

Hybridized Superficially Porous Particles with Broad pH Applicability and Different Selectivities

Wu Chen¹, Leszek Gora², William Long¹, Ta-Chen Wei¹, Xiaoli Wang¹, William Barber¹,

¹Agilent Technologies, Wilmington, DE, USA; ²Agilent Technologies, Middelburg, THE NETHERLANDS



Agilent Technologies

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Introduction

The most significant advancement in LC column technology in the past several years is the new generation of superficially porous silica particles. These particles provide similar efficiency as smaller size totally porous particles but significantly lower back pressure. The initial introduction of 2.6-2.7 μm superficially porous particles in 2006 was a huge success and this is followed by the introduction of either smaller (e.g. $\leq 1.7 \mu\text{m}$) or larger (e.g. $\geq 4 \mu\text{m}$) superficially porous particles.

While chromatographers enjoy the ultrahigh efficiency of these superficially porous particles, they also desire more selectivity options to facilitate method development. One way to achieve this is to use different bonded phases. Another way is to use different pH mobile phases but this requires particles that can withstand extreme pH conditions. In this regard, there are very limited options in superficially porous particles compared to totally porous particles. In this presentation, we report a novel approach to synthesize superficially porous particles that are stable in a wider pH range. Different selectivity can be achieved on the same column for various applications at low, medium and high pH.

Experimental

Synthesis of hybridized superficially porous particles (Poroshell HPH):

2.7 μm hybridized superficially porous particles (Poroshell HPH) were synthesized by our proprietary process. The final 2.7 μm Poroshell HPH particle consists of a solid core of 1.7 μm in size and a porous shell of 0.5 μm thickness (Figure 1), and have surface area of 95 m^2/g , pore volume of 0.3 cm^3/g , and pore size of 110Å. The surface of Poroshell HPH particles is chemically modified to form a thick organic layer to resistant to silica dissolution at high pH condition.

The Poroshell HPH particles were bonded with C18 and endcapped as normal. The particles were loaded into the columns, and the columns were compared with the columns packed with original Poroshell 120 as well as other commercially available totally porous particles with hybrid surface.

QC test and van Deemter Curve:

The 2.7 μm Poroshell HPH-C18 particles were loaded into 4.6x50 mm column. QC test method was following:

- QC Method: Mobile phase: 60% $\text{CH}_3\text{CN}/40\% \text{H}_2\text{O}$; Flow: 2 mL/min; Injection: 1 μL of naphthalene sample; Temperature: 25°C; Detector: 254 nm, 80 Hz.
- Van Deemter curve were generated at different flow rates (Figure 2).

Test of peak shape for strong bases:

The chromatographic performance of Poroshell HPH particles were evaluated by peak shape of strong bases at pH7.0 under following condition (for Figure 3):

- Column: 4.6x50 mm Poroshell HPH-C18 column
- Mobile: 60% $\text{CH}_3\text{CN}/40\% 20 \text{ mM NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ at pH7.0; Flow: 1.5 mL/min; Injection: 2 μL of anti-depressant sample; Temp.: 25°C; Detector: 254 nm, 80 Hz.

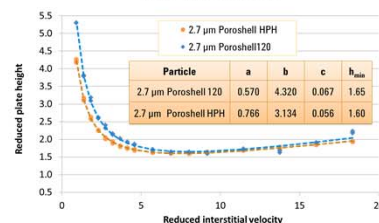
High pH lifetime test:

The hybridized Poroshell particles were tested at different extreme high pH mobile phase conditions, to evaluate their high pH stability.

- In 10 mM Bicarbonate pH10 and 50 degree isocratic condition:**
 - Stress buffer: 10 mM Ammonium Bicarbonate
 - Mix 1200 ml Acetonitrile and 800 ml Water in 2 Liter reservoir. (QC solvent)
 - Run 5 QC runs (Uracil/Butyl Benzene) at 0.4 mL/min and 50°C
 - Run Stress buffer for 120 minutes at 0.4 mL/min and 50 degree
 - Column: 2.1 x 50 mm, 1) 2.7 μm Poroshell 120 EC-C18 (part# 699975-702), 2) 2.7 μm Poroshell HPH-C18, 3) non-Agilent C18 column with hybrid surface.
- In 10 mM bicarbonate pH10 gradient condition:**
 - Mobile phase: A: 10 mM Ammonium Bicarbonate adjusted to pH 10.0 in water, B: Acetonitrile.
 - Time 0 min. 5% B
 - Time 5 min. 95% B
 - Time 5.1 min 5% B
 - Total method run time was 7 min..
 - Flow rate: 0.4 mL/min.
 - Column: 2.1 x 50 mm, 1) 2.7 μm Poroshell HPH-C18, 2) non-Agilent C18 column with hybrid surface

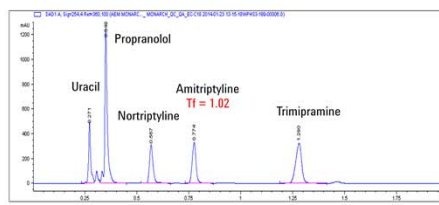
Results and Discussion

Figure 2: van Deemter plot of Poroshell 120 EC-C18 and Poroshell HPH-C18 particles



Poroshell HPH particles maintain high efficiency and low back pressure of superficially porous particles like Poroshell 120 particles.

Figure 3: Chromatogram of strong base test at pH7.0



Because of reduced surface silanol activity, the Poroshell HPH column showed excellent peak shape for strong bases at neutral pH mobile phase condition.

Figure 4: Batch-to-batch reproducibility of strong base separation

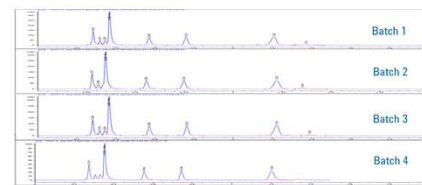
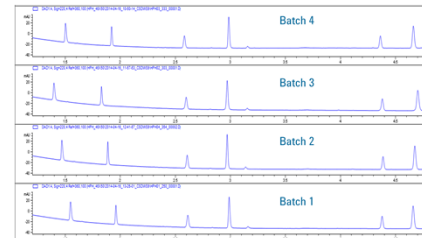


Figure 5: Batch-to-batch reproducibility test at high pH condition



Column: 4.6x50 mm; Mobile phase: A: 10mM ammonium bicarbonate; B: Acetonitrile; Gradient: 5%B to 95%B in 5 min; Flow: 1.5 mL/min; Compounds: 1) 3-methyl-4-nitrobenzoic acid, 2) Cl-cinnamic acid, 3) acetophenone, 4) quinine, 5) hexanophenone, 6) amitriptyline

Figure 4 and 5 showed excellent batch-to-batch reproducibility of Poroshell HPH-C18.

Results and Discussion

Figure 6: Comparison of high pH lifetime test in bicarbonate pH10 isocratic condition

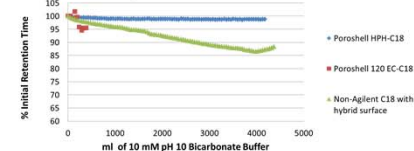


Figure 7: Comparison of high pH lifetime test in bicarbonate pH 10 gradient condition

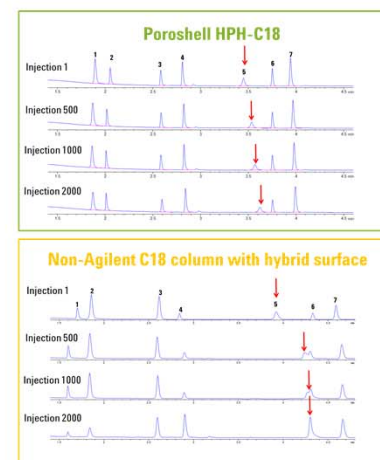


Figure 6 and 7 showed Poroshell HPH lifetime outperformed non-Agilent C18 column with hybrid surface at both pH10 isocratic condition and gradient condition, as well as Poroshell 120 EC-C18 which is silica based particles.

Figure 8: Selectivity change of Poroshell HPH-C18 at different pH

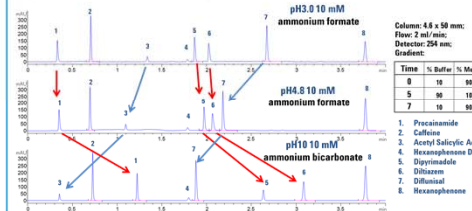


Figure 8 showed different selectivity of Poroshell HPH-C18 at different pH's. With good stability at high pH, Poroshell HPH is ideal to utilize the selectivity difference at different pH for method development.

Conclusions

- The new hybridized superficially porous particles (Poroshell HPH) with a chemically modified organic layer on the pore surface have been successfully developed with good batch-to-batch reproducibility.
- The Poroshell HPH column not only maintains the advantages of superficially porous particles, but also provides chemical stability at high pH mobile phase condition.
- Poroshell HPH-C18 particles showed better lifetime at high pH and better performance than the similar competitor's C18 particles
- Different selectivity is shown on the same column at low, medium and high pH.