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Fractionation and Solvent-Free MALDI-MS Analysis of Polymers Using Liquid Adsorption Chromatography at Critical Conditions in Combination with a Multisample On-Target Homogenization/Transfer Sample Preparation Method

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A solvent-free homogenization/transfer matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) method is described for the preparation and precise transfer of up to 100 samples simultaneously on a single MALDI plate. This method is demonstrated using a poly(ethylene oxide) (PEO) mixture consisting of different molecular weights (500−6000) and end groups (PEO, dimethoxy-PEO, monomethoxy monomethacrylate-PEO, and dimethacrylate-PEO) that was fractionated using liquid adsorption chromatography at critical conditions. Off-line fractionation is performed prior to the on-target homogenization/transfer solvent-free sample preparation and MALDI mass analysis. The miniaturization of the solvent-free MALDI approach allowed analysis of less than 2 μg per PEO component per fraction corresponding to ~200 pmol for PEO 6000. The amounts of polymer sample used for LC separation and the quality of the MS results are equivalent to the "dry spray" method; however, three times more fractions were collected and analyzed with the newly developed hyphenated approach. The off-line method eliminates optimization of, for example, spray conditions or spreading of organic solvents on the MALDI plate that occurs with droplet deposition methods. The widespread applications of MALDI make this solvent-free, multisample method particularly important as it expands the capabilities for obtaining mass measurements with great efficiencies in areas with increased sample numbers. In addition, the solvent-free method is well suited for automated MALDI analysis as it virtually eliminates the "dead-spot" phenomenon.

Polymers are a complex mixture of components made up of single repeat units as in homopolymers or multiple repeat units as in co- and terpolymers in various order such as random or block with pendant or end groups and containing additives such as initiators, antioxidants, flame retardants, and finishes. Chemical complexity increases further with polymer blends. Matrix-assisted laser desorption/ionization (MALDI) and in some cases electrospray ionization mass spectrometry (MS) have been demonstrated to be powerful methods for characterizing the chemical compositions and in some instances the molar mass distributions for certain polymer classes. However, the complexity of synthetic polymer samples can be so large that it is impossible to analyze a sample directly so that hyphenated analytical approaches have to be employed. Liquid chromatography (LC) fractionation of polymer samples has been successfully used to reduce complexity using on-line, e.g., refs 1−4 and off-line methods, e.g., refs 5−8.

Traditional polymer analysis employing size exclusion chromatography (SEC) uses ~100 μL of 1−2 mg mL^{-1} polymer solution with detection of byproducts or impurities only when present in excess of a few percent. Limitations of SEC fractionation combined with solvent-based MALDI detection for higher molecular weight polymers have been reported.[8] Byproducts of a polymer reaction are expected to be present in small amounts and difficult to detect by any SEC approach. On the other hand, adsorption chromatography at critical conditions

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(1) Prokai, L.; Simonsick, W. J., Jr. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 853−856.

(2) Falkenhagen, J.; Friedrich, J. F.; Schulz, G.; Krüger, R.-P.; Much, H.; Weidner, S. *Int. J. Polym. Anal. Charact.* **2000**, *5*, 549−562.

(3) Aaserud, D. J.; Prokai, L.; Simonsick, W. J. *Anal. Chem.* **1999**, *71*, 4793−4799.

(4) Fei, X.; Murray, K. K. *Anal. Chem.* **1996**, *68*, 3555−3560.

(5) Montaudo, G.; Garozzo, D.; Montaudo, M. S.; Puglisi, C.; Samperi, F. *Macromolecules* **1995**, *28*, 7983−7989.

(6) Kassis, C. E.; DeSimone, J. M.; Linton, R. W.; Remsen, E. E.; Lange, G. W.; Friedman, R. M. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1134−1138.

(7) Hanton, S. D.; Liu, X. M. *Anal. Chem.* **2000**, *72*, 4550−4554.

(8) Trimpin, S.; Eichhorn, P.; Räder, H. J.; Müllen, K.; Knepper, T. P. *J. Chromatogr., A.* **2001**, *938*, 67−77.

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(LACCC) efficiently separates components of a mixture differing by as little as a single end group so that even components present in low quantities are detected.

Various difficulties can accompany fractionation of polymers for subsequent analysis using mass spectrometric methods. The polymer concentration in the eluent can vary greatly (e.g., front, center, or back of a LC peak) so that with MALDI analysis, for example, the analyte/matrix ratios are less than optimal, which might create difficulties in the subsequent mass measurements as well as in the analytical result. Direct deposition of eluent onto a MALDI target plate using organic solvents, which are necessary for most polymers, is problematic because of the low surface tension that results in uncontrolled spreading of the solvent/analyte mixture. The use of on-line spraying methods is also problematic, requiring a set of conditions (e.g., heat, nebulizing gas flow, distance from spray tip to MALDI plate as well as rotating speed of the target) that can vary in fractionation methods in which the solvent composition changes during the analysis. Furthermore, many polymers rely on the use of difficult to spray solvents during, for example, gel permeation chromatography fractionation so that off-line coupling approaches are more applicable. In such a case, fractions are collected, concentrated (or dried to completeness), and redissolved in a MALDI-appropriate solvent. In addition to the time required to prepare a large number of samples, sample loss to the wall of the container might occur during the concentration step and give rise to analytical inaccuracies.

Solvent-free MALDI MS has been shown to provide qualitative and quantitative improvements in the analysis of synthetic polymers, organic macromolecules, and certain peptides and proteins.[9−14] The most obvious advantage is that solvent-free MALDI-MS has been shown to improve the analysis of insoluble and solubility-restricted macromolecules such as synthetic polymers,[9,10] nanosized polycyclic aromatic hydrocarbon-based material,[10,11] fullerenes,[10,14] β-amyloid peptides,[12] and bacteriorhodopsin,[13] an integral membrane protein. Therefore, solubility properties of matrix or analyte molecules are expected to be less important in solvent-free MALDI analyses; solvents used prior to sample preparation become unimportant. Furthermore, compatibility of analyte and matrix and sample loss to the wall are less of an issue.[10,12,13]

The solvent-free MALDI MS method was shown to provide improved results, especially for polymer characterization.[9,10] It was further simplified with the introduction of a simple laboratory vortex device[15] and a miniball mill[16,17] along with inexpensive disposable vials and metal balls for homogenization of sample with matrix that avoided carryover and intensive cleaning. The vortex approach was recently multiplexed with simultaneous transfer of the matrix/analyte to the MALDI target using either a TissueLyzer device in combination with Eppendorf tubes for 16 samples or a vortex device in combination with a 96-well bacti plate for 36 samples.[17] The multisample on-target homogenization/transfer solvent-free method is an important extension to "dry" MALDI analysis as it encompasses homogenization of multiple samples while simultaneously transferring the matrix/analyte mixture to the MALDI target plate. Here we extend this methodology to increase the number of samples that can be prepared/transferred simultaneously and substantially reduce the sample amounts required for solvent-free MALDI analysis. This multisample on-target homogenization/transfer MALDI strategy was applied to the analysis of fractionated synthetic polymers by comparing results of poly(ethylene oxide) PEO mixtures (Scheme 1) previously analyzed by fractionation approaches using the "dry spray" method employing *trans*-2-[3-(4-tert.-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) as MALDI matrix.[18]

EXPERIMENTAL SECTION

Materials. DCTB, 2,5-dihydroxybenzoic acid (DHB), dithranol, and sodium trifluoracetate were obtained from Sigma Aldrich (Milwaukee, WI) and used without further purification. PMMA 5250, PEO 4250 (**1**), are from Polymer Laboratories (Church Stetton, UK). Compounds shown as peak 1, PEO 1000 (**2**) and PEO 6000 (**3**), were obtained from Polymer Standards Service (PSS, Mainz, Germany) whereas functionalized PEO samples were synthesized and supplied by Dr. H. Budde (Martin-Luther-University of Halle-Wittenberg, Germany) including those of peak 2, dimethoxy-PEO 500 (**4**); peak 3, monomethoxy-monomethacrylate-PEO 1000 (**5**); and peak 4, dimethacrylate-PEO 1000 (**6**) (structures in Scheme 1)." peak 2, dimethoxy-PEO 500 (**4**); peak 3, monomethoxy-monomethacrylate-PEO 1000 (**5**); and peak 4, dimethacrylate-PEO 1000 (**6**) (structures in Scheme 1).

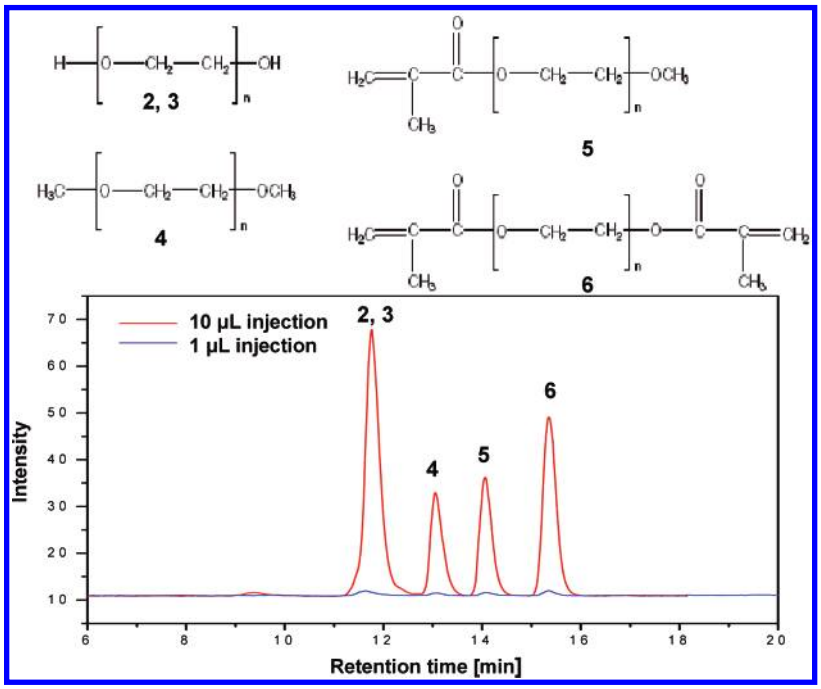
Adsorption Chromatography at Critical Conditions of Adsorption (LACCC) for Off-Line Fractionation. The following system was employed (BAM laboratories): column, 2 X YMC RP 18 (150, 300 Å); eluents, methanol/water 83/17 (v/v); detection, ELSD, SEDEX 45 (Sedere, ERC); temperature, 45 °C; flow rate, 0.5 mL min^{-1}; concentration, 5 samples PEO (2−6), total amount, 5.41 mg mL^{-1}; injection amount, 10 and 1 μL (Scheme 1, LACCC chromatogram). One fraction per 3 s was collected in a 200-μL microcentrifuge PCR tube (Molecular Bioproducts, San Diego, CA) containing three 1.2-mm stainless steel beads (BioSpec Products, Inc, Bartlesville, OK). For each injection, 43 fractions were collected and dried to completeness in a fumehood.

MALDI Instrumentation. The mass measurements on the fractionated samples were performed at DuPont on a Perseptives Biosystems (Framington, MA) Voyager-DE STR MALDI mass spectrometer using a nitrogen laser and a laser power level slightly above threshold for analyte signal. A Bruker Reflex III MALDI mass spectrometer (Bruker-Daltonik, Bremen, Germany) operated with a nitrogen laser and at 20-kV acceleration voltage was used for the unfractionated polymer mixture (BAM Laboratories). The laser power was varied systematically.

Production of Custom-Made Sample Holders Suitable for Multisample Solvent-Free Sample Preparation. Two different

(9) Trimpin, S.; Grimsdale, A. C.; Räder, H. J.; Müllen, K. *Anal. Chem.* **2002**, *74*, 3777−3782.
(10) Trimpin, S.; Keune, S.; Räder, H. J.; Müllen, K. *J. Am. Mass Spectrom.* **2006**, *17*, 661−671.
(11) Räder, H. J.; Rouhanipour, A.; Talarico, A. M.; Palermo, V.; Samori, P.; Müllen, K. *Nat. Mater.* **2006**, *5*, 276−80.
(12) Trimpin, S.; Deinzer, M. L. *J. Am. Mass Spectrom.* **2007**, *18*, 1533−1543.
(13) Trimpin, S.; Deinzer, M. L. *Anal. Chem.* **2007**, *79*, 71−78.
(14) Kotsiris, S. G.; Vasil'ev, Y. V.; Streleetskii, A. V.; Han, M.; Mark, L. P.; Boltalina, O. V.; Chronakis, N.; Orfanopoulos, M.; Hunerbuehler, H.; Drewello, T. *Eur. J. Mass Spectrom.* **2006**, *12*, 397−408.
(15) Hanton, S. D.; Parees, D. M. *J. Am. Mass Spectrom.* **2005**, *16*, 90−93.
(16) Trimpin, S.; Deinzer, M. L. *J. Am. Mass Spectrom.* **2005**, *16*, 542−547.
(17) Trimpin, S.; McEwen, C. N. *J. Am. Mass Spectrom.* **2007**, *18*, 377−381.
(18) Falkenhagen, J.; Weidner, S. M. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3724−3730.

Scheme 1. Structures of PEO Samples 2−6 Used as a Model Mixture and Separated by LACCC Chromatography[a]



[a] The traces of the two different injection volumes and the assignment of the peaks with the respective end group are provided.

custom-made sample holders were produced that precisely allow the homogenization/transfer of 100 samples to the exact MALDI well on the Perseptives Biosystems 100 spot MALDI plate. Holder 1 (Supporting Information photograph of the sample holder) has 100 holes (<10 μL) drilled into a metal plate allowing collection of fractions directly. Holder 2 (Figure 1A.I) has 100 3/16-in. holes entirely drilled through the metal plate and a Teflon spacer that permitted the use of cut vials (200 μL) to be placed into the holes. All holes were cut to match the manufacturer's target positions on a Perseptives 100 spot MALDI plate. In either case, the fractionated samples and matrix/salt mixture in the holes or vials were capped with a MALDI plate. The MALDI plate was held tightly against the sample holder using metal brackets.

Solvent-Free MALDI-MS Based on an On-Target Homogenization Method. A protocol was developed by adapting an existing on-target homogenization/transfer method.[17] Briefly, matrix and salt were added premixed (≤10:1) to the PEO analytes (Scheme 1) that had been dried in the respective slot of sample holder 1 or vial of sample holder 2 along with three metal beads (1.2 mm); this matrix/salt addition was accomplished by shuffling with a small spatula a small amount (<1 mg) from a folded weighing paper. In the case of the LACCC-fractionated samples, because of concerns about sample condition after transportation from Germany to Oregon, all 86 fractions were redissolved in CH_2Cl_2 and redried to ensure that the sample was on the bottom of the vials before use of holder 2. The custom-made sample holders, 1 or 2, were covered by a MALDI plate (Supporting Information, photographs schematically describe the individual steps for holder 2). Each of the custom-made sample holder/MALDI plate sets was tightly held together to homogenize and transfer the sample to the MALDI plate by holding the set on a vortex device for the desired time (10 to 1 min); the set was flipped from time to time to aid transfer of a representative sample onto the MALDI plate.

Solvent-Based MALDI-MS Method. DCTB matrix (10 mg mL^{-1}) and PEO (6.5 mg mL^{-1}, i.e., 5 × 1.3 mg of each PEO) were dissolved in THF. Three matrix/analyte mixtures were prepared by adding to 50 μL of matrix solution 50, 20, and 2.5 μL of sample solution. The best spectra were obtained with the 50:2.5 mixture.

RESULTS

Mixture Analysis Using Standard Polymer Analysis Procedures. Experiments of the polymer mixture using standard solvent-based MALDI analysis and DCTB as matrix detected the lower molecular weight component with good signal intensity but failed to detect the high-mass component at low and medium laser power. At high laser power, however, the high-mass component is detected with poor intensity but the low-mass components fragmented under these high laser power conditions. The mixture analysis is not satisfactory. The suppression of the detection of high-mass components in standard solvent-based MALDI analysis is a well-described problem.[18,19]

Development and Validation of Custom-Made Sample Holder Plates. The purpose of these experiments was to evaluate whether the solvent-free MALDI method could be adapted to 100 position MALDI target plates and to determine whether reduction in sample preparation volume results in reduced analyte requirements. Two custom-made sample holders were machined and also evaluated for sufficient matrix/analyte homogenization and to determine if cross-contamination between sample spots occurred. Adapting the multisample preparation method to deposit the matrix exactly on the prearranged MALDI target spots has the advantage of allowing direct use of automation software to acquire mass spectra. Figure 1A.I shows sample holder 2 with beads, matrixes (DHB and dithranol), and salt. The MALDI plate after

(19) Martin, K.; Spickermann, J.; Räder, H. J.; Müllen, K. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1471−1474.

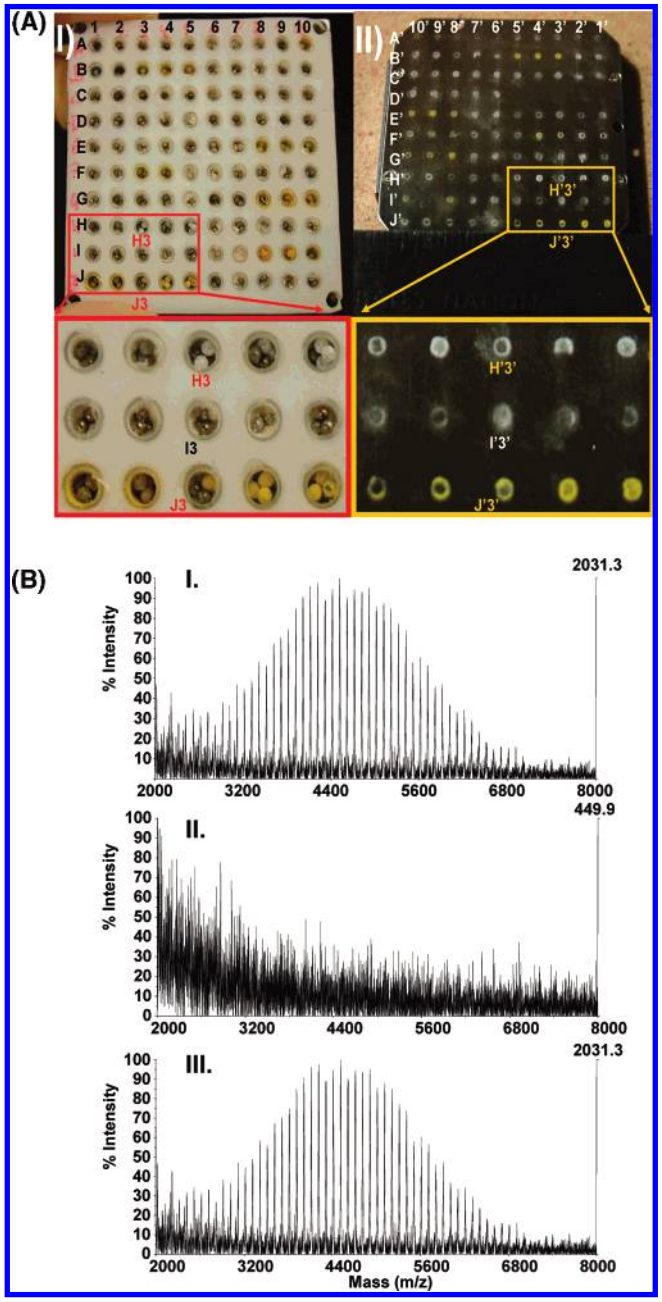


Figure 1. (A) Photograph of on-target homogenization/transfer device of the multisample (100) MALDI method using a laboratory vortex device: (I) custom-made sample holder 2 loaded with sample, beads, salt, and MALDI matrix (II) and the mirror image MALDI plate of the homogenized/transferred MALDI samples using the vortex device for 10 min after excess material was removed from the plate, as well as their respective enlargements (bottom). To allow the evaluation of cross-contamination, each row with sample (e.g., H3 and J3) was always separated by a row of only matrix (e.g., I3) added to each well. Dithranol and DHB were used as matrixes. (B) Mass spectra of PMMA 5200 narrow polydisperse polymer using on-target homogenization/transfer of the multisample (100) MALDI method applying the custom device shown in Figure 2A: (I) sample spot H'3′, DHB and PMMA; (II) sample spot I'3′, DHB only; (III) sample spot J'3′, dithranol and PMMA. The sample spot (I'3′) in which only matrix was added to the custom-made sample holder does not provide polymer signal in the acquired mass spectrum so that cross-contamination between sample spots can be excluded.

homogenization for 10 min is displayed in Figure 1A.II showing the transferred analyte/matrix. The analyses of a PMMA 5250

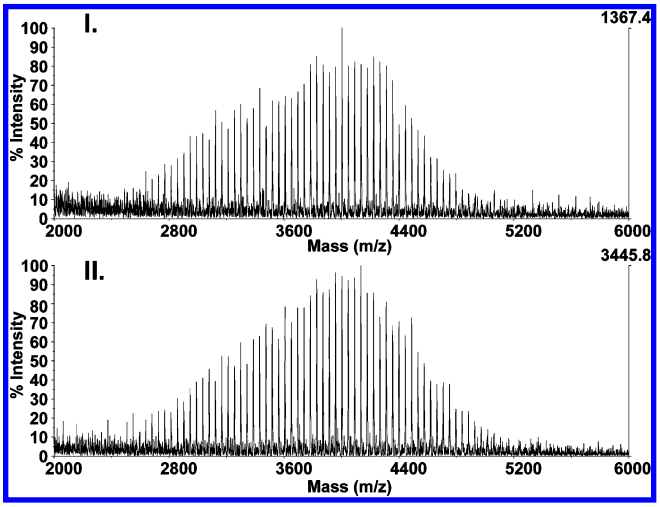


Figure 2. Mass spectra of PEO 4250 standard evaluating appropriate salt addition (sample holder 1). With sodium acetate added (I) and with no salt (II). These results indicate that the addition of excess salt is not necessary.

standard (Figure 2B.I and III) provide evidence that the homogenization is sufficient. In addition, cross-contamination is not observed in this experiment (Figure 2B.II).

A subsequent set of experiments, using sample holder 1, demonstrates that sufficient sodium salt is present in the dithranol matrix or on surfaces to provide good ionization of PEO 4250 (Figure 2). Adding additional sodium salt in the form of sodium acetate did not result in improved results. Sample holder 2 provided similar results and did not require loading and cutting vials but required cleanup after sample preparation. Ideally, disposable sample holder plates would be employed. These results demonstrate that the 100-sample method provides sufficient homogenization and transfer of matrix/analyte for successful MALDI analysis.

Application of the On-Target Homogenization/Transfer Solvent-Free MALDI (100) Method to LACCC-Fractionated PEO Mixtures. The LACCC method has been shown to separate polymers according to end groups.[20] In these experiments, narrow distribution PEO standards having different end groups were separated using LACCC and collected in 3-μL increments into 200-μL Eppendorf tubes. This analysis was selected because nearly identical experiments using a "spray" method for analyte/matrix deposition are published,[18] thus providing a straightforward comparison of methods. The homogenized/transferred samples from each LACCC run are precisely transferred to the manufactured target spots on the MALDI plate (Figure 3A). Results from injection of 10-μL are shown in Figure 3B.I and II as well as the 1-μL injection in Figure 3B.III and IV. Each provided the unequivocal mixture analysis including PEO end groups independent of the polymer molecular weight (Scheme 1). Both the 10- and 1-μL injections provided mass spectra from wells corresponding to polymer elution. The 10-μL injection, however, provided more abundant polymer ions and could be observed in wells in which collection occurred at the base of the elution peak. Although the amount of polymeric material for analysis is usually not a limiting factor, sensitivity issues are nevertheless important in

(20) Entelis, S. G.; Evreinov, V. V.; Gorshkov, A. V. *Adv. Polym. Sci.* **1986**, *76*, 129−317.

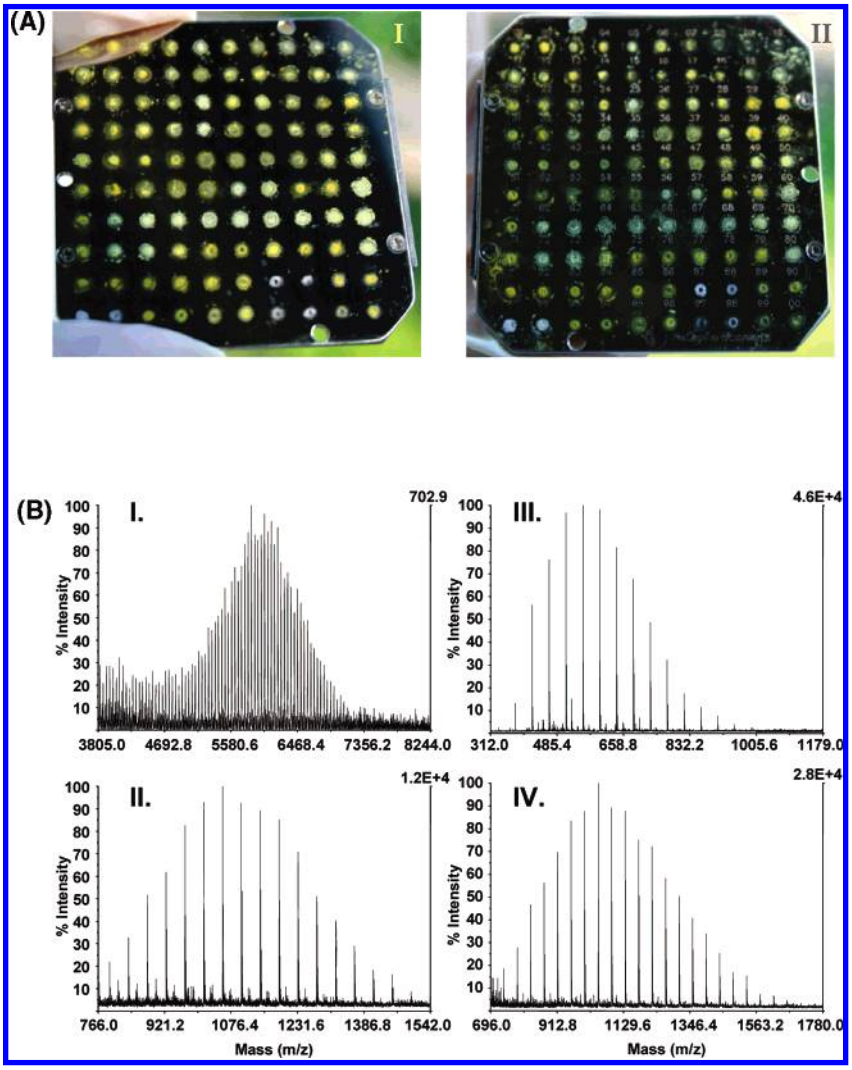


Figure 3. (A) Photographs of on-target homogenization/transfer of fractionated samples using the multisample (100) MALDI method after excess material was removed from the plate: MALDI plate after 10 min vortexing (A) and preparation of a second plate after vortexing of sample (I) for an additional 1 min (II). (B) Mass spectra of the LACCC fractionated samples and prepared by the multisample approach using sample holder 2 (A). The 10-μL injection of the mixture detecting component (I) PEO 6000 in peak 2,3 Figure 1, (II) PEO 6 in peak 6 Figure 1; 1-μL injection of the mixture detecting component III PEO 4 in peak 4 Figure 1; (IV) PEO 5 peak 5 Figure 1.

detecting minor components and impurities. The increase in dynamic range of detection using a hyphenated separation/MALDI MS approach is directly dependent on the sensitivity of the MS method. Another important feature of this hyphenated approach is the ease of detecting PEO 6000 when it is separated from the lower-mass polymers.

After the first MALDI plate was removed from the custom-made sample holder, another MALDI plate was affixed and the set was vortexed for an additional 1 min (Figure 3A.II) and reanalyzed. The spots on the MALDI plate appeared smeared; however, equally good mass spectra were obtained. The option of easy reanalyses is a valuable asset to this method, as is the ability to transfer or distribute collected fractions between laboratories, even over great distances as was done in this study.

In this novel off-line approach, 43 fractions of each of the respective injection volumes were collected. In the dry spray approach previously reported for the same samples and injection concentrations, 13 different MALDI spots were sprayed with the on-line eluting samples.[18] Consequently, the observed sensitivity of the off-line method presented here is comparable to the previously reported on-line approach. Calculating the amount of sample in successful MALDI spots shows that, for the 1 μL of injected PEO samples, as little as 1 μg of polymer injected provides acceptable results, which for PEO 6000 represents ~200 pmol. This clearly demonstrates that the reduction in matrix used in the miniaturization resulted in less analyte requirement than previous high-volume solvent-free methods.[9−11,14,15] Because the samples were dried before analysis, it also shows that the solvent-free method is efficient in recovering sample from the container walls during the homogenization process.

CONCLUSION

Four PEO samples differing in molecular weight from about 500 to 6000 and in end groups were cleanly separated using LACCC chromatography. Injection of <2 μg per polymeric fraction was sufficient to detect each of the polymers using a multisample, solvent-free MALDI approach in which samples are homogenized with a matrix/salt mixture and simultaneously transferred to the precise manufactured target spots of a 100-well MALDI plate. The simplicity of this off-line collection method with subsequent

homogenization/transfer of matrix/sample to the MALDI plate for automated MALDI analysis, as well as the inherent advantages of solvent-free MALDI of synthetic polymers should make this technique useful not only for LC/LACCC separations but for the analysis of wide polydisperse polymers in combination with gel permeation chromatography. The LACCC/solvent-free MALDI approach was shown to provide high sensitivity detection of fractionated polymers with different end groups and having M_n values from 500 to 6000.

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SUPPORTING INFORMATION AVAILABLE

Photograph of sample holder 1; schematic representation of the procedure used for the off-line fractionation for the PEO mixture analysis using the multisample solvent-free MALDI analysis using the sample holder 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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