A simple interface based on an oscillating capillary nebulizer (OCN) is described for direct deposition of eluate from a thermal field-flow fractionation (ThFFF) system onto a matrix-assisted laser desorption/ionization (MALDI) probe. In this study, the polymer-containing eluent from the ThFFF system was mixed on-line with MALDI matrix solution and deposited directly onto a moving MALDI probe. The result was a continuous sample track representative of the fractionation process. Subsequent off-line MALDI-mass spectrometry analysis was performed in automated and manual modes. Polystyrene samples of broad polydispersity were used to characterize the overall system performance. The OCN interface is easy to build and operate without the use of heaters or high voltages and is compatible with any MALDI probe format.

The analysis of complex mixtures by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) can be hindered by signal suppression effects. For polymer samples of high polydispersities, discrepancies in the calculated molar masses from MALDI-MS measurements have been observed when compared to measurements using other techniques. In addition, selective signal loss can occur when samples containing different composition polymers are analyzed. To circumvent these problems, polydisperse and complex samples have been fractionated and the collected fractions individually analyzed by MALDI-MS. Size exclusion chromatography (SEC) has often been used to separate polymer samples into narrow polydispersity fractions prior to off-line MALDI-MS analysis. More recently, thermal field-flow fractionation (ThFFF) was shown to be compatible with off-line MALDI-MS.

Thermal FFF generally has a higher selectivity (larger change in retention time per unit change in molecular weight) and thus a lower efficiency than SEC. However, in terms of resolution, ThFFF has an advantage as resolution varies linearly with selectivity but with the square root of efficiency. An additional advantage of ThFFF over SEC is its sensitivity to both polymer molecular weight and chemical composition. These capabilities were demonstrated by the separation of complex polymer samples into fractions of more uniform molecular weight, chemical homogeneity, or both. Each fraction was subsequently analyzed with different matrixes and MALDI-MS and individually optimized.

The MALDI-MS analysis of collected fractions can be accomplished either on-line or off-line. Off-line separation and MALDI-MS analysis is particularly attractive because it is experimentally simpler than the on-line approach and allows for the independent optimization of both the separation and MALDI-MS analyses. However, manual collection and sample preparation for subsequent MALDI-MS analysis of each fraction can be tedious and time-consuming. The collection of fractions also has intrinsic disadvantages in that set intervals of effluent are analyzed by MALDI and separation efficiency can be lost as components remix.
in the collected volume. On the other hand, on-line analysis is more difficult to implement in MALDI since the sample is dried before introducing it into the mass spectrometer (although the incorporation of atmospheric pressure MALDI to on-line analysis would simplify this process\textsuperscript{24–26}). As a result, continuous deposition of the sample–matrix solution directly onto the MALDI probe is an attractive alternative.

Studies have been performed that prove the versatility of continuous deposition of fractionated polymer sample directly onto a MALDI plate.\textsuperscript{4,27} In these investigations, the eluent from a gel permeation chromatography system was deposited directly onto matrix precoated MALDI probes. The deposition system consisted of a nebulizer with a heated sheath gas (temperature was adjusted to allow complete solvent evaporation prior to sample spraying onto a moving MALDI probe). Both of these studies were successful in outlining the advantages of direct sample deposition onto a MALDI probe precoated with matrix. This last point may not be ideal for complex samples as different matrices and sample/matrix ratios may be needed to circumvent selective signal suppression. Moreover, both interfaces use heat to remove solvent, which increases the complexity of the interface and makes incompatible with heat-labile analytes, and it may require optimization when using an elution gradient. On the other hand, an interface such as that described in this work has the potential for introducing different matrices and sample/matrix ratios to the effluent stream depending on the properties of the eluting sample component.

This technical note describes a simple interface based on a modified oscillating capillary nebulizer (OCN) where the eluent from a ThFFF system is premixed with matrix and deposited directly and continuously onto a MALDI probe. The OCN has been used to apply uniform matrix coatings on MALDI probes\textsuperscript{28,29} and to deposit the output of a reversed-phase HPLC onto a MALDI probe for the analysis of intact proteins by MALDI-MS.\textsuperscript{30,31} These studies determined that the OCN was effective in handling liquid compositions from 100% aqueous to 100% organic (reversed-phase LC gradient elution). The direct and continuous sample deposition with the OCN interface allows rapid fraction collection and sample-preparation times after ThFFF separation. The uniform nature of the deposited sample trace on the MALDI probe also makes automated MALDI-MS acquisition possible (i.e., no “hot spot” search is needed).\textsuperscript{32}

**EXPERIMENTAL SECTION**

**Chemicals.** Tetrahydrofuran (THF, HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA). The 28.5 kDa ($M_w/M_n = 1.03$) and the wide polydispersity polystyrene standard PSBR35K ($M_w = 14.5$ kDa, $M_n = 33.0$ kDa) were purchased from Polymer Laboratories (Amherst, MA) and American Polymer Standards Corp. (Mentor, OH), respectively. These polymer standards were selected because they fall in an optimum region of separation for ThFFF, and second, the MALDI MS of higher MW polymers can be quite challenging due to reduced desorption/ionization and detection efficiencies.\textsuperscript{3} The matrix, all-trans-retinonic acid, and the cationization agent, silver trifluoroacetate, were purchased from Aldrich, (Milwaukee, WI).

**Oscillating Capillary Nebulizer Interface Construction.** Figure 1a illustrates a detailed diagram of the modified OCN interface. The OCN\textsuperscript{28} was built with the following modifications.

**Figure 1.** (a) Detailed diagram of the modified oscillating capillary nebulizer for liquid sample deposition. (b) Setup of the thermal field-flow fractionation system with the oscillating capillary interface for direct effluent deposition onto the MALDI probe.

**Figure 1a.** Detailed diagram of the modified oscillating capillary nebulizer for liquid sample deposition. (b) Setup of the thermal field-flow fractionation system with the oscillating capillary interface for direct effluent deposition onto the MALDI probe.
tip, and 127 μm in thickness with a resulting channel volume of 0.62 ± 0.02 mL. In all experiments, the flow rate was set at 0.1 mL/min.

A diagram of the overall ThFFF–OCN system is illustrated in Figure 1b. The 100 μL/min flow from the ThFFF system was reduced to 30 μL/min with a microsplitter valve (Upchurch Scientific; part P-451). A capillary of internal diameter and length equivalent to the one used in the OCN (inner capillary) was placed at the split flow output (i.e., going to waste) of the microsplitter valve in order to maintain adequate back pressure on the ThFFF system (∼180 psi). The 30 μL/min split flow from the ThFFF system was mixed on-line with matrix solution in a microstatic mixing Tee (Upchurch Scientific; part M-540). The matrix solution was delivered with a syringe pump (Fisher Scientific) at a rate of 3 μL/min. The mixed sample–matrix flow was connected to the OCN and directly deposited onto the MALDI probe. With THF as the solvent, the OCN tip was set at a distance of 1 cm from the MALDI probe. This distance allowed for complete solvent evaporation during sample deposition, while maintaining a narrow (<2 mm wide) sample–matrix trace width on the probe. The MALDI probe was placed on the paper of a stripchart recorder to provide constant MALDI probe motion during the deposition process. The chart recorder speed was set at 1 cm/min for continuous sample deposition. When the trace reached the end of each row in the MALDI probe during sample deposition, the probe was manually moved and aligned to the next row for further sample collection.

For the signal strength versus sample–matrix thickness study, the OCN was directly connected to a syringe pump loaded with a premixed solution of 28 kDa polystyrene (5 mg/mL in THF), retinoic acid (10 mg/mL in THF), and silver trifluoroacetate (10 mg/mL in THF) at a ratio of 190:10:1. Film thickness was measured with a surface profilometer (Tencor P-10, San Jose, CA).

MALDI-MS Analysis. MALDI-MS analyses were performed on a Voyager DE-STR + MALDI-TOF-MS system (Applied Biosystems, Foster City, CA) operated in the linear mode. Both a nitrogen laser (337-nm laser wavelength) and a frequency tripled neodinium:yttrium-aluminum-garnet (Nd:YAG, 355-nm laser wavelength) were used in these analyses.

Automated data acquisition was set up through the instrument Sequence Control software. Three spectra were collected at each position on the MALDI probe (each consisting of 50 laser shots) and averaged. The resulting average mass spectrum from these spots (a total of 150 laser shots at constant laser intensity) represented a “fraction” of the continuously deposited trace of the sample. A plate file was set up (through the instrument software; file extension “.plt”) such that a mass spectrum from each “fraction” was acquired every 2 mm along the MALDI probe (this is equivalent to every 0.2 min in the fractogram), for a total of 20 fractions/row (each row ∼4 cm in length). Using these parameters, each “fraction” was equivalent to ∼6.5 μL of total volume (eluent + matrix). Manual data acquisition was also performed by accumulating and averaging spectra from different locations (within a region 2 mm²) in sets of 10 shots/position for a total of 100 shots/spectrum. All data were plotted with a low mass range cutoff determined by the matrix clusters’ background signals.

Figure 2. S/N versus matrix film thickness for a standard polystyrene (28 kDa) sample with retinoic acid matrix. The polymer/matrix molar ratio and laser irradiance were kept constant for all measurements.

RESULTS AND DISCUSSION

OCN MALDI Matrix Deposition Characterization. For optimal sample deposition (matrix + analyte), it was determined that the sprayed solution should be completely dry before reaching the MALDI probe, otherwise the deposited sample trace is wider than 2 mm and shows a nonuniform morphology. Depending on the solvent composition and flow rate, the OCN-MALDI probe distance should be optimized for complete solvent evaporation prior to sample deposition. For 100% organic solvents, distances of 1–1.5 cm were found to be adequate for complete solvent evaporation even at flow rates as high as 50 μL/min. For 100% aqueous solutions and flow rates less than 10 μL/min, larger distances (2–2.5 cm) must be used to allow for complete solvent evaporation without the need of sheath gas heating.

Matrix deposition onto a stainless steel surface with the OCN was near quantitative, as more than 99% of the retinoic acid delivered by the OCN was deposited on the MALDI probe surface (determined by gravimetric measurements). Also, the thickness of the deposited matrix film (μm) increased linearly with solution flow rate through the OCN in the range of 1–10 μL/min, and this film thickness could be varied reproducibly from 1 to 10 μm (data not shown: plot of film thickness vs OCN flow rate, slope 0.92 ± 0.03 μm/μL min⁻¹; y-intercept 0.008 ± 0.132 μm; r² = 0.997). The full width at half-maximum (fwhm) of the deposited matrix tracks varied from 1.5 to 2.5 mm over this thickness range.

Signal strength and signal-to-noise ratio (S/N) of a polystyrene (28 kDa) standard were measured with respect to the deposited MALDI matrix film thickness (and matrix flow rate into the OCN), while keeping the sample–matrix molar ratio constant. As illustrated in Figure 2, the S/N for this sample reached a maximum value at 2-μm film thickness and decreased rapidly at film thicknesses above 3 μm. Based on these results, all subsequent experiments were performed to obtain a film thickness between 2 and 3 μm. The observed trend in Figure 2 in this study is similar to that reported by Axellsson et al. (Figure 3 in ref 34) in which signal intensity was plotted as a function of sample thickness.

deposition time (electrospray time a matrix thickness). The same trend and maximum (~2 μm matrix thickness) was observed for a 110-kDa polystyrene standard.

ThFFF-OCN-MALDI-MS Polymer Analysis. A broad polydisperse polystyrene sample (MW = 35 000) was analyzed with this methodology. From a previous study using ThFFF-MALDI,8 the polydispersities of the ThFFF fractions were determined to between 1.01 and 1.03 (by both MALDI and reinjection into the ThFFF system). A 40-min ThFFF separation was directly deposited onto the MALDI probe. The deposited sample was analyzed by MALDI-MS using automated and manual acquisition modes. Previous studies have found that MALDI-MS analysis performed using the frequency tripled Nd:YAG laser detected polystyrene fractions over a wide range of MW.8 Hence, automated acquisition (every 2 mm) was performed with the 355-nm laser wavelength only in order to obtain a general picture of the location of the eluted polymers on the MALDI probe. Subsequent manual data acquisition was performed using both the 337- and 355-nm laser wavelengths in order to obtained optimized spectra of all the collected polymer fractions.

Spectra illustrated in Figure 3 correspond to locations separated by 10 mm along the deposited matrix—sample trace on the MALDI probe (average of data acquired every 2 mm). As expected, an increase in MW is observed with increasing retention time or distance along the MALDI probe. Automated acquisition was successful in generating a general picture of the spatial separation and location of the fractionated polymer on the MALDI probe. However, poor resolution of the monomeric unit in the low mass range was observed.

Our experience with polymer analyses using the 355-nm laser wavelength for the entire mass range has been the loss of resolution of the monomeric units for the low molecular weight fractions (<20 kDa). However, resolution of monomeric units is easily achieved with the use of the 337-nm laser wavelength. Hence, a combination of 337-nm laser wavelength for low molec-
ular weight fractions and 355-nm laser wavelength for higher molecular weight fractions would yield optimum spectra for this entire mass range of the fractions collected. Mechanistic reasons for this difference in resolution between these two lasers may be related to laser pulse energy and beam shape differences, among others.

Optimized manual acquisition at either 337- or 355-nm wavelengths of the eluted and deposited polymer resulted in a series of mass spectra representing "fractions" of the separated polymer. Analyses on the first row of the MALDI probe (first 4 min of elution) showed that polymers in the MW range between 2 and 20 kDa eluted in this region (Figure 4). The 337-nm laser wavelength enabled resolution of the polystyrene monomer units as shown by the inset in the t = 9 min plot in Figure 4. The poor apparent separation for these fractions is due to their proximity to the void peak where sample retention is low. As fractions of polymer with increasing MW were eluted and deposited onto the MALDI probe, the signal-to-noise ratio of the polymer signal decreased (data not shown). From this point on, the 355-nm laser wavelength was used. Figure 5 shows the MALDI spectra resulting from the manual data acquisition of the eluted and deposited polymers using the 355-nm laser. Manual laser intensity adjustments were required in order to obtain optimum signal intensities at retention times above 10 min. This was not due to matrix hot spots, but rather an increase in the laser threshold levels for polymer ion formation with increasing retention times. Approximate elution time, calculated $M_n$, and absolute position within the MALDI probe are also reported with each spectrum in Figure 5. As expected, an increase in the polymer MW with increasing MALDI probe position was observed. The separation efficiency was maintained during the deposition step. This is illustrated by the spatial resolution of polymers $M_n$’s varying 59–81 kDa in a section spanning 2.6 cm across the MALDI probe.

CONCLUSIONS

Work presented here illustrated the ability of the OCN interface to continuously deposit samples eluting from a ThFFF system onto a MALDI probe without compromising separation resolution. Both manual and automatic mass spectra acquisitions were possible with this approach, generating spectra of resolved polymeric fractions as a function of MALDI probe position. Automated data acquisition was used to generate a preliminary picture of the spatial separation of the fractionated polymer. This acquisition mode is simplified by the uniform nature of the deposited sample—matrix film. Manual acquisition was performed subsequently to optimize the MALDI-MS measurement at regions of interest on the MALDI probe. In principle, these “fractions” could be removed from the probe, brought back into solution, and reanalyzed by other techniques. It is worth keeping in mind that the amount of sample predicted to be in a 2-mm² section of the probe is expected to be in the submicrogram range. The OCN was found to be a viable approach for the direct sample—matrix deposition from a ThFFF system onto a MALDI probe. Finally, the MALDI probe displacement can be fully automated for high-throughput analyses. The chart recorder-based MALDI probe movement used in this study is obviously a semiautomated option, albeit inexpensive.

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