Recent Advances in MALDI Mass Spectrometry of Polymers

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Summary: Matrix-Assisted Laser Desorption/Ionization (MALDI) allows the identification of repeat units and end groups, the structural analysis of linear and cyclic oligomers, and the estimate of composition and sequence for copolymers. MALDI has also been applied to the measurement of molar mass distributions in polymers and to the study of thermal and oxidative processes in polymers. This paper illustrates the detection of self-association in macromolecules made by coupling MALDI and Size Exclusion Chromatography (SEC), the investigation of polymer oxidation phenomena, and the characterization of copolymers formed in the processing of reactive polymer blends.

Keywords: copolymers; MALDI; molecular association; photo oxidation; reactive blending; thermal degradation

Introduction

Modern mass spectrometry (MS) offers the opportunity to explore the finest structural details in polymers. Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI) mass spectrometry has dramatically increased the mass range of MS; it provides mass-resolved spectra up to 50-70 kDaltons and above, allowing the detection of quite large molecules (10^6 Da) even in complex mixtures. Peaks observed in the spectra originate from ions of intact polymer chains and therefore allow the structural identification of single oligomers. The last few years have witnessed outstanding progress in the application of MALDI to several open problems concerning the characterization of polymers. MS yields information on the mass of individual oligomers, a remarkable difference with respect to NMR, which is an averaging

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method. Therefore, in addition to providing unequivocal information on the chemical structure of polymeric materials, MALDI allows the identification of chain end groups, including species present to minor amounts in a polymer sample. This is so crucial in polymer analysis that its importance cannot be overemphasized, and it has been one of the most popular applications of MS to polymer analysis. It is now possible to perform end group analysis on intact polymer samples, thus identifying the synthetic procedures used in the synthesis of research and industrial polymers and to define the structure of eventual capping agents and additives.

Features and applications of the MALDI technique to the characterization of synthetic polymers have been reviewed to some extent,[1] however new and relevant progress has been reported recently.

In the following, we shall illustrate the detection of self-association in macromolecules made by coupling MALDI and Size Exclusion Chromatography (SEC), the investigation of polymer oxidation phenomena, and the characterization of copolymers formed in the processing of reactive polymer blends.

Detection of Molecular Association by SEC/MALDI

The ionization process of a neutral macromolecule in MALDI proceeds through the capture of a proton or a metal ion (usually Lithium, Sodium, Potassium), which forms a charged adduct with the molecular species.[1] Since MALDI allows desorption and ionization of very large molecules without producing ion fragmentation, one can perform the measurement of molar masses (MM) in high polymers.[1] It has been recently shown that molar mass estimates provided by MALDI measurements agree with the values obtained by conventional techniques only in the case of polymer samples possessing narrow MM distributions (MMD).[1] At high dispersions, MALDI spectra fail to yield reliable MM values, and the MM measured were much lower than those obtained by conventional methods.[1] The MALDI instrument can be used as a detector for size exclusion chromatography (SEC), collecting the SEC fractions and analyzing them off-line by MALDI. The mass spectra obtained allow the calibration of the SEC curves against absolute MM, and thereafter to calculate the MM averages of the original samples from the SEC trace.[1,2] The SEC fractionation of polydisperse polymers provides nearly monodisperse fractions, and MM measurements by

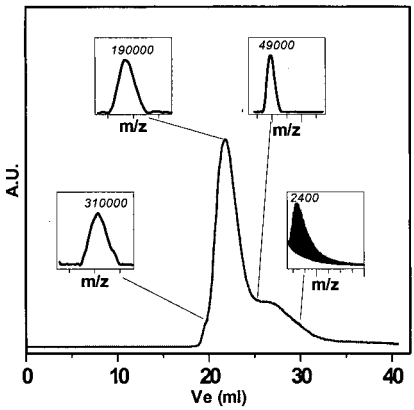


Figure 1. SEC trace of poly(dimethylsiloxane) (PDMS) in THF as eluent. The insets display the MALDI mass spectra of four selected fractions.

MALDI on the samples collected can be used to calibrate the molar mass versus retention volume in SEC plots. Thus, average MM of polydisperse polymers can be determined. At the same time, the MALDI analysis of the fractions obtained by SEC allow the identification of polymer end groups as a function of the MM.[1] A further advantage of collecting SEC fractions of a polymer sample is that one can use them not only for performing the SEC/MALDI coupling, but also for SEC/NMR, or any other suitable coupling, to obtain complementary structural information.[1] Determination of MM and composition distributions (bivariate distribution), was achieved for several copolymers by performing SEC/MALDI and SEC/NMR experiments.[1]

In Figure 1 is shown the SEC trace of a polydimethylsiloxane (PDMS) sample, together with the MALDI mass spectra of four fractions obtained collecting the SEC fractions. These data allowed the calibration of the SEC curve against absolute molar masses and, thereafter, to compute the MM averages from the SEC curve according to the standard procedure adopted in the SEC work.[1,2] The MALDI spectra of the SEC fractions containing the lowest molecular species show the oligomers present as mass resolved signals, allowing the identification both of the polymer structure and of the end groups. It is also possible to identify the presence of cyclic and open chain oligomers, a recurrent structural problem in polymer synthesis. In Figure 2 are shown the mass spectra corresponding to SEC fractions of very low mass of the DPMS polymer reported in Figure 1. One can see that peaks in Figure

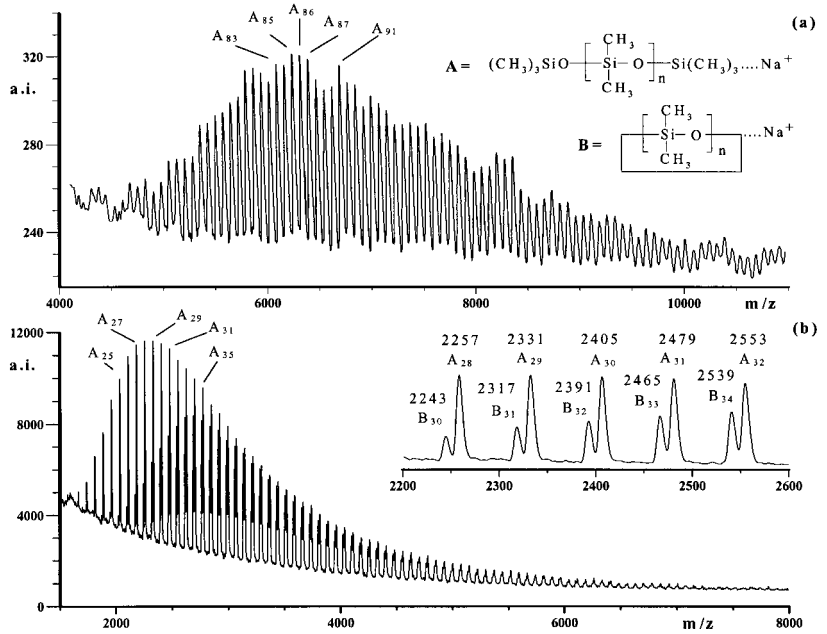


Figure 2. Reflectron MALDI-TOF mass spectra of two PDMS SEC fractions of low MM.

2a correspond only to linear oligomers, whereas in Figure 2b a distribution of peaks corresponding to the cyclic oligomers can be detected beside to that of the linear chains.

The insight gained in these investigations has been extended to explore the MALDI response to the phenomenon of molecular association in poly (bisphenol A carbonate) (PC).[2] Chain self association was observed when a 20 mg/ml solution was injected onto SEC columns, using chloroform ($CHCl_3$) or tetrahydrofuran (THF) as eluent. The presence of self association in PC was revealed by the difficulty of obtaining SEC fractions with the usually narrow MM distribution. In fact, the MALDI spectrum of one of these PC fractions (Figure 3b) shows a bimodal distribution of peaks. The low mass peaks are due to PC chains terminated with OH groups, whereas the ions at high mass correspond to PC chains capped at both ends (see oligomer structures depicted in Figure 3). Thus, the SEC fractionation produced spectra containing PC chains of widely different size, and the MALDI analysis showed that PC chains terminated with hydroxyl (OH) groups undergo self-association by hydrogen bonding. Figures 3a-c show the MALDI spectra of PC fractions collected at the

same elution volume in three different SEC runs, in order to monitor the effect of varying the sample concentration and solvent on the PC self-association.[2] As shown in the spectra, a higher sample dilution (Figure 3a), or the addition of a polar solvent such as ethanol to the $CHCl_3$ eluent (Figure 3c), are able to suppress self-association in PC samples, in fact both MALDI spectra show a narrow distribution of peaks centered around 15 Kda, corresponding to the PC chains of type A (Figure 3). Chain association of PC was found to produce molecular aggregates of relatively small molecules showing a high hydrodynamic volume, that were therefore eluted through SEC columns at the same elution volume a the higher molar mass chains. However, the molecular aggregates were broken when the SEC fractions, containing an heterogeneous mixture of PC chains of different size, were diluted in the matrix used for the MALDI sample preparation. Some common MALDI matrices contain the carboxylic acid units, which is able to break the hydrogen bonds responsible for the formation of the chain aggregates.

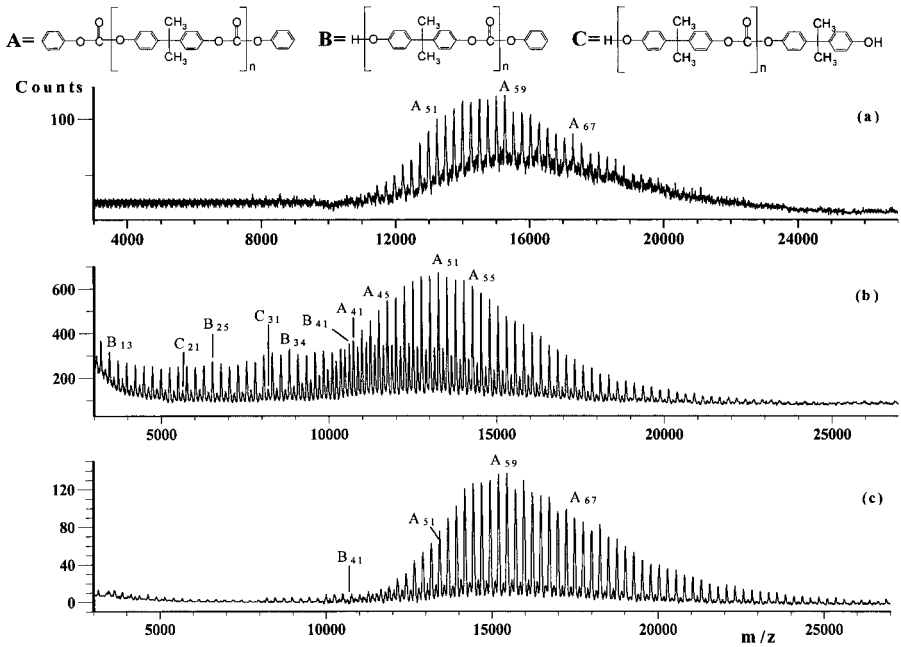


Figure 3. MALDI-TOF spectra of PC fractions collected at the same elution volume (31.3 mL) in four different SEC runs: **(a)** sample PC1 injected at a concentration of 2.5 mg/mL in $CHCl_3$; **(b)** sample PC1 injected at a concentration of 20 mg/mL in $CHCl_3$; **(c)** sample PC1 injected at a concentration of 20 mg/mL in $CHCl_3/C_2H_5OH$ 95/5 v/v.

Thermal and Oxidative Degradation

Recent studies have shown that MALDI is an incredibly useful method to analyze the oligomers produced in the degradation of synthetic polymers.[3-6] The study of polymer degradation phenomena by MALDI involves the collection of several MALDI spectra at different times and/or temperature to observe the structural changes induced by heat, light under inert and/or oxidising atmosphere.

The isothermal pyrolysis of BPA-polycarbonate (PC) was studied at a temperature between 300°C and 450°C under a nitrogen stream, and the MALDI-TOF spectra showed that a rearrangement of the carbonate group leads to the formation of several adjacent xanthone units in PC chains of sizeable molar mass.[3] Xanthones are considered to be precursors of graphite-like structures in the char residue that is produced at temperatures higher than 450°C under inert atmosphere.[3] The structure of the species produced in the thermal oxidative degradation of PC has been also analysed.[4] The PC samples were heated at 300°C and 350°C under atmospheric air up to 180 min producing a THF insoluble gel at the longer heating times. The MALDI spectra of the thermal oxidized samples showed the presence of oligomers containing acetophenone, phenyl substituted acetone, phenols, benzyl-alcohol terminal groups. The presence of biphenyl units among the thermal oxidation products confirms the occurrence of cross-linking processes, which are responsible for the formation of the insoluble gel fraction.[4]

Applications of MALDI to the study of polymer photo oxidation are quite recent.[5,6] The results obtained for the systems so far investigated are surprisingly high informative, as compared with previous studies based on conventional techniques as UV and IR. Molecules formed in the photo oxidation processes are often very reactive, do not accumulate, and are present only in minor amounts among the reaction products. Nevertheless, MALDI spectra yield precise information on the size, structure and end groups of molecules originated in the oxidation process, allowing the discrimination among possible oxidation mechanisms. In a recent investigation, Ny6 films subjected to photo ageing were analysed by MALDI.[5] The MALDI spectra show the presence of over 40 compounds, as compared to only three in the blank Ny6 sample (Figure 4). The spectra in Figure 4 carry an extremely rich structural information on the photo-oxidation products of Nylon 6. Three photo-oxidation processes are occurring in Ny6, as summarised in Scheme 1. The first process consists in a hydrogen

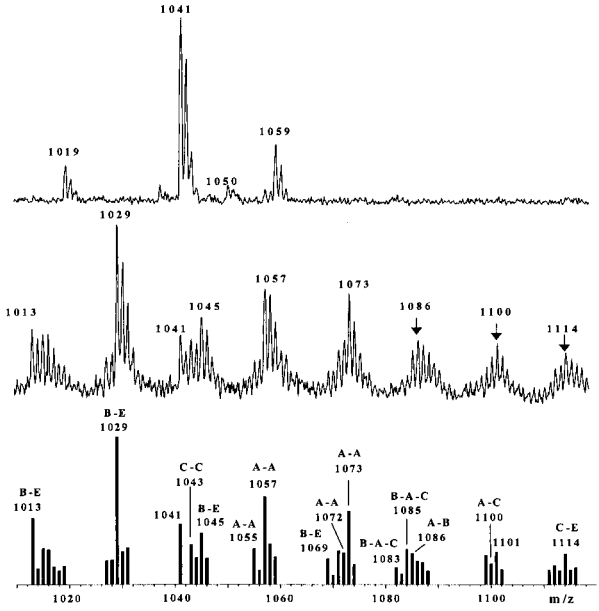
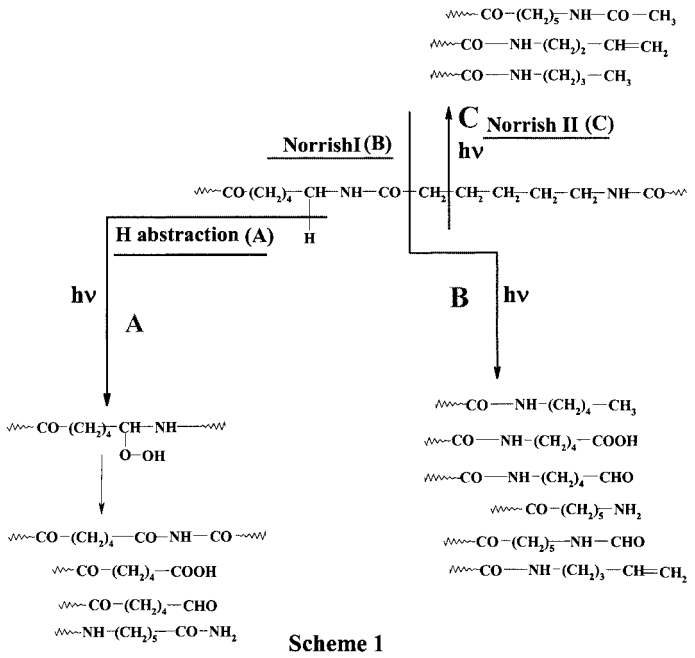


Figure 4. MALDI spectra, in the mass range 1010-1120 Da obtained in reflectron mode, of 40mm Ny6 photo-oxidized for (a) 0 h and (b) 289 h. In (c) is reported the deisotiping mass spectrum of last sample.

abstraction from the methylene group adjacent to the amide NH, leading then to the formation of a hydroperoxide intermediate. The decomposition of this hydroperoxide generates the actual products of Ny6 photo-oxidation by radical rearrangement reactions (Scheme 1A). Beside to the hydrogen abstraction and subsequent hydroperoxide formation, which had been established in previous studies, two other major processes appear to be operating in Ny6, i.e., chain cleavage reactions Norrish type I and Norrish type II (Scheme 1B and 1C). In Figure 4c each peak carries a label; the capital letter A specifies any end group generated by the decomposition of the hydroperoxides (Scheme 1A); B specifies any end group generated by the Norrish type I chain cleavage (Scheme 1B); C specifies any end group generated by the Norrish type II chain cleavage (Scheme 1C); whereas E indicates just one of the end groups present in the original Ny6 sample. Since each oligomer has two ends, the notation B-A, for instance, means that a Norrish type I chain cleavage occurred at one end and that hydroperoxide decomposition occurred at the other end. There are five oligomers are originating exclusively from Norrish I, and four oligomers exclusively from Norrish II chain



Scheme 1

cleavage reactions. Furthermore, nine peaks are exclusively due to Ny6 oligomers originating only from hydroperoxide decomposition reactions. The presence of the majority of these oligomers had not been revealed before.

In another study, the commercial polyetherimide ULTEM was subjected to photo ageing.[6] The MALDI spectra showed the presence of polymer chains containing acetophenone, phenyl acetic acid, phenols, benzoic acid, phthalic anhydride and phthalic acid end groups. The mechanisms accounting for the formation of photo oxidation products of Ultem involve several reactions, which could be identified: i) photo-cleavage of methyl groups of the N-methyl phthalimide terminal units; ii) photo-oxidative degradation of the isopropylidene bridge of BPA units; iii) photo-oxidation of phthalimide units to phthalic anhydride end groups: iiii) hydrolysis of phthalic anhydride end groups.

Overall, a remarkable advance has been provided by these investigations and it should be expected that future MALDI studies might have an impact on the current views on photo oxidation processes of other polymer systems.

Reactive Polymer Blends

The melt mixing of polymer blends is a promising route for the synthesis of block, segmented, or random copolymers, which are produced through chemical exchange reactions occurring between opportune functional homopolymers.[7-9] Furthermore, the formation of sizeable amounts of copolymer is frequently observed in the melt processing of polymer blends. Since the copolymer may act as a compatibilizer and influence the blend properties, in order to ensure reproducible results, some knowledge of the reaction pathways and of the parameters involved in the process would be required. However, the reactive polymer blends technology currently encounter problems in controlling the reaction parameters, partially due to the practice of mixing polymers looking only at the repeating units but not at their chain ends, and own also to the lack of adequate analytical protocols.

The material produced by reactive blending of two homopolymers is a mixture of the two unreacted homopolymers and of the copolymer formed. As the reaction goes on, the two homopolymers disappear and the copolymer becomes abundant. NMR methods, which are able to determine directly the copolymer sequence and composition, have found ample use to analyze the blends, thus avoiding complicated and tedious wet chemistry methods involving solvent separation of unreacted homopolymer components from the copolymer formed. However, being an averaging method, NMR yields safe results only when the homopolymers blend has reacted nearly completely, and the copolymer is the only component left. In case of partial conversion, NMR data provide only an averaged estimate of the blend composition, which may not be coincident with the actual copolymer composition.[7-9] For the same reason, NMR does not provide information on copolymer yield. Furthermore, NMR is often unable to detect low amounts of reactive end groups and, furthermore, it is not possible to establish to what molecule these groups are attached. On the contrary, the application of MS to the study of reaction mechanisms in melt mixing processes, has allowed solving important problems: structure of the end groups present in the reacting polymers, copolymer yield as a function of the melt mixing time, composition and sequence of the copolymer formed.[7-9] The current trend follows still the practice of mixing polymers without considering the chain ends, looking only at the repeating unit of each polymer. This is because the initial studies started on ester/ester blends, where the ester linkage can react by ester/ester exchange (in presence of a catalyst). Therefore the role of end groups was often neglected, although it is now

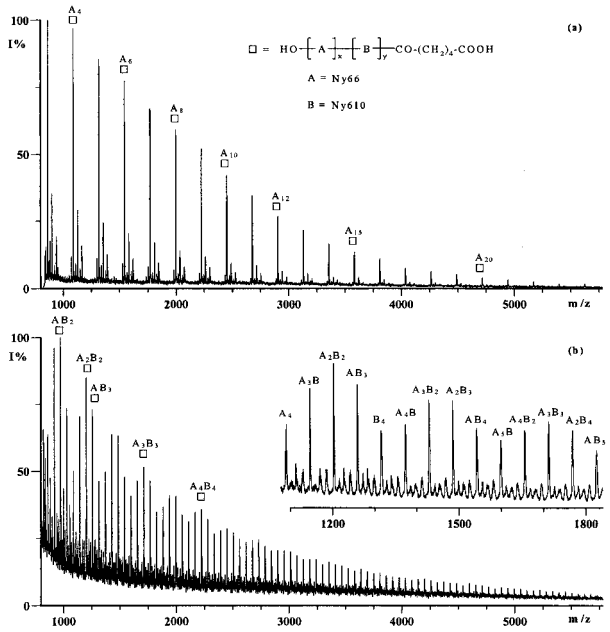


Figure 5. MALDI-TOF mass spectra of an equimolar mixture of Ny6,6-COOH (M_v=7,200) and high MM Ny6,10 (M_v =36,100): (a) physical blend, (b) melt mixed at 290°C for 30 min.

ascertained that the exchange in absence of catalysts occurs through the attack of reactive end groups to the polyester main chain. Remarkably, in the case of ester/amide and amide/amide blends there are no exchange catalysts, and the presence of reactive end groups is essential to the occurrence of exchange in these blends.[7-9]

In Figure 5 are shown the MALDI spectra of an equimolar mixture of carboxyl terminated Ny6,6 (Ny6,6-COOH) and high MM Ny6,10, either for the physical blend (Figure 5a) and for the melt mixed blends at 290°C for 30 min (Figure 5b). Ny6,6-COOH oligomers predominate in Figure 5a, whereas a drastic change is observed in the MALDI spectrum of the heated blend (Figure 5b), hinting that the formation of Ny6,6/Ny6,10 copolymers by exchange reactions has occurred.[7] In fact, the most intense peaks are due to copolymer oligomers formed in the process of melt mixing. From the MALDI spectra it is possible to characterise directly the copolymer structure. In fact, the problem of decoding the intensity of peaks appearing in the mass spectra of copolymers and of relating them to the comonomer sequence and composition has been approached, providing a method for deducing the sequence

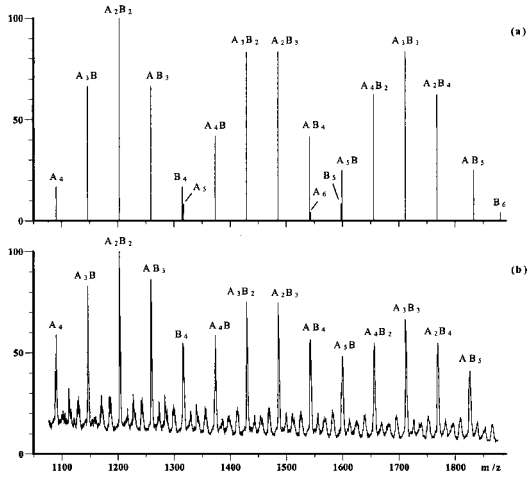


Figure 6. MALDI spectra in the mass region 1050-1890 Da, of a random copolymer containing a equimolar units of Ny66 (A) and Ny610 (B) units: (a) Calculated mass spectrum and (b) experimental mass spectrum.

distributions and composition of comonomers in copolymers by MS techniques.[1] Looking at copolymers by MS, the relative abundance of all the oligomers of a defined chain length reflects the composition and monomers sequence present in the copolymer. Chain statistics (Bernoullian, first or second order Markoffian), allows to generate any arrangement of comonomer units along the chain. Starting from any sequence, a theoretical mass spectrum can be generated, based on the assignment of each spectroscopic peak to a set of sequential arrangements of monomers. The intensity of each peak is related to the relative abundance of the sequential arrangements present in the copolymer, and their relative intensities reflect the comonomer distribution.[1] This means that one has the possibility to build a theoretical mass spectrum for any given copolymer sequence, and that this can be compared with the experimental mass spectrum corresponding to the copolymer sample being investigated. In the case just discussed (Ny66/Ny610), theoretical matching of the experimental peak intensities (Figure 6a-b), was obtained for a Bernoullian distribution in the copolymer and for a molar ratio of 50/50 between the two comonomers. The statistical analysis was also applied to the MALDI spectra of the equimolar Ny66-COOH/Ny610 blend melt mixed for 10 and 15 min at 290°C, showing that a random copolyamide containing 64 mol% of Ny66 and 59 mol% of Ny66, were formed after 10 and 15 min heating, respectively. This analysis has

revealed also that 46, 54 and 100 percent % of copolyamide were formed by heating the Ny66/Ny610 blend for 10, 15 and 30 min, respectively. The determination of the extent of Ny66/Ny610 copolyamide formation, sequence, and composition proved to be unfeasible by ^{13}C-NMR analysis of the melt mixed materials, because the chemical shift of the copolyamide are close to that of the Ny66 and Ny610 homopolymers.[7]

In another study, the MALDI analysis allowed the structural identification of the copolyesteramide formed during the melt mixing of the Ny6/PET and of the Ny6/PBT blends.[8,9] It revealed the essential role of carboxyl end groups in the exchange reaction, and provided a detailed mechanism for this reaction.[8,9] The composition, sequence distribution, degree of randomness and the yield of the copolyesteramides formed, as a function of the melt mixing time, were also calculated.[8,9]

Conclusions

In the last few years Mass Spectrometry has rapidly become indispensable in polymer analysis and complements in many ways the structural data provided by NMR. Mass Spectrometry of polymers is emerging as a revolutionary technique, capable of challenging the techniques and protocols established for years for the characterisation of synthetic polymers. The primary aim of this review is to illustrate some recent advances in the study of macromolecular systems by MALDI. In the foregoing sections we have discussed the striking detection of self-association in macromolecules, the surprising detailed analysis of thermal and oxidative processes in polymers, and the remarkable progress in the understanding of the exchange reactions occurring in the reactive blends processing. The examples given in the text are drawn from work of the authors laboratory, and perhaps it should be remarked that they are not meant to illustrate all the recent advances in the field.

A detailed review of MALDI work on polymers can be found in reference.[1]

[1] G. Montaudo, M.S. Montaudo, F. Samperi in: "Mass Spectrometry of Polymers", G. Montaudo, R.P. Lattimer, Eds., CRC Press, Boca Raton 2002, Chapter 10.
[2] C. Puglisi, F. Samperi, S. Carroccio, G. Montaudo, *Rapid Commun. Mass Spectrom.* **1999**, 13, 2268.
[3] C. Puglisi, F. Samperi, S. Carroccio, G. Montaudo, *Macromolecules* **1999**, 32, 8821.
[4] S. Carroccio, C. Puglisi, G. Montaudo, *Macromolecules* **2002**, 35, 4297.
[5] S. Carroccio, C. Puglisi, G. Montaudo, *Macromolecules* **2003**, 36, 7499.
[6] S. Carroccio, C. Puglisi, G. Montaudo, *Polym. Deg. & Stab.* **2003**, 80, 459.
[7] C. Puglisi, F. Samperi, S. Di Giorgi, G. Montaudo, *Macromolecules* **2003**, 36, 1098.
[8] F. Samperi, C. Puglisi, R. Alicata, G. Montaudo, *J. Polym. Sci. A: Polym. Chem.* **2003**, 41, 2778.
[9] F. Samperi, M.S. Montaudo, C. Puglisi, R. Alicata, G. Montaudo, *Macromolecules* **2003**, 36, 7143.