

Coupling Thermal Field-Flow Fractionation with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for the Analysis of Synthetic Polymers

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Thermal field-flow fractionation (ThFFF) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) have been coupled to yield a powerful combination of techniques for polymer analysis. Thermal FFF's high molecular weight (MW) selectivity and sensitivity to chemical composition are used to separate polydisperse polymers and polymer mixtures into the narrow polydispersity and homogeneous chemical composition fractions essential for MALDI-TOFMS analyses. On the other hand, MALDI-TOFMS's ability to directly measure molecular weight alleviates the need for polymer standards for ThFFF. In this first-time coupling of ThFFF and MALDI-TOFMS, compatibility issues were addressed and optimum conditions and procedures were identified and developed to maximize the capabilities of the combined technique. Depending on the polymer MW and the method of MALDI sample deposition, fractions from 1–10 ThFFF runs were combined for MALDI-TOFMS analysis. Binary solvents were used to enhance ThFFF retention and resolution of low-MW (<15-kDa) polymers, and methods were developed to allow routine MALDI-TOFMS analyses of polystyrene polymers up to 575 kDa. Overall, the MW compatibility of the two techniques was extended from several kilodaltons to several hundred kilodaltons. Polymer fractions were collected after separation by ThFFF and analyzed either by MALDI-TOFMS or reinjection into the ThFFF system. Good agreement was observed between the MW distribution data obtained by MALDI-TOFMS and ThFFF. The application of ThFFF/MALDI-TOFMS to polydisperse polymers and polymer mixtures was demonstrated. This combined technique was also shown to be a viable means for preparing standards from the original polymer sample.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) has become a widely used method of polymer analysis.^{1–6} For narrow polydispersity polymers, it is

capable of directly measuring molecular weight (MW) and molecular weight distribution (MWD). For lower-MW polymers, it can also provide resolution of the oligomers and, hence, information about polymer chemical composition. However, in the case of wide polydispersity (PD > 1.2) polymers, MALDI-TOFMS experiences significant mass discrimination for the higher MW components and can consequently provide erroneous MW information.^{2,3,7–12} Selective signal loss is also encountered in the analysis of samples composed of polymers with different chemical composition because of their significant differences in ionization probability and other discrimination effects. For instance, preferential desorption of poly(methyl methacrylate) has been observed for mixtures of polystyrene and poly(methyl methacrylate).² Generally, MALDI-TOFMS analyses of wide-polydispersity polymers and polymer mixtures require preliminary fractionation of the sample according to MW or chemical composition. Since the mid-1990s, size-exclusion chromatography has been used often to obtain narrow polydispersity fractions for MALDI-TOFMS analysis.^{3,13–17} However, because of a dearth of capable separation methods, only a few studies have addressed fractionation by chemical composition prior to MALDI.^{18–20}

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Table 1. Summary of Nominal and MALDI-TOFMS Data for Polystyrene Standards

no.	company	MWD data (kDa)	
		manufacturer	MALDI -TOFMS ^a
1	Polymer Lab.	$M_p = 11.3; M_w/M_n = 1.02$	$M_p = 11.2; M_w/M_n < 1.01$
2	Polymer Lab.	$M_p = 28.5; M_w/M_n = 1.03$	$M_p = 26.7; M_w/M_n < 1.01$
3	Polymer Lab.	$M_p = 66.0; M_w/M_n = 1.03$	$M_p = 67.0; M_w/M_n < 1.01$
4	Pressure Chem.	$M_p = 90.0; M_w/M_n \leq 1.04$	$M_p = 87.0; M_w/M_n < 1.01$
5	Pressure Chem.	$M_p = 110; M_w/M_n < 1.06$	$M_p = 115; M_w/M_n < 1.01$
6	Polymer Lab.	$M_p = 170; M_w/M_n = 1.03$	$M_p = 167; M_w/M_n < 1.01$
7	Polymer Lab.	$M_p = 200; M_w/M_n = 1.02$	$M_p = 190; M_w/M_n < 1.01$
8	Polymer Lab.	$M_p = 290; M_w/M_n \leq 1.06$	$M_p = 288; M_w/M_n < 1.01$
9	American Polym. Stds	$M_p = 470; M_w/M_n = 1.05$	$M_p = 470; M_w/M_n < 1.01$
10	Pressure Chem.	$M_p = 575; M_w/M_n \leq 1.06$	$M_p = 575; M_w/M_n < 1.01$

^a For MW < 100 kDa, sample preparation I (dithranol matrix) was used. For MW > 100 kDa, sample preparation II (all-*trans*-retinoic acid) was used.

In this work, thermal field-flow fractionation (ThFFF) is used to separate polymers according to MW or chemical composition prior to MALDI-TOFMS. In addition to the sensitivity to chemical composition,^{21–25} ThFFF has other attractive features, such as the wide MW range that can be analyzed within a single channel,¹⁶ the open channel (devoid of packing material) that results in low shear forces and applications that extend to gels and particles,^{25–29} and a mass selectivity that is higher than that of size-exclusion chromatography (SEC) for MW > 50 kDa.³⁰ As with SEC, ThFFF requires calibration with MW standards or connection to an absolute detection method that measures molecular weight.

When ThFFF and MALDI-TOFMS are coupled, their complementary characteristics result in a powerful tool for MW and chemical composition characterization of complex polymer samples. For instance, ThFFF can separate the original sample into narrow polydispersity or more chemically homogeneous fractions. MALDI-TOFMS conditions can then be optimized for each fraction to obtain representative MWD data. Chemical information can also be extracted from the MALDI spectra of lower-MW fractions. Furthermore, the well-characterized fractions can subsequently be used as standards for the absolute calibration of ThFFF.

An important issue addressed in this first ThFFF/MALDI-TOFMS work is the compatibility between the two techniques, particularly with respect to sample amount, applicable MW range, ThFFF carrier liquid, and MALDI sample preparation, and ThFFF's ability to produce sufficiently narrow polydispersity fractions for subsequent MALDI analysis. Another important focus is the optimization of ThFFF and MALDI-TOF conditions for analyzing polymers over a wide range of molecular weights. For

ThFFF, the effect of binary solvents on retention and resolution of polymers is investigated further, and the findings are used in separations of low-MW polydisperse polymers and polymer mixtures. For higher MW polymers, different MALDI parameters, such as sample composition, sample deposition methods, and laser type, are examined with the objective of increasing the signal intensity and, thus, the accuracy of the MWD determination. Finally, the fractions collected from the separation of two polydisperse polymer samples are analyzed by MALDI-TOFMS and by reinjection into ThFFF, and MWD data obtained by both techniques are compared.

EXPERIMENTAL SECTION

ThFFF. The experimental ThFFF system used in this study has been described in detail elsewhere.³¹ The channel dimensions were 2 cm in breadth, 27.4 cm in length tip-to-tip, and 127 μm in thickness, with a resulting channel volume of 0.62 ± 0.02 mL. For all experiments, the flow rate was 0.1 mL/min. Temperature drops of 60–100 K were maintained between the hot and cold walls to within ± 1 K. The temperature of the cold wall was maintained in the range of 292–299 K (± 0.5 K). The relative standard deviation (RSD) of retention time measurements was <2%. A UV detector (SPD-6A, Shimadzu Corp., Columbia, MD) was used to monitor polymer elution from the channel. A 100 psi ultralow-volume back-pressure regulator (Upchurch Scientific, Oak Harbor, CA) was used to elevate the boiling point of the solvent.

The narrow polystyrene standards used in this study are listed in Table 1, along with the nominal MWs provided by manufacturers and the MWs measured in our laboratory using MALDI. Wide polydispersity polystyrene standards PSBR35K ($M_n = 14.5$ kDa, $M_w = 33.0$ kDa) and PSBR250K ($M_n = 100$ kDa, $M_w = 250$ kDa) were purchased from American Polymer Standards Corp. (Mentor, Ohio). Poly(2-vinylpyridine) standards with nominal MWs of 10.5 kDa and 110 kDa were obtained from Pressure Chemical Co. (Pittsburgh, Pa). The organic solvents used in this study were HPLC-grade tetrahydrofuran, *n*-heptane (Fisher Scientific Pittsburgh, PA), 1,4-dioxane, cyclohexane, and 99.5+% trichloroethylene (Aldrich, Milwaukee, WI).

Dynamic light-scattering data for 66- and 110-kDa polystyrenes in 40% dioxane/60% heptane were kindly provided by Particle

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Sizing Systems (Langhorne, PA) using a Nicomp 380 instrument. The hydrodynamic diameters were 13.8 and 19.1 nm, and the diffusion coefficients were 5.32 and $3.84 \times 10^{-11} \text{ m}^2/\text{s}$ for 66- and 110-kDa polystyrenes, respectively. The viscosity of the 40% dioxane/60% heptane solvent that was used for low-MW polymer separations was measured previously as 0.593 cP at a temperature of 293 K .³¹

MALDI-TOFMS. Mass spectra were acquired using a Voyager-DE STR+ MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA) in the linear mode if not otherwise stated. This instrument was equipped with nitrogen and Nd:YAG lasers, operating at wavelengths of 337 and 355 nm, respectively. The acceleration voltage was maintained at 25 kV. Data acquisition was performed using Voyager supporting software and Poly32 software (Sierra Analytics, Modesto, CA).

Two sample preparation procedures were used for polystyrene (PS) studies:

Sample preparation I, for $\text{MW} < 100 \text{ kDa}$, utilized dithranol (Aldrich, Milwaukee, WI) as the matrix, silver trifluoroacetate (AgTFA) (Aldrich, Milwaukee, WI) as the cationization agent, and tetrahydrofuran (unstabilized, HPLC grade; Fisher Scientific, Pittsburgh, PA) as the solvent. Equal volumes of a 10 mg/mL matrix solution and 10^{-5} – 10^{-4} M polymer solution were combined and vortexed for 2 min (Vortex Genie-2, Fisher Scientific, Pittsburgh, PA). This was followed by addition of 2.5% (v/v) of 5 mg/mL AgTFA and 2 min vortexing. A $0.8\text{-}\mu\text{L}$ aliquot of sample was spotted on a gold-coated MALDI plate and allowed to air-dry before analysis.

Sample preparation II, for $\text{MW} > 100 \text{ kDa}$, utilized all-*trans*-retinoic acid (Aldrich, Milwaukee, WI) as the matrix, AgNO_3 (99.999% pure, Aldrich, Milwaukee, WI) as the cationization agent, and tetrahydrofuran as the solvent. A 0.05 M (for PS with $\text{MW} < 300 \text{ kDa}$) or 0.075 M (for $\text{MW} > 300 \text{ kDa}$) matrix solution was combined with 10^{-6} – 10^{-5} M polymer solution in a volume ratio of 19:1 and vortexed for 2 min. Next, 1% (v/v) AgNO_3 saturated solution in methanol (HPLC grade; Fisher Scientific, Pittsburgh, PA) was added, followed by 2 min vortexing. The sample was spotted on a flat stainless steel MALDI plate (Applied Biosystems, Framingham, MA) using a commercial perfume atomizer with vial volumes of 0.1, 0.5, or 1 mL.

Other salts, such as AgTFA , $\text{Cu}(\text{NO}_3)_2$ (Mallinckrodt, Inc., St. Louis, MO) and $\text{Cu}(\text{TFA})_2$ (Advanced Materials, New Hill, NC) were also studied for use with PS samples that had $\text{MW} \geq 470 \text{ kDa}$.

RESULTS AND DISCUSSION

The first part of this study focused on the use of ThFFF/MALDI-TOFMS to analyze wide polydispersity polymers over a broad MW range. A series of compatibility and optimization studies were performed to achieve this objective.

Sample Amount. Each analysis began with the injection of $200 \mu\text{g}$ of wide-polydispersity polystyrene into the ThFFF channel. The 1-min or $100\text{-}\mu\text{L}$ fractions collected at the channel outlet contained 2–7 μg of polymer. We have observed that a larger amount of polymer was needed for MALDI analyses of fractions with $\text{MW} > 40 \text{ kDa}$. Fractions from several ThFFF runs were collected and combined as needed. The reproducibility of fraction collections was determined by ThFFF analysis of fractions

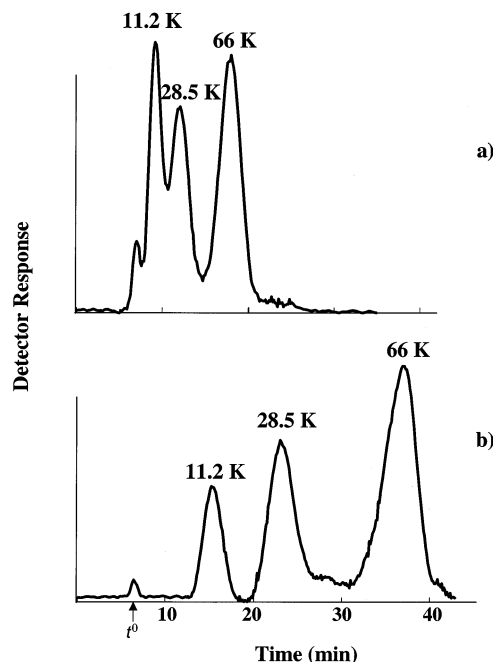


Figure 1. Separation of polystyrene standards in (a) tetrahydrofuran and (b) 40% dioxane/60% heptane. Conditions: $\Delta T = 100 \text{ K}$, $T_c = 297 \text{ K}$. The void time is t^0 .

collected from different runs. It was found that for all fractions, the relative standard deviation (RSD) in retention times did not exceed 2%.

It should be mentioned that the injection of $200 \mu\text{g}$ of polymer results in overloading of the ThFFF channel and a subsequent increase in the retention time.³² The shift in retention time does not affect this work, because the MW of each ThFFF fraction is subsequently determined by MALDI-TOFMS. In addition, the fractions that are reinjected into the ThFFF system contain polymer amounts that range from a fraction of a microgram to several micrograms.

Solvent Compatibility. Tetrahydrofuran (THF) is commonly used as the carrier liquid in ThFFF separations of polystyrene and other polymers with $\text{MW} > 20 \text{ kDa}$. For lower-MW polymers, binary solvents are used to obtain enhanced retention and resolution.^{31,33–36} Figure 1 compares the effects of using a single-component solvent (THF) and a binary solvent (40% dioxane/60% heptane) in the separation of polystyrene standards in the lower MW range of ThFFF. The retention time is almost doubled, and close to baseline resolution is observed in the latter case. Successful binary solvents have a common characteristic in that they are composed of a thermodynamically “good” solvent and a “poor” solvent.^{31,33–36} However, the presence of poor solvents, particularly nonsolvents, in the MALDI sample can affect the signal reproducibility and MW data.³⁷ Thus, the ThFFF solvent has to be removed from the collected fractions, for example, by evaporation, prior to sample preparation for MALDI analysis. This

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requirement restricts the solvent choice for ThFFF analysis. For example, the binary solvents that produced the highest retention for low MW polystyrenes dioxane/dodecane and tetrahydrofuran/dodecane³¹ could not be used in this study because of the low evaporation rate of dodecane. Instead, we use the next best binary solvent (40% dioxane/60% heptane) in separations of samples with MW < 100 kDa and single-component solvents for cases in which MW > 100 kDa.³¹ In this manner, narrow polydispersity fractions can be generated across a wide MW range.

Molecular Weight Range. ThFFF can be used to separate polymers with molecular weights ranging from several to 10⁴ kDa. As alluded to in the previous section, certain binary solvents significantly enhance retention and resolution of the lower-MW polymers. Polystyrene standards with MWs of 2.6 kDa and 4.4 kDa have been resolved using a carrier liquid that is 15% THF and 85% dodecane and standard operating conditions.³¹

MALDI-TOFMS is generally used for polymers below 100 kDa with resolution to oligomers often obtainable for polymers below 30 kDa. The upper MW limit is due, in part, to the lower sensitivity of the ion detectors and efficiency of desorption-ionization processes.^{1–12} These effects can be partially offset through optimization of the sample preparation procedure and MALDI parameters enhancing ion yield.

While MALDI conditions with dithranol as a matrix, dried-drop deposition, and desorption by a N₂-laser are effective for lower-MW polystyrenes,³⁸ we have observed that they are not optimum for polymers with MW > 100 kDa. Using these conditions, the signal intensity for the 170-kDa PS was very low with a S/N < 3. To improve signal intensity, MALDI processes, such as sample preparation and deposition, and UV laser parameters were varied. First, dithranol was replaced with all-*trans*-retinoic acid whose potential in analyses of high MW polystyrenes have already been shown.³⁹ Next, the dried-drop deposition method was replaced by spray deposition. The former produces a nonuniform sample layer that leads to the search for “sweet” or “hot” spots and low reproducibility of spectra. Several spray deposition techniques have been shown to produce homogeneous sample layers that resulted in improved signal reproducibility^{40–42} and intensity for polymers <8 kDa.^{40,41} Sample homogeneity is especially important for higher-MW polymers, which are more prone to aggregation. Another advantage of spray deposition is the thin sample layers that are formed. It was previously noted for dried-drop deposition³⁹ that thinner layers were beneficial for high MW polymer analysis. In this study, we used a commercial perfume atomizer to obtain sample film thicknesses under 1 μm (data courtesy of Dr. F. Basile of Colorado School of Mines; obtained with profilometer, model P-10, KLA-Tencor, San Jose, CA). The use of spray deposition and retinoic acid matrix produced more than a 10-fold increase in signal intensity, as compared with dithranol and dried-drop deposition. Another

significant improvement was achieved by using a Nd:YAG laser instead of an N₂-laser. The signal intensity was increased by >10-fold for all sample preparations. This enhancement was initially attributed to the coincidence of the wavelength of the Nd:YAG laser (355 nm) with the absorbance maxima of dithranol and retinoic acid. However, a similar increase in signal intensity was also observed in the analysis of polyvinylpyridine using indole-acrylic acid whose absorbance maximum at 320 nm is closer to the N₂-laser wavelength of 337 nm. The origin of this effect may be partially related to the area of the laser spot impinging on the MALDI plate, which was several times larger for the Nd:YAG laser than the N₂-laser. The study by Dreisewerd et al.⁴³ showed that the matrix signal intensity was very sensitive to the laser spot area and exceeded the expected linear dependence. Laser power has also been observed to affect the signal intensity of high MW polymers.⁴⁴

The retinoic acid matrix, spray deposition, and Nd:YAG laser, along with optimization of polymer/matrix/salt ratio (sample preparation II), produced an overall increase of >100 times in signal intensity and 20 times in S/N, in comparison to sample preparation I with dithranol, dried-drop deposition, and N₂-laser. Moreover, the polymer amount used in preparation II was ~5 times less than in preparation I. These conditions have enabled us to do routine analysis of polymers with MW as high as 575 kDa.

For MALDI experiments with dithranol as the matrix, it has been shown that the most favorable cation–polymer interaction occurred with either silver or copper.⁴⁵ The counterion can also be important, because ion yields were observed to decrease as the anion of the alkali metal hydride was changed from I[–] to Br[–] to Cl[–].⁴⁶ In our experiments with retinoic acid, further improvement of signal intensity and S/N for high-MW polymers, for example, 575-kDa, was achieved by using Cu(TFA)₂. We observed that silver produced similar signal intensities, regardless of whether AgNO₃ in methanol or AgTFA in THF was used. However, the results for copper depended significantly on the type of salt. The use of Cu(NO₃)₃ in water led to a 2-fold decrease in signal intensity compared to silver, while Cu(TFA)₂ in THF produced 40% higher signals compared to silver. Using Cu(TFA)₂, a S/N of 10 was obtained for 470- and 575-kDa polystyrenes, which allowed the determination of peak molecular weight (*M_p*) and polydispersity (*M_n*/*M_w* or PD) values summarized in Table 1. The *M_p* values measured by MALDI-TOF are in good agreement with the manufacturer's data, but the polydispersity values are lower for MALDI-TOFMS.

ThFFF/MALDI-TOFMS Analyses of Wide-Polydispersity PS Polymers. A 40% dioxane/60% heptane solvent mixture was used to fractionate a wide polydispersity polystyrene PSBR35K with a nominal *M_w* of 33 kDa. One-minute fractions, each containing several micrograms of polymer, were collected. The fractions that were selected for further analysis are shown as shaded areas in Figure 2a.

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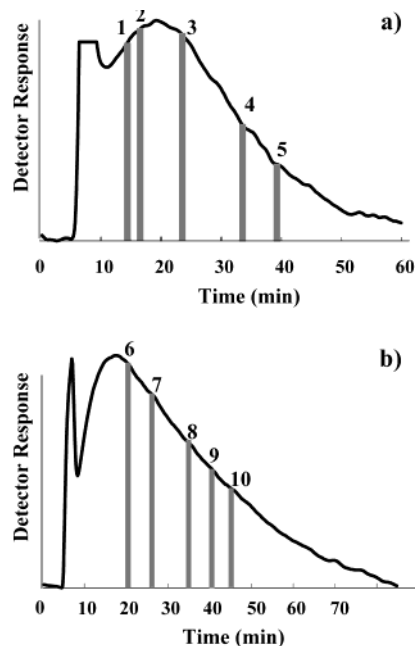


Figure 2. ThFFF fractionation of the wide polydispersity polystyrene. (a) PSBR35K in 40% dioxane/60% heptane. Conditions: $\Delta T = 100$ K, $T_c = 296$ K. (b) PSBR250K in tetrahydrofuran. Conditions: $\Delta T = 80$ K, $T_c = 295$ K. In both cases, sample amount injected was 200 μ g.

The collected ThFFF fractions of PSBR35K were further analyzed by both MALDI-TOFMS and ThFFF. The latter was performed to verify the accuracy of MALDI results and involved reanalysis of the fractions under ThFFF conditions identical to those implemented in the initial separation of the whole sample. Molecular weight distribution data were then calculated using a calibration curve (retention time vs MW) derived from the MW standards listed in Table 1. Since the fractions had MWs < 100 kDa, MALDI sample preparation I with dithranol matrix, dried-drop deposition, and an N_2 -laser was used. Fractions 1–3 were analyzed as collected, whereas material from up to four runs were combined prior to MALDI analysis of fractions 4 and 5. ThFFF fractograms and MALDI spectra of fractions 1–5 are presented in Figures 3 and 4, respectively. These results show that ThFFF can separate the polydisperse polymer into narrow fractions, which could be easily analyzed and accurately characterized by MALDI over the MW range of 10^3 – 10^5 Da. For fractions 1 and 2, MALDI-TOF resolution was sufficient to detect oligomer peaks. The molecular weight distribution data obtained by both techniques are summarized in Table 2. The first column represents MALDI data, and the second column is ThFFF data where the calibration curve was obtained by plotting retention time versus the MWs determined by MALDI (standards listed in Table 1). The MWD for fraction 1 analyzed by ThFFF is not shown because of the inaccuracies introduced by the proximity of the fraction to the void peak. For fractions 2–5, the ThFFF data are comparable (within 6%) of the MALDI values. The data presented in Table 2 will be discussed in detail in a later section. Figure 4 confirms that fractionation has taken place, because the MW increases with increasing fraction number. The intensity of MALDI signals for higher-MW fractions 4 (MW \sim 45 kDa) and 5 (\sim 60 kDa) is observed to decrease significantly, even though the overall MALDI sample amount was similar to or higher than in fractions 1–3. It

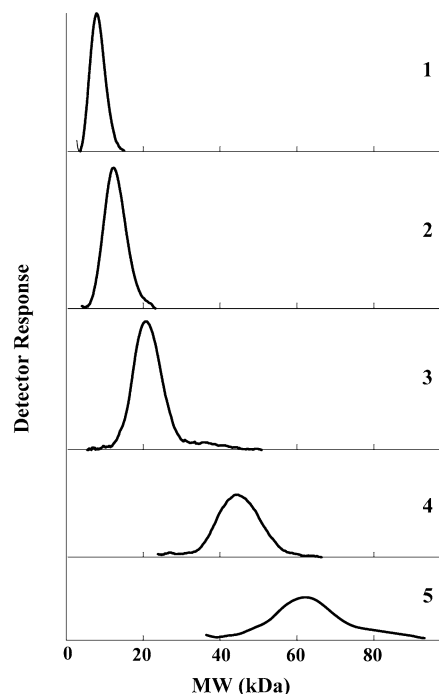


Figure 3. ThFFF analysis of fractions 1–5. Conditions are as in Figure 2a.

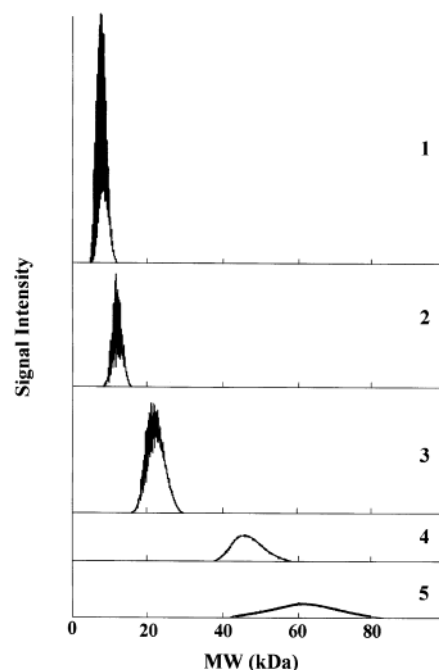


Figure 4. MALDI-TOFMS analysis of fractions 1–5. Sample preparation I with dithranol as the matrix.

is evident that the analysis of even higher MW fractions (≥ 100 kDa) requires the use of a different set of conditions, namely MALDI sample preparation II.

The ThFFF fractogram of a second wide polydispersity polystyrene (PSBR250K, $M_n = 100$ kDa and $M_w = 250$ kDa) is shown in Figure 2b. One-minute fractions, containing several micrograms of polymer each, were collected, and fractions 6–10 were further analyzed. Spray deposition with the disposable perfume atomizer required more sample than the dried drop method, because the spray is deposited over a larger area. Hence,

Table 2. MW Data for Fractions

fraction	MALDI-TOFMS ^a (kDa)	ThFFF ^b (kDa)
PSBR35K		
1	$M_n = 7.8$; $M_w = 8.0$ PD = 1.03	
2	$M_n = 12.0$; $M_w = 12.2$ PD = 1.02	$M_n = 12.1$; $M_w = 12.8$ PD = 1.03 (1.06)
3	$M_n = 21.4$; $M_w = 21.5$ PD < 1.01	$M_n = 20.7$; $M_w = 21.8$ PD = 1.03 (1.05)
4	$M_n = 46.3$; $M_w = 46.4$ PD = 1.01	$M_n = 43.7$; $M_w = 44.7$ PD = 1.01 (1.02)
5	$M_n = 61.5$; $M_w = 61.8$ PD < 1.01	$M_n = 62.0$; $M_w = 63.4$ PD = 1.01 (1.02)
PSBR250K		
6	$M_n = 109$; $M_w = 112$ PD = 1.03	$M_n = 108$; $M_w = 111$ PD = 1.03 (1.03)
7	$M_n = 166$; $M_w = 170$ PD = 1.02	$M_n = 164$; $M_w = 169$ PD = 1.03 (1.03)
8	$M_n = 262$; $M_w = 266$ PD = 1.02	$M_n = 255$; $M_w = 265$ PD = 1.02 (1.04)
9	$M_n = 309$; $M_w = 314$ PD = 1.02	$M_n = 292$; $M_w = 313$ PD = 1.02 (1.07)
10	$M_n = 359$; $M_w = 365$ PD = 1.02	$M_n = 342$; $M_w = 385$ PD = 1.05 (1.13)

^a Fractions 1–5 obtained using sample preparation I; fractions 6–10, with sample preparation II. ^b ThFFF calibration was obtained using MALDI MW data for standards listed in Table 1. PD values in parentheses are uncorrected for band broadening.

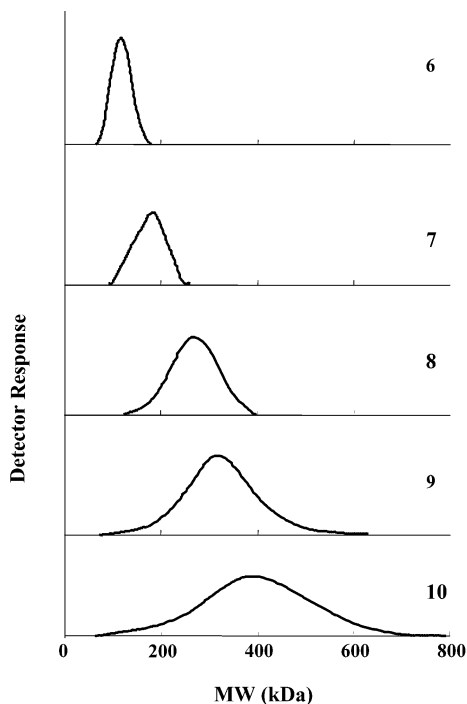


Figure 5. ThFFF analysis of fractions 6–10. Experimental conditions for fractions 6 and 7 are the same as in Figure 2b. For fraction 8, the solvent is chloroform, $\Delta T = 80$ K, $T_c = 290$ K. For fraction 9, chloroform, 70 K, 293 K; and for fraction 10, chloroform, 60 K, 295 K.

fractions from 10 ThFFF runs were combined for MALDI analysis. Figures 5 and 6 are of the ThFFF and MALDI-TOFMS analyses of the fractions, respectively. In this case, ThFFF analyses of the fractions were performed under conditions different from those used in the initial separation of the whole sample so as to minimize the sample overloading effect. This effect becomes more pro-

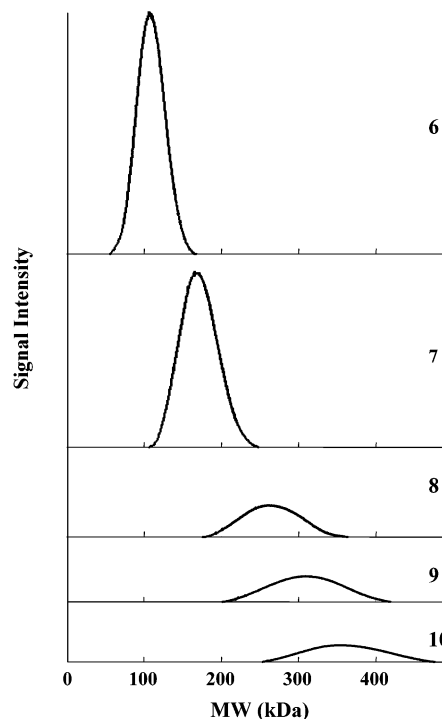


Figure 6. MALDI-TOFMS analysis of fractions 6–10. MALDI sample preparation II with retinoic acid as the matrix.

nounced for polymers with MW > 100 kDa and can lead to overestimation of MW.³² The sample amount injected into the ThFFF channel was maintained below 1 μ g. High retention times were also avoided, because the sample overloading effect tends to increase with retention time for the same sample amount.³² ThFFF conditions were selected such that each fraction was retained just enough to be baseline-separated from the void peak (important for accurate MWD calculations). This was achieved at a retention ratio R of ~ 0.3 .

MALDI sample preparation II, consisting of spray deposition, all-*trans*-retinoic acid matrix, and the Nd:YAG laser, was used in the analysis of these MW > 100-kDa fractions. Significantly higher signal intensities were achieved in comparison to signals from fractions 4 and 5 analyzed using sample preparation I. The presence of ~ 359 -kDa PS is clearly observed in fraction 10. MWD data for fractions 6–10 are summarized in the second part of Table 2. The agreement between the average MWs determined by MALDI and ThFFF is within 5%.

As with size-exclusion chromatography (SEC), column dispersion processes occur in ThFFF and can lead to an overestimation of polymer polydispersity.^{47,48} However, ThFFF dispersion mechanisms are better understood and can thus be estimated analytically⁴⁹ or determined experimentally.^{48–50} The main contribution to the channel dispersion is the hydrodynamic or nonequilibrium

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dispersion, H_h , that can be calculated as⁴⁹

$$H_h = \chi \frac{w^2 \langle v \rangle}{D} \quad (1)$$

where w is the channel thickness, $\langle v \rangle$ is the average channel flow velocity, D is the molecular diffusion coefficient of a polymer, and χ is the dimensionless parameter, $\chi \approx (R^3/9)(1 - R/3 - 4R^2/9 - 10R^3/27)$. Errors in the determination of χ are <1% for $R < 0.41$ and <3% for $R < 0.74$. Molecular diffusion coefficients of polystyrene fractions 6–10 in pure solvents were calculated using reference data.^{51–53} Because of a lack of reference data for molecular diffusion coefficients in 40% dioxane/60% heptane, D values for fractions 1–5 were estimated from dynamic light scattering (DLS) data for two PS standards with MWs of 66 kDa and 110 kDa. The resulting relationship $D = 4.35 \times 10^{-4} M^{-0.6}$ (D is in cm^2/s , M is in Da) was extrapolated to lower MWs. The power -0.6 is in reasonable agreement with the -0.59 to -0.62 values determined previously for PS in 50% dioxane/50% heptane and 60% dioxane/40% heptane mixtures using ThFFF.³³ The ThFFF polydispersity data, corrected for nonequilibrium dispersion, are shown in Table 2 and are in good agreement with MALDI results. The uncorrected PD values are given in parentheses and illustrate the importance of such corrections.

These results confirm the accuracy of MWD determination by MALDI and hence, in further studies, the fractions characterized solely by MALDI can be considered well-characterized standards and used for the absolute calibration of ThFFF or SEC in analysis of polydisperse polymers.

ThFFF/MALDI-TOFMS Analyses of Polymer Mixtures. A second important application of the combined ThFFF/MALDI method is the analysis of polymer mixtures. The MALDI process is quite sensitive to the chemical composition of polymers, and thus, MALDI conditions optimized for one polymer are generally not suitable for another polymer.² In our initial experiments, MALDI conditions suitable for individual analyses of 11.2-kDa PS and 10.5-kDa PVP yielded only a PS signal when a 2:1 (w/w) ratio of a PS/PVP mixture was analyzed. Upon increasing the PS/PVP ratio to 19:1, the PS signal could be discerned in addition to PVP, as demonstrated in Figure 7. Three sections of the spectrum have been expanded, and the PS and PVP oligomers have been labeled. Sections 1 and 3 corresponding to the tails on each side of the peak showed clear evidence of the presence of both PS and PVP. However, the PS and PVP peaks located in section 2 overlap to such an extent that they cannot be resolved from one another. If not for the tails, which could be indistinguishable from the noise in less optimized experimental conditions, one could mistakenly conclude that this is a single-component sample. Polymer analysis software applied to the entire spectrum estimated a mixture composition of 6PS/4PVP, which is significantly different from that of the sample analyzed. It is evident that even in this idealized case of a mixture of two narrow standards, MALDI experiences problems and preliminary ThFFF separation would be very helpful.

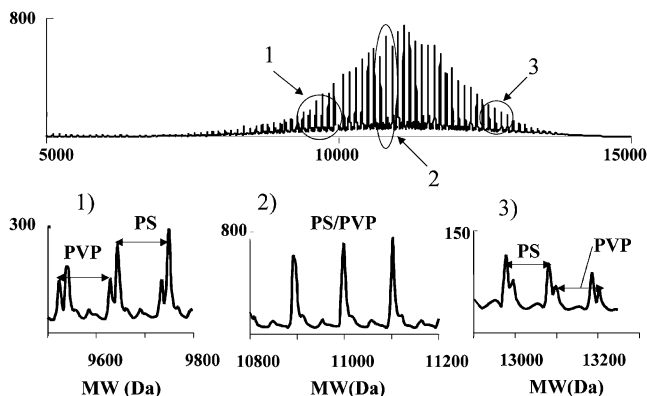


Figure 7. MALDI-TOFMS analysis of 95:5(w/w) mixture of polystyrene ($M_p = 11.2$ kDa, PD = 1.02) and polyvinylpyrrolidone ($M_p = 10.5$ kDa, PD = 1.06 kDa) in reflector mode. Conditions: solvent, THF; matrix, 10 mg/mL dithranol; polymer mixture, 1 mg/mL; salt, 5 mg/mL silver trifluoroacetate; matrix/polymer/salt volume ratio is 100:3; spray deposition; N_2 laser.

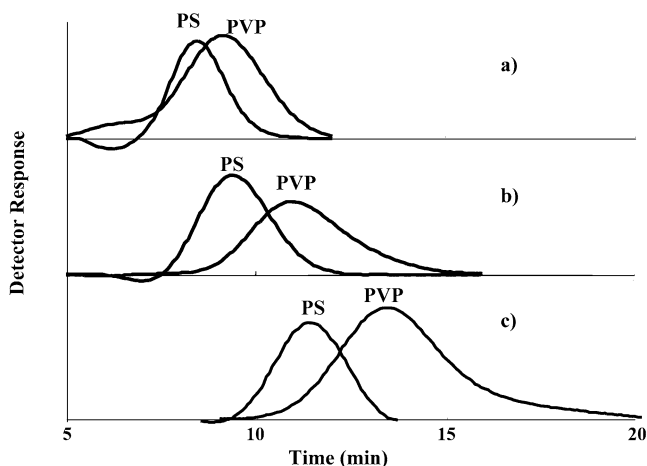


Figure 8. ThFFF separation of 11.2-kDa PS and 10.5-kDa PVP in (a) THF, (b) 50% dioxane/50% cyclohexane, and (c) 60% dioxane/40% heptane. Conditions: $\Delta T = 100$ K, $T_c = 297$ K.

Using pure THF, the ThFFF retention of 11.2-kDa PS and 10.5-kDa PVP was too low to achieve a satisfactory separation (Figure 8a). Binary solvents were again effective in enhancing ThFFF retention and resolution, as shown in Figure 8b and c. A 60% dioxane/40% heptane carrier liquid yielded improved resolution and allowed more chemically homogeneous fractions to be collected for MALDI analyses. Fractions collected before 11 min will be substantially enriched in PS, while fractions collected after 14 min will contain predominantly PVP.

These ThFFF separations on the basis of polymer composition will allow subsequent MALDI analyses under conditions optimum for each polymer type. In addition, MALDI's ability to provide oligomer level resolution in this MW region will lead to further important information about the composition of the eluted polymer. This is important in the case of mixtures that contain polymers for which there are no calibration standards for ThFFF and the identity of the separated polymers must be verified.

The ThFFF/MALDI-TOFMS approach was also used to analyze higher MW (>100-kDa) PS and PVP. The ThFFF fractogram in Figure 9 shows almost baseline resolution of the two polymer components. For this MW region, pure THF yielded sufficient retention and resolution. The MALDI spectra of the PS

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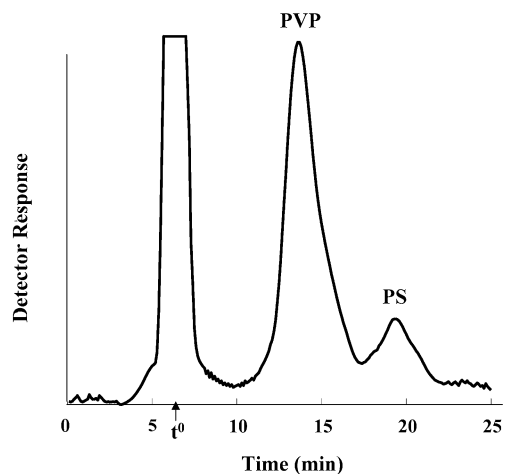


Figure 9. Separation of mixture of polystyrene and poly(vinylpyridine) with nominal MW of 110 kDa. Conditions: solvent, tetrahydrofuran, $\Delta T = 70$ K, $T_c = 299$ K.

and PVP polymers exhibited in Figure 10 were obtained using different conditions optimized for each polymer. PVP is a polar polymer and does not require the addition of a silver salt for optimum spectra. It interacts with trace amounts of sodium and potassium that are usually present in glassware. Interestingly, the spray deposition and Nd:YAG laser also significantly improved the signal for this polymer.

CONCLUSIONS

Binary solvents were shown to have a significant effect on ThFFF retention and to be useful in separations of low-MW polymers and polymer mixtures. Different sample preparations and lasers were found to be effective for MALDI-TOFMS analyses of polymers in different molecular weight ranges, for example, above or below ~ 100 kDa. Following the extension of capabilities of each individual technique, the combined ThFFF/MALDI-TOFMS was successfully applied to the analysis of polydisperse polymers in the MW range of several kilodaltons to several hundred kilodaltons and polymer mixtures. ThFFF served not only as a sample preparation technique for MALDI-TOFMS but also as a method for verifying the accuracy of MALDI-TOF MWD results. ThFFF's ability to produce fractions that possess narrow polydispersity and are more chemically homogeneous allows subsequent MALDI-TOFMS at different optimum conditions. The good agreement observed between the MWD data obtained by

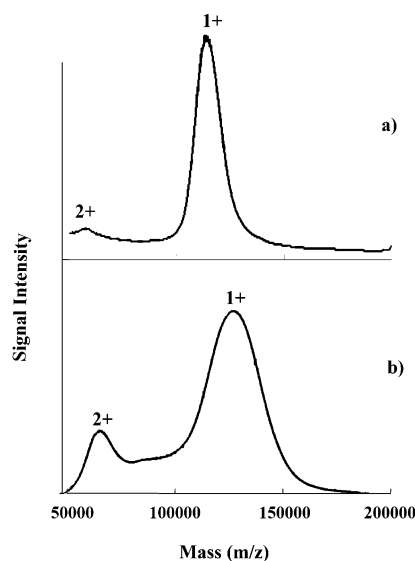


Figure 10. MALDI spectra of the separated PS and PVP polymers obtained under different optimized conditions. (a) Polystyrene. MALDI conditions: sample, 5 mg/mL polymer in THF; matrix, 15 mg/mL all-*trans*-retinoic acid in THF; salt, saturated solution of AgNO_3 in methanol; mixing, 10:190:1; spray deposition; Nd:YAG laser. (b) Poly(vinylpyridine). MALDI conditions: sample, 4 mg/mL polymer in THF; matrix, 18.7 mg/mL *trans*-3-indoleacrylic acid in THF; no salt added; mixing, 5:1; spray deposition; Nd:YAG laser.

MALDI-TOFMS and ThFFF is an indication that this combined technique can be extended to various polymer systems.

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