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Comprehensive two-dimensional liquid chromatography for the characterization of functional acrylate polymers

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Abstract

Comprehensive two-dimensional liquid chromatography–size-exclusion chromatography (LC × SEC) was investigated as a tool for the characterization of functional poly(methyl methacrylate) (PMMA) polymers. Ultraviolet-absorbance and evaporative light-scattering detection (ELSD) were used. A simple method to quantify ELSD data is presented. Each data point from the ELSD chromatogram can be converted into a mass concentration using experimental calibration curves. The qualitative and quantitative information obtained on two representative samples is used to demonstrate the applicability of LC × SEC for determining the mutually dependent molar-mass distributions (MMD) and functionality-type distributions (FTD) of functional polymers. The influence of the molar mass on the retention behavior in LC was investigated using LC × SEC for hydroxyl-functional PMMA polymers. The critical conditions, at which retention is – by definition – independent of molar mass, were not exactly the same for PMMA series with different end-groups. Our observations are in close agreement with theoretical curves reported in the literature. However, for practical applications of LC × SEC it is not strictly necessary to work at the exact critical solvent composition. Near-critical conditions are often sufficient to determine the mutually dependent distributions (MMD and FTD) of functional polymers.

Keywords: Critical liquid chromatography; Comprehensive 2D-LC; RAFT; Polymer functionality; LC × SEC; ELSD quantification

1. Introduction

Synthetic polymers are very complex mixtures of many different chemical compounds [1]. In "simple" homopolymers the individual molecules vary unavoidably in the number of polymer repeat units. The individual molecules in synthetic polymers may be built up from several different repeat units (copolymer), and possibly be initiated by different compounds or terminated in different ways, to give rise to various end groups. Polymeric chains may be linear, branched to variable extents, or even cyclic. In addition, some polymers exhibit variations in chain (stereo-) regularity or "tacticity". Variations in the chemical structure, such as the number of functional groups or end-groups present or the chemical composition of copolymers, can have dramatic effects on the properties of the polymer. Clearly, in order to establish relationships between molecular structure and material performance of polymers, we need to obtain information on the average molecular structure, as well as on the underlying distributions.

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Liquid chromatography (LC) is eminently suitable for separating soluble polymers. A number of different mechanisms (size exclusion, adsorption, partition, etc.) can be exploited [1]. Size-exclusion chromatography (SEC) is the most commonly applied technique for separating polymers based on the size (hydrodynamic volume) of molecules in solution and the extent to which they are excluded from porous particles. The molar-mass averages and the molar-mass distribution (MMD) can be obtained using a calibration curve that relates the (logarithm of the) molar mass to the retention time or volume, or using on-line molar-mass detectors such as light scattering or viscosimetry. "Interactive" LC, which is based on molecular interactions between the polymer molecules and the mobile and stationary phases in the column, can be used to separate polymers based on chemical composition or functionality (functional groups or end-groups). As in conventional LC techniques, the composition of the mobile phase is varied to achieve the desired separation. Gradient elution is often needed to elute a variety of polymer molecules within a reasonable time, because the molecular interactions vary dramatically with the size and structure of polymer molecules. Between (or beside) the SEC mode and the interactive mode, there is a specific mode of isocratic LC, in which retention is independent of molar mass and solely influenced by the chemical composition or functionality of the molecules. These so-called critical conditions are hard to achieve and maintain, but they are extremely useful for separating polymer molecules according to the number of functional groups present.

Complex polymers feature several simultaneous distributions. For example, all functional polymers exhibit functionality-type distributions (FTD) and all copolymers exhibit chemical-composition distributions (CCD). As a rule, the different distributions are mutually dependent. To characterize multiple, mutually dependent distributions, multi-dimensional separations are indispensable. In this work, functional polymers featuring an MMD and an FTD will be analyzed. To characterize these two dependent distributions, we need a two-dimensional separation. Ideally, but not necessarily, one separation step distinguishes between molecules of different molar mass, while the other step reveals differences in functionality.

Two-dimensional liquid chromatographic (2D-LC) systems have been used for many years to separate and characterize synthetic polymers, biomolecules and complex mixtures [2]. The most common form of 2D-LC in the earlier studies was an off-line approach [3–5]. In this so-called "cross-fractionation" or "heart-cut" method, a few fractions from the first-dimension column were collected and re-injected into a second liquid-chromatographic system. The resulting data are two or more chromatograms. This technique requires knowledge of the retention of specific sample components, before the fractionation can take place. It is very useful for the separation of (a) specific component(s) in a polymer or copolymer.

During the 1970s Erni and Frei [6] were probably the first to explore the on-line approach, which has become known as "comprehensive" two-dimensional LC. In this method, sequential aliquots from the first column are transferred on-line to the second one using an automated switching valve [5–10]. The transfer volume is taken sufficiently small, so that each chromatographic peak from the first dimension is divided into several fractions of equal volume. The resulting data is a three-dimensional matrix, usually represented as a contour plot, with each chromatographic retention time along one axis and the detector signal as the intensity parameter. Comprehensive two-dimensional operation greatly increases the peak capacity of LC systems. Consequently, the information content of the resulting chromatogram is greatly enhanced. Several other research groups have contributed significantly to the development of two-dimensional separations of polymers. Especially relevant in the context of the present work are the studies from the group of Pasch [11,12]. With respect to nomenclature, heart-cut two-dimensional liquid chromatography is usually referred to as LC–LC, whereas for on-line coupling with complete transfer of the eluate from the first dimension (i.e. comprehensive 2D-LC) the notation LC × LC is preferred [13].

In LC × LC of polymers several different separation mechanisms can be exploited in the first and second dimensions. The choice for either dimension is dependent on the distributions of interest. Following the method of van der Horst et al. [5], we used LC × SEC in this work to investigate the FTD and MMD of functional polymers. The total analysis time is the product of the analysis time in the second dimension and the number of fractions collected from the first dimension effluent. To limit the total analysis time in LC × SEC and to conserve the chromatographic separation (resolution) obtained in the first dimension, it is very important that the second dimension be fast. We opted for size exclusion in the second dimension and fast SEC analyses were performed, using short columns packed with small particles.

In a comprehensive set-up, a 10-port switching valve equipped with two loops was used in a symmetrical configuration [5]. While one loop is being filled with the first-dimension eluate, the fraction that has previously been collected in the second loop is analyzed in the second-dimension separation. The collection time for each fraction in the first dimension is equal to the analysis time in the second dimension. As a consequence, the analysis time in the second dimension and the loop volume together determine the (maximum) flow rate for the first-dimension separation. Therefore, to realize truly comprehensive LC × LC (i.e. without splitting after the first column), the flow rate of the first-dimension separation cannot be very high. We prefer to use a micro-bore LC column for the first-dimension separation. This ensures comprehensive operation of the system [5].

In this work, we demonstrate the use of comprehensive two-dimensional LC (specifically LC × SEC) to obtain the MMD and FTD for functional poly(methyl methacrylate)

(PMMA) polymers. Also, the influence of the molar mass on the so-called critical conditions in LC was investigated for hydroxyl-functional polymers.

2. Experimental

2.1. Chemicals

Dichloromethane (DCM) and acetonitrile (both HPLC grades), were from Rathburn Chemicals (Walkerburn, Scotland). Non-stabilized tetrahydrofuran (THF, Biosolve, Valkenswaard, The Netherlands) was used as the mobile phase in size-exclusion chromatography. Poly(methyl methacrylate) standards were obtained from Polymer Laboratories (Church Stretton, Shropshire, UK). These standards are terminated with hydrogen end-groups and they are referred to as "non-functional polymers" in this paper. The molar-mass (M_n, M_p) and polydispersity-index (PDI) values were specified by the manufacturer. The ("RAFT") polymers with one hydroxy (OH) end-group were synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization, using a hydroxy-functional initiator and a hydroxy-functional RAFT chain-transfer agent [14]. A commercial telechelic PMMA (TEGO DIOL MD-1000X) with two OH groups was obtained from Tego Chemie Service (Essen, Germany). The molar masses and molar-mass distributions were measured by SEC using a Waters (Milford, MA, USA) instrument equipped with a Waters model 510 pump and a model 410 differential refractometer (40 °C). A set of two linear columns (Mixed-C, Polymer Laboratories, 300 mm × 7.5 mm i.d., 40 °C) was used. The calibration curve was prepared with polystyrene (PS) standards and the molar masses were estimated based on the universal-calibration principle and Mark-Houwink parameters [PS, $K = 1.14 \times 10^{-4}$ dL g^{-1} and $a = 0.716$; PMMA, $K = 0.944 \times 10^{-4}$ dL g^{-1} and $a = 0.719$] [15–17]. The effect of the hydroxyl end-groups on the Mark-Houwink parameters was neglected. All the PMMA standards and samples used are summarized in Table 1. Numbers after PMMA (or PMMA-OH, or PMMA-2OH) refer to the peak molar mass. All the samples injected in the first dimension were dissolved in DCM, which is a good solvent for PMMA, but a weak eluent on bare silica. In this way breakthrough peaks were avoided [18]. All the samples analyzed by SEC (as a stand-alone technique) were dissolved in THF (Samples analyzed in SEC as a second-dimension separation were dissolved in the first-dimension effluent.

Table 1
PMMA samples used in this study

Sample name	M_n (kg/mol)	M_p (kg/mol)	PDI	Intended number of OH end groups
PMMA 620[a]	5.50	6.20	1.35	0
PMMA 1310[a]	1.16	1.31	1.12	0
PMMA 1680[a]	1.33	1.68	1.15	0
PMMA 1990[a]	1.84	1.99	1.09	0
PMMA 2990[a]	2.76	2.99	1.08	0
PMMA 3800[a]	3.44	3.81	1.07	0
PMMA 5270[a]	4.98	5.27	1.06	0
PMMA 6950[a]	–[e]	6.95	1.05	0
PMMA 9200[a]	8.50	9.20	1.06	0
PMMA 13,930[a]	12.49	13.93	1.06	0
PMMA-OH 3310[b]	2.43	3.31	1.22	1
PMMA-OH 13,950[b]	10.93	13.95	1.21	1
MD-1000X	1.49	2.32	1.64	2
VL37A[c]	2.59	3.75	1.29	1
VL37B[d]	2.85	3.68	1.29	1

The molar-mass (M_n, M_p) and polydispersity-index (PDI) values of samples were measured by SEC. The values for PMMA standards were supplied by the manufacturer.

[a] PMMA standards obtained from Polymer Laboratories.

[b] Polymers with one OH group synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization using a hydroxy-functional initiator and a hydroxy-functional RAFT chain-transfer agent.

[c] Synthesized via end-group modification of well-defined RAFT polymers [14].

[d] Synthesized by RAFT polymerization using a 2,2′-azobisisobutyronitrile (AIBN) initiator and a hydroxy-functional RAFT chain-transfer agent [14].

[e] M_n value not known.

2.2. Instrumentation

The first-dimension LC system used consisted of a Shimadzu LC-10ADvp solvent-delivery unit (Shimadzu, 's-Hertogenbosch, The Netherlands) and a Rheodyne two-position six-port injection valve (Berkeley, CA, USA) equipped with a 1 or a 10 μl loop. Two home-packed Hypersil "bare" silica columns (150 mm × 1.0 mm i.d., 3 μm particles; 100 Å pore size; ThermoQuest, Breda, The Netherlands) were used in the first (LC) dimension at room temperature. The second-dimension (SEC) system consisted of a Kratos Spectroflow 400 pump (ABI, Ramsey, NJ, USA), a Kratos Spectroflow 757 UV-absorbance detector (ABI) operated at a wavelength of 220 or 300 nm, and a Sedex 55 evaporative light-scattering detector (ELSD; temperature 62 °C, N_2 pressure 2.2 bar). The ELSD is positioned in the flow line after the UV detector. This causes a shift of a few seconds in the chromatograms (at 0.9 ml/min). The two-dimensional contour plots are slightly shifted, but this does not affect the interpretation. One or two 50 mm × 4.6 mm i.d. PLgel columns (Polymer Laboratories, 5 μm particles with 100 Å pore size and/or 6 μm oligoPore particles with 100 Å pore size, 25 °C) were used with THF at a flow rate of 0.9 ml/min in the SEC systems. The LC and SEC systems were coupled with an air-actuated VICI two-position 10-port valve (Valco, Schenkon, Switzerland) [5]. This valve was operated using a high-speed switching accessory (switching-time of 20 ms using nitrogen) and dual injection loops of equal volume (various sizes between 4 and 40 μl) were used.

2.3. Instrument control

A personal computer with Windows NT was equipped with a Keithley KNM-DCV 12 Smartlink interface (Cleve-

land, OH, USA). Two-dimensional plots and distribution data were calculated with an in-house program written in a Matlab (Natick, MA, USA) software environment [5]. This program enabled us to register and control the valve-switching time for the two-dimensional separations. The program options also allowed us to extract LC and SEC chromatograms at any positions in the LC × SEC contour-plot. Furthermore, the software was able to carry out quantification by computing peak volumes for specified retention ranges and to calculate MMDs for different polymers, using SEC calibration curves, provided that the detection response was linear or linearized (see the text in Section 3.3).

3. Results and discussion

3.1. Optimization of SEC flow rate

The second-dimension separation in LC × SEC needs to be fast, while sufficient separation efficiency needs to be maintained. Because a conventional SEC analysis, for example, using two 300 mm × 4.6 mm i.d. columns at 0.5 ml/min, shows a typical analysis time of 10–15 min, it is not useful for LC × SEC. Instead, fast-SEC column(s) should be selected and relatively high flow rates should be employed. There is a trend to perform fast-SEC separations on short (typically 50 mm) columns with analysis times of 1–3 min. The flow rate is dependent on the column diameter (e.g. 0.5 ml/min for a 4.6 mm i.d. column or 1.0 ml/min for a 7.5 mm i.d. column). However, recent studies from our group [19–21] provide theoretical and practical support for the use of longer columns and higher flow rates in fast SEC. To keep the analysis time in the second-dimension SEC separation similar (within 2 min), we selected one column at a flow rate of 0.45 ml/min, or two columns of the same dimension at a flow rate of 0.9 ml/min. For comparison of the SEC resolution, a mixture of Standards PMMA 620 and PMMA 6950 was injected using the second-dimension part of the LC × SEC set-up (same 10-port switching valve equipped with two 40 μl loops) with the UV and ELSD detectors as a stand-alone system. As can be seen clearly from Fig. 1, better resolution was obtained when two columns were used at a flow rate of 0.9 ml/min. The PMMA 620 peak (2) was baseline separated from the solvent peak (3) (UV at 220 nm, see Fig. 1b). The former was also nearly baseline separated from the PMMA 6950 peak (1). Therefore, longer columns with higher flow rates are seen to provide better resolution in fast SEC. However, when using even longer columns, the pressure drop may become prohibitive. Also, the higher flow rates required would induce higher solvent costs and might impart the sustained stable operation of the pump [22].

In the present case, the selection of columns (one regular PLGel 100 Å column and one 100 Å OligoPore column) was also tailored to the separation of low-molar-mass prepolymers. Therefore, the two 50 mm columns were used in series

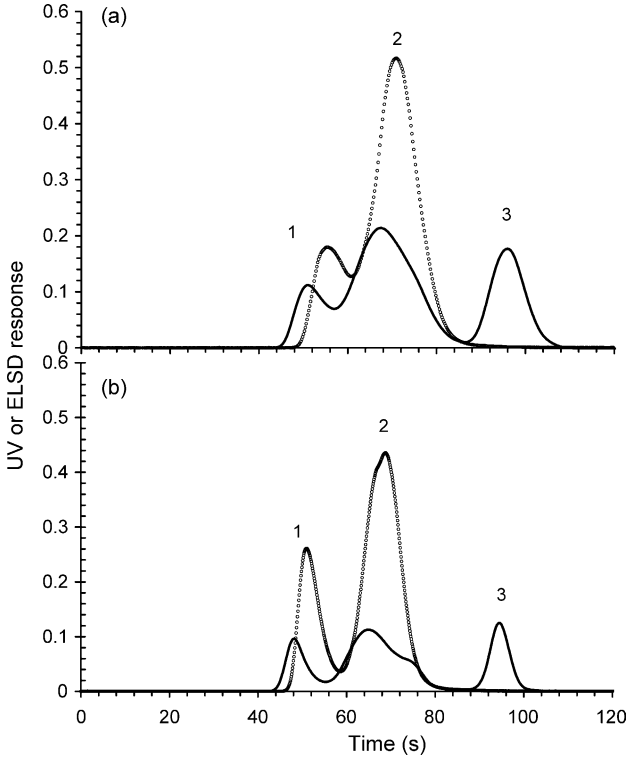

Fig. 1. SEC chromatograms obtained from a standard mixture of PMMA 620 and PMMA 6950. Dotted line, ELSD response; drawn line, UV response at 220 nm. (a) One 50 mm × 4.6 mm i.d. PLgel column (5 μm particles with 100 Å pore size), flow rate 0.45 ml/mim. (b) Two 50 mm × 4.6 mm i.d. PLgel columns (5 μm particles, 100 Å pore size and 6 μm oligoPore particles, 100 Å pore size), flow rate 0.9 ml/min. Peak 1: PMMA 6950; peak 2: PMMA 620; peak 3: THF. Mobile phase was fresh non-stabilized THF.

in the second-dimension SEC separation for the LC × SEC experiments reported in this work.

3.2. Molar-mass effects at near-critical LC

In previous work [23] we have shown the robust critical separation of PMMA using a conventional bare-silica column (150 mm × 4.6 mm). The PMMA samples could be separated according to the number of hydroxyl end-groups with negligible influence of the molar mass [23]. However, this column cannot be coupled directly on-line to electospray ionization-mass spectrometry (ESI-MS) without splitting. Two microbore columns (150 mm × 1.0 mm) were used to study the effect of molar mass on retention time at near-critical conditions [24]. Because of the limited (low) molar-mass range of ESI-MS and its limited range of applicability to specific (polar) types of polymers [25], LC × SEC was investigated in this work as an alternative approach to study the molar-mass effect. LC × SEC is applicable to all soluble polymers across a broad range in molar mass. It also allows quantitative data to be obtained on mutually dependent distributions, such as the FTD and MMD of functional polymers.

As shown in Fig. 2, a mixture of non-, mono- and difunctional PMMA polymers of various (relatively low) molar

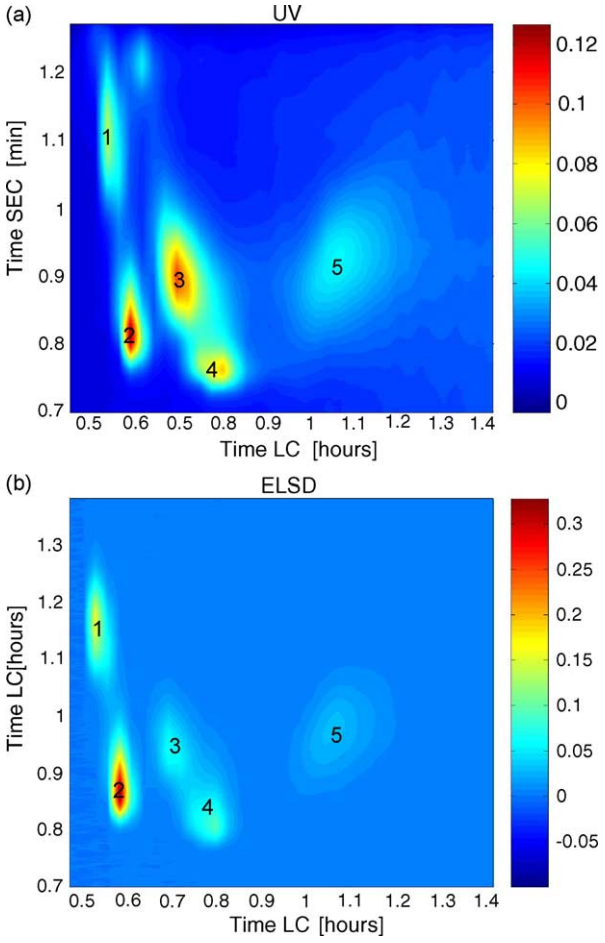

Fig. 2. LC × SEC chromatograms of a mixture of PMMA standards (peak 1: PMMA 620, peak 2: PMMA 5270; non-functional), mono-functional RAFT polymers (peak 3: PMMA-OH 3310, peak 4: PMMA-OH 13,950) and di-functional PMMA (peak 5: MD-1000X) at near-critical conditions. (a) UV220 nm, (b) ELSD. LC columns: two 150 mm × 1.0 mm i.d., 3 μm, 100 Å bare silica; 48% ACN in DCM, 8 μl/min. SEC columns: 5 μm 100 Å plus 6 μm oligopore, two times 50 mm × 4.6 mm i.d.; fresh non-stabilized THF, 0.9 ml/min.

masses yielded clearly separated peaks using 48% acetonitrile in DCM as the mobile-phase in the first dimension (LC). Some variation in the retention time with molar mass can be observed in Fig. 2. The retention times of non- and mono-functional PMMA polymers increased slightly with increasing molar mass, displaying a "banana" shape, which indicated that the mobile-phase was on the adsorption side of the critical point. This is in agreement with what van der Horst et al. have reported [5]. However, the retention of di-functional PMMA polymers seemed to decrease slightly with increasing molar mass, suggesting an opposite molar-mass effect at the same mobile-phase composition. Despite the slight molar-mass effect, we could obtain the molar-mass and MMD information for individual peaks using the SEC calibration curve. Subsequently, we could obtain the FTD and MMD for the functional polymers by LC × SEC at a near-critical composition (see discussion below in Section 3.4). If only one dimensional chromatography (either LC or SEC) were used, we

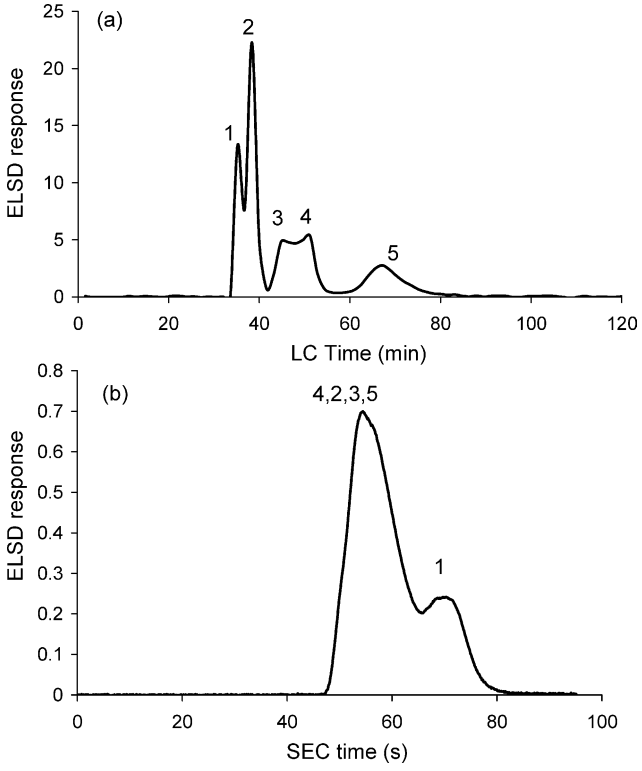

Fig. 3. (a) Reconstructed first-dimension LC-ELSD chromatogram and (b) reconstructed second-dimension SEC-ELSD chromatogram from Fig. 2b (see Fig. 2 for samples and conditions).

would have obtained overlapping peaks, as is evident from the one-dimensional projections of the chromatograms in the LC dimension (shown as Fig. 3a) and in the SEC dimension (shown as Fig. 3b). A small peak was observed in the UV chromatogram ($^1t_R \approx 0.6$ h, $^2t_R \approx 1.2$ min), but not seen with ELSD detection. This was probably an unreacted OH-RAFT agent (see discussion in Section 3.4).

Acetonitrile is a more-polar solvent than DCM. It can desorb PMMA from the silica column. When the concentration of acetonitrile in the mobile phase increases, the retention of non-functional and hydroxyl-functional PMMA polymers will decrease. When the concentration of acetonitrile in the mobile phase was 52% in DCM, the two mono-functional PMMA polymers with different molar masses showed the same retention time, which suggested that this was the critical solvent composition for mono-hydroxyl PMMA samples (results not shown). However, the retention of non-functional PMMA standards still increased slightly with increasing molar mass, displaying a "banana" shape. This suggested that the mobile phase was still on the adsorption side of the critical point. At the same time the retention of di-functional PMMA polymers decreased slightly with increasing molar mass. One should note that this is not typical exclusion behavior, because the retention volumes far exceed the total volume of mobile phase in the column.

As shown in Fig. 4, with a higher acetonitrile concentration (56% ACN in DCM), the retention of non-functional PMMA

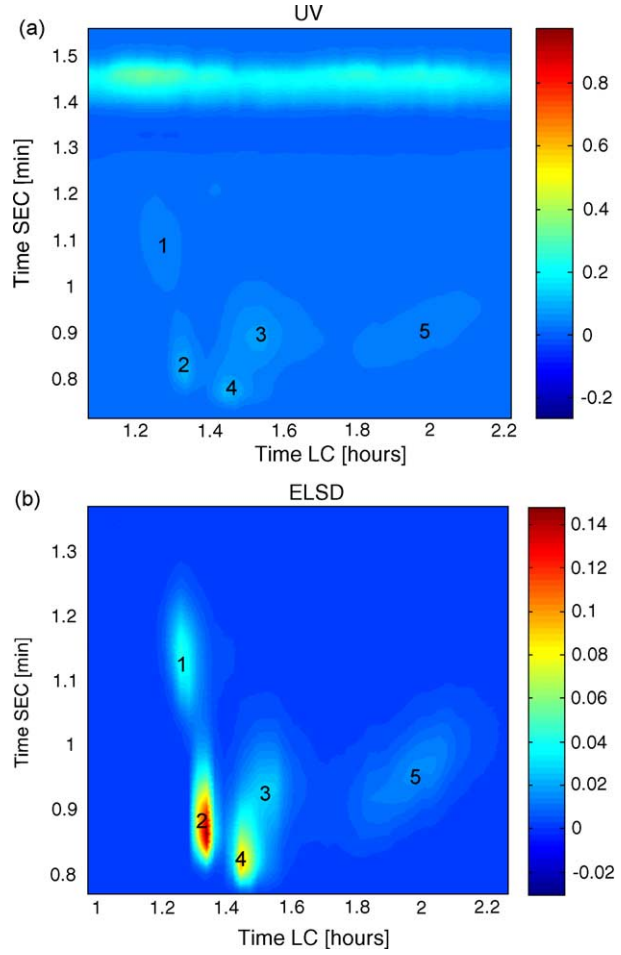

Fig. 4. LC × SEC (a) UV (220 nm) and (b) ELSD chromatograms of the same mixture of PMMA samples as in Fig. 2. The conditions were identical as for Fig. 2 except that the LC mobile phase was 56% ACN in DCM and the flow rate was 4 μl/min in the first dimension.

standards in the first dimension still increased slightly with increasing molar mass, displaying a "banana" shape, which indicated that the mobile phase was still on the adsorption side of the critical point. However, the retentions of mono- and di-functional PMMA polymers decreased slightly with increasing molar mass. When the concentration of acetonitrile in the mobile phase was 70% or above, the retention of all PMMA polymers decreased with increasing molar mass and typical exclusion behavior was observed.

We may conclude that the critical conditions, at which retention is independent of molar mass, are not the same for PMMA series with different end-groups. Apparently, the critical composition is (slightly) less than 48% ACN for di-functional PMMA, about 52% for mono-functional PMMA and more than 56% ACN for non-functional PMMA. This may be surprising, because, in principle, the critical composition for the PMMA backbone should not change with the end-groups. Variations in the exact critical composition with different end-groups has also been observed for poly(*n*-butyl acrylate) polymers [26].

Gorbunov and Trathnigg [27] and Skvortsov and Fleer [28] developed a unified theory of the combination of interaction (adsorption) LC and SEC for polymers. Theoretical models suggested that the retention of di-functional polymers at the critical point (Fig. 15 in ref. [28]) or at conditions of very weak adsorption (Fig. 14 in ref. [28]) for non-functional polymers should depend on the molar mass. Gorbunov and Trathnigg [27] reported some experimental and theoretical results indicating that the retention of di-functional polymer at the critical conditions of the non-functional polymer decreased with increasing molar mass. They stated that the distribution coefficient of functional polymers could exceed unity and that it would decrease with increasing radius of gyration (molar mass) if the interaction of end-groups with the stationary phase was strongly attractive. Our observations in Fig. 2 are in close agreement with the theoretical curves reported in Fig. 14 of ref. [28] for the low-molar-mass range. The results shown in Fig. 4 of the present paper are similar to the data published in Fig. 4 of ref. [27]. It would be interesting to study the molar-mass effect for high-molar-mass polymers by LC × SEC, if samples with various molar masses and end-groups were available.

At this point it is worth noticing that for practical applications of LC × SEC it is not strictly necessary to work at the exact critical solvent composition. Near-critical conditions often suffice to determine the mutually dependent MMD and FTD for functional polymers.

3.3. Quantitative aspects

In LC × SEC, the detector monitors the signal after SEC. Therefore, the detectors used in common SEC can, in principle, be used in LC × SEC. The most frequently used detectors in SEC of polymers are refractive-index (RI) and ultraviolet absorbance (UV) detectors. However, both of them have their limitations. For instance, RI detectors exhibit a low sensitivity. Because two-dimensional separation gives rise to a strong dilution of the analytes, it is not easy to use RI detectors in LC × SEC. Moreover, a significant dependence of RI on molar mass was observed for samples with low molar mass [29], due to influence of the end-groups. Since in our case low-molar-mass RAFT-polymers and the corresponding non-RAFT polymers (lost RAFT end-group) must be analyzed, the effect of the RAFT end-group on the RI response greatly complicates the quantitative analysis.

UV detection is limited to UV-active polymers. It can only be used when chromophores, which may be the repeating unit, the end-groups, or both, are present in the analyte. In case both the polymer backbone and the end-groups show a high UV absorbance at the selected wavelength, it is also difficult to obtain accurate quantitative results for low-molar-mass samples. Tetrahydrofuran is a common solvent in SEC, but it is not transparent at short wavelengths, especially due to its oxidization in air. Acrylate polymers exhibit UV absorbance only at short wavelengths (210–235 nm). The UV absorbance at 220 nm for fresh non-stabilized THF was 0.47

AU with water as a reference. As an example, the UV absorbance at 220 nm was 0.83 AU for standard PMMA 1680 at a concentration of 0.5 mg/ml with non-stabilized THF as the reference. When fresh, helium-covered non-stabilized THF was used as SEC mobile phase, UV detection with a selected wavelength of 215–233 nm clearly showed peaks of acrylate polymers. As shown above in Figs. 1 and 2a, the UV signal at 220 nm provided a good qualitative impression of the PMMA samples. However, this signal could not be used to obtain accurate quantitative results on functional PMMA polymers, because the response was due to the polymer backbone as well as to end-groups. Both contributions are significant and variable in case of low-molar-mass samples with different end-groups. The response neither reflects the sample mass, nor the number of the polymer chains. For example, the UV absorbance at 220 nm was 0.53 AU for RAFT polymer PMMA-OH 3310 at a concentration of 0.1 mg/ml with non-stabilized THF as the reference. However, it was approximately 3 for the OH-containing RAFT agent (with the same end-groups as those of PMMA-OH 3310 polymer, but without MMA units) at the same concentration. In the functional RAFT polymer VL37A every polymer chain has a RAFT group, which exhibits UV absorbance at high wavelengths (300 nm). It is reasonable to assume that the UV absorbance at this wavelength is proportional to the number of RAFT polymer chains with a negligible effect of molar mass. For such a polymer we can obtain quantitative information by LC × SEC (see Section 3.4).

Infrared (IR) spectrometry has proven to be a powerful tool for the selective detection of (either UV-active or non-UV-active) functional groups in polymers [22]. However, the practical use of IR detection in LC is still quite limited, because of the possible occurrence of inconsistent absorption-band intensities in solvent-elimination LC-IR spectra, the low detection limits for flow-cell interfaces and other reasons [22].

Evaporative light-scattering detection (ELSD) has become increasing popular in HPLC, due to its "universal" applicability and high sensitivity for all non-volatile analytes [30]. However, quantitative analysis using an ELSD is not easily achieved [30–32], because the ELSD response does not usually increase linearly with the polymer concentration. Calibration curves should be established and applied carefully. An exponential calibration curve, such as in Eq. (1), is often used [32–34]:

$$A = a \times m_{\mathrm{i}}^{b} \tag{1}$$

where A is the ELSD response area, m_{i} is the injected mass of sample, and a and b are constants. The values of a and b can easily be determined from a logarithmic plot, in which the exponent b is obtained from the slope and the constant a from the intercept of the regression line.

It should be noted that Eq. (1) was established and proven in one-dimensional LC. There is a serious difficulty in deriving quantitative data from comprehensive two-dimensional LC using a non-linear detector. In this discussion we assume that the chromatographic profile (analyte concentration vs. time) is Gaussian, an approximation that is often satisfactory. If the detector response is such that at any time the signal, y, is related to the analyte concentration (C) (see plateau method below) by

$$y = a' \times c^{b'} \tag{2}$$

where a' is the response factor, then the recorded peak profile by ELSD is also a Gaussian curve, because of the properties of the exponential [35]. Only the standard deviation observed by ELSD (σ_{ELSD}) is changed from the standard deviation σ obtained by using a linear detector to

$$\sigma_{\mathrm{ELSD}}^{2} = \frac{\sigma^{2}}{b'} \tag{3}$$

The power constants b used in Eq. (1) and b' in Eq. (2) are identical [34,35]. The constants a in Eq. (1) and a' in Eq. (2) are related by [34,35]

$$a = \frac{a'}{[\sqrt{b} \times F^{b} \times (\sigma \times \sqrt{2\pi})^{b-1}]} \tag{4}$$

where F is the flow rate. The constant a will not vary when the LC conditions do not change. Therefore, the calibration curves can be established based on the measured individual response (Eq. (2), see plateau method below) using ELSD (column is not necessary), on the integrated peak area (Eq. (1), see method 2 below) or on the integrated peak volume (see method 1 below). As a consequence, the calibration curves can be established in three different ways in LC × SEC. First, by individually injecting each standard in various amounts into the LC × SEC, we obtain a series of integrated peak volumes (equivalent to summing the peak areas obtained from each fraction of the first dimension in LC × SEC, similar to summing all data points in one-dimensional LC; the power constant b will not change). Second, by injecting each standard in various amounts only into the second-dimension SEC system we can obtain the calibration curves based on integrated peak areas from SEC. Third, by injecting each standard in various concentrations directly into the ELSD to get the stationary (plateau) signal using the 10-port switching valve equipped with two big loops, such that a flat peak (plateau) can be obtained. The height of the plateau refers to the real injected analyte concentration, without dispersion in the connecting capillary tubes between the injector and the detector. Thus, the ELSD calibration curves can be obtained (Eq. (2)). Each response (data point) from the ELSD chromatogram can be converted into the analyte concentration using the above calibration curves. The same power constant b or b' should be obtained in any case. However, it is time-consuming to obtain the calibration curves by the first method due to the long analysis time in LC × SEC. The third method is fast and allows easy data processing. Therefore, it is recommended.

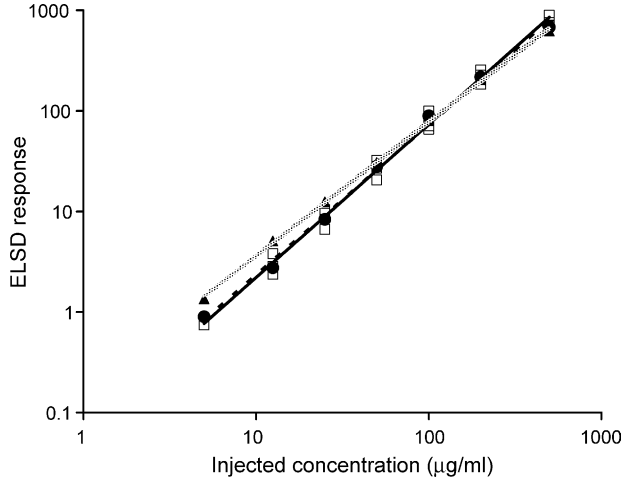

Fig. 5. ELSD calibration curves (plateau height versus injection concentration, logarithmic scale) for PMMAs with different end-groups. Open squares, drawn line: PMMA standards (non-funtional); circles, dashed line: PMMA-OH 3310 (mono-funtional); triangles, dotted line: MD-1000X (di-funtional). Mobile phase: THF, flow rate 0.9 ml/min at 25 °C; injection loop volume 800 μl; no column used.

The above discussion refers to one compound. However, polymers are mixtures of (large) series of molecules with different molar masses, which strongly overlap even after elution from the SEC column. It is impossible to calculate the responses for the individual molecules and add them up, due to the non-linear characteristics of the ELSD. Therefore, we assume polymers to consist of one compound, neglecting the molar-mass effect on the ELSD response. This can be justified, because no obvious molar-mass effect was observed in previous studies [23,32,36–39]. Most of these papers deal with high-molar-mass polymers. In our work we deal with relatively low-molar-mass polymers, where the effect of molar mass on the response may be greater. In our own work, some variation in response was discerned, but no systematic trend could be observed.

We selected the third method described above to obtain calibration curves. Fig. 5 shows ELSD calibration curves for PMMAs with different end-groups. The values of a' and b' are shown in Table 2. It can be seen from Fig. 5 and Table 2 that the OH end-groups had a smaller influence on the ELSD detection in SEC than on the observed (peak area) in critical LC [23]. This is mainly due to the use of integrated peak areas in the latter case. The standard deviation (Eq. (3)) observed for the di-functional polymer was larger than those of the non- and mono-functional polymers. The calibration curves in Fig. 5 were used for quantitative analysis of functional PMMA prepolymers under the specified SEC conditions (see Section 3.4).

Table 2
End-group effect on ELSD calibration curves. LC conditions as in Fig. 5

Sample	a'[a]	b'[a]	R^2
PMMA standards[b]	0.066	1.52	0.9929
PMMA-OH 3310	0.076	1.49	0.9964
HO-PMMA-OH (MD-1000X)	0.167	1.33	0.9995

[a] Parameters in Eq. (2).
[b] PMMA standards used include PMMA 620, PMMA 1990, PMMA 3800, PMMA 9200.

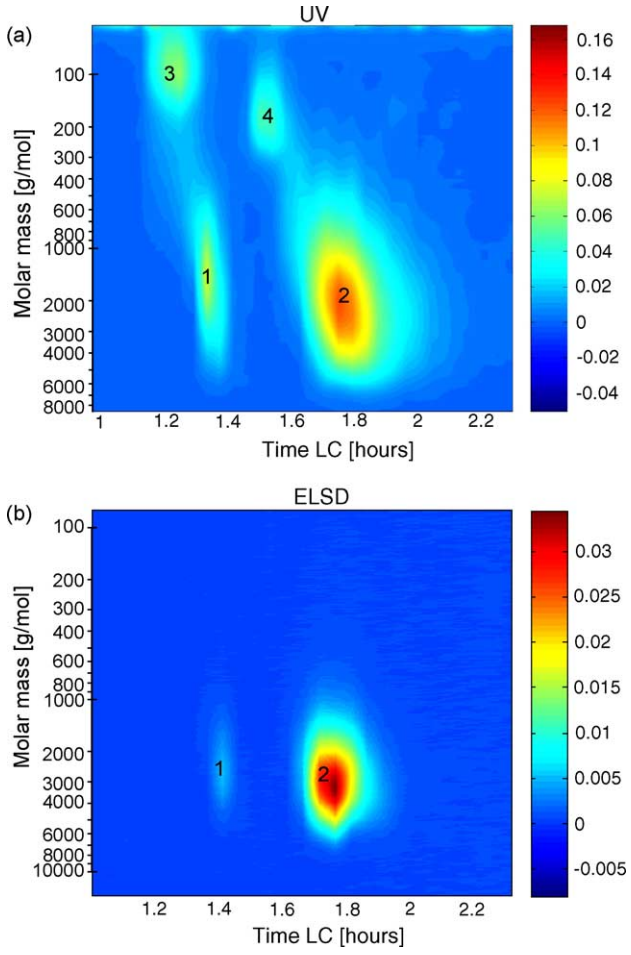

Fig. 6. LC × SEC chromatogram of sample VL37A. (a) UV detection at 300 nm. (b) ELSD. The conditions were identical as for Fig. 2 except that the LC mobile phase was 50% ACN in DCM and the flow rate was 4 μl/min (for peak identification see text).

3.4. Application

Fig. 6 shows an example of LC × SEC for a real RAFT sample (VL37A). In the ELSD trace of Fig. 6b two peaks are observed. Peak 1 represents non-functional PMMA and peak 2 mono-OH PMMA. Both peaks are in agreement with the observations in one-dimensional near-critical LC [24]. However, four separate peaks were detected by UV at 300 nm, as shown in Fig. 6a (similar results were obtained using UV at 220 nm). Peaks 1 and 2 in Fig. 6a are similar to those in Fig. 6b obtained using ELSD. Peak 3 in Fig. 6a represents molecules with a very low molar mass and without OH end-groups; peak 4 may represent residual (unreacted) RAFT agent used in the polymerization process. This latter peak was not observed if PMMA standards were subjected to LC × SEC. If only one-dimensional LC or SEC is used, peaks 3 and 4 are

Table 3
Quantitative results obtained for sample VL37A using UV at 300 nm and calibrated ELSD

Peak name	UV (300 nm)				Calibrated ELSD			
	M_n (kg/mol)	M_p (kg/mol)	PDI	Conc. (mole%) weight%	M_n (kg/mol)	M_p (kg/mol)	PDI	Conc. (mole%) weight%
Peak 1 (non-OH)	2.3	2.3	1.28	16 (13[a])	2.1	2.5	1.24	(10[a]) 9
Peak 2 (mono-OH)	2.7	3.2	1.28	84 (87[a])	2.4	2.9	1.27	(90[a]) 91
Peaks 1 and 2 (combined)	2.6	3.0	1.30	N/A	2.3	2.9	1.28	N/A

The conditions and the peak numbers are as in Fig. 6.
[a] Indirect estimate.

hard to separate completely. Therefore, LC × SEC provides more information and is demonstrated to be useful in polymer analysis.

On-line LC–ESI-MS confirmed that non-functional RAFT (*m/z* 244 + 100*n* with sodium cation) and OH-RAFT polymers (*m/z* 288 + 100*n*) existed in the VL37A sample. On-line LC–ESI-MS showed that the 288 peak (without MMA unit) had a higher intensity than the 388 (1 MMA unit) and 488 peaks (2 MMA units) and that the 444 peak had a higher intensity than the 244, 344, and 544 peaks. We also injected the pure non-functional RAFT and mono-functional OH-RAFT agents. The results support the peak identification above.

The quantitative results obtained for sample VL37A by LC × SEC are summarized in Table 3. Because every polymer chain possesses a RAFT end-group, which exhibits UV absorbance at high wavelengths (300 nm), we can calculate the relative molar concentration for each peak shown in Fig. 6a directly from the UV chromatogram at this wavelength. The concentration of molecules in peaks 3 and 4 in Fig. 6 combined was only about 1 mole% in sample VL37A. The average molar masses (M_n and M_w) and the molar-mass distribution (MMD or PDI) calculated from all four peaks together (1, 2, 3 and 4, in Fig. 6) were very close to those obtained summing only peaks 1 and 2 (results not shown). Since we want to compare the results from ELSD and UV and since the lowest molar mass of the PMMA standards used in the second-dimension calibration is 620, quantitative results for peaks 3 and 4 in Fig. 6a are not included in Table 3. As seen from Table 3, there was a small difference between the molar mass information (M_n, M_p and PDI) obtained for the non-functional PMMA (peak 1) and for the mono-OH RAFT (peak 2). The concentration of non-functional PMMA (peak 1) was 16 mole% in sample VL37A, calculated directly from the UV chromatogram. The second-dimension molar-mass calibration (retention time converted into molar mass) can be used to convert this number to a weight%. A value of 13 weight% was obtained for the non-functional PMMA (peak 1).

To compare UV and ELSD detection, quantitative results were obtained from the ELSD chromatogram. Each data point of the ELSD chromatogram was converted into a concentration using the ELSD calibration curves shown in Fig. 5 and Table 2. Similarly, we obtained the molar mass information (M_n, M_p and PDI) for each peak and for the total sample, as well as relative amounts in weight% or mole% (indirectly) as shown in Table 3. It can be seen from Table 3 that the molar mass values (M_n, M_p and PDI) for each peak and for the total sample obtained from the UV chromatogram were close to those obtained from the calibrated ELSD chromatogram. However, there were some differences between the calculated relative amounts of non-functional PMMA in mole% when calculated directly using UV and when calculated indirectly using ELSD and the second-dimension molar-mass calibration. Likewise, there were some differences when calculating the weight% directly (from ELSD) and indirectly (from UV). This is likely due to uncertainties in the SEC calibration curves.

The relative amount of non-functional PMMA in weight% is close to that obtained by (one-dimension) critical LC (10%, obtained using ELSD, see ref. [23]). When the ELSD response was assumed to be proportional to the mass (an assumption that is likely to be incorrect), the calculated average molar masses (M_n and M_p) from the original ELSD chromatogram were close to those obtained from the UV chromatogram or from the calibrated ELSD chromatogram. However, the PDI value calculated from the original ELSD chromatogram (1.21) was much lower than that from the UV chromatogram (1.30) or that from the calibrated ELSD chromatogram (1.28). This is logical when we consider Eq. (3), because the power constant *b* is usually larger than 1 (see Table 2). Similar results were observed for other samples (results not shown). Therefore, we can use the original ELSD chromatogram to get the approximate molar-mass values (M_n and M_p), but we have to bear in mind that the PDI (and, thus, also the weight-average molar mass, M_w) is somewhat underestimated.

Fig. 7 shows an example of LC × SEC for a real sample without UV-active groups (VL37B). Only the ELSD chromatogram could be used for quantitative analysis, as summarized in Table 4. As seen from this table, there were small difference between the molar mass characteristics (M_n, M_p and PDI) of the various functional polymers [non-functional (peak 1), mono-OH (peak 2) and di-OH PMMA (peak 3)]. This was especially true for the di-functional PMMA, which showed the highest molar mass. The relative amounts of non-, mono-, and di-functional polymers in weight%, calculated directly from the calibrated ELSD chromatogram, are close to those obtained by (one-dimension) critical LC (non-funct. 6%, mono-funct. 83% and di-funct. 11% obtained us-

Table 4
Quantitative results obtained for sample VL37B using calibrated ELSD

Peak name	M_n (kg/mol)	M_p (kg/mol)	PDI	Conc. (mole%) weight%
Peak 1 (non-OH)	2.4	2.5	1.25	(7[a]) 6
Peak 2 (mono-OH)	2.6	3.0	1.31	(86[a]) 85
Peak 3 (di-OH)	3.2	5.2	1.35	(7[a]) 9
Peaks 1, 2 and 3 (combined)	2.7	3.0	1.32	–

The conditions and the peak numbers are as in Fig. 7.
[a] Indirect estimate.

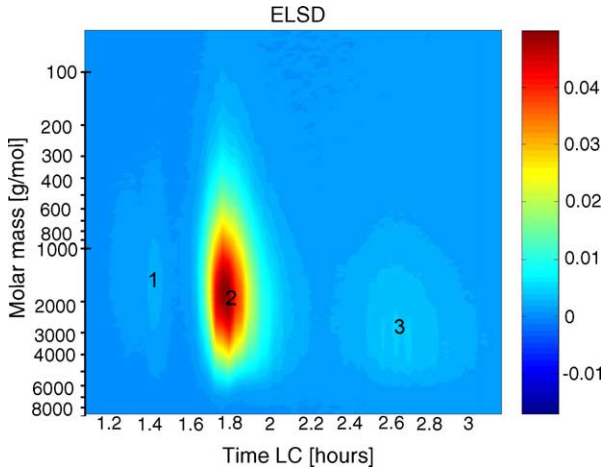

Fig. 7. LC × SEC chromatogram of sample VL37B detected by ELSD. The conditions were identical to those of Fig. 6.

ing ELSD, see ref. [23]). Using the second-dimension molar-mass calibration, the concentrations by weight can be converted into molar concentrations for individual peaks. As seen in Table 4, the molar concentration was larger than the weight concentration for non-functional polymer (and vice versa for the di-functional polymer), because the molar mass of the former was lower than that of the latter. It also can be seen from Tables 3 and 4 that the molar mass of the non-functional fraction was very similar for samples VL37A and VL37B. It was also similar for the mono-OH PMMA fraction. Therefore, we established a method to calculate the molar mass information (M_n, M_p and PDI) and the relative amount (mole or weight percentages) for any fraction or peak(s) in LC × SEC to obtain the MMD and FTD for functional polymers.

4. Conclusions

Comprehensive two-dimensional liquid chromatography (LC × SEC) was investigated to determine the mutually dependent molar-mass distributions and functionality-type distributions of functional poly(methyl methacrylate) polymers. Experimental results confirmed that LC × SEC may benefit from the use of longer columns and higher flow rates, to maintain sufficient separation efficiency in the second (fast-SEC) dimension. The complications of quantitative analysis for functional low-molar-mass PMMA polymers by LC × SEC was discussed for various detection techniques, including refractive-index, ultraviolet-absorbance (UV), and evaporative light-scattering detection. A simple method to establish ELSD calibration curves was presented. Each response (data point) from the ELSD chromatogram could be converted into the corresponding mass concentration, using calibration curves obtained by injecting each standard directly into the ELSD without a column. The height of the flat area (plateau) is related to the injected concentration.

Qualitative and quantitative information was obtained on real samples (VL37A and VL37B). This demonstrated the usefulness of LC × SEC in determining the MMD and FTD for functional polymers. The peak capacity was greatly enhanced by LC × SEC in comparison with one-dimensional separations and accurate molar-mass information (M_n, M_w, M_p and PDI) could be obtained for individual peaks or for combinations of peaks. Experimental results suggested that the original (uncorrected) ELSD chromatograms could be used to obtain the approximate molar-mass values (M_n and M_p), but that the resulting PDI and M_w were somewhat underestimated.

The influence of the molar mass on the retention behavior in LC was also investigated for hydroxyl-functional PMMA polymers using LC × SEC. The critical conditions – by definition – independent of molar mass were not exactly the same for PMMA series with different end-groups. Our observations are in close agreement with theoretical curves reported in the literature. However, for practical applications of LC × SEC it is not strictly necessary to work at the exact critical solvent composition. Near-critical conditions often suffice to determine the mutually dependent MMD and FTD of functional polymers.

Quantitative results obtained by LC × SEC are still subjected to some error, especially in case ELSD is required. This is the subject of ongoing research.

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