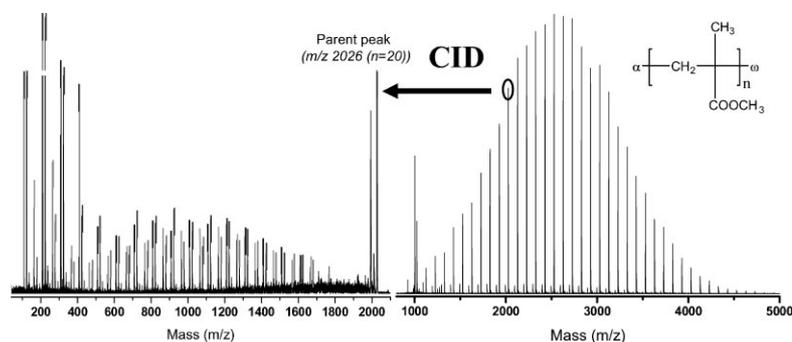


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MALDI-TOF MS Coupled with Collision-Induced Dissociation (CID) Measurements of Poly(methyl methacrylate)^a

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was chosen for an in-detail analysis of poly(methyl methacrylate) (PMMA) in order to determine the possible fragmentation mechanism with the help of collision-induced dissociation (CID). All experiments were performed on a well-defined PMMA standard and were optimized for sample preparation and measurement conditions of both MS and MS/MS. In order to investigate the fragmentation pathways, two parent peaks—both charged with sodium ($m/z = 1625.9$ and 2226.2 Da, respectively)—were selected, thus permitting the examination of possible cleavages, and reaction pathways. For both chosen peaks, the MALDI-TOF MS/MS spectra revealed four fragmentation series that could be explained by single or multiple main chain scissions and secondary reactions of the PMMA side groups. According to the molar mass of the fragments, a loss or migration of the side group to the end of the free radical, followed by a β -scission, was favored. These insights are the first steps toward the construction of a library with fragments and fragmentation pathways, complementary to proteomics libraries, in order to obtain fast and automated identification of substances.



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Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is one of the most fundamental analysis techniques for proteins, peptides, and synthetic polymers to date.^[1] Along with field desorption (FD), fast atom bombardment (FAB), laser desorption coupled with time-of-flight (LD-TOF), secondary ion mass spectrometry coupled with time-of-flight (SIMS-TOF), and electron spray ionization (ESI), it is one of the softest ionization methods available.^[2] The main limitation for most mass spectrometry techniques consists in their applicability being limited to synthetic polymers with relatively low molar masses (<5 000 Da). MALDI, on the

^a Supporting information for this article is available at the bottom of the article's abstract page, which can be accessed from the journal's homepage at <http://www.mrc-journal.de>, or from the author.

other hand, enables the analysis of polymers with higher molecular weights (up to 50 000 Da).^[3,4] A successful characterization with MALDI-TOF MS provides the number-average molecular mass, the weight-average molecular mass, and the polydispersity index of the investigated macromolecules, as well as information concerning the end groups and the molar mass of the repeating units.^[5]

The coupling of MALDI-TOF MS with post-source decay (PSD) or collision-induced dissociation (CID) delivers more information on the structure of the parent ions and fragment peaks. During the last decade, the introduction of tandem mass spectrometry (MALDI-TOF MS/MS) overcame the limitations of the conventional coupled techniques such as PSD or CID.^[6] In the instrument utilized for the present study, a LIFTTM (CID mode) unit was integrated to be able to gain this additional structural information. The unit is located in the flight tube, thus rendering it possible to obtain a full-fragment ion spectrum with a single scan.^[7] It has to be realized that the parent ion and resulting fragments are formed in the drift region of the MALDI-TOF MS/MS system. After selection of the target species by the precursor ion selector (PCIS) that works as a mass filter for the parent ion and fragments, the ions and fragments could be simultaneously detected, when the energy difference between the parent ion and the smallest fragment was not higher than 30%. Figure 1 schematically illustrates the principle of the instrument.

Poly(methyl methacrylate) (PMMA) was chosen for the characterization because of its versatile physical and chemical properties rendering it suitable for many applications, e.g., in the field of medicine, optics, or as safety glasses. In industry, PMMA is routinely produced through radical polymerization methods in emulsion, solution, or in the bulk. Alternatively, PMMA can be synthesized under controlled conditions by different initiating systems [screened anionic polymerization (SAP), group transfer polymerization (GTP), and catalytic chain transfer polymerization (CTTP)].^[2] In the SAP process, the polymerization of PMMA can be carried out with the use of lithium aluminum alkyl/phenoxide as an initiator,^[8] but depending on the reaction conditions, polymers with different end groups are produced. The GTP technique gives rise to a single type of end groups as a result of a polymerization

mechanism proceeding via a Michael addition and a defined silyl ketene acetal initiator.^[9] The third technique, CTP, requires a chain transfer agent as the initiator in order for the end groups to be well defined.^[8] PMMA is one of the most thoroughly studied polymers, also by MALDI-TOF MS, since the end groups are of considerable practical importance.^[10] The knowledge of the polymerization mechanism and a combination of characterization approaches by MALDI-TOF MS and ¹H NMR spectroscopy have, up until now, provided reasonable results for the end group determination of the polymers.^[10]

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry coupled with a collision cell has been demonstrated for some synthetic polymers such as poly(ethylene glycol) (PEG),^[11,12] polystyrene (PS),^[13–15] poly(ethylene terephthalate),^[16] and poly(butylene adipate) (PBAD)^[6], for direct determination of the end groups of the polymers. Furthermore, various fragmentation pathways of the polymers are observable under high energy levels. Motivated by these first results, PMMA was selected in order to study the fragmentation of the polymer by use of MALDI-TOF MS/MS further. The aim was to gain more information of the structural composition of PMMA and its behavior under high energy level conditions, which should permit a better understanding of the applied polymerization techniques and a detailed investigation of the polymerization mechanism.

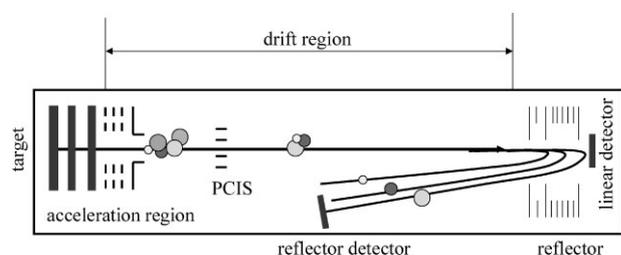
Experimental Part

Materials

A PMMA calibration standard ($\overline{M}_{n,SEC} = 2\,250$ Da; $\overline{M}_{n,MALDI} = 2\,720$ Da) was purchased from Polymer Standards Services (PSS) GmbH (Mainz, Germany). The molecular weight and the two end groups were certificated, through size-exclusion chromatography (SEC) and MALDI-TOF MS, by the manufacturer. A quality certificate of the polymer is provided as Supporting Information. A typical ¹H NMR spectrum for PMMA in CDCl₃ was obtained displaying the –CH₃ protons at 0.8–1.1 ppm, the –CH₂ protons at 1.5–2.2 ppm, and the –OCH₃ protons at 3.6 ppm. Furthermore, a ratio of 3:2:3 could be observed in the measured ¹H NMR. These three characteristic values were similar to those that can be theoretically calculated and found in the literature.^[17] The matrix material, *trans*-[2-3-(4-*tert*-butylphenyl)-2-methyl-2-propen-ylidene]malononitrile (DCTB), and sodiumiodide (NaI) were obtained from Sigma–Aldrich and Acros Organics, respectively. Tetrahydrofuran (THF), chloroform, and acetone of HPLC grade were purchased from Roth and used as received.

Instrumentation

An Ultraflex III TOF/TOF (Bruker Daltonics, Bremen, Germany) was used for the analysis. The instrument was equipped with a Nd:YAG laser and a collision cell. All spectra were measured in the



■ Figure 1. The principle of a PSD measurement.

positive reflector mode. For the MS/MS mode, argon was used as the collision gas at a pressure of 2×10^{-6} mbar. The instrument was calibrated prior to each measurement with an external standard PMMA from PSS. MS and MS/MS data were processed using PolyTools 1.0 (Bruker Daltonics) and Data Explorer 4.0 (Applied Biosystems). For the characterization by ^1H NMR, an Avance 250 from Bruker was used at 250 MHz and under automated standard conditions. A GPC characterization was performed during the certification process by the manufacturer.

Sample Preparation

For the sample preparation, PMMA 2500 ($10 \text{ mg} \cdot \text{mL}^{-1}$) in chloroform, DCTB ($10 \text{ mg} \cdot \text{mL}^{-1}$) in THF, and the doping salt (NaI) dissolved in acetone at a concentration of $100 \text{ mg} \cdot \text{mL}^{-1}$ were used, and a multiple-layer spotting technique (matrix, salt, and analyte) was applied. A detailed description of the preparation can be found elsewhere.^[19] Each layer was air dried before the next layer was spotted onto the previous one.

Results and Discussion

The method for synthesizing the PMMA calibration standard was reported to be a GTP. Both end groups were referred to as being hydrogen, and there was said to be no heterogeneity in the end group functionality. The MALDI-TOF MS spectrum of the certificated PMMA was measured in reflector mode and is displayed in Figure 2. The investigated sample exhibited a molar mass distribution from 1000 Da up to 4200 Da with a nominal peak maximum mass of 2526.3 Da. This corresponded to 25 repeating units of MMA and 1 sodium ion. The molar mass increment between the peaks in the main distribution was found to be 100.1 Da which was an exact fit of the molar mass of the monomeric repeating unit. The corresponding

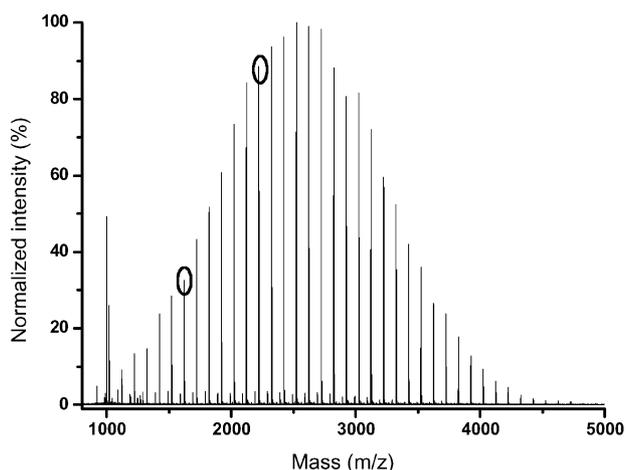


Figure 2. A MALDI-TOF MS spectrum of the PMMA calibration standard (matrix: DCTB in THF, ionization salt: NaI in acetone, solvent: CHCl_3). The selected parent peaks (1625.9 and 2226.2 Da) are marked with circles.

molar mass (M , in the formula) of the peaks in the distribution were due to the PMMA being charged with a sodium cation, and the molar masses of the polymer chains could thus be calculated from the following formula, $M + \text{Na}^+ = 100.1n + 23$, where n represents the number of repeating units in the polymer. The typical isotopic pattern of the PMMA was calculated by the isotope calculator and was found to be consistent with the pattern obtained from the main distribution in the spectrum. Homogeneity of the end groups in the investigated polymer could thereby be proven. The polydispersity index was calculated with a value of 1.05, whereas the number-average and the weight-average molecular masses were calculated as 2624 and 2762 Da, respectively (a mass tolerance of 1 Da was chosen for the evaluation in the range of 1025 Da–4128 Da). These results differed slightly from the certified values ($\bar{M}_n = 2720$ Da; $\bar{M}_w = 2910$ Da; $\text{PDI} = 1.07$), and a possible reason for this discrepancy could be the different software utilized for the calculation of the average molecular masses. In addition, the presence of the second distribution with a low intensity in the MALDI-TOF MS spectrum may have caused a deviation. Pasch and Gores explained the occurrence of these species with the formation of cyclic products during the GTP process of methyl methacrylate.^[9] These cyclic structures can be formed due to the back-biting reactions.^[18] The molar mass difference between these peaks was 100.1 Da and the intensities were found to be higher for lower molar masses. Nevertheless, the second distribution had a relative intensity of the main distribution of less than 4%, and there was no influence on the selection of the parent ion peaks from the spectrum for the LIFT mode measurements.

Each peak in the MALDI-TOF MS spectrum, up to the molar mass of 5000 Da, could be selected for the MALDI-TOF CID measurements. However, a sufficient intensity of the selected peak in the MALDI-TOF MS spectrum was required in order to obtain a CID spectrum of high intensity. A majority of the peaks from the MALDI-TOF MS spectrum were investigated in the CID mode, and two of them are discussed in the present communication. The representative parent ion peaks displayed molar masses of 1625.9 and 2226.2 Da. They are marked with circles in Figure 2. The corresponding CID spectra of each of the two parent peaks are displayed in Figure 3. At a first glance, the relative intensities of the monomer, dimer, and trimer regions exhibited high values in both spectra, whereas the fragments in the area between these regions and the parent peak demonstrated a relative intensity of less than 4%. Moreover, four groups of peaks (A, A', B, B') were observed with a molar mass difference of 100.1 Da. The possible mechanism for the formation of the corresponding structures in these distributions is discussed later.

The intensity of the peaks in the fragmentation region is known to be greatly affected by several parameters in the MALDI-TOF MS measurements. Therefore, most of these parameters, i.e., the applied sample preparation technique, the used matrix compound, and the sample concentration, have previously been investigated in detail for various polymers.^[19–21] On the other hand, the collision gas type and the collision gas pressure represent important factors in the MALDI-TOF CID measurement and have been thoroughly investigated by Owens and coworkers in the case of PEG.^[11] Based on these literature reports, optimal parameters for both the sample preparation as well as the measurement conditions for PMMA could be applied. Besides, Jackson et al. examined the end groups and the fragmentation pathways of PMMA under different conditions using several mass spectrometry techniques, like liquid secondary ion mass spectrometry (LSIMS), ESI, and MALDI and some of these techniques were coupled with a CID cell in order to investigate the fragmentation series of the macromolecules. Moreover, Laine et al. observed similar structures for the different fragment series.^[22]

Obviously, there exist several possibilities for a fragmentation pathway of a macromolecule at the high energy level conditions during a CID measurement. Recently, Gies et al. published a report on the combination of MALDI-TOF CID and pyrolysis GC/MS for examining the fragmentation reactions of a telechelic PS.^[13] One of the conclusions that were reached was that free radicals were initially formed through the polymer chain, which subsequently gave rise to multiple chain scissions, similar to what was observed in the present study. With the aim of providing a better explanation, a PMMA chain with the length of 16 repeating units is schematically displayed in Scheme 1. The potential single or multiple main chain fragmentations are indicated as an overview of the possible structures that can be obtained through CID measurements. In addition, one or more repeating units of PMMA can be cleaved by the high CID energy and form a radical center that will promote the multiple main chain scissions. It should be noted that all these possible fragments were charged with sodium. Furthermore, according to our observations, an unzipping of the polymer chain was believed to be unlikely since the intensity of the peaks in the monomeric region was much lower than otherwise expected. Besides, the relatively high intensities of the dimer and trimer peaks were indications of multiple chain fragmentation reactions.

As mentioned above, four series of fragment ions were determined in the MALDI-TOF CID spectrum. The molar mass difference between these series was 100.1 Da, i.e., the mass of one repeating unit of MMA. Figure 3(c) demonstrates a zoom of the molar mass range of 500–600 Da for the MALDI-TOF CID spectrum of the m/z 1625.9 Da species in order to identify each of the four observed fragmenta-

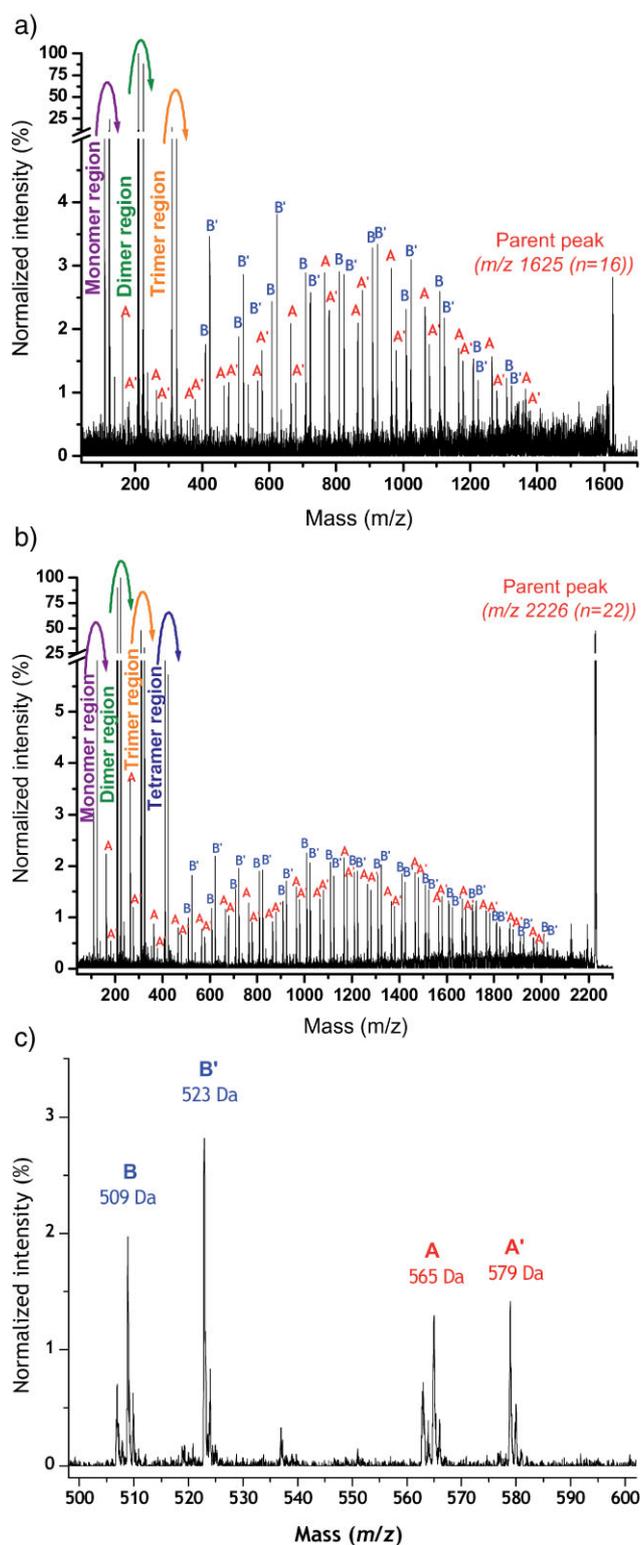
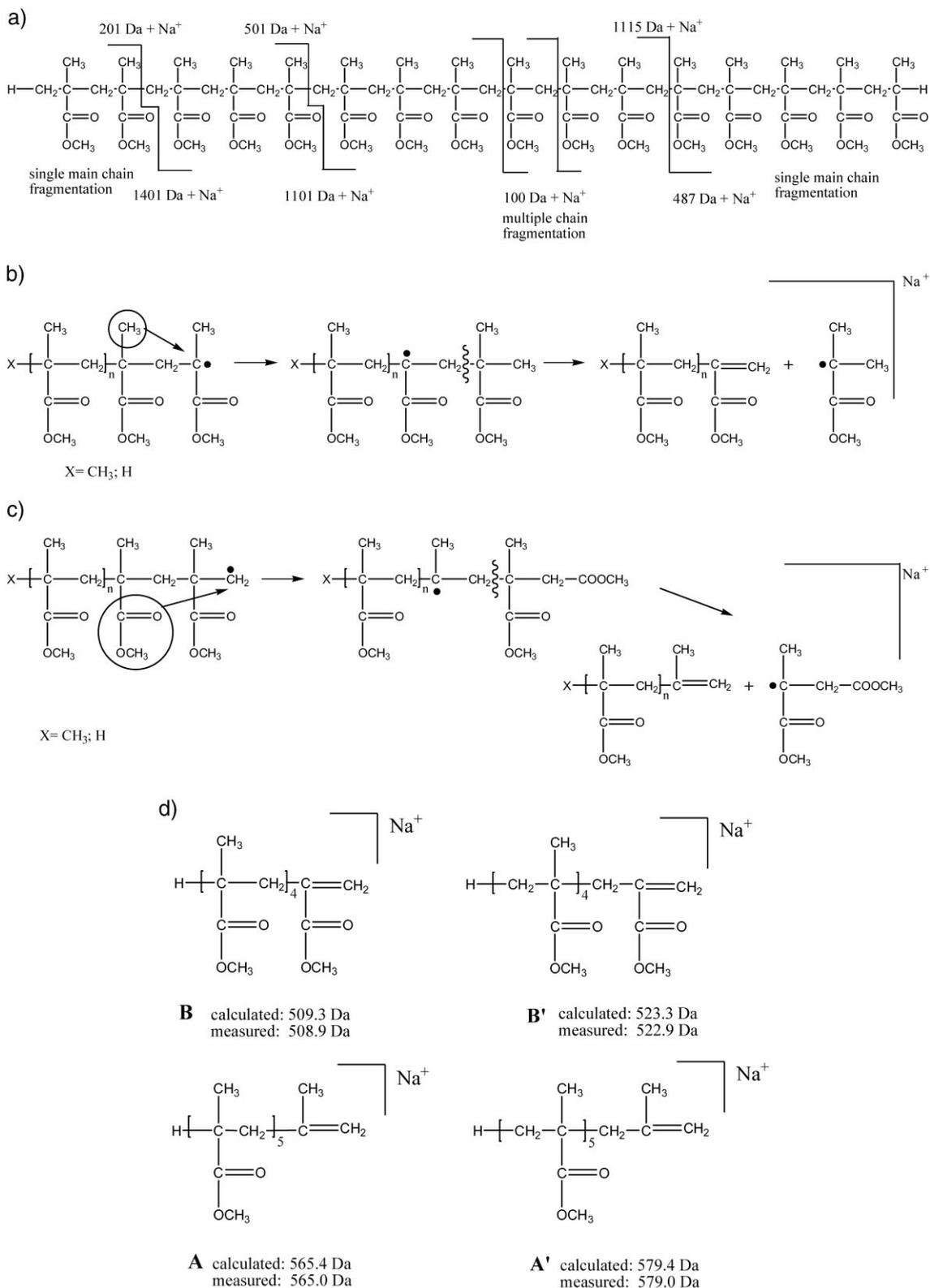


Figure 3. A MALDI-TOF MS/MS spectrum of the selected parent peaks at molar masses of (a) 1625.9 Da and (b) 2226.2 Da. A, A', B and B' indicate four different fragment series. (c) A zoom of the MALDI-TOF MS/MS spectrum of PMMA 2500 (region from 500 to 600 m/z).



Scheme 1. (a) Suggested fragmentation pathways for sodiated PMMA oligomers leading to the four series of fragment ions observed in Figure 3a. (b) Methyl-migration and β -scission. (c) Ester-migration and β -scission. (d) Proposed chemical structures of the fragments in the MALDI-TOF MS/MS spectrum in Figure 3c.

tion series. The peak tagged as B' had a molar mass of 523 Da and fit to the molar mass of a fragment that could be formed by the loss of a methyl group or migration in the backbone followed by a β -scission (1,4 - transfer) and a subsequent main chain fragmentation reaction. The molar mass difference between B' and B was 14, which corresponded to the molar mass difference between the two possibilities of a single main chain scission of the α or ω end of the polymer. As shown in Scheme 1, cleavages from both sides of the polymer could create a couple of radicals that could undergo secondary reactions. The proposed fragmentation mechanism for B' and B could also be observed in the determination with LSIMS and MALDI, which was reported by Jackson et al.^[23–25] They had discussed the generation of the fragment ions as an intramolecular rearrangement process followed by chain scission reactions. For the other two fragment series, it was suggested that the loss of carbon dioxide along with a 1,5 hydrogen rearrangement^[24] or carbon monoxide and methanol^[23] might be possible. These peaks, which are tagged as A and A', were also observed in our studies and corresponded to fragments without a methyl ester group. Most probably, these fragments were obtained through a cleavage of the bond between the carboxyl group and the carbon atom on the backbone. Similar observations for the loss of this group in PMMA during the pyrolysis GC-MS measurements was reported by Tsuge and coworkers.^[26] Finally, the last discussed species, A', corresponded to the complementary of fragmentation A due to the 14 Da difference in molar mass. The proposed chemical structures of all four fragments are displayed in Scheme 1(d). The knowledge obtained from this study will be used in future experiments for a detailed investigation of PMMA, synthesized by various controlled radical polymerization techniques, i.e., reversible addition-fragmentation chain transfer (RAFT) polymerization, atom transfer radical polymerization (ATRP), and nitroxide-mediated radical polymerization (NMP).

Conclusion

A detailed characterization of a defined PMMA sample was demonstrated using the MALDI-TOF MS technique coupled with a CID unit, which allowed the fragmentation of single peaks from the MALDI-TOF MS spectrum. Various distributions were observed in the MS/MS spectra depending on the end groups of the macromolecule as well as the fragmentation process. Possible fragmentation routes in the CID unit were proposed and correlated with the obtained molar masses. It is believed that the characterization of macromolecules using MALDI-TOF MS/MS technique will provide an up until now unrivaled level of information for each single chemical unit of a complex

macromolecule. With this knowledge, it should be possible to investigate the molecular composition, end groups, sequence, as well as specific reaction mechanisms in great detail. The collected fragmentation patterns and species should, in a later stage, permit the construction of MS/MS data libraries for a fast and automated analysis of synthetic polymers in a manner similar to the methods known for proteins and peptide sequences.

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