980947

OBSERVATIONS ON THE WETTING OF REVERSED-PHASE HPLC PACKINGS

<u>T. Walter</u>, P. Iraneta, and M. Capparella Waters Corporation, 34 Maple Street, Milford, MA 01757 USA

Presented at HPLC'97, Birmingham, UK June 1997 Poster #WT208

Abstract

The behavior of reversed-phase HPLC packings is strongly dependent on the interaction of the mobile phase with the stationary phase. One important aspect of this interaction is wetting, or spreading of the mobile phase on the stationary phase surface. The penetration of the mobile phase into the pores of porous packings is strongly affected by the degree of wetting, as well as by the pressure difference across the interface, and the pore size distribution of the packing. Because most of the surface area of porous packings resides in the pores, the extent of penetration of mobile phase into the pores affects retention times. We have developed chromatographic protocols to observe the wetting of reversed-phase packings, and determined the extent of wetting using water/methanol mobile phases of varying water content. Results are presented for a range of C18- and C8-silica packings, showing the effects of different pore size distributions and bonded phase coverages. Results will also be presented for novel reversed-phase packings containing an internal polar group (prepared by bonding silica with 3-(chlorodimethylsilyl)propyl-N-octylcarbamate or -N-octadecylcarbamate), which are wetted even by completely aqueous mobile phases.

Observations

Chromatographers have observed complete loss of retention when working with low organic mobile phases.



- When we investigated this phenomenon we found that retention times were stable for over 20 hrs (77 injections) using 100% aqueous mobile phase.
- However, when flow was stopped, then restarted, retention was lost. This observation suggested that the mobile phase is extruded from the pores when pressure is released from the column.

Conclusion

- Flow must be stopped for an extended period of time to dewet the stationary phase.
- Once wet, stationary phases remain wet in lower methanol content mobile phases than are required to rewet once dewetting has occurred.
- Three stationary phase characteristics that reduce the degree of dewetting after stopping flow in low organic mobile phases:
 - Iow coverage (however, this often results in poor peak shapes for basic compounds)
 - larger pore size
 - embedded polar groups (which can also provide unique selectivity)

Stationary phases containing polar embedded groups do not dewet even when flow is stopped for extended periods of time.

Wetting Theory: Pressure Required to Force a Fluid into Pores

Young and Laplace Equation:

$$P_c = -\frac{4\gamma}{d}\cos\theta$$

Contact Angle:



non-wetted surface

where:

- P_c = capillary pressure
- γ = surface tension
- d = capillary or pore diameter
- θ = contact angle



wetted surface

Wetting Theory: Water vs. Methanol on C₁₈-Silica



*B. Janczuk, T. Bialopiotrowicz and W. Wojcik, Colloids and Surfaces 36 (1989) 391-403

Effect of Dewetting on $V_0(D_2O)$



Testing Resistance to Dewetting



Effect of Flow Stop Time on Dewetting

The largest change in retention occurs within the first 1 min of flow stoppage.

However, for low coverage materials retention continues to decrease as flow stoppage times increase.



Experimental

Sample:

- 100 µg/mL Sulfanilamide
- 100 µg/mL Procainamide in 80:20 D₂O/methanol

Structures:



HPLC Analysis Conditions:

- I mL/min flow rate except for high pressure runs
- 25°C column temperature
- 254 nm detection wavelength
- 5 µm particles in 3.9 x 150 mm columns
- 10 µL injection volume (n=3)
- D₂O used as V₀ marker
- Mobile Phases
 - 20 mM K₂HPO₄, pH 6 (100%)
 - Methanol/ 20 mM K₂HPO₄, pH 6 (5:95)
 - Methanol/20 mM K₂HPO₄, pH 6 (10:90)

Calculations:

Retention Factor =
$$\frac{V_r - V_o}{V_o}$$

% Dewetting =
$$\frac{k_{before} - k_{after}}{k_{before}} x100$$

% Rewetted =
$$\frac{k_{after}}{k_{before}} x 100$$

notes: before stopping flow (before) after stopping flow (after)

Effect of Pore Diameter on Stationary Phase Dewetting



Packing Material: C18 Bonded High Purity Silica 3.21-3.53 µmol/m²

Mobile Phases:

- 100% Aqueous
- 🔶 95% Aqueous
- **~ 90% Aqueous**



Effect of C₁₈ Coverage on Stationary Phase Dewetting



Effect of Embedded Polar Group



Less dewetting with an embedded polar group(Shield RP) even with:
high coverage (3.1 µmol/m²)
small pore size (65 Å)

Mobile Phases:



To Rewet a Stationary Phase Once Dewetted:

- Use a mobile phase containing > 40 % methanol or other polar organic solvent
 - this works by reducing the contact angle
- Use pressure to force aqueous mobile phase back into pores
 - not practical because column outlet is at atmospheric pressure

Rewetting Stationary Phase by Changing the Contact Angle



% Methanol in Water used to Rewet Stationary Phase

Rewetting C₁₈ Stationary Phase using Pressure



Pressure Gradient across Column



Stationary Phase Remains Wet until Flow is Stopped (Dewetting Hysteresis)



Packing Materials used in Evaluation

Packing Material	Base Silica			Bonded Silica			
	Surface	Pore	Avg. Pore	C18	Surface	Pore	Avg. Pore
	Area ¹	Volume ¹	Diameter ¹	Coverage	Area ¹	Volume ¹	Diameter ¹
	(m²/g)	(cm³/g)	(Å)	(µmol/m²)	(m²/g)	(cm³/g)	(Å)
A -C18	332	0.878	93	3.21	136	0.31	67.4
B -C18	203	0.868	155	3.50	114	0.517	121
C -C18	145	0.838	215	3.41	91.2	0.578	175
D -C18	128	0.831	245	3.53	85.1	0.609	199
E -C18	319	0.839	91	2.00	184	0.467	69.5
F -C18	319	0.839	91	2.30	170	0.444	69.4
G -C18	337	0.880	93	2.62	155	0.425	70.9
H -C18	337	0.880	93	2.80	143	0.394	70.5
l - RP18	344	0.861	91	3.07	106	0.274	64.9
J - RP8	331	0.816	88	3.31	168	0.405	63.0
K - C8	329	0.860	93	3.41	201*	0.513*	67.0*

* values obtained for similar batches

¹measured by multipoint N₂ sorption

Symmetry and SymmetryShield are trademarks of Waters Corporation. © 1997 Waters Corporation. Printed in the U.S.A. 6/97 WT208