Reports of the Death of Fully Porous Particles are Greatly Exaggerated

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The use of superficially porous silica particles in liquid chromatography dates back to the 1960s. Recently, there has been renewed interest in these particles due to several reports of their lower reduced plate heights as compared to conventional fully porous particles.[1-5] The performance claims of superficially porous particles are briefly reviewed. We demonstrate that the efficiencies and backpressures of 2.1 x 50mm columns packed with 2.6/2.7µm superficially porous particles are very similar to those of columns packed with 2.5µm fully porous particles. We also show that the efficiency of a representative 2.1 x 50mm column packed with 2.7µm superficially porous particles is significantly lower than that of the same size column packed with 1.7µm fully porous particles. We demonstrate the range of selectivities currently available for columns containing fully porous particles, as well as the ability of hybrid organic/inorganic particles (currently only available as fully porous particles) to operate with a wide mobile phase pH range, enabling dramatic changes in selectivity for ionisable compounds. We discuss the benefits of fully porous particles for purification applications and for retaining polar compounds by reversed-phase chromatography. We also demonstrate the excellent performance of columns containing 1.7µm fully porous particles for size exclusion chromatography. These results show that there are several chromatographic goals that may currently only be achieved using modern, fully porous particles.

In the early days of HPLC, pellicular (superficially porous) particles were developed to overcome the speed and efficiency limitations of large (30 – 80µm) fully porous particles.[6-8] However, these particles were soon superseded by smaller (< 10µm) fully porous particles.[9] Since the mid-1970s, columns containing fully porous particles have been used for the majority of HPLC separations. One of their principal advantages is that they generally have higher retentivity and loading capacities than superficially porous particles. In addition, in size exclusion chromatography, it is the higher pore volume of fully porous particles that enables higher resolution of various large molecular weight biological or synthetic polymers.[10]

The recent renewal of interest in superficially porous particles began with the introduction of Halo™ columns, containing 2.7µm superficially porous silica particles with 0.5µm thick porous shells.[11] Columns containing these particles showed lower reduced plate heights than columns containing fully porous particles of similar size, primarily for 4.6mm diameter columns. This was initially attributed to the more rapid solute mass transfer into and out of the porous layer. The fact that the Halo particles have a narrow particle size distribution was also claimed to contribute to the lower reduced plate heights.[4] These columns are positioned as an alternative to columns packed with fully porous 1.7µm particles, used in UPLC.

A number of studies of Halo columns have shown that the lower reduced plate heights for small molecules are not due to faster diffusion into and out of the porous layer.[12] One contribution to the lower reduced plate heights is from decreased longitudinal diffusion resulting from the lower pore volume of the superficially porous particles. A second contribution is from a decreased external film mass transfer resistance across the thin layer of mobile phase surrounding the external surface of the particles. A third contribution is from reduced eddy dispersion, at least for 4.6mm diameter columns.[13] There is no evidence that the reduced eddy dispersion arises from the narrow particle size distribution of the superficially porous particles. It appears more likely that the rougher external surfaces of the superficially porous particles are the cause.[14] It has been found that 2.1mm diameter columns packed with superficially porous particles don't show the same reduced eddy dispersion as do 4.6mm diameter columns.[4,15,16] 2.1mm diameter columns are becoming increasingly popular because they require much less solvent usage than larger diameter columns and are well suited for use with electrospray mass spectrometry.

The goals of this study were to compare the performance of columns containing superficially porous and fully porous particles and to highlight the advantages of the latter. The advantages include the broader range of selectivities currently available, compatibility with a wide mobile phase pH range, greater retention of polar compounds in reversed-phase chromatography, scalability to preparative columns and utility for size exclusion chromatography.

Results and Discussion

It has been shown that 2.6/2.7µm superficially porous particles come close to providing the efficiency of 1.7µm particles when comparisons are performed in 2.1mm diameter columns.[4] Testing in our laboratories indicates that the chromatographic performance differences between 2.1mm diameter columns containing superficially porous and fully porous particles of similar size (e.g., 2.5µm) are minimal. As can be seen in Figure 1, van Deemter curves

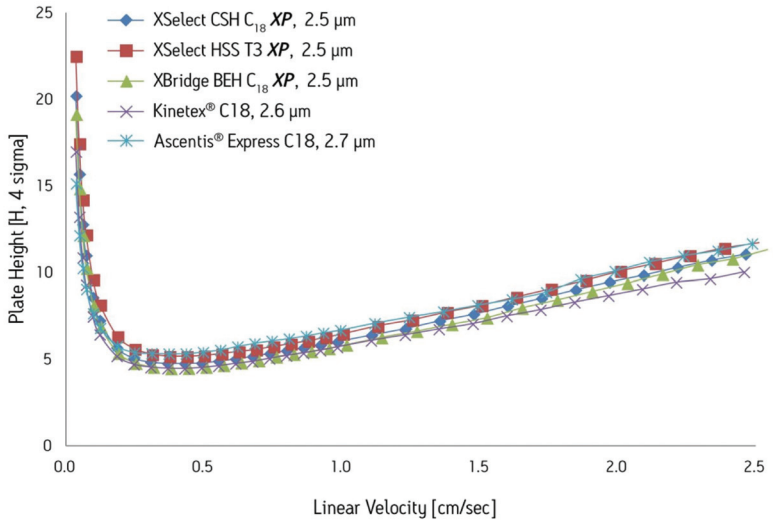

Figure 1: van Deemter curves for five 2.1 x 50mm columns. The analyte is acenaphthene, the mobile phase is a mixture of acetonitrile and water (75/25, v/v), the temperature is 30°C and the system is an ACQUITY UPLC with a tunable UV (TUV) detector. Efficiencies were corrected for system dispersion.

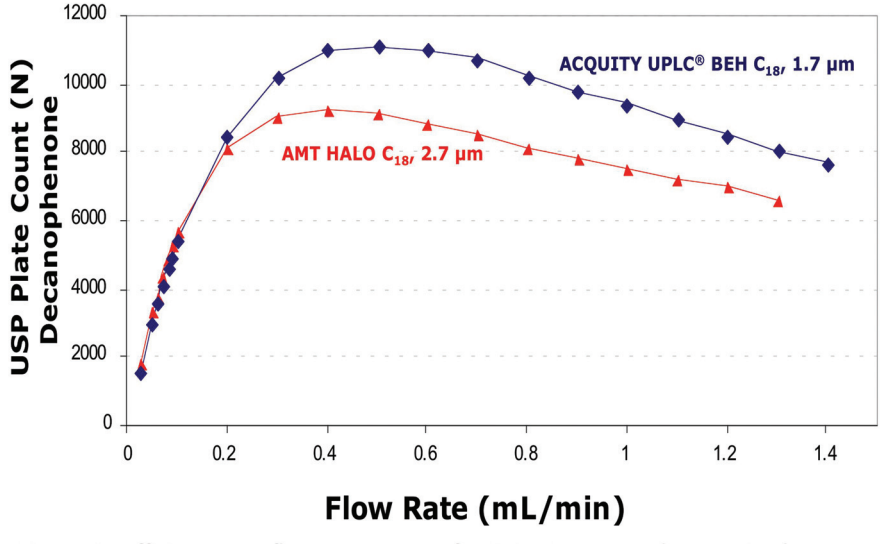

Figure 2. Efficiency vs. flow rate curves for 2.1 x 50mm columns. The analyte is decanophenone, the mobile phase is a mixture of acetonitrile and water (70/30, v/v), the temperature is 30°C and the system is an ACQUITY UPLC with a TUV detector. Efficiencies were corrected for system dispersion.

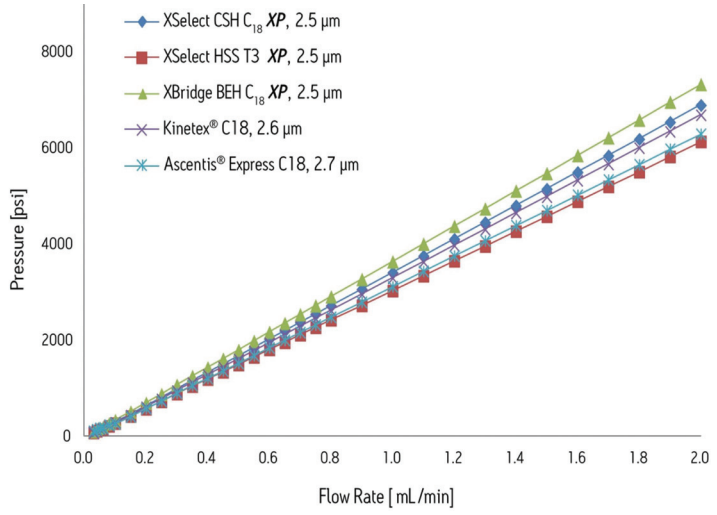

Figure 3. Backpressure vs. flow rate for five 2.1 x 50mm columns. The mobile phase is a mixture of acetonitrile and water (75/25, v/v), the temperature is 30°C and the system is an ACQUITY UPLC with a TUV detector. Pressure values were corrected for system backpressure.

for three fully porous particle columns (XSelect CSH C_{18}, XSelect HSS T3 and XBridge BEH C_{18}) and two superficially porous particle columns (Kinetex C_{18} and Ascentis Express C_{18}) were generated on the same ACQUITY UPLC system under a single set of conditions. These data indicate that all five columns produce similar efficiencies for the neutral probe acenaphthene.

In Figure 2 we show the efficiencies of a 2.7µm superficially porous particle column compared to a 1.7µm fully porous particle column on the same ACQUITY UPLC system under a single set of conditions. As expected, the smaller particle column generated higher efficiencies and had a higher optimal linear velocity.

Generally speaking, under a given set of chromatographic conditions, the backpressures generated by LC columns are a function of the particle size and how tightly the particles are packed into the column.[17] In other words, smaller particles packed at higher pressures generate higher backpressure. Factors such as particle size distribution and bonded phase have a smaller influence on column backpressure. As illustrated in Figure 3, there were small differences in observed backpressures between the 2.5µm fully porous particle columns (XSelect CSH C_{18}, XSelect HSS T3, XBridge BEH C_{18}) and the two 2.6/2.7µm superficially porous particle columns (Kinetex C_{18} and Ascentis Express C_{18}). These data indicate that the particle porosity (full versus superficial) and the narrower particle size distribution of the superficially porous particles have little effect on backpressure.

One of the primary objectives of liquid chromatography is to resolve one peak from another. Resolution (R_s) can be expressed mathematically in the form of the fundamental resolution equation (see Figure 4).
From this equation we can 'break' resolution (R_s) into two parts: mechanical (N, efficiency) and chemical/physical (α, selectivity and k, retentivity).[18] Starting from the left side of the equation there has been a great deal of attention focused on increasing the efficiency of the chromatographic separation: specifically, lower dispersion chromatographic systems capable of operating at the optimal linear velocity (and resulting pressures) of smaller (< 2µm) particle columns. Focusing on increasing efficiency alone is akin to overcoming only part of the challenge. As the arithmetic of the equation indicates, resolution can best be achieved by combining efficiency with selectivity and retentivity. This is where fully

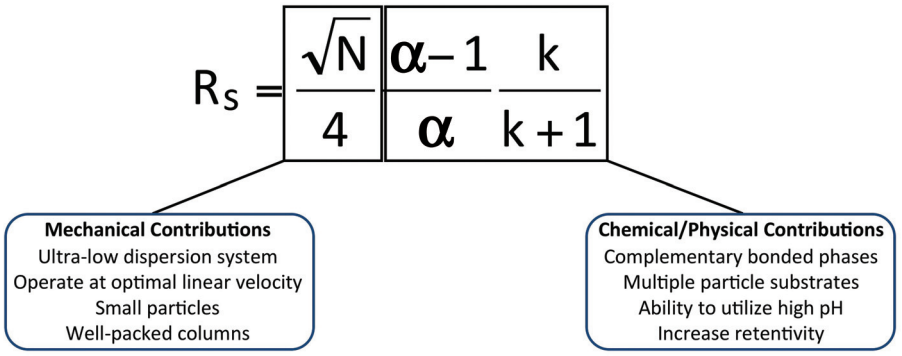

Figure 4. Fundamental resolution equation split into mechanical and chemical/physical contributions to resolution (R_S).

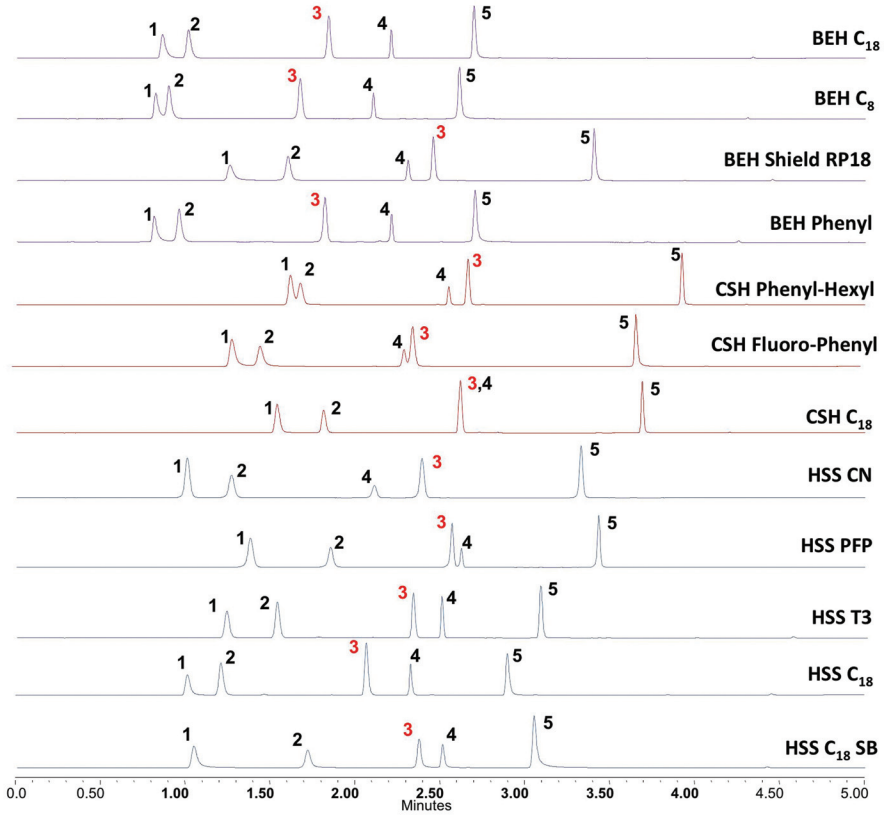

Figure 5. Reversed-phase gradient separations of caffeic acid derivatives on 2.1 x 50mm 1.7µm (BEH and CSH) and 1.8 µm (HSS) columns. The five components are caftaric acid (1), chlorogenic acid (2), cynarin (3), echinacoside (4) and cichoric acid (5). Note the retention differences for analyte 3 (cynarin) on the twelve different UPLC stationary phases. Mobile phase A is 0.1% trifluoroacetic acid in water, mobile phase B is acetonitrile and the gradient is 8 to 50% B in 4.45 min then 50 to 90% B in 0.41 min. The flow rate is 0.5mL/min, the temperature is 30°C and the system is an ACQUITY UPLC with a TUV detector (set to 330nm).

porous particle columns currently have a distinct advantage over superficially porous particle columns. Fully porous particles are available in a wide variety of bonded phases (e.g., C_{18}, C_8, Embedded Polar, Phenyl, CN, PFP, Amide, HILIC, Diol, etc.), particle substrates (e.g., ethylene bridged hybrid, charged surface hybrid, silica) and fully scalable particle sizes (e.g., 1.7, 1.8, 2.5, 3.5, 5 and 10µm). This wide variety of stationary phases allows the separation scientist to optimise selectivity and retentivity while also enjoying the efficiency benefits of modern low dispersion, high pressure capable LC systems.

An example is shown in Figure 5. The separation of caffeic acid derivatives was performed on twelve fully porous < 2µm stationary phases on a low dispersion ACQUITY UPLC system. The twelve phases shown are bonded to three different (fully porous) particle substrates: BEH (ethylene bridged hybrid), CSH (charged surface hybrid) and HSS (high strength silica). Each of these particle substrates was designed and optimised to perform slightly different chromatographic tasks. BEH particle columns are designed for pH stability;[19-21] CSH particle columns also offer improved pH stability while providing improved peak shape for basic compounds under acidic, low ionic strength conditions;[22] HSS particle columns are generally more retentive and provide additional selectivity choices.[23]

One especially powerful chromatographic tool to optimise the selectivity and retentivity of ionisable compounds is the ability to reliably operate over a wide mobile phase pH range. This was facilitated by the introduction of hybrid organic/inorganic particles.[20, 21] These hybrid based packing materials offer an extended usable pH range compared to silica-based (e.g., superficially porous) packing materials as well as excellent peak shape and high efficiency. The effect of pH on the separation of a mixture of basic, acidic and neutral analytes is illustrated in Figure 6. At pH 10 the basic compounds imipramine and amitriptyline are no longer positively charged. In their neutral state they are more hydrophobic and are retained longer on reversed-phase columns. The opposite behaviour is observed for the acidic compounds 1-pyrenesulfonic acid, fenoprofen and diclofenac which are more retained at pH 3. The neutral compounds are unaffected by pH.

One of the key benefits of fully porous particles is their utility for purification applications. Fully porous particles are available in a wide range of particle sizes and column diameters. The manufacturing procedures used for these particles are generally amenable to the production of relatively inexpensive large (≥ 5µm) particles, enabling affordable preparative columns. In contrast, the manufacturing processes used for superficially porous particles such as Halo involve a layer-by-layer growth process, where the number of layers would need to increase to produce large (≥ 5µm) particles with the same shell to core diameter ratio as the < 3µm particles.[24] This makes it difficult to produce inexpensive large (≥ 5µm) superficially porous particles. Consequently, these particles are not likely to become competitive with fully porous particles for purification applications.

In Figure 7, the benefits of scalable, high pH tolerant particles for the screening and mass-directed purification of 5-hydroxyindole acetic acid after acetylation are shown. Here, the speed and efficiency of < 2µm particle columns is used to quickly develop a screening separation. Next, after calculating the focused gradient from the UPLC screening run, the UPLC separation can be directly transferred to a preparative HPLC column format packed with larger particles (5µm) with the same stationary phase and selectivity as the screening run. This allows

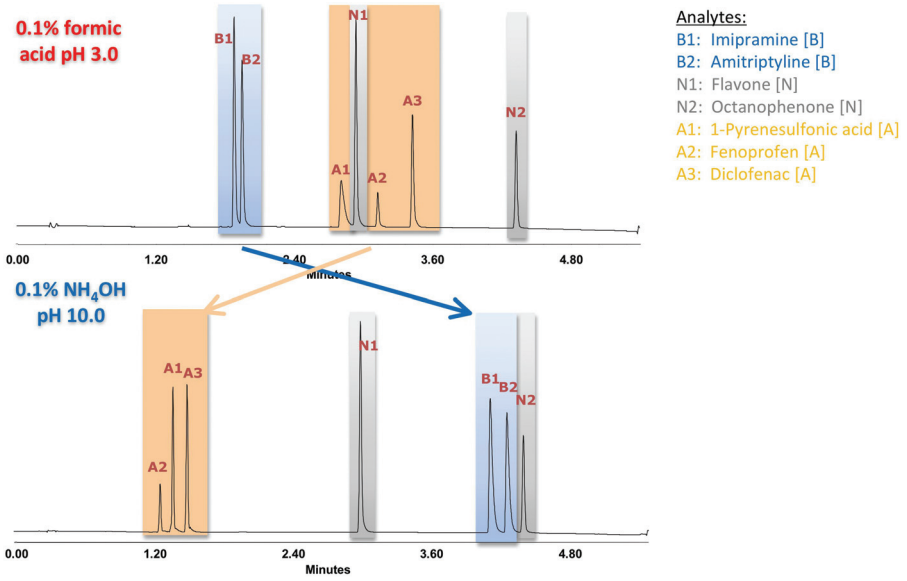

Figure 6. Effect of pH on selectivity (α) and retentivity (k) in the gradient separation of a mixture of acidic [A], basic [B] and neutral [N] analytes at pH 3 (top) and pH 10 (bottom). The column is a 2.1 x 50mm 1.7µm ACQUITY UPLC CSH C_{18}. Mobile phase A is 0.1% formic acid or 0.1% ammonium hydroxide in water, mobile phase B is acetonitrile and the gradient is 5 to 95% B in 5.0 min. The flow rate is 0.5mL/min, the temperature is 30°C and the system is an ACQUITY UPLC with a TUV detector (set to 254nm).

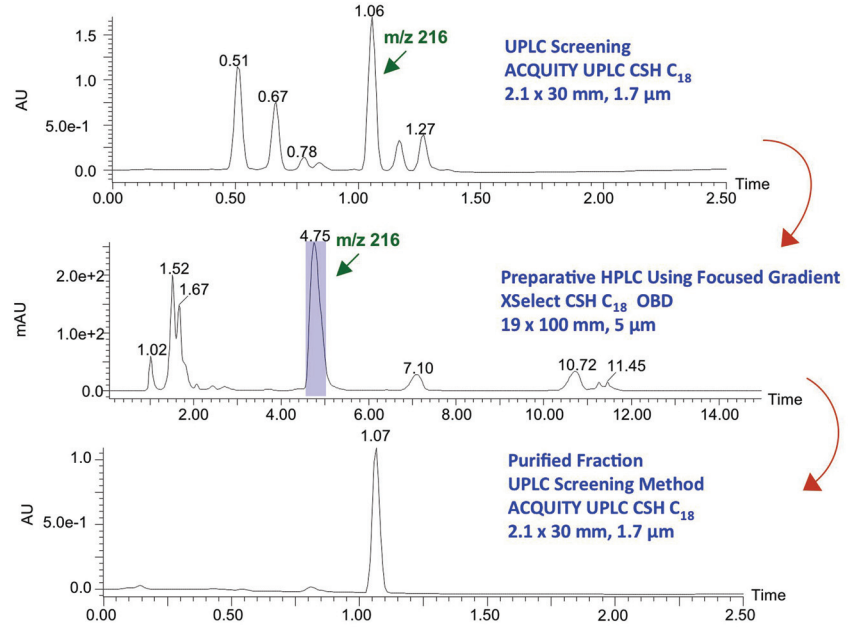

Figure 7. Screening (top), purification (middle) and purity confirmation (bottom) for 5-hydroxyindoleacetic acid reaction products. The UPLC screening result with the target peak at 1.06 min (m/z 216) was transferred to preparative HPLC using a focused-gradient (50mg loading). Mass-directed purification of the target peak results in a pure product, confirmed by analysing the purified product using the UPLC screening protocol. Mobile phase A is 0.1% ammonium hydroxide in water and mobile phase B is 0.1% ammonium hydroxide in acetonitrile. The UPLC gradient conditions are 2 to 98% B in 2.0 min, then hold for 0.5 min. The flow rate is 0.9mL/min, the temperature is 30°C and the system is an ACQUITY UPLC with a TUV detector (set to 210 and 254nm) and ToF-MS (in ESI+ mode). The preparative gradient conditions are 2 to 30% B in 0.81 min, 40% B at 10 min, 98% B at 11 min, hold for 1.0 min, reset to initial conditions for 3.0 min. The flow rate is 25.0mL/min, the temperature is ambient and the system is an Autopurification Preparative HPLC with FractionLynx (detection by UV at 210 and 254nm and ESI+ MS).

for rapid purification of the compound of interest and alleviates the need for method re-development. The use of focused gradients in preparative HPLC allows for cleaner purification of the target compound in a shorter run time compared to the full gradient, ultimately resulting in higher sample purification throughput. The mass-directed purification of the targeted m/z 216 peak results in a pure product, confirmed by analysing the purified product using the UPLC screening protocol. This is a good example of how the well understood, 'manufacturing friendly' synthesis process of fully porous particles benefits chromatographers in small molecule pharmaceutical discovery laboratories.

Another application area where fully porous particles outperform superficially porous particles is in the retention of polar compounds using highly aqueous mobile phases. The retention of polar analytes can be a challenge under reversed-phase conditions.[25] Although hydrophilic interaction chromatography (HILIC) is becoming a more popular chromatographic technique for retaining very polar compounds, there often are times when the analysis requires the simultaneous separation of very polar compounds (e.g., metabolites) along with moderately/very hydrophobic species (e.g., APIs). In these cases, a reversed-phase separation is desired. The use of 100% aqueous mobile phases can provide the retention needed to make the reversed-phase separation work. For more hydrophobic analytes, retention can be increased by decreasing the percent organic modifier in the mobile phase for isocratic separations and in the starting composition for gradient separations. However, because the shell of superficially porous particles occupies a range of ~62% to ~77% of the particle volume, depending on the particular column brand, the loss in surface area due to the non-porous core decreases the retention volume relative to columns packed with fully porous particles of a similar stationary phase. As shown in Figure 8, the retention factors (k') for the five very polar analytes are about 4 to 9 times greater on the fully porous HSS T3 column compared to the superficially porous particle column that was designed for polar retention.

An additional separation technique for which fully porous particles are superior to superficially porous particles is gel permeation chromatography (GPC) or size exclusion chromatography (SEC). The technique is used to characterise polymers, proteins and other macromolecules. SEC has been performed on a commercial basis using fully porous polymer and silica particles since the early 1960s.[26] In SEC, analytes are separated based on size. SEC columns utilise fully porous particles with a pore size or combination of pore sizes chosen for the particular analysis. In SEC, unlike other forms of liquid chromatography, the analytes elute within a very narrow volume range: typically two column volumes.[10] Therefore, the elution volume should be made as large as possible in order to maximise resolution in SEC. As stated previously, superficially porous particle columns have lower elution volumes than fully porous particle columns due to the solid, non-porous core of the packing material. Therefore, this shortcoming would need to be overcome in order to

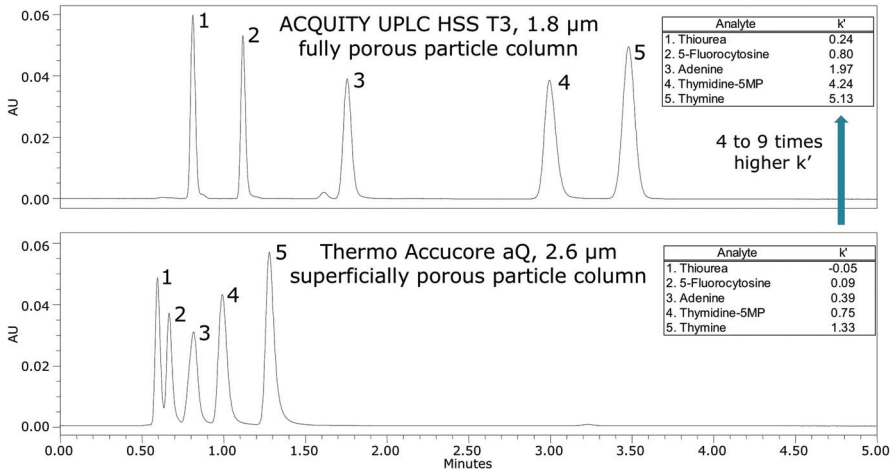

Figure 8. Comparison of retention factors for two 2.1 x 50mm reversed-phase columns designed to retain polar compounds using highly aqueous mobile phases. Note the increased retention factors (k) for the fully porous particle ACQUITY UPLC HSS T3 column (top). The mobile phase is 10mM ammonium formate, pH 3.0, the flow rate is 0.2mL/min, the temperature is 30°C and the system is an ACQUITY UPLC with a TUVe detector (set to 254nm).

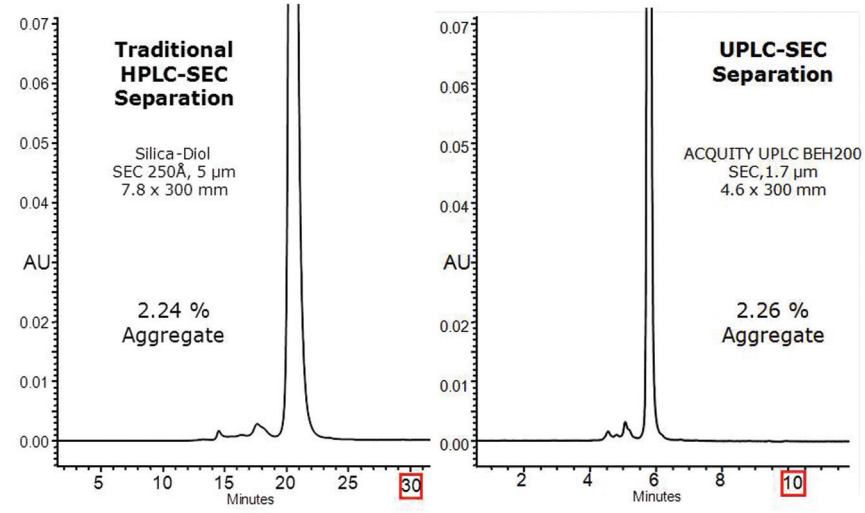

Figure 9. Comparison of traditional HPLC (left) vs UPLC (right) SEC separation of murine mAb. A 70% time savings is realised using the 1.7µm SEC column. The (same) flow rate used for each separation offered the best balance between resolution and analysis time(s). The mobile phase is 25mM sodium phosphate buffer, 0.15M sodium chloride, pH 6.8. The flow rate is 0.4mL/min, the temperature is ambient and detection is by UV at 280nm. The systems are an Alliance 2796 (HPLC) and an ACQUITY UPLC H-Class Bio (UPLC).

commercialise superficially porous particle columns for SEC. Presently, there are no superficially porous particle SEC or GPC columns commercially available. Fully porous < 2µm particle columns improve the throughput, sensitivity and resolution of SEC separations of proteins.[27-29] An example of these benefits is shown in Figure 9. A murine monoclonal antibody (mAb) SEC separation is performed using an HPLC column (left) and a UPLC column (right). The UPLC SEC separation is 70% faster than the traditional HPLC SEC separation and the resolution is significantly higher.

Conclusions

The results presented in this article provide both generic and specific examples of the advantages of fully porous particles over currently available superficially porous particles. The benefits of fully porous particles such as the much wider range of stationary phase selectivity choices, the ability to reliably operate over a wide mobile phase pH range, scalability between particle sizes and analysis platforms, increased polar compound retention and the ability to perform SEC outweigh the improved reduced plate heights exhibited by today's silica-based superficially porous particles in 4.6mm diameter columns. It is the authors' view that fully porous particles will always have a place in liquid chromatography.

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