Analysis of complex polymers by MALDI-TOF mass spectrometry

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SUMMARY: Since the introduction by Karas and Hillenkamp in 1987 for the analysis of biomolecules, MALDI mass spectrometry has emerged to a powerful method for characterizing synthetic polymers. This soft ionization technique combined with time-of-flight mass analyzers is capable of providing a complete mass spectrum per event in a virtually unlimited mass range. Up to molar masses of about 30 000 g/mol mass-resolved polymer chains can be detected. From the oligomer masses the mass of the repeat units and the endgroups can be determined. For functionally heterogeneous systems detailed information on the functionality type distribution is available, while for copolymers sequence analysis can be conducted. The application of MALDI-TOF mass spectrometry to the analysis of homopolymers and heterogeneous polymers with respect to molar mass and chemical composition is demonstrated. It is shown that MALDI-TOF can be used efficiently for molar mass-sensitive detection in liquid chromatography.

Introduction

The chemical structure of a complex polymer is characterized by its chemical composition, the sequence of the monomer units in the polymer chain, the functionality and the molar mass. Typically, polymer analysis has to deal with the overlaying effects of different types of molecular heterogeneity in one macromolecular system. As for functional homopolymers (telechelics, macromonomers), the molar mass distribution is superimposed by a functionality type distribution. In the ideal case, such a complex system is separated into the individual oligomers, which then may be analysed with respect to mass and composition.

The power of mass spectrometry is the fast and accurate determination of molar masses, the sequence of repeat units, polymer additives and impurities. The main barriers for mass spectrometry of high molar mass compounds, caused by the low volatility and thermal instability of polymers, have been overcome by the development of soft ionization techniques. A new method for the separation of large molecules according to their molar mass and chemical composition has been introduced several years ago. Matrix-assisted laser

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desorption/ionization mass spectrometry (MALDI-MS) was developed by Karas and Hillenkamp in 1987 for the analysis of large biomolecules[1-3], however, it was demonstrated not until 1992 that synthetic polymers can be analysed with molar masses above 100 000 $g \cdot mol^{-1}$ [4-6]. Compared to other mass spectrometric techniques, the accessible mass range has been extended considerably, and the technique is fast and instrumentally very simple. Moreover, relatively inexpensive commercial instrumentation has become accessible.

In principle, the sample to be investigated and a matrix solution are mixed in such a ratio that matrix separation of the sample molecules is achieved. After drying, a laser pulse is directed onto the solid matrix to photo-excite the matrix material. This excitation causes the matrix to explode, resulting in the expulsion and soft ionization of the sample molecules. Once the analyte is ionized, it is accelerated and analysed in a time-of-flight (TOF) mass spectrometer. As a result, the analyte is separated according to the molar mass of its components, and in the case of heterogeneous polymers additional information on chemical composition may be obtained. In a number of papers it was shown that polymers may be analysed up to and above relative molar masses of about 500 000 $g \cdot mol^{-1}$ [7-11].

One of the most interesting features of MALDI-TOF mass spectrometry is, that single polymer chains can be resolved up to masses of about 30 000 $g \cdot mol^{-1}$. This enables determination of repeat units and endgroups in chemically heterogeneous polymers[12-14]. It was shown recently that MALDI-TOF is superiour to all other techniques in analysing functionally heterogeneous polymers with respect to the degree of polymerization and the type of functional endgroups in one experiment[15,16].

The present article describes some recent results on the functionality and molar mass analysis of complex polymers. In particular, it shall be demonstrated that matrices can be found that are applicable to a wide variety of different polymer systems. For a number of applications it is useful to combine liquid chromatographic separation with mass spectrometric detection. It will be shown that MALDI-TOF mass spectrometry can be efficiently used as an off-line detector. Finally, the application of pulsed extraction fields for improving the resolution in MALDI-TOF will be discussed.

Functionality and molar mass analysis of complex polymers

The key feature of the MALDI process is, that the sample to be investigated is embedded in a matrix material, which absorbs the laser energy and is responsible for the transfer of the sample molecules into the gas phase. The matrix has to meet the following requirements: high absorption coefficient at the laser wavelength (typically 337 nm), good solubility in the sample solvent, good miscibility with the sample in the solid state. Due to the fact, that the polymers to be analysed can be very different in polarity [for example polystyrene vs. poly(ethylene glycol)] a selection of different matrices must be available to meet the above mentioned requirements for each particular polymer. Up to now there is no general rule how to select the appropriate matrix for a given polymer.

One matrix which has been shown to be useful for the analysis of nonpolar as well as polar polymers is 1,8,9-trihydroxy anthracene (dithranol). This matrix exhibits absorption maxima at about 250, 285 and 350 nm. Due to its polycyclic aromatic character combined with three hydroxy groups, dithranol is sufficiently soluble in polar (acetone, tetrahydrofuran, hexafluoro propanol) and nonpolar solvents (chloroform, methylene chloride, toluene). Similarly, dithranol is miscible with polymers of different polarity.

The analysis of polystyrenes of different molar masses is presented in Fig. 1. Using dithranol as matrix, good quality spectra are obtained when silver trifluoroacetate is added to the polymer/matrix solution. At sufficient laser power, cations can also be attached by desorption from a probe tip made of an appropriate metal. We used dithranol and a copper target for MALDI-MS measurement of PS, and were able to produce Cu^+-attached PS oligomers. Fig. 1A displays the MALDI-MS spectrum of a PS sample with an average molar mass of 8000 $g{\cdot}mol^{-1}$, using dithranol and a copper target. Base line separated, well resolved signals indicate the Cu^+-adducts of individual oligomers and confirm the repeating unit of polystyrene. Due to the observed m/z values, a butyl function can be identified as the end group of PS chains, showing that butyl lithium was used for the anionic polymerisation of styrene in this case. From MALDI-MS data, a M_w value of 7600 $g{\cdot}mol^{-1}$ was calculated for this sample. Fig. 1B shows the dithranol-assisted spectrum of a PS sample with a given M_p of 67 000 $g{\cdot}mol^{-1}$. AgTFA was used as Ag^+-donor. The main peak around 70 000 shows the distribution of the singly charged PS chains. Besides, doubly charged macromolecules and singly charged clusters are obtained.

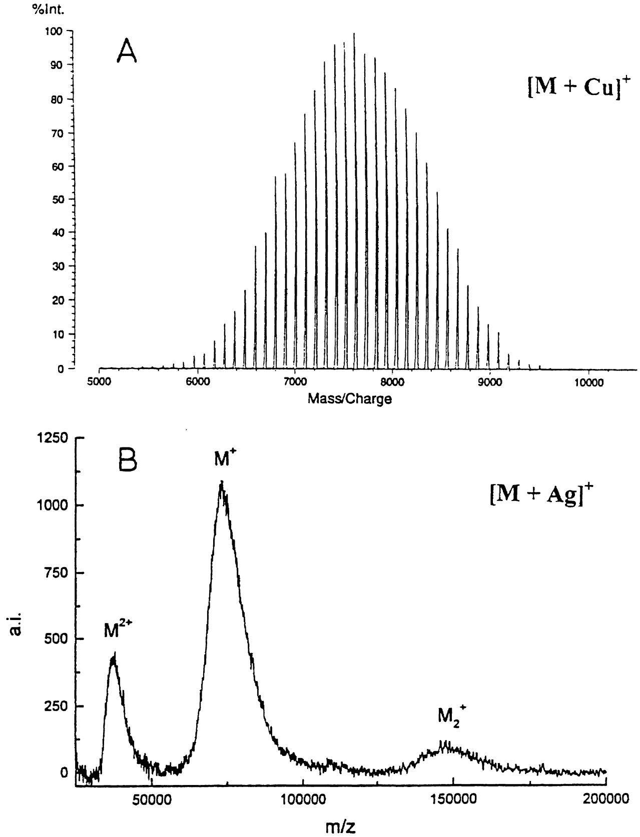
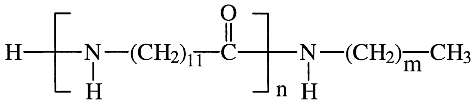


Fig. 1: MALDI-TOF mass spectra of two polystyrene samples, A: Cu^+-attached oligomers are produced by using a copper sample slide; B: AgTFA is used as the Ag^+-donor

Dithranol shows its efficiency also for difficult samples like polyamides, which are normally not soluble in common organic solvents. Spectra can be obtained by using acetic acid and trifluoroacetic acid as solvents. Fig. 2A displays the spectrum of polyamide 12, where trifluoroacetic acid is used as solvent and sodium trifluoroacetate served as Na^+-donor. For this sample, an octadecyl amine function was expected as one chain endgroup. It was known that the octadecyl amine which was used as terminating agent contained 10 to

15 vol.-% hexadecyl amine (manufacturer's data). Accordingly, two homologous series are expected to appear in the MALDI mass spectrum of this sample. For these two end groups, not only Na^+-attached oligomers, but also protonated species are identified in the mass spectrum. Besides, ion signals from Na^+-cationized and protonated cyclic oligomers can be observed in the spectrum. The following structures can be identified by the MALDI-MS analysis of this sample (see numbered signals in the inset of Fig. 2A).

M



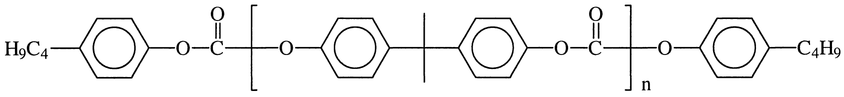
$$H\left[\underset{\displaystyle H}{N}-(CH_2)_{11}-\overset{\displaystyle O}{\overset{\|}{C}}\right]_n\underset{\displaystyle H}{N}-(CH_2)_m-CH_3$$

m = 15, 17

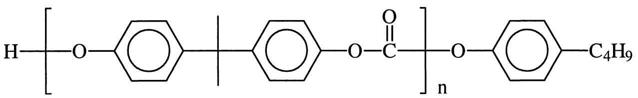
1:	M (octadecyl) + Na^+	1a:	M (octadecyl) + H^+
2:	M (hexadecyl) + Na^+	2a:	M (hexadecyl) + H^+
3:	M (cyclus) + Na^+	3a:	M (cyclus) + H^+

The usefulness of dithranol for analysing further polar polymer classes can be shown by introducing bisphenol A-polycarbonate to MALDI-MS, see results displayed in Fig. 2B. In this case, LiCl was used for cationisation. The spectrum shows well resolved, base line separated ion signals of Li^+-attached oligomers, providing information about molar mass, repeating unit and end group in each case. The following structures are identified by comparing the expected and the resulted m/z values:

1



2



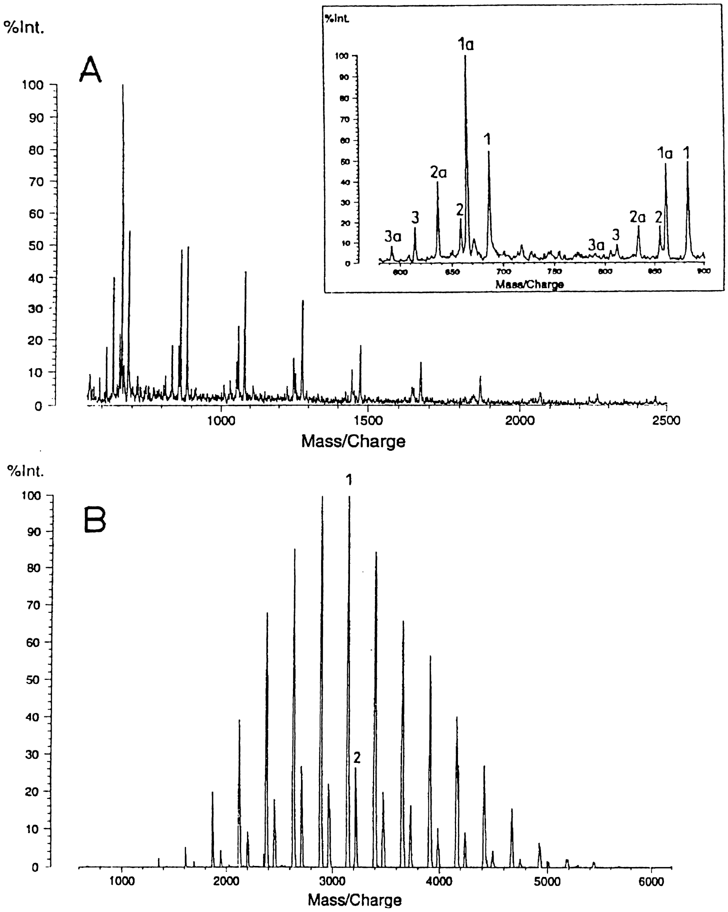


Fig. 2: MALDI-TOF mass spectra of polyamide-12 (A) and bisphenol-A-polycarbonate (B)

Furthermore, good quality spectra can be obtained for poly(dimethyl siloxane), polybutadiene, polyisoprene, poly(alkylene glycol), polytetrahydrofuran, aliphatic and aromatic polyesters, poly(phenylene oxide) and carbohydrates such as inuline using dithranol as matrix.

MALDI-TOF as a detector in liquid chromatography

The combination of liquid chromatography and MALDI-TOF-MS is of great value for polymer analysis. In particular, for chemically or functionally heterogeneous polymers liquid chromatography can provide separation with respect to chemical composition, while MALDI-TOF can analyse the fractions with respect to oligomer distribution or molar mass. Unfortunately, MALDI-TOF is based on the desorption of molecules from a solid surface layer and is, therefore, *a priori* not compatible with liquid chromatography. In an attempt to take advantage of the MALDI-TOF capabilities, a number of research groups carried out off-line LC separations and subjected the resulting fractions to MALDI-TOF measurements. Although this is laborious, it has the advantage that virtually any type of chromatographic separation can be combined with MALDI-TOF.

The different options for using MALDI-TOF as an off-line detector in liquid chromatography have been discussed by Pasch and Rode17). In SEC of low molar mass samples, the separation into individual oligomers and the quantitative determination of the molar mass distribution via an oligomer calibration can be achieved. The SEC separation can be conducted at the usual analytical scale and the oligomer fractions are collected, resulting in amounts of 5-20 ng substance per fraction in the eluent. The solutions are directly mixed with the matrix solution, placed on the sample slide and subjected to the MALDI experiments. As a large number of fractions may be introduced into the mass spectrometer at one time, sample preparation and MALDI-MS measurements take a very short period of time.

The analysis of a methoxylated melamine-formaldehyde resin by SEC and MALDI-TOF is shown in Fig. 3. Such resins are prepared by condensation of melamine and formaldehyde in the presence of methanol. The products are polydisperse with respect to molar mass and the number of functional groups ($-CH_2OH$, $-CH_2OCH_3$). The SEC separation yields oligomer fractions C-G and a not resolved higher molar mass part. The oligomer fractions are subjected to MALDI-TOF and give well resolved mass spectra, which can be analysed with respect to degree of polymerization (n) and functional heterogeneity. As can be seen, fractions D-G are rather homogeneous in chain length. However, within each oligomer fraction up to 15 different mass peaks are obtained, clearly indicating the functional heterogeneity of the oligomers. These mass peaks result from oligomers having different numbers of hydroxymethyl and methoxymethyl groups bound to the oligomer chain. The assignment of the oligomer fractions C-G to a certain degree of polymerization is then used for constructing a melamine-based calibration curve for the SEC, while the detailed analysis of the MALDI-TOF spectra gives information on the functionality type distribution.

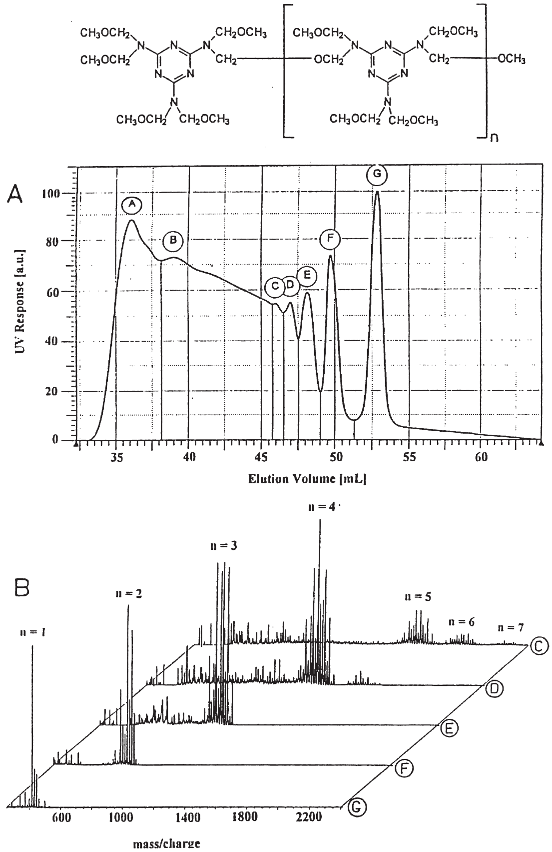


Fig. 3: Size exclusion chromatogram (A) and MALDI-TOF spectra (B) of a methoxylated melamine-formaldehyde resin

The analysis of carboxy-terminated polystyrenes by combination of gradient HPLC and MALDI-TOF mass spectrometry has been recently described by Braun, Henze and Pasch[18].

Using silica gel as the stationary phase and THF-hexane as the eluent, samples are separated with respect to the number of carboxy groups, see Fig. 4. While fraction 1 has a carboxy functionality of f=0, the subsequent fractions 2, 3, and 4 can be assigned to f=1, f=2, and f>2, respectively. The MALDI-TOF analysis of the functionality fractions reveals that in addition to the distribution in carboxy functionality other functional group distributions are present. These result in up to three different oligomer series in each fraction 1-4.

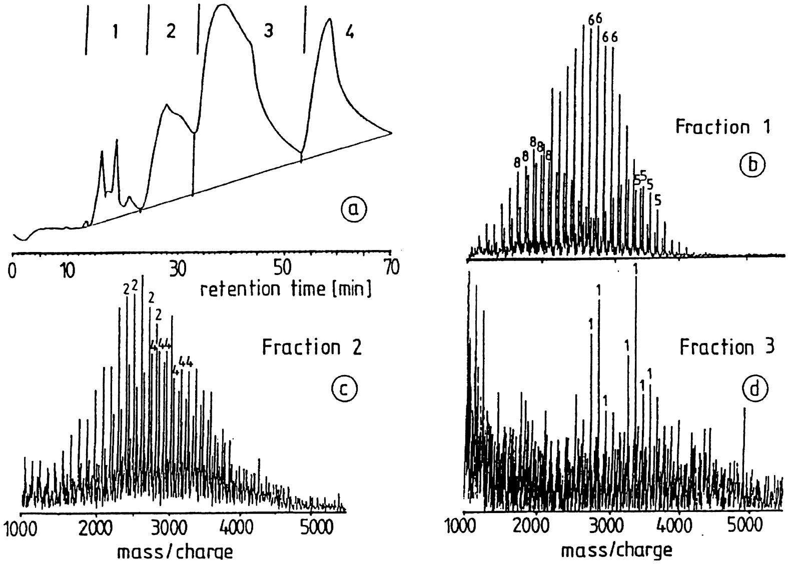


Fig. 4: Gradient HPLC chromatogram (a) and MALDI-TOF spectra (b,c,d) of a carboxy-terminated polystyrene

Other examples of successful combinations of liquid chromatography and MALDI-TOF were given in literature [19-22].

Considering the potential of MALDI-TOF in terms of versatility and sensitivity, on-line coupling with liquid chromatography would be a highly attractive possibility. Given the experiences with the direct introduction of small matrix-containing liquid streams into high-vacuum instruments, it took surprisingly long before a device for liquid introduction to MALDI was described. In some recent papers Murray et al. discussed the on-line coupling of SEC and MALDI-TOF-MS[23-25)]. In an aerosol MALDI/SEC experiment, the effluent from the SEC column was combined with a matrix solution and sprayed directly into a TOF-MS. The ions were mass separated in a two-stage reflectron TOF instrument, and well resolved MALDI-TOF spectra were obtained from commercial PEG 1000 and PPG 1000.

Delayed extraction MALDI-TOF

Key instrumental parameters of MALDI-TOF mass spectrometry are mass resolution and mass accuracy which allow the separation of single oligomers and endgroup determination.

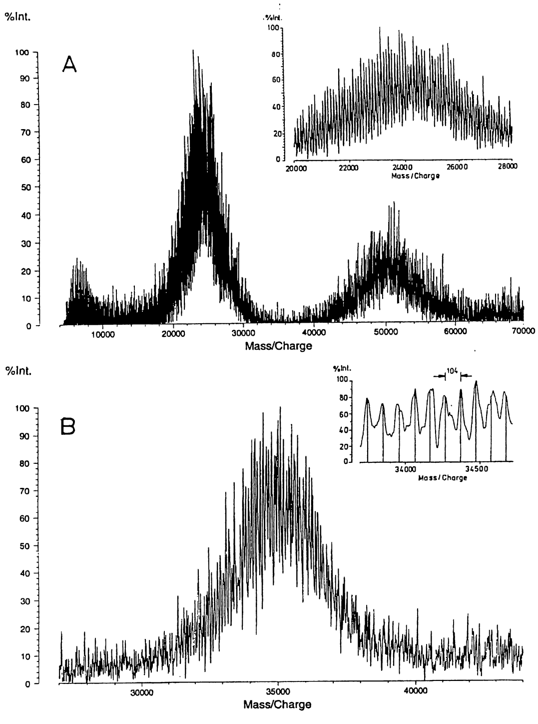


Fig. 5: Delayed extraction MALDI-TOF spectra of a PMMA (A) and a polystyrene (B)

For conventional instruments resolution of single oligomers is limited to approx. 15 000 g·mol^{-1}. Factors for the limited mass resolution include the temporal spread of the laser pulse and the initial velocity and spatial distribution of the ions. Other factors like the ion formation time and the occurrence of collisions can also contribute to this problem$^{26)}$.

The use of time-dependent accelerating fields, originally introduced by Wiley and Mc Laren$^{27)}$ can considerably improve the resolution ($m/\Delta m$) of TOF instruments. The technique involves the introduction of an appropriate time-delay between the ion formation and ion acceleration. In this way, correction for the initial ion velocity distribution is achieved.

A number of groups have reported on dramatic improvements in resolution and quality of MALDI mass spectra by application of time-delayed ion extraction techniques $^{26,28-33)}$. For example, PMMA and polystyrene with peak maximum molar masses above 20 000 $g \cdot mol^{-1}$, give well resolved oligomer distributions, see Fig. 5. While without delayed ion extraction a "polymer hump" is obtained, the mass resolution with a time-delay is still sufficient to obtain well resolved oligomer peaks with the appropriate peak-to-peak mass increments.

Acknowledgement

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