Supporting Information

High Speed Electroseparations inside of Silica Colloidal Crystals
Suping Zheng, Eric Ross, Michael A. Legg, Mary. J. Wirth*
Department of Chemistry, University of Arizona, 1306 E University Blvd. Tucson AZ 85721

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1) Relation between velocity, electrophoretic mobility and capacity factor.

The separation is electrically driven, with no flow of mobile phase, therefore, the velocity of the analyte is determined by its electrophoretic velocity, \( v_e = \mu_e E \), and the fraction of time, \( f \), that the analyte spends in the mobile phase. The fraction of time spent in the mobile phase depends on the capacity factor, \( k' \), which is the ratio of moles in the stationary phase to moles in the mobile phase. Since a time-average and an ensemble average are equivalent, \( k' \) is also the ratio of time spent in the stationary and mobile phases.

\[
\frac{1}{1 + k'} = \frac{\text{time}_{\text{mob}}}{\text{time}_{\text{stat}} + \text{time}_{\text{mob}}} = f
\]

By substitution, the migration velocity of the analyte has contributions from both electrophoretic mobility and capacity factor.

\[
v = \mu_e E \cdot \frac{1}{1 + k'}
\]

Eq. d corresponds to Eq. 1 in the paper.

2) The colloidal crystals and device.

Uniform strips of colloidal crystals were prepared by first depositing a few cm\(^2\) of calcinated colloids over the bottom of a fused silica microscope slide by evaporation of ethanol overnight. After deposition, most of the colloidal crystal was scraped off with a razor blade to allow bare glass to adhere to the PDMS cover plate to avoid leakage. The resulting strip was 1.6 cm in length and several mm wide. The strip was sintered at 900 °C for three hours. Photograph show a typical strip in Figure S1a, and a similar strip under a PDMS sheet in Figure S1b.

Figure S2 shows that a micrograph of a typical colloidal crystal, showing that it is quite uniform, with no breaks or cracks.

An atomic force micrograph (Nanoscope IV) of a typical colloidal crystal is shown in Figure S3. The individual 200-nm colloids are evident on this scale. The image reveals the material to be very tightly packed. Cracks are entirely avoided by calcinating the particles before deposition, as has been shown in reference 12 of the paper. More
crystalline materials can be grown by sedimenting the calcinated particles before deposition to eliminate aggregates, as shown in the cited references, and also by evaporating the colloids more slowly. The imperfections could contribute to lead to the $A$ term of the van Deemter equation, but the more rapid process of making the crystals provides sufficient performance and enables many crystals to be prepared rapidly.

A fluorescence image (Nikon TE2000) shows in Figure S4 that the zone are injected evenly into the material. The separation of the three Dils spatially is shown in Figure S5, illustrating the uniformity of the colloidal crystal.

Figure S4. The green (false color) is a band of Dil beginning its migration through the material from left to right. The width of the injected zone is 50 µm (4 σ), revealing $H_{inj}=12.5^2/L=0.15$ µm for the 1 mm separation length.

Figure S5. Fluorescence image in false color showing the spatial separation of the three Dils.

3) Electrophoretic mobilities of the Dils and the peptides.

The results of the capillary electrophoresis (Beckmann Coulter P/ACE) of the Dil mixture is shown in Figure S6. The migration times of the dyes were assigned by injecting each one individually. The separation length is 30 cm and the applied field is 25 kV. The migration time for Dil C12 (peak 1) is 4.24 min and for DilC16 and Dil C18 (peak 2) is 4.55 min. This gives electrophoretic mobilities of $4.7 \times 10^{-6}$ for Dil C12 and $4.4 \times 10^{-6}$ cm$^2$/V·s for DilC16 and Dil C18.

Figure S6. Capillary electropherogram of the mixture of the three Dils using 90:10 MeOH:water with 0.1% TFA. The concentration of each dye was 10 µM.

The capillary electropherograms (HP 3D Capillary Electrophoresis) of the three peptides are shown in Figure S7. These establish that the separation of the peptides by the colloids is based on their electrophoretic mobility. A bare fused silica capillary was used, with $L=20$ cm and dia.=50 µm. The running buffer was 90:10 MeOH:water with 0.1% TFA. The electroosmotic flow rate was confirmed to be zero by virtue of no migration of mesitylene oxide. The noisier appearance of the electropherogram compared to Figure S6 is due to a combination of three-fold lower molar absorptivity of the rhodamine label compared to the Dil chromophore, and the fact that the three peptides are resolved rather than overlapped.

Figure S7. Capillary electropherogram of a mixture of the three peptides using 90:10 MeOH:water and 0.1% TFA and a bare silica capillary. The separation length was 20 cm, the field strength was 1200 V/cm, and the concentration of each peptide was 10 µM.

4) Voltage dependence of the migration velocities of Dil and Rhodamine 6G.

Figure S8 shows how the velocity of Dil C12 varies with applied electric field. Velocity is super-linear with respect to applied field. Heating could cause this either by lowering viscosity or lowering adsorption equilibrium constant.

Figure S8. Plot of migration velocity vs. electric field for the three Dils. The dotted lines are lines fit to the data points at low electric field.
The dependence of the velocity of Rhodamine 6G on electric field is shown in Figure S9. The mobility is so high that it is difficult to detect at high electric fields due to the need for a finite acquisition time of the ICCD camera.

![Figure S9](image)

**Figure S9.** Plot of migration velocity vs. electric field for Rhodamine 6G. The dotted lines are lines fit to the data points at low electric field.

Comparing Figures S8 and S9 allows one to conclude that the Rhodamine dye would elute in approximately 0.9 s in the separation of Figure 2 in the paper. The elution times of Dil-C16 and Dil-C18 are slightly shorter in Figure 2 of the paper than one would predict from the migration studies of Figures S8 and S9. This could be due to progressive heating of the material before the separation.

5) **List of additional references.**

Silica colloidal crystals were used previously by our group to investigate the migration rate of DNA chains of varying length. Although these studies did not involve a physical separation, they did point to the possibility of using colloidal crystals as possible media to replace polyacrylamide and agarose gels for crossed-field electrophoretic separations of DNA. Silica colloidal crystals have been used and characterized for this ability to block access of ionic species to an electrode by virtue of the pore sizes being on the order of the Debye length. The diffusion in colloidal crystals has been shown to be anisotropic due to their hexagonal symmetry. This could be an important point in designing media for separations because electrically driven transport will also be anisotropic.

Silica monoliths, polymer monoliths, and ultrahigh pressure liquid chromatography have all been reviewed recently. The basic principles underlying fast electrophoresis, including the contributions of the injected and detected widths, were developed thoroughly by Moore and Jorgenson.