UPLC: New Boundaries for the Chromatography Laboratory

Beyond HPLC

Over the past 30 years high performance liquid chromatography (HPLC) has become one of the most widely used technologies in laboratories worldwide. This is due to the fact that HPLC is a dynamic and versatile technique. It is dynamic in its scope of application from capillary scale to preparative scale and it is versatile in the range of detection techniques that can be associated with it, providing analytical capabilities over a broad spectrum of compounds. Fundamentally, HPLC has evolved during this period in three primary areas: chemistry, detectors, and system/data management. Together, advances in these aspects of chromatography have expanded the technology to widespread acceptance. Even so, there is opportunity for further advances in the technique. What does the future hold for HPLC? What are the continued developments that can extend and expand the utility of this well-established technique?

Defining UPLC™

New frontiers in liquid chromatography are under investigation. Both Dr. J. Jorgensen at the University of North Carolina[1] and Dr. M. Lee at the Brigham Young University[2] have recently published studies that provide insight into future capabilities for the chromatography laboratory. Their research paints a new landscape for separation sciences. By utilizing much smaller particle sizes, a new end point for the separation process can be realized. The underlying principles of this approach are exemplified in the van Deemter equation. The van Deemter equation is an empirical formula that describes the relationship between linear velocity and plate height (column efficiency).

It considers particle size as one of the variables and therefore, it can be used to characterize theoretical performance across various particle sizes (Figure 1). From the information in Figure 1 it can be realized that once moving below 2 µm in particle size, a realm of chromatography is entered where not only are higher efficiencies gained, but also these efficiencies no longer diminish with flow rate.

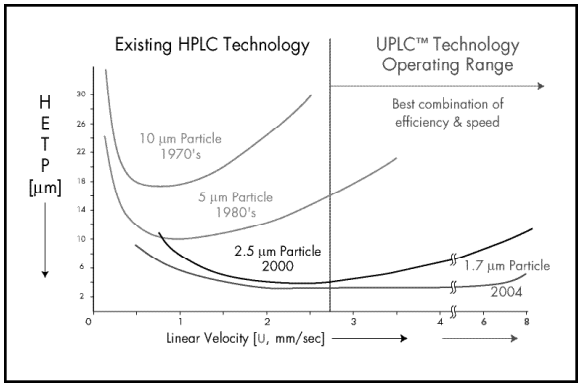

Figure 1: These van Deemter plots illustrate the evolution to smaller particle sizes over the last three decades. Lower HETP means higher efficiency. But even the efficiency of 2.5 µm particles begins to decrease at higher flow rates, with a corresponding increase in system backpressure. With UPLC™ technology and particle sizes below 2 µm, the efficiency is maintained even at higher linear velocities. It is now possible to take full advantage of chromatographic principles to run separations, using shorter columns and/or higher flow rates for increased speed, with superior resolution and sensitivity.

Waters

This presents a situation where both the peak capacity and speed of a separation can be pushed to new limits. Peak capacity is simply stated as the number of peaks that can be resolved per unit time of chromatography. These increased performance levels can be defined as Ultra Performance Liquid Chromatography or UPLC™. Figure 2 demonstrates what increased peak capacity brings to a chromatographic separation by moving from a 5 µm particle to a 1.7 µm particle.

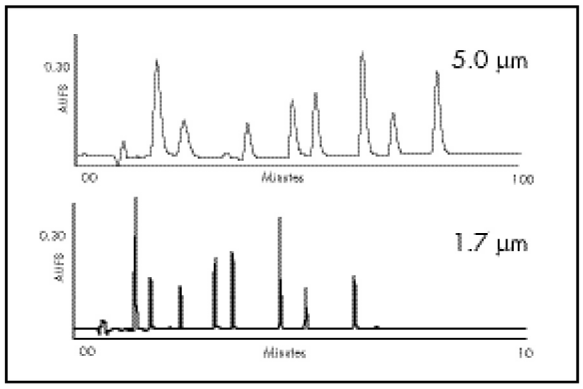

Figure 2: Comparison of 5 µm vs. 1.7 µm particles. Peak capacity and resolution increase significantly.

Speed, sensitivity and resolution

With this added efficiency from smaller particles come additional significant benefits. Speed, through shorter columns or with higher flow rates, and resolution. These variables can be manipulated to find the best combination of speed and resolution for any given separation. Figure 3 demonstrates the use of UPLC relative to HPLC in the quest for speed. The experiment was designed to generate equal resolution on a 3 µm particle and a 1.7 µm particle and expose the speed benefits of the smaller particle. An additional benefit derived from the increased efficiency is sensitivity. Due to the reduced band spreading during the separation process, the analytes will be more concentrated at the point of detection. This will be discussed in more detail when the instrument design requirements are considered for UPLC.

It should be noted that 3 µm is the limiting particle size for conventional 15 cm long columns. This is the smallest particle size that can be used when operating the system at the optimum flow rate of the column, but within the pressure limit of commercially available equipment using any composition of a methanol-water solvent mixture (N.B. The smallest particle reported to date for use in HPLC applications is 0.67 µm [3]). As mentioned above, the Van Deemter equation (and plots) show that it is necessary to go below 2 µm particles to realize the advantages of increased efficiency. Therefore, what technological advances are required to allow the UPLC vision to manifest itself in the chromatography laboratory?

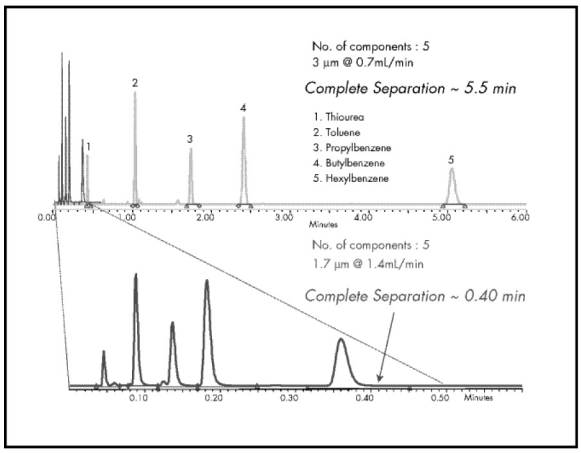

Figure 3: The top chromatogram is an overlay of both conventional (3 µm) HPLC and 1.7 µm UPLC for a five-component sample mixture. The bottom chromatogram is an expansion of the first 0.6 minutes of the separation to show the increased speed and maintained resolution of UPLC. Solvent use is also greatly reduced with UPLC.

Smaller particle, high peak capacity

Developing and packing a sub 2 µm particle in reproducible and rugged columns is a challenge in itself. Figure 4 provides some insight into the significant differences between a 5 µm particle, common in today's laboratory, and the proposed smaller 1.7 µm particle to be used in UPLC columns. Currently, non-porous 1.5 µm particles are commercially available. Although these particles demonstrate high efficiency, they suffer from low surface area. This low surface area leads to poor loading capacity and low retention. To maintain similar retention and loading capacity to conventional HPLC, UPLC must use porous particles. A novel porous particle is required that can withstand the high pressures. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while reaching the goal of faster separations. Another requirement is that the interior surface of the column hardware be much smoother to facilitate packing of the smaller particles. The end frits should be redesigned, enabling them to retain the small particles but resist clogging.

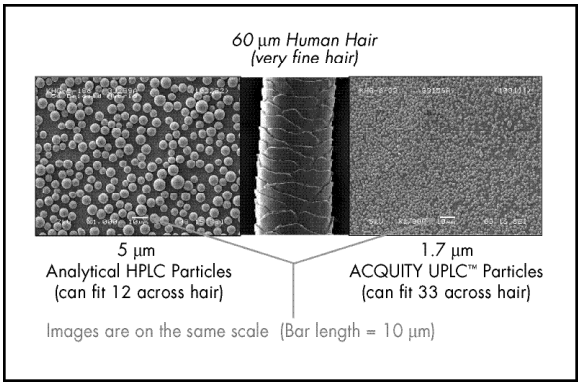

Figure 4: To demonstrate the significant difference between 5 µm and 1.7 µm particles, this Scanning Electron Micrograph compares the particles to a 60 µm human hair. The diameter of the hair is equal to approximately 12 of the 5 µm particles versus 33 of the 1.7 µm particles required to span the same distance.

UPLC system design requirements

Achieving small particle, high peak capacity separations requires a greater pressure range of current HPLC instrumentation. The calculated pressure drop at the optimum flow rate for maximum efficiency across a 10 cm long column packed with 1.7 µm particles is ~15,000 psi. Therefore, a pump that can deliver solvent at these pressures is required. Another consideration is the fact that solvent compressibility will be significant under these conditions, especially with multi-solvent and gradient separation conditions. A solvent delivery system for UPLC needs to accomplish more than simply high pressure pumping. A solvent delivery system must compensate for solvent compressibility across a wide range of potential pressures to achieve smooth and reproducible flow using a variety of solvents in both isocratic and gradient separation modes.

Sample introduction is also critical. Conventional injection valves, either manual or automated, are not designed and hardened to work at these extremes of pressure. Typical performance standards for injection reproducibility and linearity are required for analytical use but other features are also necessary. To protect the column from experiencing extreme pressure fluctuations, the injection process should be relatively pulse-free. The swept volume of the device needs to be small to reduce potential band spreading of the injected sample. A fast injection cycle means the potential speed of UPLC can be realized, and with a high sample capacity, unattended use is possible for long periods. Low volume injections with minimal carryover are required to accommodate the increased sensitivity benefits.

In theory, with 1.7 µm particles, UPLC systems could generate peak widths < 1 second at half height. This poses challenges for UPLC detection. First and foremost, the detector must have a high sampling rate to capture enough data points across the peak to perform accurate and reproducible recognition (and integration) of the analyte peak. Secondly, the detector cell must have minimal dispersion (volume) to preserve the efficiency of the separation. The detector optics must also provide performance that takes advantage of UPLC sensitivity benefits. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations depending on detection technique (Figure 5). MS detection, for example, should benefit most from the performance characteristics of this technique. Increased peak concentrations with reduced chromatographic dispersion at lower flow rates (no flow splitting required) will promote increased source efficiencies, resulting in at least 3X improvements in sensitivity with a UPLC interface to mass spectrometers.

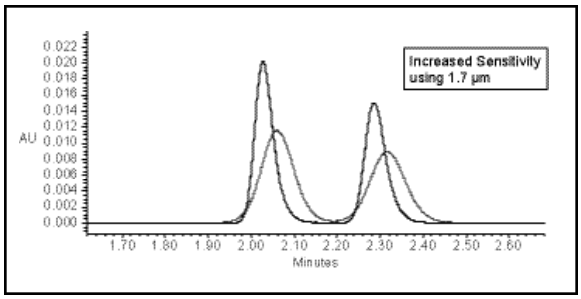

Figure 5: Reduced analyte dispersion during high peak capacity chromatography provides potential for increased sensitivity.

Total system approach

By thinking through the chromatographic process and how to apply the principles to UPLC, it becomes apparent that there are system considerations beyond the chemistry, pump, injector and detector. System volume is critical. The total system volume must be reduced well below where HPLC systems are today if the fidelity of the UPLC separation is to be maintained throughout the chromatographic process. It is crucial to carefully consider not only the system component performance specifications as noted, but also the connecting tubing and fittings. To make UPLC performance a reality for widespread use, a total system approach providing both the required performance specifications and effective management of the ancillary variables, is essential.

UPLC system advantages can be leveraged in the chromatography lab to deliver great benefits. High-throughput library screening for characterization purposes can be accomplished either faster or with better resolution, both of which translate into improved efficiency. Metabolite identification and bioanalysis will benefit from speed, resolution and sensitivity. Peptide mapping can potentially be accomplished in significantly shorter time for characterization purposes. For peptide research a much more definitive map than is generated with HPLC today can be produced with the increased peak capacity provided by UPLC systems. Stability-indicating methods, and method development in general, will yield more information per injection with faster separations. And, quantitative analysis, the most prevalent objective of chromatography separations, will be faster with baseline separations that are more easily and reproducibly integrated to increase productivity and confidence in the laboratory.

UPLC™: Redefining the chromatography laboratory

At a time when many scientists have reached separation barriers and are pushing the limits of conventional HPLC, Ultra Performance Liquid Chromatography presents the possibility to extend and expand the utility of this widely used separation science (Figure 6). When properly commercialized, the UPLC technique will gain wide acceptance in the laboratory due to its ability to provide more information per unit of work as it fulfills the promise of increased speed, resolution and sensitivity predicted for liquid chromatography.

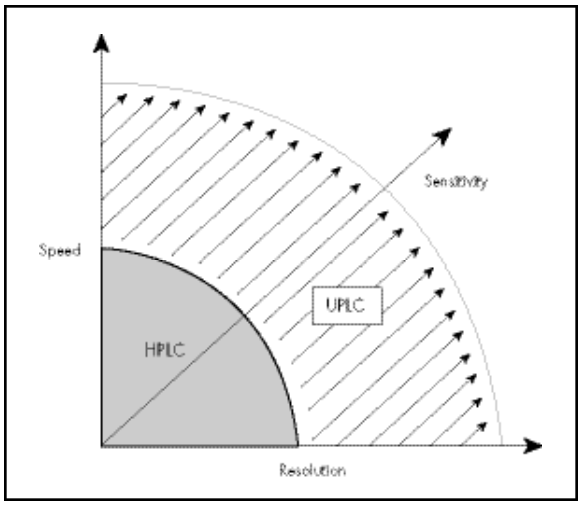

Figure 6: UPLC presents new capabilities in speed, resolution and sensitivity that will redefine the chromatography laboratory.

References

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[3] J. M. Cintron, L. A. Colon, "Organo-silica Nanoparticles Used in Ultrahigh-Pressure Liquid Chromatography," *The Analyst,* **127** (2002) 701 - 704.

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