# Sub-2µm Particles and Beyond: Effect of Particle Size on Efficiency, Back Pressure, and Analysis Time for HPLC and UHPLC Separations

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## Abstract

Recently considerable attention has been paid to improving chromatographic efficiency by using smaller particle size silica for liquid chromatography. While decreasing particle size does increase efficiency (N) and provide faster analysis times, it does so at the cost of increased column back pressure. As a result, the speed of the analysis and total number of theoretical plates that can be achieved by reducing particle size is limited by the column back pressure. This presentation will demonstrate the chromatographic impact of mean particle size on theoretical plates, analysis time, flow rates, and column back pressure. Several different columns will be discussed which have mean particle size diameters ranging from 1.9 to 5 microns. The limits of using smaller particles, and/or longer columns, to achieve greater efficiencies will be explored and discussed in terms of back pressure and the number of theoretical plates as a function of time. The trends shown will help in determining the optimum column configuration, given the practical limitations of the current HPLC and UHPLC instrumentation.

## Introduction

Next to selectivity, the two most fundamental factors that limit any HPLC separation are efficiency and column back pressure. The total number of theoretical plates that can be obtained are governed by column length and particle diameter. By increasing column length or decreasing particle diameter greater resolution can be achieved, however these measures also increase column backpressure. Recently there has been increased interest in increasing efficiency by the use of smaller particle stationary phases for HPLC. This presentation will demonstrate the chromatographic impact of mean particle size on;

1) Column efficiency
2) Column back pressure
3) Analysis time

## Experimental

**Equipment:**
Jasco XLC liquid chromatograph consisting of binary gradient pump, auto sampler, and variable wavelength detector.

**Columns:**
Pinnacle DB C18 1.9um 50x3.0mm
Pinnacle DB C18 2.2um 50x3.0mm
Pinnacle DB C18 3.0um 50x3.0mm
Pinnacle DB C18 5.0um 50x3.0mm

**Mobile Phase:**
Isocratic 55% Acetonitrile 45% Deionized Water

**Test Sample:**
Uracil, Toluene, Ethylbenzene, Propylbenzene, Butylbenzene, Pentylbenzene
Concentration: Uracil 0.05mg/mL, all others 10mg/mL

Calculation of efficiency:
Calculation of efficiency was performed on the toluene peak of the test sample using the USP method in EZChrom Elite data acquisition and analysis software.

## Reduced Plate Height, h and Column Efficiency

To compare columns packed with particle of different diameters, Calvin Giddings introduced the concept of reduced plate height ***h***. [1] The reduced plate height is a dimensionless number that is defined as the ratio of the plate height ***H*** divided by the particle diameter ***dp***. This allows us to estimate the performance of a "good" column independent of particle size. [2] Thus we have a normalized way to compare the performance of columns packed with different particle sizes. The best column efficiency is achieved as the reduced plate height approaches the theoretical limit of 2 particle diameters.

$$h = \frac{H}{d_p} \qquad (1)$$

$H$ = Heigth Equivalent of a Theoretical Plate
$h$ = Reduced plate height
$d_p$ = Particle Diameter

The reduced plate heights for "good" commercially available columns are typical in the range of 2.25 to 3.00. To be in this range, the reduced plate height h was set equal to 2.4 to determine a predicted efficiency for columns with various particle sizes. The number of theoretical plates N for a 50mm column where then calculated using equation (2).

$$N = \frac{L}{h * d_p} \qquad (2)$$

$N$ = Number of theoretical plates
$L$ = Column length

## Column Backpressure

Equation (3) describes the variables that contribute to column backpressure. It is important to note that backpressure is inversely proportional to the square of the particle diameter

$$P = \frac{250 L \eta F}{d_p^{\,2} d_c^{\,2}} \qquad (3)$$

$\eta$ = Mobile phase viscosity
$d_c$ = Column ID
$F$ = Flow rate

To determine a predicted backpressure for columns with various particle sizes, pressures for both a 3 and 5 micron columns were empirically measured. Then equation (3) was solved for the $\frac{250 L \eta F}{d_c^{\,2}}$ term as all terms other than particle size remain constant. The predicted backpressures were then calculated for the other particle sizes.

## Linear Velocity / van Deemter Curve

It is well established that plate height will vary with the linear velocity according to the van Deemter equation:

$$H = A' + \frac{B'}{u} + C'u \qquad (4)$$

$A'$ = Contribution from eddy diffusion
$B'$ = Contribution from logitudinal diffusion
$C'$ = Contribution from mass transfer
$u$ = Mobile phase velocity

To determine the optimum linear velocity 50 x 3.0mm columns were packed with 1.9, 2.2, 3.0 and 5.0um particles. The columns were then tested at various flow rates to generate the van Deemter plot shown in Figure 1. As expected the smaller particles have lower plate heights, higher efficiency, higher optimum linear velocity, and a flatter curve at higher linear velocities.

An unexpected result occurred in the plate height values of the 2.2µm column below 2 mm/sec. It is believed to be due to some sort of experimental error, but the cause of this anomaly is currently unknown. It is not believed to be a simple measurement error as numerous repeat injections were made and consistently reproduced the results for that area of the curve. Further testing with additional columns is needed to confirm the actual trend. Also of interest is that at the slower linear velocities the B term become significant and the curves for the smaller particles become steeper and the plate heights are approach similar values. This means that a 1.9µm column run below the optimum linear velocity does not provide the increase in efficiency as one might expect.

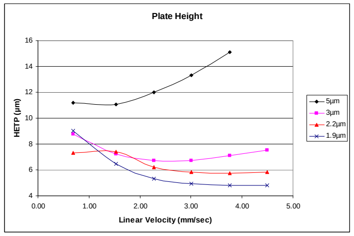

Figure 1. *The van Deemter curves for the various size particles.*

## Measured Column Backpressure

Figure 2 shows the empirically measured column backpressures for these columns at the various linear velocities.

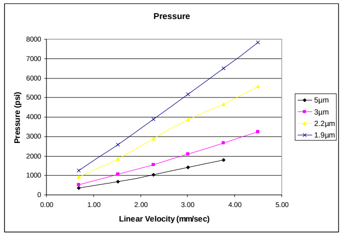

Figure 2. *Empirically measured column backpressures for the columns at various linear velocities.*

## Measured Column Efficiency

Figure 3 shows the actual column efficiencies measured at the optimum linear velocity compared to the predicted the efficiencies of a "good" column with a reduced plate height of 2.4. The 3 and 5µm columns showed slightly better efficiency than predicted where as the 1.9 and 2.2µm columns showed slightly lower efficiency. This variation is caused by slight differences in how well the columns were packed and extra-column band broadening which becomes more significant as the peak widths become narrower with the smaller particle sizes.

## References

1. J.C.Giddings,"Dynamics of Chromatography", Marcel Dekker, New York, (1965) 125.
2. L. R. Snyder and J. J. Kirkland "Introduction to Modern Liquid Chromatography" John Wiley & Sons, Inc. (1979) p.234
3. L. R. Snyder, J. J. Kirkland and J. L. Glajch, "Practical Method Development", John Wiley & Sons, Inc. (1997) pp.43-44

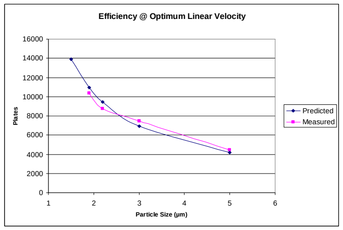

Figure 3. *Efficiencies measured at the optimum linear velocity.*

## Backpressure at Optimum Linear Velocity

Figure 4 shows the comparison of the predicted column backpressures in blue versus the actual measured pressure at the optimum linear velocity in pink. The green curve show the pressure if all column were run at 1.53 mm/sec. This is a key factor when transferring methods from 3 and 5µm to smaller particle as the optimum linear velocity is much higher resulting in higher backpressure. This is important to note because as we saw in the van Deemter plots, small particle columns used below the optimum linear velocity have higher plate heights resulting in lower efficiencies than desired.

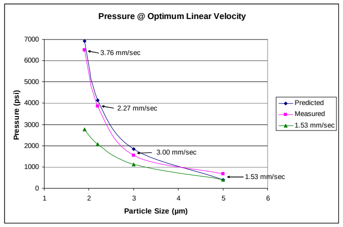

Figure 4. *Backpressure at the optimum linear velocity.*

## Backpressure Per Plate

Figure 5 shows the pressure in psi required to generate one theoretical plate or the Pressure Equivalent of a Theoretical Plate (PETP) for the various particle size columns.

$$\text{PETP} = \frac{P}{N} \qquad (5)$$

The pink curve shows the pressure/plate when run at the optimum linear velocities and the green shows the pressure/plate when all column were run at 1.53 mm/sec. This expression allows for the comparison of pressure requirements when changing from one particle size to another. The implications are that shorter columns utilizing small particles require higher pressure to generate the same number of plate as longer columns that utilize larger particles. The tradeoff however is longer analysis time for larger particles.

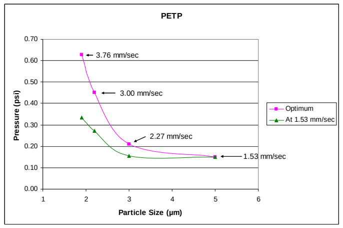

Figure 5. *Pressure required to generate one theoretical plate.*

## Time Per Plate

Figure 6 shows the time in milliseconds required to generate one theoretical plate or the Time Equivalent of a Theoretical Plate (TETP) for the various particle size columns at the optimum flow rate. This was determined by empirical measurements of tr and efficiency at the optimum linear velocity.

$$\text{TETP} = \frac{t_r}{N} \qquad (6)$$

This expression allows for the calculation of the analysis time required when changing from one particle size to another.

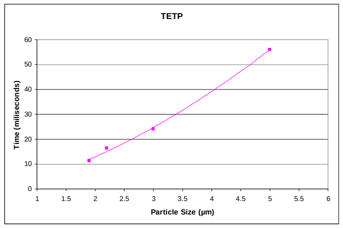

Figure 6. *Time required to generate one theoretical plate.*

Equation 6 can be written as:

$$\text{TETP} = \frac{(1+k) * h * d_p}{u} \qquad (7)$$

$k$ = k for most retained peak
$h$ = reduced plate height
$d_p$ = Particle diameter
$u$ = Linear velocity of the mobile phase

k is independent of column length, linear velocity and particle size. [3] Therefore equation 7 can be used to calculate the TETP of different size particles at any designated linear velocity. This is done by substituting the k for the most retained peak of an existing analysis along with the particle diameter, reduced plate height and optimum linear velocity of a column with a different particle size. Comparison of the TETP however is specific to the most retained compound of the analysis when using the same stationary and mobile phase under isocratic conditions.

## Analysis time

Figure 7 shows show the analysis time required to perform a separation requiring 10,000 theoretical plates N on the various particle size columns. This is

$$\text{Analysis time} = N_{required} * TETP \qquad (8)$$

In reality to generate 10,000 plates on columns of different particle sizes would require that the columns be of different lengths. In this particular case the 1.9um column would need to be 50mm in length where as a 5um column would need to be 112mm in length. Obviously columns are only available in discrete lengths and thus the optimum combination of particle size and column length may not be available for the requirements of each analysis. However this graph is based on empirically derived values of TETP and represents the realistic trend of possible analysis times for this particular separation when using different particle size columns.

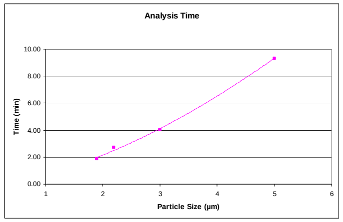

Figure 7. *Analysis time at the optimum linear velocity.*

## Conclusions

Optimum linear velocity increases with smaller particle diameters. To fully realize the advantages of the decrease plate height HETP and decreased plate time TETP when using smaller particle columns, it is necessary to operate under higher linear velocities.

Column efficiency is proportional to column length and inversely proportional to particle diameter. Column backpressure is inversely proportional to the square of particle diameter and proportional to the column length and linear velocity. Therefore as efficiency increases so to does backpressure. Furthermore, as we reduce particle size the backpressure generated per plate PETP also increases. Thus for any separation requiring a minimum number of theoretical plates, columns utilizing smaller particle will result in a higher PETP and operating back pressure.

Analysis time is somewhat more complex as it is dependant on particle size and linear velocity as well as column length. As we go to smaller particles we can use shorter columns. The flat nature of the van Deemter curves for smaller particles allow us to run at linear velocities well beyond the optimum allowing dramatically faster separation but resulting in much higher backpressures.

These relationships are mathematically defined continuums on to which a column of any particle size and length will fall. Ultimately the limiting factor for the total number of theoretical plates and analysis time is pressure. In other words there is no "ideal" particle size, column length or operating pressure. Rather there compromise between desired efficiency, analysis time and pressure limitations.

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