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Comparison of Matrix Assisted Laser Desorption/ Ionization Mass Spectrometry with Electrospray Ionisation Mass Spectrometry for the characterisation of semi-telechelic polyethylene oxide

Julien De Winter,[1,2] Magali Vachaudez,[2] Olivier Coulembier,[2] Philippe Dubois,[2] Robert Flammang,[1]Pascal Gerbaux[1]*

[1*]Mass Spectrometry Research Group, Centre Interdisciplinaire en Spectrométrie de Masse (CISMa), University of Mons – UMons, Place du Parc 20, Mons, 7000, Belgium; e-mail : Pascal.Gerbaux@umons.ac.be

[2]Center of Innovation and Research in MAterials and Polymers (CIRMAP), Laboratory of Polymeric and Composite Materials, University of Mons – UMons, Place du Parc 20, Mons, 7000, Belgium.

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Abstract: MALDI-MS methodology is nowadays considered as a very reliable technique to determine the chemical structures and the molecular weight distribution of a synthetic polymer. At variance, ESI-MS occupies only a small segment in the analysis of polymers mainly because of the formation of multiple charged ion distributions, which interfere with the molecular distribution in the mass spectra of synthetic polymers. In the present communication, we want to demonstrate that, when applied to fragile polymer samples, both techniques can lead to misinterpretation since in source decomposition of the ionized oligomers can sometimes not be avoided. An accurate knowledge of the source parameters and their influences is therefore a prerequisite prior to start the analysis of the MS data. Moreover, we would like to propose that the best MS methodology to analyze fragile synthetic polymers is to associate the data obtained by both the MALDI-MS and ESI-MS techniques.

Keywords: MALDI, ESI, Time of Flight, MSMS, Synthetic polymers characterization

Introduction

Polymers are intrinsically complex materials characterized by different distributions, amongst which the average molecular weights M_n and M_w are the most commonly examined. In practice no single method can be used to investigate all these distributions. Size Exclusion Chromatography (SEC) is a very successful method to measure molecular weight and molecular weight distributions of polymer samples when calibrated with proper standards and used with appropriate detectors [1]. Nevertheless, no information regarding the oligomer repeat unit and the nature of the end groups can be obtained starting from a SEC analysis. Nuclear Magnetic Resonance (NMR) methodology can also afford valuable pieces of information but the determination of the number average molecular weight relies on the preliminary knowledge of the nature of both the repeat unit and the end-groups. Moreover, NMR analyses are only reliable when dealing with polymer samples of high purity.

Because of its high sensitivity, broad dynamic range, specificity and selectivity, mass spectrometry can be intuitively considered as the ideal tool for the determination of the structure of organic polymeric materials [2]. Two significant developments in ionization techniques in the late 1980s – namely Matrix-assisted Laser Desorption/Ionization (MALDI) [3] and Electrospray Ionization (ESI) [4] – greatly enhanced the use of mass spectrometry for polymer characterization. These techniques enable ionization of large non volatile molecules with little or no fragmentation, thus enabling the measure of the molar mass of intact polymers by mass spectrometry (MS).

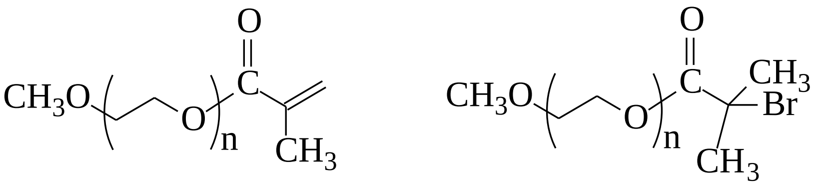
The MALDI methodology involves embedding the polymer under investigation in a matrix which strongly absorbs at the wavelength of the laser. The transfer of the energy from the matrix to the analyte induces the desorption and the subsequent ionization of the isolated oligomer chains. The ionization mechanisms are still under debate and consequently are a topic of active research [5]. In the context of the present work, we just would like to emphasize that the strength of MALDI-MS for synthetic polymers analysis mainly lies in the simplicity of the recorded mass spectra, which contain primarily singly-charged ions [6]. Despite the success of this technique, several drawbacks have been encountered when trying to measure the average molecular weights of polymer materials [2]. First of all, molecular mass determination by MALDI-MS depends on how accurately the ion abundance over the mass range represents the composition of the polymer. Indeed, the efficiency of the desorption/ionization processes should be independent of the chemical composition of the polymer and the masses of the oligomers, if accurate average molecular mass values are the aim of the analysis. In addition, the ions produced must be transmitted and detected without any mass discrimination in order to adequately represent the abundance of the observed ions. Everything together, the intensity of each peak can be considered as representative of the molar concentration of the particular molecular species if discrimination in ionization, transmission and detection is avoided. These requirements are fulfilled for narrow-distribution polymers (polydispersity index < 1.2), for which molecular masses and molecular mass distributions are accurately determined [7]. For polydisperse polymers, given the fact that mass discrimination is often really pronounced, the determination of the average molecular weights is totally not relevant. Nevertheless, state of the art MALDI-MS instruments, associating the MALDI source with a high resolution and high mass accuracy mass analyzer, nowadays allow the qualitative determination of the chemical composition of ionized oligomers, including end-groups identification, whatever the polydispersity of the polymer.

Electrospray Ionization (ESI) denotes the overall process by which an intense electric field transforms a solution in polar solvent in a fine spray constituted by highly charged droplets [4]. The subsequent evaporation of these charged droplets produces *in fine* gas phase ions that are driven to the mass analyzer. Although ESI-MS has become one of the most widely method used in analysis [4], it is interesting to note that polymer molecular mass and structural analysis by ESI-MS has been less fruitful than MALDI-MS and is not considered as the right choice to characterize polymer materials by mass spectrometry methodologies. As for a practical explanation, the insolubility of polymers in ESI-compatible polar solvents sometimes prohibits ESI analysis [8]. This is of course at variance with the MALDI-MS methodology that only requires the dissolution of the polymer in a suitable solvent [7]. The unique ability of ESI to generate multiply charged ions facilitates the detection of higher mass species, even for analyzers with a limited mass range. Unfortunately, this specificity of the ESI source over the MALDI source is often considered as one of the main disadvantage of the ESI-MS methodology, since even for polymers with narrow polydispersity, the result of multiple charging gives rise to very complex mass spectra [9]. Moreover, the lack of resolution when using a common mass spectrometer prevents the analyst from identifying the charge state of the detected ions. Consequently, determination of the real mass of the observed species could be quite problematic. This is particularly verified in the context of mass spectrometry analysis of high mass polymers since, for technical reasons, mass analyzers that offer high resolution capabilities often propose quite a limited mass range.

In the present paper, we would like to present MS results obtained when analyzing semi-telechelic polyethylene oxide polymers by MALDI and ESI-ToF mass spectrometry. In particular, we would like to demonstrate that MALDI and ESI results must be handled with great care since both methodologies afford data that must be carefully analyzed in the light of the source parameters.

Results and discussion

The samples used in the present work were low mass semi-telechelic poly(ethylene oxide) based polymers, namely α-methoxy, ω-methacrylate poly(ethylene oxide) (**PEOMA**) and α-methoxy, ω-bromoisobutyrate poly(ethylene oxide) (**PEOBr**), presented in Scheme 1.



PEOMA **PEOBr**

Scheme 1. Molecular structure of PEOMA and PEOBr.

For the sake of comparison, the average molecular mass and the molecular mass distributions of both polymers have first been characterized by SEC or ^{1}H-NMR.

PEOMA has been characterized by ^{1}H-NMR (300 MHz, in $CDCl_3$) spectroscopy and a M_n of 530 g.mol^{-1} was determined, whereas, for **PEOBr**, a M_n SEC of 700 g.mol^{-1} was measured.

1° MALDI-ToF analyses of PEOMA and PEOBr

Both **PEOMA** and **PEOBr** polymer samples were analyzed by MALDI-ToF mass spectrometry in exactly the same experimental conditions as far as the source conditions - matrix, sample deposition, laser fluence - are concerned. The recorded mass spectra are presented in Figures 1 and 2.

The MALDI mass spectrum of **PEOMA**, presented in Figure 1, features two distinct ion series that are readily assigned to **PEOMA** oligomers, mainly cationized by a sodium ion (●) or a potassium ion (**o**).

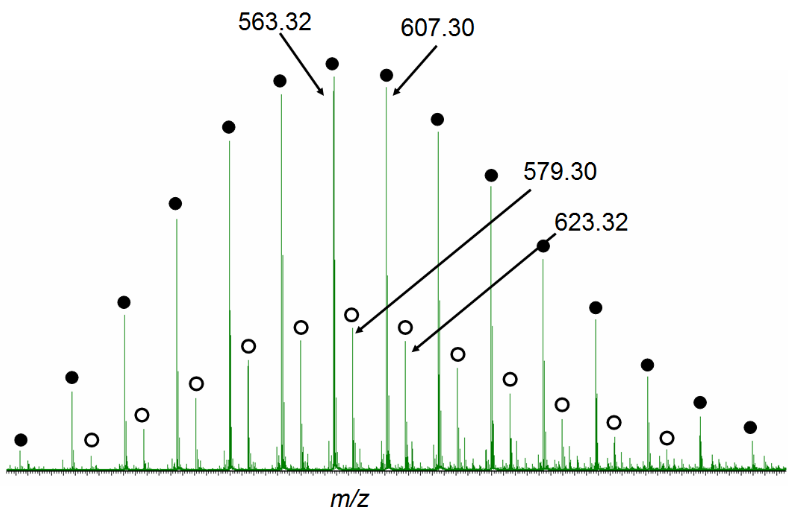


Fig. 1. PEOMA MALDI-ToF analysis (M_n NMR 530 g.mol^{-1}) using α-cyano-4-hydroxycinnamic acid as the matrix and a laser fluence of 350 J.m^{-2} : global mass spectrum.

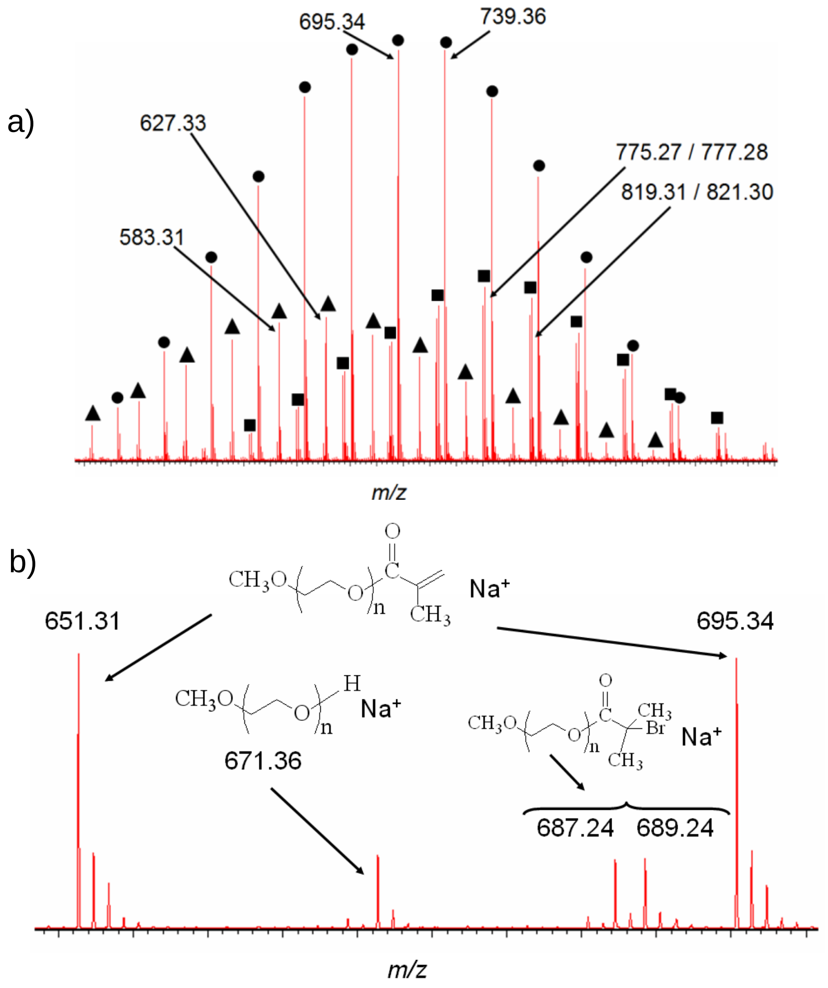


Fig. 2. MALDI-ToF analysis of PEOBr (M_n SEC 700 g.mol^{-1}) using α-cyano-4-hydroxycinnamic acid as the matrix and a laser fluence of 350 J.m^{-2} : (a) global mass spectrum and (b) partial view from *m/z* 650 to 700.

Hence, the polymer base peak being at *m/z* 563.32 corresponds to a molecule with a correct molecular weight of 540.33 Da. The polymer distribution was then determined on the basis of the recorded spectrum (Figure 1) and the calculated values of M_n and M_w amounts to respectively 546 and 563 leading to a calculated polydispersity index (PDI or M_w/M_n) of 1.03. The obtained parameters are in close agreement with data derived from other methodologies, e.g. ^{1}H-NMR (M_n ^{1}H-NMR = 530 g.mol^{-1}), confirming then the reliability of the MALDI-MS measurements in the case of low PDI polymer samples. For the sake of information, in the case of broad polymer distributions, it is now advised to combine on-line or off-line mass spectrometry with, for instance, SEC fractionation [10].

The MALDI-ToF mass spectrum of **PEOBr** is presented in Figure 2a and appears more complicated to analyze. Indeed, at least, three ion series can be observed in the recorded spectrum. The by far major ion series surprisingly corresponds to Na-cationized **PEOMA** oligomers (● in Figure 2a). This observation was unexpected since as attested by ^{1}H-NMR (not show here) no **PEOMA** polymer is present in the **PEOBr** sample at least within the experimental error of the NMR technique. As exemplified in Figure 2b, both other minor ion series correspond either to Na-cationized α-methoxy, ω-hydroxyl poly(ethylene oxide) (PEO, see for instance *m/z* 671.36 and ▲ in Figure 2a) or the expected Na-cationized PEOBr (■ in Figure 2a). The MALDI QToF Premier instrument used in this work allows the clear-cut resolution of the isotopic pattern and the subsequent identification of both the isotopes of bromine atom, namely ^{79}Br (50.7% natural abundance) and ^{81}Br (49.3 %). Nevertheless, at this stage, a misinterpretation of the MS data could lead to the conclusion that the polymer sample is mainly constituted by **PEOMA**, with some traces of **PEO** and **PEOBr**.

In order to obtain additional pieces of information on the **PEOBr** sample, we turned to an ionization technique softer than the MALDI methodology by using an Electrospray ionization.

2° ESI-ToF analysis of PEOBr

The ESI-ToF mass spectrum of the **PEOBr** sample (cone voltage : 20 V) is presented in Figure 3a and is dominated by an ion series that is unambiguously attributed to singly charged Na^+-cationized **PEOBr** oligomers (■). At variance with the MALDI spectrum depicted in Figure 2a, no characteristic **PEOMA** signals are detected. The maximum of this **PEOBr** ion distribution is observed at *m/z* 599-601 and corresponds to an oligomer constituted by 8 repeating units and cationized by a sodium ion. Beside this major ion series, **PEOBr** oligomers are also observed in the mass spectrum cationized by two sodium ions (♦). The maximum of this doubly charged ion series is detected at *m/z* 422.15 and corresponds to an oligomer constituted by 14 repeating units. The difference between the maxima of the singly and doubly charged ion distributions (**8 *vs* 14**) is readily explained since a longer oligomer can stabilize more sodium ions than a shorter one [11]. The third observed population (▲) is attributed to Na-cationized **PEO**.

In the present case, since the ESI-ToF spectrum features intense signals for **PEOBr** oligomers, the ESI-ToF methodology seems then *a priori* more suitable for the identification and the characterization of the **PEOBr** sample than the MALDI experiment. This assumption is no longer verified when increasing the cone voltage parameter. Indeed, the ESI mass spectrum presented in Figure 3b was obtained at a cone voltage value of 70 V. Clearly, beside the ion series characteristic of the **PEOBr** polymer (■), the spectrum is now dominated by ions readily identified as Na^+-cationized **PEOMA** oligomers (●) that originates from gas phase decomposition of Na^+-cationized **PEOBr** oligomers. Indeed, as proposed in Scheme 2, the loss of a molecule of HBr from **PEOBr.Na$^+$** cations is likely to afford **PEOMA.Na$^+$** ions that are mass-measured and detected by the mass spectrometer instead of the decomposed **PEOBr.Na$^+$** cations.

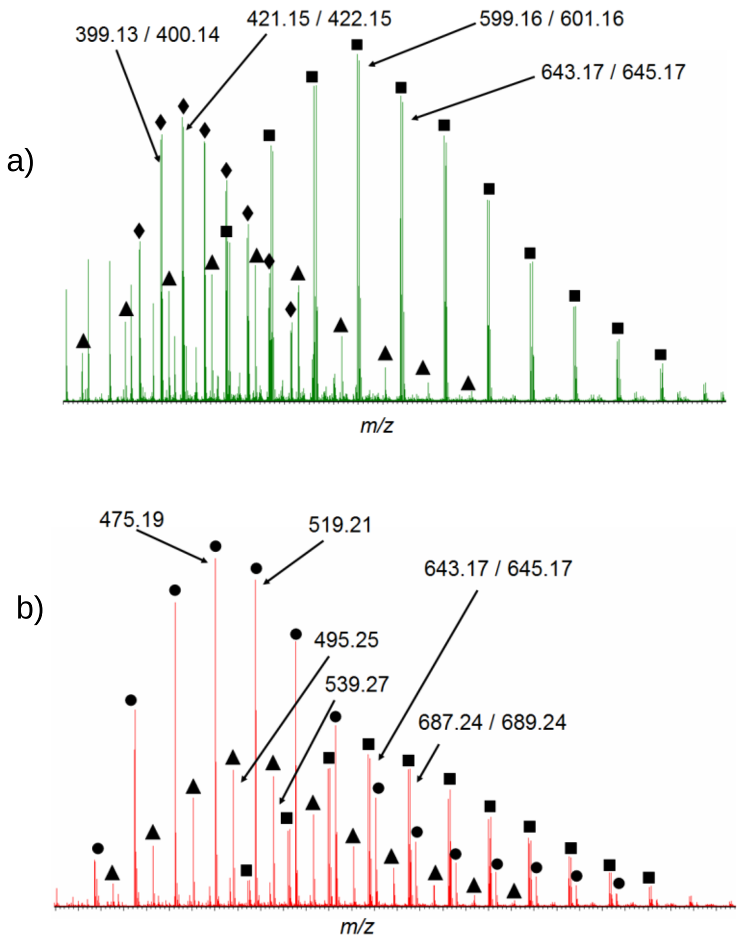
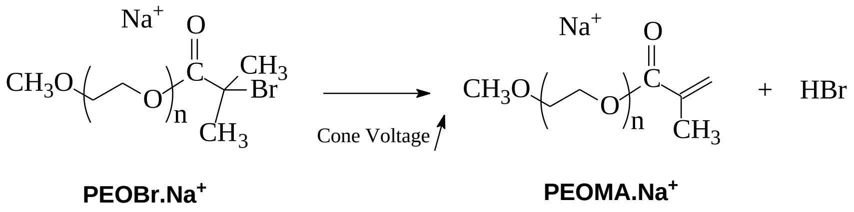


Fig. 3. ESI-ToF analysis of PEOBr (M_n SEC 700 $g.mol^{-1}$) : (a) global mass spectrum recorded at 20 V and (b) recorded at 70 V cone voltage.



Scheme 2. In Source collision-induced HBr elimination from Na^+-cationized PEOBr.

An experimental demonstration that the observed **PEOMA-Na$^+$** ions originate from the dissociation route depicted in Scheme 2 is obtained by measuring the collision-induced dissociation (CID) spectrum of **PEOBr-Na$^+$** ions. Indeed, when submitted to collision against argon in the collision cell of our hybrid QToF instrument, mass-selected **PEOBr-Na$^+$** ions suffer from a fast HBr loss exclusively leading to **PEOMA-Na$^+$** ions, see Figure 4.

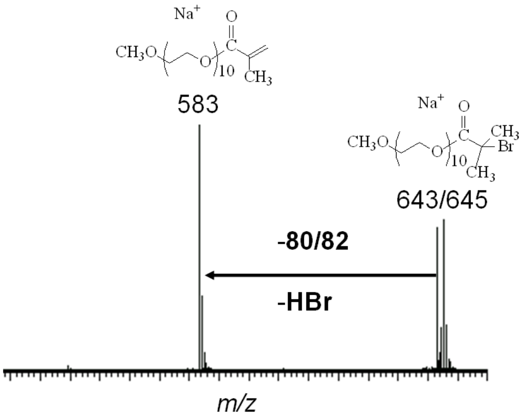


Fig. 4. CID mass spectrum of *m/z* 643/645 at 25 eV.

Such a process is also expected to occur inside the ion source of the mass spectrometer when increasing the kinetic energy of the ions by increasing the cone voltage. This voltage is a component-dependent ESI source parameter that must be optimized during an ESI analysis towards a value that corresponds to the maximum ion currents for the analyte peaks [4]. However, increasing the cone voltage gives the ions more kinetic energy in the ion source and consequently collisions between ions and residual gas molecules are likely to induce decomposition of the ions [4]. Gas phase decomposition reactions of collision-excited ions are complex unimolecular processes that can be described by the use of several basic theories [12]. Indeed, a given fragmentation reaction is defined by a threshold energy, E_0, that corresponds to the minimum energy needed for the decomposition reaction to occur. Basically, the fragment ions will be observed if the rate constant (*k* in Scheme 3a) of the corresponding decomposition reaction reaches a certain point that depends on the instrument characteristics. The RRKM (Rice–Ramsperger–Kassel–Marcus) [12] theory provides an expression describing the internal energy-dependent rate constant for a fragmentation reaction; see Scheme 3(a).

$$k = \nu \left[\frac{E - E_0}{E} \right]^{N-1} \qquad E_{CM} = E_{lab} \left[\frac{M_{gas}}{M_{gas} + M_p} \right]$$

(a) (b)

Scheme 3. (a) RRKM equation and (b) center-of-mass equation [12].

In the presented expression, *k* corresponds to the rate constant of the gas phase unimolecular decomposition, *N* represents the number of vibrational degrees of freedom, E stands for the internal energy obtained by the ions upon activation and υ is a frequency factor that depends on the decomposition mechanism. The RRKM theory relies on several assumptions. In the context of the present discussion, it is important to emphasize that it is *inter alia* assumed that the statistical partition of the internal energy excess over all the vibrational degrees of freedom is faster than the dissociation reactions. This aspect is definitively relevant when discussing the fragmentation processes of polymer ions since, for a given internal energy (E), the available energy per vibrational degrees of freedom is decreasing with the increase of the monomeric unit number within a given ion series.

As far as the excess of internal energy deposited in ions after the inelastic collisional event is concerned, the total available energy for the transfer of kinetic energy to internal energy is the so-called center-of-mass energy (E_{CM}) and depends on the collision partners' masses through the expression in Scheme 3(b). In this expression, E_{lab} is the kinetic energy of the ion prior to the collision, whereas M_{gas} and M_p represent the masses for the neutral gas and the precursor ion, respectively. Again, this aspect determines the mass-dependent extent of decomposition for ionized oligomers for a given cone voltage. Indeed, for increasing masses, the center-of-mass energy is decreasing and then high mass ions are less prone to decomposition than low mass ions. This discrepancy is moreover reinforced since higher mass ions possess more vibrational degrees of freedom.

All those aspects determine the distribution of ionized oligomers in an Electrospray mass spectrum for a given cone voltage.

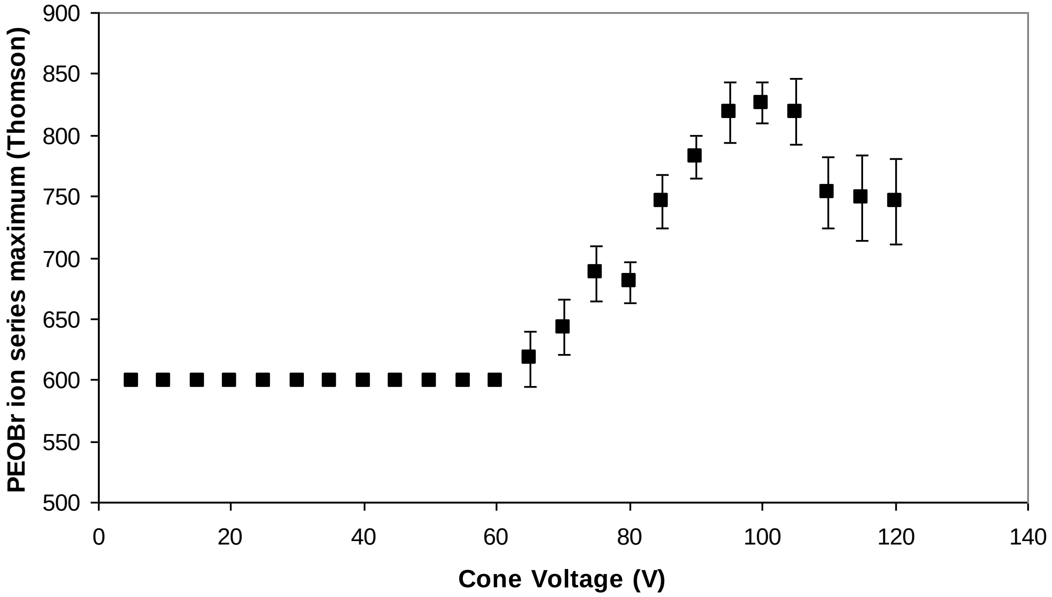


Fig. 5. ESI-ToF analysis of PEOBr (M_n SEC 700 g.mol^{-1}): influence of the cone voltage (V) on the PEOBr ion series maximum.

This is clearly observed in Figure 5 where the dependence of the cone voltage on the maximum of the **PEOBr** ion distribution is depicted. Indeed, the maximum of the ion series distribution is gradually moved towards larger *m/z* values when increasing the cone voltage. This is clearly explained by the fact that the decomposition reactions of the low mass ions are faster than the fragmentation reactions of the high mass ions. As a consequence, the low mass ions are progressively removed from the ion distribution and the maximum of the distribution is shifted towards higher *m/z* ratios. Nevertheless, when the energy transferred to the ions reaches a certain point, the maximum of the distribution seems to reach a stage before going back to smaller mass to charge ratios. This observation can be explained by the fact that, at the corresponding cone voltages, the rate constants for the decomposition reactions of all the ions are high enough to allow the decomposition of the high mass ions to be observed. Basically, we can then imagine that the initial ion distribution could be recovered, except that, upon such high energy conditions, low mass ions are definitively excluded from the distribution and contribute to the PEOMA.Na^+ distribution.

Conclusions

MALDI-MS methodology is nowadays considered as a very reliable technique to determine the chemical structures and the molecular weight distribution of a synthetic polymer. ESI-MS occupies only a small segment in the analysis of polymers mainly because of the formation of multiply charged ion distributions, which interfere with the molecular distribution in the mass spectra of polymers. In the present communication, we wanted to demonstrate that, when applied to fragile polymer samples, both techniques can lead to misinterpretation since in source decomposition of the ionized oligomers can sometimes not be avoided. The knowledge of the source parameters and their influences is therefore a prerequisite prior to start the analysis of the MS data. Moreover, we would like to propose that the best MS methodology to analyze synthetic polymers is to associate the data obtained by both the MALDI-MS and ESI-MS techniques.

Experimental

Materials

α-Methoxy,ω-methacrylate poly(ethylene oxide) (PEOMA, Aldrich, M_n ~475 g.mol^{-1}) was used as received. α-Methoxy,ω-bromoisobutyrate poly(ethylene oxide) (PEOBr, M_n ~700 g.mol^{-1}) was synthesized according to a procedure described in the literature [13].

Techniques: MALDI-ToF, ESI-ToF & Size Exclusion Chromatography (SEC)

MALDI mass spectra were recorded using a Waters QToF Premier mass spectrometer equipped with a nitrogen laser, operating at 337 nm with a maximum output of 500 J/m^2 delivered to the sample in 4 ns pulses at 20 Hz repeating rate. Time-of-flight mass analysis were performed in the reflectron mode at a resolution of about 10 000. All the samples were analyzed using α-cyano-4-hydroxycinnamic acid (α - cyano), that matrix was prepared as 10 mg/mL solution in acetone. The matrix solution (1 µL) was applied to a stainless steel target and air dried. Polymer samples were dissolved in acetonitrile to obtain 1 mg/mL solutions. 1µL aliquots of these solutions were applied onto the target area already bearing the matrix crystals, and then air dried. This procedure (air drying) is used to prevent thermal degradation of the fragile polymer chains to occur already during the sample preparation. Finally, 1µL of a solution of NaI (2 mg/mL in acetonitrile) was applied onto the target plate.

Electrospray mass spectra were recorded using a Waters QToF2 mass spectrometer. The polymer solutions (1 mg.mL^{-1} in acetonitrile / 2 mg.mL^{-1} NaI in acetonitrile : 1 / 1) were delivered to the ESI source by a Harvard Apparatus syringe pump at a flow rate of 5 µL.min^{-1}. Typical ESI conditions were : capillary voltage, 3.1 kV ; cone voltage, 20 – 105 V ; source temperature, 80 °C ; desolvation temperature, 120 °C. Dry nitrogen was used as the ESI gas.

Size Exclusion Chromatography (SEC) was performed in tetrahydrofuran on a Waters chromatograph equipped with four 5 µm Waters column (800 mm X 7.7 mm) connected in series with increasing pore size (10, 100, 1000, 10^5, 10^6 Å).

Poly(ethylene oxide) samples of known molecular weight were used as calibration standards. A Waters 410 Differential refractometer and 996 photodiode away detector were employed.

^{1}*H-NMR* spectrum was recorded on a 300 MHz spectrometer, with chemical shifts reported in parts per million downfield from tetramethylsilane (^{1}H-NMR) used as internal reference. The sample was prepared by dissolution of 30 mg in 0.6 mL of $CDCl_3$.

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