Journal of Colloid and Interface Science 418 (2014) 103–112

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Size histograms of gold nanoparticles measured by gravitational sedimentation

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ARTICLE INFO

Article history:
Received 15 August 2013
Accepted 27 November 2013
Available online 5 December 2013

Keywords:
Sedimentation
Gold nanoparticles
Histogram

ABSTRACT

Sedimentation curves of gold nanoparticles in water were obtained by measuring the optical density of a suspension over time. The results are not subject to sampling errors, and refer to the particles in situ. Curves obtained simultaneously at several wave lengths were analyzed together to derive the size histogram of the sedimenting particles. The bins in the histogram were 5 nm wide and centered at diameters 60, 65, ..., 110 nm. To get the histogram, we weighted previously calculated solutions to the Mason–Weaver sedimentation–diffusion equation for various particle diameters with absorption/scattering coefficients and size (diameter) abundances $\{c_j\}$, and found the $\{c_j\}$ which gave the best fit to all the theoretical sedimentation curves. The effects of changing the number of bins and the wave lengths used were studied. Going to smaller bins would mean determining more parameters and require more wave lengths. The histograms derived from sedimentation agreed quite well in general with the histogram derived from TEM. Differences found for the smallest particle diameters are partly due to statistical fluctuations (TEM found only 1–2 particles out of 103 with these diameters). More important is that the TEM histogram indicates 12% of the particles have diameters of 75 ± 2.5 nm, and the sedimentation histogram shows none. We show that this reflects the difference between the particles in situ, which possess a low-density shell about 1 nm thick, and the bare particles on the TEM stage. Correcting for this makes agreement between the two histograms excellent. Comparing sedimentation-derived with TEM-derived histograms thus shows differences between the particles in situ and on the TEM stage.

1. Introduction

There are a number of applications for gold nanoparticles (AuNPs) in which size and size distribution are significant. A broad list includes catalysis [1], assembly [2,3], "smart" particle systems [4], sensors [5], and biomedical applications [6]. It is therefore useful to have one or even multiple means of accurately measuring the size distributions of AuNP samples. In this work, we provide an approach for determining AuNP size distribution, complementary to more conventional techniques such as transmission electron microscopy (TEM) and dynamic light scattering (DLS). By measuring the gravitational sedimentation of polydisperse AuNP in suspension, we are able to generate size distribution histograms reflecting the entire particle population.

One application in which AuNP size has been shown to be critical is catalysis. In general, smaller particles exhibit superior catalytic activity due to their greater surface area and enhanced surface effects [7–10]. However, the relationship between AuNP size and catalytic activity is often complex. The catalytic rate of eosin reduction, for example, was found to be strongly dependent on the diameter of AuNP catalysts ranging from 10 – 46 nm. The kinetics were a complex function of size effects involving surface area, mass per area, and particle concentration [11]. Clearly, the design of AuNP-based catalysts benefits from a complete understanding of AuNP size and size distribution.

In many applications, the monodispersity of particles is an important goal for quality control purposes. This is particularly so when linker-functionalized AuNP are assembled into structured arrays. In 2D or 3D arrays, the AuNP sizes can affect the interparticle distance, array uniformity, optical, and electronic properties [3,12]. For example, Kim showed that the interparticle spacing parameter in a 2D array was a direct function of AuNP size, and that spacing and size influenced the optical properties (e.g., λ_{spr}) of the array [3].

Gold nanoparticle size is critical for biomedicine in a number of ways. Size-selective accumulation of nanoscale particles in the leaky vasculature of tumor tissue (i.e., passive targeting) occurs via the enhanced permeability and retention (EPR) effect [6]. The AuNP size also strongly determines cellular uptake [13,14], nanoparticle toxicity [15,16], and ligand loading capacity [17]. Optimizing AuNP design for diagnostic or therapeutic purposes requires consideration of these size-dependent properties. For example, we recently developed DNA-capped AuNP for the delivery of the anticancer

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http://dx.doi.org/10.1016/j.jcis.2013.11.074

drugs doxorubicin and actinomycin D [18,19]. With an AuNP core size of 11.4 ± 0.6 nm and hydrodynamic diameter, D_h, of 32.9 ± 1.5 nm, the vehicles are within the size range requirements for passive targeting (D_h = ~6–200 nm[6]) and cellular uptake (optimized at D_h = ~50 nm[13]), and their monodispersity permits consistent loading of the drug cargo.

The current gold standard for determining the size and shape of AuNP is transmission electron microscopy (TEM). Here, particle size is measured directly from an image of a sample of particles. While the directness of the measure is an advantage, there may be several problems. First, because a relatively small number, typically one or two hundred, particles are measured, there may be only one or two particles in some size ranges. The statistical errors may be large for these. Second, experimental artifacts may affect the sampling process which brings the particles to the TEM stage. Third, the particles are not being measured in situ, and there may be differences between the particles in suspension and the particles on the TEM stage.

In previous work, we showed that useful information about the sizes of gold nanoparticles in suspension could be obtained using ordinary gravitational sedimentation [20]. The measured quantity was the "sedimentation curve," which is the optical density of the sedimenting system, at a fixed height, as a function of time. The contribution of particles of a given size to the optical density is proportional to the absorption/scattering coefficient and the number density of those particles. These measurements see *all* gold nanoparticles in a sample, and do not require removal of the particles from the system. They are also free from possible effects of shear forces which may result from the high gravitational fields used in ultracentrifugation. These forces could affect the structure and properties of the particles, and small changes would not necessarily be seen in TEM. More important, the density profile from ultracentrifugation is less detailed than from sedimentation under ordinary gravitational fields. Although the measurement time is long, the experiment is simple and unambiguous.

In previous work, we calculated [20] sedimentation curves for particles of various diameters by solving the Mason–Weaver equation [21]. Then we showed that, if these curves were weighted with the known size-dependent absorption/scattering coefficients and the relative numbers of particles of various diameters (from TEM measurements), we could reproduce the experimental sedimentation curve for the collection of particles. The agreement was good for three different samples, with particle diameters 65.0 ± 5.2, 82.5 ± 5.2, and 91.8 ± 6.2 nm. In the last case, discrepancies between calculated and measured sedimentation curves pointed to inhomogeneities in the system. The overall good agreement for all three samples showed that the particles sediment individually, without agglomeration

We also pointed out that the process could be inverted, that is, the measured sedimentation curve could be analyzed, given calculated sedimentation curves for various sized particles, to give the histogram. As an example, three particle abundances were calculated in reasonable agreement with the TEM results. Since the measured sedimentation curve is essentially featureless, the number of such parameters one can extract is limited. However, it was suggested that much more information could be obtained if the sedimentation curve was measured at several wave lengths. The absorption/scattering coefficient for a particle of a given size depends on the wave length in a known way, so particles of different sizes are weighted differently in the sedimentation curves for different wave lengths.

In the present work, we develop this approach, to show that sedimentation curves of AuNP monitored at multiple wavelengths can provide more information about a polydisperse AuNP suspension and generate extremely accurate size histograms. We start from a set of sedimentation curves measured at different wave lengths, and analyze them in terms of previously calculated sedimentation curves for particles of various sizes to determine up to ten parameters in the size histogram (numbers of particles of various sizes). After summarizing the theory relating sedimentation rate to particle diameter, we show how one generates the theoretical sedimentation curves for particles of given sizes. These curves are derived from solutions to the Mason–Weaver sedimentation–diffusion equations, starting from a uniform density at time zero. We also show how absorption/scattering coefficients are calculated. Then, if $T_j(t)$ is the calculated sedimentation curve for particles of size j and α_j^k is the absorption/scattering coefficient for a particle of size j at wave length λ_k, $T_j^k(t) \equiv \alpha_j^k T_j(t)$ is the contribution of one such particle to the optical density. The total optical density as a function of time, which is the measured sedimentation curve, is $\Sigma_j\, c_j\, T_j^k$, where c_j is the concentration of particles of size j. Given a number of sedimentation curves for different λ_k, the c_j can be calculated by determining the c_j to give the best fit between the theoretical and the measured sedimentation curves.

We show how many histogram numbers can be obtained, and how many different wave lengths should be considered. Finally, calculated histograms are compared with the TEM-derived histogram. Differences between the two which do not disappear when more wave lengths are used point to actual physical effects which make the particles in suspension differ from the bare particles on a TEM grid. These are considered in the Discussion.

2. Theory

2.1. Mason–Weaver equation

We consider a cylinder of height h containing a uniform suspension of nanoparticles of a given size at $t = 0$. If the concentration of the nanoparticles at height x is represented by $c(x,t)$, at $t = 0$ we have $c(x,0) = c_o$, $0 \leqslant x \leqslant h$ and $c(x,0) = 0$ elsewhere. When sedimentation occurs, the concentration of nanoparticles in the medium becomes non-uniform, larger for smaller x. 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 $x = h$ at all times $t > 0$.

It has been previously shown [21,22] that there are two contributions to J: the sedimentation current density, J_{sed}, and the diffusion current density, J_{dif}, such that $J = J_{sed} + J_{dif}$. Then

$$J = -Dm\left(1 - \frac{\rho_1}{\rho_2}\right)\frac{gc}{kT} - D\frac{\partial c}{\partial x}$$

Here m is the mass of a nanoparticle, g is the acceleration of gravity, ρ_1 is the density of the suspending fluid (water), and ρ_2 is the density of a nanoparticle and D is the diffusion coefficient. Then the equation of continuity, (1), becomes

$$\frac{1}{D}\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left[m\left(1 - \frac{\rho_1}{\rho_2}\right)\frac{gc}{kT} + \frac{\partial c}{\partial x}\right] = \frac{\partial}{\partial x}\left[ac + \frac{\partial c}{\partial x}\right] \tag{2}$$

where

$$a = m\left(1 - \frac{\rho_1}{\rho_2}\right)\frac{g}{kT} \tag{3}$$

We write the diffusion coefficient D as kT/f with the friction factor $f = 3\pi\eta d$ where $\eta = 9.0 \times 10^{-4}$ Pa s (viscosity of water at 25 °C) and d is the particle diameter. At 25 °C, $D = (4.53 \times 10^{-19}\ \mathrm{m^3/s})/\mathrm{d}$, and $g/kT = 2.382 \times 10^{21}\ \mathrm{kg^{-1}\,m^{-1}}$.