Waters

HPLC 2004

COMPARISON OF DIFFERENT REVERSED-PHASE PACKING MATERIALS BASED ON BRIDGED HYBRID PARTICLES

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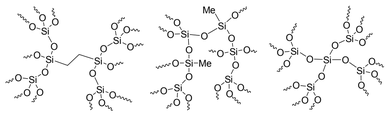
OVERVIEW

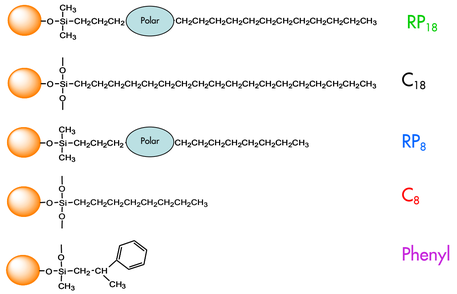
Since the launch of our first generation Methyl Hybrid particle (XTerra®)[1] we have continued to explore the use of hybrid organic/inorganic particles as reversed-phase HPLC packing materials. We recently developed the use of ethyl bridged alkoxysilanes as particle precursors.[2] By employing a 4:1 molar ratio of inorganic (SiO_2) to organic substituent groups ($SiO_{1.5}CH_2CH_2SiO_{1.5}$), novel silsesquioxane-based materials have been shown to be excellent base particles for the preparation of efficient and resilient reversed-phase (C_{18}, C_8) packing materials.



Bridged Hybrid	Methyl Hybrid	Silica
$(SiO_{1.5}CH_2CH_2SiO_{1.5})(SiO_2)$	$(CH_3SiO_{1.5})(SiO_2)_2$	(SiO_2)

In this report we present the synthesis and characterization of different reversed-phase bondings on Bridged Hybrid particles. We will also evaluate the reversed-phase chromatographic performance of these new packing materials, and compare these results with Methyl Hybrid and conventional monofunctional C_{18} and sterically protected C_{18}-bonded silica columns.

BRIDGED HYBRID BONDING CHEMISTRIES



- Surface derivatization of 5 µm materials by reaction with alkylchlorosilanes and endcapping. (SA= 189 m^2/g; APD = 148 Å)
- Bridged Hybrid particles contain 6.4 %C before bonding
- Bridged Hybrid materials have 2.9-3.3 µmol/m^2 ligand coverage

CHROMATOGRAPHIC EVALUATION

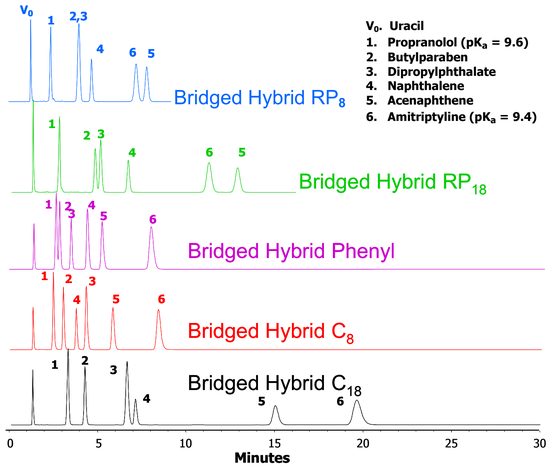


Figure 1: Chromatographic comparison of different bondings on 5 µm Bridged Hybrid particles. Column: 4.6 x 150mm. Mobile Phase: 65 / 35 methanol / 20 mM KH_2PO_4 / K_2HPO_4, pH 7.00, 1.4 mL /min, 23.4 ± 0.1°C.

- Chromatographic comparison tests performed using standard conditions and a standard set of test analytes.[3]
- Bridged Hybrid columns have high resolution and unique selectivities.
- Bridged Hybrid columns show similar selectivity as XTerra® MS C_{18} and Symmetry® C_{18} columns.
- Bridged Hybrid columns have excellent peak shape for all analytes (USP Tailing Factors = 0.9-1.3).

COLUMN STABILITY UNDER ACIDIC CONDITIONS

- Generally accepted failure mechanism for silica columns at low pH is acid-catalyzed bonded phase hydrolysis.
- Accelerated low pH aging test was developed to compare chemical stability of different columns.[2]

Test Protocols (3.0 x 50 mm columns, 80°C):

Challenge:	1% TFA in water (0.6 mL/min, 240 min)
Wash Step:	1% TFA in Acetonitrile (1.3 mL/min, 90 min)
Test:	1% TFA in water
Repeat:	until a 50% loss in original ethyl paraben retention

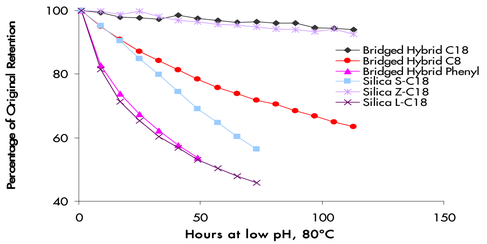


Figure 2: Accelerated low pH aging of different 5 µm Bridged Hybrid columns and conventional mono-functional Silica C_{18} columns.

- Acid stability increases with chain length and trifunctional bondings.
- Bridged Hybrid C_{18} bonded particles show < 10% loss in k at 113 h, which is comparable to the sterically protected Silica Z-C_{18}.

COLUMN STABILITY AT PH 10, 50°C

- Generally accepted failure mechanism for silica columns at high pH is base-catalyzed particle dissolution.
- Accelerated alkaline pH aging test was developed to compare chemical stability of different columns.[1]

Test Protocols (4.6x150 mm columns, 50°C):

Challenge:	50 mM NEt_3 in water (pH 10, 2 mL/min, 60 min)
Wash Step:	water (2 mL/min, 10 min), methanol (2 mL/min, 10 min)
Test:	methanol/pH 7.0 K_2HPO_4 (65/35 v/v)

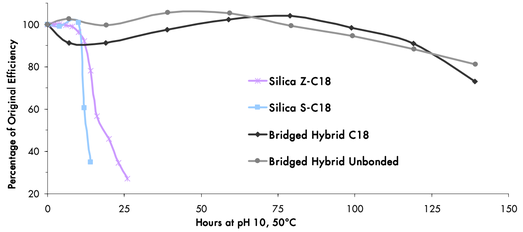


Figure 3: Loss of original efficiency (5 sigma method) for acenaphthene on different 5 µm Hybrid columns and conventional monofunctional Silica C_{18} columns. For unbonded Bridged Hybrid the efficiency loss of the void marker, uracil, was reported.

- Silica C_{18}-columns fail within 15-17 hours.
- Bridged Hybrid columns are still good after 135 hours (Unbonded included).
- Drastic increase in high pH stability attributed to chemical hydrolytic stability of the base hybrid particle.

COLUMN STABILITY AT pH 12.3 (0.02 N NaOH), 50°C

Test Protocols (3.0 x 30 mm columns, 50°C):

Challenge:	0.02 N NaOH in water (pH 12.3, 0.85 mL/min, 60 min)
Wash Step:	water (2 mL/min, 10 min), methanol (2 mL/min, 10 min)
Test:	Acetonitrile/Water (1/1 v/v)
Repeat:	until a 50% loss in original decanophenone efficiency

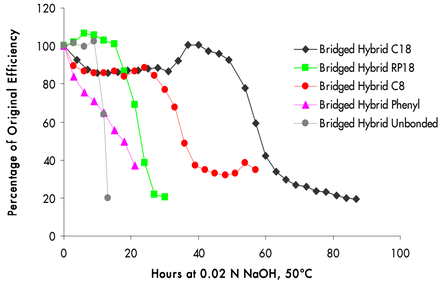


Figure 4: Loss of original efficiency for different 5 µm Bridged Hybrid columns at pH 12.3.

- Bridged Hybrid C_{18} bonded particles last for 60 hours before 50% loss in efficiency.
- Bridged unbonded particles last for 12 hours under these vigorous conditions.

CONCLUSIONS

- Chromatographic evaluation of Bridged Hybrid columns show excellent peak shape, unique selectivity, and great efficiency.
- Bridged Hybrid columns show great performance at both high and low pH:
  - C_{18} bonded phase shows great retention stability with 1% TFA (80°C).
  - Bonded and unbonded Bridged Hybrid columns display excellent chemical stability at pH 10-12.3, 50°C.

REFERENCES

1) *Am. Lab.* **1999**, *31*, 36.
2) *Anal. Chem.* **2003**, *24*, 6781
3) *LC-GC* **1994**, *12*, 468.