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Evaluation of Second Generation Hybrid Organic/Inorganic Particles: New Reversed-Phase Materials that Excel Under Extremes

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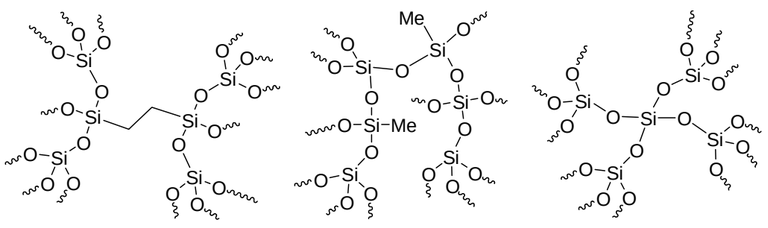
OVERVIEW

Since the launch of our first generation Methyl Hybrid particle[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 bridged alkoxysilanes as particle precursors. Novel Bridged Hybrid materials containing a 4:1 molar ratio of inorganic (SiO_2) to organic substituent groups ($SiO_{1.5}CH_2CH_2SiO_{1.5}$), have been shown to be excellent base-particles for the preparation of efficient and chemically resilient reversed-phase packing materials.



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

In this report we present the synthesis and bonding process for three different Bridged Hybrids (having surface areas varying between 145 and 281 m^2/g). We will also compare the chemical and chromatographic performance of these 5 µm packing materials with Methyl Hybrid and Silica materials, and describe the implications of hybrid particle technology for improved RP-HPLC applications.

UNBONDED PARTICLE SYNTHESIS

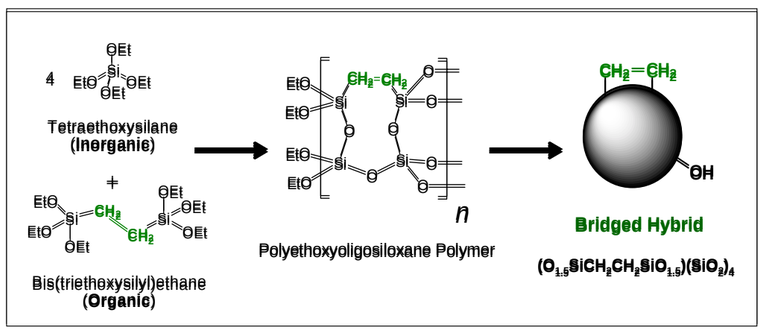
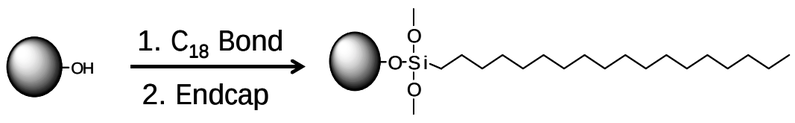


Figure 1. Synthesis of Bridged Hybrid particles

- Multi-step process using hybrid precursors, prepared through a Sol-Gel process.[2]
- Yields highly spherical porous particles.
- Can control surface area by modifying synthetic process (Table 1).

BONDED PARTICLE SYNTHESIS



	Bridged Hybrid A	Bridged Hybrid B	Bridged Hybrid C	Methyl Hybrid	Silica
Unbonded %C	6.2	6.4	6.3	6.9	0.0
Surface Area (m^2/g)	145	189	281	182	340
Pore Volume (cc/g)	0.72	0.76	0.84	0.72	0.85
Pore Diameter (Å)	185	148	109	137	100
C_{18} Coverage (µmol/m^2)	3.3	3.3	3.2	2.5	3.3
Final %C	15.0	17.3	21.4	15.6	19.8

Table 1. Characterization Data for C_{18}-Bonded Particles

- Surface derivitization of 5 µm materials by reaction with $C_{18}H_{37}SiCl_3$, endcapped with $(CH_3)_3SiCl$.
- Hybrid particles contain 6.2 - 6.9 %C before bonding
- Bridged Hybrid and Silica materials have 3.3 µmol/m^2 C_{18}-surface coverage
- Methyl Hybrids have lower coverage — Lower surface silanol concentration

CHROMATOGRAPHIC EVALUATION

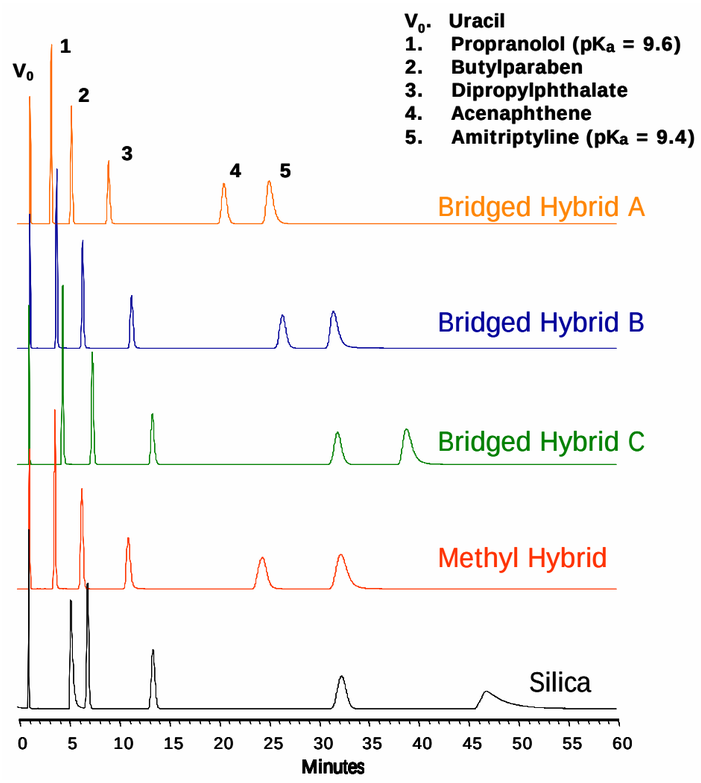


Figure 2. Chromatographic comparison of similarly C_{18}-bonded 5 µm particles. Column: 4.6 x 150mm. Mobile Phase: 60/40 methanol / 20 mM KH_2PO_4 / K_2HPO_4, pH 7.00, 1.4 mL/min, 23.4±0.1°C.

- Chromatographic comparison tests performed using a standard set of test analytes.[3]
- Hybrid columns have similar efficiencies and elution orders as Silica.
- Hybrid columns show reduced retention for analytes due to lower surface area and reduced concentration of surface silanol groups.
- Peaks obtained for bases (propranolol, amitriptyline) are sharper and more symmetric for hybrid columns.
- Higher surface area Bridged Hybrids show greater retentivity.

VAN DEEMTER CURVES

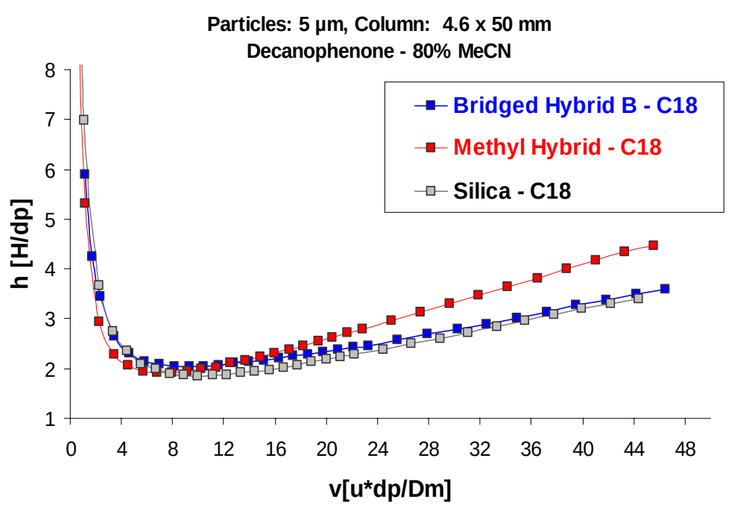


Figure 3. Van Deemter analysis of C_{18}-bonded 5 µm particles.

- Bridged Hybrid columns are very efficient and have similar Van Deemter curves to Silica.
- Improved mass transfer over Methyl Hybrids.

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 developed to compare chemical stability of different columns.[1]

Test Protocols:

(4.6 x 150 mm columns, 50°C)

Challenge: 50 mM NEt_3 (pH 10, 2 mL/min, 60 min)

Wash Step: water (2 mL/min, 10 min), methanol (2 mL/min, 10 min)

Test: methanol/pH 7.00 K_2HPO_4 (65/35 v/v)

Repeat until a 50% loss in original efficiency

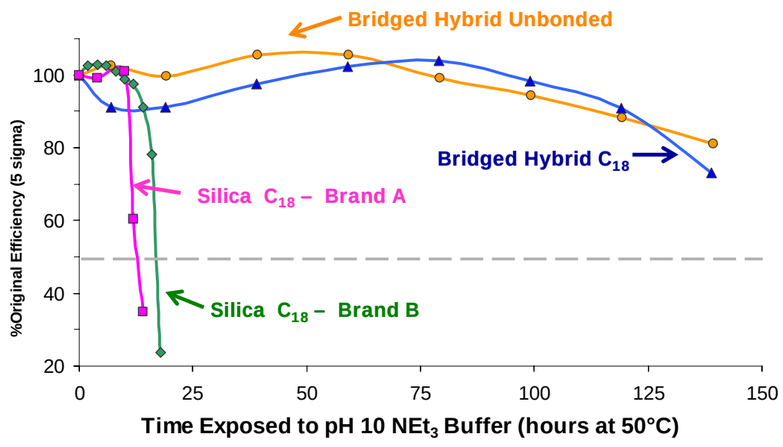


Figure 4. Loss of original efficiency (5 sigma method) for acenaphthene on C_{18}-bonded Bridged Hybrids and Silica. For unbonded Bridged Hybrid the efficiency loss of the void marker, uracil, was reported.

- Silica C_{18}-columns fail within 15-17 hours.
- C_{18}-bonded and Unbonded Bridged Hybrid columns are still good after 135 hours.
- Drastic increase in high pH stability attributed to chemical stability of the base hybrid particle.
- Bridged Hybrid materials allow for an order of magnitude increase in high pH column stability.

COLUMN STABILITY WITH NaOH, 50°C

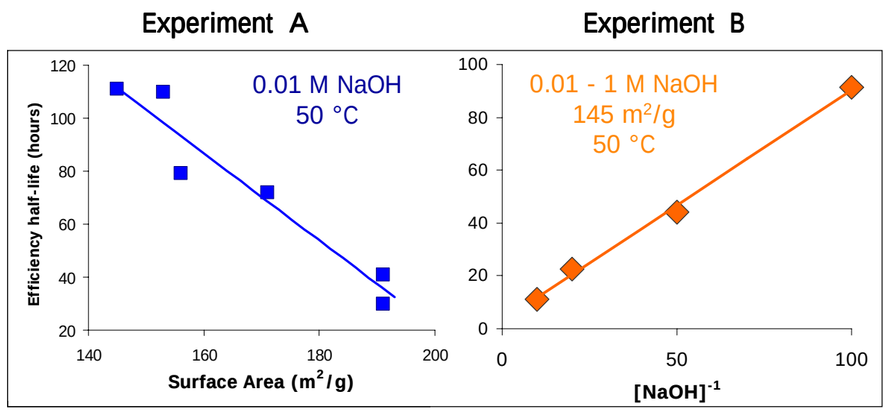


Figure 5. Loss of original efficiency for C_{18}-bonded Bridged Hybrid materials as a function of particle surface area and NaOH concentration.

- Further explored column stability of Bridged Hybrids using NaOH mobile phases at 50°C.
- **Experiment A**: Efficiency lifetime is inversely proportional to surface area.
- **Experiment B**: Efficiency lifetime is inversely proportional to [NaOH]

CONCLUSIONS

Chromatographic properties of Bridged Hybrid C_{18} columns were evaluated:

- Similar performance as C_{18}-silica columns
- Comparable selectivity and dramatically reduced peak tailing factors for basic analytes.
- Significant improvement in mass transfer for the second generation hybrid columns

Bridged Hybrid columns show great performance at elevated pH.

- Stable toward high pH mobile phases at 50°C
- Can use NaOH as a mobile phase additive
- Column stability increases with decreasing surface area.

REFERENCES

1) *Am. Lab.* **1999**, *31*, 36.
2) Patent Pending. *PMSE Preprints* **2002**, *87*, 274.
3) *LC-GC* **1994**, *12*, 468.

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