

## Studying the size/shape separation and optical properties of silver nanoparticles by capillary electrophoresis

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### Abstract

This paper describes the feasibility of employing capillary electrophoresis (CE) to separate silver particles in nanometer regimes. We have found that the addition of an anionic surfactant, sodium dodecyl sulphate (SDS), to the running electrolyte prevents coalescence of the silver particles during the process, which improves the separation performance; the concentration of SDS required for optimal silver nanoparticle separation is ca. 20 mM. By monitoring the electropherograms using a diode-array detection (DAD) system, we have also investigated the separation of suspended silver nanorods with respect to their shapes. Our results demonstrate that the combination of CE and DAD is a powerful one for the separation and characterization of various silver nanoparticles.

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### 1. Introduction

Nanoparticles continue to attract widespread attention in many scientific fields [1]. The sizes and shapes of nanoparticles are important factors that determine their physical and chemical properties [2]. It is well known that silver nanoparticles have superior properties relative to other nanostructured metal particles, such as their electrical conductivities [3], antimicrobial effects [4], optical properties [5], and applications in oxidative catalysis [6]. Methods for the preparation of silver nanoparticles are discussed in a number of publications [7,8].

In addition to studying methods for their synthesis, new tools are also required for characterizing and determining the sizes of nanoparticles. To date, scientists who wished to characterize the size distribution of solution-grown nanoparticles

have relied almost exclusively on TEM [9]. Unfortunately, it is difficult to infer ensemble average properties, such as average diameter or shape, based on the limited regions examined in typical TEM images. This statistical uncertainty occurs partly because of human subjectivity when deciding which areas of the grid to image and photograph, but it is due also to size segregation effects during the drying process, which may give rise to a non-representative sample of clusters in a given region. An additional difficulty, often ignored, occurs during the process of drying the clusters on a grid. This process can cause changes to the clusters' sizes, induce aggregation, or lead to chemical changes (e.g., oxidation) of the clusters. Furthermore, this technique does not involve any separation process and, therefore, it cannot allow any size- or shape-dependent properties to be determined.

Capillary electrophoresis (CE) has emerged recently as one of the most powerful separation techniques [10] and has been used successfully to separate a variety of differently sized materials, including inorganic oxide [11], latex [12,13], polystyrene, silicate [14,15], and gold particles [16,17]. The

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separation mechanism relies upon these particulate materials having surface charges and, therefore, their separation behavior is similar to that of charged molecular species in CE. For example, the charge of a gold nanoparticle arises from the sorption of ions onto the nanoparticle's surface during the preparation process, which results subsequently in the formation of an electric double layer [17]. The electric potential associated with the double layer prevents agglomeration of the nanoparticles and increases their stability. The differently sized particles display specific mobilities in a capillary column under the presence of an external applied voltage because they have different charge-to-size ratios; therefore, CE can be used to characterize their sizes.

Although we are by no means advocating that TEM approaches to qualitative cluster analysis be abandoned, we hope to demonstrate that CE, an approach traditionally used to separate molecules, can be used to analyze nanoparticles in terms of both their sizes and shapes. If size- or shape-based separation of silver nanoparticles could be established, the widespread use of modern CE systems could make this method particularly attractive for the routine characterization of nanometer-sized silver particles. An additional attractive feature is that the optical spectra of nanoparticles, which depend on both their size and shape, can be readily obtained when using a CE system equipped with a diode-array detection (DAD) system. Thus, a single sample can be analyzed using a volume of only several nanoliters and a rapid analysis time (ca. 10 min) to characterize the size- and shape-dependent properties of silver nanoparticles.

## 2. Experimental

### 2.1. Apparatus

All silver nanoparticles were synthesized using a microwave heating system (MARS-5, CEM, Matthews, NC, USA). The microwave instrument was operated in the range from 0 to 100% of its full power (1200 W). The scanning electron microscopy (SEM) image was taken using a JEOL JSM-6500F instrument (Tokyo, Japan). The particle sizes of the sample were determined by two-dimensional (2D) grain analysis after digitizing the SEM image. The UV–vis spectra of samples were recorded using a Shimadzu UV 2501PC spectrometer (Shimadzu, Tokyo, Japan).

All CE separations were performed using an Agilent capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany). Uncoated fused-silica capillaries (i.d.: 75  $\mu\text{m}$ ; length: 48.5 cm) were obtained from Polymicro Technologies (Phoenix, AZ, USA). A detection window was fabricated ca. 8.5 cm from the capillary outlet. Injections at a pressure of 50 mbar for 1 s were employed throughout. Positive polarity was applied at the capillary inlet. The values of the pH of the electrolytes were measured using an Orion 420A pH meter (Boston, MA, USA). Before use, the capillary was pretreated by flushing sequentially, for 3 min each time, with

1 M NaOH, 0.1 M NaOH, water, and buffer. The capillary was also rinsed with running buffer for 3 min between each run. All CE experiments were performed at a temperature of 15 °C.

### 2.2. Reagents

All chemical materials were GR grade. Sodium citrate and silver nitrate ( $\text{AgNO}_3$ ) were purchased from Acros Organics (Geel, Belgium). SDS surfactant was obtained from Tokyo Chemical Industry (Tokyo, Japan). Tris(hydroxymethyl)aminomethane (Tris) and sodium citrate were obtained from Merck (Darmstadt, Germany). Buffer solutions of Tris and sodium citrate were prepared and adjusted to the desired pH using either 0.1 N sodium hydroxide or 0.1 N hydrochloric acid solutions. All electrolytes were prepared freshly each day and filtered through a 0.2- $\mu\text{m}$  membrane filter (Alltech Associated, Deerfield, IL, USA) before use.

### 2.3. Preparation of suspended silver nanoparticles

We prepared aqueous suspensions of silver nanoparticles having different sizes and shapes by using our newly developed microwave irradiation method [18]. Briefly, for the preparation of the spherically shaped silver nanoparticles (36.3-nm diameter), an aqueous mixture of 1.0 mM  $\text{AgNO}_3$  and 3.5 mM sodium citrate (10 mL) was placed in a microwave (MW) heating system. The MW heating program involved raising the temperature from 20 to 100 °C over 20 min and then maintaining that reaction temperature for 10 min. For the preparation of the spherically shaped silver nanoparticles (17.0- and 49.7-nm diameters), an aqueous mixture of 1.0 mM  $\text{AgNO}_3$ , 3.5 mM sodium citrate (10 mL), and 0.1 M NaOH (50  $\mu\text{L}$ ) were placed in the MW heating system and reacted at 100 °C: the rise in temperature from 20 to 140 °C occurred over 20 min and this reaction temperature was then maintained for 10 min. To prepare the sample comprising a mixture of silver nanorods and nanoparticles, an aqueous mixture of 1.0 mM  $\text{AgNO}_3$  and 3.5 mM sodium citrate (10 mL) was placed in a microwave heating system. The MW heating program in this case involved raising the temperature from 20 to 100 °C over 10 min and then maintaining this reaction temperature for a further 10 min. After cooling to room temperature, the products were collected for their further characterization.

## 3. Results and discussion

### 3.1. SEM images and UV–vis spectra of silver nanoparticles

Fig. 1a displays the UV–vis absorption spectrum of a spherically shaped Ag colloid solution. The appearance of a typical silver plasmon absorption peak at 430 nm indicates

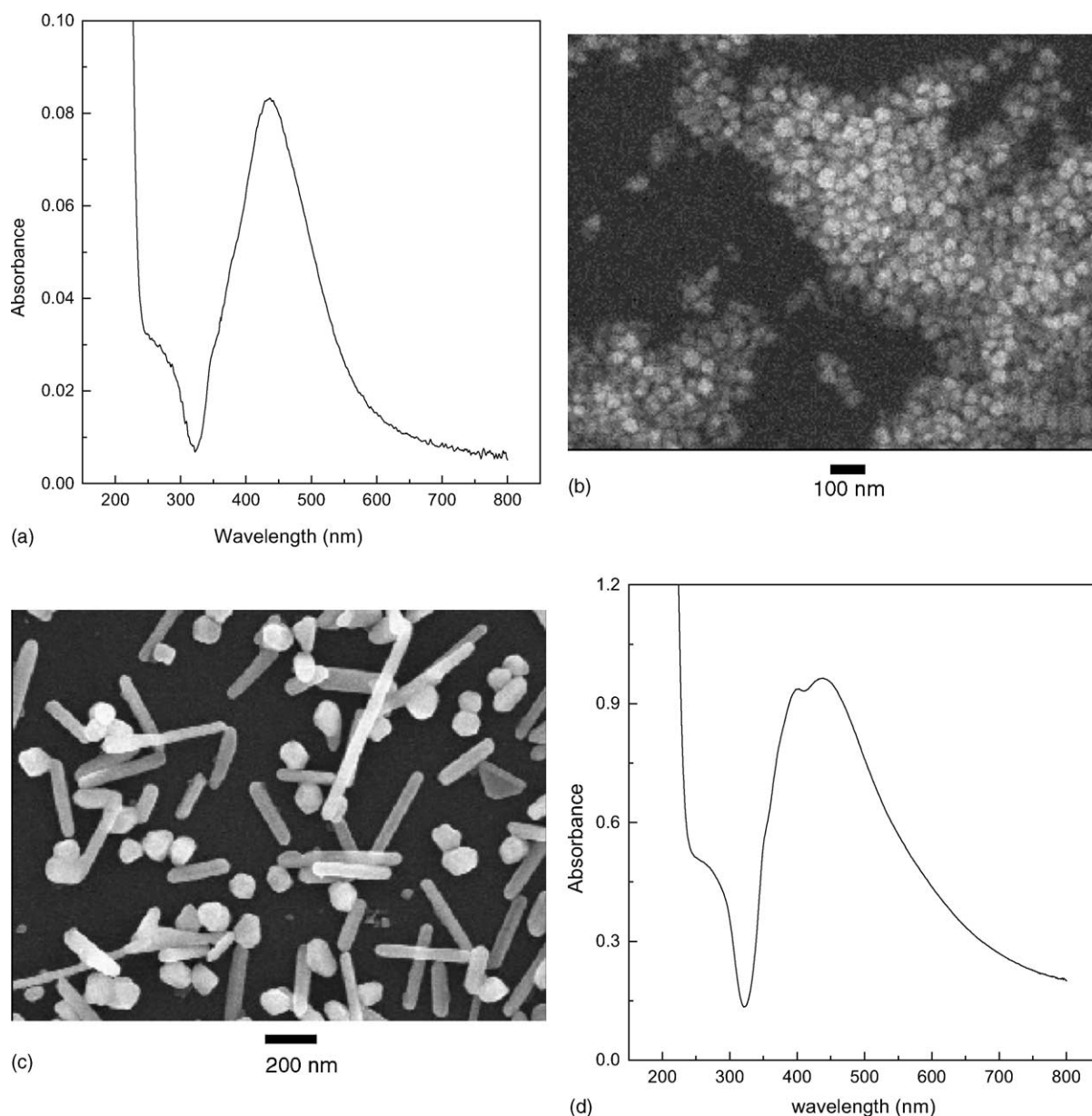


Fig. 1. (a) UV-vis absorption spectrum and (b) an SEM image of silver nanoparticles obtained by microwave heating. (c) SEM image and (d) UV-vis absorption spectrum of a mixture of silver nanoparticles and nanorods obtained by microwave heating.

the formation of spherically shaped silver nanoparticles. The small shoulder absorption peak at 350 nm is likely to be contributed by the silver nanorods that are also present in the solution. The SEM image of a typical sample employed in this study is presented in Fig. 1b; analysis of this image indicates that the sample contains mainly spherically shaped silver nanoparticles (ca. 98%) that have diameters of ca.  $36.3 \pm 5.9$  nm. In addition, the image reveals that the sample contains a relatively small overall quantity (ca. 2%) of nanorods.

The SEM image of a sample employed in the shape separation process is displayed in Fig. 1c; this sample contains both rod-shaped (ca. 51%) and spherical silver nanoparti-

cles. The rod-shaped particles have a mean aspect ratio (rod length: minor axis diameter) and an average minor axis diameter of ca. 6.2 and 55.5 nm, respectively, while the mean diameter of the spherical particles is ca. 80.2 nm. Fig. 1d displays the UV-vis absorption spectrum of a sample that we employed in the shape separation process; three absorption bands, located at ca. 350, 395, and 440 nm, characterize this spectrum. We attribute these absorption peaks to three different surface plasmon resonances of the silver particles: that of the longitudinal mode of the nanowires, that of the transverse mode of both the nanowires and the nanorods, and that of the nanoparticles, respectively; these assignments are consistent with those reported previously [19,20].

### 3.2. Reagents that stabilize silver nanoparticles

In this study, the citrate ion performs multiple tasks during the synthesis of the silver nanoparticles: not only does it complex strongly to the silver ions but also it is responsible for their reduction and subsequent capping on the resulting silver metal. The solutions of the citrate-capped silver nanoparticles were clear and stable for weeks, but, because citrate represents as an extra additive in the matrix of the as-prepared

silver nanoparticles, the citrate concentration has a strong influence on the size and morphology of the silver particles. The effects of its concentration can be observed clearly in Fig. 2a: the intensity of the surface plasmon absorption peak of the silver nanoparticles located at 435 nm decreases upon increasing the citrate ion concentration. At concentrations of citrate in the sample solution >10 mM, the silver particles begin to precipitate from the solution and a black material settles on the bottom of the sample vial. An SEM image

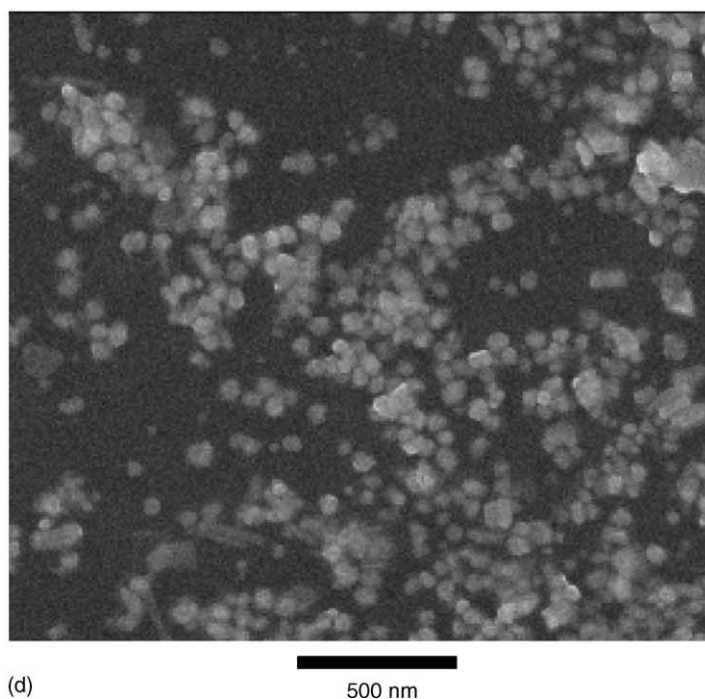
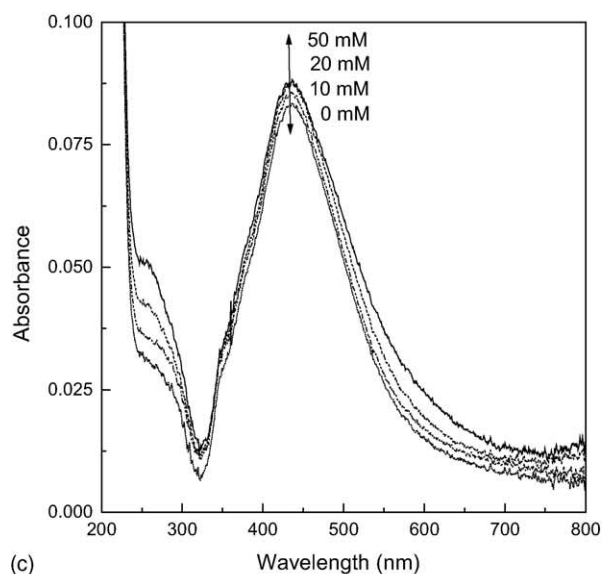
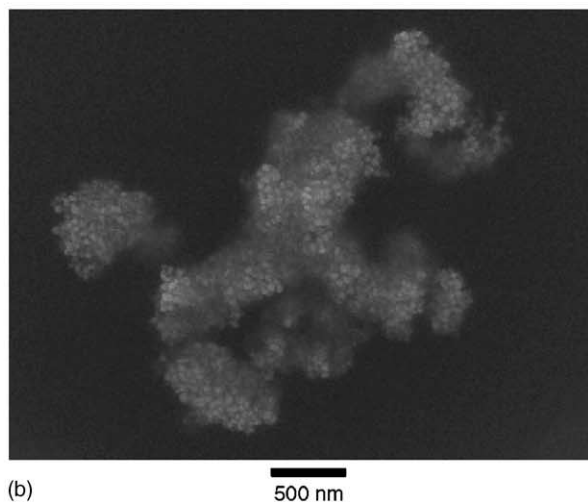
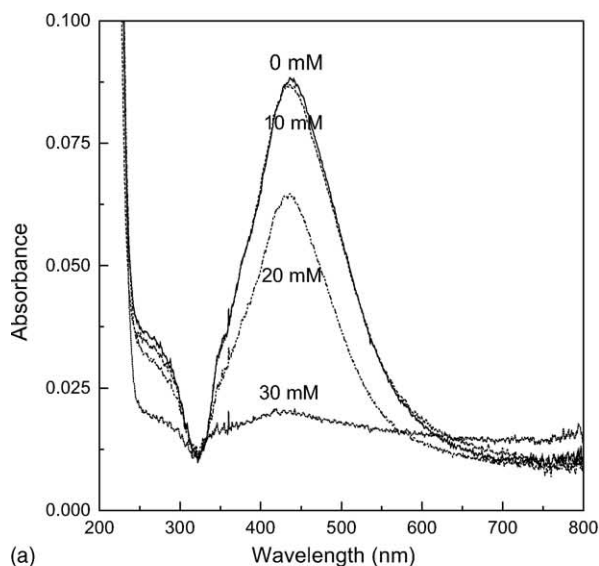


Fig. 2. (a) The effect of citrate concentration on the UV-vis absorption spectra of the silver nanoparticles characterized in Fig. 1a and b. The particle solution was diluted with different citrate concentrations in a 1:1 ratio. (b) SEM image of as-prepared silver nanoparticles after the particle solution had been diluted with 20 mM citrate (1:1 mixture). (c) The effect of the SDS concentration on the UV-vis absorption spectra of silver nanoparticles characterized in Fig. 1a and b. The particle solution was diluted with different SDS concentrations in a 1:1 ratio. (d) SEM image of the as-prepared silver nanoparticles after they had been diluted with 40 mM SDS (1:1 mixture).

obtained under these conditions (Fig. 2b) reveals that the silver nanoparticles have merged together. This phenomenon occurs as a result of the double layer acting as a coating on the surfaces to prevent coagulation [21]: when this protecting layer grows smaller, which occurs when the citrate concentration increases, the particles begin to coalesce. During separation by CE of nanoparticles having different diameters, the presence of a high concentration of ions in the running electrolyte may be required to tune the charge-to-size parameters of the particles and yield the optimal separation efficiency [17]. Our finding above suggests that CE is not effective for the separation or characterization of the sizes of silver nanoparticles when citrate is used alone as the running electrolyte; the size distribution of the silver nanoparticles is influenced significantly by the separation conditions and, therefore, the electrophoretic results may not represent the true migration behavior of the suspended silver particles. To overcome this problem, we sought a suitable reagent that is better at dispersing the silver nanoparticles during their separation.

Recently, surfactants have been employed as stabilizers for the size-selective preparation of metal particles [22,23]. Kondow and co-workers [24] and Chen and Yeh [25] have demonstrated that the surfactant SDS has a positive stabilizing effect on silver nanoparticles. It has been demonstrated that ionic surfactants surround the metal cores and prevent their agglomeration by electrostatic repulsion between the nanoparticles. We became interested in discovering whether the use of SDS as an additive anionic surfactant in the sample matrix would improve the stability of the silver particles.

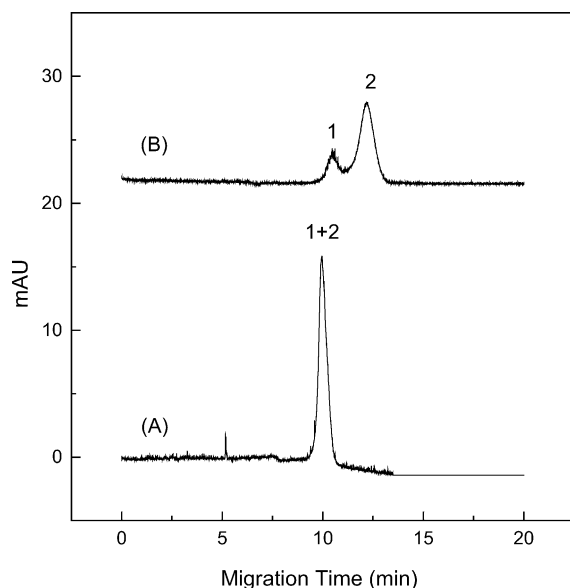


Fig. 3. Electropherograms of silver nanoparticles (“1” = 17.0-nm diameter particles; “2” = 49.7-nm particles) as a function of SDS surfactant concentration. The separation was obtained using a running electrolyte of SDS at (A) 0 mM, (B) 20 mM. CE separation conditions: 10 mM Tris (pH 8.5); applied voltage: 20 kV.

The UV–vis spectra presented in Fig. 2c reveals that when SDS surfactant was introduced into the sample matrix of the nanoparticles, the silver nanoparticles remained quite well dispersed, which is a significant difference from their appearance in the spectra presented in Fig. 2a. There are no signs of decay of the surface plasmon absorption intensity of the silver nanoparticles when the SDS surfactant was present at concentrations as high as 50 mM. The SEM image presented in Fig. 2d confirms the stability of the silver nanoparticles under such conditions; the particle sizes calculated from this image are  $36.0 \pm 5.7$  nm. A statistical *F*-test, with a 95% confidence interval, indicates that there is no significant difference between the size distributions the samples presented in Figs. 1b and 2d. Therefore, the SDS surfactant seems to be a good stabilizing additive that would allow the characterization of the silver nanoparticles’ sizes during their separation by CE. Taken together, the results presented in Fig. 2 suggests that SDS surfactant is a good stabilizer for silver nanoparticles and that its presence prevents them from coalescing. The SDS surfactant protects the suspended silver nanoparticles so that they have longer lifetimes relative to the suspensions in sodium citrate.

### 3.3. Surfactant effects on the size separation of silver nanoparticles

The choice of the method in which the SDS surfactant should be utilized for the size-related separation of silver nanoparticles is an interesting one. During the CE separation process, samples that are prepared in a solution that differs from that of the running electrolyte are immediately enveloped by the running electrolyte. This blending offers the possibility of bringing particles into a defined environment; any subsequent changes in the effective charge-to-size ratios of the particles can be monitored by changing the composition of the running electrolyte. In this study, we have also investigated the effect that running electrolytes possessing different concentrations of surfactant have on the CE separation of silver nanoparticle solutions of various particle sizes. We used concentrations of SDS surfactant in the range 0–40 mM to evaluate the concentration dependence of the separation of a mixture of 17.0- and 49.7-nm diameter silver nanoparticles in a single run. Fig. 3 demonstrates the effect that different concentrations of SDS surfactant solution in running electrolyte have on the separation of these silver nanoparticles. The data indicate that no separation occurs when a running electrolyte is used that does not contain any SDS. The separation of the silver nanoparticles is improved, however, upon the addition of SDS to the running electrolyte. We found that an SDS concentration of 20 mM is close to optimal: it provides almost fully resolved Gaussian-shaped peaks ( $R_s > 1.5$ ). By obtaining electropherograms of the individual sets of the two nanoparticles, we assign the peak that migrates more quickly (10.5 min) to the smaller-diameter silver nanoparticles (17.0 nm); the more slowly eluting peak (12.2 min) corresponds to the larger-diameter silver nanoparticles (49.7 nm).

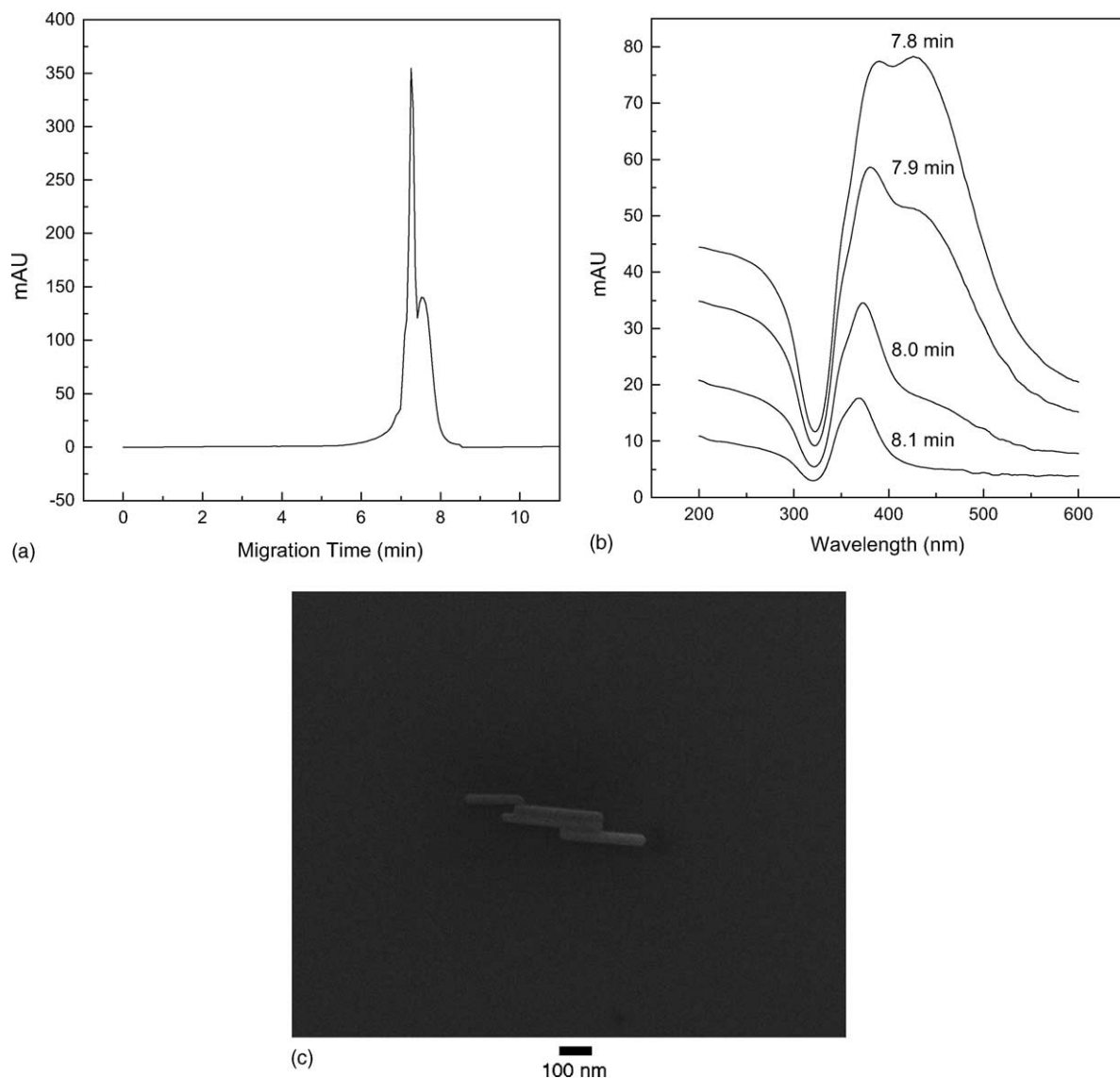


Fig. 4. (a) Electropherogram of the mixture of silver nanoparticles and nanorods characterized in Fig. 1c and d. The separation was obtained using a running electrolyte of 20 mM SDS and 10 mM Tris (pH 8.5); applied voltage: 30 kV. (b) UV-vis absorption spectra of the silver nanoparticles obtained at separation times of 7.8, 7.9, 8.0, and 8.1 min. (c) SEM image of the nanorods collected from a fraction obtained at a separation time of 8.1 min.

If this method is to be used for the routine separation and characterization of the sizes of nanoparticles, it requires further validation. For this purpose, we evaluated the reproducibility of the size-based separation by measuring the relative standard deviation of the elution times over seven consecutive runs: the reproducibilities of the elution times for the 17.0- and 49.7-nm diameter silver nanoparticles are 1.2 and 1.5%, respectively.

### 3.4. Shape separation

Using 20 mM SDS as the optimal running electrolyte, we also attempted to use this CE technique to separate the differently shaped silver nanoparticles presented in Fig. 1d. Fig. 4a indicates that although the differently shaped particles are not baseline-resolved, they can be identified on the CE trace by

examining the UV-vis spectra obtained at different elution times using the DAD. The DAD system, which offers the advantage of allowing spectra to be obtained at all wavelengths simultaneously, can be used to track the morphological evolution in the electropherogram because the different silver nanostructures exhibit their surface plasmon resonance (SPR) bands at different frequencies [19,20]. Fig. 4b displays a comparison of the UV-vis absorption spectra obtained when sampling the electropherogram at different separation times. At a separation time of 7.8 min, we observe the absorption spectra to be a mixture of silver nanorods and nanoparticles. The plasmon peak at 430 nm is indicative of the silver nanoparticles; this peak exhibits a broad full-width at half-maximum (FWHM) of ca. 100 nm as well as a tailing effect, which indicates a broad distribution in the size and morphology of these nanoparticles [20]. At the same time, the shoulder

peaks at 350 and 388 nm are considered to be optical signatures of relatively long silver nanowires. As the lengths of these nanorods increase-with a corresponding increase in the separation time-the two SPR peaks at 350 and 388 nm increase further in intensity relative to the peak located at 430 nm. Eventually, at separation time of ca. 8.1 min, the UV-vis absorption spectrum clearly reveals that the samples passing through the detection window of the capillary consist almost entirely of nanorods and/or nanowires because the peak located at 430 nm can hardly be observed. The two SPR peaks (350 and 370 nm) displayed after 8.1 min are characteristic optical signatures of both silver nanorods and nanowires. Fig. 4c indicates an SEM image of the sample eluted at a separation time of ca. 8.1 min. Because the sample solution collected from this fraction is highly dilute, only a few silver particles are visible, but this image confirms quite convincingly that we have collected purely rod-shaped nanoparticles. These observations suggest that pure nanorods and nanowires can be separated from a mixture of differently shaped silver particles by using a CE system. In addition, this study demonstrates clearly that DAD can be employed to investigate the spectroscopic properties of the nanoparticles that have been separated by CE according to their different sizes and shapes.

#### 4. Conclusions

Our results demonstrate the potential of CE to be used for the analytical separation of silver nanoparticles in nanometer-size regimes. This work illustrates that SDS surfactant in the running electrolyte can prevent silver nanoparticles from coagulating during the separation process. Furthermore, the SDS surfactant enhances the resolution of the separation of the silver nanoparticles by CE. We have also demonstrated that CE has the potential to separate differently shaped silver particles. The UV-vis spectra provided by a DAD system can be utilized to characterize the differently shaped silver particles based on their SPR bands and, therefore, the combination of DAD with CE is a powerful one that allows simultaneous separation and spectroscopic analysis of these silver particles. We believe that this CE technique also will be ap-

plicable to the separation and characterization of size- and shape-dependent nanomaterials other than silver.

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