Capillary Electrophoresis Measurements of Electrophoretic Mobility for Colloidal Particles of Biological Interest

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The electrophoretic mobilities of three bacterial strains were investigated by capillary electrophoresis (CE) and were compared with results obtained by microelectrophoresis (ME). The CE measurements yielded bimodal electropherograms for two of the strains, thus illustrating for the first time that surface charge variations within a monoclonal population can be probed by CE. Intrapopulation variations were not detected by ME. The mobilities of three chemically distinct types of latex microspheres were also measured. Differences between the mean mobilities obtained by CE and ME were not statistically significant (P ≤ 0.50); the standard deviations of the CE measurements were typically 2 to 10 times smaller than those obtained by comparable ME measurements. The reproducibility of CE permitted batch-to-batch mobility variations to be probed for the bacteria (one of the strains exhibited such variations), and aggregation was evident in one of the latex suspensions. These effects were not measurable with ME.

Over the past decade, capillary electrophoresis (CE) has been developed into a standard technique in biotechnology. CE is used for analytical separation of charged solutes, such as carbohydrates, proteins, DNA, pharmaceuticals, herbicides, and pesticides, as well as lower-molecular-weight ions (1, 19, 27). CE is a powerful clinical diagnostic tool for profiling, screening, and detecting drugs, carbohydrates, lipids, enzymes, proteins, and nucleic acids (46). Variants of CE, known as micellar electrokinetic capillary chromatography (27) and capillary electrophoresis (36), allow solutions of nonionic analytes to be resolved.

Recently, CE has been applied to suspensions involving particles of biological interest, including viruses (19), bacteria (9, 19), silica sols (31), and polystyrene nanoparticles (45). An advantage of electrophoretic separation compared with filtration or centrifugation is that electrophoresis is characteristically gentle and suitable for labile compounds and microorganisms. For example, Ebersole and McCormick (9) found that more than 90% of the bacteria that they tested remained viable after CE, and other workers (13, 35) have obtained similar viability results for cells separated dielectrophoretically in electric fields having comparable strengths (ca. 100 V/cm).

While the capabilities of CE to separate compounds and particles are well-established (for reviews see references 2, 26, and 27), the method has not been widely used to measure electrophoretic mobilities. Accurate measurements of electrophoretic mobilities are important in many biological and environmental sciences and technologies, ranging from clinical diagnostics (40, 41) to biocolloid adhesion (8, 10, 12, 17, 33, 39, 43, 44). Tobacco mosaic virus (6, 15) and polymer latex mobilities (6, 15, 23) have been characterized by CE, but Zhu and Chen (47) found that for human erythrocytes, the electrophoretic mobility measured by CE differed by 21% from previously published values. As there have been no direct comparisons between CE and other techniques, we determined electrophoretic mobilities by CE and by microelectrophoresis (ME), and we report the results obtained here. Our objectives were essentially to examine whether CE could be used to measure the electrophoretic mobility of larger particles (diameter, ca. 1 μm) of biological interest and to assess the accuracy of the CE method compared with ME (8, 29, 42). We present results for the electrophoretic mobilities of three strains of bacteria and three chemically distinct microspheres (used in biocompatibility determinations) at two pHs and three ionic strengths. The method that we used was based on the work done with tobacco mosaic virus by Grossman and Soane (15). Differences between CE and ME mobility measurements were not statistically significant, and the CE data typically showed lower variances than the ME data. In addition, it was possible by using CE to detect electrophoretic heterogeneities in two of the bacterial species, as well as aggregation in one of the latex suspensions. To our knowledge, this was the first use of CE to resolve mobility variations within a monoclonal population.

MATERIALS AND METHODS

Bacterial strains and preparation. The bacterial strains investigated in this study were A1264, a gram-negative, motile, ellipsoidal bacterium (ratio of length to width, 2.5) that was isolated from the Savannah River deep subsurface environment, and two strains isolated from the Department of Energy site at Oyster, Va. The Oyster groundwater isolates were CD1 (a gram-negative, nonmotile, ellipsoidal bacterium with a ratio of length to width of 1.5) and PL2W31 (a gram-positive, nonmotile, ellipsoidal bacterium with a ratio of length to width of 2.5). The equivalent spherical radii of the bacteria used, based on their projected areas, were all approximately 0.5 μm. Cultures were grown to the early stationary phase (absorbance at 600 nm, approximately 1.5) in 250-ml Erlenmeyer flasks containing 100 ml of 10% FTYG (0.25 g of peptone per liter, 0.25 g of tryptone per liter, 0.5 g of yeast extract per liter, 0.6 g of MgSO4·7H2O per liter, 0.07 g of CaCl2·2H2O per liter) at room temperature (22 to 24°C) with constant agitation (150 rpm). The cells were separated by centrifugation (20 min, 104 g) and resuspended in 100 ml of filter-sterilized 3-(N-morpholino)propanesulfonic acid (MOPS) buffer containing 4.186 g of MOPS (Sigma; titrated to pH 7.02 with 1 N NaOH; conductivity, 760 μS/cm; ionic strength, 10 mM) per liter. The washing procedure was repeated to produce each final suspension (concentration, 1010 to 1012 cells/ml), which was then incubated at room temperature for 18 h. (The bacterial species and stage of growth were predicated on the results of a related bacterial attachment study (3); the CE technique which we describe is robust and adaptable to species at all stages of growth.) At that point, cell density was measured by acridine orange direct counting (20). Throughout the process, culture purity was repeatedly verified by direct visual observation (approximately 104 cells were observed), plating, and comparisons of colony mor-
buffer. The optimum detector wavelength for all of the measurements was 214 nm. For the bacteria, the lower detection limit corresponded to an injected bacterial concentration of approximately 10⁹ cells/ml (data not shown) or 2,500 cells (for an injected volume of 25 nl). For the microspheres, the detection limit was 10⁷ particles/ml.

Actual measurements were preceded by a prerinse with buffer (5 min for bacteria and 2 min for latex microspheres). Samples were introduced into the capillary with a 2- to 10-s standard high-pressure (20-hr/min) injection. The injection time was a function of the sample absorbance. Typically, a 5-s injection was used, and for a 57-cm-long capillary with an inside diameter of 75 µm the sample volume injected was approximately 25 nl. CD1 was also injected electrokinetically by using a 175-V/cm applied electric field for 10 s to introduce the sample into the capillary. Heating effects were avoided by maintaining the capillary at 25°C during the measurements and by performing an Ohm’s law test (32) to establish the operating conditions for which there was a linear relationship between the applied electric field and the current within the capillary. The applied electric fields used ranged from 88 to 426 V/cm.

A neutral dopant, mesityl oxide (15, 28), was added to each sample (2 µl of mesityl oxide in 400 µl of suspension) to determine the electroosmotic velocity. Measurements were determined for the sample with and without the marker to verify that addition of the marker did not affect colloid migration. Electrokinetic measurements were also determined for the background buffer and (for bacterial samples) a bacterial filtrate, which was obtained by passing the bacterial suspension through a 0.2-μm-pore-size syringe sterile filter. The measurements were determined at least three times per sample.

A series of high-pressure rinses (5 min with 0.1 N HCl, 5 min with H₂O, 5 min with Beckman capillary regenerator solution A, and 5 min with H₂O for bacteria; 5 min with Beckman capillary regenerator solution A and 5 min with H₂O for latex spheres) were used between successive measurements. The contents of the cathode and anode buffer reservoirs were replaced after three measurements to minimize the effect of electrode reactions on buffer composition (4). At the end of each period of experimentation, the capillary was prepared for storage by repeating the rinse procedure twice (for a total of three rinses). This standard rinse procedure was followed by successive 10-min high-pressure rinses with methanol and air, and then the capillary was stored dry.

**RESULTS**

**Bacteria.** Capillary electropherograms for the carrier buffer and the bacterial filtrate exhibited no electrokinetic peaks. The strength of the electroosmotic flow (EOF) was 7.13 ± 0.55 μm · cm/V · s (n = 36). The marker did not affect the magnitude or distribution of the electrokinetic mobilities for the bacteria. Preliminary experiments showed that the results were also independent of the injection technique employed.

Electropherograms of A1264 and CD1 exhibited two electrokinetic peaks, as shown in Fig. 2. Neither A1264 nor CD1 exhibited significant batch-to-batch variation in their electrokinetic mobility modes, but some variation was observed in the fractions of the populations associated with these modes (Table 1). Note that the mode mobilities, M₁ and M₂, varied little from batch to batch, and there was little variation in M_avg, the area-weighted average of M₁ and M₂. Similar results were obtained for A1264 (data not shown).

The third bacterium tested, PL2W31, exhibited only one electrokinetic peak (Fig. 2) and batch-to-batch variation in mobility. The mobilities of the batches were −0.51 ± 0.04 μm · cm/V · s (n = 5), −0.45 ± 0.04 μm · cm/V · s (n = 2), and −0.49 ± 0.02 μm · cm/V · s (n = 3). The CE and ME results are compared in Table 2. Differences in the mobilities obtained with the two techniques are shown in Table 2. The CE data for A1264 and CD1 are reported as the mode mobilities and relative peak areas, averaged over all of the measurements; the standard deviations shown are the mean standard deviations for the batches. No
bimodal distribution was detected by ME for A1264 and CD1. Since batch-to-batch variability was evident with PL2W31, comparisons between the measurement techniques were based on results from a single culture of this microorganism. CE and ME measurements were not obtained with cells from the same culture for A1264 and CD1, as these organisms showed no significant batch-to-batch variability in $M_{\text{avg}}$ (Table 1).

**Microspheres.** Light microscopy of the microspheres revealed monodisperse suspensions except for the PMMA particles, which exhibited significant aggregation. No electrophoretic peaks were observed with the baseline. The strength of the EOF was $10.77 \pm 0.17 \mu m \cdot cm/V \cdot s$ ($n = 9$) in 2 mM borate buffer and $6.60 \pm 0.18 \mu m \cdot cm/V \cdot s$ ($n = 8$) in 100 mM borate buffer. The marker had no effect on the magnitude or distribution of the electrophoretic mobilities for the latex spheres. Typical electropherograms for amidine and CML particles are shown in Fig. 3. The results of the CE and ME experiments are summarized in Table 2.

CE measurements obtained with the heterogeneous PMMA suspension revealed a bimodal distribution in mobility (Fig. 4; Table 2). Aggregates of microspheres were apparent when the suspension was inspected by light microscopy. After sonication for 1 h, the PMMA aggregates were disrupted (i.e., the suspension was monodisperse, as determined by light microscopy). CE measurements obtained for the monodisperse suspension yielded one electrophoretic peak (Fig. 4). Additional CE measurements were determined after time intervals ranging from 0 to 2.5 h to test for reaggregation of the microspheres. Time-dependent aggregation was apparent as determined by both visual inspection (light microscopy) and the redevelopment of a bimodal distribution in the electrophoretic mobility data (Fig. 4).

**DISCUSSION**

The general agreement between the data obtained with the CE and ME mobility measurement techniques is indicated in Fig. 5. Statistical differences in the mean electrophoretic mobilities determined by CE and ME were investigated by using a paired $t$ test ($P = 0.40$) (7), a sign test ($P = 0.50$), and a Wilcoxon signed rank test ($P = 0.47$) (14); differences between the means were insignificant. Systematic differences associated with the choice of colloid, pH, or ionic strength were not observed.

The finding that CE results agree with ME results is not surprising. Electrokinetic theory shows that, as long as the particle size is no more than a few percent of the capillary diameter, the influence of hydrodynamic interactions on mobility measurements is negligible (25). Colloidal (e.g., van der Waals) and/or hydrophobic interactions between the analyte and the capillary wall can also bias mobility measurements. Such interactions are well-documented for proteins and other organic analytes (18, 24, 30), which tend to adsorb to quartz capillaries. Similar effects have been observed for 0.2-$\mu m$-diameter hydrophobic polystyrene and PMMA particles in capillaries with inside diameters of 25 $\mu m$ (6). Analyte-wall interactions can be diminished by using capillaries with larger cross-sectional areas (5, 6), by modifying the carrier buffer, or by using coated capillaries (34). Coatings that reduce wall interactions, however, often reduce EOF and increase analysis time.

Based on similar numbers of replicate measurements, the standard deviations for CE results were typically much lower than the standard deviations for ME measurements (Table 2). This was probably because a single CE mobility measurement

<table>
<thead>
<tr>
<th>Batch</th>
<th>No. of measurements</th>
<th>$M_1$ (µm · cm/V · s)$^a$</th>
<th>$M_2$ (µm · cm/V · s)$^a$</th>
<th>% of total area as peak 1</th>
<th>$M_{\text{avg}}$ (µm · cm/V · s)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>$2.29 \pm 0.11$</td>
<td>$2.60 \pm 0.12$</td>
<td>31.8 ± 10.8</td>
<td>$2.51 \pm 0.11$</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>$2.27 \pm 0.09$</td>
<td>$2.52 \pm 0.13$</td>
<td>47.7 ± 10.7</td>
<td>$2.40 \pm 0.10$</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>$2.27 \pm 0.05$</td>
<td>$2.71 \pm 0.12$</td>
<td>61.2 ± 7.4</td>
<td>$2.44 \pm 0.07$</td>
</tr>
</tbody>
</table>

$^a$ $M_1$ and $M_2$ are the mobilities for the first and second modes in the electropherogram, respectively.

$^b$ $M_{\text{avg}}$ is the area-weighted average of $M_1$ and $M_2$. 

**FIG. 2.** Typical electropherograms for bacterial cells. Measurements were obtained in 10 mM MOPS buffer with an injection interval of 5 s. Unless indicated otherwise, an electric field of 175 V/cm was applied over a 57-cm column. (a) Neutral marker peak (2.2 min) and two bacterial peaks (2.8 and 3.3 min) for strain A1264. An electric field of 426 V/cm was applied over a 47-cm column. (b) Neutral marker peak (8.9 min) and two bacterial peaks (12.5 and 13.6 min) for CD1. The column length was 77 cm. (c) Neutral marker peak (7.1 min) and a single bacterial peak (7.7 min) for PL2W31.

**TABLE 1.** Batch-to-batch variation in CD1 electropherograms

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was based on (i) analysis of a relatively large number of particles (approximately $10^4$ particles) and (ii) the time required for the analyte to move distances on the order of 0.1 m in an electric field stronger than 100 V/cm. By contrast, ME and related measurement techniques (22) detect particle translation over short distances (typically less than 100 μm) in weak fields (10 V/cm or less) and often rely on observation of far fewer particles.

Two of the bacteria used, A1264 and CD1, and one of the microsphere types used, PMMA, exhibited multimodal behavior during CE measurements. Similar distributions were not obtained with ME. CE is a sensitive analytical separation technique tailored to resolve mixtures of charged solutes on the basis of their electrophoretic mobilities. The sensitivity stems from the strength of the applied field and the uniformity of the particle velocities over the capillary cross section. Thus, when a large number of particles is sampled, an electrophoretic histogram and a more detailed picture are obtained.

The results of related studies on bacterial sorption (3) suggest that the bimodal electrophoretic mobilities of the CD1 and A1264 populations were due to intrapopulation heterogeneities in surface charge rather than aggregation, contamination, or artificial peak splitting. Bimodal mobilities of cells have been observed in other contexts, and in these cases the bimodality has been attributed to variations in surface structure (the presence or absence of appendages or capsular material) (8) and surface antigens (38). Ebersole and McCormick (9) found that differences in chain morphology can cause multimodal behavior in bacterial electrophoretic mobility. Visual inspection confirmed that the cells used here were monodisperse. Spread plating revealed no major contaminant. The detection limit for bacteria was approximately 2,500 cells or roughly 1/10th the standard number of cells injected. The presence of a significant contaminant (observable with CE as a separate peak) should have been recognized by direct visual observation or by differences in colony morphology following growth on solid media. The multiple peaks were in no way due to the carrier buffer, cell exudates, or mesityl oxide. Ermakov et al. (11) concluded (based on both theory and experiments) that artificial peak splitting is avoided by buffering solutions at least 1 pH unit from the particle isoelectric point. Since bacterial isoelectric points are generally in the range from 2 to 4, depending on species and growth stage (16), and since the pH of the MOPS buffer was 7.02, artificial peak splitting is an unlikely source of multimodal mobilities observed.

The PMMA microspheres also exhibited an electrophoretic mobility distribution. Direct visual observation revealed both

**TABLE 2. Comparison of electrophoretic mobilities as determined by CE and ME**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_1$ (μm · cm/V · s)$^a$</th>
<th>$M_2$ (μm · cm/V · s)$^a$</th>
<th>% of total area as peak 1</th>
<th>$M_{CE}$ (μm · cm/V · s)$^b$</th>
<th>$M_{ME}$ (μm · cm/V · s)$^c$</th>
<th>$M_{CE} - M_{ME}$ (μm · cm/V · s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1264$^d$</td>
<td>-1.48 ± 0.05 (7)$^f$</td>
<td>-2.31 ± 0.10 (7)</td>
<td>28.9 ± 3.6 (7)</td>
<td>-2.07 ± 0.07 (7)$^g$</td>
<td>-1.95 ± 0.65 (10)$^h$</td>
<td>-0.12</td>
</tr>
<tr>
<td>CD1$^i$</td>
<td>-2.25 ± 0.07 (18)</td>
<td>-2.62 ± 0.11 (18)</td>
<td>48.2 ± 8.6 (18)</td>
<td>-2.44 ± 0.08 (10)$^i$</td>
<td>-2.24 ± 0.32 (10)$^i$</td>
<td>-0.20</td>
</tr>
<tr>
<td>PL2W31$^i$</td>
<td></td>
<td></td>
<td></td>
<td>-0.51 ± 0.04 (5)$^i$</td>
<td>-0.78 ± 0.35 (10)$^i$</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Microspheres</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amidine$^j$</td>
<td>-4.41 ± 0.08 (3)</td>
<td>-5.17 ± 0.11 (3)</td>
<td>78.8 ± 3.1 (3)</td>
<td>-1.79 ± 0.18 (3)</td>
<td>-1.98 ± 0.12 (10)</td>
<td>0.19</td>
</tr>
<tr>
<td>PMMA$^j$</td>
<td>-4.57 ± 0.09 (3)</td>
<td>-4.73 ± 0.39 (10)</td>
<td>0.16</td>
<td>-3.42 ± 0.14 (10)</td>
<td>-2.95 ± 0.25 (10)</td>
<td>-0.47</td>
</tr>
<tr>
<td>CML$^j$</td>
<td>-1.95 ± 0.17 (3)</td>
<td>-1.78 ± 0.21 (10)</td>
<td>-0.17</td>
<td>-3.69 ± 0.06 (3)</td>
<td>-3.82 ± 0.20 (10)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

$^a$ $M_1$ and $M_2$ are the mobilities for the first and second modes in the electropherogram, respectively.

$^b$ $M_{CE}$ is the electrophoretic mobility as determined by CE.

$^c$ $M_{ME}$ is the electrophoretic mobility as determined by ME.

$^d$ Measurements were determined in 10 mM MOPS buffer, pH 7.02.

$^e$ The numbers in parentheses are the numbers of times that measurements were determined.

$^f$ $M_{CE}$ is the weighted average of $M_1$ and $M_2$.

$^g$ Mobility was determined by measuring multiple cultures of bacteria.

$^h$ Mobility was determined by measuring a single culture of bacteria.

$^i$ Measurements were determined in 2 mM borate buffer, pH 8.35.

$^j$ Measurements were determined in 100 mM borate buffer, pH 8.39.
individual particles and aggregated clumps in suspensions used for the original CE and ME measurements. Sonication subsequently produced a monodisperse suspension of PMMA microspheres that yielded a single peak in the corresponding electropherograms. Subsequent CE measurements obtained with the same suspension revealed the time-dependent evolution of a second peak. Within 2.5 h, the suspension was comprised of mainly aggregated particles, as indicated by visual inspection. Over the same period the area of the peak containing the monodisperse microspheres decreased, while the second peak grew, presumably due to aggregation.

Despite the noticeable difference in the areas under the peaks in their respective electropherograms, the numbers of amidine and CML (Fig. 3) particles injected were actually similar. A likely explanation for this phenomenon is the differences in the specific absorbances of the particles, as the area of an electrophoretic peak is a function of both the number of particles injected and the specific absorbance of the particles. Since both colloid volume fractions (less than 0.01 for both microorganisms and microspheres) and UV absorbance (typically less than 0.1 absorbance unit) were low, detection artifacts associated with high particle concentrations are unlikely.

In the CE measurements, electroosmotic flow was characterized by doping the samples with a neutral marker, mesityl oxide. EOF measurements were reproducible for all of the carrier buffers tested. The standard deviation of the EOF in 10 mM MOPS buffer (pH 7.02) was roughly three times the standard deviation of the values obtained at both ionic strengths in the borate buffer (pH 8.35). This probably was due to the neutral pH of MOPS solutions, since electroosmosis in uncoated capillaries is extremely sensitive to pH changes in the range from pH 4 to 8 (27).

CD1 samples were injected both electrokinetically and by high pressure. Electrokinetic injections have been known to bias samples (27), since analytes with lower (more negative) electrophoretic mobilities tend to remain in the sample vial. No difference in either the distribution or the magnitude of the electrophoretic mobilities exhibited by CD1 was observed in response to the change in injection technique (data not shown).

CD1 exhibited multimodal behavior in electrophoretic mobility at relatively low field strengths (175 V/cm). Higher applied electric fields were required for A1264 because at low field strengths, microbial motility distorted the CE peaks. Attempts to uncover multimodal electrophoretic behavior in bacterial strain PL2W31 by increasing the applied electric field to approximately 350 V/cm were unsuccessful.

The reproducibility of the CE data allowed batch-to-batch variations to be studied in the bacteria. A1264 and CD1 showed variations in the distributions of their populations between the respective mobility modes, although this had only a minor effect on the average mobilities of the populations. PL2W31 exhibited batch-to-batch variability in its (unimodal) mean electrophoretic mobility. Investigators should be able to use CE to help assess the influence of such variability on biophysical processes (e.g., attachment to surfaces) or, alternatively, to help evaluate connections between variations in surface charge density and outer membrane structure and composition.

In summary, CE measurements of electrophoretic mobility were not distinguishable from measurements of electrophoretic mobility obtained by ME. Based on similar numbers of replicate measurements, the standard deviations for CE results were typically much lower than the standard deviations for ME measurements. Distributed mobilities, which were readily observed with CE for two bacterial species and one of the polymer latices, could not be detected with ME.

![FIG. 4. Electropherograms for the PMMA suspension. Measurements were obtained in 2 mM borate buffer with an applied electric field of 175 V/cm over a 57-cm column. The injection interval was 5 s. (a) Neutral marker peak (4.2 min) and two microsphere peaks (7.0 and 7.7 min) for the polydisperse PMMA particles (prior to sonication). (b) Evolution of bimodal electrophoretic mobility in PMMA suspensions. Electropherograms based on suspensions were obtained immediately after sonication (dashed line) and 2.5 h after sonication (solid line).](http://aem.asm.org/)

![FIG. 5. Comparison of the electrophoretic mobilities determined by CE (M_{CE}) and ME (M_{ME}). The error bars are 2 standard deviations wide. The diagonal is a 1:1 line representing complete data agreement. The data are labeled according to sample type.](http://aem.asm.org/)
REFERENCES


