

Supplementary Information: Coupling Isotachophoresis with Affinity Chromatography for Rapid and Selective Purification with High Column Utilization. Part I: Theory

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In this supplementary information document, we present additional information on the following topic:

SI 1. Solution to the simplified advection-reaction equations

SI 2. Separation resolution of ITP-AC

SI 1. Solution to the simplified advection-reaction equations

We solve the simplified advection-reaction equations derived in Section 2.2 of the main text. We start by finding the zeroth order solution for c and n following Thomas¹ but using the boundary and initial conditions pertinent to our problem. We then follow a similar procedure to find first and second order solutions. Similar procedure can be applied to obtain higher order solutions. However, in the main text we discuss and explain the trends given only by the zeroth order solution (accurate to $O(\varepsilon)$), as it is the simplest engineering approximation to this problem. Fortunately, the results of the zeroth order solution agree well with experimental measurements of key ITP-AC parameters at our experimental conditions (presented in Part II of this two-paper series). At the end of this section we present the solution accurate to $O(\varepsilon^3)$ for situations where knowledge of spatiotemporal behavior of ITP-AC is needed to greater accuracy. While we continue to work with the non-dimensionalized equations (as we have in Section 2.2 of the main text), we here drop the asterisks above the variables for clarity of presentation.

SI 2.1 Solution of zeroth order equations

We begin by transforming the two zeroth order equations into a coordinate moving with the LE-TE interface velocity (for the non-dimensionalized equations this velocity equals to unity). We then convert the result into a potential function form, thereby collapsing the two equations into a single equation. We then solve the resulting equation using Laplace transforms.

We write the zeroth order non-dimensionalized equations and their respective boundary and initial conditions here again for convenience:

$$\frac{\partial c_0}{\partial t} + \frac{\partial c_0}{\partial z} + \frac{\partial n_0}{\partial t} = 0, \quad (1)$$

$$\frac{\partial n_0}{\partial t} - c_0(1 - n_0) + \beta n_0 = 0, \quad (2)$$

$$\begin{aligned} c_0(z, 0) &= 0, \\ n_0(z, 0) &= 0, \end{aligned} \quad (3)$$

$$c_0(0, t) = \left(\alpha/\sqrt{2\pi}\right) \exp\left[-(t/Da - 3)^2/2\right]. \quad (4)$$

We begin by transforming (1) through (4) into new independent variables, $x = z$ and $y = (t - z)$:

$$\frac{\partial c_0}{\partial x} + \frac{\partial n_0}{\partial y} = 0, \quad (5)$$

$$\frac{\partial n_0}{\partial y} - c_0(1 - n_0) + \beta n_0 = 0, \quad (6)$$

$$\begin{aligned} c_0(x, 0) &= 0, \\ n_0(x, 0) &= 0, \end{aligned} \quad (7)$$

$$c_0(0, y) = \left(\alpha/\sqrt{2\pi}\right) \exp\left[-(y/Da - 3)^2/2\right]. \quad (8)$$

Next we introduce a potential function f such that

$$\begin{aligned} n_0 &= \frac{\partial f}{\partial x}, \\ c_0 &= -\frac{\partial f}{\partial y}. \end{aligned} \quad (9)$$

The potential function f automatically satisfies equation (5). Therefore, we substitute (9) into (6) and obtain

$$\frac{\partial^2 f}{\partial y \partial x} + \frac{\partial f}{\partial y} + \beta \frac{\partial f}{\partial x} - \frac{\partial f}{\partial y} \frac{\partial f}{\partial x} = 0. \quad (10)$$

Equation (10) is non-linear and we linearize it by substituting in

$$f(x, y) = x + y\beta - \ln \phi(x, y), \quad (11)$$

after which we obtain

$$\frac{\partial^2 \phi}{\partial y \partial x} = \beta \phi. \quad (12)$$

In this way, we have condensed equations (5) and (6) into a single equation (12). Next we transform the initial and the boundary conditions. We note that we use the only initial condition for c_0 . The initial condition on n_0 was applied much earlier, when we tacitly assumed that there is no bound target initially in the affinity region. See the main text when we first introduce the reaction equation in terms of N (eq. (2) of main text). The transformed initial and boundary conditions are

$$\frac{1}{\phi(x, 0)} \frac{\partial \phi}{\partial x}(x, 0) = 1 - \frac{\partial f}{\partial x} = 1 - c(x, 0), \quad (13)$$

$$\frac{1}{\phi(0, y)} \frac{\partial \phi}{\partial y}(0, y) = \beta - \frac{\partial f}{\partial y} = \beta + c(0, y). \quad (14)$$

We solve the differential equations (13) and (14) and obtain

$$\phi(x, 0) = \exp \left[x - \int_0^x c(x', 0) dx' \right] = H(x), \quad (15)$$

$$\phi(0, y) = \exp \left[y\beta + \int_0^y c(0, y') dy' \right] = G(y). \quad (16)$$

Next we apply the Laplace transform to equation (12) and use the result (16) to obtain

$$p \frac{d\bar{\phi}}{dx} = \beta \bar{\phi} + \frac{dH(x)}{dx}, \quad (17)$$

where the overbar denotes the Laplace transform of a function with respect to y (e.g., $\bar{\phi}(x, p)$ is the Laplace transform of ϕ and p is the Laplace transform variable). Also,

$$\bar{\phi}(0, p) = \bar{G}(p). \quad (18)$$

We solve the differential equation (17) by variation of parameters to obtain

$$\bar{\phi}(x, p) = \bar{G}(p) \exp \left[\frac{\beta x}{p} \right] + \frac{1}{p} \int_0^x \frac{dH(x')}{dx} \exp \left[\frac{\beta(x-x')}{p} \right] dx'. \quad (19)$$

We then take the inverse transform of (19) to obtain

$$\phi(x, y) = \int_0^y G(y-y') F_1(x, y') dy' + \int_0^x \frac{dH(x-x')}{dx} F_2(x', y) dx', \quad (20)$$

where

$$\begin{aligned} L[F_1(x, y)] &= \exp \left[\frac{\beta x}{p} \right], \\ L[F_2(x, y)] &= \frac{1}{p} \exp \left[\frac{\beta x}{p} \right]. \end{aligned} \quad (21)$$

$L[F(x, y)]$ designates the Laplace transform of $F(x, y)$. Taking the inverse Laplace transform of (21)² we obtain

$$F_1(x, y) = \sqrt{\frac{\beta x}{y}} I_1(2\sqrt{\beta yx}) + \delta(y), \quad (22)$$

$$F_2(x, y) = I_0(2\sqrt{\beta yx}).$$

Here I_0 and I_1 are modified Bessel functions of the first kind of zeroth and first order respectively. We substitute (22) into (20) and obtain

$$\phi(x, y) = G(y) + \int_0^y G(y - y') \sqrt{\frac{\beta x}{y'}} I_1(2\sqrt{\beta y'x}) dy' + \int_0^x \frac{dH(x - x')}{dx} I_0(2\sqrt{\beta yx'}) dx'. \quad (23)$$

We then simplify (23) by evaluating the right hand side term via integration by parts and noticing that $G(0) = 1$

$$\phi(x, y) = I_0(2\sqrt{\beta yx}) + \int_0^y \frac{dG(y - y')}{dy} I_0(2\sqrt{\beta y'x}) dy' + \int_0^x \frac{dH(x - x')}{dx} I_0(2\sqrt{\beta yx'}) dx'. \quad (24)$$

This is the general solution to the zeroth order equations in terms of the potential function ϕ . Next we apply the initial and boundary conditions specific to ITP-AC. We begin by substituting (7) into (15) and obtain

$$H(x) = \exp(x), \quad (25)$$

and

$$\frac{dH(x)}{dx} = \exp(x). \quad (26)$$

Substituting, this simplifies (24) to

$$\phi(x, y) = I_0(2\sqrt{\beta yx}) + \int_0^y \frac{dG(y - y')}{dy} I_0(2\sqrt{\beta y'x}) dy' + \int_0^x \exp[(x - x')] I_0(2\sqrt{\beta yx'}) dx'. \quad (27)$$

We next apply the boundary condition by substituting (8) into (16) and obtain

$$G(y) = \exp\left[y\beta + \frac{\alpha Da}{2} \left(\operatorname{erf}\left(\frac{y/Da - 3}{\sqrt{2}}\right) - \operatorname{erf}\left(\frac{-3}{\sqrt{2}}\right)\right)\right], \quad (28)$$

and

$$\frac{dG(y)}{dy} = \left(\frac{\alpha}{\sqrt{2\pi}} \exp\left[-\frac{(y/Da-3)^2}{2}\right] + \beta \right) \exp\left[y\beta + \frac{\alpha Da}{2} \left(\operatorname{erf}\left(\frac{y/Da-3}{\sqrt{2}}\right) - \operatorname{erf}\left(\frac{-3}{\sqrt{2}}\right) \right) \right]. \quad (29)$$

Therefore

$$\phi(x, y) = I_0\left(2\sqrt{\beta yx}\right) + \int_0^y \frac{dG(y-y')}{dy} I_0\left(2\sqrt{\beta y'x}\right) dy' + \int_0^x \exp[(x-x')] I_0\left(2\sqrt{\beta yx'}\right) dx', \quad (30)$$

where dG/dy is given by (29). Now c_0 and n_0 can be easily obtained by substituting (30) into (11) and substituting the result into (9). For convenience, we evaluated solutions numerically using custom MATLAB scripts as we analyzed the spatiotemporal behavior of the target in ITP-AC.

SI 2.2 Solution of first order equations

We solve the first order equations following a procedure similar to the zeroth order equations. We again begin by transforming the two first order equations into a coordinate moving with the LE-TE interface velocity. We then convert the result into a potential function form, thereby again collapsing the two equations into a single equation. We again solve the resulting equation using Laplace transforms.

We write the first order non-dimensionalized equations and their respective boundary and initial conditions here again for convenience:

$$\frac{\partial c_1}{\partial t} + \frac{\partial c_1}{\partial z} + \frac{\partial n_1}{\partial t} + \frac{\partial(\tilde{u}c_0)}{\partial z} = 0, \quad (31)$$

$$\frac{\partial n_1}{\partial t} - c_1 + \beta n_1 = 0, \quad (32)$$

$$c_1(z, 0) = 0, \quad (33)$$

$$n_1(z, 0) = 0,$$

$$c_1(0, t) = 0. \quad (34)$$

We then transform (31) through (34) into new independent variables, $x = z$ and $y = (t - z)$:

$$\frac{\partial c_1}{\partial x} + \frac{\partial n_1}{\partial y} + \frac{\partial(\tilde{u}c_0)}{\partial x} - \frac{\partial(\tilde{u}c_0)}{\partial y} = 0, \quad (35)$$

$$\frac{\partial n_1}{\partial y} - c_1 + \beta n_1 = 0. \quad (36)$$

Next we define new variables

$$\begin{aligned} C_1 &= c_1 + \tilde{u}c_0, \\ M_1 &= n_1 - \tilde{u}c_0, \end{aligned} \quad (37)$$

and recast (35) and (36) in these variables:

$$\frac{\partial C_1}{\partial x} + \frac{\partial M_1}{\partial y} = 0, \quad (38)$$

$$\frac{\partial M_1}{\partial y} + \frac{\partial(\tilde{u}c_0)}{\partial y} - (C_1 - \tilde{u}c_0) + \beta(M_1 + \tilde{u}c_0) = 0. \quad (39)$$

We also recast the initial and boundary condition in these variables

$$C_1(z, 0) - \tilde{u}(z, 0)c_0(z, 0) = 0, \quad (40)$$

$$M_1(z, 0) + \tilde{u}(z, 0)c_0(z, 0) = 0,$$

$$C_1(0, t) - \tilde{u}(0, t)c_0(0, t) = 0. \quad (41)$$

Next we introduce a potential function f such that

$$\begin{aligned} M_1 &= \frac{\partial f}{\partial x} \\ C_1 &= -\frac{\partial f}{\partial y} \end{aligned} \quad (42)$$

and substitute this into (38) and (39). The potential function f automatically satisfies equation (38), and (39) becomes

$$\frac{\partial^2 f}{\partial y \partial x} + \frac{\partial f}{\partial y} + \beta \frac{\partial f}{\partial x} + \frac{\partial(\tilde{u}c_0)}{\partial y} + (1 + \beta)\tilde{u}c_0 = 0. \quad (43)$$

Now we apply f to the initial and boundary conditions

$$\frac{\partial f}{\partial x}(x, 0) = M_1(x, 0) = n_1(x, 0) - \tilde{u}(x, 0)c_0(x, 0), \quad (44)$$

$$\frac{\partial f}{\partial y}(0, y) = -C_1(0, y) = -c_1(0, y) - \tilde{u}(0, y)c_0(0, y). \quad (45)$$

We integrate (44) and (45)

$$f(x, 0) = \int_0^x n_1(x', 0) - \tilde{u}(x', 0)c_0(x', 0) dx', \quad (46)$$

$$f(0, y) = \int_0^y -c_1(0, y') - \tilde{u}(0, y')c_0(0, y') dy'. \quad (47)$$

Noticing that $n_1(x', 0) = 0$ and $c_1(0, y') = 0$ we simplify the above equations to

$$f(x, 0) = -\int_0^x \tilde{u}(x', 0)c_0(x', 0) dx' = H_1(x), \quad (48)$$

$$f(0, y) = -\int_0^y \tilde{u}(0, y')c_0(0, y') dy' = G_1(y). \quad (49)$$

Equations for initial and boundary conditions (48) and (49) are to be evaluated using the result from the zeroth order equations. \tilde{u} is of the form of an error function (see main text), i.e., $\tilde{u} = \text{erf}[(t - z - 3Da)/Da]$.

Next, we take the Laplace transform of (43) to yield

$$\frac{\partial \bar{f}}{\partial x} + \frac{p}{p + \beta} \bar{f} = \frac{1}{p + \beta} \left[\frac{\partial H_1(x)}{\partial x} + H_1(x) - (1 + \beta + p)\bar{w} + w(x, 0) \right], \quad (50)$$

where $w = \tilde{u}c_0$. We solve differential equation (50) using variation of parameters and obtain

$$\begin{aligned} \bar{f} &= \bar{G}_1(p) \exp\left[\frac{-xp}{p + \beta}\right] \\ &+ \exp\left[\frac{-xp}{p + \beta}\right] \int_0^x \exp\left[\frac{x'p}{p + \beta}\right] \frac{1}{p + \beta} \left[\frac{\partial H_1(x')}{\partial x} + H_1(x') - (1 + \beta + p)\bar{w} + w(x', 0) \right] dx'. \end{aligned} \quad (51)$$

We expand this to

$$\bar{f} = \bar{G}_1(p) \bar{F}_1 + \int_0^x \left[\frac{\partial H_1(x' - x)}{\partial x} + H_1(x' - x) + w(x' - x, 0) \right] \bar{F}_2 dx' - \int_0^x \bar{F}_3 \bar{w} dx', \quad (52)$$

where

$$\begin{aligned}
\bar{F}_1 &= \exp\left[\frac{-xp}{p+\beta}\right], \\
\bar{F}_2 &= \frac{1}{p+\beta} \exp\left[\frac{px'}{p+\beta}\right], \\
\bar{F}_3 &= \frac{(1+\beta+p)}{p+\beta} \exp\left[\frac{p(x'-x)}{p+\beta}\right].
\end{aligned} \tag{53}$$

We recommend finding the inverse Laplace transforms of equations in (53) numerically. We then take the inverse Laplace transform of (52) to obtain

$$\begin{aligned}
f &= \int_0^y G_1(y-y') F_1(x, y') dy' \\
&+ \int_0^x \left[\frac{\partial H_1(x-x')}{\partial x} + H_1(x-x') + w(x-x', 0) \right] F_2(x', y) dx' \\
&- \int_0^x \int_0^y w(x', y-y') F_3(x, y') dy' dx'.
\end{aligned} \tag{54}$$

Now the functions c_1 and n_1 can be obtained fairly easily by substituting (54) into (42), and using the appropriate inverse Laplace transforms for (53) and the result from the zeroth order equations (as inputs for G_1 , H_1 , and w). We recommend evaluating this numerically using MATLAB.

SI 2.3 Solution of second order equations

We solve the second order equations following a similar procedure to the zeroth and first order equations. We once again begin by transforming the two first order equations into a coordinate moving with the LE-TE interface velocity. We then convert the result into a potential function form, again collapsing the two equations into a single equation. We then solve the resulting equation using Laplace transforms.

We write the second order non-dimensionalized equations and their respective boundary and initial conditions here again for convenience:

$$\frac{\partial c_2}{\partial t} + \frac{\partial c_2}{\partial z} + \frac{\partial n_2}{\partial t} + \frac{\partial(\tilde{u}c_1)}{\partial z} = 0, \tag{55}$$

$$\frac{\partial n_2}{\partial t} - c_2 + \beta n_2 + c_1 n_1 = 0, \tag{56}$$

$$\begin{aligned}c_2(z, 0) &= 0, \\n_2(z, 0) &= 0,\end{aligned}\tag{57}$$

$$c_2(0, t) = 0.\tag{58}$$

We transform (55) through (58) into new independent variables, $x = z$ and $y = (t - z)$:

$$\frac{\partial c_2}{\partial x} + \frac{\partial n_2}{\partial y} + \frac{\partial(\tilde{u}c_1)}{\partial x} - \frac{\partial(\tilde{u}c_1)}{\partial y} = 0,\tag{59}$$

$$\frac{\partial n_2}{\partial y} - c_2 + \beta n_2 + c_1 n_1 = 0.\tag{60}$$

Next, we define new variables

$$\begin{aligned}C_2 &= c_2 + \tilde{u}c_1, \\M_2 &= n_2 - \tilde{u}c_1,\end{aligned}\tag{61}$$

and recast (59) and (60) in these variables

$$\frac{\partial C_2}{\partial x} + \frac{\partial M_2}{\partial y} = 0,\tag{62}$$

$$\frac{\partial M_2}{\partial y} + \frac{\partial(\tilde{u}c_1)}{\partial y} - (C_2 - \tilde{u}c_1) + \beta(M_2 + \tilde{u}c_1) + c_1 n_1 = 0.\tag{63}$$

We also recast the initial and boundary condition in these variables

$$\begin{aligned}C_2(z, 0) - \tilde{u}(z, 0)c_1(z, 0) &= 0, \\M_2(z, 0) + \tilde{u}(z, 0)c_1(z, 0) &= 0,\end{aligned}\tag{64}$$

$$C_2(0, t) - \tilde{u}(0, t)c_1(0, t) = 0.\tag{65}$$

Next we introduce a potential function f such that

$$\begin{aligned}M_2 &= \frac{\partial f}{\partial x}, \\C_2 &= -\frac{\partial f}{\partial y},\end{aligned}\tag{66}$$

and substitute this into (62) and (63). The potential function f automatically satisfies equation (62), and (63) becomes

$$\frac{\partial^2 f}{\partial y \partial x} + \frac{\partial f}{\partial y} + \beta \frac{\partial f}{\partial x} + \frac{\partial(\tilde{u}c_1)}{\partial y} + (1 + \beta)\tilde{u}c_1 + c_1n_1 = 0. \quad (67)$$

Now we apply f to the initial and boundary conditions and obtain

$$\frac{\partial f}{\partial x}(x, 0) = M_2(x, 0) = n_2(x, 0) - \tilde{u}(x, 0)c_1(x, 0), \quad (68)$$

$$\frac{\partial f}{\partial y}(0, y) = -C_2(0, y) = -c_2(0, y) - \tilde{u}(0, y)c_1(0, y). \quad (69)$$

We integrate (68) and (69) and obtain

$$f(x, 0) = \int_0^x n_2(x', 0) - \tilde{u}(x', 0)c_1(x', 0) dx', \quad (70)$$

$$f(0, y) = \int_0^y -c_2(0, y') - \tilde{u}(0, y')c_1(0, y') dy'. \quad (71)$$

Noticing that $n_2(x', 0) = 0$ and $c_2(0, y') = 0$, we simplify the equations above to

$$f(x, 0) = -\int_0^x \tilde{u}(x', 0)c_1(x', 0) dx' = H_2(x), \quad (72)$$

$$f(0, y) = -\int_0^y \tilde{u}(0, y')c_1(0, y') dy' = G_2(y). \quad (73)$$

Equations for initial and boundary conditions (72) and (73) are to be evaluated using the result from the first order equations.

Next, we take the Laplace transform of (67) to obtain

$$\frac{\partial \bar{f}}{\partial x} + \frac{p}{p + \beta} \bar{f} = \frac{1}{p + \beta} \left[\frac{\partial H_2(x)}{\partial x} + H_2(x) - (1 + \beta + p)\bar{w}_1 + w(x, 0) - \bar{w}_2 \right], \quad (74)$$

where $w_1 = \tilde{u}c_1$, $w_2 = n_1c_1$. We solve differential equation (74) using variation of parameters and obtain

$$\bar{f} = (\bar{G}_2(p) + \Theta) \exp\left[\frac{-xp}{p + \beta}\right], \quad (75)$$

where

$$\Theta = \int_0^x \exp\left[\frac{x'p}{p + \beta}\right] \frac{1}{p + \beta} \left[\frac{\partial H_2(x')}{\partial x} + H_2(x') - (1 + \beta + p)\bar{w}_1 + w_1(x', 0) + \bar{w}_2 \right] dx'. \quad (76)$$

We expand (75) to

$$\bar{f} = \bar{G}_2(p)\bar{F}_1 + \int_0^x \left[\frac{\partial H_2(x'-x)}{\partial x} + H_2(x'-x) + w_1(x'-x, 0) \right] \bar{F}_2 dx' - \int_0^x \bar{F}_3 \bar{w}_1 dx' + \int_0^x \bar{F}_4 \bar{w}_2 dx', \quad (77)$$

where

$$\begin{aligned} \bar{F}_1 &= \exp\left[\frac{-xp}{p + \beta}\right], \\ \bar{F}_2 &= \frac{1}{p + \beta} \exp\left[\frac{px'}{p + \beta}\right], \\ \bar{F}_3 &= \frac{(1 + \beta + p)}{p + \beta} \exp\left[\frac{p(x'-x)}{p + \beta}\right], \\ \bar{F}_4 &= \frac{1}{p + \beta} \exp\left[\frac{p(x'-x)}{p + \beta}\right]. \end{aligned} \quad (78)$$

We then take the inverse Laplace transform of (78) to obtain

$$\begin{aligned} f &= \int_0^y G_2(y - y') F_1(x, y') dy' \\ &+ \int_0^x \left[\frac{\partial H_2(x - x')}{\partial x} + H_2(x - x') + w_1(x - x', 0) \right] F_2(x', y) dx' \\ &- \int_0^x \int_0^y w_1(x', y - y') F_3(x, y') dy' dx' \\ &+ \int_0^x \int_0^y w_2(x', y - y') F_4(x, y') dy' dx'. \end{aligned} \quad (79)$$

Now functions c_2 and n_2 can be obtained by substituting (79) into (66) using the appropriate inverse Laplace transforms for (78) and the result from the first order equations (as inputs for G_2 , H_2 , w_1 , and w_2). Again, we recommend evaluating this numerically using MATLAB.

SI 2.4 Third order accurate solutions

Finally, a third order accurate solution is obtained by combining results obtained from (30), (54), and (79) into

$$\begin{aligned} c &= c_0 + \varepsilon c_1 + \varepsilon^2 c_2 + O(\varepsilon^3), \\ n &= n_0 + \varepsilon n_1 + \varepsilon^2 n_2 + O(\varepsilon^3), \end{aligned} \tag{80}$$

where once again, ε is defined as

$$\varepsilon = \frac{\mu_{a,TE} - \mu_{TEion,TE}}{\mu_{TEion,TE}}. \tag{81}$$

Higher order equations can be obtained in a manner similar to that for first and second order equations and hence even higher order of accuracy solutions can be obtained.

SI 2. Separation resolution of ITP-AC

As described in the main text, the ideal case for ITP-AC separation is that $\beta_{cn} \ll \beta_{target}$ (where subscript “cn” refers to contaminant). That is, target should be captured approximately irreversibly while contaminant is removed and transported downstream. For this regime, the target is captured in time p_t while contaminants migrate through the column. We present an experimental demonstration of such separation in the main text of Part II of this two-part series. We consider here a regime where the target is completely captured (e.g., $\beta < 10^{-6}$) while the contaminant species remains focused in ITP and migrating at the ITP velocity. For this regime, we define a simple criterion for the time required to achieve sufficient separation between captured target and contaminant. We apply a common definition of resolution, R , given by Giddings³ as follows:

$$R = \frac{\Delta L}{2\sigma_1 + 2\sigma_2}, \quad (82)$$

where ΔL is the distance between the two peaks and, σ_1 and σ_2 are the corresponding standard deviations of the two peaks. We let σ_1 be the standard deviation width of the captured (immobile) target peak and σ_2 the standard deviation of the contaminant peak. In ITP-AC the target attains zero velocity at time approximately p_t , and thereafter the width of the target distribution scales as p_z . After the target attains zero velocity, the distance between the target and the analyte simply scales as ut . Using the scaling for the ITP peak width from MacInnes and Longsworth^{4,5}, we obtain the scaling for resolution for ITP-AC, as we did in equation (42) of the main text. We write it here again for convenience:

$$R_{ITP-AC} \approx \frac{ut}{\frac{u}{k_1 N} p_z^* + \frac{1}{u} \left(\frac{\mu_{L \text{ in LE}} \mu_{T \text{ in TE}}}{\mu_{L \text{ in LE}} - \mu_{T \text{ in TE}}} \right) \frac{k_B T}{e}}, \quad (83)$$

where again, k_B is the Boltzmann's constant, T is the absolute temperature, e is the electron charge, and $\mu_{L \text{ in LE}}$ and $\mu_{T \text{ in TE}}$ are the mobilities of the LE ion in the LE and the TE ion in the TE respectively. We note that the resolution for ITP-AC scales as t . This is in contrast to the resolution of traditional electrophoresis or of AC which scale as \sqrt{t} .⁶ The resolution of ITP-AC increases with increasing ITP velocity u and it asymptotes to a value of $tk_1 N / p_z^*$ for large u . We can achieve 95% of this resolution with

$$u_{95} \approx \sqrt{\frac{0.95}{0.05} \left(\frac{\mu_{L \text{ in LE}} \mu_{T \text{ in TE}}}{\mu_{L \text{ in LE}} - \mu_{T \text{ in TE}}} \right) \frac{k_B T}{e} \frac{k_1 N}{p_z^*}}. \quad (84)$$

Operating at ITP velocity lower than u_{95} results in loss of resolution. Operating at a velocity much higher than u_{95} leads to larger capture lengths with little gain in resolution and therefore loss in column utilization (see Section 3.2.2 of the main text). For a good compromise between resolution and column utilization, we therefore recommend operating at around u_{95} . For an ITP velocity of u_{95} and a convenient resolution, $R_{\text{ITP-AC}}$, value of, say, 10 (for well separated capture target and contaminant zone), the time to capture and purify, $t_{c,p}$ is approximately $10p_z^*/(k_1N)$. If αDa is also less than unity, then $t_{c,p}$ is approximately $28/(k_1N)$. For typical ITP-AC conditions such as those we employed in Part II of this two-paper series ($\mu_{L \text{ in } LE} \approx -60 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$, $\mu_{T \text{ in } LE} \approx -20 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$, $k_I = 10^3 \text{ M}^{-1} \text{ s}^{-1}$, $N \approx 30 \text{ }\mu\text{M}$, and $p_z^* \approx 2.8$), u_{95} is on the order of 0.01 mm/s and $t_{c,p}$ is approximately 15 min.⁷

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