SUPPORTING INFORMATION

Gone in Seconds: Praxis, Performance, and Peculiarities of Ultrafast Chiral Liquid Chromatography with Superficially Porous Particles.

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1. Calculation of Peak Parameters in the ChemStation Software

The reported theoretical plates (N, by half height method), peak asymmetry factors (USP tailing factor $T = \frac{W_{0.05}}{2f}$; where $W_{0.05}$ = peak width at 5% peak height, f = distance from the leading edge of the peak to the peak maximum at 5% peak height), peak variance σ^2 (by tangent method), and retention times (t_R) were obtained from ChemStation. Resolutions $\left(R_S = \frac{2.35\ (t_{R_2} - t_{R_1})}{2\ (W_{0.5,1} + W_{0.5,2})}\right)$ were calculated using the half-height method. To assess the extra-column contribution to the observed plate heights, the column was removed and the tubings were connected by a zero dead volume union. See later section for calculations.

2. Column packing

Each stationary phase was slurry packed with a pneumatically driven Haskel pump (DSTV-122) into 10 x 0.46, 5 x 0.46 and 3 x 0.46 cm i.d. stainless steel columns (IDEX Health and Science, Oak Harbor, WA). The slurry optimization procedures, such as reduction of shear thickening, optical microscopy, and settling vial tests were conducted for several organic solvents before packing.[1] A 2 µm frit cap (IDEX) was used to cap the column ends quickly after the packing procedure. The column specific permeability (K_s) was determined by plotting the pressure drop against the linear mobile phase velocity.

To accomplish ultrafast separations, enantiomeric selectivity must be sufficient and column efficiencies must be as high as possible. The quality of the column packing affects all these parameters except enantiomeric selectivity. It is well known that unique packing procedures are often needed for different stationary phases as well as for the same stationary phase packed into columns of different dimensions.[2] For commercial columns, these procedures are usually treated as trade secrets. In a recent academic study on packing of SPPs, Jorgenson, et al. indicated that the best packed capillary column used a slurry of agglomerated particles.[3] For the SPP-CSPs in this study, the opposite was found. Figure S1 shows the difference in performance of two identically packed columns where the only difference was in the slurry medium. The “well dispersed” slurry (Figure S1B) produced a column with > 2.3x higher efficiencies and with more symmetrical peaks. However, this is only part of the story. The backpressure and permeability also can be significantly different. Using Darcy’s law

$$\Delta P = \left(\frac{\eta L}{K_S}\right) u \qquad (1)$$

where ΔP is the pressure drop (Pa), η is the viscosity (Pa.s) of the mobile phase (70:30 heptane: ethanol), L is the column length (m), K_S is the specific permeability (m^2), and u is the superficial linear velocity (m/s), one can calculate K_S for the two columns (see below).[4] The permeability of the column from the agglomerated suspension and the dispersed suspension were 4.64 x 10^{-15} m^2 and 4.33 x 10^{-15} m^2 respectively. As expected, both permeabilities are significantly greater (~ 63% to ~ 67%) than reported for 1.7 µm FPP-CSP.[5]

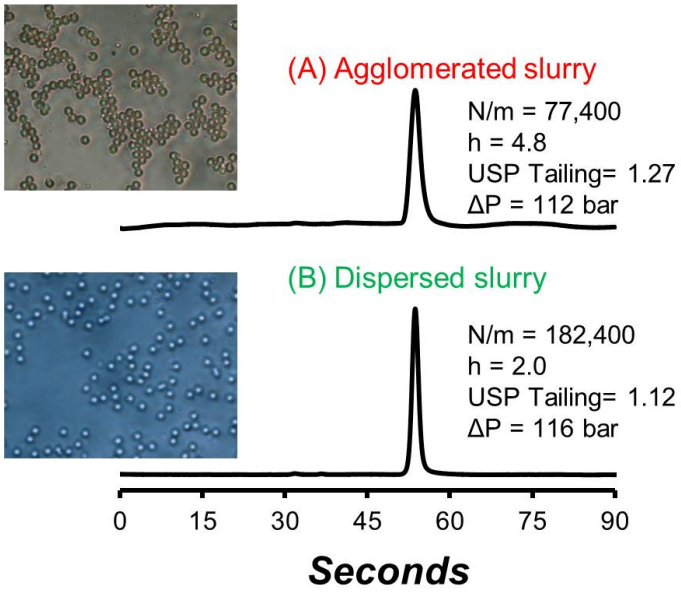


Figure S1. Comparison of the efficiency of 2.7 µm CF7-DMP SPPs packed using different slurry solvents. Conditions: 5% (w/v) slurry packed in 5 x 0.46 cm columns using constant pressure mode; pushing solvent: methanol. Both columns were packed at the same pressure and conditioned equally. Probe: 1,3-dinitrobenzene, m.p. = 70:30 heptane:ethanol, 1.0 mL/min, 230 nm. Top (A) shows the appearance of agglomerated suspension and (B) shows a dispersed suspension under an optical microscope at 500x zoom.

3. Column Specific Permeability (K_S) Calculations

One of the useful tests for assessing the quality of packed beds is to calculate the specific permeability of the chromatographic column using the Darcy's law for pressure drop across a porous medium. A detailed analysis of column quality parameters is presented in the classic work by Bristow and John H. Knox.[6] According to Darcy's law:

$$\Delta P = \frac{u\eta L}{K_s} \quad (2)$$

where ΔP is the pressure drop across the column (Pa) , *u* is the superficial linear velocity (m/s) calculated by dividing the volumetric flow rate (m^3/s) by the cross sectional area of the cylindrical column (m^2), η is the viscosity of the mobile phase (Pa.s), L is the length of the column (m), and K_s is the specific permeability (m^2). From the slope of pressure drop vs. linear velocity, the specific permeability of the packed bed can be obtained (Figure S2).

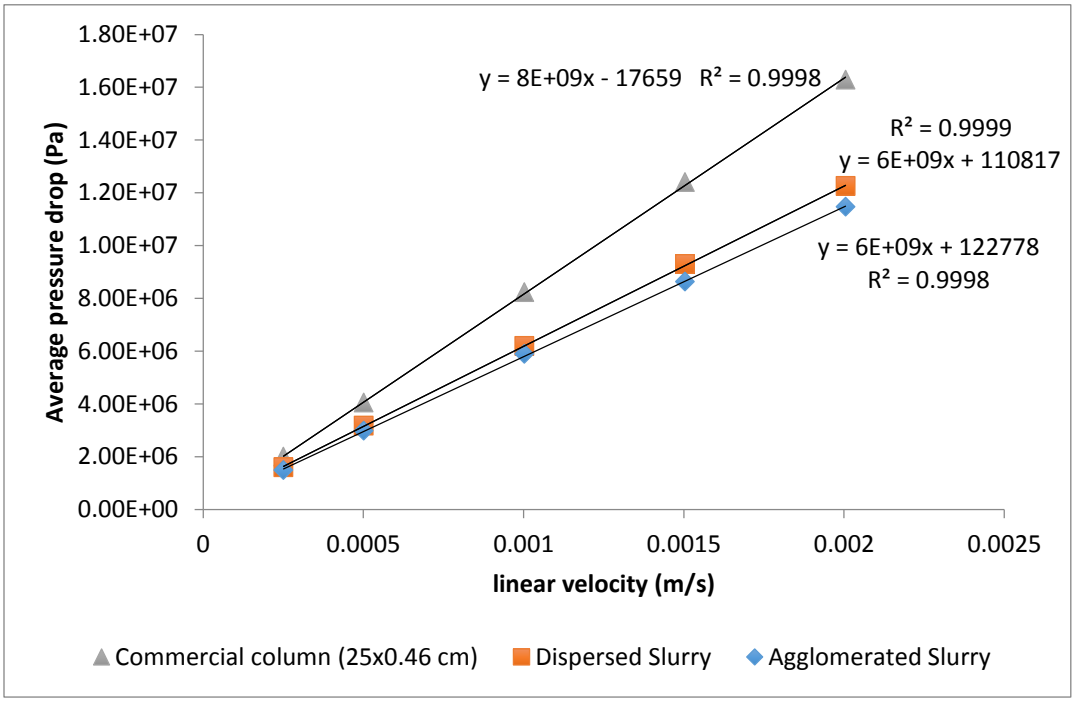


Figure S2. Comparison of specific permeability of commercial CF7-DMP column (5 µm FPP, 25 cm x 0.46 cm i.d.) vs. SPP CF7-DMP (2.7 µm SPP, 5 cm x 0.46 cm i.d.). Mobile phase: 70% Heptane-30% Ethanol.

4. Determination of Extra-Column Contribution in a Chromatographic Peak

The efficiency of a chromatographic (Gaussian and non-Gaussian) peak is defined as:

$$N_{obs} = \frac{t_R^2}{\sigma_{obs}^2} \tag{3}$$

where N_{obs} is the apparent efficiency, t_R is the mean time of the peak and σ_{obs}^2 is the observed peak variance. The observed peak variance includes the band broadening contribution from the column and other components from the injector port to the detector flow cell. For any true measurement of the efficiency and high efficiency separations, extra-column effects should be minimized in the chromatography system.

Assuming Gaussian peaks, the extra-column band broadening can be estimated by removing the column and connecting the injection port directly to the detector by a zero dead volume union using the same tubing connections. The peak widths at the baseline (4σ) can be determined by tangent method and converted into σ (in unit time) by dividing by 4.

$$Width\ at\ the\ base\ w = 4\sigma$$

The widths at the base (4σ) for first peak in Figure 4A and 4B are 0.01677 min and 0.01310 min respectively. When the column was removed and the tubings were connected by a zero dead volume union, the peak widths were 0.00719 min and 0.00298 min for Figure 4A and 4B configuration respectively. This approach gives a simple way of estimating the variance. It is desirable that the ratio of the variance of the system and the peak variance (σ_{ratio}^2) observed with a column and the system be kept as small as possible.

$$\sigma_{\text{ratio}}^2 = \frac{\sigma_{\text{system}}^2}{\sigma_{\text{column+system}}^2} \tag{4}$$

In our case, the moment analysis was used to account for the exact peak shapes of retained peaks and the peak without any column. By using moment analysis, same intrinsic efficiencies were obtained.

The Agilent Chemstation Software calculates the second moment by the following relation:

$$\sigma^2 = M2 = \frac{d_t^2}{X}\sum_{i=1}^{N}\left(\left(i - 1 - \frac{Y}{X}\right)^2 A_i\right) \quad (5)$$

N = number of area slices,

A_i = value of area slice indexed by *i*

$\sum_{i=1}^{N}$ = sum of starting index 1 to final index N for discrete observations

d_t = time interval between adjacent area slices

t_0 = time of first area slice

X = $\sum_{i=1}^{N} A_i$

Y = $\sum_{i=1}^{N}((i-1)A_i)$

It is desirable that the ratio σ^2_{ratio} of the variance of the system and the peak variance observed with a column and the system should be kept as small as possible.

The corrected peak variance was calculated as follows:

$$\sigma^2{}_{intrinsic} = \sigma^2{}_{obs} - \sigma^2{}_{extra} \quad (6)$$

Finally, $N_{intrinsic}$ was calculated using the $\sigma^2{}_{intrinsic}$ in the efficiency equation as follows:

$$N_{intrinsic} = \frac{t_R^2}{\sigma^2_{intrinsic}} \quad (7)$$

Table S1: Instrumental parts that contribute to extra-column band broadening and their dimensions

Parts	**Dimensions**
Agilent 1290 UHPLC stock needle	6.5 cm x 130 µm
Agilent 1290 UHPLC stock needle seat	11 cm x 130 µm
Agilent ultralow dispersion kit needle	6.5 cm x 75 µm
Agilent ultralow dispersion kit needle seat	11 cm x 75 µm
Stock steel tubings (total)	22 cm x 170 µm
nanoViper capillaries (total)	22 cm x 75 µm
Detector flow cell	0.6 µL

5. Surface Coverage and Loading of Chiral Selectors

The elemental analysis for each stationary phase, the surface coverage of the chiral selectors, and the selector loading on the SPP silica are given in a table S1 below. The size for SPP silica was 2.7 µm average particle diameter. The pore size and the surface area of these silica particles are 120 Å and 120 m^2/g respectively.

Table S2: Elemental analysis and the surface coverage for the SPP based chiral stationary phases.

Stationary phase	%C	%N	Surface coverage µmol/m^2	Selector loading %
CF6-P	6.2	0.88	0.88	13.1
CF7-DMP	3.05	0.21	0.11	5.0
teicoplanin	4.73	0.57	0.34	8.8
vancomycin	5.32	0.94	0.48	10.0
hydroxypropyl-β-cyclodextrin	4.17	Not detected	0.50	8.5
teicoplanin aglycone[a]	8.34	1.74	N/A	N/A

[a]The details required to calculate surface coverage values for this stationary phase are unavailable.

6. Temperature Data on Viscous Frictional Heating in Polar Organic Mode, Normal Phase, and Reversed Phase Mode

Table S3: CF6-P (10 x 0.46 cm) packed with 2.7 µm SPP. Mobile phase = 80/20/0.3/0.2 ACN/MeOH/TFA/TEA. Temperature measured at the column outlet (flow averaged temeprature). Inlet temperature (~ 22 °C) was the same as ambient temperature in each case.

Flow rate mL/min	Pressure (bar) of the System	Temperature (°C) of the Eluent at the Outlet
0.5	45	23
1.0	97	24
1.5	151	26
2.0	209	27
2.5	271	29
3.0	336	31
3.5	408	32
4.0	497	35
4.5	582	37
5.0	668	39

Table S4: CF7-DMP (10 x 0.46 cm) packed with 2.7 µm SPP. Mobile phase = 90 Heptane-10 EtOH. Temperature measured at the column outlet (flow averaged temperature). Inlet temperature (~ 22 °C) was the same as ambient temperature in each case.

Flow rate mL/min	Pressure (bar) of the System	Temperature (°C) of the Eluent at the Outlet
3.0	354	34
3.5	426	36

Table S5: Teicoplanin (5 x 0.46 cm) packed with 2.7 µm SPP. Mobile phase = 90/10 Water/MeOH. Temperature measured at the column outlet (flow averaged temperature). Inlet temperature (~ 22 °C) was the same as ambient temperature in each case.

Flow rate mL/min	Pressure (bar) of the UHPLC System	Temperature (°C) of the Eluent at the Outlet
0.5	109	25
1.0	226	27
1.5	346	28
2.0	468	30
2.5	579	32
3.0	681	34
3.5	780	36
4.0	> More than packing pressure	N/A

7. First Order Approximation for Radial Temperature Gradient

$$\Delta T_R = \frac{u\left(\frac{dP}{dz}\right)R^2}{4\lambda_{rad}} \quad (8)$$

where u is the superficial flow velocity in m/s (obtained by dividing the volumetric flow rate by the total cross sectional area of the column), d*P*/d*z* the change in pressure in the direction of the column axis per unit length in N/m^3, *R* the column radius in m and λ_{rad} is the approximate thermal conductivity of the mobile phase in the radial direction in W/m°C.

The equation 8 can also be written as the following[7]

$$\Delta T_R = \frac{F_v \Delta P}{4\pi\lambda_{rad} L} \quad (9)$$

A) Mobile phase (v/v): 90 Heptane – 10 Ethanol
Column: CF7-DMP SPP
Dimensions: 10 cm x 0.46 cm i.d.

Table S6: First Order Approximation for Radial Temperature Gradient for Normal Phase

Flow rate								
mL/min	m^3/s	P (system+column), bar	P(sys), bar	P (column), bar	P(column), Pa	Length m	Thermal Cond[8] (W/m ^{o}C)	T Max (^{o}C)
1.0	1.67E-08	105	25	80	8000000	0.1	0.1253	1
2.0	3.33E-08	225	61	164	16400000	0.1	0.1253	3
3.0	5.00E-08	364	107	257	25700000	0.1	0.1253	8
3.5	5.83E-08	433	136	297	29700000	0.1	0.1253	11

B) Mobile phase (v/v): 90 Water – 10 Methanol
Column: Teicoplanin bonded SPP
Dimensions: 5 cm x 0.46 cm i.d.

Table S7: First Order Approximation for Radial Temperature Gradient for Reversed Phase

Flow rate								
mL/min	m^3/s	P (system+column), bar	P(sys), bar	P (column), bar	P(column), Pa	Length m	Thermal Cond (W/m ^{o}C)	T Max (^{o}C)
1.0	1.67E-08	178	66	112	11200000	0.05	0.555	1
2.0	3.33E-08	348	140	208	20800000	0.05	0.555	2
3.0	5.00E-08	517	221	296	29600000	0.05	0.555	4
3.5	5.83E-08	601	266	335	33500000	0.05	0.555	6

8. Selectivity values for Figure 5 van Deemter plots.

The following table shows the selectivity values between the enantiomers in each van Deemter plot at 0.2 mL/min and 3 mL/min. Frictional heating at high flow rates impacts the retention of analytes and consequently the selectivity.

Table S8: Selectivity Values for Figure 5.

van Deemter plot	Selectivity α	
	0.2 mL/min	3 mL/min
Figure 5A (CF6-P, 10cm SPP, thermostated at 25 °C)	1.37	1.36
Figure 5B (CF7-DMP, 10cm SPP, thermostated at 25 °C)	1.35	1.32
Figure 5C (teicoplanin, 5cm SPP, thermostated at 25 °C)	1.95	1.82
Figure 5D (teicoplanin, 5cm SPP, non thermostated)	1.93	1.77

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