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Nanocrystal Separation

Separation of Nanoparticles in a Density Gradient: FeCo@C and Gold Nanocrystals**

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Size and geometric control of nanomaterials are important to the discovery of intrinsic size/shape dependent properties and bottom up approaches for the fabrication of functional nanodevices.[1–10] Two general strategies have been employed to create size-uniform nanocrystals. One method is direct particle size control during synthesis by adjusting growth parameters;[1–3,5,7–9] the other is post-synthesis separation.[11–19] Much capacity exists to improve size separation efficacy in the latter case. Differential centrifugation can remove large and unstable particles from colloidal systems, but lacks precise control over particle size.[9,11] Addition of adjustable amounts of "anti-solvent"[19] (including CO_2)[12] into colloidal systems may make precipitation processes more controllable. Other methods include filtration[14] (including diafiltration[15]), electrophoresis,[16,17] and chromatographic methods[11,18] that can produce particle fractions with narrow shape and size distributions.

To maintain or improve the quality of nanoparticle (NP) separation, whilst addressing the issues of adhesion and clogging in liquid–solid phase separation processes, a completely liquid phase separation method is highly appealing. Isopycnic centrifugation, which is often used for biomacromolecule separation,[20] relies upon a density gradient and ultracentrifugation to separate components according to subtle density differences, and has been applied for diameter and electronic-dependent separation of single-walled carbon nanotubes (SWNT).[13,21] However, the isopycnic density-gradient centrifugation method reaches a limitation when it is extended to the separation of metal nanoparticles. Such a method requires that the components for separation have densities within a gradient range. Aqueous density gradient media usually have densities less than 1.4 $g\,cm^{-3}$, which is much less than the density of metal nanoparticles. Size or shape separation of such heavy nanocrystals remains an issue, both in their preparation and utility for various applications.

In contrast to isopycnic separation, ultracentrifugal rate separation can utilize density gradients to separate nanocrystals with higher densities than the gradient media itself. We have previously applied such a method to achieve length separation of suspended SWNTs[22] and pegylated graphene oxide.[23] In this report, the method was extended to metallic NP size separation. Nanoparticles of various size, suspension chemistry, and composition, including FeCo@C[24] and gold nanoparticles (Au NPs), were separated using the method.

FeCo nanocrystals coated in graphitic shells have superior magnetic properties, and have shown promise for applications in biolabeling and magnetic resonance imaging (MRI).[24] However, the chemical deposition method used for their preparation produces nanocrystals with wide size distribution.[24] They are thus ideal candidates for post-synthesis separation. FeCo@C NPs with average diameters of about 4 nm were separated first by our density gradient rate (DGR) separation method by using a 10 + 20 + 30 + 40 % gradient and centrifugation for 3.5 h. TEM results of typical fractions (Figure 1 A) indicate that fraction 8 (labeled as "f8" in Figure 1 B) contained circa 1.5 nm NPs. The average particle diameter of subsequent fractions (f11, 15, 19, 24, and 27) gradually increased from 2.5 to 5.6 nm.

By varying the step gradient densities and centrifuge exposure time, this method could be used for separation of nanoparticles of a larger size range, which was demonstrated by (on average) 7 nm FeCo@C NP separation (see TEM images of initial 7 nm FeCo@C NPs in the Supporting Information). A gradient of higher density steps (20 + 30 + 40 + 60 %) was used. The use of higher density gradient media helps to control the sedimentation rate by reducing the density difference between the NPs and the environmental medium, and increasing the medium viscosity. After centrifugation for 2.5 h, several bands formed in the centrifuge vessel (Figure 2 A), just as in the 4 nm NP case. Sampling fractions along the centrifuge vessel yielded nanoparticles of increasing size (with increasing density), as revealed by TEM (Figure 2 B). From f5 to f16, the average particle size increased from 2 to 6.5 nm. It is noteworthy that the Fe/Co atomic ratios of f8 and f16 were measured by energy dispersive spectra (EDS) and found to be different (Figure 2 B, bottom right). For f8, the ratio Fe/Co = 48:52; for f16, Fe/Co = 40:60. Previously such stoichiometry was analyzed by calcination/burning of the graphitic shells at 500 °C, dissolving the metal species in an HCl solution, and measuring the iron and cobalt concentrations on the basis of the ultraviolet-

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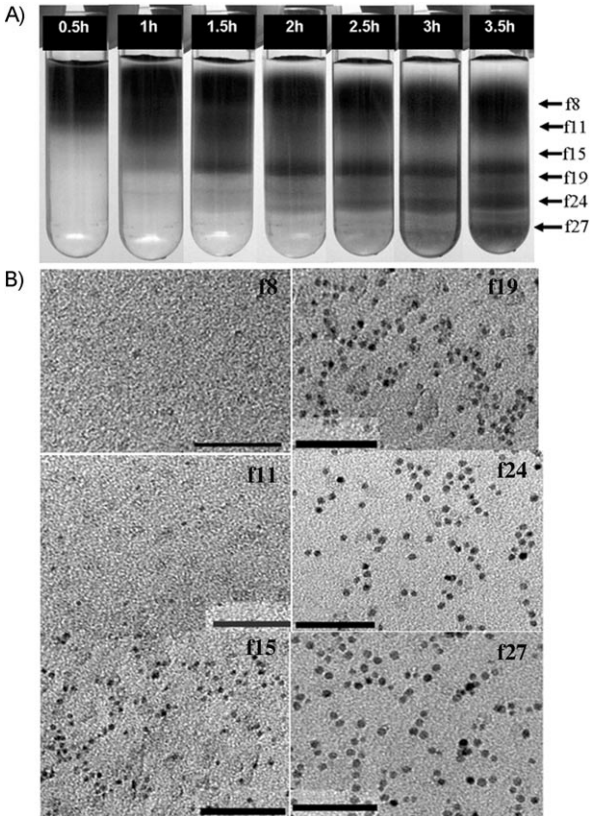

Figure 1. Optical and TEM images showing the separation of 4 nm FeCo@C nanoparticles. A) Digital camera images of ultracentrifuge tubes taken at 30 min intervals. B) TEM images of different fractions labeled in (A). Scale bars: 50 nm.

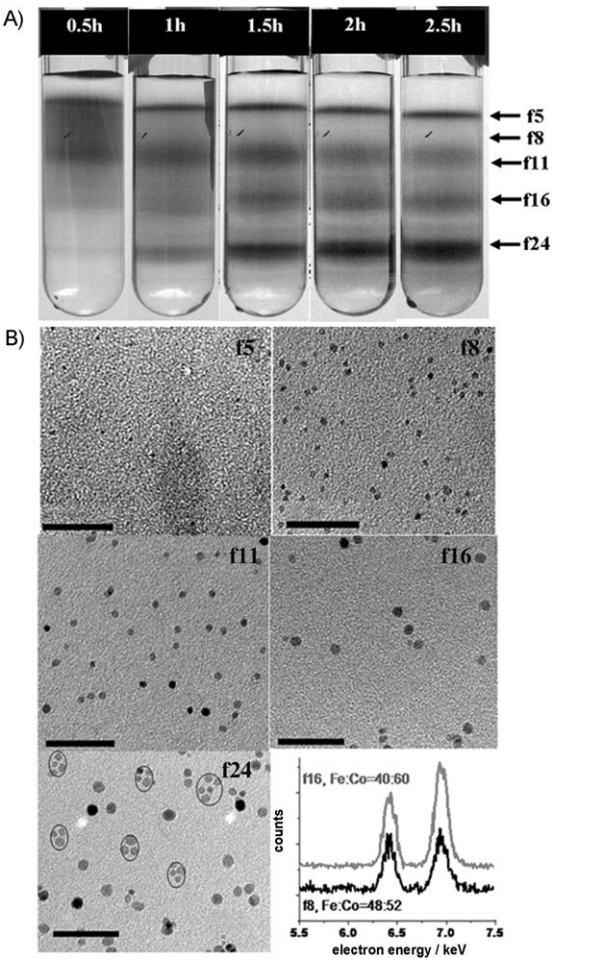

Figure 2. Separation of 7 nm FeCo@C nanoparticles. A) Digital camera images of ultracentrifuge tubes taken at 30 min intervals. B) TEM images of different fractions labeled in (A). The energy dispersive spectra shown at the bottom right of (B) shows the Fe/Co atomic ratio difference of fractions 8 and 16. Scale bars: 50 nm.

visible absorbance of Fe^{3+} and Co^{2+}.[24] Such compositional intricacy would be lost amongst the ensemble of FeCo@C NPs without applying the described separation methodology. NPs of greater diameter were found in f24 that coexist with aggregates of three to five primary smaller particles. This result implied that such a procedure could also separate aggregated clusters from individual NPs, as further evidenced by AFM characterization (Figure 3).

All the samples were calcined at 350 °C to remove organic components before AFM. This process sintered the FeCo@C nanoparticles together if they were clusters. The tapping mode heights of features in f24 and f28 were obviously higher than f16, suggesting that the small nanoparticles observed by TEM in f24 and f28 were always contained in a cluster.

AFM results indicate the existence of a small amount of SWNTs in f5 (Figure 3), which have a diameter of about 1.5 nm owing to the CVD synthesis method used.[25] The SWNTs coexist with some very small FeCo@C NPs, which were observed by AFM, but not by TEM, because of the low contrast. The SWNTs were all found at the top layer (lowest media density) because they had almost the same net density as a 20% iodixanol solution (ca. 1.1 $g\,cm^{-3}$). This result indicates that the density gradient method could be used for separation of particles with different compositions, provided their net densities were very different.

It is notable that the method is versatile for various NPs, and can be done in a significantly shortened time (e.g. 15 min), if the separation resolution is not critical, and if the size variation between colloidal particles is large. As an example, we applied the rate sedimentation method to three commercially available gold nanoparticles with diameters of 5, 10, and 20 nm, and their respective mixture. The resulting centrifuge vessels containing Au NPs after ultracentrifugation for 15 min are shown in Figure 4A. As the three standard Au NPs have highly uniform sizes, they were observed in the gradient as single bands, at the interfaces of 0–30, 30–40, and 50–60% iodixanol, respectively. The sharp density/viscosity increase at step gradient boundaries was found to concentrate the nanoparticles locally. As expected, the mixture of Au NPs separated into visible bands along the centrifuge tube after 15 min of centrifugation. The locations corresponded to individual 5, 10, and 20 nm samples, as shown in Figure 4A. Further characterization and comparison by TEM analysis of unseparated Au NP standard samples (Figure 4B–D) and fractions after separation (Figure 4E–G) showed the recovery of the standard samples.

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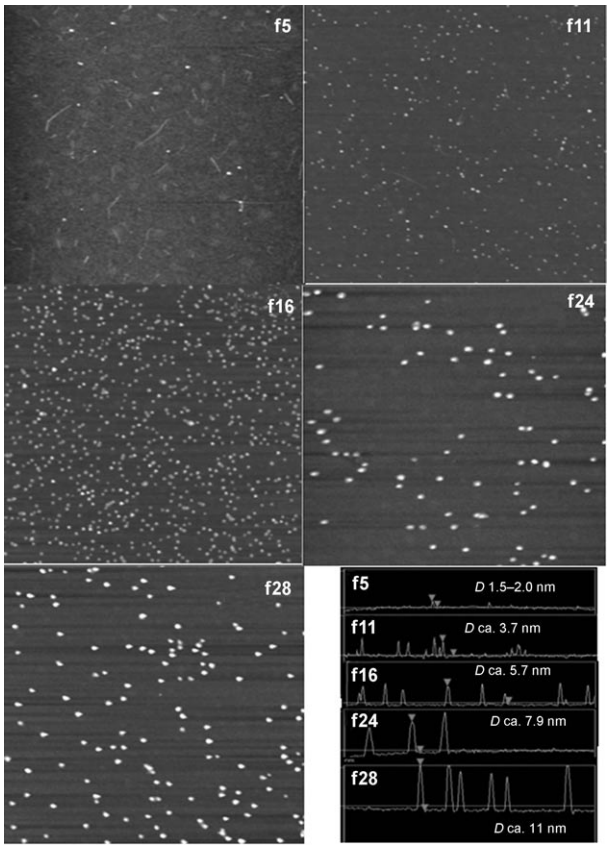

Figure 3. AFM characterization of separated 7 nm FeCo@C NP fractions. Note that the image of f5 is flattened, with total height 10 nm, whereas other images were obtained by flattening with total height 20 nm, to show contrast (full image size: 2 μm). The topographical height profiles of the fractions are compared bottom right. ▼ = measurement positions, D = nanoparticle diameter.

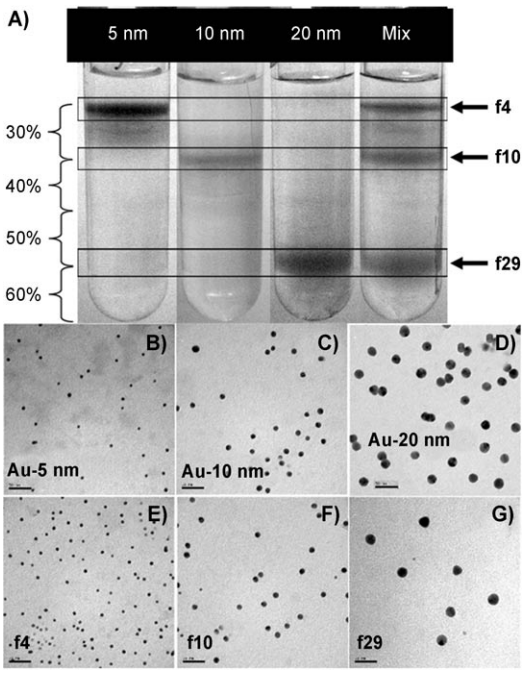

Figure 4. DGR separation of Au NPs and recovery of mixed Au NPs. A) Digital camera images of ultracentrifuge vessels containing Au NPs. From left to right: 5 nm, 10 nm, 20 nm, and mixed. B)–D) TEM images of standard 5 nm, 10 nm, and 20 nm Au NPs; E)–G) TEM of images of fractions f4, f10, and f29 following separation of a mixed Au NP colloidal suspension, demonstrating unmixing of the original Au NPs. Scale bars: 50 nm.

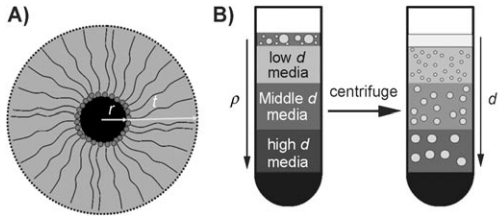

Figure 5. Illustration of the separation mechanism. A) A hydrated colloidal nanoparticle structure. B) The separation of different-sized colloids in a multilayer step density gradient. d = sedimentation distance.

In this experiment, the NP separation was realized far from equilibrium density, as the colloid net density was much higher than that of the medium. This result shows three outstanding features of DGR separation in comparison with the isopycnic method: First, density of the colloid of interest can be much higher than that of the gradient, significantly broadening the number of applicable systems for separation. Second, much shorter time is required for separation (e.g. 15 min), compared with a typical isopycnic separation, requiring about 12 h of ultracentrifugation. Third, parameters such as centrifuge time and gradient densities can be adjusted to target a specific size range.

It is noteworthy that the results of Au NP rate separation suggest the possibility of utilizing ultracentrifuge rate separation as a novel method to perform analysis on colloid size distributions in a suspension. Given appropriate standards the technique may be quantitative, in a similar fashion to, for example, chromatography, in which unknown molecules must be compared to molecules with standard molecular weights.

Quantitatively, a theoretical assay would be important for improving and directing such separation and analysis applications. Current data can be understood by the density [Figure 5 A, Equation (1)] and sedimentation rate difference of NPs when forced through a medium with given density and viscosity. For ideal spherical nanoparticles with core density ρ_c, radius r, and hydrated shell thickness t (Figure 5 A), and given that the hydrated shell has the density of water, 1 g cm^{-3}, the net density ρ_p may be estimated as:

$$\rho_p = 1 + (\rho_c - 1)r^3/(r+t)^3 \quad (1)$$

It can be deduced from the above formula that the net density of a colloidal system would increase when the core size increases with respect to hydration shell thickness, and the particle density becomes equal to the core material density when the NP is large enough (i.e. $r \gg t$).

All NPs would have a tendency to sediment when they are layered on top of step gradient layers and driven by centripetal force. Their terminal velocity is determined by particle radius r, net density ρ, and centrifugal force g' in a

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given layer by balancing the centrifugal force against buoyancy and viscous drag.[20] An increase in one or more of these parameters (r, ρ, g') would make colloidal nanocrystals sediment faster, and favor separation.

Step-layer gradients are used herein (Figure 5B, left) to afford the following effects: 1) The sharp increase in density and viscosity at the boundaries can work like a screen to slow down the NPs with small radius and small densities. Large NPs can go through to the succeeding layers, which affords separation; 2) the increased medium density and viscosity in the succeeding layers can slow down the sedimentation of larger NPs, allowing most NPs to be captured within the layer without aggregation or pelletting; and 3) ideally, a linear dependence on particle radius increase can be obtained along the centrifuge tube (with increasing density), when appropriate layers are used and the centrifugation is stopped at an appropriate time point (Figure 5B, right), affording the highest resolution among fractions sampled along the centrifuge tubes. Under our optimized conditions (Figure 1A, 2A, and 4A), the curves showing the increase in NP diameter along the centrifuge tubes were roughly linear (See Supporting Information, Figure S2).

In summary, a DGR separation method was used for separation of nanocrystals by taking advantage of the difference in their sedimentation rate. The method provides resolution for separation of colloid particles of different compositions, in different size ranges, and of different aggregation states in much shorter time. The method is versatile, scalable, efficient, and non-destructive. Furthermore, the separation provides a potential novel analytical method to identify the colloid size distribution in a suspension by comparison against standards.

Experimental Section

Separation of FeCo@C NPs (a more detailed procedure is given in the Supporting Information): FeCo@C nanoparticles with average sizes of 4 nm and 7 nm were thouroughly sonicated in an aqueous solution of the surfactant PL-PEG (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyetheyleneglycol)-5000]), yielding surfactant-wrapped, mostly individual particles. A four-layer gradient (10 + 20 + 30 + 40% for 4 nm, 20 + 30 + 40 + 60% for 7 nm NPs) was used for separation. A layer of 0.2 mL of NP suspension was added on top of the multilayer aqueous iodixanol density step gradient, and centrifuged at ultrahigh speed (ca. 240 kg) for several hours (see photos of the ultracentrifuge tubes after separation in Figures 1A and 2A). The transparent centrifuge vessels were imaged every 30 min, and the centrifuge was stopped after 3.5 or 2.5 hours because this time point yielded maximal displacement resolution of the nanoparticles. The gradient media containing separated NPs was manually sampled and fractioned (100 μL each) from the centrifuge-vessel for characterization.

Separation of Au NPs:Three commercially available Au NPs with diameters of 5 nm, 10 nm, 20 nm, and their mixture were wrapped with thiolated PEG by sonication. A four-layer density gradient (30 + 40 + 50 + 60%) was used for separation, and following sample loading, was centrifuged for only 15 min (see resulting centrifuge tubes in Figure 4A).

Characterization: After purification, fractions were characterized with tapping mode AFM and TEM (Philips CM20, 120 kV) following the typical procedure,[22,24] as shown in the Supporting Information.

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