Supplementary Information
Ionic Strength Effects on Electrophoretic Focusing and Separations
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We here present further details on the injection protocol of the isotachophoresis experiments presented in Figure 4 of the main paper. We also present Table S1 which summarizes key published models for the effects of ionic strength on fully-ionized mobility.

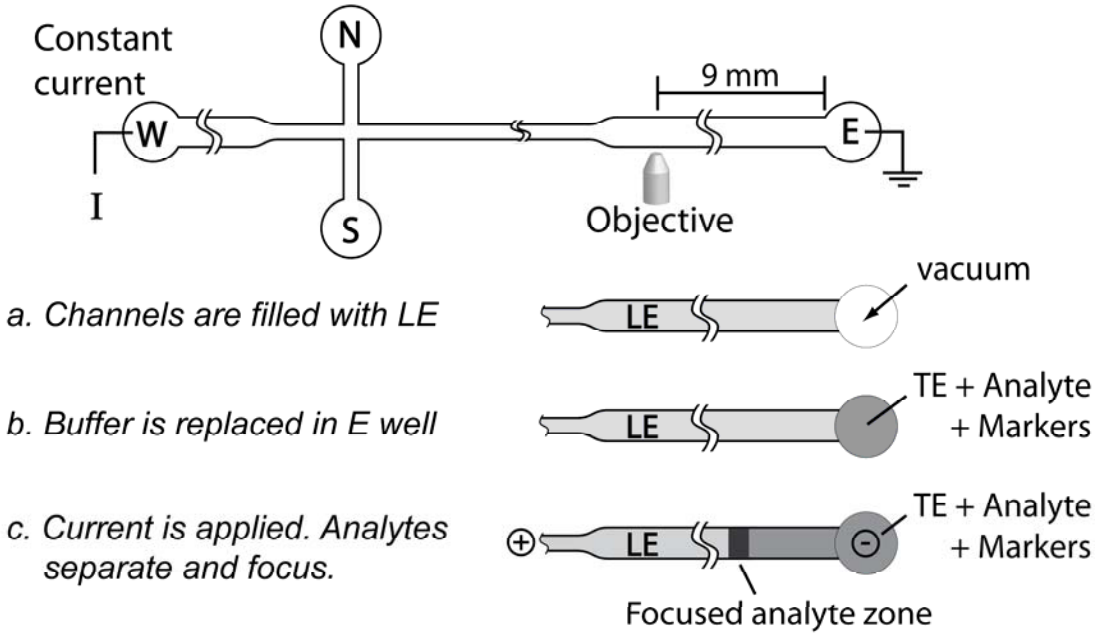


Figure 1. Schematic of the isotachophoresis (ITP) injection protocol applied to a Caliper NS-95 chip. (a) We filled the North, South and West wells of the chip with leading electrolyte (LE) and applied vacuum to the East well until all channels are filled with LE. (b) We rinsed the East well several times with distilled water and filled it with the trailing electrolyte (TE), and the analyte and markers mixture. (c) We placed the electrodes in the East and West wells and applied constant current. We centered the field of view of the microscope at a fixed distance of 9 mm from the (East) TE well, on the wide region of the microfluidic channel (74 μm wide by 12 μm deep). Note the experiments do not require/use the channel intersection.

Table S1. Summary of models describing the effect of ionic strength on the fully ionized electrophoretic mobility of ions. These models were developed to predict the conductance of electrolytes. The equations represent these models for conductance in terms of mobility.

| Model* | Assumptions | Equation | Description |
|---|---|---|---|
| Kohlrausch [1] | Dilute and strong electrolytes | $\mu_i = \mu_i^0 - k\sqrt{c}$ | Empirical model |
| Onsager [2,3] | Dilute, strong binary electrolytes, treats ions as point charges | $\mu_i = \mu_i^0 - (A\mu_i^0 + B)\sqrt{c}$ | Theoretical model. *A* represents relaxation effect and *B* takes into account the electrophoretic effect. |
| Onsager-Fuoss [4] | Electrolyte with mixture of fully ionized ionic species, neglects finite ion size | $\mu_i = \mu_i^0 - (A\mu_i^0 + B)\sqrt{\Gamma}$, $A = z_i \frac{e^2}{12\pi}\sqrt{\frac{N_{AV}}{(\varepsilon kT)^3}}\sum_{n=0}^{\infty} C_n R_i^n$. $B = \mid z_i \mid \frac{e^2}{6\pi\eta}\sqrt{\frac{N_{AV}}{\varepsilon kT}}$, $\Gamma = \sum_{i=1}^{s}\Gamma_i$. $\Gamma_i = c_i z_i^2$. | Extension of Onsager's model [2, 3], with electrolytes having any number of ionic species. Valid for dilute mixtures as ions are assumed to be point charges. |
| Pitts [5] | Symmetrical binary electrolytes. | See Pitts [5]. | Takes into account the finite ionic radius |
| Extended Onsager-Fuoss [6] | Electrolyte with mixture of fully ionized ionic species. Approximately accounts for finite ionic radius | $\mu_i = \mu_i^0 - (A\mu_i^0 + B)\frac{\sqrt{\Gamma}}{1+\frac{aD}{\sqrt{2}}\sqrt{\Gamma}}$, $D = \sqrt{\frac{2e^2 N_{AV}}{\varepsilon kT}}$. | Additional denominator term, compared to Onsager-Fuoss model [4], approximately accounts for finite ion size. |

Table S2: A comparison of SPRESSO and PeakMaster calculations involving ionic strength corrections. . Calculations for HEPES-NaOH buffer for different ionic strengths. The concentrations of HEPES and NaOH were both changed while preserving a constant HEPES-to-NaOH concentration ratio 2:1 (to approximately fix pH).

| SPRESSO | | | | | SIMUL | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ionic Strength (mM) | pH | Mobility HEPES $10^{-9}\,m^2V^{-1}s^{-1}$ | Mobility Sodium $10^{-9}\,m^2V^{-1}s^{-1}$ | Conductivity S/m | Ionic Strength (mM) | pH | Mobility HEPES $10^{-9}\,m^2V^{-1}s^{-1}$ | Mobility Sodium $10^{-9}\,m^2V^{-1}s^{-1}$ | Conductivity S/m |
| 10 | 7.457 | -10.15 | 48.13 | 0.066 | 10 | 7.457 | -10.16 | 48.14 | 0.066 |
| 20 | 7.443 | -9.60 | 46.84 | 0.128 | 20 | 7.443 | -9.61 | 46.86 | 0.128 |
| 30 | 7.433 | -9.22 | 45.94 | 0.186 | 30 | 7.433 | -9.23 | 45.96 | 0.186 |
| 40 | 7.426 | -8.92 | 45.23 | 0.244 | 40 | 7.426 | -8.93 | 45.25 | 0.244 |
| 50 | 7.420 | -8.67 | 44.64 | 0.299 | 50 | 7.420 | -8.68 | 44.67 | 0.299 |
| 60 | 7.415 | -8.45 | 44.14 | 0.354 | 60 | 7.415 | -8.47 | 44.16 | 0.354 |
| 70 | 7.411 | -8.26 | 43.69 | 0.407 | 70 | 7.411 | -8.28 | 43.72 | 0.407 |
| 80 | 7.407 | -8.10 | 43.30 | 0.460 | 80 | 7.407 | -8.11 | 43.32 | 0.460 |
| 90 | 7.404 | -7.94 | 42.93 | 0.511 | 90 | 7.404 | -7.96 | 42.96 | 0.511 |
| 100 | 7.401 | -7.80 | 42.61 | 0.562 | 100 | 7.401 | -7.82 | 42.63 | 0.562 |

Results agree with maximum error of 0.2%. This may be because Peakmaster uses slightly different values of the basic properties of the solvent (here, water) as they appear in the Onsager-Fuoss relation. We do not know the values used by Peakmaster, and they are

not listed in [6]. For our calculations we use assume a value of 78.36 for the relative permittivity of water, and assume a viscosity of water of $0.8903\times10^{-3}$ Pa.s at 25°C.

Table S3: List of chemical species mobility (in $10^{-9}\,m^2V^{-1}s^{-1}$) and p$K_a$ values, as used in this paper.

| Analyte | $\mu_{-1}$ | $\mu_{-2}$ | $\mu_{-3}$ | p$K_{-1}$ | p$K_{-2}$ | p$K_{-3}$ | $\mu_1$ | $\mu_2$ | p$K_1$ | p$K_2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| histidine | 28.3 | | | 9.33 | | | 28.8 | 44.7 | 6.04 | 2 |
| Hydrocholoric acid | 79.1 | | | -2 | | | | | | |
| 3-phenylpropionic acid | 26.5 | | | 4.664 | | | | | | |
| Sodium | | | | | | | 51.9 | | 13.7 | |
| MOPS | 26.9 | | | 7.2 | | | | | | |
| OGCA | 43 | | | 4.7 | | | | | | |
| Fluorescein | 19 | 36 | | 4.4 | 6.8 | | | | | |
| TRIS | | | | | | | 29.5 | | 8.076 | |
| Boric Acid | 30 | | | 9.24 | | | | | | |
| Hydrofluoric acid | 57.4 | | | 3.173 | | | | | | |
| Phosphoric acid | 34.6 | 61.4 | 71.5 | 2.16 | 7.21 | 12.67 | | | | |
| AlexaFlour 488 | 47.2* | | | | | | | | | |

*Fully ionized mobility, since Alexa Fluor 488 is fully ionized above pH 5 [7].

**Measurement electrophoretic mobility of Fluorescein at different ionic strengths**

The following section describes the experiments associated with the electrophoretic mobility data of fluorescein shown in Figure 2. These data are part of an experimental study of the mobility of sodium-fluorescein, Alexa Fluor 488 succinimidyl ester and Rhodamine 6G performed by Denitsa Milanova , Robert D. chambers, and Juan G. Santiago. A manuscript describing this study is currently under preparation by the latter authors. We here provide a brief description of their experimental method as a brief documentation for the part of that data used in this work. We shall refer to this study as that of Milanova *et al.* [7].

Milanova *et al.* [7] performed controlled capillary elecrophoresis (CE) experiments to measure the electrophoretic mobility of fluorescein for ionic concentrations between 30 mM and 90 mM at pH of 9.4 and 7.2. They used anionic sodium-fluorescein (Molecular Probes, Eugene, OR) at 300 µM and rhodamine B dye (Sigma-Aldrich, St. Louis, MO) at 200 µM. The latter served as a neutral marker of electroosmotic flow. All assays were analyzed on a commercial NS-95 borosilicate microchip purchased from Caliper Life Sciences (Mountain View, CA) with a simple cross pattern, which consists of relatively narrow and wide channels (10 and 50 µm mask widths) and 12 µm in depth. Images of fluorescent peaks were obtained using an inverted epifluorescent microscope (IX70, Olympus, Hauppauge, NY) equipped with a mercury lamp, a U-MWIBA filter-cube from Olympus (460-490 nm excitation, 515 nm emission) and a 10× (NA of 0.4) UPlanApo objective for fluorescence imaging. Images were captured using a 12 bit, 1300 by 1030 pixel array CCD camera (Coolsnap, Roper Scientific, Trenton, NJ) and controlled with $\mu$-Manager microscopy software (available for free at: micro-manager.org). High voltage was applied in the microchip wells using a computer-controlled Labsmith HVS-3000D (Livermore, CA) power supply. A schematic of experimental procedure is shown in Figure 2. The electrokinetic injection experiment consisted of: 1) a pinching step during which the analyte and the neutral marker were pulled from sample to waste wells; 2) injection of the sample zone during which a high voltage difference is applied from the West to East wells; and 3) a pull back (simultaneous with step 2) to retract the analyte back to sample and waste wells. A point detector was placed 15 mm down the separation channel and the data were postprocessed with custom MATLAB scripts.

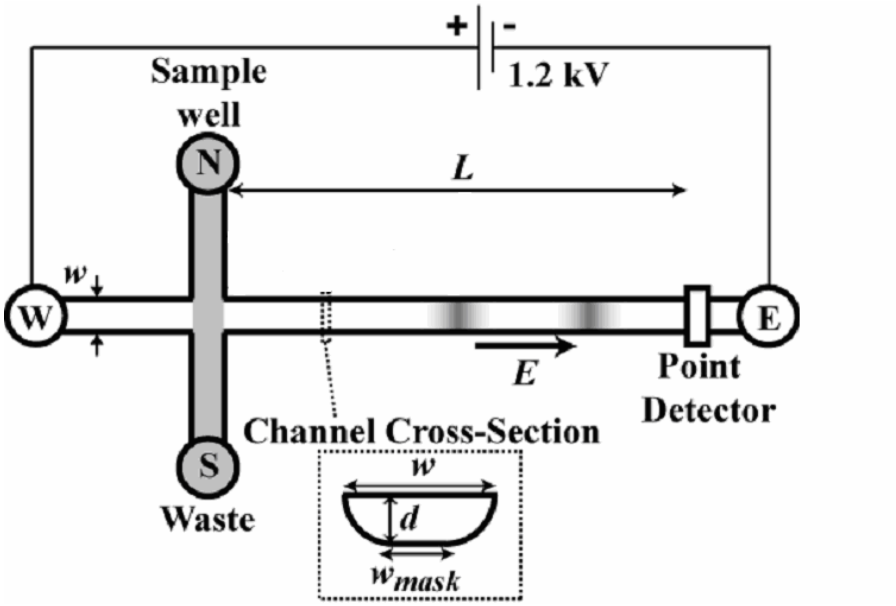


Figure 2: The experimental apparatus of Milanova *et al.* [7] for capillary electrophoresis includes microfluidic chip, epifluorescence microscope, CCD camera, high voltage switching system, 1.2 kV DC power supply, and DAQ system.

References cited here:


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