

The effect of tetrahydrofuran as solvent on matrix-assisted laser desorption/ionization and electrospray ionization mass spectra of functional polystyrenes

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Received 14 September 2005; Revised 9 November 2005; Accepted 9 November 2005

It is demonstrated that tetrahydrofuran (THF) should be used with caution as a solvent in polymer sample preparation for matrix-assisted laser desorption/ionization (MALDI) analyses. The presence of peroxides in THF may cause the oxidation of chemically active groups. This effect is illustrated in the case of S-containing polystyrene derivatives. The oxidation of the trithiocarbonate group resulted in the formation of poly(styrene)sulfonic acids, $R(\text{CH}_2\text{CHPh})_n\text{SO}_3\text{H}$, which was detected in negative mode by MALDI and electrospray ionization (ESI) methods after the samples remained in THF for several hours. Copyright © 2005 John Wiley & Sons, Ltd.

Sample preparation is a critical step in a matrix-assisted laser desorption/ionization (MALDI) analysis of polymers.^{1–3} The success of such analyses depends on a choice of matrix, correct matrix-to-analyte ratio, and the use of proper ionization promoters. One of the most common ways of sample preparation for MALDI analysis consists of dissolving all components in the same solvent. The nature of the solvent is not considered to be essential if it serves the purpose of properly mixing all components and providing good co-crystallization of the mixture. Some polymers have very restricted solubility and the choice of solvents may be extremely limited. For example, many polystyrene (PS) polymers can only be dissolved in solvents such as tetrahydrofuran (THF), methylene chloride or chloroform. The latter two have high volatility making it difficult to prepare and handle solutions. THF is a solvent of choice for such polymers and it dominates the list of recipes for MALDI analysis of PS derivatives suggested by NIST.⁴ In the present work we report our observations that the use of THF may cause chemical transformations of polymers having functional groups that are sensitive to oxidation.

EXPERIMENTAL

MALDI mass spectra were obtained using a ToF Spec 2E time-of-flight mass spectrometer (Micromass, UK). Ionization was achieved using a pulsed N₂ laser at a wavelength of 337 nm. Mass spectra were recorded in reflectron mode. Data acquisition and data processing were performed using MassLynx 3.5 software. Polymer solutions were prepared by dissolving ~2 mg of a PS derivative in 1 mL of a solvent (THF, chloro-

form or methylene chloride). The solutions were immediately used for MALDI analyses or stored for up to 3 days before MALDI or electrospray ionization (ESI) analyses were performed. THF (purchased from Aldrich and not containing stabilizer) which was used in the experiments was exposed to air for a long period of time. Sample solutions were mixed with dithranol (10 mg/mL) in a 1:1 ratio. To induce the production of positively charged ions, 1 mL of 0.01 M Ag(I) trifluoroacetate (AgTFA) solution was added to samples analyzed in *positive ion* mode. Dithranol and AgTFA solutions were always freshly prepared. Final solutions (0.5–1 µL) were applied to the target plate.

ESI mass spectra were obtained on an Agilent 1100 series LC/MS-SL ion trap system (Germany). Samples were dissolved in THF and introduced into the ion source using a syringe pump at a flow rate of 400 µL/h. A mixture of methanol/isopropanol/ammonium hydroxide (70:20:10) was added to sample solutions in a ~1:10 ratio to induce the production of negatively charged ions. The data were collected in a mass range from m/z 300–2200. The ion trap parameters were optimized for the most efficient storage of ions of interest. Instrument control, data acquisition and data processing were performed using ChemStation 10.01 and IonTrap 5.2 softwares.

RESULTS AND DISCUSSION

One of the major requirements of solvents that are used in sample preparation for MALDI analysis is their ability to dissolve all components of the mixture, including the analyte, matrix, and any ionization enhancement additives. It is often assumed *a priori* that solvents are chemically inert, i.e., they do not react with the analyte. In this communication, we report that the commonly used solvent tetrahydrofuran

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(THF) can cause chemical transformations of functionalized polystyrenes.

We subjected to MALDI analysis a series of PS-based polymers generated by reversible addition/fragmentation chain transfer (RAFT) polymerization.⁵ The polymers were prepared by the 2,2'-azobisisobutyronitrile (AIBN)-initiated RAFT polymerization of styrene using $\text{HOOC-CMe}_2\text{SC(=S)SCMe}_2\text{COOH}$ as a chain transfer reagent.^{6,7} The formation of $\text{RCMe}_2(\text{PS})_n\text{SC(=S)S(PS)}_m\text{CMe}_2\text{R}'$ (R, R' = CN, CN; CN, COOH and COOH, COOH) oligomers had been anticipated. Mass spectrometry methods, ESI and MALDI, have been used for the characterization of products of RAFT polymerization.^{8–14} The presence of carboxylic end group(s) in the products motivated us to use negative ion MALDI in addition to traditional positive ion mode experiments. Negative ion MALDI has been successfully applied for polymers having acidic (COOH, SO_3H) end groups.^{14–18} Examining our polymers in negative ion mode was also challenging because MALDI(+) mass spectra did not show intact oligomers displaying only fragments formed by (laser-induced) dissociation of C–S bonds.

MALDI(–) mass spectrum of one of the polymers tested in the present work is shown in Fig. 1(a). The sample was prepared by dissolving the matrix and the polymer in THF, placing the mixture on the MALDI plate, and performing the analysis immediately after the spot had dried. Peaks of the

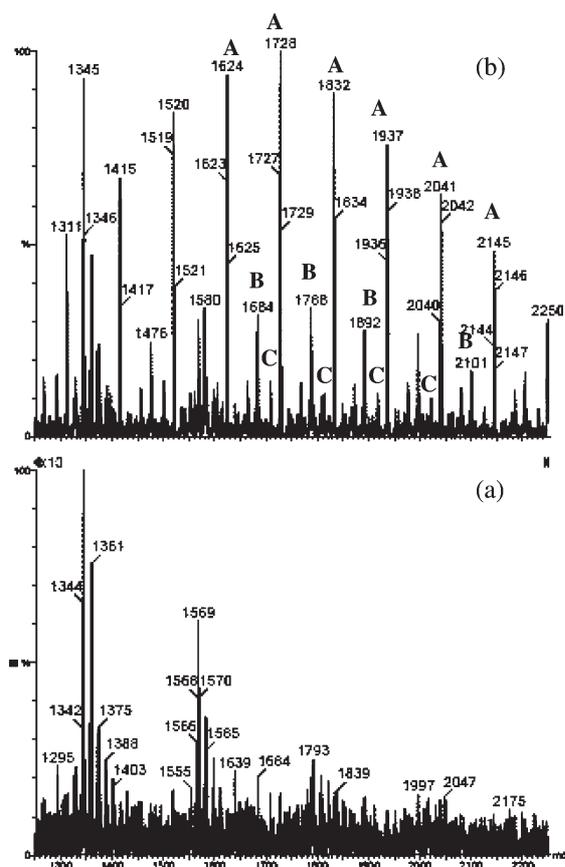


Figure 1. MALDI(–) mass spectra of a polymer dissolved in THF and analyzed (a) within a few minutes and (b) after storing the solution for 48 h. Peaks of A, B and C series correspond to $[\text{RCMe}_2(\text{PS})_n\text{SO}_3]^-$ ions with R=HOOC, NC, and H, respectively.

matrix clusters dominated the mass spectrum. A few signals separated by 104 Da could be noticed but their intensity was extremely low. A surprising result was obtained when the same sample was spotted on the MALDI plate after it had been stored in THF overnight or for longer periods of time (up to 3 days). The mass spectrum shown in Fig. 1(b) displayed several distinctive series of signals corresponding to newly formed PS derivatives. The signals were assigned to PS derivatives having a sulfonic acid end group, $[\text{RCMe}_2(\text{PS})_n\text{SO}_3]^-$, where R=H, CN, or COOH.

The origin of these ions was investigated by using different solvents. MALDI(–) mass spectra of freshly prepared samples using methylene chloride or chloroform samples did not show peaks corresponding to any newly formed PS derivatives. Only matrix cluster peaks were detected for samples dissolved and stored in methylene chloride for 2–5 days. These results pointed to THF as the cause for the appearance of $[\text{RCMe}_2(\text{PS})_n\text{SO}_3]^-$ ions.

In another set of experiments, a THF solution of a polymer was analyzed by the ESI(–) method. Mass spectra of a freshly prepared solution and a solution left overnight are shown in Fig. 2. In both cases ammonium hydroxide in MeOH/i-PrOH

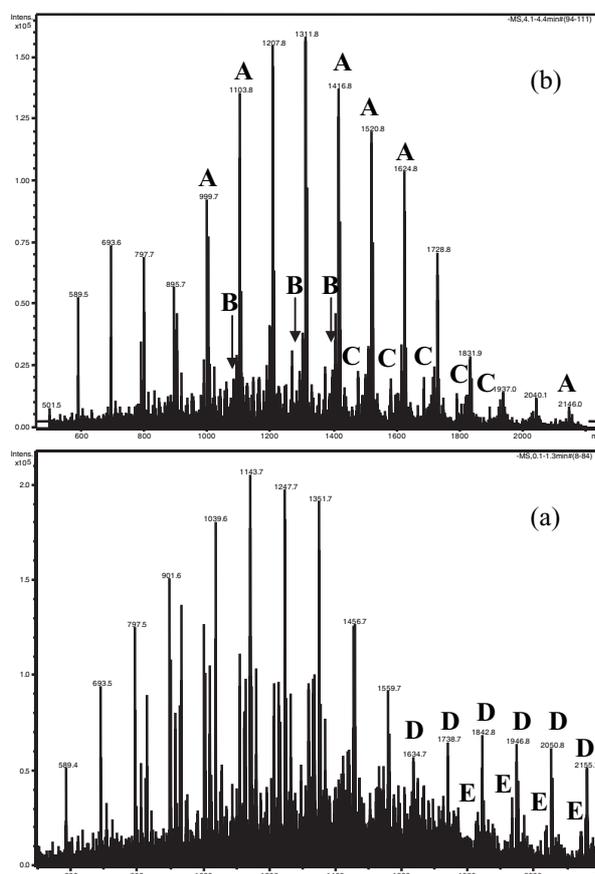


Figure 2. ESI(–) mass spectra of a polymer (a) freshly dissolved in THF and (b) after 48 h of storing in solution. Peaks of A and C series are the same as in Fig. 1. Peaks of series D and E correspond to $[\text{M}-\text{H}]^-$ ions of intact oligomers $\text{HOOCMe}_2\text{C(PS)}_n\text{SC(S)S(PS)}_m\text{CMe}_2\text{COOH}$ and $\text{HOOCMe}_2\text{C(PS)}_n\text{SC(S)S(PS)}_m\text{CMe}_2\text{CN}$, respectively. Lower mass region peaks are assigned to $[\text{HS(PS)}_n\text{CMe}_2\text{COOH}-\text{H}]^-$, $[\text{NC(PS)}_n\text{S}]^-$, $[\text{CH}_2\text{CPh(PS)}_n\text{CMe}_2\text{COO}]^-$ and some unidentified series of ions.

was added before the analyses to assist the formation of negatively charged ions. The ESI mass spectrum of the 'aged' sample (Fig. 2(b)) was completely different from that of the 'fresh' sample (Fig. 2(a)) and displayed peaks similar to those in the MALDI(-) mass spectrum (Fig. 1(b)). Tandem mass spectrometry (MS/MS) experiments with mass-selected ions showed a neutral loss of SO₂, 64 Da, confirming the presence of the SO₃ group. Perhaps the most remarkable and important difference between the two ESI mass spectra was the disappearance of peaks of intact oligomers, [NCCMe₂(PS)_nSC(=S)S(PS)_mCMe₂COO]⁻ and [HOCCMe₂(PS)_nSC(=S)S(PS)_mCMe₂COO]⁻, in the mass spectrum of the 'aged' sample. These peaks were present in the ESI(-) mass spectrum of the 'fresh' sample (Fig. 2(a)).

All experimental data obtained in this study undoubtedly indicate that the storage of S-containing PS derivatives in THF causes the oxidation of the trithiocarbonate groups to sulfonic acid groups. The detection of these products could only be accomplished by recording MALDI or ESI mass spectra in negative ion mode. The oxidation process seemed to occur quickly as the sulfonate derivatives were detected after just a few hours at room temperature. The formation of sulfonates does not appear to affect seriously the results obtained in positive ion mode for samples retained in THF for 2–3 days. After that it was difficult to detect signals due to PS derivatives in both negative and positive ion modes indicating additional chemical transformations of the polymer samples.

CONCLUSIONS

THF is a commonly used solvent, in which peroxides are easily formed. Their presence should not be ignored when THF is used for dissolving polymers having easily oxidized groups such as those containing sulfur atoms. Oxidation processes may result in chain cleavage within the polymer backbone and the inability of mass spectrometry techniques to detect the intact molecules. The oxidation may also interfere with the accurate determination of end-group chemistry. The effect of the origin and age of THF was not investigated in the present study. Analysis of peroxide concentration in the solvent may not be needed unless mass spectrometry data are in unexplainable discrepancy with predicted structures. In such cases, use of an alternative solvent (including a different batch of THF) for sample preparation is recommended for more comprehensive polymer structure determination.

It should be noted that backbone or end-group oxidation by the solvents may provide an alternative method of determining polymer structures by using ESI and MALDI techniques in negative ion mode. The production of negative ions does not require adding metal salts as ionization enhancers and can be performed on simple polymer/matrix mixtures.

Acknowledgements

The Department of Chemistry and Chemical Biology is acknowledged for continuing support of the Mass Spectrometry Facility. The National Science Foundation is gratefully acknowledged for the purchase of the MALDI-TOF instrument (NSF Grant CHE-0078056) and the LC/MS system (NSF Grant CHE-0091892).

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