

## Mixing in Microfluidic Gradient Chromatography Systems

In gradient chromatography, it is important to mix the mobile phases fully prior to reaching the injector/column. Mixing can be accomplished by simply adding the components together and providing a sufficiently long section of tubing to allow diffusion to take its course. The length of tubing required to mix to better than 99.999% is approximately equal to 2.5 cm times the flow rate in units of  $\mu\text{l}/\text{min}$ .

For a pumping system running at 1 ml/min, this requires a tube length of about 25 meters, which is clearly unacceptable. Using 20 mil (0.5 mm) internal diameter tubing this gives about 33 psi pressure drop and represents a delay volume of 5 ml, or a delay time of 5 minutes. To avoid this large delay volume and delay time, traditional pumping systems use a so-called 'mixer' followed by a short section of tubing. This mixer serves to repeatedly fold the liquids and the following short section of tubing allows the blended liquids to mix by diffusion.

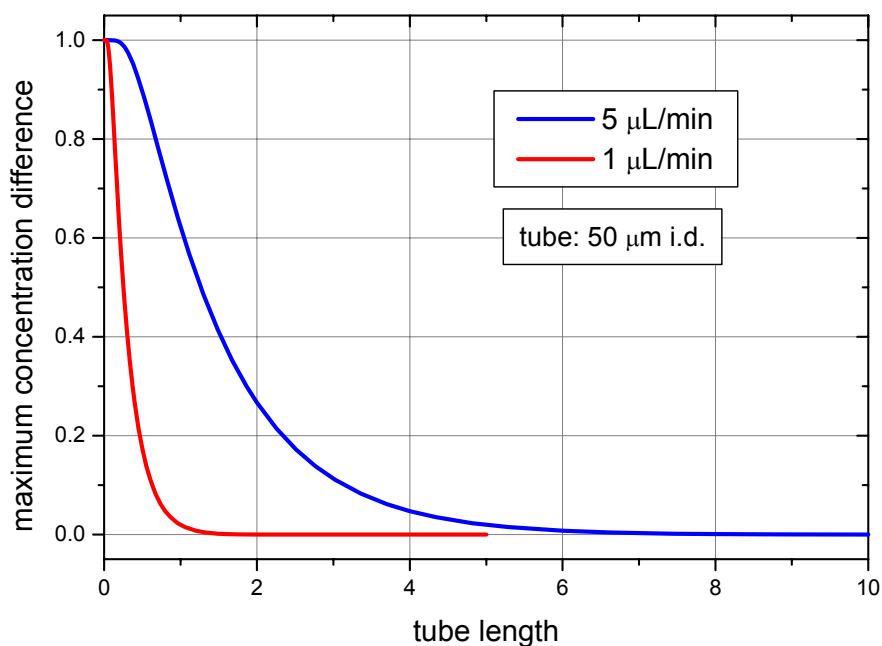
In a microfluidic HPLC system the flow rates are much lower and thus the tubing length can be greatly reduced. For example, running at 4  $\mu\text{l}/\text{min}$  requires a tube length of about 10 cm. Using 50 micron i.d. capillary this gives about 6 psi pressure drop and represents a delay volume of 200 nl or a delay time of 3 seconds. Thus in a microfluidic HPLC system, a simple short length of capillary provides complete mixing and negligible delay volume.

There are many imaging results in the literature of apparently slow mixing in microfluidic systems. However, the images only show the concentration field near the point where the streams join, thus accounting for the lack of thorough mixing. For a 50-micron i.d. capillary, a 10 cm length is 2000 diameters long. If image data at 2000 diameters downstream were shown, mixing would be complete.

The scaling rule discussed above- that the length of tubing required to mix to better than 99.999% is about equal to 2.5 cm times the flow rate in  $\mu\text{l}/\text{min}$ - was established by numerically solving the convection-advection equation for steady creeping flow of a conserved scalar through a tube. The entrance condition had one-half the tube labeled with the scalar with the other half of the tube unlabeled. The diffusion coefficient was taken as that for ACN (acetonitrile) into water (about  $1.25\text{E}-9 \text{ m}^2/\text{s}$ ). Figure 1 shows the results of the simulation plotting the maximum concentration difference (the maximum deviation from the average) along the axis of the tube. At 4  $\mu\text{l}/\text{min}$ , the plot shows the concentration difference dropping below 1% at about 6 cm along the length and below 0.001% at 10 cm along the length. For the case of 1  $\mu\text{l}/\text{min}$  flow rates (and lower), full mixing is achieved significantly earlier- in less than 2 cm. The scaling rule given above was found by running the simulation for a range of flow rates and tube diameters.

The simulation assumes perfect entry conditions and ignores differences in viscosity between the two liquids. Non-perfect scalar entry conditions (i.e. some amount of pre-stirring) are inevitable in any real system and this will produce more rapid mixing. Differences in viscosity between the two liquids produce a flow that is inherently unstable and this also will enhance the mixing. The simulation actually considers a worst possible case.

In conclusion, microfluidic HPLC systems can achieve complete gradient mixing in a short length of capillary with minimal delay volume. The benefit of this rapid diffusional mixing is that there is no need for a separate mixer, further reducing the system's internal delay volume. This results in very short mixing delay times and rapid system response. These features allow capabilities such as very fast (<1 minute) gradients in high throughput assays, and peak parking to increase MS/MS acquisition time in proteomics research.



**Figure 1-** Concentration difference as a function of distance from the point where both mobile phases come in contact. Both cases are for water and acetonitrile in a 50 µm internal diameter capillary. At 1 µl/min and below, complete mixing is achieved in less than 2 cm.