A novel software tool for copolymer characterization by coupling of liquid chromatography with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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The results of copolymer characterization by coupling of chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) techniques and subsequent calculation of copolymer composition using a novel software tool ‘MassChrom2D’ are presented. For high-resolution mass analysis copolymer samples were fractionated by means of liquid adsorption chromatography (LAC). These fractions were investigated off-line by MALDI-TOF MS. Various mono-n-butyl ethers of polyethylene oxide-polypropylene oxide copolymers (PEO-co-PPO) were investigated. As well as the copolymer composition presented in two-dimensional plots, the applied approach can give additional hints on specific structure-dependent separation conditions in chromatography. Copyright © 2007 John Wiley & Sons, Ltd.
standards for calibrating the SEC. For this reason a completely different approach was recently presented. The chromatographic separation in LACCC was shifted to LAC (near ‘critical’, LACnearCC) mode. This resulted in a broadening of peaks due to increased interactions of polymer molecules with the stationary phase. However, these separation conditions have to be very carefully adjusted, because the risk of precipitation of molecules always exists. Simultaneously, the second chromatographic dimension (SEC) was replaced by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry mass spectrometry (MALDI-TOF MS). For MALDI coupling, fractions taken from LACnearCC were transferred by means of a spray device onto the MALDI sample target.

Using the new MassChrom2D software tool the copolymer composition of each fraction can be calculated from mass spectra. This software was developed to replace the inconvenient and time-consuming calculation procedure previously performed in Microsoft Excel. The practicability of the chosen mathematical procedure for the analysis of copolyesters was presented recently. The procedure is based on the assignment of observed masses by MALDI-TOF MS to calculated masses in a matrix formed by both monomer units, normalization and subsequent correction by chromatographic intensity information. Depending on the peak resolution in MALDI-TOF MS, which can be significantly improved by a previous chromatographic separation, copolymers with masses up to \( m/z \) 11 000 could be investigated. Due to inhomogeneities in the solid sample/matrix mixture as well as structure-dependent ionization probabilities in MALDI-TOF MS, the signal intensity information provided by MALDI-TOF spectra cannot be used (see Fig. 2). Therefore, after baseline subtraction, each MALDI spectrum was normalized in a second step to an absolute intensity of 100%. For the construction of 2D plots

**Figure 1.** Elution of PEO and PPO standards at ‘critical’ conditions of the second unit (a: RP system, C18 phase, THF/H2O solvent mixture; b: NP system, NH2 phase, THF/methanol/n-hexane solvent mixture) and resulting calibration curves (c, d), used for the determination of molar masses and mass distributions of PEO-co-PPO polymers.

**Figure 2.** Evaporative light scattering detector (ELSD) signal of a LACnearCC chromatogram of a PEO-co-PPO polymer and total ion current (TIC) of a series of 667 MALDI mass spectra (each sum of 200 shots) of the copolymer after spray deposition onto the MALDI target.
intensity information was exclusively gained from chromatography. Therefore, this principle cannot be directly compared with other 2D approaches of copolymer characterization.  

Various mono-n-butyl ethers of polyethylene oxide-polypropylene oxide copolymers (PEO-co-PPO) were investigated. This class of copolymers is widely used for lubricants, emulsifiers and surfactants. Due to the poor mass resolution of the method in the lower mass region these seemed to be ideal substances to be checked by the new approach.

EXPERIMENTAL

‘MassChrom2D’ software
The graphic user interface of the program is shown in Fig. 3. For an accurate calculation of copolymer composition molar masses of copolymer units as well as end groups and added

Figure 3. Graphic user interface of the ‘MassChrom2D’ software.

Figure 4. MALDI-TOF mass spectrum of a PEO-co-PPO polymer before separation and fractionation in gradient chromatography.

Figure 5. Gradient chromatography of a PEO-co-PPO polymer (arrows indicate retention times where MALDI-TOF mass spectra were recorded; dashed line corresponds to the amount of isopropanol).
 salts for cationization are essential. Finally, the chromatogram and single MALDI-TOF mass spectra of all fractions have to be imported in ASCII format. After assignment of the start and end points of the fractionation, depending on the number of spectra, the average intensity shown in marked slices is used for the calculation of the intensity of single 2D plots. The final 2D plot is composed of the sum of single 2D plots.

**Liquid adsorption chromatography (LAC)**

A Hewlett Packard HP 1090 liquid chromatograph (Palo Alto, CA, USA) was used for chromatographic separation.
For detection an evaporative light scattering detector (ELSD) SEDEX 45 (Sedere, Alfortville, France) was employed. The chromatographic system was kept at a constant temperature of 45 °C. The eluent flow was 0.5 mL/min. The injected volume was 20 μL. The sample concentration of PEO-co-PPO mono-n-butyl ether polymers was 2–3 mg/mL.

In isocratic mode two YMC RP 18 columns (300 Å with 5 μm pore size, 250 + 125 mm length and 4.6 mm i.d.) were used for separation. The solvent composition of 85:15 v/v tetrahydrofuran (THF)/water, which represents the ‘critical’ mode for the separation of PPO, was changed to 65:35 v/v ratio.

In gradient mode ‘critical’ conditions of separation of PEO (84:16 v/v methanol/water) were initially adjusted. These conditions were shifted into LAC mode by adding a third, thermodynamically poor solvent (isopropanol).

MALDI-TOF MS

A REFLEX III MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen, Germany) operating in reflectron mode at 20 kV was used. Matrix and polymers were sprayed separately on the MALDI target by means of a LC 500 spray interface (LabConnections, Carrboro, NC, USA). trans-2-(3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene)malononitrile (DCTB) has been shown to be a suitable matrix for PEO and PPO. Therefore, for matrix preparation a 3 mg/mL solution of DCTB (Fluka, Seelze, Germany) and 1 mmol/mL sodium trifluoroacetate (Merck, Darmstadt, Germany) in THF (Sigma-Aldrich, Buchs, Switzerland) was sprayed at a temperature of 120 °C at a flow rate of 1 mL/min, simultaneously applying a 20 L/min N2 stream. After the MALDI target had been pre-coated with matrix the chromatographic run was deposited onto the target. Due to the use of water in the eluent mixtures, polymer samples were sprayed at higher temperatures (180–220 °C). The N2 flow rate was again 20 L/min.

Tandem mass spectrometric (MS/MS) fragmentation experiments were performed by means of an Autoflex III TOF/TOF mass spectrometer (Bruker Daltonik) equipped with a gridless delayed extraction ion source, precursor ion selector, collision cell, cell for product ion post-acceleration (so-called LIFT cell), and gridless ion reflector. A frequency-tripled modulated, diode pumped Nd:YAG laser (SmartBeam; Bruker) working at 355 nm was used for the MALDI process.13 Ions were accelerated in a high-voltage field of 6 kV from the ion source. Fragmentation was carried out using argon as collision gas. The pressure in the collision cell was adjusted to 5 × 10⁻⁸ mbar. Formed product ions were accelerated at 19 kV in the LIFT cell.

Figure 7. MALDI-TOF mass spectrum of a PEO-co-PPO polymer before separation and fractionation in isocratic mode.

Figure 8. Chromatography (isocratic) of a PEO-co-PPO polymer (arrows indicate retention times where MALDI-TOF mass spectra were recorded.)
RESULTS

The MALDI-TOF mass spectrum of a non-fractionated PEO-PPO copolymer is shown in Fig. 4. The enlargement of the mass spectrum clearly illustrates one major problem of copolymer characterization using MALDI-TOF MS. The spectrum is characterized by a series with typical peak-to-peak distances of 44 (EO units) and 58 (PO units) m/z units. The overlapping of these peak series inevitably causes peak-to-peak distances of 2 m/z units. The resulting peak clusters consist of multiple distributions of carbon isotopes overlaying each other. These peaks can only be separated from each other by means of chromatography. A separation at ‘critical’ conditions would lead to narrow peaks eluting from the column. Therefore, conditions for a separation at ‘critical’ conditions of adsorption were changed into near ‘critical’ conditions either by an isocratic alteration of the solvent composition or by applying a gradient mode. Due to increasing interactions of dissolved molecules and the stationary phase much broader chromatograms were observed. These chromatograms offer better resolution and could be used for a subsequent fractionation by means of the spray device. Figure 5 shows the chromatogram of a lower mass PEO-co-PPO mono-n-butyl ether polymer after

Figure 9. MALDI-TOF mass spectra (left) and corresponding 2D composition plots (right) of fractions 8, 10 and 12 recorded after separation in isocratic chromatography.

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gradient chromatography starting at ‘critical’ separation conditions for PEO. The chromatographic run between 8 to 13 min was sprayed onto the MALDI-TOF target and 14 MALDI mass spectra were recorded continuously. By means of three selected mass spectra, attributed to fractions 5, 7 and 9, very good peak resolution after fractionation can be demonstrated (see Fig. 6). In fractions 5 and 7 only two mass distributions, both representing series with peak-to-peak differences of 44 m/z units, can be seen. In fraction 9 a third distribution with a maximum at m/z 1100 is visible but even in this fraction the carbon isotope patterns of different series are clearly separated from each other. This enabled a clear assignment of masses to copolymer compositions by means of the ‘MassChrom2D’ software. The resulting 2D plots of fractions 5, 7 and 9 are also shown in Fig. 6. These plots indicate a shift of the copolymer composition during fractionation that seems to be only little affected by the number of EO units, whereas the number of PO units simultaneously increases from 3 and 4 in fraction 5 to 5 and 6 in fraction 9. They clearly reveal that PEO units still elute in a ‘critical’ mode of adsorption, while PPO elutes in a typical LAC mode.

A completely different behaviour can be found for a PEO-PPO copolymer with an average molar mass of 2100. The mass resolution, shown in the MALDI-TOF mass spectrum of the original sample (Fig. 7), is very poor but, after fractionation in LAC mode using an isocratic solvent mixture (Fig. 8), the MALDI-TOF mass spectra of fractions 5, 7 and 9 (see Fig. 9) exhibit well-resolved peaks again. However, the progression of 2D plots, also shown in Fig. 9, reveals an astonishing separation mechanism in the chromatographic system. While the number of EO units decreases with increasing retention time, the number of PO units increases. This indicates a parallel separation of PEO in SEC mode and PPO in LAC mode.

The sum 2D plots of both polymers are shown in Fig. 10. Each sum plot consists of 14 single fractions plots, which were corrected for chromatographic intensities at the corresponding retention times. A comparison clearly indicates differences due to different separation modes in chromatography. Nevertheless, both 2D plots reflect the overall copolymer composition, which is independent of the chromatographic separation.

Due to the generally lower mass accuracy of MALDI-TOF MS compared with that of other MS methods (e.g. electrospray ionization TOF), some ions in the spectrum (Fig. 10(b)) could not be unequivocally attributed to certain copolymer compositions. An ion at m/z 2216.5 can be theoretically attributed to a copolymer either with EO₂PO₃₅ (m/z 2216.59) or EO₃PO₁₃ (m/z 2216.41). For this reason some additional spots in the 2D plot representing regions of so-called isobars were obtained. A differentiation between these isobaric structures was performed by MS/MS fragmentation analysis. The corresponding MALDI-MS/MS mass spectrum for a precursor ion at m/z 2216.41, seen in Fig. 11, shows a fragmentation pattern typical for block copolymers. Chain fragments consisting of more than 15 EO and 12 PO units could be determined. This result can only be explained by a fragmentation of a block-like EO₁₃PO₁₃ copolymer and excludes the formation of EO₂PO₃₅.

A mapping of isobars within the 2D composition plots can only be avoided by better mass resolution combined with excellent calibration of the mass spectrometer.

CONCLUSIONS

The composition of various PEO-co-PPO mono-butyl ether polymers was determined using coupled chromatographic and mass spectrometric techniques. The need for a previous chromatographic separation, which is essential for high resolution in mass spectrometry, has been shown. Using the ‘MassChrom2D’ software the copolymer composition can be visualized in 2D plots, which can be used as fast fingerprint identification. The software allows us to detect changes in molar mass and mass distribution as a result of changing manufacturing conditions or aging processes. A general change of the copolymer composition can be easily monitored. In addition, the character of the separation of each copolymer unit in various chromatographic systems can be easily detected and evaluated. This would allow adapting the conditions of copolymer separation to the most useful chromatographic parameters.
Figure 11. MALDI-TOF MS/MS spectrum recorded for a precursor ion at m/z 2216.4 of a PEO-co-PPO polymer after chromatographic separation (top) and sections showing EO (middle) and PO distributions (bottom).
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REFERENCES