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Synthesis and Characterization of Hybrid Organic/Inorganic Particles Containing Organo-Reactive Groups for Reversed-Phase HPLC

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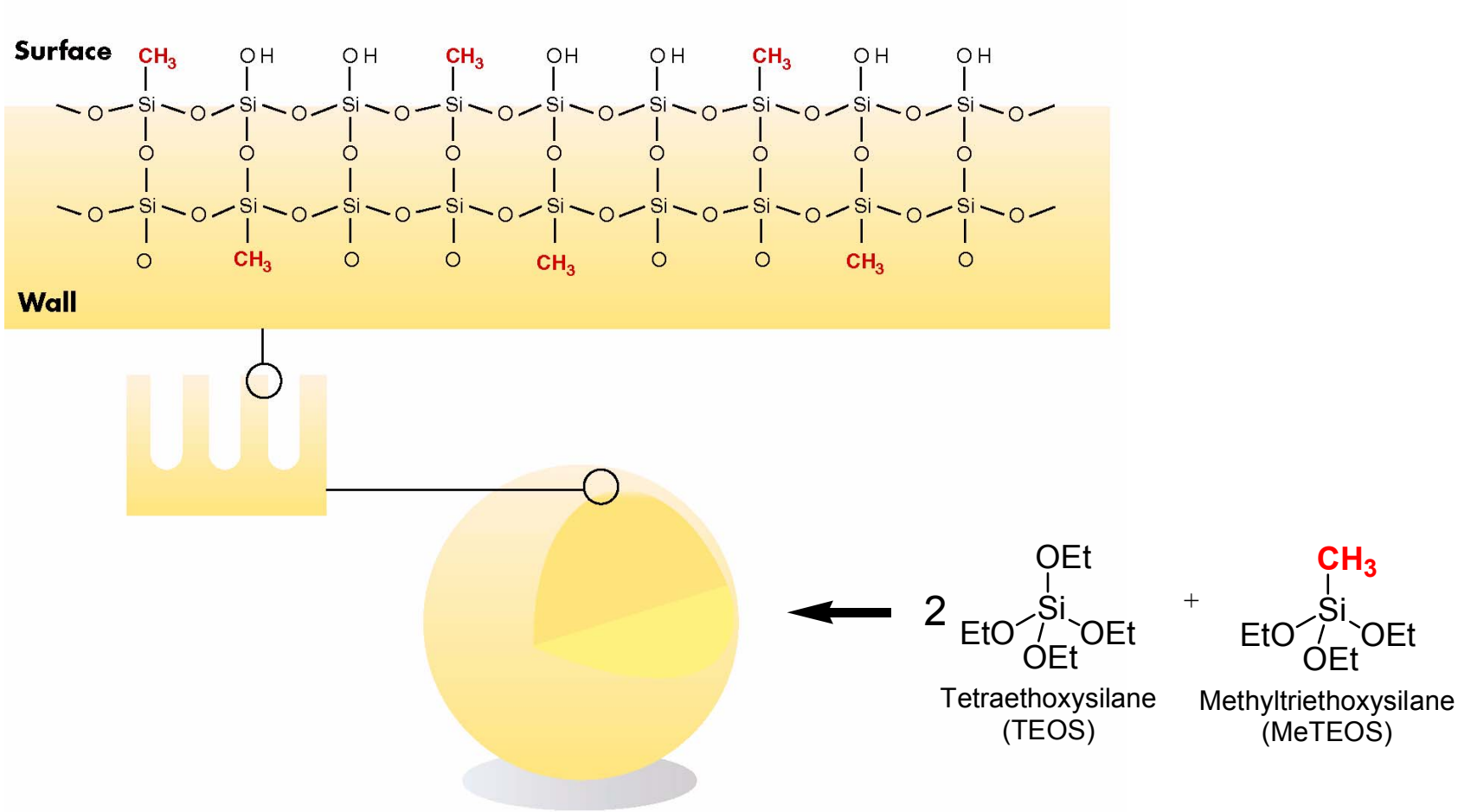
I. Introduction

In reversed-phase HPLC separations, hybrid organic-inorganic particles have been shown to give improved peak shape for bases and exceptional stability in alkaline (pH 12) mobile phases. To date, first generation hybrid particles have consisted of inorganic SiO_2 units and essentially inert organic units, such as the methyl group ($-CH_3$).

In the present report, we will introduce a new generation of hybrid particles, having organo-reactive groups incorporated throughout the particles. These organo-reactive groups, such as propanol and vinyl groups, are directly derivatized using non-silane chemistry. In comparison to conventional bonded silica particles, we will show how these novel HPLC stationary phases can offer excellent combined particle and bonded phase stability.

Hybrid Technology - First Generation

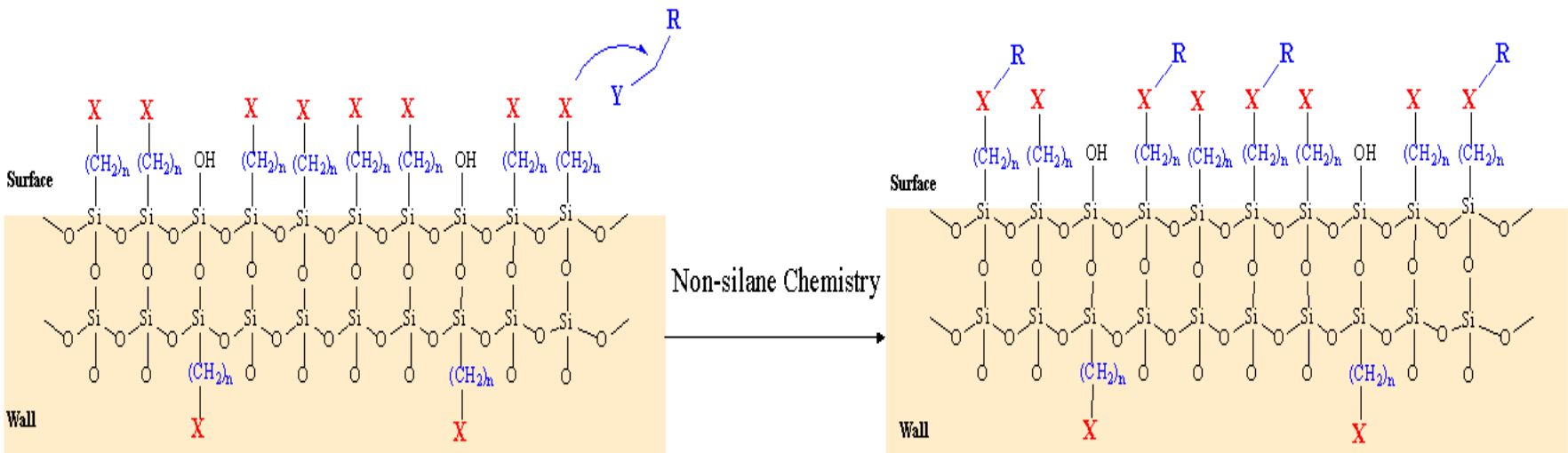


Reverse Phase HPLC Attributes:

- Excellent peak shape for basic analytes: *attributed to reduced surface silanol groups*

- Bonded methyl hybrids are 2-4 fold more stable at pH 10 than bonded silicas: *attributed to a molecular level blend of CH_3 groups in the particle matrix*

New Generation –Organo-reactive Hybrid Technology



Organo-reactive Group: X

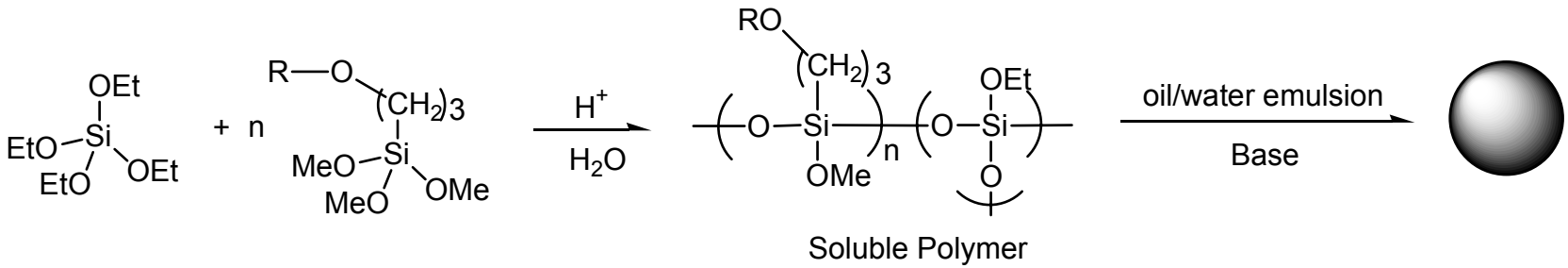
Novel Bonded Phase on Organo-reactiveHybrid Particles

Second Generation Expected Additional RP-HPLC Attributes:

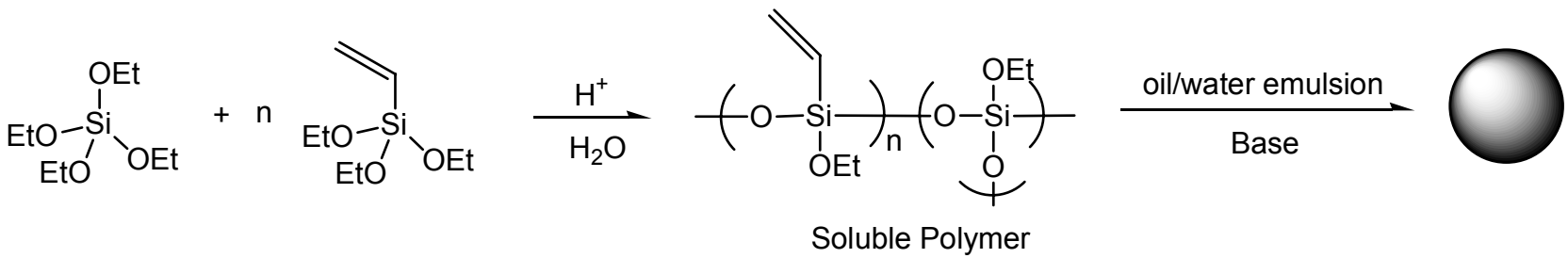
- Excellent bonded phase stability: *attributed to novel surface chemistry*
- Low LC-MS Bleed: *attributed to combined advantages of particle and bonded phase stability*

II. Particle Synthesis and Characterization

1. Propanol Particle Synthesis:



2. Vinyl Particle Synthesis:



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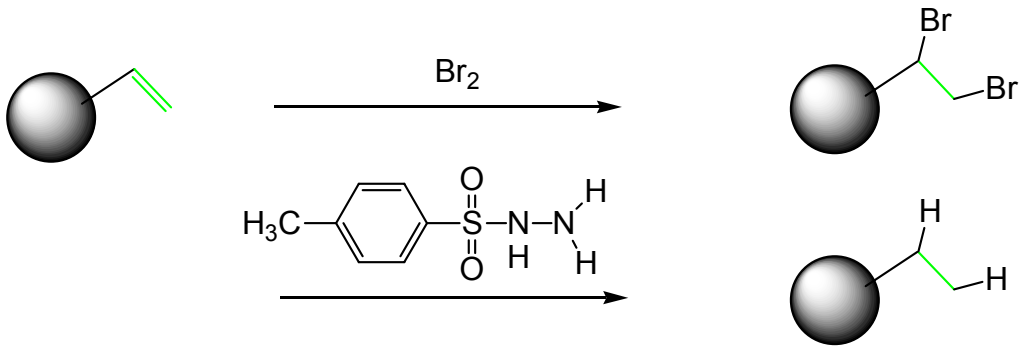
3. Particle Characterization:

Hybrid (organic/inorganic mole ratio)	Propanol Hybrid (1/4)	Vinyl Hybrid (1/2)	Methyl Hybrid (1/2)	Leading Silica Packing
%C	12.0	12.55	6.9	0
SA (m^2/g)	305	197	173	340
PV (cc/g)	0.68	0.60	0.72	0.85
APD (Å)	90	144	145	100

III. Novel Surface Chemistry Synopsis

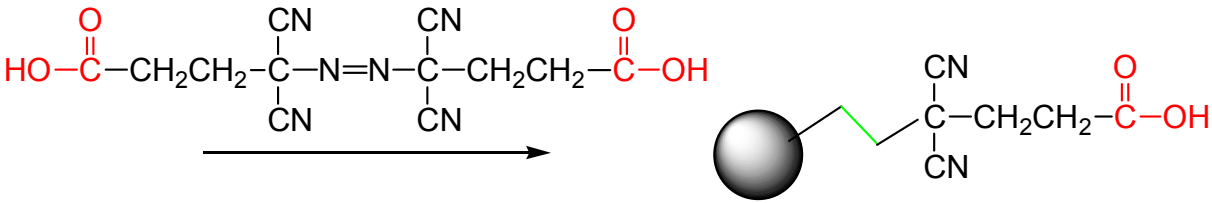
1. Vinyl Particles:

Surface Coverage

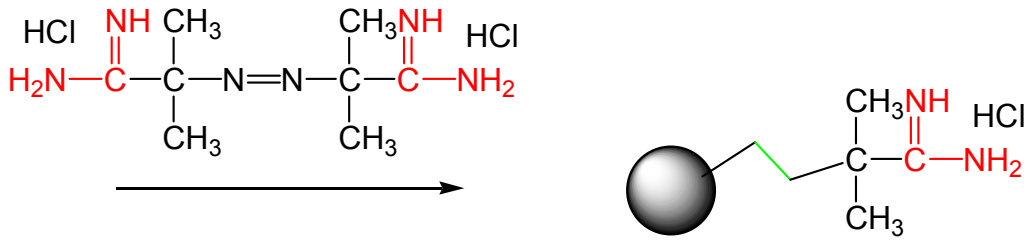


20% vinyl brominated

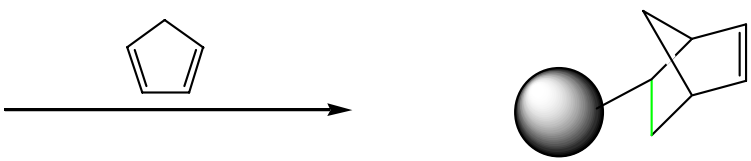
100% vinyl reduced



1.83 µmol/m²



2.86 µmol/m²



1.36 µmol/m²

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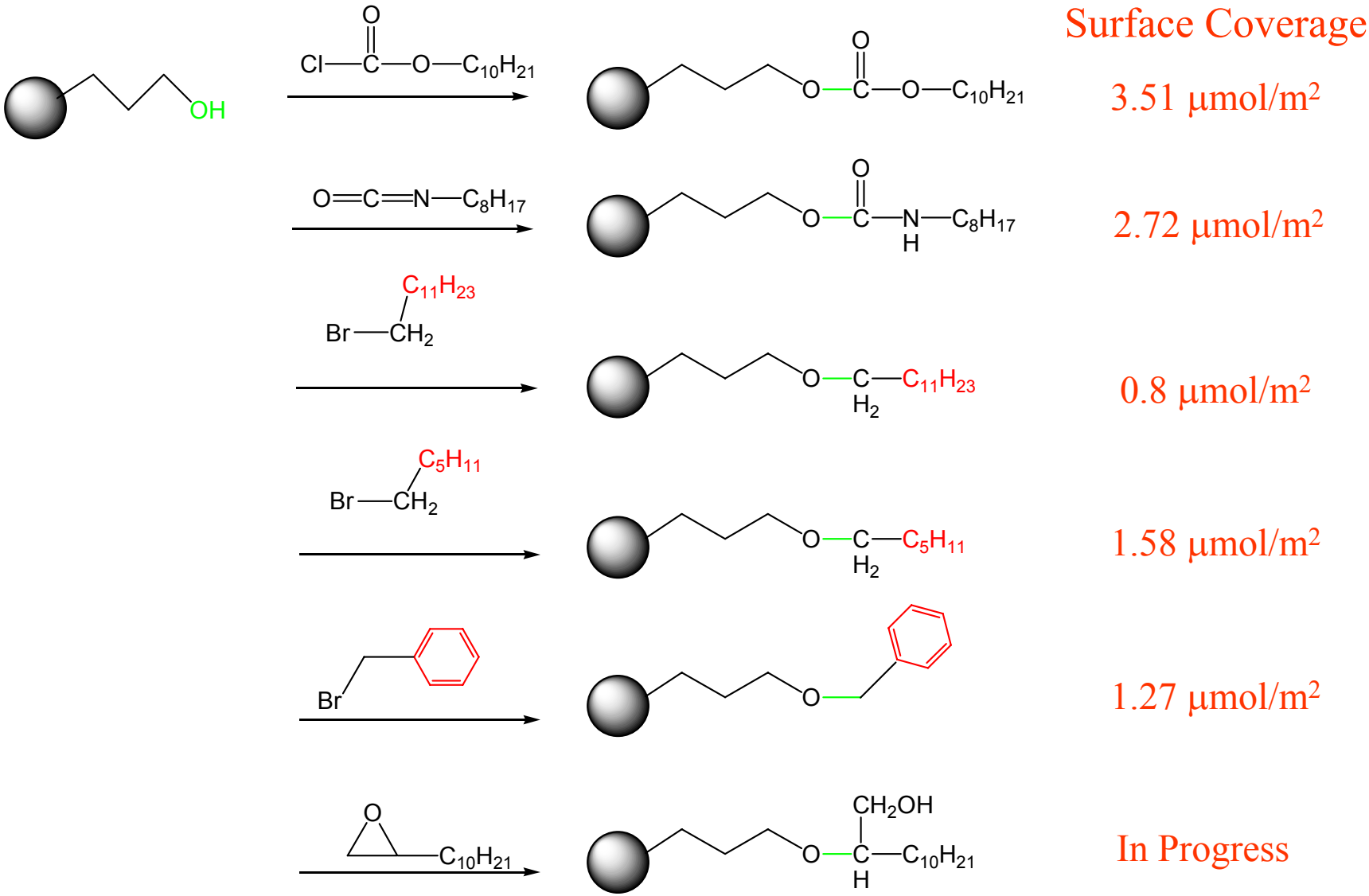
2. Propanol Particles:



Surface Coverage

3.51 µmol/m²

2.72 µmol/m²

0.8 µmol/m²

1.58 µmol/m²

1.27 µmol/m²

In Progress

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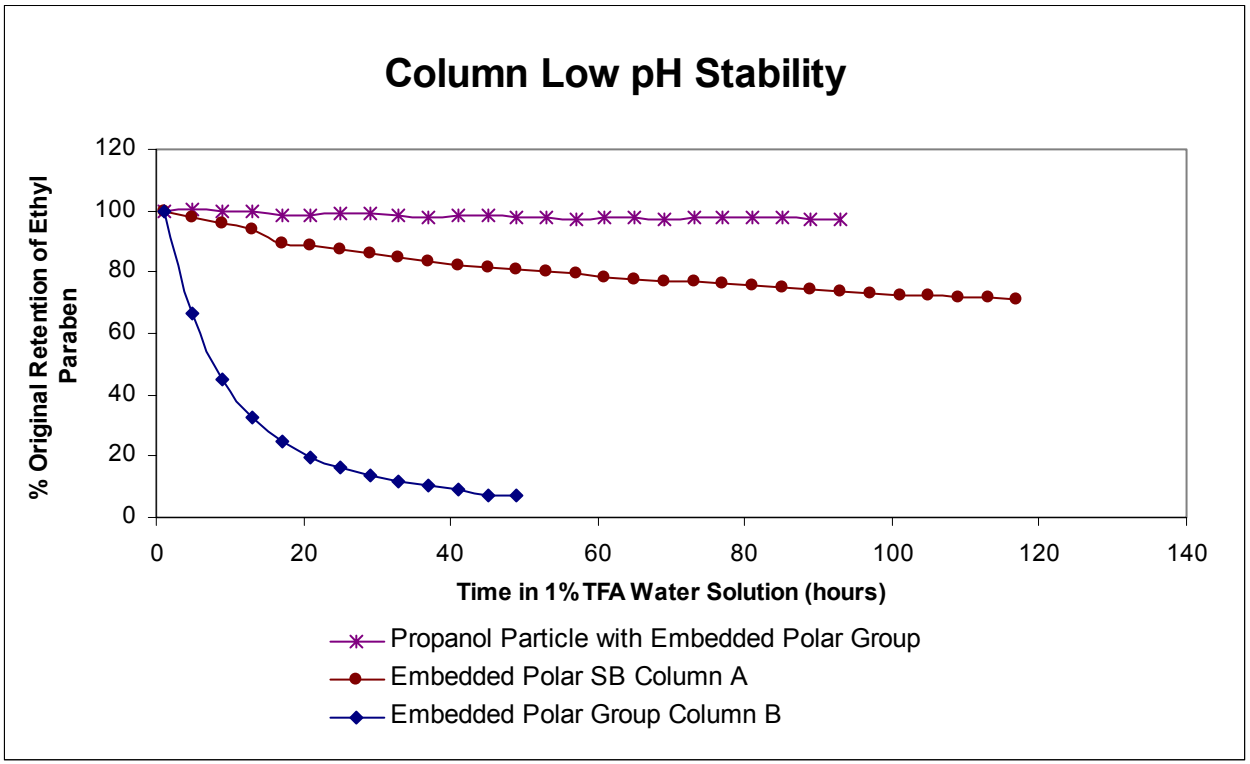
IV. Reverse Phase HPLC Attributes

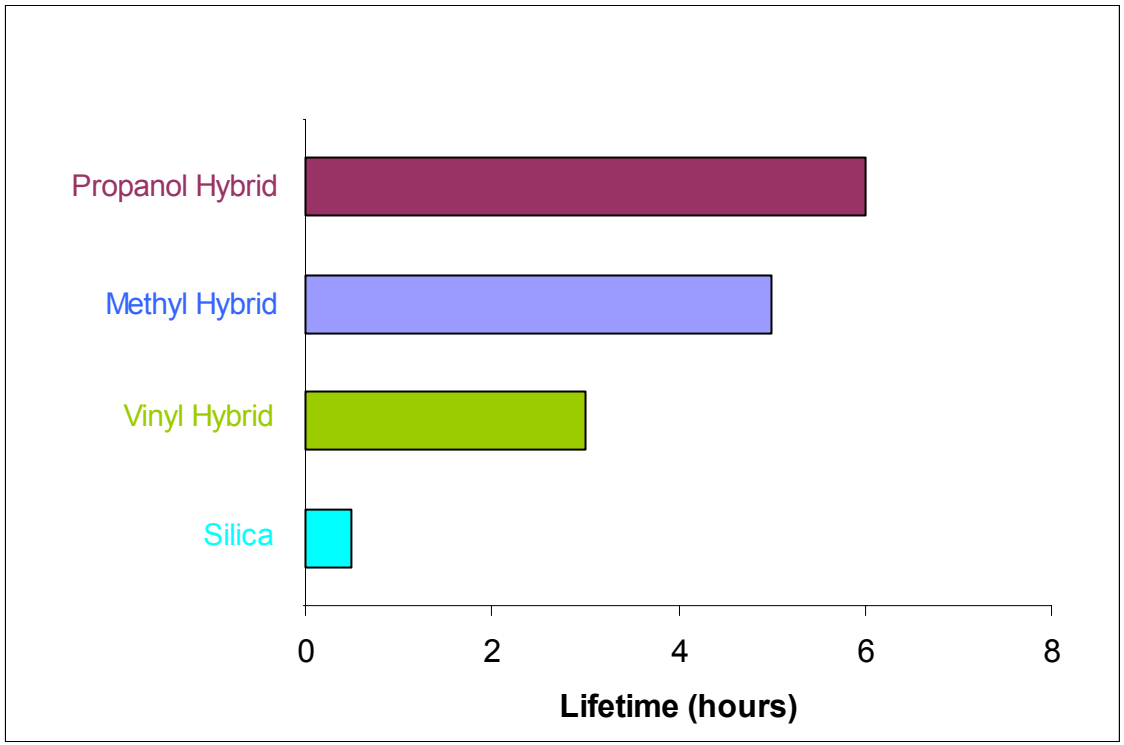
1. Bonded Phase Stability in an Acidic Mobile Phase



Test Conditions: 80 ºC

- Challenge: 1% TFA in water 240 min 0.6 mL/min
- Purge: 1% TFA in acetonitrile 40 min 1.3 mL/min
- Repeat until column fails:.defined as 50% original retention time on ethyl paraben

2. Particle Stability in Alkaline Mobile Phase



- Challenge: 50 mM $N(CH_2CH_3)_3$ pH 10.0 at 50ºC 2 mL/min for 60 min
- Purge: water at 2 mL/min for 10 min, methanol at 2 mL/min for 10 min
- Test: 40/60 acetonitrile / pH 7.0 K_2HPO_4 at 1mL/min
- Repeat until column fails: defined as system went high pressure > 6000psi.

3. Electrospray LC-MS Bonded Phase Bleed

Column	Peak Area ($x10^6$)		Relative Peak Area
	IS	Ligand Peak	
Propanol Embedded-polar	15	1.44	0.1
Silica Embedded-polar	9.9	52	5.25

LC Conditions:

Column dimensions: 2.1×150 mm.

Flow rate: 0.2 mL/min.

Mobile phase: A. 0.1% formic acid in water; and B. 0.1% formic acid in acetonitrile

Gradient: Change from 5% to 100% B in 5 min., hold 5 min. at 100% B, change from 100% to 5% B in 1 min., and hold 15 min. at 5% B.

Injection volume: 10 mL

Internal Standard: 10 ppm amitriptyline in water containing 0.1% formic acid.

MS Conditions (Micromass ZMD):

Inonization Mode: ES^+

Capillary Voltage: 3.50 kV

Cone Voltage: 20V

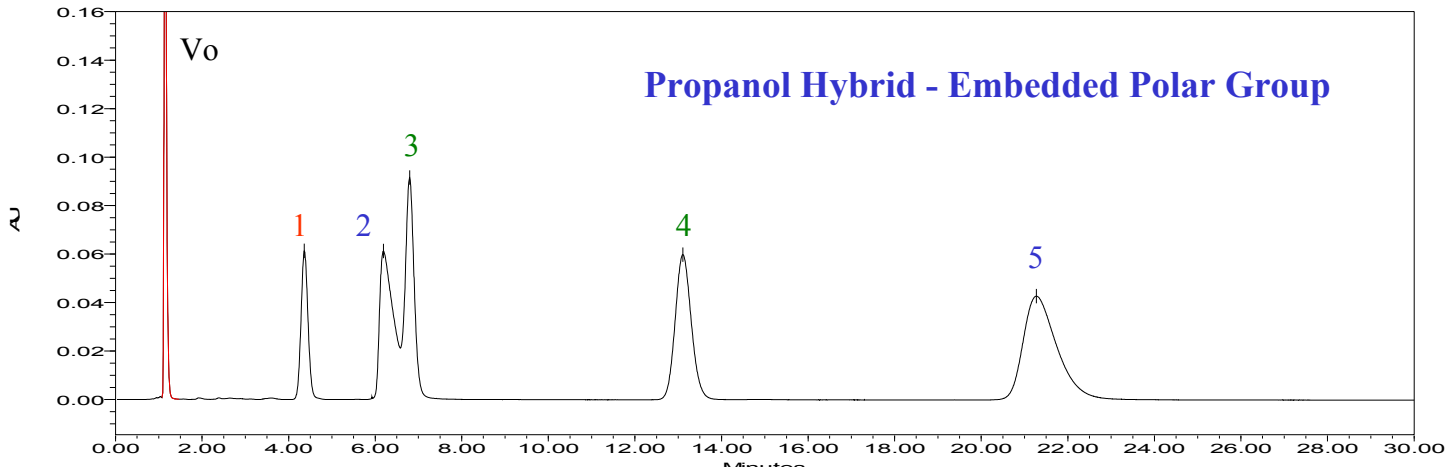
Source Block Temperature: 150 ºC

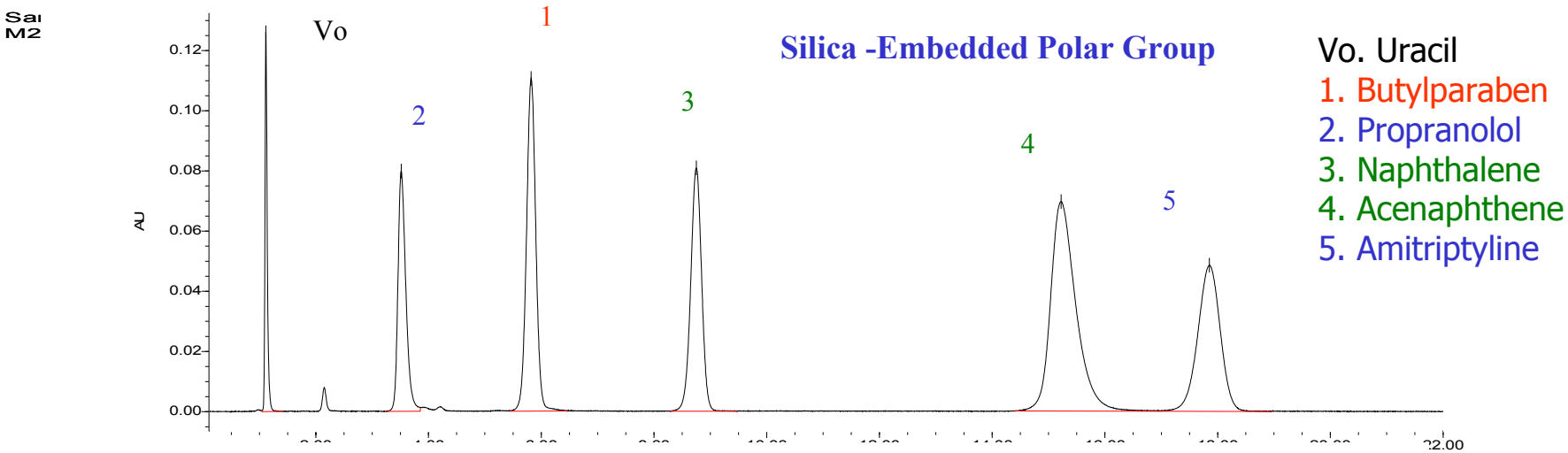
Desolvation Temperature: 250 ºC

Mass Range: 100 to 1000 Dalton

4. Selectivity and Peak Shape

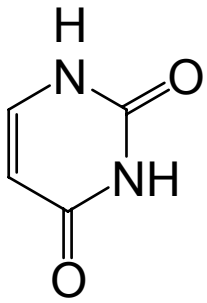
Batch Test: Propanol Hybrid vs. Silica



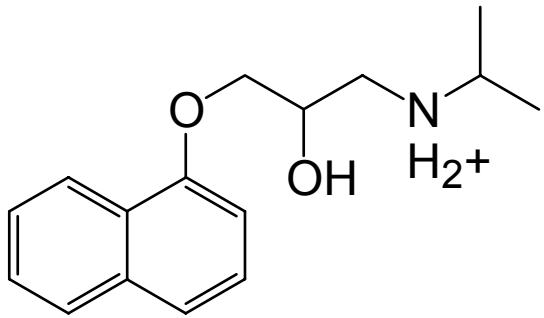


Vo. Uracil
1. Butylparaben
2. Propranolol
3. Naphthalene
4. Acenaphthene
5. Amitriptyline

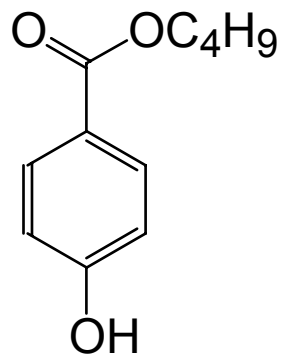
Analytes



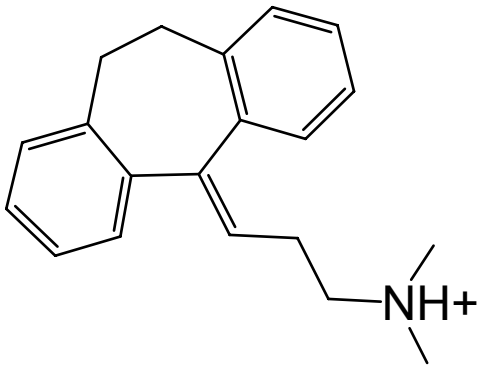
Uracil



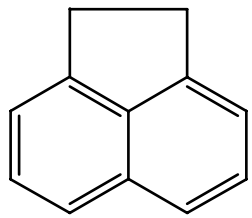
Propranolol pK_a = 9.6



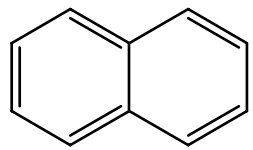
Butyl Paraben



Amitriptyline pK_a = 9.4



Acenaphthene



Naphthalene

Mobile Phase: MeOH/20 mM K_2HPO_4, pH 7.00 (65/35)

V. Conclusions

Novel organo-reactive hybrid particles, such as propanol and vinyl particles, have been introduced. Surface modifications using non-silane chemistry were performed on these particles. We have shown that these modified particles give excellent bonded phase stability in an acidic mobile phase. Particle stability in a high pH mobile phase is comparable to the first generation - methyl hybrid particle. All three hybrid particles are more stable than pure silica particles.

These novel bonded particles also give a much lower LC-MS bleed than conventional bonded phases made through silane chemistry. In the future, organo-reactive surface bonding may lead to particles with novel selectivity in comparison to silica bonded with classical silane chemistry.