Fundamentals, Optimization and Practical Aspects of UHPLC Part 2

Kinetic plot theory

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Review Important Insights from Part 1

- UHPLC delivers extended abilities for HPLC through
  - New column technologies (in particular, sub-2-micron and superficially porous particles)
  - Next generation HPLC equipment with extended operating pressure and reduced extra-column band broadening
- The premier goal in UHPLC is to obtain the best possible resolution in the shortest possible time.
- Despite being powerful, chromatographic selectivity has practical limitations; with complex samples, peak overlap will be unavoidable. The column plate number remains the ultimate handle to improve resolution.
- Kinetic optimization according to Knox and Saleem, showed in principle how to obtain the plate number N_{req} for best resolution in the shortest possible time by choosing the proper particle size and column length.
- As a rule of thumb:
  - For $N_{req} \leq 50000$ (which satisfies 95% of cases) this is achieved by using columns with new particles and shortest length operating at max. pressure achievable with next generation UHPLC systems.
  - Very large N_{req} is obtained by using columns with large particle size and long length at maximum pressure but at the cost of time.

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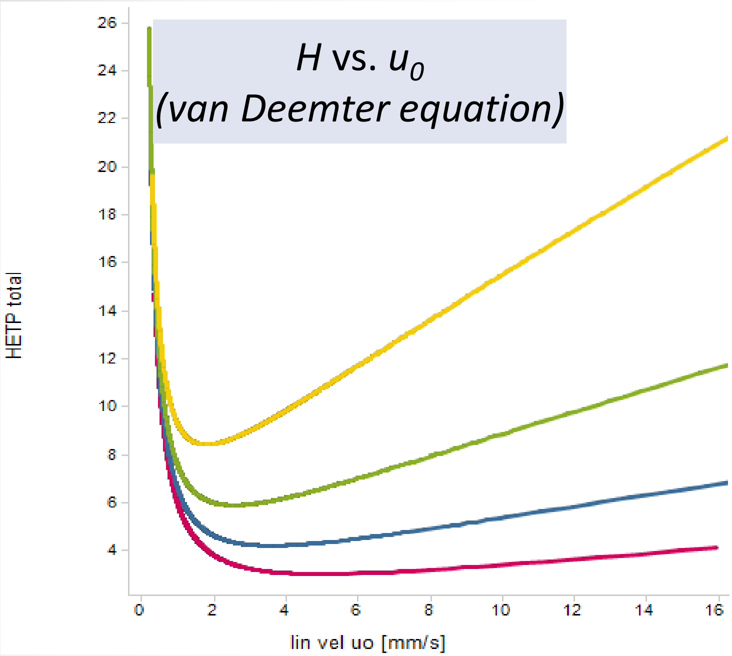
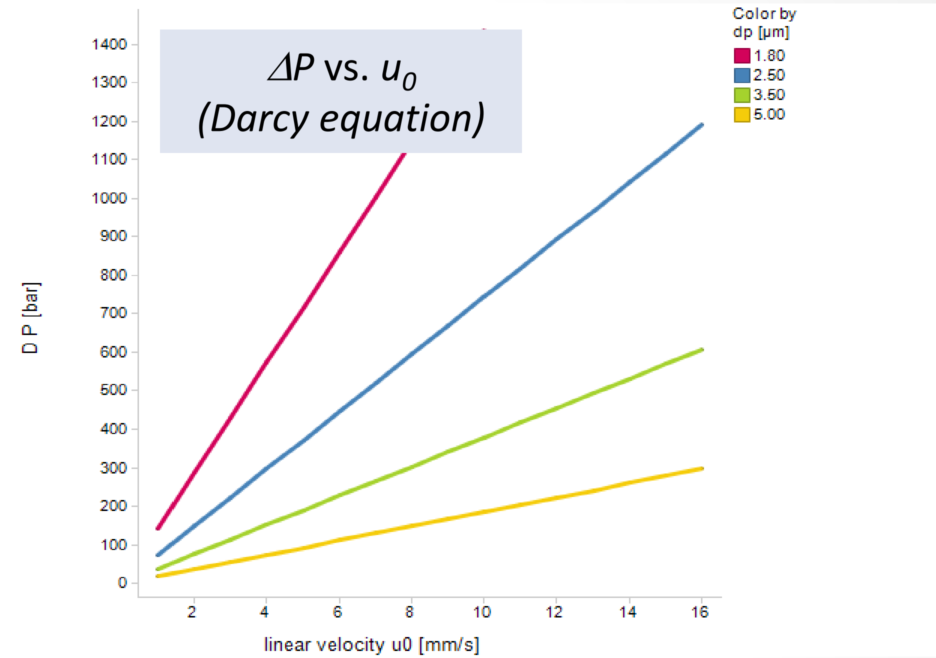
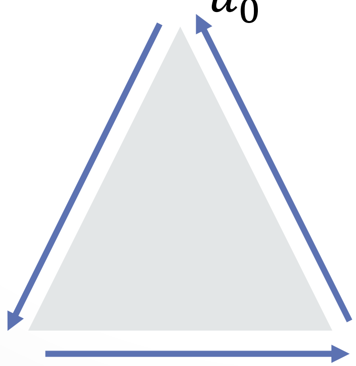
HETP and Pressure Drop versus Solvent Velocity





$$t_0 = \frac{L}{u_0}$$

Column length = *100 mm*,
viscosity = *1 cP*



$$N = L / H$$

$$\Delta P_{\max} = \frac{u_0 . \eta . L}{B_0} = u_0 \cdot \eta \cdot L \cdot \frac{\Phi_0}{d_p^2}$$

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Knox & Saleem* Approach for Kinetic Optimization

- In practice it is impossible to vary column length and particle size continuously to obtain N_{req} in the shortest possible time since in practice, HPLC columns have fixed dimensions and the particle size is not a continuous variable

- How to minimize the time (t_0) to obtain N_{req} given the constraint of ΔP_{max} and the dependence of the *HETP* on the velocity of the solvent?

***J.H. Knox and M. Saleem. J. Chromatogr. Sci., 7 (1969), p. 614**

K&S Approach for Kinetic Optimization

- In practice it is impossible to vary column length and particle size continuously to obtain N_{req} in the shortest possible time since in practice, HPLC columns have fixed dimensions and the particle size is not a continuous variable

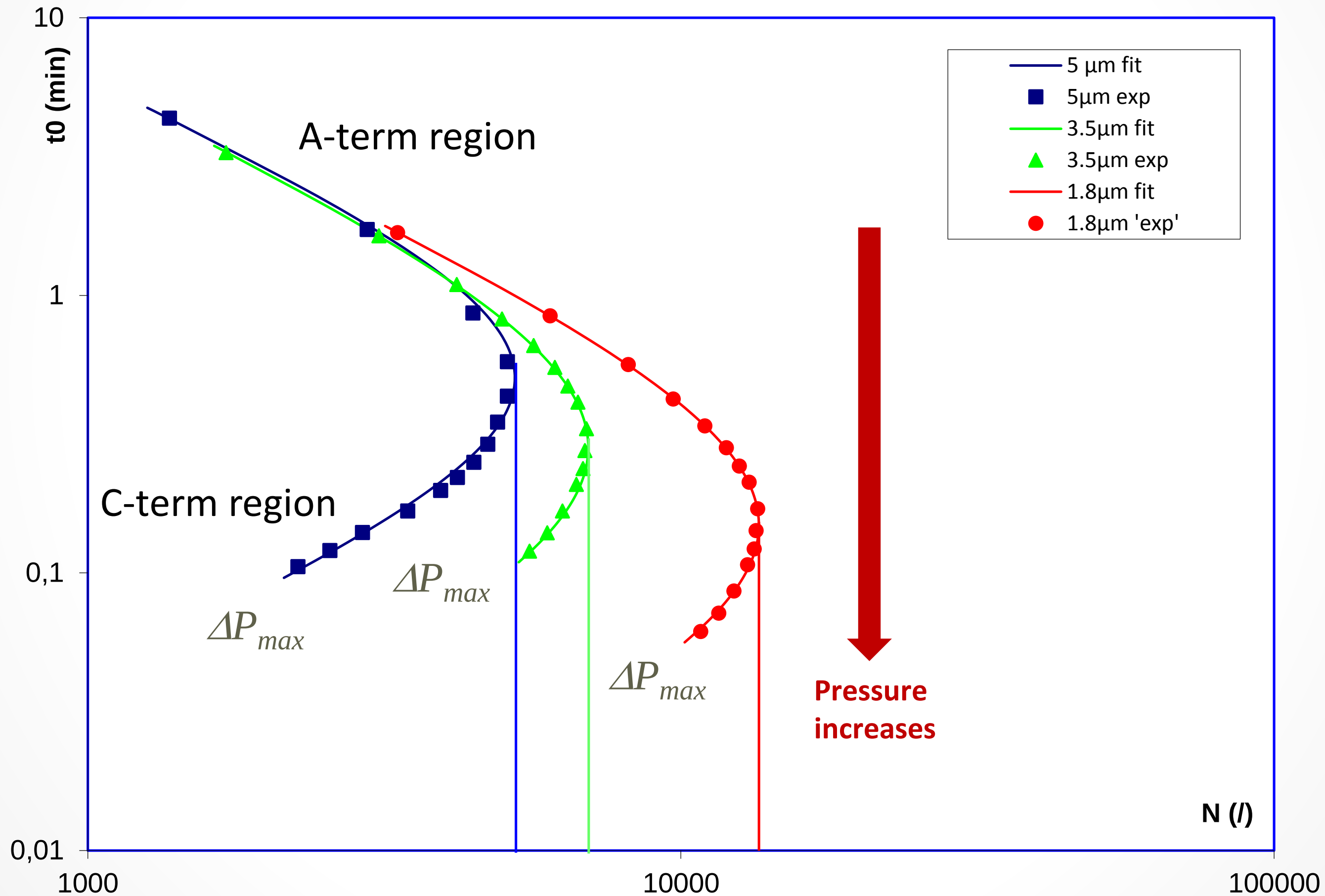
- How to minimize the time (t_0) to obtain N_{req} given the constraint of ΔP_{max} and the dependence of the *HETP* on the velocity of the solvent?

➔ Kinetic Plot theory:
plot of separation quality (efficiency, peak capacity, resolution, separation impedance) versus time required to achieve this

UHPLC - Essentials

1 - Parameter Kinetic Plot*

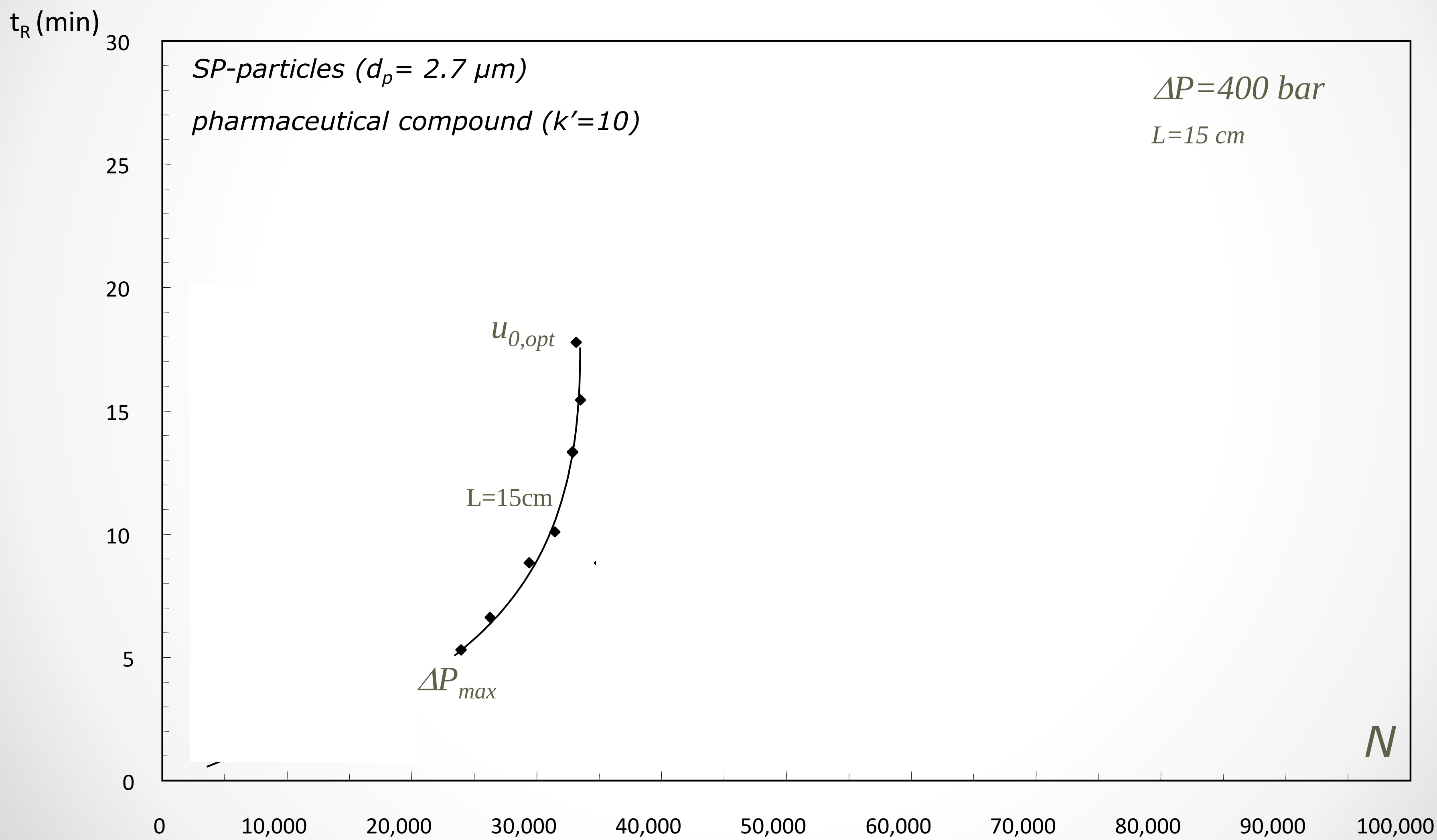
L (50 mm) and d_p fixed, select optimal u_o



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

UHPLC - Essentials

1 - Parameter Kinetic Plot - Fixed and Variable Length Kinetic Plots*



*Desmet, G. and Cabooter, D. LC-GC Europe, 2009, 22, 70-77

UHPLC - Essentials

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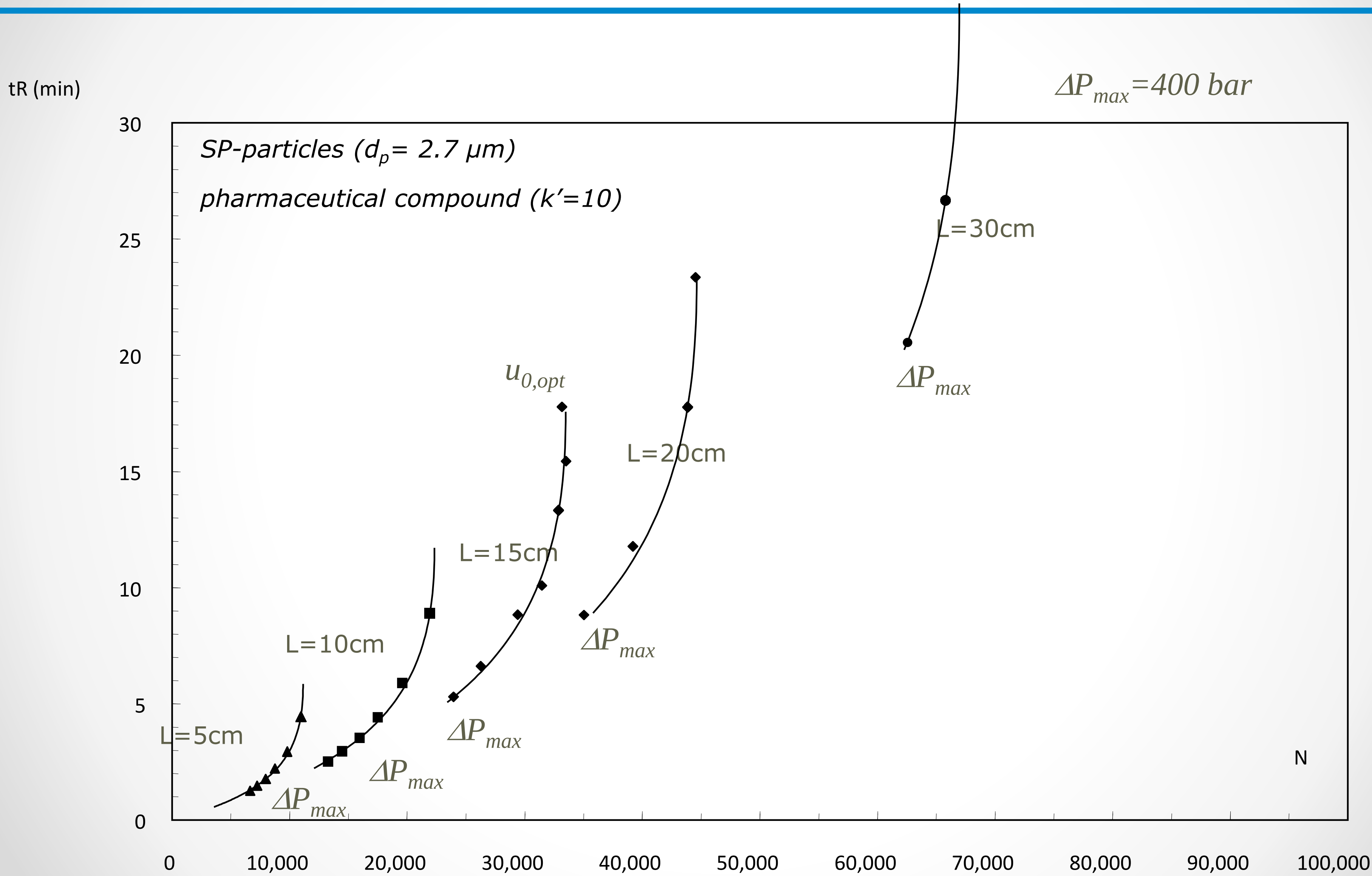

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UHPLC - Essentials

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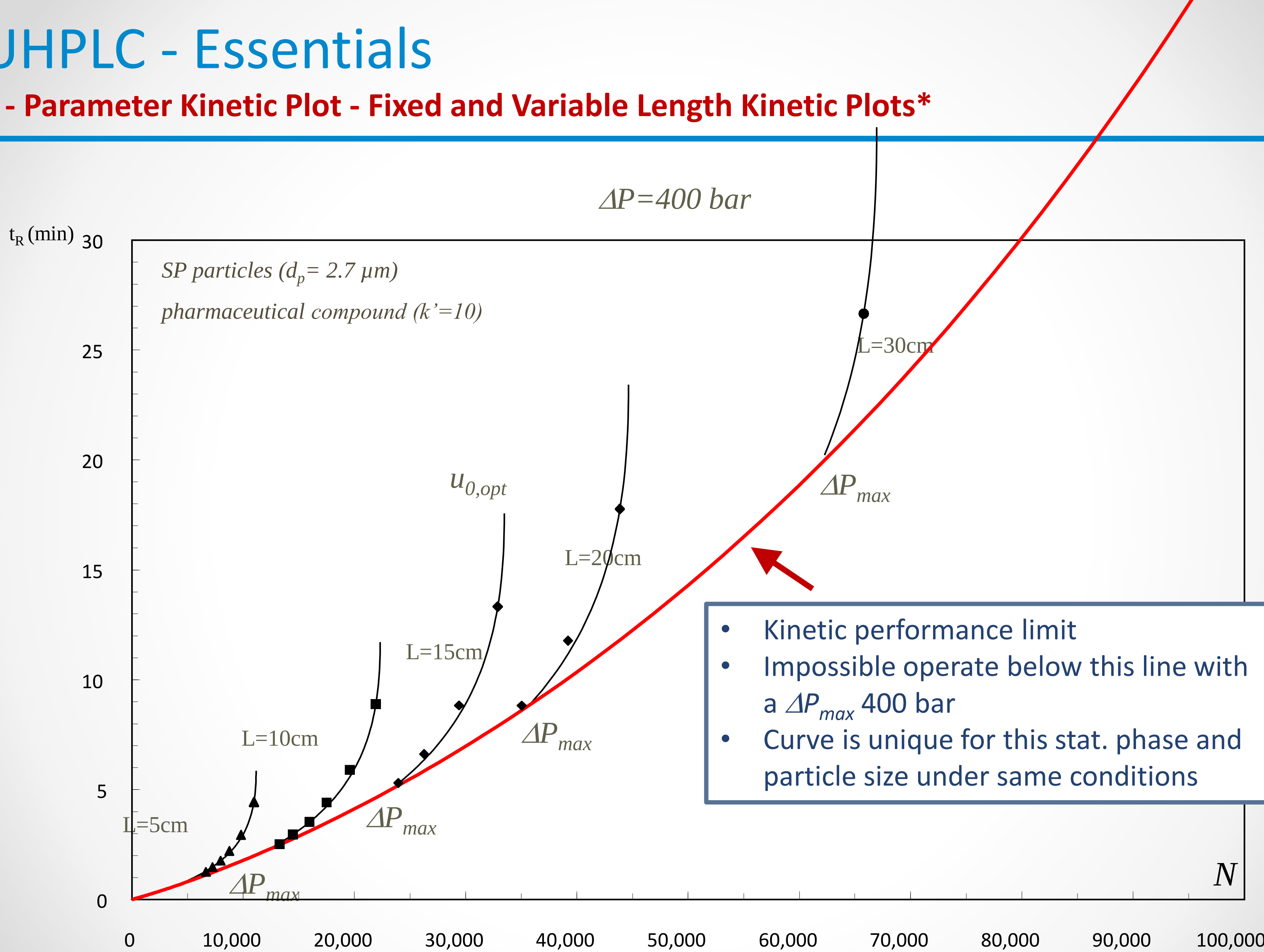

- Kinetic performance limit
- Impossible operate below this line with a ΔP_{max} 400 bar
- Curve is unique for this stat. phase and particle size under same conditions

*Desmet, G. and Cabooter, D. LC-GC Europe, 2009, 22, 70-77

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Kinetic Plot Theory

Two approaches

- "Poppe" plot[1]
  - Find the shortest time (u_0 and L) to obtain the required plate number at given maximum pressure
- "Kinetic Performance Limit" plot[2,3]
  - Find the maximum plate number that one can obtain at given maximum pressure

[1] H Poppe, J. Chrom., **778**, 3 (1997)
[2] J.C. Giddings, Anal. Chem. **37**, 60 (1965)
[3] G. Desmet et al., LCGC Europe, **18** (7), 403-409 (2005)

Kinetic Plot Theory

*"Poppe" Plot Approach

The following relationship exists between t_0 and N_r

$$t_0 = \frac{L}{u_0} = N_r \cdot \frac{H(u_0)}{u_0}$$

To minimize t_0, the value of $H(u_0)/u_0$ needs to be minimized.

$$\frac{H(u_0)}{u_0} = \frac{A \cdot d_p}{u_0} + \frac{B \cdot D_m}{u_0^2} + C \cdot \frac{d_p^2}{D_m}$$

As H/u_0 becomes smaller with increasing values of u_0, the largest value for u_0 is best, but constrained by ΔP_{max}

Poppe's approach: find $u_{0,max}$ and $L(u_{0,max})$ corresponding to a given N_r and ΔP_{max} at a fixed particle size

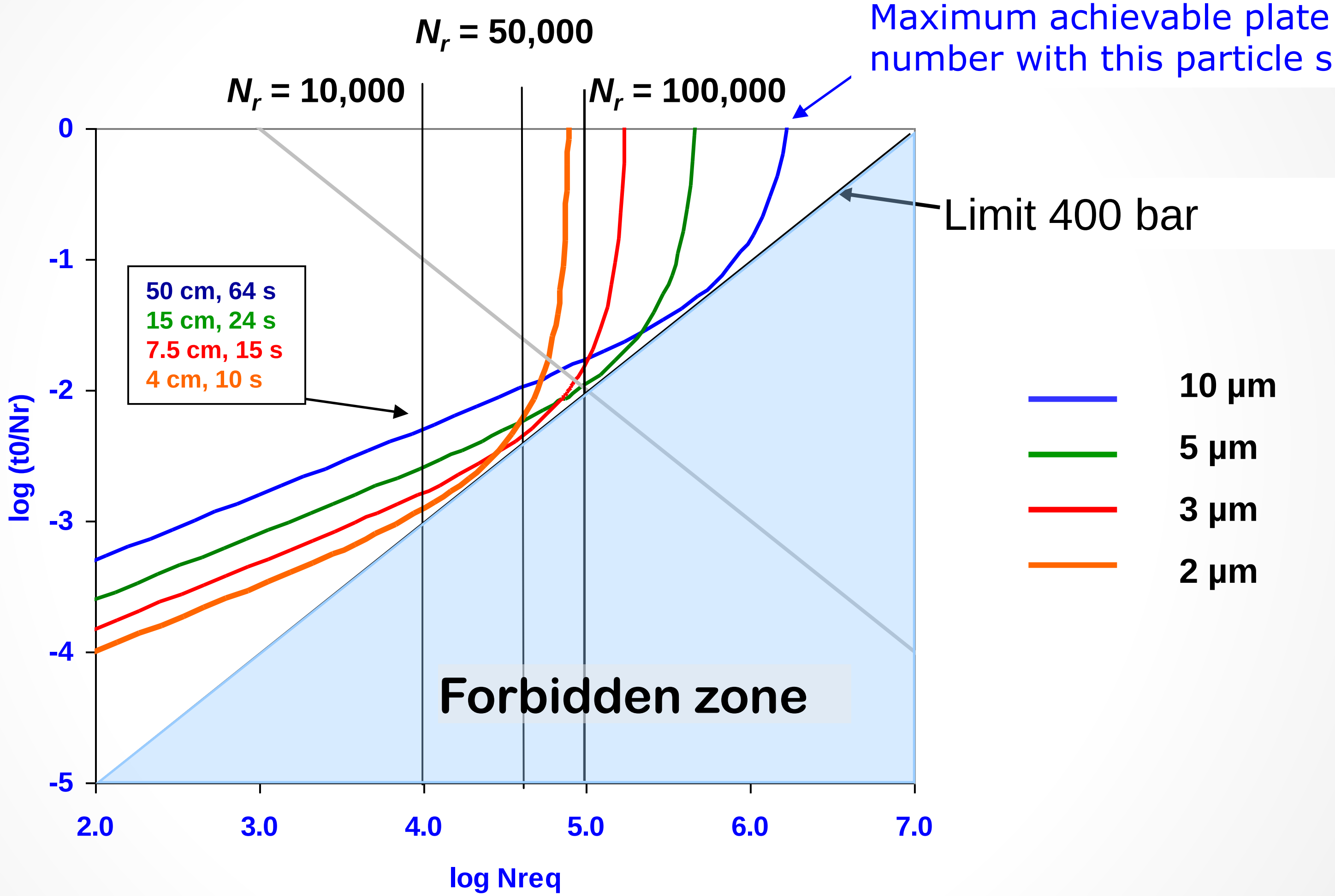
In order to construct the plot of the plate time in dependence of N_{req} after obtaining the coefficients of the van Deemter equation by non-linear regression (or from the Knox equation by iteratively calculation) , the Poppe plot can be calculated after substitution of the appropriate value for ΔP_{max}.

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

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

Kinetic Plot Theory

"Poppe" Plot Approach

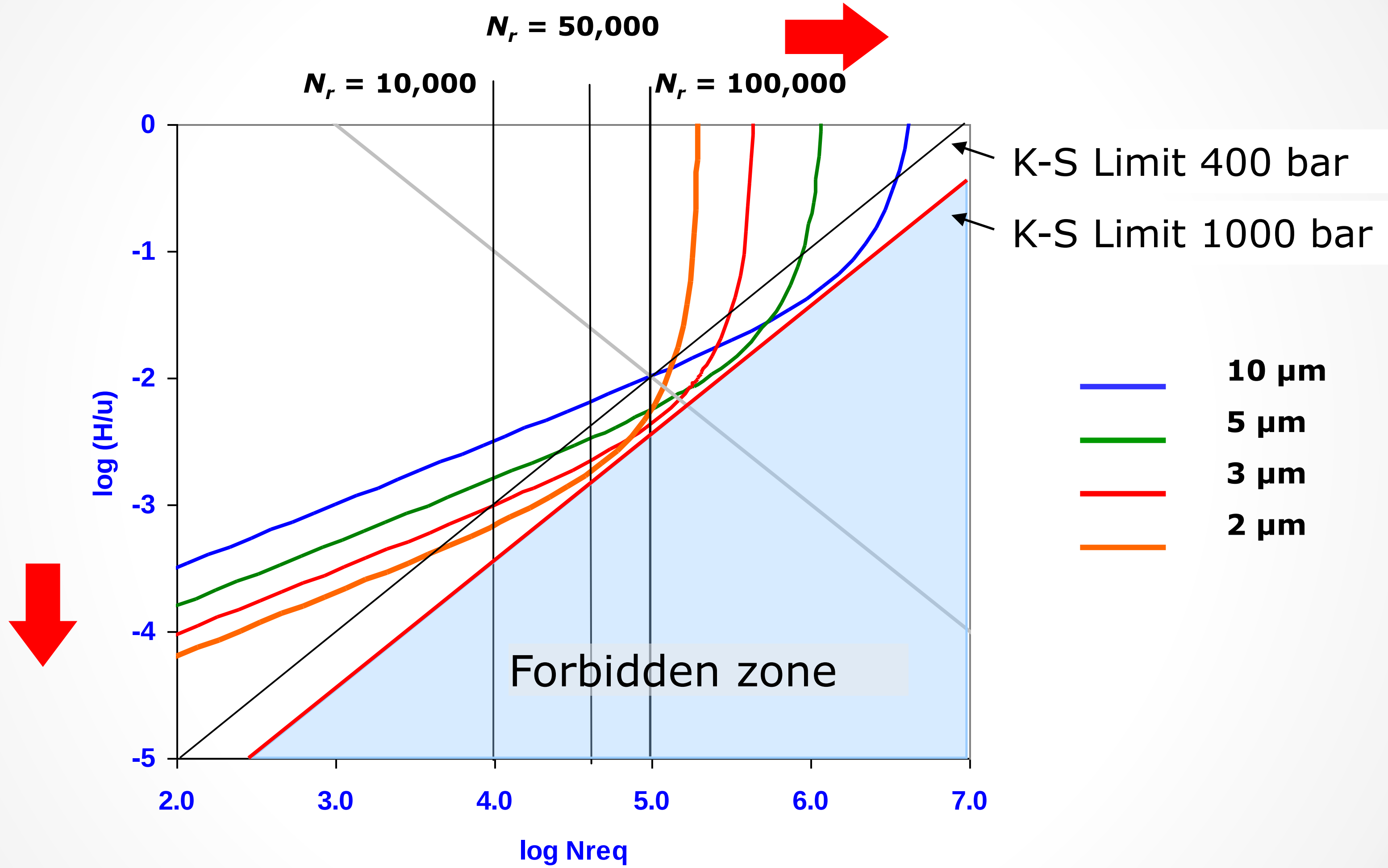


All parameters default for small molecules. Non-linearity by pressure increase neglected

*Plot courtesy of Prof. Peter Schoenmakers, University of Amsterdam

Kinetic Plot Theory

"Poppe" Plot Approach



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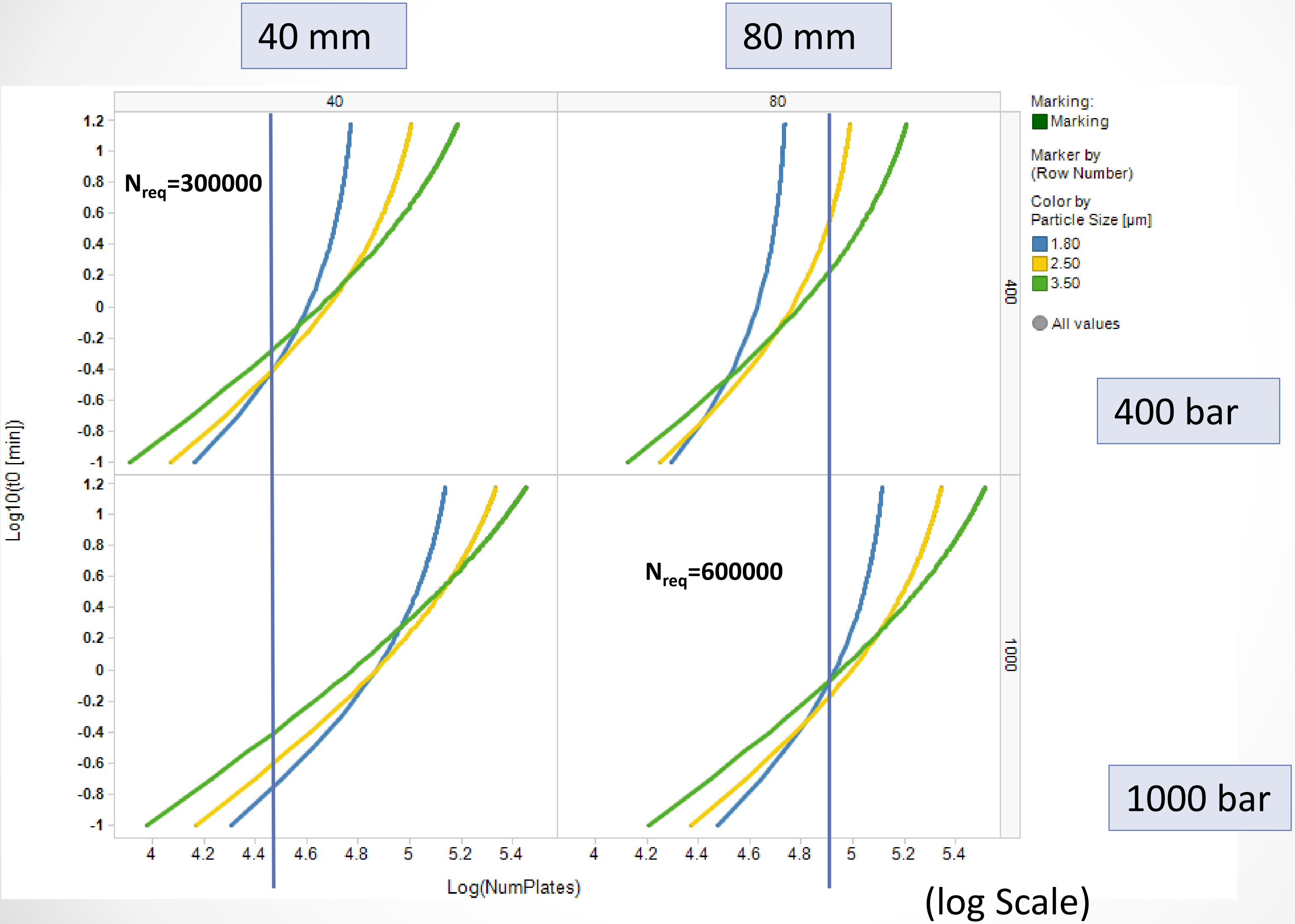
Kinetic Plot Theory

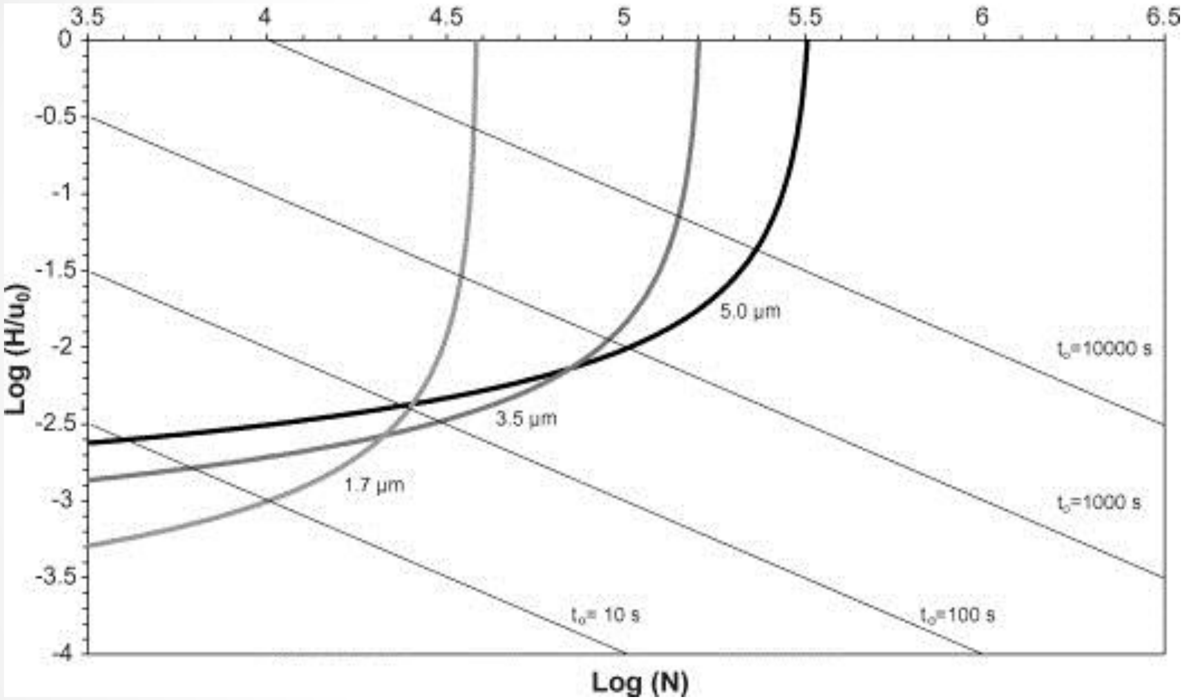
*"Poppe" Plot Approach



*Plot courtesy of Dr. Monika Dittmann, Agilent Technologies, Waldbronn, Germany

Kinetic Plot Theory

*"Poppe" Plot Approach

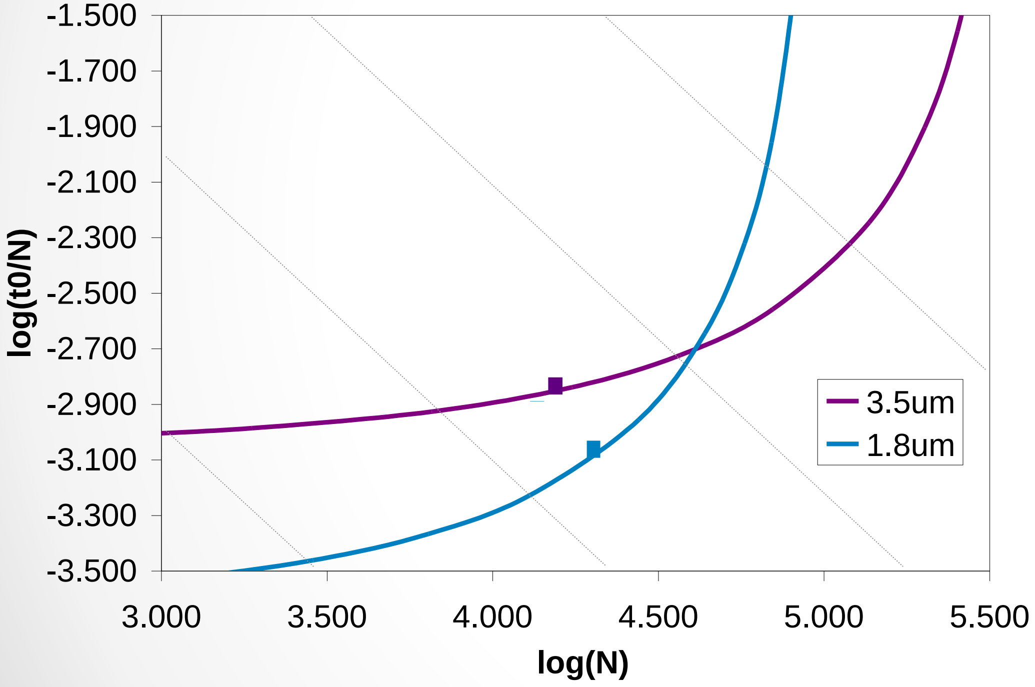


Theoretical Poppe plots for 1.7 (grey), 3.5 (dark grey) and 5 µm (black) particles at a maximum pressure of ΔP = 400 bar (40 MPa)....

***F. Lestremau et al., J. Chrom. A, Volume 1138, 2007, 120 - 131**

Poppe Plots – Try it yourself

Authors: Dwight Stoll, Peter Carr and Xiaoli Wang
http://homepages.gac.edu/~dstoll/calculators/web_opt_2p_pair_1.1.html



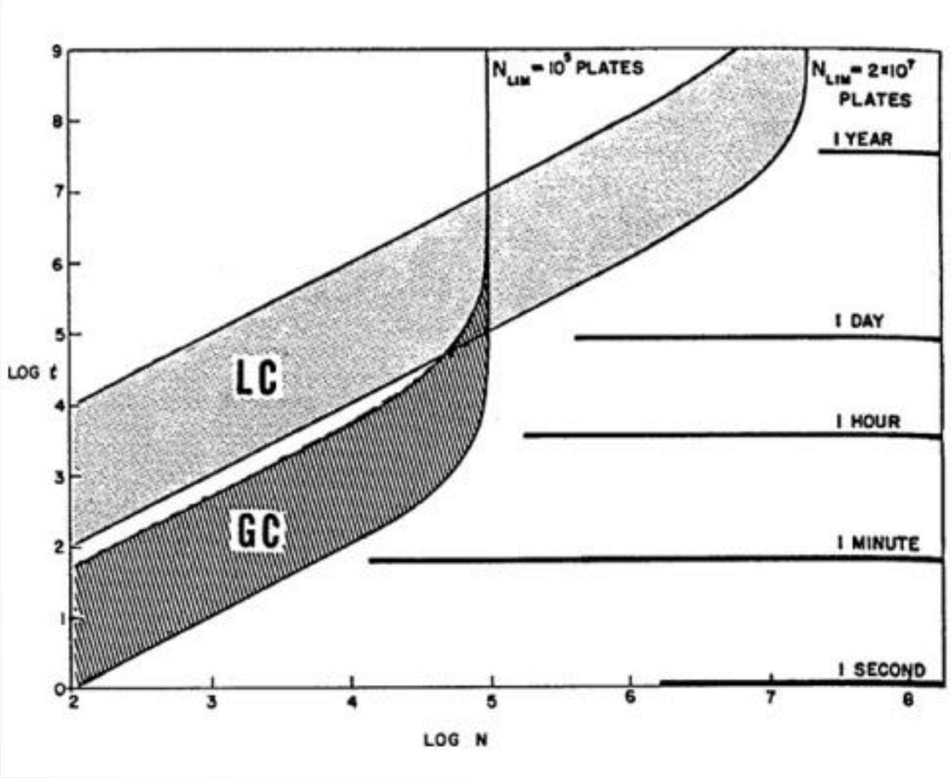
ΔP_{max} = 400 bar, T = 40 °C,
Column 50x2.1 mm,
van Deemter equation
A = 1.0, B = 5.0, C = 0.05
$D_m = 1 \times 10^{-5}$ cm²/s.
η = 6.6 × 10-4 Pa/s, Φ_0= 500, ε_e = 0.38,
ε_e = 0.30

Example taken from: W. Barber et al., presented at Pittsburg Conference 2010 available at:
http://www.chem.agilent.com/Library/posters/Public/High_Speed_vs_High_Resolution_Analysis_HPLC.pdf

Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach

Originally proposed by Giddings*



*J.C. Giddings, Anal. Chem. **37**, 60 (1965)

Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach

As proposed by Desmet et al.*

find L_{max} and N_{max} corresponding with t_0 (so at a fixed u_0) and ΔP_{max}

$$L = N \cdot H \qquad N = \left(\frac{\Delta P_{\max}}{\eta}\right)\cdot\left[\frac{B_0}{u_0 H}\right] \qquad t_0 = \left(\frac{\Delta P_{\max}}{\eta}\right)\cdot\left[\frac{B_0}{u_0^2}\right]$$

Not a theoretical exercise, but based on your own experimental data, obtained for your own compound(s) of interest and your own columns!!

G. Desmet et al., LCGC Europe, **18 (7), 403-409 (2005) and G. Desmet et al., J. Chrom. A, **1228**, 20 (2012);

Kinetic Plot Theory

“Kinetic Performance Limit” Plot Approach

Equations required:

$$\begin{cases} L = N.H \\ t_0 = \dfrac{L}{u_0} = \dfrac{N.H}{u_0} \\ u_0 = B_0.\dfrac{\Delta P}{\eta.L} = B_0.\dfrac{\Delta P}{\eta.H.N} \end{cases}$$

Eliminate L from these equations

*J.C. Giddings, Anal. Chem. **37**, 60 (1965)
G. Desmet et al., LCGC Europe, **18 (7), 403-409 (2005) and G. Desmet et al., J. Chrom. A, **1228**, 20 (2012);

Kinetic Plot Theory

“Kinetic Performance Limit” Plot Approach

Equations required:

$$\begin{cases} L = N.H \\ t_0 = \dfrac{L}{u_0} = \dfrac{N.H}{u_0} \\ u_0 = B_0.\dfrac{\Delta P}{\eta.L} = B_0.\dfrac{\Delta P}{\eta.H.N} \end{cases}$$

$$\Rightarrow \quad t_0 = \left\{\frac{\Delta P}{\eta}\right\}.\left\{\frac{B_0}{u_0^2}\right\}$$

$$\Rightarrow \quad N = \left\{\frac{\Delta P}{\eta}\right\}.\left\{\frac{B_0}{u_0 H}\right\}$$

*J.C. Giddings, Anal. Chem. **37**, 60 (1965)

G. Desmet et al., LCGC Europe, **18 (7), 403-409 (2005) and G. Desmet et al., J. Chrom. A, **1228**, 20 (2012);

Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach*

Example:

Maximum pressure	400 bar
Column	Zorbax 3.5µm 10cm

Experimental data			
F (ml/min)	*ΔP* (bar)	u_0 (mm/s)	*H* (µm)
3,5	228,1	6,95	8,72
3	196,5	5,97	8,40
2,5	164,6	4,98	7,92
2	132,8	4,00	7,51
1,75	117,0	3,51	7,31
1,5	101,2	3,01	7,26
1,25	85,2	2,52	7,21
1	69,1	2,02	7,46
0,875	61,1	1,77	7,75
0,75	53,0	1,51	8,15
0,625	45,0	1,26	8,86
0,5	36,7	1,01	10,02
0,375	28,4	0,76	11,93
0,25	19,6	0,51	16,15
0,125	10,4	0,25	29,21

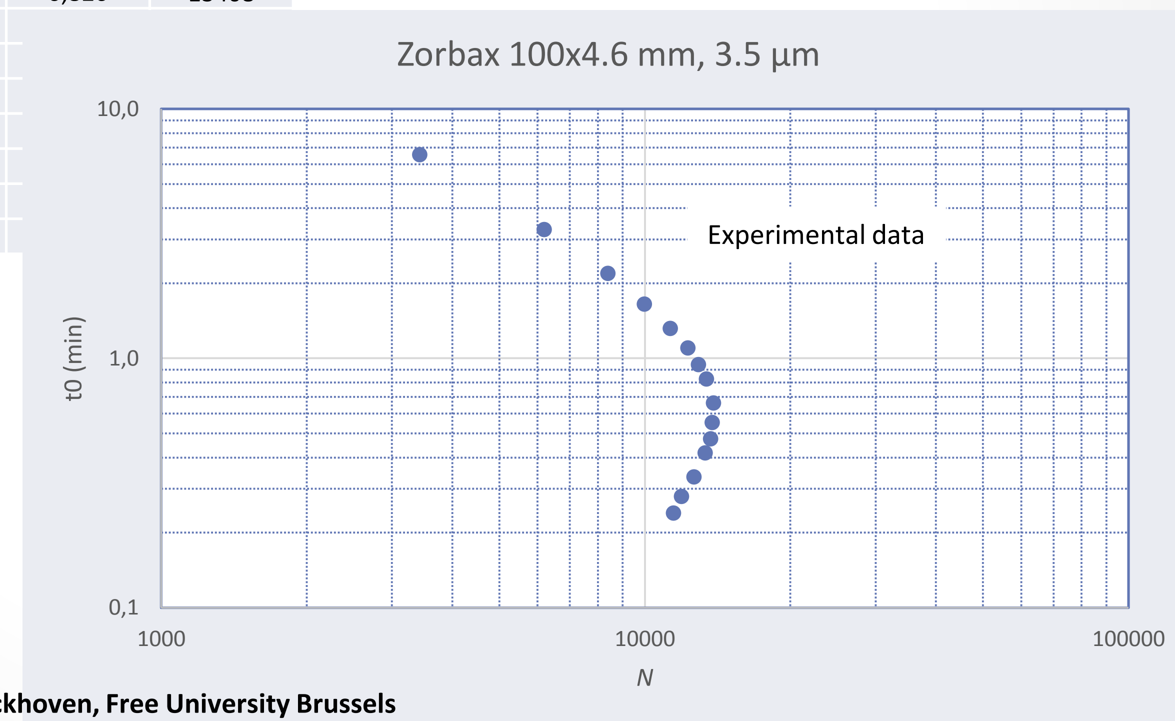
Data courtesy of Profs. Gert Desmet & Ken Broeckhoven, Free University Brussels

Kinetic Plot Theory

“Kinetic Performance Limit” Plot Approach

Experimental data

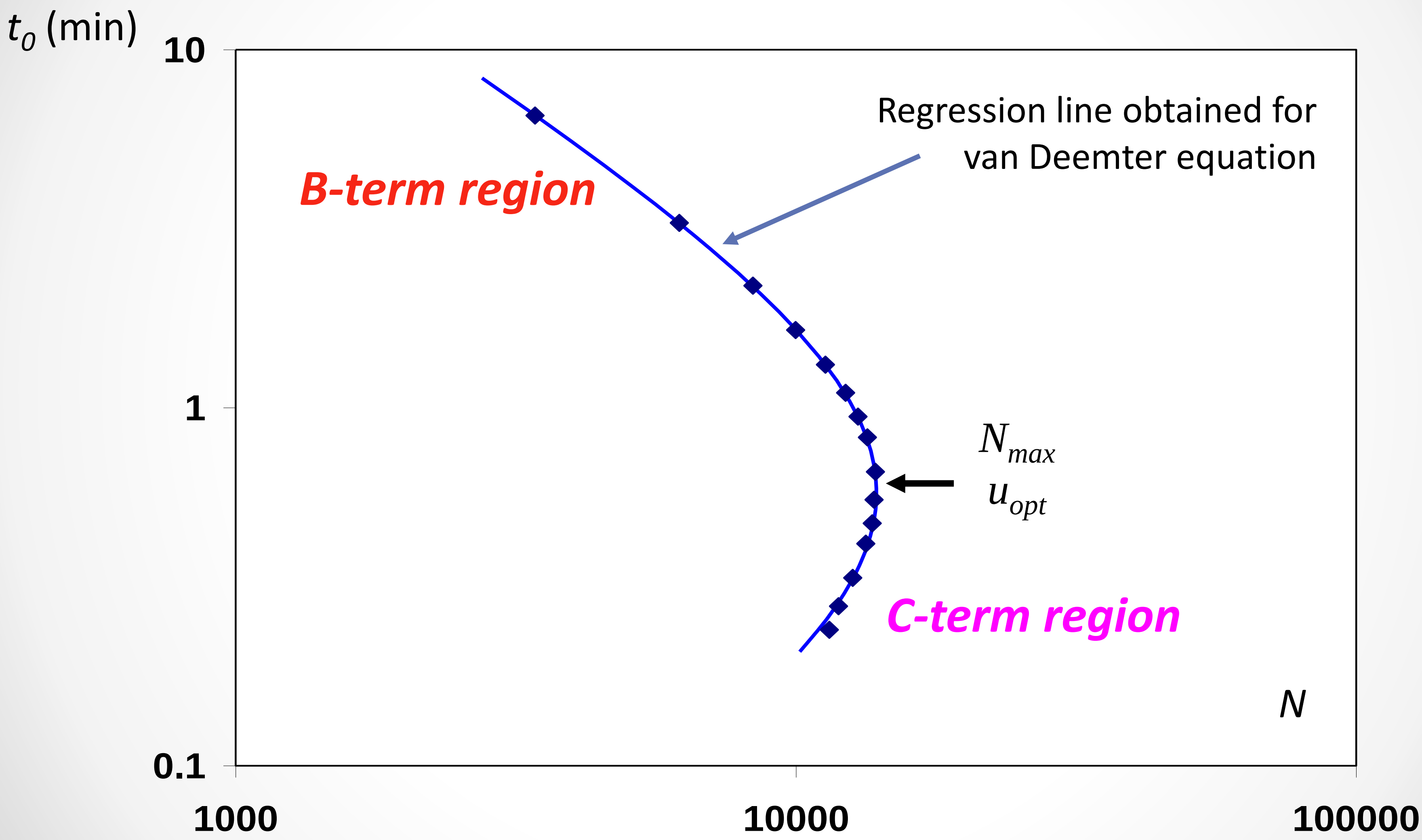
Experimental data					
F (ml/min)	*ΔP* (bar)	u_0 (mm/s)	*H* (µm)	t_0 (min)	*N*
3,5	228,1	6,95	8,72	0,240	11466
3	196,5	5,97	8,40	0,279	11911
2,5	164,6	4,98	7,92	0,335	12633
2	132,8	4,00	7,51	0,417	13323
1,75	117,0	3,51	7,31	0,475	13675
1,5	101,2	3,01	7,26	0,553	13779
1,25	85,2	2,52	7,21	0,662	13860
1	69,1	2,02	7,46	0,826	13408
0,875	61,1	1,77	7,75		
0,75	53,0	1,51	8,15		
0,625	45,0	1,26	8,86		
0,5	36,7	1,01	10,02		
0,375	28,4	0,76	11,93		
0,25	19,6	0,51	16,15		
0,125	10,4	0,25	29,21		



Data courtesy of Profs. Gert Desmet & Ken Broeckhoven, Free University Brussels

Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach



Data courtesy of Profs. Gert Desmet & Ken Broeckhoven, Free University Brussels

Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach*

Extrapolate the experimental data *N*, t_0 (or H, u_0) obtained by measuring a "van Deemter" plot, to the kinetic performance limit (KPL) obtained at ΔP_{max}

Elongation factor: $\lambda = \dfrac{\Delta P_{\max}}{\Delta P_{\exp}}$

$$N_{KPL} = \lambda \cdot N_{\exp}$$

$$t_{0,KPL} = \lambda \cdot t_{0,\exp}$$

$$L_{KPL} = \lambda \cdot L_{\exp}$$

$$t_{R,KPL} = \lambda \cdot t_{R,\exp}$$

*** K. Broeckhoven, et al., J. Chromatogr. A, 1217 (2010) 2787-2795.**

Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach

ΔP_{max} = 400 bar

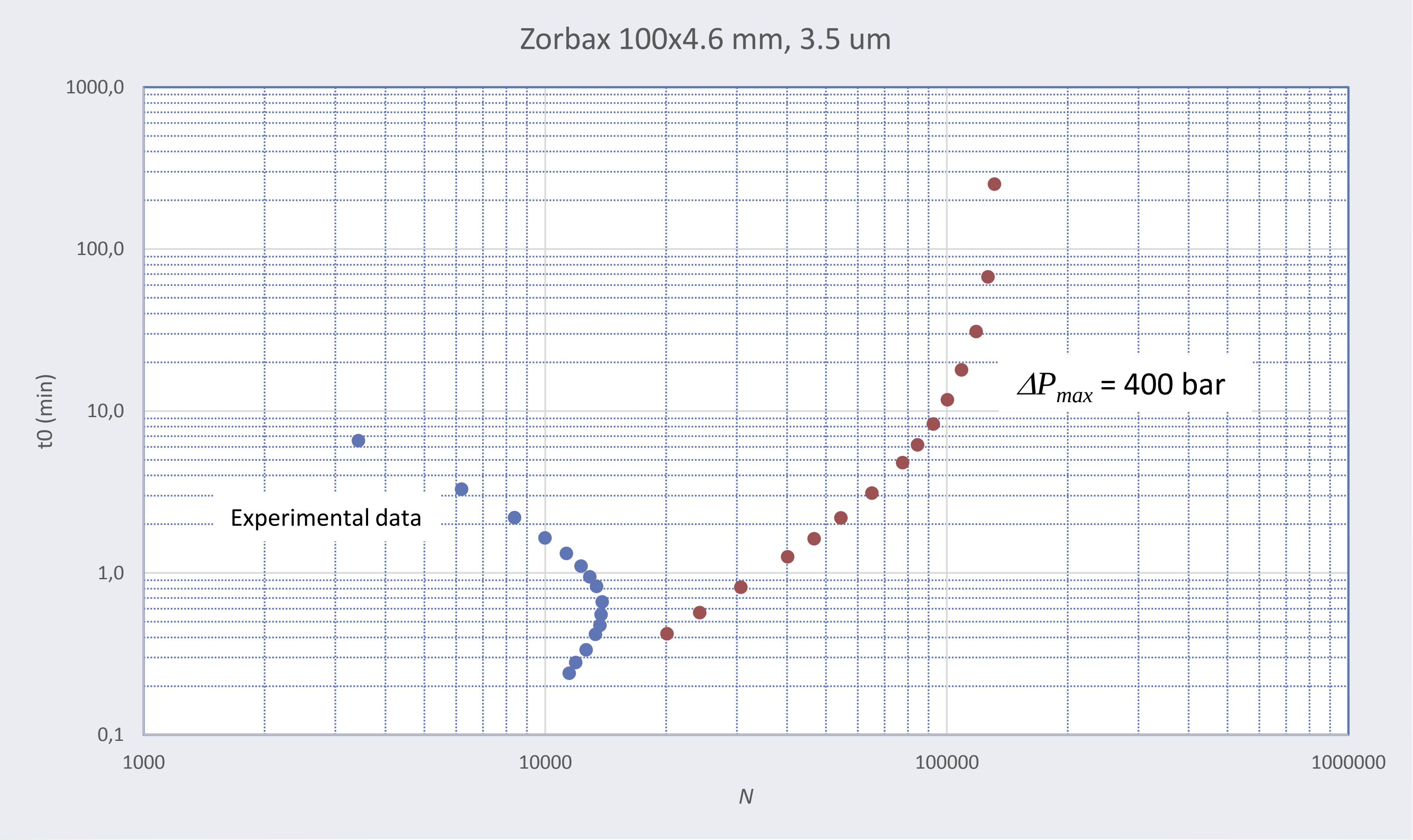
Experimental data						Kinetic plot data			
F (ml/min)	ΔP (bar)	u_0 (mm/s)	H (µm)	t_0 (min)	N (/)	λ (/)	t_0 KPL (min)	N KPL (min)	t_R KPL (min)
3,5	228,1	6,95	8,72	0,240	11466	1,75	0,420	20109	2,98
3	196,5	5,97	8,40	0,279	11911	2,04	0,568	24247	4,03
2,5	164,6	4,98	7,92	0,335	12633	2,43	0,814	30699	5,78
2	132,8	4,00	7,51	0,417	13323	3,01	1,256	40118	8,92
1,75	117,0	3,51	7,31	0,475	13675	3,42	1,625	46745	11,54
1,5	101,2	3,01	7,26	0,553	13779	3,95	2,187	54464	15,53
1,25	85,2	2,52	7,21	0,662	13860	4,69	3,108	65047	22,07
1	69,1	2,02	7,46	0,826	13408	5,79	4,786	77654	33,98
0,875	61,1	1,77	7,75	0,944	12902	6,55	6,182	84490	43,89
0,75	53,0	1,51	8,15	1,101	12268	7,54	8,309	92560	59,00
0,625	45,0	1,26	8,86	1,319	11293	8,89	11,727	100382	83,26
0,5	36,7	1,01	10,02	1,647	9983	10,89	17,946	108754	127,42
0,375	28,4	0,76	11,93	2,192	8382	14,11	30,928	118265	219,59
0,25	19,6	0,51	16,15	3,284	6191	20,46	67,199	126663	477,11
0,125	10,4	0,25	29,21	6,553	3423	38,40	251,635	131456	1786,61

Elongation factor λ

Data courtesy of Profs. Gert Desmet & Ken Broeckhoven, Free University Brussels

Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach



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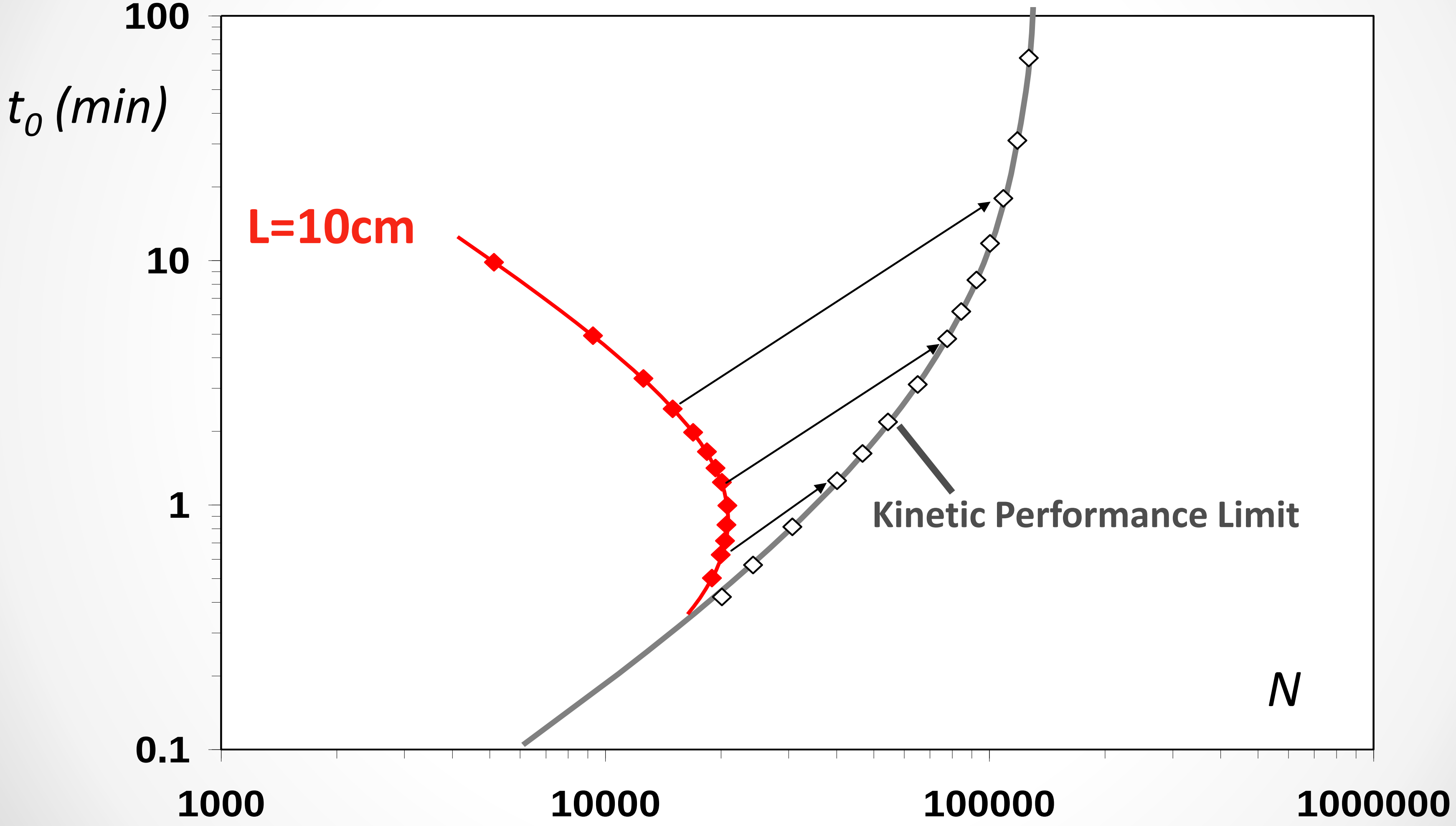

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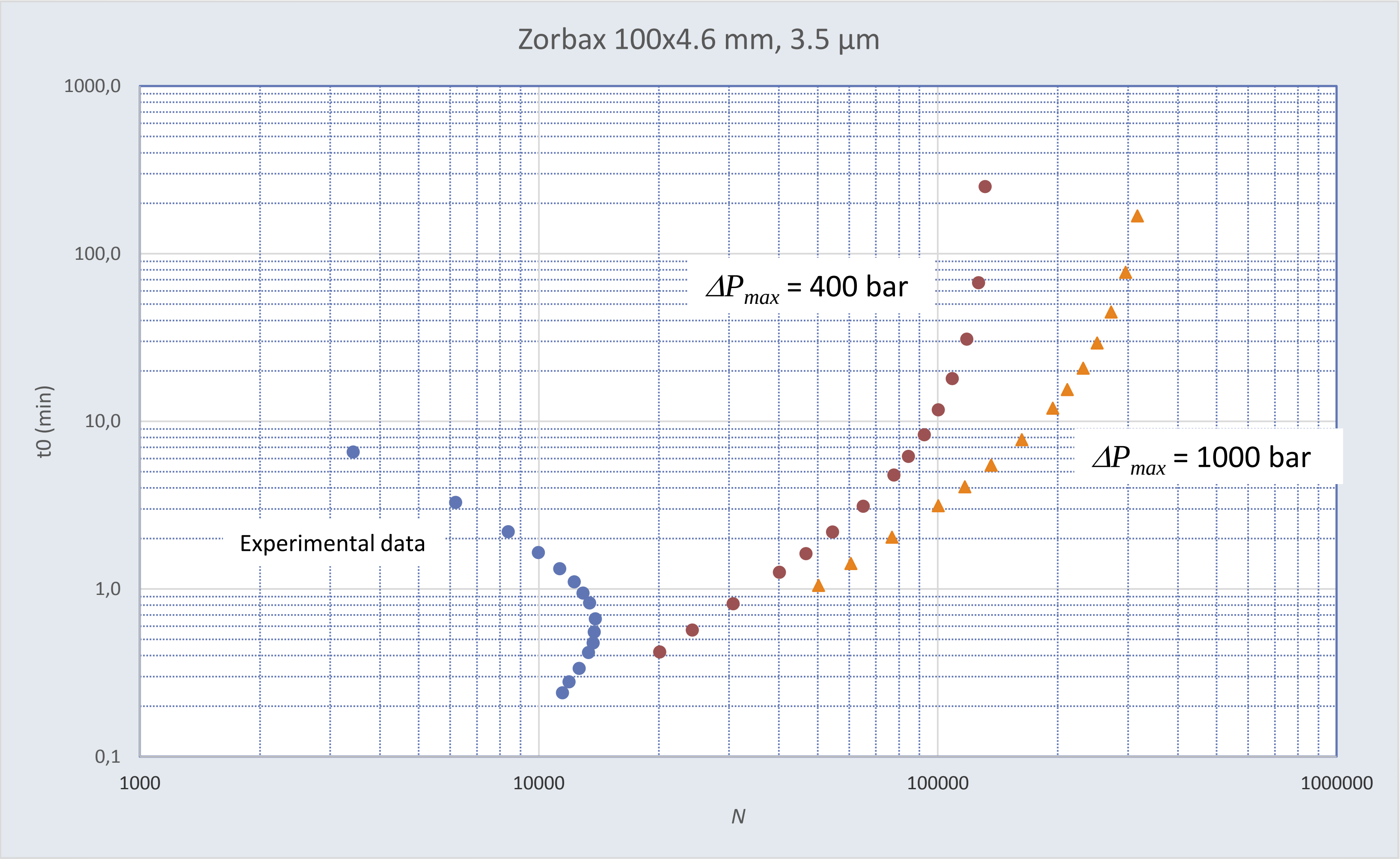
"Kinetic Performance Limit" Plot Approach



Kinetic Plot Theory

"Kinetic Performance Limit" Plot Approach

ΔP_{max} = 400 bar

Experimental data						Kinetic plot data				
F (ml/min)	ΔP (bar)	u_0 (mm/s)	H (µm)	t_0 (min)	N (/)	λ (/)	t_0 KPL (min)	N KPL (min)	tR KPL (min)	Length (cm)
3,5	228,1	6,95	8,72	0,240	11466	1,75	0,420	20109	2,984	17,5
3	196,5	5,97	8,40	0,279	11911	2,04	0,568	24247	4,032	20,4
2,5	164,6	4,98	7,92	0,335	12633	2,43	0,814	30699	5,780	24,3
2	132,8	4,00	7,51	0,417	13323	3,01	1,256	40118	8,916	30,1
1,75	117,0	3,51	7,31	0,475	13675	3,42	1,625	46745	11,536	34,2
1,5	101,2	3,01	7,26	0,553	13779	3,95	2,187	54464	15,528	39,5
1,25	85,2	2,52	7,21	0,662	13860	4,69	3,108	65047	22,069	46,9
1	69,1	2,02	7,46	0,826	13408	5,79	4,786	77654	33,979	57,9
0,875	61,1	1,77	7,75	0,944	12902	6,55	6,182	84490	43,890	65,5
0,75	53,0	1,51	8,15	1,101	12268	7,54	8,309	92560	58,996	75,4
0,625	45,0	1,26	8,86	1,319	11293	8,89	11,727	100382	83,265	88,9
0,5	36,7	1,01	10,02	1,647	9983	10,89	17,946	108754	127,420	108,9
0,375	28,4	0,76	11,93	2,192	8382	14,11	30,928	118265	219,587	141,1
0,25	19,6	0,51	16,15	3,284	6191	20,46	67,199	126663	477,110	204,6
0,125	10,4	0,25	29,21	6,553	3423	38,40	251,635	131456	1786,610	384,0

Elongation factor λ

Data courtesy of Profs. Gert Desmet & Ken Broeckhoven, Free University Brussels

Kinetic Plot Theory

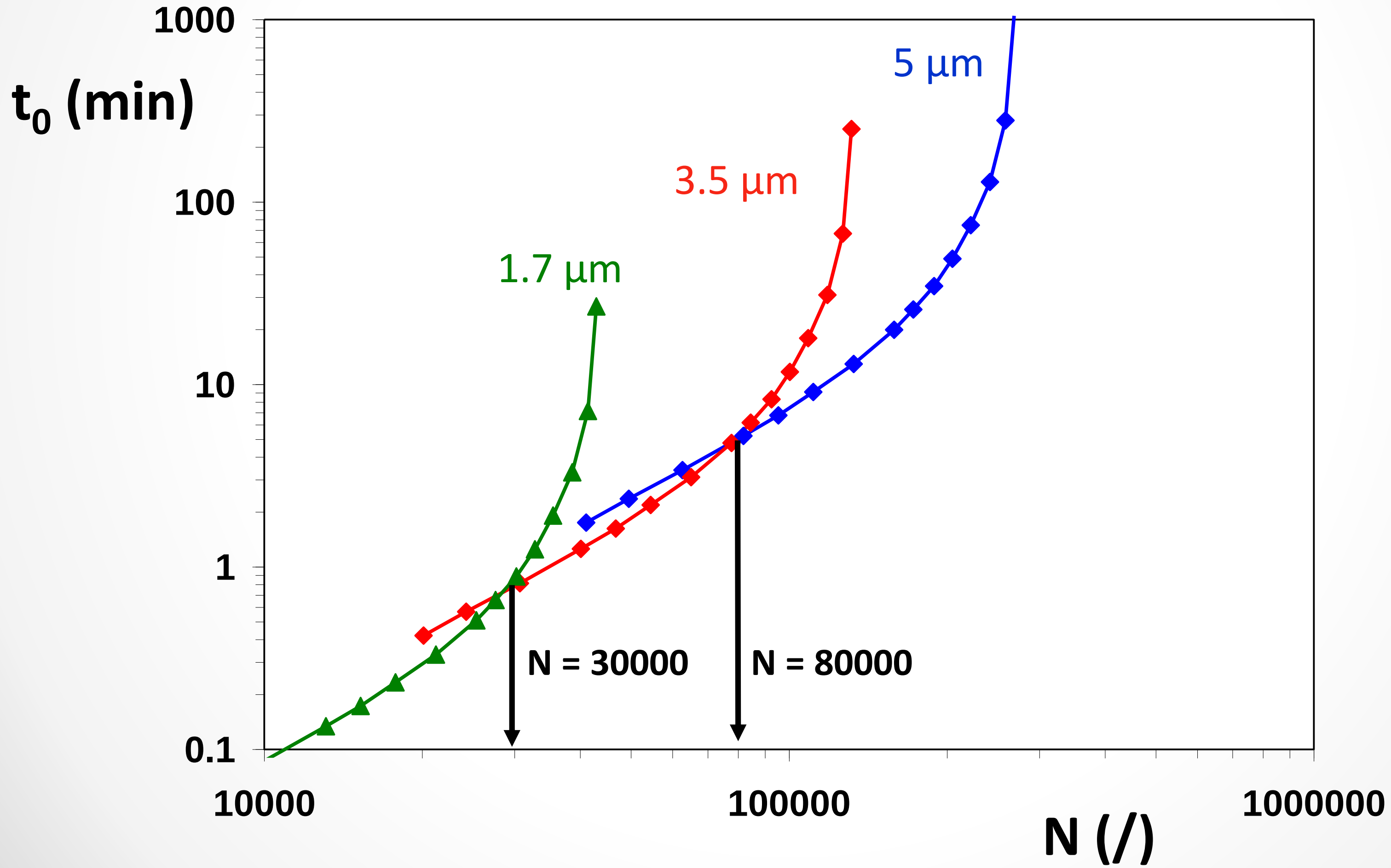
Underlying Assumptions for Column Length Elongation

- *HETP* is independent of *L*
- Viscosity is independent of *L*
- Permeability B_0 is independent of *L*
- Retention factor is independent of *L*
- No effect of pressure on physical properties of solutes and solvent
- No effect of frictional heating

How to verify? Extrapolating from data obtained from a column with a different length and see whether same KPL is being found

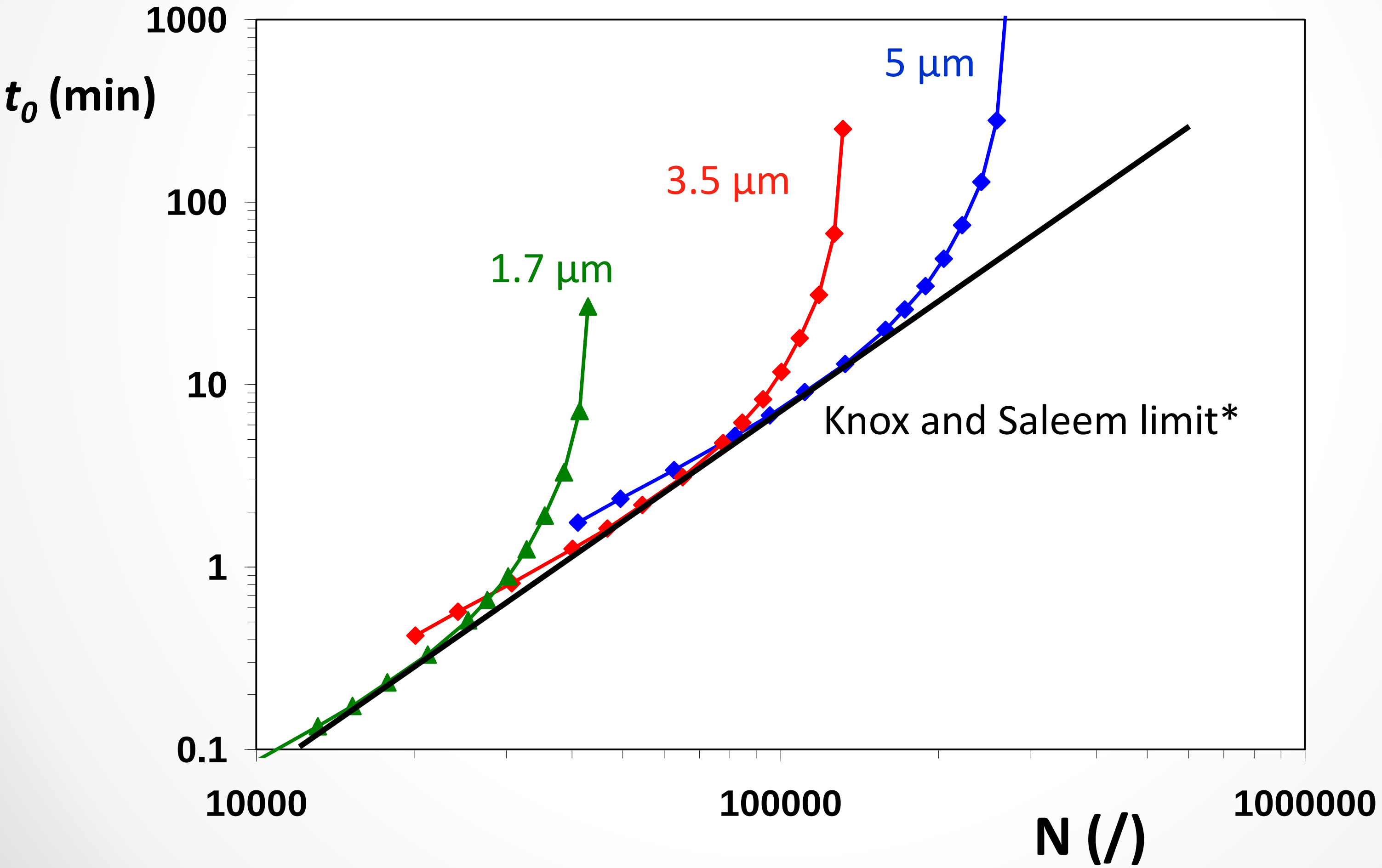
KPL Plot Example

Effect of particle size



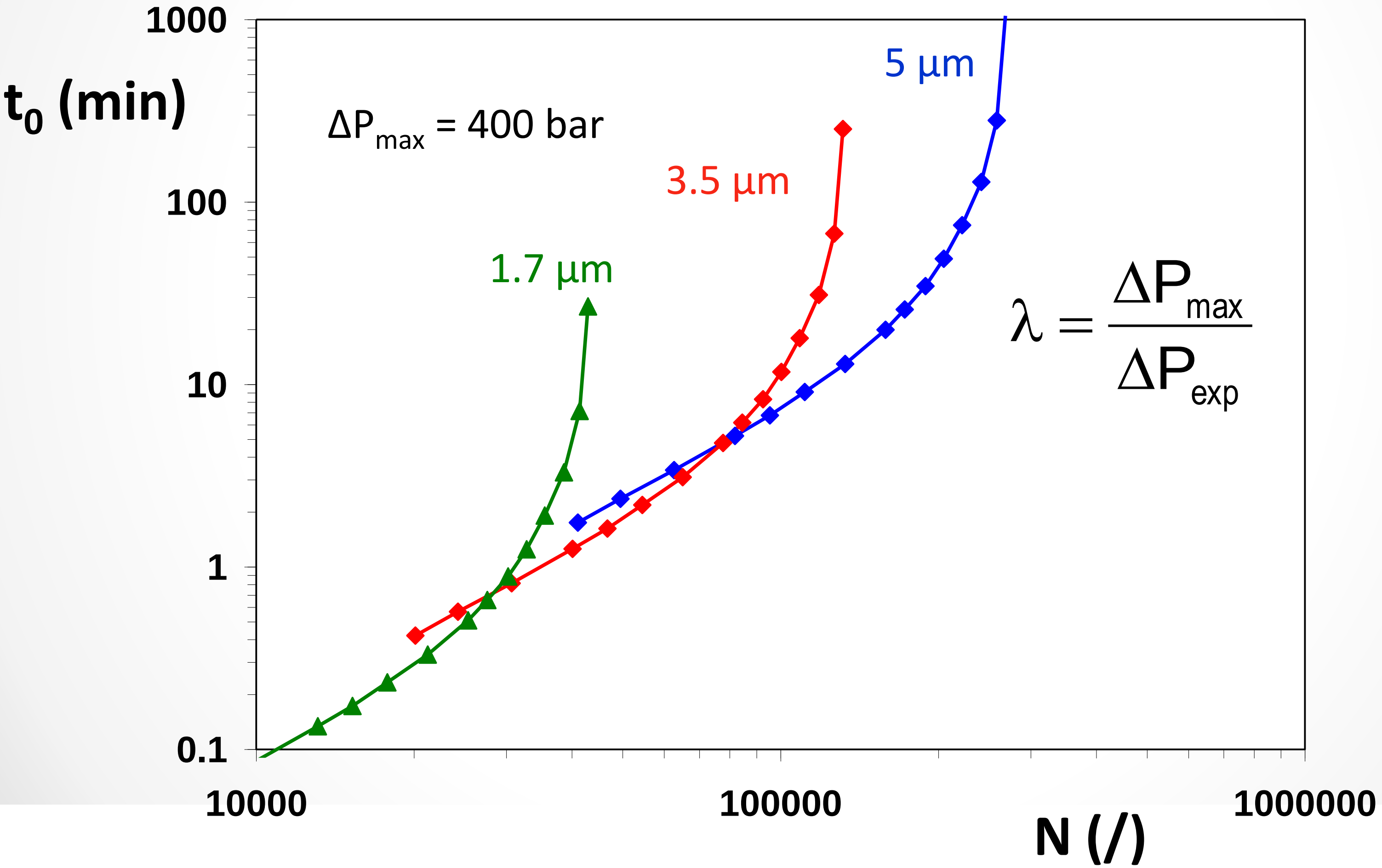
KPL Plot Example

Effect of particle size



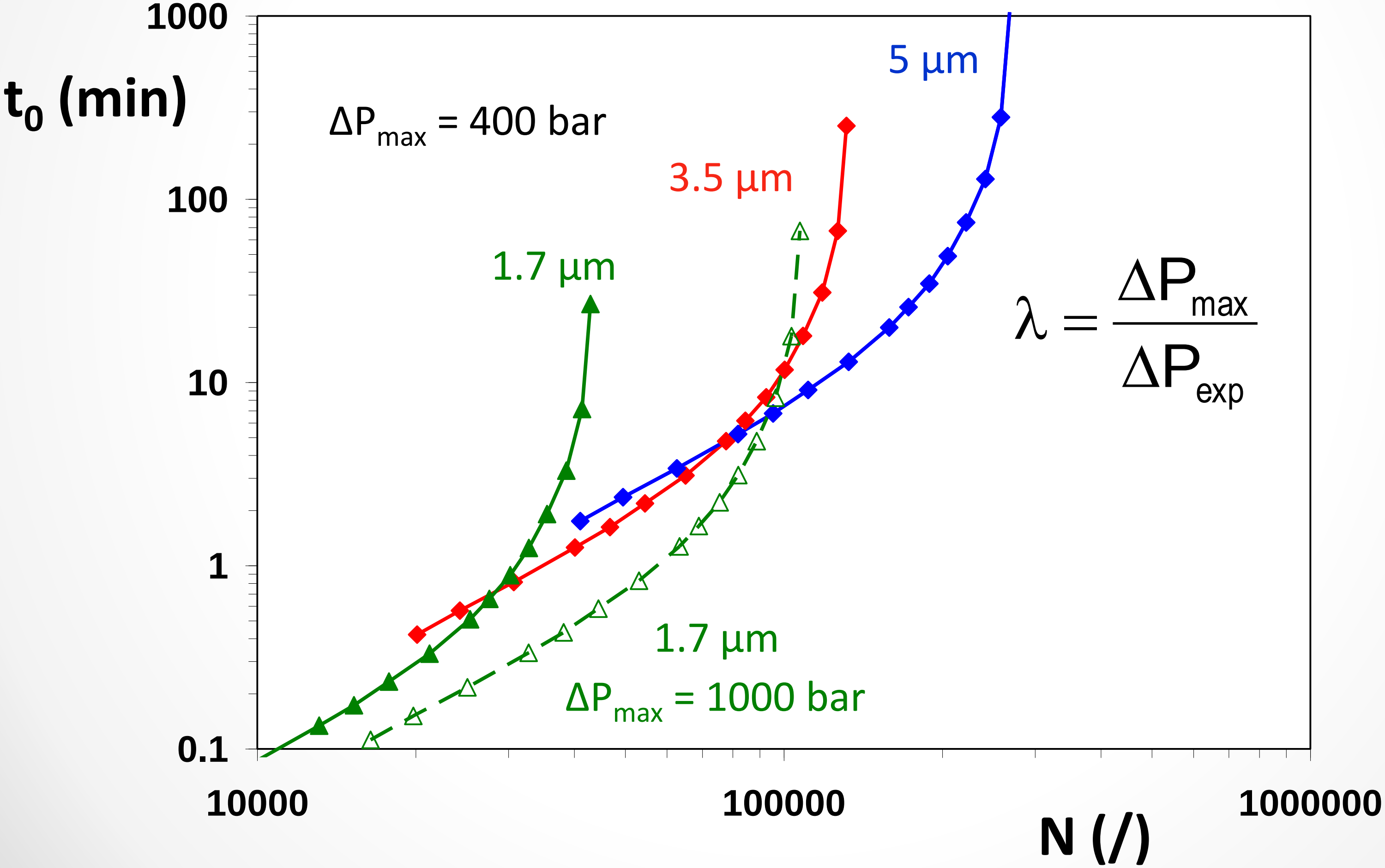
KPL Plot Example

Effect of max. pressure limit



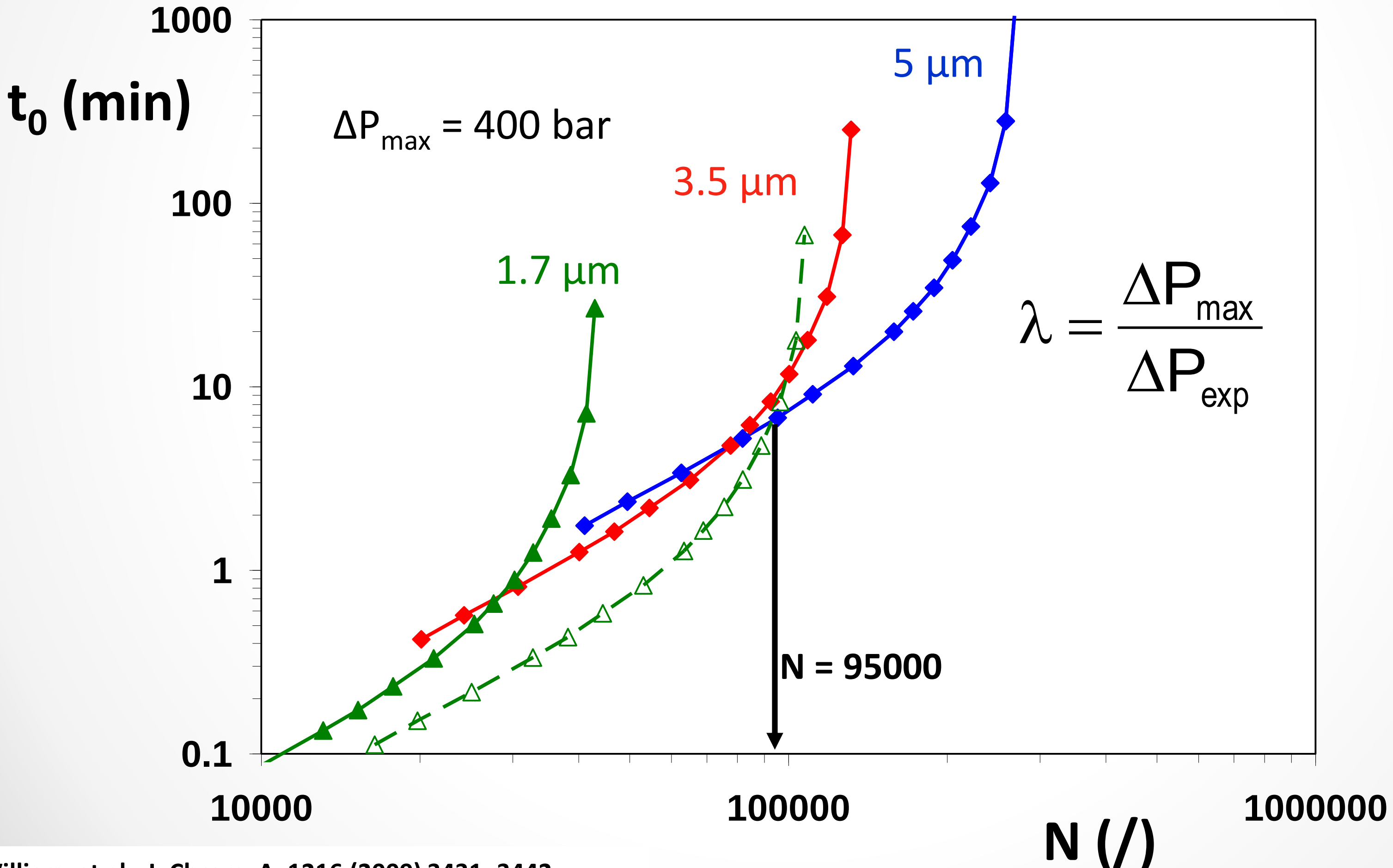
KPL Plot Example

Effect of max. pressure limit



KPL Plot Example

Effect of max. pressure limit

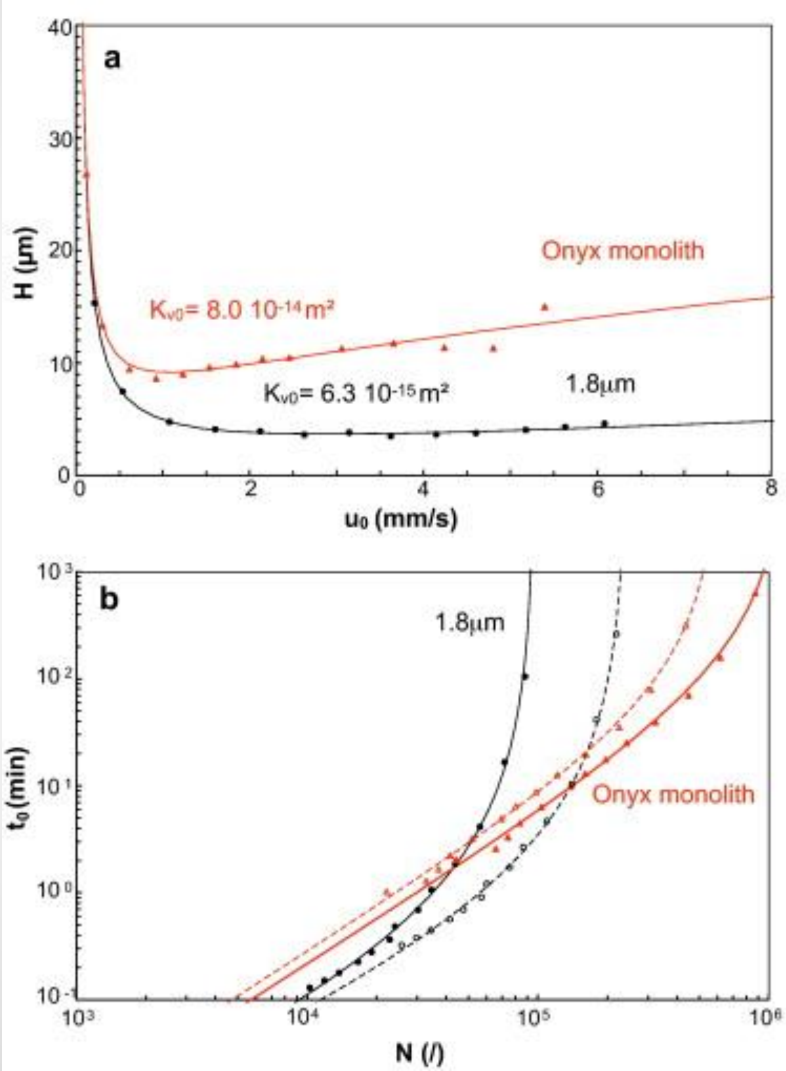


$$\lambda = \frac{\Delta P_{max}}{\Delta P_{exp}}$$

A. de Villiers, et al., J. Chrom. A, 1216 (2009) 3431–3442.

KPL Plot Example

Effect of particle morphology



Comparison of a particle (Zorbax SB 2.1 mm × 50 mm, d_p = 1.8 µm) and a monolithic column (Onyx 2.1 mm × 100 mm, d_{dom} = 3 µm)
(a) experimental Van Deemter data
(b) a kinetic plots

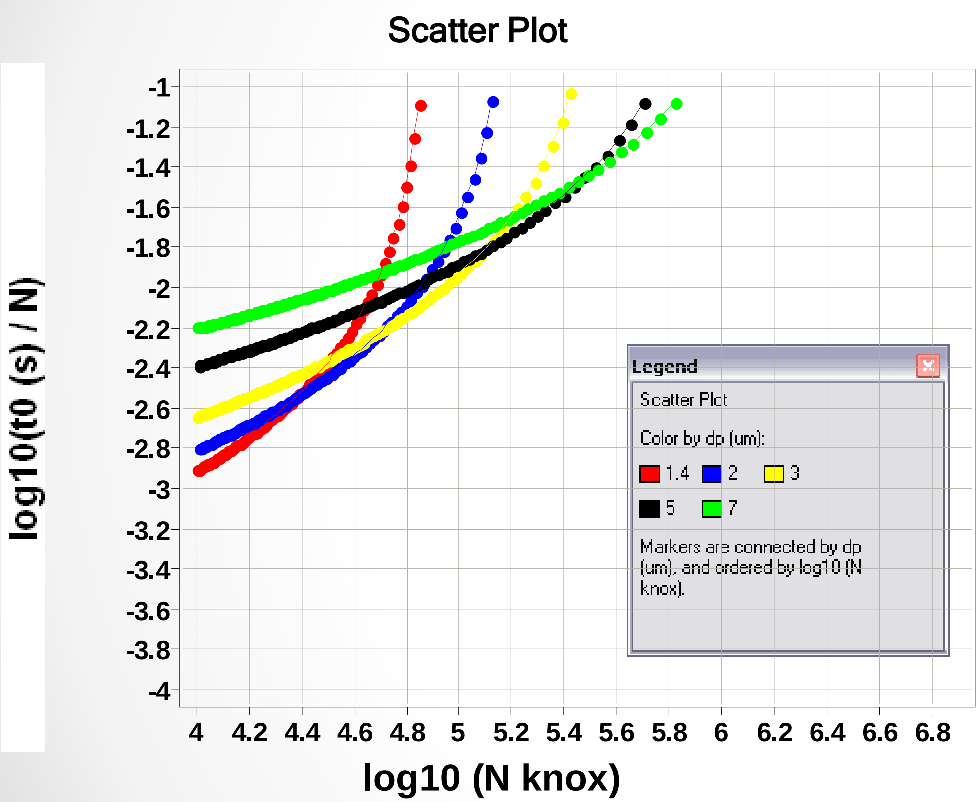
The straight line data in (b) compare both systems at identical pressure (P = 400 bar), the dashed line data compare each support type at its proper operation limit (DP = 200 bar for the monolith and DP = 1000 bar for the Zorbax column).

Experimental conditions: T = 30 °C, test compound: 10 ppm methyl paraben (k ≅ 2), mobile phase: 35%/65% (v/v) ACN/H2O for the sub-2 µm column () and 27%/73% (v/v) ACN/H2O for the monolith

K. Broeckhoven et al., J. of Chrom. A, 1228, 2012, 20 - 30

Comparison of Kinetic Plot Methods

Kinetic Plot constructed by Desmet procedure with same parameters as in Poppe's paper



Kinetic Plot constructed by Poppe**

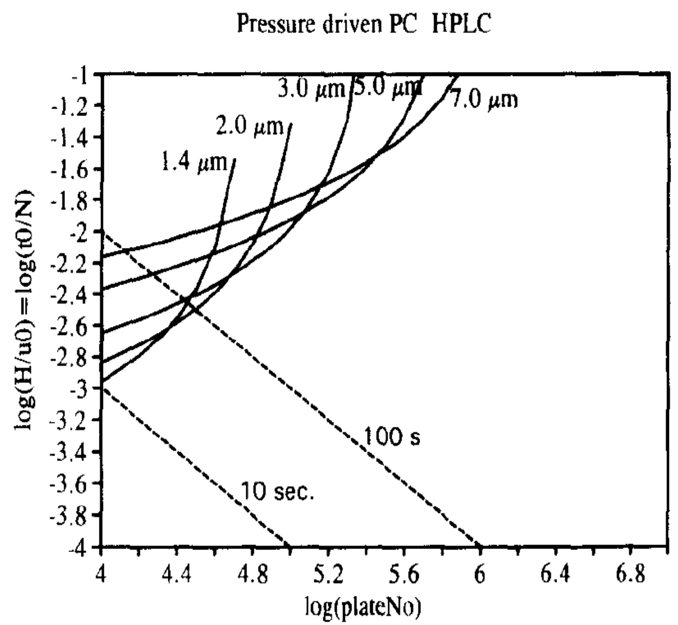


Fig. 3. Plot of plate time, H/u_0 vs. required plate number in conventional HPLC (PD-PCLC) with various particle sizes. Assumed parameters: maximum pressure $\Delta P = 4 \cdot 10^7$ Pa, viscosity $\eta = 0.001$ Pa/s, flow resistance factor $\phi = 1000$, diffusion coefficient $D = 1 \cdot 10^{-9}$ m^2/s, reduced plate height expression Eq. (9) with $A = 1.0$, $B = 1.5$ and $C = 0.05$.

Left diagram courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

**** J. Chrom. A, 778, (1997), 3**

3-Parameter Kinetic Optimization (Knox & Saleem)

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

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

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

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

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

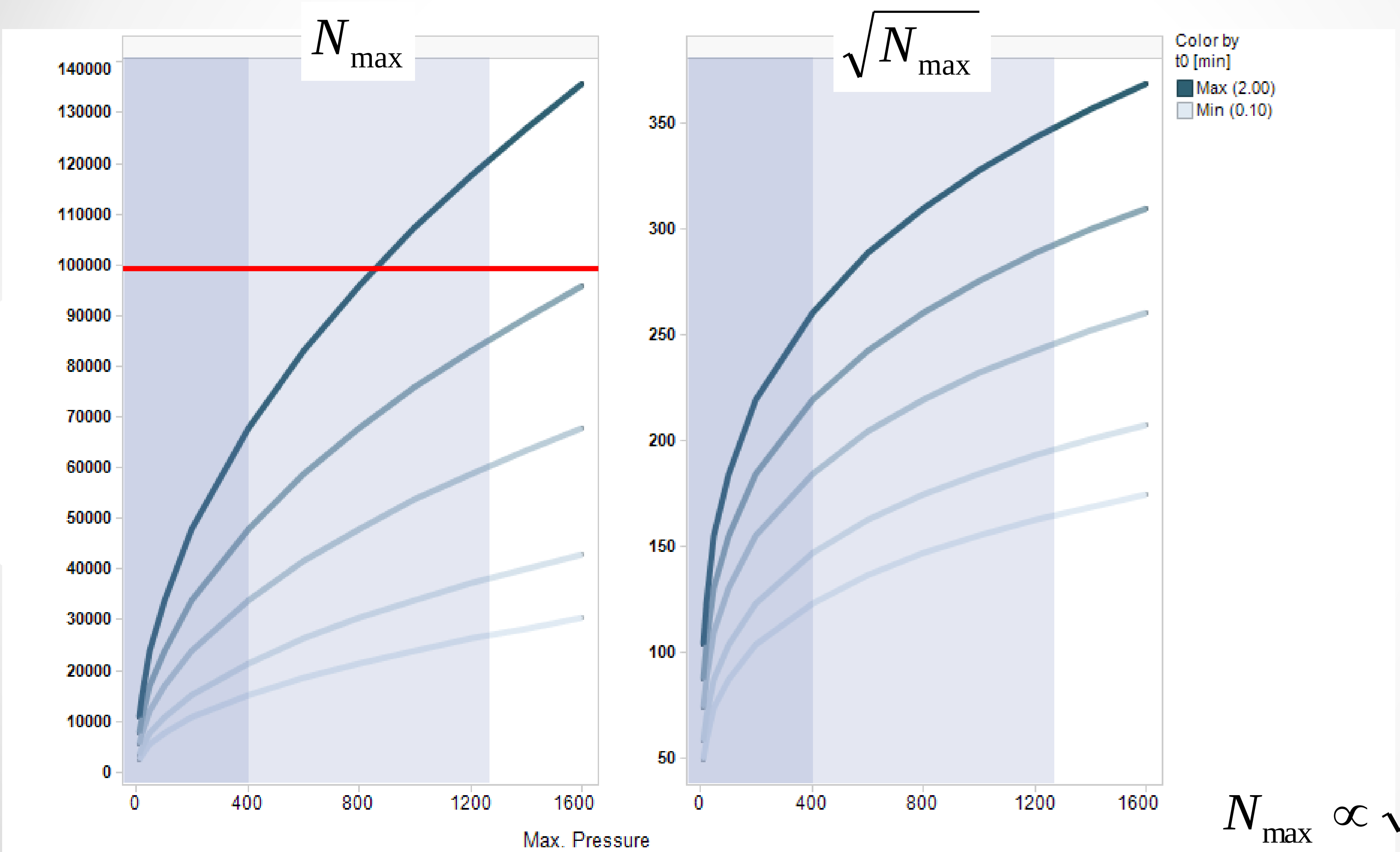
⬇

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

UHPLC –Essentials

Maximum Achievable Plate Number vs. Pressure*



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

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

Lessons learned so far

Kinetic Plots

- Poppe Plot aims to calculate u_0 and L_{max} at ΔP_{max}. Requires non-linear regression or an iterative calculation method or to determine the coefficients of the van Deemter/Knox equation
- Kinetic Performance Limit (KPL) plot according to Desmet et al. aims to calculate the plate number of an imaginary set of columns with different length, operated at the maximally allowable pressure.
- Both methods allow to compare column performance unambiguously and independent of dimensions, particle size and particle morphology.
- The KPL plot method can be done by straightforward spreadsheet calculations and allow comparison of multiple columns under different operating conditions.
- A simulation tool allows to compare two columns by Poppe Plots and is available (see http://homepages.gac.edu/~dstoll/calculators/web_opt_2p_pair_1.1.html)
- Increase of the max. pressure limits increases the max. achievable plate number by square root relationship (and the resolution with quadruple root) but will allow to achieve the required plate number faster. However an over proportional effort in engineering will be required to provide equally robust and reliable equipment (see part 4)
- All relations apply under the assumption that the physics and chemistry of separation do not change under increased pressure (see part 4)

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Thank You for Your Attention

谢谢

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