Separation of Nanoparticles in Different Sizes and Compositions by Capillary Electrophoresis

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Nano-sized particles have received considerable interest in the past two decades.[1] Due to the quantum confinement effect, the nanoparticles show distinct physical and chemical properties depending on the sizes and shapes, particularly when the sizes are close to or smaller than the dimensions of exciton of the corresponding bulk materials.[2] However, their wide distributions of sizes always give rise to the major limitations for precise investigation of their unique physical and chemical characteristics. Therefore, the separation of the nano-sized particles has brought considerable attention in many scientific areas recently.[3-8]

Many methods such as transmission electron microscopy (TEM) and size-exclusion chromatography were introduced so far for identifying and separating nanoparticles. These methods, however, have some inherent problems in the detection processes involving the degradation of sample, irreversible adsorption, etc.[9-12] Recently, several attempts to use capillary electrophoresis (CE) as a novel separation method for nanoparticles of inorganic and polymer materials have been made.[11-18] The principle of electrophoretic separation is based on the fact that when an external electric field is applied to a solution of charged species, each ion moves toward the electrode of opposite charge.

The electrophoretic mobility (μ_p) of ionic species is expressed as

$$\mu_p = \frac{q}{6\pi\eta r} \quad (1)$$

where q is the charge of the ion, η is the coefficient of viscosity of the fluid, and r is the hydrodynamic radius of the ion. The apparent mobility (μ_a), which gives rise to the net driving force for the separation in CE, is given by the sum of electroosmotic mobility (μ_o) and electrophoretic mobility (μ_p). Overall, the migration time t of ionic species can be expressed by

$$t = \frac{I}{\mu_a E} = \frac{IL}{(\mu_o + \mu_p)V} \quad (2)$$

where I and L are the effective and total lengths of capillary, respectively, E is the electric field strength, and V is the applied potential. Thus the separation in CE can be achieved by the mobility of the species depending on not only the solvent medium, but also the charges, sizes, and shapes of nanoparticles.

In this Letter we report the use of capillary zone electrophoresis (CZE) to separate nanoparticles according to the sizes of samples as well as to the nature of materials in same sizes.

Experimental Section

The CZE system constructed for this work is similar to that used conventionally. A 30 kV high voltage power supply with a reversible polarity output (Model CZR 100R, Spellman, U.S.A) was employed as a potential source for the separation. The detection of the sample was achieved with an UV-VIS absorbance detector (Model Spectra 100, Thermo Separation Products, U.S.A). Electropherograms were collected through a Data Acquisition Device (NI4351, National Instruments, U.S.A.) and a personal computer using Multimicro 2000 data acquisition software (Yullin Technology, Korea). Fused silica capillaries (75 μm i.d. and 363 μm o.d.) were purchased from Unimicro Technology, U.S.A.

All chemicals were obtained from Aldrich Chemical Co. (Milwaukee, U.S.A) and were used without further purification. The concentration and the pH of the buffer solution were systematically varied to find the optimal conditions for each experimental runs. In the experiments reported here, 50 mM Tris solution at pH 9.2 as a buffer and 3 mM benzyl alcohol as an electroosmotic flow (EOF) neutral marker were employed, where freshly deionized and distilled water was used to prepare the solutions. Polystyrene nanosphere standards (20-300 nm, Duke Scientific Corporation, Palo Alto, U.S.A) and gold nanoparticles (5-20 nm, Sigma, St. Louis, U.S.A) were used as standard analytes.

Samples containing nanospheres were dissolved in buffer solutions before the injection. In typical experimental runs, samples were introduced hydrodynamically with the injection time of 5 s and height at 28 cm, providing the approximate injection volume of 7.2 nL. Throughout this work, the total length and the effective length of 75 μm i.d. capillary were 70 cm and 55 cm, respectively.

Results and Discussion

Two approaches have been performed in this work to demonstrate the separation of the nanosphere size samples, *i.e.*, the mixture of same materials in different sizes and the mixture of different nanoparticles in same sizes. Firstly the

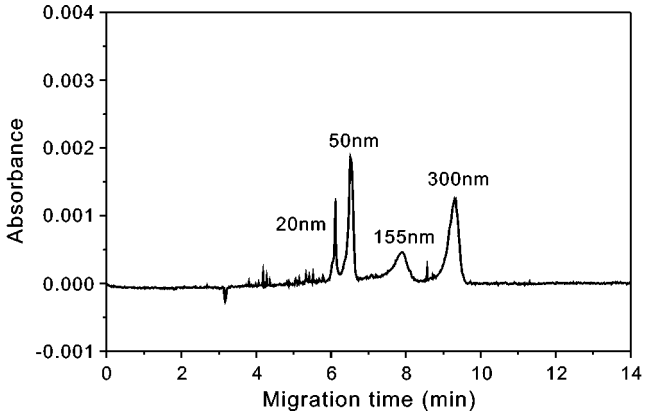

Figure 1. Capillary electropherogram of a mixture of four-component polystyrene nanospheres measured with a UV absorption detector at 254 nm and separation potential of 23 kV.

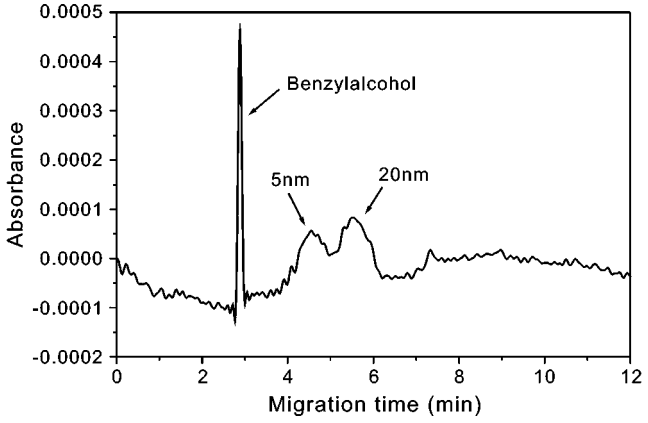

Figure 2. Capillary electropherogram of standard gold nanoparticle mixture measured with a UV absorption detector at 520 nm and separation potential of 28 kV.

various polystyrene nanosphere standard samples with diameters of 20, 50, 155 and 300 nm were mixed and the separation was conducted at the separation voltage of 23 kV and the detection wavelength of 254 nm. The results for polystyrene standard samples are shown in Figure 1. In the electropherogram, each component appears well-separated peaks with quite flat baseline. By the use of precise control of the separation voltage and pH of the buffer solution, the efficiency of separation seems somewhat better than the previous result.[13] The number of theoretical plates for the 20 nm peak was calculated to be 1.3×10^6.

Figure 2 illustrates the separation of the mixture of gold nanoparticles (5 nm and 20 nm in diameter) with the same system. This electropherogram was obtained by the injection time of 10 s and the induced potential of 28 kV at the detection wavelength of 520 nm. It is notable that although the separation of gold nanoparticles mixture was not as good as that of polystyrene mixture, metal nanoparticles even with small size difference could be separated by CE method. It would be possible to increase the separation efficiency by further optimization of the experimental conditions such as the injection methods, separation media, buffer composition, etc.

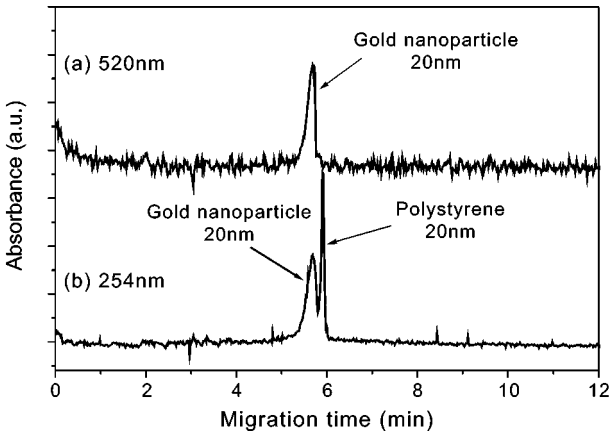

Figure 3. Capillary electropherogram of two-component mixture with polystyrene and gold nanoparticles (both 20 nm). The electropherograms were obtained at the separation voltage of 20 kV and the detection wavelengths of (a) 520 nm and (b) 254 nm.

In order to test the separation capability for mixed species of different chemical origins, experiments were carried out with a mixture of two different components with same sizes. Figure 3 shows an electropherogram of two analytes, gold and polystyrene particles of both 20 nm in size. The separation voltage was 20 kV and the effective length of the capillary was 55 cm. As shown in Figure 3, the separation between two species was successively achieved. The separation was characterized as to numbers of theoretical plates of 1.4×10^3 and 2.4×10^4 for gold and polystyrene nanoparticles, respectively. Owing to the fact that gold naoparticles absorb light in a wide range of wavelength (200-550 nm, λ_{max}=514 nm), the 520 nm wavelength was used for detecting only gold nanoparticles while the UV wavelength (254 nm) was used for detecting both gold and polystyrene nanoparticles.

As mentioned in equations (1) and (2), the migration times of the nanoparticles depend upon both the sizes and charges of the species. However, the hydrodynamic radii of nano-particles used in this work cannot be exactly specified due to their wide variance of mean diameters, and also ill-defined surface charges and conditions which are sensitive to the separation media and buffer solutions containing stabilizers and preservatives. Thus, the exact separation mechanisms for two different nanoparticles are still unclear. Nonetheless, these results suggest that CE method combined with standard absorption and fluorescence detection techniques can be utilized to separate and detect nano-sized species in a mixture of various species in different sizes and chemical origins.

In conclusion, we have demonstrated in this work the possibility of capillary zone electrophoresis as a novel separation tool for mixed nanoparticles. Although only two different materials were used in this work, this method should be applicable to the separation and detection of other nano-sized particles in a mixture. It is believed that the analytical schemes utilizing capillary zone electrophoresis

would be important for further investigation on the origin of size-, shape-dependence of physicochemical properties of nanoparticles, and possibly for future applications.

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