

Poppe plots for size-exclusion chromatography

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Abstract

Poppe plots provide a clear and unambiguous way to discuss the performance limits of separation systems. The effects of particle size, pressure drop and column permeability can be illustrated using such plots. The performance limits of size-exclusion chromatography are of interest, due to developments in combinatorial chemistry and high-throughput experimentation. In these fields, fast separations of high-molecular-weight analytes are required. In this paper, Poppe plots will be presented for size-exclusion chromatography. Because of the very high-reduced velocities encountered, the Poppe plots are found to be significantly different from those commonly observed in HPLC. Fast separations in size-exclusion chromatography are not as unfavourable as suggested by conventional theory. The results are based on experimental data obtained for a wide range of polystyrenes (1.7–3.25 kDa) using THF as mobile phase, but may be equally valid in other cases. © 2004 Elsevier B.V. All rights reserved.

Keywords: Poppe plots; Size-exclusion chromatography; Fast SEC; High-throughput screening; Polymers

1. Introduction

In 1997, Hans Poppe [1] published a review paper entitled “*Some reflections on speed and efficiency of modern chromatographic methods*”. In this study, he graphically displayed the logarithm of “plate time” (time required to realize one theoretical plate) as a function of the logarithm of the total number of plates required for realizing a given separation. From such a “Poppe plot” the limitations of specific chromatographic systems become instantly apparent. This has led to an increased use of such plots, for example by Tanaka et al. [2] to compare the performance of monolithic silica columns with that of packed columns and by Gzil et al. [3] in computational fluid dynamics studies of various configurations for micro-machined separation systems.

Fig. 1a shows a Poppe plot calculated for conventional packed columns with common particle sizes (3, 5, and 10 μm) at a maximum column-inlet pressure of 200 bar (42×10^7 Pa), assuming a viscosity of 5×10^{-3} Pa/s (i.e. the

value for THF). Details on the construction of such a plot are provided in Section 2.

The lines in this plot reflect the separations that can be achieved at the maximum pressure. Fast separations have a short “plate time” and are located towards the bottom of the figure. High-resolution separations (high numbers of theoretical plates) are located towards the right in the figure. The diagonal dashed lines indicate a constant analysis time (product of plate time and number of plates). The shortest separations are located at the left bottom, the longest ones at the top right.

Any separations above the lines (top-left corner) can be achieved at lower column-inlet pressures. The bottom left and right parts of the figure are inaccessible under the specified conditions. High-resolution separations in HPLC (top right) are slow and require (relatively) large particles. Fig. 1b illustrates the current trend towards very-high-pressure (or “ultra-performance”) liquid chromatography [4,5]. A series of lines are drawn, which correspond to increasing maximum inlet pressures (top to bottom). A particle size of 2 μm is assumed. Clearly, by increasing the maximum permissible pressure drop we progress from the top left to the bottom right of the figure, i.e. towards more efficient separations in shorter times. Fig. 1b suggests that increasing the pressure is

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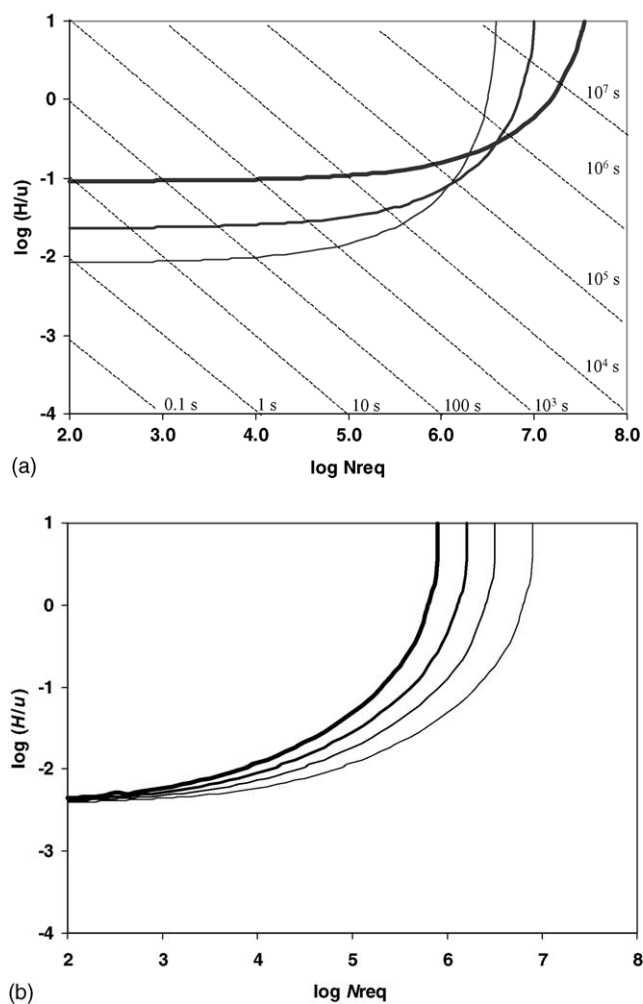


Fig. 1. (a) Conventional Poppe plot for packed columns in HPLC. Column-inlet pressure, $\Delta P = 2 \times 10^7$ Pa; eluent viscosity, $\eta = 5 \times 10^{-4}$ Pa/s; flow-resistance parameter, $\phi = 1000$; solute diffusion coefficient, $D_m = 5.42 \times 10^{-11}$ m²/s; coefficients in reduced-plate-height equation $A = 1$, $B = 1.5$, $C = 0.05$; particles sizes, 10 μm (heaviest line), 5 μm (middle line), and 3 μm (thinnest line). (b) Poppe plot for UHPLC at varying column-inlet pressures, from left to right 100, 200, 400 and 1000 bar; eluent viscosity, $\eta = 5 \times 10^{-4}$ Pa/s; flow-resistance parameter, $\phi = 1000$; solute diffusion coefficient, $D_m = 5.42 \times 10^{-11}$ m²/s (for a $M = 100$ kDa); coefficients in reduced-plate-height equation $A = 1$, $B = 1.5$, $C = 0.05$; particle sizes, $d_p = 2 \mu\text{m}$.

most useful for complex separations that require very large plate counts.

The lines in Fig. 1 depend on the quality of the columns in two different ways. The flow-resistance parameter, ϕ , plays a role equivalent to that of the maximum pressure. Columns with a higher permeability allow higher flow rates to be achieved at equal pressures. This may be a fundamental advantage of using monoliths, as pointed out by Tanaka et al. [2] and others [6,7]. In a Poppe plot, monolithic silica columns are found lower than packed columns at equal pressure drops [2]. The other way in which column quality is reflected in the curves in a Poppe plot is through the coefficients in the (dimensionless) plate-height equations. Typical

values for HPLC [8] have been used in Fig. 1. However, some columns may be packed better than other ones. Thus, individual columns may have higher or lower values of the coefficients A (eddy diffusion) and C (mass transfer).

We have recently discussed efficiency in size-exclusion chromatography (SEC). The experimentally observed plate-height curves are quite different from those commonly encountered in HPLC [9]. SEC is a separation technique compatible with high-molecular-weight analytes, which show slow molecular diffusion. As a result, SEC is typically performed at very much higher reduced velocities (explained in Section 2), where the appearance of the plate-height curve is dominated by the coupling term [10]. As a result, the Poppe plots for SEC are also expected to be quite different than for conventional HPLC. In recent years, the possibilities and limitations of fast size-exclusion separations are being explored in practice [11,12], spurred on by developments in combinatorial chemistry and high-throughput experimentation. This makes the construction and discussion of Poppe plots for SEC both relevant and timely.

2. Theory

The two factors that feature on the axes of a Poppe plot are the number of theoretical plates (the “plate count”, N) and the time required to realize one theoretical plate (the “plate time”, t_p). The latter can be related to the plate height (H) and the interstitial mobile-phase velocity (u_0), or to the unretained time (t_0) and the plate count.

$$t_p = \frac{H}{u_0} = \frac{L/N}{L/t_0} = \frac{t_0}{N} \quad (1)$$

The relationship between H and u_0 in chromatography is given by a so-called plate-height equation. The van-Deemeter equation is most common.

$$H = A + \frac{B}{u_0} + C u_0 \quad (2)$$

or

$$\frac{H}{u_0} = \frac{A}{u_0} + \frac{B}{u_0^2} + C \quad (3)$$

The three different terms in Eqs. (2) and (3) represent different contributions to the band broadening, viz. eddy diffusion or tortuosity (A), molecular diffusion (B) and inertia to mass transfer C [9,13], and references cited therein. At high linear velocities this becomes:

$$\frac{H}{u_0} \approx C \quad (4)$$

which is the limiting value for fast separations in conventional HPLC. In Fig. 1a and b, this limit results in the horizontal parts in the Poppe curves. Thus, high values of u_0 are favourable for fast separations. The maximum attainable velocities are

limited by the maximum pressure drop across the column (ΔP_{\max}), as can be seen from the Darcy equation:

$$u_{0,\max} = \frac{\Delta P_{\max} d_p^2}{\phi \eta L} = \frac{\Delta P_{\max} d_p^2}{\phi \eta N H} \quad (5)$$

For a given column, the flow-resistance factor (or column-resistance factor), ϕ , and the particle size, d_p , are fixed. The viscosity, η , is fixed once a mobile phase is selected. When the required number of plate (N) increases, the maximum permissible flow rate decreases. When N is low the velocity (u_0) can be high and the plate time stays constant (Eq. (4)). When the required number of plates increases u_0 must decrease. Eventually, it becomes so low that Eq. (3) must be used instead of Eq. (4). The plate time (H/u_0) then increases drastically, as can be clearly seen in Fig. 1.

To construct a Poppe plot, we must establish the maximum value of u_0 corresponding to a given number of plates on a given column (ϕ , d_p) and using a given mobile phase (η). In case the van-Deemter equation (Eq. (2)) is used to relate H to u_0 , a quadratic equation must be solved to find the maximum value of u_0 .

$$\begin{aligned} \Delta P_{\max} &= \frac{\phi \eta N}{d_p^2} u_{0,\max} H(u_{0,\max}) \\ &= \frac{\phi \eta N}{d_p^2} (A u_{0,\max} + B + C u_{0,\max}^2) \end{aligned} \quad (6)$$

Any kind of $H(u_0)$ relationships can be accommodated using numerical calculation. For size-exclusion chromatography of high-molecular-weight analytes, we have recently established that Eq. (2) is not applicable. To explain the difference between low-molecular-weight and high-molecular-weight analytes, it is necessary to introduce reduced (dimensionless) parameters [1,13], i.e. the dimensionless plate height:

$$h = \frac{H}{d_p} \quad (7)$$

and the dimensionless velocity:

$$v_0 = \frac{u_0 d_p}{D_m} \quad (8)$$

In Eq. (8) D_m is the diffusion coefficient of the analyte in the mobile phase. Note that Eqs. (7) and (8) are limited to packed columns with particle size d_p . For open-tubular columns, the column diameter d_c can be substituted for d_p . Extending the concept of reduced parameters to monolithic columns is more difficult, but possible [6].

The great difference between low-molecular-weight and high-molecular-weight analytes is in the value of the diffusion coefficient. Typically, D_m decreases with increasing analyte molecular weight. For example, for polystyrene polymers in THF:

$$D_m = 0.0386 M^{-0.57} \quad (9)$$

where M is the polymer molecular weight [14]. Because the diffusion coefficient is much smaller for high-molecular-

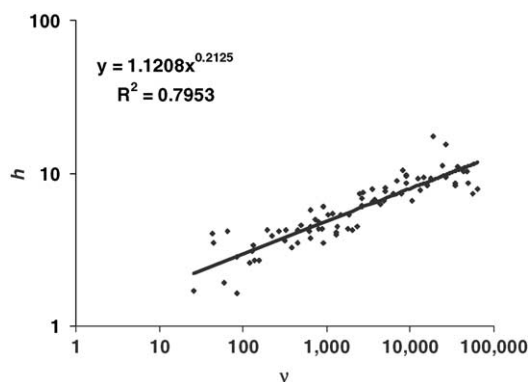


Fig. 2. Experimental plate-height curve for polystyrenes in size-exclusion chromatography. The PS standards ($M=1.7$ kDa, specified PDI=1.06, Supplier PL=Polymer Labs; $M=3.25$ kDa, PDI=1.04, PL; $M=10.9$ kDa, PDI=1.03, Supplier PSS=Polymer Standards Service; $M=39.2$ kDa, PDI=1.03, PSS; $M=117$ kDa, PDI=1.03, PSS; $M=325$ kDa, PDI=1.03, PSS; $M=2.2$ MDa, PDI=1.04, MN; $M=3.25$ MDa, PDI=1.13, PSS), sample concentration 1 mg/ml in THF; 50 mm \times 7.5 mm i.d. mixed-C column.

weight analytes than for low-molecular-weight ones, the reduced velocity (v_0 , Eq. (8)) is much higher. At high values of v_0 Eq. (2) is no longer valid [10]. Fig. 2 summarizes a large amount of experimental data on h versus v_0 obtained for polystyrenes of varying molecular weight on a short SEC column. Although there is significant scatter around the calculated regression line, it is obvious that the slope of the $\log h$ versus $\log v_0$ line is much smaller than unity. Therefore, h increases much more slowly with increasing v_0 than suggested by Eq. (2). The regression line is described by:

$$\log(h) = \log(1.12) + 0.21 \log(v_0) \quad (10)$$

or

$$h = A_C v_0^{0.21} = 1.12 v_0^{0.21} \quad (10a)$$

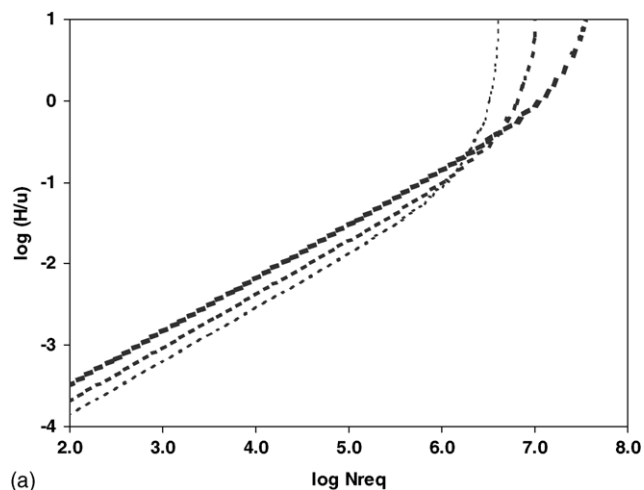
In the completed plate-height equation, the B term (molecular diffusion) is retained, while the A and C terms (eddy diffusion and mass transfer, respectively) are 'coupled' in the A_C term. This coupling arises, because eddy diffusion contributes greatly to the radial diffusion of analyte molecules, thus reducing the dispersion due to local differences in the flow velocity (for a detailed discussion, see [13]). This yields,

$$h = \frac{B}{v_0} + A_C v_0^{0.21} \quad (11)$$

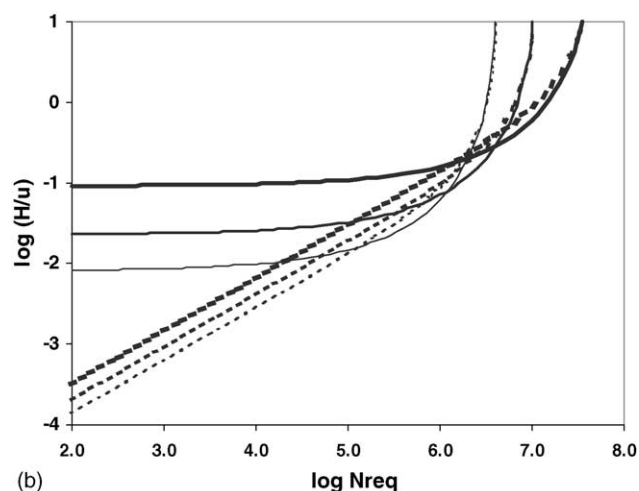
This equation has been used to construct Poppe curves for size-exclusion chromatography.

3. Results and discussion

Fig. 3 shows a typical Poppe plot for size-exclusion chromatography using Eq. (10) to describe the relationship between h and v_0 . The maximum pressure was 200 bar (2×10^7 Pa), the viscosity 5×10^{-4} Pa/s (a typical value for THF) and the diffusion coefficient was calculated from Eq.



(a)



(b)

Fig. 3. (a) Poppe plot for size-exclusion chromatography. *Conditions:* $\Delta P = 200$ bar (2×10^7 Pa); $\eta = 5 \times 10^{-4}$ Pa/s (THF); $D_m = 5.42 \times 10^{-11}$ m²/s (for PS, $M = 100,000$; see Eq. (9)). Coefficients in reduced-plate-height equation $B = 1.5$, $A_C = 1.12$, $n = 0.21$. (see Eq. (11)). Particle sizes, 10 μm (heaviest line), 5 μm (middle line), and 3 μm (thinnest line). (b) Superposition of a conventional Poppe plot for HPLC (as in Fig. 1a) and a plot for SEC (as in (a)).

(9) for polystyrene with a molecular weight of 100 kDa. Note that because water has a viscosity that is about twice as high as that of THF, Fig. 3a is also approximately valid for aqueous SEC at 400 bar (4×10^7 Pa), provided that in this case Eq. (10) is equally valid. No experimental data are available yet to support the latter assumption.

The difference between Fig. 1a and Fig. 3a is striking. To facilitate a clear comparison, Fig. 3b shows an overlay of the lines from Fig. 1a and those from Fig. 3a. When applying Eq. (10) rather than Eq. (2), fast separations with relatively low plate counts become very much more attractive. Instead of a constant plate time at high flow rates (Eq. (4)) the plate time decreases continuously with increasing linear velocity (from Eq. (10a): $H/u_0 \sim u_0^{-0.79}$). “Quick and dirty” separations can apparently be carried out for high-molecular-weight analytes at relatively high flow-rates.

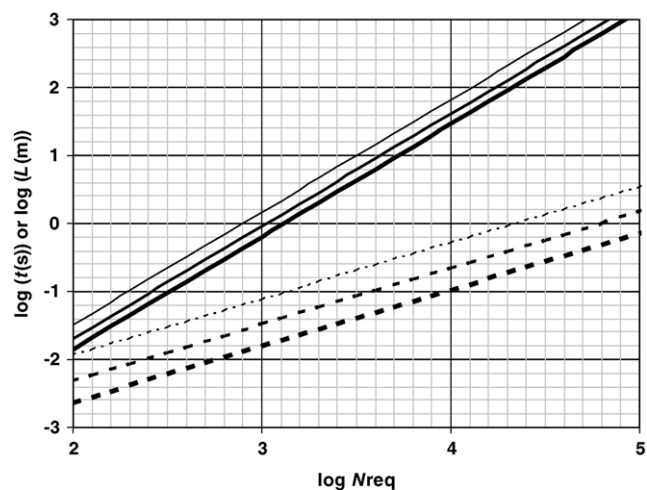


Fig. 4. Optimum column lengths (dashed lines) and resulting analysis times (drawn lines) corresponding to the Poppe plot for size-exclusion chromatography. *Conditions:* $\Delta P = 2400$ bar (42×10^7 Pa); $\eta = 5 \times 10^{-4}$ Pa/s (THF); $D_m = 5.42 \times 10^{-11}$ m²/s (for PS 100 kDa standard). Coefficients in reduced-plate-height equation $B = 1.5$, $A_C = 1.12$, $n = 0.21$ (see Eq. (11)). Particle sizes, 10 μm (thinnest line), 5 μm (middle line), and 3 μm (heaviest line).

Interestingly, the effect of the particle size on the speed of analysis is much smaller for fast SEC (high- M analytes) than it is for fast HPLC (low- M analytes). In the latter case, the horizontal parts of the curve are determined directly by the value of the mass-transfer coefficient (C ; see Eq. (4)). In SEC, this effect is suppressed because the plate height increases much more slowly with increasing velocity (see Eq. (11) and Fig. 2).

It is interesting to investigate the column lengths and analysis times associated with the separations in Fig. 3. Both are readily available once Eq. (6) is solved ($L = NH$, where H can be obtained by substituting the known value of u_0 in the appropriate plate-height equation; $t_0 = L/u_0 = NH/u_0$). Very short (50 mm) columns have become popular for fast-SEC separations. These are operated at relatively high flow rates, but the pressure drops are quite moderate. Fig. 4 shows the calculated column lengths corresponding to the SEC separations in Fig. 3a. The optimum column lengths may be longer than the current fashion dictates. For example, 10,000 plates can be obtained in about 40 s on a 250-mm long column packed with 5- μm particles. The corresponding flow rate is also higher than the values currently used in fast SEC. The above example implies a flow rate of 2.5 ml/min on a 4.6-mm i.d. column. The superiority of longer columns for fast SEC, have since been corroborated by experimental results [12,15].

Fig. 5 shows a Poppe plot obtained for SEC at various column-inlet pressures using the empirical plate-height curve of Eq. (10). In comparison with Fig. 1b, the effect of high pressures is especially different in the region of fast separations with moderate plate counts. While the benefit of using very high pressures is conventionally perceived to be mainly in the domain of complex separations requiring high plate

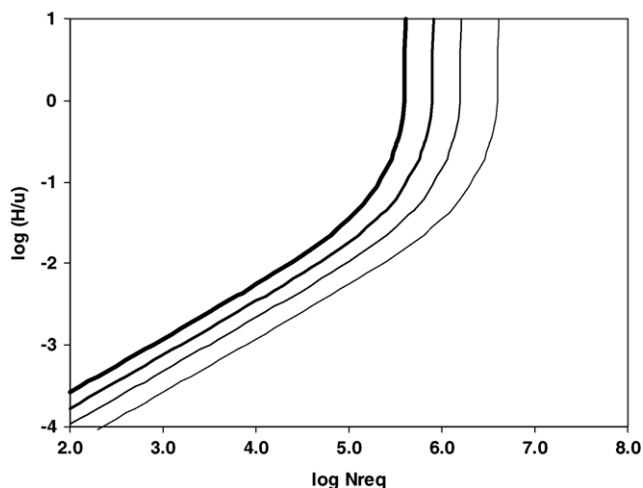


Fig. 5. UHPLC: Comparison between the conventional Poppe plots (Eq. (2)) and the plot obtained using the coefficients from Eq. (10) column-inlet pressures, are (from left to right) 100, 200, 400 and 1000 bar. Coefficients from Eq. (11) $B = 1.12$, $A_C = 0.21$, $n = 0.21$. $\Delta P = 24 \times 10^7$ Pa; eluent viscosity, $\eta = 5 \times 10^{-4}$ Pa/s; flow-resistance parameter, $\phi = 1000$; solute diffusion coefficient, $D_m = 5.42 \times 10^{-11}$ m²/s (for PS, $M = 100$ kDa; see Eq. (9), particle size, $d_p = 2$ μ m).

count (Fig. 1b), Fig. 5 demonstrates that very high pressures may also be substantial benefit for the fast separations of (especially) high- M -analytes.

4. Conclusions

The plate height increases only slowly at very high reduced velocities, as was predicted by Knox [13] based on their “coupling-term” concept. As a result, fast separations of high-molecular-weight analytes are much more favourable than suggested by conventional theory. A good way to illustrate the possibilities and limitations of size-exclusion chromatography is by constructing Poppe plots, which relate the “plate time” (time required to realize one theoretical plate) with the “plate count” (the total number of required plates). The appearance of the Poppe plot is dramatically affected when an empirical plate-height curve is used that is based on experimental SEC data. The experimental data are limited to polystyrene, using THF as the mobile phase. However, a similar favourable effect is expected to be found for other fast separations of high-molecular-weight analytes, e.g. for the high-throughput screening of proteins. Based on the present analysis, the optimum column length for fast SEC is thought to be significantly greater than the 50 mm that is currently in fashion.

Very high pressures are not only interesting for highly efficient separations (high plate numbers), but also for very fast separations (moderate N values, very low H/u_0 values) of high- M analytes.

In the present treatment, some factors have been excluded. The availability of column packing materials for SEC has not been discussed. Typically, smaller particles (3 μ m) are used for relatively small polymers, while larger particles (10 or 20 μ m) are used for the largest polymers amenable with SEC (molecular weights exceeding 1 MDa). However, the effect of the particle size is found to be much smaller for fast SEC separations than it is for fast HPLC separations. At ultra-high pressures the separation becomes more efficient and shorter.

Another factor that has not been discussed is the effect of high flow rates and high pressure drops on the analytes. Shear degradation and shear deformation have been a subject of concern in the literature on size-exclusion chromatography. Our experience with the separation of polystyrene suggests that the former effect is absent and that the second effect is small. However, both effects should be studied more carefully.

Acknowledgements

The authors thank Polymer Laboratories (particularly Dr. Greg Saunders) for providing the Fast-SEC columns, Gert Desmet and Pjotr Gzil from the Free University of Brussels for helpful discussions, and Hans Poppe for continued inspiration.

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