for a 20 nm citrate-stabilized gold nanoparticle obtained from this study, the experimental and theoretical study by El-Sayed et al. are $8.8E8$, $1.36E9$, and $7.6E7 M^{-1} cm^{-1}$, respectively. Between the three values, there is approximately an order of magnitude difference. Our data is between the experimental and theoretical value as reported by El-Sayed et al.

From Table 1, another important result to notice is that the increase in core diameter of gold nanoparticles introduced dramatic and continuous increase in the extinction coefficients. From a core diameter of ~ 4 – 35 nm, the extinction coefficient increased three orders of magnitude. From the double logarithm plot of extinction coefficient against the nanoparticle size in diameter, (Fig. 3) a good linear relationship was found and can be expressed in Eq. (4), where ε is extinction coefficient

in $M^{-1} cm^{-1}$, D is the core diameter of the nanoparticles, and $k = 3.32111$, $a = 10.80505$. The linear fitting of the experimental data gives a correlation coefficient of 0.99754 and standard deviation of 0.21983.

$$\ln \varepsilon = k \ln D + a \quad (4)$$

This finding is also in accordance with the Mie theory as well as the study reported by El-Sayed and coworkers [14,15].

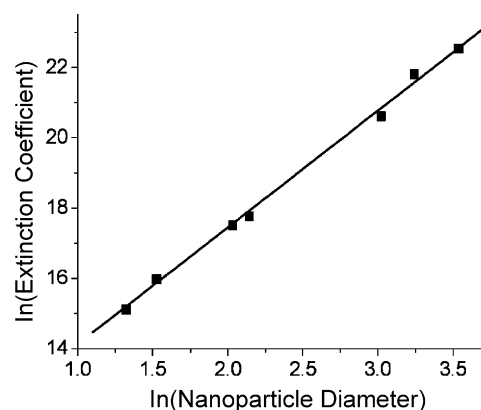


Fig. 3. Experimental data and linear fitting curve of natural logarithm of extinction coefficients vs. logarithm of average nanoparticle core diameters.

The k and a value obtained from our study is 3.32 and 10.8, respectively, while the values for these two parameters are 2.75 and 11.7 from the experimental study, and 3.36 and 8.1 from the theoretical study as reported by El-Sayed et al. Comparing these values, it appears that our experimental result is closer to the theoretical value as calculated by El-Sayed et al. The discrepancy between different studies is most likely due to the slight difference in the average core size and size dispersity of the nanoparticles [15].

The experimental study reported by El-Sayed was on citrate-stabilized gold nanoparticles that were soluble in water. Our results suggest that the linear relationship of the double logarithm of extinction coefficient versus nanoparticle core diameter can be extended to most gold nanoparticles independent of the capping ligands used to protect the nanoparticles and the solvents used to dissolve the nanoparticles. The solvents used to dissolve the three types of nanoparticles synthesized in this study, toluene for decanethiol-protected nanoparticles, THF for oleylamide-protected nanoparticles, and water for citrate-stabilized nanoparticles, have very different dielectric constants (as listed in Table 1), varying from 2.4 to 78. The stabilization effect by the capping agents for these three types of nanoparticles is also quite different. The citrate-stabilized nanoparticles rely on electrostatic interactions between the ligands and the nanoparticle core. The decanethiol-protected gold nanoparticles are stabilized by covalent bonding between thiolate groups and surface gold atoms, while the oleylamide-protected gold nanoparticles depend on hydrogen bonding network formation between amide groups to create a stable environment for nanoparticle core [22]. Although Mie theory indicates that the plasmon resonance absorption of a nanoparticle is related to the dielectric constant of the environmental media around the nanoparticles (which include both solvents and the capping ligands), these effects do not appear to make significant contribution to the extinction coefficient of the surface plasmon resonance band [7,23].

Because of the general applicability of the relation of nanoparticle extinction coefficient versus nanoparticle size, one can use the extinction coefficient-core diameter double logarithm curve established in this study as a calibration curve to calculate or estimate the nanoparticle concentration or average diameter of a nanoparticle solution sample. For example, by determine the average size of a nanoparticle sample using TEM, one can obtain the extinction coefficient of the sample. By taking UV–vis absorption spectra of the sample, the concentration of the sample can be calculated. This will be useful for determining the concentration of biomolecules such as proteins when labeled with gold nanoparticles [24]. Most organic molecules and biomolecules have much lower extinction coefficient than gold nanoparticles. By labeling biomolecules with gold nanopar-

ticles, the biomolecules can be detected at concentrations as low as sub-nanomolar range. This could lead to a very powerful tool at detection of biomarker molecules using a simple spectrophotometer. On the other hand, if the concentration and extinction coefficient of a nanoparticle sample can be determined, the size of the particle may be estimated without imaging analysis by TEM.

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