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Comprehensive two-dimensional liquid chromatography with on-line Fourier-transform-infrared-spectroscopy detection for the characterization of copolymers

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Abstract

The on-line coupling of comprehensive two-dimensional liquid chromatography (liquid chromatography × size-exclusion chromatography, LC × SEC) and infrared (IR) spectroscopy has been realized by means of an IR flow cell. The system has been assessed by the functional-group analysis of a series of styrene-methylacrylate (SMA) copolymers with varying styrene content. Ultraviolet (UV) detection was used as a detection technique to verify the detection with IR. The LC × SEC–IR functional-group contour plots (comprehensive chromatograms) obtained for styrene were in agreement with the contour plots constructed from the UV signal. In addition, contour plots can be obtained from non-UV-active groups. One such plot, for the carbonyl-stretching vibration of methylacrylate (MA), is shown. Selective detection of MA proved possible using flow cell IR detection. The combination of the contour plots for styrene and MA allowed a full characterization of the copolymer and it was revealed that the present series of SMA copolymers exhibited homogeneous chemical-composition distributions (CCDs). In addition, commercially available fast-SEC columns have been assessed in this study with respect to their potential to serve as second-dimension separation columns.

Keywords: Two-dimensional liquid chromatography; Size-exclusion chromatography; Solvent-elimination interfaces; Flow cell; Polymer analysis; Fourier-transform-infrared-spectroscopy; Copolymers

1. Introduction

The molecular structure of polymers is complex and they can show a dispersity in more than one property. Next to the molar-mass distribution (MMD) other distributions can be distinguished. For example, a chemical-composition distribution (CCD) and a functionality-type distribution (FTD) can also be present. In addition, a topological distribution which leads to a variation in polymer architecture (e.g. linear, branched, grafted) and a structural variation for copolymers can be discerned, leading to random, block or alternating copolymers [1,2]. All these distributions may affect the physical properties of the polymer and knowledge of these distributions is crucial for the synthetic polymer chemist, for product development, and for product control.

Size-exclusion chromatography (SEC) is a well-established chromatographic technique to characterize polymers. It separates polymers according to their hydrodynamic volume (which, for a homopolymer, can be related to the molar mass). When combined with the appropriate detectors (e.g. ultraviolet/visible, UV–vis, or infrared spectroscopy, IR), SEC can be used to obtain chemical-composition information as function of the molar mass. However, only the average composition at a certain molecular size is obtained. In addition to SEC, non-exclusion based chromatographic techniques can be used to characterize polymers. Gradient-elution liquid chromatography (LC) can be used to characterize chemical composition distributions. Furthermore, isocratic LC can be used to determine block-length distributions or the numbers and types of functional

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groups (FTD; [3]). In both cases, any effect of the polymer molar mass on the chromatographic retention should be avoided, as this would confound the CCD or FTD information. This is achieved under critical (isocratic) or pseudo-critical (gradient) conditions. At these conditions no molar-mass information can be obtained.

Obviously, a two-dimensional distribution cannot be determined using just one separation mechanism and a two-dimensional separation is required [4,5]. When combining two different separation mechanisms an increase in resolution and selectivity can be obtained. Systems separating into two completely independent directions are called orthogonal [6]. With such separation systems, fractions can be obtained that are homogeneous in terms of two parameters.

In practice, two-dimensional chromatography can be carried out in an off-line [7–13] or on-line manner [2,5,14–19]. A trend can be observed towards the on-line approach. Although the on-line approach requires a dedicated instrumental set-up and often home-written software, it is preferred to the off-line approach for a number of reasons. In principle an on-line coupling allows complete quantification from second-dimension data. Furthermore, the entire analysis takes places in a closed system and no contamination or oxidation can take place during fraction collection and pre-concentration, as is the case in the off-line approach. Finally, on-line analysis can be performed fully automated and unattended. Off-line fractionation has the advantage that beside injection into the second-dimension column other analytical techniques can be used for characterization, e.g. IR, or nuclear-magnetic-resonance (NMR) spectroscopy, (pyrolysis-) gas chromatography–mass spectrometry (GC–MS), or matrix-assisted laser-desorption ionization time-of-flight-MS (MALDI-TOF-MS). Also, after the fractions have been collected the polymers can be dried and redissolved in a suitable solvent for the second-dimension separation, thus avoiding eluent-miscibility problems.

For the full characterization of a polymer sample by on-line LC × SEC, an eight-port valve containing two sample-storage loops is often used [2,15–19]. Recently, it has been demonstrated that the best results are achieved when a 10-port valve is used in a symmetrical configuration (see Fig. 1) [5]. While one loop is being filled with the first-dimension effluent, the fraction that had previously been collected in the second loop is analyzed in the second-dimension separation. In a comprehensive set-up, the fraction collection time is equal to the analysis time in the second dimension. As a consequence, the second-dimension analysis time and the loop volume together determine the (maximum) flow rate for the first-dimension separation. When choosing an LC × SEC set-up, the SEC analysis time must be reduced to a minimum, while maintaining an adequate separation efficiency. In addition, the eluents used in the first- and second-dimension separations must be completely miscible.

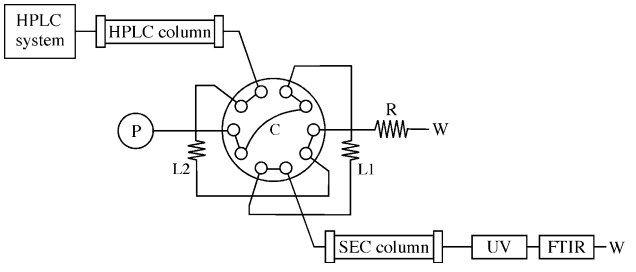

Fig. 1. Set-up for the LC × SEC-UV–FTIR system comprising a 10-port switching valve. *Abbreviations*: P, SEC-eluent pump; L1–L2 fraction-collection loops (200 μl each); C, connection tubing; R, restriction; UV, UV detector; FTIR, FTIR spectrometer and flow cell; W, waste.

In general, on-line detection in LC or SEC is accomplished by ultraviolet, refractive-index (RI), evaporative-light-scattering (ELS) or MS detection. All of these techniques have their limitations. For instance, UV detection can only be used when chromophores are present in the analyte and is therefore limited to UV-active polymers. RI detectors exhibit a low sensitivity, while an ELS detector yields a non-linear response. Both these latter types of detectors are universally applicable. They do not respond selectively to specific polymers. In addition, on-line LC–MS is limited to rather polar and relatively small polymers and mass-spectra can be extremely complex. In contrast, infrared (IR) spectroscopy is better suited for the selective detection of (either UV-active or non-UV-active) functional groups. IR detection has already been applied in the characterization of polymers during many years [20–26] and it has proven to be a powerful tool. Two types of interfacing can be distinguished, viz. coupling via a solvent-elimination interface or coupling via a flow cell. In the first (off-line) approach the eluent is removed prior to IR detection, while in the second case spectra are acquired during the elution in an on-line manner. The advantages and disadvantages of each technique have been described extensively and the reader is referred to [26–29] for an in-depth discussion on this topic. The coupling of comprehensive two-dimensional LC and IR can reveal information about the polymer that cannot be obtained when either one of the selected LC modes is coupled separately to IR detection.

Recently, IR detection was used in the analysis of a grafting product of butylacrylate onto poly(styrene-*block*-butadiene) by coupling of critical chromatography (CC) and SEC (CC–SEC) via a commercial solvent-elimination interface [15]. The authors showed IR spectra at several elution times, which can be used for identification purposes. Furthermore, plots of absorption-band intensities were constructed to reveal distribution inhomogeneities. However, the authors mentioned that the inherent spray characteristics of the interface could result in overlapping depositions of chromatographic peaks that are only marginally separated. The circular deposition substrate used in this study offers only a confined space for the deposition of a large number of second-dimension fractions. Therefore, solvent-elimination and sample deposition is restricted to a limited number of individually discernable second-dimension fractions, unless large (expensive) rectangular substrates are used. When an IR flow cell is used, the entire chromatogram can be recorded and the total analysis time is not restricted by the required spatial resolution on a particular substrate and by the size of the substrate.

In this work we therefore chose to investigate the use of an IR flow cell in combination with comprehensive two-dimensional LC. Hereto, a series of styrene-methylacrylate copolymers with varying fractions of styrene were selected as model compounds. The chemical compositions and molar masses of the model

compounds were determined with gradient-elution LC as the first dimension and SEC as the second dimension (LC × SEC). During the LC × SEC optimization process, two commercially available fast SEC columns were assessed with respect to their potential to serve as second-dimension separation columns. The performance of the IR flow cell was verified by the construction of functional-group chromatograms (contour plots). Such a plot, that was specific for styrene, was compared to the signal obtained by UV detection.

2. Experimental

2.1. Chemicals

Mobile phases consisted of methanol (MeOH, Biosolve, Valkenswaard, The Netherlands), unstabilized tetrahydrofuran (THF, Biosolve), chloroform (Biosolve), dichloromethane (DCM, J.T. Baker, Deventer, The Netherlands) and *n*-heptane (Fluka, Buchs, Switzerland). All mobile phases used were of HPLC grade or better. In the experiments for the assessment of SEC columns, narrow polystyrene standards with different peak molar masses (M_p) were used, which were purchased from Polymer Standards Service (Mainz, Germany) in the form of a ReadyCal mixture. The mixture was diluted to 2 mg/ml in THF. In the LC × SEC experiments, narrow polystyrene standards from Polymer Laboratories (Church Stretton, Shropshire, UK) were used to construct a relative calibration curve. Polymer stock solutions were prepared by weighing the polymer and dissolution in DCM (20 mg/ml). Combining these solutions resulted in a concentration of 5 mg/ml for each standard. Styrene-*co*-methylacrylate (SMA) copolymers were prepared at the Technische Universiteit Eindhoven (Eindhoven, The Netherlands) and a solution was prepared in DCM (ca. 75 mg/ml). Molar-mass data given in this study are expressed as PS equivalents. All standard solutions were stored in the dark at 6 °C.

2.2. Chromatography

The LC system consisted of a Waters 2695 Separations Module (Milford, MA, USA), equipped with a vacuum degasser and a thermostatted column compartment. The sample volumes injected were between 10 and 100 μl and all standards and samples were analyzed in duplicate. UV detection was performed with a Waters photodiode-array (PDA) detector model 996. A computer using Waters Millenium32 (version 3.2) software controlled the system and was used to record the UV detector signal. This system served as first-dimension LC system operating in the gradient mode. Isocratic second-dimension eluent was delivered by a Dionex model P580 pump (Germering, Germany), operating at 0.8 ml/min and preceded by a vacuum degasser (Alltech, Deerfield, IL, USA). Chloroform was selected as SEC eluent. It exhibits adequate IR-transparency windows in regions of functional-group vibrations of the two co-monomers of interest. A schematic of the experimental set-up is shown in Fig. 1. First-dimension separations were carried out on a 150 mm × 3.9 mm I.D. Waters NovaPak silica column (particle diameter 4 μm, specified pore diameter 60 Å). During the gradient-optimization process, the flow rate was 1.0 ml/min (linear gradient: 90–10% *n*-heptane–DCM to 93–7% DCM–MeOH in 17.5 min). In the two-dimensional set-up, the flow rate was reduced by a factor 25 to 40 μl/min and the gradient slope was reduced correspondingly, resulting in a total gradient time of 437 min. This in order to obtain a comprehensive LC × SEC set-up. The gradient was started upon injection of the sample.

Three SEC columns, with total analysis times of 5 min or less were selected for potential use in the second dimension (for column details, see Table 1). Experiments were performed using the Waters LC system with THF as eluent (see Table 1 for flow rates). In all experiments, the LC and SEC columns were thermostatted at 35 °C.

Comprehensive coupling of LC and SEC was achieved by means of a helium-actuated VICI two-position 10-port valve (Valco, Schenkon, Switzerland) with a port diameter of 0.25 mm. A high-speed switching accessory (Valco) was used to obtain a valve-switching time of 8 ms in order to minimize pressure build-up for the first-dimension column and to reduce pressure pulses in the second-dimension column. The 10-port valve was switched every 5 min by means of an electronic pulse from the LC system. Two injection loops of 200 μl each were connected to the 10-port valve. This resulted in four or five fractions per first-dimension peak, which was considered acceptable. We have recently found that the valve configuration as shown in Fig. 1 gives best results and yields constant retention times for the second-dimension separation. For a detailed discussion on this topic, the reader is referred to [5]. An in-house 2D-LC program written in a Matlab (Natick, MA, USA) environment was used for the calculation of two-dimensional contour plots.

2.3. Spectroscopy

The FTIR system consisted of a Perkin-Elmer (Norwalk, CT, USA) spectrometer model Spectrum GX, equipped with

Table 1
Characteristics of fast SEC columns selected for this study

Packing, particle diameter (μm)	Dimensions (mm) (L × I.D.)	Molar mass range (g/mol)	Flow rate (μl/min)	Mobile phase velocity (mm/min)	Supplier
HighSpeed SDV linear S, 3	50 × 20	100–150,000	6000	47.7	Polymer Standards Service
HSPgel-RT MB-L/M, 3	150 × 6.0	500–400,000	800	70.7	Waters
PLgel MiniMix-D, 5	50 × 4.6	200–400,000	300	45.1	Polymer Laboratories

The Waters HSPgel column was used in LC × SEC experiments. The mobile phase velocity (v) was calculated as: $v = F/\pi r_c^2 \varepsilon$, where F is the volumetric flow rate (μl/min), r_c the column radius (mm) and ε the interparticle porosity (typical value, 0.4) [30].

a medium-band mercury/cadmium/telluride (MCT) detector. The FTIR sample and detector compartments were continuously purged with nitrogen, which was dried using a Zander Adsorbtion Dryer, Type KM5 TE (Essen, Germany) to minimize the interference from carbon dioxide and water vapor present in the atmosphere. To ensure a stable and constant background, the sample and detector compartments were purged for 60 min before data acquisition. Single-beam spectra (range, 3300–1300 cm^{-1}), consisting of eight scans at a scan resolution of 8 cm^{-1}, were acquired continuously using Perkin-Elmer Spectrum TimeBase 1.1. This resulted in a data interval of 4.69 s and 64 spectra per second-dimension SEC run of 5 min. Data acquisition was started by means of an electronic pulse from the LC system 124 min after injection of the sample. Online IR detection was accomplished using a high-pressure flow cell (Reflex Analytical, Ridgewood, NJ, USA), which was connected in series after the UV detector. Two calcium-fluoride cell windows of 13 mm diameter (2 mm thickness) were used (transmission range, 50,000–1100 cm^{-1}), which were separated by a circular 0.39 mm thick Teflon spacer (8 mm clear aperture), resulting in a cell volume of 19.6 μl. The inlet and outlet connections of the flow cell were adapted by the manufacturer allowing to connect 1/16 in. × 0.010 in. I.D. tubing at the flow cell inlet and 1/16 in. × 0.020 in. I.D. tubing at the flow cell outlet.

The acquisition of single-beam spectra instead of absorption spectra has the advantage that a single suitable reference spectrum can be selected for the calculation of absorption spectra for the complete IR chromatogram. In the present case, a single-beam spectrum at 126.99 min was used, which was free of first-dimension eluent interferences. Subsequently, IR absorption spectra were calculated using a Matlab routine. The resulting absorption spectra between 125 and 300 min were used for the construction of two-dimensional functional-group contour plots using the 2D-LC program mentioned earlier. The integrated absorption within a selected IR window was used to construct the contour plots. For methylacrylate, the integrated absorption of the carbonyl-stretch vibration was used (1748–1708 cm^{-1}), while for styrene the integrated absorption of the aromatic-ring C–C stretching vibration was selected (1510–1482 cm^{-1}).

An increase in retention time (by ca. 0.7 min at a flow rate of 40 μl/min) was observed for the IR-chromatograms, due to the extra system volume between the UV detector and the flow cell interface.

3. Results and discussion

3.1. Selection of size-exclusion column

The second-dimension separation in LC × SEC needs to be completed in a short time, while providing sufficient chromatographic resolution for the application of choice. Conventional SEC columns show a typical analysis time of 10–15 min at conventional flow rates. Preferably, such columns are not used to realize a second-dimension separation. Therefore, two commercially available SEC columns, especially developed to achieve an analysis time shorter than 5 min were evaluated (fast-SEC columns). Additionally, a miniaturized (small-bore) SEC column containing conventional packing material was assessed. Such a column is normally used as a pre-column. All SEC columns were selected for a wide molar-mass range in order to be generically applicable in future experiments.

All the columns were operated at the (maximum) flow rate recommended by the manufacturer, keeping the second-dimension separation time within 5 min. Details on column dimensions, packing materials and flow rates for the three columns are given in Table 1.

For the assessment of the columns the chromatographic resolution was determined by analyzing a mixture containing four PS standards with UV detection. As can be seen from Fig. 2C, simply shortening the column length severely reduces the separation efficiency. Consequently, reliable molar-mass data cannot be obtained with such a column. It should be mentioned that the Polymer-Laboratories column dimensions and packing materials were not optimized for use in fast SEC. Better separation efficiencies were obtained when fast-SEC columns were used. All PS standards could be distinguished when a PSS high-speed column was used. The high-molar-mass PS standards (i.e. 2,950,000 and 465,000 g/mol) were not baseline separated, as the molar-mass separation range of this column was from 100 to 150,000 g/mol. Furthermore, the high flow rate required to operate this column places demands on the LC pump. We noticed an increased pump-seal wear after seven days of operation at 6 ml/min. Additionally, the high flow rate results in high eluent costs. Best results in terms of chromatographic resolution were obtained when the Waters HSPgel column was used and baseline separation for all PS standards was obtained. Although this column is relatively long, the better separation efficiency is, at least partly, due to the small particle diameter and not only due to the longer column length. Actually, a shorter column would have been preferred, but this was not commercially available. The high separation efficiency combined with a 5 min run time made us select this column for the LC × SEC experiments reported in this study.

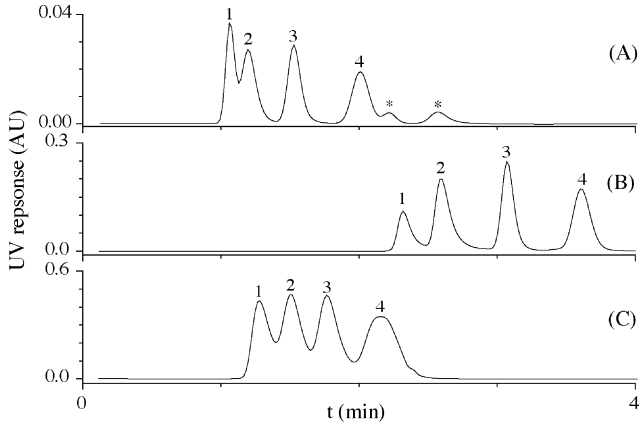

Fig. 2. SEC-UV chromatograms obtained from the analysis of a polystyrene standard mixture (c = 2 mg/ml; injection volume, 10 μl). The following SEC columns were used: (A) Polymer Standards Service High Speed; (B) Waters HSPgel; (C) Polymer Laboratories MiniMix-D. For additional column details and conditions, see Table 1. Peak molar masses: (1) 659,000 g/mol, (2) 67,500 g/mol, (3) 9130 g/mol and (4) 374 g/mol. Peaks marked with an asterisk are not identified. UV detection was performed at 261 nm.

3.2. Comprehensive LC × SEC-UV–IR of SMA copolymers

The SMA copolymers are preferably separated by normal-phase LC (NPLC) [31,32], because the introduction of large amounts of reversed-phase eluents in the IR flow cell may complicate IR detection. The SMA copolymers were dissolved in DCM. This is a solvent for both PS and PMA, but a weak eluent on silica ($\varepsilon^{\circ}_{SiO_2} = 0.32$). Thus, it prevents breakthrough of the injected sample [33]. A non-solvent (*n*-heptane) was selected as the starting (weak) eluent. By adding an increasing amount of methanol ($\varepsilon^{\circ}_{SiO_2} = 0.73$) to DCM in the gradient step, the elution strength was gradually increased and a separation according to chemical composition was accomplished.

The maximum first-dimension flow rate ($^1F_{max}$) for genuinely comprehensive two-dimensional LC is defined by the injection volume in the second dimension ($^2V_{inj}$) and by the maximum (full permeation) second-dimension analysis time ($^2t_{R,max}$) according to Eq. (1).

$$^1F_{max} \leq \frac{^2V_{inj}}{^2t_{R,max}} \quad (1)$$

A low flow rate for the first-dimension column is a prerequisite to fulfill Eq. (1). This can be accomplished by the use of a small-bore or microbore column [5]. Alternatively, a conventional sized column can be used and an effluent splitter can be incorporated between the first-dimension column and the switching valve. However, a varying split ratio (caused by a varying viscosity of the first-dimension effluent) is a potential obstacle and a large part of the sample is discarded to waste. Therefore, we have chosen an alternative set-up, employing an analytical sized (i.e. 3.9 mm I.D.) column operating at an unconventionally low flow rate of 40 μl/min. The large diameter permits a high sample load, favoring the sensitivity of the method. For high-molar-mass polymers the optimal flow rate in the van-Deemter (*H* versus *u*) curve is very low, because molecular diffusion decreases significantly with increasing molar mass (e.g. for polystyrene in THF D_m ($mm^2\,s^{-1}$) = $0.0386M^{-0.549}$ [34]). Also, the use of low flow rates reduces the risk of column blocking upon the injection of concentrated polymer solutions, because the redissolution of the precipitated polymer is a time-dependent process. Proper functioning of the first-dimension column at the selected low flow rate was verified in practice and we still obtained baseline separation for all copolymers at 40 μl/min.

Because the analytes experience an additional dilution in the second-dimension separation, a high sample load is desirable in the first dimension. In the current experiment $^2V_{inj}$ = 200 μl and $^2t_{R,max}$ = 5 min, which is in agreement with Eq. (1). It can be foreseen that reducing the column length of the second-dimension SEC column by a factor of two will still lead to an acceptable resolution. In that case, even smaller fraction volumes ($^2V_{inj}$) can be used at the same first-dimension flow rate, increasing the overall resolution obtained by LC × SEC, or equal fraction volumes can be used at a higher first-dimension flow rate, reducing the total analysis time to approximately 2.5 h.

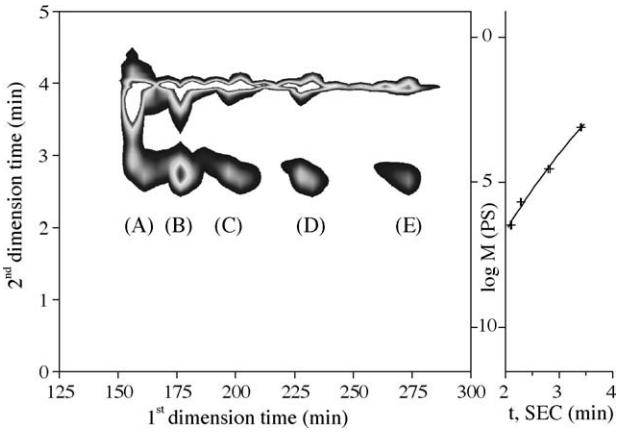

Fig. 3. LC × SEC-UV contour plot of a mixture containing SMA copolymers with: (A) 90%; (B) 80%; (C) 60%; (D) 40% and (E) 20% styrene. UV detection was performed at 261 nm. A PS calibration curve is indicated on the right and was constructed from narrow PS standards with peak molar masses of 2,950,000, 465,000, 34,500 and 1250 g/mol.

For molar-mass calibration of the second-dimension SEC column, the first-dimension eluent was changed to 100% DCM (isocratic) and a series of PS standards was analyzed by UV and FTIR detection. All other parameters (i.e. valve switching time, eluent flow rates) were held constant. In this way, the first-dimension column only served as a diluter and all PS standards eluted at the column hold-up time. Subsequently, 200 μl injections were made into the second-dimension column. Afterwards, calibration curves were constructed from the UV trace at 261 nm (inserted in Fig. 3, right-hand side) and from the IR trace (C=C stretching vibrations at 1491 cm^{-1}; calibration curve not shown).

The LC × SEC-UV chromatogram (contour plot) obtained for the SMA copolymers is shown in Fig. 3. It can be seen that all SMA copolymers used in this study are of a similar molar mass. A one-dimensional separation according to hydrodynamic volume, would not have revealed any compositional information. UV detection allows only the styrene part of the copolymer to be studied. Employing an IR flow cell allowed the selective detection of both co-monomers in the SMA copolymer samples and the contour plot of MA obtained by IR demonstrates the usefulness of IR for non-UV absorbing polymers.

The IR contour plots for the carbonyl-stretching vibration (characteristic for MA) and the C=C stretching vibration (characteristic for styrene) are given in Figs. 4 and 5, respectively. The contour plot characteristic for styrene is in agreement with the UV contour plot (compare Figs. 3 and 5). The two functional-group contour plots clearly reveal information on the chemical composition (*x*-axis) and the molar masses (*y*-axis).

The copolymer homogeneity was determined from the ratio of the functional-group contour plots for styrene and methylacrylate (cf. Figs. 4 and 5). The ratios have been calculated for a limited molar-mass range from the SEC fractions corresponding to the elution region of the SMA copolymers. The results are shown in Fig. 6. The fact that the ratios approach zero at the side of the polymer elution window in Fig. 6 is caused by a very small numerator. No evidence was found for chemical

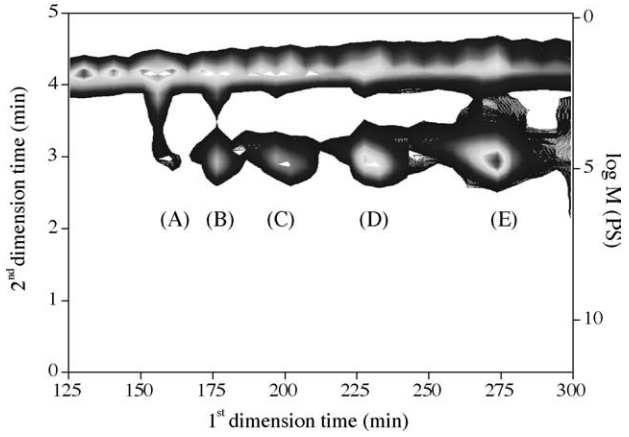

Fig. 4. LC × SEC–IR functional-group contour plot for the carbonyl-stretching vibration of a mixture of SMA copolymers. Styrene content: (A) 90%; (B) 80%; (C) 60%; (D) 40% and (E) 20%. The response was constructed from the integrated absorption from 1708 to 1748 cm^{-1}.

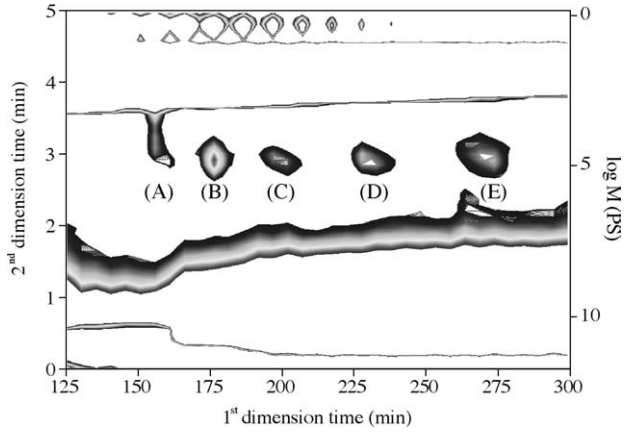

Fig. 5. LC × SEC–IR functional-group contour plot for the aromatic-ring C—C stretching vibration of a mixture of SMA copolymers. Styrene content: (A) 90%; (B) 80%; (C) 60%; (D) 40% and (E) 20%. The response was constructed from the integrated absorption from 1510 to 1482 cm^{-1}.

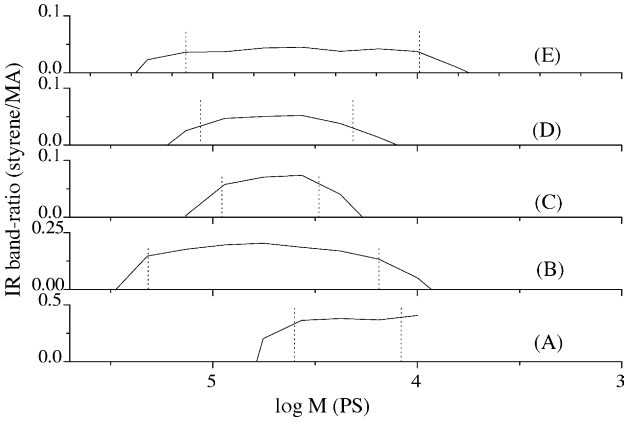

Fig. 6. IR absorption-band ratios for SMA copolymers determined from the functional-group contour plots in Figs. 4 and 5. The dotted lines mark a constant ratio within the elution window of the respective SMA copolymer. Styrene content: (A) 90%; (B) 80%; (C) 60%; (D) 40% and (E) 20%.

inhomogeneities in the comprehensive two-dimensional distributions (chemical-composition distribution versus molar-mass distributions, or CCD × MMD) of the copolymers.

All the contour plots shown here, exhibit broad horizontal bands at approximately 4 min SEC elution time. These bands represent the injection-solvent peaks (indicating the total permeation volume or $^2t_{R,max}$), arising from the successive injections of first-dimension eluent. A decreasing response with increasing gradient time is observed. As the injection volume is 200 μl, the signals obtained for the injection-solvent peaks can be very intense compared with the analyte response. A sample amount of about 750 μg per SMA copolymer is injected into the LC column. This amount is diluted upon elution in the first-dimension column and experiences a further dilution in the second-dimension separation step. For example, 750 μg of a SMA copolymer elutes from the LC column in a 25 min retention window, which is equal to five second-dimension fractions. Assuming a Gaussian peak profile, the amount of SMA in the fraction taken around the apex is 350 μg. The resulting peak width from the second-dimension SEC-column is about one-sixth of the elution volume (i.e. 50 s). At the current sampling rate, approximately 11 spectra can be acquired and the central fraction of a Gaussian peak represents 84 μg of sample. The signal-to-noise obtained at the apex of the SEC-elution peak for SMA (for example 80% styrene) was 5 and 18 at 1510–1482 cm^{-1} and 1748–1708 cm^{-1}, respectively. This yields respective limits of detection (LOD) of 50 and 14 μg for the styrene and MA absorption bands in the IR spectrum taken at the chromatographic peak top. However, in this simple calculation we have assumed a Gaussian-profile for the eluted analytes in both dimensions.

4. Conclusions

Comprehensive coupling of LC and SEC with on-line spectroscopic IR detection via a flow cell offers the possibility for selective detection of functional groups for a variety of complex polymers. This was successfully demonstrated by the analysis of a series of styrene-methylacrylate (SMA) copolymers with varying styrene content. UV detection was used as reference to verify the analysis with IR detection. The LC × SEC–IR functional-group contour plot obtained for methylacrylate proved that selective detection was possible using a flow cell and IR detection. The use of a commercially available fast-SEC column in the second dimension was possible with respect to flow rate, chromatographic resolution and flow cell compatibility.

Application of the currently described LC × SEC-UV–IR system is not limited to SMA copolymers. Recently, we have demonstrated the usefulness of an IR flow cell for the analysis of a blend consisting of aromatic and aliphatic polyesters and of a polyester/polydimethylsiloxane copolymer [26]. Provided that an eluent can be selected that exhibits an IR-transparent window in the region where functional-group vibrations of interest are present, the application area of comprehensive two-dimensional chromatography can be extended to include many classes of non-UV-active (co)polymers when an IR flow cell is used. Using LC × SEC and IR spectroscopy, it is now possible to selec-

tively detect differences in functional groups between polymer samples. This can be of high value in the determination of the changes in chemical composition due to aging of polymers [35].

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