Adding sodium dodecylsulfate to the running electrolyte enhances the separation of gold nanoparticles by capillary electrophoresis

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Abstract

This paper describes employing capillary electrophoresis (CE) for the separation of gold colloids in nanometer-size regimes. Adding sodium dodecylsulfate (SDS) surfactant to the running buffer enhances the capability of CE to separate gold nanoparticles. We found that the optimized separation conditions involved SDS (70 mM), 3-cyclohexylammoniumpropanesulfonic acid (CAPS) buffer (10 mM), pH 10.0, and an applied voltage of 20 kV. We propose that the charged surfactants associate onto the surface of the gold nanoparticles and cause a change in the charge-to-size ratio of gold nanoparticle, which is a function of the surface area of nanoparticle and the surfactant concentration of running electrolyte. At high concentrations of the surfactant in the running electrolyte—i.e., when the surface of the gold nanoparticles is fully occupied with SDS—a linear relationship exists between the electrophoretic mobility and nanoparticles having diameters ranging from 5.3 to 38 nm. Based on the results of separating the 5.3 and 19 nm nanoparticles, we estimate that the size resolution ($R_s$) is 5.0 nm.

The relative standard deviations of the electrophoretic mobilities of the 5.3 and 19 nm gold nanoparticles are 0.97 and 0.54%, respectively.

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1. Introduction

Nanometer-sized gold particles have attracted broad attention in various research fields [1–4]. In this regime, size is an important factor that may dramatically affect a particle’s physical and chemical properties, such as its ability to be involved in catalysis [5,6]. Extensive investigations continue to demonstrate that both the size and shape of gold nanoparticles affect their properties [7–9]. During the last two decades, various procedures for the manufacture of nanoparticles have been introduced that allow gold nanoparticles to be obtained with a monodisperse nature (e.g., size, shape, and coefficient of variance) [10–12].

Conventionally, transmission electron microscopy (TEM) is used to measure the sizes of metal particles [13–15]. TEM, however, is a time-consuming technique that does not include a separation process by which size-dependent properties can be deduced. In addition, it is difficult to infer an ensemble’s average properties, such as average diameter or shape, based on the limited regions typically examined by TEM. This statistical uncertainty arises partly because of human subjectivity when deciding which areas of the grid to image and photograph, as well as size segregation effects that may occur during the drying process, which may provide a non-representative sample of clusters in a given region.

The use of separation techniques for the characterization of nanoparticles has the advantage of allowing particle size distributions to be measured simultaneously with the physical properties (e.g., absorbance, conductivity) of the particles. In addition, such an analysis can be performed using a small sample volume (e.g., 10 nl for CE over a short analysis time (<30 min) to obtain the true, unbiased, ensemble average of the size and optical properties of the solution.

High-performance liquid chromatography (HPLC) and size-exclusion chromatography (SEC) have been utilized and combined with TEM to successfully characterize nanometer-sized metal clusters [13–15]. The reported resolutions, however, of HPLC and SEC are not high enough; they are estimated to be in the range 20–60 nm. CE has emerged recently as one of the most powerful separation...
techniques [16–18]. Generally, CE has better separation efficiency than either SEC or HPLC. CE is not limited to separating small molecules; it has been used to separate particulate materials, such as inorganic oxides [19], latex particles [20,21], polystyrene, and silicates [22–24]. These particulate materials all have surface charges. Therefore, particles behave in a manner similar to that of charged species in CE when the separation is based on their charge-to-size ratio. CE has been employed to characterize nanometer-sized gold particles [25]. In that investigation, however, a linear relationship was obtained between the electrophoretic mobility and reciprocal of the core radius for gold nanoparticles having diameters between 5.2 and 14.6 nm, but this dependence contradicted theoretical expectations. The charge of a gold nanoparticle arises from the sorption of citrate ions onto the nanoparticle’s surface during the preparation process and the subsequent formation of an electric double layer (EDL) [25]. The electric potential associated with this double layer stabilizes the nanoparticles and prevents their agglomeration. Surfactants have been employed as stabilizers for the size-selective preparation of metal nanoparticles [26,27]: ionic surfactants surrounding the metal cores prevent the agglomeration of nanoparticles by electrostatic repulsion. These nanoparticles behave in a manner similar to charged particles. Hence, the use of nanoparticles stabilized by ionic surfactants may be more feasible for separation by CE.

The purpose of this study was to use sodium dodecylsulfate (SDS) surfactant as an additive to the CE running electrolyte for the separation of gold nanoparticles. We hoped that if a relationship exists between electrophoretic mobility and nanoparticle size, then CE might provide a simple means of characterizing the size of gold nanoparticles.

2. Experimental

2.1. Apparatus

All CE separations were performed using a Waters Quanta 4000 CE system. An Hg lamp with a 546 nm filter was employed to detect the gold nanoparticles. Uncoated fused-silica capillaries of 75 µm i.d. and 50.6 cm length were obtained from Polymicro Technologies. A detection window was fabricated ca. 7.5 cm from the capillary outlet. Throughout this study, we employed hydrodynamic injections lasting 10 s. We used positive polarity at the capillary inlet. Data acquisition was achieved using a Hewlett-Packard ChromStation and an HP 35900E interface card. The pH of the electrolytes was measured using an Orion model 420A pH meter. The capillary was pretreated before use: the capillary was flushed sequentially with 1 N NaOH and 0.1 N NaOH for 3 min, rinsed with water for 3 min, and then flushed with buffer for 3 min. The capillary was also rinsed with running buffer for 3 min between each run. All CE experiments were performed at an ambient temperature of ca. 20 ◦C.

2.2. Samples of nanoparticles and chemical reagents

Gold nanoparticles with mean diameters of 5.3, 9.8, and 19 nm were obtained from Sigma, and those with mean diameters of 29.3 and 38.3 nm were obtained from British BioCell. The concentration of the gold nanoparticles in solution was 50 mg l−1. SDS and 3-cyclohexylamino propylsulfonic acid (CAPS) were obtained from Tokyo Chemical Industry (TCI). Tris(hydroxymethyl)aminomethane (Tris) and sodium citrate were obtained from Merck. The 5 µm C18-capped (BondaPack™) and untreated silica gels used for the study of the adsorption of gold nanoparticles were obtained from Waters and Merck, respectively. Buffer stock solutions (100 mM) of CAPS, Tris, and sodium citrate were prepared and adjusted with 0.1 N sodium hydroxide or 0.1 N hydrochloric acid solutions to the desired value of pH. All electrolytes were prepared freshly each day and filtered through a 0.2 µm membrane filter before use.

3. Results and discussion

3.1. Effects of SDS surfactant on the separation

Gold nanoparticles with different diameters have different electrophoretic mobilities [25]. Electrophoretic mobility depends on the electrolyte’s ionic strength, i.e., the buffer concentration, because ionic strength influences the thickness of the EDL. When considering how the buffer concentration affects the thickness of the EDL, the electrophoretic mobility of gold nanoparticles should increase upon increasing the buffer concentration because a high buffer concentration reduces the thickness of the EDL and, thus, leads to a smaller apparent particle size [25]. Similar results (data not shown) were obtained in this study for the effects of different buffer (CAPS, Tris, and sodium citrate) and concentrations on the separation of gold nanoparticles. Even under the optimized separation conditions, a mixture of 5.3 and 19 nm gold nanoparticles could not be separated well when using buffer solutions that lack SDS.

Recently, SDS surfactant was introduced in SEC to improve its efficiency at separating gold nanoparticles [6,28]. In this current study, we introduced SDS surfactant into the running electrolyte of a CE system to examine the separation of gold nanoparticles. Fig. 1 reveals the effect of SDS concentration on the electrophoretic mobilities of gold nanoparticles. Increasing the SDS concentration increases the electrophoretic mobility of a gold nanoparticle. This phenomenon can be attributed possibly to the interaction of SDS with gold nanoparticles during the separation process, which leads to a change in the charge-to-size ratio of the nanoparticle. Hence, the electrophoretic mobility of a gold nanoparticle depends on the SDS concentration.
In our current study, the gold nanoparticles are capped with citrate ions. The interaction between the hydrophobic tail of SDS and the gold surface causes the adsorption of SDS onto the surface of the nanoparticles. This process results in an exchange from citrate to SDS of the stabilizing reagent on the gold nanoparticles. To further support the supposition of hydrophobic interaction between SDS and gold nanoparticles, we employed C18-capped silica gel (5 μm), which is generally used as a separation medium in the reversed-phase liquid chromatography (RPLC), to mimic the association of SDS with gold nanoparticles. First of all, two glass tubes containing unmodified silica gel and C18-capped silica gel (0.1 g), respectively, were washed with MeOH and then rinsed with water. Secondly, a solution of gold nanoparticles (3 ml) was added to each tube, which was agitated ultrasonically for 10 min before being filtered through a 0.45 μm membrane. Finally, the unmodified and C18-capped silica gels remaining on the membrane were rinsed with water and then dried. Fig. 2 displays the absorptions of the two eluent fractions from the second step. The intensity of the absorption signal of the solution that had been passed through the unmodified silica gel (Fig. 2B) is slightly reduced relative to the unfiltered solution. In contrast, however, the intensity of the absorption signal of the solution that had been passed through the C18-modified silica gel (Fig. 2C) is dramatically reduced. The latter spectrum suggests that absorption of gold nanoparticles has occurred on the C18-capped silica gel, presumably though some kind of interaction existing between the gold nanoparticles and the long-chain hydrophobic tails of the C18-capped silica gel. We propose, therefore, that gold nanoparticles associate with SDS probably through the oil of hydrocarbon tail of the surfactant being oriented directly toward the surface of the nanoparticles.

Since SDS surfactants in the CE buffer electrolyte may associate with gold nanoparticles and form a protective layer surrounding them, the charge of the nanoparticles will, therefore, be related to the number of molecules of SDS on the surface. In CE, the electrophoretic mobility is proportional to the charge-to-size ratio of analytes. At a high surfactant concentration, the charge-to-size ratio of the nanoparticle is larger than that at a low concentration. Consequently, for gold nanoparticles having constant diameter, the electrophoretic mobility is larger at high surfactant concentrations. Fig. 1 indicates that a 5.3 nm gold particle has a larger electrophoretic mobility than does a 19 nm one in the absence of SDS or in the presence of a low concentration of surfactant (<20 mM), which is consistent with a previous finding [25]. However, the mobility sequence is reversed at high surfactant concentrations. Since the charge-to-size ratio of particle is a function of particle size, Fig. 1 implies that, at low surfactant concentrations, large particles have smaller

![Fig. 1](image1.png)

**Fig. 1.** Effect of SDS concentration on the electrophoretic mobility of gold nanoparticles. CAPS, 10 mM; pH 10.0; applied voltage, 20 kV: (A) 5.3 nm and (B) 19 nm.

![Fig. 2](image2.png)

**Fig. 2.** Absorption spectra of gold nanoparticles: (A) original sample; (B) after passing original sample through a syringe filter containing 0.1 g of unmodified silica gel and (C) after passing through a syringe filter containing 0.1 g of C18-modified silica gel.
charge-to-size ratios than do small particles. This suggests that the charge of nanoparticle is limited to the number of SDS molecules available to adsorb onto the surface of a gold nanoparticle. Therefore, the charge-to-size ratio of 5.3 nm particles is larger than that of 19 nm particles at low SDS concentrations. However, the electrophoretic mobility of the former is higher than that of the latter. Increasing the SDS concentration in the running electrolyte increases the total charges of all the gold nanoparticles because there are more SDS molecules available for adsorption. Thus, an increase in the SDS concentration increases the electrophoretic mobility of gold nanoparticles. Furthermore, the amount of charge on a particle is no longer limited to the number of SDS ions available once the concentration of the surfactant reaches a certain level. Instead, at high surfactant concentration, the amount of charge on a particle depends on the surface area available to associate with the SDS molecule. At high SDS concentration, a greater amount of charge is expected for the 19 nm particles because these particles have a larger surface area to associate with more surfactant molecules than do the smaller particles.

Equations related to the proposed separation mechanism are described as follows. The number of SDS molecules associated with gold nanoparticles is proportional to the surface area of gold nanoparticles:

\[ n_{SDS} \propto A \]

where \( n_{SDS} \) denotes the number of SDS molecules, \( A \) is the surface area of a gold nanoparticle, and \( r \) is the radius of a gold nanoparticle. Therefore, larger gold particles have larger electrophoretic mobilities at high SDS concentrations, as illustrated in Fig. 1. High surfactant concentrations induce long migration times and result in lower heights and wider widths of peaks. An SDS concentration of 70 mM offers a satisfactory separation and, therefore, we employed it as a standard in the following studies.

### 3.2. Optimizing separation

Fig. 3 displays the effect of different buffers on the separation of 5.3 and 19 nm gold nanoparticles. When using 10 mM CAPS buffer and 70 mM SDS in the running electrolyte, the difference in the electrophoretic mobility between these two particles is 0.46 cm² s⁻¹ V⁻¹. With 10 mM Tris buffer and 70 mM SDS, the difference is 0.39 cm² s⁻¹ V⁻¹. Since the charge-to-size ratios of the 5.3 and 19 nm gold nanoparticles do not change significantly over the range of values of pH from 8.2 to 11.0 and over the range of CAPS concentrations from 5 to 30 mM, it is probably the case that the zeta potential no longer significantly changes when pH > 8.0 [31] and, consequently, the electroosmotic mobility changes only slightly. For values of pH below 8.0, we utilized another type of citrate buffer having better buffer capacity. Since the long migration times exhibited when using the citrate buffer system lead to broadened bands, the exact migration time of each band is difficult to locate. Therefore, the effects of pH on the separation of nanoparticles are limited to values above 8.2, and we employed a value of 10.0 for the following experiments. In addition, CAPS provides a buffer possessing low ionic strength and, thus, increasing the buffer concentration does not significantly increase the overall ionic strength of electrolyte.

### 3.3. Separation performance of gold nanoparticles by CE

Fig. 4 displays an electropherogram, obtained under our optimal separation conditions, showing the separation of a mixture of 5.3 and 19 nm gold nanoparticles. This figure depicts clearly that nanoparticles having different diameters can be separated well. Baseline-resolved separation of the same mixture is impossible without the addition of SDS to the running electrolyte. We calculate the resolution between the 5.3 and 19 nm nanoparticles from Eq. (4) to be 3.0. The size resolution (\( R_s = 1.0 \)) is ca. 5.0 nm, which is better than the results obtained using SEC [13–15]. One point that we emphasize is that particles whose sizes differ even by <5 nm can be distinguished as resolved peaks. Since the CE
Fig. 3. Effects of buffer types on the separation of gold nanoparticles. SDS, 70 mM; applied voltage, 20 kV: (a) CAPS, 10 mM; pH 10.0 and (b) TRIS, 10 mM; pH 8.5. (A) 5.3 nm and (B) 19 nm.

Fig. 4. Electropherogram of the separation of differently sized gold nanoparticles. SDS, 70 mM; CAPS, 10 mM; pH 10.0; applied voltage, 20 kV. (A) 5.3 nm and (B) 19 nm.
technique offers better separation resolution than other techniques, it can be applied to assist in characterizing the size distribution of solution-grown nanoparticles in a simpler and easier operation relative to that employing TEM. Our results demonstrate that using CE to separate gold nanoparticles is feasible.

We examined the reproducibility of determining the electrophoretic mobility of a mixture of the 5.3 and 19 nm gold nanoparticles from fifteen consecutive runs. The calculated precisions on the electrophoretic mobilities of these nanoparticles are 0.97 and 0.54%, respectively. These results indicate that there is good reproducibility in the migration behavior of gold nanoparticles in CE. We applied a sample of a mixture of five differently sized gold nanoparticles to the optimized separating conditions to investigate whether a correlation exists between electrophoretic mobility and nanoparticle diameter. As displayed in Fig. 5, a good linear relationship ($r^2 = 0.99$) does exist between electrophoretic mobility and nanoparticle size. This figure also reiterates that the CE technique is capable of characterizing gold particles in nanometer-size regimes.

4. Conclusions

This work illustrates that using SDS surfactant as a running electrolyte can enhance the separation capability of CE for nanoparticles. Adding SDS to the running buffer allows the surfactant to associate with gold nanoparticles and, consequently, change its charge-to-size ratio. The concentration of the surfactant plays an important role in enhancing electrophoretic mobilities and has different effects depending on the diameter of the nanoparticles. In addition, a linear relationship exists between the electrophoretic mobility and nanoparticle size. The precision of the calculated electrophoretic mobility is <1%. Our results demonstrate the potential that CE has for the analytical separation of gold nanoparticles in nanometer-size regimes.

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References
