2011 年 1 月 色 谱 Vol. 29 No. 1
January 2011 *Chinese Journal of Chromatography* 3 ~ 5

Special Dedication DOI：10.3724/SP.J.1123.2011.00003

Cutting through the confusion in high performance liquid chromatographic column technology

Robert STEVENSON*

(*International Scientific Communications, Inc., Shelton, CT 06484, USA*)

Abstract：Column packings continue to evolve as the needs of users for high efficiency, high resolution and highly sensitive high performance liquid chromatographic (HPLC) analysis drive further developments. In comparing and contrasting modern HPLC columns technologies, diameters of column packings and particle materials are covered. Some products and applications of modern HPLC columns are provided. Future directions in packing developments are predicted in this introductory article.

Key words：high performance liquid chromatography (HPLC); column packings; silica particles

CLC number：O658 **Document code**：A **Article IC**：1000-8713(2011)01-0003-03

The last six years have seen three competing column technologies (sub 2 μm column packings, Halo column packings and monolithic column technology) try to supplant the status quo in high performance liquid chromatographic (HPLC) columns. For the record, the baseline until early 2005 was packed bed columns using 3 μm spherical porous column packings. These can provide about 10 000 plates in a 15 cm long reversed phase column. Other phase chemistries, or modes such as ion exchange often offer less efficiency and lower peak capacity. However, most chromatographers would prefer to use columns with the best available efficiency, since this is directly related to the ease in achieving a separation of interest. Yes, one can often improve separations by looking for optimum separation selectivity, but the current state of the art makes this search mostly empirical and hence time consuming. Most of the computer aided simulation software such as DryLab [1] starts with an experimental run or two with a particular stationary and mobile phase, and then expands this empirical base case using well developed extrapolations. Ab initio prediction of chromatographic separation without resorting to empirical or calibration reference points is still mostly a dream, especially as the molecular complexity increases.

To date, most of the leading research on new column packings occurs in the occidental world. For LC instruments, China is probably the second largest market behind the USA, but the number of cutting edge research papers is small, although these are increasing significantly. So let's look at the consensus on column technology that is developing in the occidental world. This is based primarily upon lectures presented by Mr. Jupille [2] (LC Resources, Walnut Creek, CA) and Prof. Paull [3] (Dublin City University, Dublin, IR).

The general correlation of improved efficiency and speed with reducing the diameter of column packings is well known (Table 1). Reducing the size by a factor of two usually improves efficiency by more than twice, but at the expense of back pressure, which quadruples with the two fold size reduction. In the mid 1990's firms such as Micra Scientific (Now Eichrom, Darien, IL) and Bischoff Chromatography (Stuttgart, Germany) introduced column packings with diameter as small as 1.5 μm. These failed to win user acceptance, since the instrumentation (fittings, pumps and injectors) was too limited in pressure and detector response time. In 2004, Waters introduced the ultra high performance liquid chromatographs

* **Corresponding author**：Robert STEVENSON, PhD, editor of Separation Science for International Scientific Communications, Inc. E-mail：rlsteven@comcast.net.

Received date：2010-11-17

(ACQUITY UHPLC systems) that provided a complete user friendly chromatograph that could take advantage of the performance offered by the columns. ACQUITY had one conspicuous figure of merit. It could operate reliably at any pressure up to 15 000 psi. This pressure was required by short columns packed with 1.7 and 1.8 μm diameter particles. Detectors needed to operate with sampling rate of 50 Hz or higher to keep up with the peaks. Over the last five years, the major occidental vendors have all responded to Waters' lead with instruments capable of operating at more than 10 000 psi and at least 50 Hz sampling rate. Premium price is the other key metric of these instruments. Even with the advanced column technology the practical limit for plates is about 50 000. To get more plates, one must take the counter intuitive approach and use long columns packed with 5 μm or larger particles, and then wait and wait some more.

Table 1 Scale factors for a typical reversed phase liquid chromatographic separation using packed bed columns with the listed particle size (d_p)

d_p/μm	R_S ($F=1/d_p^{1/2}$)	N ($F=1/d_p$)	p/psi ($F=1/d_p^2$)
10	1.08	6050	105
5	1.62	13850	425
3	2.04	22200	1180
1.7	2.46	32250	3660

R_s: resolution; N: number of theoretical plates; p: pressure; F: scale factor. note: The particle size of the column packing is the primary determinant governing the separation. All columns have the same dimension (150 mm × 4.6 mm, eluted with 60% acetonitrile at 1 mL/min, 35 ℃). In practice most HPLC separations require only 10 000 plates. Thus, it is often possible to reduce the column length as the particle size decreases. This saves time and reduces the required pressure.

What about more efficiency from even smaller particles than 1.7 μm? A recent report by Prof. Wirth (Purdue University, Lafayette, IN) at HPLC 2010 in Boston, reported 100 μm i.d. capillaries packed with 50 nm silica particles. These generated very high efficiency (plate heights of 100 nm for proteins and 300 nm for small molecules) using electrochromatography (CEC). Since the columns are also very short (~1.5 cm), the pressure drop and frictional heating are manageable. Column plate count is 150 000 to 50 000 plates.

The global installed base of UHPLC capable instruments is about 18 000 and growing by several thousand per year. In contrast, the number of active HPLC Instruments with pressure rating of 6 000 psi or so, is about 300 000. These are called legacy instruments since the technology really dates from the 1970s, but these give very good performance and excellent reliability, especially if one does not need the ultimate in detection sensitivity and speed offered by the UHPLCs.

Dr. Kirkland [4] of Advance Material Technologies (Wilmington DE) dusted off some technology from the 1960s called Zipax® [5] which featured a porous layer of stationary phase around a solid core of 35 μm diameter silica. The thin active surface provided rapid mass transfer, and the large particles made the columns easy to pack. Typical columns were 1 m long and ran at only a few hundred psi. He shrunk the size of the solid core to 1.7 μm and added a porous surface silica surface. These are now called "Halo" column packings. They sacrifice some of the column capacity due to the solid core, but mass transfer in and out of the column packing is fast, so efficiency is high. The main feature is that the particle diameter is very close to 3 μm, which is about the limit that is useful with legacy (6 000 psi) instruments. Halo columns provide a significant improvement in efficiency and resolution over the corresponding column packed with 3 μm porous particles, and at the same pressure drop. And, with the large installed base of legacy instruments, these columns are quite popular.

Monoliths are the third emerging, potentially competitive HPLC column technology. The open bed structure of both the silica and polymeric monoliths offers excellent efficiency (comparable to good quality columns packed with 3 μm particles) but require only about 15% of the pressure for comparable length and flow rate. This means that one can use longer columns before running up against the pressure limit of the instrument. And, longer columns give more plates and hence resolution.

In Sep. 2010, Prof. Paull, received the International Ion Chromatography Symposium (IICS) Award for his work on monoliths for stationary phase in IC. The title of his award lecture was "Polymeric Monolithic Phases: The Future or a Fading Novelty?" is both provocative and timely. Despite the efforts of several development teams, problems persist. He cited poor column-to-column reproducibility as a fundamental problem. Ultra high efficiency is another. Monoliths do offer very low pressure drop but have difficulty competing with the column efficiency provided by columns packed with sub two micron porous particles. Apparently, as one reduces the unit cell size of monoliths to get more efficiency, the through pores are choked off, thus the back pressure increases very rapidly. This diminishes the potential advantage of monoliths to provide long high resolution columns for rapid separation of complex samples. Another issue is the lack of multiple vendors for silica and polymeric monoliths. Potential customers are cautious about relying on single source vendors. The primary patents on organic monoliths start to expire in 2012, so more competitors well certainly appear. Some will probably offer technology that is very similar to today's products.

Going back to the performance issues, Prof. Paull reports that monolithic beds polymerized in situ are amenable to making engineered structure along the bed by photografting bonding sites and then attaching ligands. Masks can control the bonding of the surface chemistry providing varying type and density. This facilitates creating novel devices such as open tubular capillaries with different monolithic films along the surface. Another possibility is taking advantage of the low pressure required for existing monolithic columns to instruments made with common plastics. As an example, he pointed to the SIChrom™ from FIAlab (Seattle, WA). This instrument is rated to 700 psi, which should be more than sufficient for today's monolithic columns. Prof. Paull concluded that monoliths will certainly find a niche in separation science.

The take home message is, select your columns for HPLC to fit your particular needs and also capabilities. One can certainly do good work with legacy (6 000 psi) instruments. This is especially true if the assay results are not time critical as in closed loop control systems. One should avoid falling under the influence of the promoters of new technology for its own sake. Certainly, it is nice to have the prestige of having the premium performance instrument, but it is easier to rest at night if you know that the probability of getting an urgent call from the lab late at night is not very likely. The choice is yours.

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