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Method of Determining Nanoparticle Core Weight

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Polymer-coated metal or metal oxide nanoparticles have a variety of uses in industry, biological research, and medicine. Characterization of nanoparticles often includes determination of the dimensions of the electron-dense core by transmission electron microscopy (TEM), with the weight of the core determined from core volume and core density. However, TEM is labor intensive, has a long turnaround time, and uses equipment that is sometimes not readily available. Here we present an alternative method for determining the weight of nanoparticle cores termed the viscosity/light scattering method, which uses (i) measurements of viscosity over a wide concentration range to obtain the partial specific volume, (ii) measurements of particle diameter by light scattering, to obtain the volume of an individual particle, and (iii) the concentration of nanoparticles (w/v). We have applied this method to determine the weights of nanoparticle cores (iron of amino-CLIO and ferritin), the weights of globular proteins (molecular weight of IgG and albumin), and the weight of polystyrene microspheres. The viscosity/light scattering method is nondestructive of the sample and can be performed with a variety of materials on a routine basis.

Nanoparticles such as quantum dots,[1,2] colloidal gold,[3] and magnetic iron oxides[4−6] are important materials for diverse applications in biology, in medicine, and for industrial applications. Often these materials consist of a core of metal or metal oxide surrounded by a polymer or surfactant, to make the surface of the nanoparticle compatible with the solvent and achieve dispersion. Figure 1 provides a schematic representation of a nanoparticle used frequently in this laboratory, consisting of a stabilizing coating of cross-linked dextran surrounding a core of superparamagnetic iron oxide. Determination of the overall volume of a nanoparticle (dextran plus iron oxide) can be accomplished by many methods such as size exclusion chromatography, light scattering, sedimentation velocity, or atomic force microscopy, which can visualize the non-electron-dense polymer. However, these methods yield no information on the weight of the nanoparticle or weights of its various components. Since the polymer layer can be thicker than the core diameter in some cases,[7] measurements over the overall size in solution are not informative regarding the dimensions of the core.

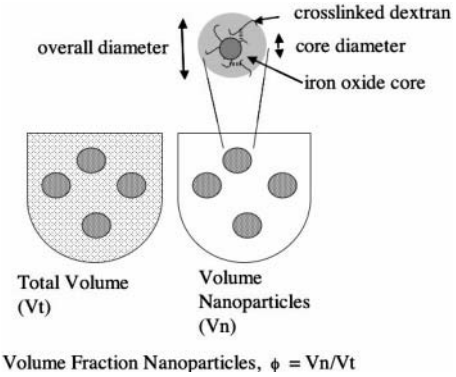

Figure 1. Viscosity/light scattering method for the determination of the core size of polymer-coated nanoparticles.

Transmission electron microscopy (TEM) is often used to obtain the dimensions of the electron-dense cores of polymer-coated nanoparticles. From core volume and density, core weight may then be obtained. However, TEM requires the immobilization of nanoparticles on a grid at an appropriate density, the accumulation of micrographs where the dimensions of hundreds or thousands of particles can be measured, and the tabulation of the mean nanoparticle diameter and nanoparticle volume. Line broadening by X-ray diffraction can be employed when crystalline compounds form the core of nanoparticles.[7] However, X-ray diffraction requires a dry powder sample and, like TEM, requires equipment that is not routinely available. Because of these difficulties, the size of nanoparticle cores is infrequently determined, both during the research and development phase and on materials made during routine manufacture.

We present here a method for determining the weight of the core of polymer-coated nanoparticles based on viscosity and light scattering (LS) measurements (viscosity/light scattering method). Both measurements are nondestructive to the sample, utilize widely available instrumentation, and require little sample preparation or data analysis. We also show that viscosity, light scattering, and sample concentration can give the weight of the iron core of

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(1) Murphy, C. *Anal. Chem.* **2002**, *74*, 520A−526A.
(2) Dirk, B. H. D.; Colm, M.; Sorin, A.; Thomas, M.; Horst, W.; Alexander, E. *J. Phys. Chem. B* **2003**, *107*, 7486−7491.
(3) Reynolds, I.; Robert A.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **2000**, *122*, 3795−3796.
(4) Josephson, L. P., J. M.; Weissleder, R. *Angew, Chem,, Int, Ed.* **2001**, *40*, 3204.
(5) Perez, M. J. L.; O'Loughlin, T.; Hogemann, D.; Weissleder, R. *Nat. Biotechnol.* **2002**, *20*, 816−820.
(6) Harisinghani, M. G.; Barentsz, J.; Hahn, P. F.; Deserno, W. M.; Tabatabaei, S.; van de Kaa, C. H.; de la Rosette, J.; Weissleder, R. *N. Engl. J. Med.* **2003**, *348*, 2491−2499.
(7) Jung, C. W.; Jacobs, P. *Magn. Reson. Imaging* **1995**, *13*, 661−674.

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amino-CLIO, a nanoparticle used for biosensor applications,[5] loading cells for tracking by MRI,[8] and targeted MR contrast agents.[9]

MATERIALS AND METHODS

Amino-CLIO was prepared as described.[5] Briefly, a solution of ferrous and ferric chloride and T-10 dextran was reacted with ammonium hydroxide and free T-10 dextran removed by ultrafiltration. The dextran coat was cross-linked with epichlorohydrin and reacted with ammonia to yield amino-CLIO. Bovine serum albumin, bovine IgG, and ferritin solution were from Sigma-Aldrich (St. Louis, MO). Polystyrene calibration beads were from Duke Scientific (Palo Alto, CA). Proteins were dissolved and diluted in phosphate-buffered saline. Polystyrene beads were diluted with 0.01% sodium dodecyl sulfate in water. MION and amino-CLIO nanoparticles were obtained in a solution of 0.15 M NaCl and 0.02 M citrate, pH = 8, and diluted using the same solvent.

The concentration of the polystyrene nanoparticles was determined by weight after ultrafiltration to remove an absorbed surfactant. Specifically, a YM-100 centrifugal ultrafiltration tube was weighed and a known volume of nanoparticle solution was added. After the particles were washed with distilled water (5 times), the ultrafiltration tube was lyophilized and reweighed. IgG and albumin concentrations were determined as the dry weight of protein. Ferritin protein concentration was obtained from the mass given by the manufacturer. Iron concentrations for amino-CLIO and ferritin were determined spectrophotometrically at 410 nm against a standard of $FeCl_3$, after digestion of materials with 0.03% H_2O_2 in 6 N HCl, for 1 h at room temperature. Viscosity was determined using a cross-arm capillary poiseuille viscometer at 25 °C (Cole Parmer, Vernon Hills, Il).

Particle size was determined by dynamic light scattering (photon correlation spectroscopy) using a Zetasizer (Malvern Instruments, Marlborough, MA) after solutions underwent 0.45-μm filtration. This instrument uses a 5-mW HeNe laser (633 nm) and measures particle sizes from 2 to 3000 nm. Fluctuations in the intensity of scattered light (at 90° to the incident) are analyzed through the use of first-order and second autocorrelation functions. The volume-weighted average diameter obtained by the manufacturer's software was used for the calculation of the average nanoparticle volume. Instrument calibration was verified weekly using polystyrene latex standards from Duke Scientific. A refractive index of 1.57 was used for nanoparticles (the refractive index of dextran), while 1.59 was used for polystyrene standards, as per the manufacturer's instructions. A refractive index of 1.42 was used for proteins, which is a value typical of proteins and polyamides.

For TEM, quantifoil micromachined carbon grids (Structure Pobe, Inc. Westchester, PA) containing 2-μm perforations in the support film were used as the substrate. Four microliters of amino-CLIO nanoparticles (1 mg Fe/mL) was placed on the grid in a humidity-controlled chamber, wicked partially dry for 3 s, and then plunged into liquid nitrogen-cooled liquid ethane. Samples were stored in liquid nitrogen prior to imaging. This technique traps the sample within a thin (30−40 nm) film of vitreous ice. All grids were kept at −175 °C in the microscope by use of a Gatan cryoholder and photographed under low-dose electron conditions ($\sim 5e^-/A^2$). Images were then digitized at 2000 dpi and analyzed with ImageJ (NIH, Bethesda MD). The areas of irregular-shaped particles were obtained by tracing and converted to circles of equivalent area. Crystal sizes were expressed as the mean volume of the equivalent sphere ± one standard deviation.

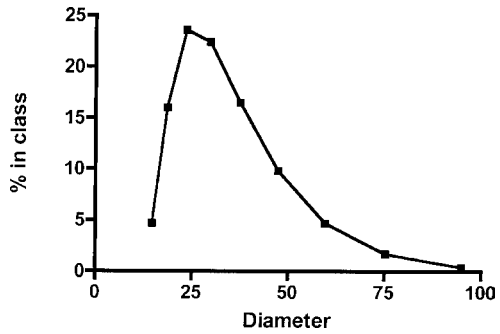

Figure 2. Determination of volume-weighted size distribution of the amino-CLIO nanoparticle.

RESULTS AND DISCUSSION

The viscosity/light scattering method of determining the weight of nanoparticle cores uses three values. They are as follows: (i) ϕ, a unitless number equivalent to the volume fraction of nanoparticles; (ii) average volume of a nanoparticle (core plus polymer) obtained from light scattering; (iii) the weight of nanoparticles or a component thereof per unit volume (concentration as w/v).

The theoretical relationship between the viscosity of a dilute solution of spherical nanoparticles and the volume fraction of suspended nanoparticles (ϕ) was derived by Einstein in 1906; see eq 1. The equation has been verified for materials ranging from individual molecules to spheres as large as hundreds of micrometers.[10]

$$h/h_0 = 1 + 2.5\phi \quad (1)$$

where h is the viscosity of the nanoparticle suspension, h_0 is the viscosity of the solvent without nanoparticles (so h/h_0 is the relative viscosity), and ϕ is the volume fraction of nanoparticles.

To determine values of ϕ for nanoparticles, viscosity measurements over a wide range of concentrations were fitted to the Einstein viscosity equation. Nonlinear curves from excessively high concentration of particles were analyzed by looking at the linear section of the curve at low concentration.

To determine the weight of the iron core of the amino-CLIO nanoparticle (present as iron oxide), the number of nanoparticles per volume is obtained by dividing the total volume of nanoparticles per volume solution, Φ, by the average volume of a nanoparticle obtained from light scattering, an example of which is shown in Figure 2. Hence

$$N = \phi/[4/3\pi(d/2)^3] \quad (2)$$

where N is the number of nanoparticles/volume, ϕ is the volume

(8) Lewin, M.; Carlesso, N.; Tung, C. H.; Tang, X. W.; Cory, D.; Scadden, D. T.; Weissleder, R. *Nat. Biotechnol.* **2000**, *18*, 410−414.

(9) Hogemann, D.; Josephson, L.; Weissleder, R.; Basilion, J. P. *Bioconjugate Chem.* **2000**, *11*, 941−946.

(10) Hiemenz, P. C. *Principles of colloid and surface chemistry*, 2nd ed.; Marcel Dekker: New York, 1986.

Table 1. Weights of Nanoparticles and Reference Materials by Viscosity/Light Scattering

particle	$\Phi \times 10^3$	vol-weighted diam (LLS)	particle diam (lit. ref)	nanoparticle wt[a] (viscosity−light scattering)	nanoparticle wt (ref methods)
amino-CLIO	20/mg Fe	31.3 nm	none	8.1×10^{-19} g of Fe or 8700 Fe (core)	8.6×10^{-19} g or 9300 Fe (Figure 3)
ferritin	24/mg Fe	14 nm	13 nm [12]	7.0×10^{-20} g of Fe or 750 Fe (core)	6.1×10^{-20} g or 660 Fe (see text)
polystyrene sphere	1.03/mg polystry	113 nm	110 nm[b] (TEM/LLS)	7.32×10^{-16} g (total)	7.93×10^{-16} g (calcd)
albumin	2.4/mg protein	6.9 nm	6 ± 1 nm (TEM)[13]	1.09×10^{-19} g or 65 800 (total)	1.10×10^{-19} g or 66 200[13]
IgG	3.2/mg protein	11.7 nm	12 nm (X-ray)[14] 10.5 ± 1 (TEM)[15]	2.61×10^{-19} g or 157 000 (total)	2.57×10^{-19} g or 155 000[14]

[a] Weight may be that of a component, like iron core (Figure 1), or the total weight of the molecule. [b] Value from TEM provided by the manufacturer.

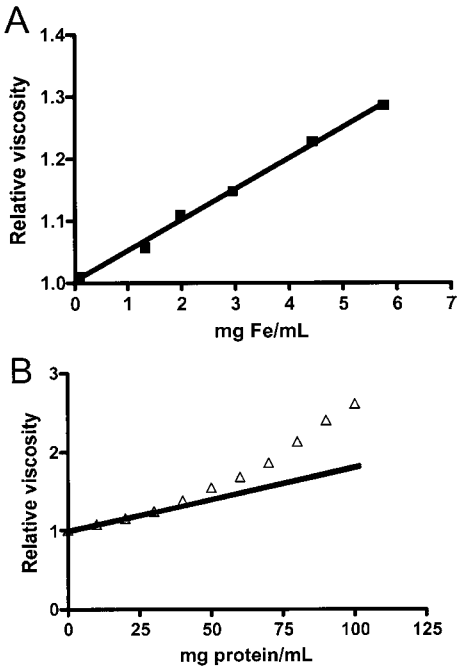

Figure 3. Determination of partial specific volume.

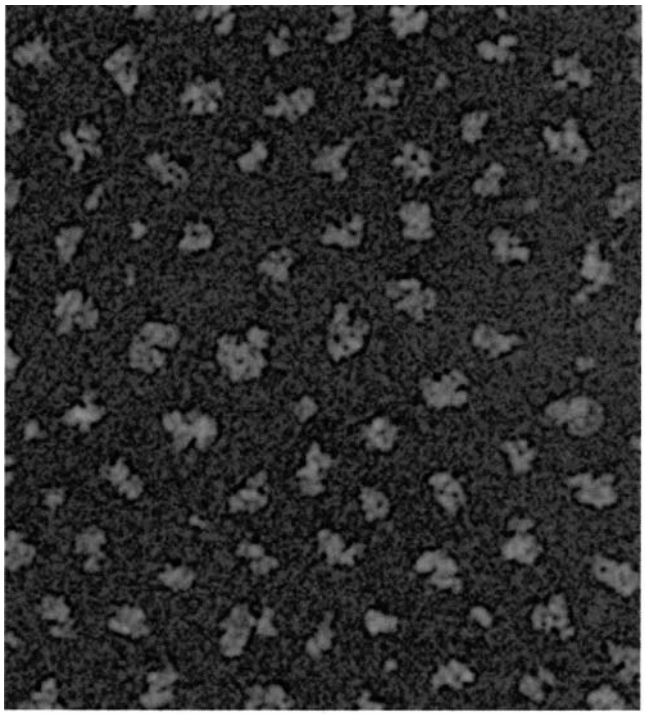

Figure 4. Transmission electron microscopy of the amino-CLIO nanoparticle.

fraction of particles determined by viscosity, $4/3\pi(d/2)^3$ is the average volume of a nanoparticle, and d is the volume-weighted diameter determined by light scattering.

The weight of iron per nanoparticle is then equal to the iron weight per unit volume (iron concentration, w/v), divided by the number of nanoparticles per unit volume.

We compared the weights of different materials (ferritin, polystyrene spheres, albumin) determined by the viscosity/light scattering method with the weight determined by reference methods (Table 1) as explained below. Figure 3A shows the viscosity of amino-CLIO solutions as a function of iron concentration, demonstrating the typical precision of the viscometer. Figure 3B shows the effect of excessive concentration on the viscosity/concentration plots, using IgG. The linear portion of the plot at low concentrations was used to derive a ϕ of 0.032/mg of protein.

Amino-CLIO. We compared the weight of the iron core obtained by the viscosity/light scattering method with that obtained by TEM. The TEM of amino-CLIO used a vitreous ice technique and showed the electron-dense iron oxide crystals with highly regular spacing between them (Figure 4). The cross-linked dextran shell was not sufficiently electron dense to be visible, but its presence is evident from the even spacing between the cores. Image analysis of 2500 iron oxide cores revealed an average diameter, as spheres, of 8.74 ± 3.09 nm. However, since the micrograph showed highly irregular shapes, which were presumed to entrap some water, a packing factor of 0.68,[11] appropriate for a random packing of spheres, was used. This yielded 9300 Fe atoms/nanoparticle for the TEM reference method versus 8700 Fe atoms by the viscosity/light scattering method.

Ferritin. Ferritin consists of a core of ferric oxide surrounded by protein subunits.[12] The interior of the protein shell can hold up to 4500 iron atoms.[12] However, using the measured iron

(11) Peters, M. S. a. T.; Klaus, D. *Plant design and economics for chemical engineers*, 3rd ed.; McGraw-Hill: New York, 1986.

concentration with the protein concentration given by the manufacturer, and using a molecular weight of 500 000 for the protein portion of the molecule,[12] our ferritin sample was determined to contain 6.1×10^{-20} g of Fe/molecule. This yielded a value of 660 Fe atoms/protein, which compared well to the 750 Fe/protein obtained by the viscosity/light scattering technique.

The weight per nanoparticle of any component of the nanoparticle (core, polymer) can be obtained with the viscosity/light scattering method, by the determining the number of nanoparticles per unit volume and then using the appropriate value of weight per unit volume. The method can be used to obtain the weight of materials, like globular proteins, consisting of a single component.

Albumin and IgG. With proteins, the viscosity/light scattering method yields the weight of a single component, the protein. We obtained diameters of 6.9 and 11.7 nm by LS for albumin and IgG, respectively. With a spherical assumption, we yielded weights of 65 800 and 157 000 for albumin and IgG, respectively. These were close to the literature values of 66 200 for albumin[13] and 155 000 for IgG.[14,15]

Polystyrene Calibration Spheres. We obtained the overall diameter of 113 nm by LS, which can be compared with an overall diameter of 110 nm given by the manufacturer. The difference is due to the use of a volume-weighted average for this study versus a number-weighted average by the manufacturer. The viscosity/light scattering method gave a value of nanoparticle weight similar to that calculated from the size of the particles and the density of bulk polystyrene.

The day-to-day reproducibility of the method proved to be excellent. The same sample, run on subsequent days, had a coefficient of variation (SD/mean) of 4−5%, which was almost entirely attributable to the variation in the diameter as determined by light scattering.

The viscosity/light scattering method can be further modified and improved in a number of ways. While a capillary viscometer was used in this study, more sophisticated viscometers based on rotating concentric cylinders or cone and plate geometries could greatly reduce the time required for viscosity measurements without sacrificing accuracy. In addition, a wide range of methods can be used instead of light scattering to determine overall nanoparticle size.

CONCLUSION

The viscosity/light scattering method has been used to determine the weight of iron in the core of the amino-CLIO nanoparticle through the use of viscosity, light scattering, and concentration measurements. The method has been validated using nanoparticles, proteins, and polystyrene spheres.

(12) Harrison, P. M.; Arosio, P. *Biochim. Biophys. Acta* **1996**, *1275*, 161−203.
(13) Slayter, E. M. *J. Mol. Biol.* **1965**, *14*, 443−452.
(14) Davies, D. R. S., V. R.; Labaw, L. W.; Silverton, E. W.; Terry, W. D. In *Progress in Immunology*; Amos, B., Ed.; Academic Press: New York, 1971; pp 27−32.
(15) Valentine, R. C. a. G., N. M. *J. Mol. Biol.* **1967**, *27*, 615−618.

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