File ID	uvapub:234
Filename	3143y.pdf
Version	unknown

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type	article
Title	Separation speed in thermal field flow fractionation.
Author(s)	A.C. van Asten, G. Stegeman, W.Th. Kok, H. Poppe
Faculty	FNWI: Van 't Hoff Institute for Molecular Sciences (HIMS), FNWI
Year	1994

FULL BIBLIOGRAPHIC DETAILS:
  http://hdl.handle.net/11245/1.102219

Separation Speed in Thermal Field Flow Fractionation

A. C. van Asten, G. Stegeman, W. Th. Kok, R. Tijssen, and H. Poppe*

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV, Amsterdam, The Netherlands

The effects of the temperature drop, channel thickness, solvent choice, and molecular mass and chemical nature of the polymer species on the separation speed in thermal field flow fractionation are investigated. The trends observed in the experiments are in good agreement with theoretical predictions. Separation speed was found to increase with the temperature gradient and the molecular mass of the polymer samples. Furthermore, the separation speed depends strongly on the chemical composition of the polymer and the solvent. The ability to fractionate polymer samples according to their chemical nature is demonstrated with the separation of four different polymer standards of approximately the same molecular mass.

Field flow fractionation (FFF) was introduced by Giddings in 1966.[1] Since then FFF has been steadily developed, primarily at the Field Flow Fractionation Research Center at the University of Utah. FFF can be used for the analysis of virtually all classes of polymers and particles. FFF techniques are usually carried out in a thin ribbonlike flow channel in which a stationary phase is absent. An external field, applied perpendicular to the laminar flow of a carrier liquid in an open channel, is used to concentrate the solutes at one of the channel walls. Retention and separation are based on the coupling of the solute concentration profile to the large velocity gradient of the fluid near the accumulation wall.

A number of different fields can be used to obtain FFF separations. In thermal field flow fractionation (ThFFF), a temperature gradient is used to drive the polymer molecules to the cold wall. This migration of compounds under the influence of a temperature gradient is called thermal diffusion. Thermal diffusion is opposed by molecular diffusion. Therefore, retention in ThFFF is determined by both the molecular and thermal diffusion coefficients. The thermal diffusion effect has been known for over a century.[2] However, thermal diffusion in liquids is still a poorly understood process. As demonstrated by Schimpf and Giddings,[3] none of the existing theories is able to describe thermal diffusion of polymers in solution. If ThFFF is combined with another technique for the measurement of molecular diffusion coefficients, thermal diffusion coefficients can be determined. Therefore, ThFFF is an excellent tool for the study of thermal diffusion of polymer species, and with the use of this technique, the understanding of the thermal diffusion phenomenon has increased significantly.[3–6]

Over the years, ThFFF has proven to be a valuable tool for the fractionation and analysis of synthetic polymers in various organic solvents.[7–12] Samples of a broad molecular mass range can effectively be analyzed by using temperature programming.[13,14] Because retention is not only determined by the extent of molecular diffusion, reflecting mainly the size of the polymers, but also by the thermal diffusion coefficient, ThFFF offers unique separation possibilities compared to other polymer fractionation methods. If the difference in thermal diffusion is sufficient, it is possible to separate different polymer species that are equal in size.[15] Because thermal diffusion depends on the chemical nature of the polymer, ThFFF data contain structural and chemical information of polymer species. Very recently it has been demonstrated that ThFFF is also suitable for the separation of particles in aqueous and non-aqueous solutions.[16,17] However, the use of ThFFF for the analysis of water-soluble polymers has been very limited due to the fact that only very few polymer species show thermal diffusion in aqueous solutions.[18]

In this work the effect of the various operating parameters on the separation speed in ThFFF is investigated both experimentally and theoretically. First, the effect of the temperature gradient and the molecular mass of the polymer compounds on the separation speed in ThFFF is studied. However, in ThFFF separation speed is not solely determined by these two parameters. Because the extent of thermal diffusion depends on the chemical nature of both the polymer and the solvent, these two factors also influence the separation speed in ThFFF. Therefore, the effect of the solvent choice and the polymer type is also investigated. Finally, the possibilities of fractionating polymer species according to thermal diffusion and thus to chemical nature are demonstrated with the separation of four chemically different polymer standards of the same molecular mass.

(1) Giddings, J. C. *Sep. Sci.* **1966**, *1*, 123.
(2) Tyrell, H. J. V. *Diffusion and Heat Flow in Liquids*; Butherworths: London, 1961.
(3) Schimpf M. E.; Giddings J. C. *J. Polym. Sci., Part B* **1989**, *27*, 1317.
(4) Schimpf M. E.; Giddings J. C. *J. Polym. Sci., Part B* **1990**, *28*, 2673.
(5) Brimhall, S. L.; Myers, M. N.; Caldwell, K. D.; Giddings, J. C. *J. Polym. Sci., Polym. Phys. Ed.* **1985**, *23*, 2443.
(6) Schimpf, M. E.; Giddings, J. C. *Macromolecules* **1987**, *20*, 1561.
(7) Brimhall, S. L.; Myers, M. N.; Caldwell, K. D.; Giddings, J. C. *Sep. Sci. Technol.* **1981**, *16*, 671.
(8) Giddings, J. C.; Caldwell, K. D.; Kesner, L. F. *Determination of Molecular Weight*; J. Wiley and Sons: New York, 1989; Chapter 12.
(9) Schimpf, M. E.; Myers, M. N.; Giddings, J. C. *J. Appl. Polym. Sci.* **1987**, *33*, 117.
(10) Giddings, J. C.; Myers, M. N.; Janča, J. *J. Chromatogr.* **1979**, *186*, 37.
(11) Gao, Y. S.; Caldwell, K. D.; Myers, M. N.; Giddings, J. C. *Macromolecules* **1985**, *18*, 1272.
(12) Kirkland, J. J.; Rementer, S. W. *Anal. Chem.* **1992**, *64*, 904.
(13) Giddings, J. C.; Smith, L. K.; Myers, M. N. *Anal. Chem.* **1976**, *48*, 1587.
(14) Kirkland, J. J.; Yau, W. W. *J. Chromatogr.* **1990**, *499*, 655.
(15) Gunderson, J. J.; Giddings, J. C. *Macromolecules* **1986**, *19*, 2618.
(16) Liu, G.; Giddings, J. C. *Anal. Chem.* **1991**, *63*, 296.
(17) Liu, G.; Giddings, J. C. *Chromatographia* **1992**, *34*, 483.
(18) Kirkland, J. J.; Yau, W. W. *J. Chromatogr.* **1986**, *353*, 95.

THEORY

In the following discussion the temperature dependence of the solvent viscosity, the solvent thermal conductivity, and the Soret coefficient is neglected. The consideration of these effects increases the complexity of the theoretical description of retention and plate height in ThFFF.[19,20] Although it is known that these temperature dependencies are often significant, we believe that the conclusions regarding selectivity and separation speed are unaffected when these effects are ignored.

The theoretical framework describing retention and plate height in FFF can be found in several papers and text books.[21-23] The retention ratio R, defined as the ratio of the channel void volume and the retention volume of the polymer, can be expressed as

$$R = 6\lambda[\coth(1/2\lambda) - 2\lambda] \qquad (1)$$

where the dimensionless parameter λ is equal to the ratio of the mean layer thickness of the compressed solute zone and the channel thickness.

As λ approaches zero, the term in brackets on the right-hand side of eq 1 approaches 1 and R is approximately equal to 6λ.[8] In ThFFF λ is given by

$$\lambda = D/D_T\Delta T \qquad (2)$$

where ΔT is the temperature drop across the channel thickness and D and D_T are the molecular and the thermal diffusion coefficients, respectively.

The plate height equation in FFF is given by

$$H = 2D/R\langle v \rangle + \chi w^2\langle v \rangle/D \qquad (3)$$

where $\langle v \rangle$ is the linear velocity of the carrier liquid, w is the channel thickness, and χ is a complicated function of λ, which can be approximated by $24\lambda^3$ as λ approaches zero.[24]

The first term on the right-hand side of eq 3 corresponds to longitudinal diffusion and is negligible under normal working conditions.[25] The second term describes zone broadening due to slow mass transfer in the presence of a velocity gradient and is often denoted as the nonequilibrium contribution.[25] External peak broadening caused by injection and detection can usually be neglected in FFF. Consequently, the plate height is exclusively determined by the nonequilibrium contribution.

The polydispersity of polymer samples can contribute significantly to the peak width of the observed signal. However, polymer polydispersity should be considered separately from system dispersion because it directly reflects the mass selectivity of the separation method. In order to

determine the mass distribution of a polymer sample from the observed fractogram, system dispersion should be negligible compared to peak broadening caused by polydispersity.[26] Therefore, in the following discussion on time optimization and separation speed in ThFFF, only absolute molecular masses will be considered.

The concept of time optimization in FFF can be treated in the same way as in chromatography.[27] The analysis time t_a, needed to separate two polymer fractions with a given resolution R_s, can be expressed as

$$t_a = 16R_s^2[M/\Delta M]^2 (t_p/S^2) \qquad (4)$$

where t_p is the time needed to evolve one plate, S is the mass selectivity of the separation system, and M and ΔM are the mean molecular mass and the difference in molecular mass of the two polymer fractions, respectively.

From eq 4 it follows that t_p/S^2 is a crucial parameter when the separation capacities of a fractionation method are discussed, R_s and $[M/\Delta M]$ are parameters that define the extent of separation desired.

The mass selectivity of a polymer fractionation method is generally defined as $|d \ln V_r/d \ln M|$, where V_r is the retention volume.[10] For FFF this definition can be written as

$$S = \left|\frac{d \ln R}{d \ln M}\right| = \left|\frac{d \ln R}{d \ln \lambda}\frac{d \ln \lambda}{d \ln M}\right| \qquad (5)$$

The term $d \ln R/d \ln \lambda$ can be evaluated by use of eq 1. Schimpf and Giddings[3] found that the thermal diffusion coefficient is independent of the molecular mass of the polymer. Therefore, the molecular mass will influence λ only through its effect on the molecular diffusion coefficient. The empirical relationship $D = AM^{-b}$ is often used to describe polymer diffusion as function of the molecular mass.[3] The constant b is usually in the range of 0.5–0.6 for most polymer–solvent systems. From eq 2, it can be shown that the term $d \ln \lambda/d \ln M$ equals b. The mass selectivity in FFF can now be found by combining eqs 1 and 5:

$$S = b\left[1 + \frac{\coth^2(1/2\lambda) - 4\lambda^2 - 1}{2\lambda\coth(1/2\lambda) - 4\lambda^2}\right] \qquad (6)$$

In the case of high retention ($\lambda \rightarrow 0$), the term in brackets on the right-hand side of eq 6 approaches 1 and the selectivity is equal to b. However, under normal working conditions, the selectivity will be somewhat less, as demonstrated in Figure 1.

The time needed to evolve one plate is equal to the ratio of the plate height and the mean velocity of the solute zone. Therefore, when the plate height is determined by the nonequilibrium term, t_p/S^2 in ThFFF can be expressed as

$$\frac{t_p}{S^2} = \frac{H}{R\langle v \rangle}\frac{1}{S^2} = \frac{1}{b^2}\left[\frac{x}{R(S/b)^2}\right]\frac{w^2}{D} \qquad (7)$$

Note that the flow rate of the carrier liquid has no effect on

(19) Gunderson, J. J.; Caldwell, K. D.; Giddings, J. C. *Sep. Sci. Technol.* **1984**, *19*, 667.
(20) van Asten, A. C.; Boelens, H. F. M.; Kok, W. Th.; Poppe, H.; Williams, P. S.; Giddings, J. C. *Sep. Sci. Technol.* **1994**, *29*, 513.
(21) Giddings, J. C. *Unified Separation Science*; J. Wiley and Sons: New York, 1991.
(22) Giddings, J. C. *J. Chem. Educ.* **1973**, *50*, 667.
(23) Janca, J.; Kleparnik, K. *J. Liq. Chromatogr.* **1993**, *634*, 149.
(24) Giddings, J. C.; Yoon, Y. H.; Caldwell, K. D.; Myers, M. N.; Hoving, M. E. *Sep. Sci.* **1975**, *10*, 447.
(25) Smith, L. K.; Myers, M. N.; Giddings, J. C. *Anal. Chem.* **1977**, *49*, 1750.

(26) Schimpf, M. E.; Williams, P. S.; Giddings, J. C. *J. Appl. Polym. Sci.* **1989**, *37*, 2059.
(27) Giddings, J. C.; Martin, M.; Myers, M. N. *J. Chromatogr.* **1978**, *158*, 419.
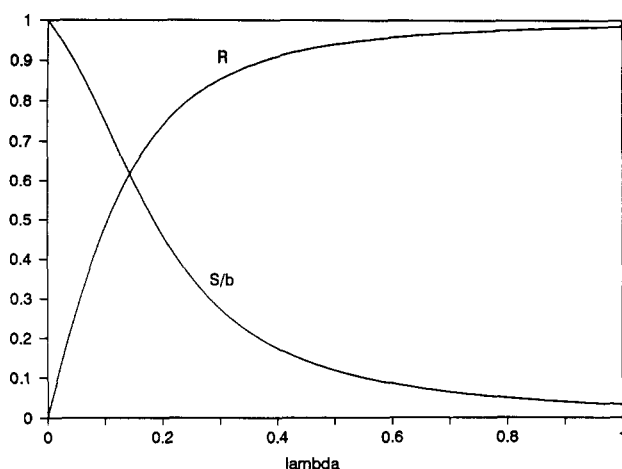
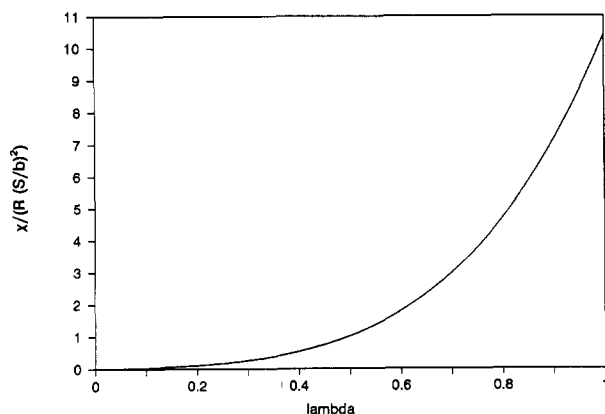**Figure 1.** S/b ($= d \ln R/d \ln \lambda$) and R as functions of λ.

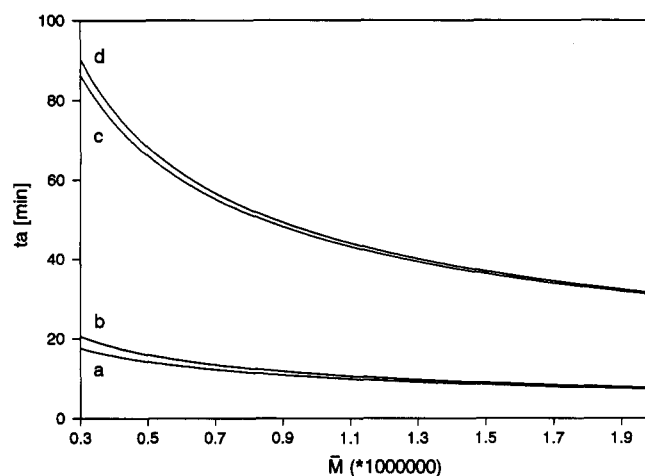**Figure 2.** $\chi/(R(S/b)^2)$ as a function of λ.

Figure 3. Analysis time as function of the mean molecular mass of two polystyrene fractions dissolved in THF, with $R_s = 1$, $M/\Delta M = 5$, $D_T = 1 \times 10^{-7}$ cm^2 s^{-1} K^{-1}, and $D = 3.861 \times 10^{-4}$ M$^{-0.571}$ cm^2 s^{-1}: (a) $w = 25$ μm, $\Delta T = 25$ K; (b) $w = 50$ μm, $\Delta T = 50$ K; (c) $w = 150$ μm, $\Delta T = 75$ K; (d) $w = 300$ μm, $\Delta T = 150$ K.

the separation speed, a situation similar to chromatographic optimization under conditions where the C term in the plate height equation predominates. For minimum analysis time (i.e., maximum separation speed), t_p/S^2 should be as small as possible. The term in brackets in eq 7 is only a function of λ and, as is demonstrated in Figure 2, approaches zero when λ approaches zero. As stated by Giddings et al.,[27] an increase in separation speed can be expected with decreasing channel thickness and increasing temperature drop. Equation 7 clearly demonstrates that a gain in separation speed is obtained with decreasing channel thickness. Since the parameter λ is not a function of the channel thickness, a change in w does not lead to a change in the retention ratio.[28] Therefore, the channel thickness can be regarded as an independent parameter. With the use of eq 2, the effect of the temperature drop on the separation speed can be clarified. An increase in ΔT will result in a decreasing λ value. Therefore, an increase in the temperature drop will decrease the term in brackets in eq 7 and will thus decrease the analysis time. In sharp contrast to the channel thickness, the diffusion coefficient (or rather the mean diffusion coefficient of the two polymer fractions) cannot be seen as an independent parameter. From eq 2 it follows that the diffusion coefficient also has an influence on the λ value and, therefore, also on the λ-dependent term in eq 7. On first sight, a high diffusion coefficient seems beneficial for the separation speed in ThFFF. However, high diffusion will result in a high λ value and thus in low retention.

The effect of the various parameters on the separation speed in ThFFF becomes much clearer when high retention ($\lambda \rightarrow 0$) is considered. In this case, R can be expressed as 6λ, χ can be approximated by $24\lambda^3$ and S/b is equal to 1. Using eqs 2 and 7, it can be shown that

$$\frac{t_p}{S^2} = \frac{4}{b^2} \frac{D}{D_T^2} \left[\frac{w}{\Delta T}\right]^2 \qquad (8)$$

As can be seen from eq 8, a decrease in molecular diffusion is expected to increase the separation speed in ThFFF. Since an increase in molecular mass corresponds to a decrease in the diffusion coefficient, the use of ThFFF becomes more advantageous when polymer samples of very high molecular mass are analyzed. This is an important feature of ThFFF because other polymer fractionation methods such as size exclusion chromatography (SEC) and hydrodynamic chromatography (HDC) are more suited for the analysis of polymers of lower molecular mass.[29] Equation 8 also demonstrates that high thermal diffusion is needed for a rapid ThFFF analysis. As thermal diffusion depends strongly on the chemical nature of both the polymer and solvent, the polymer–solvent system will severely influence the separation speed in ThFFF. Finally, it can be seen that the analysis time is strongly decreased with an increase in ΔT or a decrease in w. It is noteworthy that, ultimately, only the temperature gradient is of importance for the separation speed in ThFFF. So for a ThFFF setup with $w = 50$ μm and $\Delta T = 50$ K, approximately the same separation speed is obtained as for a system with $w = 100$ μm and $\Delta T = 100$ K. This is illustrated in Figure 3.

The effects of the various parameters on the separation speed in ThFFF will be discussed in more detail in the Results and Discussion section. The effects will be demonstrated with the separation of various polymer standards under different conditions. In order to illustrate the difference in separation speed when a certain parameter value is changed, related

(28) Myers, M. N.; Caldwell, K. D.; Giddings, J. C. *Sep. Sci.* **1974**, *9*, 47.

(29) Stegeman, G.; van Asten, A. C.; Kraak, J. C.; Poppe, H.; Tijssen, R. *Anal. Chem.* **1994**, *66*, 1147.
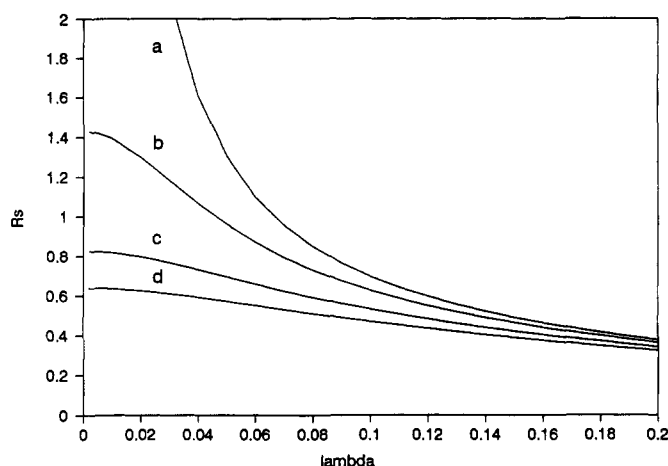
Figure 4. Resolution at constant analysis time as function of λ, with $t_a = 20$ min, $M = 175\,000$, $\Delta M = 100\,000$, $w = 127$ μm, and D = 4×10^{-7} cm^2 s^{-1}: (a) $\mu = 1$; (b) $\mu = 1.01$; (c) $\mu = 1.03$; (d) $\mu = 1.05$.

separations were performed at constant analysis time (or rather constant run time as explained in the Results and Discussion section). Because the separation speed parameter t_p/S^2 is independent of the flow rate of the carrier liquid, the analysis time can be kept constant by adjusting the solvent flow. In this way, a change in separation performance caused by the variation of a system parameter is visualized by an increase or decrease of the resolution. An increase in resolution at constant analysis time corresponds to a decrease in t_p/S^2 or, in other words, an increase in separation speed.

However, polymer standards do not represent an absolute molecular mass but rather a narrow molecular mass distribution. Therefore, it is important to assess in what way the polydispersity of the polymer standards will influence the outcome of the experimental results. When the polydispersity, μ, is smaller than 1.05, which is true for the polymer standards used in this study, the apparent plate height contribution can formally be approximated by[9]

$$H_{pd} = LS^2(\mu - 1) \tag{9}$$

where L is the channel length.

If the separation of two polymer standards with Gaussian mass distributions and an identical polydispersity is considered, the observed resolution can be expressed as

$$R_s = \left[\frac{\Delta M}{4M}\right]\left(\frac{t_a}{[\chi/RS^2](w^2/D) + t_a(\mu - 1)}\right)^{1/2} \tag{10}$$

The effect of the polydispersity is illustrated in Figure 4. When system dispersion is negligible compared to peak broadening caused by polydispersity, the resolution will be determined completely by the molecular mass distribution of the two polymer standards (e.g., $R_s = \Delta M/(4M(\mu - 1)^{1/2})$). Figure 4 demonstrates that polydispersity does not alter the trends in the observed separation speed. With increasing polydispersity, the effects of the changing parameters on the resolution will only become less visible. Nevertheless, if the results for different polymer standards have to be compared, care has to be taken. The different standards may possess different polydispersities. However, it has been shown[9,30] that polymer

standards usually have much lower polydispersities (typically $\mu < 1.01$) than the values specified by the manufacturers. Furthermore, the separations were performed under medium retention conditions (typically $\lambda > 0.05$). As can be seen from Figure 4, an increase in λ diminishes the influence of the polydispersity of the polymer standards on the resolution. Therefore, under these conditions, trends in the separation speed can be investigated by comparing the results for different polymer standards.

EXPERIMENTAL SECTION

Instrumentation. The T100 thermal FFF system used in this work was obtained from FFFractionation (Salt Lake City, UT). Separations were performed by use of channels with a thickness of 127 and 254 μm. A sheet of Mylar, from which the channel space was cut, was clamped between the two chrome-plated copper bars to form the channel. The channels were 46 cm long and 1.6 cm wide. To allow a smooth fluid flow at the channel inlet and outlet, the channel ends were tapered. Void volumes of 0.76 and 1.55 mL were measured for the two channels with thicknesses of 127 and 254 μm, respectively. To avoid peak broadening due to insufficient relaxation, the solvent flow was stopped for 2 min after injection. For the experiments performed with the 254-μm-thick channel, a relaxation time of 5 min was used. Various temperature drops, ranging from 20 to 80 K, were used in this work. The temperature drops and corresponding cold wall temperatures are specified in the legends of the Figures. Solvent flow was provided by a Spectroflow 400 HPLC pump (ABI, Ramsey, NJ). All organic solvents were continuously degassed with helium. Pulsation of the solvent flow was minimized by means of a pulse damper (Model 812, Tegimenta, Rotkreuz, Switzerland) and a small column packed with silica particles, which served as a flow restrictor. A six-port valve with an injection loop of 20 μL was used to introduce the sample solutions in the channel. Polymer concentrations, which never exceeded 1 mg/mL, are specified in the legends of the Figures. The eluting polymer zones were detected by a Varex evaporative light scattering detector (ELSD) (Model 2A, Burtonsville, MD). A fused silica capillary with 100-μm i.d., 360-μm o.d., and a length of 1 m was used to connect the outlet of the channel to the detector. This setup ensured a small dead volume (0.008 mL) and a sufficiently large back pressure to increase the boiling points of the organic solvents in the channel. When a channel with a thickness of 254 μm was used, a capillary length of only 50 cm was chosen due to the high flow rates used in this case.

Materials. All solvents were of analytical-reagent grade. Tetrahydrofuran (THF) was obtained from Janssen Chimica (Geel, Belgium), and benzene from Baker (Deventer, The Netherlands); methyl ethyl ketone (MEK) and dioxane were from Merck (Darmstadt, Germany). The polymer standards used in this work are listed in Table 1.

RESULTS AND DISCUSSION

In this section, results from the theoretical part are exemplified with separations of various polymer standards under varying conditions. The effects of the temperature drop,

(30) Stegeman, G.; Kraak, J. C.; Poppe, H. *J. Chromatogr.* **1993**, *634*, 149.

Table 1. Polymer Standards Used for This Study.

polymer	M_p	μ $(=M_w/M_n)$	source
polystyrene	580	<1.18	Merck
polystyrene	19 800	<1.06	Pressure Chemical Co.
polystyrene	34 500	<1.05	Merck
polystyrene	68 000	<1.05	Merck
polystyrene	160 000	<1.06	Pressure Chemical Co.
polystyrene	310 000	<1.05	Merck
polystyrene	700 000	<1.05	Merck
polyisoprene	60 000	1.04	Polymer Laboratories
polyisoprene	295 000	1.04	Polymer Laboratories
polybutadiene	120 000	1.03	Polymer Laboratories
polybutadiene	950 000	1.03	Polymer Laboratories
polytetrahydrofuran	67 000	1.08	Polymer Laboratories
polytetrahydrofuran	282 300	1.08	Polymer Laboratories

channel thickness, mean molecular mass of the polymer standards, solvent choice, and polymer type on the separation speed in ThFFF were investigated. In order to illustrate the difference in separation speed when a certain parameter value is changed, related separations were performed at a constant run time. As can be seen in the Figures displayed in this section, the run time (indicated by the dotted lines in the Figures) is not fully identical to the analysis time, which was introduced in the theoretical section. Some of the fractograms show the separation of more than two polymer standards. Furthermore, the run time is defined as the time needed to elute all polymer standards completely, whereas the analysis time is defined as the mean retention time of two polymer fractions of absolute molecular mass. However, analysis time and run time are two closely related parameters and the latter can be seen as the experimental analog of the analysis time. Due to the fact that the run time was kept constant by adjusting of the flow rate, differences in resolution indicate the differences in separation speed when one of the system parameters is altered.

Effect of the Channel Thickness and the Temperature Drop. As demonstrated by Giddings et al.,[27] and as can be seen from eq 8, the separation speed in ThFFF increases with decreasing channel thickness and increasing temperature drop. Equation 8 also demonstrates that, ultimately, the temperature gradient is the only important instrumental parameter regarding separation speed in ThFFF. The highest temperature gradient ever used in ThFFF is equal to 1.18 K μm^{-1} ($w = 51$ μm and $\Delta T = 60$ K).[27] A further increase has been impeded by instrumental difficulties. The use of a channel thickness below 50 μm requires an extreme smoothness of the channel walls. Higher temperature drops can only be used if the enormous amount of heat can be dissipated more efficiently. It should be noted that the upper limitation to the temperature gradient is purely of an instrumental nature. Therefore, it is conceivable that a new design of the ThFFF channel could increase the separation speed to some extent.

In Figures 5 and 6 the effect of the temperature drop at constant channel thickness can be seen. Three polystyrene (PS) standards were fractionated in a constant run time using different temperature drops. The fractograms in Figure 5 were obtained with a 127-μm-thick channel, whereas for the fractograms displayed in Figure 6, a channel thickness of 254 μm was used. The run time was set at 18–20 min. From eqs 1 and 2 it follows that retention increases with the temperature drop. Therefore, for the fractograms obtained at higher
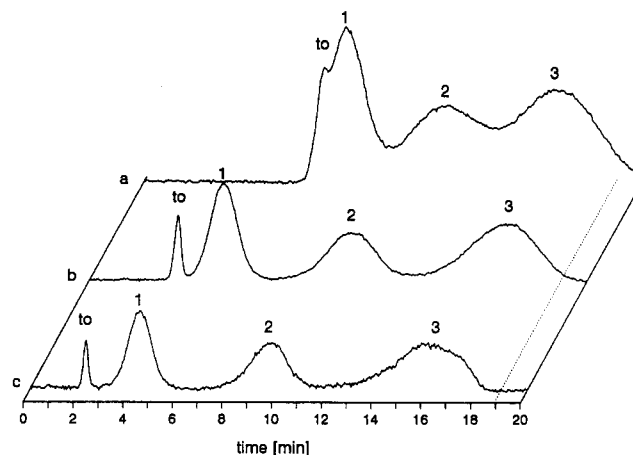

Figure 5. Separation of three PS standards in THF for different temperature drops at constant run time, with $w = 127$ μm, $t_r = 18$–20 min, $t_0 = $ PS 580 (0.05 mg/mL), (1) PS 68 000 (0.25 mg/mL), (2) PS 310 000 (0.3 mg/mL), and (3) PS 700 000 (0.5 mg/mL): (a) $\Delta T = 20$ K, $T_c = 289$ K, flow rate 0.11 mL/min; (b) $\Delta T = 40$ K, $T_c = 293$ K, flow rate 0.22 mL/min; (c) $\Delta T = 60$ K, $T_c = 296$ K, flow rate 0.33 mL/min.
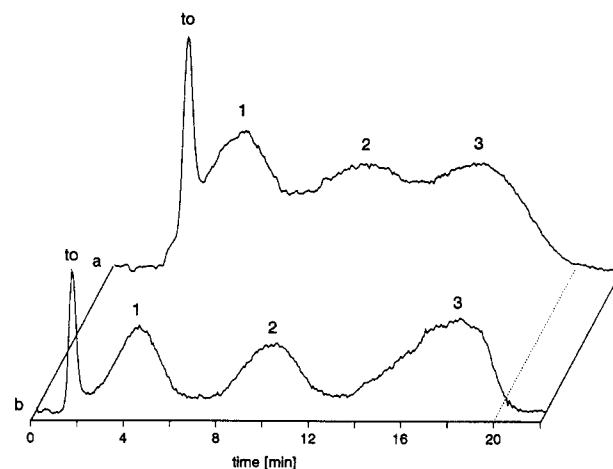

Figure 6. Separation of three PS standards in THF for different temperature drops at constant run time, with $w = 254$ μm, $t_r = 20$ min, $t_0 = $ PS 580 (1 mg/mL), (1) PS 68 000 (0.5 mg/mL), (2) PS 310 000 (0.6 mg/mL), and (3) PS 700 000 (1 mg/mL): (a) $\Delta T = 40$ K, $T_c = 295$ K, flow rate 0.5 mL/min; (b) $\Delta T = 80$ K, $T_c = 297$ K, flow rate 1 mL/min.

temperature drops, higher flow rates had to be used in order to maintain a constant run time. Indeed, Figures 5 and 6 clearly illustrate that an increase in ΔT (at constant w) is very beneficial for the separation speed in ThFFF. When fractograms 5b and 6a are compared, the effect of decreasing the channel thickness at a constant temperature drop can be assessed. As expected, a significant increase in resolution and, thus, separation speed is obtained with a decrease in w (at constant ΔT). However, the theoretical prediction that the separation speed is only determined by the temperature gradient is of course the most interesting one to test. Fractograms 5a and 6a and fractograms 5b and 6b were performed under identical temperature gradients, respectively, 0.16 and 0.31 K μm^{-1}. When these fractograms are compared, it can indeed be seen that the separation speed is more or less constant for a given temperature gradient.

Although the channel thickness is theoretically an independent parameter (it does not influence the λ value), it is in practice coupled to the maximum temperature drop that can

be employed. When thin channels are used, the need for efficient heat dissipation limits the maximum temperature drop. Temperature drops up to 158 K have been reported in combination with a channel thickness of 245 μm,[31] whereas for a channel thickness of 51 μm, only a maximum ΔT of 60 K could be used.[27] Bearing in mind that the temperature gradient is the only instrumental parameter of importance regarding separation speed, one could conclude that a decrease in channel thickness does not bring about any effect. However, there are three important factors which favor a decrease in w rather than a corresponding increase in ΔT. First, the use of a thinner channel decreases the dilution of the polymer zones in the channel. The increase in dilution with channel thickness is also demonstrated in Figures 5 and 6. Even though higher sample concentrations were used to obtain the fractograms given in Figure 6, a lower signal-to-noise ratio was observed compared to that of the fractograms in Figure 5. Therefore, a decrease in w decreases the detection limit, and lower sample concentrations can be injected. This is an important feature because concentration overload can be a severe problem in ThFFF.[32,33] Second, with the use of a thin channel, high temperature gradients are obtained with moderate temperature drops. Therefore, no excessive pressurization of the ThFFF channel is needed to avoid boiling of the organic solvents. Finally, systematic errors in the conversion of retention data into λ values, caused by neglecting the temperature dependence of the solvent viscosity and thermal conductivity, will be smaller with the use of lower temperature drops.

Effect of the Molecular Mass. Schimpf and Giddings[3] found that the thermal diffusion coefficient is independent of the molecular mass of the polymer for 17 polymer–solvent combinations. As yet, no results have been published which indicate a molecular mass dependence of the thermal diffusion coefficient for other polymer species and solvents. Therefore, only the effect of the molecular mass on the molecular diffusion coefficient has to be considered. As can be seen from eq 8, a decrease in molecular diffusion is expected to be beneficial to separation speed in ThFFF. This feature, combined with the fact that ThFFF can be considered as a gentle separation method, makes this technique, in contrast to HDC and SEC, especially suited for the analysis of materials of very high molecular mass.[11]

The effect of the molecular mass on the separation speed in ThFFF is illustrated in Figure 7. Each of the five fractograms displayed in this Figure corresponds to the separation of two polystyrene standards in THF. The temperature drop was kept constant at 40 K, and the run time was set at 18 min. Note that as a high molecular mass corresponds to a low molecular diffusion coefficient and thus to high retention (see eqs 1 and 2), the flow had to be increased with molecular mass to maintain the run time at 18 min. When the five fractograms are compared it can be noticed that the mean molecular mass of the two standards increases going from a to d, while the ratio of molecular masses is constant ($M/\Delta M \approx 0.77$). Because a significant increase in resolution can be observed going from fractogram a to
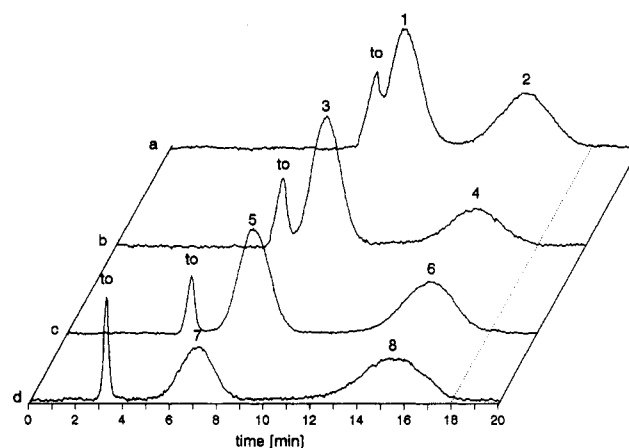
(31) Giddings, J. C.; Smith, L. K.; Myers, M. N. *Anal. Chem.* **1975**, *47*, 2389.
(32) Schimpf, M. E. *J. Chromatogr.* **1990**, *517*, 405.
(33) Caldwell, K. D.; Brimhall, S. L.; Gao, Y; Giddings, J. C. *J. Appl. Polym. Sci.* **1988**, *36*, 703.

Figure 7. Separation of PS standards of different molecular mass in THF at constant run time, with w = 127 μm, ΔT = 40 K, T_c = 293 K, t_r = 18 min, t_0 = PS 580 (0.05 mg/mL), (1) PS 19 800 (0.25 mg/mL), (2) PS 97 000 (0.25 mg/mL), (3) PS 34 500 (0.25 mg/mL), (4) PS 160 000 (0.25 mg/mL), (5) PS 68 000 (0.25 mg/mL), (6) PS 310 000 (0.3 mg/mL), (7) PS 160 000 (0.3 mg/mL), and (8) PS 700 000 (0.45 mg/mL). Flow rate: (a) 0.09, (b) 0.11, (c) 0.15, and (d) 0.23 mL/min.

fractogram d, Figure 7 clearly demonstrates that an increase in the (mean) molecular mass of the polymer sample enhances the separation speed in ThFFF. From the theoretical and experimental results, it is expected that the run time would approach zero when ultrahigh molecular mass compounds have to be analyzed. An infinitely small run time corresponds to an infinitely small λ value or, in other words, extremely high retention. To achieve a short run time under high retention conditions, very high flow rates are required. Unfortunately, our treatment breaks down for such extreme conditions. Giddings et al.[34] showed that under these circumstances a so-called hyperlayer operation mode is achieved. In this mode, sufficiently high shear gradients lead to polymer chain deformation, and as a result, retention and peak broadening cannot be described by the original equations. However, under these conditions still very high separation speeds were obtained.

Effect of the Solvent Choice. Giddings and Schimpf[3] found that the solvent has a profound influence on the thermal diffusion coefficient. Since molecular diffusion is also dependent on the solvent, it can be expected that the chemical nature of the solvent will significantly influence the separation speed in ThFFF. From eq 8 it can be seen that for a given molecular mass it is profitable to have a low molecular diffusion coefficient combined with a high thermal diffusion coefficient. However, as thermal diffusion is still an uncomprehended phenomenon, it is not possible to predict theoretically which solvent can be used best for the ThFFF analysis of a given polymer species. To test whether ThFFF is a suitable technique for the fractionation of a specific polymer type, retention and plate height measurements have to be performed in all possible solvents.

The fact that the choice of the solvent can be an important factor regarding separation speed in ThFFF is demonstrated in Figure 8. A mixture of three polystyrene standards was fractionated in three different solvents. The channel thickness

(34) Giddings, J. C.; Li, S; Williams, P. S.; Schimpf, M. E. *Makromol. Chem., Rapid Commun.* **1988**, *9*, 817.

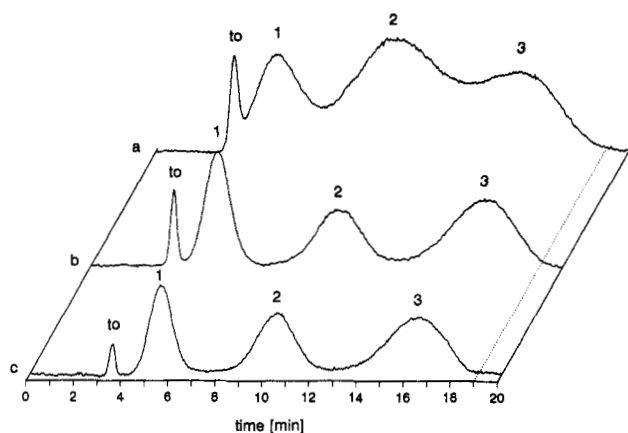**Figure 8.** Separation of three PS standards in different solvents at constant run time, with $w = 127~\mu m$, $\Delta T = 40$ K, $T_c = 293$ K, $t_r = 18$ min, $t_0 =$ PS 580 (0.05 mg/mL), (1) PS 68 000 (0.25 mg/mL), (2) PS 310 000 (0.3 mg/mL), (3) PS 700 000 (0.5 mg/mL), and flow rate 0.22 mL/min. Solvent: (a) dioxane, (b) THF, and (c) MEK.

Figure 9. Separation of two PS, PI, PB, and PTHF standards in THF at constant run time, with $w = 127~\mu m$, $\Delta T = 40$ K, $T_c = 293$ K, $t_r = 18–20$ min, $t_0 =$ PS 580 (0.05 mg/mL), (1) PB 120 000 (0.25 mg/mL), (2) PB 950 000 (0.5 mg/mL), (3) PTHF 67 000 (0.25 mg/mL), (4) PTHF 282 300 (0.5 mg/mL), (5) PI 60 000 (0.25 mg/mL), (6) PI 295 000 (0.3 mg/mL), (7) PS 68 000 (0.25 mg/mL), and (8) PS 310 000 (0.3 mg/mL). Flow rate: (a) 0.12, (b) 0.1, (c) 0.11, and (d) 0.15 mL/min.

and temperature drop were constant, and the run time was kept at 19 min. Highest resolution and thus separation speed was found when MEK was used as the solvent; only very poor resolution was found when dioxane was used. Note that in each fractogram the same flow rate was used to obtain a run time of 19 min. This indicates that the retention ratios and, therefore, the λ values of the polystyrene standards are comparable. With the use of eq 2, it follows that the ratio of D and D_T for a polystyrene standard of given molecular mass must be similar in all three solvents. The differences in resolution can be explained from the fact that the specific values of D and D_T are different in the various solvents. Since the λ value at a fixed molecular mass and ΔT is constant, the selectivity and the parameter χ also are constant. As seen in eq 7, an increase in molecular diffusion *without* altering the λ value increases the separation speed in ThFFF. Therefore, it can be concluded that polystyrene displays highest thermal diffusion in MEK and lowest thermal diffusion in dioxane. Schimpf and Giddings[3] indeed determined that the thermal diffusion coefficient of polystyrene was higher in MEK (1.39×10^{-7} cm^2 s^{-1} K^{-1}) than in THF (1.00×10^{-7} cm^2 s^{-1} K^{-1}).

From Figure 8 it can be concluded that, of the three solvents, MEK is the best choice for the ThFFF analysis of polystyrene samples. However, in most ThFFF experiments involving polystyrene, THF has been used as the solvent. This is because THF, in contrast to MEK, is UV transparent. So the use of THF makes it possible to detect the polystyrene samples with an UV detector. The ELSD can be used for almost any solvent and polymer, and as this detector also displays a very high sensitivity, it can be regarded as an essential tool to fully utilize the separation capabilities of ThFFF.

Effect of the Polymer Type. In addition to the chemical nature of the solvent, the composition of the polymer also has a profound influence on the thermal diffusion effect.[3] For a given molecular mass and solvent, molecular diffusion can also differ for different polymer species. The discussion concerning the effect of the solvent choice on the separation speed in ThFFF also applies for the effect of the polymer type. Low molecular diffusion for a given molecular mass and high thermal diffusion will lead to high separation speeds. However, the magnitude of the thermal diffusion effect for
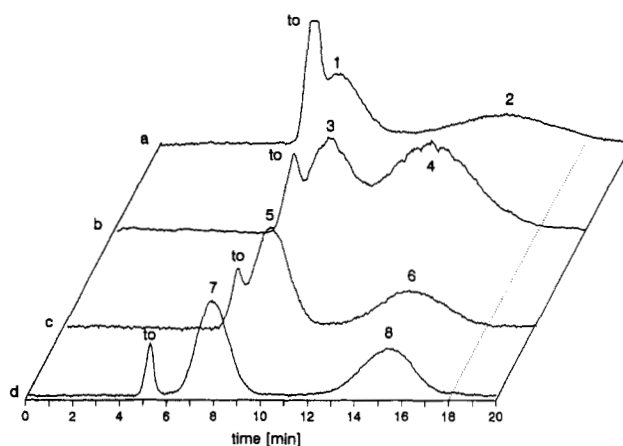
a specific polymer–solvent system cannot be predicted and has to be determined empirically.

The effect of the polymer composition on the separation speed is demonstrated in Figure 9. All separations were performed in THF, w was equal to 127 μm, the temperature drop was set at 40 K and, except for polybutadiene, the molecular masses of the standards of the different polymer species were similar. The run time was kept at 18–20 min. The huge differences in resolution indicate the profound influence of the chemical nature of the polymer on the separation speed in ThFFF. When THF is used as the solvent, it can be seen that only for the analysis of polystyrene and polyisoprene (PI) is a satisfactory separation speed obtained. For polybutadiene (PB) and poly(tetrahydrofuran) (PTHF) samples, analysis should be performed in a different solvent. For polybutadiene in THF, the separation speed was so low that polymer standards with a higher molecular mass and a larger difference in molecular mass had to be chosen to see any separation at all.

Figure 9 demonstrates that, for efficient use of ThFFF, a sufficiently high thermal diffusion coefficient is necessary. In comparison with SEC and HDC, this can be considered as a disadvantage. ThFFF cannot be used for the fractionation of polymer samples that display weak thermal diffusion in all possible solvents. The use of ThFFF for the analysis of water-soluble polymers has, for example, always been minimal because very few polymer species show thermal diffusion in aqueous environments.[18]

However, the fact that retention in ThFFF is also determined by the thermal diffusion coefficient offers unique separation possibilities. As demonstrated by Gundersson and Giddings[15] and recently also by our group,[35] ThFFF can be used for the separation of polymer species which are equal in size but differ in chemical nature. This feature of ThFFF is demonstrated in Figure 10, which shows the separation of four polymers standards of approximately the same molecular mass but different composition. The diffusion coefficients of

---

(35) van Asten, A. C.; Venema, E.; Kok, W. Th.; Poppe, H. *J. Chromatogr.* **1993**, *644*, 83.
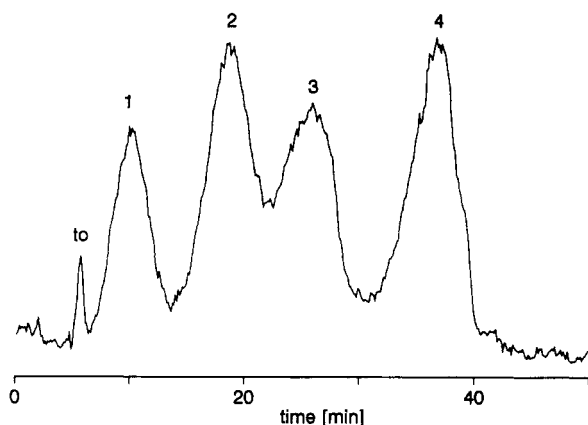
Figure 10. Separation of PB 330 000 (1), PI 295 000 (2), PTHF 282 300 (3), and PS 310 000 (4) in benzene, with $w = 127\ \mu m$, $\Delta T = 80$ K, $T_c = 303$ K, flow rate 0.14 mL/min, $t_0 = $ PS 580 (0.0033 mg/mL), and polymer concentrations in the range from 0.15 to 0.35 mg/mL.

the standards will not be exactly the same in benzene. However, the degree of resolution cannot be fully accounted for by the differences in molecular diffusion and is therefore mainly brought about by differences in thermal diffusion. Although at first it does not seem very useful to separate polymer standards of the same mass but different chemical nature, Figure 10 illustrates a much more important underlying principle. Namely, the fact that ThFFF data contain chemical and structural information of polymer molecules. In combination with SEC or HDC, the ThFFF analysis of complex polymer samples can produce qualitative information on the chemical structure of such samples. This has already been demonstrated by Schimpf and Giddings,[4] who showed that block and random copolymers display distinct differences in thermal diffusion.

CONCLUSIONS

The performance of a polymer fractionation method can be described effectively by the time optimization method as suggested by Giddings et al.[27] Use of this method demonstrates that separation speed in ThFFF depends on many parameters, including the temperature gradient, the molecular mass and chemical nature of the polymer sample, and the choice of the solvent.

As predicted by theory, the separation speed increases with the temperature gradient and the molecular mass of the polymer molecules. As adsorption phenomena are minimal inside the fractionation channel, ThFFF is ideally suited for the analysis of materials of very high molecular mass. The highest temperature gradient ever used in ThFFF is equal to $1.2\ \mathrm{K}\ \mu m^{-1}$, with further increases impeded by the experimental design of the channel. If an instrument could be designed to achieve higher temperature gradients, an increase in separation speed would be obtained.

Both molecular and thermal diffusion depend on the chemical nature of the solvent and the polymer species. As no theoretical understanding of the thermal diffusion phenomenon exists, the solvent that yields the highest separation speed for the ThFFF fractionation of a given polymer sample has to be determined empirically. It is shown in this study that the separation speed differs greatly for different polymer–solvent systems.

Because retention in ThFFF is also determined by the thermal diffusion coefficient, polymer species are not only fractionated according to size but also according to chemical nature. Therefore, qualitative information on the chemical composition of complex polymer samples can be obtained with ThFFF. In this respect, the use of ThFFF in combination with techniques that produce information on polymer size (SEC, HDC, or a multiangle laser light scattering detector) seems promising.

GLOSSARY

A, b	empirical constants relating diffusion to molecular mass
D	molecular diffusion coefficient, $m^2\ s^{-1}$
D_T	thermal diffusion coefficient, $m^2\ s^{-1}\ K^{-1}$
H	plate height, m
H_{pd}	polydispersity plate height contribution, m
L	channel length, m
M	mean molecular mass, g mol^{-1}
M_n	number-average molecular mass, g mol^{-1}
M_p	average molecular mass as determined by SEC, g mol^{-1}
M_w	weight-average molecular mass, g mol^{-1}
R	retention ratio
R_s	resolution
S	selectivity
t_0	unretained time, s
t_a	analysis time, s
T_c	cold wall temperature, K
t_p	time needed to evolve one plate, s
t_r	run time, s
V_r	retention volume, m^3
$\langle v \rangle$	mean linear velocity of the carrier liquid, m s^{-1}
w	channel thickness, m
ΔM	difference in molecular mass, g mol^{-1}
ΔT	temperature drop, K
λ	dimensionless zone thickness
μ	polydispersity
χ	λ dependent function in the nonequilibrium term of the plate height equation