Do Sub 2um Particles Offer the Best Performance for Short Fast Gradients?

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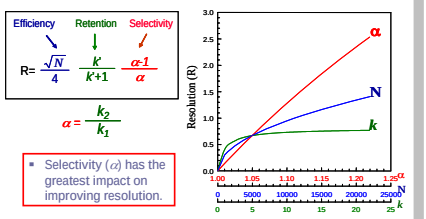
Introduction

The current trend towards using high pressure in LC is well documented, high efficiencies, good resolution and fast throughput being the goal that has driven the move towards the use of sub 2um particles.
Analysts however can achieve much if not all of these variables by correct use of current systems. By optimising the column packed bed and utilising an optimised column hardware design, excellent separation, speed efficiency and resolution can all be achieved. Sub 1min run times can be achieved by the correct use of 3um particles without the associated pressure issues, allowing analysts high throughput on regular systems.

Improving Resolution

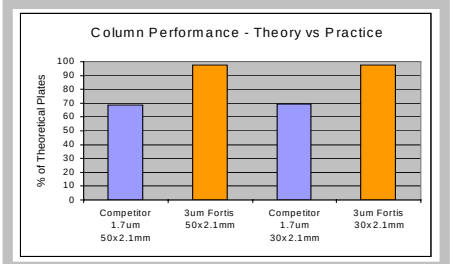
Approaches to improving resolution involve making changes to one or more of three variables; efficiency, retention and selectivity. Recent advances in HPLC instrumentation have been driven by the requirement to run with high backpressure whilst using sub 2um particles as the stationary phase. The move towards using sub 2um particles has been driven by the theory that the resultant jump in efficiency will lead to significant improvements in resolution. As can be seen in figure 1 efficiency (N) does play a significant part in improving resolution, however by far the greatest factor is column selectivity. Although the majority of chemistries produced and sold in column format by manufacturers are C18 it should not be assumed that they have the same selectivity. Therefore rather than switching to sub 2um particles and the expense of an instrument capable of running at extreme pressures it might be wise to test some alternative C18's or alternative chemistries.

FIGURE 1. Contributions to Resolution



The degree to which resolution is improved by the use of sub 2um particles is dependent on the efficiency gained by switching to the smaller particle columns. However the smaller the particle the greater the difficulty in preparing a well packed column bed, when we then compare the actual column efficiency versus what would in theory* be possible to achieve we see large deviations in performance.

FIGURE 2. Column Performance vs. Theory



*Snyder, L. R.; Kirkland, J. J.; Glajch, J. L. Practical HPLC method development. 2nd Edition, Wiley, New York, 1997. N=3500x L / dp

In figure 2 we can see the efficiency for two different column formats both containing 3um Fortis™ C18 particles and a competitor sub 2um particle. Both columns were tested under isocratic conditions using Naphthalene as a test probe. The results show that the sub 2um particle column falls considerably short of its theoretical potential, whereas the columns packed with 3um particles achieve the level of performance expected.

When studying the factors affecting resolution shown in figure 1 we can see that choosing efficiency as the factor to improve resolution means that relative resolution gain can be interpreted as the ratio of the square root of any efficiency gained. The fact is that in certain column geometries the packed bed deficiency seen for sub 2um particles means that resolution improvement gained by moving to sub 2um particles can be as little as 10%.

Experiment 1: Resolution Gain versus Theory

Column:50x2.1mm

Mobile phase: Acetonitrile/$H_2$0 60/40 0.5ml/min

Test Compound: Naphthalene

Instrument: HP1200

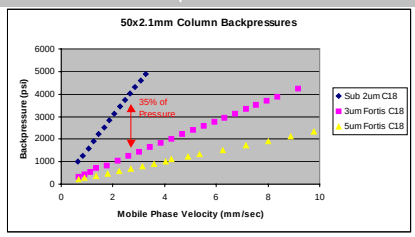
Results:

	3um Fortis™ C18	Sub 2um C18	Sub 2um Theory
Column Plates	6368	7640	10294
√N/4	19.95	21.85	25.36
Resolution Gain >3u	-	9.5%	27.0%
Back pressure	122 Bar	347 Bar	-

The results of experiment 1 show that, for the 50x2.1mm geometry tested, the gain in resolution achieved by the sub 2um particles is in fact only 9.5%. This is a very low gain considering the back pressure increase generated by the small particles and the associated issues that come with running at increased pressure. Furthermore when the use of gradient conditions are applied to the columns this small resolution gain will be further diminished.

Figure 3 shows a comparison of backpressure values for a range of particle sizes in a Water/Acetonitrile mobile phase, it can be seen that the sub 2um particle generates a threefold increase in backpressure.

FIGURE 3. Column Backpressure



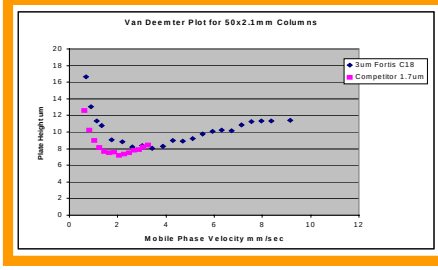
Real Life Van Deemter Curves

Van Deemter curves have been extensively published in the promotional data put forward for the application of sub 2um particles. These plots are generated under isocratic conditions and the column diameter typically being 4.6mm as these tend to provide a better packed bed. However this can mislead the chromatographer into believing that they themselves will see large improvements in their chromatography by switching to a sub 2um column. In reality each Van Deemter plot is specific to the set of conditions, column geometry and instrument used to generate the data.

Experiment 2:
Two 50x2.1mm columns containing different particle size C18 phases were tested under identical conditions on two separate HPLC systems, one designed to run at higher than standard pressures (up to 600 Bar) and the other not (up to 400 Bar only).

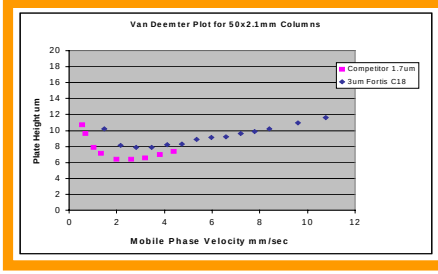
Results:
As can be seen in figure 4a. when using standard pressure ranges on a system that has not been optimized to reduce system volume,apart from backpressures generated, there is very little if any difference in the optimum performance of the 3um and 2um materials.

FIGURE 4a. HP1100 System Result



However when the same columns are tested on a system that has had the system volume reduced in order to run small particles there is an improvement seen in the performance of the 2um column as seen in figure 4b.

FIGURE 4b. HP1200 System Result



This shows how the extra system volume is detrimental to the performance seen for small particle columns and that the use of these columns on standard HPLC systems is not likely to offer the chromatographer any improvement in performance.

Peak Capacity

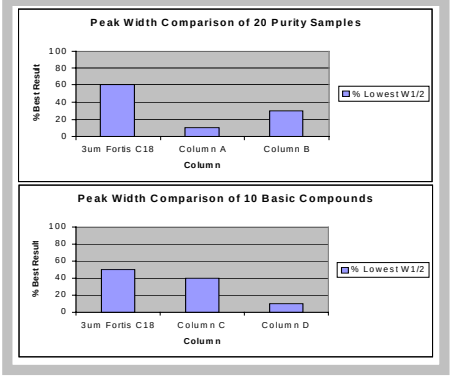
Peak capacity is often used as a measurement of performance, by calculating the theoretical number of peaks that could fit within a gradient based on the average peak widths of a set of compounds run across that gradient. For sub 2um particles to offer an increase in peak capacity they would need to provide a significant decrease in mean peak width.

Experiment 3:
Using a short acetonitrile/TFA gradient on a HP1200 a range of 20 pharmaceutical purity samples and 10 basic compounds were analysed. Analytical columns containing sub 2um and 3um particles were compared, and the peak widths at half height were measured for comparison.

Column A Sub 2um, 50x2.1mm (Manufacturer 1)
Column B Sub 2um, 30x2.1mm (Manufacturer 2)
Column C Sub 2um, 50x2.1mm (Manufacturer 3)
Column D 3.5um, 50x2.1mm (Manufacturer 2)
Fortis™ C18 3um, 50x2.1mm (Fortis™ Technologies)

Results:

FIGURE 5. Peak Widths



As figure 5 shows, when compared against three different manufacturers sub 2um products for the analysis of 20 purity samples the Fortis C18 3um column gave the narrowest peak width for 60% of the samples. For the 10 basic compounds the Fortis C18 3um column gave the narrowest peak width for 50% of the samples.

Short Fast Gradients

Figure 6 shows how little or no advantage is gained by using a sub 2um particle C18 for the analysis of alkylphenones in 1.5mins, the pressure increase of 50% in order to reduce peak width by 0.1 seconds would seem to be unjustified.

FIGURE 6. Alkylphenones C2–C8

Short well packed 3um columns offer the opportunity to obtain equivalent resolution at greatly reduced backpressure, alternatively this reduced backpressure can be used to increase flow and speed up analysis.

FIGURE 7a. Speed - Equivalent Pressures

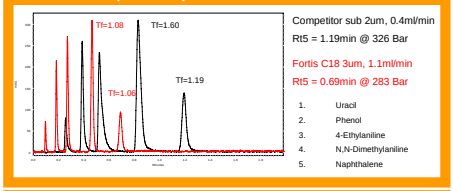
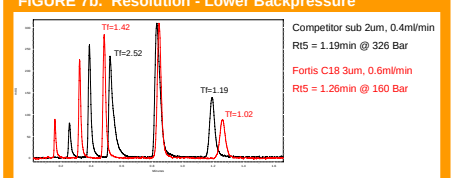


FIGURE 7b. Resolution - Lower Backpressure



Conclusion

Long columns with small particles can provide extremely high resolution or peak capacity. However when studying the use of small particles for fast chromatography in short column formats they appear to offer no improvement when run on standard HPLC systems. Under isocratic conditions it is possible to achieve 85% of the efficiency of sub 2um particle columns with only 1/3rd of the back pressure by using a well packed 3um column.
When run under fast gradient conditions it is difficult to see any performance benefit by moving to sub 2um particles, even on optimised systems, gradient conditions negate any small efficiency gains seen from sub 2um particles.
The decision as to whether the analyst moves to use sub 2um particles should be judged on an individual case by case basis.