Hybridized Superficially Porous Particles with Broad pH Applicability and Different Selectivities

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 efficiencies as smaller size totally porous particles but significantly lower back pressure. The initial introduction of 2.7 μm superficially porous particles in 2008 was a huge success and this is followed by the introduction of either smaller (i.e., 1.7-3.0 μm) or larger (e.g., 2.4 μm) superficially porous particles.

We conducted chromatographies using these superficially porous particles, they all show more selectivity options to facilitate method development. One way to achieve this is to use different bonded phases. Another way is to use different eluents or secondary phases but this requires particles that can withstand extreme pH conditions. In this regard, there are very few 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 wide 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 155 m²/g, pore volume of 0.33 cm³/g, and pore size of 11.6 Å. The surface of Poroshell HPH particles is chemically modified to form a thick organosilane layer to resist silica dissolution at high pH conditions.

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

GC test and van Deemter Curve:

The 2.7 μm Poroshell HPH/C18 particles were packed into 4.6x100 mm column. GC test method was performed:

- GC Method (Mobile phase): 60% CH3CN/40% H2O, Flow: 2 ml/min; Injection: 1 μl of sulfadimidine sample; Temperature: 250 °C; Detection: 254 nm, 80 s FID.
- Van Deemter curves were generated at different flow rates (Figure 2).

Test of peak shape for strong bases:

The chromatographic performances of Poroshell HPH particles were evaluated by peak shape of strong bases at pH 7.0 under following conditions (for Figure 3):

1. Column: 4.6x46 mm Poroshell HPH C18 column
2. Mobile: 60% CH3CN/40% H2O
3. Flow: 1.5 ml/min
4. Injection: 2 μl of test solution
5. Temperature: 25 °C
6. Detection: 254 nm, 83 s FID.

High pH lifetime test:

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

1. In 10 mM bicarbonate pH 11.0 and 50 degree isocratic condition:
   - Mobile phase: 10 mM Ammonium Bicarbonate
   - Mix 1500 mM Acetate and 800 mM Water in 2 liter reservoir (GC solvent)
   - Run 6 hours using (Liquid State Multiplexer) at 0.4 ml/min and 30 °C
   - Run between buffer for 10 minutes at 0.4 ml/min and 90 degree
   - Column: 2.1×50 mm, 2.7 μm Poroshell 120 EC-C18 (p/n G9X278-700) 2.7 μm Poroshell HPH C18, 3 non-Agilent C18 column with hybrid surface.

2. In 10 mM bicarbonate pH 11.0 gradient condition:
   - Mobile phase: A: 10 mM Ammonium Bicarbonate adjusted to pH 11.0 in water; B: Acetate: A: Time 0 min: 50:50 B: Time 5 min: 90:10 B: Time 5.1 min: 50:50 B: Total gradient time was 7 min.
   - Flow rate: 0.4 ml/min.
   - Column: 2.1×50 mm, 2.7 μm Poroshell HPH C18 2.7 μm Poroshell HPH C18 column with hybrid surface.