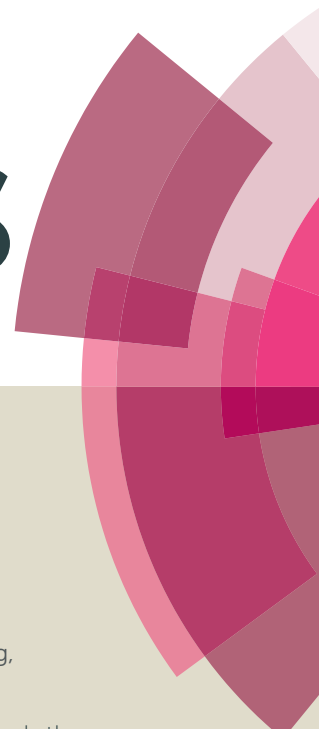


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Rapid Separation of Gold Nanorods in Multilayer Aqueous Systems via Centrifugation

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In this work, we demonstrate the multilayer aqueous systems as media to separate gold nanoparticles of different shapes and sizes. The multilayers aqueous systems are composed of CTAB/EG solutions with different viscosities. By centrifugation, gold nanoparticles with different shapes and sizes can be dispersed in different layers of the aqueous systems. Gold nanorods synthesized by different conditions, such as changing the amount of AgNO₃ or different aging times, are used to examine the multilayer aqueous systems. Gold nanorods with higher aspect ratios can be obtained by increasing the concentration of AgNO₃, as confirmed by UV-Vis spectra and TEM studies. The separated gold nanorods are also characterized by surface-enhanced Raman scattering (SERS), showing lower activity for gold nanorods with higher aspect ratios, due to the size-dependent absorption of the longitudinal plasmon band. The multilayer aqueous systems can effectively separate gold nanorods prepared by different conditions and can be applied to separate other types of nanoparticles.

Noble metal nanoparticles have the unique ability to guide and localize light at the nanometer scale, a feature at the heart of the field of plasmonics. Upon exposure to light, nanoparticles exhibit free electron oscillations referred to as localized surface plasmon resonances.¹ The resonance energy of the nanoparticle plasmon is sensitive to the particle size, shape, local environment, and crucially, proximity to other plasmon-sustaining nanoparticles.^{2,3}

Gold nanorods (Au NRs) are anisotropic nanoparticles that exhibit well-defined optical properties and have attracted much

attention in biomedical applications such as bioimaging,⁴ biosensing,⁵⁻⁷ and photo-thermal therapy.⁸ They can support a longitudinal surface plasmon with a strong extinction peak in the red end of the visible or the near-infrared region of the spectrum. For each distinct axis, nanorods support a unique plasmon mode, where the energy of each resonance is dependent on the sizes and aspect ratios of the nanorods. For prolate nanorods, there are two distinct resonances, the transverse and longitudinal, corresponding to electron oscillations along the short and long axes of the nanorods. For nanorods aligned end-to-end and spaced less than their width, the individual longitudinal dipole plasmons couple strongly, resulting in a lowering of the resonance energy.^{9,10}

In the past years, significant progress has been made on controlling the sizes and shapes of gold nanorods.¹¹⁻¹³ Bottom-up methods are usually used, and the size and shape control of gold nanorods is at scales of nanometers to tens of nanometers. For adjusting the aspect ratios, there are two main approaches including anisotropic oxidation and transverse overgrowth. In the anisotropic oxidation approach, the aspect ratios of gold nanorods can be tuned by shortening the lengths selectively via laser heating, thermal heating, or cyanide dissolution. For changing the shapes of the gold nanorods, the seed-mediated growth method combining with subsequent chemical modifications can be used, resulting in gold nanorods with various head shapes.¹¹

Although many efforts have been made for optimizing the conditions to synthesize gold nanorods, the as-produced dispersions usually contain polydisperse gold nanorods and spherical nanoparticles. For example, the commonly used seed-mediated synthesis usually produce gold nanostructures with different shapes.¹² In a representative synthesis, gold nanospheres, rather than gold nanorods, may comprise 5-20% (w/w) of the synthesized nanostructures.¹⁴ To establish the relationship between shapes and properties of gold nanorods and to explore the usage of nanorods in technological applications, it is highly desirable to have subpopulations of purified nanorods from a synthesized mixture.

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Sedimentation by centrifugation using suitable solvents, such as water and toluene, is a straightforward method to collect the reaction products generated in the synthesis of gold nanorods.¹⁵ Multiple rounds of centrifugation, however, are usually necessary to separate byproducts from nanorods.^{16, 17} Other techniques used to separate monodisperse nanorods from mixtures are either time-consuming (e.g., size exclusion chromatography) or require secondary chemical modification of the nanoparticles (e.g., gel electrophoresis).^{18, 19}

In this work, we report a simple and efficient method to separate gold nanoparticles of different shapes and sizes using multilayer aqueous systems. The multilayer aqueous systems are composed of hexadecyltrimethylammoniumbromide and ethylene glycol (CTAB/EG) solutions with different viscosities. After centrifugation, gold nanoparticles with different shapes and sizes can be dispersed in different layers of the aqueous systems. Gold nanorods synthesized by the seed-mediated growth method under different experimental conditions are used as a model example and purified through the multilayer aqueous systems. The separated gold samples are examined by UV-Vis spectrometer, SEM, and TEM. The separated gold nanorods shows size and aspect ratio dependent plasmon resonance absorption and surface enhanced Raman scattering (SERS) activity. This approach of using the multilayer aqueous systems provides a facile way to separate gold nanorods with desired aspect ratios with narrow size distributions.

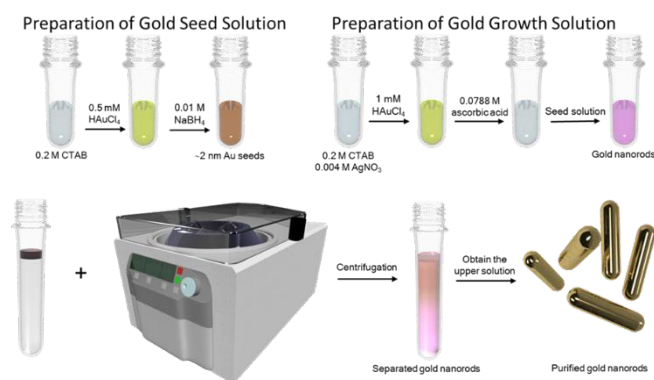


Figure 1. Experimental scheme to synthesize and separate gold nanorods.

Figure 1 shows the experimental scheme to synthesize the gold nanorods using the seed-mediated growth method.²⁰ The seed-mediated surfactant-assisted synthesis of the gold nanorods relies on the initial preparation of gold nanoparticles formed by mixing aqueous solutions of hydrogen tetrachloroaurate (III) hydrate, CTAB, and sodium borohydride.²⁰ These gold nanoparticles serving as seeds are then added to a growth solution which contains concentrated hydrogen tetrachloroaurate (III) hydrate, CTAB, silver nitrate, and *L*-ascorbic acid.²¹ *L*-Ascorbic acid is a weak reducing agent that can induce heterogeneous deposition of gold on the surface of the seed nanoparticles. The facet-selective deposition of gold is promoted by silver ions, which can be described by an underpotential deposition

(UPD) mechanism, resulting in the growth of anisotropic gold nanorods.²² It has been reported that the aspect ratios of the gold nanorods can be increased up to 4.5 by increasing the silver concentration.²¹ Without the presence of silver ion from the reactions, only a very low yield of gold nanorods can be obtained. The surface of the nanorods are covered by CTAB, which prevents the aggregation of nanorods;²³ it is also believed that CTAB assemblies around the gold nanorods in the form of micellar or bilayer structures. After the synthesis process, the gold solution is concentrated and added on top of the multilayer aqueous systems. Upon centrifugation, the gold nanostructures with different shapes and sizes can be separated in the multilayer aqueous systems.

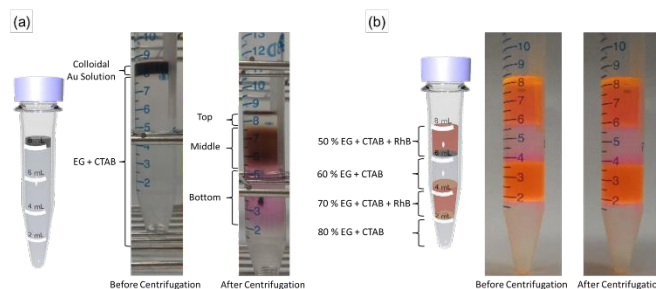


Figure 2. (a) Separation process of gold samples using the multilayer aqueous systems. (b) Demonstration of the multilayer aqueous systems using a rhodamine dye (RhB 6G).

The detail of the separation process using the multilayer aqueous systems is demonstrated in Figure 2a. The multilayer aqueous systems are composed of CTAB solutions mixing with EG at different ratios (50, 60, 70, and 80 %), providing a density gradient for separating the gold nanostructures. For example, the 80% CTAB/EG solution is prepared by mixing CTAB and EG with the volume ratio of CTAB:EG = 1:4. EG is selected among common solvents because it is water soluble and has a higher density than water ($\rho_{EG} = 1.11 \text{ g/cm}^3$); CTAB is also added in each solution to prevent aggregation of gold nanostructures during the separation process. The four layers of CTAB/EG solutions form the multilayer aqueous systems with a gradient of density and viscosity. The gold solutions are added on the top of the multilayer aqueous systems. After the centrifugation process for 10 min, the gold nanostructures can be dispersed in different layers of the aqueous systems. Later, different portions of the aqueous systems can be extracted. In this work, the purified gold nanorods are mainly located at the middle section and can be easily extracted.

To confirm the feasibility of the multilayer aqueous systems and that the interlayer diffusion in the systems is restricted, a rhodamine dye (RhB 6G) is added in the first and the third layers (50% and 70%) of the multilayer aqueous systems as a probe material, as demonstrated in Figure 2b. Before centrifugation, the first and the third layers with the RhB 6G in the centrifuge tube can be observed. After the centrifugation process at 12000 rpm for 10 min at 20 °C, the layered solution is still maintained, indicating that the interlayer diffusion layer in the multilayer aqueous systems is highly restricted

and that the concept of using the multilayer aqueous systems is feasible.

The formation of the gold nanostructures can be measured by the UV-Vis spectrometer with wavelengths ranging from 300 to 1100 nm, as shown in Figure 3. Figure 3a shows the UV-Vis absorption spectra of gold seed solution, in which a shoulder at ~ 500 nm is observed, similar to the results by previous studies.²¹ The particle sizes of the gold seeds are believed to be less than 4 nm when a similar experimental condition is implemented.²¹ After the seed solution is aged for 10 min, 20 μ L of the seed solution is added to 10 mL of the growth solution and the mixed solution is maintained at 30 $^{\circ}$ C without stirring for different times. The gold nanorods prepared by different conditions are purified using the multilayer aqueous systems. The UV-Vis spectra of the purified gold nanorods prepared at different aging times are shown in Figure 3b. Two absorption bands are observed for the gold nanorod samples. One is caused by the transverse oscillations of the electrons (transverse absorption band), which is typically located in the visible wavelength region; the other is caused by the longitudinal oscillations of the electrons (longitudinal absorption band).²⁴ The transverse absorption band can interfere with the absorption of spherical gold particles, while the longitudinal absorption band is shifted from the visible region to the near-infrared region with increasing aspect ratios of the gold nanorods. In Figure 3b, the higher intensities of the transverse absorption band at longer aging times without obvious shifting in the peak position indicate the formation of more gold nanorods with similar aspect ratios at longer aging times.

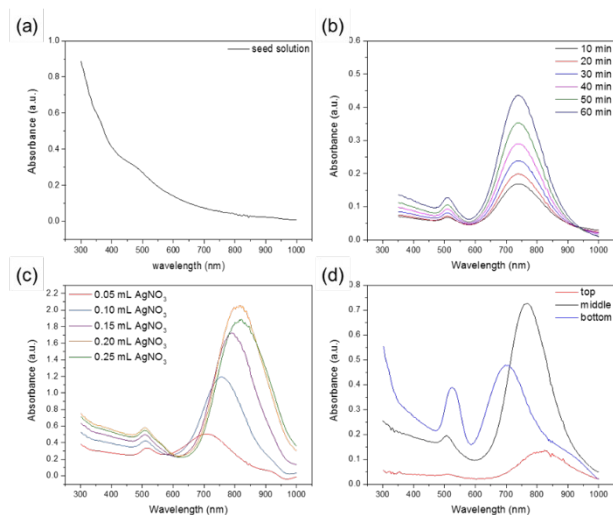


Figure 3. UV-Vis spectra of gold samples: (a) seed solution, (b) aging for different times, (c) using different amounts of AgNO_3 , and (d) extracting from different proportions (top, middle, and bottom).

The amount of silver ions also plays a critical role in the formation of gold nanostructures. The UV-Vis spectra of the purified gold nanorods prepared at different amounts of silver ions in the growth solutions using 0.004 M AgNO_3 are shown in Figure 3c. When the amount of the AgNO_3 solutions is increased from 0.05 to 0.25 mL,

the longitudinal absorption band is redshifted with a higher intensity, indicating the formation of gold nanorods with higher aspect ratios. The effect of the silver ions on the aspect ratios of gold nanorods using the seed-mediated growth method has been studied and discussed previously.²¹ The cause of the size dependence on the silver ions, however, are still unclear.

In our experiment, the CTAB-capped seed is used in the synthesis process and gold nanorods are formed. For other works using citrate-capped seed in the synthesis process, gold nanoparticles with other shapes can be prepared, whose UV-Vis spectra are different from the results we observed here because of the different geometries of the gold nanostructures.¹⁴

To further confirm that the multilayer aqueous systems can effectively purify the gold samples, UV-Vis measurements are conducted for different proportions of the layered solutions. For example, the gold solution prepared using 0.02 mL of 0.004 M AgNO_3 and aged for 60 min is purified by the multilayer aqueous systems and the different proportions (top, middle, and bottom) are extracted and examined by UV-Vis spectrometer, as shown in Figure 3d. For the top layer, the peak position and lower intensity of the UV-Vis spectrum indicate that the top layer contains fewer gold nanoparticles and gold nanorods with short lengths. For the middle layer, the near-IR absorption with the highest intensity in the UV-Vis spectra demonstrates that there are much more gold nanorods in this proportion. For the bottom layer, two absorption peaks, one at ~ 520 nm and the other at ~ 710 nm, are observed, indicating that there are a lot of gold particles in the bottom proportion. The UV-Vis spectrum of the bottom proportion is in agreement with the purple color exhibited in the bottom proportion of the layer solution shown in Figure 2a. The purple color of the bottom proportion also implies that there are at least 50% byproducts, according to previous studies.²⁵

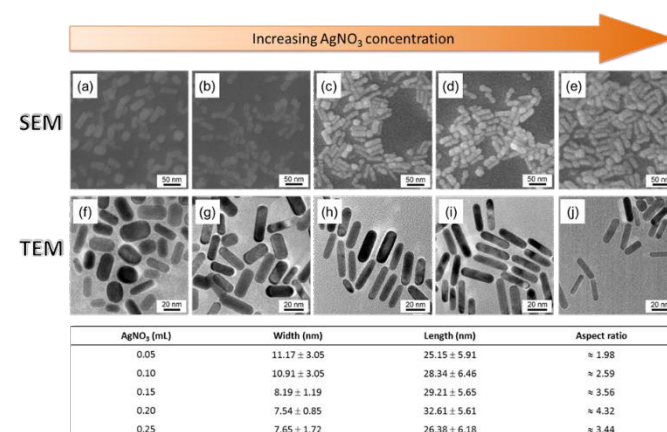


Figure 4. (a-j) SEM and TEM images of gold nanorods using different amounts of AgNO_3 : (a, f) 0.05, (b, g) 0.10, (c, h) 0.15, (d, i) 0.20, and (e, j) 0.25. The widths, lengths, and aspect ratios of gold nanorods synthesized using different amounts of silver ions are also summarized.

In addition to UV-Vis absorption measurements for studying the effect of different experimental conditions on the formation of gold nanorods, SEM and TEM measurements are also conducted. For example, Figure 4 shows the SEM and TEM images of purified gold nanorods prepared using different amounts of silver ions (0.004 M AgNO₃, 0.05 to 0.25 mL). Both the SEM and TEM data show that the aspect ratios of the gold nanorods increase with the amounts of silver ions. The widths, lengths, and aspect ratios of gold nanorods synthesized using different amounts of silver ions are also summarized in Figure 4. The lengths of the gold nanorods increase from ~25.1 to ~32.6 nm as the amount of AgNO₃ increases from 0.05 to 0.20 mL. The length, however, decrease slightly to ~26.3 nm at 0.25 mL of AgNO₃. It has to be noted that the data shown in Figure 4 are obtained from the middle layers of the aqueous systems. The SEM images and size distributions of gold nanostructures in different layers using 0.15 mL AgNO₃ are also included in the supporting information.

It has been studied that the silver ions have a negative effect at higher concentrations on the size of the gold nanorods, which is probably due to the interactions of the silver ions with the bromide counterions of the surfactants.²¹ But the ability of the silver ions in the mechanism of the seed-mediated growth method is still unclear. If the rod-like gold is caused by the catalytic activity of the silver ions, only the number of gold nanorods is affected and the length of gold nanorods should not be increased. Still, the length of the gold nanorods can be controlled by the concentration of the silver ions.

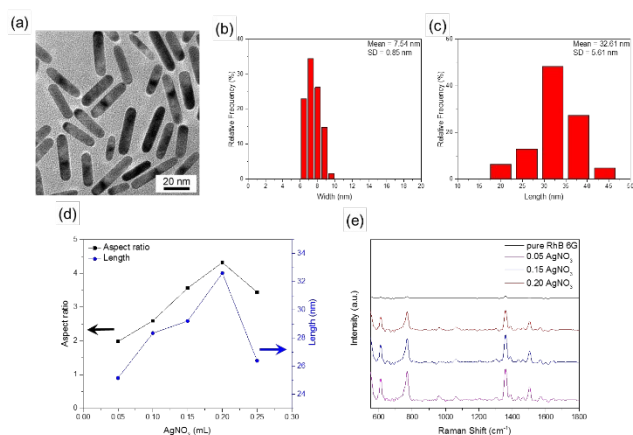


Figure 5. (a-c) Gold nanorods using 0.20 mL AgNO₃: (a) TEM image, (b) distribution of the width of the gold nanorods, and (c) distribution of the length of the gold nanorods. (d) Plot of the aspect ratios and length of the gold nanorods versus the amount of AgNO₃. (e) SERS data of RhB 6G (10⁻⁶ M) on different gold samples, in which the SERS spectra are collected from five different regions for each sample.

For the different amounts of AgNO₃, the gold nanorods with the highest average aspect ratio (~4.32) are obtained using 0.20 mL AgNO₃, whose TEM image and size distributions of the gold nanorods are shown in Figure 5a-c. The average width and length of the gold nanorods are ~7.56 and ~32.6 nm, respectively. The TEM images and size distributions of the gold nanorods using other amounts of AgNO₃

are shown in the supporting information (Figure S2-S5). Figure 5d shows the plot of the aspect ratios and lengths of the gold nanorods versus the amount of AgNO₃. The aspect ratio increases from ~1.98 to ~4.32 when the amount of AgNO₃ increases from 0.05 to 0.20 mL; when the amount of AgNO₃ further increases to 0.25 mL, the aspect ratio decreases to ~3.44. The dependence of the aspect ratios on the amount of the silver ion is similar to that of the lengths. The lengths increase from ~25.15 to ~32.61 nm when the amount of AgNO₃ increases from 0.05 to 0.20 mL; when the amount of AgNO₃ further increases to 0.25 mL, the length decreases to ~26.38 nm. The negative effect at higher concentration of AgNO₃ is probably due to the interactions between the silver ions and the bromide counterions of the surfactants.²¹

For the gold nanorods, it can also be seen that the highest intensity of the maximum absorption peaks occurs at samples with the highest aspect ratio (~4.3), where the wavelength is extended to ~820 nm, as shown in Figure 3c. Because of the strong absorption in the near-infrared region, the prepared gold nanorods are used for SERS experiments. RhB 6G is chosen as the probe material for its well defined Raman spectrum. Figure 5e shows the SERS data of RhB 6G (10⁻⁶ M) on different gold samples, in which the SERS spectra are collected from five different regions for each sample. For RhB 6G (10⁻⁶ M) without gold nanorods, the blank sample, only weak signals are observed. The Raman signals, corresponding to the characteristic peaks of RhB 6G,²⁶ are strongly enhanced when the dyes are deposited on gold nanostructures. It can be seen that the degrees of enhancement are higher for gold nanorods with lower aspect ratios, similar to the previous work.²⁷

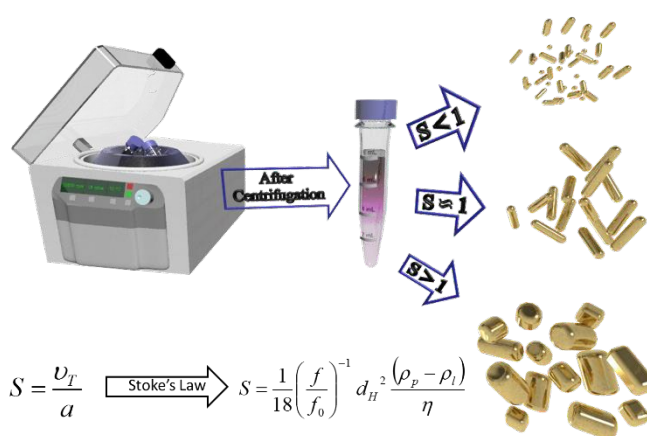


Figure 6. Operation mechanism of separating gold nanostructures by the multilayer aqueous systems.

The formation mechanism of the multilayer aqueous systems is related to the sedimentation coefficient of the solutions, as illustrated in Figure 6. The gold nanostructures can be redispersed into different layers during the centrifugation process. The ratio of the sedimentation coefficients of gold nanoparticles with two

different shapes are considered.²⁸ The sedimentation coefficient (S) can be defined by eq 1:

$$S = \frac{V_T}{a} \quad (1)$$

where V_T is the terminal velocity of the gold nanostructures in the multilayer aqueous solution and a is the acceleration of the gold nanostructures which is supplied by gravity or centrifugation. According to the Stokes' law, the sedimentation coefficient of gold nanospheres (S_s) can be defined by eq 2:

$$S_s = \frac{1}{18} d_H^2 \frac{(\rho_p - \rho_l)}{\eta} \quad (2)$$

where η is the viscosity of the medium, d_H is the diameter of the gold nanosphere, ρ_p is the density of the objects, and ρ_l is the density of the liquid medium. Eq 2 can be further modified to eq 3 for obtaining the sedimentation coefficients of anisotropic gold particles:

$$S = \frac{1}{18} \left(\frac{f}{f_0}\right)^{-1} d_H^2 \frac{(\rho_p - \rho_l)}{\eta} \quad (3)$$

where f/f_0 is the frictional coefficient of anisotropic particles and d_H is the diameter of a nanosphere of equivalent volume to the object. Hubbard and Douglas have calculated the frictional coefficient for a rod with a diameter of d and a length of l :

$$\frac{f}{f_0} = 0.55 \left(\frac{l}{d}\right)^{-\frac{1}{3}} (1 + 0.869 \left(\frac{l}{d}\right)^{0.76}) \quad (4)$$

where the effective values of l/d are between 0 to 8.²⁹ Therefore, gold structures with two different shapes can be separated using centrifugal force when their sedimentation ratios are different than 1.

The separations behavior of the gold nanostructures in the multilayer aqueous systems depends on the differences of the hydrodynamic components of the nanostructures via centrifugal force. For two different gold nanostructures to pass through a layer of multilayer aqueous systems, the densities of the two objects (ρ_1 and ρ_2) have to be higher than that of the layer (ρ_l). In addition, the interfacial tensions between different layers of the multilayer aqueous systems have to be taken into consideration for the separation process.³⁰ For gold nanostructures moving through the interfaces of different layers in the multilayer aqueous systems, the interfacial tensions can pose non-trivial barriers for continuous sedimentation process. Such energy barriers can be overcome by choosing appropriate object densities (ρ_p), particle sizes (d_H), and

relative centrifugal forces (RCF). The relationships between the variables for ensuring that the gravitational energies (E_g) are much greater than the surface energies (E_s) can be estimated using a simplified comparison of energies.³⁰

$$E_g \gg E_s \quad (5)$$

The gravitational energies (E_g) and the surface energies (E_s) can be approximated as the following.

$$E_g \approx (\rho_p - \rho_l) RCF \times g d_H^4 \quad (6)$$

$$E_s \approx \gamma d_H^2 \quad (7)$$

Therefore, larger RCF is critical in the separation process of the gold nanostructures to overcome the effect of the interfacial energies. In this work, the centrifugation processes are conducted at 10000 rpm, in which the RCF is large enough for effective separation of the gold nanorods.

Conclusions

In conclusion, we develop a hyper process to effectively separate gold nanostructures with different sizes and shapes using multilayer aqueous systems. The multilayer aqueous systems contain stable layers of CTAB/EG solutions with different viscosities, allowing good dispersion of gold nanostructures with different shapes. CTAB plays a critical role in the multilayer aqueous systems for preventing the diffusion of the different layer of solutions. The purified gold nanostructures are examined by UV-Vis spectrometer, SEM, and TEM images, confirming the formation of the gold nanorods with different aspect ratios. SERS experiments are also conducted using RhB 6G dyes on different gold nanorod samples, showing that higher SERS intensity can be obtained for gold nanorods with lower aspect ratios. The multilayer aqueous systems can be applied to large scale separation process and to separate other types of nanoparticles.

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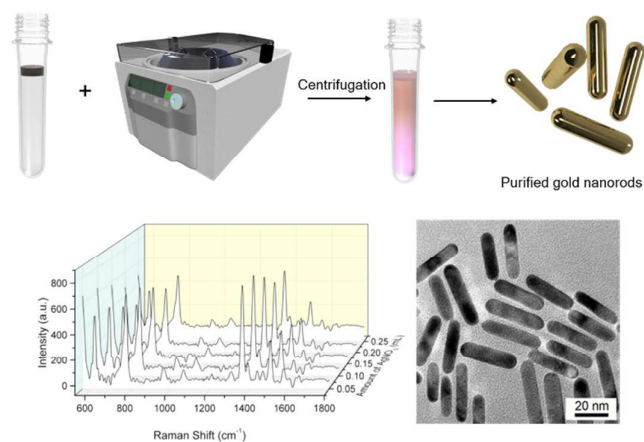
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A rapid strategy to separate gold nanorods in multilayer aqueous system via centrifugation.