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Matrix-assisted laser desorption/ionization mass spectrometry of synthetic polymers. 4. Coupling of size exclusion chromatography and MALDI-TOF using a spray-deposition interface

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Abstract

Size exclusion chromatography and MALDI-TOF mass spectrometry can be effectively coupled via a robotic interface, where chromatographic fractions are sprayed directly onto a moving MALDI-TOF target through a heated capillary nozzle. The target is precoated with an appropriate matrix, such as dithranol. For polydisperse synthetic polymers a continuous track of matter is deposited onto the matrix surface. After deposition of the sample fractions, the MALDI-TOF target is introduced into the spectrometer, and spectra are taken from different positions of the polymer track. As a result, well-resolved spectra are obtained which are characteristic for different molar mass fractions of the sample. In the case of copolymers, information on molar mass and copolymer composition is accessible.

Keywords: MALDI-TOF mass spectrometry; Size exclusion chromtography; Hyphenated techniques

1. Introduction

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is a particularly effective tool for the molar mass determination of natural and synthetic polymers. The ability to ionize a broad range of materials, its high sensitivity, large mass range, fast sample preparation, and the absence of fragmentation are characteristics of this powerful technique. In a MALDI-TOF instrument, short-duration laser pulses are directed at a sample dispersed in a specific matrix. The laser energy causes a portion of the sample/matrix mixture to be desorbed from the surface and ionized. The produced ions are analysed in a TOF mass analyser. Initially developed for use with large biomolecules in 1988 by Karas and Hillenkamp [1,2], MALDI-TOF has advanced very rapidly into a powerful technique for synthetic polymer analysis [3–10].

One major concern is the ability of MALDI-TOF to provide accurate molar mass measurements. It has been shown that for polydispersities $M_w/M_n > 1.1$ there is a significant discrepancy between molar masses calculated from MALDI-TOF vs. that calculated from size exclusion chromatography (SEC). Polymers with higher polydispersities may be completely inaccessible to analysis by MALDI-TOF [11–14]. This is caused by the fundamental difference between recording of the number fraction vs. molecular mass (correctly *m*/*z*) as in mass spectrometry and the weight fraction vs. logarithm of molecular mass as in SEC. In addition, the discrepancies between MALDI-TOF and SEC are affected by the different detection efficiency for low and high molecular masses [15] and experimental parameters in MALDI-TOF.

A solution to this problem has been proposed recently by combining a SEC prefractionation with a subsequent MALDI-TOF analysis of the resulting fractions. This combination can be “off-line” as has been discussed in Refs. [11,13,16] or “on-line” as has been shown by Fei [17], Kassis [18], Nielen [19], and Whittal [20]. While the “off-line” approach is experimentally very simple, it is rather time-consuming and laborious. The feasibility of direct deposition has been shown in Ref. [18], where the SEC effluent was sprayed onto a rotating matrix-coated substrate using a modified LC-Transform Series 100 IR interface of Lab Connections. Alternatively, Nielen described the coupling of SEC to a robotic interface Probot of Bioanalytical Instruments, where the matrix is coaxially added to the SEC effluent and spotted dropwise onto the MALDI target.

In this paper, the application of a new interface

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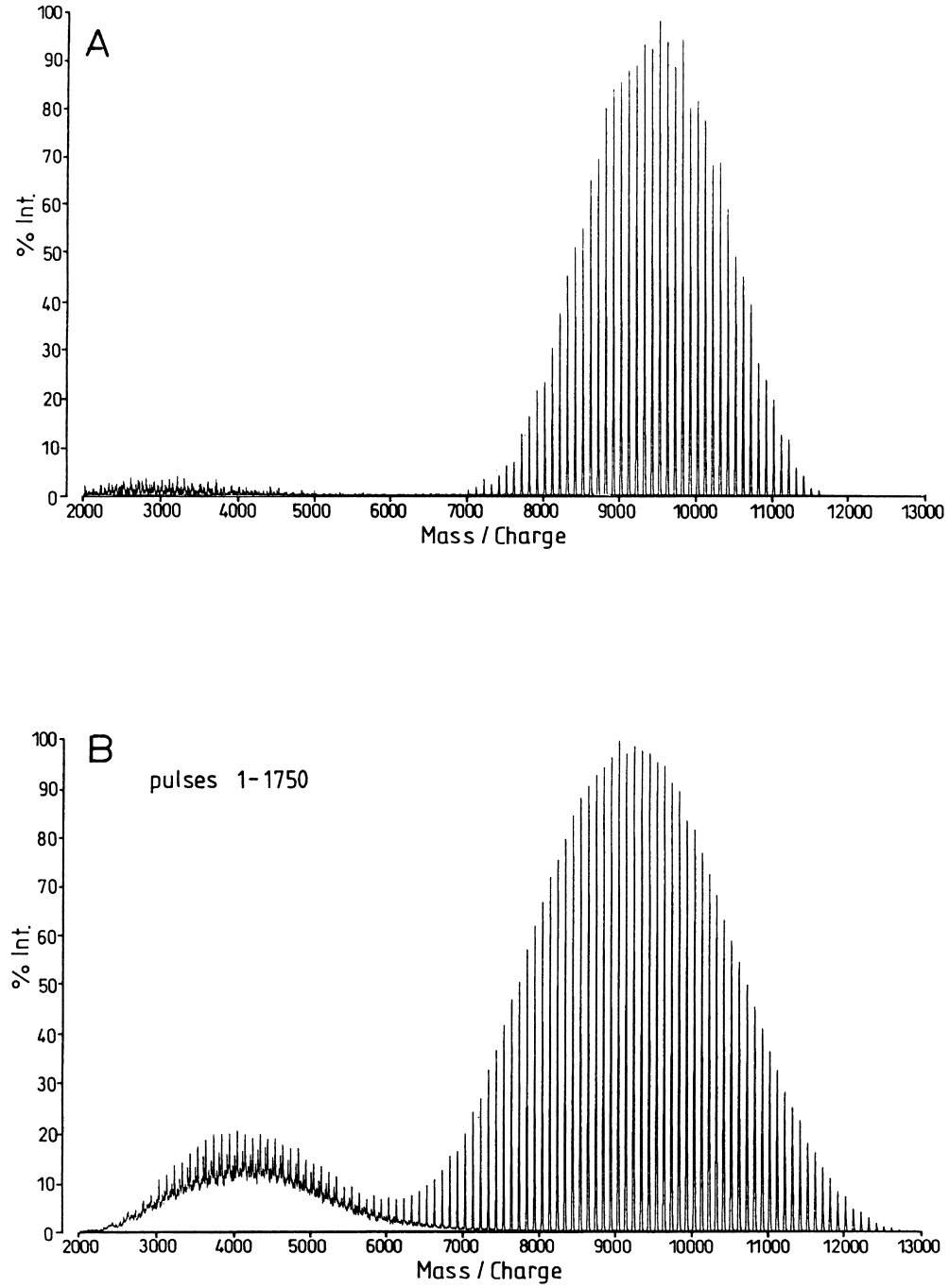


Fig. 1. MALDI-TOF spectrum of PMMA 10 900 measured in (A) the off-line; and (B) the on-line mode, matrix: dithranol, LiCl.

LC-Transform 501 in SEC–MALDI-TOF coupling shall be discussed. The advantage of this interface is that sample fractions can be deposited on any MALDI target geometry. In the first step, the matrix is deposited on the target followed by the deposition of the SEC fractions.

2. Experimental

SEC: The measurements were conducted using a modular system, comprising a Waters HPLC pump Model 510, a Rheodyne six-port injection valve, a Waters tunable UV detector Model 486 and a Waters refractive index detector Model 410. The columns were Polymer Laboratories 10^5 Å, Mixed-D, Mixed-E and 50 Å, 300 × 7.8 mm^2 i.d. The eluent was HPLC grade tetrahydrofuran (THF).

MALDI-TOF-MS: The spectra were recorded on a KRATOS Kompact MALDI 4 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The

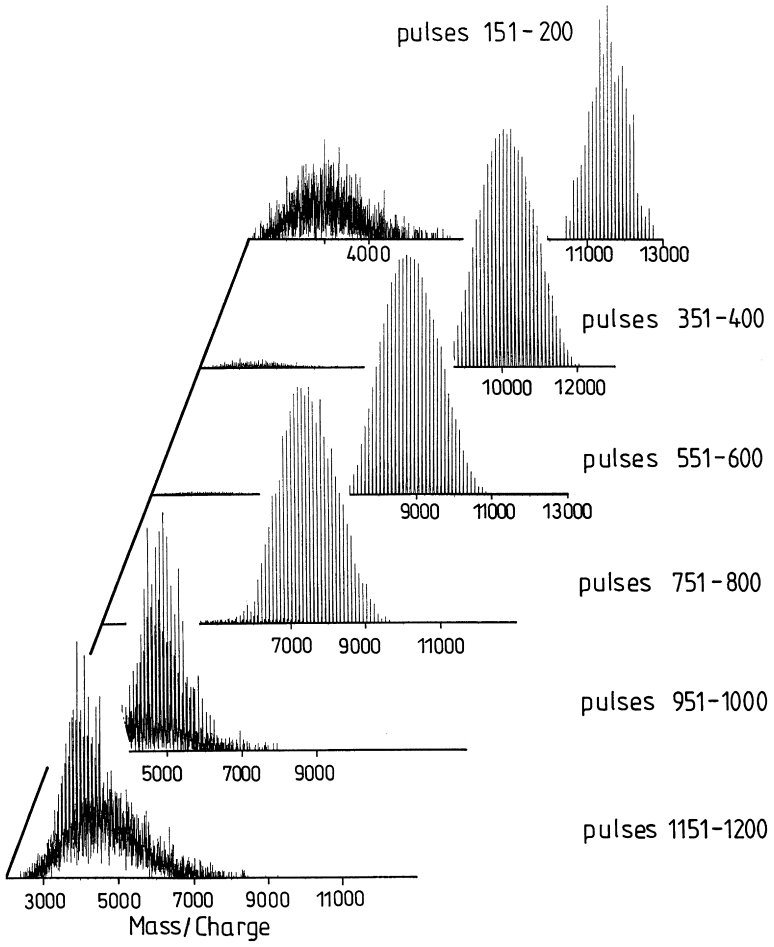


Fig. 2. MALDI-TOF spectra of fractions from SEC separation of PMMA 10 900, on-line SEC–MALDI-TOF analysis, matrix: dithranol, LiCl.

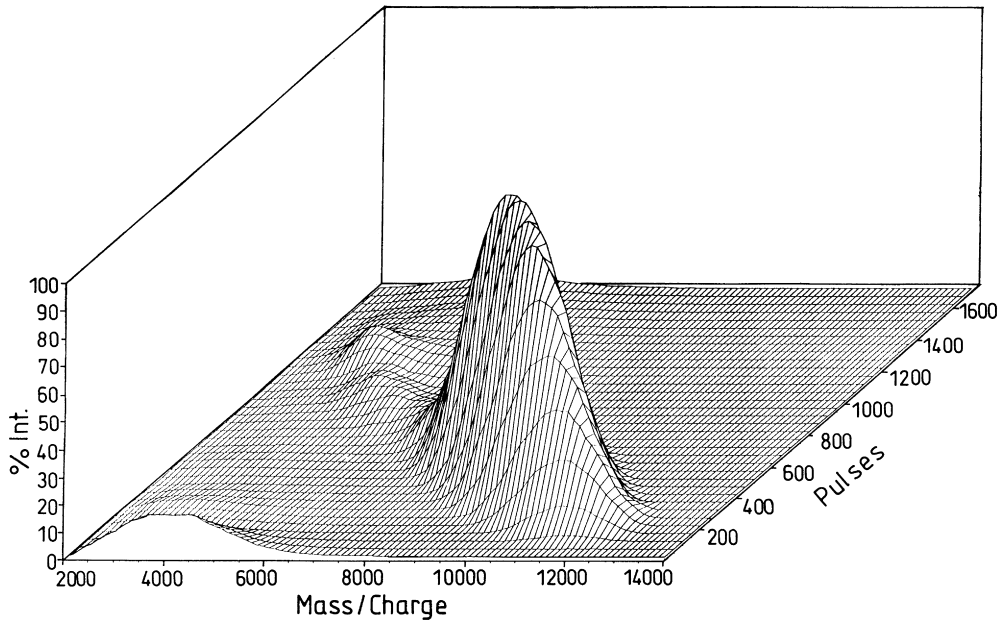


Fig. 3. 3D plot for PMMA 10 900 obtained from on-line SEC–MALDI-TOF analysis.

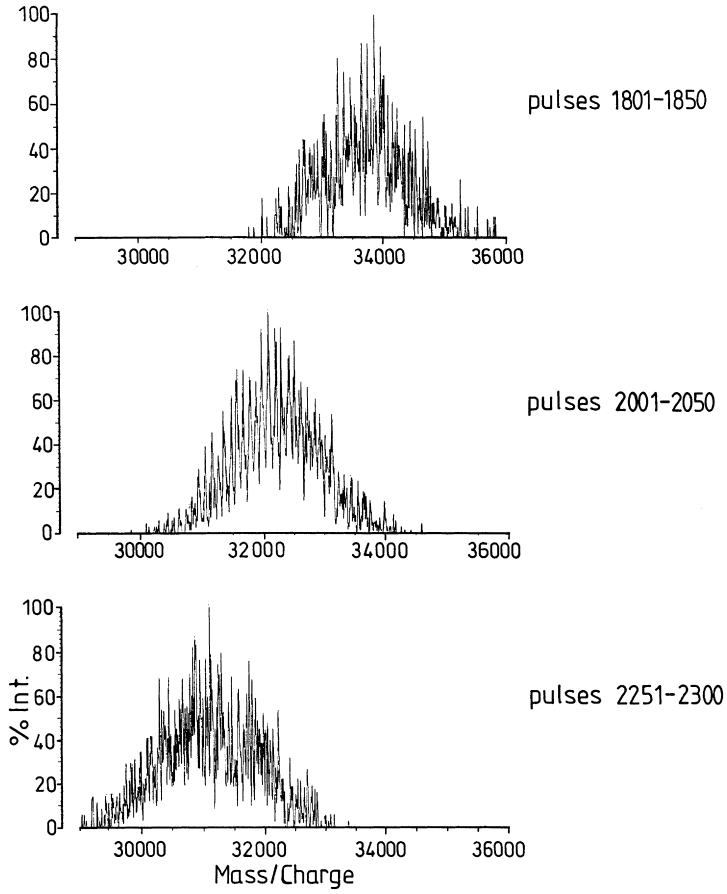


Fig. 4. MALDI-TOF spectra of fractions from SEC separation of PS 32 500, on-line SEC–MALDI-TOF analysis, matrix: dithranol, copper target.

measurements were carried out using the following conditions: polarity—positive; flight path—linear; mass—high (20 kV acceleration voltage); and 100–150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200–800 ns.

MALDI-TOF sample preparation: 10% of the SEC effluent was introduced via a capillary into the interface LC-Transform Series 501 of Lab Connections (Marlborough, MA, USA). The effluent was sprayed through a heated capillary nozzle continuously on a slowly moving Kratos MALDI target precoated with the appropriate matrix, resulting in a uniform surface layer of sample fraction and matrix. As matrices 1,8,9-trihydroxy anthracene (dithranol) and 2,5-dihydroxy benzoic acid (DHB) were used. The matrix was

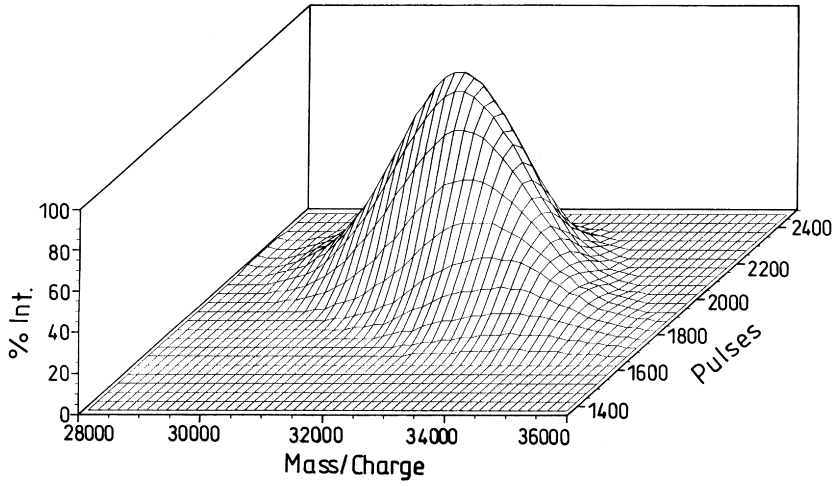


Fig. 5. 3D plot for PS 32 500 obtained from on-line SEC–MALDI-TOF analysis.

manually deposited on the MALDI target from the THF solution. When necessary, a salt was added to the matrix solution.

3. Results and discussion

In the first set of experiments, the results of a conventional MALDI-TOF measurement ("off-line") were compared to the results of a coupled SEC–MALDI-TOF investigation ("on-line"). The off-line spectrum of a polymethyl methacrylate (PMMA) calibration standard with a nominal molar mass of 10,900 g/mol using dithranol as the matrix is given in Fig. 1A. Well-resolved mass peaks in a mass range of 7000–12,000 Da are obtained. In addition, the spectrum indicates lower molar mass peaks around 3000 Da. These peaks, however, are not well resolved due to the low peak intensity.

The same sample is separated by SEC and the fractions are automatically deposited on the MALDI-TOF sample target. Prior to fraction deposition the target was precoated with the matrix dithranol and a small amount of LiCl to enhance the formation of $[M + Li]^+$ molecular ions. Since the fraction deposition is carried out through a heated capillary nozzle, a solid fraction/matrix film is obtained on the MALDI-TOF target. The spray-deposition procedure must be optimized very carefully in order to assure that a uniform sample/matrix track is formed. If the nozzle temperature is too low, the aerosol stream is too wet, resulting in partial desolving and blowing away of the matrix. If the nozzle temperature is too high, the aerosol stream is too dry, resulting in a bilayer film where the matrix and the sample molecules are not mixed at all. In the present experiments where solely THF was used as the eluent, a spray nozzle temperature of 70°C has been found to be the optimum. In the same manner, the distance between the spray nozzle and the MALDI-TOF target has to be optimized.

The MALDI-TOF target had a length of 70 mm and was scanned continuously with 3500 laser pulses. Every set of 50 pulses was summarized to give a complete MALDI-TOF spectrum. With SEC as the preseparation technique, low positions on the target correspond to high molar masses, while high positions are equivalent to low molar masses. Selected spectra from different positions of the polymer/matrix track of the PMMA sample are given in Fig. 2. In the present experiment, a sample amount of 10 μg (100 μl of a 0.1 mg/ml solution) is injected into the SEC. An amount of 10% of the total effluent is sprayed onto the MALDI target resulting in a total amount of deposited sample of 1 μg. As can be seen, for all fractions high quality spectra are obtained giving the oligomer distributions of the different fractions. As expected, the lowest fraction corresponds to the highest molar mass. For this fraction a small amount of fragmented molecules is obtained.

Now the MALDI-TOF spectra of the different SEC fractions can be processed in different ways. First of all,

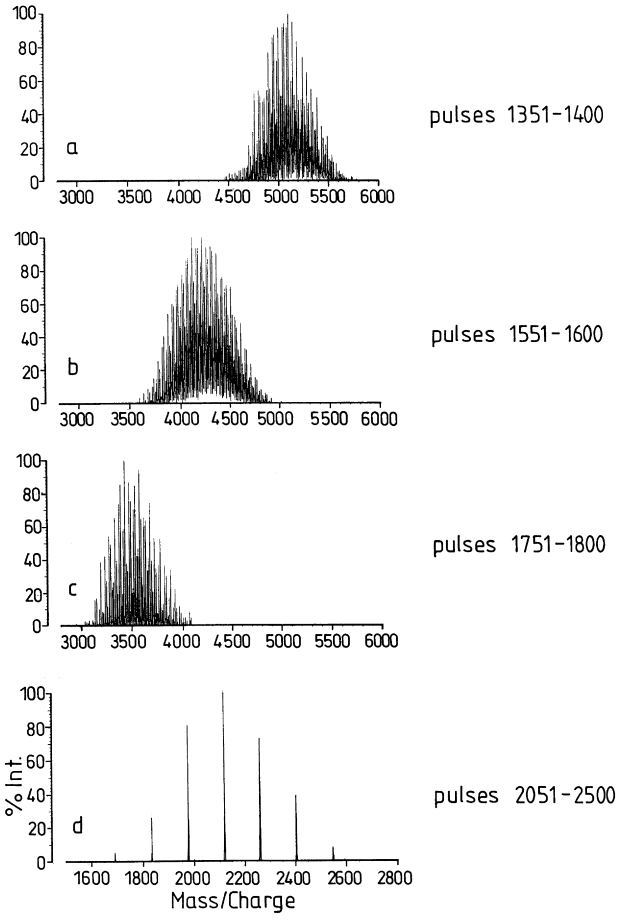


Fig. 6. MALDI-TOF spectra of fractions from SEC separation of P*n*BMA-*b*-PMMA, on-line SEC–MALDI-TOF analysis, matrix: dithranol, Na-TFA.

the peak maximum molar masses can be used to calibrate the SEC system. Using this approach, SEC can be calibrated with only a few calibration standards or even only one broadly distributed PMMA sample. To compare the results of the off-line and on-line experiments, all fraction spectra can be summarized to give one (calculated) total spectrum of the sample, see Fig. 1B. As is obvious, the quality of the calculated on-line spectrum is much better in particular with respect to the high and low molar mass tail of the distribution. In addition, the low molar mass peaks are much clearly presented as compared to Fig. 1A. Finally, the results of the coupled experiment can be presented in a three-dimensional (3D) plot, where one axis corresponds to the mass range, one axis corresponds to the position on the MALDI-TOF target (equivalent to elution volume in SEC) and one axis gives the mass peak intensity, see Fig. 3. Such a 3D plot can be regarded as a finger print for a specific sample.

The separation of a polystyrene (PS) calibration standard of a nominal molar mass of 32,500 g/mol is demonstrated in the next experiment. The matrix is again dithranol, however, for PS samples LiCl cannot be used for cation formation.

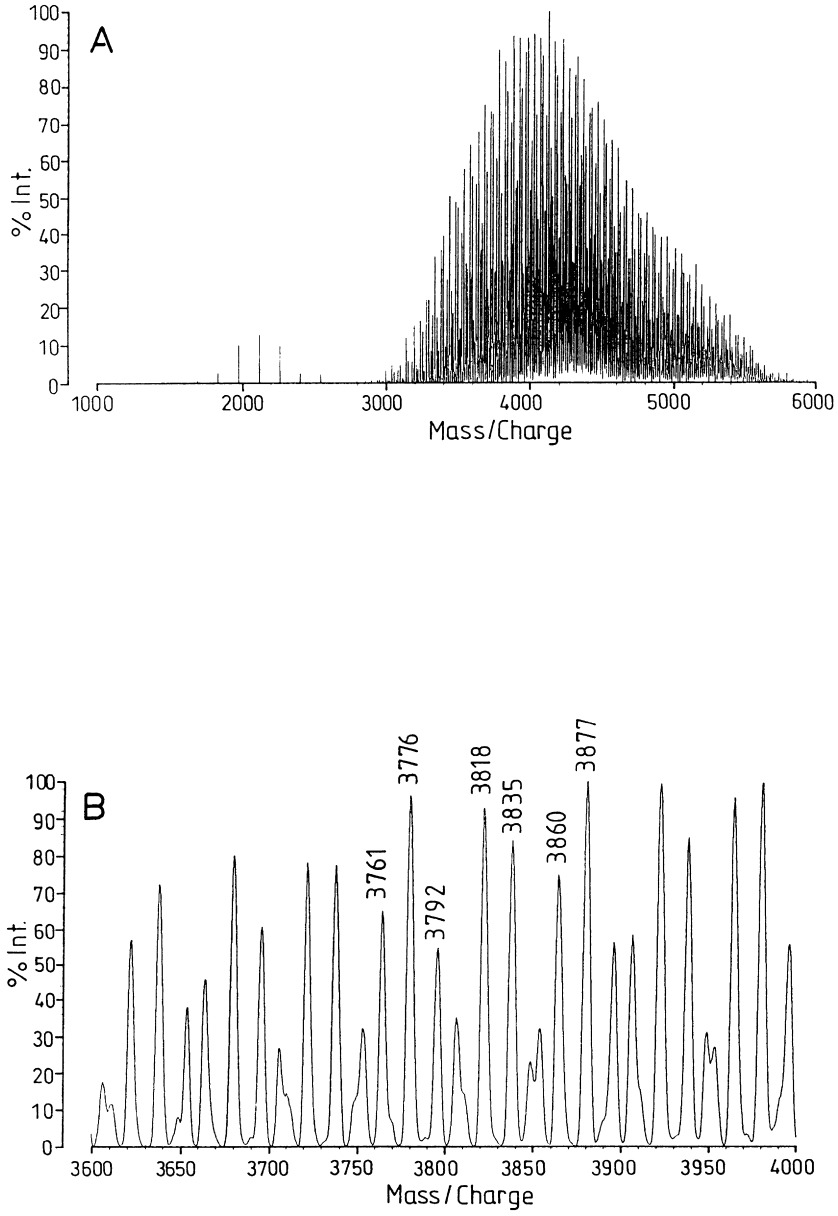


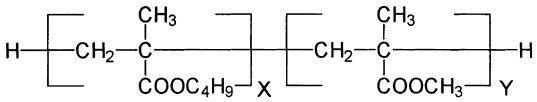
Fig. 7. (A) Calculated MALDI-TOF spectrum from on-line analysis of P*n*BMA-*b*-PMMA; and (B) zoomed part of the spectrum.

Typically, silver trifluoroacetate is used for cationization, however, we found in previous investigations that an effective formation of $[M + Cu]^+$ molecular ions can be achieved when a copper MALDI-TOF target is used. Under otherwise similar experimental conditions mass spectra for SEC fractions are obtained which demonstrate the limits in mass resolution of the present experimental set-up, see Fig. 4. However, even from these poorly resolved spectra a perfect 3D plot can be generated, fingerprinting the sample, see Fig. 5.

Depending on the complexity of a specific sample, MALDI-TOF is more or less capable of resolving different chemical structures. While this technique is very powerful in determining different endgroups in macromonomers and telechelics, it has its limitations when it comes to analysing copolymers. Due to the fact that the number of possible oligomers increases exponentially with the degree of polymerization, even for low molar masses very complex product mixtures are obtained which cannot be analysed solely by MALDI-TOF. In these cases it is unavoidable to combine a chromatographic prefractionation with a MALDI-TOF analysis.

The usefulness of such a combination shall be demonstrated for a diblock copolymer of *n*-butyl methacrylate and methyl methacrylate (P*n*BMA-*b*-PMMA). The sample under investigation is a commercial sample of Polymer Standards Service GmbH (Mainz, Germany). It was prepared by group transfer polymerization (GTP) starting with *n*BMA followed by addition of MMA to the reaction mixture. The average molar mass determined by SEC is

4200 g/mol, the comonomer ratio is roughly 1:1. The chemical structure of the block copolymer can be presented as follows:



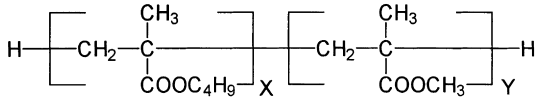
The size exclusion chromatogram of the sample is uniform and does not indicate any high or low molar mass by-products. For investigating the chemical composition of the block copolymer, fractions from SEC are automatically collected on the MALDI-TOF target and analysed. Typical spectra for fractions of different molar masses are given in Fig. 6. The higher molar mass fractions in a–c are characteristic for copolymer structures exhibiting typical mass increments of 100 Da for the MMA repeat unit and 142 Da for the *n*BMA repeat unit. Even these narrow disperse fractions exhibit a multitude of different mass peaks (usually more than 100) indicating the high complexity of the fractions. Different from a–c, the lower molar mass fraction in d is very uniform with respect to composition. For this fraction, only peak-to-peak mass increments of 142 Da are observed which are typically for P*n*BMA. Accordingly, this fraction can be assigned to an unwanted by-product, namely butyl methacrylate homopolymer. In the present case, sodium trifluoroacetate is used for promoting the cationization and, accordingly the mass peaks correspond to the $[M + Na]^+$ molecular ions.

The total spectrum of the sample is obtained when the individual spectra of all pulses are summarized, see Fig. 7a. This spectrum clearly indicates the presence of P*n*BMA having an average molar mass which is about 50% of the total molar mass of 4200 g/mol. This result is in very good agreement with the expectations. The monomer ratio of the sample under investigation is about 1:1 resulting in average block lengths of 2100 g/mol for the P*n*BMA and the PMMA blocks. Since the polymerization was started with *n*-butyl methacrylate, the formation of a small amount of P*n*BMA has to be expected. The molar mass of this homopolymer must be of the same magnitude as the P*n*BMA block in the copolymer. This is indeed the case as is shown by the MALDI-TOF measurement.

The chemical composition of the block copolymer can be studied in detail by analysing the different mass peaks, see zoomed part of the spectrum in Fig. 7B. Each peak in the spectrum can be assigned to one individual oligomer composition $(n\text{BMA})_X(\text{MMA})_Y$. For example, the mass peak at 3760 Da corresponds to an oligomer with 15 *n*BMA and 16 MMA units. Its gross structure is H–$(n\text{BMA})_{15}$–$(\text{MMA})_{16}$–H. The mass peak at 3834 Da is due to the oligomer H–$(n\text{BMA})_{12}$–$(\text{MMA})_{21}$–H. The calculated and observed molar masses for selected oligomers are summarized in Table 1.

To summarize, the combination of SEC and MALDI-TOF mass spectrometry via a spray nozzle interface is an efficient tool for analysing polydisperse and complex polymers. Samples are separated into different molar mass fractions by SEC and directly deposited on a precoated MALDI-TOF target. A uniform continuous polymer/matrix track is obtained which can be directly analysed by MALDI-TOF. As a result, well-resolved mass spectra are obtained which bear information on molar mass and chemical composition. In forthcoming experiments this approach shall be used to combine different modes of interaction chromatography with MALDI-TOF.

Table 1
Calculated and observed molar masses for individual oligomers of sample PMMA-*b*-P*n*BMA



X	*Y*	*M* (calculated)	*M* (observed)
15	16	3760	3761
13	19	3776	3776
11	22	3792	3792
16	15	3802	3803
14	18	3818	3818
12	21	3834	3835
10	24	3850	3850
15	17	3860	3860
13	20	3876	3877

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