# Microfluidics-Based Separations Technology for the Analytical Laboratory

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**A seminal paper was written by Manz et al. (1) identifying the use of possibilities of using 'chip based technology' within an analytical instrument. When did you as a scientist first think that 'chip technology' could be a useful addition to analytical instrument design and separation science in particular?**

In the early 1990's we saw a lot of enthusiasm for the "Lab-on-a-Chip" concept. And at the time, the concept of integrating multiple laboratory processes like sample prep, cell lysing, digestion, separation and detection onto a single microfluidic device looked technically possible.

However, as researchers began to develop these microfluidic systems, integrating these various functions onto a single device proved difficult. Each lab process used different technologies, such as sample prep or digestion, compared to technology used for separation and detection. What started out as a single device became a hybridisation of a variety of technologies with a host of complex interfaces.

Early microfluidic devices showed promise until they were applied to real world applications. The small volumes, picolitres rather than microlitres, made maintaining chromatographic resolution and transporting the analytes to an external detector difficult.

While I shared much of this early enthusiasm for microfluidics, it was not until the early 2000's that I started to see real utility for microfluidics in LC, particularly to improve the usability of nano-scale chromatography. Rather than implement the full "Lab-on-a-Chip," the best approach seemed to be replacing the typical nano-LC consumable package (i.e. trap column, analytical column, electrospray tip) with one integrated consumable device.

**What particular aspects of 'chip technology' offered the possibilities of moving separation science forwards in your eyes? Were there any particular elements within the workflow of a typical lab that needed the advantages that this new technology could bring?**

At the time we began working in microfluidics, interest in proteomics was on the rise which was best performed with nano-scale chromatography using fused silica tubing in order to get the sensitivity required. Our goal has been to retain the attributes of fused silica capillaries in a highly usable microfluidic device.

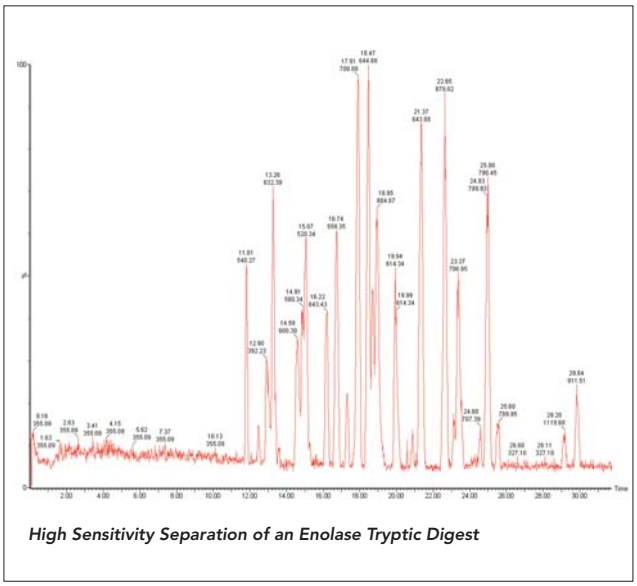
Proteomics was an area that seemed ripe for integrating all the fluidic components that make up a typical system: a trap column, analytical column, and electrospray tip onto one microfluidic platform or cartridge so the user wouldn't need special skills to plumb together these fragile fused silica parts.

**Was there such a thing as 'first generation chips' and what were the limitations restricting their usefulness in instrument design?**

When we were developing our microfluidic platform, there were many "first generation chips" that had limitations. One thing we discovered is that at this nano-scale, surfaces become very important. The surface-to-volume ratio at this scale is significantly higher than at, say, the analytical scale (i.e. 2.1 mm I.D. columns). For a proteomics application, a digested protein sample contains a wide range of chemical species: basic, acidic, hydrophobic, hydrophilic peptides, and phosphopeptides, that will adhere to any metal-oxide surface. We learned quickly that we had to create inert surfaces to minimise any interactions of analytes with the surface. Early ceramic formulations we investigated were not inert enough and caused tailing for some basic and phosophopeptides. Through a combination of ceramic formulation and coating technologies, we engineered an inert and benign surface similar to that found in fused-silica tubing.

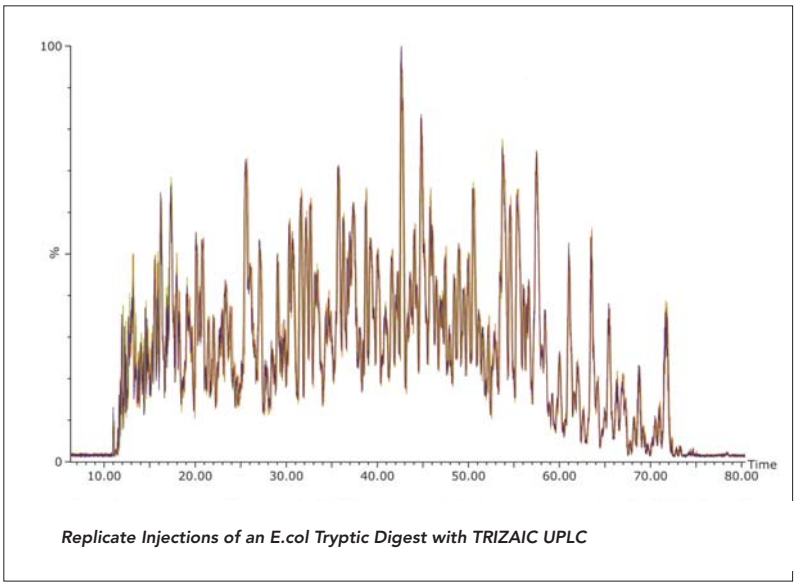
**When did Waters first start to investigate the usefulness of the chips and consider how to integrate them into their instruments? How were they successfully interfaced?**

In the early 2000's we began working with Sandia Laboratories to develop an electrokinetic (EK) pump. By taking a bulky, reciprocating mechanical HPLC pump and replacing it with a small "solid state" disposable pump, we could take it to extremely high pressures. Essentially, an EK pump is a packed column where flow is



*High Sensitivity Separation of an Enolase Tryptic Digest*

*Figure 1: High sensitivity separation of an enolase tryptic digest as performed on a Waters nanoACQUITY UPLC System and a TRIZAIC nanoTile at 450 nl/min. flow rate.*




*Replicate Injections of an E.col Tryptic Digest with TRIZAIC UPLC*

*Figure 2: TRIZAIC UPLC nanoTile separations for triplicate injections of 700 ng of a tryptic digest of E.coli. The separation conditions were three percent A to 40 percent A over 90 minutes at a flow rate of 450 nl/min.*

generated by applying a voltage across this packed bed. An EK pump seemed perfect for a microfluidic system.

At the same time, we began looking at high pressure microfluidic platforms. Previously, microfluidics had been used for low pressure applications at pneumatic-type pressures (<1,000 psi) in silicon, glass, polymers, or other materials which are not appropriate for high-pressure LC/UPLC pressures up to 15,000 psi. These materials would burst at the hydraulic pressures that exist inside a typical LC channel. We didn't want the materials of construction used in an LC microfluidic device to limit the pressure/performance envelope. Not coincidentally, it was about this same time we began working on UPLC, so 15,000 psi was the goal we set for a system operating pressure.

About 2004, we constructed microfluidic prototypes with multilayer ceramic platforms in LTCC and HTCC (low/high temperature co-fired ceramic). These ceramics have been used for years in the electronics industry to create rugged multilayer circuit boards. We learned that the internal channels on the ceramic devices could withstand extreme hydraulic pressures. And since the manufacturing process for these was quite straightforward, we decided to bring the manufacturing process in-house, and we refined the process to create microfluidic features and develop a ceramic that had the strength and inertness needed for many applications.

**Which components within a 'typical' LC system stand to benefit most from integrating 'chip technology' into their design? Are there any other components which could benefit from utilising this technology but for one reason or another have not yet done so? How far away from seeing 'full system benefits' owing to this technology are we?**

While we thought we could put an entire LC system on a microfluidic device by leveraging EK pumping technology, we quickly learned that this might be too big an initial step. Instead, replacing the consumable package that makes up a typical nanoLC system - the analytical and trap columns, and electrospray tips - and gaining

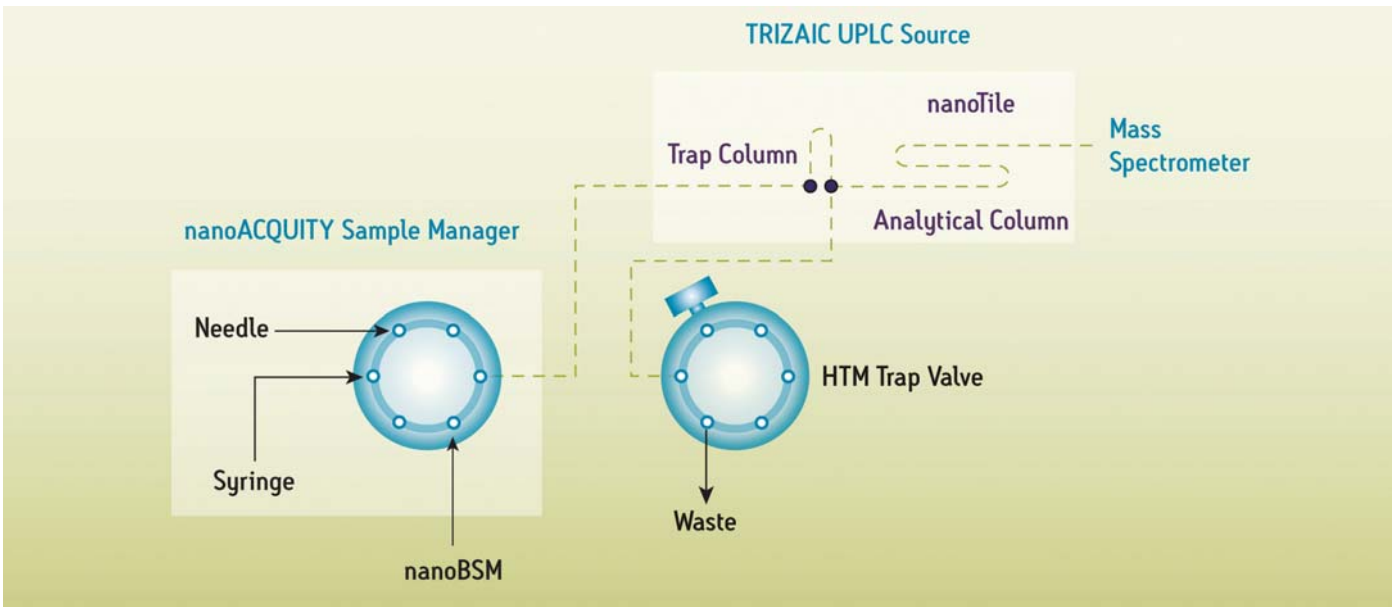



*Figure 3: The TRIZAIC UPLC nanoTile incorporates traditional fittings, columns and electrospray emitters into a single device for performing nanoscale LC separations.*

*Figure 5: TRIZAIC UPLC source for Waters Xevo and Synapt mass spectrometers.*

a significant improvement in usability became our initial goal.
We focused on the development of an integrated cartridge, what became the TRIZAIC UPLC nanoTile, that could be inserted into a clamp assembly. After inserting the nanoTile into the clamp assembly and rotating a lever 80° this clamp assembly makes all the connections into the nanoTile: high pressure fluidic fittings, low voltage connections for a heater, temperature sensor, an EPROM for storing data, an electrospray tip as well as gas connections for the nebulisation gas. It was a big challenge to integrate all those features onto a single, integrated device and maintain UPLC performance.

**Where do you see this 'chip based technology' in 5 years time? Has the technology reached a plateau, maybe waiting for other components within an Instrument to be able to realise the improvements in instrument design?**

As I mentioned earlier, the primary rationale for Waters developing a microfluidic LC system has been to improve usability, while maintaining UPLC performance. Currently, the primary users for nano-scale microfluidic LC are doing discovery proteomics, and are sample limited. While there are real sensitivity benefits to working at the nano scale, even with improvements brought on by microfluidics, this scale lacks the robustness most LC users are accustomed to.

While we initially focused on proteomics applications and nano-scale LC, the microfluidic platforms we have been developing are capable of going to higher pressures, and larger channel diameters. Moving up to the 150-300 µm scale, flow rates get quite a bit faster (2 to15 µL/min). At these flow rates the benefits become become obvious: a typical LC system performs faster, electrospray performance is more robust and requires less tuning and larger sample volumes can be introduced.

So, while microfluidic LC has primarily been used for proteomics up until now, over the next five years, I think we will see implementation of capillary-scale microfluidic systems that will attract LC users who typically work at the analytical scale. I expect that we will push the performance of separations on a microscale past that of analytical scale. With analytical scale LC we are currently using 1.7 micron particles at 15,000 PSI. Benefits of reduced solvent consumption, higher sensitivities will be realised with the same or better robustness as analytical scale.

**For all of the advantages (perceived and realised) that chip based technology brings to the industry are there any application areas, e.g. small molecule, proteomics, environmental which could directly benefit from the technology?**

As I mentioned, we have been developing microfluidic platforms that will allow us to expand microfluidic LC beyond typical nano-scale applications into applications traditionally performed at the analytical scale.

Examples are microsampling for bioanalysis and dried blood spot analysis where you are no longer using a test tube of blood to draw a sample, but where the sample is drawn from a few microlitres of blood spotted onto a piece of paper. These small sample volume applications require higher sensitivity, and with a microfluidic format, we can provide these sensitivity improvements while maintaining the robustness and ease-of-use of an analytical-scale LC system.

Aside from the performance benefits that can be achieved with microfluidic LC, using microfluidic LC can also improve the usability of LC/MS systems. We see that most scientists view LC as a tool, and as such, it should be easier to use and easier to train people on. If cartridge-like consumables can be integrated into more LC/MS systems, researchers can spend more time acquiring data than setting up the instrumentation. When I was a practicing analytical chemist in the 1990's at the Canadian Food Inspection Agency, the lab I was part of was responsible for monitoring Canada's meat for drug residues. Typically, we used LC/UV systems for screening, and then if we got a positive result, that sample would be taken to the mass spectrometry technician to run a confirmatory analysis.

Those days are over. The lab I used to work in now has LC/MS systems for every chemist. That's because the technology had become so robust and easy enough to use that mass spectrometry specialists are no longer needed to run samples.

Further improvements in usability and integration can be achieved with microfluidics that will allow LC/MS technology to extend beyond the analytical lab. Right now, we are being asked to create "Open Access" systems where anyone can walk up and load samples for analysis.
The ultimate goal for any analytical technology is to allow it to be viewed as simple analyser that can deliver an answer. While we are a long way from creating an LC/MS "analyser" that can be used by an untrained user, this is the ultimate goal, and I think microfluidic technology will play an important role in realising this.

(1) A. Manz*, , J.C. Fettinger, E. Verpoorte, H. Lüdi, H.M. Widmer and D.J. Harrison Trends in Analytical Chemistry 1991, Micro machining of monocrystalline silicon and glass for chemical analysis systems. A look into next century's technology or just a fashionable craze?


*Figure 4: TRIZAIC UPLC nanoTile encases everything needed for nanoUPLC separations.*