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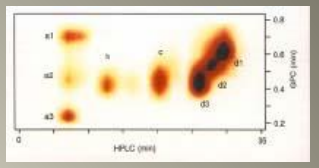

Size exclusion chromatography (SEC) with superficially porous (core-shell) particles

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Talk outline

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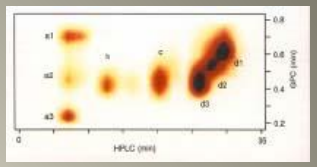

Introduction

- Size exclusion chromatography (SEC) and superficially porous particles (SPPs)

Experimental results of polystyrene separations

- Chromatograms, retention volume, plates, temporal plate production (speed), specific resolution
- Peak capacity, dilution volume

Optimization of shell thickness for plates and peak capacity

Applications

- Gold nanoparticle separations

Closing remarks

SEC basics

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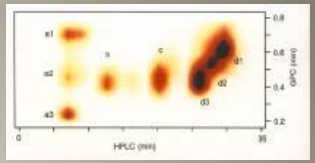
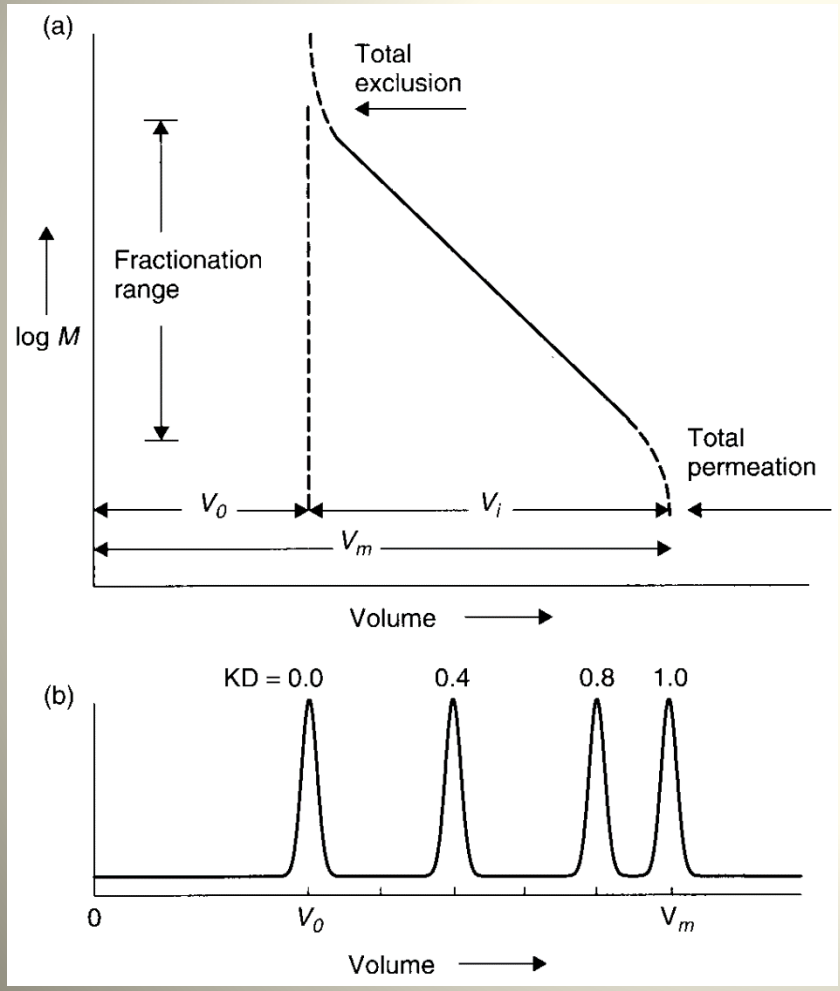


Hypothetical SEC calibration curve (a) and chromatogram (b).



Molecular weight of unknown compounds can be estimated accurately only if their structures are similar to the standards used to generate the calibration curve.

The molecular weight range can be extended by coupling columns with particle pore sizes that differ.

$$V_R = V_0 + K \cdot V_i$$

If V_i is larger then the retention volume, V_R is larger at constant K. This is the case for fully porous particles (FPPs). V_R is less for superficially porous particles (SPPs).

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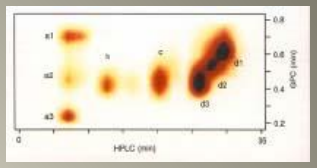

SEC: the good and bad

Problems with SEC

- Limited peak capacity
  - typically ≈10 peaks; maybe more maybe less
  - for biomolecules there is always retention effects which reduce efficiency
    - typically run in water so phase must be hydrophilic
- Limited elution volume
  - peaks elute between V_0 and V_M so elution range is limited
- Generally a low resolution technique
- High shear force can (allegedly) tear up sensitive biomolecules, more later.

Good things about SEC

- Easy to use if adsorption is not a problem
- Compatible with multiangle light scattering (MALS) detectors
- Sometimes it's the only game in town
- Works well with 2D chromatography

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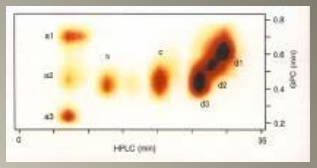
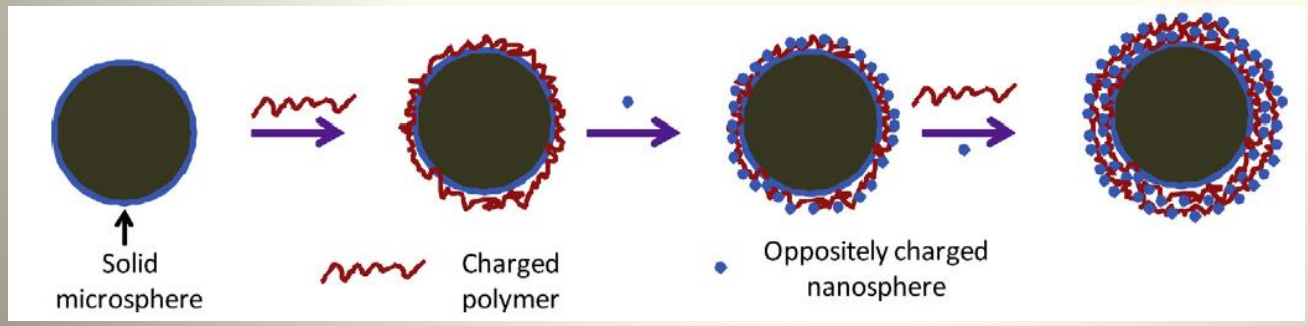
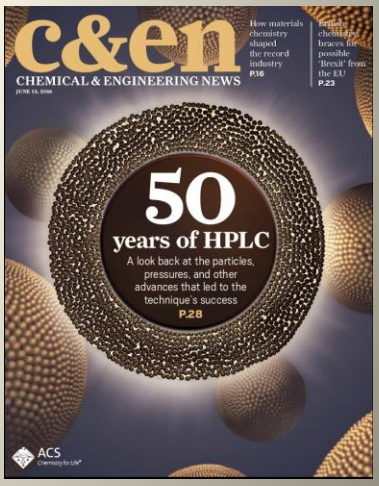


Superficially porous particles (SPPs)

- Originally introduced by Horvath and Lipsky (J. Chrom. Sci. 7(2) (1969) 109-116.)
- Developed extensively by Jack Kirkland and staff at AMT
- The idea is simple: put a thin porous shell on a nonporous core
  - The shell thickness determines mass transport characteristics in the particle
  - Performance of a 2.7 µm particle rivals ≤ 2 µm particles with lower pressure drop

Core shell particle synthesis – the "layer by layer" approach:





picture from R. Hayes, A. Ahmed, T. Edge, H. Zhang J. Chromatogr. A 1357 (2014) 36-52

Chromatograms

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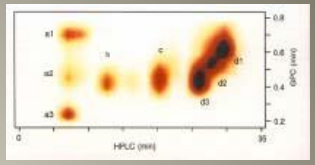
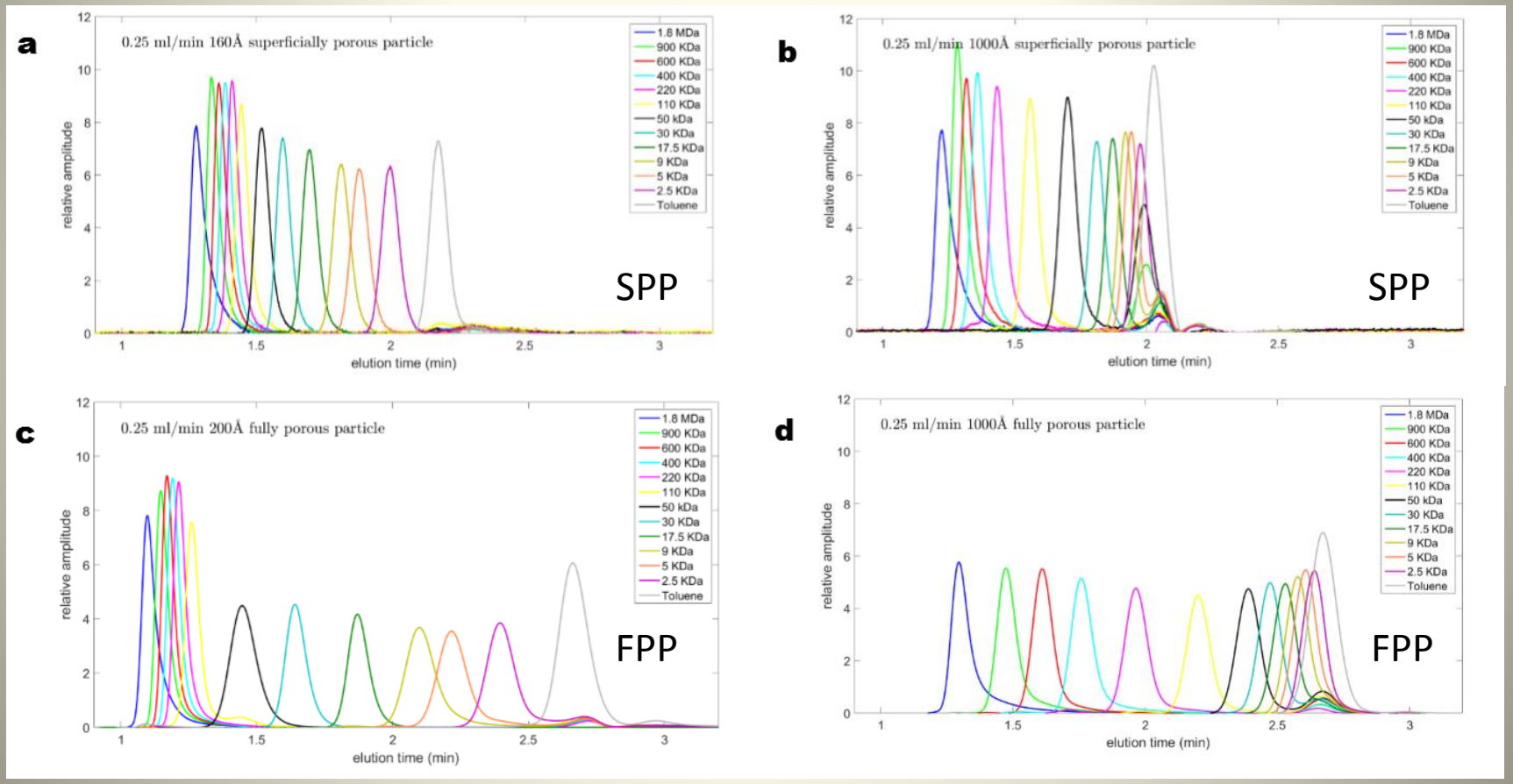


a,c: Chromatograms for small pore: 160 and 200 Å SPPs and FPPs
b,d: Chromatograms for wide pore: 1000 Å SPPs and FPPs



- Things to notice: SPPs – thinner zones and less retention time (volume) range

Retention volume

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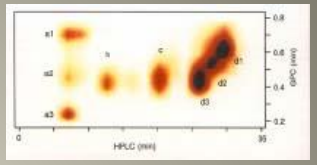
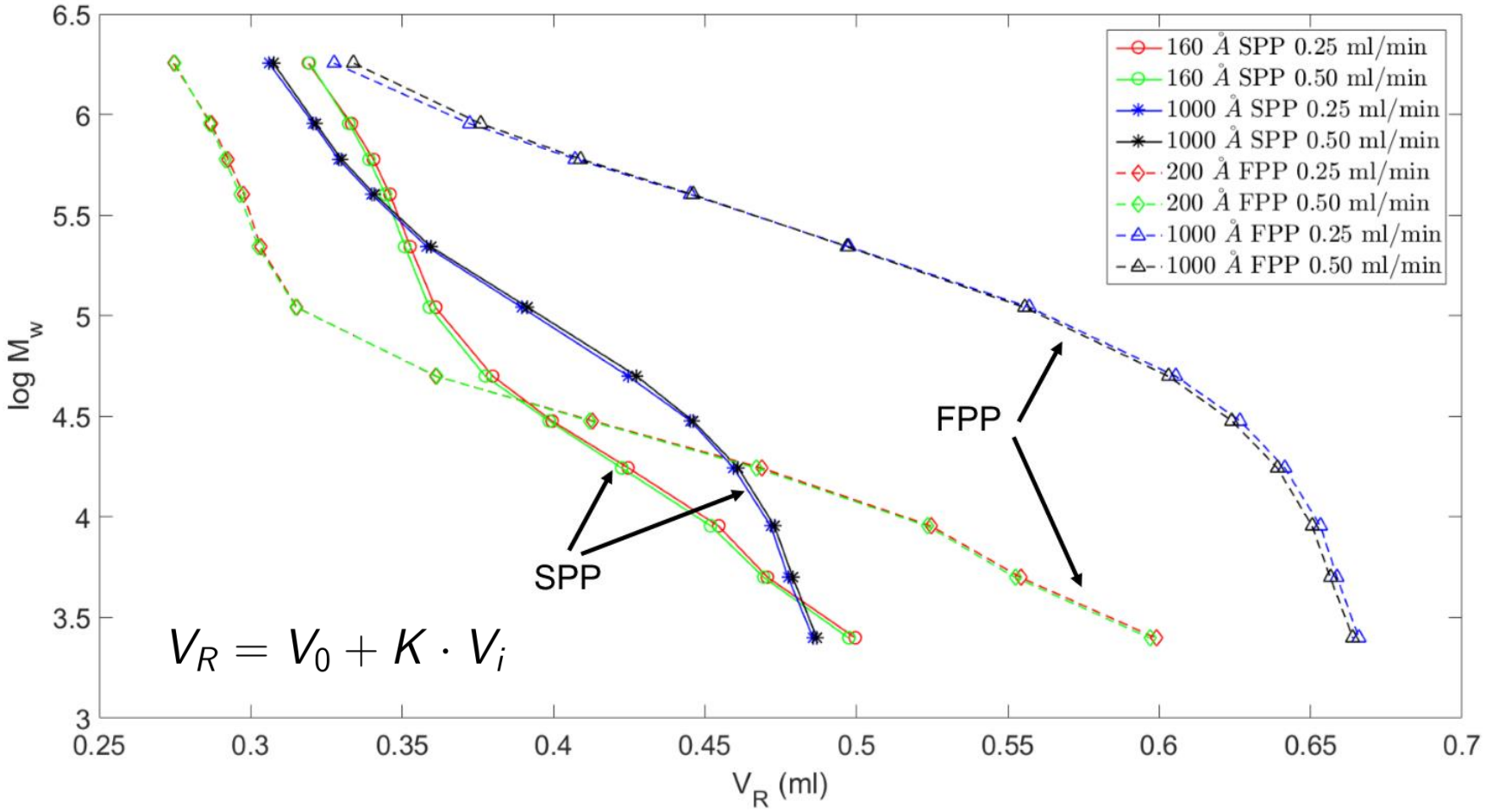


The retention volume, V_R, for SPPs and FPPs



Smaller retention volume range because V_i is smaller

This suggests faster elution with SPPs

Plates

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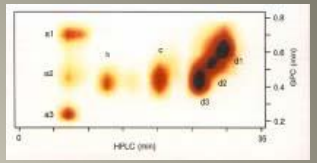
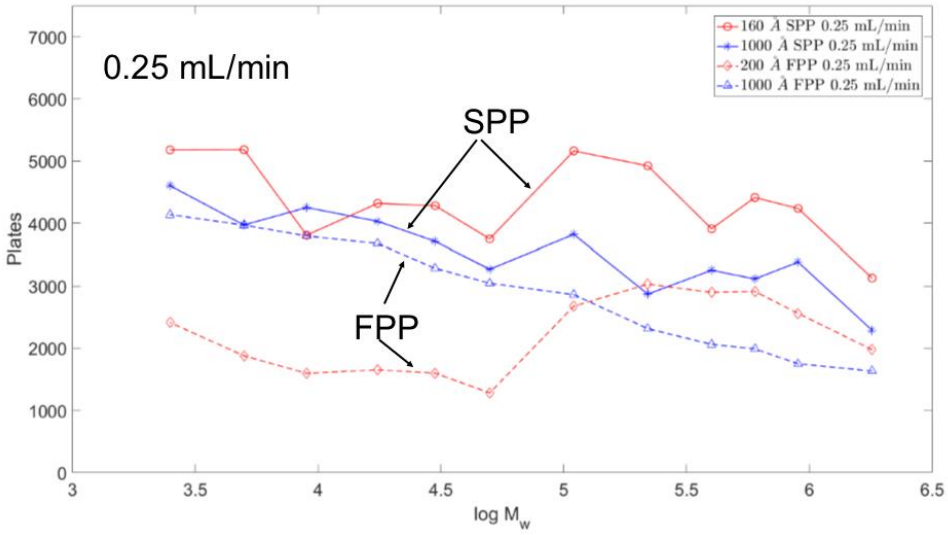
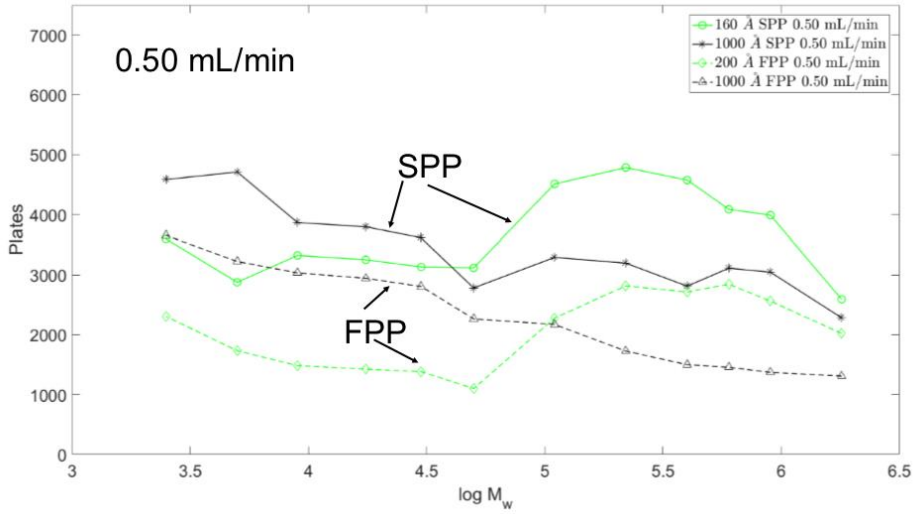


Plates comparison





SPPs promote higher number of plates

$N=(t/\sigma)^2$ - this is a balance between thermodynamics and kinetics

Kinetics wins out with SPPs

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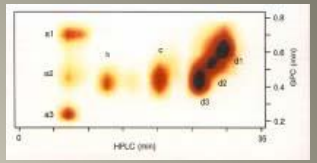

Plates per unit time

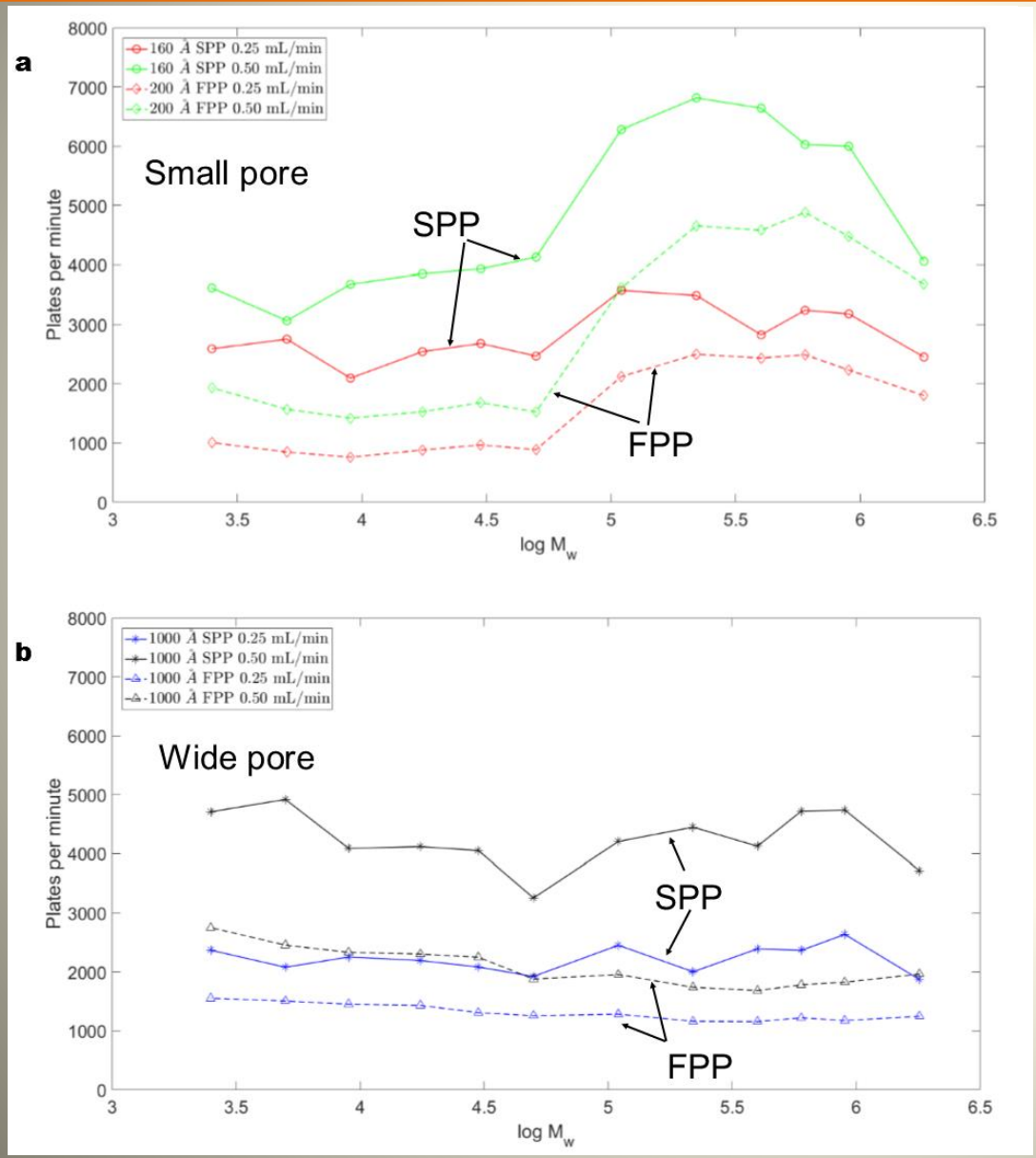

Plate production per unit time is faster for SPPs than FPPs

Plate production per unit time is often referred to as speed[1]

[1]P. W. Carr, D. R. Stoll, X. Wang, Anal. Chem. 83 (2011) 1890-1900.

Resolution

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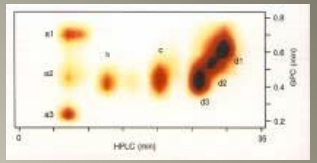
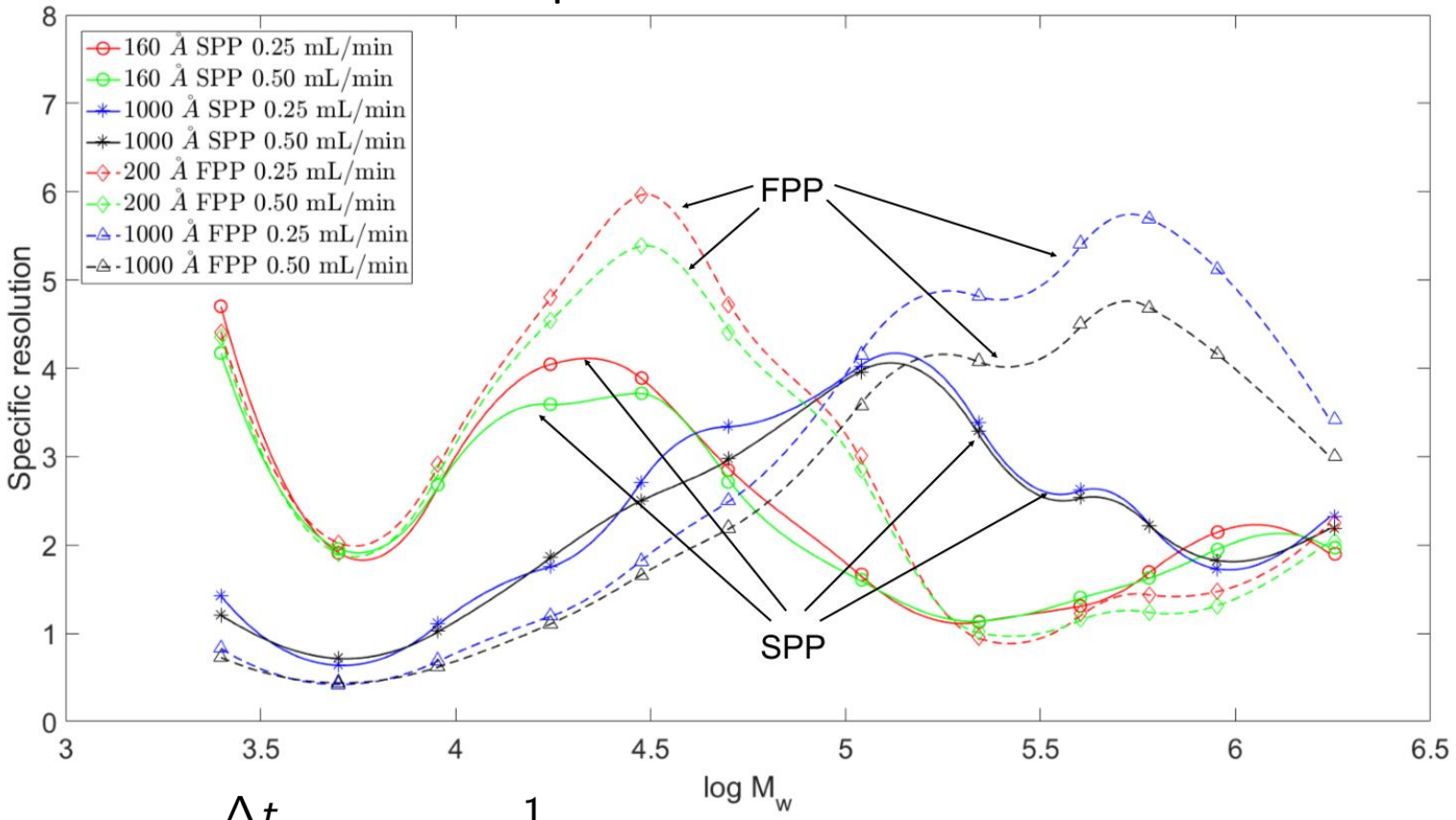


Specific resolution



$$R_{sp} = \frac{\Delta t}{2(\sigma_1 + \sigma_2)} \cdot \frac{1}{\log(M_{w2}/M_{w1})}$$

FPP's have higher resolution than SPP's

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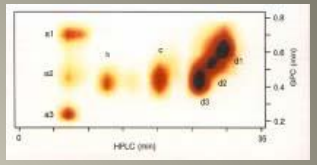
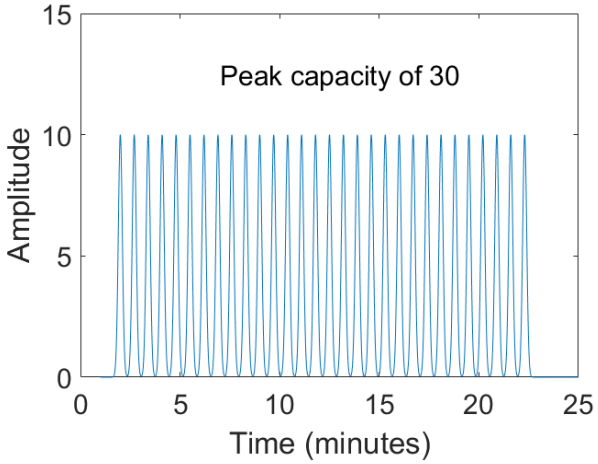


Peak capacity in SEC: SPPs versus FPPs

Peak capa**c**ity is the number of peaks that can be put next to each other with constant spacing between peaks

$$n_c = \frac{t_f - t_1}{4\sigma R_s} \qquad n_c = 1 + \frac{V_p / V_t}{4}\sqrt{N}$$

from Lars Hagel, J. Chromatogr. A 591 (1992) 47-54.



Morphology	Pore size (A)	flow rate (mL/min)	V_p	V_t	V_p / V_t	N	$\sqrt{N} = t/\sigma$	n_c
SPP	160	0.25	0.164	0.544	0.301	4907	70.0	6.3
SPP	160	0.5	0.164	0.542	0.303	7056	84.0	7.4
SPP	1000	0.25	0.176	0.497	0.354	3410	58.4	6.2
SPP	1000	0.5	0.176	0.497	0.354	5399	73.5	7.5
FPP	200	0.25	0.379	0.666	0.569	2658	51.6	8.3
FPP	200	0.5	0.376	0.663	0.567	3731	61.1	9.7
FPP	1000	0.25	0.303	0.675	0.448	2717	52.1	6.8
FPP	1000	0.5	0.299	0.675	0.443	3926	62.7	7.9

- Peak capacity in SEC is never large (≤ 10)
- The SPP and FPP peak capacity are ≈ the same with a slight advantage to FPPs

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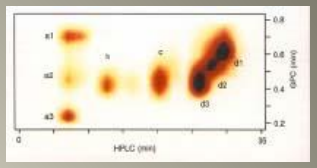


Zone Dilution

$$C_{max} = m_{inj} / V_{dil}$$

smaller dilution volume → larger concentration max
C_{max} is proportional to chromatographic peak height

$$V_{dil} = \frac{V_R \sqrt{2\pi}}{\sqrt{N}}$$

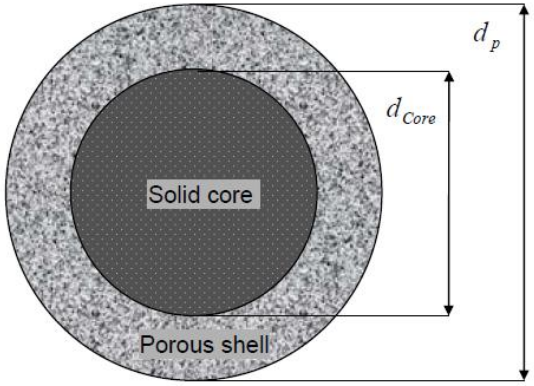
smaller retention volume & larger plate count → minimize V_{dil} **advantage: SPP**

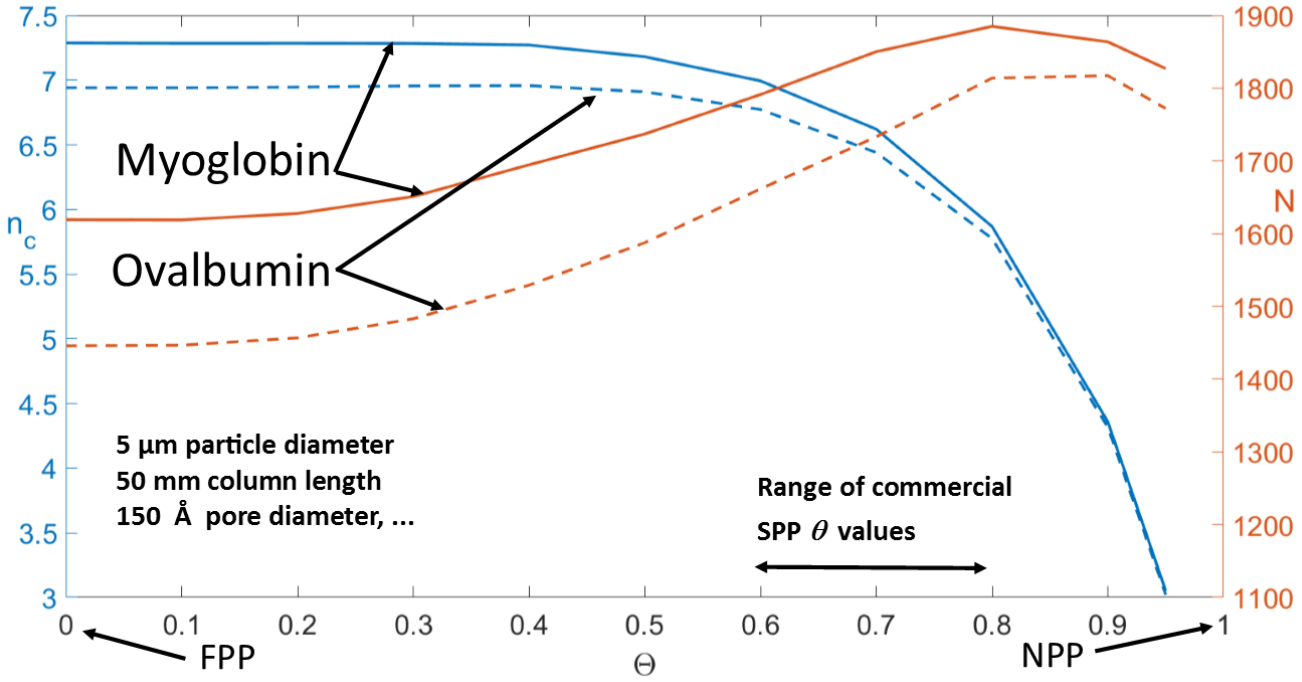
These equations are pertinent to 1D
and to 2D when SEC is in the second dimension

V_{dil} is very important in biomarker research
high V_{dil} can be bad in 2D chromatography – you can lose the signal

Optimization of shell thickness for SEC SPP's

1) Use general rate model for different shell thickness θ, calculate moments.
2) Use Hagel's expression $n_c = 1 + \frac{V_p/V_t}{4}\sqrt{N}$





$$\theta = \frac{d_{core}}{d_p}$$

$$\phi_{porous} = 1 - \theta^3$$

$$\epsilon_p' = \epsilon_p \cdot \phi_{porous}$$

$$\frac{V_p}{V_t} = \frac{1}{1 + \frac{\epsilon_e}{\epsilon_p'}}$$

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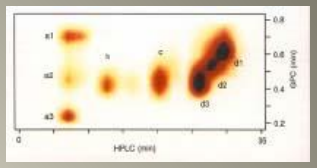

Some applications of SPP/SEC technology

- Industrial polymer separations
- Fast mAb separations from digests using 2D chromatography in the second dimension
- Isolation of extracellular vesicles (EVs) including exosomes from human plasma
- Nanoparticles: gold, silver, quantum dots
- Organic and biocolloids, liposomes for drug delivery

Gold nanoparticles

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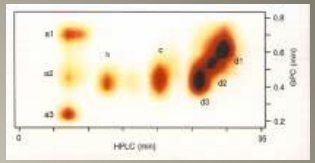
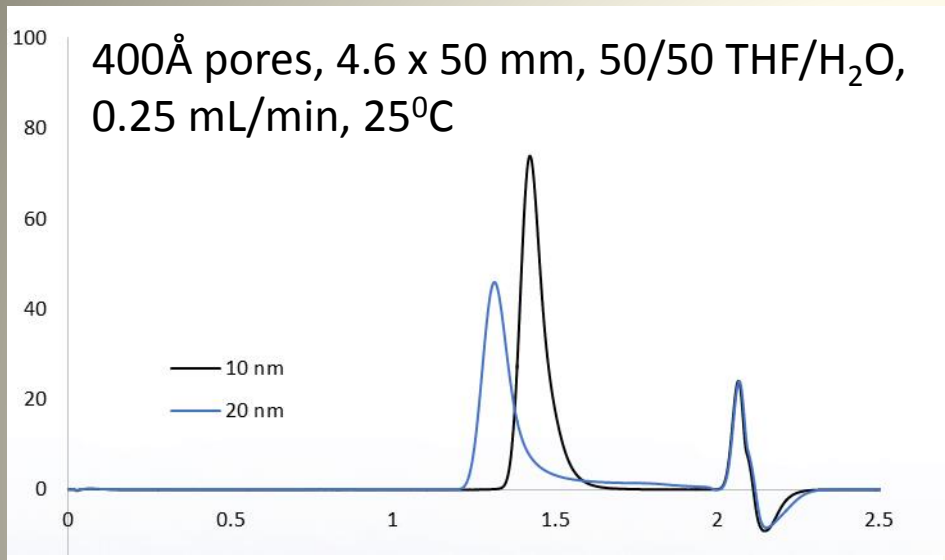
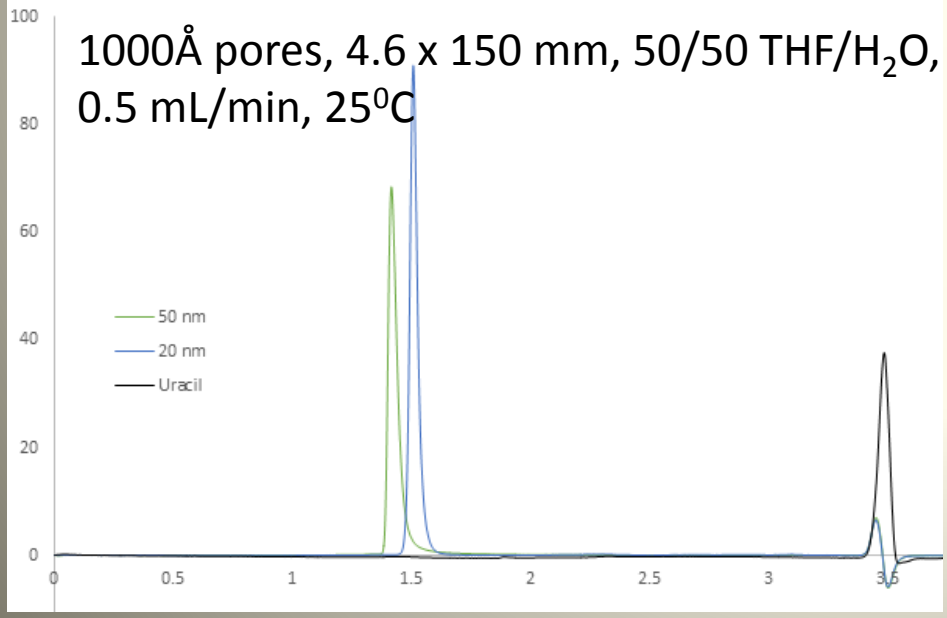


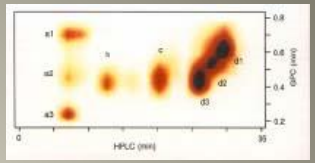




nanoComposix gold nanoparticles with citrated surfaces

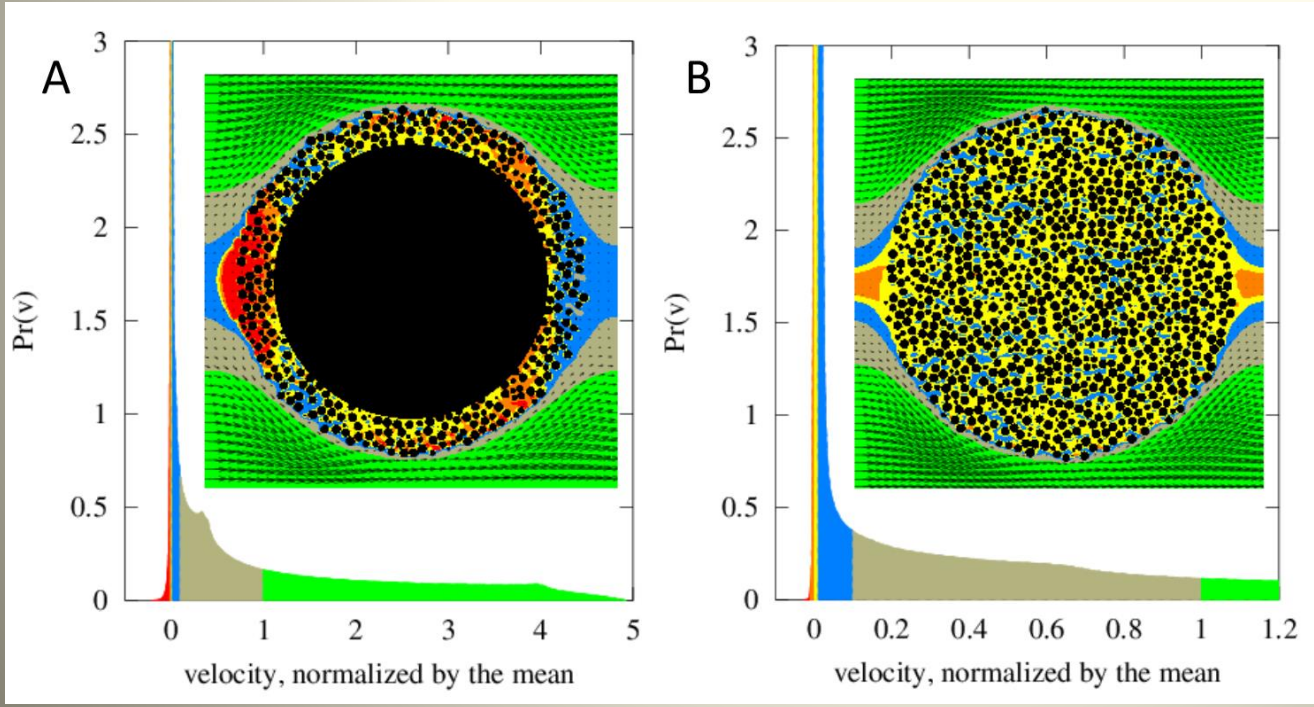
Both chromatograms use bare silica
Detection (UV) at 520 nm
Solvent systems are not obvious
- surfactants
- citrate buffers
- other salts

Need multiple columns for wide range
Stabilizers are often added



Shear and flow

- 10% of mass flux goes through the SPP particle
- Shear, the spatial derivative of the velocity field, is calculated from previous work.
- Most of the shear field is weak; strongest at the hull; molecules can break just by adsorption[1]



[1]S. S. Sheiko, F. C. Sun, A. Randall, D. Shirvanyants, M. Rubinstein, H. Lee, K. Matyjaszewski

Adsorption-induced scission of carbon–carbon bonds

Nature 440, (2006) 191-194

SPPs vs FPPs for SEC

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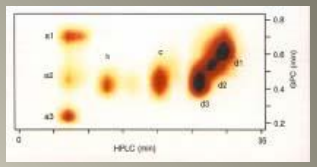

Advantages for SPPs:

- Small diffusion distance, faster kinetics **especially for large molecules**
- More efficient (plates)
- More plates per unit time (faster)

Advantages for FPPs:

- More resolution – use FPPs when time is not an issue

Wide pore (1000 Å) SPP's

- Flow in the shell (perfusion chromatography)
  - Computational fluid dynamics shows this
  - Really important for high efficiency in large molecule analytical separations – "even faster" kinetics

Reading

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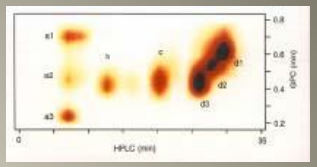

1. J. Luo, W. Zhou, Z. Su, G. Ma, T. Gu Comparison of fully-porous beads and cored beads in size-exclusion chromatography for protein purification Chem. Eng. Sci. 102 (2013) 99-105.

2. M. R. Schure, R. E. Moran Size exclusion chromatography performed with superficially porous particles J. Chromatogr. A 1480 (2017) 11-19.

3. B. W. J. Pirok, P. Breuer, S. J.M. Hoppe, M. Chitty, E. Welch, T. Farkas, S. van der Wal, R. Peters, P. J. Schoenmakers Size-exclusion chromatography using core-shell particles J. Chromatogr. A 1486 (2017) 96-102.

4. B. M. Wagner, S. A. Schuster, T. J. Shields, B. E. Boyes, T. J. Shields, W. L. Miles, M. J. Haynes, R. E. Moran, J. J. Kirkland, M. R. Schure Superficially porous particles with 1000 Å pores for large biomolecule HPLC and polymer SEC J. Chromatogr. A 1489 (2017) 75-85.

5. R. S. Maier, M. R. Schure, T. J. Shields, C. M. Wunder, B. M. Wagner Intraparticle and interstitial flow in wide-pore superficially porous and fully porous particles, submitted to Chemical Eng. Science.

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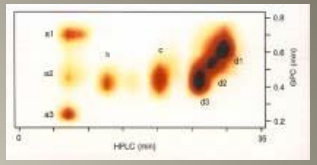

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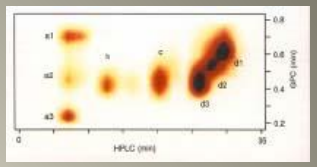

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