Supporting Information

Sample Zone Dynamics in Peak Mode Isotachophoresis

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This document contains the following supporting information for the article:

- Figure S-1: Inverted intensity images of ITP sample zone at various locations.
- Figure S-2: Electrophoretic mobility of Alexa Fluor 488 as a function of ionic strength.
- Analytical solution of sample concentration growth a LE-TE interface
- Corrections for electrophoretic mobility and activity coefficient
- Scaling argument for effective dispersion coefficient of species in ITP interface.



Figure S-1: Raw greyscale intensity images of sample zone at five locations downstream of the TE well in an ITP experiment. Here, the leading and trailing electrolytes were 240 mM histidine-HCl and 2.5 mM Na-phenylpropionic acid respectively and current was held constant at 30 μ Amp. The curvature in the sample zone is evidence of dispersion due to residual (non-uniform) EOF. The curvature changes direction after x = 21 mm (station 4).



Figure S-2: Plot of the electrophoretic mobility of Alexa-Fluor 488 as a function of the ionic strength of histidine-HCl buffer (pH = 5.3), as obtained from independent, uniform electrolyte injection experiments. Solid line is a power law fit to the data. The electrophoretic mobility by determined by electrokinetic injection of Alexa-Fluor 488 inside a simple-cross geometry microchip and we monitored electroosomotic flow in the channel using Rhodamine B as the neutral marker.

Analytical solution of sample concentration growth a LE-TE interface:

Simplified flux-balance analysis

For a univalent ITP system (weak/strong electrolyte), the concentration of trailing ion in the regulated TE zone is given by (from Jovin's and Alberty's relations)

$$C_{T}^{te} = C_{L}^{le} \frac{\mu_{T}^{te}}{\mu_{L}^{le}} \left(\frac{\mu_{C}^{le} + |\mu_{L}^{le}|}{\mu_{C}^{te} + |\mu_{T}^{te}|} \right)$$
(S.1)

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We use Jovin's relation to arrive at the counterion concentration in the trailing zone

$$C_{C}^{te} = C_{C}^{le} - C_{L}^{le} + C_{L}^{le} \frac{\mu_{T}^{te}}{\mu_{L}^{le}} \left(\frac{\mu_{C}^{le} + |\mu_{L}^{le}|}{\mu_{C}^{te} + |\mu_{T}^{te}|} \right)$$
(S.2)

Using these trailing ion and counterion concentrations, we can obtain the conductivity of the regulated TE zone as:

$$\sigma_{te} = F\left(\alpha_T^{te} \left| \mu_T \right| C_t^{te} + \alpha_c^{te} \mu_c C_c^{te}\right)$$
(S.3)

where α_T^{te} and α_C^{te} are the degree of dissociation of the trailing and the counterion in the TE zone. These are obtained from their respective acid equilibrium constants ($K_{a,T}$ and $K_{a,C}$) and hydronium ion concentration (or pH) as:

$$\alpha_{T}^{te} = \frac{K_{a,T}}{K_{a,T} + C_{H^{+}}}$$
(S.4)
$$\alpha_{C}^{te} = \frac{C_{H^{+}}}{K_{a,C} + C_{H^{+}}}$$

 C_{H^+} can be obtained by using the electroneutrality condition $\alpha_T^{te} C_T^{te} = \alpha_C^{te} C_C^{te}$ and substituting for α_T^{te} and α_C^{te} from eq (S.4).

$$C_{H^{+}} = -\frac{K_{a,T}}{2} \left(1 - \frac{C_{T}^{te}}{C_{C}^{te}} - \sqrt{\left(1 - \frac{C_{T}^{te}}{C_{C}^{te}}\right)^{2} + 4\frac{K_{a,C}C_{T}^{te}}{K_{a,T}C_{C}^{te}}} \right)$$
(S.5)

Since the concentration of trailing ion and counterion is known apriori, we can obtain the conductivity of the TE well (σ_{tewell}) in similar way (equations (S.3)-(S.5)) The sample concentration in the regulated TE zone is related to the initial sample concentration in the well through the flux balance condition across the initial stationary boundary as:

$$C_{S}^{te} = \frac{\alpha_{S}^{tewell} \mu_{s}^{tewell}}{\alpha_{S}^{te} \mu_{s}^{te}} \frac{\sigma_{te}}{\sigma_{tewell}} C_{S}^{tewell}$$
(S.6)

The net influx of sample ions at the interface between the LE and TE zone is equal to the accumulation rate of sample ions. The diffusive fluxes are dominant only at the interface and only govern the width of the interface as well as the sample zone. Hence, we can also express the same accumulation rate as the net influx of sample ions into the sample zone, migrating at speed V_{TTP} between the LE-TE interface.

$$\frac{dN_S}{dt} = \left(\mu_S^{te} E_{te} - V_{ITP}\right) C_S^{te} \tag{S.7}$$

The speed of the interface V_{ITP} is given by:

$$V_{ITP} = \frac{\alpha_L^{le} \mu_L^{le} j}{\sigma_{le}} = \frac{\alpha_T^{te} \mu_T^{te} j}{\sigma_{te}}$$
(S.8)

We now substitute for V_{ITP} in eq (S.7) with the expression in eq (S.8) and also substitute expression for C_S^{te} from eq (S.6) to arrive at

$$\frac{dN_S}{dt} = \left(\alpha_S^{te} \mu_S^{te} - \alpha_T^{te} \mu_T^{te}\right) \frac{\alpha_S^{tewell} \mu_S^{tewell}}{\alpha_S^{te} \mu_S^{te}} \frac{C_S^{tewell}}{\sigma_{tewell}} j$$
(S.9)

Since the ITP interface moves at constant speed over time, i.e. $x = V_{ITP}t$, we replace time *t* with distance *x* in eq (S.9)

$$\frac{dN_S}{dx} = \left(\alpha_S^{te} \mu_S^{te} - \alpha_T^{te} \mu_T^{te}\right) \frac{\alpha_S^{tewell} \mu_S^{tewell}}{\alpha_S^{te} \mu_S^{te}} \frac{C_S^{tewell}}{\sigma_{tewell}} \frac{j}{V_{ITP}}$$
(S.10)

Again, substituting for V_{ITP} from eq (S.8), we obtain

$$\frac{dN_S}{dx} = \frac{\left(\alpha_S^{te} \mu_S^{te} - \alpha_T^{te} \mu_T^{te}\right)}{\alpha_L^{le} \mu_L^{le}} \frac{\alpha_S^{tewell} \mu_S^{tewell}}{\alpha_S^{te} \mu_S^{te}} \frac{\sigma_{le}}{\sigma_{tewell}} C_S^{tewell}$$
(S.11)

As none of the parameters on the RHS of eq (S.11) change with distance x, the number of sample species (per unit cross-section area) accumulated at the interface is simply

$$N_{S} = \frac{\left(\alpha_{S}^{te} \mu_{S}^{te} - \alpha_{T}^{te} \mu_{T}^{te}\right)}{\alpha_{L}^{le} \mu_{L}^{le}} \frac{\alpha_{S}^{tewell} \mu_{S}^{tewell}}{\alpha_{S}^{te} \mu_{S}^{te}} \frac{\sigma_{le}}{\sigma_{tewell}} C_{S}^{tewell} x$$
(S.12)

The approximate length scale of the interface can be obtained by solving for the distribution of the leading and trailing electrolyte at the interface. In the lab frame of reference, the convective diffusion equation for a univalent (weak/strong) electrolyte is given by:

$$\frac{\partial C_i}{\partial t} = \frac{\partial}{\partial x} \left(-\alpha_i \mu_i C_i E + D_i \frac{\partial C_i}{\partial x} \right)$$
(S.13)

Here α_i is the degree of dissociation of species *i*.

We apply the following transformation to the convective diffusion equation to translate the frame of reference with the interface.

$$\tau = t$$

$$\xi = x - V_{TTP}t$$
(S.14)

In the transformed set of variables, the convective diffusion equation is given by:

$$\frac{\partial C_i}{\partial \tau} - V_{ITP} \frac{\partial C_i}{\partial \xi} = \frac{\partial}{\partial \xi} \left(-\alpha_i \mu_i C_i E + D_i \frac{\partial C_i}{\partial \xi} \right)$$
(S.15)

The LE and TE species acquire reach a steady state distribution in short time scale (~ 1-2 sec for interface width ~100 μ m), we can neglect the species evolution term on the LHS

of eq (S.15) and solve for the steady state distribution. The simplified equation is then written as

$$\frac{\partial}{\partial\xi} \left(-V_{ITP} C_i \right) = \frac{\partial}{\partial\xi} \left(-\alpha_i \mu_i C_i E + D_i \frac{\partial C_i}{\partial\xi} \right)$$
(S.16)

or,

$$-V_{ITP}C_i = -\alpha_i \mu_i C_i E + D_i \frac{\partial C_i}{\partial \xi} + F_i$$
(S.17)

where F_i is a constant determined by the boundary conditions. For the leading and trailing ion, $F_i = 0$ since the LE and TE interfaces move at identical speed and far away from the interface,

$$V_{ITP} = \alpha_L \mu_L C_L E_{LE} = \alpha_T \mu_T C_T E_{TE}$$
(S.18)

Therefore, the flux balance equations for leading and trailing ion simplify to

$$-V_{ITP}C_L = -\alpha_L \mu_L C_L E + D_L \frac{\partial C_L}{\partial \xi}$$
(S.19)

and

$$-V_{ITP}C_T = -\alpha_T \mu_T C_T E + D_T \frac{\partial C_T}{\partial \xi}$$
(S.20)

We eliminate E from eq (S.19) and (S.20) arrive at the following equation

$$-\frac{V_{TTP}}{\alpha_L \mu_L} + \frac{V_{TTP}}{\alpha_T \mu_T} = \frac{D_L}{\alpha_L \mu_L C_L} \frac{\partial C_L}{\partial \xi} - \frac{D_T}{\alpha_T \mu_T C_T} \frac{\partial C_T}{\partial \xi}$$
(S.21)

Next we assume negligible change in the pH across the interface such that α_T and α_L do not change significantly (i.e. $\alpha_T = \alpha_T^{te}$ and $\alpha_L = \alpha_L^{te}$) and we use Nerst-Einstein relationship to relate the diffusion coefficient of a species to its mobility

$$\frac{D_L}{\mu_L} = \frac{D_T}{\mu_T} = \frac{kT}{e}$$
(S.22)

We then integrate eq (S.21) obtain the following relationship between C_L and C_T .

$$\left(\frac{C_L}{C_{L,x=0}}\right)^{1/\alpha_L} \left(\frac{C_{T,x=0}}{C_T}\right)^{1/\alpha_T} = \exp\left(\frac{\xi}{\delta}\right)$$
(S.23)

where δ is the length-scale of the interface width, given by

$$\delta = \frac{kT}{e} \frac{\alpha_L \mu_L \alpha_T \mu_T}{\left(\alpha_L \mu_L - \alpha_T \mu_T\right) V_{ITP}}$$

$$= \frac{kT}{e} \frac{\alpha_T \mu_T}{\left(\alpha_L \mu_L - \alpha_T \mu_T\right)} \frac{\sigma_{le}}{j}$$
(S.24)

The total length scale over which E changes at the interface is apprx. equal to the sum of lengthscale over which C_L and C_T drop.

$$w = \left(\frac{2\delta}{\alpha_L} + \frac{2\delta}{\alpha_T}\right) = \frac{2kT}{e} \left(\frac{1}{\alpha_L} + \frac{1}{\alpha_T}\right) \frac{\alpha_T \mu_T}{\left(\alpha_L \mu_L - \alpha_T \mu_T\right)} \frac{\sigma_{le}}{j}$$
(S.25)

Noting that $\alpha_L = \alpha_L^{le}$, $\mu_L = \mu_L^{le}$ and so on, the scale for concentration of sample ions in the interface can now be obtained using the accumulated moles sample species and interface width length-scale obtained in eq (S.12) and (S.24).

$$C_{S} \approx \frac{N_{S}}{w}$$

$$\approx \left(\frac{e}{2kT}\right) \frac{\alpha_{L}^{le} \alpha_{T}^{le}}{\alpha_{L}^{le} + \alpha_{L}^{le}} \frac{\left(\alpha_{S}^{te} \mu_{S}^{te} - \alpha_{T}^{te} \mu_{T}^{te}\right) \left(\alpha_{L}^{le} \mu_{L}^{le} - \alpha_{T}^{te} \mu_{T}^{te}\right)}{\alpha_{T}^{te} \mu_{T}^{te} \alpha_{L}^{le} \mu_{L}^{le}} \frac{\alpha_{S}^{te} \mu_{T}^{te}}{\alpha_{S}^{te} \mu_{S}^{te}} \frac{j}{\sigma_{tewell}} C_{S}^{tewell} x$$
(S.26)

Corrections for Electrophoretic Mobility and Activity Coefficient:

The fully ionized mobility of background electrolyte species (leading, trailing and counterion) is corrected to accommodate the influence of ionic strength using the Pitts equation.^{40,41}

$$\mu_{i,z} = \mu_{i,z}^{0} - \left(\left| z_{-} z_{+} \right| c_{1} \mu_{i,z}^{0} + \left| z_{-} \right| c_{2} \right) \frac{\sqrt{I}}{1 + c_{3} \sqrt{I}}$$
(S.27)

where $\mu_{i,z}^0$ is the fully ionized mobility of the species *i* with valence *z*, *I* is the ionic strength and c_1, c_2 and c_3 are constants, $c_1 = 0.23, c_2 = 31.4e - 9$ and $c_3 = 1.5$ (obtained after simplification of Pitts equation for symmetric electrolytes).

Similarly, we correct for the dependence of activity coefficient of the electrolyte species on the ionic strength using the Truesdell-Jones⁴² model of the form:

$$\log \gamma_{i,z} = \frac{-Az^2 \sqrt{I}}{1 + Ba\sqrt{I}} + cI \tag{S.28}$$

where $\gamma_{i,z}$ is the activity coefficient of species *i* with valence *z*, *I* is the ionic strength and constants A = 0.51, Ba = 1.5 and c = 0.1.

Scaling Argument for Effective Dispersion Coefficient:



Figure S-3. (a) Schematic of the distorted ITP interface showing the bulk velocity field (as black arrows in the frame of reference moving with the plug) and electric field (grey arrows) in the vicinity of the plug. (b) Schematic of the net velocity (bulk + electrophoretic) of LE and TE ions in the frame of reference moving with the ITP plug). In b), arrows indicate net velocity of LE ions and dashed arrows indicate net velocity of TE ions.

The non-uniform bulk (EOF) velocity in the LE and TE zones induces an axially varying pressure gradient. This distorts the ITP interface since the centerline bulk velocity is higher than the bulk velocity near the wall. In the frame of reference moving with the interface, the bulk velocity vector at the interface is shown in dark arrows in Figure S-3(a). The interface distorts due to this induced pressure driven flow thereby creating a radially non-uniform conductivity and electric field. The electric field vectors, in the vicinity of the interface, are shown in gray arrows in Figure S-3(a). In this modified flow-field, the plug acquires a new steady state shape. The velocity (and net flux) of the LE and TE ions near the LE-TE interface in the stationary frame of reference,

is positive at the centerline and negative near the walls, as shown in Figure S-3(b). This mismatch in the axial flux at the LE-TE interface must be balanced by radial flux of the species. The species will diffuse, advect and electromigrate in the radial direction and in suppressed EOF conditions, we hypothesize that radial electromigration is the dominant mechanism contributing to the radial mass flux. For radial electromigration to balance axial dispersion, the two phenomena must occur over the same time scale.

For the given case, the time scale for axial dispersion is given by:

$$\tau_{axial} \sim \frac{V_{eof}}{\delta_{eof}}$$
(S.29)

where $\,\delta_{\scriptscriptstyle \! e\!o\!f}\,$ is the stretched interface width due to EOF

Similarly, time scale for radial electromigration:

$$\tau_{radial} \sim \frac{\mu E_y}{a} \tag{S.30}$$

where a is the width of the channel and E_y is the radial electric field component.

Therefore, equating eq. (S.29) and (S.30), we arrive at an expression for δ_{eof}

$$\delta_{eof} \sim \frac{V_{eof}a}{\mu_L E_y} \tag{S.31}$$

Now, we express the total width of ITP plug as the sum of original interface width (only diffusion) and stretched interface width

$$\Delta = w + \delta_{eof} \tag{S.32}$$

Here
$$w = \frac{D_L}{\mu_L} \frac{\mu_T}{(\mu_L - \mu_T)E_x}$$
 (S.33)

Next we express Δ in the same form as w in terms of effective dispersion coefficient as:

$$\Delta \approx \frac{D_{L,eff}}{\mu_L} \frac{\mu_T}{(\mu_L - \mu_T)E_x}$$
(S.34)

Substituting expressions for Δ, δ_0 and δ_{eof} in eq (S.32), and simplifying to obtain $D_{L,eff}$ we get:

$$D_{L,eff} \approx D_L + V_{eof} a \frac{E_x}{E_y} \frac{(\mu_L - \mu_T)}{\mu_T}$$
(S.35)

or,

$$D_{L,eff} \approx D_L (1 + \beta P e)$$
(S.36)
where, $\beta \sim \frac{E_x}{E_y} \frac{(\mu_L - \mu_T)}{\mu_T}$