Particle Electrophoresis in Closed-both-end Capillary and Like Charged Particle Aggregation Induced by AC Electric Field

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ABSTRACT

An alternative solution to the zero electroosmotic flow (EOF) capillary electrophoresis (CE) is proposed. First, flows in a capillary are decomposed and measurement results reconstructed. Second, CE “markers” are developed as reference. Third, the system is calibrated and cross-checked. Finally, costs of the alternative solution are evaluated. This report marks the beginning of these orchestrated efforts. A closed-both-end bare fused silica capillary as a model system has created informative data of DC or AC particle CE with the dilute suspension of carboxylated polystyrene spheres in deionized water. The apparent electrophoresis mobility of a particle strongly depends on the DC electric field. In an electric field range from 5 to 10 V/cm, the particle motion becomes at random; the particle mobility first increases to $7 \times 10^{-8}$ m²/(sV)⁻¹ and then levels off in the DC electric field of 10 to 110 V/cm. These particles are induced to aggregate into clusters by an AC electric field in some frequency bands.

Keywords: US health care cost, zero electroosmotic flow, decomposing flows, capillary electrophoresis “marker”, negatively charged particle attraction

1 ALTERNATIVE TO ZERO EOF CE

Capillary electrophoresis (CE) based DNA sequencers accelerated the Human Genome Project (HGP) by 10 years over the initial projection, and dramatically reduced the cost from $1.00 per base pair by gel methods to $0.01 per base pair today [1]. With the completion of HGP [2], the focus has shifted from genomics to proteomics and metabolomics, with a particular interest in the identification of proteins and metabolites involved in diseases. So far, the mixed-mode electrophoretic method is proven to be the only available method to separate 3,000-15,000 individual proteins present in a single cell or tissue sample.

Some researchers and engineers prefer CE for proteome-level separations because of its outstanding success in HGP. CE has the potential to displace gel methods for proteome-level separations, with concomitant quality and cost reductions seen in capillary based DNA sequencing, but only if electroosmotic flow (EOF) can be completely eliminated. The following sections will address the difficulties of achieving zero EOF CE and an alternative is developed.

CE separation techniques have the separation capacity unsurpassed by any other chromatographic methods. The capillary geometry allows much more efficient cooling of separation bed than in slab gel electrophoresis. Thus, higher electric field strengths can be applied in CE, which translates into significantly better resolution and shorter separation times. CE also allows much longer reading lengths in DNA sequencing than that in slab gel.

One dimensional capillary electrophoresis does not allow the separation of thousands of analytes, which led to the development of multi-dimensional separations. CE offers three nearly orthogonal separation dimensions of capillary isoelectric focusing (CIEF), capillary zone electrophoresis (CZE), and capillary gel electrophoresis (CGE). In CIEF proteins migrate in a pH gradient under an electric field to their respective isoelectric points. The focused proteins are subsequently mobilized by pressure,
residual EOF, or chemical displacement of the pH gradient. In CZE proteins migrate from a starting point in the capillary at a velocity that is proportional to the ratio of the intrinsic net charge to hydrodynamic radius to the 1/3 to 2/3 power. In CGE the charge on the protein is dominated by the addition of an ionic surfactant and so the separation is based solely on the hydrodynamic radius of the protein.

However, with the shift of focus onto the proteome, the deficiencies of these separation techniques, rooting in EOF, are becoming more visible. In protein or metabolite separations, EOF reduces the CE separation capacity in two aspects. First, EOF drains the capillary contents, limiting the time and distance over which protein or metabolite separations may occur. Second, even very small EOF differences within a capillary lead to band broadening, even separation failure. The magnitude of the EOF problem and the difficulties of its solution are demonstrated by about 40 US Patents on different methods to suppress or eliminate EOF in CE in the past two decades.

EOF is the result of electroneutrality constraints. Walls of capillaries contain the fixed charges. These fixed charges attract a layer of counterions creating an electric double-layer near the wall. When an electric field is applied across the flow channel, the counterions move, but the fixed charges on the wall don’t. The viscous drag caused by movement of the counterions in the capillary transfers momentum to the bulk fluid inside the capillary. The bulk liquid begins to move with the same velocity as the ions. This induced bulk flow is called electroosmotic flow (EOF).

According to the Helmholtz-Smoluchovski equation (1), the induced bulk liquid velocity (V_{EOF}) is written as

$$V_{EOF} = \mu_{EOF} E = \frac{\varepsilon\zeta \pi \eta}{4\varepsilon_0}$$

where \(\mu_{EOF}\) is the apparent mobility of ions, \(\zeta\), the zeta-potential of the wall, \(\varepsilon\), the permittivity of liquid, \(\eta\), the viscosity of liquid, and \(E\), the applied electric field strength.

The year 2007 is the 40th anniversary for the foundation of CE [4]. Researchers of one generation after another have been persistently pursuing ways to eliminate or reduce EOF through new capillary materials such as Teflon, static and dynamic wall coatings, and other physical or electrical methods. One of the latest efforts is to provide a barrier to momentum transfer within the capillary, which prevents bulk fluid movement but still allows ionic mobility. So far, the EOF mobility has been reduced by two orders from 10^{-8} to 10^{-10} m^2(sV)^{-1}, or more specifically, 4.6X10^{-10} m^2(sV)^{-1}, over the bare fused silica capillary. Unfortunately, it is still far away from the requirement for proteome-level separations, even with the multi-dimensional CE separation techniques.

With the channel size shrinking to the order of nanometer, it is no longer possible to suppress EOF via conventionally coating inner walls of channels with a diameter of 10nm or even bigger. This kind of nano-pore and nano-channel based techniques [5][6] have the potential to act as a new generation of separation tools for proteins. Thus, it is important to fostering technological innovation in protein separation to get deeper insight into EOF. It is necessary and feasible to develop the below alternative solution to the zero EOF CE. First, flows in a capillary are decomposed and measurement results are reconstructed. Second, some CE “marker” is developed as reference for protein separation. Third, the system is calibrated and cross-checked. Finally, costs of the alternative solution are evaluated. This report marks the beginning of these orchestrated efforts. A closed-both-end capillary as a model system has generated the informative data of DC or AC electrophoresis to be presented here.

2 EXPERIMENTAL

2.1 Setup Description

Shown in Figure 2 is the device for the closed-both-end capillary electrophoresis. The main component is a 41.5-45.0 mm long bare fused silica capillary with an inner diameter (ID) of 50\(\mu\)m (MicroSolv Technologies Inc., NJ, Catalogue No. 04051-C25). Both ends of the capillary are closed by inserting two copper wires as two electrodes. Every capillary tested has a 1.022-1.060 mm long clear window for observation and recording of particle motion within the capillary. The capillary is secured on the stage and its clear window is exposed to the view field of the microscope (Nikon SMZ-2T, Japan) with a CCD camera (COHU Solid State Camera, Model No 322272) that is connected to a VCR (Panasonic AG-1960).

The power sources used are combinations of a 30/15A DC power source (Model DIGI 35A, Electro Industries), a 5V/1MHz function generator (Wavetek Meterman, Model: FG2C), and a \(\pm10kV/0-2kHz\) high voltage DC and AC amplifier (Trek, Inc., Medina, NY, Model 10/40) as needed.

A commercial suspension of carboxylated polystyrene spheres with a density of 1.05 g/cm^3 and a mean diameter of 4.13 \(\mu\)m in deionized water at 1\% by volume (Bangs Laboratories, Inc) is diluted in deionized water as the experiments require. The diluted suspension has a particle volume fraction of 1/4000; its pH value is close to that of

Figure 2: Setup for the closed-both-end CE with an AC or DC excitation voltage.
The suspension is carefully loaded into the capillary by a syringe and two copper wires are inserted into the capillary from both the ends. Special measures are taken to prevent tiny air bubbles from entering the capillary. When the clear window of the capillary is illuminated by a light source (Moritex Corporation, MHF-G150LR), particles become shining stars on a dark background. When an AC or DC voltage is applied to the two ends of capillary through the copper wires; the movie of particle motion is recorded by VCR.

The apparent particle electrophoresis mobility is defined as a ratio of a particle velocity relative to the ground to the applied electric field strength. The capillary inner diameter is verified to be 50 µm and uniform, allowing the inner wall to act as a ruler in the movie. Based on the distance particles move given a period under an electric field, a particle velocity relative to the ground is calculated. Each capillary is used ten times for one sample of suspension. Velocities of 200 to 500 particles are measured for one externally applied electric field. Finally, the mobility distribution and their average value are obtained.

2.2 Particle Characterization

The carboxylated polystyrene spheres are characterized on a laser scattering zeta potential analyzer (Brookhaven Instrument Corporation, Model: ZetaPlus). Electrophoresis mobility of the spheres in deionized water has a very sharp distribution with a mean value of $3.49 \times 10^{-8} \text{m}^2(\text{sV})^{-1}$, as shown in Figure 3 and so does their zeta potential distribution with an average value of -44.7 mV, not shown here. These measurements demonstrate that the particles in deionized water have extremely uniform electric properties. The parameters of particle measured on ZetaPlus will be used as reference in the closed-both-end CE experiment.

2.3 DC Particle CE Data and Discussion

In the DC particle CE, the particle mobility in the closed-both-end capillary very strongly depends on the electric field, as shown in Figure 4. When the electric field < 5 V/cm, the particles move along one direction and their average mobility is $10^{-8} \text{m}^2(\text{sV})^{-1}$. EOF in a bare fused silica capillary root in the double layer formed against the fixed negative charges formed by deprotonation of the surface silanol groups at any pH greater than 3. Compared with $3.49 \times 10^{-8} \text{m}^2(\text{sV})^{-1}$ measured with ZetaPlus, the large difference of the mobility reflects effects of EOF and closing both the ends of a capillary. More interesting is that when an externally applied electric field is between 5 and 10 V/cm, the particle motion becomes at random. It indicates that in a closed-both-end capillary, all the factors including EOF influence the apparent mobility in a collective way. When the electric field > 10 V/cm, the particles move along one direction, and the apparent mobility reaches its maximum value of $7 \times 10^{-8} \text{m}^2(\text{sV})^{-1}$ at 65 V/cm, two times the measured value of $3.49 \times 10^{-8} \text{m}^2(\text{sV})^{-1}$ on ZetaPlus.

Although the closed-both-end capillary is a simple system, the flows inside it under a DC electric field cover all kinds of flows that appear in the most advanced capillary separation systems, including EOF, charged particle induced liquid flow, pressure or vacuum building and their driven flows, as well as their mutual effects and coupling. A numerical technique to decompose these flows in this model system will be developed. It is anticipated that this technique can be used for other systems.

2.4 Like Charged Particle Aggregation

The AC particle CE is run in the closed-both-end capillary with excitation voltage of 700 Vrms/1~3000 Hz. At low frequencies, usually < 50 Hz, the particles follow the electric field; at frequencies > 50 Hz, particles seem “static”. However, these negatively charged particles are induced to aggregate into clusters within certain frequency bands. The characteristic time for the aggregation is on the order of 10s. A typical time sequence of particle aggregation pictures is shown in Figure 5. This finding could be used to control the local particle concentration of a suspension in micro- or nano-fluidic systems.
Figure 5: A typical time sequence of particle aggregation induced by an applied AC voltage of 694V_{RMS}/1576Hz in a 41.5mm long closed-both-end capillary. The numbers to the right denote time particles are exposed to the electric field.

When an AC electric field is applied to the suspension, the charge carriers are conducted and diffuse, see Figure 6. The charge densities, potential, and current density satisfy the Poisson’s equation and two equations of conduction and diffusion that are derived from the charge conservation. The counter-ions in the double layer are driven to accumulate in pole areas of the particle. The diffusion cloud in turn arises from the enhanced population of the counter-ions in the double layer, giving rise to predominately counter-ionic normal current, which cannot be convected away from the double layer by a conduction process alone. Diffusion cloud carries diffusion currents circumferentially and radially.

and is out of phase with the applied field in general. The diffusion cloud greatly enhances the perturbed counterion density in the double layer which increases the circumferential diffusion current in the double layer. This out-of-phase diffusion current in the double layer induced an out-of-phase dipole field outside the particle, which would be responsible for the observed aggregation.

3 SUMMARY

An alternative solution to the zero electroosmotic flow (EOF) capillary electrophoresis (CE) is proposed. First, flows in a capillary are decomposed and measurement results reconstructed based on numerical simulations. Second, some CE “marker” is developed as reference for protein separation. The marker will be used to check the measurement results reconstructed. Third, the system is calibrated and cross-checked. Finally, costs of the alternative solution are evaluated. This report marks the beginning of these orchestrated efforts. A closed-both-end bare fused silica capillary as a model system in a DC electric field has all kinds of flows that appear in the most advanced capillary separation systems, including EOF, charged particle induced liquid flow, vacuum or pressure building and their driven flows. This model system has created informative data of DC or AC particle electrophoresis with the dilute suspension of carboxylated polystyrene spheres in deionized. The apparent electrophoresis mobility of a particle strongly depends on the DC electric field. In the electric field of 5 to 10 V/cm, the particles move at random; in the electric field of 10 to 110 V/cm, the particle mobility first increases up to 7\times10^{-8} m^2(sV)^{-1} at 65V/cm and then levels off. It is unexpectedly found that particles are induced to aggregate into clusters by an AC electric field in some frequency band and this can be used to control the local particle concentration of a suspension in fluidic systems.

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