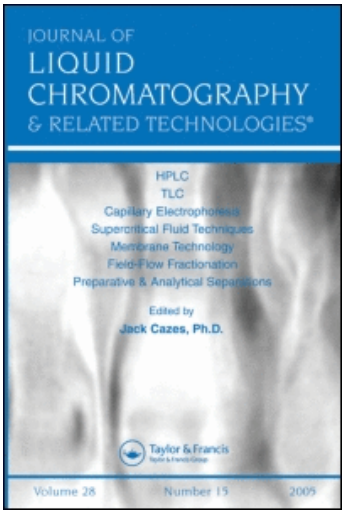

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Effect of Sub-2-Micron Particle Size on Peak Efficiency, Capacity, and Resolution in Preparative Liquid Chromatography

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Effect of Sub-2-Micron Particle Size on Peak Efficiency, Capacity, and Resolution in Preparative Liquid Chromatography

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Abstract: Near and above overload concentrations of seven separate compounds were injected onto three separate C_{18} columns containing 1.7, 3.5, and 5.0 μm particles to determine "loadability" of the column during preparative chromatography. The columns differed in particle size only, and assay conditions were consistent for all three columns. The flow rate was chosen so as to maximize efficiency (plates) for each particle size according to the Van Deemter curves. Theoretical plates for each peak, along with the resolution between peaks, were compared for each particle size before and at overload concentrations. It was found that for each compound, both the number of theoretical plates and resolution were consistently superior for the 1.7 μm particles compared to the 3.5 and 5.0 μm particles before and after overload concentrations. It was also observed that at no time did the 5.0 μm particles offer a greater loading capacity than the 1.7 μm particles.

Keywords: Preparative HPLC, VHPLC, Particle size, Efficiency, Capacity, Resolution

INTRODUCTION

The desire for superior column efficiency, resolution, and sensitivity in combination with shorter analysis time has led to the development of columns packed with decreasingly smaller particle sizes. Although the advantages of sub-2-micron particles is clearly illustrated by Van Deemter plots, the back pressures associated with these smaller particles cannot be tolerated by

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typical HPLC instruments.[1–7] This dilemma led to the development of the ultra performance liquid chromatography (UPLC®) and related very high pressure liquid chromatographic (VHPLC) techniques that can easily handle the pressures generated by these smaller particles, giving much faster analysis times thanks to increased efficiency, resolution, and sensitivity.[8]

Preparative chromatography, unlike analytical chromatography, typically involves overloading the column with analytes as much as possible, while maintaining resolution. Therefore, any system that could supply improved peak resolution and efficiency would lead to higher sample load and, consequently, a shorter isolation time. Also, a system that could operate at higher flow rates without loss of efficiency would present a definite advantage in preparative chromatography.

There are now several vendors offering analytical scale VHPLC equipment but none offering preparative scale equipment. This will remain the case until an advantage over typical preparative HPLC equipment can be demonstrated. Previous studies demonstrate superior loading capacity in 20 μm versus 80 μm particle size,[9] as well as superior capacity in 3 μm versus 8 μm particle size.[10] In this study the possible advantages of smaller (sub 2 μm) particle size in preparative chromatography were evaluated on an analytical scale by gradually overloading three separate analytical columns packed with 1.7, 3.5, and 5.0 μm particles, while monitoring peak efficiency and resolution.

EXPERIMENTAL

Chemical and Reagents

HPLC grade acetonitrile, trifluoroacetic acid, and high purity water were purchased from Mallinckrodt (Paris, KY. USA). 2-Methyl-1-pyridone, *N*,*N*-dimethylbenzamine, 4-chloroaniline, p-toluic acid, and toluene were purchased from Sigma-Aldrich. All other compounds were obtained from Pfizer Compound Management.

Instrumentation and HPLC Methods

Separations were performed using an Agilent 1200 Series quaternary pump (Agilent Technologies, USA), equipped with a 1200 autosampler and diode array detector. Three 150 × 2.1 mm columns were packed with 1.7, 3.5, and 5.0 μm Waters Xbridge C_{18} particles (Waters Corporation, Milford, MA). The HPLC mobile phase consisted of gradient elutions of acetonitrile:0.1% trifluoroacetic acid in water, in ratios ranging from 5:95 to 65:45. Data was collected and analyzed using Agilent Chemstation software.

Loading

Increasing amounts of each compound were injected individually onto each column until peak integrity was lost completely. Theoretical plates were then calculated (tangent method) for each injection on each column, and plotted versus amount injected. The compounds used were chosen to give a range of hydrophobicity and loading capacity. To prevent detector saturation, a wavelength of 280 nm was chosen so that the peak at maximum loading remained on-scale. A flow rate of 0.3 mL/min was chosen to equally maximize the efficiency of each particle size. A resolution mix containing each compound was injected onto each column in increasing amounts, until either peak integrity and/or resolution was lost. The resolution between each compound and its closest eluter was then calculated and plotted versus the amount injected.

RESULTS AND DISCUSSION

Efficiency and Load Capacity

The compounds chosen for this study were selected based on their low loadability and high solubility in a water:acetonitrile mixture. This allowed large sample amounts to be injected while keeping the injection volume small, thus avoiding loading problems found previously.[11] As the amount of analyte injected onto each column increased, the peak efficiency decreased correspondingly for each column. However, as illustrated in Figure 1 (a-f), the 1.7 μm particle column demonstrated the highest efficiency over the entire load range, followed by the 3.5 μm particle column, then the 5.0 μm particle column for each compound. Also, the load capacity for each compound was found to be the same on all the columns regardless of particle size.

Since the load capacity is about the same regardless of particle size, the advantage of the increased efficiency associated with the 1.7 μm particles at and above overload conditions is clearly seen in terms of resolution. To demonstrate this, increasing concentrations of a six component mixture were injected onto each column, and the resolution between each component and its closest eluter was calculated and plotted versus amount injected (Figure 2, a-e). In each case, the resolution was superior in the 1.7 μm column. On average, the resolution values observed in the 1.7 μm particle column near or at overload conditions were 1.7 times greater than the resolution values observed in the 3.5 μm particle column and 2.1 times greater than the resolution values observed in the 5.0 μm particle column.

While the sample load at which each column exhibits overload characteristics is about the same for all three particle sizes, the 1.7 μm particle columns present an advantage in preparative chromatography due to superior efficiency and, therefore, superior resolution and loadability. In general, the more

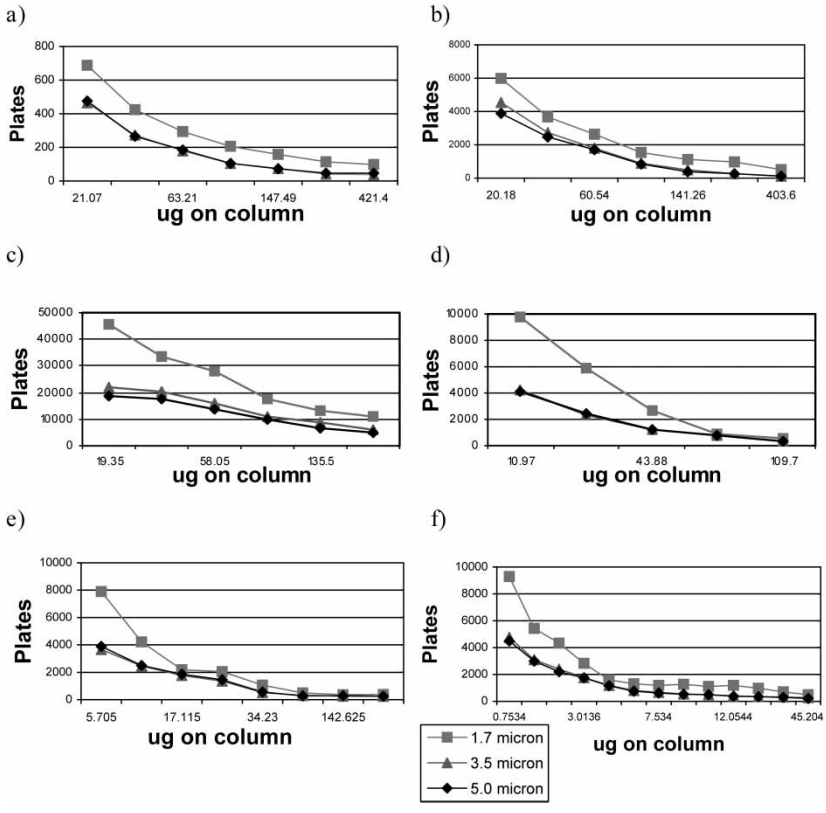

Figure 1. Theoretical plates versus column load of analyte (μg) on Waters Xbridge C18 columns packed with 1.7, 3.5, and 5.0 μm particle sizes: a) quinapril, b) p-toluic acid, c) carboxylic acid compound A, d) methyl pyridone, e) dimethylbenamine, f) 4-chloroaniline.

compound injected onto a column above the overload limit, the greater the peak broadening that leads to coelution of compounds. However, with greater peak resolution, more broadening is allowed before coelution occurs, thus allowing a larger sample load. Also, increased resolution allows for the use of shorter column lengths, resulting in shorter run times, shorter equilibration times, and less solvent consumption.

Another clear advantage of the 1.7 μm particle column can be seen in the comparison of Van Deemter curves for different particle sizes (Figure 3). This comparison illustrates that the plate height increases (and efficiency decreases) as the flow rate increases past an optimum point. However, the rate of increase in plate height observed for the 1.7 μm particle column is much lower than the rate observed for larger particles. Using a 1.7 μm particle column, a faster flow rate can be used without a noticeable loss in efficiency, resulting in shorter run times.

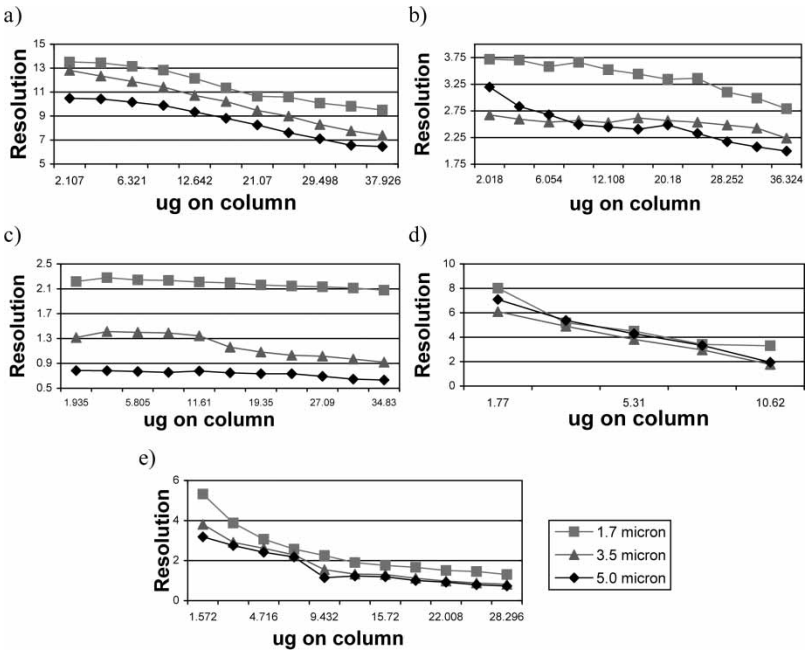

Figure 2. Resolution between indicated analyte and its closest eluter versus column load (μg) on Waters Xbridge C18 columns packed with 1.7, 3.5, and 5.0 μm particle sizes: a) quinapril, b) p-toluic acid, c) carboxylic acid compound A, d) dimethylbenzamine, e) 4-chloroaniline.

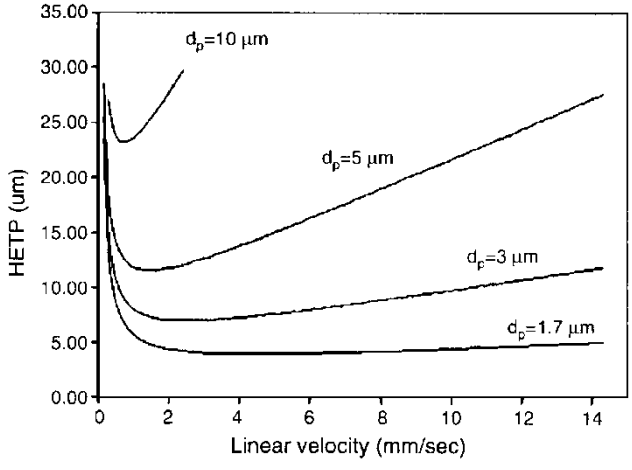

Figure 3. Van Deemter curves for 1.7, 3.5, 5, and 10 μm particle sizes (Waters Xbridge C18 columns).

CONCLUSIONS

No trade-off between smaller particle size and loading capacity in preparative chromatography was observed in our studies. In fact, an increased loading capacity in 1.7 μm particles versus larger particle sizes (3.5 and 5 μm) was demonstrated, suggesting that the use of these smaller particle sizes in preparative chromatography has promising potential.

The use of smaller particle columns and higher flows lead to a very large increase in pump backpressure. However, analytical systems, which can handle such pressures, are currently available (VHPLC), and since the backpressure associated with preparative columns and flow rates is no greater than for analytical systems, the construction of a preparative VHPLC should not be any more difficult. The backpressure could also be controlled to some extent through the use of a column oven along with a mobile phase preheater. This would not only lower the backpressure but also shorten runtimes without a loss in resolution.

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