On-chip chromatography: the last twenty years

Andrew de Mello reviews the development of chip-based chromatography systems over the past twenty years

At its most fundamental level, the great interest in lab-on-a-chip technology stems from the inherent performance gains that arise when most analytical systems are downsized to the micron scale. It can be argued that the reduced physical size of many chip-based systems is attractive and important in niche areas, such as 'point-of-care' diagnostics and 'in-the-field' analysis, but even then performance gains determine ultimate applicability. Over the past decade, the most active field (as judged by publication output) of microsystem development has been in transferring conventional, macroscale separation techniques to planar chip formats. This in large part has been directly due to the unmistakable improvements in separation performance as system features are diminished. For example, electrophoretic separations performed on microfabricated devices can yield distinct advantages when compared to conventional capillary and slab-gel formats. Analysis times can be reduced to sub-second timescales and extremely high separation efficiencies can be achieved (due to the application of high field-strengths).[1] The use of cross-type injectors provides a simple and efficient means of reproducibly introducing picoliter volumes into the separation channel,[2] and photolithographic printing techniques afford the creation of multiple or parallel devices on a single substrate.[3] Many of these benefits equally apply to downsized chromatographic techniques, however a cursory survey of the literature uncovers relatively few examples of chip-based chromatographic instruments compared to chip-based CE devices. This is perhaps not surprising due to the fact that CE is almost perfectly suited to miniaturization. On the other hand, the miniaturization of chromatographic systems involves some technical challenges, which are generally not faced in CE. Although CE is a powerful analytical tool, chromatography is the most widely used separation technique due to its outstanding separation power and versatility. Consequently, the ability to successfully fabricate a diversity of chip-based chromatographic systems should have a huge impact on the way complex chemical and biological media may be analyzed. The aim of this mini review is to highlight some of the major developments in the area of chip-based chromatography over the past two decades and assess the potentials of the current generation of chromatographic instruments. For simplicity, the development of gaseous and liquid methods will be addressed independently.

How it all began

In December 1979 Stephen Terry and co-workers at the Stanford Electronic Laboratories, Stanford University reported what is generally considered the first example of a microfabricated device for chemical analysis.[4] The paper, published in *IEEE Trans. Electron Devices*, described the design, fabrication and testing of a gas chromatograph integrated on a planar silicon wafer. The device, pictured in Fig. 1, was fabricated using standard photolithographic and wet-etching techniques and incorporated a sample injection system, a 1.5 m column and a thermal conductivity detector (separately batch fabricated and directly mounted on the substrate surface). To create an enclosed chromatographic column, a Pyrex coverplate was anodically bonded to the structured silicon substrate. The column walls were then lined with an organosilane adhesion layer, and a standard OV-101 liquid stationary phase dissolved in a volatile solvent was forced through the channel network. Using helium as a carrier gas, the completed device was subsequently used to separate and detect gaseous hydrocarbon mixtures in less than ten seconds. The elegance of both the design and operation of the GC chip was clear to see, but unfortunately the resolving power of the microfabricated column was poor when compared to standard columns of the day. The authors realized that a reduction in the column depth could be used to increase plate numbers (at the expense of higher pressure drops), but the critical limitation of this initial design originated from the introduction of the stationary phase layer. Since, the stationary phase material was introduced into the pre-fabricated column in a liquid form, condensation at corners and curvatures resulted in a non-homogeneous layer coverage, and thus poor column

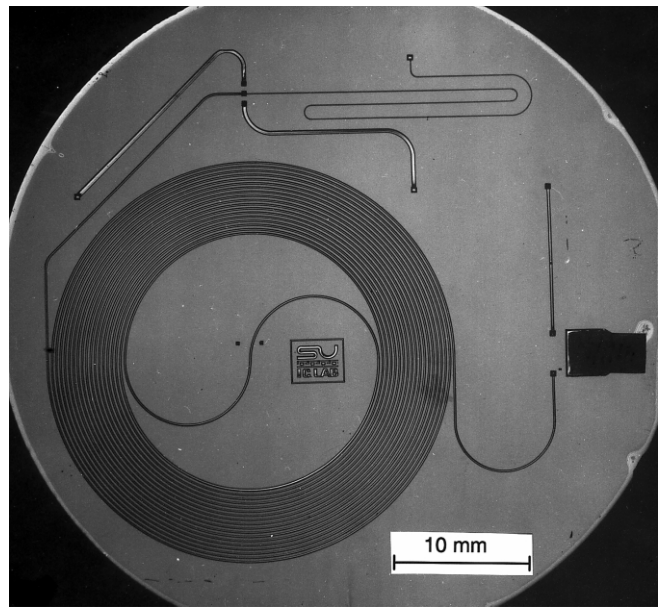

Fig. 1 Photograph of a gas chromatograph integrated on a planar silicon wafer fabricated by Terry and co-workers at Stanford University.

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performance. Consequently, subsequent attempts to create microfabricated GC systems have focussed on the development of superior stationary phases within microchannels. For example, Edward Kolesar and Rocky Reston have reported the fabrication of a functional GC microsystem that integrates silicon micromachining with conventional integrated circuit (IC) processing techniques.[5–7] Their silicon micromachined gas chromatography system (SMGCS) incorporated a miniature sample injector, a 90 cm long, rectangular-shaped (300 μm × 10 μm) capillary column and a dual detector (based upon a chemiresistor and a thermal conductivity detector (TCD) bead). To facilitate superior column efficiencies the stationary phase material was deposited onto column walls prior to the bonding process. Specifically, the authors used conventional IC patterning techniques to sublime a 200 nm thick copper phthalocyanine layer onto the surfaces of the etched channels of the silicon substrate. The structured silicon wafer was then bonded to a Pyrex coverplate using a low temperature (< 300 °C) anodic bonding process. The combination of these two techniques ensured the creation of a nearly-homogeneous thin-film stationary phase within the microchannel column. The efficacy of the approach was demonstrated by the isothermal separation of ammonia and nitrogen dioxide within 30 minutes. Furthermore, the integrated system design yielded detection limits in the parts-per-million range, and column dead volumes of only 20 nL. An alternative approach to the development of high-efficiency columns within microfabricated columns is through the use of plasma-deposited amino acid films. As stationary phase materials these films provide good chromatographic performance and are resistant to both chemical etchants and high temperatures.[8] Importantly, this allows for facile deposition prior to silicon micromachining and anodic bonding. Shinsuke Hannoe and co-workers at the NTT Integrated Information & Energy Systems Laboratories in Tokyo have reported the use of such amino acid films as stationary phases in chip-bed GC columns. By plasma sputtering of D-phenylalanine onto the walls of rectangular-shaped (100 μm × 10 μm) capillary columns etched in silicon, the authors were able to demonstrate a high efficiency chromatogram of methane in 600 ms. In a similar manner, Uwe Lehmann and co-workers at the Technical University Hamburg have described an integrated, chip-based GC system consisting of a sample injector, separation column and TCD.[9,10] The separation column is formed by reactive ion etching a silicon substrate. An organosilicon stationary phase is then deposited by plasma enhanced chemical vapour deposition to yield a homogeneous (500 nm) film thickness. A four-valve sample injection system allowing precise introduction of sample gas, and a micromachined TCD having an extremely low thermal capacity (and thus short response time and high sensitivity) complete the set-up. A prototype device was subsequently shown to separate a C1–C5 natural gas sample in less than one minute with high-efficiency. Furthermore, since microfabrication methods are used to structure all components, the authors expect to demonstrate complete system integration on a monolithic device in the very near future.

The development of chip-based GC systems has in large part been motivated by the performance gains facilitated by miniaturisation and integration. However, the ability to create portable and robust instruments for 'in-the-field' and 'on-site' chemical measurements has also been a major incentive for recent developments in the area. This has particular significance in applications such as the detection of biological and chemical warfare agents and pollution control.[11] Realizing these needs, Sandia National Laboratories initiated funding of a 'Microanalytical Systems' program in 1997.[12] The primary aim being to develop highly integrated, portable μTAS for the analysis of chemical warfare agents and explosives. A significant part of this initiative had focussed on the creation of microscale GC technology. The 'μChemlab' gas analysis system, shown in Fig. 2, incorporates a number of distinct micromachined components. These include a microfabricated sample pre-concentrator, a chromatographic column and polymer-coated, surface acoustic wave (SAW) detectors.[13] The sample pre-concentrator incorporates a thermally isolated (and sol–gel coated) membrane and resistive heater to effect rapid sample heating. Since, the membrane has a small thermal mass it can be heated to 200 °C within 10 ms, resulting in concentration enhancements of over 2 orders of magnitude and the creation of well-defined injection volumes. As with previous examples, the chromatographic column is structured in silicon and coated with a thin-film of stationary phase material. Finally, detection of separated species is performed using an array of chemically modified SAW sensors. These provide for high sensitivity and unique response patterns (which aid analyte identification). The entire, integrated system has been shown to successfully separate and detect a wide range of chemical species at parts-per-billion concentrations,[13] and has more recently been applied to the rapid detection of gaseous nerve agents.[12] Despite the problems associated with packing microfabricated chromatography columns, the Sandia group have also demonstrated the successful operation of packed, microfabricated GC columns.[14] Using reactive ion etching, relatively large channels (300 μm × 300 μm) could be fabricated and packed with porous materials. These were then used as

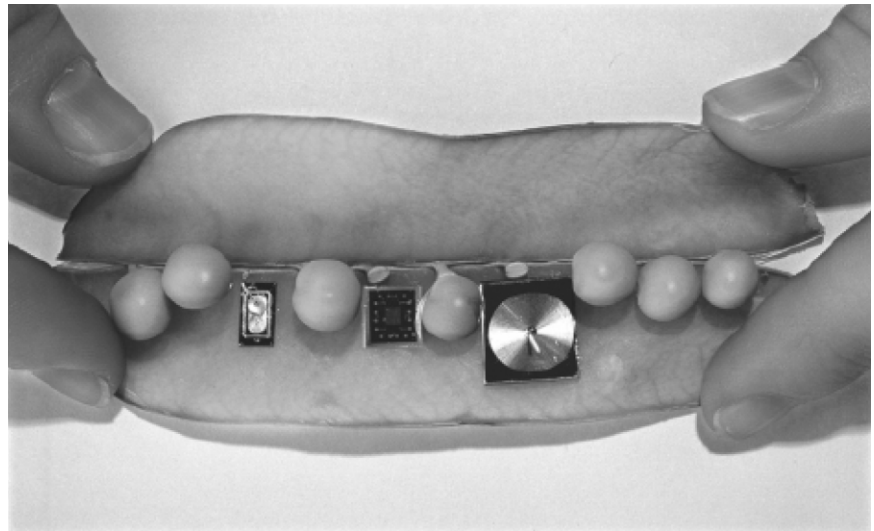

Fig. 2 Components of the μChemLab gas analysis subsystem. From left to right the SAW detector (an array of four), the sample collector/pre-concentrator, and a 1 m gas chromatography column.

chromatographic columns in the separation of gases and volatile organic compounds.

As in all separation techniques, definition of a small volume of injected sample is crucial in preventing adverse broadening of peaks and a consequent loss in component resolution. As has been seen, injection of gaseous samples into microfabricated GC columns normally involves the integration of solenoid or membrane valves on chip. Although successful, these methods necessarily increase the complexity of the fabrication process. Omar Naji and Andreas Manz at Imperial College of Science, Technology & Medicine have addressed this issue by proposing a novel method to inject gaseous samples onto microfabricated columns without the use of integrated valve technology.[15] The authors demonstrate that if a sample is continuously introduced into a chromatographic column and passed through a plasma generator, the sample is modified (*via* ionisation and fragmentation processes). If the plasma is then interrupted for a brief period, a short unmodified sample plug is introduced into the separation column and subsequently separated. The injection volume can be precisely controlled by variation of either the sampling flow rate or the time that the plasma is interrupted. The simplicity of the approach and the ability to use a second plasma as an integrated detector will surely make the general approach extremely attractive for highly-integrated chip-based GC systems.

As a final comment, it is apparent from the few studies described herein that the precise cross sectional geometries and lengths of microfabricated columns vary greatly. This is, in large part, due to the availability of appropriate fabrication techniques and the precise nature of the separation medium used. Furthermore, although successful operation of microfabricated GC systems has clearly been demonstrated, performance variability has been poor when compared to conventional GC technology. As has been seen, this is often associated with the ability to deposit or pack stationary phase materials in a homogeneous fashion. However, it is also noted that the rectangular cross-sectional geometries of typical microfabricated channels are very different from the circular geometries encountered in conventional micro- and megabore capillaries. Standard theories for open-tubular GC columns generally assume circular cross-sections, and thus are not wholly applicable to most microfabricated columns. The lack of theoretical models that can be used to predict the performance characteristics of rectangular columns has almost certainly been detrimental to effective optimization of system parameters such as channel geometries and volumetric flow rates. Recently, Glenn Spangler has addressed this issue by developing more robust theoretical models for GC separations in rectangular columns.[16,17] By modifying Golay's theory[18] for the measurement of performance of a column (the height-equivalent-to-a-theoretical-plate) Spangler was able to correctly predict experimental results described by Terry in ref. 4 for microfabricated GC columns. General predictions from this model suggest that resolution can be optimized by selecting the column height (provided the channel posses a low aspect ratio), and the flow of carrier gas adjusted by selecting the column width (or cross-sectional area). The success of this model will be especially useful when combined with modern deep-etching methods to create highly efficient microfabricated GC systems.

Liquid phase chromatography

As with GC, liquid chromatography (LC) on chip-based structures were first proposed before microfabricated CE systems. In 1990 Andreas Manz and colleagues at the Hitachi Central Research Laboratories described the design and fabrication of an open-tubular liquid chromatograph on a silicon wafer.[19] Although physically smaller, the silicon/glass device (5 × 5mm) was similar in structure to Terry's gas chromatograph made a decade earlier (Fig. 3). The device incorporated a micromachined open-tubular separation column (6 μm × 2 μm × 150 mm) and an integrated platinum electrode detector. Although no testing of the complete device was presented in this initial publication, the authors clearly appreciated the potential advantages that miniaturization could effect. These included superior efficiency per unit time when compared to conventional LC, facile positioning of detection cells and low unit cost. In addition, the use of an open tubular system rather than a more normal packed column was deemed advantageous due to the short analysis times and low pressure drops for a given performance. The more normal approach to LC is through the use of columns packed with particles. The particles (which are normally porous) support the stationary phase, provide a large interfacial area for analyte partitioning and form a homogeneous medium for the transport of mobile phase. In conventional systems, this approach is generally more effective since in open-tubular methods ultra-small

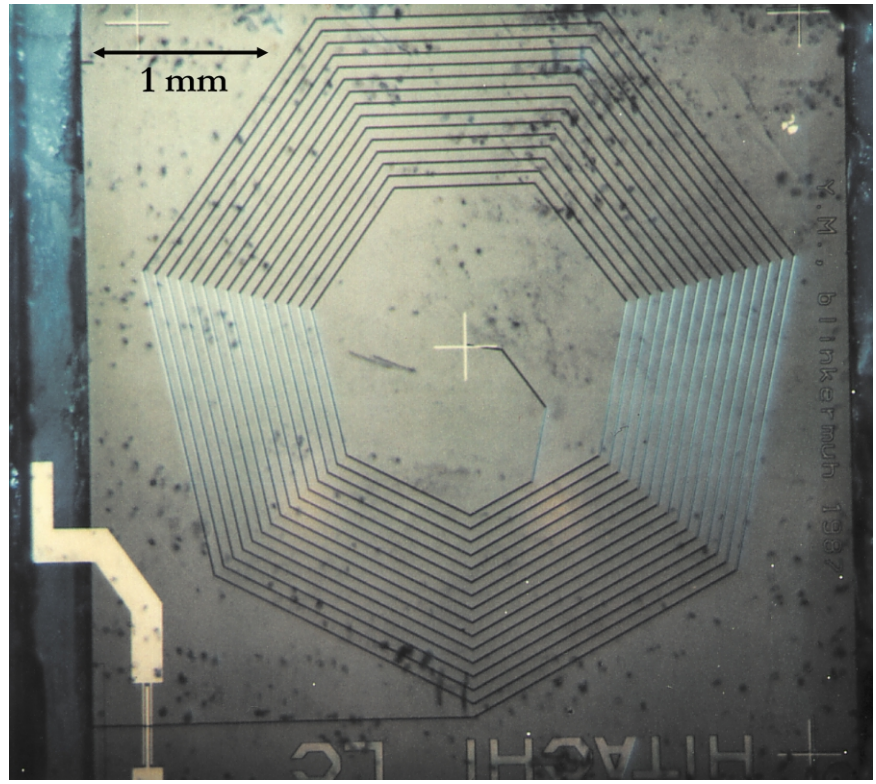

Fig. 3 Micrograph of Liquid Chromatograph chip manufactured by Manz and co-workers at Hitachi Ltd.

column widths are required to counteract the limited rates of mobile phase transfer. These small dimensions lead to reduced loading capacities and a propensity to blockages. To this end, in 1995 Andreas Manz and colleagues at Ciba Geigy, Basel demonstrated the successful functioning of a liquid chromatograph partially integrated onto a silicon chip.[20] The device incorporated a microfabricated split injector, a small-bore column (packed with 5 μm C_8 particles), a retaining frit and optical detector. The device was successfully used to separate two fluorescent dyes, yielding a maximum of 200 theoretical plates in 3 minutes. More recent examples of packed bed systems include a poly(dimethylsiloxane) (PDMS) chip-based chromatograph, filled with 30 micron anion-exchange beads, for the pressure-driven separation of proteins,[21] and a 330 pL chromatographic bed integrated within an electroosmotically pumped microsystem.[22] In the latter device, Jed Harrison and co-workers at the University of Alberta, utilized weirs within a microfabricated channel to trap coated silica beads (1.5–4 μm diameter). These were then used to perform both solid phase extraction and electrochromatography of small molecules. Concentration enhancements of up to 500 times, and separation times of 20 seconds were demonstrated for two fluorescent dyes.

Although packed columns may be desirable, the introduction of stationary phase material into microfabricated channels is a non-trivial process. Frits must be fabricated within the channel structure to retain the packing, and a high-pressure interface between the chip and an external pump must be made. In addition, due to the reduced channel dimensions and the complexities of the manifold pattern, the packing process is difficult and can often lead to non-uniform particle densities at channel walls and thus reduced separation efficiencies. Consequently, the majority of initial chromatographic methods within microfabricated columns employed an open-tubular approach. An early example included a silicon based open-tubular liquid chromatograph, by Simon Cowen and Derek Craston at the Lab of the Government Chemist.[23] This device, incorporating a modified separation column and amperometric detector was used to analyze iron complexes in solution. Additionally, in 1994 Mike Ramey and co-workers at the Oak Ridge National Laboratory reported the fabrication of a chip-based device for performing open channel electrochromatography (OCEC).[24] Channel surfaces were modified with octadecylsilane and electroosmotic flow used to pump the mobile phase during separation. Using this approach, the authors were able to separate a number of dye molecules with plate heights as low as 4.1 μm. More recently, the same group described OCEC in combination with solvent programming on chip.[25] Isocratic and gradient elution conditions were easily established on-chip, and the control of system parameters allowed effective tuning of selectivities and elution times. It is important to note that electrically-driven chromatographic separations are especially attractive within chip-based systems due to a lack of pressure gradients and reduced fabrication complexity. Furthermore, since the mobile phase is motivated by electroosmosis, the flat flow profile significantly reduced band-broadening when compared to standard HPLC methods. Other examples of open tubular research for microchannel systems include, chiral separations using membrane chromatography,[26] the development of self-containing open-tubular CEC columns by sol–gel techniques[27] and ion-exchange chromatography.[28]

The primary difficulties associated with packing microfabricated channels can be eliminated if the packed bed is replaced by a continuous, porous bed of support formed by *in-situ* polymerization of organic monomers.[29–31] The process of bed formation is facile, since a low-viscosity monomer solution can be introduced by vacuum or pressure into the microfluidic channel prior to initiation. In addition, the continuous polymer bed is attached to the channels walls, making a retaining frit redundant. Although, this approach was initially developed for standard chromatographic columns, a variety of different chemistries and stationary phase have now been applied to microfabricated channels. For example, Stellan Hjertén and co-workers at University of Uppsala described both electroosmosis- and pressure-driven chromatography in planar quartz chips using a continuous polymer bed.[32] The authors used three kinds of derivatized polymer beds for both reversed-phase electrochromatography and anion-exchange chromatography, and successfully performed separations of low-molecular weight compounds and acidic proteins in short times. More recently, the authors described similar separations in diamond chromatographic chips.[33] Diamond possesses a number of attractive characteristics, such as the high thermal conductivity, chemical inertness, optical transparency and controllable electrical conductivity. Using sacrificial silicon etching, high aspect ratio channels were formed in diamond (Fig. 4), and in addition the hydrophilicity of the channel surfaces could be varied *via* wet- or dry-oxidation. The structured channels were then filled with a porous continuous bed and used for fast anion-exchange chromatography of proteins. Although, successful as substrate material for chromatographic chips, the application of diamond in chip-based CE may prove even more advantageous, due to its extremely high thermal conductivity and dielectric strength (which should allow for the application of ultra-high field strengths).

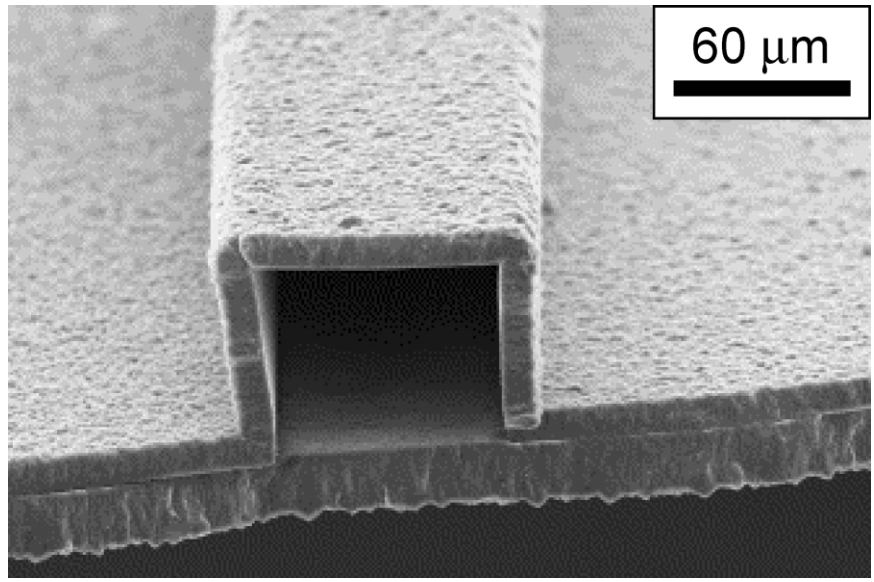

Fig. 4 SEM image of a cleaved diamond microchip without a continuous bed. Adapted with permission from ref. 33. © 2001 Elsevier Science BV.

An interesting variation of the continuous bed theme has been the development of porous polymer monoliths (PPMs) that are formed *via* photo-initiated polymerization (rather than chemical or thermal initiation).[34] Photo-initiated methods are ideally suited to monolith formation within chip-based systems, since polymerization can be restricted to specific areas through the use of a lithographic mask. Anup Singh and co-workers at Sandia National Laboratories have recently reported the fabrication of microchips containing porous polymer monoliths for the separation of peptides and amino acids.[35,36] The authors described UV-initiated polymerization of acrylate-based PPMs within microchannels, with control of separation parameters such as hydrophobicity and pore size. The resulting chip devices were used to separate bioactive peptide mixtures in short times (< 45 seconds) and with high efficiency (up to 6 × 105 plates/metre). Interestingly, the authors were also able to remove the PPM after use (*via* thermal incineration) and regenerate the monolithic glass chips. The Sandia group has also demonstrated the use of the same acrylate-based PPMs for reversed-phased CEC separations of polycyclic aromatic hydrocarbons on planar glass chips.[37]

Different perspectives

One of the primary advantages associated with micromachining analytical instrumentation is the ability to facilitate processes or fabricate structures that are either extremely difficult or even impossible to recreate on the macroscale. Fred Regnier and colleagues at Purdue University have used this facility to describe a novel and elegant approach to the manufacture of liquid chromatography columns. The method, at a fundamental level, is highly attractive since it negates the problematic packing process and more importantly allows system features to positioned and arranged within the column 'by design'. The concept, which can be thought of as *in-situ* micromachining, involves the creation of micron-sized, particle-like support structures on the surface of a planar quartz wafer.[38–40] Using reactive ion etching methods, arrays of high aspect ratio, support structures can be directly fabricated to form columns with volumes ranging from 10–1000 nL. Fig. 5 illustrates such a column composed of 5 × 5 × 10 μm collocated support structures (that mimic particles in a conventional column) and channels of width 1.5 μm. The generic structure is advantageous for a number of reasons, including the fact that components, such as reservoirs and mixers, are fabricated simultaneously and *in situ*, flow channels can be made small and highly uniform, and no mechanical packing of support material is necessary. Initial testing of the approach demonstrated isocratic elution of Rhodamine 123 from an octadecylsilane reversed-phase column with an efficiency of 7.7 × 10^5 plates per metre. More recent studies by Regnier's group have included the demonstration of reversed-phase liquid chromatography of tryptic peptides on similar devices.[41] It was found that peak capacity was comparable to that of conventional HPLC, and more interestingly mixtures could be resolved isocratically rather than in gradient elution mode. A key feature of these chromatography chips is the small channel widths (~ 1 μm). Although these allow for efficient analyte partitioning during separation, they are unsurprisingly prone to blockages. Consequently, the Purdue group has since developed second-generation structures fabricated in PDMS.[42] This elastomeric polymer is optically transparent, flexible, cheap and can be structured using a variety of soft-lithographic techniques.[43] Devices fabricated by direct moulding from a negative relief master were used to separate peptides from a tryptic digest. Importantly, the surface chemistry of PDMS is well-suited to modification by standard silica silanization procedures, thus allowing peptide separations with efficiencies up to 4 × 105 plates/metre. However, the authors also demonstrated the use of unmodified PDMS as a stationary phase material. More recent studies have investigated the modification of oxidized PDMS columns by cerium (IV) catalyzed polymerizations to prepare high-efficiency media for peptide separations.[44]

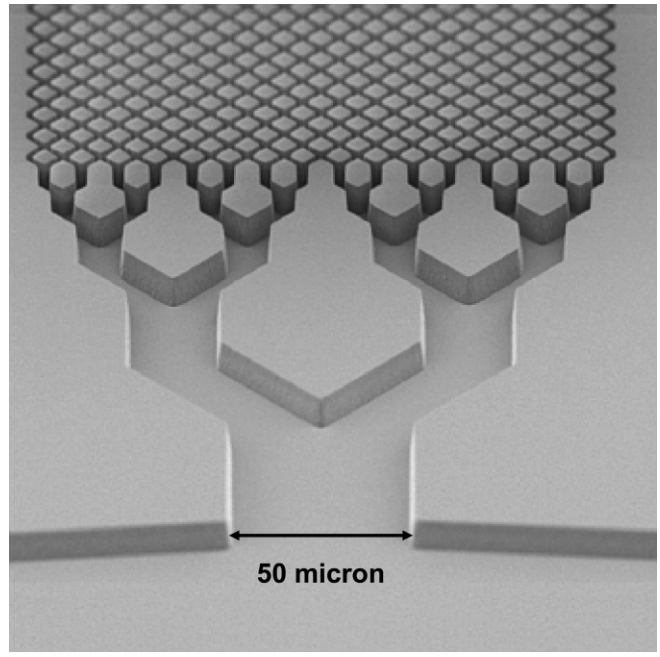

Fig. 5 SEM image of the inlet splitter and initial part of a micromachnined stationary phase support. Adapted with permission from ref. 39.© 1998 American Chemical Society.

Another unusual alternative to conventional on-chip chromatography has recently been described by Robert Tijssen and co-workers at the Universities of Amsterdam and Twente. Their approach utilizes hydrodynamic chromatography (HDC) to separate macromolecules and particles within microfluidic channels. The separation mechanism is purely based on flow and does involve the use of a stationary phase material. Liquids hydrodynamically flowing through narrow conduits exhibit a parabolic flow profile. This means that the average flow velocity of a molecule at a position close to the channel walls will be lower than that at the center of the channel. In HDC this effect, which leads to band broadening in conventional chromatography, is used to discriminate between molecules of different sizes. Since large molecules (ranging from 0.002 to 0.2 of the channel size) cannot fully access 'slow-flow' regions near the channel walls, they are transported more quickly than smaller molecules that can access interfacial regions. In a number of feasibility studies, the authors demonstrate the fabrication, design and testing of an HDC chip for the

separation of small molecules, nanoparticles and biopolymers.[45–47]

As previously stated large inlet pressures often required in chromatographic techniques present significant technical issues for microfabricated devices. Gert Desmet and colleagues at the Vrije Universiteit in Brussels have recently proposed the innovative concept of 'shear driven' chromatography (SDC) as a means to circumventing the inlet pressure limitation.[48,49] Their approach uses channels whose walls are split axially into two parts (one much longer than the other). If the 'long part' is dragged past the 'short part', fluids can be dragged through the channel opening without the need for an increased inlet pressure (or voltage gradient). This means that channels with very small depths (< 1 micron) can be used in separations, without any restrictions on the mobile phase velocity. For example, the authors demonstrate high-speed flows (up to 2cm s^{-1}) through 100 nm thick channels, that correspond to required inlet pressures in pressure driven systems of over 30000 bar.[50] Using commercially available reversed-phase HPLC beads, prototype devices were successful in separating mixtures of small molecules,[49] indicating that the future use of sub-micron thick channels should allow for the development of a high-efficiency separation technique.

Outlook

Although the development of chip-based chromatography systems has occurred at a more modest rate than electrophoretic systems, it is clear that over the past ten years a number of distinct technologies have been introduced and matured. The transferal of chromatographic methods to chip-based formats, although advantageous, is not trivial and imaginative solutions to the problems of stationary phase introduction and mobile phase motivation have defined much of the documented progress. It seems likely that future chromatographic chip systems will incorporate stationary phases in the form of PPMs, due to their facile fabrication and localization within microchannels. In addition, it is also evident that much new chemistry for PPM formation is currently being developed specifically for microfluidic environments.[51] Furthermore, the 'direct fabrication' strategies described by Fred Regnier and co-workers will almost certainly become especially useful when integrated with other components such as picoliter volume mixers (for mobile phase formation)[52] and detection cells on monolithic substrates.

Finally, with the rapid accumulation of genome sequence data we are entering the so-called 'post-genomic' era. This represents the beginning of a fundamentally new period of biological research, in which the development of high-efficiency techniques for the separation and analysis of thousands of proteins and peptides present in cells will be key. Due to the outstanding separation power inherent in chromatographic methods, chip-based systems will undoubtedly play a very important role in this rather important field.

References

1 S. C. Jacobson, C. T. Culbertson, J. E. Daler and J. M. Ramsey, *Anal. Chem.*, 1998, **70**, 3476.
2 D. J. Harrison, K. Flurii, K. Seiler, Z. H. Fan, C. S. Effenhauser and A. Manz, *Science*, 1993, **261**, 895.
3 B. M. Paegel, C. A. Emrich, G. J. Weyemayer, J. R. Scherer and R. A. Mathies, *PNAS*, 2002, **99**, 574.
4 S. C. Terry, J. H. Jerman and J. B. Angell, *IEEE Trans. Electron Devices*, 1979, **ED–26**, 1880.
5 R. R. Reston and E. S. Kolesar, *IEEE/ASME J. Microelectromech. Syst.*, 1994, **3**, 134.
6 E. S. Kolesar and R. R. Reston, *IEEE/ASME J. Microelectromech. Syst.*, 1994, **3**, 147.
7 E. S. Kolesar and R. R. Reston, *IEEE Trans. Compon. Packag. Manuf. Technol., Part B*, 1998, **21**, 324.
8 I. Sugimoto, M. Nakamura and H. Kuwano, *Anal. Chem.*, 1994, **66**, 4316.
9 U. Lehmann, A. Feustel, J. Hall and J. Müller, *Proc. Sensor '97*, 1997, **2**, 151.
10 U. Lehmann, O. Krusemark and J. Müller, in *Proceedings MicroTotal Analysis Systems 2000*, ed. A. Van Den Berg, W. Olthius and P. Bergveld, Kluwer, Boston, 2000, pp. 167.
11 http://www.nn.doe.gov/cbnp/.
12 D. Lindner, *Lab Chip*, 2001, **1**, 15N.
13 G. C. Frye-Mason, R. J. Kottenstette, P. Lewis, E. J. Heller, R. P. Manginell, D. R. Adkins, G. Dulleck, D. Martinez, D. Y. Sasaki, C. Mowry, C. Matzke and L. F. Anderson, in *Proceedings MicroTotal Analysis Systems 2000*, ed. A. Van Den Berg, W. Olthius and P. Bergveld, Kluwer, Boston, 2000, pp. 229.
14 G. C. Frye-Mason, R. J. Kottenstette, C. Mowry, C. Morgan, R. P. Manginell, P. Lewis, C. Matzke, G. Dulleck, L. F. Anderson and D. R. Adkins, in *Proceedings MicroTotal Analysis Systems 2001*, ed. J. M. Ramsey and A. Van Den Berg, Kluwer, Boston, 2001, pp. 658.
15 O. P. Naji, F. G. Bessoth and A. Manz, in *Proceedings MicroTotal Analysis Systems 2001*, ed. J. M. Ramsey and A. Van Den Berg, Kluwer, Boston, 2001, pp. 655.
16 G. E. Spangler, *Anal. Chem.*, 1998, **70**, 4805.
17 G. E. Spangler, *J. Microcolumn Sep.*, 2001, **13**, 285.
18 M. J. E. Golay, in *Gas Chromatography 1958*, ed. D. H. Desty, Academic Press, New York, 1958, pp. 36.
19 A. Manz, Y. Miyahara, J. Miura, Y. Watanabe, H. Miyage and K. Sato, *Sens. Actuators, B1*, 1990, 249.
20 G. Ocvirk, E. Verpoorte, A. Manz, M. Grasserbauer and H. M. Widmer, *Anal. Methods Instrum.*, 1995, **2**, 74.
21 M. Seki, M. Yamada, R. Ezaki, R. Aoyama and J. W. Hong, in *Proceedings MicroTotal Analysis Systems 2001*, ed. J. M. Ramsey and A. Van Den Berg, Kluwer, Boston, 2001, pp. 48.
22 R. D. Oleschuk, L. L. Shultz-Lockyear, Y. Ning and D. J. Harrison, *Anal. Chem.*, 2000, **72**, 585.
23 S. Cowen and D. H. Craston, in *Proceedings MicroTotal Analysis Systems 1995*, ed. A. Van Den Berg and P. Bergveld, Kluwer, Boston, 1995, pp. 295.
24 S. C. Jacobson, R. Hergenröder, L. B. Koutny and J. M. Ramsey, *Anal. Chem.*, 1994, **66**, 2369.
25 J. P. Kutter, S. C. Jacobson, N. Matsubara and J. M. Ramsey, *Anal. Chem.*, 1998, **70**, 3291.
26 P.-C. Wang, J. Gao and C. S. Lee, *J. Chromatogr., A*, 2002, **942**, 115.
27 S. Constantin, R. Freitag, D. Solignac, A. Sayah and M. A. M. Gijs, *Sens. Actuators, B*, 2001, **78**, 267.
28 J. P. Murrihy, M. C. Breadmore, A. Tan, M. McEnery, J. Alderman, C. O'Mathuna, A. P. O'Neill, P. O'Brien, N. Advoldvic, P. R. Haddad and J. D. Glennon, *J. Chromatogr., A*, 2001, **924**, 233.
29 S. Hjerten, J.-L. Liao and R. Zhang, *J. Chromatogr.*, 1989, **473**, 273.
30 S. Hjerten, Y.-M. Li, J.-L. Liao, J. Mohammad, K. Nakazato and G. Pettersson, *Nature*, 1992, **356**, 810.
31 F. Svec and J. M. J. Frechet, *Anal. Chem.*, 1992, **64**, 820.
32 C. Ericson, J. Holm, T. Ericson and S. Hjertén, *Anal. Chem.*, 2000, **72**, 81.
33 H. Björkman, C. Ericson, S. Hjertén and K. Hjort, *Sens. Actuators, A*, 2001, **79**, 71.
34 C. Viklund, E. Pontén, B. Glad, K. Irgum, P. Hörstedt and F. Svec, *Chem. Mater.*, 1997, **9**, 463.
35 A. K. Singh, D. J. Throckmorton and T. J. Shepodd, in *Proceedings MicroTotal Analysis Systems 2001*, ed. J. M. Ramsey and A. Van Den Berg, Kluwer, Boston, 2001, pp. 649.
36 D. J. Throckmorton, T. J. Shepodd and A. K. Singh, *Anal. Chem.*, 2002, **74**, 784.
37 S. M. Ngola, Y. Fintschenko, W.-Y. Choi and T. J. Shepodd, *Anal. Chem.*, 2001, **73**, 849.
38 B. He and F. Regnier, *J. Pharm. Biomed. Anal.*, 1998, **17**, 925.
39 B. He, N. Tait and F. Regnier, *Anal. Chem.*, 1998, **70**, 3790.
40 F. E. Regnier, B. He, S. Lin and J. Busse, *TIBTECH*, 1999, **1**, 101.

41 B. He, J. Ji and F. Regnier, *J. Chromatogr., A*, 1999, **853**, 257.
42 B. E. Slentz, N. A. Penner, E. Lugowska and F. Regnier, *Electrophoresis*, 2001, **22**, 3736.
43 A. J. de Mello, *Lab Chip*, 2002, **2**, 31N.
44 B. E. Slentz, N. A. Penner, E. Lugowska and F. Regnier, *J. Chromatogr., A*, 2002, **948**, 225.
45 E. Chmela, M. T. Blom, H. J. G. E. Gardeniers, A. Van Den Berg and R. Tijssen, in *Proceedings MicroTotal Analysis Systems 2001*, ed. J. M. Ramsey and A. Van Den Berg, Kluwer, Boston, 2001, pp. 646.
46 E. Chmela, R. Tijssen, M. T. Blom, H. J. G. E. Gardeniers and A. Van Den Berg, *Anal. Chem.*, 2002, **74**, 3470.
47 M. T. Blom, E. Chmela, H. J. G. E. Gardeniers, R. Tijssen, M. Elwenspoek and A. Van Den Berg, *Sens. Actuators, B*, 2002, **82**, 111.
48 G. Desmet and G. V. Baron, *J. Chromatogr., A*, 2001, **855**, 57.
49 G. Desmet, N. Vervoort, D. Clicq and G. V. Baron, *J. Chromatogr., A*, 2001, **924**, 111.
50 G. Desmet and G. V. Baron, *Anal. Chem.*, 2000, **72**, 2160.
51 J. P. Kutter, *TrAC, Trends Anal. Chem.*, 2000, **19**, 352.
52 B. He, B. J. Burke, X. Zhang, R. Zhang, R. Regnier and F. E. Regnier, *Anal. Chem.*, 2001, **73**, 1942.