Introduction

In the April edition of this magazine¹ Ron Majors, in his annual report on new columns and accessories from the Pittsburgh conference, reported how at the 2002 conference the largest number of product introductions were for biomolecule separations and for liquid chromatography–mass spectrometry (LC–MS). He went on to state that speciality HPLC columns for chiral compounds, biomolecules, combinational chemistry, carbohydrate analysis and LC–MS represented the largest category of entries. Supporting these products was an increase in capillary and nano-LC columns for LC–MS. But more important the support accessories (e.g., mixers, valves and connecting tubes) were also shown for the new capillary columns. Our immediate response was to say “about time”; at last we are starting to realize the advantages of moving to capillary systems and at last instrument and column manufacturers are starting to introduce the products.

We have read and understood many articles over the last 35 years talking about the advantages of capillary columns; this includes the first paper we are aware of in microcolumn LC by Horvath² who used 0.5–1.0 mm i.d. stainless steel columns packed with large pellicular materials (15 µm) for the separation of ribonucleotides. This like many other classic papers was ignored until the mid 1970s when Ishii demonstrated the use of slurry-packed PTFE columns.³ This work was performed with 1.0 mm i.d. columns, which in the late 1970s was really pushing the instrumentation boundaries, particularly for pumps, micro-injectors and, especially, detectors. From these developments we then saw the classic work that really laid down the foundations of the miniaturization of LC columns by Novonty.⁴ But here we are in the 21st century and we are only just starting to see the expansion of capillary and maybe nanocolumns into the laboratory and out of the hands of researchers. Why is this so when all the work of Novonty and others has shown so many advantages in terms of sample size, small volumetric flow-rates, and the enhanced detection performance that can be obtained by using concentration-sensitive detection devices.⁵ The answer must lie in the old “Catch 22” circular argument of needs versus instrumentation versus new applications versus existing applications and that it is only now with the new breed of mass spectrometer and the new requirements in the fields of genomics that manufacturers are developing the instrumentation for miniaturization in LC columns.

Miniaturization and the Manufacture of Capillary and Nano LC Columns

By miniaturization we are referring to the reduction of the internal diameter (i.d.) of a column and also to the reduction in the extra-column dispersion sources. It is this extra-column dispersion that has caused so many problems in the miniaturization of columns and may have caused the lag in the uptake of capillary and nanocolumns. Today more work is required on the fittings, interconnects and external interfaces that support any form of miniature column.

In general the miniaturization of LC columns has gone along the lines of packing conventional LC column material, developed for the 4.6 mm i.d. columns, into small i.d. tubes manufactured from steel, PEEK and fused silica. Now we are also seeing the combination of these materials (e.g., PeekSil,⁶ SteelLined fused silica⁷) and in the very latest developments the integration of the columns onto a silicon substrate. The capillary columns are still packed using variations on the packing methods developed for the larger format columns, in
that gases, supercritical fluids, liquids or electro osmosis methods are used to transport the particles into the columns. In general terms the most common particle size for capillary columns is 3 µm based on area distributions, but we are unaware of any specially developed packing in the 2–3 µm range. The efficiency of the capillary columns is influenced by the same parameters as those affecting the efficiency of conventional columns. Research groups such as ours8 and others9 have tried to gain a better understanding of the packing process by optimizing empirical methods based on trial and error. There is still a great deal of work to be done in this area, but groups are now producing, by their own individual in-house methods, optimal columns with a reduced plate height near two. Columns with i.d.s of less than 200 µm are commonplace in capillary electrochromatography10 and the research from these and the use of even smaller i.d. columns has shown some very interesting results. A number of studies11 have shown how, when the Knox-Parcher ratio (i.e., the column-to-particle ratio) falls below six then the reduced plate height of a packed column in LC reduces by a factor of two. Research by the Jorgenson12 group explained this observation by suggesting that the inhomogeneities in the mobile phase flow paths are reduced by the more uniform cross sectional packing structures of small i.d. columns because the packing is wall ordered. The decrease in the packing structure variation also leads to a more uniform retention factor, across the column, which in turn reduces column band broadening. Also, smaller i.d. columns allow for rapid transcolumn diffusion between all possible flow paths. These observations and results must be applied into the development of nanocolumns. The use of exotic particles can also be considered in capillary columns (Figure 1). As a 4.6 mm × 25 cm column will use in the order of 2 gm of packing material, a capillary column will use in the order of mg. Hence, the large production method used to manufacture supports for classic columns can be discarded and new methods can be used that only produce gram quantities of material but which are monodispersed (Figure 2). In particular, solid ceramic materials as manufactured by the Stober-type processes can be used and if employed in conjunction with the packing research described above will result in the development of very efficient columns.

However, over the last few years we have seen a new type of support gaining popularity in both capillary columns for CEC and LC; the monolithic support. The manufacture of monolithic columns for LC can be divided into three general classes: organic polymer, inorganic and hybrid organic–inorganic monoliths. An excellent review on monoliths has recently been published by Zou13 that outlines many different manufacturing methods. It is unfortunate nonetheless that very little data is provided in papers regarding the reproducibility of monoliths, in that each individual casting into a capillary or nanocolumn is in effect a different batch. Economics suggest that a full QC in terms of surface area, pore size, pore volume cannot be done on each column. However, manufacturing a 1 kg batch of particles allows in depth QC to be performed and batch-to-batch reproducibility maintained. This is clearly required and as a 320 µm i.d. capillary column will use in the order of 70 mg; even accounting for column loss this is sufficient for over 10 000 columns all with the same batch. We suggest that packed capillary and especially nanocolumns have considerable advantages over monoliths.

When we consider columns we must also look at open tubular columns. Knox pointed out that by using columns with inner diameters smaller than 5 µm very high efficiencies could be achieved.14 An added advantage of the open tube is that it has a 20–30 times higher permeability than a packed tube so that the length of the capillary can be increased without the penalizing high back pressure of a packed column. So, on the one hand we are reducing the i.d. of the column, certainly miniaturization, but on the other we are also increasing the total length of the column. However, the two do become compatible when the open column is manufactured on a silicon wafer.

This is maybe the way forward for the miniaturization of LC columns. To date we have seen very little work and no commercial products as it is outside of our normal chromatography base and requires very specialist instrumentation. However, this aspect is developing and new wafer-based columns are being manufactured using a variety of techniques borrowed from the electronics industry. The most exciting of these methods is dry etching and in particular dry reactive ion etching (DRIE).

In this technique a flux of chemically reactive gas is directed towards the wafer surface in a reactor. This can be in the form

**Figure 1:** Monodispersed silica beads.

**Figure 2:** Monodispersed silica beads packed into fused-silica tube.
of plasma; such techniques can give microstructures with vertical sidewalls, which are independent of the crystallographic structure. So, almost any material can be dry etched by choosing the appropriate combination of plasma chemistry and ion bombardment energy as can be seen in Figures 3 and 4, where vertical pillars are used as frits and flow directors. Using this technique we are only starting to see the form factors that are available and how by designing new channels new columns both packed and open can be developed.

**Column Design**

The basic column design of a length of tubing in which the packing material is held in place by frits, which are in turn held in position by compression fitting, has changed very little since 1977. In capillary columns the same principles are applied except that the tubing is commonly of fused silica, but the end fittings are still of a compression type. This has led to many of the difficulties in the use of capillary systems, the reason being that in using the scaled-down type of end fittings it is still very difficult to connect capillary columns into a capillary instrument and ensure the connectors do not introduce any extracolumn band-broadening effects. For any LC system the loss in column plates based on extracolumn effects should not exceed 10%. The variance, of course, is proportional to the retention volume $V_r$ and inversely proportional to the plate count $N$. If it is assumed that the plate count is constant over a chromatogram then the retention volume is influenced by the column volume and the retention factor such that:

$$\sigma^2_{\text{acc}} \leq 0.10 \sigma^2_{\text{column}} \leq 0.10(\pi r^2 \alpha_c)^2(1+k)^2HL$$  \[1\]

Taking a retention factor of 1 and a column length of 15 cm, then extracolumn variances for micro, capillary and nanocolumns are given in Table 1.

So, to make nanocolumns feasible the extracolumn effects must be reduced by over 30 000, from micro-sized columns. Quite a challenge, and something we do not think can be achieved using conventional, although miniaturized, end fittings!

It is however pleasing to note that manufactures are now taking up this challenge and are addressing the points of real zero dead volume connections, small frit volumes, the ability to obtain square cut tubing so as to obtain clean butt connections; and of course the columns must be tough and easy to use. An interesting solution to these considerations has been offered by SGE, by integrating the connecting tubing with the column as shown in Figure 5.

Here 50µm PEEK-lined, fused-silica tubing is used as direct connections from both the injector and then to the detector so minimizing the dead volume. The column end connection incorporates a pressed steel mesh again to keep the dead volume down to an absolute minimum. New work has also started to appear on the use of cartridge-type columns. These cartridges have the appearance of a PCMCIA card as used in portable computers. Internally they appear to be of two basic types: (1) a tightly coiled, fused-silica column or (2) a column etched from a silicon substrate and then packed with either a conventional packing material or monodispersed beads as shown in Figure 6.

Research is still ongoing with these columns, with emphasis in particular on the effect of the tight bends on band broadening. However, there are also reported to be intelligent columns that can carry information on the packing material including methods that can then be read by the instrument. This places the separation column at the centre of an instrument’s logic, surely the true place for it to be.

**Mass Spectrometry**

The availability of atmospheric pressure ionization (API) techniques such as electrospray and chemical ionization has been a real driving force behind the requirement for micro LC columns because MS is arguably the best chromatographic detector available for both qualitative and quantitative analysis. API has always been a technique that is very well suited to very...
low flow-rates as flows greater than 10 µL/min cause noticeable performance loss. By using micro or nano capillaries\textsuperscript{15,16} it was shown that by taking the capillary end to a very fine tip, of the order of microns real benefits could be seen in the gain of sensitivity. This is believed to come from the fact that small droplets are produced in the nm range compared with microns for conventional ESI, so leading to improved ionization efficiency from the higher surface to volume ratio, and also to the fact that a greater proportion of the ions enter the mass spectrometer because the capillary is placed much closer to the ion source. However, the main problem with these nanotips is that they are extremely fragile and can block very easily. In our own work\textsuperscript{17} we have packed 3 µm spherical Spherisorb particles into 100 µm capillaries with an outlet taper of approximately 10 µm i.d. No outlet frit was required as the material self blocks. Electrical contact was made at the end of the taper using either gold or silver paint. However, these tips were still very fragile and not at all robust. We have made attempts to overcome this by developing a very open porous silica bead that is gold plated. This bead is 120 µm in diameter and has pores of 1–2 µm. This bead, by careful micro-manipulation, can be placed at the end of a micro-column and act as both a retaining frit for the LC packing material and also as the nano tip, because each individual 1–2 µm pore acts as an individual tip. But maybe the best and most robust interface between the LC and MS has been reported by the group of Karin Markides,\textsuperscript{18} who use a conductive polypropylene/graphite mixture to coat fused-silica capillaries for sheathless ESI. These have shown excellent chemical inertness and long-term stability.

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**Conclusions**

The new requirements for detection of pico and femtomole amounts of proteins from numerous studies is leading to the development of capillary and nano LC columns coupled to the detector of choice for such studies, the mass spectrometer. However, today there are still bottlenecks and drawbacks in the robustness of the systems and the technical skills of the users. We are sure such words were written in the early 1970s when 4.6 mm columns started to appear on the market!

Now instrument manufactures have taken up the research ideas and are commercializing new capillary instruments and columns and addressing the issues of connectors and dead volumes. Hopefully, in another few years we will be wondering why there was such a delay in the use of capillary and nanocolumns coupled to mass spectrometers. The exact format of the new miniature columns still has to be assessed; whether they will be of a conventional packed type using 1–3 µm particles, manufactured from monoliths, manufactured from etching silicon, or even using open tubular principles is impossible to say. But it is a very exciting area and one that will see major developments over the next few years.

**References**


Until 1995 Peter Myers was the technical director at Phase Separations, responsible for the Spherisorb product range. He now runs his own consultancy company X-tec, specializing in the optimization and miniaturization of separation techniques. Academically, he has visiting chairs at the University of Leeds and York.

Keith Bartle is an Emeritus Professor at the University of Leeds where for the last ten years he has led the Leeds separations group. This research group has developed new methods for separating complex mixtures, usually on microcolumns, and using gas, liquid and supercritical fluids as mobile phases.