Simulation of Chromatographic Band Transport

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Abstract:
Central to all chemical separation techniques, including high-performance liquid chromatography (HPLC) is the need to minimize band spreading or band broadening. We use Comsol to analyze two classes of problems in HPLC: 1) extra band broadening in a planar microfluidic device, 2) thermal effects in high-pressure separations with sub-2 um particles.

Keywords: HPLC, chemical separation, microfluidics.

1. Introduction

HPLC is a standard method in the pharmaceutical industry for the separation and identification of chemical compounds, used in basic research as well as in manufacturing and QC. Separation typically occurs in a column packed with micron-sized particles coated with a functional layer, referred to as the stationary phase. In the most common approach, reverse-phase chromatography, this stationary phase is non-polar and a polar solvent, referred to as mobile phase, such as water, methanol, acetonitrile, or a mixture of these, percolates through the packed bed formed by the particles. A small amount of the mixture of chemical species to be analyzed, referred to as the solute or analyte, is injected at the head of the column. Different species in the mixture will bind with different strength to the stationary phase. Non-polar analytes will bind more strongly than polar analytes and therefore migrate more slowly through the column. The different analytes elute the column at different times and are detected using for example UV absorbance. The result is a chromatogram, a plot of UV absorbance vs time showing the different analytes as distinct peaks.

Central to chromatography is the process of convection and diffusion of a chemical species in a packed bed and in fluid conduits (tubing, mixers, heat exchangers, detectors). Fundamentally, the eluent peaks should be as sharp as possible. Although diffusion is unavoidable, HPLC instrument designers seek to minimize extra dispersion associated with factors such as large dead volumes, poorly packed columns, thermal mismatches, etc. We describe in this paper our ongoing effort to use Comsol to study this fundamental problem of band transport in a chromatographic column, both in the case of a standard 2.1 mm column and in the case of a microfluidic HPLC column.

2. Theory and numerical method

Three physical problems are coupled using Comsol Multiphysics: flow of mobile phase in open tubes and packed beds, transport of solute, and thermal effects due to viscous friction in the packed bed.

2.1 Fluid flow (mobile phase)

A chromatographic column consists of a packed bed of micron-sized particles held at both ends by frits and connected at both ends to tubes. Solvent flows through the tubes and percolates through the packed bed and frits, which can both be treated as porous media. The equation describing the solvent motion in the tubes is the Navier-Stokes equation. Due to the characteristic dimensions, the flow is expected to be laminar everywhere and no turbulence model was included.

Flow in the packed bed and frits is modeled using Darcy's law:

$$\nabla.\left(\rho\frac{\kappa}{\mu}\nabla P\right)=0 \qquad \textbf{Eq 1}$$

where ρ and μ are the density and viscosity and $\kappa=\frac{d_p^2\,\varepsilon^3}{180\left(1-\varepsilon\right)^2}$ is the permeability of the bed, d_p being the average particle diameter and ε the interparticle porosity. In the packed bed, typical values are d_p=1.7 or 3.0 um and ε=0.40. Note that the particles are themselves porous and contain an intraparticle porosity that is typically 0.3 so that the total porosity is ε_t =0.7. In the frits, which are typically made out of larger size particles sintered together, d_p and ε were typically taken to yield a significantly larger permeability than in the bed. Once the pressure is

determined, the solver calculates the superficial velocity

$$\vec{U}_s = -\frac{\kappa}{\mu}\nabla P, \qquad \textbf{Eq 2}$$

which is the flux of fluid across a unit area but not the actual velocity of the fluid between the particles. The linear velocity is then defined from the superficial velocity as the speed at which an unretained compound is transported through the column:

$$\vec{u} = \frac{\vec{U}_s}{\varepsilon_t}. \qquad \textbf{Eq 3}$$

Originally, two different Comsol application modes, "incompressible Navier-Stokes" and "Darcy's law" were used together and appropriate boundary conditions were defined at the interface between the porous media and the open tubes. Later on, the development of an incompressible Navier-Stokes application mode that integrates the Brinkman equation as an option for porous media allowed us to use a single application mode to simulate the entire fluid domain without having to deal with boundary conditions between fluid subdomains.

2.2 Analyte transport

The solute or analyte is transported by convection-diffusion through the column. In the open fluid subdomains, the governing equation is simply:

$$\frac{\partial C}{\partial t} + \vec{u}.\nabla C = D_{mol}\nabla^2 C, \qquad \textbf{Eq 4}$$

where D_{mol} is the molecular diffusion coefficient of the solute in the solvent. In the packed bed, a convection-diffusion equation is likewise solved:

$$\frac{\partial C}{\partial t} + \frac{\vec{u}}{1+k'}.\nabla C = D_{eff}\nabla^2 C, \qquad \textbf{Eq 5}$$

but the transport process is more complex. First, the advection velocity, which is based on the linear velocity of the solute through the porous medium, also takes into account the fact that the solute interacts with the stationary phase attached to the particles through the retention factor k'. For a compound that is not retained, k'=0. For a well-retained compound, we took k'=8.0 as a typical value.

More importantly, the diffusion process of the analyte through the space between particles and in the network of pores inside the particles, is very complex and can only be accounted for in a lumped model sense through an effective diffusion coefficient D_{eff}. Following [1], we define for an unretained compound this effective diffusion coefficient by

$$D_{eff} = \frac{H(u)\,u}{2}, \qquad \textbf{Eq 6}$$

where u is the absolute value of the linear velocity and H is the plate height, modeled here by a classical Van Deemter equation [2]:

$$H(u) = 1.5\,d_p + \frac{D_{mol}}{u} + \frac{d_p^2}{6\,D_{mol}}u. \qquad \textbf{Eq 7}$$

In [1], the effective diffusion coefficient is meant to be a mean value that applies either to an entire column or to a slice of a column. Here, we extend the definition to each point of the packed bed. At each point, a local plate height is calculated from the norm of the local linear velocity. The expressions for retained compounds are modified but follow the same idea.

The boundary conditions are defined as follows. At the inlet, a Gaussian peak is input. At the outlet, the BC is convective flux ($\nabla C = 0$) and the average concentration is measured from the simulation results. Typical inlet and outlet peaks are shown in Fig. 1. The band broadening due to the column is defined by the increase of the variance between the inlet and outlet peaks. This is the key insight that we seek with Comsol.

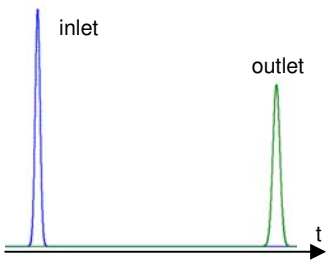

Figure 1 Typical chromatogram with Gaussian peak at inlet and outlet

2.3 Thermal effects

Normally fluid flows rarely induce shear stresses large enough to create appreciable heating through viscous friction. This is however precisely what happens in beds packed with particles smaller than 2 um when the flow rate is relatively large. Substantial self-heating can then occur [3]. This is of particular interest in large

diameter columns, such as the 2.1 mm columns that are standard in analytical chromatography.

The heat transfer equation is

$$\rho C_p \vec{U}_s . \nabla T = \nabla . (k \nabla T) + Q \qquad \textbf{Eq 8}$$

Note that the advection velocity is the superficial velocity $\vec{U}_s$. Frictional forces create a heat source in the bed

$$Q = \vec{U}_s . \nabla P \qquad \textbf{Eq 9}$$

This heat is removed to the ambient atmosphere through convection by the solvent flowing through the column and through conduction through the solvent, packed bed, and stainless steel column walls. The result is an increase in the temperature of the solvent and solute. The increase of the solvent's temperature causes a reduction in the fluid density and viscosity. The increase of the solute's temperature causes an increase of the molecular diffusion coefficient, and hence also of D_{eff} in the bed.

As in the case of the solute transport equation, the thermal conductivity k in the bed is not that of the pure mobile phase. It is a composite value that models heat diffusion through the silica particles, the polymeric bonded phase, the liquid in the intra-particle pores, and the liquid in the interstitial space between particles.

Two extremes cases are of particular interest. In the first case, the column is in a liquid bath at a fixed temperature, which determines the BC at the exterior wall. This is referred to as the isothermal case. In the second case, the column is insulated so that the BC is zero heat flux at the exterior wall. In this case, heat is only removed from the column by the solvent that flows through it. This is referred to as the adiabatic case. In practice, the column is typically placed in a chamber surrounded by air, so that heat is removed by natural convection at the column wall.

2.4 Solution method

Two types of elution modes are used in chromatography. In isocratic mode, the mobile phase is a mixture of water and organic solvent with a fixed composition. In gradient mode, the fraction of organic solvent is ramped up over a period of time, for example, from 10 to 60% over 1 hour. In our simulations so far, for the sake of simplicity, we have only considered isocratic elution, with a mobile phase consisting of 40% water and 60% acetonitrile.

The velocity and temperature fields are steady-state and therefore calculated once only. They are coupled through temperature- and pressure-dependent viscosity and density. The heat transfer equations themselves are non-linear because the heat capacity C_p is itself a function of temperature and to a smaller degree of pressure.

Once the velocity and temperature fields have been obtained, the unsteady solute transport equation is solved for. The molecular diffusion coefficient D_{mol} depends on P and T. Furthermore, the local plate height H(u) (eq. 7) depends on the local velocity u and on D_{mol}, and from that the effective diffusion coefficient in the bed D_{eff} (eq. 6)

2.5 Validation of model

The model for band transport without thermal effect was validated by comparison with one-dimensional analytical solutions in packed bed [1] and in open fluid tubes (Aris-Taylor theory, [4]). Grid refinement studies were performed to ensure that the solution is independent of the finite element mesh.

We expanded significant effort to minimize the amount of numerical diffusion arising from the discretization of the equations over a finite element mesh and from other numerical artifacts. If numerical diffusion is not brought under control, it can easily overwhelm physical diffusion and dramatically skew the band broadening results.

3. Results

3.1 Band broadening in microfluidic HPLC

Waters is developing a microfluidic-based planar HPLC column that is equivalent to a fused silica capillary column of 75 um diameter. A planar device presents a number of geometric features that are different from the ideal geometry of a fused silica capillary (circular cross-section, straight channel) and that have been explored using Comsol simulations. Two are presented here: The presence of inlet and outlet vias perpendicular to the channel and the channel turns necessary to fit a 10 cm long

separation channel within a small rectangular format.

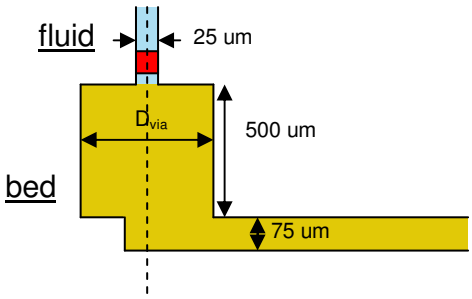

Figure 2: Geometry of inlet via

Fig. 2 shows the geometry of the inlet via that was modeled in Comsol. The separation channel has a square cross-section with width and height 75 um. The via is a 500 um high cylinder of diameter D_{via}. Both the channel and the via are packed with 3.0 um particles. A 25 um ID capillary makes the fluidic connection to the device. In this case, the initial band, marked in red in fig. 2, is placed in the capillary right above the entrance of the via.

Fig. 3 shows the evolution of the band as it enters the via and travels around the 90° bend from the via into the channel.

Several values of via diameter were evaluated: 75, 150, 225 microns. The key insight from the simulation results, shown in fig. 4, is that vias introduce only a small penalty when the via is small. For the capillary column, the plate height is H=7.0 um. A lower value of H means better separation performance. For D_{via}=75 um, H is only 1.5% larger. For D_{via}=150 um, the penalty is 22%. For D_{via}=225 um, the penalty is huge as the plate height is more than 3 times larger than that of the capillary column.

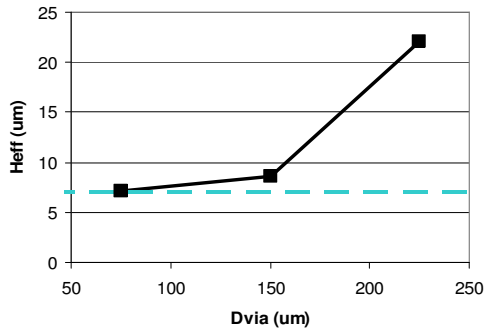

Figure 4: Effective plate height as a function of via diameter compared to a straight column without via

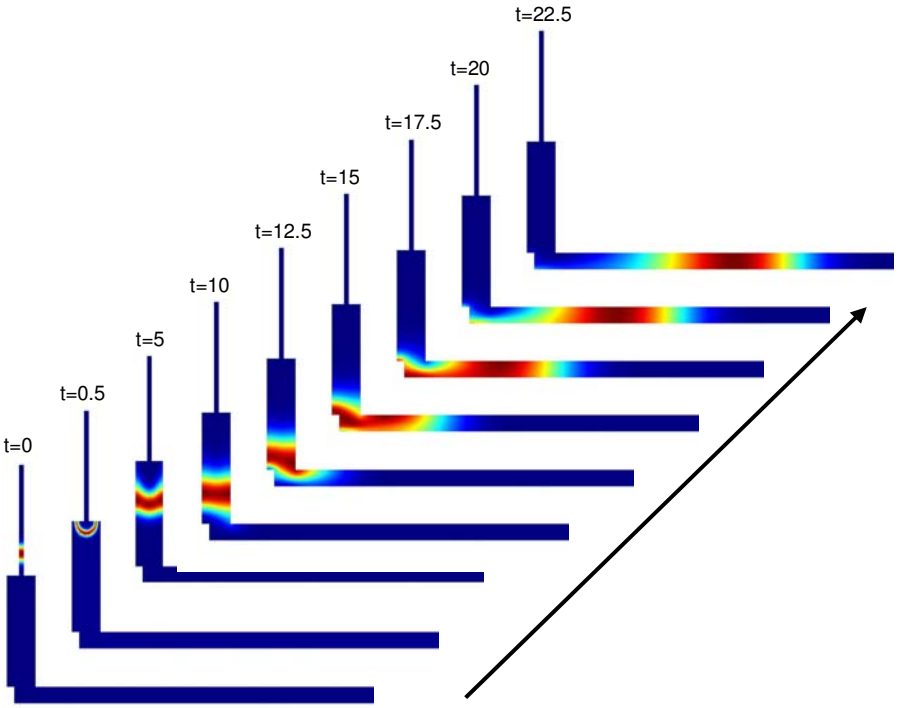

Figure 3: Motion of analyte band through inlet via for a via with diameter D_{via}=150 um

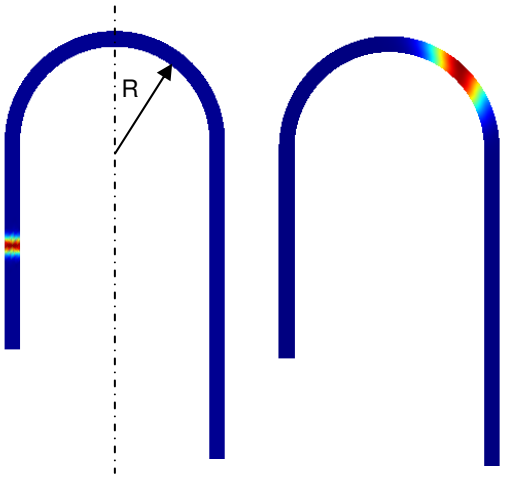

Figure 5: Band motion around a 180° channel turn of radius R=0.5 mm.

The second geometric feature characteristic of separation channels in microchip HPLC device, as in capillary electrophoresis separation, is the presence of channel turns due to the need to fit a long separation channel in a limited footprint. The geometry shown in Fig. 5 was simulated in Comsol with different radii R=0.1, 0.5, and 1.5 mm. The results, in Fig. 6, show that channel turns lead to very small extra band broadening, provided R≥0.5 mm. The simulation results were compared with analytical expressions derived from a study of band spreading in turns with electrokinetic transport [5].

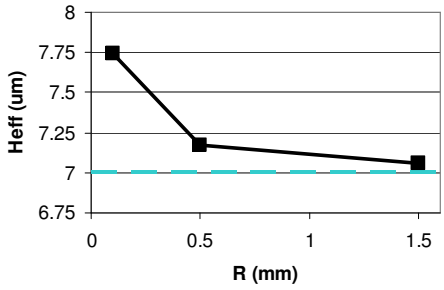

Figure 6: Effective plate height of a 10 cm separation channel with 5 turns a a function of channel radius R, compared to a straight channel.

3.2 Thermal effects in 2.1 mm columns packed with sub 2 um particles

The trend in the HPLC industry is to use smaller and smaller particles to speed up separations and improve separation efficiency.

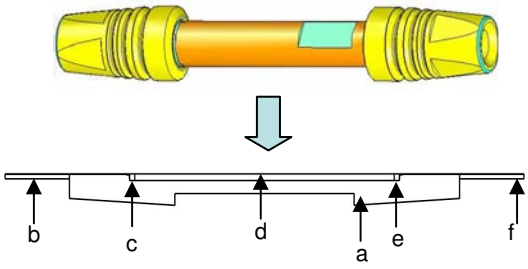

Figure 7: Solid model of the body of a 2.1 mm ID column showing the end-fittings. The axisymmetric model with simplified geometry below consists of a) a stainless steel column body, b) a stainless steel inlet tube with 0.005" ID, c) a 1 mm thick, 2.1 mm diameter inlet frit, d) the 2.1 mm diameter, 5 cm long packed bed, e) a 1 mm thick, 2.1 mm diameter outlet frit, f) a PEEK outlet tube with 0.005" ID.

From 3.5 or 5.0 um particles, which are used commonly, the trend is towards 1.7 um particles and 1.0 or 1.2 um particles are being evaluated for the future.

As the particle size decreases, the mobile phase velocity that results in the optimum separation performance increases. The combination of these two trends causes a large increase in the shear stress, to the point that frictional heating becomes significant.

A standard 2.1 mm diameter column is shown in fig. 7, along with the axisymmetric model used to model it in Comsol. The body of the column and the end fittings are made out of stainless steel. The packed bed is held between two frits. 0.005" ID tubes provide fluid connections at the inlet and outlet.

The heat generated in the packed bed is carried away by conduction through the bed and the column and by convection in the packed bed. In the case where the column is completely insulated (adiabatic boundary condition), the only way for heat to be removed from the column is through the mobile phase. In contrast, in the case where the column exterior wall is at the ambient temperature, 303 K, (isothermal BC), heat can also be removed very efficiently by conduction through the stainless steel column wall. Consequently, the maximum temperature in the column is much larger in the adiabatic case than in the isothermal case. See fig. 8 for an example.

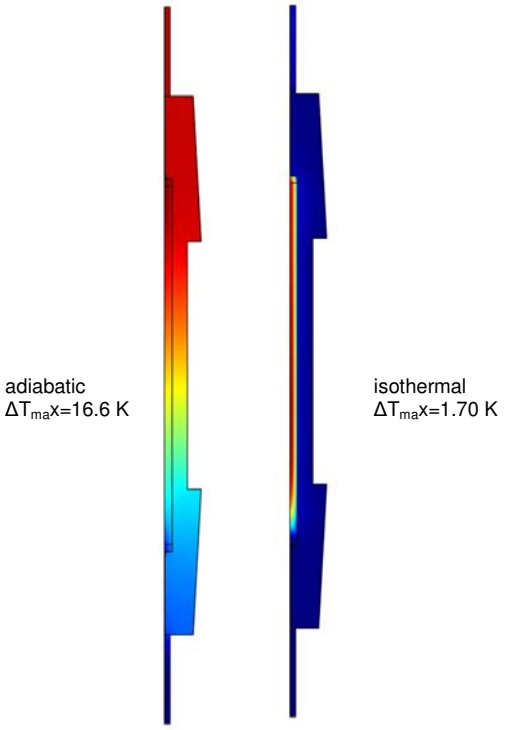

Figure 8: Temperature profile in the column. The pressure drop between inlet and outlet is the same but due to the effect of temperature on viscosity, the flow rates are different. Left: adiabatic BC, Q=0.952 ml/min. Right: isothermal BC, Q=0.780 ml/min.

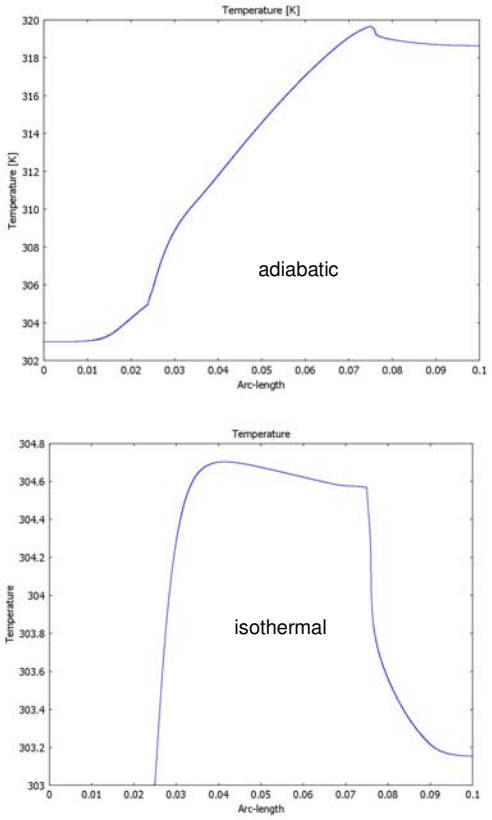

Figure 9: Temperature profile along the column axis.

Fig. 9 shows the temperature profile along the column axis for the two cases of fig. 8. In the adiabatic case, the temperature increases gradually along the column. If the viscosity were independent of the temperature, heat generation would create a linear rise in the temperature along the packed bed, but the stainless steel redistributes heat generated downstream towards the front of the column.

In the isothermal case, heat is removed by conduction through the stainless steel wall as soon as it is generated. The temperature reaches its maximum as soon as the mobile phase enters the packed bed. Downstream, an equilibrium establishes itself between heat generation and radial heat conduction, so that the temperature is approximately constant along the axis.

The results of band transport simulations are summarized in fig. 10. The cases with heat generation are compared with the case, physically unrealizable, where no heat is generated. The plate count is defined as $N = L / H$, L being the column length.

A higher plate count means lower band broadening and better performance. We see that the curves for the adiabatic case and for the no heating case overlap approximately and that the isothermal BC leads to increasingly poor performance as the flow rate increases. In spite of a much smaller temperature rise, the plate count is much worse in the isothermal case. What explains this?

The explanation lies in the radial temperature profiles, not shown here. In the adiabatic case, the temperature is radially almost uniform. While the viscosity and diffusion coefficient may vary by more than 50% between the beginning and end of the column, they are about the same through a radial slice of the column, so that the velocity of the mobile phase and the diffusion behavior of the solute are about the same. When a band of analyte is introduced into the column, it is not distorted by the velocity field.

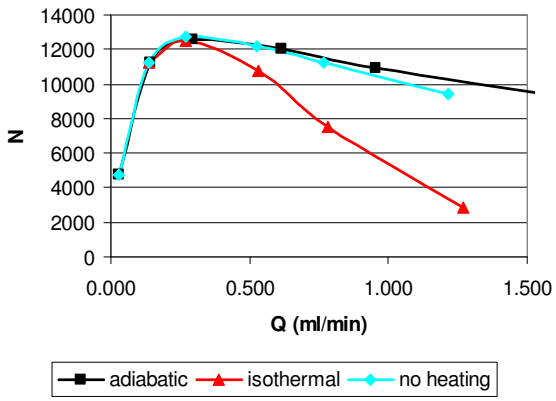

Figure 10: Plate count as a function of flow rate

In contrast, the radial temperature profile in the isothermal case is parabolic, with a maximum at the axis. As a result, the viscosity of the mobile phase is lower in the center of the column than close to the wall and the analyte travels faster in the center of the column. ΔT_{max} is small, at most a few degrees, and the variation of the viscosity is at most a few percents, but this is sufficient to cause significant band distortion and extra band broadening.

4. Conclusions

Modelling of band transport, or band broadening phenomena, can provide tremendous insight into problems of chromatography that are difficult or impossible to measure experimentally, because of very small dimensions, opaque materials, or environments experiencing very high pressures. This was illustrated with a few examples here. Other problems of interest that were analyzed by simulations are band spreading in UV absorbance detectors, construction of frits, performance of binary solvent mixers. The critical issue in these simulations of convection-diffusion phenomena is to minimize numerical diffusion. Comsol was able to cope with most of the problems we looked at. The only numerical convergence difficulties that we encountered were when we looked at problems with high fluid velocities.

5. References

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