Coupling of Liquid Chromatographic Polymer Separations with Time-of-Flight Mass Spectrometry


Coupling of liquid chromatography (LC) techniques with matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI) time-of-flight (TOF) mass spectrometry (MS) provides both MS-based structural information and LC-based quantitative data in polymer analysis. In one experimental set-up three different LC modes can be interfaced with MS: size-exclusion chromatography (SEC)–MS, gradient polymer elution chromatography (GPEC)–MS and liquid chromatography at the critical point of adsorption (LCCC)–MS. In SEC–MS both absolute mass calibration of the SEC column based on the polymer itself as well as determination of monomers and end-groups from the mass spectra are achieved. GPEC–MS shows detailed chemical heterogeneity of the polymer and the chemical composition distribution within oligomer groups. In LCCC–MS the retention behaviour is primarily governed by chemical heterogeneities, such as different end-group functionalities, and quantitative end-group calculations can easily be made.

In this article experimental details of the couplings are presented, both for LC–ESI-TOF and LC–MALDI-TOF MS, and the potential of these methods is illustrated by two polymer applications.

The Complexity of Polymer Samples

In comparison with biopolymers, such as proteins and peptides, the analysis of synthetic polymers is even more complex because of the coexistence of several distributions.

- Instead of a single molecular weight, we must deal with a molecular weight distribution (MWD) as a result of polymer synthesis.
- The polymer chains might have different end-group chemistries because of different initiation and termination processes, thereby creating a functionality-type distribution (FTD).
- In the instance of random copolymers, the polymer chains also show a chemical composition distribution (CCD).
- In the instance of block copolymers, additional sequence and block-length distributions are present.

Polymers and copolymers show an architecture distribution and might be linear, cyclic, branched or dendritic etc. Despite this complexity, ESI and MALDI MS can contribute to the characterization of all these distributions but so far most studies are limited to MWD and FTD analyses. Unfortunately direct mass spectrometric characterization of synthetic polymers is not very quantitative and even under optimized conditions mass discrimination in the analysis of polydisperse polymers and specific oligomer discrimination might occur (1). Hence, hyphenated techniques have been developed for polymer analyses in which the reliable quantitative features of LC are combined with the identification power and structure analysis of MS.

How to Couple Polymer Separations and Electrospray MS

The rather non-polar solvents used in polymer separations are problematic for on-line coupling with ESI MS. The first complication is the solvent compatibility: commercial ESI probes developed for biopolymers often contain tubing, unions or fittings made of polyetheretherketone (PEEK). As a result we have not encountered any incompatibilities since we started SEC–ESI MS in 1993. The second complication is about ion formation. In contrast to biopolymers, synthetic polymers are hardly ionized by protonation, but typically by cationization. Polar solvents usually contain sufficient residual sodium or potassium cations to support the cationization process. Less polar solvents, such as THF or dichloromethane, do not supply these cations and the mobile phase should either be doped with a salt (2) or, preferably, a salt solution should be added postcolumn (3). The latter can also be done conveniently using the make-up solvent channel of a triaxial ESI probe (2).

In Figure 1 a generic experimental set-up is presented for the coupling of any LC mode in polymer analysis with an ESI mass spectrometer (3). Depending on the specific LC mode, only columns and mobile phases need to be exchanged for the purpose of SEC–MS, GPEC–MS or LCCC–MS. The column effluent is split: the majority of the flow towards an ultraviolet (UV)-absorbance diode array detector placed in series with a refractive index detector, and only 30 µL/min towards a second T-piece in which a compatibilizer liquid is added consisting of 250 µM sodium iodide in stabilized THF/1-propanol (9:1) at 10 µL/min. The sodium salt supports the ionization process and the propanol component suppresses adsorption of oligomers having polar end-groups on the inner wall of the fused-silica transfer capillary. We prefer the use of an ESI-TOF mass spectrometer for polymer analysis.
because of the significant improvements over the quadrupole MS system used in the past (1): increased signal caused by the much higher duty cycle; increased m/z range which is particularly relevant for less polar synthetic polymers that show only moderate multiple charging upon ESI; and high mass accuracy that allows exact mass measurements and calculation of elemental composition as recently shown in the accurate end-group determination of low molecular weight polymers (4).

How to Couple Polymer Separations and MALDI MS

Because of the high mass range of MALDI-TOF MS, couplings are made mostly with SEC. Still, many articles describe off-line coupling matters. SEC. Still, many articles describe off-line TOF MS, couplings are made mostly with MALDI-MS or MALDI MS. How to Couple Polymer Separations

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Figure 1: Experimental set-up for three different LC–MS modes in polymer analysis. A–C = solvents for mobile phase; D = gradient HPLC pump (HP 1050, Agilent Technologies); E = autosampler (Basic Marathon, Spark Holland); F = 2 µm stainless steel precolumn filter; G = LC column (Symmetry C18, Waters); H = 0.010 in. i.d. T-piece; I = 5 cm × 0.007 in. i.d. stainless steel capillary; J = diode-array UV absorbance detector (AB1 1000S, Applied Biosystems); K = refractive index detector (Shodex RI-71, Showa Denko); L = 60 cm × 75 µm i.d. fused silica capillary; M = syringe pump (Model 11, Harvard Apparatus) with compatibilizer; N = electrospray-TOF mass spectrometer (LCT, Micromass). Flows are indicated in µL/min. (Reproduced from reference 3 with permission from the American Chemical Society.)

Figure 2: (a) Set-up for µSEC–MALDI-TOF MS using a robotic interface, consisting of a model 1408 µpump (Applied Biosystems); model C4-1004-0.5 injection valve (Valco Cheminert); µSEC column packed with MiniMixed-D (LC Packings); a model Lambda 1000 µUV-absorbance detector (Bischoff); a Probot robotic interface (BAI); a Baby Bee matrix pump (BAS) and a Biflex XRF 5 µm i.d. fused silica capillary. (b) Close-up of the needle tip. (Reproduced from reference 8 with permission from the American Chemical Society.)

Figure 3: µSEC–UV chromatogram of polybisphenol A carbonate. Inset: MALDI MS-based calibration plot. Log M = 9.836 – 79.62 Ve (r² = 0.999). (Reproduced from reference 8 with permission from the American Chemical Society.)
only, it can be similarly used in the µGPEC–MS or µLCCC–MS mode by using a µ-gradient LC pump and a reversed-phase or normal-phase µLC column. Ultimately, two-dimensional polymer separation systems consisting of both an LCCC and a SEC column can be coupled with MALDI MS in a similar way.

**Selected Applications**

An important application of coupled polymer separations/mass spectrometry is SEC–MS in which the mass spectra are used to generate absolute mass calibration points for the SEC chromatogram, based on the polymer itself instead of a narrow standard, such as polystyrene calibrants. In this example µSEC–MALDI-TOF MS of polybisphenol A carbonate (PC) is shown using indoleacrylic acid as a MALDI matrix (8). Eight spots were selected for MALDI analysis and the ion distributions measured were used to determine the $M_p$ values for the calibration of the µSEC column with subsequent calculation of the absolute MWDs. The MS-based absolute calibration curve of the µSEC system is shown in Figure 3. The absolute MWD data were calculated from the calibrated chromatogram and are given in Table 1, and compared with reference data.

It can be concluded that both direct MALDI MS (i.e., without fractionation by SEC) and SEC alone (regular polystyrene calibration curve) yield incorrect results. Only data obtained by coupled µSEC–MALDI MS are comparable with the manufacturers’ data (8). Of course structural information is included in these data: the isotopically resolved mass spectra of the lower oligomer fractions/spots confirmed the monomer mass of the polycarbonate and after subtraction of the cation mass and $n$ times the monomer mass, the end-groups could be inferred from the mass residues yielding the structure proposals phenyl(bisphenol A carbonate),$n$phenyl, cyclic (bisphenol A

![Figure 4: GPEC of dipropoxylated bisphenol A/dipropionic acid polyester](image)

**Table 1: Molecular Weight Distribution Data of Polybisphenol A Carbonate.**

<table>
<thead>
<tr>
<th>Method</th>
<th>$M_w$</th>
<th>$M_n$</th>
<th>$M_z$</th>
<th>$M_p$</th>
<th>$M_w/M_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEC, without MS, calibrated</td>
<td>53100</td>
<td>28500</td>
<td>80500</td>
<td>55400</td>
<td>1.86</td>
</tr>
<tr>
<td>MALDI MS, without SEC</td>
<td>11500</td>
<td>9600</td>
<td></td>
<td></td>
<td>1.20</td>
</tr>
<tr>
<td>µSEC–MALDI MS</td>
<td>26400</td>
<td>15800</td>
<td>38300</td>
<td>27900</td>
<td>1.67</td>
</tr>
<tr>
<td>Manufacturer data</td>
<td>28800</td>
<td>17300</td>
<td></td>
<td></td>
<td>1.66</td>
</tr>
</tbody>
</table>

![Time (min)](time)

![Figure 4: GPEC of dipropoxylated bisphenol A/dipropionic acid polyester](image)
A second application of coupled polymer separations/mass spectrometry is GPEC–MS in which the mass spectrometer is mainly used to identify the complex pattern of peaks in the chromatogram (3). The sample is dissolved in a good solvent and a small volume is injected into the gradient LC system, which starts with a non-solvent, causing precipitation of the sample. The point of redissolution in the gradient, (i.e., a specific composition of non-solvent and good solvent), depends both on chemical heterogeneities, such as chemical composition and end-group functionality, and on the molecular weight of the oligomers. In this selected application a sample of dipropoxylated bisphenol A/adipic acid (DA polyester) was analysed by GPEC with parallel UV and ESI-TOF MS detection (3). The UV-absorbance signal and the identified total ion current chromatogram from ESI MS are given in Figure 4. Despite the use of a postcolumn splitter and the addition of the compatibilizer solvent, detailed oligomer resolution has been maintained in the MS signal and oligomers can be easily recognized up to \( n = 20 \) (i.e., \( M \approx 9000 \) Da). Following identification by ESI MS the UV-absorbance chromatogram can be used for quantification provided that a correction is made for the additional UV absorbance of the end-group in (DA),D-type oligomers. The chemical composition distribution can be calculated and is shown in Table 2.

It can be concluded that significant cyclization of lower oligomers occurs and that the quantitative end-group composition of the sample tends to stabilize at \( n = 5 \) towards a polyester with equal hydroxyl and carboxylic ends.

**Conclusion**

Coupling of polymer LC separations with electrospray or MALDI mass spectrometry is straightforward and can be achieved both in a conventional or in a µLC system. An overlap exists between the use of both MS techniques: the final choice is typically based on instrument availability, expected mass range and expected degree of multiple charging. The recent introduction of benchtop ESI TOF instruments having a typical mass range beyond 12,000 for single-charged molecules covers the characterization of many low molecular weight resins without the necessity of matrix optimization as in MALDI.

**References**

7. LC-Transform Series 500, Lab Connections, Inc., Marlborough, Massachusetts, USA.

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