Current Perspectives on UHPLC; Requirements for Improved Abilities.

Gerard Rozing,

ROZING.COM Consulting, Karlsruhe, Germany

2004 ➔ HPLC evolved towards UHPLC

Two key ingredients for this evolution

- Introduction of columns with new ultra-high pressure stable sub-2-µmeter diameter (STM) totally porous particles followed by the introduction of superficially porous, low diameter particles in 2006

- Next generation HPLC instrumentation capable to deliver solvents at ultra-high pressure and able to conserve the ultra high efficiency separation of columns packed with these new particles

Primary Goals in any HPLC Separation*

- 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

$$R_s = \frac{\sqrt{N_2}}{4} \cdot \left[\frac{\alpha - 1}{\alpha} \right] \cdot \left[\frac{k_2}{k_2 + 1} \right]$$

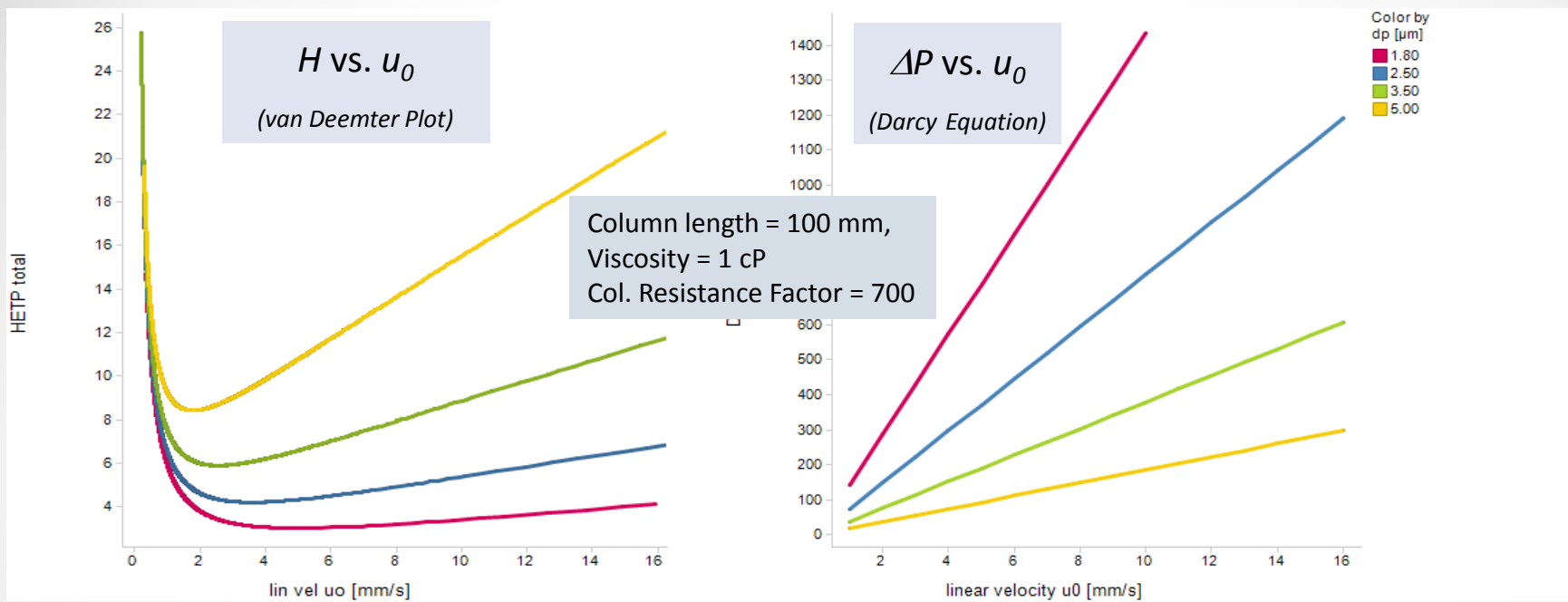Purnell equation for resolution for the chromatographic separation of two bands
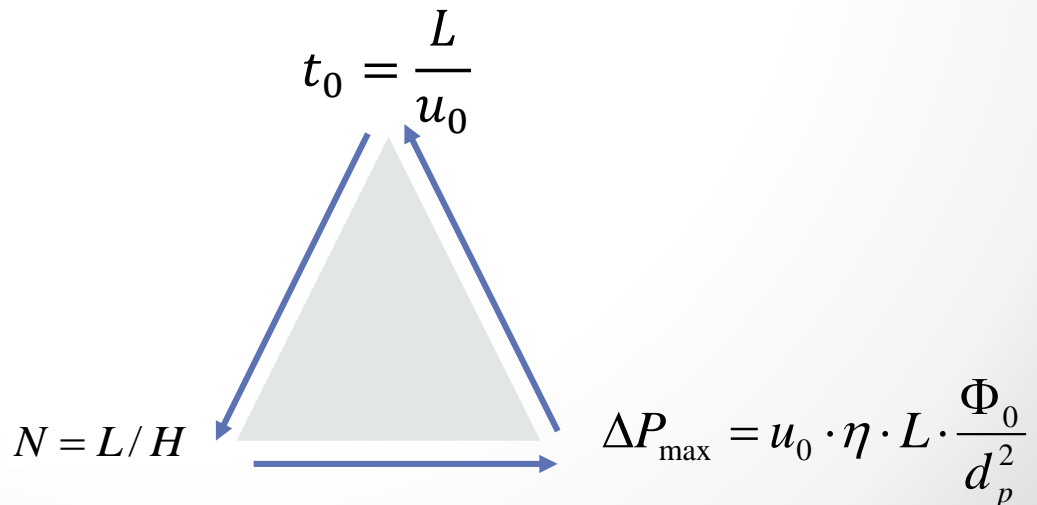
Transport Kinetics   Selectivity   Retention

*Quoted from Guiochon & Gritti, J. Chrom. A, 1228, 2 (2012)

UHPLC – Trilemma
HETP and Pressure Drop vs. Solvent Velocity

H vs. u_0

(van Deemter Plot)

ΔP vs. u_0

(Darcy Equation)

Column length = 100 mm,
Viscosity = 1 cP
Col. Resistance Factor = 700

Color by
dp [µm]
- 1.80
- 2.50
- 3.50
- 5.00

HETP total

lin vel uo [mm/s]

linear velocity u0 [mm/s]

Find the best values of L, d_p and u_o to obtain the highest N or (N/t_0) under one constraint: ΔP_{max}
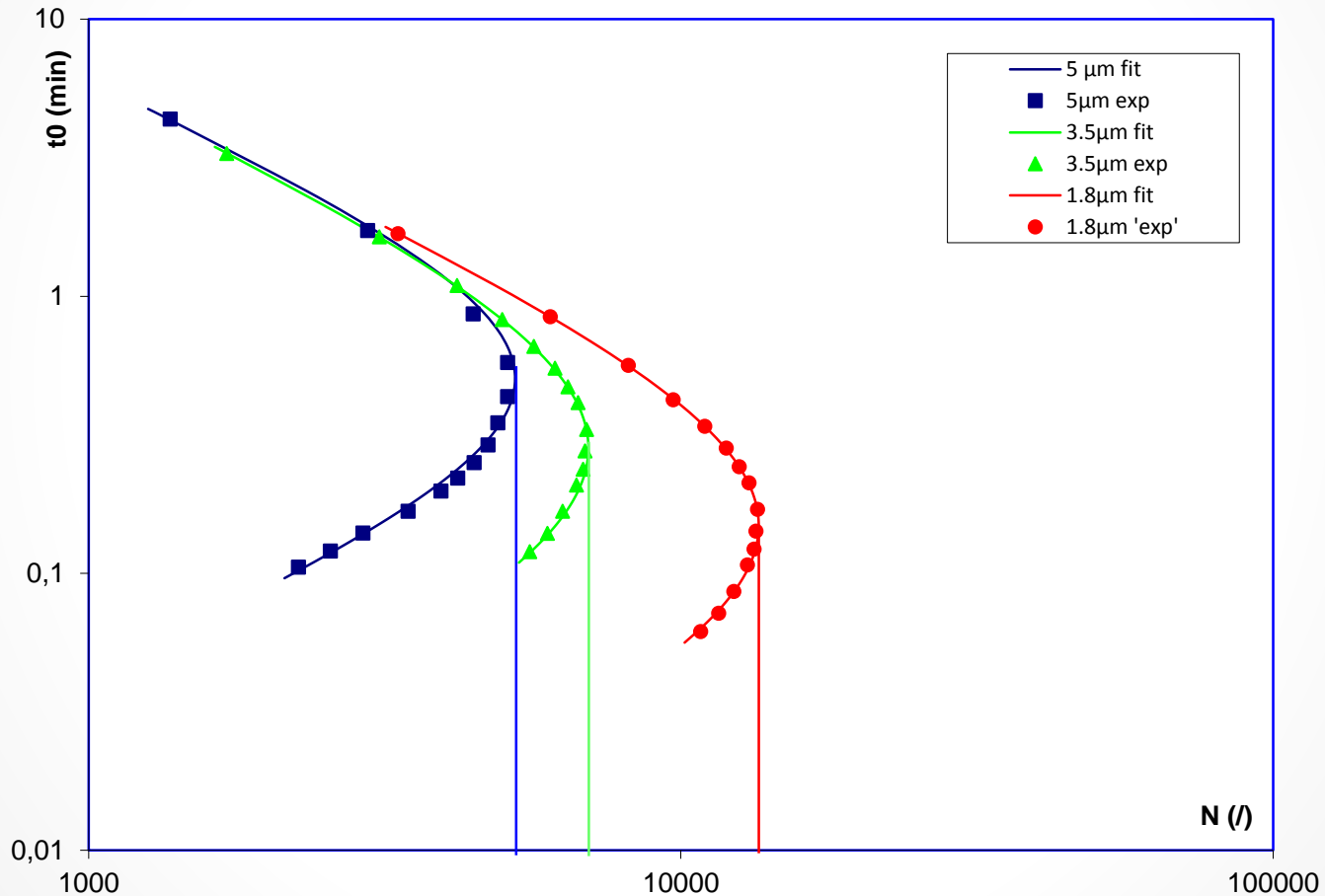
$$t_0 = \frac{L}{u_0}$$

$$N = L/H$$

$$\Delta P_{max} = u_0 \cdot \eta \cdot L \cdot \frac{\Phi_0}{d_p^2}$$

UHPLC – Essentials of Kinetic Optimization
1 - Parameter Kinetic*

L (50 mm) and d_p fixed, select optimal u_o

*Slide courtesy of Prof. Ken Broeckhoven, Free University of Brussels

UHPLC – Essentials of Kinetic Optimization
2- Parameter Approach (e.g. "Poppe Plot" approach)

Poppe's approach: find $u_{0,max}$ and $L(u_{0,max})$ corresponding to a given N_{req} and ΔP_{max} at a <u>fixed particle size</u>

The following relationship exists for "<u>plate time</u>":

$$\frac{t_0}{N} = \frac{H(u_0)}{u_0}$$

$$\frac{H(u_0)}{u_0} = \frac{A \cdot d_p}{u_0} + \frac{B \cdot D_m}{u_0^2} + C \cdot \frac{d_p^2}{D_m}$$
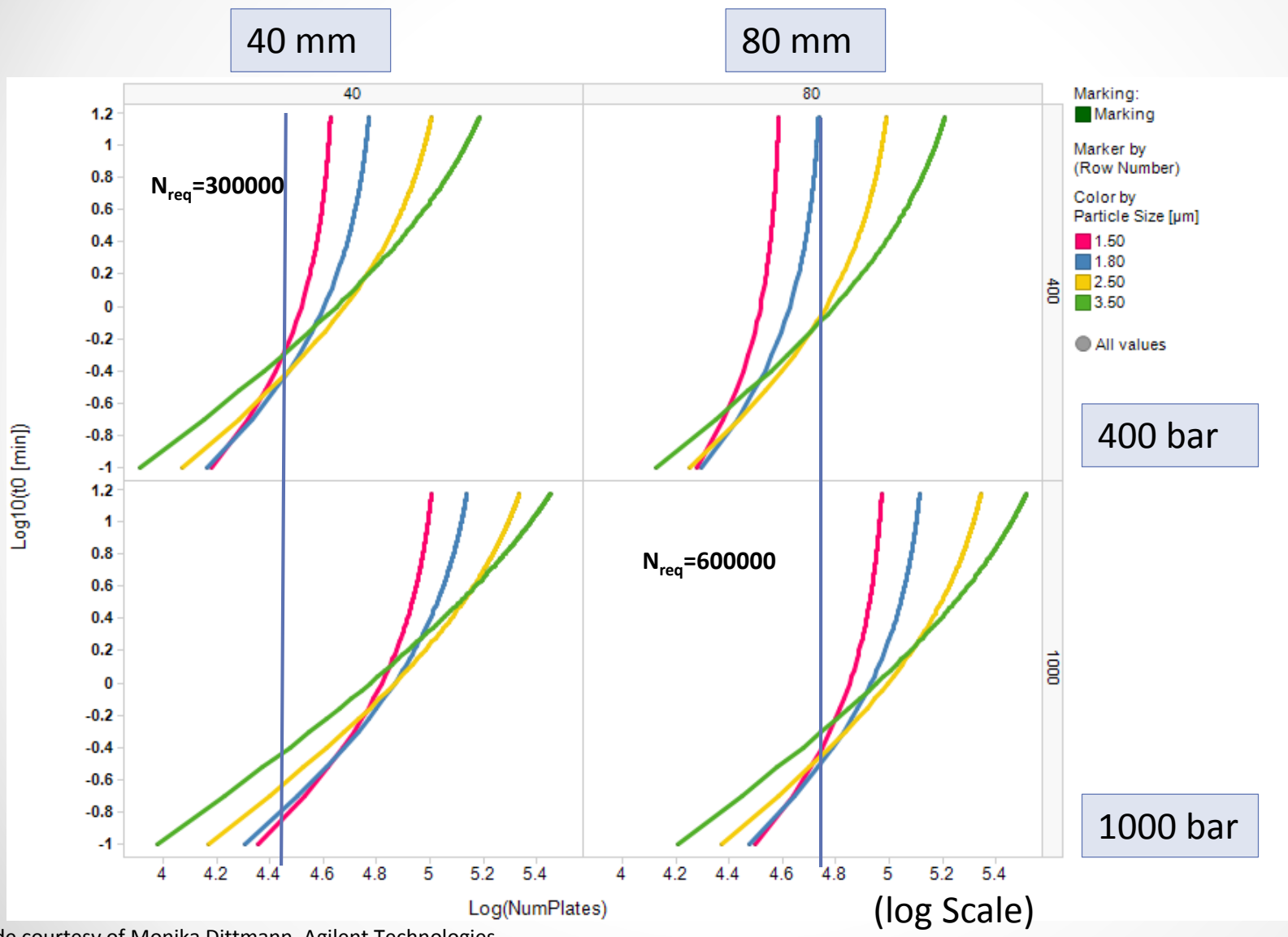
After obtaining the coefficients of the van Deemter equation (or e.g. the Knox equation) by non-linear regression the plot of the plate time in dependence of N_{req}, the Poppe plot, can be calculated by using the appropriate value of ΔP_{max}.

$$\log(t_0 / N_{req}) = \log C_1 N_{req} \qquad C_1 = \left[h^2 \Phi_0 \eta / \Delta P^{max} \right] \qquad h = H(u_0)/d_p$$

So, when one wants to decrease the plate time and does not want to sacrifice separation time, the column resistance factor (Φ) should be reduced (e.g. monoliths!), viscosity decreased (η) (e.g. increase temperature!) or increase the max. available pressure

H Poppe, J. Chrom. A, **778**, 3 (1997), G. Desmet et al., Anal. Chem., **77**, 4058 (2005), F. Gritti and G. Guiochon, J. Chrom. A, **1228**, 2, (2012)

UHPLC – Essentials of Kinetic Optimization
2-Parameter Approach Example

*Slide courtesy of Monika Dittmann, Agilent Technologies

3-Parameter Approach (Knox & Saleem)

Find the <u>maximum plate number possible in the shortest time,</u> when, u_0, L and d_p are varied to reach any ΔP_{max}

$$u_0^* = \left(\frac{\Delta P_{max} \cdot v_{opt}^2}{\Phi \cdot \eta} \right)^{0.25} \cdot t_0^{-0.25} \cdot D_m^{0.5}$$

$$d_p^* = \left(\frac{\Phi \cdot \eta \cdot v_{opt}^2 \cdot}{\Delta P_{max}} \right)^{0.25} t_0^{0.25} \cdot D_m^{0.5}$$

$$L^* = \left(\frac{\Delta P_{max} \cdot v_{opt}^2 \cdot}{\Phi \cdot \eta} \right)^{0.25} t_0^{0.75} \cdot D_m^{0.5}$$

$$N_{max}^* = \left(\frac{\Delta P_{max} \cdot t_0}{\Phi \cdot \eta} \right)^{0.5} \cdot \frac{1}{h_{min}}$$

Knox-Saleem limit

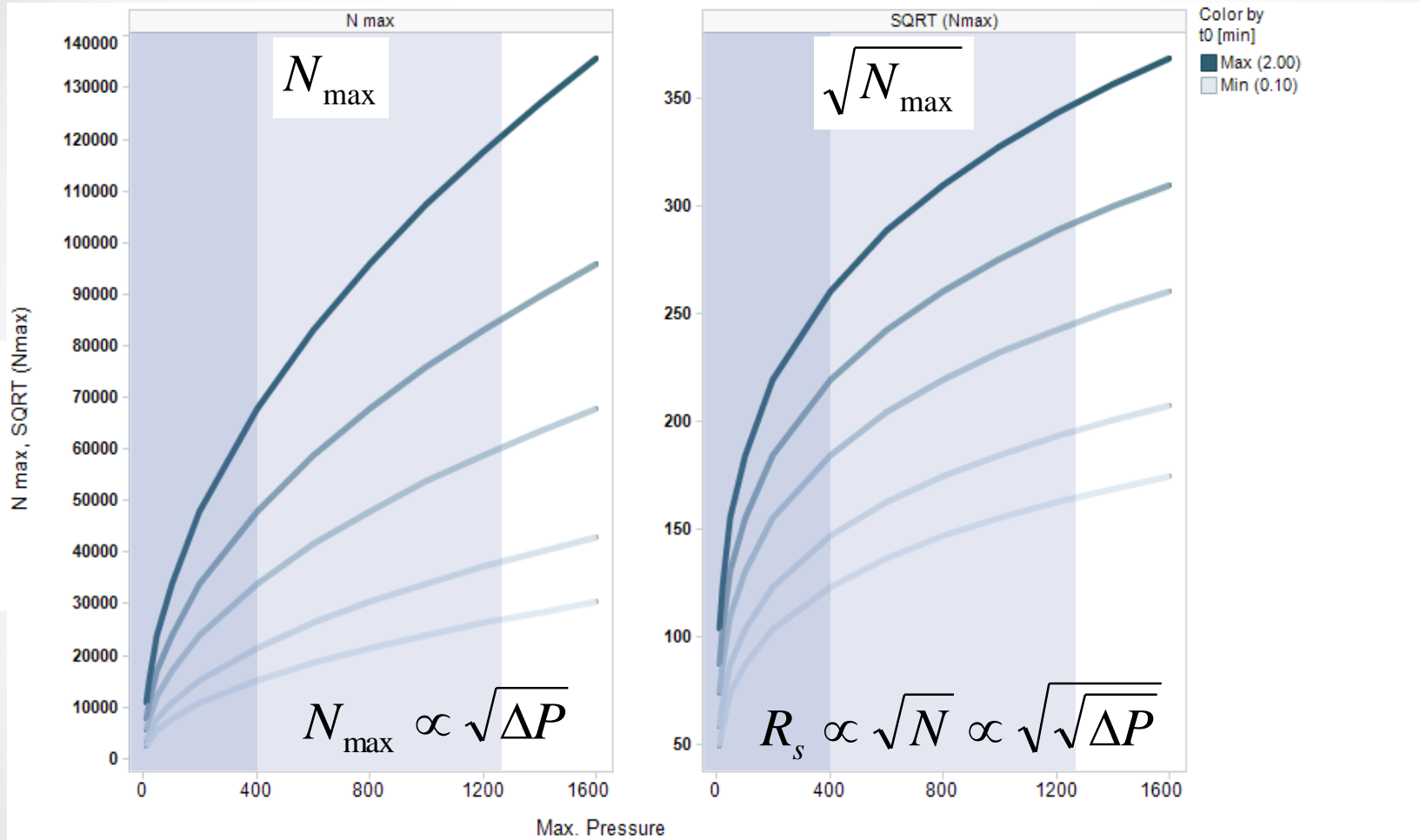**J.H. Knox and M. Saleem. J. Chromatogr. Sci., 7** (1969), p. 614
P. W. Carr, X. Wang, *Anal. Chem.* 2009**, 81,** 5342–5353
K. Broeckhoven, G. Desmet, Tr. Anal. Chem., accepted for publication

UHPLC – Kinetic Optimization
Maximum Achievable Plate Number vs. Pressure*

$$N_{\max}$$

$$\sqrt{N_{\max}}$$

$$N_{\max} \propto \sqrt{\Delta P}$$

$$R_s \propto \sqrt{N} \propto \sqrt{\sqrt{\Delta P}}$$

Quoted from Broeckhoven & Desmet** *"the possible gain by moving from 1200 bar to 2400 bar instruments, would maximally lead to a 40% increase in efficiency and only 20% in resolution or peak capacity, whereas analysis time decreases by twofold"*

*Slide courtesy of Monika Dittmann, Agilent Technologies   **K. Broeckhoven, G. Desmet, Tr. Anal. Chem., accepted for publication

Preliminary Conclusions and Assumptions

✳ Kinetic optimization predicts that the max. plate number achievable increases with square root of pressure

✳ But more pressure will result in shorter time to obtain N_{req} *

But ......

Inferences from kinetic plots only apply if**:

■ HETP is independent from column length

■ physical and chemical properties of solvents and solutes, particle properties and column dimensions are independent of pressure change

■ frictional heating can be neglected

*K. Broeckhoven and G. Desmet, accepted for publication in Trends in Analytical Chemistry

**F. Gritti and G. Guiochon, J. Chrom., 1228, 2, (2012)

UHPLC – Constraints
Effect of High Pressure on*

- Solvent properties
  - density (ρ), specific volume, viscosity (η), compressibility (χ) and melting point

- Solute properties
  - diffusion coefficient (D_m), molar volume, conformation/3D-structure changes (in particular with high MW substances)

- Retention factors (k) and in particular of ionizable molecules and HMW substances**

- Particle porosity and total porosity of the packed bed (ε_T)

- Column dimensions length and diameter (L and d_c)

> **Under ultra-high pressure conditions, solute and solvent properties change but not independently which complicates method optimization!!**
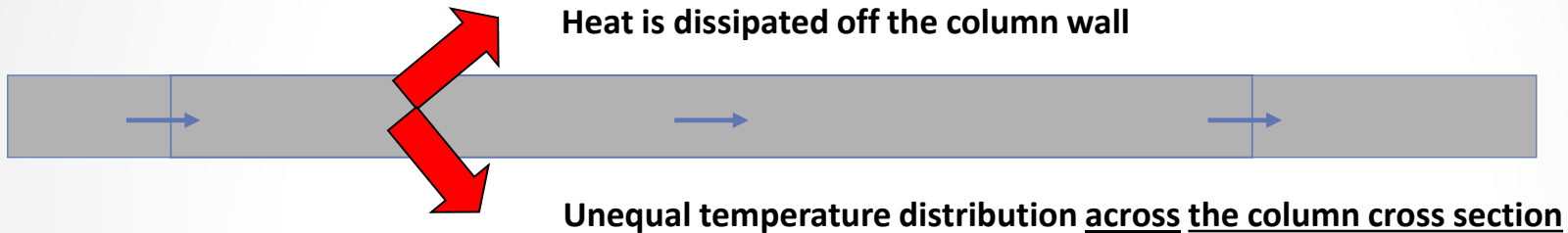
*M. Martin & G. Guiochon, J. Chrom. A, 1090, 16 (2005)
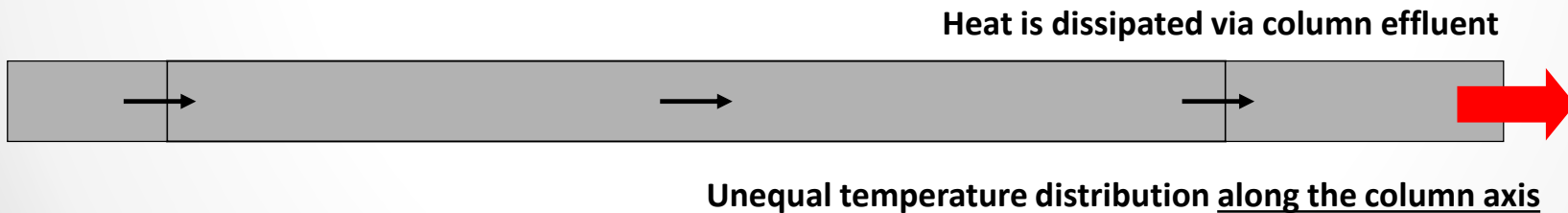**M.M. Fallas, U.D. Neue, M.R. Hadley, D.V. McCalley, J. Chrom. A, 1209, 195–205 (2008)

$$Power = \Delta P \times F$$

Isothermal: fast heat exchange with constant temperature environment

Heat is dissipated off the column wall

Unequal temperature distribution across the column cross section

Adiabatic: no heat exchange with environment

Heat is dissipated via column effluent

Unequal temperature distribution along the column axis

Slide courtesy of Prof. G. Desmet et al, Free University of Brussels, CHIS

UHPLC – Constraints
Frictional Heating and Column Environment*

○ Column in still air
○ Column in water bath

ACN/water 60:40
Octanophenone
k' = 10
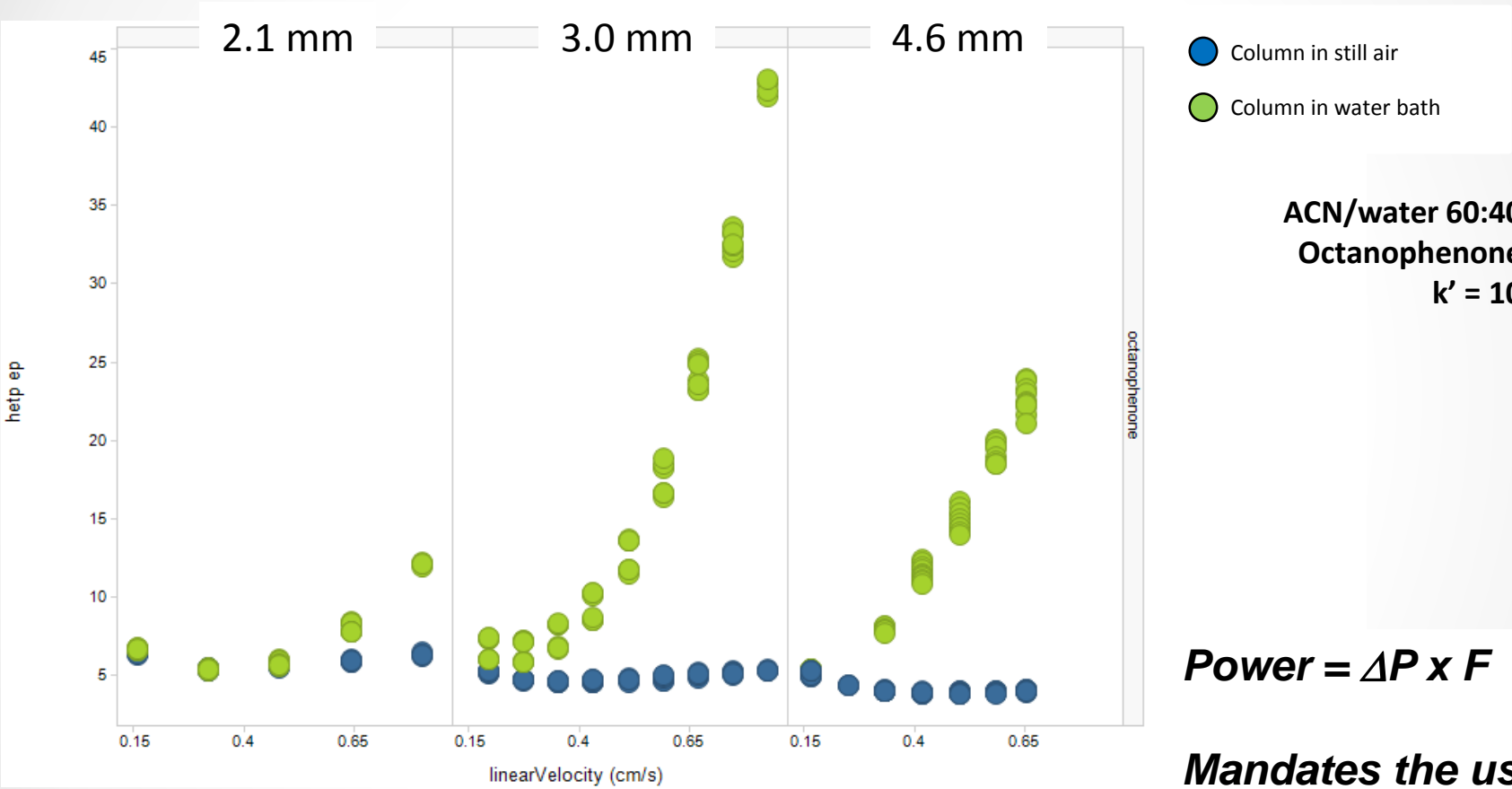
Power = ΔP x F

Mandates the usage of lower i.d. columns

*Slide courtesy of Monika Dittmann, Agilent Technologies

# UHPLC – Mechanical & Hydraulic Engineering Challenges at Ultra-High Pressure

- Switching and sealing interfaces, like piston-seal, check and rotary valves will become difficult to realize especially when the material strength limits are reached or materials more readily fatigues

- Pressure cycles will blow and relax the tubing in (sub)micrometer dimensions may damage bed structure and/or particles

- Narrower i.d. columns mandate lower flow rates and lower system volume and ultra-high pressure. Leaks will not noticeable, harder to find and eliminate. Significant influence on flow rate and composition precision and accuracy

- Reliable pumps at ultra high pressure ➔ example
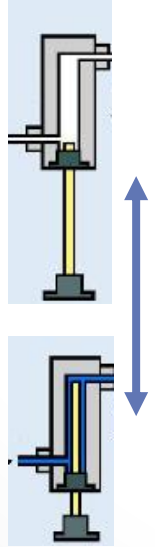
UHPLC – Engineering Requirements Pump

What is the force required to deliver solvents @ 1000 bar?

Desired flow rate 0.05 - 5 ml/min

Piston diameter e.g. 3.1 mm, cross-section 7.6 mm^2

Stroke volume up to 100 µl = 100 mm^3
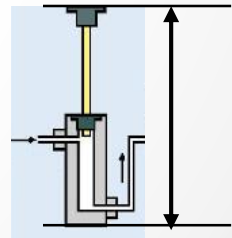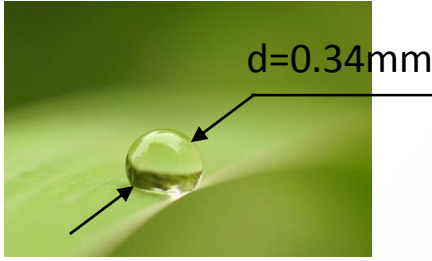
Desired flow rate precision (per channel) ~0.2%

**Force
~ 800N**
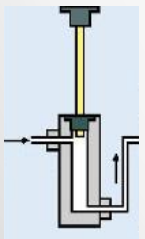
**Linear
displacement
~13 mm**

**Required displacement
precision:
20 nl vol.
or ~2 µm linear
or better**

d=0.34mm

~ 10mm/s
X(t)**±1µm**

UHPLC – Engineering Requirements Pump
What is the force required to deliver solvents @ 1500 bar?
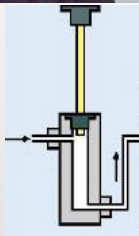
Desired flow rate 0.05 - 5 ml/min

Piston diameter e.g. 3.1 mm, cross-section 7.6 mm^2

Stroke volume up to 100 µl = 100 mm^3

Desired flow rate precision (per channel) ~0.2%

Force
~ 1200N

UHPLC – Extra Column Band-Broadening

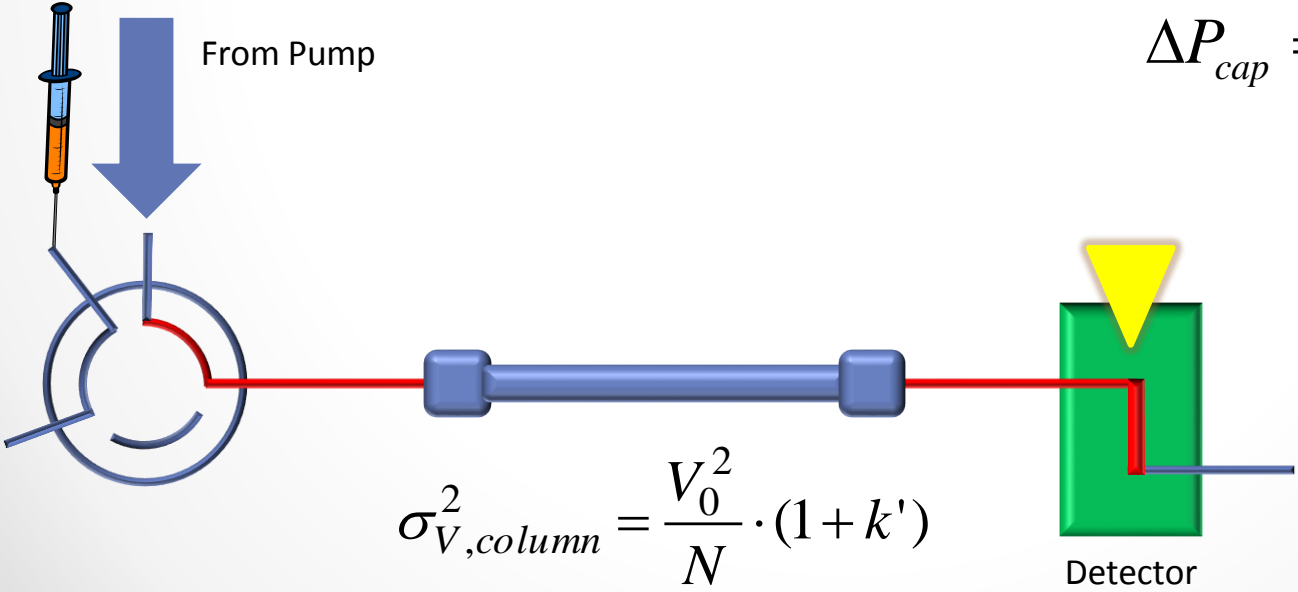$$\sigma^2_{v,tot} = \boxed{\sigma^2_{v,col}} + \sigma^2_{v,cap} + \sigma^2_{v,inj.} + \sigma^2_{v,cell}$$

$$\sigma^2_{v,cap} = \frac{V_{cap}r^2u}{24D_m}$$

$$\sigma^2_{v,cell} = \frac{V^2_{cell}}{X}$$

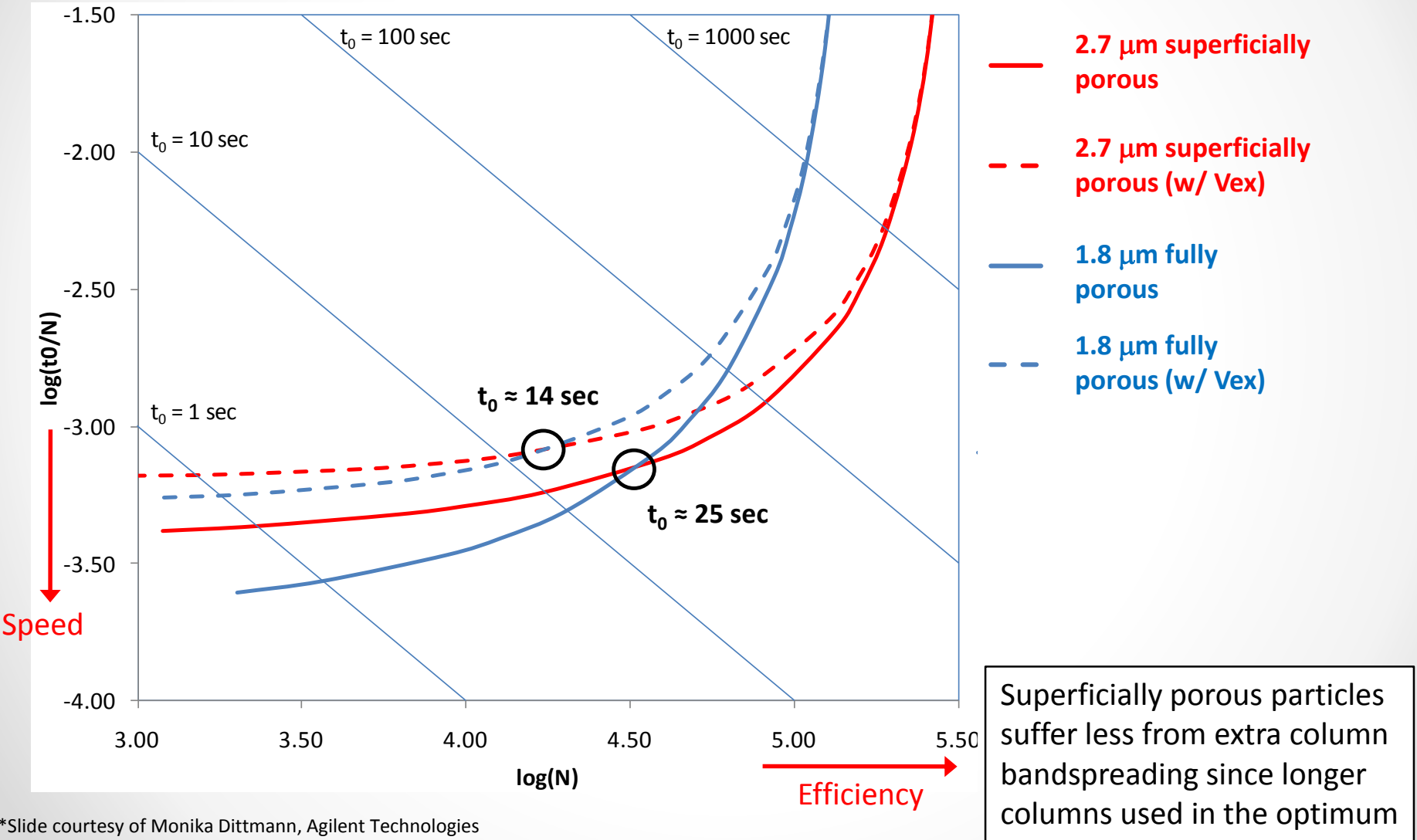X=dispersion factor,
1 (ideal mixer case) -
12 (plug flow case)

Typical values for variance	
Injector:	1 µl²
Capillaries (500x0.12 mm)	7 µl²
Capillaries (500x0.08 mm)	4 µl²
Column (2.1x50 mm, k' =1)	3 µl²
Column (2.1x50 mm, k' =5)	33 µl²

$$\Delta P_{cap} = \frac{F \cdot 8\eta \cdot L_{cap}}{\pi r^4_{cap}}$$

From Pump

$$\sigma^2_{V,column} = \frac{V^2_0}{N} \cdot (1+k')$$

Detector

Instrument Effect on Column Efficiency

2.7 μm superficially porous

2.7 μm superficially porous (w/ Vex)

1.8 μm fully porous

1.8 μm fully porous (w/ Vex)

Superficially porous particles suffer less from extra column bandspreading since longer columns used in the optimum

*Slide courtesy of Monika Dittmann, Agilent Technologies

Relation between Peak Height in HPLC and Extra-Column Dispersion*

For a solute eluting from the column with a Gaussian peak shape

$$c_{i,\max} = \frac{Q_{inj,i}}{\sqrt{2\pi} \cdot \sigma_{v_i,col}}$$

In practice however:

$$\sigma^2_{v_i,tot} = \sigma^2_{v,inj} + \sigma^2_{v_i,col} + \sigma^2_{v,cap} + \sigma^2_{v,det}$$

minimize   negligible, eliminate

$$\sigma^2_{v_i,tot} \approx \sigma^2_{v_i,col} + \sigma^2_{v,det}$$

$$\sigma^2_{v_i,tot} \approx \frac{V^2_{R,i}}{N} + \frac{V^2_{cell}}{X}$$

$$c_{\max,i} \approx \frac{Q_{inj,i}}{\sqrt{2\pi} \cdot \sqrt{\dfrac{V^2_{R,i}}{N} + \dfrac{V^2_{cell}}{X}}}$$

$A_{i,\lambda}$	= measured extinction of solute i at λ
$\varepsilon_{i,\lambda}$	= molar absorption coefficient of solute i
c_i	= concentration of the solute i
L_{cell}	= optical/sample path length
$Q_{inj,i}$	= amount of sample injected of solute i
$\sigma_{v_i,tot}$	= standard deviation of measured peak profile
$\sigma^2_{v_i,tot}$	= total variance = sum of injector, column, connection capillaries and detector
$V_{R,i}$	= retention volume of solute i = f (k')
N	= column plate number
V_{cell}	= volume of detector flow cell
X	= factor describing dispersion behavior of detector flow cell

X=1 : flow cell is ideal mixer

X=12: non-dispersing flow cell, plug flow

*K. Kraiczek et al., Anal. Chem., 2013, 85 (10), pp 4829–4835

Signal-to-Noise Ratio in Spectrophotometric Detectors
Extending Baumann's Principle*,*,*

Lambert Beer's law

$$A_{i,\lambda} = \varepsilon_{i,\lambda} \cdot c_{i,\max} \cdot L_{cell}$$

Photon flux converted into electrical signal

$$m_{0,\lambda} = M_{\lambda} \cdot \Delta\lambda \cdot G \cdot T_{optic,\lambda} \cdot \eta_{\lambda}$$

Optical Signal ~ chromatographic Signal

$$S = (m_{0,\lambda} - m_{\lambda}) \cdot \tau$$

Standard deviation of photon flux = rms Noise

$$N_{rms} = \sqrt{m_{0,\lambda} \cdot \tau}$$

The rms-value of the Signal-to-Noise Ratio

$$SNR_{rms} = \frac{(m_{0,\lambda} - m_{\lambda}) \cdot \tau}{\sqrt{m_{0,\lambda} \cdot \tau}}$$

M_{λ}	= spectral output of light source
$\Delta\lambda$	= spectral bandwidth
G	= (lowest) light conductivity of optical system
$T_{optic,\lambda}$	= overall transmission of optic
η_{λ}	= quantum efficiency of photo detector at λ
τ	= detectors time constant
$m_{0,\lambda}$	= fraction of photon flux converted into electrons
m_{λ}	= reduced photon flux caused by sample absorption
$\varepsilon_{i,\lambda}$	= molar extinction coefficient of solute i
c_i	= concentration of the solute i
L_{cell}	= optical, sample path length of detector flow cell

$m_{0,\lambda}$ → m_{λ}

1st order of Taylor expansion
Lambert-Beer's law

$$\frac{m_{0,\lambda} - m_{\lambda}}{m_{0,\lambda}} = 2.3 \cdot \varepsilon_{i,\lambda} \cdot c_i \cdot L_{cell}$$

*Z. Anal. Chem. 284, 31-38 (1977)

*,*K. Kraiczek et al., Anal. Chem., 2013, 85 (10), pp 4829–4835

Slide courtesy of Karsten Kraiczek, Agilent Technologies

The Dilemma in Spectrophotometric HPLC detection*,*

Signal Parameters
Solute and Chromatography

Noise Parameters
Optics and Detector Settings

$$SNR_{ptp}(L_{cell}, V_{cell}) = \frac{2.3}{6} \cdot \varepsilon_{i,\lambda} \cdot c_{\max,i} \cdot L_{cell} \cdot \sqrt{M_\lambda \cdot \Delta\lambda \cdot G \cdot T_{optic,\lambda} \cdot \eta_\lambda \cdot \tau}$$ *

Flow Cell Geometry and Flow Cell Dispersion Behavior

$$c_{\max,i} \approx \frac{Q_{inj,i}}{\sqrt{2\pi} \cdot \sqrt{\frac{V_{R,i}^2}{N} + \frac{V_{cell}^2}{X}}}$$

◆ $c_{i,max}$ highest if dispersion is absent i.e. cell volume → 0:
<u>then pathlength → 0; no change in absorbance is measured</u>

◆ Absorbance is highest with high L_{cell} and maximum light throughput (diameter, maximum light intensity optics, spectral bandwidth) cell volume → increases:
<u>then dispersion increases, $c_{i,max}$ decreases and resolution is lost</u>

◆ Hence there must be a compromise in flow cell dimensions for best SNR →
<u>Total Internal Reflection (TIR) flow cell is the best compromise</u>

*,*K. Kraiczek et al., Anal. Chem., 2013, 85 (10), pp 4829–4835

*Slide courtesy of Karsten Kraiczek, Agilent Technologies

UHPLC – Perspectives and Requirements

- Kinetic optimization predicts that the max. plate number achievable with square root of pressure. More pressure will result in shorter time to obtain N_{req}

- At ultra-high pressure, physical, thermal and engineering constraints will make requirements for systems extremely challenging especially since columns have to be of lower i.d.

- Total internal reflection flow cells offer the best compromise between minimizing dispersion and maximizing absorbance for spectrophotometric detectors.

Acknowledgements

- Former colleagues at Agilent Technologies, Monika Dittmann, Karsten Kraiczek, Konstantin Choikhet

- Ken Broeckhoven and Gert Desmet, Free University of Brussels

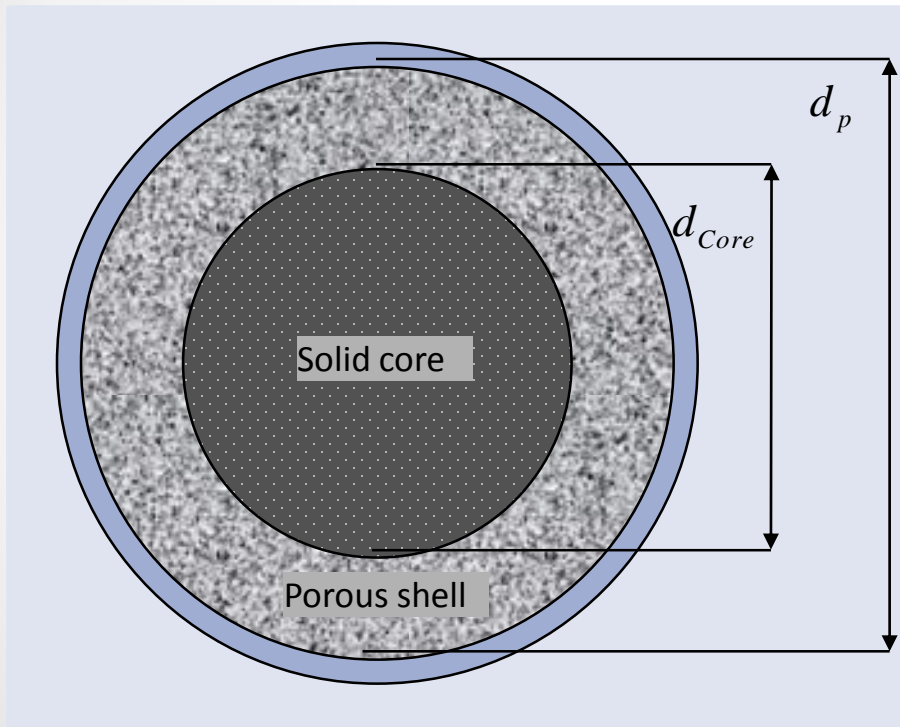A PDF copy of this talk will be available on my website http://www.rozing.com after the talk (registration required)

For the discussion sessions
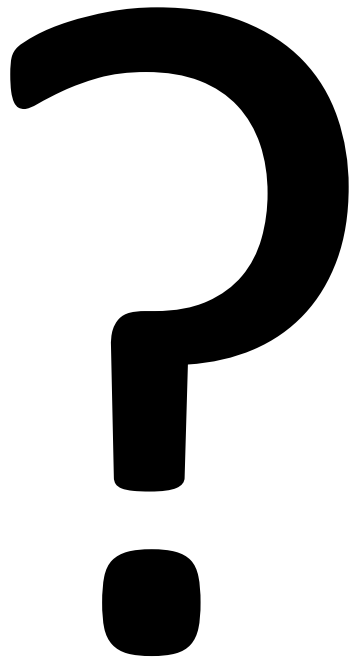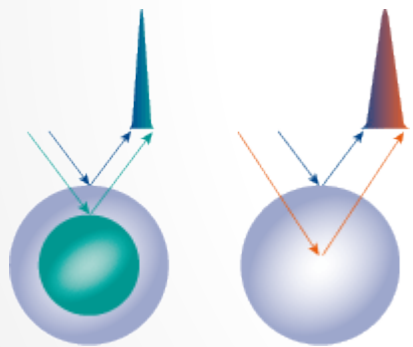
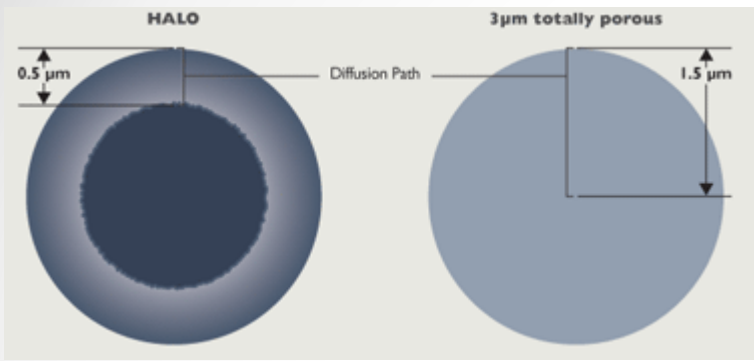# Morphology of Superficially Porous Particles

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 <u>internal porosity</u> ε_i of a superficially porous particle is assumed to be

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

Columns packed with superficially porous particles will deliver significantly higher efficiencies than columns packed with totally porous particles of the same diameter[1,2,3]

[1] Quoted from http://www.phenomenex.com/Kinetex/CoreShellTechnology
[2] Quoted from http://www.advanced-materials-tech.com/halo.html
[3] Quoted from http://www.mn-net.com/tabid/11635/default.aspx

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 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)

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 µm (core 1.7 µm), $\varphi = 0.75$

Conditions:

Column dim.:	50x4.6 mm
Solvent:	Acetonitrile/water 60:40
Temperature :	25 °C
Sample:	series of homologous alkyl phenones

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

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)

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

$$h = H / d_p$$

$$v = \frac{u \cdot D_m}{d_p}$$

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
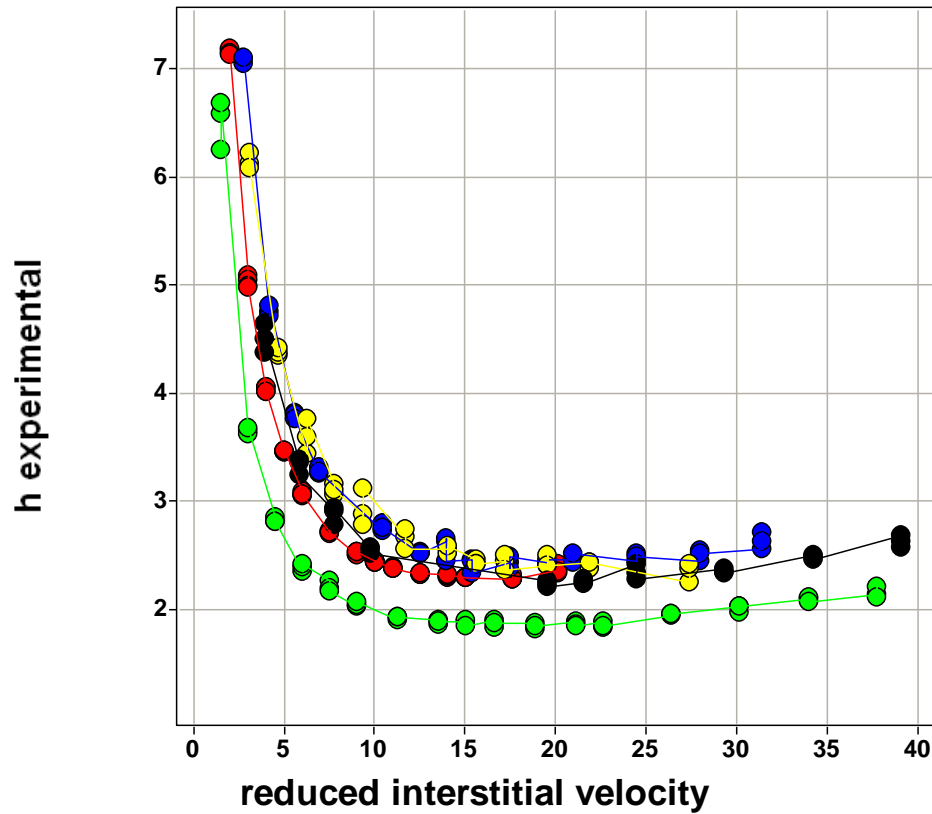
Reduced van Deemter Curves for Octanophenone and Valerophenone

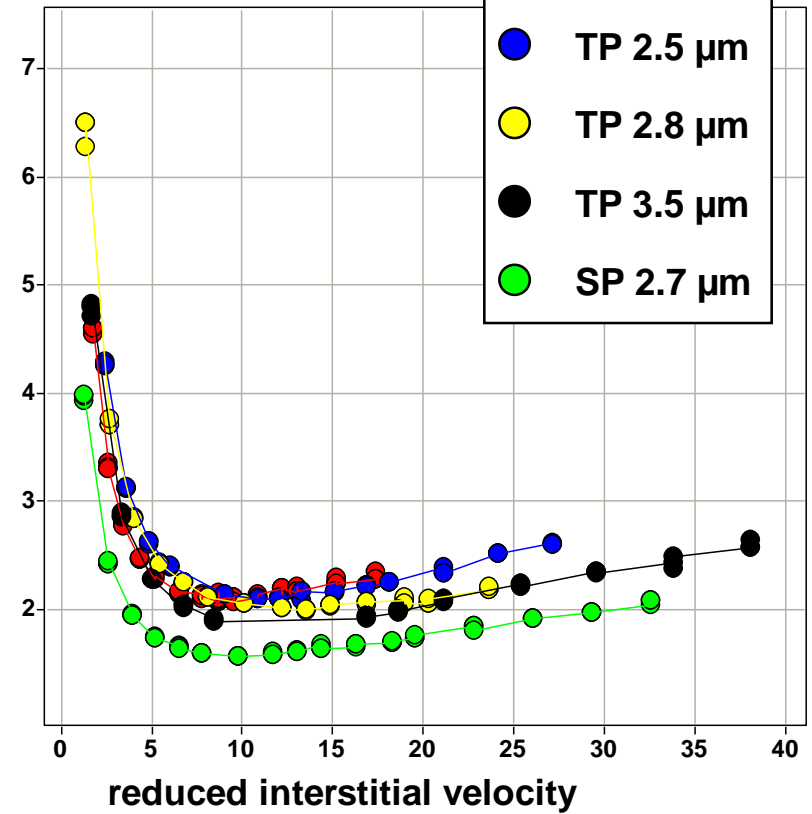$$u_e = u_0 \cdot \frac{\varepsilon_T}{\varepsilon_e}$$

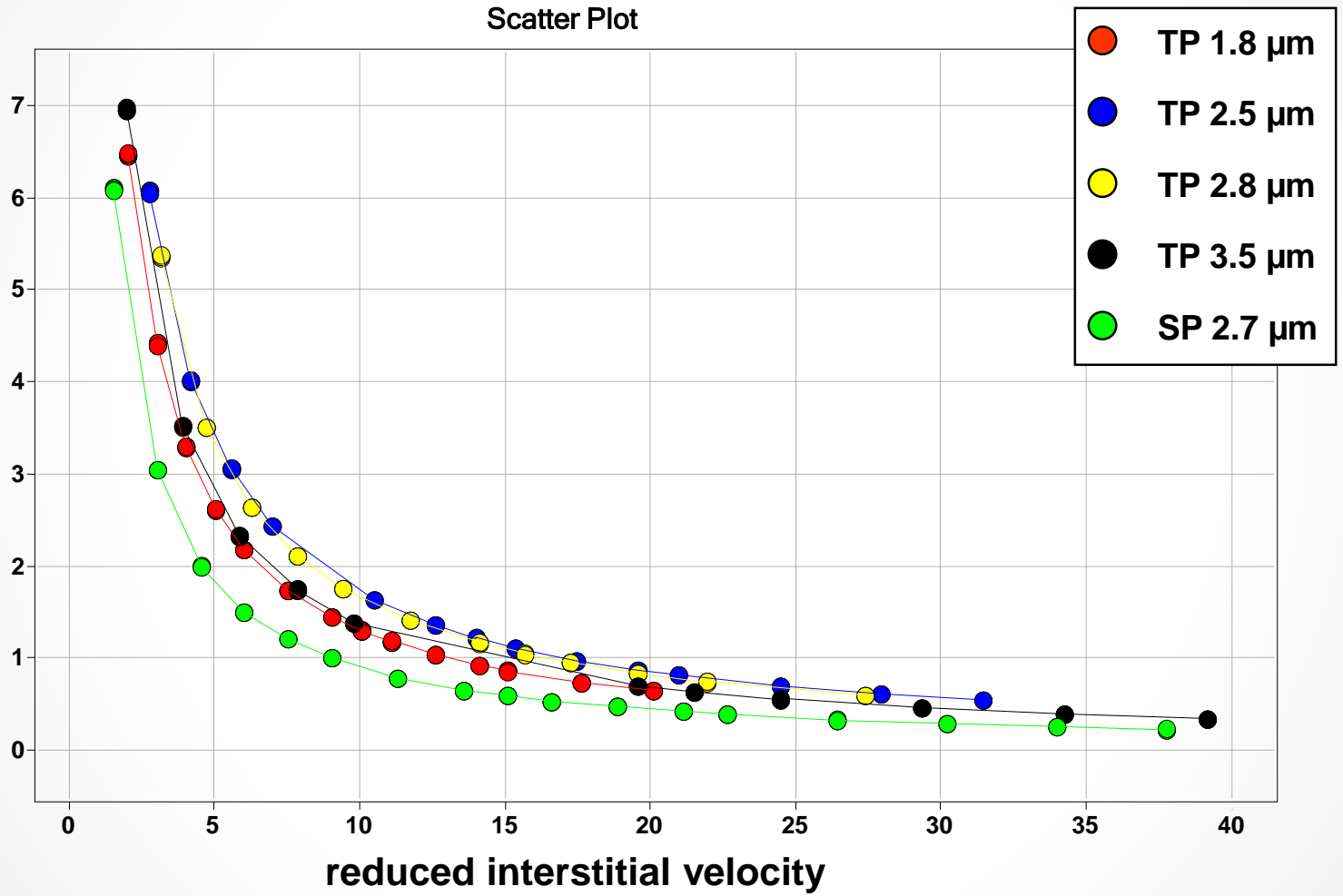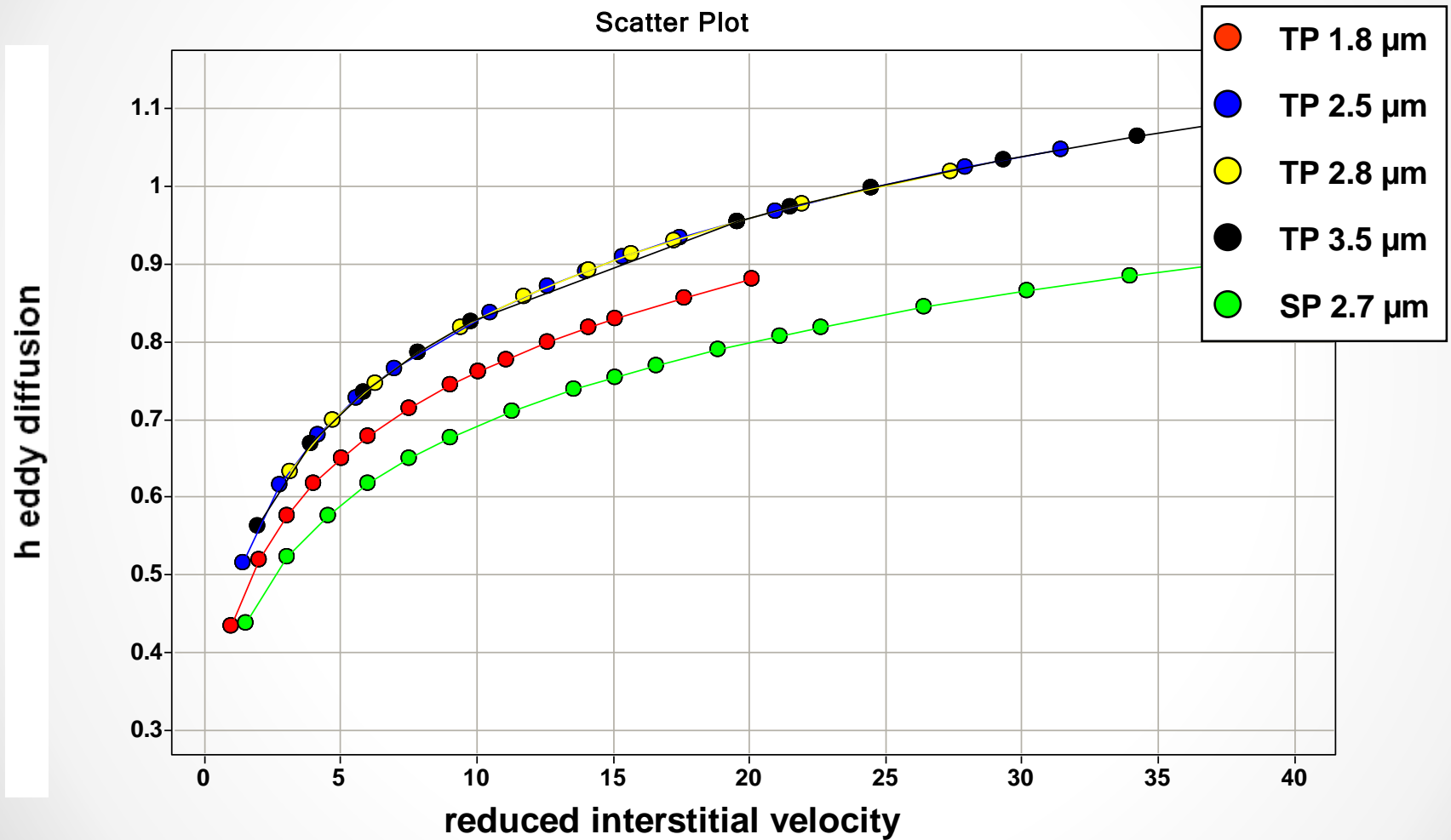$$v_e = u_e \cdot \frac{d_p}{D_m}$$

$$h = \frac{H}{d_p}$$

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

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

A PDF copy of this talk will be available on my website
http://www.rozing.com after the talk (registration required)