Onset of the Chromatographic Mode Transition from Hydrodynamic Chromatography to Slalom Chromatography: An Effect of Polymer Stretching

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In packed-column hydrodynamic chromatography (HDC) of polymers, the parabolic flow profile in the interstitial space between the packing particles, which is conceptually very similar to the familiar Poiseuille flow in a capillary, is used for polymer separation based on molecular size.[1,2] The solute molecules are excluded from the low-velocity regions near the particle surface with larger macromolecules being more excluded from the low-velocity regions than smaller ones. Hence, the larger macromolecule experiences a higher mean eluent velocity and elutes earlier than the smaller macromolecule; i.e., the elution order of macromolecules in HDC is the same as in size exclusion chromatography (SEC).

Another chromatographic mode, slalom chromatography (SC), was observed at certain conditions when SEC or HDC columns were employed to separate relatively large DNA molecules.[3−5] It was found that in SC the larger macromolecule eluted later than the smaller macromolecule; i.e., the elution order in SC was opposite to SEC and HDC. This chromatographic mode transition from SEC or HDC to SC was found to be dependent on the packing particle size, the flow rate, molecular size, temperature, and solvent viscosity.[6,7] Although the precise separation mechanism of SC was still not clearly understood, it was proposed that SC separation in based on a hydrodynamic principle and that a coil−stretch transition is accompanied with this chromatographic mode transition.[3−7]

For a polymer molecule in elongational flow, a coil−stretch transition occurs when the Deborah number (*De*), which is the product of the longest relaxation time of the polymer, τ, and the strain rate of the flow, $\dot{\epsilon}$, is larger than a threshold value of 0.50.[8]

In our previous study, the chromatographic mode transition from HDC to SC was observed for high molar mass polystyrene (PS).[9] The onset of the chromatographic mode transition, i.e., the critical polymer size $R_{g,c}$ of the HDC → SC transition, was determined at different flow rates. The dependence of $R_{g,c}$ on flow rate strongly supported that the HDC → SC transition in a packed column was related to the coil−stretch transition of the polymer chain in elongational flow through the packed column. However, it was found that the Deborah number *De* of the polymer at the onset of the HDC → SC transition was lower in columns packed with 3 μm particles than that in columns packed with 15 μm particles (Figure 1). This suggests that the onset of the coil−stretch transition, which is expected to occur at $De = 0.50$ according to theory,[8] can only give a rough estimation of the onset of the HDC → SC transition.

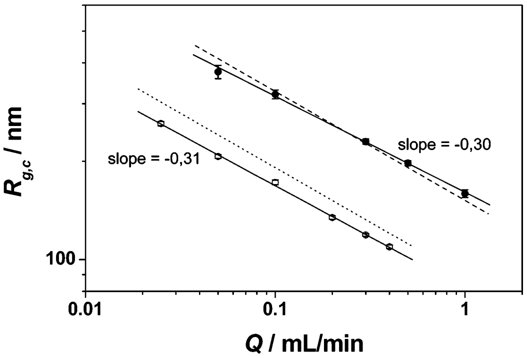

Figure 1. Onset of the HDC → SC transition for PS in columns packed with 15 μm (●) and 3 μm (○) particles. The dashed line and the dotted line were calculated with $De = 0.50$ for columns packed with 15 μm particles and 3 μm particles, respectively.

In this Note, we correlate the extension of a polymer stretched in elongational flow through packed columns, with the onset of the HDC → SC transition.

Therefore, the elution behavior of high molar mass PS in two columns (300 × 4.6 mm) packed with 15 and 3 μm cross-linked polystyrene−divinylbenzene nonporous particles (Polymer Laboratories Ltd., Shropshire, UK) was studied at different eluent flow rates using a Wyatt DAWN EOS multiangle laser light scattering detector (Santa Barbara, CA). The experimental details have been described elsewhere.[9]

The stretching of polymer is a result of the equilibrium of the hydrodynamic force and the entropic force. Theoretical calculations show that the force−extension diagram of a DNA molecule is well described by a wormlike chain (WLC) model. Marko and Siggia deduce a useful approximate interpolation formula[10]

$$\frac{FA}{kT} = \frac{1}{4(1 - x/L)^2} - \frac{1}{4} + \frac{x}{L} \tag{1}$$

where *F* is the force, *A* is the persistence length, *x* is the steady-state extension, *L* is the contour length of the polymer, *k* is Boltzmann's constant, and *T* is the temperature.

The Marko−Siggia formula has the nice property of reducing to the exact solution as either $x \to 0$ or $x \to L$. However, it differs from the exact solution in the middle *x* region by as much as 10% for $x/L \sim 0.5$. Instead, Bouchiat et al. proposed an improved formula with a relative error in force of 0.01% for any given extension by[11]

$$\frac{FA}{kT} = \frac{1}{4(1 - x/L)^2} - \frac{1}{4} + \frac{x}{L} + \sum_{i=2}^{7} a_i \left(\frac{x}{L}\right)^i \tag{2}$$

where $a_2 = -0.516\,422\,8$, $a_3 = -2.737\,418$, $a_4 = 16.074\,97$, $a_5 = -38.876\,07$, $a_6 = 39.499\,44$, and $a_7 = -14.177\,18$.

The drag force *F* on a sphere particle with radius of *R* suspended in a viscous medium at a constant velocity v is

$$F = \zeta v \tag{3}$$

where $\zeta = 6\pi\eta R$ is the friction coefficient with η the solvent viscosity. The linear eluent velocity v in the column can be calculated from flow rate *Q* by $v = Q/(\pi r^2 \epsilon)$, where ϵ is the column porosity and *r* is the column radius. For a polymer coil with radius of gyration of R_g, the friction coefficient is identical

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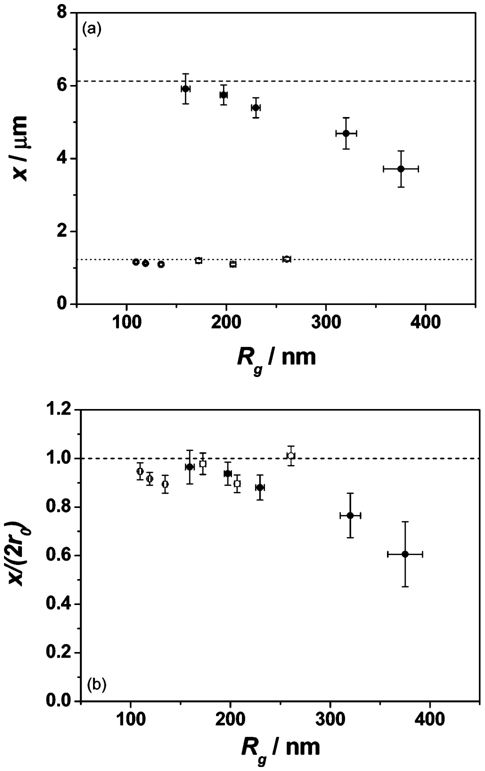

Figure 2. (a) Extension of PS at the onset of the HDC → SC transition in columns packed with 15 μm (●) and 3 μm (○) particles. The dashed line and the dotted line indicate $2r_0 = 6.129$ and 1.226 μm for columns packed with 15 and 3 μm particles, respectively. (b) Ratio between extension of PS and the average channel diameters between particles at the onset of the HDC → SC transition in columns packed with 15 μm (●) and 3 μm (○) particles.

with that of a sphere with radius of R_h; R_h is the hydrodynamic radius of the polymer coil. The hydrodynamic radius R_h of PS in tetrahydrofuran with radius of gyration R_g can be calculated according to the following relations:[9,12] R_g (nm) = $0.0139M^{0.588}$ and R_h (nm) = $0.0094M^{0.588}$.

For a sphere particle with radius of R confined in a channel of radius r_0, the friction coefficient ζ_C is a function of R/r_0 by[13]

$$\zeta_C = f\zeta \tag{4}$$

where

$$f = \frac{1 - \frac{2}{3}\frac{R^2}{r_0^{\,2}} - 0.20217\frac{R^5}{r_0^{\,5}}}{1 - 2.1050\frac{R}{r_0} + 2.0865\frac{R^3}{r_0^{\,3}} - 1.7068\frac{R^5}{r_0^{\,5}} + 0.72603\frac{R^6}{r_0^{\,6}}} \tag{5}$$

For columns packed with particles of diameter d_p, the average channel radius r_0 is[14]

$$r_0 = \frac{d_p}{3}\frac{\epsilon}{1-\epsilon} \tag{6}$$

The drag force on PS with $R_{g,c}$ at different flow rates in the columns can be calculated according to eqs 3−6; then the extension of polymer can be calculated according to eq 2, in which L was calculated from $R_{g,c}$ and a literature value of A = 0.9 nm was used.[15]

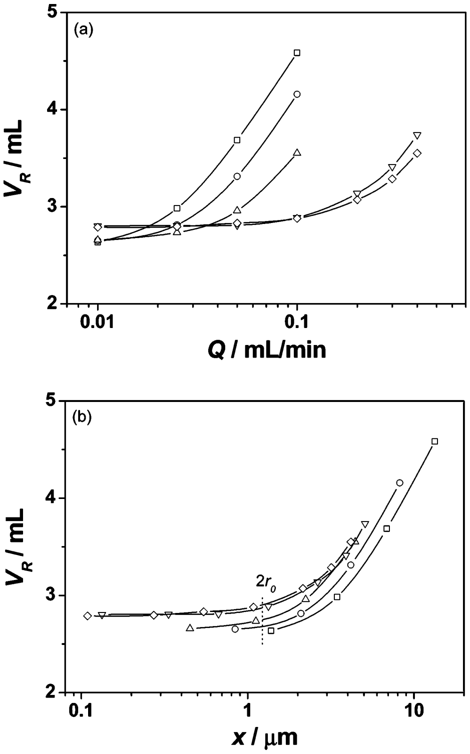

Figure 3. V_R as a function of Q (a) and x (b) for PS with sizes of 340 (□), 300 (○), 257 (△), 178 (▽), and 167 nm (◇) in columns packed with 3 μm particles; the dotted line is $2r_0 = 1.226$ μm.

Figure 2a shows the calculated extension of PS at the onset of the HDC → SC transition in columns packed with 15 and 3 μm particles. It is observed that the extensions of PS at the onset of the HDC → SC transition in columns packed with 3 μm particles are less than in columns packed with 15 μm particles, indicating that for the onset of the HDC → SC transition in columns packed with larger particles a larger extension of a given polymer is required than in columns packed with smaller particles. This explains our previous results that the Deborah number De of PS at the onset of the HDC → SC transition varies with the size of the packing particles. A closer inspection of the data shows that the extension of PS at the onset of the HDC → SC transition is very close to the average channel diameters $2r_0$ for both columns packed with 15 and 3 μm particles. This is shown in Figure 2b, where the ratio between extension of PS and the average channel diameters, $x/(2r_0)$, at the onset of the HDC → SC transition is plotted as a function of the coil size of the polymer. In both columns, the ratio of polymer extension to the average channel diameter is close to 1.0, indicating that the polymer will be retarded when it is stretched to a critical length equal to the average channel diameter between the particles.

In our previous paper, it was shown that the chromatograms of PS10M, a PS narrow standard with M_w of 10M, shift to higher elution volume with increasing flow rate in columns packed with 3 μm particles (Figure 10 in ref 9). The retention volume V_R of the main peak with R_g = 178 nm and that of the shoulder peak with R_g = 257 nm in PS10M at different flow rates are listed in Table 1. The retention volumes of the main peak with R_g = 167 nm in PS8M, with R_g = 300 nm in PS16M, and with R_g = 340 nm in PS14M at different flow rates are also summarized in Table 1. V_R as a function of Q for PS with different sizes are shown in Figure 3a. It can be seen that there exists a critical flow rate Q_c in the V_R−Q plot: at flow rate less than Q_c, V_R of PS nearly did not change with flow rate. While at flow rate larger than Q_c, V_R of PS increased rapidly with increasing flow rate. The larger the polymer size, the lower

Table 1. Peak Retention Volume V_R for PS at Different Flow Rates in Columns Packed 3 μm Particles

sample	R_g (nm)	V_R (mL) at flow rate (mL/min) of						
		0.010	0.025	0.050	0.10	0.20	0.30	0.40
PS8M	167	2.788	2.795	2.832	2.880	3.072	3.288	3.550
PS10M	178	2.802	2.806	2.809	2.888	3.138	3.412	3.740
	257	2.657	2.735	2.959	3.550			
PS16M	300	2.653	2.813	3.311	4.158			
PS14M	340	2.637	2.983	3.686	4.585			

the value of Q_c. The extensions of PS are calculated for the different flow rates and are visualized in Figure 3b, where V_R is plotted as a function of x for PS with different sizes. It can be seen that V_R of PS increased rapidly when PS was stretched to the critical length equal to $2r_0$, which agreed with the above conclusion (Figure 2). That is to say, the onset of the HDC → SC transition is when polymers are stretched to the critical length equal to the average channel diameter between particles.

The critical flow rates for the onset of the HDC → SC transition were calculated for PS of different sizes in columns packed with particles of different sizes. The calculated dependences of log $R_{g,c}$ vs log Q are shown in Figure 4, in comparison with the HDC → SC transitions observed experimentally. The calculated log $R_{g,c}$−log Q curves agreed well with the experimental results. The slope of the middle portion of the log $R_{g,c}$−log Q curves are very close to $-^1/_3$, which was predicted from the onset of the coil−stretch transition at $De = 0.50$ and was verified in our previous study. However, the log $R_{g,c}$−log Q curves deviate from the linear relations at both ends. When the ratio between polymer size and $2r_0$ is relatively small, the HDC → SC transition occurs at a high flow rate. This is due to the fact that a high flow rate is needed to stretch a relatively small polymer to a relatively large extension of $2r_0$. A polymer with contour length less than $2r_0$ will never experience the HDC → SC transition. On the other hand, when the ratio between polymer size and $2r_0$ is relatively large, the flow rate at the onset of the HDC → SC transition is extremely low because for a relatively large polymer, only a low flow rate is enough to stretch it to a relatively small extension of $2r_0$. Hence, to avoid the HDC → SC transition, an extremely low flow rate must be employed to analyze large polymers in columns packed with small particles.

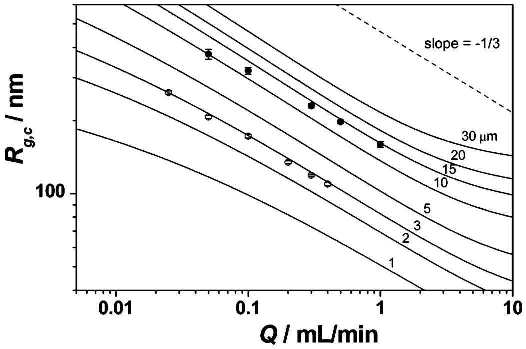

Figure 4. Onset of the HDC → SC transition for PS in columns packed with 1, 2, 3, 5, 10, 15, 20, and 30 μm particles. The experimental data for PS in columns packed with 15 μm (●) and 3 μm (○) particles are also shown. The dashed line corresponding to a slope of $-^1/_3$ serves as a guide to the eye.

In conclusion, by calculating the extension of PS stretched in elongational flow through columns packed with 15 and 3 μm particles, the onset of the HDC → SC transition is found to occur when polymers are stretched to the length of the average channel diameter between particles. The onset of the HDC → SC transition in columns packed with particles of different sizes was calculated, which agreed well with the experimental results. To avoid the HDC → SC transition, an extremely low flow rate must be employed to analyze large polymers in columns packed with small particles. The results might also be applied to predict the onset of the SEC → SC transition.

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