Separation and characterization of functional polymers
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State-of-the-art techniques for the mass-spectrometric characterization of synthetic polymers have been applied to functional poly(methyl methacrylate) (PMMA), synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization. The polymers were first separated effectively according to functionality by liquid chromatography (LC) at the critical conditions (i.e., almost no influence of molecular weight on retention). The separated polymers were characterized off-line by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and both off-line and on-line by LC-electrospray-ionization-quadrupole-TOF-MS (LC-ESI-QTOF-MS). The on-line ESI experiments confirmed a clear baseline separation of the hydroxyl-functional prepolymer according to the number of hydroxyl groups. Labile end-groups of PMMA, such as the dithioester group, were lost in the MALDI-TOF-MS experiments, while they were observed intact in the ESI-QTOF-MS spectra. This indicates that in the present case ESI is a much softer ionization technique than is MALDI. The ESI-MS experiments provided direct evidence that the RAFT polymers still exhibited living characteristics in the form of the dithio moiety.

**Keywords:** Critical liquid chromatography, hydroxyl group, telechelic polymers, RAFT polymers, LC-ESI-MS, MALDI, fragmentation.

3.1 Introduction

Functional (pre-)polymers can be used as cross-linking components in coating formulations to create hard, durable films. For example, telechelic prepolymers (containing two hydroxyl end-groups) with narrowly distributed molecular weights can react with trifunctional isocyanates to form a well-defined polyurethane network. It is expected that the study of these systems will provide better insights into the relations between the network structure and the properties of cross-linked coating systems. Eventually, this should also lead to coatings with better properties. However, it is very difficult to produce the required telechelic polymers by conventional polymerization methods.

Fortunately, the recent development of controlled-'living' radical polymerization has created possibilities for the synthesis of many well-defined polymers with designed architectures and predictable molecular weights [1,2]. The most important methodologies include nitroxide-mediated radical polymerization [3], atom-transfer radical polymerization (ATRP) [4], and lately reversible addition-fragmentation chain-transfer (RAFT) polymerization [5]. Among these, RAFT polymerization arguably has the greatest commercial impact, because it only involves organic substances, it has a high tolerance to impurities, and it can be applied for a wide range of monomers, including acrylic acid [2]. Therefore, we have selected RAFT polymerization for the preparation of bifunctional telechelic (meth)acrylate polymers with a low polydispersity index.

RAFT polymerization relies on the use of thio carbonylthio-compounds (or dithioesters) of general structure \( Z-C(=S)S-R \). \( R \) is the homolytic leaving group of the RAFT agent; \( Z \) is an activating group. The dithioesters act as reversible addition-fragmentation chain-transfer agents, allowing the formation of polymers with functional end-groups [5,6]. A simplified mechanism of the RAFT process is given in scheme 3.1. Initiator-derived primary radicals \([I^*]\) react with monomer units to form oligomeric (propagating) radicals \([P_n^*]\), which undergo addition to thio carbonylthio compounds to form adduct radicals. The resulting species fragments into a polymeric thio carbonylthio compound and a homolytic leaving group. The latter (\([R^*]\)) is capable of reinitiating the polymerization to give a new propagating radical \([P_{n+1}^*]\). Equilibrium is then established between all the active propagating radicals \([P_n^* \text{ and } P_{m+1}^*]\) and the dormant polymeric thio carbonylthio-compounds by way of the intermediate radicals. Most of the polymers obtained at the end of a RAFT polymerization will contain the leaving group of the RAFT agent, but if initiators are used a (small) fraction will contain the initiating radical fragments (I). However, in some experiments [7,8,9] the amount of initiator-derived polymer was too low to be detected. Destarac et al. [10] and Schilli et al. [11] did observe signals corresponding to polymers with initiator end-groups in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) of RAFT polymers.
Mass spectrometry plays an increasingly important role in polymer analysis, because of its high sensitivity, broad dynamic range, specificity, and selectivity [12,13,14,15]. Two significant developments in ionization techniques in the late 1980s – MALDI and electrospray ionization (ESI) – greatly enhanced the applicability of MS for polymer characterization. These techniques enable the ionization of large nonvolatile molecules with good integrity, thus allowing determination of the weight of intact polymer molecules by MS. However, to study the heterogeneity of complex polymer systems at the molecular level, it is vital that MALDI or ESI-MS is combined with a suitable separation technique [16,17,18,19]. MALDI is the newest and reputedly most promising ionization method for synthetic polymers [13]. Thanks to the emergence of MALDI, polymer scientists have taken a greater interest in MS [13]. In publications relating to the characterization of synthetic (co-)polymers by mass spectrometry, MALDI is favored as the ionization method, because only singly charged ions are observed [15], producing much clearer spectra.

However, only a few references contain MALDI mass spectra of polymers obtained by RAFT polymerization [7,8,10,11,20,21]. The spectra obtained in these reports do not provide direct structural information on, for example, end-groups of the RAFT polymers. Vosloo et al. [20] presented a MALDI-TOF-MS spectrum of RAFT polystyrene, which contained several series of peaks. They stated that it was difficult to obtain useful MALDI-TOF-MS spectra of these polymers. Destarac et al. [10] published a MALDI-TOF-MS spectrum of RAFT poly(vinyl acetate). They observed initiator-derived and hydrogen-terminated polymers. D’Agosto et al. [21] reported the
MALDI-TOF-MS spectrum of poly(N-acryloylmorpholine), synthesized by RAFT polymerization. They described that in the MALDI instrument the RAFT polymer experienced fragmentation in the laser beam to lose the dithioester moiety. Subsequently, the macroradical formed could abstract a hydrogen from the matrix, giving rise to hydrogen-terminated polymers. Ganachaud et al. [7] and Schilli et al. [11] reported MALDI mass spectra of RAFT poly(N-isopropylacrylamide). Schilli et al. gave a good discussion on the fragmentation of RAFT polymers during ionization. Vana et al. [8] ascribed the multiple peaks within one repeat unit of RAFT poly(methyl acrylate) (PMA) to the complex MALDI ionization and fragmentation processes. Although MALDI was initially described as a “soft ionization” process, a significant extent of fragmentation may occur for MALDI-generated ions [22,23]. This fragmentation usually occurs not only for the RAFT polymers mentioned above, but also for polymers obtained by other controlled-'living' radical-polymerization methods, such as nitroxide-mediated radical polymerization [24,25,26] and ATRP [27-33]. Nonaka et al. [32] suggested that the terminal C-Cl group of PMA obtained by ATRP is relatively stable in comparison with that of poly(methyl methacrylate) (PMMA) during MALDI-TOF-MS analysis.

Musat et al. [23] analyzed some labile low-molecular-weight polyesteramides by field desorption (FD) MS, ESI-MS, and MALDI-MS. They found that ESI yielded the least ion fragmentation and sample decomposition; in other words, they found ESI to be the “softest” ionization method. FD-MS rated second (less soft) and MALDI-MS third (least soft). Because of the reasonable expectation that ESI-MS would yield less fragmentation and therefore clearer spectra, we tried to apply ESI-MS to analyze RAFT polymers and to compare the results with those obtained by MALDI-TOF-MS. Our objective was to obtain mass spectra containing peaks representative of intact molecular ions, to unambiguously assign the polymer end-groups.

In polymer LC, critical conditions refer to a separation in which retention is independent of the number of monomeric units in a homopolymer chain [19]. Under critical conditions, differences in retention (i.e. selectivity) are solely caused by the end-groups and by other (functional) groups present in the polymer molecules. Critical separations are of great potential interest, but critical conditions are typically hard to realize and very hard to maintain through extended series of LC experiments. In a previous study [34], robust critical LC conditions were established for PMMA in normal-phase LC using mixtures of acetonitrile and dichloromethane as the mobile phase on a bare silica column. Baseline separations were obtained for low-molecular-weight RAFT prepolymers, either with or without end-group modification. Separation was thought to be solely based on the number of hydroxyl groups present in the molecules. In this work, off-line LC/MALDI-TOF-MS and both off-line and on-line LC-ESI-QTOF-MS have been used to identify the repeating units and, especially, the end-groups of the polymers. This should allow us to confirm the critical separation of non-functional, mono-functional, and di-functional polymers.
3.2 Experimental

3.2.1 Chemicals

Dichloromethane (DCM), tetrahydrofuran (THF), and acetonitrile (all HPLC grade), were from Rathburn Chemicals (Walkerburn, Scotland). Polymer sample V37A was synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization using a 2,2'-azobisisobutyronitrile (AIBN) initiator and a hydroxyl-functional RAFT chain-transfer agent [35]. Sample V37B was obtained via end-group modification of V37A, with cleavage of the RAFT activating group [35]. The molecular-weight distributions were measured by size-exclusion chromatography (SEC). Calibration is based on polystyrene standards and the molecular weights were recalculated using the universal-calibration principle and Mark-Houwink parameters [34]. The SEC data of samples V37A and V37B are summarized in table 3.1. Sample V37A0 is the non-functional fraction of V37A collected between 1.7 and 2.2 min, V37A1 is the mono-functional fraction of V37A collected between 2.3 and 3.3 min, V37B0 is the non-functional fraction of V37B (1.7-2.2 min), and V37B1 is the mono-functional fraction of V37B (2.3-3.3 min; see Fig. 3.1).

<table>
<thead>
<tr>
<th>Sample name</th>
<th>$M_n$</th>
<th>$M_p$</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>V37A</td>
<td>2,587</td>
<td>3,754</td>
<td>1.29</td>
</tr>
<tr>
<td>V37B</td>
<td>2,853</td>
<td>3,678</td>
<td>1.29</td>
</tr>
</tbody>
</table>

* Synthesized by RAFT polymerization using a 2,2'-azobisisobutyronitrile (AIBN) initiator and a hydroxyl-functional RAFT chain-transfer agent [35].
* Synthesized via end-group modification of sample V37A with cleavage of the RAFT activating group [35].

3.2.2 Equipment

The HPLC equipment used was the same as described in the previous paper [34]. The MALDI-TOF-MS measurements were performed with a Voyager-De Pro instrument (PerSeptive Biosystems, Framingham, MA, USA) equipped with a 337-nm nitrogen laser. Spectra were acquired by summing the data obtained from 200 laser shots in the reflector mode. The laser energy per pulse was tuned to yield sufficient ionization, while minimizing fragmentation. Absolute intensities were similar to the value of 80 $\mu$J reported by Scriven et al. [36]. $\alpha$-Cyano-4-hydroxycinnamic acid (about 20 mg/ml in THF) was used as the matrix. The concentration of the polymer sample was about 1 mg/ml in THF.

The off-line ESI-MS experiments were carried out using a Q-TOF Ultima™ Global mass spectrometer (Micromass, Manchester, UK) equipped with an atmospheric-pressure-ionization electrospray interface. The instrument was calibrated with phosphoric acid in the mass range of 98 to 2058 amu. Polymer samples were dissolved in a 4:1 v/v mixture of dichloromethane/methanol at
a concentration of 0.5 mg/ml. The flow rate of the sample introduced into the electrospray interface was 20 µl/min. The sampling cone potential was 31 V and the capillary voltage 3.0 kV. The electrospray-source temperature was 80°C and the desolvation temperature 100°C. Mass spectra were scanned over the range m/z 500-3000 in positive-ion mode. More than 100 scans were summed to produce the final spectrum.

The on-line LC-ESI-QTOF-MS experiments were carried out with an HPLC system consisting of two Shimadzu LC-10ADvp pumps (high-pressure gradient system to prepare the mobile phase in-situ). A post-column addition of methanol at a flow rate of 20 µl/min was delivered by another Shimadzu LC-10ADvp pump to enhance the ionization efficiency. The concentration of the polymer injected was about 1 mg/ml in DCM. Care was taken to avoid breakthrough peaks [37]. The other ESI operation parameters were the same as in the off-line process described above.

Tandem-MS (ESI-MS/MS) experiments were carried out on the selected precursor ions by collision-induced dissociation (CID) using helium as collision gas and a collision energy of about 50 V to obtain the fragmentation-ion spectrum.
3.3 Results and discussion

In a previous study [34] PMMA prepolymer were successfully separated according to hydroxyl functionality under the critical LC conditions. These conditions were used to analyze the functional RAFT polymers. The representative chromatograms of two low-molecular-weight functional-polymer samples are shown in Fig. 3.1. It is relatively straightforward to collect fractions (identified in Fig. 3.1) and to perform off-line mass spectrometry.

![Chromatogram](image)

**Fig. 3.1** Separation of functional RAFT polymers according to the number of OH groups. Evaporative light-scattering detector, mobile phase 40% acetonitrile in dichloromethane, home-packed silica column (150 mm x 4.6 mm i.d.; 3-μm particles; 100-Å pore size), flow rate 1 ml/min.

### 3.3.1 Comparison of MALDI-TOF-MS and ESI-QTOF-MS

Because low-molecular-weight materials yield very high MALDI signals, even if present at very low concentrations, the complete spectra are not very useful. This is indicated in Appendix A Fig. Ap-1, where spectra are shown across a broader range, especially for sample V37B1. Fig. 3.2 shows the most useful range of the MALDI-TOF-MS spectra of four different fractions. It can be seen from the spectra in Fig. 3.2 that two different series were observed with a repeating unit of 100.1 amu, which clearly corresponds to a single methyl methacrylate (MMA) monomeric unit, in all of the mass spectra for the fractions V37A0, V37B0, V37A1, V37B1. The main series of peaks result from adducts with one sodium cation (Na⁺). The minor series (seen in Fig. 3.3a and Appendix A Fig. Ap-3a) can be assigned to the potassium-cation (K⁺) series (with mass increments of 16 amu relative to the Na⁺ series). The results are summarized in table 3.2. In addition, we were unable to assign another minor series (with masses 25 amu lower than those of the main peaks) for V37A0 (Fig. 3.3a), for which there were many possibilities. There is another minor series (with masses 16
amu lower than those of the main peaks) that can be seen in Fig. 3.2 for V37B1. These peaks are not likely to be due to the loss of an OH group, as they are not observed for any of the other structures, which contain the same OH moiety. Also, a difference of 16 amu is not expected to result from the loss of a hydroxyl group. More likely, these peaks may originate from oligomeric species B1S undergoing cyclisation to yield oligomers with structure B1RI (see table 3.2 for abbreviations). Such a mechanism would be analogous to the loss of BrCH$_3$ followed by cyclisation of the final two methyl methacrylate monomer repeat units to yield a lactone end-group, as demonstrated by Borman et al. [30] for PMMA prepared by ATRP.

![MALDI-TOF mass spectra](image)

**Fig. 3.2** MALDI-TOF mass spectra for the first two fractions of sample PMMA V37A and V37B. (V37A0) Non-functional fraction of V37A collected at 1.7-2.2 min; (V37A1) Mono-functional fraction of V37A (2.3-3.3 min); (V37B0) Non-functional fraction of V37B (1.7-2.2 min). (V37B1) Mono-functional fraction of V37B (2.3-3.3 min). LC separation conditions were the same as in Fig. 3.1, except a flow rate of 0.5 ml/min.
### Table 3.2

Structural assignment of the peaks displayed in the MALDI-TOF and ESI-QTOF mass spectra of the PMMA samples reported in this work.

<table>
<thead>
<tr>
<th>Sample name (abbr.)</th>
<th>Structure (abbr.)</th>
<th>Slope</th>
<th>Intercept</th>
<th>End-group, m/z</th>
<th>Representative ions (m/z)</th>
<th>Associated ion</th>
<th>Ionization method</th>
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<tr>
<td>V37A0</td>
<td>A0H</td>
<td>100.07</td>
<td>92.05</td>
<td>69.06</td>
<td>1492.96, 1509.00</td>
<td>Na⁺</td>
<td>MALDI</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1492.75 (14), 1508.86 (14)</td>
<td>K⁺</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1467.93, unknown</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>A0R</td>
<td>100.05</td>
<td>244.03</td>
<td>221.04, 221.03</td>
<td>1644.79, 1644.76 (14)</td>
<td>Na⁺</td>
<td>ESI</td>
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<td></td>
<td></td>
<td>983.98, 983.95 (17)</td>
<td>(2Na)⁺</td>
<td></td>
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<tr>
<td></td>
<td>A1H</td>
<td>100.06</td>
<td>136.18</td>
<td>113.19, 113.08</td>
<td>1537.08, 1536.78 (14)</td>
<td>Na⁺</td>
<td>MALDI</td>
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<td></td>
<td></td>
<td></td>
<td>1553.00, 1552.89 (14)</td>
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<tr>
<td>V37A1</td>
<td>A1R</td>
<td>100.06</td>
<td>288.05</td>
<td>265.06, 265.06</td>
<td>1688.84, 1688.78 (14)</td>
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<td>MALDI</td>
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<td>1006.01, 1005.97 (17)</td>
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<td>1045.19, 1045.17 (28)</td>
<td>(3Na)⁺</td>
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<tr>
<td></td>
<td>B0H</td>
<td>100.03</td>
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<td>1492.75 (14), 1508.86 (14)</td>
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<td>973.83, 973.96 (18)</td>
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<td>B1H</td>
<td>100.02</td>
<td>136.40</td>
<td>113.41, 113.08</td>
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<td>Na⁺</td>
<td>MALDI</td>
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<td>B1Ri</td>
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<td>120.09</td>
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<td>1520.82, 1520.75 (14)</td>
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<td>MALDI</td>
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<td>V37B1</td>
<td>B1H</td>
<td>100.06</td>
<td>136.05</td>
<td>113.06, 113.08</td>
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<td>Na⁺</td>
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<td>1005.22, 1005.16 (28)</td>
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<td></td>
<td>B1S</td>
<td>100.05</td>
<td>168.06</td>
<td>145.07, 145.06</td>
<td>1005.22, 1005.16 (28)</td>
<td>Na⁺</td>
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1 Abbreviations: 
- CH₃
- [MMA]ₙ
- CN
- S
- HO

A0H, B0H: CH₃-C-[MMA]ₙH

A0R: CH₃-C-[MMA]ₙS

B0S: CH₃-C-[MMA]ₙSH

A1H, B1H: HO-(CH₂)₃C-[MMA]ₙH

A1R: HO-(CH₂)₃C-[MMA]ₙS

B1Ri: HO-(CH₂)₃C-[MMA]ₙS

2 The molar mass of the monomer calculated from peak series in MS, see Appendix A.
3 The total molar mass of polymer-chain end-groups and associated cations calculated from peak series in MS, see Supporting Info.
4 Experimental m/z value of end-groups: intercept minus associated cation.
5 Theoretically calculated m/z value.
Surprisingly, the MALDI-TOF mass spectrum of the fraction V37B0 is very similar to the one of V37A0, and V37B1 is roughly identical to V37A1. Yet, the end-groups of the polymers are known to be different. The UV (ultraviolet) spectrum of sample V37A showed strong absorbance with a maximum at 300 nm in dichloromethane, which is ascribed to the RAFT dithioester moiety \([S=\text{C(C}_6\text{H}_3)\text{S}-]\). The UV spectrum of sample V37B, which was prepared by removing the RAFT dithioester moiety via end-group modification of the RAFT polymer [35], showed no UV absorbance at 300 nm.

Panels a and b of Fig. 3.3 compare the MALDI and ESI spectra for non-functional PMMAs V37A0 in the mass range of 1,400 to 2,050 amu. The ESI-QTOF mass spectrum is different from the MALDI-TOF spectrum for the same sample. End-groups calculated from the MALDI spectrum of V37A0 are different from those obtained from the ESI spectrum. In the former case the total end-group mass is consistent with hydrogen and initiator moiety \([\text{CH}_3\text{CCN-}]\) as the end-groups. In the latter case the end-groups of V37A0 can be assigned to the RAFT dithioester moiety and the initiator moiety, which correspond to the non-functional structure. The fragments lost in the MALDI experimental process account for 152 amu.

We also compare the ESI and MALDI spectra for mono-functional PMMA V37A1 (see Appendix A Fig. Ap-3). Again, the ESI mass spectrum is different from that obtained by MALDI for the fraction V37A1. The total end-group mass calculated from the MALDI spectrum of V37A1 is different (152 amu lower) than that obtained from the ESI spectrum. From the ESI spectra we can conclude that sample V37A1 does contain PMMA with the RAFT dithioester moiety and the leaving group of the RAFT agent \([\text{HO(CH}_2)_3\text{CH}_3\text{CCN-}]\), which together result in a mono-functional structure (one hydroxyl group).

There are also differences between MALDI-MS and ESI-MS spectra for samples V37B0 and V37B1 (see discussion below). The end-groups observed in ESI-MS are different from those observed in MALDI-MS, as shown in table 3.2.

In conclusion, the weakly bonded end-groups were cleaved in the MALDI-MS experimental process, while the polymers were observed intact in ESI-MS. This indicates that in the present case ESI is a much softer ionization technique than MALDI. This is in agreement with the results obtained by Musat et al. [23].

3.3.2 Off-line LC//ESI-QTOF-MS and MS²

Fig. 3.4a shows an expanded portion of the ESI spectrum for V37A1 in the range of 980 to 1100 amu. It can be seen clearly from Fig. 3.4a and table 3.2 that singly charged ions, doubly charged ions (difference between isotopic peaks 0.5 amu) and triply charged ions (isotopic peaks separated by 0.33 amu) occur in the ESI spectrum. These different series of peaks arise from the same series of PMMA (same end-groups).
Fig. 3.3  Comparison of MALDI and ESI mass spectra for non-functional RAFT polymer (V37A0).
(a) MALDI-TOF-MS; (b) ESI-QTOF-MS.
Fig. 3.4  ESI-QTOF mass spectra of mono-functional RAFT polymer (V37A1). (a) Enlarged part of ESI-QTOF-MS; (b) ESI-MS$^2$ selected at 1088 amu; (c) the proposed fragmentation of the 8-mer in ESI-CID-MS$^2$. 

(a) ESI (V37A1) 

(b) ESI-MS$^2$ (V37A1) 

(c) (+Na$^+$) 235 335 934

CH$_3$ CH$_3$ CH$_3$ CH$_3$ CH$_2$ H S

HO-(CH$_2$)$_3$ CN C=O C=O C=O C=O

OCH$_3$ OCH$_3$ OCH$_3$ OCH$_3$
To verify the obtained structure, multiple-stage MS (ESI-MS²) experiments were carried out on selected precursor ions with helium as collision gas. This technique takes advantage of the ion-trap mechanism and allows for the fragmentation of a selected precursor ion by collision-induced dissociation (CID) and results in a product-ion spectrum. Fig. 3.4b shows a representative MS² spectrum of the precursor ion with mass-to-charge ratio m/z 1088 corresponding to n = 8 (V37A1) with a collision voltage of 50 V. The RAFT dithioester moiety (152.98 amu) was observed to split off easily. The resulting macroradical (the polymer chain without the RAFT group) loses an additional hydrogen to give rise to the most abundant peak at m/z 934.50. Also a series of fracture ions with m/z 135, 235, 335, and 445 were observed. These correspond to PMMA chains with the RAFT leaving group and a sodium cation. The fragmentation scheme is shown in Fig. 3.4c.

The ESI-CID-MS² spectrum (Appendix A Fig. Ap-4) for the m/z=1044 (n=8) molecular-ion peak of V37A0 shows similar results. The RAFT dithio moiety (152.98 amu) was again observed to split off easily. An additional hydrogen is abstracted from the polymer chain, to give rise to the most abundant peak at m/z 890.63. A series of fracture ions with m/z 191, 291, 391, etc. were observed, which correspond to PMMA prepolymer with an initiator moiety as an end-group.

All our results can be explained from the RAFT polymerization mechanism (Scheme 1). Our results provide direct evidence that the RAFT polymers still present “latent living” (or dormant) characteristics in the form of the dithioester end-group. It can be used for further-chain-extension if new batches of initiator and monomer are added. We can conclude that the dithio moiety very easily splits off from the RAFT polymer, as shown in MALDI-TOF-MS as well as in ESI-CID-MS² experiments.

We proved directly that the RAFT polymer V37A contained not only a fraction V37A1 featuring the leaving group of the RAFT agent, but also a fraction V37A0 featuring initiator moiety. Therefore, an initiator with a radical fragment (I) that has the same structure as the leaving group of the RAFT agent should be used to synthesize RAFT polymers with a well-defined functionality. Based on this knowledge, a new hydroxyl-functional initiator was used to obtain a well-defined mono-functional RAFT polymer with a narrow molecular-weight distribution (see references 34, 35).

In order to characterize sample V37B, fractions V37B0 and V37B1 were also subjected to ESI-QTOF-MS. For the di-functional fraction of V37B2 collected at 3.3-4.3 min, highly complex ESI-MS spectra were obtained, which will not be discussed in the present paper. Fig. 3.5a shows an enlarged part of the ESI spectrum for mono-functional polymer V37B1 in the mass range of 935 to 1040 amu. Despite the rather low ionization efficiency, which is apparent from the relatively high base-line noise, the qualitative information from this spectrum is clear. It can be seen from Fig. 3.5a and table 3.2 that singly, doubly and triply charged ions are all present in the spectrum of V37B1, (somewhat) enhancing the complexity. There are two dominant structures B1H and B1S (see table 3.2 and Appendix A table Ap-1). The ESI-CID-MS² spectrum for the m/z 1036 (n=9) molecular-ion peak, which is shown by way of example in Fig. 3.5b, provides further evidence for the structure of B1H. A series of fracture ions was identified with m/z 135, 235, 335, and 445, which correspond to PMMA polymers with the RAFT leaving group as a chain-end. The most abundant peak at m/z 210
and the associated series correspond to hydrogen as another end-group in sample V37B1. The fragmentation scheme is shown in Fig. 3.5c (see reference 36). The analysis of sample V37B0 via ESI-MS is similar and the results are also summarized in table 3.2.

Fig. 3.5 ESI-QTOF mass spectra of mono-functional polymer V37B1. (a) Enlarged section (935-1040 amu); (b) ESI-MS$^2$ with 1036 as the parent ion; (c) the proposed fragmentation of the 9-mer in ESI-CID-MS$^2$. 

![ESI-QTOF mass spectra of mono-functional polymer V37B1.](image-url)
3.3.3 On-line LC-ESI-QTOF-MS

In order to further confirm the hydroxyl-based separation, we used on-line LC-ESI-QTOF-MS to identify the repeating units and, especially, the end-groups. Two narrow-bore columns (2 × 150 mm × 1.0 mm i.d) were used, as these were compatible with the ESI interface without post-column splitting of the effluent (LC eluent flow rate 0.1 ml/min). However, a mobile phase containing 40% acetonitrile in dichloromethane could not be used to elute the PMMA samples from the two columns. The critical solvent composition for the two small columns was about 5% higher in acetonitrile concentration compared with the conventional column (150 mm × 4.6 mm i.d).

An example of the summed mass chromatogram of sample V37A using (near) critical conditions (45% acetonitrile in dichloromethane) is shown in Fig. 3.6. Fig. 3.6a shows the summed chromatogram of masses set at 244+100.06n and 288+100.06n (n is an integral number, which represents the degree of polymerization of the polymer, from 0 to 22 in the present case). These two series correspond to non-functional (244 series, Fig. 3.6c) and mono-functional (288 series, Fig. 3.6b) RAFT polymers, respectively (see Appendix A). It can be seen from Fig. 3.6 that the non-functional polymer (no OH group) eluted earlier (2.17 min.) than the mono-functional polymer (one OH group; 2.67 min). This confirms the base-line separation of polymers according to functionality as seen in Fig. 3.1.

The mass spectra of the two peaks at elution times of 2.2 and 2.6 min (Appendix A Fig. Ap-5) are similar to those in Figs 3a and Ap-3a. The only observed difference in these mass spectra is that next to the sodium-cation (Na\(^+\)) series also a high-intensity potassium-cation (K\(^+\)) series (with a mass difference of 16 amu) was observed in the on-line mass spectra. This is probably due to traces of potassium present in the mobile phase.

On-line MS monitoring of elution profiles of individual oligomeric species can also facilitate a better understanding of LC separation mechanisms [16]. For example, thanks to the high mass accuracy of mass spectrometry [18], on-line LC-ESI-QTOF-MS can be used to investigate the effect of the molecular weights of the polymer on the elution behavior. Fig. 3.7 shows an example of mass chromatograms for various selected masses for sample V37A. It can be seen that the retention times of PMMA oligomers with different molecular weights were almost identical. However, it also can be seen from Fig. 3.7 that the retention time of the polymer increased slightly with increasing molecular weight (from 2.53 to 2.85 minutes for mono-functional PMMAs with \(n\) increasing from 0 to 22). The retention time increased from 2.10 to 2.24 minutes for non-functional PMMAs with \(n\) between 0 and 20 (Appendix A Fig. Ap-6). This indicates a slight adsorption effect of PMMA, so that the LC conditions are not perfectly critical. We therefore speak of near-critical conditions. The original RAFT agent (\(n = 0\)) was also observed in the ESI-QTOF-MS spectra of the low-molecular-weight RAFT polymers.
Fig. 3.6 LC-ESI-QTOF-MS summed mass chromatogram of sample 37A. (a) Summed masses set at 244+100.1n and 288+100.1n (n: 0-22); (b) 288 series; (c) 244 series (see explanation in text and Appendix A Fig. Ap-3). Mobile phase: 45% acetonitrile in dichloromethane, two home-packed silica columns (150 mm × 1 mm i.d.; 3-μm particles; 100-Å pore size), flow rate 0.10 ml/min.
Fig. 3.7 Representative (near-critical) LC × MS mass chromatograms for various selected masses of mono-functional PMMA from sample V37A. LC conditions as in Fig. 3.6.

### 3.4 Conclusions

MALDI-TOF-MS and on-line LC-ESI-QTOF-MS were performed to analyze hydroxyl-functional PMMA samples synthesized by RAFT polymerization. The end-groups of a non-functional fraction containing the RAFT dithioester moiety \([S=C(C_6H_5)S-]\) and the initiating-radical fragment \([(CH_3)2CCN-]\) could be assigned directly from the ESI spectrum. However, the dithioester moiety was lost in the MALDI experimental process. It was also observed to split off easily in collision induced dissociation (ESI-CID-MS²) experiments, which further verified the structure of the non-functional fraction of the RAFT polymer. Similar results were obtained with ESI-MS for the mono-functional fraction of the RAFT polymer, which was shown to contain the leaving group of the RAFT agent \([HO(CH_2)_2CH_3CCN-]\) and the RAFT dithioester moiety. The latter was not observed in the MALDI-MS spectrum. These results provide direct evidence that the RAFT polymers still present latent living character through the presence of the dithioester moiety. This property can be used for further chain extension with addition of fresh monomer and fresh initiator. The structures of non-functional and mono-functional fractions of a derivatized RAFT polymer were also established and discussed. All these results are in agreement with a contemporary interpretation of the RAFT polymerization process and the derivatization reaction. The active (weakly bonded) end-groups, such as the dithioester moiety, were lost in the MALDI-MS experiments, while the
polymeric molecules were observed intact in the ESI-MS spectra. This indicates that in the present case ESI is a much softer ionization method than MALDI.

On-line LC-ESI-QTOF-MS was used to provide further evidence for the structure of the end-groups and to study the mechanism of the LC separation. Specifically the effect of the polymer molar mass on the elution behavior was studied. The results confirmed the base-line separation of the functional prepolymer according to the number of hydroxyl groups. They also revealed a slight adsorption effect of PMMA under the experimental (near-critical) conditions.

Acknowledgments

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References

Appendix A

Additional ESI-MS spectra, assignment, mass chromatograms as well as explanatory texts are provided in this appendix.

Fig. Ap-1  MALDI-TOF mass spectra for the first two fractions of sample PMMA V37A and V37B. (V37A0) Non-functional fraction of V37A collected at 1.7-2.2 min; (V37A1) Mono-functional fraction of V37A (2.3-3.3 min); (V37B0) Non-functional fraction of V37B (1.7-2.2 min). (V37B1) Mono-functional fraction of V37B (2.3-3.3 min). LC separation conditions were the same as in figure 1, except a flow rate of 0.5 ml/min.
The polymer end groups can be calculated from a polymeric series in a mass spectrum using equation 1

\[ M_{\text{peak}} = n \times M_{\text{monomer}} + M_{\text{end group}} + M_{\text{counterion}} \]  

where \( M_{\text{peak}} \) is the molar mass value of the selected peak, \( M_{\text{monomer}} \) the molar mass of the monomer, \( n \) the (integral) number of monomer repeat units, \( M_{\text{end group}} \) the total molar mass of chain end groups (including the initiating group and the end-capping group), and \( M_{\text{counterion}} \) the molar mass of the counterion attached in the ionization process.

Plot \( M_{\text{peak}} \) vs. \( n \) should yield a straight line. The slope of the line represents the value of \( M_{\text{monomer}} \). The intercept of the line is the sum of \( M_{\text{end group}} \) and \( M_{\text{counterion}} \). Figure SI-1 shows an example of the calculation of the total end-group mass (\( M_{\text{end group}} \)). All the calculation results and structures are summarized in Table 2.

**Fig. Ap-2** Representative calculation of sum of the two end-groups and the counterion in sample V37A1 from MALDI-TOF-MS and ESI-QTOF-MS data.

Fig. Ap-4a shows an expanded portion of the ESI spectrum of V37A0 in the range of 930 to 1050 amu. It can be clearly seen from Fig. Ap-4a that singly charged ions exist next to doubly charged ions based on the isotopic peaks separated by 0.5 amu.

Fig. Ap-4b displays the MS\(^2\) spectrum for the \( m/z \) 1044 (\( n = 8 \)) molecular ion peak. The fragmentation scheme is shown in Fig. Ap-4c.
Fig. Ap-3  Comparison of MALDI and ESI mass spectra for mono-functional RAFT polymer (V37A1). (a) MALDI-TOF-MS; (b) ESI-QTOF-MS.
**Mass Spectrometric Characterization of Functional PMMA in Combination with Critical LC**

(a) ESI (V37A0)

(b) ESI-MS² (V37A0)

(c) ESI-QTOF mass spectra of non-functional RAFT polymer (V37A0). (a) Enlarged part of ESI-QTOF MS; (b) ESI-MS² selected at 1044 amu; (c) the proposed fragmentation of the 8-mer in ESI-CID-MS².
Table Ap-1 Assignments of the peaks in the ESI-QTOF-MS spectrum of V37B1 (Fig. 3.6a).

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<th>Entry</th>
<th>n</th>
<th>z</th>
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<th>(m/z) (experimental)</th>
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<tr>
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</tr>
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<td>28</td>
<td>3</td>
<td>1005.16</td>
<td>1005.22</td>
<td></td>
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</table>

\(^\text{a}\)The calculated value of B1H: 

\[m/z = [112.08 \times (\text{HO(CH}_2)_2\text{CCN})] + n \times 100.06 \times \text{PMMA backbone} + 1.01 \times (-\text{H}) + z \times 22.99 \times (\text{Na}^{+})/z,\]

where \(n\) refers to the degree of polymerization, and \(m\) to the number of charges \((\text{Na}^{+})\) on the polymers.

The calculated value of B1S: 

\[m/z = [112.08 \times (\text{HO(CH}_2)_2\text{CCN})] + n \times 100.06 \times \text{PMMA backbone} + 32.98 \times (-\text{SH}) + z \times 22.99 \times (\text{Na}^{+})/z,\]

where \(n\) refers to the degree of polymerization, and \(m\) to the number of charges \((\text{Na}^{+})\) on the polymers.

Fig. Ap-5 shows ESI-MS spectra of the non-functional peak eluted at 2.2 min (a) and monofunctional peak eluted at 2.6 min (b) for sample V37A. Two different series were observed with a repeating unit of 100.1 Da (PMMA unit). They result from the counter-ions, sodium cation \((\text{Na}^{+})\) and potassium cation \((\text{K}^{+})\) respectively, with mass difference of 16 amu. The calculated mass of the end groups \((M_{\text{end group}} = 221 \text{ amu})\) at an elution time of 2.2 min corresponds to polymeric structures with \((\text{CH}_3)_2\text{CCN}\) as one end group and \(\text{SCSC}_6\text{H}_5\) as the other end group (Fig. Ap-5a). The calculated mass of the end groups \((M_{\text{end group}} = 265 \text{ amu})\) at an elution time of 2.6 min corresponds to structures with \(\text{HO(CH}_2)_2\text{CH}_2\text{CCN}\) as one end group and \(\text{SCSC}_6\text{H}_5\) as the other end group (Fig. Ap-5b). These confirm the results obtained in off-line ESI-QTOF-MS and are in agreement with our understanding of the RAFT polymerization process. On this basis, the summed chromatograms selected masses of \(244+100.06n\) and \(288+100.06n\) are shown in Fig. 3.6.
**Fig. Ap-5**  ESI-MS spectra of the non-functional peak eluted at 2.2 min (a) and mono-functional peak eluted at 2.6 min (b) from sample V37A.
Fig. Ap-6 illustrates the effect of the molecular mass on retention time for non-functional PMMAs. The selected mass-to-charge ratio is shown in the second line of the top-right corner. The value shown in the curve is the retention time.

Fig. Ap-6  Representative (near-critical) LC × MS mass chromatograms for various selected masses of non-functional PMMA from sample V37A. Mobile phase: 45% acetonitrile in dichloromethane, two home-packed silica columns (150 mm × 1 mm i.d.; 3-μm particles; 100-Å pore size), flow rate 0.10 ml/min.