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Quantitation of functionality of poly(methyl methacrylate) by liquid chromatography under critical conditions followed by evaporative light-scattering detection
Comparison with NMR and titration

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Abstract

Atom transfer radical polymerisation (ATRP) is a versatile 'living' controlled polymerisation technique for the synthesis of well-defined architectures such as block copolymers, gradient copolymers, hyperbranched polymers and telechelic polymers. ATRP provides control over molecular mass and molecular mass distribution and is suitable for the polymerisation of a wide variety of monomers, including methyl methacrylate. A chromatographic method was developed for an endgroup-based separation of low-molecular-mass poly(methyl methacrylate) (PMMA), based on liquid chromatography under critical conditions. With this method the PMMA, irrespective of its low-molecular-mass, is separated according to endgroups (functionality) due to interactions of the polar endgroups with the non-modified silica based stationary phase. The different series were identified using on-line atmospheric pressure ionisation electrospray mass spectrometry and quantified by evaporative light scattering detection. These results were compared with those obtained by NMR and titration.

Keywords: Liquid chromatography under critical conditions; Poly(methyl methacrylate)

1. Introduction

(Meth)acrylate-based polymers are currently produced using oligo(meth)acrylates that contain a random distribution of OH-functional moieties along the backbone. This statistically defined presence of functional groups also implies a random crosslinking process, and hampers a detailed insight into the relation between network formation and mechanical properties. To be able to improve the knowledge with respect to this relationship, the synthesis of oligo(meth)acrylates with predetermined molecular mass, which contain hydroxyl functional groups at both ends of the polymer chain (so-called telechelic materials) is needed. Until recently, it was very difficult to produce these hydroxyl-functional telechelic poly(methyl methacrylate) (PMMA) polymers, because of the absence of suitable polymerisation

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techniques. With the advance of 'living'/controlled radical polymerisations, however, these materials have come within reach. Especially, atom transfer radical polymerisation (ATRP) is very well suited for the construction of these well-defined polymers, because of its robustness and tolerance towards a large number of functional groups [1–3]. Therefore, this is the method of choice for the preparation of hydroxyl-functional telechelic PMMA of low molecular mass (2 kg/mol). One synthetic approach to these materials is to use the hydroxyl functional initiator 2,2,2-trichloro ethanol for the polymerisation of methyl methacrylate (MMA), which results in mono-functional polymer. The second OH functionality is introduced by an endcapping reaction with 3-methyl-3-buten-1-ol. The degree of OH functionalisation (see Fig. 1) is of course crucial to the interpretation of the structure–property relationship of the polymer formed. Although, based on experience, the OH endcapping of methacrylates does not proceed with full conversion, the materials obtained are very useful as model compounds for the development of analytical techniques that allow reliable determination of OH functionality of these types of polymers.

Three different analytical techniques can be used to obtain the OH functionality of synthesised PMMA; liquid chromatography under critical conditions (LC-CC), nuclear magnetic resonance (NMR) and specific OH titration.

With ^{1}H-NMR spectroscopy the OH functionality can be quantitatively determined. Information about the level of introduction of the second OH function could be obtained indirectly, by the disappearance of the methyl ester resonance of the final methacrylate moiety. However, the value for quantification by this method is doubtfully, mostly because of the difficulty of interpretation. The functionality of PMMA can also be quantitatively determined by specific endgroup titration, which seems to be the most logical choice of techniques. The determination of the endgroup functionality of PMMA with specific titration can be performed by the reaction between hydroxyl groups of the PMMA and acetic acid anhydride. The hydroxyl groups react to form an ester and an equivalent amount of acetic acid, which is therefore a measure for the OH functionality. Water, phenols, primary and secondary amines and oximes react with acetic acid anhydride and can negatively influence the result of the titration.

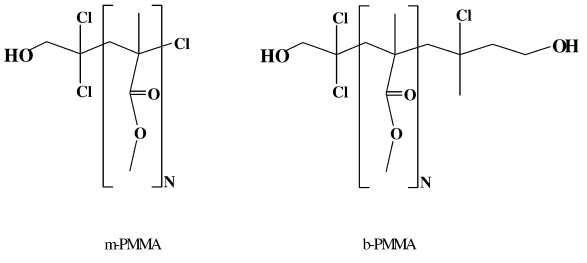

Fig. 1. Structure of poly(methyl methacrylate) with one OH functionality (mono-PMMA) and two OH functionalities (bi-PMMA).

The OH functionality of PMMA can also be determined with a relatively new analytical method, based on LC-CC. This critical mode of chromatography is based on two separation mechanisms; size exclusion and adsorption. In the exclusion mode the polymers are separated based on their molecular mass and information can be obtained about the number-average molecular mass (M_n) and molecular mass distribution (M_w/M_n); M_w=weight-average molecular mass. Adsorption chromatography is based on adsorption, therefore the polymers will separate based on their molecular mass and their polarity. Often only the lower-molecular-mass compounds will elute [4–6], because the higher-molecular-mass material will adsorb irreversibly onto the stationary phase. At the critical point of adsorption, entropy and enthalpy interactions of the polymer backbone and the stationary phase exactly compensate each other so excess retention depends only on the inhomogeneity of the polymer chain, functionality, branching sites, etc. Because the chain length of a polymer does not contribute to the retention at critical conditions, LC-CC, as developed by Entelis et al. [7], is an excellent technique for the determination of the functionality type distribution of polymers. The elution of polymer is very sensitive to temperature, compositions of the mobile phase, stationary phase and pore size. Several papers have been published about the principles, problems and applications of LC-CC [8–10].

The compositional analysis of PMMA after the LC-CC separation, by identifying the repeating units

and end-groups, on-line atmospheric pressure ionisation electrospray mass spectrometry (API–ESI–MS) can be used. In general, API–ESI–MS has shown to be excellently suited to give molecular mass and structure information about soluble 'low' mass polymers. This technique is also compatible with liquid chromatography separations like LC-CC. By the use of potassium ion (K^+) as a counter ion in combination of high ionisation and low fragmentation voltage, multiple charging and fragmentation will be avoided [11]. ESI–MS is very suitable for identification/verification but is not suitable for quantitative analyses because several factors affect the intensity of the MS signal. This makes quantification with ESI–MS not accurate and easy to perform. In general the quantification method is the use of UV detection, but if the background adsorption is too high, another detection method can be used; refractive index detection (RI) or evaporative light-scattering detection (ELSD). RI detection is not very sensitive and it is very time consuming to perform a good calibration [12]. For the quantification with ELSD a universal calibration with peak width correction is recently developed by Mengerink et al. for the quantification of cyclic and linear structures of nylon-6 [13] after separation by LC-CC. With the use of this calibration method no mass dependency was observed for nylon-6. This universal calibration makes ELSD favoured for quantification of samples of PMMA with different functionality after LC-CC separation.

In this paper the separation by LC-CC of PMMA according to it is functionality was described. The separated peaks are identified using LC-CC–API–ESI–MS and are quantified using ELSD. The quantification was performed with a universal calibration method that included peak-width correction. A comparison of the functionality obtained by ELSD quantification with the functionality obtained by ^{1}H-NMR and specific OH titration was made.

2. Experimental

The quantification experiments were performed on a HP1100 quaternary pump, including a degasser and control module (Agilent, Waldbronn, Germany). The mobile phase, 16.8% (w/w) *n*-hexane and 83.2% (w/w) tetrahydrofuran (THF), was premixed and pumped with a flow-rate of 0.5 ml/min. Two 250×4 mm Nucleosil 50-5 (Machery–Nagel) columns were used to perform the critical separation. The injector (Rheodyne, Cotati, CA, USA) was equipped with a 55 μl loop, mounted into the column oven that was operated at 40°C (Mistral, Spark, Netherlands). Approximately 6 m of 0.25 mm I.D. stainless steel capillary tubing was used to thermostat the mobile phase before it reached the injector. Detection with a Sedex 55 ELSD system (Sedere, Vitry/Seine, France) was performed with 1.9 bar air pressure at the nebuliser and a drift tube temperature at room temperature. The detector signal was collected with an X-Chrom version 2.11b data management system (Labs-system, Manchester, UK). Data calculations were performed using a spreadsheet program (EXCEL 97, Microsoft).

The identification experiments where performed on a HP1100-MS system (Agilent) which consisted of a degasser, quaternary pump, column oven, large volume autosampler, diode array detection (DAD) system with on-line a HP1100 single quadrupole mass spectrometer equipped with an atmospheric pressure ionisation electrospray interface. ChemStation revision A.08.03 software was used to run the system and collect data. The same critical LC conditions mentioned before were used to separate the samples. The MS system was run in the positive mode at an ionisation voltage of 5.5 kV, fragmentor voltage of 200 V, scan range of m/z 250–2500, step size 0.2 and condensed data storage. To assure a flow of approximately 0.25 ml/min into the ESI interface the LC effluent flow (0.50 ml/min) was split (1:3) by means of a zero dead volume T-piece. After the DAD and before the split a post-column addition of potassium iodide (KI) in acetone (5 mg KI/10 ml acetone) at a flow of 0.25 ml/min was delivered with a Gynkotek pump (Gynkotek HPLC, Germering, Germany) to perform co-ordination mass spectrometry [11]. The interface gas temperature was held at 250°C, and nebuliser pressure of 40 p.s.i. (1 p.s.i. = 6894.76 Pa). As nebulising gas nitrogen at a flow-rate of 10.0 l/min was used.

^{1}H-NMR was performed on a 500 MHz NMR Spectrometer (Bruker, Germany). The sample was dissolved in $C_2^2H_2Cl_4$ and measured at a temperature of 70°C. Thirty two scans were accumulated using a

20 s relaxation delay and a 30° pulse angle. The acquisition time was 3.2 s resulting in a digital resolution of 0.15 Hz.

The determination of the endgroup functionality with specific titration was performed with a Metrohm type 670 titroprocessor with a 665-piston burette in the dynamic titration mode. The drift was 15 mV and the delay time is 43 s. The pH electrode was a Yokogawa SM21AL4 electrode and the reference electrode, filled with a saturated solution of LiCl in ethanol, was a Yologawa SR20-AS52. An amount of sample which contains approximately 0.5 mmol hydroxy groups is dissolved in 2 ml of 4-dimethyl-aminopyridine-solution in dimethylformamide (DMF) by stirring. A 2 g amount of a solution of acetic acid anhydride in DMF is added and the hydroxyl groups react to form a ester and an equivalent amount of acetic acid. After 2 h the excess amount of acetic acid anhydride is hydrolysed with water. The amount of acetic acid is titrated under a flow of nitrogen with tetrabutylammonium hydroxide, which is a measure for the concentration hydroxylic groups (mmol/g).

Low average molecular mass PMMA samples, with mono- and bi-functionality, were prepared using ATRP [1–3], in the laboratory at DSM Research. The different samples were dissolved in mobile phase; 16.8% (w/w) *n*-hexane and 83.2% (w/w) THF.

3. Results

3.1. Separation under critical conditions and indentation by ESI(+)–MS

Low-molecular-mass PMMAs were separated according to endgroups (functionality) with LC-CC. The ELSD chromatogram of a representative low-molecular-mass sample PMMA, which exists of mono- and bi-functional PMMA, eluted at the critical point of adsorption, is shown in Fig. 2. The PMMA elutes with a retention volume slightly larger than the volume of the column, almost independently of its molar mass but in order of its functionality. When a high concentration of PMMA (>2 mg/g) was injected a third peak was observed (Fig. 2), which may be caused by some contamination of the PMMA with initiator (trichloroethanol), MMA or endcapping material [3-methyl-3-buten-1-ol (MBO)]. Both main peaks in the PMMA samples were identified using on-line LC-CC–API–ESI–MS. Potassium ion, added post-column to the mobile phase before splitting, is used as counter-ion in combination with high ionisation voltage. Fig. 3 show a representative mass chromatogram (total ion current) of a PMMA sample, after separation under critical conditions. The mass-spectrum of the peak at 10–12 min is shown in Fig. 4. Three different series, were observed with a repeating unit of 100.1 u (repeating unit of PMMA). The endgroups of these series can be determined by extrapolation to zero repeating units; which gives residual masses of 186.9, 151.0 and 115.0 u. The residual mass of 186.9 u is the summation of the end-group masses and the mass of the counter-ion, which corresponds with the end-groups of mono-functional PMMA and K^+ as counter-ion ($[M+K]^+$). The residual masses of 151.0 and 115.0 u correspond with the end-groups of mono-functional PMMA whereby one hydrochloric acid is split off in the ESI interface of the MS system ($[M-HCl+K]^+$) respectively with the endgroup of mono-functional PMMA whereby two hydrochloric acid groups are split off ($[M-2HCl+K]^+$). This is confirmed by the chloro-isotope pattern of these series. The mass-spectrum of the second peak gives a similar picture; a repeating unit of 100.1 from PMMA and a residual mass of 273.1, 237.1 and 201.1 u. The residual masses of 273.1 correspond with the end-groups of bi-functional PMMA and K^+ as counter-ion ($[M+K]^+$). The other residual masses show also the deletion of one respectively two hydrochloric acid groups from bi-functional PMMA. In Fig. 5 a macro reconstructed chromatogram is shown; a summation of each molecule with endgroup 186.9 u is made (m/z $186.9+287+37.1+\cdots+2489.1$). This shows that mono- and bi-functional PMMA are separated with LC-CC; retention only depends on the functionality of the PMMA.

3.2. Quantification with the use of ELSD

The quantification of mono- and bi-functional PMMA is performed with ELSD. When the responses for the different compounds are similar and

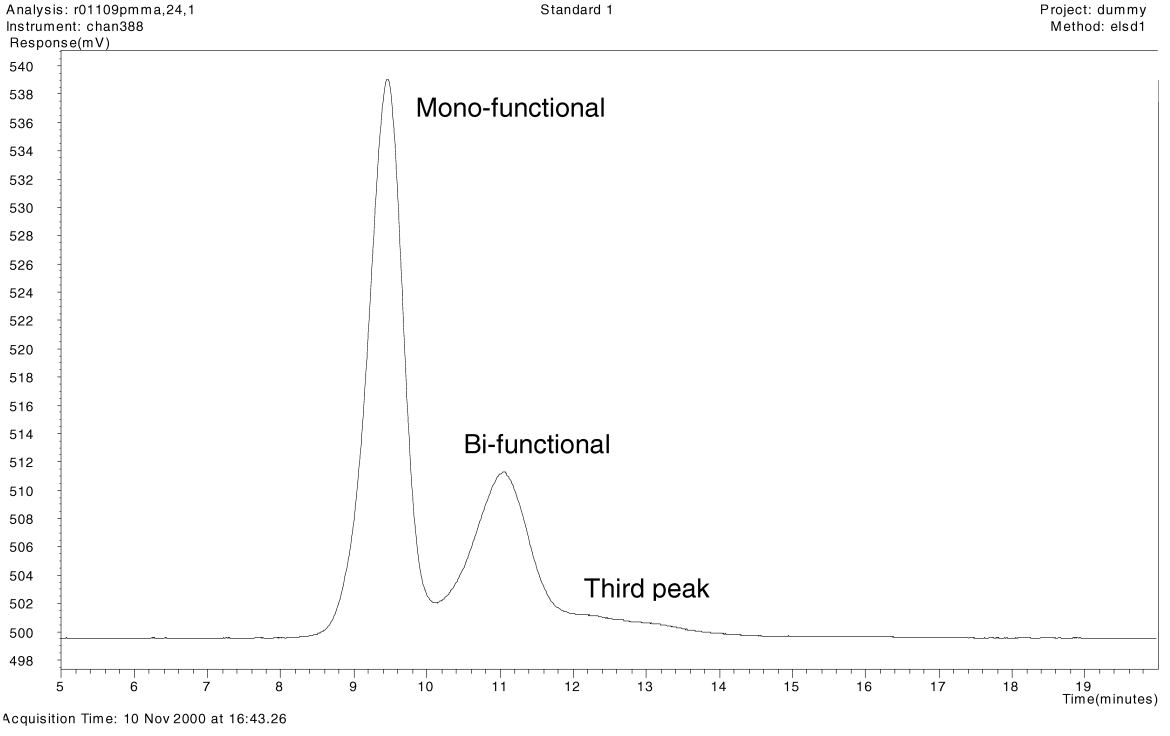

Fig. 2. LC-CC chromatogram of a representative PMMA sample dissolved in mobile phase. Conditions: Mobile phase: 16.8% (w/w) *n*-hexane and 83.2% (w/w) tetrahydrofuran; flow: 0.5 ml/min, column: Two columns of 250×4 mm each Nucleosil 50-5 at 40°C; V_{inj} 55 μl; detection: ELSD, drift tube at room temperature and nebulisation at 1.9 bar air.

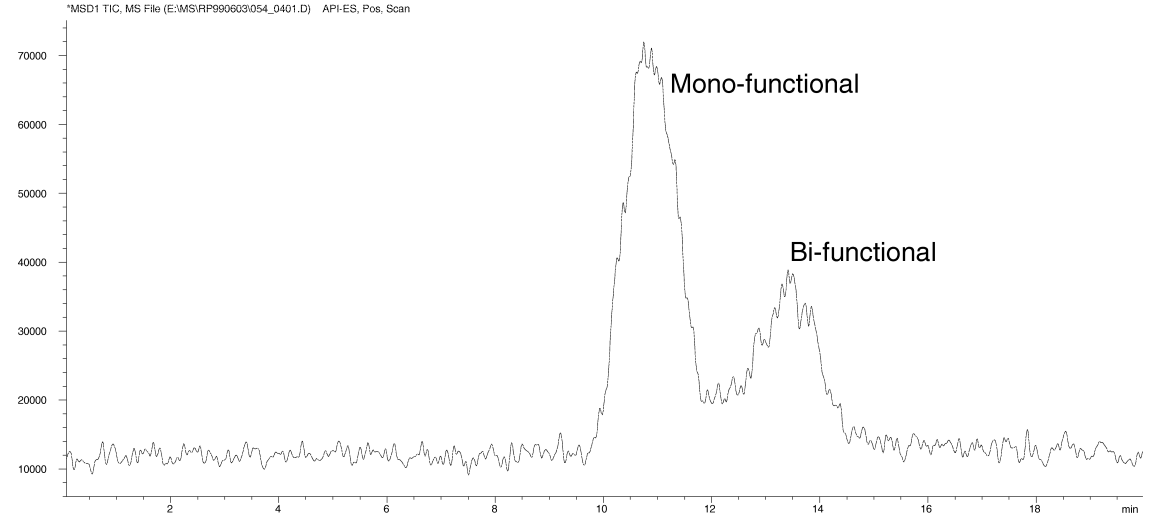

Fig. 3. LC-CC–API–ESI–MS chromatogram of a representative PMMA sample dissolved in mobile phase. Conditions: Mobile phase: 16.8% (w/w) *n*-hexane and 83.2% (w/w) tetrahydrofuran: Flow: 0.5 ml/min; two columns of 250×4 mm each Nucleosil 50-5 at 40°C; V_{inj} 55 μl; detection: ESI(+)–MS, ionisation 5.5 kV, fragmentation 200 V with post-column addition of 5 mg KI/10 ml acetone.

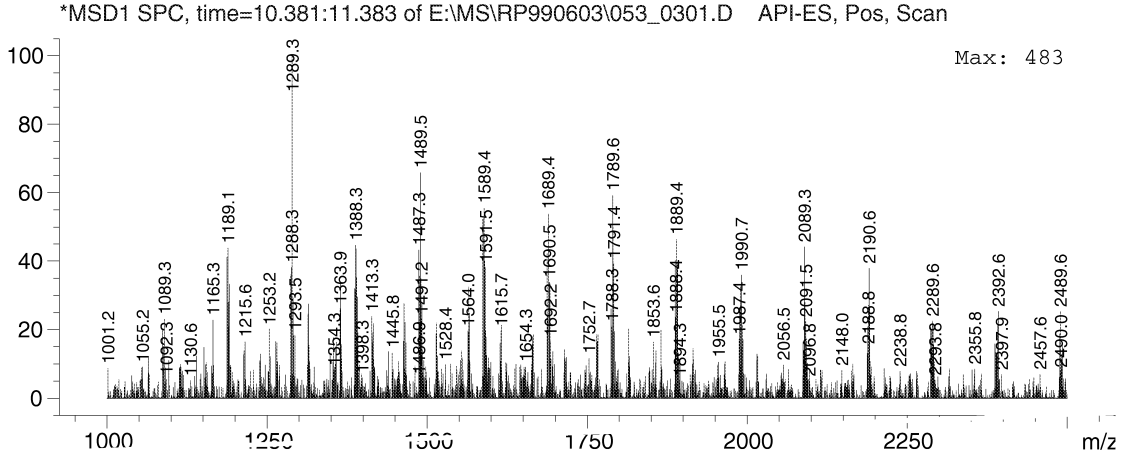

Fig. 4. ESI(+)–MS spectrum of the mono-functional peak at 10–12 min.

the peak-width is equal, the general response equation [13] can be used:

$$\text{Response}\,(t) = \text{A0}c(t)^{\text{A1}} \quad (1)$$

The response (t) is the detector response at time t, $c(t)$ is the concentration at time t and A0 and A1 are constants. When the peak width of the different compounds is not equal, a peak width correction [13] should be performed:

$$\text{Area}\,(a) = \text{Area}\,(b) \cdot (s_a/s_b)^{(1-\text{A1})} \quad (2)$$

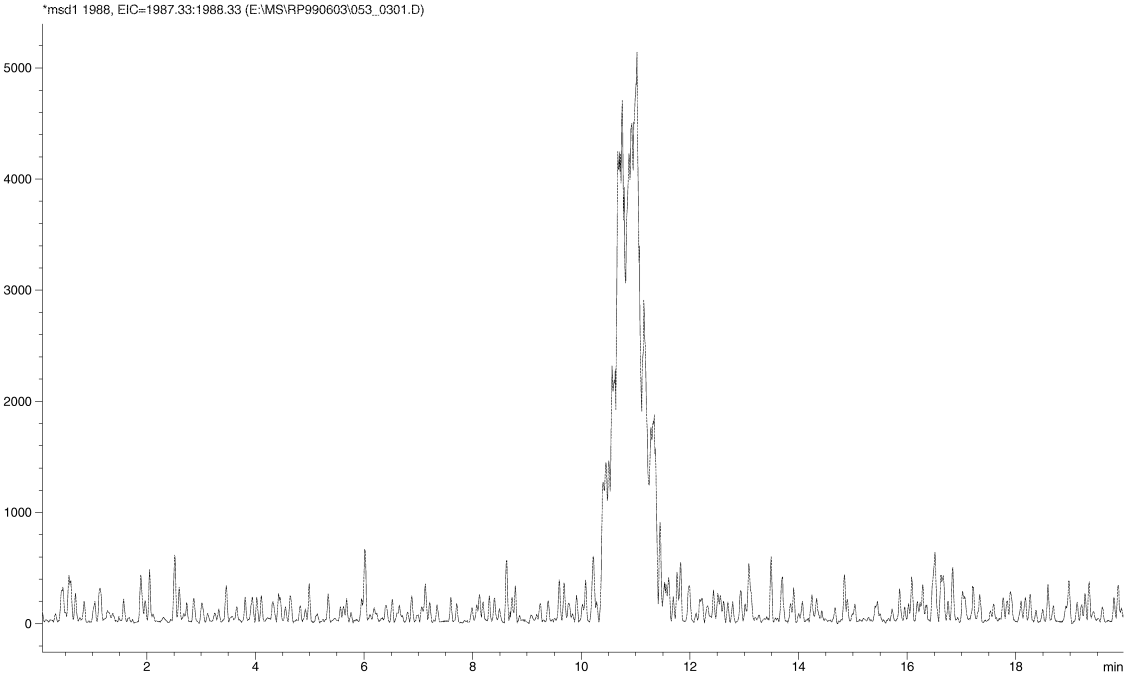

Fig. 5. Reconstructed chromatogram of the mono-functional peak at 10–12 min.

The area (a) is the normalised are, area (b) is the area which has to be normalised, s_a is the standard deviation, s_b is the standard deviation of the peak which has to be normalised and A1 is a constant of the response curve [Eq. (1)].

The response curve was made by injecting a mono-functional PMMA, with an average M_n of 2090 g/mol and a M_w/M_n value of 1.33, which were determined with size-exclusion chromatography (SEC), which was calibrated based on polystyrene standards. The experimental response curve is: {[log Response $(t)] = 1.3136 \cdot [\log\ c(t)] + 3.0874$, $R^2 = 0.9969$}. The OH functionality of two PMMA samples, m-PMMA and b-PMMA (=m-PMMA end-capped with MBO) with an average (M_n value of 2360 g/mol and a M_w/M_n value of 1.29, also determined with SEC, were investigated. Each sample was dissolved at three concentrations between 0.9 and 1.2 mg/g into the mobile phase; 16.8% (w/w) *n*-hexane and 83.2% (w/w) THF, at a concentration and was analysed under critical conditions with ELSD. By using the response curve and peak width correction [Eq. (2)] the concentration of mono- and bi-functionality PMMA was determined in both samples, see Table 1. When the concentration of mono- and bi-functionality is known, the OH functionality (mol OH/mol polymer) calculation can be performed:

$$\text{OH functionality} = [c(\text{mono}) + 2 \cdot c(\text{bi})]/c(t) \qquad (3)$$

The c(mono) is the concentration mono-functional PMMA, c(bi) is the concentration bi-functional PMMA and $c(t)$ is the concentration of the sample that was investigated. The practical sample of mono-functional PMMA (m-PMMA) gives an average OH functionality of 0.89 and the practical sample of bi-functional PMMA (b-PMMA) gives an average OH functionality of 1.19 mol OH/mol polymer.

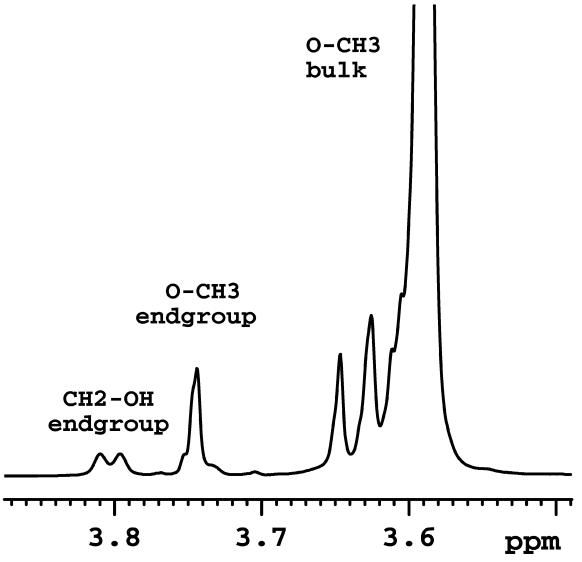

Fig. 6. ^{1}H-NMR spectrum of mono-functional PMMA.

3.3. Quantification by NMR and specific titration

The OH functionality of both mono- and bi-functional PMMA was performed after dissolving both samples in $C_2^2H_2Cl_4$ and measured at a temperature of 70°C with the use of 500 MHz ^{1}H-NMR. In the mono-functionalised PMMA, the OCH_3 and CH_2OH end group signals could be assigned [14] at 3.75 and 3.8 ppm in the 500 MHz ^{1}H-NMR spectrum as indicated in Fig. 6. Based on the known M_n, the OH

Table 1
Results of the quantification of OH functionality of two PMMA samples determined with LC-CC–ELSD

Sample	C (t) (mg/kg)	Area mono (mV/s)	Area bi (mV/s)	W-mono (s)	W-bi (s)	Corr-bi (mV/s)	C (mono) (mg/kg)	C (bi) (mg/kg)	OH functionality (mol/mol)
m-PMMA-1	1161	11 041 184	–	26	–	–	1026	–	0.88
m-PMMA-2	1231	12 306 872	–	26	–	–	1115	–	0.91
m-PMMA-3	1162	10 675 810	–	26	–	–	1000	–	0.86
b-PMMA-1	1041	5 586 690	2 331 722	28	49	2 777 918	722	359	1.22
b-PMMA-2	986	5 034 500	2 147 910	27	47	2 540 374	679	335	1.19
b-PMMA-3	1065	5 442 408	2 299 861	27	46	2 703 259	711	352	1.17

C, concentration; mono, mono-functional; bi, bi-functional; W, width of peak at half height; Corr, corrected area.

Table 2
Results of the quantification of OH functionality of two PMMA samples determined with the three different analytical techniques

Sample	M_n ([a]) (g/mol)	M_w/M_n ([a])	OH functionality		
			LC-CC–ELSD	^{1}H-NMR	Titration
m-PMMA	2360	1.29	0.89	0.83	0.87
b-PMMA	2360	1.29	1.19	1.42	1.20

[a] Determined with SEC, calibration based on polystyrene standards.

functionality for practical sample of mono-functional PMMA was calculated. The functionality of the practical sample of bi-functional PMMA was derived from the loss of signal intensity at 3.75 ppm (OCH_3), see Table 2. The OH functionality based on NMR for the mono-functional PMMA sample has an OH functionality equal to that determined by LC-CC–ELSD, but the bi-functional PMMA shows a slightly higher functionality compared to LC-CC–ELSD

The determination of the endgroup functionality of PMMA with specific titration was performed by the reaction between hydroxyl groups of the PMMA and acetic acid anhydride in DMF. The hydroxyl groups react to form an ester and an equivalent amount of acetic acid, which is a measure for the concentration of hydroxylic groups (mmol/g). The samples, mono- and bi-functional PMMA, were quantified using this titration (for results see Table 2). From experience with samples of polybutylene adipate a standard deviation of 0.8% was obtained for this titration, but this tells nothing about the accuracy of the titration because phenols, primary and secondary amines, oximes and water can influence the quantification. The results of the OH functionality of both PMMA samples corresponded with the results obtained from LC-CC–ELSD and ^{1}H-NMR. These similar results for both samples obtained from three different analytical techniques gives an indication of the performance of the OH functionality quantification with LC-CC–ELSD.

4. Conclusions

Hydroxyl-endcapped low-molecular-mass PMMA, synthesised with ATRP, was separated under critical conditions due to interactions between the polar hydroxyl endgroups and the non-modified silica based stationary phase. The different separated peaks were identified using on-line LC-CC–API–ESI–MS and the OH functionality was quantified with ELSD, with the use of peak width correction. The OH functionality of mono- and bi-functional PMMA samples was also estimated with ^{1}H-NMR, by quantifying the disappearance of the methyl ester resonance of the final methacrylate moiety. The functionality was also quantified with the use of specific OH titration. The OH functionality results obtained with these three different analytical techniques show a good agreement, except for the OH-functionality of bi-functional PMMA obtained with ^{1}H-NMR. The agreement between these different techniques gives an indication that the quantification of the OH functionality of mono- and bi-functional PMMA with LC-CC–ELSD is correct.

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