# Superficially Porous Particle UHPLC Columns; Assessment, Requirements and Examples

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- To identify the maximum number of analytes in the sample
- In the shortest time possible
- Obtain an accurate quantitative estimation of the concentration of each analyte in the sample

\*Quoted from Guiochon & Gritti, J. Chrom. A, <u>1228</u>, 2 (2012)

#### The HPLC Optimization Triangle



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#### The HPLC Optimization Triangle



**Resolution** 

#### **UHPLC →** Obtain the best <u>resolution</u> in the shortest possible <u>time</u>

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$$R_{s} = \frac{2(t_{R,2} - t_{R,1})}{(w_{b,1} + w_{b,2})}$$

Which can be reformulated\*:

$$R_{s} = \frac{\sqrt{N_{2}}}{4} \cdot \left[\frac{\alpha - 1}{\alpha}\right] \cdot \left[\frac{k_{2}}{k_{2} + 1}\right]$$



#### \*<u>Purnell equation</u>!

- $N_2$  plate number of the second peak
- $\alpha$  selectivity for two adjacent peaks ( $\alpha = k_2 / k_1$ )
- $k_2$  capacity factor for the 2<sup>nd</sup> peak

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#### **Increase Resolution**

- Normalized Plot of Individual Terms



Normalized range 1 -20 for N, k and  $\alpha$ 

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#### Improve Resolution; Selectivity



Selectivity ( $\alpha$ ) helps best but:

- Is driven by difference in molecular properties (polarity, charge, hydrophobicity, size etc.)
  - "Know your molecules"!!
- Is somewhat difficult to predict and optimize
  - Experience
  - Retention modeling of all molecules one is interested in
- Use of optimization software for the separation of multi-component mixtures (ChromSword, DryLab)
  - In principle a laborious procedure especially with multicomponent samples
  - Becomes impossible to do with very complex samples

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## High plate number provides:

- Narrower and therefore higher peaks
- Better signal/noise
- Higher peak capacity
- Reduction of the separation time

#### Improve Resolution; Increase Efficiency

#### High plate number provides:

- Narrower and therefore higher peaks
- Better signal/noise
- Higher peak capacity
   Jimited by statistical peak overlap
- Reduction of the separation time
  - constrained by maximum pressure

 $\frac{\sqrt{N_2}}{4} \cdot \left[\frac{\alpha - 1}{\alpha}\right] \cdot \left|\frac{k_2}{k_2 - 1}\right|$  $R_{s}$ 

<b>N</b> 7	L
N =	$\overline{H(d_p)}$

Action	Constraint
Increase length of the column	Longer analysis time Higher column pressure
Decrease H(ETP) by using smaller particles	Higher column pressure

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$$\Delta P = \frac{\varepsilon_T \Phi}{d_p^2} \cdot \frac{\eta L}{u_0} = \frac{\Phi_0}{d_p^2} \cdot \frac{\eta L}{u_0}$$

 $\Phi_0$  chromatographic flow resistance factor (has the value of  $\cong$  700 for a good column)

Column pressure increases linearly with length and with the inverse square of the particle size!!

 $u_{o,opt} \sim 1/d_p$ Column pressure in the minimum of the van Deemter plot increases with inverse of the cubic power of particle size!

\*C. Cramers et al, Chromatographia, <u>14</u>, 439 (1981)

How to achieve a particular plate number N<sub>req</sub>, say 50,000 plates, in the shortest time possible at <u>given</u> <u>maximal pressure</u> available ?

#### According to:

J.H. Knox and M. Saleem. J. Chromatogr. Sci., <u>7</u>, (1969), p. 614
G. Guiochon, Anal. Chem., <u>52</u>, 2002 (1980)
H. Poppe, J. Chrom. A., <u>778</u>, (1997) 3-21
G. Desmet et al., Anal. Chem., <u>77</u>, 4058 (2005) and http://www.vub.ac.be/CHIS/Back-up%20Website/Research/kp.html R

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#### **Poppe Plots or Kinetic Plot Method**

$$H(ETP) = A(d_p) + B/u_0 + Cu_0(d_p^2)$$

Poppe introduced the Plate Time = time per required plate number

 $\frac{N_{req}}{t_0} = \frac{H(u_0)}{u_0}$ 

Desmet introduced the Kinetic Plot Method adapted from Giddings

$$N = \left(\frac{\Delta P_{\max}}{\eta}\right) \cdot \left[\frac{B_0}{u_0 H}\right]$$



Requires to determine the coefficients of the simplified van Deemter (or Knox) equation or of the above equation.

Not a theoretical exercise, but based on own experimental data obtained on one's columns with ones compound(s) of interest!!

#### **Problems with Ultra High Pressure\***

Physical properties depend on pressure

- Density (ρ), melting point (e.g. cyclohexane), specific volume, and viscosity
   (η) of the solvent change
- Retention factors (k') and hold-up time
- Diffusion coefficients  $(D_m)$
- Porosity of the packed bed (ɛ)
- Column efficiency (N)
- Column length and diameter (L and d<sub>c</sub>)
- Frictional heating causes temperature gradients (longitudinal:  $\Delta T_L$  and radial:  $\Delta T_R$ )

#### Mechanical stress and instrumental challenges

\*Martin & Guiochon, J. Chrom. A, 1090, 16 , (2005)

Particle Morphology	Characteristic Size	Requirements, Applications
Spheres, totally porous	2.5 – 10 μm 8 – 30 nm pores	Standard materials since many years; standard equipment
Spheres, totally porous, "STM"	< 2.0 μm 8 – 12 nm pores	High backpressure, UHPLC; mandates new equipment
Superficially Porous Shell Particles	< 3.0 μm 8 – 12 nm pores	Lower pressure/plate ratio, UHPLC; standard or new equipment
Monoliths	Interstitial distances 1 – 2 μm, 8 – 12 nm pores	HPLC equipment; not many phases

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**Superficially Porous Particles (e.g. Poroshell 120)** 

The particle has 2.7  $\mu$ m outer diameter with a solid core (1.7  $\mu$ m) and porous outer layer with a 0.5  $\mu$ m diffusion path. The average pore diameter is 120 Å. The core has 25% of the particle volume. 75% of the particle volume is porous.





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#### Morphology of Superficially Porous Particles



The <u>porous volume fraction</u> of a superficially porous particle is given by

$$\rho_{PV} = 1 - \left(\frac{d_{core}}{d_p}\right)^3 \cong 0.75$$

The internal porosity  $\mathcal{E}_i$  of a superficially porous particle is assumed to be

$$\varepsilon_i = \varepsilon_{i, fully porous} \cdot \rho_{V porous}$$

## A Brief History of Superficially Porous Particles

Subject	Reference
Pellicular beads, 40-50 μm core,	Cs. Horváth, B. Preiss, and S. Lipsky, Anal. Chem., <u>39</u> , 1422–1428 (1967).
Zipax, 37 μm glass beads, 40 nm silica layer Corasil, 37-50 μm glass bead with silica layer	J.J. Kirkland, Anal. Chem., <u>43</u> (12), 36A–48A (1971), ibid., 64, 1239 (1972). Waters
1st generation Superficially Porous particles Micro-pellicular particles, 5 μm	J.J. Kirkland, Anal. Chem., <u>64</u> , 1239–1245 (1992). J.J. Kirkland et al., J. Chrom. A, <u>890,</u> 3 (2000)
Zorbax Poroshell 300, 4.5 μm core, 0.25 μm, 300Å porous shell, $ ho$ = 0.27	Agilent Technologies, 2001.
$2^{nd}$ generation Superficially Porous particles, 2.7 $\mu m$ ,	J.J. Kirkland, T.J. Langlois, J.J. DeStefano, Am. Lab. <u>39</u> (2007) 18 J.J. DeStefano et al., J. Chrom. Sci., <u>46</u> , 254 (2008)
HALO, 1.7 μm core, 0.5 μm, 90Å porous shell, $\rho$ = 0.75 HALO-5, 3.8 μm core, 0.6 μm, 90Å porous shell $\rho$ = 0.56 Poroshell 120, 1.7 μm core, 0.5 μm, 120Å porous shell, $\rho$ = 0.75. Kinetex family, 1.3, 1.7, 2.6 ( $\rho$ = 0.60), 5 μm.	Advanced Materials Technology Inc., 2007 Ibid., 2012 Agilent Technologies, 2009 Phenomenex,

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Table II: Superficially porous particle columns for reversed-phase chromatography and HILIC						
Product	Company	Particle Size (µm)	Pore Diameter (Å)	Phases	Comments	
Accucore	Thermo Fisher Scientific	2.6	80	C18, RP-MS, aQ, PFP, phenyl-hexyl, HILIC	Average particle size distribution (D90/D10): 1.12; col- umn dimensions: 30–150 mm × 2.1, 3.0, and 4.6 mm; guard columns (10-mm length) available with sepa- rate holder, validation and method development kits available; custom columns available.	
Aeris Peptide	Phenomenex	1.7, 3.6	100	C18 (for peptides)	For peptides; shell thickness (3.6-µm particle): 0.5 µm; core diameter: 2.6-µm; shell thickness (1.6-µm particle): 0.2 µm; core: 1.25 µm; column dimensions: 50–250 mm $\times$ 2.1 and 4.6 mm.	Bonshell, 2.7 μm, 0.5 μm porous shell Bonna-Agela Technologies, Wilmington, USA
Aeris Wide Pore	Phenomenex	3.6	200	C4, C8, C18 (for poly- peptides and proteins)	For polypeptides and proteins; shell: 0.2 $\mu m$ ; core: 3.2 $\mu m$ ; column dimensions: 50–250 mm $\times$ 2.1 and 4.6 mm.	
Ascentis Express	Supelco/ Sigma Al- drich	2.7	90	C8, C18, phenyl-hexyl, RP-Amide, PFP, HILIC, ES-Cyano, OH5 (HILIC)	Shell thickness: 0.5 $\mu m;$ pore size: 90 Å; pressure limit: 600 bar; dimensions: 30–150 mm $\times$ 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available	
Ascentis Express	Supelco/ Sigma Aldrich	2.7	160	Peptide ES-C18 (for peptides)	For peptides and polypeptides; shell thickness: 0.5 $\mu m;$ 600 bar; dimensions: 30–150 mm $\times$ 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available	
Brownlee SPP	PerkinElmer	2.7	90	C8, C18, phenyl-hexyl, RP-Amide, PFP, HILIC (Silica), ES-CN	Shell thickness: 0.5 $\mu m$ ; pressure limit: 600 bar; dimensions: 20–15 mm $\times$ 2.1, 3.0, and 4.6 mm; endcapped and nonendcapped phases; guard column holder and columns (5-mm lengths) available; sold in packs of three; ultralow extracolumn volume optimization kit available for instrument modification	
Brownlee SPP	PerkinElmer	2.7	160	Peptide ES-C18 (for peptides)	For peptides or polypeptides; shell thickness: 0.5 $\mu m;$ pressure limit: 600 bar; dimensions: 30–150 mm $\times$ 1.0, 2.1, 3.0, and 4.6 mm.	
Eiroshell	Irish Separa- tion Science Cluster	1.7	100	C18 (not a commercial product)	Shell thickness: 0.35, 0.25, and 0.15 µm; these are re- search products and are not commercially available	
Halo	Advanced Material Technology	2.7	90	C8, C18, phenyl-hexyl, RP-Amide, PFP, HILIC, ES-CN, penta HILIC (Pen- tanol functionality)	Shell thickness: 0.5 $\mu m$ ; pore size: 90 Å; pressure limit: 600 bar; dimensions: 30–150 mm $\times$ 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available	
Halo	Advanced Material Technology	2.7	160	Peptide ES-C18 (for peptides)	For peptides or polypeptides: shell thickness: 0.5 $\mu$ m; pressure limit: 600 bar; dimensions: 30–150 mm $\times$ 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available	
Kinetex	Phenomenex	1.7, 2.6	100	C8, C18, XB-C18 PFP	PFP = pentafluorophenyl; shell thickness: 0.35 $\mu$ m (2.6- $\mu$ m particle) and 0.23 $\mu$ m (1.7- $\mu$ m particle); pressure limit: 800 bar (2.6 $\mu$ m), 1000 bar (1.7 $\mu$ m); dimensions: 30–150 mm $\times$ 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available	
Nucleoshell	Macherey- Nagel	2.7	90	C18, PFP, HILIC	Shell thickness: 0.5 $\mu$ m; core: 1.7 $\mu$ m; column dimensions: 50–150 mm $\times$ 2–4.6 mm; maximum pressure: 600 bar; pH range: 1–11; multiendcapped for improved peak shape with basic compounds; HILIC is zwitterionic ammonium-sulfonic acid phase	
Poroshell 120	Agilent Technologies	2.7	120	SB-C18, EC-C18, EC C8, HILIC (silica)	Shell thickness: 0.50 $\mu m$ ; core diameter: 1.7 $\mu m$ ; pressure limit: 600 bar; column dimensions: 30–150 mm $\times$ 2.1, 3.0, and 4.6 mm; double endcapped and onend-capped phases.	
Poroshell 300	Agilent Technologies	5.0	300	SB-C3, SB-C8, SB-C18, Extend (for proteins)	Extend = Bidentate C18, upper pH limit = 11; shell thickness: 0.25 $\mu$ m; core dlameter: 4.5 $\mu$ m; pressure limit: 400 bar; column dimensions: 17 mm (guard for 1.0-mm), 12.5 mm (guard for 2.1-mm), and 75 mm $\times$ 0.5, 1.0, and 2.1 mm; capillary columns available	Table taken from : R. Majors, LCGC North America, Special Supplement on HPLC Column Technology, April, 2012
Sunshell	ChromaNik Technologies	2.6	80	C18, C8, PFP	Shell thickness: 0.5 µm; core: 1.6 µm; pH limits: 1.5–10; endcapped; phase coverage C18: 7% carbon; average particle size distribution (D90/D10): 1.15; column di- mensions: 20–150 mm × 2.1, 3.0, and 4.6 mm; mar- keted in US by Nacalai USA	18-Nov-12 18

Columns packed with superficially porous particles will deliver significantly higher efficiencies than columns packed with totally porous particles of the same diameter\*



The shorter diffusion path of HALO<sup>®</sup> particles reduces axial dispersion of solutes and minimizes peak broadening. A Halo particle has only a 0.5  $\mu$ m diffusion path compared to the approximately 1.5  $\mu$ m diffusion path of a 3  $\mu$ m totally porous particle. \*\*.



Benefits of core-shell technology particles vs. totally porous silica gel\*\*\*

- <u>Short diffusion paths</u> resulting in fast mass transfer (Cterm of van Deemter equation)
- Narrow particle size distribution (d90/d10 ~ 1.1)

\* Quoted from <u>http://www.phenomenex.com/Kinetex/CoreShellTechnology</u> \*\*Quoted from <u>http://www.advanced-materials-tech.com/halo.html</u>

\*\*\* Quoted from <a href="http://www.mn-net.com/tabid/11635/default.aspx">http://www.mn-net.com/tabid/11635/default.aspx</a>

Presented November 16, Geleen, Netherlands for the WGS of the KNCV

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#### Knox Plot TP and SP Particle Columns\*



\*Results contributed by Monika Dittmann, Agilent Technologies, Waldbronn, Germany

**Results\*** 

**Knox Equation** 

$$H = A \cdot d_p \cdot u^{0.33} + \frac{B \cdot D_m}{u} + C \cdot \frac{d_p^2}{D_m} \cdot u$$
  
A-term B-term C-term

Knox Equation (reduced form)

$$h = A \cdot v^{0.33} + \frac{B}{v} + C \cdot v$$

 $h = H / d_p$  $v = \frac{u \cdot D_m}{d_p}$ 

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A-term depends on particle size and solvent velocity

B-term depends only on solute diffusivity and decreases with solvent velocity

C-term depends on particle size and solute diffusivity and increases with solvent velocity

\*Results contributed by Monika Dittmann, Agilent Technologies Waldbronn, Germany

#### **Experimental Investigation\***

H – u curves were measured on 4 TP particle and 1 superficially porous particle columns

Column A	totally porous	1.8 μm
Column B	totally porous	2.5 µm
Column C	totally porous	2.8 µm
Column D	totally porous	3.5 µm
Column E	superficially porous	2.7 $\mu$ m (core 1.7 $\mu$ m), $\phi$ = 0.75

50x4.6 mm
Acetonitrile/water 60:40
25 °C
series of homologous alkyl phenones

\*Results on the next 6 pages contributed by Monika Dittmann, Agilent Technologies, Germany; presented at HPLC2008, Baltimore, USA

# Reduced van Deemter Curves for Octanophenone and Valerophenone



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Longitudinal Diffusion ( $h_{ax}$ ) Contribution for Different Columns



#### Eddy Diffusion ( $h_{eddy}$ ) Contribution for Different Columns



Intraparticle Diffusion ( $h_{Cs}$ ) Contribution for Different Columns



#### Facts and Legends on Columns packed with sub-3 μm Porous Core-Shell Particles\*

- The assumption that the <u>shorter average diffusion path in SP particles</u> leads to better performance (supplier brochures) <u>is wrong</u>
- In many explanations, the contribution of the B-term to the optimum HETP value (25%) is <u>systematically neglected</u>
- Also it is <u>incorrectly assumed</u> that the Eddy dispersion term <u>is independent</u> <u>of solvent velocity</u>
- And it is <u>incorrectly assumed</u> that narrow particle size distribution leads to <u>lower Eddy dispersion</u>.

\*G. Guiochon & F. Gritti, LCGC North America, Vol. 30(7), 586 (2012)

## Influence of Particle Morphology on B-Coefficient\*



The reduction of the B-coefficient (axial diffusion term) in SP particle columns, contributes 50% of the reduction of *h* 

\*G. Guiochon & F. Gritti, LCGC North America, Vol. 30(7), 586 (2012)

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#### Mass Transfer Resistance Term (Cu)\*

- C-coefficient measured for 6x, 100x4.6 mm Kinetex 2.6 μm particles for naphthalene (k = 3) is 3.7 msec.
- For an equivalent TP particle column, 3.0 μm diameter the coefficient was found 8.4 msec
- At optimum reduced velocity 10, the decrease of the reduced plate height will be around 0.05

#### Quote:

"In contrast to what is usually advertised in column technology, the exceptional kinetic performance of the new sub-3  $\mu$ m core-shell particles, <u>for small molecules</u>, has very little to do with the faster solid-liquid mass transfer"

\*G. Guiochon & F. Gritti, LCGC North America, Vol. 30(7), 586 (2012)

### Eddy Dispersion Term (A)\*



- B-term is larger for TP particles than with SP particles
- Eddy dispersion term depends on solvent velocity
- Eddy dispersion term is smaller with SP particles compared with TP particles

\*G. Guiochon & F. Gritti, LCGC North America, Vol. 30(7), 586 (2012)



Taken from Phenomenex: http://flipflashpages.uniflip.com/3/41719/122473/pub/

#### Trans-Column Eddy Dispersion



Packing heterogeneity of the wall section is higher in TP particle columns! (Tallarek et al.)

Superficially porous particle columns have more homogeneous radial packing density

J.C. Giddings, Dynamics of Chromatography, (Marcel Dekker, New York, 1965).

D.J. Gunn, Trans. Instn Chem. Engrs 47, T351-T359 (1969)

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A. Daneyko, S. Khirevich, A. Holtzel, A. Seidel-Morgenstern, U. Tallarek, J. Chromatogr. A 1218, 8231-8248 (2011).

\*G. Guiochon & F. Gritti, LCGC North America, Vol. 30(7), 586 (2012)

### Influence of PSD on Column Efficiency





Normalized minimal separation impedance (using permeability of the 1.9  $\mu$ m column as a reference) as a function of the weight percentage of large particles.

Kinetic plots of the analysis time of an unretained component,  $t_0$ , versus the plate number, N for (a) the 1.9 + 3 µm mixtures and (b) the 1.9 + 5 µm mixtures

\*Anuschka Liekens, Jeroen Billen, Ron Sherant, Harald Ritchie, Joeri Denayer, Gert Desmet, Journal of Chromatography A, 1218 (2011) 6654–6662

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## Practical considerations - Instrument Broadening



Effect of extra-column broadening on 2parameter optimum of 2.7 μm superficially porous at 600 bar



Gritti et al. J Chrom A, 2010, 1217, 7677

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#### **Instrument Effect on Column Efficiency**



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#### **Lessons** Learned

- The superficially porous particles investigated show reduced plate heights which are significantly lower than that of fully porous particles in a comparable size range.
- Contributions to HETP from <u>intra</u>-particle mass transfer for low MW analytes are relatively small compared to contributions from the other HETP terms.
- The presence of an impervious core reduces the B-coefficient accounting for approx. 50% of the overall HETP improvement.
- The increased performance of superficially porous material particle columns can be attributed to a lower eddy-diffusion term at low reduced velocities as consequence of more homogeneous packing density and less wall effect.
- Narrow particle size distribution of the superficially porous particles plays a minor role in improving performance of SP particle columns.

- Gert Desmet, VUB Brussels, Belgium
- Ulrich Tallarek, Univ. Marburg, Germany
- Georges Guiochon, Univ. Knoxville USA
- Ron Majors, Agilent Technologies Inc., USA for valuable inputs and discussion
- My coauthors Monika Dittmann & Xiaoli Wang

A PDF-handout will be available at <u>http://www.rozing.com</u> soon (registration required!) R

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