Advances in Liquid Chromatography Particle Technology: Perspectives on the Development and Future of Monodispersed Silica Supports

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Over the past decade there have been a number of exciting advances in liquid chromatography particle technology. Initially, the development of commercial sub-2 μm particles in 2004 (along with the instrumentation required to effectively use them) provided improved efficiencies over common 3 μm and 5 μm materials. Novel 2.7 μm superficially porous particles (SPP) were then introduced in 2006 that provided greater efficiencies than similarly sized fully porous particles (FPP) and were comparable in performance to sub-2 μm particles. Columns packed with SPP particles could thus be utilised in place of smaller particles to obtain similar efficiencies but without the burden of high backpressures. Although the SPP architecture was initially designed to improve mass transfer kinetics, further research has revealed that the increased efficiencies observed for small molecules has been largely due to improvements in both eddy diffusion and longitudinal diffusion. This improvement may be partly due to much narrower particle size distribution resulting from the SPP construction process. These discoveries have led to a revival of research and development concerning the importance of monodispersed particle technologies for liquid chromatography. This article highlights recent efforts to understand the importance of low particle size distribution and offers perspective regarding the future of monodispersed (< 10% RSD) particle technology in separation sciences.

Introduction:

During the past decade there have been several advances in particle technology leading to higher efficiency and thus the potential for faster separations or greater resolution. The trend began in the early 2000s with the introduction of small sub-2 μm particle sizes and ultra-high pressure chromatography (UHPLC) instrumentation. As predicted in 1969 by Knox [1], these smaller particles provide increased efficiency and allow for even faster analyses as one may increase the flow rate well above optimal linear velocities without much penalty in loss of efficiencies due to the decrease in the so-called C term of the van Deemter equation. The downside to the smaller particle approach, however, is the inherent increase in backpressure that results from their use. In 2007, Kirkland [2] described an alternative approach to achieving increased efficiency in high-performance liquid chromatography (HPLC) with the introduction of a 2.7 μm superficially porous particle (SPP). SPP particles (also called core-shell, solid-core and Fused-Core®) are characterised as exhibiting a solid nonporous core surrounded by a porous shell with similar properties of the familiar fully porous particles (FPP). The advantage of the SPP architecture is that efficiencies are obtained that are similar to much smaller particles and thus at a much lower cost of backpressure. This lower operating pressure enables users with traditional HPLC systems, with few modifications, to achieve highly efficient separations [3]. Since its introduction, SPP technology has gained a strong hold in the modern analytical laboratory. Most major column manufacturers have adopted some form of the particle design and an ever increasing variety of surface chemistries have become commercially available [4]. Although the SPP architecture was initially designed to improve mass transfer kinetics, further research has revealed that the increased efficiencies observed for small molecules has been largely due to improvements in both eddy diffusion and longitudinal diffusion. This improvement may to be partly due to much narrower particle size distribution resulting from the SPP construction process [5]. These discoveries have led to a revival of research and development concerning the importance of monodispersed particle technologies for liquid chromatography [6]. This article highlights recent efforts to understand the importance of low particle size distribution and offers perspective regarding the future of monodispersed (< 10% RSD) particle technology in separation sciences.

Narrow Particle Size Distribution: A Controversy

Information found in the literature regarding the impact of narrow particle size distribution (PSD) on column efficiency is highly contradictory. Some reports indicate that a large PSD has no influence on efficiency whereas others indicate a negative impact of large PSD on chromatographic performance. The major issue in many of these evaluations seems to be the lack of ability to decouple the property of particle size distribution and the impact of the column packing process itself. Bruns, et. al. [7] utilised computerised simulation studies to independently assess the impact of particle size distribution on column morphology and thus could interpret the impact of narrow PSD on column efficiency. The authors noted a particle size segregation effect, where in larger column volumes, smaller particles were found preferentially nearer the column walls than toward the centre. The impact of this is an increase in transchannel dispersion that may lead to lower efficiencies. The authors note that the contribution of this effect on eddy diffusion in capillary columns is negligible. It is possible, however, that the impact may be greater with larger internal diameter columns. Narrow PSD may thus improve overall bed morphology. In a similar study, Daneyko, et. al. [8] measured
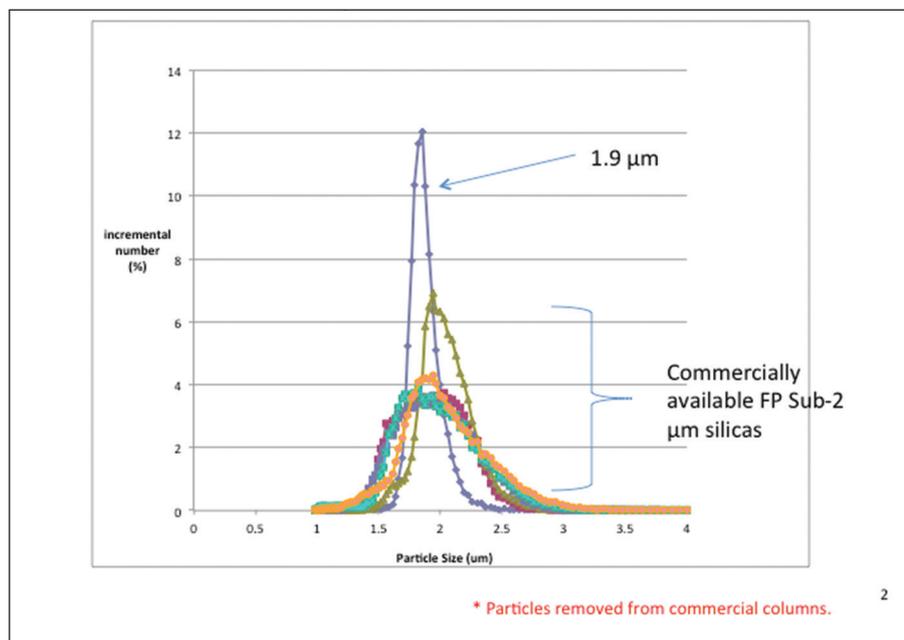
Figure 1: Particle Size Distribution Comparison – Sub-2 µm Porous Particles
This feature translates in an efficiently packed bed of with more plates in shorter columns resulting in higher efficiency, in a rugged column.
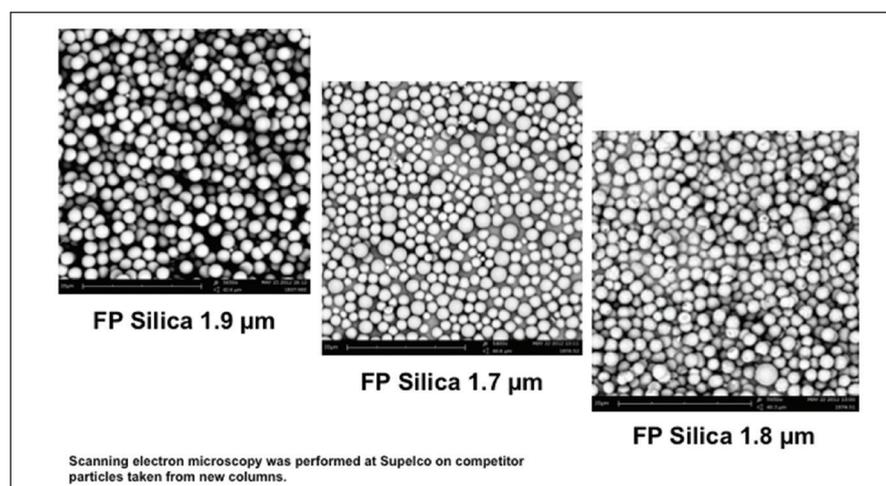

Figure 2: SEM Comparison of Narrow PSD and Commercially Available Sub-2 µm Fully Porous Particles

on separation efficiency using theoretical modelling. The group concluded that the width of the PSD does indeed impact the efficiency where the largest impact of the PSD is on the intraparticle diffusion. The authors moreover state that the impact of this would be negligible on small molecules, but should have a large effect on large molecule separations where intraparticle diffusion becomes more dominant. In addition, the group studied the impact of bimodal particle size distributions on column efficiency and found no advantage over unimodal phases. This latter result may shed some light on several of the previously discussed studies that yielded no evidence of efficiency gains with narrower PSD, where the deliberate broadening of the PSD with clearly different size particles would inherently create a bimodal distribution.

From the above discussion it is clear that there continues to be a lack of direct correlation of increased efficiency with narrow particle size distribution. Until recently only SPP particles with very narrow (~5%) PSD and nonporous silica packings were commercially available. Investigations attempting to use SPP particles as a baseline for impacts of PSD on efficiencies and then relating this to the larger PSD values of FPP is complicated by other contributing factors of the SPP architecture such as the impact of the core on the B term contribution [12]. Betz et. al. [13] introduced the first commercial fully porous material that exhibits narrow PSD in 2012 and described fully porous, 1.9 µm particle size C18 particles with a with narrow particle size distributions that were shown to generate reduced plate heights below 2. Whether the observed efficiency is due to an intrinsic property based on the narrow PSD, the packing process or a combination of both, it is clear that tightening the particle size distribution for fully porous particles is a potential means to significantly improve column performance.

Performance Investigations of 1.9 µm Titan C18

Figure 1 compares the particle size distribution of three fully porous particles which exhibit differing PSD and Figure 2 shows an SEM comparison of same particles. Both of these Figures demonstrate the 1.9 particle production process results in much narrower PSD (approximately 10% RSD) versus the other FP particles (typically 15%-30% RSD) [6]. A comparison of performance on a simple test mix between the three

the influence of particle size distribution on hydraulic permeability and eddy dispersion via simulation studies. Here again only a slight intrinsic advantage in terms of lower eddy diffusion could be attributed to the narrow PSD in the small scale models. The authors rather attribute lower eddy diffusion characteristics of columns packed with narrow PSD particles to column packing processes. Cabooter, et. al. [9] studied the relationship of particle size distribution of commercially available FPP and SPP phases and chromatographic performance. The authors noted a linear relationship between common column quality parameters of plate height, the value of the A-term (eddy diffusion) in the van Deemter equation and the minimum separation impedance with particle size distribution. Each of these quality parameters monotonically

improved with smaller PSD, suggesting that narrow PSD indeed provides increased column performance. Liekens, et. al. [10] investigated the impact of deliberately broadening the PSD of a commercially available 1.9 µm batch of particles with 3 µm and 5 µm particles on efficiency and permeability using relative performance plots, kinetic plots and impedance number analysis. The authors found that broadening of the PSD in this manner does not lead to any kinetic advantages. Although the addition of 25 wt% of 3 µm material to the 1.9 µm material showed a small effect on band broadening, the authors conclude from this work that PSD could be increased or decreased over a rather broad range without major improvement or deterioration of column efficiency. Horvath, et. al. [11] recently reported on the impact of PSD
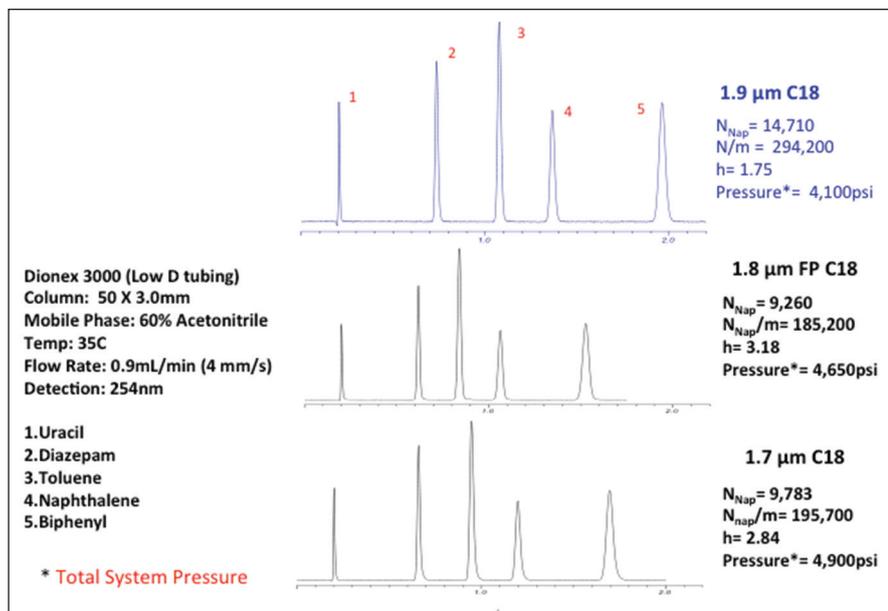
Figure 3: 1.9 μm C18 Performance Comparison to Commercially Available sub-2 μm FP Columns
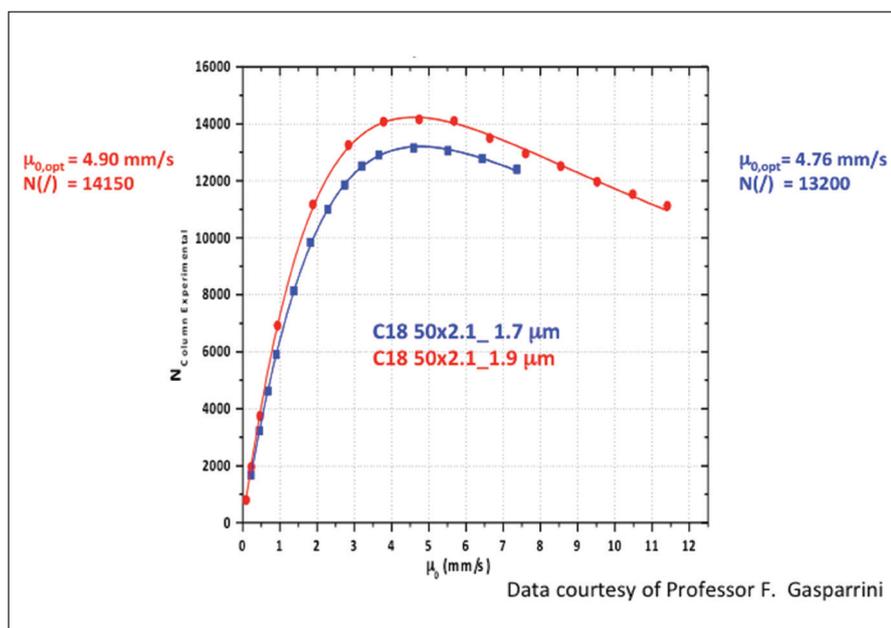

Figure 4: Column efficiency vs. Linear velocity: Comparison of 1.9 μm C18 and Commercial 1.7 μm C18

particle formats is presented in Figure 3. The narrow PSD column shows increased efficiency over the other sub-2 μm columns even though they are packed with smaller particles. Column efficiency vs. linear velocity for the particle with the narrowest PSD is compared to a 1.7 μm column in Figure 4. Although the monodispersed particle exhibits the largest particle size, the packing shows superior efficiency throughout the range of linear velocities studied.

Sommella, et. al. [14] recently reported on a comparison of sub 2-μm core shell and 1.9 μm Titan columns for use in the second dimension of two-dimensional (2D) LC/MS

analysis of peptides in expired milk. H vs. μ plots demonstrated very similar performance between the two columns. Kinetic performance data showed comparable performance in the short length/high velocity region making them both amenable to the second dimension in 2D studies where separation speed is required. The authors also noted that the greater retention observed for the narrow PSD phase resulted in improved performance where larger second dimension injection volumes (or modulation times) were employed.

Gritti and Guiochon [6] evaluated the 1.9 μm Titan C18 (80 Å) columns in detail. The authors observed reduced plate heights of 1.7 – 1.9 for 3.0 and 2.1 mm internal

diameter columns exhibiting lengths of both 50 mm and 100 mm. Benchmark commercially available columns packed with 1.8 and 1.7 μm fully porous particles and 1.7 μm superficially porous particles generated intrinsic reduced plate heights of greater than 2.0. The explanation for the performance of the columns was attributed to a small diffusion coefficient (B-term) that was found to be 30% lower than most silica C18 exhibiting broader PSD. In a second paper, Gritti and Guiochon [15] used accurate and precise measurements of plate heights, internal porosities, average pore sizes and pore size distribution as well as intraparticle diffusivities and internal obstruction factors to determine the fundamental cause of Titan's exceptional performance. The authors conclude that the high efficiency observed is again not due to the narrow PSD but to low intraparticle diffusivities and that the low diffusivity values are mainly due to a small internal obstruction factor and a relatively small average pore size. In a subsequent paper, Gritti and Guiochon [16] investigated the impact of increased average pore size for 1.9 μm Titan C18 columns where the pore size was raised from 80 Å to 120 Å. The authors observed similar intrinsic reduced plate heights of 1.7 for the 120 Å material and an approximately 10% gain in column efficiency relative to the 80 Å columns. The advantage of the wider pore structure was concluded to be most apparent in the C-term, resulting in a slightly higher optimum linear velocity for the 120 Å packing as compared to the 80 Å material.

Conclusions:

The controversy over the true impact of particle size distribution on separation efficiency is clearly not over. Fundamental research targeting this parameter and its impact on efficiency is expected to continue. The availability of the first fully porous narrow PSD particles will likely play a key role in this effort. As has occurred with sub-2 μm and SPP particles [4], it is expected that additional particle sizes, pore geometries and surface chemistries utilising the narrow PSD feature will become available in the near future. Although the reasons are yet to be clarified, the low PSD fully porous 1.9 μm columns have been shown to provide excellent chromatographic performance that is sure to add fuel to the debate.

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