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## Separation of unmodified polystyrene nanosphere standards by capillary zone electrophoresis

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### Abstract

Size separation of five unmodified polystyrene nanosphere standards with diameters between 50 and 600 nm has been achieved in phosphate buffer solutions as carrier electrolyte. The electrophoretic mobility increases with particle diameter. Optical spectra was shown to be different for particles of different size. Effects of injection time, applied voltage, pH, and phosphate concentration in carrier electrolyte on particle separation were studied. Under optimal conditions the peak efficiency ranged from 600 to 10 500 theoretical plate numbers depending on nanosphere diameter was achieved. © 2000 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

Capillary zone electrophoresis is widely applied to separation of amino acids, peptides, proteins, oligonucleotides, various small organic and inorganic ions, metal complexes, etc. The technique can be also used for the investigation of colloidal systems, in particular for the separation of particles depending on their size and type. Particles can possess surface charges and therefore can migrate in an electric field. There are some examples of successful size separation of silica sols [1], gold particles [2,3], modified

polystyrene [4] and latex [5,6] micro- and nanospheres. Polymer particles with different numbers of charged carboxylate or sulfate groups bonded to their surface were investigated and the numbers of theoretical plates of about 3000 were achieved. The order and direction of particle migration observed in [4] and [5,6] were similar. However, there are two different points of view on the mechanism of particle separation. VanOrman and McIntire [4] supposed that the separation is controlled by the particle size and the particle-wall interactions. In contrast, Jones and Ballou [5,6] considered that the particle-wall interactions are negligible and that the separation is electrophoretic in nature. It would be interesting to elucidate the separation mechanism. Separations of different metal oxide particles ( $\text{TiO}_2$ ,  $\text{Al}_2\text{O}_3$ , and  $\text{Fe}_2\text{O}_3$ ,

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including polymorphic forms  $\alpha$ - and  $\gamma$ - $\text{Al}_2\text{O}_3$ ) are also described [7,8].

The aim of our work was to test the applicability of capillary zone electrophoresis to size separation of unmodified polystyrene nanospheres. It is known [9,10] that the polymerization process leaves behind a number of acidic functional groups which, because of their polar character, tend to accumulate at the polymer-solution interface. Particles of this kind bear a slight negative surface charge due to the ionization of such groups and therefore, they can be separated by CZE. Besides, acquirement of additional charge due to ion adsorption from a carrier electrolyte is possible for these particles. It is interesting to study the influence of different factors on the separation efficiency of such polystyrene nanospheres and to compare their migration behavior with that of modified polymer particles.

## 2. Experimental

### 2.1. Chemicals

Certified size standards of unmodified polystyrene nanosphere with a density of  $1.05 \text{ g/cm}^3$  (Duke Scientific Corp., Palo Alto, USA) were used as 1% solids aqueous suspension. The synthetic particle mixtures were prepared by dilution of the original suspensions with water. Some characteristics of nanosphere size standards under investigation as well as particle concentrations in suspensions injected into the capillary are listed in Table 1. The concentrations

are given as percent of solids and as the particle number per nanoliter. Before separation particle suspensions were treated for 1 min in a low power ultrasonic bath.  $\text{NaH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ , and  $\text{NaOH}$  utilized for preparing buffer solutions were of analytical reagent grade (Merck, Darmstadt, Germany). Water purified with a Milli-Q filtration system (Millipore Corp., Bedford, USA) was used.

### 2.2. Instrumentation

Capillary electrophoresis system P/ACE™ System MDQ (Beckman Instruments, Inc., Fullerton, USA) equipped with a diode array detector was applied. P/ACE System MDQ software configured on a Pentium computer workstation provided control, collection and analysis of experimental data. Uncoated fused-silica capillaries of  $75 \mu\text{m}$  I.D.,  $360 \mu\text{m}$  O.D., with a total length of 60.2 cm, and an effective length of 50.0 cm were used. All separations were performed at positive polarity of power supply and at constant applied voltage of 20–30 kV. Temperature was maintained at  $30^\circ\text{C}$  during the all runs. Wavelengths used for nanospheres detection ranged from 190 to 400 nm. Hydrodynamic injection of samples by a pressure of 0.69–3.45 kPa were performed at the anodic end of the capillary.

A capillary was rinsed with  $0.1 \text{ M}$   $\text{NaOH}$  (5 min) and then with water (5 min) at a pressure of 138 kPa before the first experiment and after the last one. It was conditioned by rinsing with buffer electrolyte (5 min) before runs. Vials were refilled with a fresh portion of the buffer solution after each run.

## 3. Results and discussion

### 3.1. Optical spectra

Particle optical spectra at wavelengths ranged from 190 to 400 nm have been recorded. The capillary was filled with aqueous suspension containing particles of the same diameter obtained by 200-fold dilution of the origin suspension. Differences between spectra from different size particles

Table 1  
Characteristics of polystyrene suspensions

Particle diameter, nm	Cat.-Nr.	$\lambda_{\text{max}}$ , nm	Particle concentration	
			% Solids	Particles/nl
$50 \pm 2$	3050A	194	$5 \times 10^{-3}$	$7.28 \times 10^5$
$102 \pm 3$	3100A	202	$5 \times 10^{-3}$	$9.09 \times 10^4$
$204 \pm 6$	3200A	229	$5 \times 10^{-3}$	$1.14 \times 10^4$
$404 \pm 4$	3400A	–	$1 \times 10^{-2}$	$1.42 \times 10^3$
$600 \pm 5$	3600A	–	$2 \times 10^{-2}$	$4.2 \times 10^2$

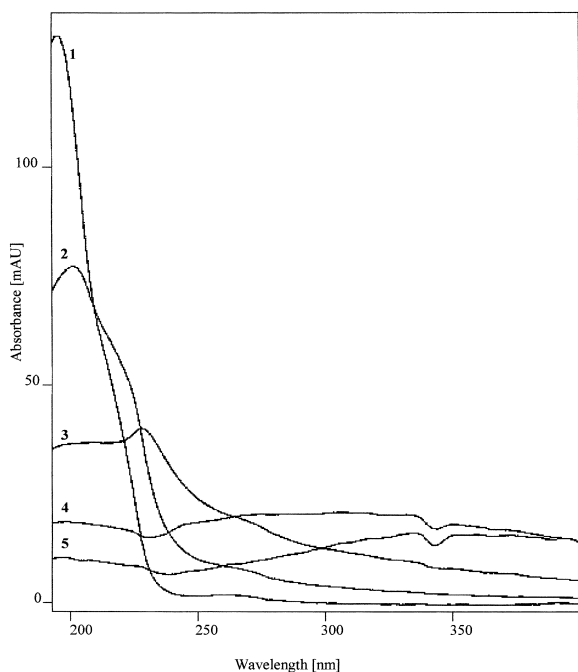


Fig. 1. Optical spectra of aqueous particle suspensions. Particle concentration:  $5 \times 10^{-3}$  % solids. Spectrum: (1) – 50 nm; (2) – 100 nm; (3) – 200 nm; (4) – 400 nm; (5) – 600 nm.

can be observed in Fig. 1. A spectrum of 50 nm polystyrene spheres has a sharp band with absorbance maximum at 194 nm. A suspension containing 100 nm polystyrene spheres also has an intensive band in its spectrum with an absorbance maximum at 202 nm. The absorbance maximum of slight broad band at 229 nm due to 200 nm polystyrene spheres is shifted to longer wavelengths compared to smaller particles. When suspensions of 400 and 600 nm particles are used the absorbance is practically independent on the wavelength. Only increasing of absorbance background is observed in this case. The same pictures were obtained when particle suspensions in a carrier electrolyte instead of water were used. Therefore, wavelengths at 200, 230, and 250 nm have been selected for the detection of particle zones in electrophoretic runs. Dependence of optical spectrum on the particle size can serve the basis for peak identification and checking peak purity in electropherograms.

### 3.2. Injection

Effect of injection parameters on particle peak height has been studied. The injection pressure between 0.69 and 3.45 kPa was applied and the injection time varied from 1 to 20 s. The dependencies of peak height on injection time were linear ( $y = a + bx$ ) for 50 ( $a = -1.8$ ,  $b = 1.408$ ), 100 ( $a = -4.0$ ,  $b = 1.250$ ), and 200 ( $a = -1.45$ ,  $b = 0.264$ ) nm particles at pH 8.1. It should be noted that the plots intercept the X-axis at different positions from the origin. This “dead time” (when particles are not injected) depends on injection pressure and particle size. It increases as the particle diameter increases and the injection pressure decreases. The phenomenon observed can be explained by inertial properties of particles. The analysis of data obtained allowed us to select the injection pressure of 1.38 kPa (0.2 p.s.i.) as optimal value.

### 3.3. Electrolyte concentration

Phosphate buffer served as a carrier electrolyte in our experiments. It is suitable due to low absorptivity in UV-range. The buffer provides a fast electroosmotic flow that is necessary to perform counterelectroosmotic mode of capillary electrophoresis. This mode seems to be the most convenient for separation of unmodified particles. The use of this buffer system allows us to vary the carrier electrolyte pH in a relatively wide range. Besides, changing an effective electrophoretic mobility of buffer anion due to mutual conversion between  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$  can be performed at pH ranged from 6.5 to 8.5 without noticeable alteration of electroosmotic flow-rate. It is of importance, when the effect of mismatching between a particle electrophoretic mobility and a buffer anion one on peak dispersion is estimated. In this case contribution owing to variations of hydrodynamic parameters should be negligible. Phosphate concentrations between 2.5 and 10 mM were tested. At 2.5 mM phosphate concentration the peak resolution was poor. At phosphate concentration more than 10 mM the operating power exceeding the value of 2.5 W/m is achieved. Besides, a danger of particle flocculation arises. It

was shown that increasing the phosphate concentration from 5.0 to 10.0 mM was accompanied with an increase in a particle migration time without a loss of peak efficiency. The values of electrophoretic mobilities are practically at the same level for all particles sizes under investigation.

### 3.4. Particle separation

A typical electropherogram obtained for a suspension containing polystyrene nanospheres of four different sizes ranged between 50 and 400 nm is shown in Fig. 2. The run was performed in 5.0 mM phosphate buffer at pH 9.0 as carrier electrolyte. It is to be seen that the peak separation is achieved for all these species. When injecting a five-component suspension with 600 nm particles, zones of 400 and 600 nm spheres co-migrate and an unresolved peak is observed. Some noise peaks rise on the front side

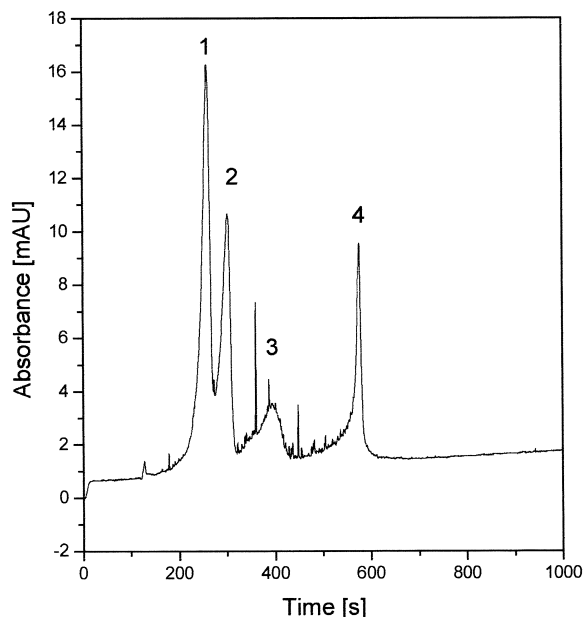


Fig. 2. Electropherogram of a four-component mixture. Carrier electrolyte: 5.0 mM phosphate buffer, pH 9.0; wavelength 200 nm; separation voltage -30 kV; injection pressure 1.38 kPa; injection time 10 s. Peak: (1) -50 nm; (2) -100 nm; (3) -200 nm; (4) -400 nm.

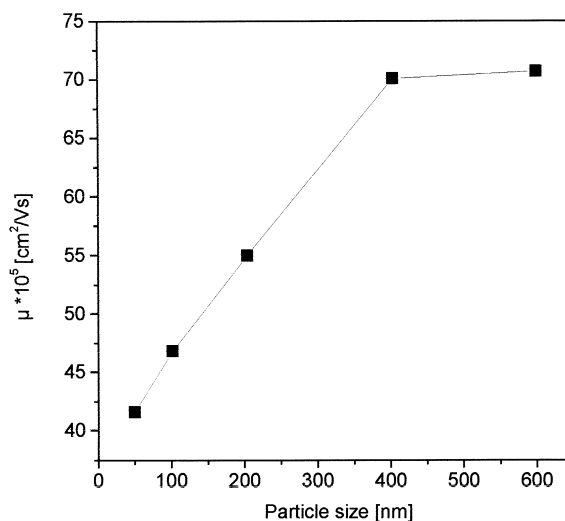


Fig. 3. Effect of particle size on the electrophoretic mobility. Carrier electrolyte: 5.0 mM phosphate buffer, pH 9.0; injection time 10 s other condition as in Fig. 2.

of the joined peak. The electrophoretic mobilities calculated for each particle size range between  $40 \times 10^{-5}$  and  $70 \times 10^{-5}$  cm<sup>2</sup>/V s. These values are high enough but they are lower than those obtained for modified nanospheres of the same sizes [5]. Fig. 3 shows that there is a proportionality between the electrophoretic mobility and the sphere radius for small size nanospheres. It allows us to suggest that the electrophoretic mobility is a function of surface charge/radius ratio as follows from the expression:

$$\mu_{ep} \sim q/a \sim 4\pi a^2 \sigma/a \sim a \quad (1)$$

where  $\mu_{ep}$  is electrophoretic mobility,  $q$  is particle charge,  $a$  is particle radius,  $\sigma$  is surface charge density. The same relationship is usually valid for various molecular ions. For large particles with the diameter more than 300 nm differences in electrophoretic mobilities are too small to provide peak resolution in electropherograms.

#### 3.4.1. Applied voltage

Variation of applied voltage between 20 and 30 kV

generates some differences in current value and analysis time but does not affect noticeably the separation efficiency.

### 3.4.2. Effect of pH

The range of pH between 6 and 10 was selected to estimate the pH effect on the migration behavior of particles depending on their size. At pH values lower than 6.5 the electroosmotic flow-rate decreases sharply and electrophoretic separation of particles takes more time or becomes impossible. This effect is more pronounced for fast large particles. At pH values higher than 10 the phosphate buffer capacity is not sufficient to maintain a constant pH value during electrophoretic runs. The data obtained are presented in Fig. 4. It is seen that the electrophoretic mobility of particles increases slightly with the pH of the carrier electrolyte. The curve shapes obtained for particles of different sizes are similar to each other. The selectivity of separated 50 and 100 nm particles increases twice when the pH value is changed from 6.0 to 6.6, and then remains almost unchanged with

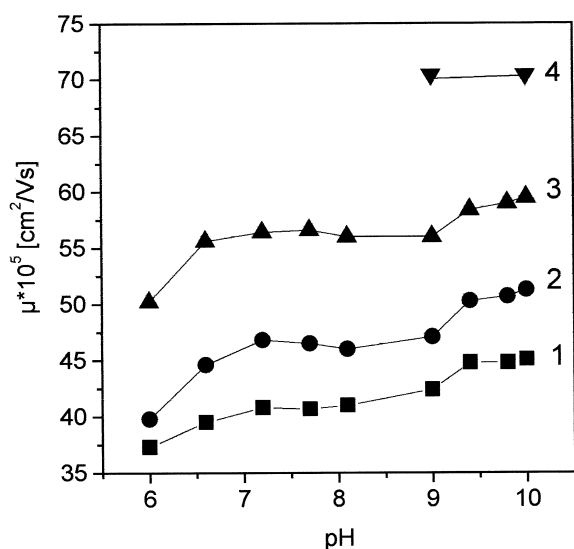


Fig. 4. Dependence of particle electrophoretic mobility on pH. Carrier electrolyte: 5.0 mM phosphate buffer; other condition as in Fig. 2. Plot: (1) – 50 nm; (2) – 100 nm; (3) – 200 nm; (4) – 400 and 600 nm.

Table 2

Some properties of polystyrene nanospheres

Particle diameter, nm	$\mu_{ep} \times 10^5$ , $\text{cm}^2/\text{Vs}$ (pH 9.8)	$w_{1/2}$ , min	$N_{max}$
50±2	44.8±0.7	0.28±0.05	2350
102±3	50.7±0.4	0.34±0.06	2150
204±6	59.0±1.0	0.66±0.06	600
404±4	~70	0.26	10 500
600±5	~70	0.33	6500

further increasing in pH. The selectivity of larger particles decreases gradually from 0.23 to 0.16 with increasing pH in the range under investigation. The peak efficiency as a function of pH value has a maximum at pH 6.6 and pH 7.7 for 50 and 100 nm particles, respectively. The peak efficiency for 200 nm particles has the lowest value and decreases with increasing pH. The highest numbers of theoretical plates per meter achieved are listed in Table 2. The contribution of longitudinal dispersion to peak broadening in case of nanospheres is small probably because their diffusivities are approximately 10 times less than those of macromolecules. To estimate the effect of mismatching between the electrophoretic mobility of a particle and that of a buffer anion, the effective electrophoretic mobilities of phosphate anion based on its dissociation constants and ionic mobilities [11] were calculated. They are near the values of particle electrophoretic mobilities and change from  $35.5 \times 10^{-5}$  to  $58.7 \times 10^{-5} \text{ cm}^2/\text{Vs}$  as pH rises from 6.0 to 10.0. The contribution due to hydrodynamic fractionation during electrophoretic run may be neglected because the electroosmotic flow-rate vectors have a flat hydrodynamic profile in a capillary. Besides, the particle sizes under investigation are too small to provide a noticeable concentration gradient in radial direction due to settling in natural gravitational field, particularly when the densities of particles to be separated are close to that of carrier electrolyte. For the largest particles (600 nm) the ratio  $L_d V_{Stokes} / 2nV_{ap}$ , (where  $L_d$  is the effective length of a capillary,  $V_{Stokes}$  is the Stokes settling rate of particles,  $n$  is the number of capillary coils in the cartridge,  $V_{ap}$  is the apparent particle rate) does not exceed a value of 0.1. The effect of particle-wall interactions is also negligible.

That is confirmed by nontailing peak shapes. Also, both the particles and capillary wall are negatively charged and they undergo mutual electrostatic repulsion. Besides, after injection no difference in electroosmotic flow-rate is observed proving that capillary walls are not modified with polystyrene nanospheres. The effect of electrophoretic heterogeneity on peak broadening of certified nanosphere standards with narrow size distribution seems to be not high. On the other hand, a large amount of polystyrene nanospheres possessing a low UV absorptivity are required for successful detection. In this case sample overloading may most dramatically influence the peak efficiency. It is proved by a noticeable peak broadening of 200 nm nanosphere standard compared to smaller ones as the sample volume injected is increased.

The influence of other parameters on the polystyrene nanosphere electrophoretic mobility can be estimated using the Henry equation for nonconducting spherical charged colloid particles:

$$\mu_{ep} = \zeta \epsilon \epsilon_0 f(\kappa a) / 1.5 \eta \quad (2)$$

Here,  $\zeta$  is the zeta potential,  $\epsilon$  is the dielectric constant,  $\epsilon_0$  is the permittivity of vacuum,  $\eta$  is the viscosity of the solution,  $a$  is the particle radius and  $\kappa$  is the Debye-Hueckel parameter, which can be calculated by formula [12]:

$$\kappa = 3.28 \sqrt{I} \quad (3)$$

where  $I$  is the ionic strength of the solution. The values of  $\kappa$ , reciprocal ( $1/\kappa$ ) named by thickness of electric double layer and product  $\kappa a$  representing the ratio of particle radius to thickness of the electric double layer have been calculated for the experimental condition used. The highest  $1/\kappa$  value of 4.0 nm is obtained when using 5.0 mM phosphate buffer solution at pH 6.0. It means that the particle diameter growth due to electric double layer exceeds size dispersion of 50 and 100 nm polystyrene nanospheres in samples under investigation, and it achieves 16% for the 50 nm particles. The 12-fold alteration of the product  $\kappa a$  is obtained on going from the particle diameter 50 to 600 nm. It is also

known, that the value of  $f(\kappa a)$  changes from 1.0 to 1.5 as the  $\kappa a$  value increases from 0 to infinity. In our experiments we observed a 1.7-fold increase in the particle electrophoretic mobility as a particle diameter increases from 50 to 600 nm. It may result from additional contribution of the frictional drag during particle separation in a narrow capillary.

#### 4. Conclusion

The results obtained show the potential of capillary electrophoresis for size separation of unmodified polystyrene nanosphere size standards with certified diameters ranging from 50 to 600 nm. Numbers of theoretical plates of about 10 000 have been achieved. This technique represents a good supplement to traditional particle size fractionation procedures such as ultrafiltration, size-exclusion chromatography or field flow fractionation. It was found that migration behavior of polystyrene spheres are mainly determined by their electrophoretic properties. The relations between peak height and injection time for different particle sizes indicate the promise of capillary electrophoresis for particle quantification.

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