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Gravitational sedimentation of gold nanoparticles

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ABSTRACT

We study the gravitational sedimentation of citrate- or ascorbate-capped spherical gold nanoparticles (AuNP) by measuring the absorption-vs.-time curve produced as the particles sediment through the optical beam of a spectrophotometer, and comparing the results with a calculated sedimentation curve. TEM showed the AuNP had gold-core diameters of 12.1 ± 0.6, 65.0 ± 5.2, 82.5 ± 5.2 or 91.8 ± 6.2 nm, and gave diameter distribution histograms. The Mason–Weaver sedimentation–diffusion equation was solved for various particle diameters and the solutions were weighted with the TEM histogram and the size-dependent extinction coefficient, for comparison with absorbance-vs.-time curve obtained from freshly prepared suspensions of the AuNP. For particles having average gold-core diameters of 12.1 ± 0.6, 65.0 ± 5.2 and 82.5 ± 5.2 nm, very good agreement exists between the theoretical and observed curves, showing that the particles sediment individually and that the diameter of the gold core is the important factor controlling sedimentation. For the largest particles, observed and calculated curves generally agree, but the former shows random effects consistent with non-homogeneous domains in the sample. Unlike TEM, the simple and unambiguous sedimentation experiment detects all the particles in the sample and can in principle be used to derive the true size histogram. It avoids artifacts of TEM sampling and shear forces of ultracentrifugation. We also show how information about the size histogram can be obtained from the sedimentation curve.

1. Introduction

Gold nanoparticles (AuNP) currently play an important role in the emerging field of nanomedicine, due in part to their tailorable sizes, morphologies, and surface chemistries [1–7]. AuNP with surface-attached doxorubicin [8,9], paclitaxel [10], cisplatin [11,12], oxaliplatin [13], or sensitizers for photodynamic therapy [14–16] are being actively investigated as new multifunctional platforms for treating cancer. Additionally, AuNP with surface- bound human tumor necrosis factor alpha have already completed Phase I clinical trials in the United States [17].

A critical property of AuNP is their size, which is not only important in the design of a vehicle for carrying drugs but is also a factor that affects the retention time of the particle in the blood, its trapping within the tumor vasculature through the EPR effect, and its uptake by the cell [18–21]. The gold standard for determining the size and shape of AuNP is transmission electron microscopy (TEM) which requires capturing an image of a group of particles, measuring the size of each and creating a histogram that shows the distribution of particle size. While this approach seems an unequivocal way to determine particle diameter, the relatively small number of particles (i.e., ~100) appearing on the TEM image means that one may not have an accurate reflection of the distribution of sizes in the entire ensemble of particles in the sample. Experimental artifacts may also affect the sampling process which brings the particles to the TEM stage.

Analytical ultracentrifugation (AUC), a technique often used to study biological molecules, has also been used to measure the sedimentation properties and size distribution of AuNP. Colvin and coworkers [22] used AUC to measure the polydispersity of AuNP and showed that the technique can detect size and shape changes produced by conjugation of the lactose repressor to the particle surface. DNA-capped AuNP have been studied using AUC by Falabella et al. [23], who found that the particle sedimentation coefficients are sensitive to DNA coverage and length, and the degree of hydration of the particle. Sedimentation and diffusion coefficients obtained by two-dimensional AUC on nanoparticles can also be transformed into precise molecular weight, density, and diameter distributions of particles in solution [24]. The growing importance of gold nanoparticles in medicine has focused attention on the state of aggregation of the particles in biological media. Zook et al. presented a theoretical framework for calculating the hydrodynamic diameter distribution of AuNP agglomerates in biological media from their sedimentation coefficient distribution [25]. They detected monomers, dimers, and trimers as well as larger agglomerates, and they suggested that the sedimentation data obtained with AUC could be used to predict how quickly particle agglomerates settle out of solution under normal gravitational force as would occur in the environment.

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In order to sample all gold nanoparticles within a sample, we measured the sedimentation of spherical AuNPs passing through the light beam of a standard spectrophotometer under normal gravitational force. Although sedimentation is slow under these conditions, so that measurement time is long, the experiment is simple, unambiguous, and requires no special apparatus. Furthermore, it is free from possible effects of shear forces which may occur at high gravitational fields in ultracentrifugation. We solve the sedimentation–diffusion equations and compare measured and calculated sedimentation rates, averaged over particle diameter. Since these equations neglect particle–particle interactions, the comparison serves to show whether they are important for these gold nanoparticles.

After summarizing the theory relating sedimentation rate to particle diameter, we show how the equations are solved and compare theory with experiment. In most cases, the measured sedimentation data are in good agreement with calculated results. Where there are discrepancies, possible reasons for their occurrence are discussed.

2. Materials and methods

2.1. Reagents

$HAuCl_4$, trisodium citrate, and all other materials unless specified otherwise were purchased from Sigma Aldrich.

2.2. UV–visible spectroscopy (UV–vis)

Absorbance spectra measurements were recorded in the 200–900 nm region using a Varian Cary 50 UV–vis spectrophotometer in scan mode, with zero/baseline correction. Ultrapure water (18.2 MΩ cm) was used as a diluent and blank. Measurements of AuNP sedimentation were collected using the same instrument in kinetics mode with a monitoring wavelength set to the measured maximum absorbance wavelength (λ_{max}) of each AuNP. Zero correction was performed prior to each kinetic experiment.

2.3. Dynamic light scattering (DLS)

Measurements of hydrodynamic diameter (D_h) were acquired using a Malvern Zetasizer Nano ZS, with a 633 nm laser source and backscattering detector at 173°. All AuNP samples were diluted in ultrapure water, filtered using a 0.22 μm filter, and measured at least three times at 25 °C in a disposable low-volume cuvette. Approximately 12 data collection runs were performed per measurement. Scattering data were analyzed using CONTIN, to give the distribution of D_h values by number for sample A (12.1 nm average diameter) and by volume for samples B–D (65.0, 82.5, and 91.8 nm average diameters).

2.4. Transmission electron microscopy (TEM)

TEM images of AuNP were acquired using a JEOL 2000EX operated at 120 kV with a tungsten filament (SUNY-ESF, N.C. Brown Center for Ultrastructure Studies). Particle diameter was manually analyzed by modeling each individual AuNP as a sphere, using >100 particles per count. ImageJ software was employed for statistical analysis, to give the distribution of AuNP spherical areas, which were then converted to AuNP diameters.

2.5. Gold nanoparticles (AuNP)

Citrate-stabilized AuNP possessing a diameter of 12.1 ± 0.6 nm were synthesized by standard citrate reduction procedures [26]. Two sizes of ascorbate-stabilized AuNP possessing diameters of 65.0 ± 5.2 nm and 82.5 ± 5.2 nm were provided as a gift from Professor M. Maye (Syracuse University). Ascorbate-stabilized AuNP possessing a diameter of 91.8 ± 6.2 nm were purchased from Nanocomposix (San Diego, CA).

2.6. Sedimentation experiments

Sedimentation experiments involved monitoring absorbance at the measured λ_{max} as a function of time, for four samples of AuNP (samples A–D). A slightly modified 2.5 mL disposable cuvette (Plastibrand, PMMA UV grade) was utilized for all sedimentation experiments. The modification allows the optical beam to pass through the AuNP sample only in the range of height from 2 to 6 mm from the base of the internal volume of the cuvette when the cuvette is placed in the spectrophotometer, Fig. 1. To ensure uniformity of the suspension, all AuNP samples were thoroughly mixed (by pumping at least three times using a pipette) before collecting absorbance/sedimentation data. AuNP samples were diluted in ultrapure water to produce absorbance spectra with absorbance at λ_{max} set equal to unity. Absorbance was measured at the λ_{max} of each AuNP sample every 4 min until completion of sedimentation. The sealed cuvette and spectrophotometer were undisturbed for the duration of each experiment, and the experiments were performed at room temperature. All absorbances (A) in sedimentation curves were divided by the absorbance for $t = 0$ and adjusted so that $A = 1.0$ at $t = 0$ and A approaches 0 as t approaches infinity, to produce a sedimentation curve, relative absorbance vs. time.

3. Theory

We consider a cylinder of height h containing a suspension of nanoparticles at $t = 0$, with the particles uniformly distributed in the medium. If the concentration of the nanoparticles at height x is represented by $c(x, t)$, at $t = 0$ we have $c(x, 0) = c_o$, $0 \leqslant x \leqslant 30$ mm. The forces acting on the particles are the gravitational force which moves the particles toward the bottom of the cylinder and the diffusion force which restores uniformity, keeping the particles suspended in the medium. At any time, the concentration obeys the equation of continuity

$$\frac{\partial c}{\partial t} = -\frac{\partial J}{\partial x} \tag{1}$$

where J is the total current density in the $+x$-direction (upward, against the gravitational force). The current density must vanish at $x = 0$ and $x = 30$ mm at all times $t > 0$.

It has been previously shown [27] that there are two contributions to J: the sedimentation current density, J_{sed}, and the diffusion

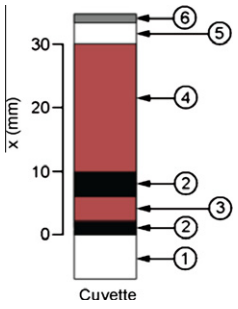

Fig. 1. Diagram of the spectrophotometer cuvette utilized for all sedimentation experiments. (1) Stage to raise cuvette in the cell compartment of the instrument. (2) Masks to restrict optical beam to a specific region of the cuvette. (3) Location of the optical beam, $x = 2$–6 mm. (4) Solution (column) of nanoparticles in cuvette, total height, $x = h = 30$ mm. (5) Air space at top of cuvette. (6) Cuvette cover.