ELSEVIER

Journal of Chromatography B, 722 (1999) 1–10

JOURNAL OF CHROMATOGRAPHY B

# Capillary electrophoresis of subcellular-sized particles

Sergey P. Radko, Andreas Chrambach*

*Section on Macromolecular Analysis, Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, Building 10, Room 9D50, Bethesda, MD 20892-1580, USA*

**Abstract**

Utilization of capillary electrophoresis (CE) for characterization and analytical separation of submicron- and micron-sized organic and inorganic particles as well as biological vesicles is reviewed. CE has been applied to charged polystyrene size standards, inorganic and organic colloidal particles, lipoprotein particles, liposomes, microsomes and viruses. These particle separations generally occur in a size-dependent manner and provide values of electrophoretic mobility which are in good agreement with those obtained by other electrophoretic techniques. 

*Keywords:* Polystyrene-size standards; Inorganic particles; Organic particles; Lipoprotein particles; Liposomes; Microsomes; Viruses

## 1. Introduction

Separation of organic and inorganic colloidal particles as well as biological vesicles within a size range of several nanometers to several micrometers is required both for analytical and preparative purposes in environmental studies, medicine and biology, biotechnology and industry. A variety of separation techniques has been developed for this purpose. Among physical separation methods, sedimentation has been traditionally used, albeit mostly for preparative fractionations (e.g., [1]). Size-exclusion liquid chromatography [2,3] and hydrodynamic chromatography [4,5] have been applied for analytical separations of different colloids of up to 1 μm diameter. Other approaches to the size-dependent separation of submicron-sized colloids using liquid flow were sedimentation field flow fractionation [6,7] and capillary hydrodynamic fractionation [8,9]. Since most colloids as well as biological vesicles possess an electric charge in buffered aqueous solutions, electrophoretic separation methods were also among those utilized for particle fractionation and characterization. Free flow electrophoresis was applied to preparative fractionation of biological vesicles (see [10] for a review) and organic colloidal particles [11]. Recently, a new approach has been introduced to separate sub-micron bioparticles and latex beads, using a non-uniform alternating electric field (dielectrophoresis) [12,13]. Gel electrophoresis was employed for fractionating biological particles of up to several hundred nanometers diameter (reviewed in [14]). The separation of subcellular-sized particles by electrophoresis in buffered polymer solutions as well as a feasibility of their isolation were also demonstrated [15–17]. Laser Doppler velocimetry [18,19] and different modifications of microelectrophoresis [20–22] were used for measuring electrophoretic mobilities of particles.

Capillary electrophoresis (CE), after emerging in the early 1980s, was immediately recognized as a promising analytical separation method for small

*Corresponding author.

0378-4347/99/$ – see front matter 
PII: S0378-4347(98)00307-7

molecules and ions (e.g., [23]). Soon it had been extended to the separation of macromolecules, mostly of biological origin, such as proteins and DNA, and nowadays CE is widely accepted as a powerful analytical technique both for macromolecular and molecular separations, providing speed, quantitation and automation to the inherently highly resolving but often labor intensive methods of electrophoresis (e.g., [24]). Over the last decade, intermittent attempts have been made to employ CE for analytical separations of ''particulate materials'' like colloidal organic or inorganic particles as well as biological subcellular vesicles. With one exception, those separations were carried out by using a particular mode of CE, viz. capillary zone electrophoresis (CZE). The review of those attempts is the subject of the present report.

## 2. Particle separations by capillary electrophoresis in free solutions

### *2.1. Polystyrene size standards*

The first CZE separation of derivatized polystyrene nanospheres of known mean diameter and narrow size distribution (polystyrene size standards) was reported by VanOrman and McIntire in 1989 [25]. The authors separated a mixture of negatively charged particles (Fig. 1): five polystyrene sulfates (PSS) and one polystyrene carboxylate (PSC) with diameters of 39 to 683 nm, using 50 μm I.D. fused-silica capillaries and 1 m*M* (acetamido)aminoethane sulfonic acid as an eluent[1]. It was found that the observed electrophoretic mobilities did not correlate with particle surface charge (provided by the manufacturer and likely to have been measured by titration) or charge-to-mass ratio but correlated well with particle size [25,26]. This size-dependent separation (Fig. 2) was ascribed to differential interaction of the polystyrenes with the capillary walls, evidenced by failure of separation to correlate with the charge/mass ratio and by an observed decrease in resolution with increasing ionic strength of electrophoretic

[1]Note: The term ''eluent'' is used for the buffer in those applications of CE where electroendoosmotic flow gives rise to an elution of buffer from the capillary.

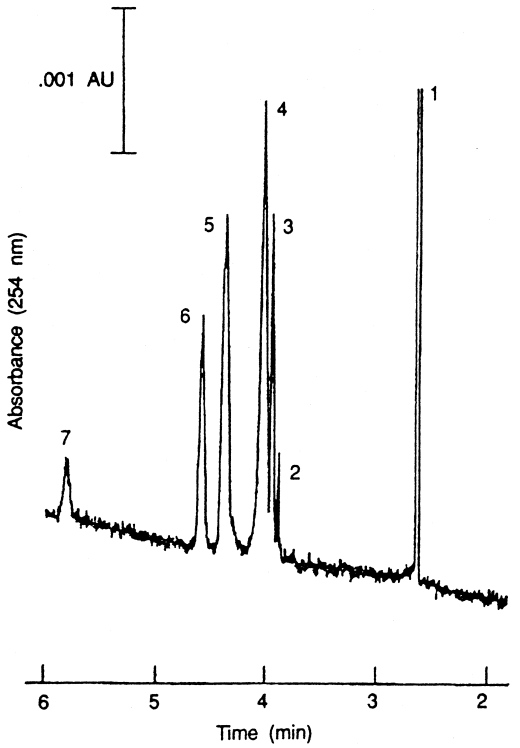


Fig. 1. CZE electropherogram of polystyrene size standards: 1=riboflavin (neutral marker), 2=39 nm, 3=72 nm, 4=132 nm, 5=308 nm, 6=488 nm, 7=683 nm particle diameter. Field strength 382 V $cm^{-1}$. Anodic sample injection and reverse order of migration due to electroendosmosis. Detection at 225 nm. (From Ref. [25] with permission).

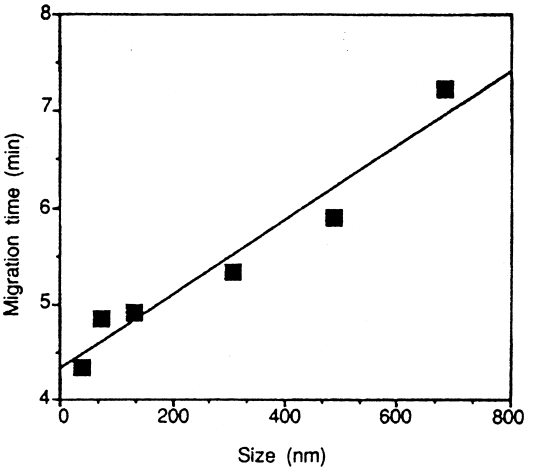


Fig. 2. Size-dependent separation of polystyrene particles by CZE: Conditions as in Fig. 1. (From Ref. [25] with permission).

buffer. Cationic detergent treatment of the capillary walls resulted in an increase in resolution, speculatively ascribed to the blocking of hydrophobic interaction sites and neutralizing the ionic interaction sites so as to diminish the charge interaction of the wall surface with the particle [25,26].

However, in a later publication by the same authors [27] the correspondence between the electrophoretic mobilities of particles determined by CZE and those determined by laser Doppler velocimetry (LDV) was demonstrated for PSS and PSC of 105 to 280 nm diameter. A good agreement between mobilities of negatively charged polystyrenes of 306 to 2010 nm diameter measured by CZE and LDV was also found by Fourest et al. [28,29]. Thus, the conclusion has to be drawn that the electrophoretic mobilities of negatively charged PSC and PSS were validly measured by CZE [27–29] and the size-dependent separation was controlled electrophoretically, i.e., by the charge density of the particle surface, rather than by particle-wall interactions.

The mobilities measured by either CZE or LDV differed from those expected according to the number of surface charged groups [26–29]. This discrepancy was likely to result from the fact that an electrophoretic mobility is a function of zeta potential defined by a net charge present within the layer of liquid enveloping the particle surface (the so-called ''surface of shear'') [30]. The resulting net charge differs from (is basically smaller than) the particle surface charge found, for instance, by titration [30]. Yet, the size-dependent separation cannot be accounted for on the basis of zeta potential since that depends on net charge density which is the same for chemically homogeneous particles of different sizes. The size-dependent separation is likely to arise from particular conditions of capillary electrophoresis, viz. a use of high electric fields and low ionic strength electrolyte buffers. Those conditions can lead to the pronounced relaxation effect [30,31], i.e., a deformation of the electric double layer around the particle which differs depending on the size of the particles and buffer ionic strength. Thus, the deformation results in a size-dependent electrophoretic migration for particles similar in zeta potential [32].

The relaxation effect is described by a dimensionless parameter $ka$ where $a$ is a particle radius and $k^{-1}$ a thickness of electric double layer. For particles of given sizes, the deformation of electric double layer is expected to slightly affect their electrophoretic mobilities in the range of very low ($ka<1$) and relatively high ($ka>>1$) ionic strengths. Under other conditions ($ka\sim1$), larger particles are expected to migrate faster than smaller ones [30–32].

Seven-component mixtures of PSS and PSC in the size range of 30 to 1160 nm diameter have been separated by CZE with optimization of column dimensions, buffer conditions and field strength [33,34]. The separation was found to be size-dependent: The larger particles exhibited higher absolute values of electrophoretic mobility. Reduction of total column (75 μm I.D.) length and distance from the inlet of capillary to detector as well as the increase in buffer pH values from 6.6 to 10.7 proved crucial to reducing peak width and improving resolution between components in the latex mixture. For a two-component latex mixture (PSS of 79 nm and PSC of 100 nm diameter) the numbers of theoretical plates, selectivity, and resolution were calculated at different pH and applied voltage values. They were found to range from 400 to 3000, 0.1 to 0.4, and 0.8 to 2.3, respectively [33]. Based on the observed independence of separation efficiencies and resolution on electric field strength, microheterogeneity of mobility (''electrophoretic heterogeneity'') was assumed to be a dominant source of zone broadening. Even under optimal conditions, that zone broadening is presumably responsible for the relatively low efficiency of only 4400 theoretical plates in a representative case of 70-nm PSC [34].

The low efficiency in CZE due to a natural electrophoretic heterogeneity of particles may be thought to ease restrictions on other zone broadening sources allowing one to employ larger injection and detection volumes, capillary inner diameters, and operating power without any significant further loss of efficiency or resolution.

The analytical CZE separation of seven negatively charged polystyrene size standards of 91 to 1100 nm diameter was also performed in the presence of the detergent, sodium lauryl sulfate [35]. 100 and 25 μm I.D. fused-silica capillaries and 1 and 100 m*M* solutions of sodium lauryl sulfate as an eluent were used. At both detergent concentrations the greater absolute values of the electrophoretic mobility of the larger particles relative to those of the smaller ones

were observed [35] in line with the increased charge equalization through reaction of the particles with the charged detergent.

While a mobility of negatively charged polystyrene latex seems to be well characterized by CZE in buffers of neutral or basic pHs [27–29,33–35], positively charged particles under the same conditions can exhibit an ''odd'' behavior in CZE experiments. In capillaries with electroosmotic flow towards the cathode, polystyrene amine nanospheres of 116 to 300 nm diameter all demonstrated exactly the same, apparently anionic mobility while possessing different values of cationic mobility according to LDV measurements [27]. It must be assumed that in this case a retention due to an electrostatic interaction of positively charged polystyrene particles with negatively charged capillary walls replaces the electrophoretic mechanism in directing the migration in CZE, making it appear as though particles possessed a negative net charge [27].

### *2.2. Inorganic and organic particles*

Seven silica sols of known mean diameter ranging from 5 to 500 nm were separated by CZE, using uncoated 50 μm I.D. fused-silica capillaries filled with pH 9.0 ammonia buffers of various ionic strengths [36]. The separation was size-dependent, with larger colloids having higher absolute values of electrophoretic mobility than the smaller ones as can be expected from the relaxation mechanism [30–32]. The ionic strength of the electrophoretic buffer was found to greatly influence the mobilities of the silica sols and their resolution, with higher ionic strengths substantially improving resolution of smaller particles. Upon increase of buffer concentrations from 2.5 m*M* $NH_4OH$, 4.65 m*M* $NH_4Cl$ to 10 m*M* $NH_4OH$, 18.6 m*M* $NH_4Cl$, an essentially baseline resolution of 5, 9, 18 and 44 nm sols has been accomplished in less than 18 min. However, it significantly increased the elution time of the larger colloids [36]. Apparently, the reported improvement in resolution results from the relaxation effect enhancing the silica sol separation upon increase of ionic strength from ''very low'' to ''low'' values (Section 2.1).

Characterization and separation of metal oxide fine particles (Al–, Ti–, Fe–oxides) ranging in size from 10 to 1000 nm diameter were carried out by CZE in 100 μm I.D. fused-silica capillaries under varying conditions of pH and ionic strength [37]. By using 5 m*M* sodium nitrate solutions with pH of 3 to 12 of constant ionic strength as an eluent, a general agreement was demonstrated between the observed isoelectric points for the metal oxide particles and the values reported in the literature. Also, a general agreement was obtained between the expected electrophoretic mobilities and those measured by CZE. Separation of titania and alumina was achieved at pHs of 11 to 12 but not at pH 9, where it is likely to suffer particle coaggregation. At pH 12, the baseline separation of a mixture of α,γ-$Al_2O_3$ and γ-$Al_2O_3$ was obtained (Fig. 3) showing the possibility to separate metal oxide particles by CZE on the basis not only of chemical differences but also of polymorphic form. Lowering of the ionic strength at a constant pH value was generally shown to result in better separation of the metal oxides [37] as expected.

The effects of phosphate, carbonate, and borate anions and of sodium ion concentration on the electrophoretic mobility and CZE separation of the metal oxide particles mentioned above have been investigated [38]. All three anion types were found

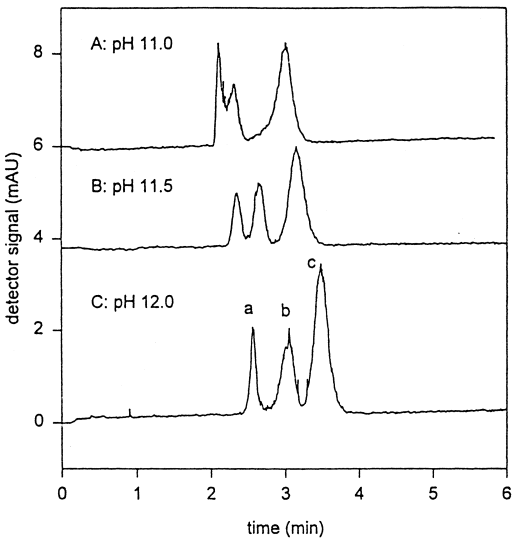


Fig. 3. CZE separation of α,γ-$Al_2O_3$, 300 nm diameter (a); γ-$Al_2O_3$, 10 nm diameter (b); $TiO_2$, 450 nm diameter (c) at various pH. Detection at 254 nm. (From Ref. [37]).

to significantly influence the mobility of each of the oxides, with borate anions yielding the highest degree of selectivity in CZE separations between titania and alumina. The best quality oxide separations for each of the buffer systems were obtained at a $Na^+$ concentration of 3 m*M* [38].

The applicability of CZE for characterization of colloidal gold particles has been studied [39], using 75 μm I.D. fused-silica capillaries and acetic acid/acetate buffers, pH 5.0, of various ionic strengths as an eluent. Five species with mean particle diameters determined by size-exclusion chromatography ranging from 5.2 to 14.6 nm were used. It was found that the mobility of the particles with a given diameter decreased with decreasing ionic strength in accordance with expectation. Surprisingly, the experimentally obtained dependence of the electrophoretic mobility on the particle size was the opposite from that expected theoretically, viz. the mobility decreased with particle diameter, showing a fairly linear dependence on its reciprocal [39]. Possibly, a size related retardation, due to some specific interaction of colloidal gold with the capillary walls, changes the order of migration similarly to that of cationic polystyrene amines (Section 2.1).

CZE has been applied to the determination of the uniformity or heterogeneity of complex nano- to micrometer sized composite colloids [40] obtained by the polymerization of aniline in the presence of water-soluble polymers such as poly(vinylpyrrolidone) or poly(vinyl alcohol). Uncoated 75 μm I.D. fused-silica capillaries and 20 m*M* sodium tetraborate buffer were used. The electrophoretic mobilities measured by CZE were found to be linearly related to values of particle zeta potential evaluated by an independent method. The clear, though not baseline, separation of the polyaniline-based composite from the silica-based composite particles was demonstrated [40]. In this case, the analytes are not ''similar'' but differ chemically. In consequence, the surface charge densities, and the corresponding zeta potentials, vary among species and give rise to separation.

Measurements of an electrophoretic mobility for nanoparticles of thorium phosphate (diameter of about 300 nm as evaluated by photon correlation spectroscopy) were performed by CZE and LDV with an essentially identical result [28,29].

Free-solution capillary electrophoresis was employed for monitoring zeta-potential of tetragonal lysozyme crystals in their growth solution (0.3 *M* NaCl), using 1 mm I.D. uncoated capillaries [41]. The lysozyme crystals are known to grow to μm-range diameters. The electrophoretic mobility was found to be unchanged in the range of 5 to 15 μm of the effective crystal radius, while it decreased sigmoidally from 0.9 to 0.3 μm cm $V^{-1}$ $s^{-1}$ between pH 3.5 and 5.7. The titration curve allowed for computation of the zeta potential of the crystal of 24 to 8 mV within that pH range. The observed size-independent mobility is thought to result from relatively high ionic strength of the electrolyte solution used and is merely a function of zeta potential.

### *2.3. Lipoprotein particles*

CE has been successful in providing a clinical assay for human serum lipoproteins [42] classified as heavy (HDL, 7–13 nm diameter), intermediate (IDL, 25–35 nm diameter), low (LDL, 18–25 nm diameter) and very low density (VLDL, 30–80 nm diameter) species, each with several components. After prestaining with the nonpolar dye Sudan Black B, lipoprotein particles were subjected to analytical capillary isotachophoresis in a discontinuous electrolyte system with a number of ''spacers'', using a 500 mm I.D. capillary at 300 V $cm^{-1}$. The lipoproteins separated into 14 well-characterized subfractions according to their net electrophoretic mobility [42]. These isotachophoretic net mobility separations constitute the sole example known to the authors where the mode of particle CE was not CZE.

HDL and LDL have also been separated by CZE in SDS-containing buffer under conventional conditions, provided that the polarity of the buffer was reduced by the addition of acetonitrile [43].

### *2.4. Liposomes and microsomes*

Liposomes, artificial phospholipid vesicles formed by a self-assembling lipid bilayer in aqueous solutions, intrinsically possess a wide size distribution. The mean diameter of liposomes can range from one hundred nanometer to the micrometer range depending on the conditions of preparation. Their behavior in CE was studied [44] with regard to the evaluation

of vesicle–wall interactions, bilayer stability in the electric field, and on-column solvent- and surfactant-induced liposome lysis. Underivatized 75 μm I.D. fused-silica capillaries and 9.5 m*M* phosphate buffer, pH 7.4, as an eluent were used. Liposomes appear to be stable under the high electric fields applied in CZE, producing a smooth broad roughly Gaussian peak with few spiking events. Although underivatized capillaries were found to adsorb liposomes until saturation of the walls occurred after three runs, mobility was unaffected by that adsorption and remained constant for at least 100 runs. It was shown that, when the column walls are saturated, liposome electropherograms can be used to determine their concentration and to provide a reasonable estimate of both bilayer charge and liposome size distribution. When exposed to conditions favoring bilayer disruption or liposome aggregation, liposome preparations exhibited greatly altered peak shapes, loss of peak area, and an increased number of spikes [44].

Ultrasonic treatment of liposome preparations for the extended period ranging from 15 min up to 4 h was found to result in a narrowing of the electrophoretic peak in the CZE of liposomes [45], using underivatized 50 μm I.D. fused-silica capillaries and 10 m*M* carbonate buffer, pH 9.0. The peak shape evolution with time of the treatment constitutes a progressive ''contraction'' of the peak towards species with the lowest mobility [45]. Since such treatment is known to greatly decrease both the relative size distribution and the mean diameter of the liposomes, the ''contraction'' indicates the lowering of mobility with particle size, in line with the size-dependent electrophoretic migration generally demonstrated for charged colloids.

To date, the sole subcellular-sized particles of some biological relevance tested by CZE were rat liver microsomes [47]. Microsomes are the membrane vesicles of about 150 nm mean diameter formed by isolated rough endoplasmic reticulum in aqueous solutions. The vesicles are extensively used as a model system for studing GTP-triggered membrane fusion [46]. Microsome preparations purified by density gradient centrifugation demonstrated a broad but single peak in CZE, using 150 μm I.D. fused-silica capillaries internally coated with linear polyacrylamide and using TBE buffer as the electrolyte solution [47] (Fig. 4). The crude preparations

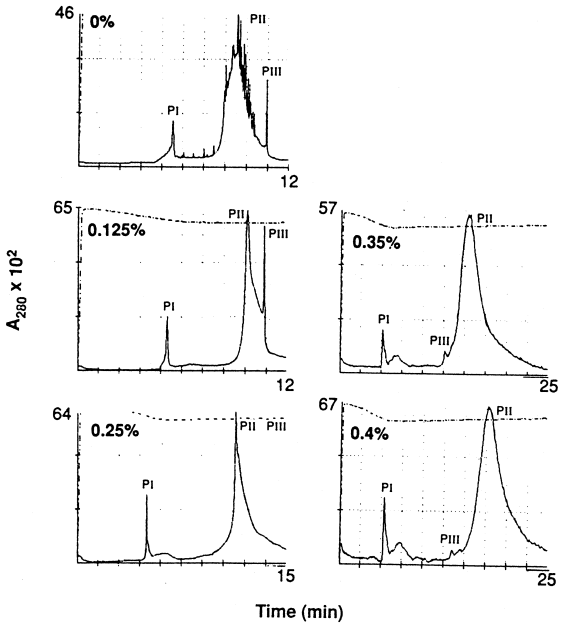


Fig. 4. CZE patterns of purified rat liver microsomes as a function of polyacrylamide (MW $5\times10^6$) concentration (%). Field strength, 270 V $cm^{-1}$. (From Ref. [47] with permission).

were found to additionally contain a minor, faster migrating component [47]. As in the CZE of liposomes, microsome electropherograms also exhibited spikes i.e., sharp peaks superimposed on the broad electrophoretic zone. The frequency of appearance and positions of spikes changed from run to run. The preincubation of the preparations with 1 m*M* $Mg^{++}$ was shown to result in a deterioration of the broad microsome peak accompanied by a formation of multiple spikes. After a short incubation under conditions promoting delayed microsome fusion, i.e., in the presence of GTP and $Mg^{2+}$, the electrophoretic pattern changes dramatically exhibiting five components [47].

Based on the high variability in the migration time of the spikes, Roberts et al. have suggested [44] that they are related to liposome aggregates forming while liposomes are migrating through the capillary. An analogous aggregation is thought to be responsible for appearance of spikes in the CZE of microsomes. It is known that aggregation of particle suspensions is promoted in the presence of an electric or centrifugal field [48]. In the case of microsomes [47], an additional contributing ag-

gregating factor is the presence of $Mg^{2+}$ and their propensity for fusion in the presence of $Mg^{2+}$ and GTP. Although commonly one attempts to suppress such aggregation, its quantitation via CZE may provide a new approach to studying fusion related phenomena.

### *2.5. Viruses*

CZE of tobacco mosaic virus (TMV), carried out by Hjerten et al. in 1987, was the first known attempt to apply CE to large particles [49]. Using 100 μm I.D. fused-silica capillaries internally coated with linear polymers and 20 m*M* Tris–HCl (pH 7.5) as an electrolyte solution, an electropherogram with a single peak, narrow by comparison with those of biological particles discussed above, was obtained (Fig. 5). It is noteworthy that this initial report on CZE of particles employed internally coated capillaries which subsequently reported work failed to do. Clearly, suppressing electroendoosmosis by wall coating with polymers makes it convenient to obtain reproducible mobilities characterizing the CZE peaks. However, it also changes the conditions of interactive dispersion to reduce separation efficiency or improve it as known for the CZE of macromolecules (e.g., [24]).

Although virus electrophoresis is a field of practical and theoretical interest [14], CE was not employed subsequently for this purpose as a conventional electrophoretic technique. However, TMV was used by Grossman and Soane [50] to study the effect of orientation of a rod-shaped polyion on its electrophoretic mobility in CZE. TMV, a rigid, cylindrically shaped particle approximatelly 340 nm in length and 1.5 nm in diameter, was electrophoresed in 50 μm I.D. fused-silica capillaries, using 2 m*M* borate, pH 8.4, as a buffer. It was found that the virus mobility exhibited a monotonic increase of about 10% with electric field strength in the range of 100 to 400 $V\,cm^{-1}$, while the mobility of spherical control particle, 364-nm polystyrene, failed to demonstrate an increase. The observed increase in mobility was shown to quantitatively correlate with the decrease in the translational frictional coefficient due to the alignment of TMV with the electric field [50]. The report accentuates the importance of considering shape factors in the CZE of particles.

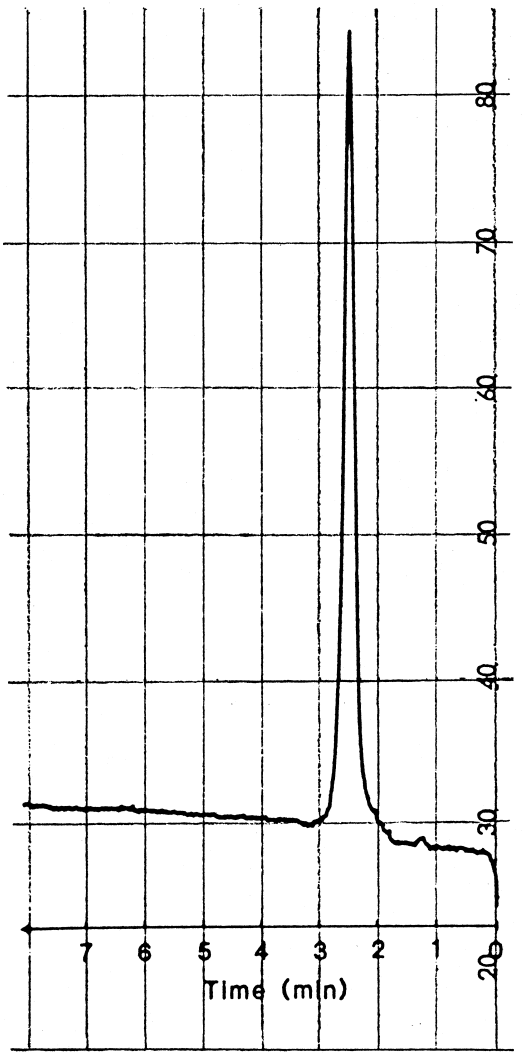


Fig. 5. CZE of Tobacco Mosaic Virus, 340 nm×1.5 nm. Fused-silica capillary of 115 mm×0.1 mm I.D.×0.26 O.D. Field strength, 73 $V\,cm^{-1}$. Detection on-line, 260 nm. (From Ref. [49]).

## 3. Capillary zone electrophoresis of particles in polymer solutions

Buffered solutions of neutral water-soluble synthetic polymers are widely employed in CZE as sieving media for the size-dependent separation of macromolecules (e.g., [51]). Their effects on electrophoretic migration of spherical nano- and micrometer diameter particles in CZE were also studied [47,52–54], using 150 μm I.D. fused-silica capillaries inter-

nally coated with polyacrylamide and using TBE or 50 m*M* Tris–HCl, pH 8.4, as an electrophoretic buffer.

It was found [52] that the presence of linear polyacrylamide (PA) of MW=$5\times10^6$ in the electrophoretic buffer can greatly influence the dependence of mobility of a 3-μm diameter PSC on sample load. While in the buffer alone the PSC electrophoretic mobility did not change appreciably over the load range of 0.3 to 6 μg of solid latex, it increased by a factor of two over the same range at a PA concentration of 0.125%. Above that concentration the dependence of mobility on the sample load became flatter [52]. The observed phenomenon was accounted for by a particle aggregation induced by the presence of polymer. Thus, the presence of polymer in electrophoretic buffer appears to impose a severe limitation on the concentration of particles analyzed by CZE.

CZE of fifteen PSS and PSC species with diameters ranging from 14 to 2170 nm, carried out in the buffered solutions of the same linear polyacrylamide, revealed an electric field dependent retardation [53]. It was shown that while for the particles of up to 30 nm in diameter the relative electrophoretic mobility ($\mu/\mu_o$, a ratio of mobility in a polymer solution, $\mu$, to that in the buffer alone, $\mu_0$) does not change with field strength, for particle sizes above that diameter the relative mobility becomes electric field- and particle size range-dependent. The inverse relative mobility (microviscosity), $\mu_0/\mu$, was demonstrated to depend on the shear rate of a polymer network deformation, due to the passage of the particle through the polymer solution, in the manner of the shear rate effect on the macroscopic viscosity of a non-Newtonian fluid. Thus, the field-dependent retardation was hypothetically accounted for by a shear stress-dependent resistance of the polymer network to passage of the particle. When the retardation of a particle by the polymer network is expressed in terms of a retardation coefficient (the slope of a linear approximation of log [relative mobility] vs. polymer concentration), a limit in the resolving capacity, due to a sharp increase in the rate of peak broadening with polymer concentration, was found once the value of the retardation coefficient exceeded 60 ml $g^{-1}$ [53].

Complex dependencies of the retardation coefficient and the rate of peak broadening on polymer MW and particle size were obtained by CZE of 30-, 91-, and 205-nm PSC in solutions of polyethyleneglycol (PEG) of 0.2 to $4\times10^6$ MW [54]. In spite of the complexity, it can be clearly seen that the retardation coefficient and the rate of peak broadening correlate, most likely due to the inherent size heterogeneity of polystyrene size standards.

Both in PA and PEG solutions the microviscosity calculated from particle retardation exhibited values far below those obtained for macroviscosity [53,54]. This may be explained by a formation of a depletion layer due to the entropic repulsion of polymer chains at the interface between polymer solution and particle. A subsequent decrease of polymer segment density near the interface results in reduced viscosity in the electric double layer region and, consequently, a higher than expected electrophoretic mobility. A theoretical description may be found in [55] as well as a review on depletion layers formed by PEG and dextran at red blood cell and liposome surfaces.

Rat liver microsomes were also subjected to CZE in solutions of various polymers [47]. Their separation is marginally improved at low concentrations and decreases when these concentrations exceed an optimal value. It is interesting to note that the number of spikes observed in microsome electropherograms in the presence of polymers was sharply reduced (Fig. 4).

## 4. Injection and detection

Though sample introduction by electromigration (electrokinetic injection) was used in a number of CE separations of nano- and micro-meter sized particles [25,29,35,44], a hydrodynamic injection seems to be preferable since samples of large particles or biological vesicles usually consist of components differing in electrophoretic mobility. Variants of hydrodynamic introduction successfully employed in CE of particles have used application of external pressure [27,34,36–40,44,52–54], application of vacuum to suck the sample into the capillary [49,50] and siphoning [33,44,45].

The injected sample volume is known to affect the peak width in CZE of macromolecules (e.g., [24]). However, in CZE of particles, due to their mobility

heterogeneity, this contribution of sample volume to the final peak width may be relatively small and negligible.

The detectability of particles during electrophoresis is an essential requirement for application of CE to analytical particle separation. Turbidity measurements, usually in the UV-range, have widely been used for on-line particle detection [25,27,29,33–40,49,50,53]. The limit of detection at 225 nm was determined as 367 pg (2000 particles) for a large 683-nm PSC and 121 pg (3.6 million particles) for a small 39-nm PSC [25]. By measuring turbidity at two [35] or three [39] wavelengths, the observed peaks could be additionally discriminated since the ratio of signals is characterictic for each particle size. Over the last years, a variety of fluorescently labeled PSC and PSS preparations has become available. A number of such fluorescent size standards was used in CZE employing fluorescence detection [52,54].

In CZE of liposomes, a detection in the visible range, at 651 nm, was achieved by including a lyophobic dye, (dioctadecyl)tetramethylindodicarbocyanine, in the lipid bilayer [44]. A method of on-line chemiluminescence detection for CZE of Eosin Y-containing liposomes has been demonstrated [45].

Lipoprotein particles in CZE were detected by both UV absorbance at 214 nm [43] and by monitoring at 570 nm after their prestaining with nonpolar dye Sudan Black B [42].

There has been an overall development of detection tools in CE to methods of ever increasing sensitivity, from absorbance to fluorescence and chemiluminescence methods of detection (e.g., [24]). This trend is important for the CE of particles in view of (i) decreased interaction and aggregation; (ii) enhanced separation efficiency at low loads; (iii) scarce supply of biological vesicles. However, for colloidal particles, the application of relatively insensitive detection methods, those based on light scattering, remains imperative.

## 5. Conclusions

CE lends itself to the analytical separation and the characterization of submicron- and micron-sized particles of different origins. Particle separations occur in a size-dependent manner, allowing one to evaluate both mean size and size distribution. Values of electrophoretic mobility provided by CE are in good agreement with those obtained by other electrophoretic techniques. An on-line detection by turbidity, absorbance or fluorescence allows for a rapid and simple quantitation of analyzed materials.

## 6. Abbreviations

| | |
|---|---|
| CE | capillary electrophoresis |
| CZE | capillary zone electrophoresis |
| LDV | laser Doppler velocimetry |
| PSC | polystyrene carboxylate |
| PSS | polystyrene sulfate |
| TBE | tris–borate–EDTA |
| GTP | guanosine 5′-triphosphate |
| PA | polyacrylamide |
| PEG | polyethyleneglycol |
| MW | nominal molecular weight |

## References

[1] C.A. Price, Centrifugation in Density Gradients, Academic Press, New York, 1982.
[2] A.E. Hamielec, S. Singh, J. Liquid Chromatogr. 1 (1978) 187.
[3] J.J. Kirkland, J. Chromatogr. 185 (1979) 273.
[4] H. Small, J. Colloid Interface Sci. 48 (1974) 147.
[5] A.W.J. Brough, D.E. Hillman, R.W. Perry, J. Chromatogr. 208 (1981) 175.
[6] J.J. Kirkland, W.W. Yau, W.A. Doerner, Anal. Chem. 52 (1980) 1944.
[7] J.J. Kirkland, C.H. Dilks Jr., W.W. Yau, J. Chromatogr. 255 (1983) 255.
[8] C.A. Silebi, J.G. Dosramos, J. Colloid Interface Sci. 130 (1989) 14.
[9] J.G. Dosramos, C.A. Silebi, J. Colloid Interface Sci. 133 (1989) 302.
[10] P.G. Righetti, M. Faupel, E. Wenisch, Adv. Electrophoresis 5 (1992) 159.
[11] M.E. Schimpf, D.D. Russel, J.K. Lewis, J. Liquid Chromatogr. 17 (1994) 3221.
[12] N.G. Green, H. Morgan, J.J. Milner, J. Biochem. Biophys. Methods 35 (1997) 89.
[13] G.H. Markx, R. Pething, J. Rousselet, J. Phys. D: Appl. Phys. 30 (1997) 2470.
[14] P. Serwer, S.A. Khan, G.A. Griess, J. Chromatogr. A 698 (1995) 251.

[15] J.A. Burroughs, A. Chrambach, Biochem. Biophys. Res. Commun. 180 (1991) 1070.
[16] H.-T. Chang, A. Chrambach, Appl. Theor. Electrophoresis 5 (1995) 73.
[17] N. Chen, A. Chrambach, Electrophoresis 18 (1997) 781.
[18] A.W. Preece, K.A. Brown, Adv. Electrophoresis 3 (1989) 351.
[19] J. Zhao, W. Brown, J. Colloid Interface Sci. 179 (1996) 255.
[20] Y. Grasselli, G. Bossis, J. Colloid Interface Sci. 170 (1995) 269.
[21] S.L. Penrod, T.M. Olson, S.B. Stanley, J. Colloid Interface Sci. 173 (1995) 521.
[22] P.J. Wit, J. Noordmans, H.J. Busscher, Colloids Surf. A 125 (1997) 85.
[23] P. Jandik, G. Bonn, Capillary Electrophoresis of Small Molecules and Ions, VCH Publishers, Cambridge, 1993.
[24] J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, 1994.
[25] B.B. VanOrman, G.L. McIntire, J. Microcolumn Sep. 1 (1989) 289.
[26] B.B. VanOrman, G.L. McIntire, Amer. Lab. (Fairfield, Conn.) 22 (1990) 66.
[27] B.B. VanOrman Huff, G.L. McIntire, J. Microcolumn Sep. 6 (1994) 591.
[28] B. Fourest, N. Hakem, R. Guillaumong, Radiochim. Acta 66–67 (1994) 173.
[29] B. Fourest, N. Hakem, J. Perrone, R. Guillaumont, J. Radioanalyt. Nucl. Chem. 208 (1996) 309.
[30] J.Th.G. Overbeek, P.H. Wiersema, in: M. Bier (Ed.), Electrophoresis: Theory, Methods, and Applications, Academic Press, Vol. II, New York, 1967, Ch. 1, p. 1.
[31] P.H. Wiersema, A.L. Loeb, J.Th.G. Overbeek, J. Colloid Interface Sci. 22 (1966) 78.
[32] K. Hannig, W. Hanns, B.-H. Meyer, K. Zeiller, Hoppe-Seyler's Z. Physiol. Chem. 356 (1975) 1209.
[33] H.K. Jones, N.E. Ballou, Anal. Chem. 62 (1990) 2484.
[34] S.L. Petersen, N.E. Ballou, Anal. Chem. 64 (1992) 1676.
[35] A.B. Hlatshwayo, C.A. Silebi, Polym. Mater. Sci. Eng. 75 (1996) 55.
[36] R.M. McCormick, J. Liquid Chromatogr. 14 (1991) 939.
[37] C. Quang, S.L. Petersen, J.R. Ducatte, N.E. Ballou, J. Chromatogr. A 732 (1996) 377.
[38] G.R. Ducatte, N.E. Ballou, C. Quang, S.L. Petersen, J. Microcolumn Sep. 8 (1996) 403.
[39] U. Schnabel, C.-H. Fischer, E. Kenndler, J. Microcolumn Sep. 9 (1997) 529.
[40] J. Janca, S. Le Hen, M. Spirkova, J. Stejskal, J. Microcolumn Sep. 9 (1997) 303.
[41] A.M. Holmes, S.G. Holliday, J.C. Clunie, J.K. Baird, Acta Crystallogr. D53 (1997) 456.
[42] G. Schmitz, C. Moellers, Electrophoresis 15 (1994) 31.
[43] I.D. Cruzado, A.Z. Hu, R.D. Macfarlane, J. Capillary Electrophoresis 3 (1996) 25.
[44] M.A. Roberts, L. Locascio-Brown, W.A. MacCrehan, R.A. Durst, Anal. Chem. 68 (1996) 3434.
[45] K. Tsukagoshi, Y. Okumura, H. Akasaka, R. Nakajima, T. Hara, Anal. Sci. 12 (1996) 869.
[46] D.W. Wilson, S.W. Whiteheart, M. Wiedmann, M. Brunner, J.E. Rothman, J. Cell Biol. 117 (1992) 531.
[47] S.P. Radko, A.V. Sokoloff, M.M. Garner, A. Chrambach, Electrophoresis 16 (1995) 981.
[48] S.C. Nichols, M. Loewenberg, R.H. David, J. Colloid Interface Sci. 176 (1995) 342.
[49] S. Hjerten, K. Elenbring, F. Kilar, J.-L. Liao, A.J.C. Chen, C.J. Siebert, M.-D. Zhu, J. Chromatogr. 403 (1987) 47.
[50] P.D. Grossman, D.S. Soane, Anal. Chem. 62 (1990) 1592.
[51] P.D. Grossman, in: P.D. Grossman, J.C. Colburn (Eds.), Capillary Electrophoresis: Theory and Practice, Academic Press, San Diego CA, 1992, Ch. 4, p. 111.
[52] S.P. Radko, M.M. Garner, G. Caiafa, A. Chrambach, Anal. Biochem. 223 (1994) 82.
[53] S.P. Radko, A. Chrambach, Electrophoresis 17 (1996) 1094.
[54] S.P. Radko, A. Chrambach, Appl. Theor. Electrophoresis 5 (1995) 79.
[55] A. Krabi, E. Donath, Colloids Surf. A 92 (1994) 175.