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Journal of Chromatography A, 699 (1995) 21–29

JOURNAL OF
CHROMATOGRAPHY A

Use of matrix-assisted laser desorption/ionization mass spectrometry for molar mass-sensitive detection in liquid chromatography of polymers

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First received 22 November 1994; revised manuscript received 13 January 1995; accepted 13 January 1995

Abstract

Matrix-assisted laser desorption/ionization mass spectrometric (MALDI-MS) detection is a powerful alternative to conventional refractive index or ultraviolet detection in the liquid chromatography of oligomers and polymers. As a molar mass-sensitive detector, MALDI-MS is capable of providing information on the molar mass and the chemical structure of fractions from liquid chromatographic separations. Although it is currently used only in the off-line mode, MALDI-MS yields fast and reliable results, consuming minimum amounts of a few nanograms. For a number of applications it is shown that MALDI-MS experiments can be carried out with fractions taken directly from the eluate after chromatographic separations on an analytical scale.

1. Introduction

Most synthetic polymers are not only heterogeneous in molar mass, but also in chemical composition or functionality. For example, copolymers exhibit a chemical composition distribution (CCD) in addition to the molar mass distribution (MMD). Telechelics or macromonomers exhibit an MMD and a functionality-type distribution (FTD). The different heterogeneities can be superimposed on one another, resulting in a very complex structure of heterogeneous polymers. The development of sophisticated methods for the analysis of heterogeneous polymers is a never-ending story. Size-exclusion chromatography (SEC) is the standard technique for MMD determination of polymers, but strictly it is only applicable to homopolymers. In the case of copolymers or telechelics, the separation according to molar mass is disturbed by the effects of chemical composition or functionality, respectively. On the other hand, when copolymers are separated with respect to chemical composition by HPLC methods, the effect of the MMD must not be neglected.

Although many types of detectors may be used to measure concentrations of polymers in solution after a chromatographic separation, differential refractometers and UV spectrophotometers are the two main types of detectors employed in both SEC and HPLC of polymers [1,2]. The differential refractometer is applicable for virtually all polymers, but for heterogeneous polymers, such as copolymers, the total refractive index change is a function of concentration

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SSDI 0021-9673(95)00061-5

and composition of the sample. UV spectrophotometers are the most widely used detectors in HPLC and are rapidly gaining favour over the differential refractometer traditionally used in SEC. The UV detector, however, is not universal because the polymer must contain chromophoric groups. Like the refractometer, for heterogeneous polymers the detector response may be influenced by the chemical composition, sequence length and conformation of the sample [3].

The differential refractometer and the UV detector in the ideal case monitor the sample concentration during a chromatographic run but do not yield information on molar mass or chemical composition of the sample. Information on molar mass may be obtained using molar mass-sensitive detectors, such as a light-scattering detector [4–6] or an on-line viscometer [7–9]. For the determination of the chemical composition of fractions after a chromatographic separation, infrared spectroscopic [10] and NMR detectors have been used [11]. Dual detection techniques, combining refractive index and UV detection [12–14] or refractive index and density detection [15,16], are also frequently used.

A most interesting alternative to the conventional detectors in HPLC and SEC is the mass spectrometric detector [17,18]. In this case, the molar mass of a particular component may be obtained, provided that fragmentation does not occur and the intact molecular ion is measured. The measured mass of the component may then be correlated with chemical composition or chain length. Although on-line HPLC–MS is a well established method in organic analysis, it is not frequently used in polymer characterization. The major drawback of all existing techniques is the limited mass range, preventing higher oligomers (M_n above 1000–2000) to be ionized without fragmentation [19–21].

A promising technique for the separation of large molecules according to their molar mass has been introduced recently. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), developed by Karras and Hillenkamp [22], has been successfully used to determine the masses of large biomolecules and synthetic polymers [23]. In a recent paper, it was shown that epoxy resins may be separated into their oligomers according to the degree of polymerization and the type of functional end-groups [24]. Minimum amounts of the magnitude of a few nanograms are sufficient for a proper analysis.

From the point of view of liquid chromatography, it would be most desirable to have a detector that is able to provide information on molar mass and chemical composition at the same time. This would give the opportunity to analyse SEC or HPLC fractions very efficiently without going into time-consuming preparative fractionations. In this paper first results are presented aimed at the use of MALDI-MS for detection in the liquid chromatography of polymers. The experiments were carried out in an off-line mode, because automatic sample introduction devices are still under development.

2. Experimental

2.1. Samples

Alkoxy-terminated polyethylene oxide was a technical product of BASF (Ludwigshafen, Germany). A triblock copolymer of ethylene oxide and propylene oxide was prepared by sequential polymerization using potassium glycolate as initiator [25]. Poly(decamethylene adipate) was prepared at Deutsches Kunststoff-Institut (Darmstadt, Germany) by polycondensation of adipic chloride and decanediol. Poly(caprolactone) was a technical product of Union Carbide Europe (Versoix, Switzerland).

2.2. SEC

SEC investigations were performed on five 300 × 8 mm I.D. columns of Ultrastyragel, 1000 Å, 2 × 500 Å and 2 × 100 Å (Waters), using tetrahydrofuran (THF) as the mobile phase at a flow-rate of 1 ml/min. A Model R-410 refractive index detector (Waters) and a Model 501 pump (Waters) were used. Volumes of 200 μl of 0.1%

(w/v) polymer solutions were injected via a Rheodyne six-port injection valve.

2.3. LC

Liquid chromatography at the critical point of adsorption was carried out on a modular HPLC system, consisting of a Waters Model 510 pump, a Waters R-401 differential refractometer, a Rheodyne six-port injection valve and a Waters column oven, keeping the temperature at 25°C in all experiments. The columns used were Nucleosil 100 RP-18 (250 × 4 mm I.D. or 125 × 4 mm I.D.) (Macherey–Nagel) and the mobile phase was acetonitrile–water at flow-rates of 1 and 0.5 ml/min, respectively. The sample concentration was 5–20 mg/ml.

2.4. MALDI-MS

MALDI-MS experiments were conducted on a Kratos Kompact MALDI 3 instrument. The samples were directly taken from the chromatographic separations and mixed with the matrix 2,5-dihydroxybenzoic acid in water. After drying the mixture of the sample and the matrix on the sample slide, the measurements were carried out using the following conditions: polarity, positive; flight path, reflection; and mass, high (20 kV acceleration voltage).

3. Results and discussion

One of the key experiments in the SEC of oligomers is the separation of a sample into its individual oligomers and the determination of the molar mass distribution via an oligomer calibration. The conventional SEC separation of an oligo(caprolactone) is shown in Fig. 1. The lower oligomers appear as well separated peaks at the high retention time end of the chromatogram. For the analysis of the peaks, i.e., the assignment of a certain degree of polymerization (n) to each peak, there are two options. First, the peaks may be identified by comparison with reference samples, i.e., individual oligomers. Second, preparative separations may be carried out, the fractions being collected and subjected to any spectroscopic method for identification. The first option is not applicable in most instances owing to a lack of appropriate reference samples. The second option is time and substance consuming and may be used only in selected cases. Therefore, most desirable would be an identification method consuming only minimum amounts of sample and providing information on the degree of polymerization (molar mass) and, if possible, on the chemical structure.

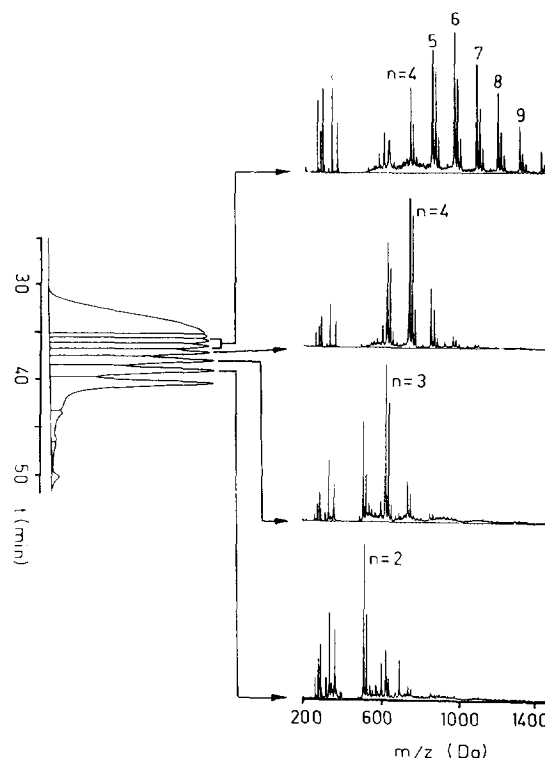


Fig. 1. SEC of an oligo(caprolactone) and analysis of fractions by MALDI-MS. Peak assignment indicates degree of polymerization (n).

The identification of oligomer peaks by MALDI-MS is shown in Fig. 1. The SEC separation is conducted on the usual analytical scale and the oligomer fractions are collected, resulting in amounts of 5–20 ng of substance per fraction in tetrahydrofuran (THF) solution. The solutions are directly mixed with the matrix

solution, placed on the sample slide and subjected to the MALDI-MS experiments. As a maximum of 20 fractions may be introduced into the mass spectrometer at one time, sample preparation and MALDI-MS measurements take less than 15 min. In total nine fractions were collected from SEC and measured by MALDI-MS. The resulting spectra of some of the fractions are shown in Fig. 1. For the lower oligomers the spectra consist of a number of peaks of high intensity, having a peak-to-peak mass increment of 114 u, which equals the mass of the caprolactone repeating unit. These peaks represent the $[M+Na]^+$ molecular ions, whereas the peaks of lower intensity in their vicinity are due to the formation of $[M+K]^+$ molecular ions. $[M+Na]^+$ and $[M+K]^+$ molecular ions are formed due to the presence of small amounts of Na^+ and K^+ ions in the samples and/or the matrix. Further peaks of low intensity indicate functional heterogeneity in the samples. From the masses of the $[M+Na]^+$ peaks, the degree of polymerization of the corresponding oligomer is calculated. By this procedure, the first peak in the chromatogram is assigned to $n=1$, the second peak to $n=2$ and so on. From the elution time and the degree of polymerization of each oligomer peak, an oligomer calibration graph of log (molar mass) vs. elution time is constructed. The conventional calibration graph based on polystyrene standards differs considerably from the oligomer calibration graph, as can be seen in Fig. 2. Therefore, molar mass calculations based on the oligomer calibration graph are expected to yield much more precise results.

In a similar way, MALDI-MS can be used for molar mass-sensitive detection in adsorption chromatography. Fig. 3 shows the separation of poly(decamethylene adipate) on silica gel using an eluent of THF–hexane (45:55, v/v). Similarly to the previous case, a separation into individual oligomers is obtained. The fractions are collected, subjected to MALDI-MS and the degree of polymerization is calculated from the major peak in the spectrum. The remarkable feature of this investigation is that, in contrast to thermospray or electrospray mass spectrometric detection, oligomers with molar masses higher

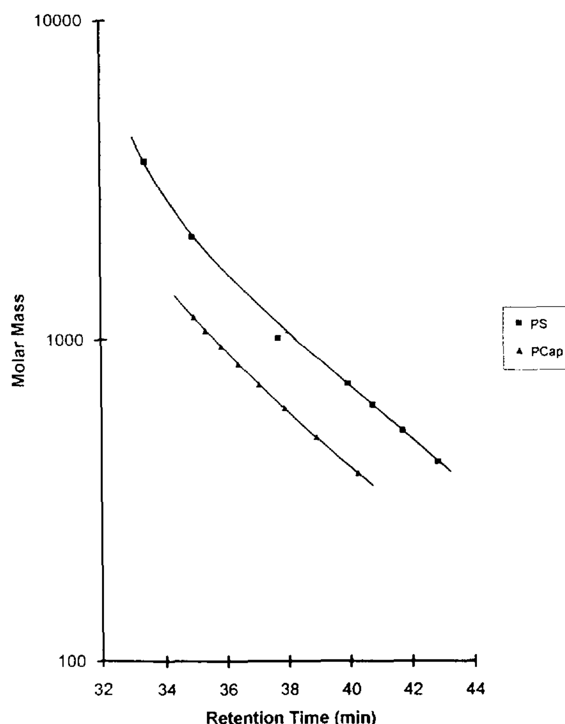


Fig. 2. SEC calibration graphs of molar mass vs. retention time for (■) polystyrene and (▲) poly(caprolactone).

than 3000 g/mol may be measured without fragmentation.

A much more demanding task is the analysis of fractions from liquid chromatography not only with respect to molar mass but also with respect to chemical structure. The separation of a technical fatty alcohol ethoxylate (FAE) by liquid chromatography under conditions such that the chain length as well as the end-groups direct the separation [26,27] is presented in Fig. 4. Using this chromatographic technique, the FAE is separated into three main fractions, the first fraction appearing as one peak at a retention time of about 60 s and fractions 2 and 3 showing oligomer separations. Fraction 1 is collected in total, whereas for fractions 2 and 3 the individual oligomer peaks are collected. The MALDI mass spectra of all three fractions give a peak-to-peak mass increment of 44 u, indicating that all fractions consist of species with an ethylene oxide-based polymer chain. From the masses

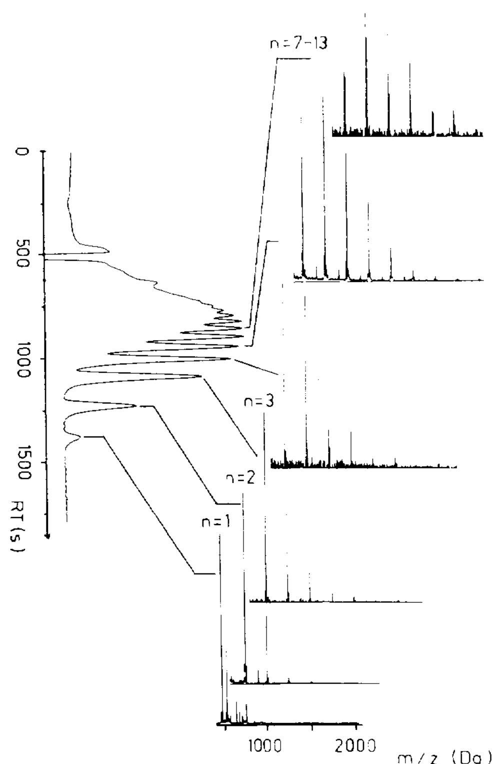


Fig. 3. HPLC of poly(decamethylene adipate) and analysis of fractions by MALDI-MS. Peak assignment indicates degree of polymerization (n). Stationary phase, Nucleosil Si-50; eluent, THF–hexane (45:55, v/v).

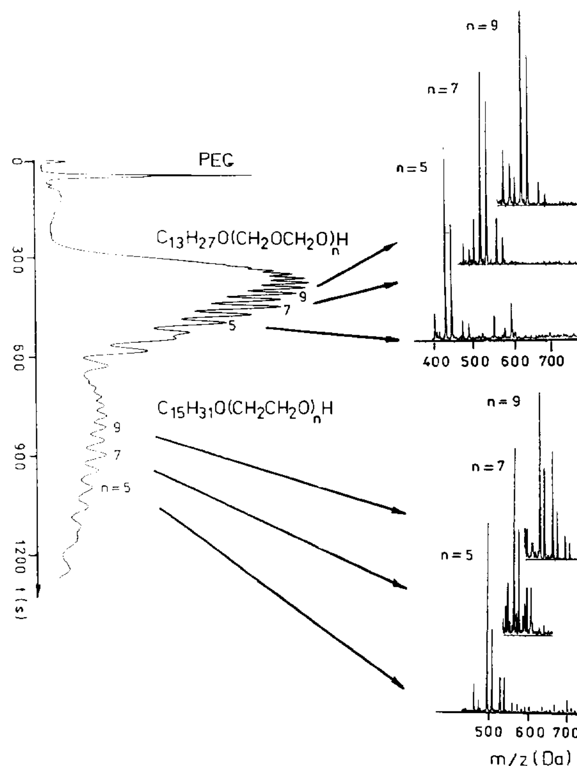


Fig. 4. Separation of a technical polyethylene oxide by liquid chromatography at the critical point of adsorption and analysis of fractions by MALDI-MS. Peak assignment indicates degree of polymerization (n). Column, Nucleosil 100 RP-18 (125 × 4 mm I.D.); eluent, acetonitrile–water (70:30, v/v).

assigned to the peaks and the peak-to-peak mass increment of the ethylene oxide repeating unit, the mass of the end-group for the different fractions may be calculated:

fraction 1:

$$[M + Na]^+ = 41 + 44n$$
$$M = 18 + 44n$$

fraction 2:

$$[M + Na]^+ = 223 + 44n$$
$$M = 200 + 44n$$

fraction 3:

$$[M + Na]^+ = 251 + 44n$$
$$M = 228 + 44n$$

Provided the sample is a pure FAE, the end-groups of fractions 1, 2 and 3 can be identified as being polyethylene glycol (PEG) (α,ω-dihydroxy end-groups), C_{13}-terminated polyethylene oxide (PEO) (α-tridecyl-ω-hydroxy end-groups) and C_{15}-terminated PEO (α-pentadecyl-ω-hydroxy end-groups), respectively. Using MALDI-MS, the oligomer distribution of the PEG fraction is measured directly. The MALDI mass spectrum of the PEG fraction (fraction 1) is given in Fig. 5. The homologous series of higher peak intensity corresponds to the $[M + K]^+$ molecular ions of the ethylene glycol oligomers. For fractions 2 and 3, by determining the degree of polymerization of the oligomer peaks oligomer calibration graphs are obtained, which are used for the molar mass calculation of the fractions. Thus, by

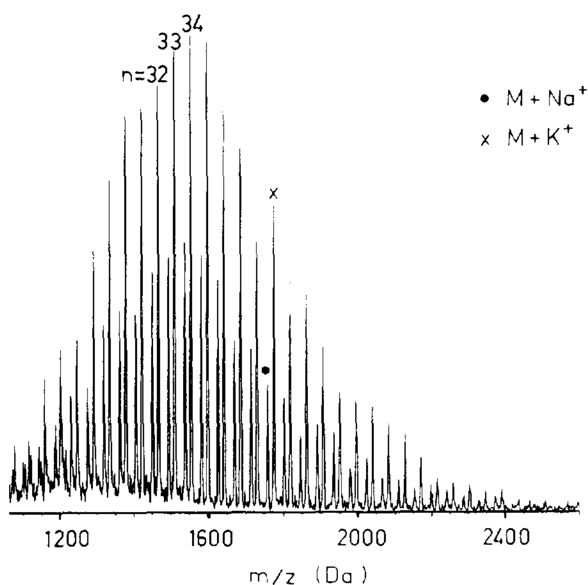


Fig. 5. MALDI mass spectrum of fraction 1 (PEG) from the chromatographic separation shown in Fig. 4.

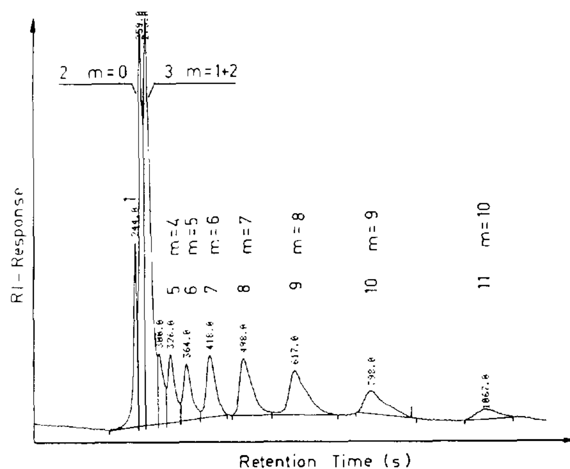


Fig. 6. Separation of a triblock copolymer $H(EO)_n(PO)_m(EO)_nOH$ with respect to the propylene oxide block by liquid chromatography at the critical point of adsorption. Peak assignment indicates fraction and degree of polymerization of the propylene oxide block (m). Column, Nucleosil 100 RP-18 (250 × 4 mm I.D.); eluent, acetonitrile–water (42:58, v/v).

combining liquid chromatography and MALDI-MS detection, complex samples may be analysed with respect to chemical structure and molar mass.

The possibility of determining molar mass distributions of polyethylene oxides or fractions thereof by MALDI-MS (see detailed discussion in Refs. [27] and [28]) prompted us to investigate block copolymers by liquid chromatography and MALDI-MS. It was shown in previous investigations that block copolymers of ethylene oxide and propylene oxide may be separated with respect to the chain length of the propylene oxide block by liquid chromatography at the critical point of adsorption [25,29]. Operating at the critical point of PEG, the ethylene oxide block behaves chromatographically "invisible" and retention of the block copolymer is solely directed by the propylene oxide block. Fig. 6 represents the separation of a triblock copolymer $H(EO)_n(PO)_m(EO)_nOH$ at the critical point of the ethylene oxide blocks. The assignment of the peaks is based on comparison with the chromatogram of a polypropylene glycol. The first and second peaks in the chromatogram at retention times of 244 and 259 s, respectively, could not be identified directly, whereas the third peak at a retention time of 275 s corresponds to $m = 1–2$, m being the degree of polymerization with respect to propylene oxide. The peak at 300 s corresponds to $m = 3$, the peak at 326 s corresponds to $m = 4$ and so on. Accordingly, every peak is uniform with respect to m but has a distribution in block length with respect to the polyethylene oxide blocks (n).

In order to identify peaks 1 and 2 and to determine n of the other fractions, they are collected and subjected to MALDI-MS. For these experiments a small amount of lithium chloride is added to the sample, thus favouring the formation of $[M + Li]^+$ molecular ions. The MALDI mass spectra of fractions 1–3 are summarized in Fig. 7. For fraction 1 a number of peaks in the mass range up to 500 is obtained, the peaks at M_r 284 and 485 being the most intense. However, a homologous series similar to Fig. 5, representing ethylene oxide oligomers, is not obtained. Therefore, these peaks are assumed to be due to impurities in the reaction product or the MALDI-MS matrix. Much more informative is the spectrum of fraction 2, show-

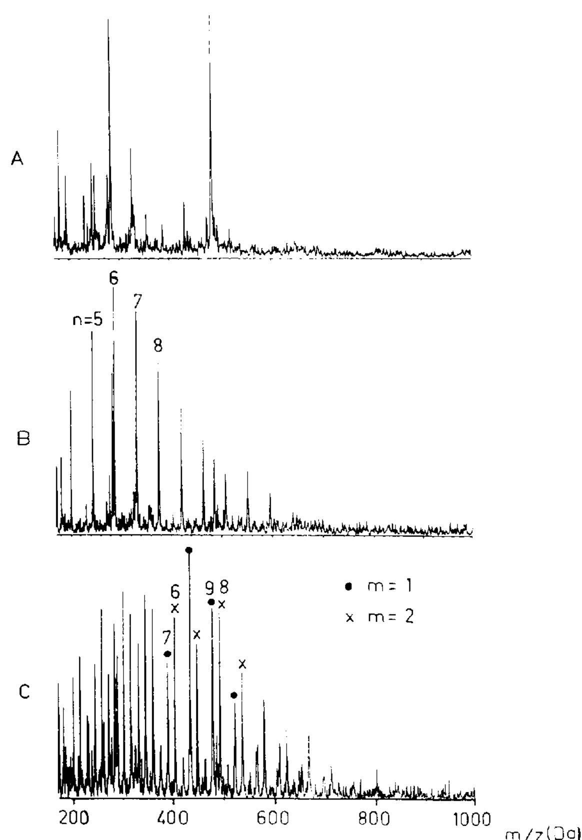


Fig. 7. MALDI mass spectra of fractions (A) 1, (B) 2 and (C) 3 from chromatographic separation shown in Fig. 6. Peak assignment indicates degree of polymerization of the ethylene oxide blocks (n).

ing the expected series of peaks having a peak-to-peak mass increment of 44 u. This increment exactly equals the mass of the ethylene oxide repeating unit and, accordingly, the observed series of peaks represents an ethylene oxide oligomer series. From the masses assigned to the peaks and the peak-to-peak mass increment of the ethylene oxide unit, the mass of the end-group is calculated to be 18:

fraction 2:

$$[M + Li]^+ = 25 + 44n$$

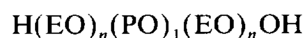
$$M = 18 + 44n$$

Therefore, fraction 2 represents polyethylene glycol ($m = 0$). Owing to a poor chromatographic separation, in addition to the PEG peaks the major peaks of fraction 1 at M_r 284 and 485 u are also detected.

The spectrum of fraction 3 is very complex. In addition to some minor PEG peaks, two other homologous series with a peak-to-peak increment of 44 u are obtained. These may be assigned to the following structures:

(1) $[M + Li]^+ = 83 + 44n$

$$M = 76 + 44n$$



$$H(EO)_n(PO)_1(EO)_nOH$$

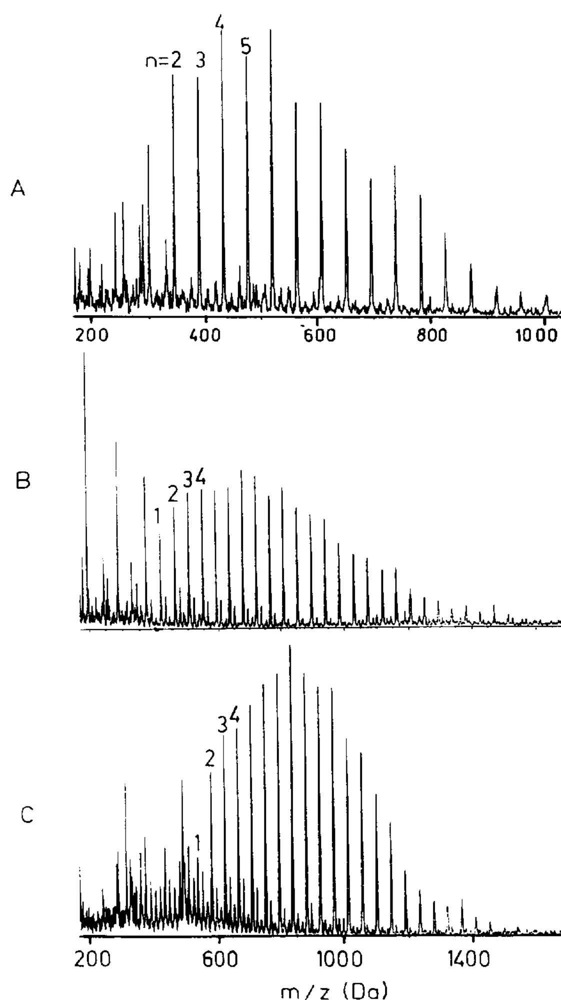


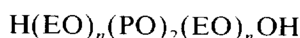
Fig. 8. MALDI mass spectra of fractions (A) 5, (B) 7 and (C) 9 from chromatographic separation shown in Fig. 6. Peak assignment indicates degree of polymerization of the ethylene oxide blocks (n).

Table 1
Assignment of fractions 4–11

Fraction	m	Structure
4	3	$H(EO)_n(PO)_3(EO)_nOH$
5	4	$H(EO)_n(PO)_4(EO)_nOH$
6	5	$H(EO)_n(PO)_5(EO)_nOH$
7	6	$H(EO)_n(PO)_6(EO)_nOH$
8	7	$H(EO)_n(PO)_7(EO)_nOH$
9	8	$H(EO)_n(PO)_8(EO)_nOH$
10	9	$H(EO)_n(PO)_9(EO)_nOH$
11	10	$H(EO)_n(PO)_{10}(EO)_nOH$

(2) $[M + Li]^+ = 141 + 44n$

$$M = 134 + 44n$$



Accordingly, fraction 3 represents a mixture of the homologous series with $m = 1$ and $m = 2$. Owing to a better separation of the subsequent fractions, their MALDI mass spectra each represent one homologous series (see Fig. 8 for fractions 5, 7 and 9). The assignment is given in Table 1.

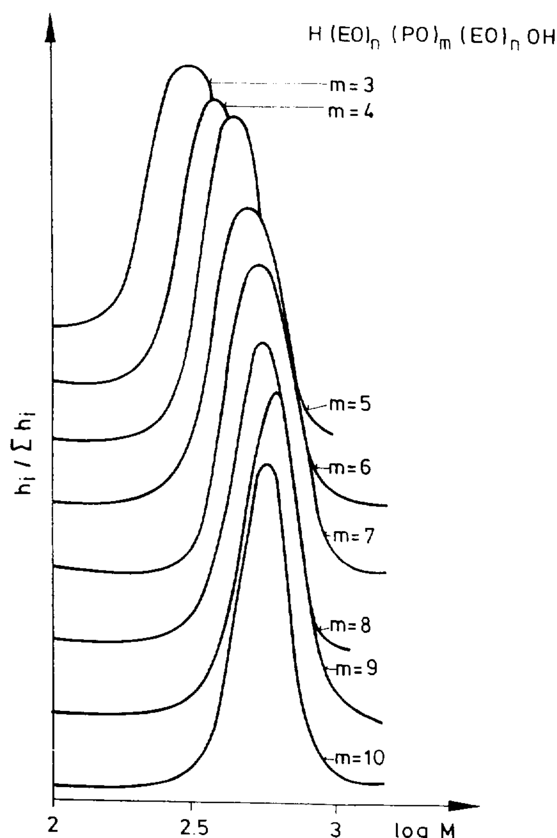


Fig. 9. Molar mass distributions of the ethylene oxide blocks of fractions 4–11 as determined by MALDI-MS.

From the intensities of the individual peaks in the MALDI mass spectra, the relative abundance of each oligomer may be determined. Thus, for each fraction the oligomer or molar mass distribution with respect to the ethylene oxide blocks may be calculated (see Fig. 9).

4. Conclusions

MALDI/MS has been shown to be a powerful detection method in the liquid chromatography of polymers. By analysing fractions taken from a chromatographic separation it is possible to obtain information on the molar mass and the chemical composition of the sample. As MALDI-MS at present can be used only in the off-line mode, a transfer step is still required. Further development will focus on developing on-line techniques, thus further increasing the efficiency of this versatile detection method.

Acknowledgements

The authors express their gratitude to V. Wilhelm for preparing the poly(decamethylene adipate) and to I. Zammert and C. Brinkmann (all of DKI) for conducting some of the chromatographic experiments. The technical support of Shimadzu Europe (Duisburg, Germany) is gratefully acknowledged.

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