Simulation Addresses Band-Broadening in HPLC Systems

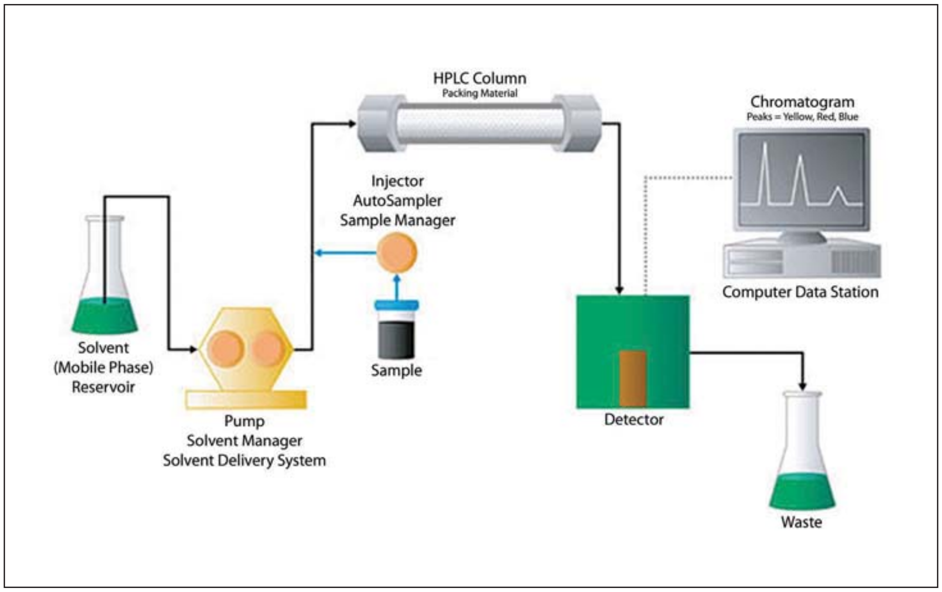
COMSOL Multiphysics helps Waters Corp. cut costs and save time in their HPLC business.

BY CATHLEEN LAMBERTSON

In the simplest terms, High Performance Liquid Chromatography (HPLC) is a separation technology — a process used to separate different chemical species that are in a mixture. And while HPLC is one of many separation technologies available, it is the most prevalent because of its versatility. These systems are most commonly used in the pharmaceutical and biotech industries, in functions as diverse as R&D, manufacturing, and quality control. They are also increasingly finding applications in food, water, and environmental monitoring. For instance, during the tragedy that occurred in China in 2008 in which baby formula was found to be contaminated with melamine, analysis of the contaminated formula was done with Waters HPLC systems.

Founded in 1958 and headquartered in Milford, MA, Waters Corp. designs and manufactures complementary analytical technologies such as liquid chromatography, mass spectrometry, rheometry, and microcalorimetry, which account for approximately $5 billion of the estimated $20-$25 billion worldwide analytical instrumentation market. Bernard Bunner, a principal engineer at Waters, said, "HPLC is the original business of Waters and still represents the largest part of the company's activity and revenues. Waters' systems account for about 20% of the worldwide HPLC market."

An Acquity UltraPerformance Liquid Chromatography (UPLC) instrument.



Schematic of an HPLC or UPLC system operating in isocratic mode. A high-pressure pump delivers a constant stream of mobile phase, typically acetonitrile in water, to the HPLC column. A plug of the sample to be analyzed is injected into this stream. At the outlet of the column, the separated components of the sample are detected and appear as peaks on the chromatogram.

The HPLC System

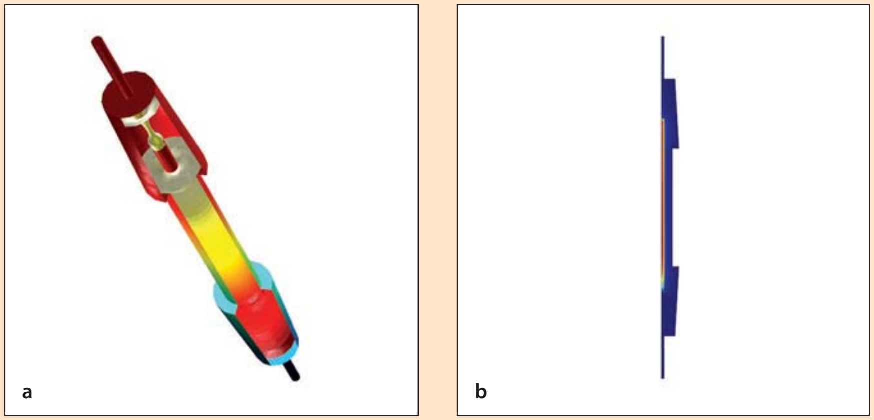
According to Bunner, an HPLC system includes what can be considered a really big desktop computer (instrument), as well as a chromatographic column — a stainless steel tube at the core of the instrument. The column typically measures ~6 mm OD and 2.1 mm ID. This column is packed with particles that measure a few microns in diameter. The solution containing the chemicals to be analyzed flows through the column. At the inlet, there is a mixture of chemical species and at the outlet, there are separated chemical species typically identified with an ultraviolet (UV) absorbance detector.

The chemical analysis of the sample results in a chromatogram — a plot of UV absorbance versus time showing the different chemical species as distinct peaks. While ideally the peaks should be as sharp as possible, diffusion is unavoidable. Therefore, HPLC instrument designers seek to lessen extra dispersion (band broadening) associated with factors such as large dead volumes, poorly packed columns, thermal mismatches, and the like. In order to minimize this problem of band broadening, Waters enlisted the help of COMSOL Multiphysics software.

Why COMSOL?

Currently, Bunner uses COMSOL for his work in the Instrument Research Group, which focuses on the development of new instruments and new products based on new technologies. Several people in Waters' Chemistry Operations — responsible for the development of the columns — use simulation too. "COMSOL is the only simulation software at Waters that has the capability of simulating complex fluid and thermal problems," said Bunner.

Waters is using COMSOL to study band transport in a chromatographic column, both in the case of a standard 2.1-mm



Temperature map in the 2.1-micron column, calculated using axisymmetric geometries, for two different boundary conditions: a) Adiabiatic BC: the column is fully insulated and heat loss only occurs through fluid that flows out of the column. The maximum temperature rise is 16.6°C; and b) Isothermal BC: The column is immersed in a liquid bath maintained at constant temperature. Heat is mainly carried away from the packed bed thorugh radial conduction through the column. The maximum temperature rise is 1.7°C.

column and in the case of a microfluidic HPLC column. Three physical problems encountered during band transport in the 2.1-mm column were addressed using COMSOL: flow of mobile phase in open tubes and packed beds, transport of solute, and thermal effects due to viscous friction in the packed bed. "In the 2.1-mm column packed with sub-2-micron particles, the geometry is not new; it's the same one that's been used in the industry for decades. Here, the physical problem becomes very complex because it adds thermal effects to what is already a complex problem," said Bunner.

"COMSOL® saved several months of development time by avoiding the need for more experiments."

Fluid Flow in Porous Media

In an HPLC system, the column consists of a packed bed of micron-sized particles held at both ends by frits and connected at both ends to tubes. The chemical species flow through the tubes and filter through the packed bed and frits. To solve for fluid flow, both Darcy's Law and Navier-Stokes equation are used. The equation describing the solvent motion in the tubes is the Navier-Stokes equation, while flow in the packed bed and frits is modeled using Darcy's law.

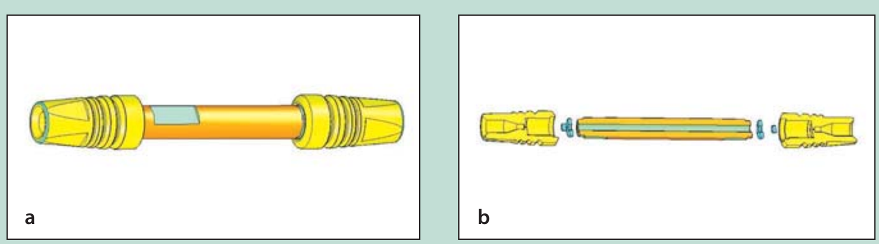
Recently, COMSOL developed an incompressible Navier-Stokes application mode that integrates the Brinkman equation as an option for porous media. Bunner stated, "Brinkman's extension combines in a single application Darcy's Law and Navier-Stokes. It is a convenience that really simplified our lives."

Equation Based Modeling

In order to evaluate band broadening, an equation of mass transport has to be solved. The equation of mass transport is used in the open fluid parts of the model, with the diffusion coefficient being the molecular diffusion coefficient of the analyte as it travels through the columns. "In a porous medium, however, diffusion is more complex and due to a number of factors having to do with the nature of the packed bed we had to resort to an ad hoc model where the diffusion coefficient was calculated using an equation," Bunner stated. "One of the nicest features of COMSOL is how easy it is to use to define your own models and tailor it to one specific problem. We are still using the standard application mode of COMSOL and we use the mass transport equation. We are just writing equations for some terms in that equation," he added.

Small Particles, High Heating

In beds packed with particles smaller than 2 um (1.7-um particles are the current state of the art) when the flow rate is relatively large, fluid flows induce shear stresses large enough to create appreciable heating through viscous friction. According to Bunner, this heating can lead to substantial differences of temperatures throughout the column, which implies that physical properties such as the viscosity of the mobile phase and the diffusion coefficient of the analytes can noticeably change. In certain circumstances, this can result in extra broadening of chromatographic peaks. "This modeling effort is especially critical because we are considering going to even smaller particles, 1.2 um. Smaller particles imply even higher pressures and higher heating. Getting a handle on



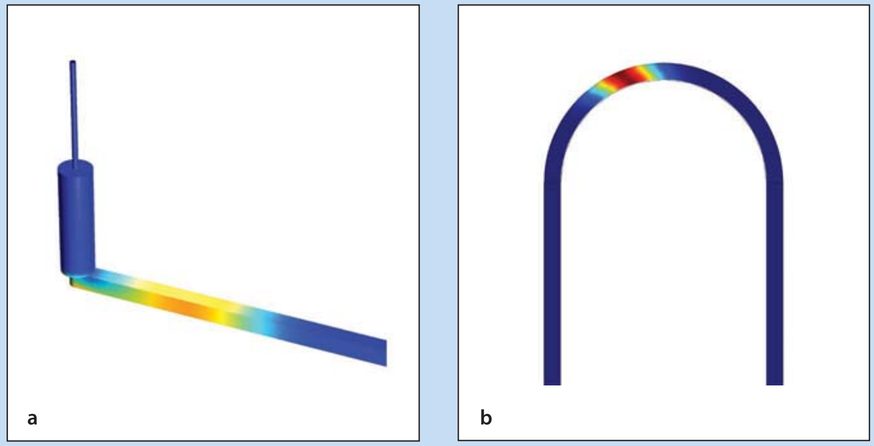
The model in assembled (a) and exploded (b) views of a 2.1-mm column. The central part contains the packing material; for example, 1.7-micron particles. The packed bed is held in place at both ends by frits and fittings for connecting tubes added at both ends.

thermal effects is even more important and COMSOL is part of this effort to see whether or not we should go forward," said Bunner.

Microfluidic HPLC

As for the microfluidic HPLC column, it features a two-dimensional chip device where fluid enters perpendicular to the chip then travels in a separation column (in the plane of the chip), and then travels back out of the chip. This geometric feature is different in the microfluidic chip format than in previous tubular formats based on stainless steel or fused silica capillaries. In addition, since a standard separation channel length is 10 cm, but a chip is typically less than 5 cm long, the separation channel cannot be straight, but must have bends or turns to fit into the chip. The questions that immediately arise are: What do these vias and 90-degree turns do to peak width, and how much extra band broadening do they create?

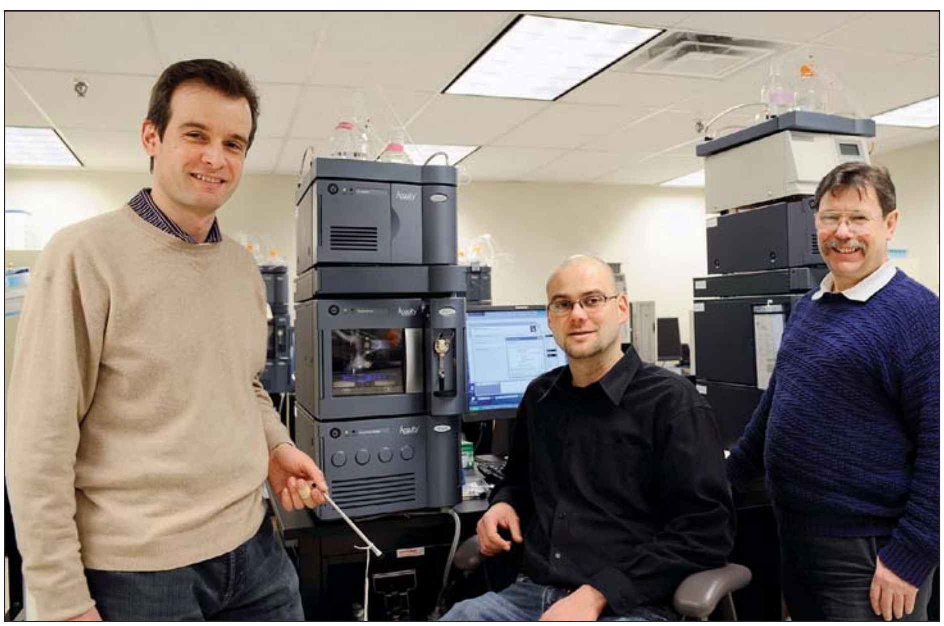
"It is difficult to evaluate the effect of each feature independently from the other since they are all part of the chip. We ran experiments with a number of different parameters (length and diameter of the via, shape of the via, bend radius of the turns), but gained most insight and understanding by using COMSOL simulations where all effects could be evaluated independently," stated Bunner. "COMSOL saved several months of development time by avoiding the need for more experiments, and gave us confidence that the trends that we saw in the experiments that we conducted were correct. We gained confidence that what we are seeing experimentally makes sense and reduced the number of designs/experiments that we had to try. Also, for these very small dimensions, it is very difficult to measure and visualize what is happening. Simulation is really the only option."



Key geometric features of microfluidic devices: a) Inlet via – A 25-micron ID fused silica capillary delivers the sample plug to the 150-micron ID inlet via, which connects perpendicularly to a 10-cm-long seoparation channel with cross-section 75 x 75 microns; and b) Channel turn with 0.5-mm turn radius. Both pictures show the distortion of the band of analyte due to the via and turn.

The Benefits

Waters has been using COMSOL now for just over four years. "People who work in R&D have seen a lot of benefits from doing simulations — from understanding fundamentally what happens to guide design, prototyping, and development. It's really about fewer prototypes; fewer design variations, which translates to shorter development time; and reduced R&D and manufacturing costs," Bunner said. "It also results in better-informed decisions. Instead of just doing one test and then having 20 people in the meeting and using very crude, preliminary and rudimentary data, you are able to study many different aspects of the problem," he said. "[In addition] you can do thought experiments, like 'what if we vary the material we are using? How would a different material affect the thermal problem? What if you used aluminum instead of stainless steel?' Now, if you are trying to evaluate the problem using experiments, you first have to find a vendor who makes this in aluminum. This doesn't exist. So you're talking about setting up a one-year program to evaluate this one experiment. If you do this in a simulation, you're done in less than a day." ■



Waters HPLC simulation team (left to right) Bernard Bunner, Markus Wanninger, and Uwe Neue.

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