Characterization of Polymers by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. End Group Determination and Molecular Weight Estimates in Poly(ethylene glycols)

Giorgio Montaudo*

Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria, 6-95125 Catania, Italy

Maurizio S. Montaudo, Concetto Puglisi, and Filippo Samperi

Istituto per la Chimica e la Tecnologia dei Materiali Polimerici, Consiglio Nazionale delle Ricerche, Viale A. Doria, 6-95125 Catania, Italy

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ABSTRACT: Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) allows mass measurements of large molecules such as those present in synthetic and natural macromolecules. We have used a self-calibrating method for the MALDI–TOF spectra of polymers, enabling us to obtain accurate mass values. Poly(ethylene glycol) (PEG) samples having two different kinds of end groups were used in our work: (i) anionic PEG, $H-(CH_2-CH_2-O)_n-H$; (ii) cationic PEG, $H-(CH_2-CH_2-O)_n-OH$. The MALDI–TOF spectra recorded in reflected mode allowed unambiguous identification of the end groups present in these PEG samples. Five anionic PEG samples with very narrow molecular weight distributions (MWD) were studied. The MALDI–TOF spectra of PEGs were recorded in both linear and reflected modes. Due to the high sensitivity and the highly linear response of HIMAS (a microchannel detector equipped with a photomultiplier), measurements of MALDI–TOF spectra were used in our work to estimate the MW and MWD of the PEG samples. Our results show that the molecular weight estimates provided by MALDI–TOF measurements agree with the values obtained by conventional techniques such as GPC, osmometry, and viscometry.

Introduction

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) is a recently introduced[1,2] soft ionization technique that allows desorption and ionization of very large molecules even if in complex mixtures, such as the wide molecular weight distributions (MWD) present in synthetic and natural macromolecules.[3–13]

The great promise of MALDI–TOF is to perform the direct identification of mass-resolved polymer chains, including intact oligomers within a MWD, measurement of MW and of MWD, and the simultaneous determination of end groups in polymer samples.

This perspective has stirred much expectation among polymer scientists. The MALDI–TOF technique was originally developed for proteins,[1,2] and its extension to the characterization of synthetic polymers is still underway.[3,6–13]

The task of obtaining accurate mass values in MALDI–TOF spectra of synthetic polymeric materials is of utmost importance, since it would make possible the direct determination of the composition of the species contained in each polymer sample and also of different end groups present in the oligomer series. End group identification and determination of the actual composition (down to species present to minor amounts) of a polymer sample are crucial in polymer analysis, and their importance cannot be overemphasized.

Problems may also be encountered in determining molecular weights of polymers from their MALDI–TOF spectra. The intensity of mass peaks in mixtures is proportional to the molar amount of each species, and therefore mass spectra of polymers yield the number average MW (although other averages may be calculated using appropriate algorithms), whereas GPC yields weight averages (although other averages may be calculated also here). This has to be taken into account when results are compared from the two techniques on polydisperse samples. Furthermore, in order to give a correct MW estimate, the MALDI–TOF detector must show a constant response to ions over a wide range of mass numbers.

In the following, we report a MALDI–TOF study of PEG samples having very narrow MWD and address some of the points listed above.

Experimental Section

Anionic PEG Samples. PEG samples were purchased by Polymer Laboratories. They were prepared by anionic polymerization of ethylene oxide initiated with potassium alcoholate in the presence of excess alcohol. Table 1 gives the average molecular weights, obtained by different methods for the five PEG samples, as given by the sample supplier.

Cationic PEG Sample. The PEG2000 sample was obtained from Fluka. It was synthesized by reacting ethylene oxide with an excess of water in the presence of acid catalyst (0.5% H_2SO_4).

MALDI Sample Preparation. 2-((4-Hydroxyphenyl)azo)-benzoic acid (HABA) was used as a matrix, and tetrahydrofuran, as a solvent. In the case of PEG2000, the solvent was ethanol and the matrix was dihydroxybenzoic acid. Probe tips were loaded with 100 pmol of polymer sample and 300 nmol of matrix.

MALDI–TOF Mass Spectra. A Bruker REFLEX mass spectrometer (Bruker Franzen Analytik, Bremen, Germany) was used to obtain the matrix-assisted laser desorption ionization–time of flight mass spectra. The spectrometer was equipped with a nitrogen laser (337 nm, 5 ns) and a flash ADC (time base 8 ns). The length of the drift tube is about 1.2 m. The detection in the linear mode was achieved by means of a HIMAS detector. It has a venetian-blind steel dynode which

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Table 1. Molecular Weight Averages and Molecular Weight Distributions for PEG Samples

sample	M_{n}	M_{n}[d]	M_{p}[e]	$M_{\mathrm{w}}/M_{\mathrm{n}}$[e]	M_{p}[f]	M_{p}[g]	M_{n}[h]	M_{w}[i]	$M_{\mathrm{w}}/M_{\mathrm{n}}$[j]
PEG4100	3927[a]	3791	4100	1.05	3900	3850	3990	4040	1.01
PEG7100	6931[a]	6874	7100	1.03	7420	7200	7190	7360	1.02
PEG8600			8650	1.03	8610	8600	8380	8530	1.02
PEG12600	11600[b]	11843	12600	1.04	12790	12750	12730	12800	1.01
PEG23600	23800[c]	21344	23600	1.06	23710	23500	21450	21900	1.02

[a] Vapor pressure osmometry, as measured from the sample supplier. [b] Membrane osmometry, as measured from the sample supplier. [c] Light scattering, as measured from the sample supplier. [d] Intrinsic viscosity, as measured from the sample supplier. [e] Estimated from GPC by the sample supplier. [f] Most probable mass estimated from the MALDI–TOF linear spectrum. [g] Most probable mass estimated from the MALDI–TOF reflection spectrum. [h] Computed from the MALDI–TOF linear spectrum by equation $M_{\mathrm{n}} = \Sigma_i N_i M_i / N_i$. [i] Computed from the MALDI–TOF linear spectrum by equation $M_{\mathrm{w}} = \Sigma_i N_i (M_i)^2 / N_i M_i$. [j] Calculated from the values in the last two columns.

performs the ion-to-electron conversion. ELectrons pass through a 6000 V potential difference and hit first a single-plate microchannel detector and then a cesium iodide scintillator which surmounts the entrance window of a photomultiplier. The conversion dynode enables the detection of high-mass ions. The scintillator avoids the detector saturation by providing a very wide dynamic range. The HIMAS detector has low temporal resolution. This is probably due to the production of secondary ions by the conversion dynode. The detection in the reflected mode was achieved by the standard double-plate microchannel detector. The accelerating voltage was 30 kV. The laser irradiance was slightly above threshold (ca. 10^6 W/cm^2). Ions below *m/z* 350 were removed with pulsed deflection, and 100 transients were summed. The resolution at 5000 Da was 300 in the linear mode and 500 in the reflected mode.

Results and Discussion

The determination of accurate masses in the MALDI spectra of synthetic polymers that may in fact be mixtures and may possess different end groups can sometimes be a difficult task.[14]

The TOF spectrum is initially obtained as a temporal spectrum, which reports the number of ions arriving at each time. The mass spectrum is then usually calibrated by recording the spectrum of a compound of known mass and computing the set (Ω) of calibration constants. The set Ω is then used to calibrate the spectra of the unknown sample. The accuracy in mass determination by this procedure is claimed[4,15] to be 0.1% (i.e. 10 mass units at 10 000). Such a low level of accuracy is related to the fact that each mass spectrum has its own set Ω, which varies when passing from one spectrum to another.

For end group determinations by MALDI–TOF spectrometry, the accuracy in mass determination by the external calibration procedure is not enough and one needs an accuracy of about 0.02%. We have used a self-calibrating method for the MALDI–TOF spectra of polydisperse polymers, which enables us to obtain an accuracy of about 0.02% for masses up to 10 000–20 000,[12] as discussed below.

The time (t_i) needed for an ion of mass $\mu_{\mathrm{calc}}(\mathrm{i})$ to fly through the tube and the mass of the ion itself are related by[4,5]

$$\mu_{\mathrm{calc}}(\mathrm{i}) = \alpha(t_{\mathrm{i}} + 10^{-6}\beta)^2 \tag{1}$$

where α and β are calibration constants, whose values must be found through a process involving tentative assignments of the peaks in the spectra and by a linear regression procedure.[5]

In the case of polymeric materials, however, one can take advantage of a unique property of the latter. Considering that the mass of a homopolymer results from the sum of identical units, eq 1 can be coupled with the condition that the mass of each oligomer results from the sum of a multiple of σ, the mass of the polymer repeat unit, and θ, the mass of the first peak considered in the process of enumerating mass spectral peaks.

Then, within the same mass series, the distance between two adjacent ions is a multiple of σ, and the exact mass of the ion $\mu_{\mathrm{multi}}(\mathrm{i})$, is

$$\mu_{\mathrm{multi}}(\mathrm{i}) = (\mathrm{i} - 1)\sigma + \theta \tag{2}$$

The calibration procedure consists in varying α and β until μ_{calc} equals μ_{multi}. This is done by minimizing the sum of squares:

$$\mathrm{SS1} = \Sigma_{\mathrm{i}}[\mu_{\mathrm{calc}}(\mathrm{i}) - \mu_{\mathrm{multi}}(\mathrm{i})]^2 \tag{3}$$

where the summation goes from 1 to n (n is the number of peaks in the mass series). The computation of the point of minimum, P, is cumbersome. In order to circumvent this obstacle, it is useful to introduce a function SS2, similar to SS1.

$$\mathrm{SS2} = \Sigma_{\mathrm{i}}\{[\mu_{\mathrm{calc}}(\mathrm{i})]^{1/2} - [\mu_{\mathrm{multi}}(\mathrm{i})]^{1/2}\}^2 \tag{4}$$

It is reasonable to assume that, if SS1 has a minimum at point P, then also SS2 takes its minimum value at point P. A computer program, called CALTOF,[16] was developed in order to find the minimum of SS2 in eq 4. It accepts as input σ (the mass of the polymer repeat unit) and the time of flight (ti) corresponding to peaks in the MALDI–TOF mass spectrum. It gives as output the vectors $\mu_{\mathrm{calc}}(\mathrm{i})$ and $\mu_{\mathrm{multi}}(\mathrm{i})$, the calibration constants α and β, and the plot of SS2 as a function of θ.

In Figure 1 are reported four minimization curves, obtained by the calibration procedure described above. As discussed above, the procedure takes advantage of the finding that SS2 takes its minimum at θ, the mass of the first oligomer appearing in the MALDI–TOF spectrum. Since σ is also known (being the repeat unit of the polymer), eqs 1 and 2 can be solved and the masses can be calculated. The mass accuracy obtained was about 0.02% at resolution 500, based on the comparison between the mass values found and those expected from the polymer formula (i.e., repeat units plus end groups plus cation). The MALDI spectra of the PEG samples were calibrated by following this procedure and are discussed below.

Parts a and b of Figure 2 report the MALDI–TOF mass spectra of PEG4100, measured in linear and reflected mode, respectively. The reflected spectrum (Figure 2b) shows a higher resolution ($m/\Delta m$ = 500) compared to that in linear mode (Figure 2a), so that two mass series can be discerned (see inset).

The anionic PEG samples investigated here (Table 1) were obtained by ring-opening polymerization of ethylene oxide initiated with potassium alcoholate in the presence of excess alcohol, acting as transfer agent and MW regulator. Assuming that the reactivities of all the OH functions in the transfer process are the same,

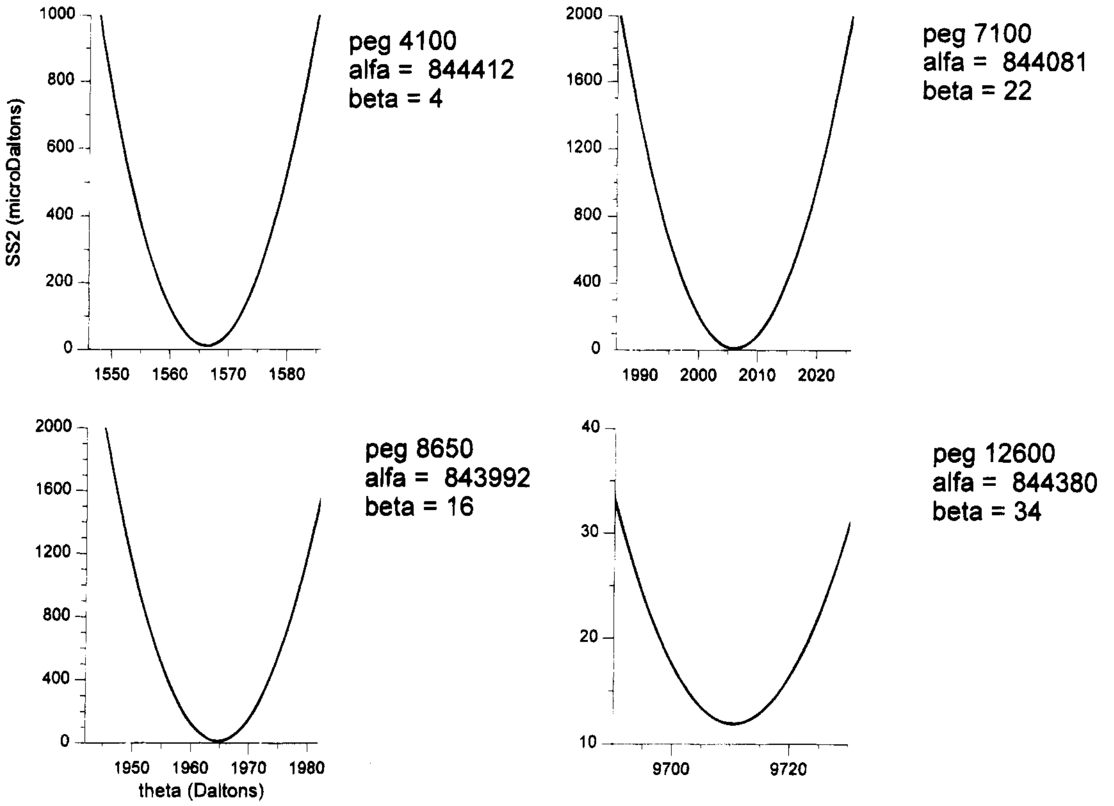
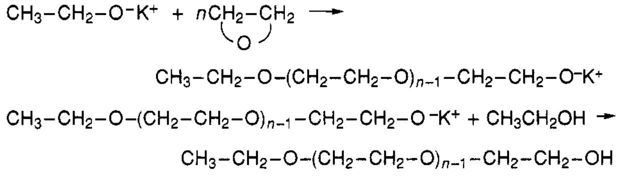


Figure 1. Plots of the function SS2 in eq 4 as a function of q for samples PEG4100, PEG7100, PEG8650, and PEG12600.

independent of the chain length, the number average degree of polymerization of the poly(ethylene oxide) formed is given by the molar ratio of monomer and alcohol involved. The reaction scheme can be written as follows:



$$CH_3-CH_2-O^-K^+ + nCH_2-CH_2(O) \rightarrow CH_3-CH_2-O-(CH_2-CH_2-O)_{n-1}-CH_2-CH_2-O^-K^+$$

$$CH_3-CH_2-O-(CH_2-CH_2-O)_{n-1}-CH_2-CH_2-O^-K^+ + CH_3CH_2OH \rightarrow CH_3-CH_2-O-(CH_2-CH_2-O)_{n-1}-CH_2-CH_2-OH$$

The latter formula can be rewritten as $H-(CH_2-CH_2-O)_{n+1}-H$, from which the MW of each PEG oligomer can be calculated: ($44.03n + 2$). If the PEG ions appear as Na^+ or K^+ adducts, then their mass shall be given by ($44.03n + 2 + 23$) or ($44.03n + 2 + 39$), respectively. The MALDI−TOF spectrum in Figure 2b was calibrated with our procedure. The CALTOF computer program gave as output $\alpha = 844\,412$, $\beta = 4.0$ (eq 1). Therefore, the most intense mass series appearing in Figure 2b is associated with ions of the type: ($44.03n + 2 + 23$), and the second with ions of the type ($44.03n + 2 + 39$). PEG ions appear therefore as Na^+ and K^+ adducts in the spectra.

In recent work,[3] the peaks appearing in the MALDI−TOF spectrum of a PEG sample were measured to have substantial mass differences from those reported above, well beyond the experimental uncertainty. For instance, the 27mer was assigned mass number 1229.7 Da (instead of 1213.8 Da), and the 37mer was assigned mass number 1670.0 Da (instead of 1654.1 Da). These mass values appear to correspond to a formula ($44.03n + 18 + 23$), where the PEG oligomers are terminated by hydroxy groups, and we thought therefore that they originated from a PEG sample prepared from ethylene oxide and water in the presence of an acidic catalyst. A sample (PEG2000), obtained by the latter synthetic method, was then analyzed (Figure 2C), and it was found to have the mass values expected for the formula ($44.03n + 18 + 23$).

This experiment shows that MALDI analysis can distinguish two PEG polymers terminated by different end groups.

In Figure 3a,b are reported the MALDI−TOF mass spectra of PEG 7100, measured in the linear and reflected modes, respectively. As in the previous case, the reflected spectrum shows a higher resolution compared to that in linear mode, so that the two mass series corresponding to Na^+ and K^+ adducts of PEGs can be distinguished. The same result was obtained in the case of PEG8650 (omitted for brevity) and of PEG12600. In the latter case, shown in Figure 4a,b, the peaks in the reflected spectrum are broader than for PEG7100. As a consequence, the peaks due to K^+ adducts are no more resolved from the peaks due to Na^+ adducts. The peak broadening at high masses is a well-known feature of MALDI−TOF.[17]

In Figure 5a,b are reported the MALDI−TOF mass spectra of PEG23600, measured in the linear and reflected modes, respectively. It can be seen that the spectrum is not mass resolved and therefore it is impossible to distinguish the peak due to one oligomer from that of another. In fact, in this mass range, peaks are quite broad (the width of peaks in the reflected spectrum were estimated to be 50−55 Da), so that the signal due to a given oligomer is not resolved from the signals of its nearest neighbors (the spacing between neighboring peaks is 44 Da). The noise level of the reflected spectrum in Figure 5b appears to be acceptable, but this result was obtained only after digital filtering (smoothing) of the raw data. In fact, the original S/N ratio was 5 times worse. As a matter of fact, ions strike against and reflected detector, but they produce a very small signal. This is due to the characteristics of the detector, and it has been shown that the sensitivity of this detector varies with the velocity

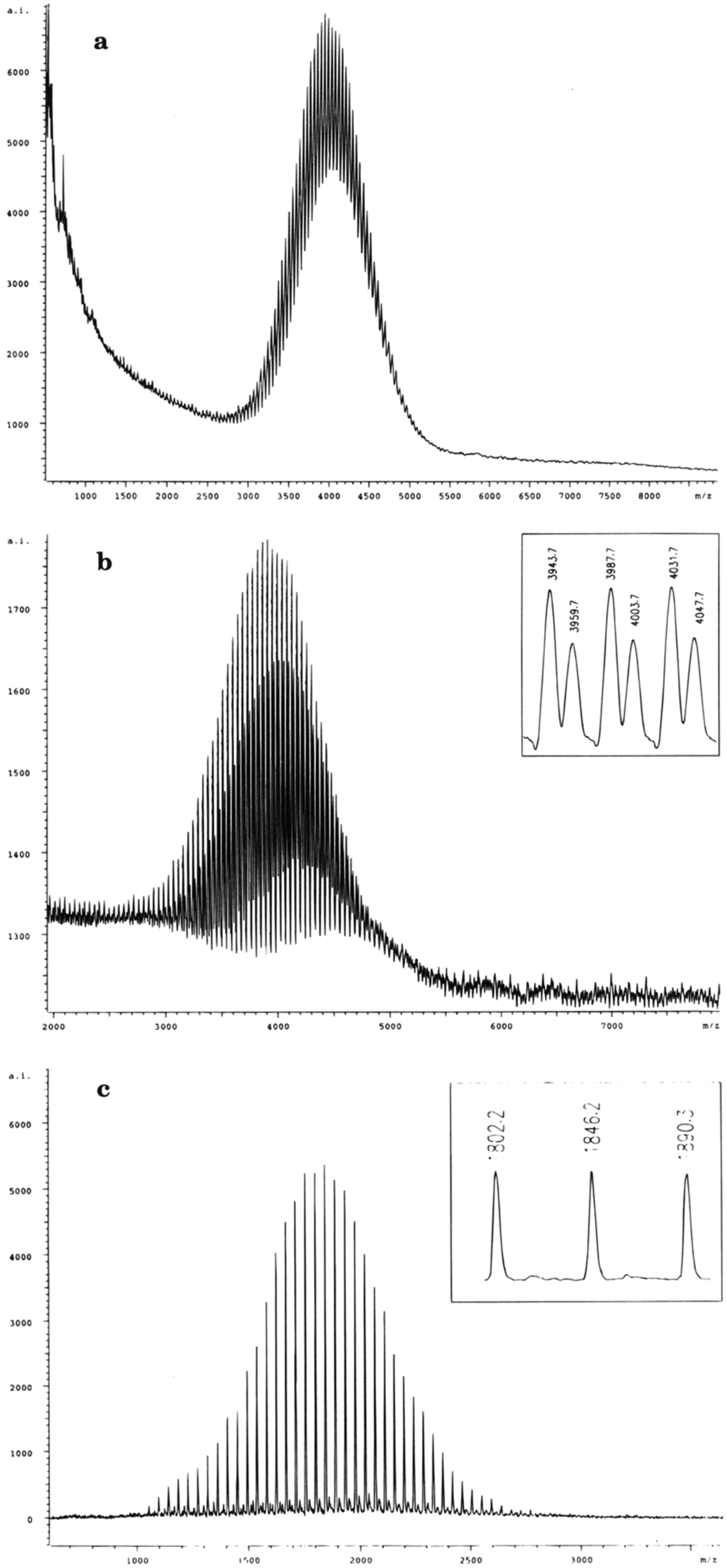


Figure 2. MALDI−TOF spectrum for sample PEG4100 in the linear mode (a), for sample PEG4100 in the reflected mode (b), and for sample PEG2000 in the reflected mode (c).

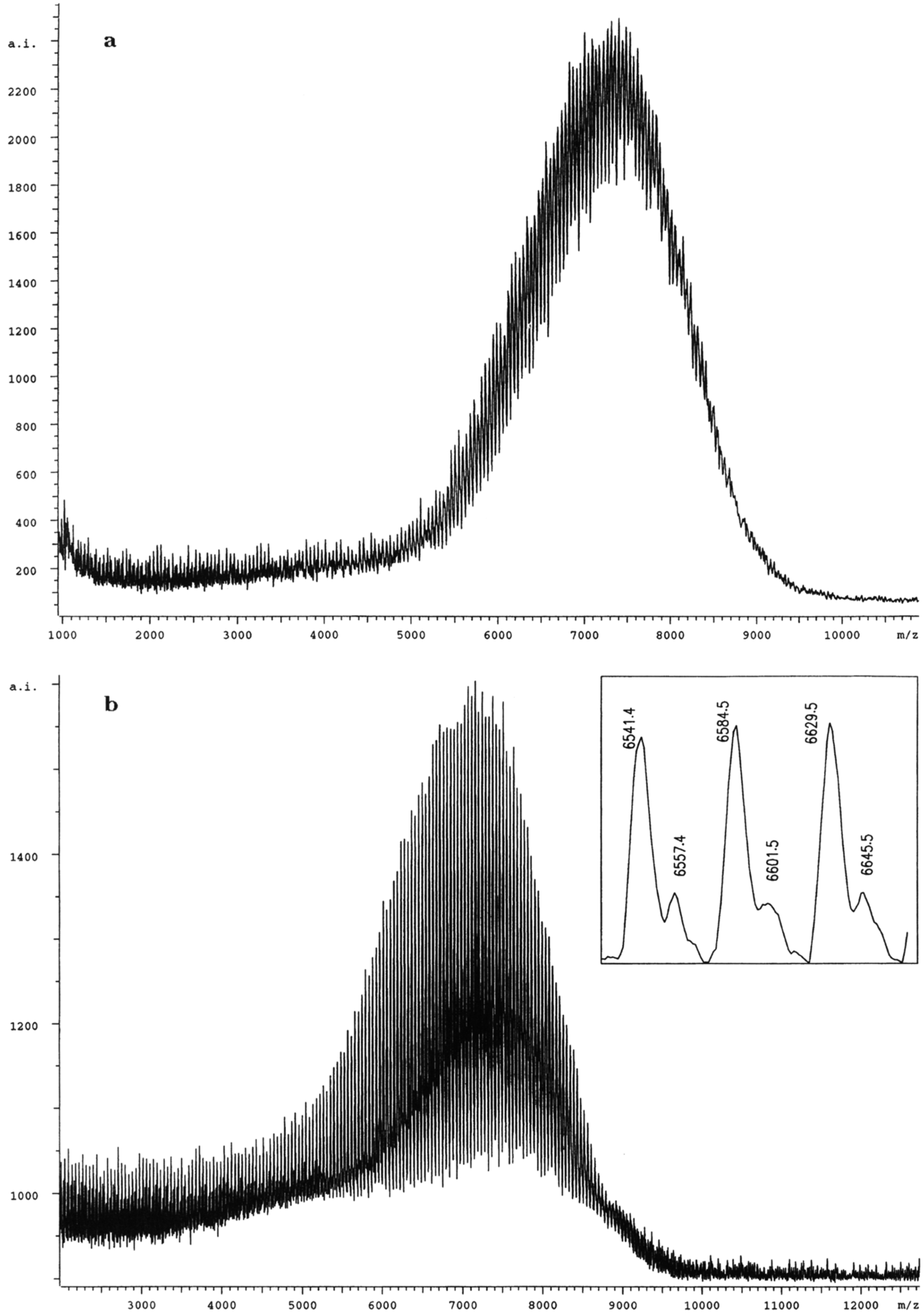


Figure 3. MALDI–TOF spectrum for sample PEG7100 in the linear (a) and the reflected mode (b).

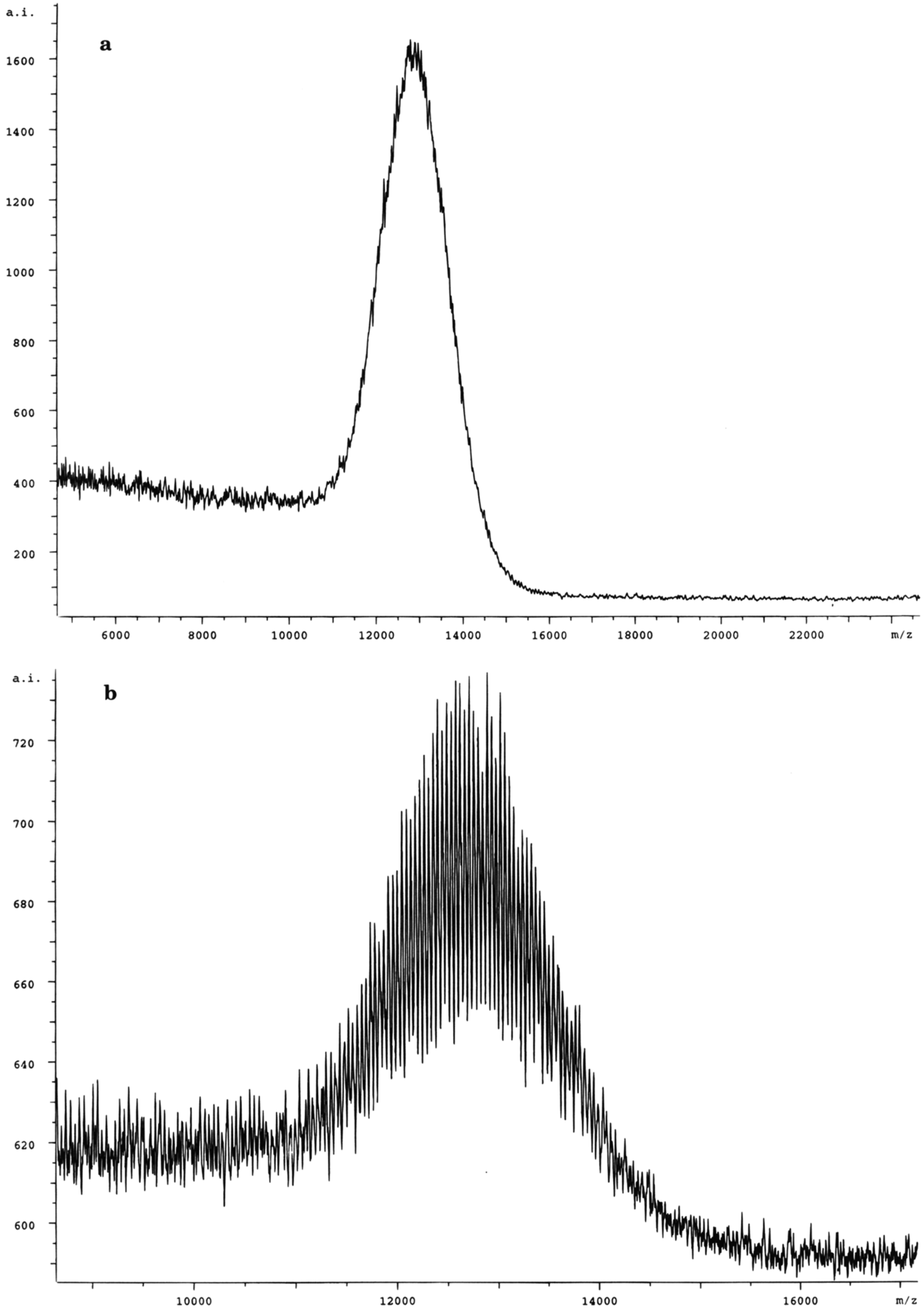


Figure 4. MALDI–TOF spectrum for sample PEG12600 in the linear (a) and the reflected mode (b).

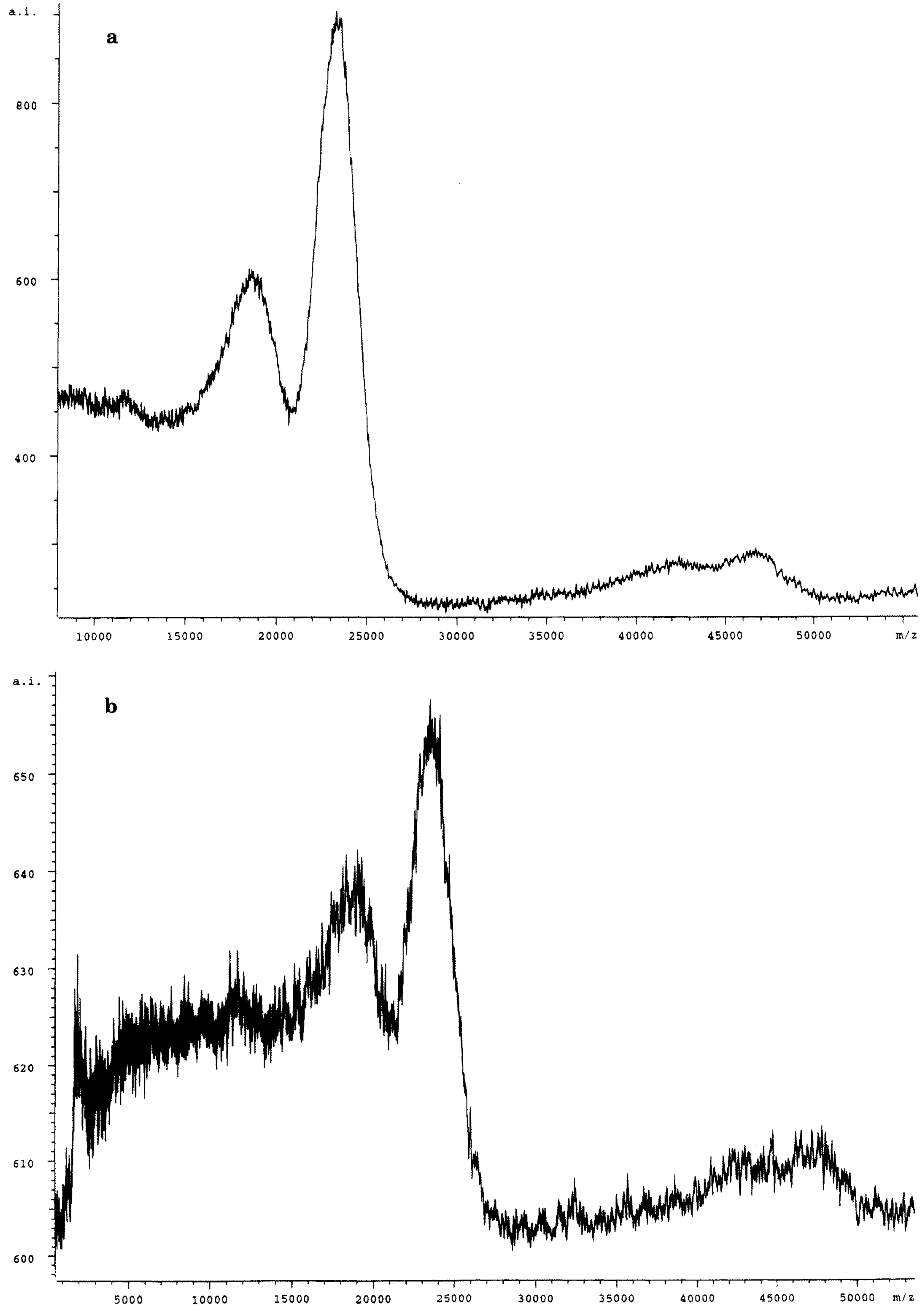


Figure 5. MALDI–TOF spectrum for sample PEG23600 in the linear (a) and the reflected mode (b).

of the incoming ions.[18] The difficulties associated with the detection of large masses can be circumvented by using a detector in which the conversion from ions into electrons is performed outside the microchannel plate.[19] The linear detector possesses this characteristic and is therefore well-suited for the detection of large masses. The MALDI spectra of PEG23600 in Figure 5a,b clearly slow also the appearance of a PEG dimer at *m/z* 47 000. Furthermore, two peaks appear in the spectra: one, less intense, at *m/z* 18 500 and another at *m/z* 23 600. This PEG sample appears to have a bimodal distribution of MW, a fact not detected by GPC (Table 1). In the linear mode, the ratio between the intensities of the peak at 23 600 and 18 500 Da is about 5. In the reflected mode, this ratio is instead about 2. This is due to the fact that the reflected detector response falls at high masses. The signal-to-noise (S/N) ratio in the linear spectrum is good. A comparison between the mass spectra in Figures 2–5 shows that the S/N ratio of the reflected spectra worsens appreciably at high masses, wheres the S/N ratio of the linear spectra remains nearly constant with varying mass.

Table 1 gives the average molecular weights for the five PEG samples investigated by MALDI–TOF together with the values given by the supplier, as obtained by different methods.

Due to the high sensitivity and highly linear response of the HIMAS detector (see Experimental Section), measurements of MALDI–TOF spectra in the linear mode were used in our work to estimate the MW and MWD of the PEG samples. The reflected detector in our instrument design has different characteristics from the HIMAS (see Experimental Section), and it is less useful for MW measurements since its sensitivity falls beyond about 30–40 kDa (but its resolution increases).

We have recorded the MALDI–TOF spectra also in the reflected mode in order to take advantage of the higher resolution of these spectra for end group identification purposes. Nevertheless, from Table 1 it can be seen that the MALDI–TOF spectra provide well-comparable estimates for the most probable (M_p) mass values, as measured independently from the linear and reflected spectra. This fact is due to the relatively low MW values of the PEG samples investigated.

Data in Table 1 also show that the molecular weight estimates provided by MALDI–TOF measurements agree with the values obtained by conventional techniques. M_n values derived from MALDI–TOF spectra in the linear mode agree well with the M_n values obtained from osmometry and viscosity measurements (Table 1). Also the M_p values estimated from GPC come close to the M_p values observed in the MALDI–TOF spectra. It ought to be remarked that M_n values are all lower than M_p. The reason is that the MWD is not symmetrical with respect to its maximum. In fact, MWD has a "tail" at low masses. In the case of sample PEG23600, the value M_p = 23 710 Da, estimated from the MALDI–TOF spectrum in the linear mode (Figure 5a), differs significantly from the calculated value of M_n = 21 450 Da because the latter was obtained by taking into account the bimodal distribution shown by the spectrum in Figure 5a.

Comparing the GPC and MALDI–TOF M_w/M_n ratios in Table 1, it appears that the MALDI–TOF spectra yield an appreciably narrower dispersity index with respect to GPC. The disagreement may not appear serious for these samples, prepared by anionic polymerization and therefore possessing quasi-Poisson distributions. However, for samples showing Flory distributions, or even wider, MALDI–TOF spectra may yield inaccurate results.[20]

Summarizing our results of the characteristics of PEGs by MALDI–TOF mass spectra, we have used a calibration procedure especially useful for end group identification and have performed a comparison of MW and MWD estimates by MALDI–TOF and conventional techniques (including GPC). The PEG samples investigated here possess a very narrow MWD; we shall report later on the characterization by MALDI–TOF of polymeric samples of wider MWD.[20]

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