Density Gradient Separation of Gold Nanorods

Using the New Avanti JXN Centrifuge & JS-24.15 Swinging Bucket Rotor

Abstract

Gold nanoparticles have enjoyed increasing popularity over the past decade in the area of biomedical sciences, especially for tumor imaging,[1] photothermal therapy,[2,3] and metal-enhanced fluorescence.[4] High-quality gold nanoparticles with monodisperse sizes and aspect ratios are needed for these applications. In this app note, we show a simple density gradient method using a high-speed Avanti JXN centrifuge to purify monodisperse gold nanorods from a polydisperse sample.

Introduction

Gold Nanorods (AuNR) hold great promise for biomedical imaging. AuNRs have very strong absorption peaks in the visible and near-infrared region due to a plasmonic effect; the aspect ratio of the AuNR directly determines the wavelength of the peak. For biomedical imaging, it is important to have optically pure samples of gold nanorods, which require physical purity as well. However, the synthesis process of AuNRs typically leads to some impurity in the form of gold nanospheres (which fail to elongate) and non-optimal AuNRs with slightly different aspect ratios. Because AuNRs and gold nanospheres (AuNS) have the same constituent element, the same surface coating from synthesis (the surfactant CTAB in most cases), and similar sizes, separation becomes a major issue.

Density Gradient Centrifugation (DGC) is highly capable of separating nanoparticles with similar sizes but varying densities due to slight shifts in surface area/volume ratios. In this application note, we worked with two samples of pure AuNRs, one 10 nm x 41 nm with 800 nm plasmon (4.1 aspect ratio); the other 25 nm x 60 nm with 650 nm plasmon (2.4 aspect ratio). The samples were mixed together and then later separated with a single-step DGC on the new Avanti JXN instrument using a JS-24.15 rotor. Based on optical spectroscopy, the separated AuNR samples were as pure as the original samples before mixing.

Protocol

Density Gradient Centrifugation of Gold Nanoparticles

Gold Nanorods (AuNR) of 10 nm diameter (808 nm plasmon peak) and 25 nm diameter (650 nm plasmon) were concentrated to 0.05 mL, by pelleting 3 mL of each in the Beckman Coulter Microfuge 16 at 10,000 x *g* for five minutes and then resuspending them in water with 0.01 CTAB. The density gradient was set up manually in 15 mL polyallomer centrifuge tubes (P/N 361707) as shown below:

Gradient Number	Material	Volume (mL)
1	0.01 M CTAB, 10% sucrose	2
2	0.01 M CTAB, 15% sucrose	4
3	0.01 M CTAB, 20% sucrose	4
4	0.01 M CTAB, 25% sucrose	4

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Both samples of AuNR were sonicated for five minutes (Branson M1800 sonicator), and then mixed and layered on top of the density gradient. They were centrifuged for 15 minutes at 10,750 x *g* at 25° C using a JS-24.15 rotor in the Beckman Coulter Avanti JXN-30. The acceleration and deceleration rates were set to 3. After the run, fractions were collected with fraction volume of 300 µl each. The fractions were scanned for peaks using Paradigm and were pooled based on the 808 nm and 650 nm peaks. Buffer exchange was done by pelleting the pooled fractions using Microfuge 16 and resuspending them in 0.01M CTAB. This step was repeated three times, and final suspension of the pellet was done in 250 µl of 0.01M CTAB. Spectrophotometer readings using a DU 800 were taken of the collected peaks, as well as the mixed sample, before centrifugation to look for the separation.

Reagent	Manufacturer	Part Number
Gold nanorods, 10 nm	Sigma	716820
Gold nanorods, 25 nm	Sigma	771686
Sucrose	Sigma	84097
CTAB	Sigma	H9151

Step 1
Gold nanorods were bought in liquid form dissolved in 0.1M CTAB (Sigma).

Step 2
Microfuge 16 was used to pellet the gold nanorods and redissolved to concentrate them.

Step 3
Avanti JXN-30 centrifuge was used for density gradient run.

Step 4
Absorption spectrum to identify the purity of each fraction (Paradigm, Molecular Devices).

Step 5
Microfuge 16 to concentrate the selected fractions.

Step 6
DU 800 to check for the absorption spectra of the isolated samples.

Results

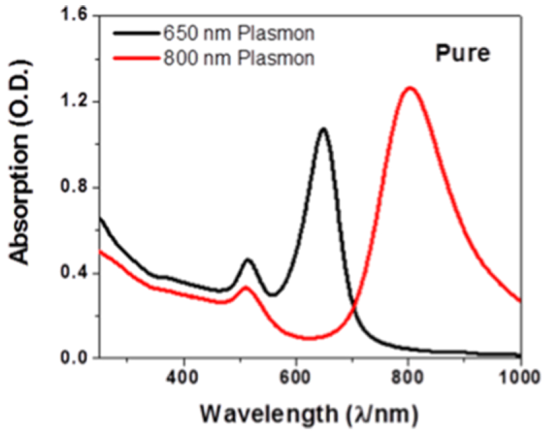


Figure 1a. Absorption spectroscopy of the samples of gold nanorods *before* mixing them together.

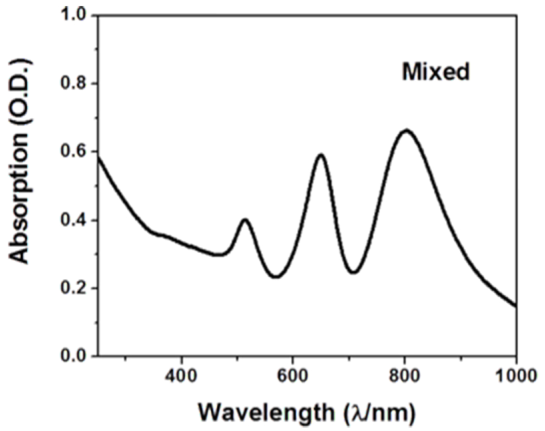


Figure 1b. Absorption spectroscopy of the pure samples of gold nanorods *after* mixing them together before Density Gradient Centrifugation.

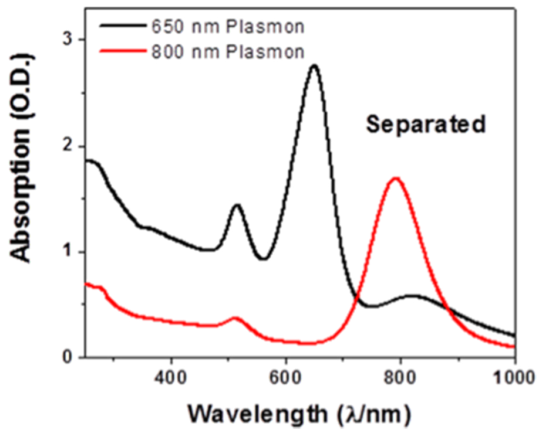
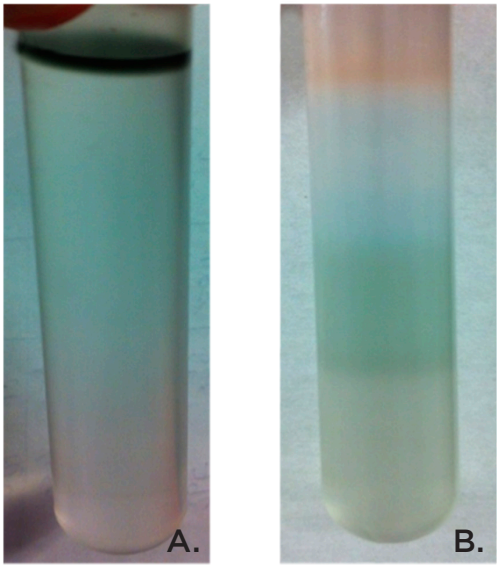


Figure 2. Absorption spectroscopy of separated samples of gold nanorods after Density Gradient Centrifugation.



Before After

Figure 3. Images of centrifuge tubes with gold nanorods: (a) *before* Density Gradient Centrifugation; and (b) *after* Density Gradient Centrifugation.

Conclusion and Discussion

After analyzing the pure samples by absorption spectroscopy (Figure 1a), we mixed the samples together and reanalyzed the absorption spectrum (Figure 1b). A test of the purity of the AuNR samples can be conducted by analyzing the longitudinal plasmon peak absorption (800 nm and 650 nm for our samples) with the transverse plasmon peak absorption (515 nm for both samples). In the case of the pure samples, the 650 nm plasmon AuNRs had an absorption ratio of 2.32 when comparing 650 nm with 515 nm. For the 800 nm plasmon AuNRs, the ratio of 800 nm to 515 nm was 3.85. After fractionating the centrifuged mixture and collecting optically pure samples, we reanalyzed the absorption spectrum (Figure 2). It was observed that the 650 nm/515 nm ratio was at 1.91—nearly as high as the pure 650 nm plasmon sample. Interestingly, for the 800 nm plasmon sample, the 800 nm/515 nm ratio was 4.54—even higher than the pure sample. This indicates that some AuNS contamination was present in the original, pure 800 nm plasmon sample that was separated out by the DGC run.

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