

# **The Effect of Contact Angle and Wetting on Performance in Solid Phase Extraction**

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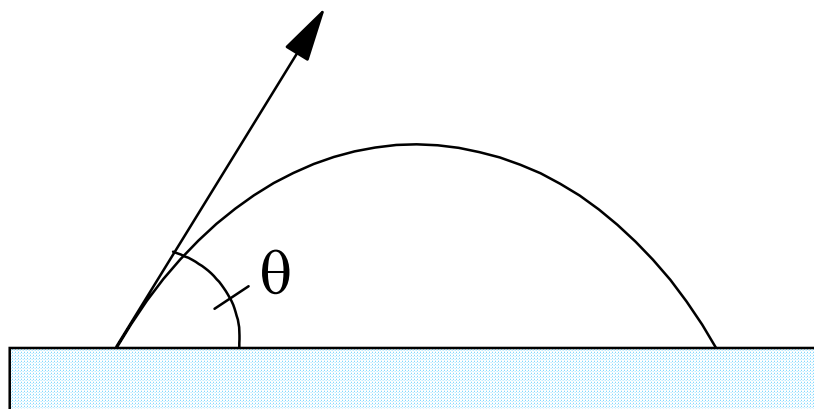
# Abstract

Solid phase extraction (SPE) is a routinely used chromatographic technique for sample enrichment and sample clean-up. Processing aqueous samples by SPE is predominantly performed using cartridges containing C18-bonded silica. Before a sample can be applied onto the cartridge, the sorbent requires a conditioning step with a water-miscible solvent. The purpose of this step is to wet the surface of the highly porous stationary phase support. This wetting depends on the contact angle of the solvent/sorbent/air system. Recently, we have introduced a porous polymeric sorbent, poly(divinylbenzene-co-N-vinylpyrrolidone) for reversed-phase solid phase extraction. This sorbent possesses greater water-wetting properties than traditional C18-silica sorbents. We have measured wetting properties for several hydro-organic mixtures on both C18-silica and the polymeric resin. We have correlated the results to performance of the sorbents in solid phase extraction.

# Introduction

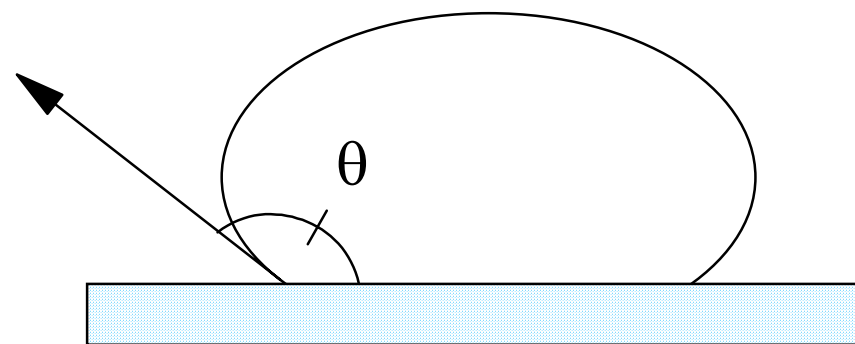
Solid phase extraction (SPE) and thin layer chromatography (TLC) are two examples of techniques in which a fluid is passed through a column or bed initially containing dry porous particles. In SPE, a conditioning step is typically performed to fully wet the porous sorbent before the sample is applied. One common theory that has been postulated to explain why this is necessary is that methanol "solvates" the alkyl-bonded phase. As a result, these C8 or C18 groups, which now have methanol associated with them, become fully extended and can thus interact better with the aqueous sample. Another theory, postulated here, is that aqueous liquids cannot penetrate into the pores of reversed-phase sorbents because of the unfavorable contact angle that the liquid has with the sorbent. Over 99.9% of the surface area of a porous silica resides within the pores, so failure to penetrate into the pores will prevent adequate adsorption of analyte. Liquid can only enter these pores if (1) the contact angle between the liquid and the surface is less than  $90^\circ$ , or (2) the external pressure is great enough to overcome the capillary pressure. When performing SPE, a vacuum ( $\sim 0.4$  bar) is typically used to effect flow. Under such conditions, a non-wetting liquid ( $<90^\circ$ ) will not penetrate the pores.

# Contact Angle Definition



$$\theta < 90^\circ$$

wetted surface



$$\theta > 90^\circ$$

non-wetted surface

A surface is wetted by a solvent when the contact angle is less than  $90^\circ$ . Wetting of pores occurs without external pressure when  $\theta < 90^\circ$ .

# Pore wetting on C18-silica for water and methanol

Equation of Young and Laplace:

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

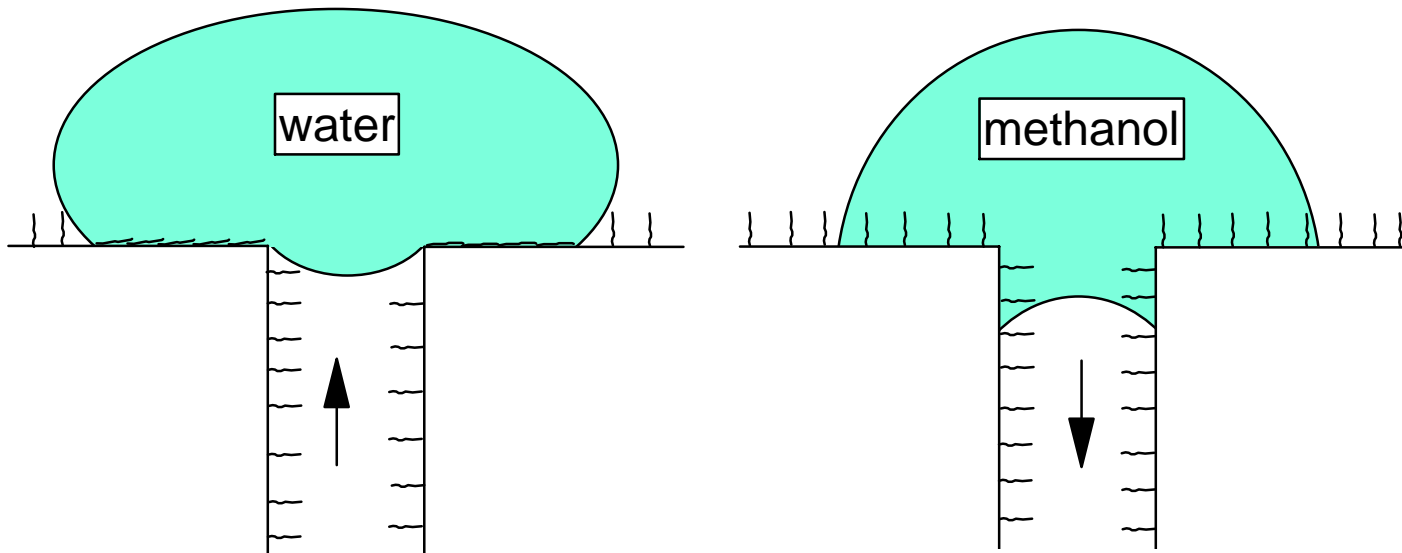
where

$P_c$  = intrusion pressure

$\gamma$  = surface tension

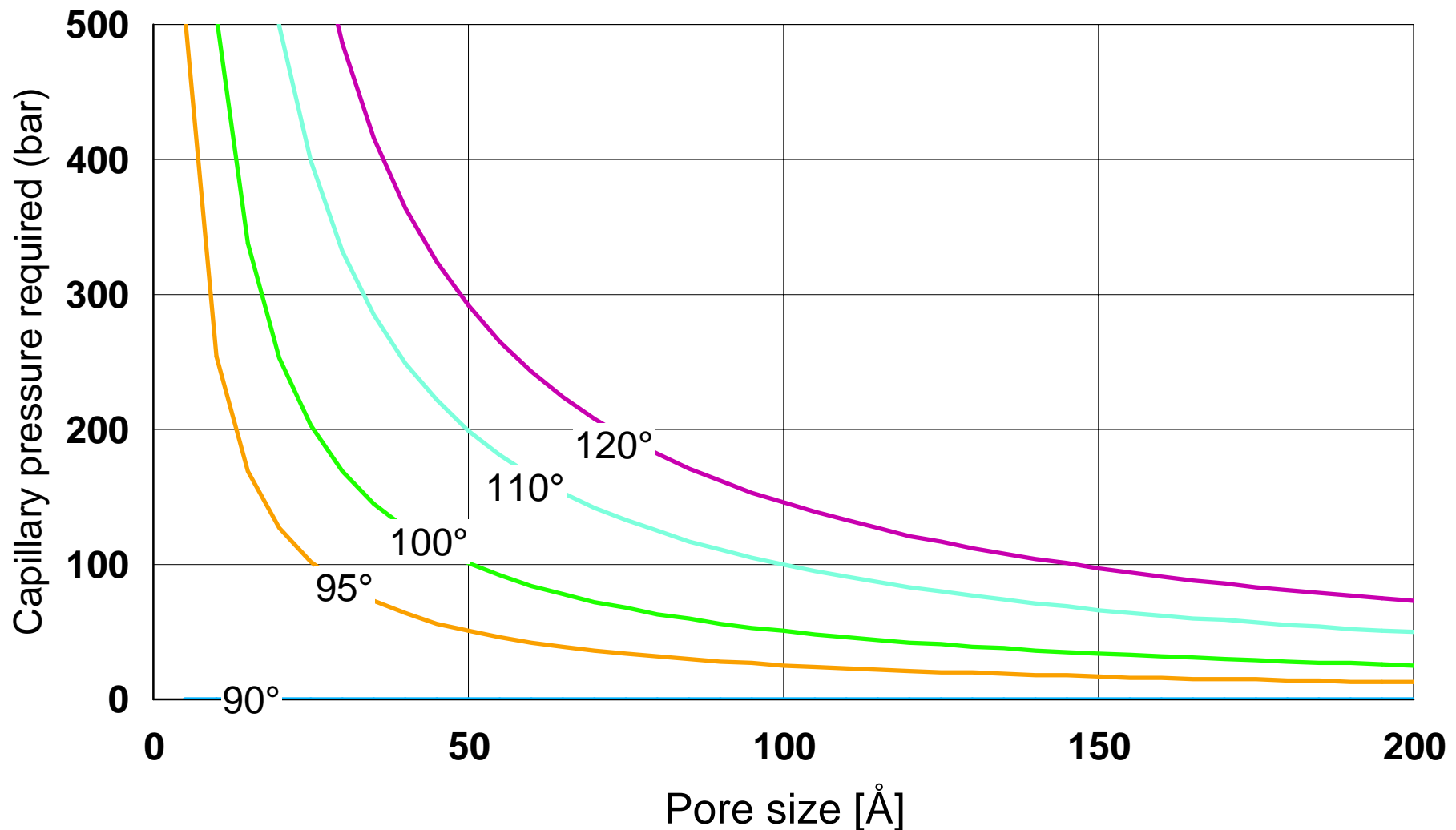
$d$  = capillary diameter

$\theta$  = contact angle



For water on C18-silica,  $\theta > 90^\circ$ . Water does not penetrate pores.  
For methanol on C18-silica,  $\theta < 90^\circ$ . Methanol fully wets pores.

# Pressure Required for Water to Penetrate Pores at Different Contact Angles



# SPE Procedure

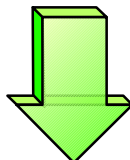
## Important considerations:

Conditioning solvent must fully wet pores (*i.e.*, intrusion contact angle must be  $< 90^\circ$ ).

Equilibration solvent, sample and wash solvents must not allow liquid to extrude from pores (*i.e.*, extrusion contact angle must be  $< 90^\circ$ ). [NOTE: Due to hysteresis, contact angle for extrusion may be less than contact angle for intrusion.]

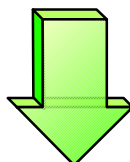
If conditioning solvent is removed from pores before application of equilibration solvent, then equilibration solvent must have *intrusion* contact angle  $< 90^\circ$  to re-wet pores.

①



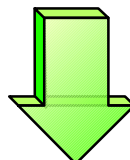
**Condition** cartridge with water-miscible organic solvent (*e.g.*, methanol or acetonitrile)

②



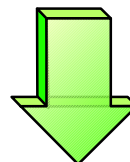
**Equilibrate** cartridge with water or aqueous buffer

③



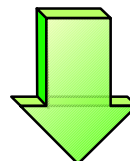
**Load** sample onto cartridge

④



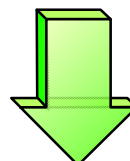
**Wash** off weakly retained interferences with weak solvent

⑤



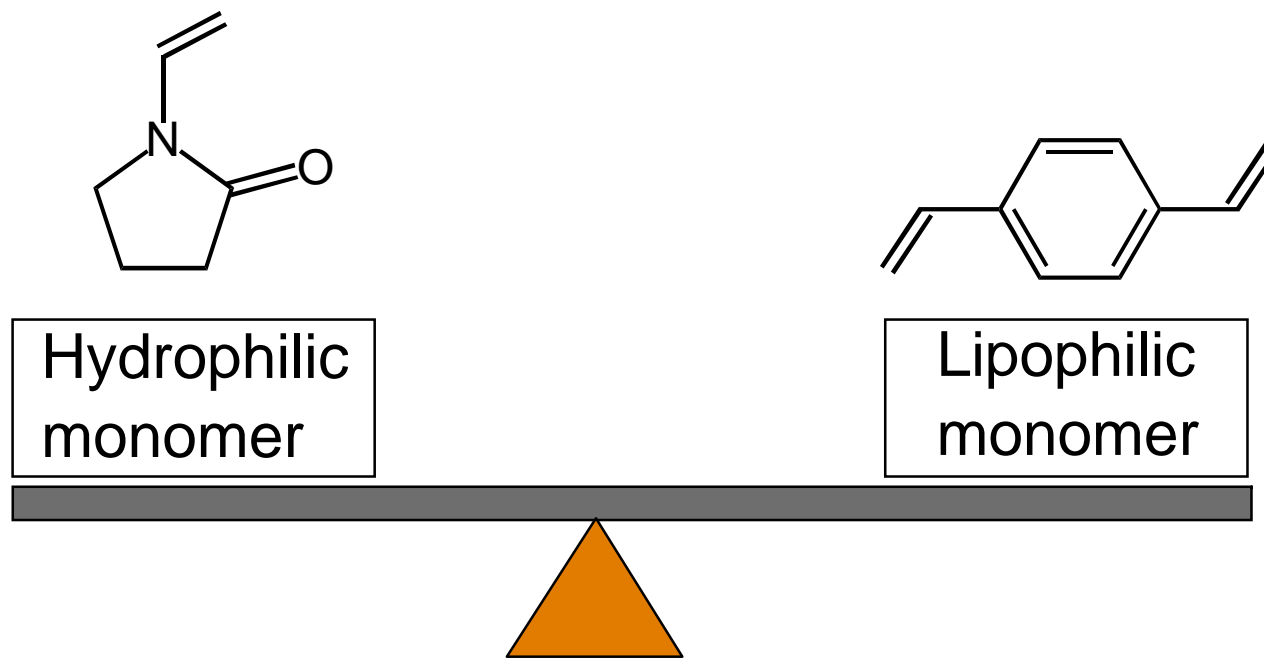
**Elute** product with strong solvent

⑥



**Evaporate, reconstitute and analyze**

# Waters Oasis™ HLB Sorbent: A Water-Wettable Reversed-Phase Copolymer

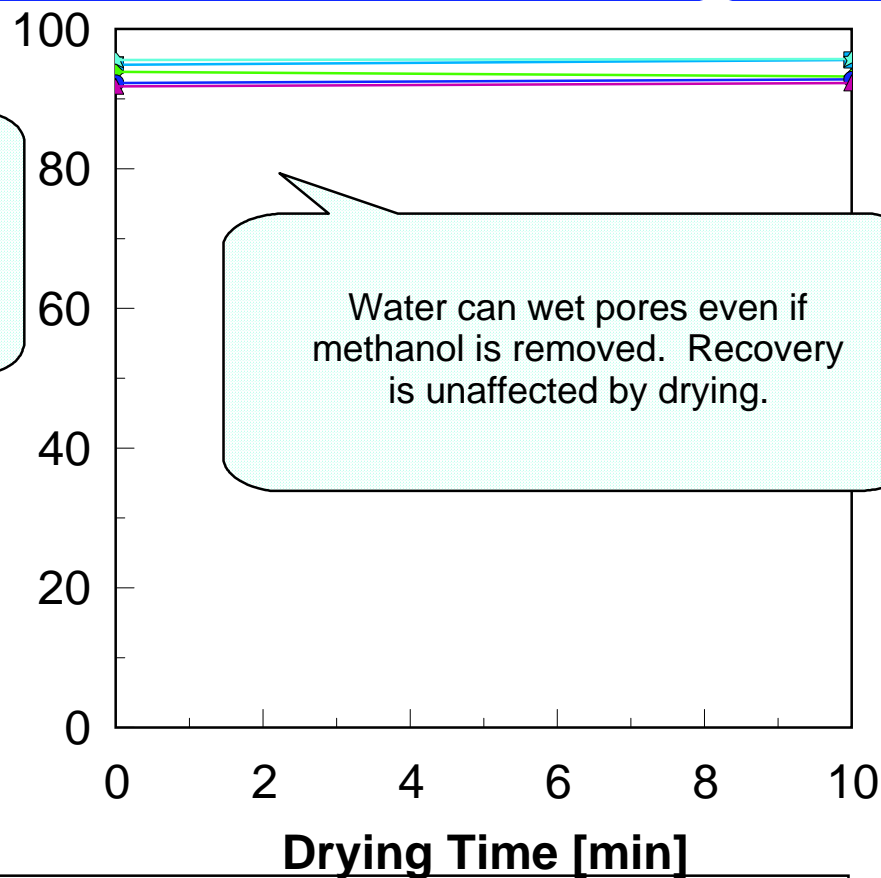
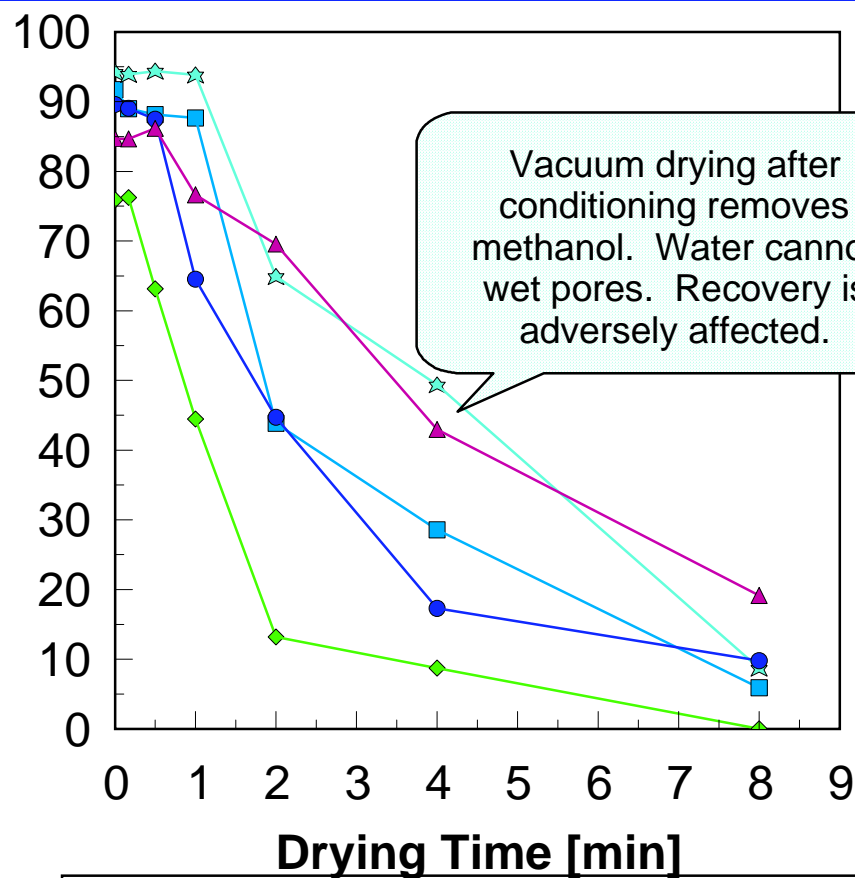


- “water loving”
- Provides wetting properties
- Reduces contact angle with water

- “fat loving”
- Provides reversed-phase property for analyte retention



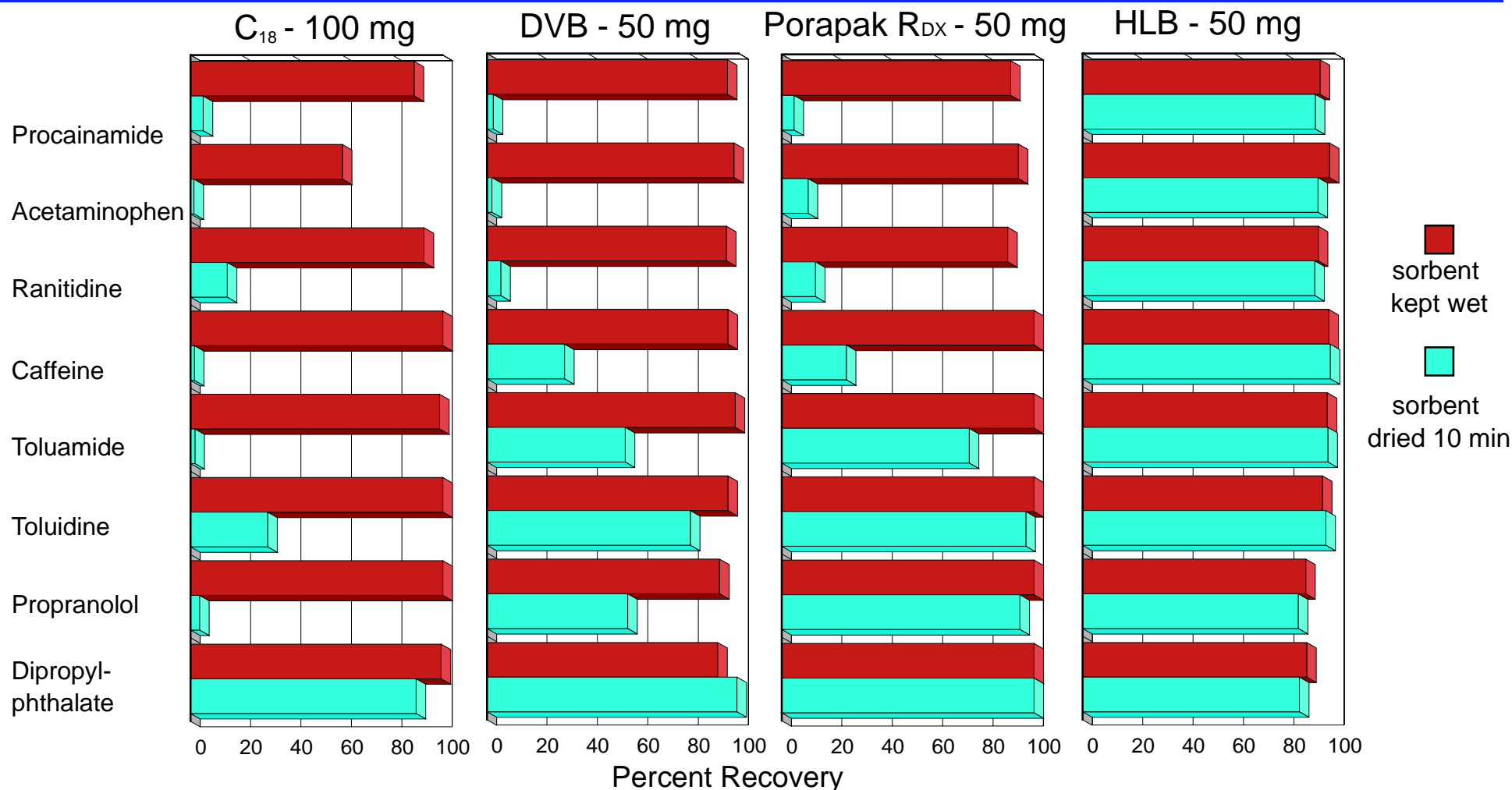
# Effect of Drying on Recovery: C<sub>18</sub> vs. Oasis™ HLB Cartridges



Procainamide	Acetaminophen	Ranitidine	Propranolol	Doxepin
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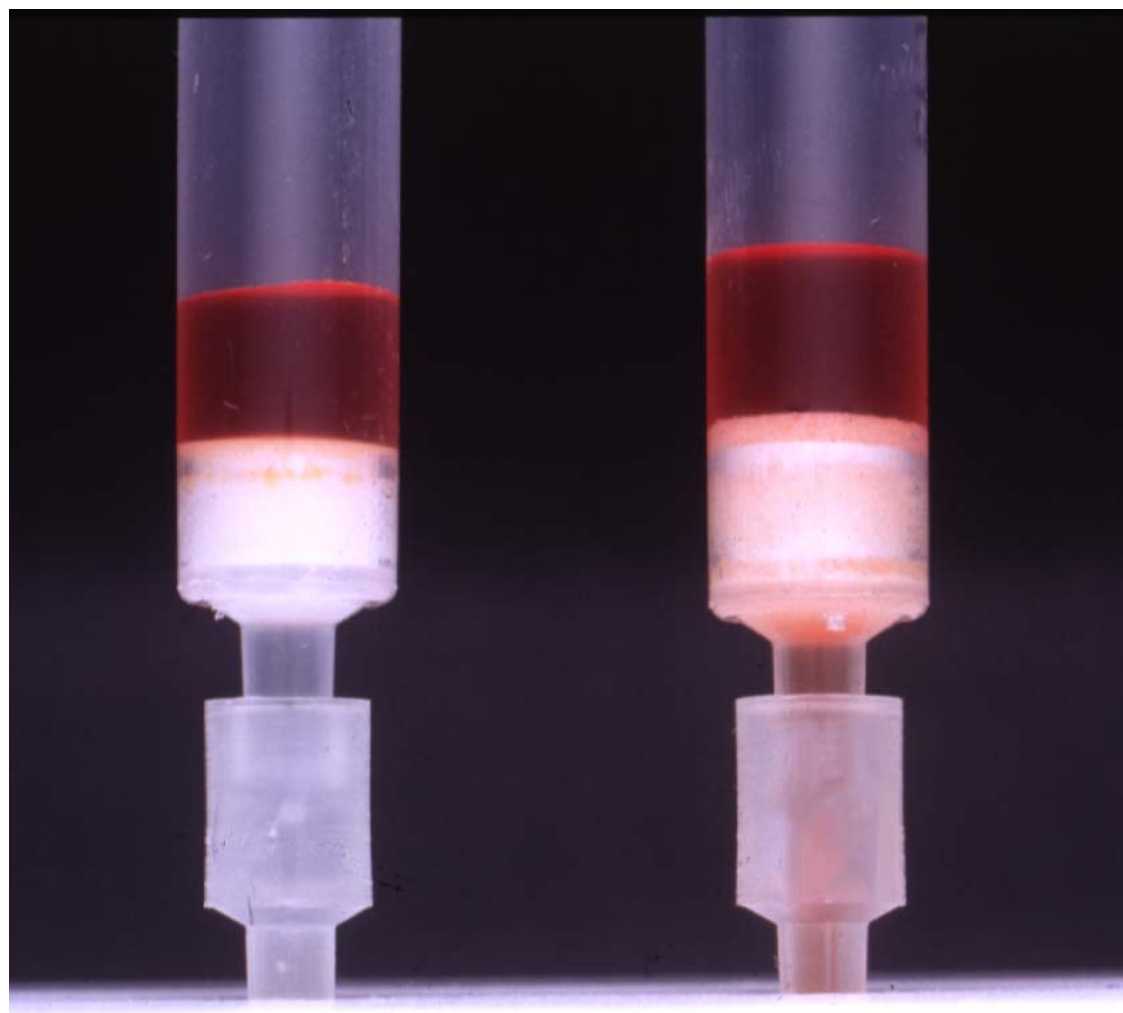
Cartridges were conditioned with 1 mL methanol, and then either (a) kept wetted, or (b) dried under vacuum for 10 minutes (0.4 bar), prior to equilibration with 1 mL water. 1 mL spiked serum was then loaded, followed by 1 mL water. Analytes were eluted with 1 mL methanol, and analyzed by HPLC.

# SPE Recoveries - Effect of Cartridge Drying



Cartridges were conditioned with 1 mL methanol, and then either (a) kept wetted, or (b) dried under vacuum for 10 minutes (0.4 bar), prior to equilibration with 1 mL water. 1 mL spiked phosphate-buffered saline solution was then loaded, followed by 1 mL water. Analytes were eluted with 1 mL methanol, and analyzed by HPLC.

# Comparison of Dye Retention: HLB vs. C<sub>18</sub>



Oasis™ HLB

C<sub>18</sub>

Shown in picture are two cartridges that have been conditioned with 1 mL of methanol and then dried for 5 minutes with vacuum (0.4 bar). 1 mL of 20 mM phosphate buffer, pH 7.0, containing phenol red was then loaded onto each cartridge. Cartridge on left (Oasis HLB) retains dye. Cartridge on right (C<sub>18</sub>) does not.

# Can an SPE Sorbent be Too Hydrophobic?

After wetting a sorbent with methanol and equilibrating with water or buffer, one of two phenomena can occur. Either (1) the pores in the hydrophobic sorbent remain wetted with water, or (2) the water extrudes from the sorbent's pores. We postulate that the *extrusion* contact angle is the driving factor here. Because of hysteresis (e.g., due to H-bonding with surface silanols), the extrusion contact angle may be significantly less than the intrusion contact angle. If the extrusion contact angle for water is  $<90^\circ$ , then the pores will remain wetted. In this case, SPE performance will be unaffected if the sorbent dries on the vacuum manifold out after applying the aqueous equilibration solvent. On the other hand, if the extrusion contact angle is greater than  $90^\circ$ , the water will be extruded from the pores, even if the sorbent bed is kept wet. In this case, low recoveries will result.

We have synthesized several different C<sub>8</sub>-silica bonded phases to measure performance in SPE. They vary in degree of surface coverage. Physical properties and surface coverage are shown in the next slide. Also included are chromatographic retention comparison for several compounds. For this data, 3.9x150 mm columns were slurry packed, and each standard was individually injected.

# C<sub>8</sub>-Silica Phases Tested:

## Effect of Bonding Density and Endcapping

Sorbent #	Chemistry	Endcap?	Coverage $\mu\text{mol}/\text{m}^2$	%MeOH for wetting ( $\theta < 90^\circ$ )
1	C8	no	2.7	55-60
2	C8	yes	3.3	55-60
3	C8	no	3.4	55-60
4	C8	yes	3.6	60-65

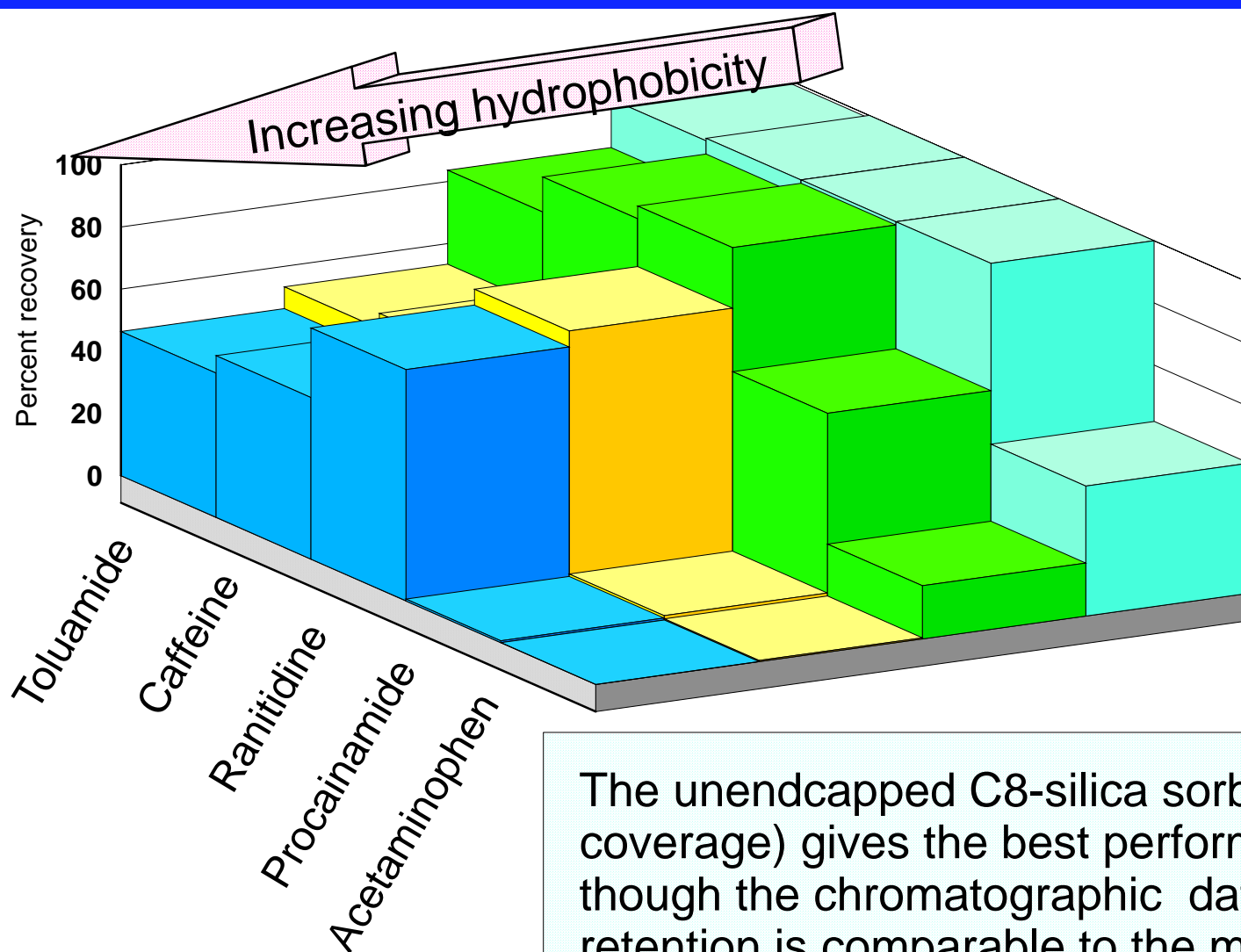
C8 bondings were carried out on 37-55  $\mu\text{m}$  Porasil. Methanol wetting was determined by observing if the sorbent wetted after mixing with different methanol/water mixtures; a wetted sorbent sinks, a non-wetted sorbent floats. For SPE, 1 mL/50 mg cartridges were conditioned with 1 mL methanol, followed by 1 mL water. After loading with 1 mL sample, and washing with 1 mL water, analytes were eluted with 1 mL methanol. *NOTE: The sorbent was not allowed to dry out during the SPE procedure.*

**Chromatographic retention factors (*k*) in 50 mM potassium phosphate, pH 7.0 with 30% methanol.**

Sorbent #	Chemistry	Acetaminophen	Procainamide	Ranitidine	Caffeine	Toluamide
1	C8	0.51	1.76	1.73	1.99	3.16
2	C8	0.41	0.27	0.26	1.02	3.10
3	C8	0.38	0.24	0.24	0.94	2.95
4	C8	0.38	0.24	0.24	0.86	3.04

# SPE Recovery on C8-Silica:

## Effect of Bonding Coverage and Endcapping



### Sorbent

	coverage	endcap?
#4 C8	high	yes
#3 C8	high	no
#2 C8	low	yes
#1 C8	low	no

The unendcapped C8-silica sorbent ( lowest coverage) gives the best performance in SPE, even though the chromatographic data indicates that retention is comparable to the more hydrophobic phases, particularly for neutral compounds.

# Conclusions

- Wetting properties play an important role in SPE performance.
  - ▶ A contact angle less than  $90^\circ$  is required for liquid to penetrate pores.
    - For C18-silica and water,  $\theta > 90^\circ$ . SPE performance will be adversely affected if the sorbent dries out after the initial conditioning step.
    - A novel reversed-phase sorbent has been designed that has  $\theta < 90^\circ$  with water. SPE performance is unaffected if sorbent dries out.
- RP-silica sorbents with high bonding coverage may give poor SPE performance.
  - ▶ Water may extrude from pores, even if pores have been previously wetted.