

# Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric Study of Bis(imidazole-1-carboxylate) Endfunctionalized Polymers

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A detailed Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric (MALDI-TOF MS) investigation of polyethylene glycol (PEG), polypropylene glycol (PPG) and polyisobutylene (PIB) bis(imidazole-1-carboxylate) esters is reported. The MS spectra of PPG and PIB bis(imidazole-1-carboxylate) esters recorded in the reflectron mode showed the presence of two additional series of peaks compared to those recorded in the linear mode, while in the case of PEG bis(imidazole-1-carboxylate) only one additional peak series appeared in the reflectron MS spectra. These additional series were attributed to the formation of fragment ions by the loss of one and two endgroups in the first field-free region of the instrument. The neutral losses for the three polymers were also supported by using the post-source decay method (PSD). The observation of decreasing mass difference between the adjacent peaks from two series of polymeric ions with the increase of oligomer mass indicates the presence of PSD ions in a reflectron MALDI spectrum. The relationship between the mass of the precursor ions and those of the PSD ions and neutral loss are also discussed. (J Am Soc Mass Spectrom 2003, 14, 117–123) © 2003 American Society for Mass Spectrometry

Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [1, 2] (MALDI-TOF MS) has received great consideration as a very sensitive and powerful tool for the characterization of large, nonvolatile molecules. The implementation of the reflectron [3] and delayed extraction [4] into MALDI-TOF mass spectrometers was proven to be effective for the correction of the initial energy distribution of the ions formed by laser desorption, thereby increasing the resolution and mass accuracy.

The extension of MALDI-TOF MS with the so-called “post-source decay” method (PSD) by Spengler et al. [5, 6] has opened the way for the structure determination of synthetic polymers [7–12]. However, there are several problems associated with MALDI-TOF MS. Biomolecules are mostly ionized through protonation, while synthetic polymers form adducts with alkali metal or transition metal ions. Due to the appearance of several peak series in the MS spectrum originating from ionization with different ions, these series may be incorrectly interpreted as polymers carrying different endgroups. Also, MALDI-TOF MS spectra are often recorded in the reflectron mode, and sometimes post-source decay oc-

curs depending on the structure of the polymers and the matrix applied, which may give rise to additional difficulties in the interpretation of the spectra.

In this paper we report a detailed MALDI-TOF MS and PSD MALDI-TOF MS/MS investigation of polyethylene glycol (PEG), polypropylene glycol (PPG), and polyisobutylene (PIB) bis(imidazole-1-carboxylates). These telechelics with imidazole-1-carboxylate endgroups were selected because they represent versatile intermediates for the synthesis of block copolymers and polymeric products [13, 14]. The three types of polymers with roughly the same molecular mass were selected to study the effect of the polarity of the backbone (which strongly decreases in the order PEG > PPG > PIB) in their MALDI-TOF MS spectra. An additional goal of this report is to establish and confirm a detailed relationship between the mass series of the precursor ions and those of the ions formed by post-source decay, which has not been reported for polymers so far.

## Experimental

### Materials

Dichloromethane (DCM) and tetrahydrofuran (THF) from Aldrich (Darmstadt, Germany) were purified as described earlier [15]. Carbonyldiimidazole (CDI), 1,8-

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**Table 1.** The number-average molecular mass ( $M_n$ ) and polydispersity ( $M_w/M_n$ ) of the starting hydroxy-terminated PEG, PPG, and PIB determined by MALDI-TOF MS and SEC

	$M_n$ (g/mol) MALDI <sup>a</sup> /SEC <sup>b</sup>	$M_w/M_n$ MALDI/SEC
PEG	1450/1300	1.02/1.17
PPG	1920/1700	1.02/1.18
PIB	2010/1900	1.03/1.18

<sup>a</sup>The calculations were based on  $M_n = \sum_i M_i / \sum_i I_i$  and  $M_w = \sum_i M_i^2 / \sum_i M_i I_i$  equations, where  $M_i$  and  $I_i$  represent the mass and the intensity of the  $i$ th polymer chain, respectively. The mass of the cation was subtracted before the calculations. Average error  $\pm 2\%$

<sup>b</sup>SEC was calibrated with polystyrene standards. Average error  $\pm 10\%$ .

dihydroxy-9(10H)-anthracenone (dithranol), potassium *t*-butoxide (*t*-BuOK), silver trifluoroacetate (AgTFA), and sodium trifluoroacetate (NaTFA) were also received from Aldrich, and were used without further purification. Polyethylene glycol (PEG) and polypropylene glycol (PPG) were provided by BorsodChem (Kazincbarcika, Hungary) and were used as received. *p*-Dicumyl chloride (pDCC) was prepared from *p*-dicumyl alcohol (Amoco, Chicago, IL) [16].  $\text{BCl}_3$  and dimethyl sulfoxide (DMSO) from Merck (Darmstadt, Germany) were purified by distillation under diminished pressure. Isobutylene received from TIFO (Tiszaújváros, Hungary) was dried by passing it through a column of 50 cm in length and 3 cm in diameter filled with  $\text{CaCl}_2$ , then distilled.

#### Preparation of Dihydroxy Telechelic Polyisobutylene

The living polymerization of isobutylene was initiated using the *p*-dicumyl chloride/ $\text{BCl}_3$  system as described in reference [17]. The dihydroxy telechelic polyisobutylene was obtained from its corresponding dichloro derivative according to reference [18].

The number of average molecular mass ( $M_n$ ) and the polydispersities ( $M_w/M_n$ ) of the starting hydroxy-terminated polymers determined by MALDI-TOF MS and

size exclusion chromatography (SEC) are summarized in Table 1.

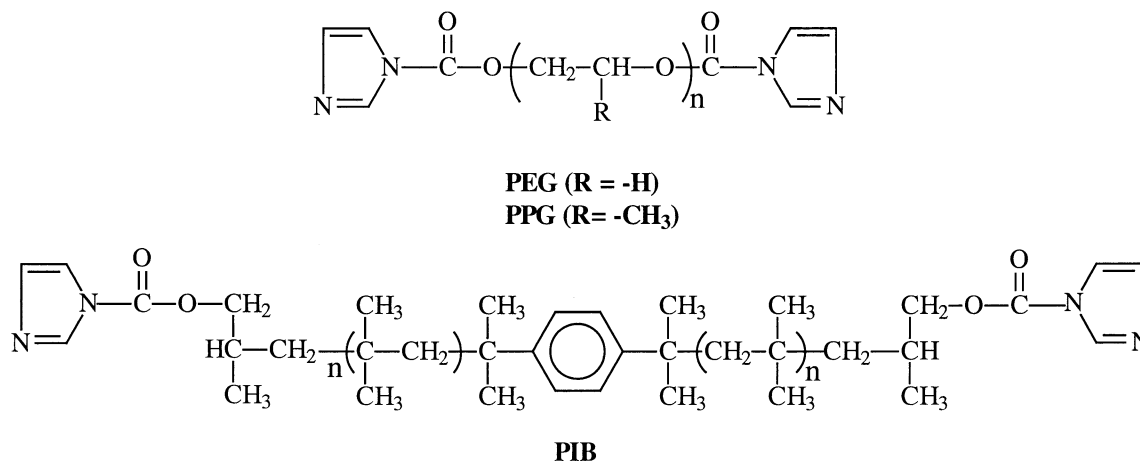
#### Preparation of PEG, PPG, and PIB Bis(imidazole-1-carboxylate) Esters

PEG, PPG, and PIB bis(imidazole-1-carboxylate) esters were prepared as described in reference [19]. The structures of esters synthesized (Scheme 1) were confirmed by  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR.

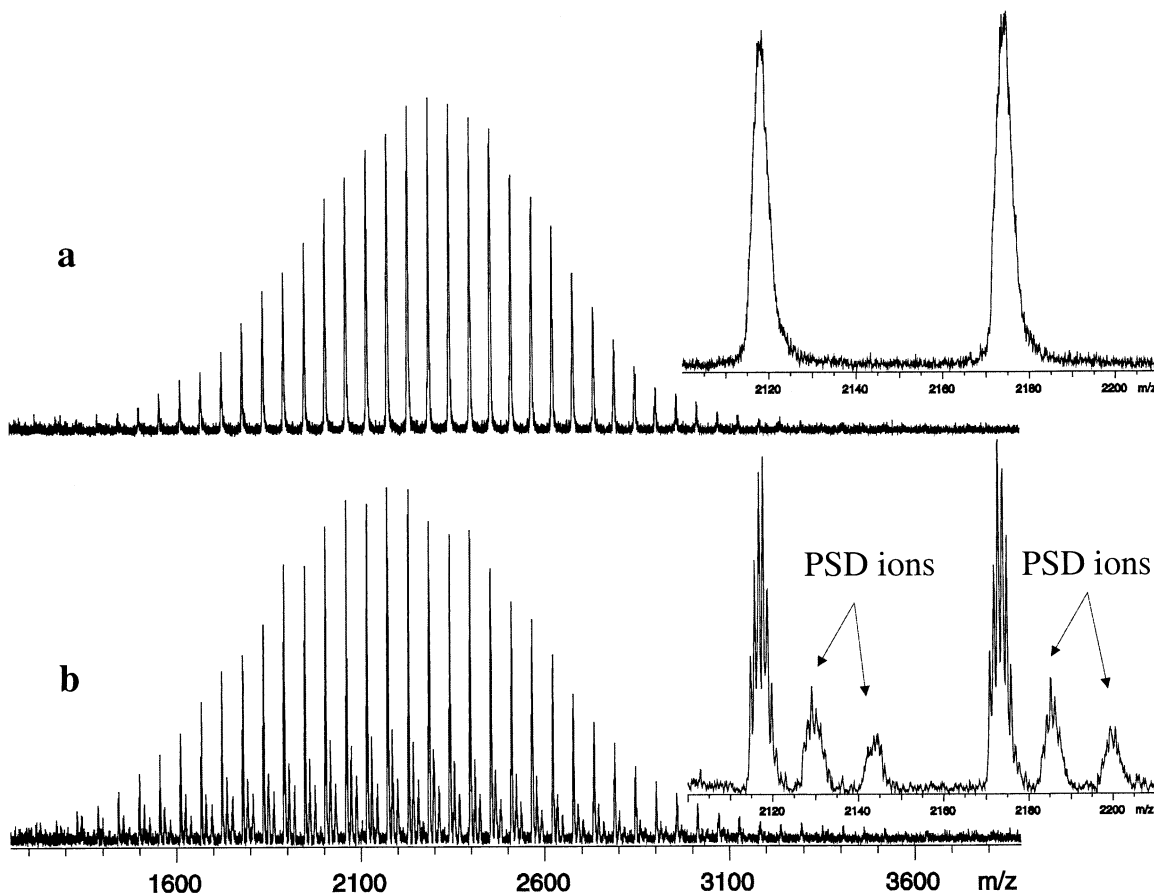
#### Characterization

**MALDI-TOF MS.** The MALDI MS measurements were performed with a Bruker (Bremen, Germany) BIFLEX III mass spectrometer equipped with a TOF analyzer. In all cases 19 kV total acceleration voltage was used with pulsed ion extraction (PIE). The positive ions were detected both in the linear and the reflectron mode. A nitrogen laser (337 nm, 3 ns pulse width,  $10^6$ – $10^7$  W/cm<sup>2</sup>) operating at 4 Hz was used to produce laser desorption and 200–250 shots were summed. Samples were prepared with dithranol matrix dissolved in THF (20 mg/ml). Bulk solutions of the polymers in THF were made at a concentration of 10 mg/mL. To enhance the cationization of PIB, AgTFA in THF (2 mg/mL), and to produce mainly sodiated peaks in the case of PEG and PPG, NaTFA (2 mg/mL) in THF were added to the corresponding matrix/analyte solutions. The solutions were mixed in a 10:2:1 vol/vol ratio (matrix:analyte:cationization agent). A volume of 0.5–1.0  $\mu\text{l}$  of these solutions was deposited onto the sample plate (stainless steel), and allowed to air-dry. The measured and the calculated masses for each corresponding oligomer peaks were within  $\pm 0.2$  Da.

**MALDI-TOF MS/PSD.** All of the PSD spectra were recorded by selection of the precursor ion to be studied using the pulser, allowing an approximately 30 Da window for selection. In each segment of spectrum



**Scheme 1.** Structure of PEG, PPG, and PIB bis(imidazole-1-carboxylate) esters.



**Figure 1.** MALDI-TOF MS spectra of PIB bis(imidazole-1-carboxylate) ester recorded in the linear (a) and in the reflectron (b) mode. The insets are zoomed spectra.

accumulation the reflectron voltage was decreased. The segments were pasted and calibrated using XMASS 5.0 software from Bruker. The PSD was calibrated using the fragmentation pattern of adrenocorticotrophic hormone (ACTH) over the mass range of 60–2450 Da.

**Size exclusion chromatography (SEC).** The  $M_n$  and molecular mass distribution (MMD) of the polymers were measured by SEC in THF at 35 °C with a Waters chromatographic system (Milford, CT) equipped with four gel columns (7  $\mu$ m ultrastylgel columns: 500,  $10^3$ ,  $10^4$ ,  $10^5$  Å), a Waters 600 HPLC pump, and with Waters (Milford, MA) 490E UV and Waters 410 refractive index detectors. The  $M_n$  and  $M_w/M_n$  values of the oligomers were calculated relative to polystyrene.

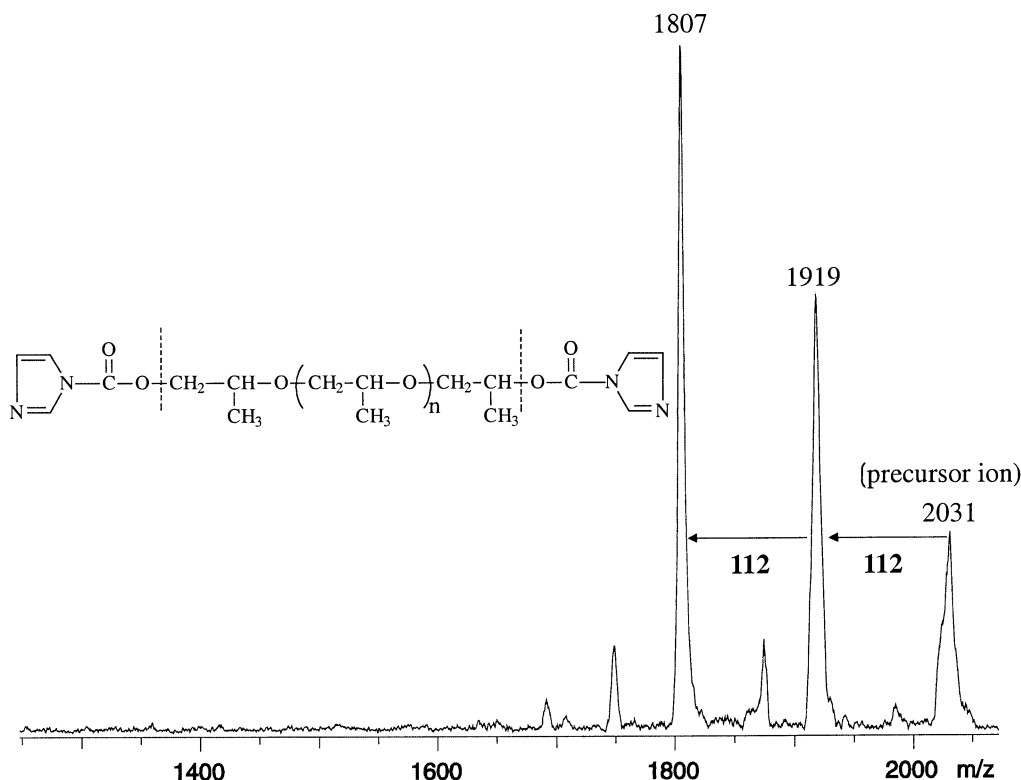
**NMR.** The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded in  $\text{CDCl}_3$  at 25 °C with a Bruker AM 360 spectrometer.

## Results and Discussion

MALDI-TOF MS spectra of PEG, PPG and PIB bis(imidazole-1-carboxylate) esters recorded in the linear mode showed the exclusive presence of oligomers with bis(imidazole-1-carboxylate) endgroups, indicating a

complete conversion of hydroxyl endgroups to imidazole-1-carboxylate termini. However, when the MS spectra were recorded in the reflectron mode, two additional series of peaks appeared in the case of both PIB and PPG bis(imidazole-1-carboxylate) esters, and one additional series of peaks occurred in the case of PEG bis(imidazole-1-carboxylate). Figure 1 shows the MS spectra of PIB accumulated in the linear (Figure 1a) and in the reflectron (Figure 1b) mode. The insets are zoomed spectra in the mass range of 2100–2220 Da.

It is also evident from the MS spectra that the two additional series of peaks are unfocused with respect to the main peaks, which corresponds to the imidazole-1-carboxylate-terminated PIB oligomers. These two poorly focussed series of peaks are attributed to the formation of fragment ions by the loss of one or two endgroups. It should be noted that the fragment ions formed by post-source decay and their precursor ions cannot be differentiated in the linear mode, since both ions have the same flight time, i.e., they occur at the same apparent mass in the linear MALDI-TOF MS spectrum. To determine the mass lost during the post-source decay, PSD MALDI-TOF MS/MS experiments were performed by selecting the precursor peaks to be studied. It should be kept in mind that the determina-



**Figure 2.** PSD MALDI-TOF MS/MS spectra of PPG bis(imidazole-1-carboxylate) esters. The breaking bonds are indicated with dotted lines.

tion of the mass of the PSD ions from the spectrum presented in Figure 1 is not straightforward due to the flight time difference caused by the reflectron. Figure 2 shows the PSD MALDI-TOF MS/MS spectra of a PPG bis(imidazole-1-carboxylate) of  $m/z$  2031.

A successive decrease of mass by 112 Da is observed, which corresponds to the loss of one and two imidazole-1-carboxylic acid molecules. A similar PSD pattern was found for the PIB bis(imidazole-1-carboxylates). However, in the case of PEG bis(imidazole-1-carboxylate) ester, as mentioned above, only one additional series of peaks appeared in the reflectron mass spectrum compared to the linear one. It was also found in the PSD MALDI-TOF MS/MS spectrum of PEG bis(imidazole-1-carboxylate) ester that the intensities of the ions formed by the loss of two imidazole-1-carboxylate termini are much lower than those formed by the loss of one end-group. This is why only one additional series appeared in the reflectron MALDI-TOF MS spectrum of PEG bis(imidazole-1-carboxylate) ester.

In some cases, when the rate of fragmentation is fast, fragmentation occurs in the source region, and the fragment ions appear as sharply focused peaks, similar to those of the precursor ions at mass  $M_p - M_o$ , where  $M_p$  and  $M_o$  are the mass of the precursor ion and the neutral loss, respectively. Fragmentation that occurs in the source region is known as prompt fragmentation or in-source-decay (ISD).

The differences between the mass series of the pre-

cursor ions (polymer carrying two imidazole-1-carboxylate endgroups) and those of the ISD ions formed by the loss of one and two imidazole-1-carboxylate termini are given as:

$$M_{n+3, \text{PEG1}} - M_{n, \text{PEG2}} = 20 \quad (1)$$

$$M_{n+6, \text{PEG0}} - M_{n, \text{PEG2}} = 40 \quad (2)$$

$$M_{n+2, \text{PPG1}} - M_{n, \text{PPG2}} = 4 \quad (3)$$

$$M_{n+4, \text{PPG0}} - M_{n, \text{PPG2}} = 8 \quad (4)$$

$$M_{n+2, \text{PIB1}} - M_{n, \text{PIB2}} = M_{n+4, \text{PIB0}} - M_{n, \text{PIB2}} = 0 \quad (5)$$

where  $n$  is the number of repeat units, and the number 0, 1, 2 after the name of the polymer represents the number of imidazole-1-carboxylate endgroups.

Seeking for the mass differences given by eqs 1-4, no sign of in-source fragmentation can be recognized, i.e., breaking of the C-O bond is a relatively slow process which takes place overwhelmingly in the first field-free region of the TOF instrument. In the case of the PIB bis(imidazole-1-carboxylate) ester in eq 5, we get isobaric mass series.

### Interpretation of the MALDI-TOF PSD MS/MS Spectra of Polymolecular Synthetic Polymers

Harvey [20, 21] et al. presented a formula in the case of well-defined carbohydrate molecules, which established a relationship between the mass of the precursor ion ( $M_a$ ), with those of the ISD ion ( $M_b$ ) and PSD ion ( $M_c$ ) as follows:

$$M_c = M_a \left[ \frac{1 + r(M_b/M_a)}{1 + r} \right]^2 \quad (6)$$

where  $r$  is defined as the instrumental constant. Following the development of Harvey [20], the instrumental constant  $r$  can be expressed by eq 7:

$$r = \frac{4R\beta}{2S_1\alpha + 2S_2\alpha/(\alpha + 1) + L_1 + L_2} \quad (7)$$

where  $S_1, S_2$  are the distances between the target and the first grid and the first grid and the second grid, respectively.  $L_1, L_2$  are the distances between the second grid and the reflectron and the reflectron and the detector, respectively.  $R$  is the length of the reflectron.  $U'_e = U_e S'_1/S_1$ ,  $S'_1 = S_1 - v_o \tau$ ,  $\alpha = [(U'_e + U_a)/U'_e]^{1/2}$ , and  $\beta = (U'_e + U_a)/U_R$ .  $\tau$  represents the delay time in the pulsed extraction (delayed extraction) mode.  $U_e, U_a$ , and  $U_R$  are the extraction (pulse voltage), the acceleration, and the reflectron voltages, respectively.

For bifunctional polymolecular synthetic polymer systems eq 6 can be applied as eq 8 and eq 9:

$$M_{n,PSD1} = M_{n,P} \left[ \frac{1 + r(M_{n,x1}/M_{n,P})}{1 + r} \right]^2 \quad (8)$$

$$M_{n,PSD2} = M_{n,P} \left[ \frac{1 + r(M_{n,x2}/M_{n,P})}{1 + r} \right]^2 \quad (9)$$

where  $n$  is the number of the repeat units, and the numbers 1 and 2 represent the series formed by the loss of one and two endgroups, respectively.  $M_{n,x1} = M_{n,P} - M_o$  and  $M_{n,x2} = M_{n,P} - 2M_o$ , where  $M_o$  is the average mass of the neutral loss, i.e., in our case  $M_o = 112.1$  Da.

Since in most cases the peaks of the precursor ions are isotopically resolved, while those of the PSD ions are not, the spectra were smoothed to obtain average masses for both types of ions. The values of  $r$  were determined from the mass series of precursor ions and those of the corresponding PSD ions using eqs 8 and 9. Eq 8 and eq 9 give a second order polynomial equation for  $r$ , which can be solved easily using a spreadsheet software. However, the determination of  $r$  in the case of polymers is not straightforward due to the appearance of a series of PSD ions. It was shown for carbohydrates that the PSD peaks appeared closely to the ISD peaks on most type of instruments [20, 21]. Therefore, the PSD peaks close to the mass  $M_n - M_o$  were considered to

**Table 2.** The measured values of  $r$ . (Experimental conditions:  $U_e = 3$  kV,  $U_a = 16$  kV,  $U_R = 20$  kV,  $\tau = 300$  ns)

Sample	Number of loss/unit <sup>a</sup>	$r$
PEG	1	$0.818 \pm 0.0007$
	2	—
PPG	1	$0.822 \pm 0.0007$
	2	$0.825 \pm 0.0008$
PIB	1	$0.820 \pm 0.0009$
	2	$0.821 \pm 0.0006$
TOF <sup>b</sup>		$0.830 \pm 0.0009$

<sup>a</sup>Indicates the numbers of lost imidazole-1-carboxylate termini.

<sup>b</sup>Based on the time of flight (TOF) by changing the reflectron voltage; for detailed explanation see the text.

originate from the oligomer having mass  $M_n$ . A confirmation based on the flight time of the series of the precursor ions was made as follows. Let the flight time of the polymer containing  $n$  repeat units  $t_{n,0}$  at the reflectron voltage  $U_{R,0}$  and  $t_{n,1}$  at reflectron voltage  $U_{R,1}$ . As an estimation, we take  $U'_e + U_a$  to be  $U_o$ . Using the flight times, eq 10 and eq 11 can be generated:

$$4R \cdot \frac{U_o}{U_{R,o}} \approx \frac{(t_{n,1} - t_{n,0})}{U_{R,o}(1/U_{R,1} - 1/U_{R,o})} \cdot \sqrt{\frac{2eU_o}{M_{n,P}}} \quad (10)$$

$$2S_1 + 2S_2 + L_1 + L_2 \approx (t_{n,0} - \tau) \sqrt{\frac{2eU_o}{M_{n,P}}} - \frac{(t_{n,1} - t_{n,0})}{U_{R,o}(1/U_{R,1} - 1/U_{R,o})} \quad (11)$$

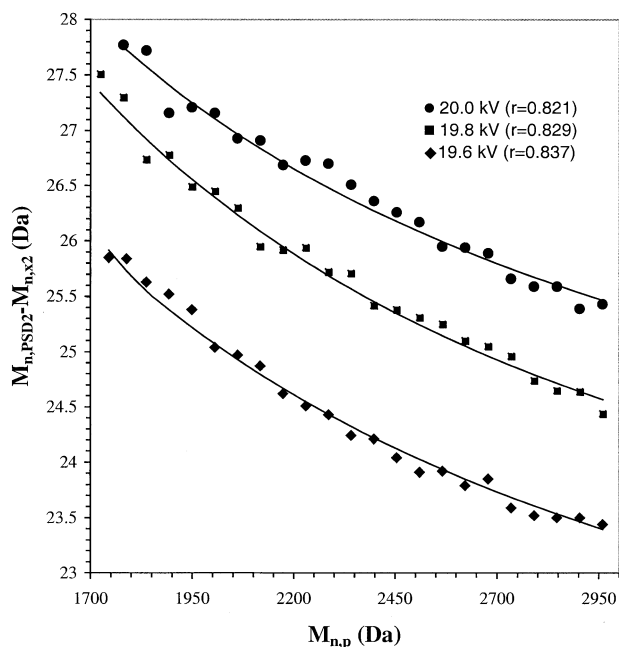
where  $U_o = U'_e + U_a$  and the value of  $r$  at  $U_{R,o}$  can be obtained by dividing eq 10 with eq 11. Table 2 summarizes the values of  $r$  obtained for the PEG, PPG, and PIB (imidazole-1-carboxylate) esters. As can be seen, there is a good agreement between the values of  $r$  of Table 2 determined with different ions.

In Figure 3, the  $M_{n,PSD2} - M_{n,x2}$  versus  $M_{n,P}$  values are plotted at different reflectron voltages. The solid lines represent the calculated curves with eq 9.

As shown in Figure 3 the  $\Delta M_{n,2} = M_{n,PSD2} - M_{n,x2}$  values decrease as the mass of the precursor ions increase in good accordance with eq 9. Also, as it is expected from eq 7, a decrease in the reflectron voltage results in the increase of the value of  $r$ , which yields smaller values of  $\Delta M_{n,2}$  according to eq 9.

The validity of eq 9 was tested using the PIB bis(imidazole-1-carboxylate) ester. Eq 9 suggests that by changing the reflectron voltage ( $U_R$ ) and keeping all of the other parameters such as  $\tau, U_e, U_a$  constant ( $U_e = 3$  kV,  $U_a = 16$  kV,  $\tau = 300$  ns) the  $r$  versus  $1/U_R$  plot should give a straight line. By doing so, we obtained a straight line with a slope of 18.43 kV and intercept of  $-0.101$  ( $R^2 = 0.995$ ).

In another set of experiments, the extraction voltage



**Figure 3.** The  $M_{n,PSD2}-M_{n,x2}$  versus  $M_{n,p}$  plots at three different reflectron voltages for the PIB bis(imidazole-1-carboxylate) ester. (Experimental conditions:  $U_e = 3$  kV,  $U_a = 16$  kV,  $\tau = 300$  ns).

was changed in a way that all of the other parameters were kept constant ( $U_o = 19$  kV,  $U_R = 20$  kV,  $\tau = 300$  ns).  $U'_e + U_a$  in eq 9 can be expressed as  $U_o + U_e(S'_1/S_1 - 1)$ , where  $U_o$  is the total voltage applied, i.e.,  $U_a = U_o - U_e$ . Therefore, taking into account that  $L_1 + L_2 \gg 2S'_1\alpha + 2S_2\alpha/(1 + \alpha)$  we can predict a linear dependence of  $r$  on the extraction voltage. The  $r$  versus  $U_e$  plot yield a straight line with a slope of  $-0.0094$   $\text{kV}^{-1}$  and intercept of  $0.848$  ( $R^2 = 0.993$ ). In addition, by taking into account the intercept and the slope of the line, the initial velocity ( $v_o$ ) of the polymer molecules can be estimated by eq 12.

$$-\text{slope}/\text{intercept} \cdot U_o = 1 - S'_1/S_1 = v_o\tau/S_1 \quad (12)$$

Using the values of  $\tau = 300$  ns,  $S_1 = 2$  mm, and  $U_o = 19,000$  V,  $v_o \approx 1.4 \cdot 10^3$  m/s was obtained. The dependence of  $r$  on the delay time ( $\tau$ ) was also investigated ( $U_e = 3$  kV,  $U_a = 16$  kV,  $U_R = 20$  kV).

According to eq 9,  $U'_e + U_a$  can be written as:  $U_o - U_e \frac{v_o}{S_1} \tau$ . By plotting  $r$  as a function of the delay time we obtained a straight line with a slope and intercept of  $-5.57 \times 10^{-5}$   $\text{ns}^{-1}$  and  $0.834$  ( $R^2 = 0.992$ ), respectively. Using the slope and the intercept, the initial velocity was estimated to be  $v_o \approx 0.8 \cdot 10^3$  m/s based on eq 13.

$$\begin{aligned} -\frac{\text{slope}}{\text{intercept}} \cdot \frac{U_o}{U_e} \cdot S_1 &= v_o (U_o = 19000 \text{ V}, U_e \\ &= 3000 \text{ V}, S_1 = 2 \text{ mm}) \end{aligned} \quad (13)$$

The value of  $v_o$  seems to be in the order of  $10^3$  m/s, which is reasonable considering that similar data have been reported for proteins [22, 23], but much lower values were reported by Juhász et al. [24].

### Analytical Applications of the Results

The presence of the peaks of the adduct ions and those of the PSD ions creates difficulties in determining what species are present in a sample. Polymers with relatively low masses are often recorded in the reflectron mode. Without alternative modes such as linear or PSD-MS/MS to check them, the appearance of additional sets of peaks due to the post-source decay may be incorrectly assigned because of the presence of polymers carrying different endgroups. Also, when the peaks of the adduct ions are poorly resolved similarly to those of the PSD ions, the recognition of the PSD peaks in the reflectron mode is hindered. Therefore, to avoid misinterpretation of the additional series of peaks, recording the MS spectra in the linear mode is highly recommended. In addition, when applying the results of the present work, the increasing mass difference between the adjacent main (precursor) and minor (PSD) series going from higher to lower masses may also be indicative of the presence of the PSD ions. It should be noted that the mass difference between the minor series (PSD series) containing  $n + 1$  and  $n$  repeat units remain practically constant, i.e., the mass of the repeat unit. Therefore, this treatment alone does not give information on the formation of the PSD ions. However, changing the reflectron voltage and monitoring the corresponding mass difference are also informative for the existence of the PSD peaks. In addition, knowledge of the instrumental constant  $r$  at a given instrumental parameter set may also help to identify the PSD series occurring in the reflectron MALDI-TOF MS spectra.

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### References

1. Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.
2. Tanaka, K.; Waki, H.; Ido, Y.; Akita, S.; Yoshida, T. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 151.
3. Mamyurin, B. A.; Karataev, V. I.; Shmikk, D. V.; Zagulin, V. A. *Sov. Phys. JETP* **1973**, *37*, 45.
4. Wiley, W. C.; McLaren, I. H. *Rev. Sci. Instrum.* **1955**, *26*, 1150.
5. Spengler, B.; Kirsch, D.; Kaufmann, R. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 198.
6. Spengler, B.; Kirsch, D.; Kaufmann, R.; Jaeger, E. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 105.
7. Seebach, D.; Herrmann, G. F.; Lengweiler, U. D.; Amrein, W. *Helv. Chim. Acta* **1997**, *80*, 989.

8. Kéki, S.; Török, J.; Deák, G.; Zsuga, M. *Macromolecules* **2001**, *34*, 6840.
9. Kéki, S.; Bodnár, I.; Borda, J.; Deák, G.; Batta, G.; Zsuga, M. *Macromolecules* **2001**, *34*, 7288.
10. Eisenberg, P.; Erra-Balsells, R.; Ishikawa, Y.; Lucas, J. C.; Nonami, H.; Williams, R. J. J. *Macromolecules* **2002**, *35*, 1160.
11. Goldschmidt, R. J.; Wetzel, J. S.; Blair, W. R.; Guttman, C. M. *J. Am. Soc. Mass. Spectrom.* **2000**, *11*, 1095.
12. Sprengler, B.; Lützenkirchen, F.; Kaufmann, R. J. *Phys. Chem.* **1992**, *96*, 9678.
13. Greenwald, R. B. J. *Contr. Release* **2001**, *74*, 159–171.
14. Bhadra, D.; Bhadra, S.; Jain, P.; Jain, W. K. *Pharmazie* **2002**, *57*, 5–29.
15. Deák, G.; Zsuga, M.; Kelen, T. *Polym. Bull.* **1992**, *29*, 239.
16. Mishra, M. K.; Mishra, B. S.; Kennedy, J. P. *Polym. Bull.* **1986**, *16*, 47.
17. Ivan, B.; Kennedy, J. P. *Macromolecules* **1990**, *23*, 2880.
18. Ivan, B.; Kennedy, J. P. *J. Polym. Sci. A: Polym. Chem.* **1990**, *28*, 89.
19. Ranucci, E.; Ferruti, P. *Synthetic Commun.* **1990**, *20(19)*, 2951.
20. Harvey, D. J.; Hunter, A. P.; Bateman, R. H.; Brown, J.; Critchley, G. *Int. J. Mass Spectrom.* **1999**, *188*, 131.
21. Harvey, D. J. *Mass Spectrom. Rev.* **1999**, *18*, 349.
22. Beavis, R. C.; Chait, R. T. *Chem. Phys. Lett.* **1991**, *181*, 479.
23. Pan, Y.; Cotter, R. J. *Org. Mass Spectrom.* **1992**, *27*, 3.
24. Juhasz, P.; Vestal, M. L.; Martin, S. A. *J. Am. Soc. Mass. Spectrom.* **1997**, *8*, 209.