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BENEFITS OF CHARGED SURFACE HYBRID MATERIALS

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INTRODUCTION

Since the introduction of high purity silica and hybrid supports, the goals of reproducibility, reduced silanol interactions, compatibility with extended mobile phase pH ranges, and the availability of sub 2 µm particles have been achieved. While these advances have proven successful, new column performance issues have emerged.

Two of the performance issues for high purity silica and hybrid supports that are most evident in acidic, low-ionic-strength mobile phases, are poor peak shape[1] for analytical mass loads of basic analytes, and slow equilibration for basic analytes when materials are exposed to alternating high and low pH mobile phases.[2] (Figure 1)

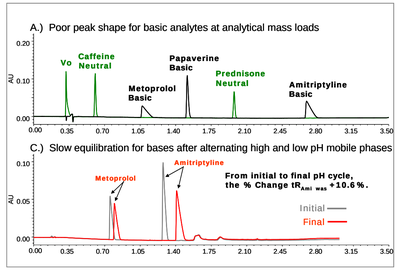

Figure 1. Performance issues observed on high purity supports using low ionic strength acidic mobile phase systems. A.) Peak Capacity Conditions, C.) High & Low pH Mobile Phase Conditions

It was hypothesized that the absence of a controlling surface charge at low pH on high purity supports was the cause of the unexpected column performance. For high purity hybrid and silica-based reversed phase materials, the surface charge of the particle is near zero at about pH 3. Near this pH, even small changes in surface charge have a large impact on the peak shape and retention of ionized compounds.

Charged Surface Hybrid columns were developed to mitigate these problems by introducing a low level controlled positive charge to the BEH particle surface (Figure 2).

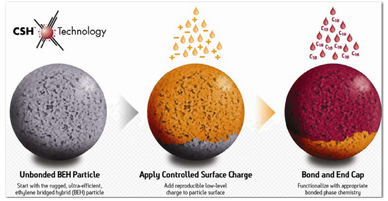

Figure 2. Schematic depiction of the CSH Technology process. Starting with an unbonded BEH particle (left), a small controlled charge is applied to the BEH particle surface (middle). The CSH particle is then bonded and sometimes end capped (right).

METHODS

A.) Peak Capacity Conditions

Gradient: 15-65% B using a linear gradient curve. The gradient time (t_g) was 4.6 minutes with a 1.4 minute hold at 65% B, and then return to initial conditions. Run time: 7.93 minutes

Flow rate: 0.4 mL/minute

Mobile Phase A: 0.1% Aqueous Formic Acid

Mobile Phase B: 100% Acetonitrile

Basic Mix [µg/mL]: Metoprolol tartrate [200], Papaverine [10], Amitriptyline [50], in Methanol/Water, (16.7/83.3)

Neutral Mix [µg/mL]: Uracil [5], Prednisone [10], Caffeine [10], in Methanol/Water, (16.7/83.3)

Injection Volume: 2 µL

B.) Loading Conditions

Isocratic Conditions: 0.05% Trifluoroacetic Acid (TFA) in 39% Acetonitrile (High Purity C_{18}) or 0.05% TFA in 37% (XSelect CSH™ C_{18}) Acetonitrile.

Flow rate: 0.2 mL/minute

Sample: Amitriptyline 0.033 - 4 mg/mL

C.) High & Low pH Mobile Phase Conditions

Gradient: 5-95% B using a linear gradient curve. The gradient time (t_g) was 2.5 minutes with a 0.4 minute hold at 95% B, and then return to initial conditions. Run time: 3.87 minutes

Flow rate: 0.8 mL/minute

Mobile Phase A: 0.1% Aqueous Formic Acid, pH~2.7 or 10 mM Ammonium Bicarbonate pH~10

Mobile Phase B: 100% Acetonitrile

Basic Mix [µg/mL]: metoprolol [300], amitriptyline [100], in Acetonitrile/Water, (5/95)

Neutral Mix [µg/mL]: dimethylphthalate [100], diethylphthalate [100], dipropylphthalate [100], in Acetonitrile/Water, (50/50)

Injection Volume: 2 µL

An ACQUITY UPLC® equipped with either an ACQUITY TUVe detector or ACQUITY PDA detector was used. The detection wavelengths were 260 nm for conditions A and C, and at 266 nm for conditions B. The columns were kept at a constant temperature of 30 °C. The column configuration used was 2.1 x 50 mm.

RESULTS & DISCUSSION

Peak Shape in 0.1% Formic Acid

A.) The performance issue of poor peak shape for basic analytes at analytical mass loads was determined using the P_c value. The P_c values were calculated for prednisone (Pred), a neutral or well-behaving analyte, and for amitriptyline (Ami), a basic or possible problem analyte under acidic, low-ionic-strength mobile phases. The prednisone on-column mass load was 0.02 µg and the amitriptyline on-column mass load was 0.1 µg.

- Comparable P_c values for Pred were obtained on XSelect CSH C_{18}, conventional high purity C_{18} **A**, and high purity C_{18} **B** (Table 1). The peak shape is sharp and nearly symmetrical (Figure 3).
- Significantly lower P_c values were obtained for Ami, a basic analyte, on the high purity C_{18} materials (Table 1). The peak shape for amitriptyline is asymmetrical and broad, a shape often associated with mass overload (Figure 3).
- XSelect CSH C_{18}, prepared using a hybrid support that incorporates a low level controlled positive surface charge, gave similar P_c values for Pred and Ami.

Table 1.

Peak Capacity 4σ	Ami	Pred	% Difference
High Purity C_{18} **A**	77	180	57
XSelect CSH C_{18}	162	160	-1
High Purity C_{18} **B**	102	182	44

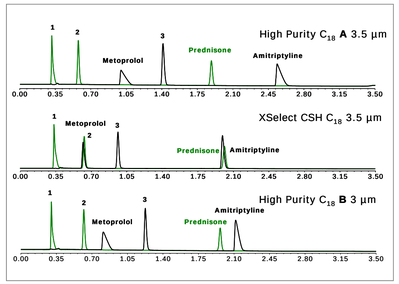

Figure 3. Peak shape comparison using peak capacity conditions. Peak ID: 1) uracil, 2) caffeine, 3) papaverine

Peak Shape in 0.05% TFA

B.) Under the peak capacity gradient test conditions, the XSelect CSH C_{18} column gave similar P_c values for prednisone (neutral analyte) and amitriptyline (basic analyte). To investigate the performance of XSelect CSH C_{18} using higher mass loads of amitriptyline, an isocratic loading study was conducted using the mass load range of 0.05—6.0 µg on-column, and 0.05 % TFA as the aqueous portion of mobile phase. The peak shape results on XSelect CSH C_{18} were compared to the results obtained for a conventional high purity C_{18} material (Figure 4). Equivalent USP tailing factors of 2 were obtained at significantly different mass loads for the two columns.

- At comparable mass loads, the high purity C_{18} column had poorer peak shape for amitriptyline than the XSelect CSH C_{18} column. The USP tailing factor was 2.12 at the mass load of 0.5 µg on-column.
- At the same mass load of 0.5 µg, the USP tailing factor was 1.49 on the XSelect CSH C_{18} column.
- At a 5X higher mass load, 2.5 µg on-column, an equivalent USP tailing factor of 2.13 was reached on the XSelect CSH C_{18} column.

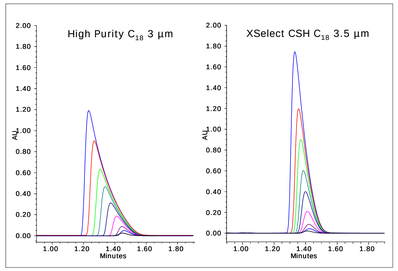

Figure 4. Peak shape and loading capacity comparison using loading conditions.

Formulas

Peak capacity, P_c: $(t_g / \text{peak width}_{13.4\%}) + 1$

% Change t_R: $(t_{R\ Final} - t_{R\ Initial}) / t_{R\ Initial} \times 100$

Equilibration in 0.1% Formic Acid

C.) Slow equilibration for basic analytes in acidic, low-ionic-strength mobile phases after exposure to a high pH mobile phase is the second performance issue. This phenomenon is characterized by the retention times for ionized analytes changing over time, and may be perceived as poor stability or poor batch-to-batch reproducibility for a material.

For neutral analytes, there is little retention time change. However, for a basic analyte, the equilibration can be slow and the retention times may not be reproducible (Figure 5). The % change for the retention time of amitriptyline after the column had been exposed to multiple cycles of alternating high and low pH mobile phases is significantly different on the three columns (Table 2).

- The XSelect CSH C_{18} column has very little retention time change for amitriptyline. Both high purity C_{18} columns have significant changes in their retention times for amitriptyline after the final exposure to high pH.
- The high purity C_{18} column **A** has a moderate retention time change, and the high purity C_{18} column **B** has a 3X greater retention time change than high purity C_{18} column **A**.

Table 2.

	% Change t_R Ami
High Purity C_{18} **A**	+7.0
XSelect CSH C_{18}	+0.4
High Purity C_{18} **B**	+25

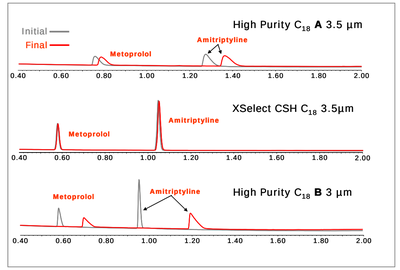

Figure 5. Equilibration for basic analytes under High & Low pH Mobile Phase Conditions.

SUMMARY

CSH Technology addresses the key issues encountered when reversed-phase columns are used with acidic, low-ionic-strength (MS-compatible) mobile phases.

- In 0.1% Formic Acid mobile phases, peak shapes for basic analytes are sharp and symmetrical.
- In 0.05% TFA mobile phase, a sharper peak shape for amitriptyline is maintained at higher mass loads than on a conventional high purity C_{18}.
- After exposure to high pH mobile phases, the re-equilibration in acidic, low-ionic-strength mobile phases is rapid, resulting in reproducible retention times for basic analytes.
- Under low pH conditions, CSH Technology provides outstanding batch-to-batch reproducibility (Figure 6). Equivalent performance was achieved across the particle sizes for all CSH chemistries.

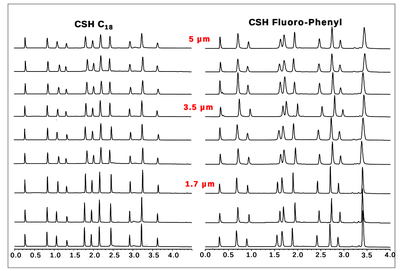

Figure 6. Batch-to-batch reproducibility for nine batches of ACQUITY CSH and XSelect phases.

SPECIAL THANKS

The results presented in this poster are from more than two years of research and development work. The tireless efforts of our in–house Column Packing Group to provide efficient columns throughout the development process have made this project a success!

References

1. McCalley, D. V. *J. Chromatogr. A* **2005**, *1075*, 57.
2. (a) Gilroy, J. J.; Dolan, J. W.; Snyder, L. R. *J. Chromatogr. A* **2003**, *1000*, 757. (b) Marchand, D.H., Williams, L.A., Dolan, J.W., L.R. Snyder, L.R. *J. Chromatog. A*, **2003**, *1015*, 53.

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