Supporting Information for:

An Advanced, Interactive, High-Performance Liquid Chromatography Simulator and Instructor Resources

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Explanation of Chromatographic Parameters and Their Calculation

We briefly discuss each chromatographic parameter and how they are calculated in approximately the same order as they are calculated in the software.

Porosity

The interparticle porosity, ε_e, is the fraction of the volume in the column that is between the stationary phase particles. It is defined by $\varepsilon_e = V_e/V$ where V_e is the volume of eluent between the particles and V is the total volume in the column if it were empty. The intraparticle porosity, ε_i, is the fraction of the stationary phase particles that is occupied by eluent. It is defined by $\varepsilon_i = V_i/V_p$ where V_i is the eluent-accessible volume within the particles and V_p is the total volume of stationary phase particles (including the porous space within them).[12] The total porosity, ε_T, is the fraction of the volume in the column that is occupied by eluent, both outside and inside the stationary phase particles. It is calculated from $\varepsilon_T = \varepsilon_e + \varepsilon_i(1-\varepsilon_e)$.

Void volume/ void time

The void volume, V_0, is the volume in the column that is accessible to the mobile phase (the space between the particles and within them). It is calculated from:

$$V_0 = \pi\left(\frac{d}{2}\right)^2 L\varepsilon_T \tag{1}$$

where d is the column diameter and L is the column length. The void time, t_0, is the time required for an entirely unretained solute to pass through the column. It is calculated from: $t_0 = V_0/F$, where F is the volumetric flow rate of eluent through the column.

Flow velocity

While the flow *rate* defines the volume of eluent moving through the column per unit time, the flow *velocity* defines the distance traveled by eluent moving through the column per unit time. The HPLC simulator displays four different representations of the flow velocity: the open tube (superficial) flow velocity, the interstitial flow velocity, the chromatographic flow velocity, and the reduced flow velocity.[12] The open tube flow velocity, u_s, is the flow velocity calculated assuming the column is completely open. It is calculated from $u_s = F/O$, where O is the cross-sectional area of the column. The interstitial flow velocity, u_e, is the flow velocity of the mobile phase assuming it moves only through the interstitial space between the stationary phase particles. It is calculated from $u_e = u_s/\varepsilon_e$. The chromatographic flow velocity, u, is the flow velocity of a completely unretained solute. It is calculated assuming the solute can travel between the particles and within them: $u = u_s/\varepsilon_T$. Finally, the reduced velocity, ν, is a dimensionless term defined by $\nu = u_e d_p/D_e$ where d_p is the diameter of the stationary phase particles and D_e is the diffusion coefficient of the solute in the eluent (see below). In physical terms, it represents the relative contributions of convection and diffusion to axial transport of the analyte through the column under laminar flow. So, when $\nu = 1$, convection and diffusion contribute equally to movement of the analyte through the column. When $\nu << 1$, axial transport is dominated by diffusion, and when $\nu >> 1$, axial transport is dominated by convection.

Eluent viscosity

The eluent viscosity, η, is calculated as a function of eluent composition and temperature. It is used for subsequent calculations of pressure drop across the column and the average

diffusion coefficient of the analyte in the eluent. For acetonitrile-water mixtures, eluent viscosity is calculated using the following empirical equation reported by Chen and Horváth:[13]

$$\eta = \exp\left[\begin{array}{l} \phi\left(-3.476 + \dfrac{726}{T + 273.15}\right) + (1-\phi)\left(-5.414 + \dfrac{1566}{T + 273.15}\right) + \\ \phi(1-\phi)\left(-1.762 + \dfrac{929}{T + 273.15}\right) \end{array}\right] \tag{2}$$

where T is the temperature (in °C) and ϕ is the volume fraction of organic modifier (in this case, acetonitrile) in the eluent. For methanol-water mixtures, η is calculated by the following equation, which we determined by a fit to experimental data reported previously:[14]

$$\eta = \exp\left[\begin{array}{l} \phi\left(-4.597 + \dfrac{1211}{T + 273.15}\right) + (1-\phi)\left(-5.961 + \dfrac{1736}{T + 273.15}\right) + \\ \phi(1-\phi)\left(-6.215 + \dfrac{2809}{T + 273.15}\right) \end{array}\right] \tag{3}$$

Backpressure

Currently, the pressure drop across the column, ΔP, is the only source of backpressure we consider in our calculation. It is calculated by combining Darcy's law[15] with the Kozeny-Carman equation[16], which estimates the permeability of porous particles:[17]

$$\Delta P = 180 \frac{u_s \eta L (1 - \varepsilon_e)^2}{\varepsilon_e^{\,3} d_p^{\,2}} \tag{4}$$

Average analyte diffusion coefficient

Diffusion coefficients in the eluent, D_e, are important as they affect the efficiency of a separation. They are calculated from the following equation:[18]

$$D_e = 7.4 \cdot 10^{-8} \frac{(T + 273.15)\sqrt{xM}}{\eta V_m^{\,0.6}} \tag{5}$$

where x is the "solvent association parameter", M is the molecular weight of the solvent, and V_m is the molar volume of the solute. The solvent association parameter is calculated from:

$$x = ((1 - \phi)(2.6 - x_{org})) + x_{org} \tag{6}$$

where x_{org} is the solvent association parameter of the organic modifier. For methanol, it was measured to be 1.9;[18] we use the same value for acetonitrile. The molecular weight of the solvent is calculated from $M = \phi\,(M_{org} - 18) + 18$ where M_{org} is the molecular weight of the organic modifier. The molar volume of each solute is estimated from its structure using the appropriate atomic volumes reported by Wilke and Chang.[18]

Each compound has a unique diffusion coefficient, but since many of the calculated chromatographic values depend on the diffusion coefficient and the differences between the diffusion coefficients are small for the set of 22 compounds currently addressed by the simulator, we average the diffusion coefficients for the selected compounds into one value that is used for all subsequent calculations.

Reduced plate height

The reduced plate height, h, is a dimensionless measure of the efficiency of an HPLC column under a particular set of conditions.[19] Unlike the Height Equivalent to a Theoretical Plate (HETP - see below), h is dimensionless because it is normalized to d_p, which allows for comparison of the efficiency of columns packed with particles of different size. The reduced plate height is calculated using the van Deemter equation:[20]

$$h = A + \frac{B}{\nu} + C\nu \tag{7}$$

where ν is the reduced flow velocity, and A, B, and C are the reduced van Deemter parameters – an empirically determined set of terms that describe the contributions of eddy dispersion, longitudinal diffusion, and slow interphase mass transfer, respectively.

Height Equivalent to a Theoretical Plate (HETP)/Number of theoretical plates

HETP is another common way of expressing the efficiency of an HPLC column. We calculate it from HETP = hd_p. The number of theoretical plates, N, is calculated from: $N = L$/HETP.

Isocratic elution mode

Isocratic retention factors/retention times

The isocratic retention factor, k, of each compound is calculated assuming the following linear relationship:

$$\log k = \log k_w - S\phi \tag{8}$$

where k_w is the isocratic retention factor of the compound in water and S is the solvent "sensitivity" factor of the solute. Although this linear relationship does not accurately represent the dependence of k on ϕ over large ranges of solvent composition, it is nevertheless a good first approximation that is useful for educational purposes.

Both k_w and S are dependent on temperature. We calculate each of them from the following two linear relationships:[21]

$$S = A' + B'T \tag{9}$$

$$\log k_w = a' + b'T \tag{10}$$

where we determined A', B', a', and b' from experimental measurements (see Experimental section). The retention time of each compound was determined from: $t_R = t_0/(1+k)$.

Isocratic peak width

The peak width, in terms of one standard deviation (σ_t) of a Gaussian peak, is calculated from the following equation:

$$\sigma_t = \sqrt{\left(\frac{t_R}{\sqrt{N}}\right)^2 + \tau^2 + \frac{1}{12}\left(\frac{V_{inj}}{F}\right)^2} \tag{11}$$

where τ is the detector time constant and V_{inj} is the injection volume. The first term accounts for broadening of the peak inside the column itself, the second term accounts for additional band broadening caused by signal filtering at the detector, and the third term accounts for the effect of injection volume (assuming a rectangular injection profile).[22]

Gradient elution mode

Gradient profile

On any real HPLC instrument, the gradient profile (ϕ vs. t) *at the column inlet* is never the same as the programmed gradient profile because of unavoidable volume that lies between the solvent proportioning valves and the column inlet. This includes volume within the pump, solvent mixer, connection fittings, connection tubing, and the column inlet itself. The sum of this volume is called the "dwell volume" (also called the "gradient delay volume"). It delays and disperses the gradient before it reaches the column inlet.

The dwell volume can be split into two theoretical portions: non-mixing volume, $V_{non\text{-}mix}$, and mixing volume, V_{mix}. One can think of non-mixing volume as an open tube (Fig S-1a), where a significant volume of eluent resides, but does not mix as it moves through it (of course, eluent under laminar flow conditions actually does mix to a relatively small degree as it moves through an open tube, but for this illustration, we ignore it). Non-mixing volume causes a delay equal to $V_{non\text{-}mix}/F$ between the time a programmed change in the gradient is set to occur and when it actually reaches the column inlet (Fig S-1b).

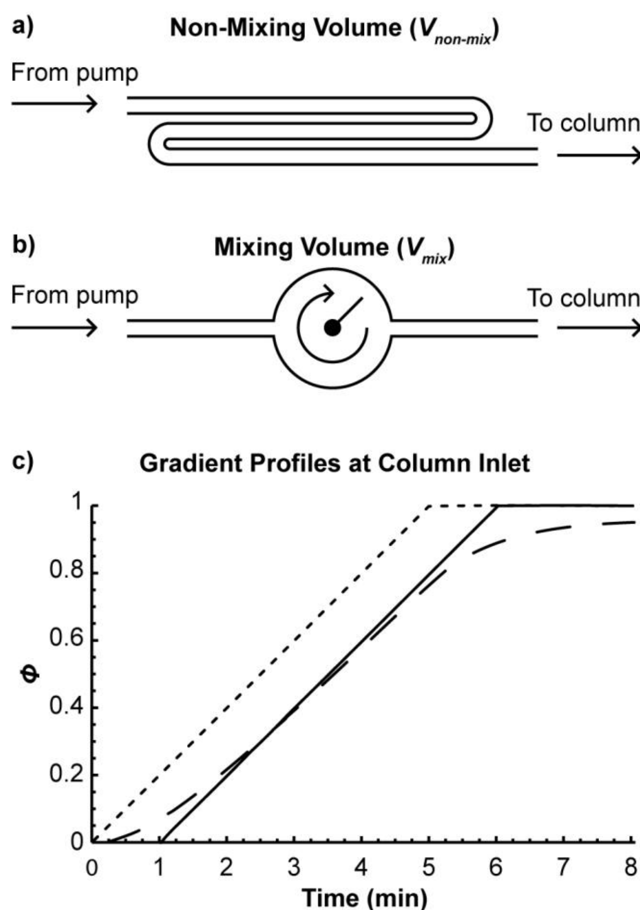

Figure S-1. a) and **b)** represent non-mixing and mixing volume between an ideal pump and the column inlet. The three plots in **c)** show 5 min gradient profiles (ϕ =0 to 1, F = 1 mL/min) as they reach the column inlet with no mixing volume (dotted), with 1 mL of non-mixing volume (solid), and with 1 mL of mixing volume (dashed).

On the other hand, one can think of *mixing* volume as a thoroughly mixed reservoir (Fig S-1b) of volume V_{mix}. To understand its effect on the gradient profile, consider a gradient in which the fraction of solvent B increases linearly over the course of the experiment. As eluent with a

higher amount of solvent B enters the mixing volume, it mixes with eluent of a lower solvent B fraction left over in the mixer. This causes the gradient profile reaching the column to not only be delayed, but also *dispersed* (Fig S-1c).

We approximate the gradient profile at the column inlet using an approach similar to that described by Quarry and co-workers.[23] First, consider a mixing chamber of a certain volume, V_{mix}, placed between an ideal pump producing an ideal gradient profile, $\phi_{ideal}(t)$, and the column inlet. The composition of the eluent in the mixing chamber starts out at a certain initial composition, $\phi_{\mathrm{mix,i}}$. Then, in each of a series of very small, successive time steps, δt, a certain amount of eluent, $F\delta t$, of composition $\phi_{\mathrm{ideal}}(t - \delta t)$, gets pumped into the mixing volume and the same amount of eluent, of composition $\phi_{\mathrm{mix}}(t - \delta t)$, gets pushed out of the mixing volume and into the column inlet. At the end of each time step, the eluent in V_{mix} is thoroughly mixed so that $\phi_{mix}(t - \delta t)$ becomes $\phi_{mix}(t)$. That change in ϕ_{mix} can be determined by adding the volume of organic modifier that *entered* the mixing chamber, $F\delta t\, \phi_{ideal}(t-\delta t)$, and subtracting the volume of organic modifier that *left* the mixing chamber, $F\delta t\, \phi_{mix}(t-\delta t)$:

$$\phi_{mix}(t) - \phi_{mix}(t - \delta t) = \frac{\left(F\delta t \phi_{ideal}(t - \delta t)\right) - \left(F\delta t \phi_{mix}(t - \delta t)\right)}{V_{mix}} \tag{12}$$

$\phi_{mix}(t)$ can then be calculated by the following summation:

$$\phi_{mix}(t) = \phi_{mix,i} + \sum_{i=1}^{n} \left(\phi_{ideal}\left((i-1)\delta t\right) - \phi_{mix}\left((i-1)\delta t\right)\right)\left(\frac{F\delta t}{V_{mix}}\right) \tag{13}$$

where n is equal to $t/\delta t$ rounded to the nearest integer. When a significant non-mixing volume, $V_{non\text{-}mix}$, is also present, the ideal gradient profile is delayed by $V_{non\text{-}mix}/F$ and equation 13 becomes:

$$\phi_{mix}(t) = \phi_{mix,i} + \sum_{i=1}^{n} \left(\phi_{ideal}\left((i-1)\delta t - \frac{V_{non-mix}}{F}\right) - \phi_{mix}\left((i-1)\delta t\right)\right)\left(\frac{F\delta t}{V_{mix}}\right) \tag{14}$$

and all values $\phi_{ideal}(t < 0)$ are substituted with $\phi_{ideal}(0)$.

Gradient retention times

Gradient retention times may be calculated by the fundamental equation of gradient elution:[24]

$$\int_{0}^{t_R - t_0} \frac{1}{t_0} \frac{\mathrm{d}t}{k_{\phi,T}} = 1 \tag{15}$$

where $k_{\phi,T}$ is the retention factor of a compound at the ϕ and T influencing it in a particular time slice. Eq. 15 calculates gradient retention times by considering gradient elution as a series of very small isocratic steps that together closely approximate the true shape of the gradient.

Since analytical solutions to Eq. 15 are not possible under all gradient profiles, we calculate gradient retention times by numerical integration, using the following summation equation which is analogous to Eq. 15:[25]

$$\sum_{i=1}^{n} \frac{1}{t_0} \frac{\delta t}{k_{\phi,T}} \geq 1 \tag{16}$$

where $k_{\phi,T}$ in each summation step is calculated the same way as for isocratic elution (see above). If n is the smallest integer that makes the inequality of Eq. 16 true, t_R can be calculated from:

$$t_R = n\delta t + t_0 \quad (17)$$

Gradient peak width

In gradient elution, we calculate the peak width from:[26]

$$\sigma_t = \sqrt{\left(t_0 \frac{(1+k_e)}{\sqrt{N}}\right)^2 + \tau^2} \quad (18)$$

where k_e is the value of k just as a compound elutes from the column. As in the isocratic calculations of peak width, the first term accounts for the effect of column efficiency on peak broadening and the second term accounts for additional band broadening from the detector time constant. However, we did not include a term for the effect of injection volume on band broadening as we did for isocratic elution. For compounds that are well-retained in the initial solvent composition, the term would be insignificant, anyway, because they would focus into a narrow band at the head of the column. On the other hand, poorly-retained compounds would still experience significant band broadening. In the future, we plan to add a means to account for such band broadening.

We also do not account for the "band compression" effect in gradient elution that should theoretically narrow peaks further.[27] Experimentally, that effect has been shown to be quite small.

Final chromatogram construction

The final chromatogram is constructed in three steps. First, individual chromatograms (Gaussian peaks) are calculated for each compound:

$$C_i(t) = \frac{W_i}{2\sqrt{\pi}\sigma_{t,i}F} \exp\left[-\left(\frac{t - t_{R,i}}{2\sigma_{t,i}}\right)^2\right] \quad (19)$$

where $C_i(t)$ is the molarity of compound i at the column outlet as a function of time and W_i is the number of moles. Second, each of n chromatograms is summed together into a total chromatogram, $C_T(t)$: $C_T(t) = \sum_{i=1}^{n} C_i(t)$. Lastly, random Gaussian noise (where the standard deviation of the random noise equals the "Noise" input divided by 10^9) is added to each time point.

References

(1) Horvath, C.; Lin, H.-J. Journal of Chromatography A. **1976**, 126, 401–420.

(2) Hong, C.; Horváth, C. Anal. Methods Instrum. **1993**, 1, 213–222.

(3) Billen, J.; Broeckhoven, K.; Liekens, A.; Choikhet, K.; Rozing, G.; Desmet, G. J. Chromatogr. A. **2008**, 1210, 30–44.

(4) Bird, R. B.; Stewart, W. E.; Lightfoot, E. N. Transport Phenomena, Revised 2nd Edition, 2nd ed.; John Wiley & Sons, Inc.; 2006.

(5) Scheidegger, A. E. The physics of flow through porous media, 3rd ed.; University of Toronto Press; 1974.

(6) Thompson, J. D.; Carr, P. W. Anal. Chem. **2002**, 74, 4150–4159.

(7) Wilke, C. R.; Chang, P. AIChE Journal. **1955**, 1, 264–270.

(8) Giddings, J. C. Dynamics of Chromatography: Principles and Theory, CRC Press; 2002.

(9) van Deemter, J. J.; Zuiderweg, F. J.; Klinkenberg, A. Chem. Eng. Sci. **1956**, 5, 271–289.

(10) Zhu, P. L.; Snyder, L. R.; Dolan, J. W.; Djordjevic, N. M.; Hill, D. W.; Sander, L. C.; Waeghe, T. J. J. Chromatogr. A. **1996**, 756, 21–39.

(11) Sternberg, J. C. Adv. Chromatogr. 2, 205–270.

(12) Quarry, M. A.; Grob, R. L.; Snyder, L. R. J. Chromatogr. A. **1984**, 285, 1–18.

(13) Snyder, L. R.; Dolan, J. W. High-Performance Gradient Elution, John Wiley & Sons, Inc.: Hoboken, New Jersey; 2006.

(14) Nikitas, P.; Pappa-Louisi, A. Anal. Chem. **2005**, 77, 5670–5677.

(15) Snyder, L. R.; Dolan, J. W. In Advances in Chromatography; Marcel Dekker, 1998; pp. 115–187.

(16) Neue, U. D.; Marchand, D. H.; Snyder, L. R. J. Chromatogr. A. **2006**, 1111, 32–39.