The shape separations of suspended gold nanoparticles were investigated using size-exclusion chromatography. The separations in shapes were identified by examining the 3-D chromatograms obtained by employing a diode-array detection system and were further confirmed by analyzing TEM images of fractional collection of particles. This shape separation was achieved by adding a mixed-surfactant system containing sodium dodecyl sulfate and polyoxyethylene (23) dodecanol (Brij-35) into the eluent, which apparently affects the adsorption behaviors of both rodlike and spherical Au nanoparticles onto the column packing materials. While the overall particle gross sizes of these two shapes were similar, the baseline resolution was unfortunately not obtainable. However, the absorption spectra from the diode-array detector could be utilized to interpret the shapes of Au nanoparticles. The potential capability for the size separation of Au nanoparticles by size-exclusion chromatography with diode-array detection was also demonstrated.

Shape Separation of Nanometer Gold Particles by Size-Exclusion Chromatography

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Nanometer-sized metal materials have attracted broad attention in the fields of physics, chemistry, and biology. The particle size in this regime is generally an important factor that may dramatically affect the physical/chemical properties, such as in catalysis. Also, the shape-dependent properties of metal nanoparticles are evident and currently under extensive study. Therefore, many efforts in nanostructured preparations have been made to gain exquisite control of the size and the shape of various types of nanoparticle systems. Unfortunately, in many cases, the resulting nanoparticles are, to a certain extent, a mixture in terms of either sizes or particle shapes. Therefore, the needs of advanced separation techniques become demanding for these new types of materials. Transmission electron microscopy (TEM) has been widely employed to characterize the size and shape of metal nanoparticles. However, it does not involve any separating process, whereas chromatographic techniques can be utilized to separate the particle mixture and obtain monodispersed nanoparticles for further studies. Also, the size-dependent optical spectra of nanoparticles can be easily obtained by chromatographic systems with diode-array detectors (DAD). Hence, chromatographic methods are potentially applicable to facilitate the characterization of size- and shape-dependent properties of metal nanoparticles.

Size-exclusion chromatography (SEC) is a well-established method for the separation of macromolecules in solution according to their sizes. This technique was combined with TEM to characterize nanometer-sized spherical gold particles ranging in size from 3 to 20 nm successfully. It was also employed for the size analysis of semiconductor particles. The problem of applying SEC for the separation of nanometer particles is the irreversible adsorption of the particles by column packing material, due to the high surface area of stationary phase and high surface activity of nanoparticles. This problem limits the types of columns that can be used for the separation of nanoparticles. Capillary zone electrophoresis (CZE) was proposed to have the potential of reducing this problem by decreasing the surface effect of the separation system. However, CZE has the disadvantage of sample collection. SEC with sample collection can be used to obtain monodispersive nanoparticles that can be applied to study the shape-dependent physicochemical properties.

Addition of surfactant in the mobile phase was shown to reduce the adsorption problem of packing materials in our recent study for the separation of different sizes of spherical gold nanoparticles. In the present work, SEC is proposed for separating different shapes of nanometer-sized gold particles. As far as we know, the use of SEC for the separation of different shapes of metal nanoparticles has not been reported yet. In this report, separation conditions for spherical and rod shapes of gold nanoparticles by SEC are investigated. The effects of mixed surfactant on the elution behavior of gold nanoparticles are discussed. Also, the separation performance is demonstrated.
EXPERIMENTAL SECTION

Apparatus. A HP 1050 chromatograph with a HP-1100 DAD detection system and a HP Chemstation datastation (Hewlett-Packard, Palo Alto, CA) were used in SEC separation of gold nanoparticles. This detection system enables a simultaneous measurement of absorption from 190 to 950 nm (UV–vis–NIR). A HP auto sampler was used to inject a 10 μL sample each time. A Nucleogel GFC 1000-8 column (Macherey-Nagel, Düren, Germany) of 300 × 7.7 mm, that has a 100-nm pore size and an 8 μm particle size with a precolumn filter (0.45 μm, Rheodyne, Cotati, CA) were employed for the separations. The typical flow rate was maintained at 1.0 mL/min. For some other flow rates, it will be specified in the figure captions. Absorption spectra of gold particles were measured by a HP 8453 UV–vis spectrometer or HP-1100 DAD system. High-resolution TEM data were acquired on a Hitachi (Tokyo, Japan) HF-2000 field emission TEM operated at 200-kV accelerating voltage. Samples containing Au nanoparticles were prepared by dip coating of colloidal solution on Formvar/carbon film Cu grids (200 mesh, 3 mm) obtained from Argar Scientific Ltd. (Essex, England). Capillary electrophoresis experiment was performed on a Waters (Milford, MA) Quanta 1F2000 capillary electrophoresis system with 20-kV positive high-voltage. A Hg lamp with a 546-nm filter was employed for gold particle detection. Uncoated silica capillaries (Polymicro Technologies, Phoenix, AZ) of 75 μm i.d. was utilized. A detector window around 7.5 cm from the outlet was fabricated. Hydrodynamic injections for 60 s were employed, and then the measurement of electrophoretic mobility was carried out in 40 mM SDS and 10 mM CAPS at pH 10.0.

Reagents. Sodium dodecyl sulfate (SDS, purity >95%) and hexadecyltrimethylammonium bromide (CTAB, purity 99%) were provided by Sigma (St. Louis, MO). Polyoxymethylene (23) dodecanol (Brij-35) was obtained from E. Merck (Darmstadt, Germany). 3-[Cyclohexylamino]-1-propanesulfonic acid (CAPS) was provided by Sigma (St. Louis, MO). Necleogel GFC 1000-8 packing material was a gift from M acherey-Nagel (Düren, Germany). All chemicals were utilized as received. Eluents were filtered through a 0.45 μm filter from Alltech. (Deerfield, IL). Stock solutions of 100 mM SDS and 100 mM Brij-35 were prepared by dissolving required amounts of SDS and Brij-35 in water. Stock solutions and water were used to obtain different compositions of mobile phase by mixing each component at the required ratio from a HP-1050 quaternary pumping system.

Preparations of Suspended Au Nanoparticles. The suspended Au nanoparticles with different shapes in aqueous solutions were prepared by a newly developed electrochemical method. Briefly, a two-electrode type electrochemical electrolysis was employed to fabricate the Au nanorods with a small amount of nearly spherical-shaped Au nanoparticles. The anodic material was a gold metal plate (1 × 1 × 0.05 cm), and a platinum plate (1 × 1 × 0.05 cm) was used as the cathode in such a synthetic process. Both electrodes were immersed in a 3-mL electrolytic solution containing cationic surfactants and several other reagents to stabilize the thermodynamically unstable rodlike nanoparticles. A control-current electrolysis was used throughout the process for a typical current of 5 mA and a typical electrolysis time of 20 min.

The absorption spectrum and a TEM image of a typical sample (sample A) employed in most parts of this study are shown in Figure 1. The analysis of TEM images, as shown in Figure 1B, indicate that sample A contains mainly rod-shaped Au nanoparticles (~85%) coexisting with a small amount of spherical particles. Two absorption bands located at about 520 and 920 nm in Figure 1A characterize its absorption spectrum. The longer wavelength absorption band (920 nm) represents the longitudinal surface plasmon resonance (SPℓong) of gold nanorods, while the absorption band at short wavelength (520 nm) is mainly corresponding to the surface plasmon band of spherical gold nanoparticles. The contribution of the transverse surface plasmon resonance (SPtrans) of gold nanorods to the 520-nm band should be relatively small. For sample A, the mean aspect ratio (rod length over minor axis diameter) and the averaged minor axis diameter of Au nanorods are about 4.8 and 9.7 nm, respectively, while the mean diameter of spherical particles is about 19.3 nm. Also, spherical gold colloid with a mean diameter of 5.3 nm is obtained from Sigma (St. Louis, MO). It was produced by the reduction of 0.005% gold chloride (KAuCl4) solution with a mixture of trisodium citrate and tannic acid mixture.

(A) Absorption spectrum and (B) TEM image of the gold nanoparticles (sample A). The mean diameter of spherical particles is estimated to be 19.3 nm, while the short-axis diameter of rod particles is about 9.7 nm and the mean aspect ratio (long axis over short axis) is about 4.8 for gold nanorod from TEM image.

![Figure 1](image_url)

Figure 1. (A) Absorption spectrum and (B) TEM image of the gold nanoparticles (sample A). The mean diameter of spherical particles is estimated to be 19.3 nm, while the short-axis diameter of rod particles is about 9.7 nm and the mean aspect ratio (long axis over short axis) is about 4.8 for gold nanorod from TEM image.
RESULTS AND DISCUSSION

Surfactant Effects on the Adsorption of Nanoparticles. The problem of employing SEC for the separation of nanometer particles is the irreversible adsorption of the particles by column packing material. Different compositions of mobile phase were employed for the separation of gold nanoparticles (sample A) to investigate the adsorption problem. The chromatograms showing the effect of different compositions of mobile phase on the adsorption of nanoparticles were overlaid for easy comparison. The adsorption was so severe that no signal was observed with H₂O as the eluent, as shown in Figure 2A. With the addition of SDS as eluent, the signals of gold particles with different shapes were observed, but the resolution was low (Figure 2B). Also, the separation of rod-shaped and spherical-shaped gold nanoparticles could be enhanced with the use of mixed surfactant, as shown in Figure 2C. In our recent study, addition of SDS in the mobile phase is able to reduce the adsorption of spherical gold nanoparticles by packing materials. It is worth noting that spherical gold nanoparticles of the previous report were prepared by the reduction of K₂AuCl₄ with trisodium citrate/tannic acid mixture. The colloidal gold particles are surrounded by an electric double layer arising from absorbed citrate and chloride ions and the counterions that are attached to them. However, gold nanoparticles employed in this study are surrounded by attached cationic surfactants. Figure 2A indicates that different shapes of gold nanoparticles stabilized in cationic surfactant still encounter irreversible sorption problems. With the addition of SDS and Brij-35 in the mobile phase, the signals of differently shaped gold nanoparticles were detected. The results imply that the adsorption problem of rod-shaped gold nanoparticles stabilized with ionic surfactant can also be solved with the addition of surfactant in the mobile phase. To further confirm the arguments mentioned above, several Au nanoparticle solutions (sample B) were prepared and then diluted with water and surfactants to obtain similar nanoparticle concentrations (judged by the absolute absorbance at SPlong) at different compositions of the solvents. The compositions of the solvents are similar to the mobile phase composition in SEC separations. They were employed to investigate the properties of adsorption of the Au nanoparticles by the packing materials. The Au colloids in sample B were prepared following the same preparation procedure as described in the Experimental Section. The mean aspect ratio and the averaged short axis diameter of rod particles of sample B are estimated to be 3.8 and 8.3 nm, respectively, while the mean diameter of spherical particles is 16.0 nm from TEM images. These Au colloidal solutions were passed though the same SEC column packing materials supported on the top of a filter paper. The absorption spectra of the filtrates were measured by a DAD system. Since each sample solution was adjusted to the same concentrations, the absorbance of each filtrate should be the same if no adsorption occurs. The results, as shown in Figure 3A, clearly indicate that a severe adsorption of nanoparticles by column packing materials occurs and results in no spectral features of Au nanoparticles observed in the filtrate without SDS. Instead, the ruby-red color of gold particles was observed on the packing materials and could be stripped down by flushing the packing materials with SDS solution. Meanwhile, there are two absorption bands recorded for the spectra of the filtrates containing SDS and Brij-35 (as shown in Figure 3B-D). The absorption spectra are apparently similar to each other except that the overall absorbances vary. Notice that the λmax’s of the SPlong bands for sample B is to the blue of that in Figure 1, which is due to the Au nanorods in this case possessing a smaller mean aspect ratio. Figure 3 confirms that the adsorption of thus prepared Au nanoparticles by column packing materials can be eliminated, or reduced, with the use of SDS solution as the mobile phase.

With SDS in the mobile phase it is possible to induce the interaction of SDS with stationary phase and nanoparticles. The interaction between surfactant and stationary phase of chromatographic column was well documented. The interaction between packing material and surfactant is the adsorption of

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surfactant on the surface of packing material. Hence, the separation performance is changed. The interaction between surfactant and nanoparticles may also occur. It is possible that SDS molecules replace the positions of cationic surfactants after mixing gold nanoparticles with SDS solution. It is also possible that the interaction between nanoparticles and surfactant induces the alignment of surfactant around gold nanoparticles. The alignment of enough SDS molecules around gold nanoparticles, which are stabilized by cationic surfactants and behave like positively charged particles, alternates the particles to be negatively charged. The possible interaction changes the surface of particles from positive to negative charge. An experiment was performed to measure the mobility of the particles by capillary electrophoresis. Gold nanoparticles with a high concentration of SDS reached the detection end after the neutral marker in the CE experiment (as shown in Figure 4) at the condition of 70 mM SDS, 10 mM CAPS, 10.0 pH value, and 20-kV positive applied voltage. Under this experimental condition (not optimized for this separation), the analyte reaches the detector in the sequence of positive, neutral, and then negative species because of high electroosmotic flow. The data indicate that gold nanoparticles with a high concentration of SDS behave like negatively charged species because they reach the detector after the neutral marker. The migration sequence from CE suggests that the interaction between nanoparticles and SDS does exist, even though the exact interaction mechanism is not yet known. On the basis of the explanation mentioned above, one can presume that the column packing material and the gold nanoparticles both possibly interact with SDS and induce a negative charge on the surface of them. The electrostatic repulsion between particle and packing material, then, prevents the adsorption of nanoparticles by column packing material.

**Surfactant Effects on the Separation of Nanoparticles.** SDS concentration effect on the retention time of gold particles is shown in Figure 5A. At low SDS concentration, increasing retention times with the increase of SDS concentration can be explained by the ionic strength effect on the double layer of particles. Ionic strength effect on the double-layer thickness and hence on the retention time was demonstrated in the separation of CdS nanoparticles. The thickness of the double layer is inversely proportional to the square root of the ionic strength of solvent, whereas the ionic strength depends on the electrolyte concentration. Increasing the SDS concentration behaves like increasing the electrolyte concentration until micelle formation where the content of SDS monomer in the solution is no longer increasing. The thickness of the electrical double layer shrinks with increasing SDS concentration at low concentration, and then the thickness no longer changes at high SDS concentration. Our previous report already shows that both the NaCl concentration and the SDS concentration effects on the retention time of gold nanoparticles are similar.

Although SDS can solve the adsorption problem of packing material, the separation resolution is still not high enough to resolve different shapes of nanoparticles. This is mainly due to the gross size difference from different shapes of gold nanoparticles prepared in the same pot is not being large enough to allow separation by SEC. The apparent radius \( C = (3a^2 l / 4)^{1/3} \), where \( a \) is the radius of short axis and \( l \) is the length of long axis, is calculated to be 9.4 nm for sample A. Therefore, the apparent

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diameter of the nanorod in terms of the sphere is about 18.8 nm that is very close to the diameter of spherical gold nanoparticles (19.3 nm).

Mixed surfactant was then employed to investigate the separation performance. Nonionic surfactant (Brij-35) was chosen to mix with SDS to form a mixed surfactant system. The effect of Brij-35 concentration, mixed with 40 mM of SDS, on the elution time of gold particles is shown in Figure 5B. Increasing retention times were found with increasing Brij-35 concentrations. Also, the difference in the retention times of rod and spherical gold nanoparticles increases with the increase of Brij-35 concentration. If the separation mechanism is pure steric effect, an increase of retention time means the decrease in the diameter of the nanoparticle. Since there is no evidence of the change of nanoparticles size from the TEM image for the addition of Brij-35 to the sample solution (not shown here), the increase of retention time with the addition of Brij-35 is not due to the size effect of particles. The other possible reason is that Brij-35 replaces the position of SDS on packing material. Once the position of SDS on packing material is replaced by Brij-35, the irreversible adsorption problem of nanoparticles occurs again. This explanation is supported by the results of Figure 3B–C. The intensities of two absorption bands for nanoparticles diluted with 30 mM Brij-35 are smaller than those for nanoparticles diluted with 5 mM SDS and 30 mM Brij 35, as shown in Figure 3C. Also the intensities of two absorption bands for gold nanoparticles diluted with Brij-35 only are far less than those for nanoparticles diluted with SDS only, as shown in Figure 3B. The ruby-red color of nanoparticles was observed on the packing material when the sample solution was diluted with Brij-35. These results clearly suggest the re-occurrence of adsorption of nanoparticles by the packing material when Brij-35 is added to the SDS mobile phase.

The arguments mentioned above imply that the separation mechanism of gold particles by SEC is the combination of steric effect and surface adsorption with the use of SDS and Brij-35 mixture as the mobile phase. In addition, the surface adsorption of gold nanoparticle is probably shape-dependent. Therefore, the difference between the retention times of rod and spherical gold nanoparticles increases with the increase of Brij-35 concentration, as shown in Figure 5B. Hence, the adsorption effect of packing material can be utilized to improve the separation resolution by combining with the size-exclusion effect for shape separation. The optimal separation conditions of mixed size exclusion and surface adsorption can be obtained by adjusting these two factors and maximizing the retention difference. The optimal condition of this separation system was obtained by fixing the SDS concentration at the plateau region of Figure 5A (40 mM SDS) and adjusting Brij-35 concentration to be as high as possible and without deteriorating the baseline. From this optimization experiment, a mixed surfactant of 30 mM Brij-35 and 40 mM SDS provided better separation performance and was employed to separate different shapes of gold nanoparticles.

Size and Shape Separation. Although different shapes of particles are not baseline-resolved (as shown in Figure 6A), the difference in retention times for different shapes of particles is improved from 0.17 to 0.50 min when the mixture of 40 mM SDS and 30 mM Brij-35 replaces 40 mM SDS as the eluent. The identification of the particles on a SEC chromatogram can be obtained by examining the spectrum of DAD at each eluted signal. The DAD system offers the advantage of obtaining the spectrum

Figure 6. (A) Chromatogram of nanoparticles eluted with 40 mM SDS and 30 mM Brij 30, (1) detected at 920 nm (solid line), (2) detected at 520 nm (dashed line), (B) UV/vis spectrum at the beginning of the first peak, and (C) TEM for the particles collected from (B).
at all wavelengths simultaneously. The spectrum of the rising part of the first signal was examined, as shown in Figure 6B. It is interesting to note that no 520-nm absorption band in the beginning of the first eluted peak is observed. Fractional sample collection of this region was performed, and the TEM image of the collected particles was attached. The TEM image (as shown in Figure 6C) reveals that pure rod-shaped gold nanoparticles were collected at the beginning of the first signal. Since the sample solution from fractional collection is highly diluted, only five gold particles are shown here. Nevertheless, pure rod-shaped gold nanoparticles were eluted from the beginning of the first signal. Also, the absorption spectrum of pure gold nanorods that were eluted at the beginning of the first peak contains a 920-nm absorption band (SP long) only. SP trans absorption band is too small to be observed by conventionally UV–vis spectrometry. This is consistent with the theoretical prediction and experimental results of gold nanorod.23–25

Ideally, pure spherical gold particles should be collected at the descent of the second signal. However, this sample contains smaller gold nanoparticles (see Figure 1), and they were eluted after the second signal. The spectrum of the last eluted signal has two absorption bands that indicate the coexistence of rod and spherical gold nanoparticles. These results clearly demonstrate the potential of SEC with DAD system for shape and size separation and characterization of nanoparticles.

To verify the capability of size and shape separation of SEC with mixed surfactants, spherical gold nanoparticles with a mean diameter of 5.3 nm were added to sample A to obtain a sample containing different sizes and shapes of gold nanoparticles. Then, this sample was separated at 0.5 mL/min flow rate with 40 mM SDS and 15 mM Brij-35 of mobile phase. Here, 5.3-nm gold nanoparticles were obtained from the reduction method. The 3D chromatogram of different sizes and shapes of gold nanoparticles is shown in Figure 7. The first peak (at 12.29 min) contains pure rod-shaped gold nanoparticles with an absorption band at 920 nm. The second signal (at 12.63 min), without baseline resolution from the first signal, comes from spherical gold nanoparticles. The third signal (at 15.01 min) is due to the doped 5.3-nm spherical gold nanoparticles. Then, finally, citrate from 5.3-nm gold nanoparticles was eluted at 16.17 min. The smaller gold nanoparticles eluted in the third peak of Figure 2C are merged with those of the second peak (not the optimal condition). Figure 7 clearly demonstrates that SEC can be employed for the separation of different shapes and sizes of gold nanoparticles.

CONCLUSIONS

This report demonstrates the potential of SEC for the separation of different shapes and sizes of gold nanoparticles. The problem of irreversible adsorption of gold nanoparticles toward packing material can be eliminated or reduced by adding SDS to the eluent. Although surface absorption takes place with the addition of Brij-35 to the SDS eluent, the adsorption factor can be added to steric factor of SEC to enhance the separation performance of differently shaped nanoparticles. Even though baseline resolution of differently shaped nanoparticles cannot be obtained, the spectrum of the DAD system still can be utilized to interpret the shape of nanoparticles. The combination of DAD detection with SEC has the advantage over TEM to investigate the spectroscopic proper-

ties of nanoparticles with different shapes. Further studies are required for determining the exact interaction mechanism between surfactant and gold nanoparticles.

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