Chromatography Products

The Role and Importance of Particle Size Distribution on Chromatographic Efficiency and Back-Pressure for Silica-Based HPLC Columns

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Abstract

There has been considerable attention to improving chromatographic efficiency by either decreasing the mean particle size of the silica, or by using nonconventional particles. While the increase in efficiency (N) is well understood to occur as the silica particle size is decreased, this does come at a cost – increased column back pressure. An overlooked variable that can affect both efficiency and back pressure is the distribution of the silica particle size. While it is customary to report mean particle size, most manufacturers do not necessarily report the distribution around this mean. This distribution may affect the manner in which the particles orient as the bed is formed during the packing process, and thus cause changes in both efficiency and column back pressure.

This presentation will demonstrate the chromatographic impact of particle size distribution. Several different columns will be evaluated which only vary in terms of the distribution around the same mean particle size. Differences in performance will be summarized so that we are better able to predict the practical relationship between particle size distribution and chromatographic performance.

Equipment

Particle Sizing:

HOSOKAWA ACUCUT® Model A-12 particle size classifier.

LECO MicroTrac LT100 particle size analyzer.

HPLC System:

Agilent 1100 HPLC consisting of a quaternary pump, autosampler, and variable wavelength detector with semi-micro flow cell.

Columns:

Six 150mm x 4.6mm columns with a controlled mean particle size of 5 microns

HPLC Conditions:

Mobile Phase: 96/4 Hexane/Isopropanol

Flow Rate: 1.0 mL/min

Wavelength: 254 nm

Temperature: Ambient

Test Probes: Restek Normal Phase Test Mix Part Number #35004 (5uL injection)

Peak #1 Benzene

Peak #2 Benzaldehyde

Peak #3 Benzylalcohol

Peak #4 4-Methoxybenzyalcohol

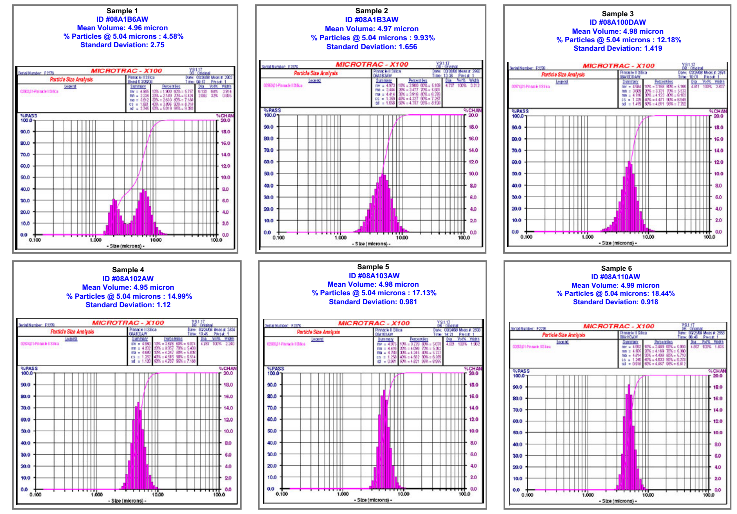
Concentration: 3mg/ml each

Samples

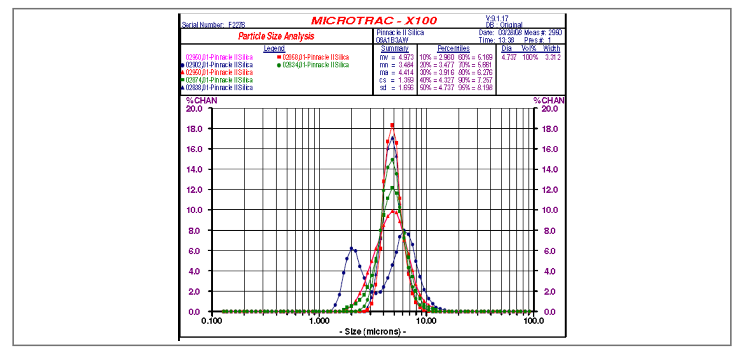
For this experiment, six 5 micron Pinnacle™ II Silica samples were selected and analyzed on a LECO MicroTrac LT100 particle size analyzer. The goal was to collect a total of six samples offering a broad particle size distribution while maintaining a particle size distribution centered on a mean of approximately 5.0 microns. Each collected silica sample was then packed into 150mm x 4.6mm ID LC column and tested chromatographically using an Agilent 1100 HPLC system with a normal phase test mix (Restek part number #35004).

Table 1 below summarizes both the physical and chromatographic data collected for these six samples. The actual individual data for these six samples can be seen on the sample figures 1-6 that follow.

Sample No.	Sample ID	Column Efficiency (plates/meter)	Column Pressure (bar)	Particle Size Standard Deviation	% Particles @5.04 microns
1	08A1B6AW	119378	102	2.745	4.58
2	08A1B3AW	104836	77	1.656	9.93
3	08A100DAW	93552	74	1.419	12.18
4	08A102AW	80302	70	1.120	14.99
5	08A103AW	77579	42	0.981	17.13
6	08A110AW	80113	36	0.918	18.44



Overlay of figures (samples 1-6) just shown.



Effect of Efficiency and Pressure on Particle Size Distribution

Graph 1 shows the effect that the particle size distribution (% particles @5.04 microns) has on column efficiency (plates/meter) when the distribution is decreased from 18.44 % to 4.58%, as was done with samples 6-1 shown above.

Upon review of the data for samples #6 thru #4, the 5.04 micron size distribution was seen to decrease from 18.44% to 14.99% with relativity no change seen in column efficiency. This factor indicates that narrowing the particle distribution near 5.04 microns in excess of a 14.99% population was not making any additional contribution towards improving column efficiency.

In samples #4 thru #1, where the distribution was widened further from 14.99% to 4.58%, while still maintaining a mean of 5 microns, there was a dramatic increase in column efficiency of nearly 50%-indicating better column efficiency. The highest efficiency is achieved with columns having the widest particle size distribution around the 5 micron mean.

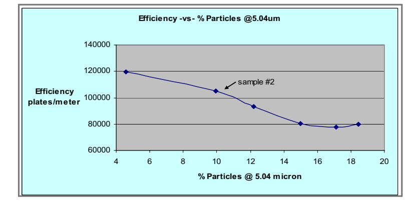
Graph 2 shows the effect of the same particle size distribution changes (% particles @5.04 microns) and the effect they had on the column back pressure (bar).

Again, examining the last three samples 6-4, the 5.04 micron size distribution decreased from 18.44% to 14.99% with the mean held at 5 microns. A near two-fold increase in column pressure was observed. Earlier in graph #1 no noticeable gain in column efficiency occurred for the same change, but the column back pressure rose from 36 to 70 bar!

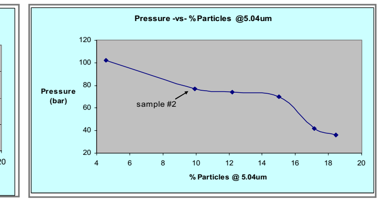
As the particle size distribution is widened further from 14.99% to 9.93% with the mean maintained at 5 microns (samples 4-2), relatively no change in column pressure occurs until the distribution (population) is decreased further to 4.58% (sample 1). Here the pressure increased nearly 33% from 77 to 102 bar.

A column such as column #1, though it offers the highest column efficiency, is not a good choice because of its high back pressure. Choosing a column similar to sample #2 is a a better choice for optimum performance. Column #2 offered the best compromise between maximum efficiency and minimal column pressure of all the 5.04 micron size distribution materials shown above.

Graph 1



Graph 2



Particle Size Distribution Comparison Typical vs. Experimental

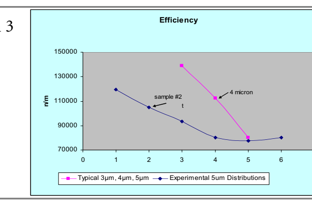
Typical Data for a 150mm x 4.6mm ID Analytical Column

Table 2 contains actual data collected from a standard 3, 4, and 5 micron, 150mm x 4.6mm ID analytical columns. This is typical data for what would be expected for a purchased commercial analytical column of that respective particle size. This data will be used for comparison purposes in the remaining slides.

Micron ACTUAL	Pressure (bar) ACTUAL	Efficiency n/m ACTUAL
3	79	139073
4	49	112231
5	36	80113

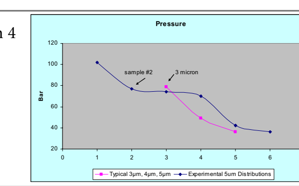
Graph 3 shows the efficiency relationship between the six distribution samples shown above compared to standard 3, 4, and 5 micron columns. As predicted, a typical 3, 4, and 5 micron analytical columns give a linear increase in column efficiency as the silica particle size is decreased. Using this information and the data from Graph 1, it can be determined that 5.04 micron sample #2 with a broad distribution of 9.93% and efficiency of ~105K n/m is very close to that for a standard 4 micron column that typically has efficiency of ~112K n/m.

Graph 3



Graph 4 shows the relationship between particle size and column pressure for the six different distribution samples and typical values for commercial 3, 4, and 5 micron columns. As predicted by using the data collected in Table 2, an increase in column pressure occurs when replacing a 5 micron column with a column pressure of 36 bar with a 3 micron column at 79 bar. The data from Graph 4 below suggests the 5.04 micron sample #2 column with a pressure of 77 bar is nearly equivalent to that of a typical 3 micron column at 79 bar.

Graph 4



Conclusion

Many chromatographers and manufacturers believe that to obtain the maximum column efficiency silica particles must have a tight particle size distribution. By broadening the particle size distribution away from the mean it was conventional wisdom that broad particle size distributions resulted in columns with low or poor efficiency.

In contrast, our data shows that a substantial increase in column efficiency can be obtained by keeping the particle size mean at 5 microns and decreasing only the particle size distribution (having fewer particles @5.04 microns and creating a broader particle size distribution). However, increasing column efficiency by changing the particle size distribution also increases column back pressure.

Therefore, to select a 5 micron particle size column, with both maximum column efficiency and minimal column back pressure from the presented data, the best compromise is sample #2 with a distribution of 9.93%. Sample #2 produces 104,836 plates/meter with only 77 bar of column back pressure. For comparative purposes this 5 micron, 150mm x 4.6mm ID column offers an efficiency similar to that of a 4 micron column, while having the pressure equivalence of a 3 micron column.

General Applications

Restek Corporation, 110 Benner Circle, Bellefonte PA 16823, 1-800-356-1688 www.restek.com