



# Lowering the molecular mass limit of thermal field-flow fractionation for polymer separations

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## Abstract

Thermal field-flow fractionation (ThFFF) is capable of separating a wide molecular mass range of polymers by their molecular mass ( $M_r$ ) and chemical composition. However, retention and resolution decrease significantly for polymers with  $M_r < 20$  kDa. Various approaches for increasing the retention of low  $M_r$  (<15 kDa) polymers were investigated. Our results showed that temperature conditions and single-component solvents had a limited effect on polymer retention and that certain binary solvent mixtures caused a dramatic increase in retention. The binary solvents approach has enabled the use of a standard ThFFF system and temperature conditions to separate 2.6 kDa PS from 4.4 kDa PS, thereby extending the applicability of ThFFF to lower molecular masses. The effect of binary solvent mixtures on polymer retention is correlated with the mixture viscosity.

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## 1. Introduction

Field-flow fractionation is a family of techniques that is characterized by separations conducted in an open channel (devoid of packing material), the use of a laminar flow of carrier liquid to effect the separation, and the application of a field perpendicular to the separation axis to drive sample components towards one channel wall [1]. For thermal field-flow fractionation (ThFFF) technique, the field is a temperature gradient established between two walls with the temperature difference between the hot and cold walls denoted as  $\Delta T$ . The temperature gradient causes thermal diffusion of sample from regions of

higher temperatures to those of lower temperatures. Thermal diffusion towards the cold wall is counteracted by molecular diffusion towards regions of lower concentrations, i.e. the hot wall. These two opposing transport processes cause the formation of equilibrium sample layers of different thicknesses,  $\ell_1, \ell_2$ , etc. Each sample component with a specific  $M_r$  has a different average distance from the cold wall and hence, occupies a different velocity streamline and elutes from the ThFFF channel at a different time.

FFF techniques can also provide information about physicochemical properties of the eluting sample components [1–3]. For ThFFF, molecular masses can be calculated from the measured retention times provided calibration constants are available. ThFFF is a separation technique that is also sensitive to

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polymer composition. Similar  $M_r$  polymers of different compositions have been shown to elute as a single peak using size-exclusion chromatography (SEC) while almost baseline resolution is obtained with ThFFF [4]. The coupling of SEC with ThFFF has resulted in a two-dimensional separation that was based initially on polymer size and subsequently on polymer chemical composition [5,6]. ThFFF has also been demonstrated to be useful in separating diblock and triblock copolymers [7].

Conventional ThFFF provides higher mass selectivity compared to size-exclusion chromatography for polymers with  $M_r > 50$  kDa [8,9]. However, for  $M_r$  less than 20 kDa, ThFFF retention and resolution drop significantly [9,10]. This has rendered ThFFF unsuitable for  $M_r$  and chemical composition analysis of many practical polymers and limited the benefits which can be gained by coupling ThFFF with methods such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The latter can provide information about polymer end groups and repeat units but mainly for polymers too low in  $M_r$  to be well separated by ThFFF. The objective of this study is to determine effective ways to enhance the retention of low  $M_r$  (<15 kDa) polymers and thus extend ThFFF applicability to lower  $M_r$  polymer analysis.

While prior studies have been published on the subject of ThFFF retention of polymers in general [11–17], the work described here differs in its focus on low  $M_r$  polymers only and specific issues relevant to lowering the  $M_r$  limit of ThFFF. Several approaches that could lead to increased retention of low  $M_r$  polymers have been evaluated. The first approach is associated with increasing the temperature drop  $\Delta T$  between the channel walls. In an early ThFFF study, it was shown that the minimum  $M_r$  is proportional to  $(1/\Delta T)^2$  and that a 600 Da polystyrene (PS) peak could be discerned from the void peak when  $\Delta T$  was raised from 80 to 158 K [11]. These high temperature studies are not currently feasible because commercial ThFFF equipment has a maximum temperature drop of 110–120 K and major modifications would have to be made to the standard ThFFF apparatus [12].

The second approach is associated with decreasing the cold wall temperature,  $T_c$ . Increased retention has been reported for higher molecular mass polymers

following a reduction in  $T_c$  [10,13]. This observation can be explained by examining the effect of  $T_c$  on molecular and thermal diffusion coefficients. For highly retained polymers, the mean temperatures of the sample zones can be approximated by that of the cold wall (because of their proximity to the cold wall). Consequently, lowering  $T_c$  while maintaining a constant  $\Delta T$  can significantly decrease the molecular diffusion coefficient of a polymer. The thermal diffusion coefficient, on the other hand, is less affected by the temperature of the cold wall [14]. The net result is a decreased sample equilibrium layer thickness and increased retention. For lower  $M_r$  polymers, additional studies are required to estimate the effect of  $T_c$  on retention because the sample zones have average distances further from the cold wall and the effect of  $T_c$  can be diminished.

The third approach is to increase retention by changing the carrier liquid composition. Since molecular and thermal diffusion of a polymer are affected by solvent composition, polymer retention time can be shifted using a different solvent. The effect of single-component solvents on retention has been observed to decrease with decreasing polymer  $M_r$  [15]. Analyses of polystyrene in 11 pure solvents showed that the retention of a 575 kDa polymer can vary by 70%, while a 23 kDa polymer experienced changes of only  $\sim 10\%$  [15]. These studies need to be extended to  $M_r < 15$  kDa.

The replacement of single-component solvents with certain binary solvent mixtures resulted in improvements in polystyrene retention of 50–80% [15–17]. It was proposed that the thermal diffusion of solvent components in a mixture can create an additional driving force on the polymer molecule [15,17]. Two requirements have been established for solvent mixtures if one is to achieve enhanced polymer retention. First, the binary mixture should consist of a thermodynamically “good” solvent and a “poor” solvent for the polymer analyzed. Second, the “good” solvent should be enriched at the cold wall as a result of thermal diffusion [17]. While several “successful” mixtures for polystyrenes with  $M_r \geq 23$  kDa have been experimentally studied, there has been very little work done on low  $M_r$  polymers (<15 kDa).

In this study, we systematically investigate various approaches of increasing retention with the specific

goal of lowering the  $M_r$  limit of ThFFF to <15 kDa using conventional equipment. While previous studies have demonstrated discernible retention of PS polymers as low as 600 and 2500 Da [11,17], these studies either employed special equipment or did not optimize conditions with a focus on lowering the  $M_r$  limit. The objectives of the present study are to use a conventional ThFFF system to: (1) examine the effect of  $\Delta T$  and  $T_c$  on retention and compare combining theoretical and experimental results over a wide range of temperature conditions and (2) use binary solvent mixtures to increase retention of low  $M_r$  polymers. Commonly used theoretical equations and assumptions that are applicable to well-retained polymers are evaluated for the case of less retained low  $M_r$  polymers in the presence of high-temperature gradients. We also attempt to empirically correlate physical properties of the solvent mixture with ThFFF retention.

## 2. Theory

The basic ThFFF equation defines the retention parameter  $\lambda$  in the presence of a linear temperature profile as [18]:

$$\lambda = \frac{D}{D_T \Delta T} \quad (1)$$

where  $\lambda$  is the ratio of thickness of the equilibrium sample layer  $\ell$  to the channel thickness  $w$ ,  $D$  is the molecular diffusion coefficient of a polymer,  $D_T$  is the thermal diffusion coefficient of a polymer, and  $\Delta T$  is the temperature drop between the hot and cold walls. Deviation of the temperature profile from a linear function is relatively small [19]. Assuming a parabolic velocity profile,  $\lambda$  is related to the retention ratio  $R$  by the equation [20]:

$$R = \frac{t^0}{t_r} = 6\lambda[\coth(1/2\lambda) - 2\lambda] \quad (2)$$

where  $t^0$  is the void time and  $t_r$  is the retention time. Different forms of the expression for  $R$  range from simpler approximations to more complex equations that correct for nonideal effects [19,20]. For example, a more accurate equation that takes into account

the variation in solvent viscosity with temperature across the channel thickness [21]:

$$R = 6\lambda\{\nu + (1 - 6\lambda\nu)[\coth(1/2\lambda) - 2\lambda]\} \quad (3)$$

is particularly important when high-temperature gradients are employed. The velocity profile distortion factor  $\nu$  is a complex function of  $\Delta T$  and  $T_c$  (units of °C) and has been numerically computed for several solvents [19] as:

$$\nu = (a_1 T_c + a_2) \Delta T + (a_3 T_c + a_4) \Delta T^2 + (a_5 T_c + a_6) \Delta T^3 \quad (4)$$

For the commonly used ThFFF carrier liquid, tetrahydrofuran,  $a_1 = 2.3140 \times 10^{-5}$ ,  $a_2 = -4.0363 \times 10^{-3}$ ,  $a_3 = -1.3965 \times 10^{-7}$ ,  $a_4 = 1.6848 \times 10^{-5}$ ,  $a_5 = 4.3405 \times 10^{-10}$ , and  $a_6 = -4.2795 \times 10^{-8}$ . Several  $R(\lambda)$  expressions, including approximations and those described by Eqs. (2) and (3), are plotted in Fig. 1. It is evident that only Eqs. (2) and (3) are applicable to poorly retained species ( $R \geq 0.7$ ). Moreover, Eq. (3) is preferable when high-temperature gradients ( $\Delta T \geq 100$  K) are employed as errors  $\geq 10\%$  can be incurred if deviations in solvent viscosity are not taken into account. Based on these observations, Eq. (3) is used throughout this work to calculate  $R$ .

In order to accurately determine  $\lambda$  (Eq. (1)), and subsequently the retention ratio for each  $M_r$ ,  $D/D_T$  of a polymer should be calculated at the local temperature of the sample zone rather than at the temperature of the cold wall [22,23]. This is especially important for poorly retained species whose equilibrium posi-

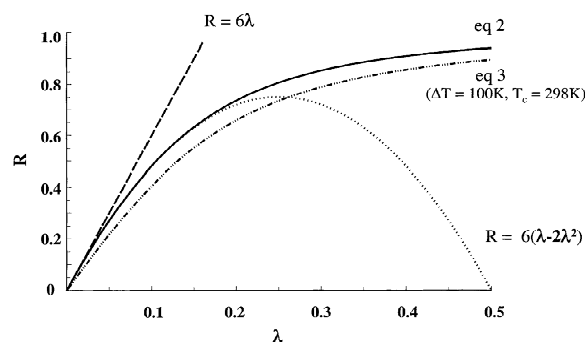


Fig. 1. Comparison of different equations for retention ratio as a function of  $\lambda$ .

tions are significantly above the cold wall and whose average temperatures are considerably higher than  $T_c$ . This discrepancy becomes further enhanced when high-temperature gradients are used. A more accurate treatment is to use temperature  $T_\ell$  at position  $\ell$ , which approximately corresponds to the center of gravity of a sample zone in basic ThFFF theory, rather than  $T_c$ . The  $T_\ell$  values corresponding to different  $\lambda$ s can be estimated using the equation [22]:

$$T_\ell = \lambda \Delta T + T_c \quad (5)$$

For example, if  $T_c = 20^\circ\text{C}$ ,  $\Delta T = 100^\circ\text{C}$ , and  $\lambda = 0.1$ , the  $T_\ell$  equal to  $30^\circ\text{C}$  will be used for  $D/D_T$  calculation.

Martin et al. [24] have introduced a ThFFF model to account for variations in the field-induced force, and hence  $\lambda$ , across the channel thickness for the case of high retention. This model allows for a more accurate determination of the equilibrium position and temperature of a sample zone. Unfortunately, this model is not applicable to the low retention region that is the focus of our present study.

The next step is to obtain  $D/D_T$  at the temperature  $T_\ell$ . As a first approximation, it can be expressed by the equation [23]:

$$\frac{D}{D_T} = \left( \frac{D}{D_T} \right)_{T_0} \left( \frac{T_\ell}{T_0} \right)^2 \quad (6)$$

where  $T_0$  is a certain temperature where  $D/D_T$  is known, i.e.  $T_c$ .

Eqs. (5) and (6) are substituted into Eq. (1) to give the following expression for  $\lambda$  that accounts for the local temperature of the sample zone:

$$\lambda = \left( \frac{D}{D_T} \right)_{T_c} \frac{(\lambda \Delta T + T_c)^2}{T_c^2 \Delta T} \quad (7)$$

This formula can be rearranged to a quadratic equation, which can be solved analytically.

For the majority of ThFFF conditions, where  $\lambda < 0.5$  and  $T_c > \Delta T$  (in K), Eq. (7) can be reduced to a simpler and more usable form:

$$\lambda \approx \frac{\lambda_{T_c}}{1 - \frac{2}{T_c} \left( \frac{D}{D_T} \right)_{T_c}} \quad (8)$$

where  $\lambda_{T_c}$  is the retention parameter determined at the temperature of the cold wall,  $T_c$ :

$$\lambda_{T_c} = \frac{1}{\Delta T} \left( \frac{D}{D_T} \right)_{T_c} \quad (9)$$

By combining Eqs. (3) and (7) (or (8)), the retention parameter  $\lambda$  and retention ratio  $R$  can be theoretically determined over a wide range of temperature and retention levels. The only experimental data required are molecular and thermal diffusion coefficients at a single temperature. The molecular diffusion coefficient of polystyrene in tetrahydrofuran (THF) at 298 K can be calculated using the relationship obtained by size-exclusion chromatography [3]:

$$D = \frac{3.861 \times 10^{-4}}{M^{0.571}} \quad (10)$$

where  $M$  is the molecular mass of the polymer in Da and  $D$  has units of  $\text{cm}^2/\text{s}$ . The thermal diffusion coefficient of polystyrene in THF is  $\sim 10^{-7} \text{cm}^2/\text{s}$  per K and is approximately independent of molecular mass [3,23,24].

Calculations of  $R$  and  $\lambda$  using Eqs. (3) and (7) will be compared with experimental data and discussed in a subsequent section of this paper.

### 3. Experimental

The ThFFF channel consisted of two copper blocks with highly polished nickel-coated internal surfaces and a PTFE-coated polyimide spacer (Fralock, Canoga Park, CA, USA) with the FFF channel shape removed. The channel was 2 cm in breadth, 27.4 cm tip-to-tip in length, 127  $\mu\text{m}$  in thickness, and had a void volume of  $0.62 \pm 0.02$  ml. Heat was supplied to the upper block using 14 equally spaced 250 W heating rods inserted perpendicular to the channel length. This design has led to a more uniform temperature along the length of the channel. Computer-controlled temperature drops of 80–110 K could be maintained between the hot and cold walls to within  $\pm 1$  K. Heat flux was removed from the cold wall using a refrigerated recirculator (Model CFT-75, Neslab, Portsmouth, NH, USA). The temperature of the cold wall was maintained in the range of 295–302 K ( $\pm 0.5$  K). A six-port valve

Table 1  
Summary of polystyrene standards' molecular mass information

Number	Company	$M_i$ data (kDa)	
		Manufacturer	MALDI-TOF MS <sup>a</sup>
1	Waters	$M_p = 2.0$ ; $M_w/M_n < 1.009$	$M_p = 2.6$ ; $M_w/M_n = 1.011$
2	Supelco	$M_p = 2.7$ ; $M_w/M_n < 1.09$	$M_p = 2.8$ ; $M_w/M_n = 1.09$
3	Waters	$M_p = 5.0$ ; $M_w/M_n < 1.009$	$M_p = 4.4$ ; $M_w/M_n = 1.009$
4	Polymer	$M_p = 11.3$ ; $M_w/M_n = 1.02$	$M_p = 11.2$ ; $M_w/M_n = 1.01$

<sup>a</sup> Dithranol matrix, silver cation, and dried-drop sample deposition [25].

(Rheodyne L.P., Rohnert Park, CA, USA) with a 50- $\mu$ l loop was partially filled with 10–30  $\mu$ l containing 2–10  $\mu$ g of sample. The relative standard deviation (RSD) for retention time measurements was less than 2%. An HPLC pump Series II (LabAlliance, State College, PA, USA) supplied the carrier liquid. The flow-rate was 0.1 ml/min if not otherwise stated. An elevated pressure was maintained in the channel using a 100-psi back-pressure regulator (Upchurch Scientific, Oak Harbor, CA, USA) to prevent the solvent from boiling. The SPD-6A UV spectrophotometric detector (Shimadzu Scientific, Columbia, MD, USA) or Optilab 903 interferometric RI detector (Wyatt Technology, Santa Barbara, CA, USA) was used to monitor peak elution. Data acquisition and system control were provided by Thermal140 software (PostNova Analytics, Salt Lake City, UT, USA).

The polystyrene standards (Waters, Milford, MA, USA; Supelco, Bellefonte, PA, USA; Polymer Laboratories, Amherst, MA, USA) used in this study are

listed in Table 1 along with manufacturers' specifications. These standards were further analyzed using matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Voyager-DE STR, Applied Biosystems, Framingham, MA, USA) and the results summarized in Table 1. Poly32 software (Sierra Analytics, Modesto, CA, USA) was used for data analysis. The MALDI-TOF sample was prepared using a dithranol matrix, silver cation, and dried-drop sample deposition [25]. Values for the most probable molecular mass,  $M_p$ , are included for comparison with nominal values. Properties of the organic solvents used in ThFFF experiments (Fisher, Pittsburgh, PA, USA; Aldrich Chemical, Milwaukee, WI, USA) are summarized in Table 2.

The solvent mixture viscosity was measured at 293 K ( $\pm 0.2$  K) with an Ubbelohde viscometer (size 25, Cannon Instruments, State College, PA, USA). In order to decrease the effect of solvent evaporation, the solvent mixture was kept in an airtight bottle at

Table 2  
Characteristics of solvents used in ThFFF experiments

Solvent	Boiling point (°C)	Density (g/cm <sup>3</sup> )	Grade	Source
<i>n</i> -Heptane <sup>a</sup> (HEP)	98.4	0.684 [20]	HPLC	Fisher
Dodecane (DOD)	215–217	0.749 [25]	99+%	Aldrich
1,4-Dioxane (DIO)	100–102	1.034 [25]	HPLC	Aldrich
Tetrahydrofuran <sup>a</sup> (THF)	67	0.889 [20]	HPLC	Fisher
Trichloroethylene (TCE)	86.7	1.463 [25]	99.5+%	Aldrich
Cyclohexane (CYH)	80.7–81	0.779 [25]	HPLC	Aldrich
Decahydronaphthalene ( <i>cis</i> and <i>trans</i> ) (DEC)	189–191	0.896 [25]	99+%	Aldrich
Toluene <sup>a</sup> (TOL)	110.6	0.867 [20]	anhydrous HPLC	Fisher

<sup>a</sup> Physical properties were obtained from Ref. [34].

the same temperature as the viscometer and a fresh sample was used for each measurement. The RSD of viscosity determinations was less than 3%.

#### 4. Results and discussion

The effect of field strength and cold wall temperature on retention were theoretically calculated and plotted in Figs. 2 and 3 for two different molecular mass (4.4 and 11.2 kDa) polystyrenes in tetrahydrofuran (THF). The polymers are more retained, as reflected by decreasing  $\lambda$  and  $R$  values, with increasing  $\Delta T$  and decreasing  $T_c$ . Figs. 2 and 3 also demonstrate the importance of using the temperature of the sample zone  $T_\ell$  rather than the temperature of the cold wall  $T_c$  to calculate  $\lambda$  and  $R$ . As noted in the theory section, low  $M_r$  polymers form diffuse

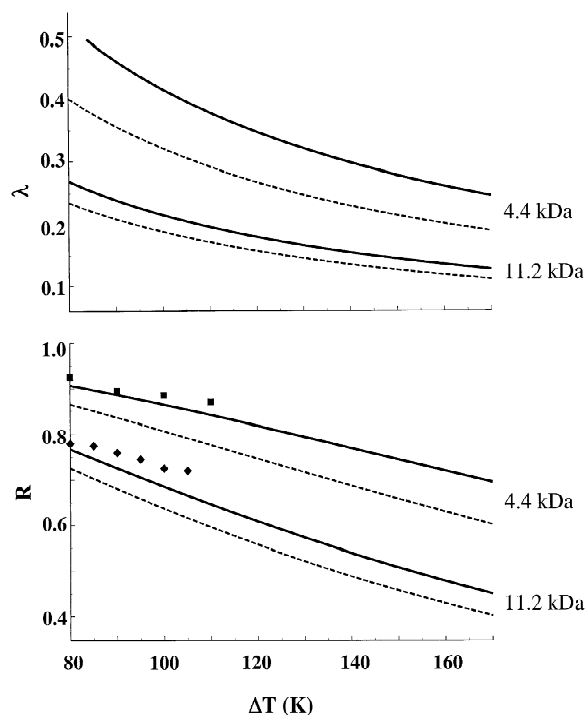


Fig. 2. Theoretical and experimental dependence of the retention parameter  $\lambda$  and retention ratio  $R$  on the temperature drop  $\Delta T$  at a cold wall temperature  $T_c = 298$  K. In all cases, Eq. (3) is used to calculate  $R$ . Eq. (7) using  $T_\ell$  (solid lines) and Eq. (9) using  $T_c$  (dashed lines) are used to calculate  $\lambda$ . Experimental data points are shown for 4.4 and 11.2 kDa PS. The injected sample volume is 10  $\mu\text{l}$ .

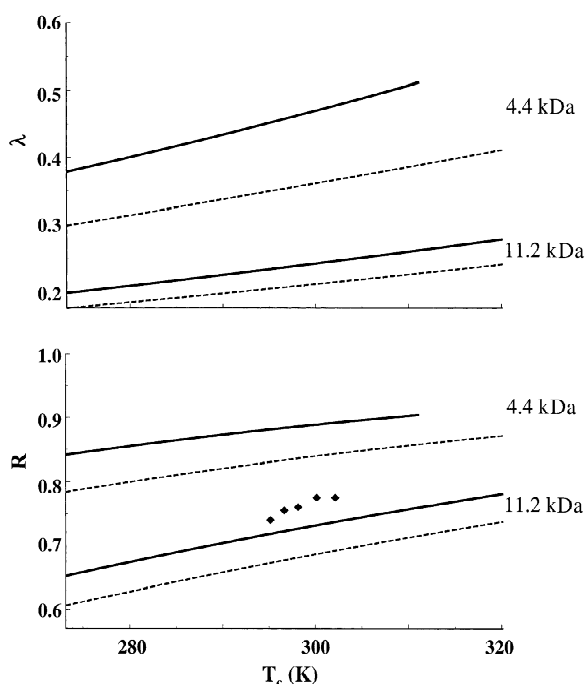


Fig. 3. Theoretical and experimental dependence of  $\lambda$  and  $R$  on the temperature of the cold wall  $T_c$  at  $\Delta T = 90$  K. In all cases, Eq. (3) is used to calculate  $R$ . Eq. (7) using  $T_\ell$  (solid lines) and Eq. (9) using  $T_c$  (dashed lines) are used to calculate  $\lambda$ . Experimental data are shown for 11.2 kDa PS. The injected sample volume is 10  $\mu\text{l}$ .

equilibrium layers that have significant average distances from the cold wall. The use of the cold wall temperature to calculate  $\lambda$  can lead to significant errors (25% for PS 4.4 kDa,  $T_c = 300$  K,  $\Delta T = 90$  K). Larger errors are observed for lower molecular masses. The  $\lambda$  values calculated at  $T_\ell$  and  $T_c$  (using Eqs. (7) and (9), respectively) were substituted into Eq. (3) for the calculation of  $R$ . It should be mentioned that over the temperature range studied the simplified Eq. (8) yields  $\lambda$  values that are  $\leq 2\%$  different from values obtained by Eq. (7). Hence, Eq. (8) can also be used, especially for practical calculations and discussion. The symbols shown in Figs. 2 and 3 are experimental data points. While these experimental results follow the trend of the theoretical plots, the absolute values are generally higher (1–4% for 4.4 kDa and 2–8% for 11.2 kDa PS) than predicted by the proposed equations. This positive deviation and its slight increase with increasing  $\Delta T$  and  $T_c$  may be due to a stronger

temperature dependence of  $D/D_T$  than the square function assumed in Eq. (6). Underestimation of the sample's local temperature (Eq. (5)) could be another possible explanation for the observed discrepancy.

Figs. 2 and 3 give good estimates of the ThFFF conditions necessary to achieve moderate polymer retention ( $R \leq 0.5$ ) in tetrahydrofuran. For example,  $\Delta T$  of 150 K and  $T_c$  of 298 K would be used to obtain an  $R \sim 0.5$  for the 11.2 kDa PS. Alternately, a cold wall temperature below 275 K at  $\Delta T$  of 90 K could be used. Higher  $\Delta T$ s and lower  $T_c$ s would be needed to retain polymers with  $M_r$ s less than 11.2 kDa. Unfortunately, these temperature-based approaches are limited by: (1) the instrumentation with commercially available systems having a maximum  $\Delta T$  of  $\sim 110$  K and cold wall temperature limited by a recirculator cooling capacity and (2) limited polymer solubility at low  $T_c$ s, especially in solvents such as cyclohexane and decahydronaphthalene [26]. The range of the data points shown in Figs. 2 and 3 and the lack of data points for 4.4 kDa PS in Fig. 3 reflect these practical limitations.

The next part of this study was to evaluate the effect of solvent composition on the retention of low  $M_r$  polymers. First, the retention of 11.2 kDa PS was studied in several commonly used single-component solvents. The difference in retention for toluene and THF was insignificant over the  $\Delta T$  and  $T_c$  range studied (Fig. 4). Pure dioxane yielded similar retention to that observed in pure THF (data point at volume fraction of poor solvent equal to zero in Fig. 5). Use of the theta-solvent decahydronaphthalene resulted in 15–20% higher retention (Fig. 4). A 15% increase was also obtained for another theta-solvent cyclohexane in comparison to THF (one data point in Fig. 5). The higher retention induced by these theta-solvents in comparison to THF has also been observed for 23 kDa polystyrene [15]. Although the effect was not considerable, these results indicate that theta-solvents may be useful for increasing retention of lower  $M_r$  polymers.

As a result of the limited influence of  $\Delta T$ ,  $T_c$ , and single-component solvents on polymer retention, a study of binary solvent mixtures was carried out. It has been shown that certain binary mixtures can produce a dramatic retention enhancement for higher  $M_r$  polystyrenes [15–17]. Rue and Schimpf [17]

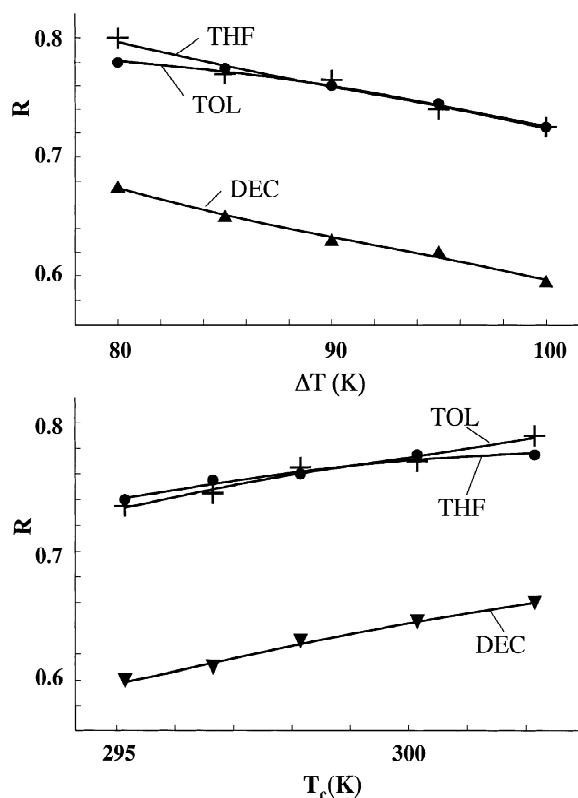


Fig. 4. Retention ratio of 11.2 kDa PS as a function of  $\Delta T$  ( $T_c = 298$  K) and  $T_c$  ( $\Delta T = 90$  K). The solvent abbreviations are tetrahydrofuran (THF), toluene (TOL), and decahydronaphthalene (DEC).

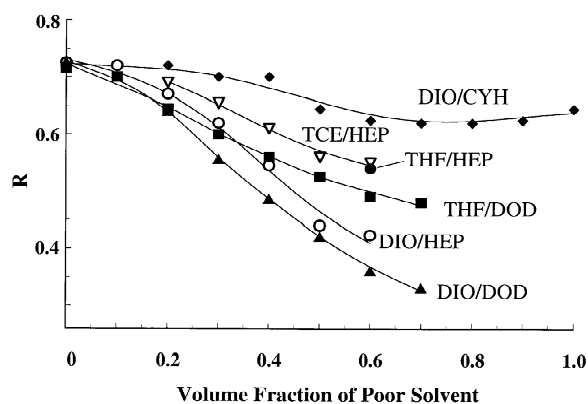


Fig. 5. Effect of the binary mixture composition on 11.2 kDa PS retention at  $\Delta T = 100$  K,  $T_c = 297$  K. The solvent abbreviations are cyclohexane (CYH), dioxane (DIO), dodecane (DOD), heptane (HEP), trichloroethylene (TCE), and tetrahydrofuran (THF).

have suggested a mechanism whereby solvent molecules undergo thermal diffusion with a net result of solvent partitioning in the ThFFF channel. If the “good” solvent partitions towards the cold wall, an additional driving force is exerted on the polymer molecules leading to enhanced retention. If the “good” solvent partitions towards the hot wall, polymer retention will be reduced. The key is to select solvent mixtures that consist of a “good” and “poor” solvent with the former undergoing thermal diffusion to the cold wall.

Several binary mixtures have been used in our low  $M_r$  study, three of which (THF/dodecane, dioxane/heptane, and dioxane/cyclohexane) have been previously demonstrated to increase the retention of higher  $M_r$  PS polymers [15–17]. New mixtures not previously studied include THF/heptane, dioxane/dodecane, and trichloroethylene/heptane. In the new set of solvent mixtures, the first two were chosen based on permutations of the previously used “successful” THF/dodecane and dioxane/heptane mixtures. The effect of binary solvent mixtures on retention of 11.2 kDa PS is shown in Fig. 5 with the data plotted as retention ratio  $R$  versus the volume fraction of poor solvent. The volume fraction was a direct reflection of the volumes of individual components. For example, a volume fraction of 0.5 means that equal volumes of good and poor solvent components were mixed. We have assumed that the volume of the mixture for solvents studied did not differ significantly from the sum of the volumes of the components [27,28].

In many cases, it was not possible to conduct studies at volume fraction of poor solvents  $>0.6$ – $0.7$  because of polymer precipitation. The exception was the dioxane/cyclohexane mixture where the second component, cyclohexane, is a theta-solvent for polystyrene. Retention is enhanced in all mixtures, but to different magnitudes. The least effective mixture, dioxane/cyclohexane gives  $R$  values similar to those obtained in pure THF. The 30% dioxane/70% dodecane mixture yielded the largest increase of 2.1 times compared to pure dioxane. It is interesting to note that all mixtures containing heptane or dodecane, non-solvents for PS, provided increased retention with increasing volume fraction. The ThFFF fractograms presented in Fig. 6 show significant improvement of 11.2 kDa PS retention time in binary mixtures compared to pure THF.

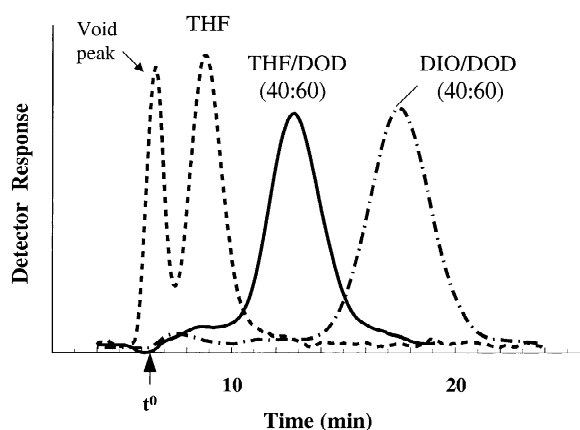


Fig. 6. Fractograms of 11.2 kDa PS obtained in different solvents at  $\Delta T = 100$  K,  $T_c = 297$  K. Solvent abbreviations are the same as in the Fig. 5 caption.

The effect of solvent density on retention was investigated by mixing heptane with “good” solvents of different densities, e.g. trichloroethylene ( $1.46 \text{ g/cm}^3$ ), dioxane ( $1.03 \text{ g/cm}^3$ ), and THF ( $0.89 \text{ g/cm}^3$ ). Significantly lower retention was observed in the higher density trichloroethylene/heptane mixture. Rue and Schimpf [17] have reported that higher density good solvents tend to partition towards the cold wall (but have not observed a direct link between “good” solvent density and polymer retention). If the density of the good solvent was the major determinant of retention, a higher retention would have been observed for the higher density trichloroethylene/heptane mixture. As this is not the case, it appears that there are additional unidentified factors that affect polymer retention in binary solvents. It should be mentioned that channel orientation has no effect on polymer retention in homogeneous solvents and solvent mixtures [29]. An additional interesting note is that further examination of the data presented in Fig. 5 reveals that within the same solvent family, i.e. cyclic ethers, the higher density dioxane produced higher retention than lower density THF in mixtures with both heptane and dodecane. These preliminary observations of the effect of solvent density on ThFFF retention suggest that more in-depth studies are needed.

The observed significant effect of solvent mixture composition on PS retention has led us to further correlate ThFFF retention mechanism with physical properties of mixtures rather than those of single-



component solvents. Solvent viscosity is an important parameter whose effect on  $D$  and  $D_T$  (and hence retention) have been reported in studies with pure solvents [2,16]. It follows that viscosity effects will also be observed in binary solvent mixtures as the mixing of two different liquids can significantly affect intermolecular interactions. Physical parameters, such as the mixture viscosity, are very sensitive to this effect [30,31]. A reduction in viscosity is associated with decreasing solvent–solvent interactions. For example, a dioxane/cyclohexane mixture with 0.1–0.5 mol fraction of dioxane has a lower viscosity than that of each individual component [32]. Due to a lack of reference data for the binary mixtures studied (except dioxane/cyclohexane), viscosity values were obtained experimentally using a Ubbelohde tube. Fig. 7 shows the relationship between dynamic viscosity and the volume fraction of the poor solvent for different mixtures. Linear relationships were observed for trichloroethylene/heptane and THF/heptane while concave relationships were obtained for other mixtures. The latter is a result of a negative deviation from the linear volume additive function of composition ( $\eta_{\text{add}} = v_1\eta_1 + v_2\eta_2$ ). Here,  $v$  is the volume fraction and  $\eta$  is the dynamic viscosity of the solvent component. Fig. 7 shows no apparent correlation between  $\eta$  and retention. A more revealing picture is given in Fig. 8 where the relative viscosity deviation from the volume additive function is plotted against volume fraction of a poor solvent. The mixtures, showing negative deviation from the additive viscosity func-

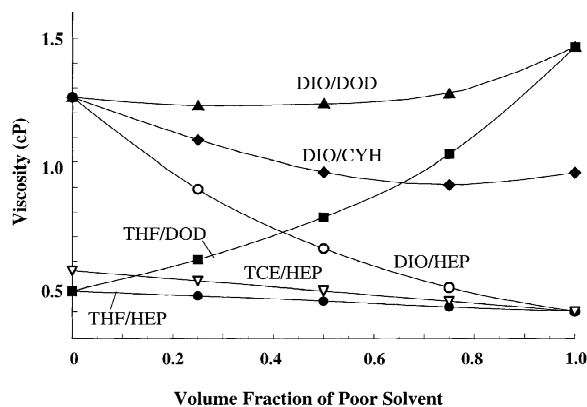


Fig. 7. Dependence of solvent mixture viscosity on composition ( $T=293$  K). Solvent abbreviations are the same as in the Fig. 5 caption.

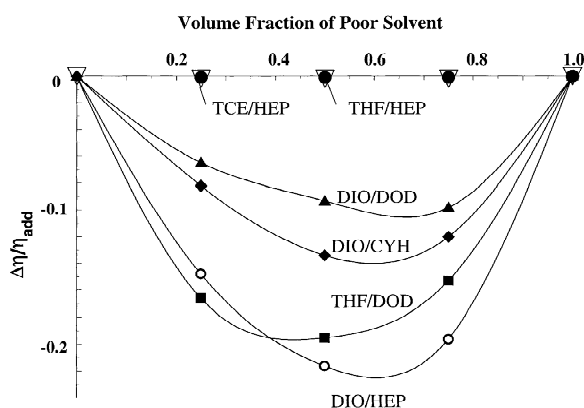


Fig. 8. Deviation of solvent mixture viscosity from the volume additive function. Solvent abbreviations are the same as in the Fig. 5 caption.

tion (except dioxane/cyclohexane), generate the highest effect on the polystyrene retention. One possible explanation for this correlation is that the decrease in interactions between the two solvent components in the mixture (reflected by the lower mixture viscosity) allows stronger interactions between the “good” solvent and the polymer molecule. These stronger “good” solvent–polymer interactions (providing that the “good” solvent partitions towards the cold wall) result in a stronger driving force on the polymer and higher retention. The fact that all mixtures studied provide polystyrene retention enhancement indicates that the “good” solvent component partitions toward the cold wall [17]. Interestingly, even the behavior of the apparent exception to this rule, the dioxane/cyclohexane mixture (which shows a significant decrease in viscosity but only a moderate effect on PS retention) can be explained using this approach. In comparison to non-solvents such as heptane and dodecane, a theta-solvent cyclohexane possesses much stronger interactions with a polymer molecule. Consequently, as cyclohexane redistributes towards the hot wall in the dioxane/cyclohexane mixture under the action of thermal diffusion [17], it diminishes the positive effect of dioxane–polymer interactions on retention. This viscosity study supports the findings from previous studies that thermal diffusion is linked with monomer–solvent interaction energies and the solvent’s activation energy for viscous flow [17,33].

The sizeable effect of binary mixtures on ThFFF retention enabled us to approach the task of retaining

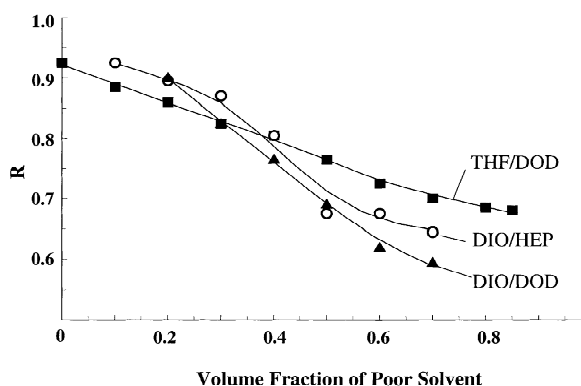


Fig. 9. Retention of 2.8 kDa PS in binary mixtures at  $\Delta T=110$  K,  $T_c=299$  K. Solvent abbreviations are the same as in the Fig. 5 caption.

very low  $M_r$  polymers. Three binary solvent mixtures that induced the highest retention were chosen for ThFFF of a 2.8 kDa PS standard. Results of this study (Fig. 9) show that significant gains in retention could also be realized for this low  $M_r$  PS when binary mixtures were used. A 1.5-fold increase in retention was obtained for a 30% dioxane/70% dodecane mixture. Finally, the mass selectivity  $S_M$  (defined as  $|d \ln R/d \ln M|$ ) is higher for separations using binary mixtures than for pure solvents. Using two  $M_r$ s, 2.8 kDa and 11.2 kDa,  $S_M$  was calculated as 0.18 and 0.28 for pure THF and 40% THF/60% dodecane, respectively.  $S_M$  was even higher for dioxane mixtures: 0.34 for 40% dioxane/60% hep-

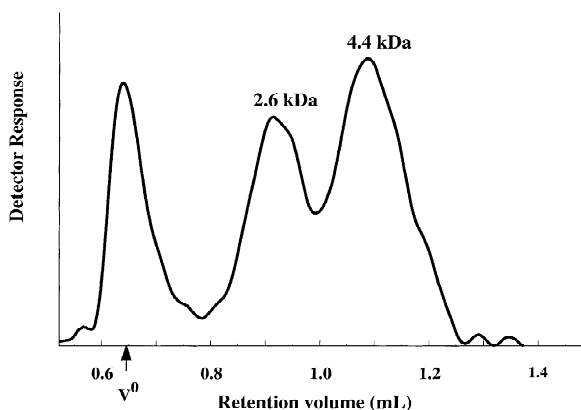


Fig. 10. Separation of PS 2.6 and 4.4 kDa in 15% tetrahydrofuran/85% dodecane at  $\Delta T=110$  K,  $T_c=299$  K, and a flow-rate of 0.03 ml/min. Solvent abbreviations are the same as in the Fig. 5 caption.

tane and 0.39 for 40% dioxane/60% dodecane. The observed increase in mass selectivity is an indication of enhanced resolution for this  $M_r$  range. Using the 15% THF/85% dodecane mixture, we were able to retain and resolve two polymer standards with  $M_r$  of 2.6 kDa and 4.4 kDa (Fig. 10) under moderate temperature conditions and, hence, extend the lower  $M_r$  limitation of ThFFF.

## 5. Conclusions

Theoretical and experimental ThFFF studies showed that the most effective way to increase retention of polymers of  $M_r < 15$  kDa is to use specially chosen binary solvent mixtures. Using this approach, polymers with  $M_r$  as low as 2.6 kDa were retained and separated from 4.4 kDa under the moderate temperature conditions available in commercial ThFFF systems. These results extend the applicability of ThFFF to an exceptionally wide  $M_r$  range spanning  $10^3$  to  $10^7$  Da using a single channel. The full advantages gained by combining ThFFF separations with absolute detection methods, such as MALDI–TOF MS, can now be realized for  $M_r$  and chemical composition analysis of polymers.

ThFFF analysis in binary mixtures requires further extensive investigation for different polymer classes. At present, the search for mixtures that enhance retention of each particular polymer is done on an empirical basis. The observation that viscosity of the solvent mixture can be correlated with ThFFF retention presents a possible key parameter in selecting mixtures that would provide the highest polymer retention. More retention versus viscosity experiments for different polymer classes and solvent mixtures are required to further support our findings.

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**References**

- [1] J.C. Giddings, *Science* 260 (1993) 1456.
- [2] M.E. Schimpf, J.C. Giddings, *Macromolecules* 20 (1987) 1561.
- [3] M.E. Schimpf, J.C. Giddings, *J. Polym. Sci. Part B Polym. Phys.* 27 (1989) 1317.
- [4] J.J. Gunderson, J.C. Giddings, *Macromolecules* 19 (1986) 2618.
- [5] A.C. van Asten, R.J. Van Dam, W.Th. Kok, R. Tijssen, H. Poppe, *J. Chromatogr. A* 703 (1995) 245.
- [6] S.J. Jeon, M.E. Schimpf, in: T. Provder (Ed.), *Chromatography of Polymers: Hyphenated and Multidimensional Techniques*, ACS Symposium Series, American Chemical Society, Washington, DC, 1999, p. 731.
- [7] K.-H. Cho, Y.H. Park, S.J. Jeon, W.S. Kim, D.W. Lee, *J. Liq. Chromatogr. Relat. Technol.* 20 (1997) 2741.
- [8] J.C. Giddings, Y.H. Yoon, M.N. Myers, *Anal. Chem.* 47 (1975) 126.
- [9] J.J. Gunderson, J.C. Giddings, *Anal. Chim. Acta* 189 (1986) 1.
- [10] M.N. Myers, K.D. Caldwell, J.C. Giddings, *Sep. Sci.* 9 (1974) 47.
- [11] J.C. Giddings, L.K. Smith, M.N. Myers, *Anal. Chem.* 47 (1975) 2389.
- [12] S.L. Brimhall, M.N. Myers, K.D. Caldwell, J.C. Giddings, *Sep. Sci. Technol.* 16 (1981) 671.
- [13] M.N. Myers, W. Cao, C.I. Chen, V. Kumar, J.C. Giddings, *J. Liq. Chromatogr. Relat. Technol.* 20 (1997) 2757.
- [14] C. Van Batten, M. Hoyos, M. Martin, *Entropie* 217 (1999) 61.
- [15] R.M. Sisson, J.C. Giddings, *Anal. Chem.* 66 (1994) 4043.
- [16] J.J. Kirkland, L.S. Boone, W.W. Yau, *J. Chromatogr.* 517 (1990) 377.
- [17] C.A. Rue, M.E. Schimpf, *Anal. Chem.* 66 (1994) 4054.
- [18] M.E. Schimpf, in: M.E. Schimpf, K.D. Caldwell, J.C. Giddings (Eds.), *FFF Handbook*, Wiley, New York, 2000, p. 239.
- [19] J.E. Belgaied, M. Hoyos, M. Martin, *J. Chromatogr. A* 678 (1994) 85.
- [20] J.C. Giddings, *J. Chem. Educ.* 50 (1973) 667.
- [21] M. Martin, J.C. Giddings, *J. Phys. Chem.* 85 (1981) 727.
- [22] A.C. van Asten, H.F.M. Boelens, W.Th. Kok, H. Poppe, P.S. Williams, J.C. Giddings, *Sep. Sci. Technol.* 29 (1994) 513.
- [23] W. Cao, P.S. Williams, M.N. Myers, J.C. Giddings, *Anal. Chem.* 71 (1999) 1597.
- [24] M. Martin, C. Van Batten, M. Hoyos, *Anal. Chem.* 69 (1997) 1339.
- [25] G.E. Kassalainen, S.K.R. Williams, *Anal. Chem.*, submitted.
- [26] J.E. Mark (Ed.), *Physical Properties of Polymers Handbook*, AIP Press, Woodbury, NY, 1996.
- [27] A. Inglese, J.-P.E. Groller, E. Wilhelm, *J. Chem. Eng. Data* 28 (1983) 124.
- [28] O.B. Rudakov, M.I. Sokolov, V.F. Selemenev, *Zh. Fiz. Khim.* 73 (1999) 1641.
- [29] J. Xu, C.A. Rue, M.E. Schimpf, *J. Liq. Chromatogr. Relat. Technol.* 20 (1997) 2703.
- [30] R.J. Martins, M.J.E. de M. Cardoso, O.E. Barcia, *Ind. Eng. Chem. Res.* 39 (2000) 849.
- [31] A.A. Abramson, M.B. Petel'skii, *Zh. Prikl. Khim.* 71 (1998) 1282.
- [32] S.L. Oswal, R.P. Phalak, *Int. J. Thermophys.* 13 (1992) 251.
- [33] M.E. Schimpf, S.N. Semenov, *J. Phys. Chem. B* 104 (2000) 9935.
- [34] *CRC Handbook of Chemistry and Physics*, 71st Edition, CRC Press, Boca Raton, FL, 1990.