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# MALDI of synthetic polymers—an update

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## Abstract

Recent advances in MALDI of synthetic polymers is reviewed. The review will be limited in scope and not comprehensive, but will focus on areas where there has been significant progress and/or where some opportunities lie. Ion formation mechanisms for polymers still remain somewhat elusive. Advances in studies of protonation mechanisms need to be extended to synthetic polymers to answer the question why the latter seem to largely prefer cationization over proton addition. There has been significant progress in the MS/MS of synthetic polymers, mostly by collision-induced dissociation and post-source decay. New and interesting results have been reported, but this area requires further investigation. The combination of MALDI with separation methods for synthetic polymers shows considerable potential and various applications are appearing. Of particular interest is coupling to size-exclusion chromatography and how to perform measurements on-line and in real time. Determination of polymer structure is possible including end-group analysis, derivatization, and analysis for embedded chain segments. Finally, novel solid-state supports have been reported, offering great potential for MALDI generally and polymer MALDI specifically.

*Keywords:* Derivatization; Biopolymers; Fourier transform mass spectrometry

## 1. Introduction

Although the introduction of the MALDI technique in 1988, as it is currently practiced [1], stimulated a flurry of activity for measuring the molecular weights of biopolymers, there was little activity in the published literature dealing with such measurements for synthetic polymers until 1992. This is surprising, given that Tanaka et al. reported a polymer spectrum in their paper using colloidal Co as a matrix [2]. Although there is considerable anecdotal evidence that MALDI of polymers was carried out before that time in industrial laboratories, three groups published the first results dealing with MALDI of polymers in that year. Wilkins and coworkers published a polymer spectrum as part of their successful coupling of MALDI to Fourier transform mass spectrometry (FT–MS) [3]. Danis et al. reported the molecular weight (MW) distributions for poly(styrene sulfonic acid) and poly(acrylic acid) [4] and Hillenkamp and coworkers reported measurements of MW distributions for a variety of polymers including poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG), poly(methyl methacrylate) (PMMA), and polystyrene [5]. Polymers with molecular weights as high as $M_n = 70{,}000$ were measured; all polymers had low polydispersity, typically 1.03.

It is of historical interest that the polymer community was not as enthusiastic as the biomedical commu-

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nity about the use of mass spectrometry for measuring high MW materials. There are probably two reasons for this. First, much could be learned about synthetic polymers from NMR and second, most forms of mass spectrometry had, historically, provided information about polymers from fragment-ion spectra; it was molecular weight information that was of interest to the polymer community. Historically, only field desorption (FD) and secondary ion mass spectrometry (SIMS) were able to provide MW distributions, and both were limited to soluble polymers having $M_n <$ 10,000 and in most cases below 5000 [6,7]. FD was plagued by serious problems with fragmentation and difficulty of use for most laboratories. SIMS could not be applied to polymers generally and, although obtaining polymer spectra was often easy, SIMS required specialized instrumentation that was not available in many laboratories. Electrospray ionization (ESI) mass spectrometry had not made its debut on the scene.

The present paper will be limited in scope and is not a comprehensive review for two reasons. First, an excellent and comprehensive review of MALDI of synthetic polymers was published by Nielen in 1999 [8]. That publication covered the literature up to 1998 with a few references from 1999. So the present review will deal mostly with publications from 1999 and forward. Second, as is typical in any area of science, progress in MALDI of polymers has been more significant in some areas than in others. Allowing for prejudicial interpretation, we will highlight the significant areas. Finally, the present review will be concerned only with MALDI using ultraviolet lasers.

Two of the most crucial aspects of any successful MALDI measurement are selection of the appropriate matrix and sample preparation. This is particularly true for synthetic polymers which can vary significantly in their solubility, polarity and affinity for different counter ions. The matrix, solvent system, preparation scheme, and ion formation conditions that are optimum for PPG and PEG will not necessarily be optimum for polystyrene. An enormous variety of different combinations of the four parameters above has been reported in the literature. Nielen has summarized successful combinations of matrices and solvent systems in an extensive table (more than six journal pages) in his review article [8]. While some progress has been made in understanding parameters which are important for the matrix selection process, it is still largely a matter of trial and error. This makes Nielen's table of considerable value to the practicing MALDI spectroscopist.

This paper will review progress over the last several years in the following areas related to MALDI of polymers: ion formation; MS/MS experiments; combination of MALDI with size-exclusion chromatography (SEC); the use of chemical reactions to enhance MALDI of polymers; and the use of solid substrates.

## 2. Ion formation

The mechanism of ion formation in MALDI still remains an interesting and fascinating puzzle; ion formation in polymers is less understood than for other types of molecules. In general, three types of ion formation processes are known in MALDI: proton transfer, cationization by metal ions, and odd-electron ion formation. All three have been observed for polymers; the topic has been reviewed recently [9]. Additionally, there is the question about whether ionization occurs mainly in the "gas phase" in the plume over the sample created by the laser, or if ablation of pre-formed ions constitutes the majority process. Similarly, the energetics of ion formation and the kinetic energies of ablated particles are important considerations. The energetics of ion formation for proton transfer reactions has received more attention than the comparable metal ion reactions.

Even a brief perusal of the literature reveals clearly that cationization is the major ion formation process in MALDI of polymers. This stands in contrast with biopolymers for which proton transfer often appears to be the preferred mechanism. So the question becomes, why is protonation less common in the MALDI of polymers? To answer this question will require careful experiments directly measuring competition for bonding with polymers between protons and metal ions. There has been much interest in proton transfer studies for a variety of MALDI matrices, and data derived from these should be of value for model compound studies. Therefore, recent work on proton transfer reactions is reviewed below.

### *2.1. Proton transfer reactions*

An important aspect of studying the mechanism(s) of proton transfer in MALDI is the availability of data on the acidity and basicity of MALDI matrices. Zenobi's group carried out a fundamental thermochemical study of the gas-phase basicities of MALDI matrix anions using FT-ICR mass spectrometry. They devised a modified bracketing method which permitted measurements to within ±11.5 kJ/mol [10]; basicities measured were over an energy range of 127 kJ/mol. Bracketing was done by using a number of anions having known basicities and was checked by measuring known standards. Ions detected in the spectra were deprotonated matrix ions, heterodimer ions, or both species simultaneously. Gas-phase ion–molecule reactions in the ICR reaction cell were mostly complete after 10 s. Unfortunately, some important MALDI matrices could not be studied by this method due to low vapor pressure. An important conclusion was that analyte deprotonation by matrix ions was energetically favorable, while proton disproportionation reactions between neutral matrix molecules and the analyte was not.

In a subsequent study, the gas-phase basicities of the anions of monomeric and dimeric ferulic and sinapinic acids were measured using the bracketing method [11]. The matrix dimer anions were less basic than the corresponding monomeric anions. This means that proton transfer reactions between matrix dimers and analyte molecules will be favorable. The energies required were estimated to be 3–4.7 eV; these are in the range of two UV photons. On this basis, matrix dimers may play a significant role in the MALDI ionization process.

Recently, Zenobi's group reported the gas-phase sodium ion basicities of 12 MALDI matrixes detected in a FT-ICR mass spectrometer by monitoring the sodium ion transfer reaction between sodiated matrix molecules and a reference base with known gas-phase basicity [12]. The data were analyzed by two methods; in the first method the equilibrium constant was detected directly from ion concentrations at equilibrium and the other was based on fitting the reaction kinetics over the full reaction time scale. Fitting of the reaction kinetics solved the problem of competing reactions, i.e., formation of sodium bound dimers, which complicates direct detection of equilibrium constants. The gas-phase sodium ion basicities of 12 MALDI matrixes were found to lie between 140 and 170 kJ/mol.

Another study reported measurement of the gas-phase basicities of the six isomeric dihydroxy benzoic acids (molecular) and their radical cations [13]. Similarly, these were measured using ICR mass spectrometry but employing the thermokinetic method. As might be expected, the basicities covered a small range, from 814 to 831 kJ/mol. The gas-phase acidities of the radical cations were 815–858 kJ/mol. Some structural effects were noted: *o*-hydroxy substitution increased basicity for the molecules, similarly the radical cations which lacked an *o*-hydroxy group were the most acidic. An important finding was that proton transfer from a radical cation to a neutral matrix molecule is not energetically favorable. However, ground-state proton transfer from matrix radical cations to analyte molecules may represent a source of protons for MALDI.

### *2.2. Cationization reactions*

A number of studies have appeared on aspects of cationization of polymers by metal ions in the MALDI process. One reported the effect of the matrix on the relative cationization efficiencies of Li and Cs (1:1 molar ratio) for PEG [14]. Relative cationization efficiency was found to depend heavily on the matrix. Cs ions showed higher efficiency for the IAA, DHB, and 2,4,6-trihydroxyacetophenone matrices, while Li ions were favored by HABA, α-CHCA and dithranol. No difference in cationization effects for different sample preparation methods was observed for HABA. This result suggests that the presence of free ions in the

MALDI plume is not the sole determinant of which cation will prevail.

The role of the oxidation state of the metal ion for MALDI cationization was also investigated [15]. It was observed that for efficient cationization of polystyrene by transition metals, reduction to the +1 formal oxidation state is essential. Comparisons considered Ag, Cu, Pd, and Cr. Another study addressed the use of metallocenes as cationizing agents for PEG and polystyrene [16]. Metallocenes of Fe, Co and Ni gave quality spectra for polymers having $M_n$ values of 2000–13,700. Based on the lack of a mass shift for a labeled ferrocene, it was concluded that bare $Fe^+$ ions were responsible for charge formation to yield polymer ions.

The effect of salt anions on the MALDI cationization process has been studied for PMMA [17]. The relative intensities of the oligomer peaks varied in the order: NaI $>$ NaBr $>$ NaCl, independently of laser power over a narrow range. When oligomer distributions were measured for the series LiI–NaI–KI–RbI–CsI there was little variation in integrated oligomer intensities. For a similar series of chlorides (LiCl–CsCl), variation was a factor of three with LiCl the most intense and CsCl the least. However, $M_n$ values calculated from the distributions did not differ statistically for chlorides. The primary reason for these effects appears to be the different abundances of cations caused by differing salt lattice energies.

Another interesting study determined the effect of polyether structure on the relative cationization efficiencies using a 1:1 mixture of LiCl and CsCl. The polyethers studied were PEG, PPG and poly(tetrahydrofuran) (PTHF) [18]. It was shown that in the IAA matrix Cs was dominant for PEG, there was about a 1:1 correspondence for PPG, and Li totally dominated for PTHF. However, Li dominated for all three polyethers in HABA. These results are discussed in terms of gas-phase structure of the polyethers, rigidity of the polymer chain, and the oxygen-atom density of the polymer.

The structures and dynamics of cationized oligomer ions of poly(ethylene terphthalate) (PET) were studied by a combination of MALDI and ion mobility spectroscopy [19]. The individual oligomer ions were mass selected by a sector instrument for ion chromatography, thus only low-number oligomers could be studied. For PET oligomers with $n = 2$ and 4 arrival time distributions were single valued, while for $n = 3$ they were bimodal. For $n = 3$ oligomers, there are two forms, open and closed, thus generating different geometries and different ion mobilities. The ionization barrier between these forms was 1.6 kcal/mol for the Na-cationized isomer and 7 kcal/mol for the Li isomer. Results observed were consistent with structures derived from molecular dynamics calculations. An ion formation mechanism was postulated which is consistent with metal ion attachment to the polymer in the “gas phase” over the sample surface.

Finding a suitable matrix for MALDI can, at times, be a significant challenge. This is particularly so in the case of synthetic polymers because of their diversity in structure, functionality and polarity. MALDI of non-polar polymers has been particularly problematic, stimulating recent interest in the use of non-polar matrices. Anthracene, pyrene and acenaphthene were used as matrices for polystyrene, polybutadiene and polyisoprene [20] having $M_n$ values of 700–5000. Results compared well with MALDI spectra taken in a traditional non-polar matrix, *trans*-retinoic acid. Cationization was with Ag and Cu; Ag gave the best results. A subsequent study investigated silver salt clusters which are problematic when non-polar polymers are run in conventional polar matrices [21]. The background signals due to these clusters were reduced significantly when non-polar matrices were used.

## 3. Tandem MS/MS

Tandem mass spectrometry is a sophisticated form of mass analysis whereby ions separated according to their $m/z$ value in a first stage analyzer are selected for fragmentation and the fragments analyzed in a second analyzer. Various fragmentation methods for the gas-phase molecular ions have been developed in the past decade (e.g., electron impact dissociation, surface-induced dissociation (SID), photodissociation, sustained-off resonance irradiation (SORI)). Among these, collision-induced dissociation (CID) [22,23] is probably the most frequently employed fragmentation technique. Fragmentation occurs due to the additional internal energy of the gas-phase molecular species. In particular, the CID mechanism involves activation of a molecular ion by collision with a target gas, resulting in the conversion of translational energy into internal energy, leading to fragmentation. The amount of internal energy imparted depends on the type of mass spectrometer. The most commonly available tandem mass spectrometers, which employ quadrupole or ion trap mass analyzers, operate in the so-called low energy regime. In this regime, kinetic energies of ions do not exceed 100 eV, resulting in the MS/MS spectra dominated by "low energy" (LE) fragments, which are formed by breaking the weakest bonds. By contrast, TOF analyzers are optimized for transmission of ions of high kinetic energies, typically up to 20 keV. Collision of these ions gives "high energy" (HE) fragments that provide more complete structural information. Therefore, there have been a number of instruments that have employed TOF analyzers in tandem mass spectrometers, sometimes as hybrids combined with sectors [24], but often as TOF/TOF [25].

Except for mass determination, MALDI processes can be also used for structural analysis. Spengler and Kaufmann [26] were the first to introduce the term "post-source decay" (PSD) to cover the possibility of metastable decomposition, caused by excess ion energy obtained during the complex MALDI processes. Compared with LE and HE-CID of ions, MALDI-PSD processes are far less understood. In general terms, the nature and extent of fragmentation observed in PSD spectra is influenced mainly by laser fluence, matrix choice, and the number of collision events in the MALDI plume. After leaving the ion source all ions have the same kinetic energy and enhanced internal energies. The internal energy gain causes ions to undergo fragmentation during their flight in the field-free region of a MALDI mass spectrometer. Since the resulting fragment ions keep the same velocity, a suitable precursor ion selector (a "first mass spectrometer") allows transmission of the selected parent ions together with their daughter ions into the reflector. Fragment ions of different masses, having different kinetic energies, can be analyzed with a second "mass spectrometer" by scanning potentials of the MALDI reflector stepwise so that a MS/MS daughter spectrum can be obtained. Therefore, in MALDI/PSD the reflector acts as a kinetic energy analyzing device in contrast to non-PSD mass spectrometry measurements where all ions have the same kinetic energy but their velocity is a function of mass.

Although charge-remote fragmentation processes that are typical for HE-CID can occur [27], PSD is considered to be a "soft" fragmentation method. The degree of fragmentation can be partially controlled by a combination of matrix selection [28], ionization facilitator, and laser fluences. However, there are analytes that do not show extensive metastable decomposition or do not undergo PSD fragmentation. This might be particularly true for certain kinds of synthetic polymers. In this case, fragmentation can be enhanced in a gas collision cell, usually located within the first field-free region of the MALDI instrument, resulting in CID of the ions. For example, our study on single molecular weight oligomers of Nylon-6 showed that in the PSD mode only end group fragments (A, B, C) are generated in contrast to the HE-CID mode in which series of sequential fragment ions (*X*) are generated in addition to end group cleavages (see Fig. 1). Differences between PSD and CID spectra of the 9-mer of PMMA were also reported by Scrivens et al. [29]. Similar fragment ions were formed, however, from metastable decay and CID processes in a hybrid sector-orthogonal acceleration time-of-flight tandem mass spectrometer [29]. The HE-CID process is a "hard" fragmentation process. An ion with a kinetic energy of several kiloelectronvolts collides with a stationary collision gas molecule. CID processes can be controlled with the pressure and nature of the gas in the collision cell. In addition, MALDI-CID processes are influenced by a matrix and ionization agent choice [28], their ion affinities, and again laser fluences. Little is currently known about the influence of matrices and cationization agents on gas-phase fragmentation processes. A better understanding of the ionization

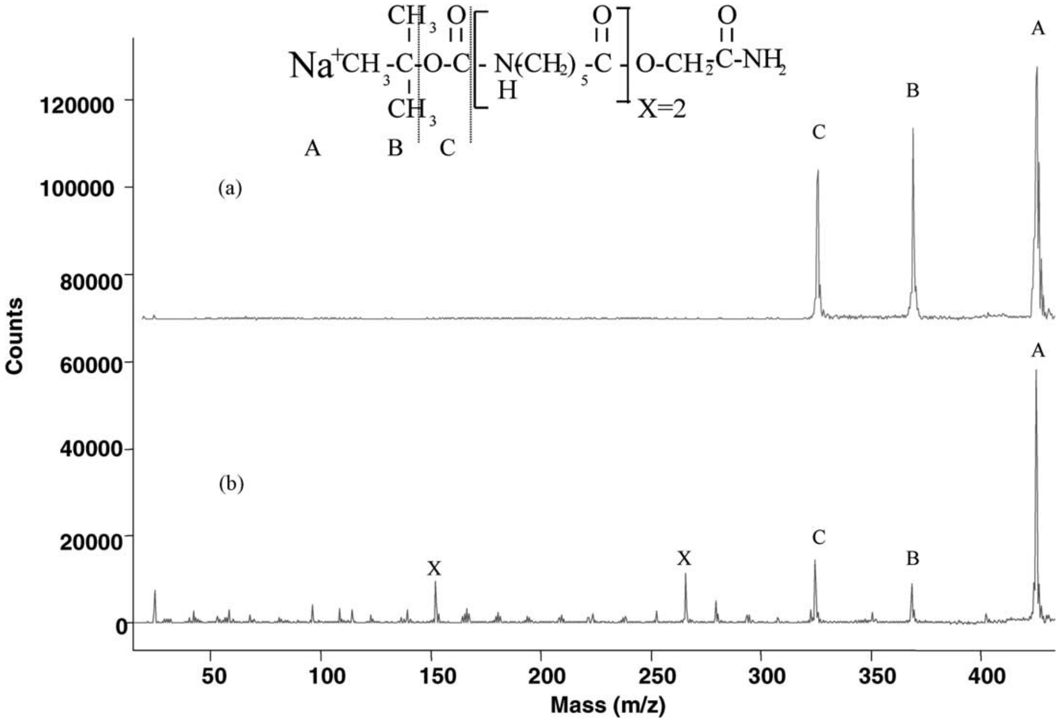

Fig. 1. MALDI-PSD (a) and MALDI-CID (b) spectra of the dimer of Nylon-6.

process in MALDI-PSD/CID is important to improve ion yields and better control fragmentation. The effect of the cation variation on the MALDI-CID spectra has been studied in a hybrid sector-orthogonal acceleration time-of-flight tandem mass spectrometer for PS and PMMA [29]. Similarly to our experiments on Nylon-6 single molecular weight oligamides, carried out in a conventional MALDI mass spectrometer [30], yields of the fragment ions in the MALDI-CID spectra were found to be dependent on the nature of the cation. The distribution of the fragment ion peaks remained essentially the same. When the matrix was varied the fragmentation patterns of the 12-mer of Nylon-6 were identical, while ion yields decreased in the order: 2,5-dihydroxybenzoic acid > *cis*-cyano-4-hydroxycinnamic acid > 2-(4-hydroxybenzylazo) benzoic acid > 2, 4, 6-trihydroxyacetophenone [30].

The use of MALDI-CID for end group determination of synthetic polymers was demonstrated on polystyrenes [31] and poly(alkyl methacrylate)s [32] in a tandem hybrid sector-TOF instrument. Mechanisms based on direct cleavage (via distonic radical species) and recombination were proposed for the formation of some of the series of ion peaks observed in the MALDI-CID spectra. The highest mass-to-charge ratio of a precursor ion, from which the polystyrene MALDI-CID spectrum was obtained with this equipment, was 4951.

Our group has carried out a MALDI-CID study of styrene-(*cis*-methyl styrene) block copolymers along with poly(styrene) and poly(*cis*-methyl styrene) homopolymers in a conventional MALDI-TOF instrument [33]. The origin of fragment ions observed in our spectra can be explained via charge remote rearrangement processes rather than direct cleavages. It should be pointed out, however, that a difference between flight times of ions in a conventional MALDI-TOF spectrometer and a hybrid instrument may influence the type, extent, and overall distribution of fragment ions in the MALDI-CID spectra. Moreover, the resolving power of current MALDI-TOF spectrometers is typically low and does not possess isotopical resolution as it is in the case of hybrid instruments.

Despite the potential of tandem mass spectrometry, particularly for microstructure determination, this technique has not been widely applied for the analysis of synthetic polymers. In fact, tandem mass spectrometry is often the only clear way to distinguish between oligomer species with the same mass and different architecture. On the other hand, synthetic polymers can possibly serve as model compounds for systematic study of gas-phase fragmentation processes as various chemical structures can be prepared by a synthetic route.

Current developments in tandem mass spectrometry TOF methods concentrate in the following areas: improvement of precursor ion selection, automation of the segmented PSD/CID spectrum, acquisition and calibration, instrument development to get full and nonsegmented daughter ion spectra, derivatization reaction and improvement of sample preparation techniques [34]. Currently, with the MALDI TOF/TOF mass spectrometer a fragment ion resolution up to 4000–5000 can be achieved [25]. Coupling of a MALDI source with an FT-ICR mass analyzer offers potentially ultrahigh mass resolving power and multistage MS$^n$ in a single instrument. The SORI-CID technique can be used for excitation and dissociation of low mass polymers providing potentially unique experiments that probe polymer striker [35].

## 4. MALDI–SEC coupling

One common feature for both the MALDI and SEC techniques is that the time a molecule takes to reach a detector is measured as a function of its mass. That means, in the case of synthetic polymers, both techniques measure the molecular weight distribution (MWD) thus providing average values of MWs. However, there are important differences between SEC and MALDI. The most obvious difference is that SEC determines the concentration of the polymer while MALDI counts the number of ions individually, thus yielding the weight- and the number-average MWDs, respectively. SEC is an entropically controlled separation technique in which molecules are separated on the basis of hydrodynamic volume, which is a relative measure of molecular weight and thus depends on chemical composition. To obtain absolute MW values by SEC, the exact relationship between hydrodynamic volume and molecular weight for the given polymer needs to be known or molecular weight-sensitive detectors must be employed.

In MALDI the mass axis is independent of the nature of the materials being analyzed. Therefore, similarly to light scattering and viscometry, MALDI can be used as an SEC detector for absolute MW measurements. However, MALDI molecular weight characterization depends on how accurately the ion abundance over the mass range represents the polymer composition. To obtain precise MW values, the efficiency of the MALDI ionization process should be independent of chemical composition or mass, and fragmentation should either not occur or not significantly alter the ion abundance. In addition, the ions that are produced must be transmitted and detected without mass discrimination so that they correctly represent the ion abundance. In other words, discrimination in ionization, transmission, or detection should be avoided so that the intensity of each peak is representative of the molar concentration of that particular molecular species. These requirements can be met only for polymers having narrow distributions (polydispersity index below $\sim$1.2), for which MW and MWD can be determined reasonably accurately [36]. For polydisperse polymers, however, the solubility, desorption probability, and detection efficiency vary significantly (decrease) with increasing mass of the analyte. Difficulties arise with samples that have a small fraction of high-mass oligomers. GPC with refractive index or light scattering detection is quite sensitive to small quantities of high mass materials. However, since the mass spectrometer operates by detecting the number of molecules, the high mass signal is often lost in the noise of the baseline. Although a procedure based on smoothing and offset subtraction can provide reliable MW and MWD estimates directly from the MALDI spectra of polydisperse polymers [37], this approach has not found wider application mainly because its reliability depends on the dynamic range of the mass spectrometer. Possibly new types of detectors for TOF-MS such as the direct ion-to-photon detector can circumvent this problem [38,39].

To overcome MALDI polydispersity discrimination, a high polydispersity sample can be fractionated by analytical or preparative SEC, yielding fractions with very narrow distributions, which can be subsequently analyzed by MALDI. An off-line variant of this approach was first reported by Montaudo's group [40] for analysis of polydisperse poly(dimethylsiloxane)s. Soon after, several applications demonstrated the use of this approach, which involves collecting fractions or depositing a sample on a prepared target prior to MALDI analysis [8]. The MALDI–SEC combination seems to be particularly useful for analysis of rigid-rod synthetic polymers such as poly(alkylthiophene)s [41].

Effort has been made to optimize and simplify the off-line MALDI–SEC technique which, as it turns out, provides reliable MW and MWD values. A direct deposition method was developed in which the SEC effluent is spray deposited onto a rotating matrix precoated substrate and the resulting track is subsequently analyzed by MALDI [42]. The $M_n$ values determined for relatively narrow polydispersity ($<1.2$) PMMA samples using the direct deposition method were in fairly good agreement with manufacturer's values, although the MWD values were somewhat lower. Recently, the direct spray deposition method was used to analyze a broadly distributed PMMA material which could not be analyzed by MALDI alone. The results were compared to a blend of five narrow MWD standards that mimic the broadly dispersed material [43].

Two different approaches were proposed to directly couple MALDI to liquid separation methods: continuous flow (CF) and aerosol. Continuous flow-MALDI [44] uses a flow probe similar to a continuous-flow fast atom bombardment probe. The maximum CF-MALDI flow rate is less than 5 μL/min; thus a conventional LC column cannot be used. There is an additional restriction that the matrix itself should be a liquid. In the aerosol method [45], the matrix and the analyte are dissolved in a solution that is sprayed directly into the mass spectrometer. Ions can be formed by irradiating the aerosol particles with pulsed 355 nm radiation from a frequency-tripled Nd:YAG laser. Flow rates in excess of 0.5 mL/min have been used. Poly(ethylene glycol) (PEG1000) and poly(propylene glycol) (PPG1000) were analyzed with this method [46]. The major advantage of off-line MALDI–SEC over on-line coupling is that the former is relatively easy to implement and does not require any instrument modification. It also allows for independent system optimization providing higher sensitivity and mass accuracy. On the other hand, on-line coupling provides fast analysis and after further refinements, may become a powerful analytical technique. Preliminary studies have demonstrated that HPLC and SEC can be coupled, on-line, to MALDI [47].

Two recent reviews discuss in detail strengths and limitations of on-line and off-line coupling of MALDI to different separation techniques as well as further technical improvement of the techniques [48,49]. Even more attractive is the attempt to combine data from SEC–NMR and SEC–MALDI, leading to a bivariate distribution with respect to molar mass and composition in copolymers. Information on the bivariate distribution is particularly important in high conversion copolymers, where the spread in composition increases with conversion. This approach was demonstrated using PMMA/poly(butyl acrylate) [50] and styrene/maleic anhydride copolymers [51]. It was also shown that interactive HPLC coupled to MALDI-TOF could yield useful information about both oligomer masses and chemical composition, thus providing complete characterization of complex polymer systems. The off-line combination of SEC and liquid chromatography under critical conditions (LCCC) was applied to poly(propylene glycol) [52], poly(L-lactide)-block-poly(ethylene oxide)-block-(L-lactide) triblock copolymers [53], polyamides-6 [54], and poly(propylene oxide) [55].

Overall, the coupling of MALDI to SEC provides advantages to both techniques. SEC provides MALDI with simplified, narrow MWD samples and the ability to detect the minor species. The molecular weights of SEC fractions measured by MALDI can be used effectively to calibrate the SEC system to give absolute values for the average molecular weight. Moreover, from the mass/retention-volume relationship, questions regarding branching, copolymer block structure, SEC column performance, and peak broadening can be studied [56]. One additional feature of this method is that at lower *m*/*z* values, the mass spectrometer provides significant compositional information.

## 5. Structure determination

The primary use of MALDI for polymer science has been its ability to measure molecular weight distributions. However, MALDI has considerable potential for obtaining additional important information. An update of some of these capabilities will be presented in this section.

Determination of end groups on polymers is of considerable importance. A recent study of poly(3-alkyl thiophenes) used MALDI to examine different end groups on polymers having both high and low polydispersities [41]. Different end groups detected were related to the synthetic method used. End group modification was also examined. In another study, halogen end groups on polystyrenes, -acrylates, and -methacrylates were substituted by other functionalities [57]. The end-group functionalized polymers were characterized by MALDI, infrared, and NMR. MALDI was used to compare polystyrenes (PS) prepared by TEMPO-mediated radical polymerization and conventional polymerization. Significant polymer chain fragmentation was observed using a conventional matrix, which was minor for conventionally prepared PS but significant for chains containing a TEMPO-based alkoxyamine end group [58]. When DHB was used without added salt, protonation occurs at the alkoxyamine functionality and no fragmentation is observed. This permits measurement of the polymer containing the alkoxyamine end groups. MALDI has been used for quantitative determination of the end groups in a small polyester [59]. The presence of carboxyl end groups was verified by their formation of carboxylate salts with metal cations. The nature of the specific cation had a significant influence on the analysis.

Derivatization is a technique that has been used widely in other analytical methods to enhance their capabilities, but has not been used extensively in MALDI. Derivatization has the capability of providing both selectivity and improved detection limits. In a recent study MALDI measurements were performed on octylphenol ethoxylates [60]. MALDI gave higher $M_n$ values by about 20% than either hydroxyl number or NMR, an effect which was attributed to MALDI discrimination against low-mass oligomers. When the ethoxylates were derivatized by reaction with phthalic anhydride, the discrimination was reduced significantly, although the MALDI results were still consistently higher than the $M_n$ values measured by the other two methods.

Another important problem in polymer analysis is obtaining information about an embedded segment of a polymer chain. This is illustrated in Fig. 2. The analytical information sought is the $M_n$ value of the embedded segment, so marked in the figure. This can be accomplished by a chemical reaction which selectively cuts the polymer chain at the ends of the desired segment (as shown by the arrows), but does not attack the segment itself. The MWD of the segment, once removed from the chain, can then be measured by MALDI.

The above approach has been used to measure $M_n$ values for embedded polyether and polyester segments of polyurethanes (PUR) [61]. The specific polyether was PTHF and the ester was poly(butylene adipate) (PBA). Two different reactions were necessary for selective degradation because of the differences in chemical stability of PTHF and PBA. Hydroxy-terminated PTHF was produced from the PUR by reaction with excess ethanolamine. An *N*-phenyl terminated polyester was produced for PBA by reaction of the PUR with excess phenyl isocyanate. The latter reaction was used because ethanolamine would degrade the polyester chain. The most successful determination of

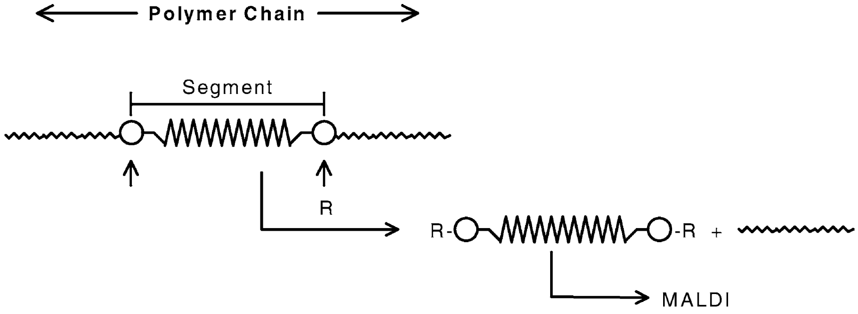

Fig. 2. Schematic diagram of removing a chain segment.

$M_n$ values for the chain segments was accomplished by the combined use of SEC and MALDI. SEC–MALDI results were compared for PTHF segments cut from the chain with those for the polymer used in synthesis of the PUR, and agreement for $M_n$, $M_w$ and polydispersity were very good. Agreement was not as good for the PBA segments, indicating the likelihood of selective reaction in the polymerization process.

## 6. Solid state supports

Several papers have appeared recently which demonstrated that high-quality MALDI spectra can be obtained from solid supports. This hearkens back to one of the two original MALDI papers [2] in which Tanaka et al. described desorption and ionization of a polymer from finely divided cobalt. The idea of a solid-phase support which is free of a matrix is an intriguing one. The two substrates of recent interest are porous silicon and carbon.

One of the potential advantages of a matrix-free approach to MALDI is that matrix background peaks will largely be absent from the spectrum. Porous silicon has the ability to trap molecules and has high optical absorption in the ultraviolet, making it a seemingly ideal matrix for use with UV lasers. Use of a porous silicon matrix for MALDI-type measurements has been termed "DIOS," desorption/ionization on silicon [62]. The Si matrix is generated from a flat silicon substrate by galvanostatic etching. The layer produced is photoetched to produce an array (either dots or a gridwork) which can be used for deposition of multiple samples on a plate which readily fits into the MALDI spectrometer. The DIOS plates produce minimal background peaks making etched silicon an ideal matrix for small molecules. The method has detection limits in the femto- to attomole range and the spectra show little fragmentation. A recent publication describes a study of the preparation and characterization of porous silicon surfaces and additional applications of the DIOS technique [63]. Etching and pre- and post-etching treatments of the Si matrix are described, along with chemical modifications. Relevant to polymer chemistry, a DIOS spectrum of PEG 1500 is presented. It is also demonstrated that quantitative analysis from the DIOS matrix is possible and post-source decay spectra can be obtained.

Another method using a solid MALDI substrate involved deposition of a thin layer of activated carbon particles onto an aluminum support [64]. Addition of glycerol made ion signals stable over hundreds of laser shots. Good quality spectra were obtained and only a few background peaks were observed. Detection limits were in the femto- to attomole range. Direct deposition onto the activated carbon (AC) substrate gave satisfactory spectra, but a TLC deposition method offered better resolution. In the latter, the analyte solution was spotted onto a TLC plate and eluted onto the AC substrate. In a related study [65], MALDI spectra of polystyrene and poly(methylsilsesquioxane) (PMSSQ) were obtained from a graphite plate in the range of 100–1000 Da. No interference from carbon clusters or other low mass fragments was observed. Products of hydrolysis and condensation of PMSSQ were studied in detail.

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## References

[1] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[2] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 2 (1988) 151.
[3] J.A. Castro, C. Koester, C. Wilkins, Rapid Commun. Mass Spectrom. 6 (1992) 239.
[4] P.O. Danis, D.E. Karr, F. Mayer, A. Holle, C.H. Watson, Org. Mass. Spectrom. Lett. 27 (1992) 843.
[5] U. Bahr, A. Deppe, M. Karas, F. Hillenkamp, Anal. Chem. 64 (1992) 2866.
[6] (a) R.P. Lattimer, H.R. Schluten, Anal. Chem. 61 (1989) 1201a;
(b) J. Saito, H. Waki, N. Teramae, S. Tanaka, Prog. Org. Coatings 15 (1988) 311.
[7] D.M. Hercules, in: G. Montaudo, R.P. Lattimer (Eds.), Mass Spectrometry of Polymers, CRC Press, Boca Raton, November 2001.
[8] M.N.F. Nielen, Mass Spectrom. Rev. 18 (1999) 309.
[9] R. Knochenmuss, E. Lehmann, R. Zenobi, Eur. Mass. Spectrom. 4 (1998) 421.
[10] K. Breuker, R. Knochenmuss, R. Zenobi, Int. J. Mass Spectrom. 184 (1999) 25.
[11] K. Breuker, R. Knochenmuss, R. Zenobi, J. Am. Soc. Mass Spectrom. 10 (1999) 1111.
[12] J. Zhang, R. Knochenmuss, E. Stevenson, R. Zenobi, Int. J. Mass Spectrom. 213 (2002) 237.
[13] M. Mormann, S. Bashir, P.J. Derrick, D. Kuck, J. Am. Soc. Mass Spectrom. 11 (2000) 544.
[14] H. Rashidzadeh, Y. Wang, B. Guo, Rapid Commun. Mass Spectrom. 14 (2000) 439.
[15] H. Rashidzadeh, K. Hung, B. Guo, Eur. Mass Spectrom. 4 (1998) 429.
[16] S.K. Poehlein, S.J. Dormady, D.R. McMillin, F.E. Regnier, Rapid Commun. Mass Spectrom. 13 (1999) 1349.
[17] A.-M. Hoberg, D.M. Hadleton, P.J. Derrick, A.T. Jackson, J.H. Scrivens, Eur. Mass Spectrom. 4 (1998) 435.
[18] Y. Wang, H. Rashidzadeh, B. Guo, J. Am. Soc. Mass Spectrom. 11 (2000) 639.
[19] J. Gidden, T. Wyttenbach, J.J. Batka, P. Weis, A.T. Jacksohjn, J.H. Scrivens, M.T. Bowers, J. Am. Soc. Mass Spectrom. 10 (1999) 883.
[20] S.F. Macha, P.A. Limbach, P.J. Savickas, J. Am. Soc. Mass Spectrom. 11 (2000) 731.
[21] S.F. Macha, P.A. Limbach, S.D. Hanton, K.G. Owens, J. Am. Soc. Mass Spectrom. 12 (2001) 732.
[22] K.R. Jenings, Int. J. Mass Spectrom. Ion Phys. 1 (1968) 520.
[23] W.F. Haddon, F.W. McLafferty, J. Am. Chem. Soc. 90 (1968) 4745.
[24] K.F. Medzihradsky, G.W. Adams, A.L. Burlingame, R.H. Bateman, M.R. Green, J. Am. Soc. Mass Spectrom. 7 (1996) 1.
[25] K.F. Medzihradsky, J.M. Campbell, M.A. Baldwin, A.M. Falick, P. Juhasz, M.L. Vestal, A.L. Burlingame, Anal. Chem. 72 (2000) 552.
[26] B. Spengler, R. Kaufmann, Analysis 20 (1992) 91.
[27] M.R.M. Domingues, M.G.O.S. Marques, C.A.M. Vale, M.G. Neves, J.A.S. Cavaleiro, A.J. Ferrer-Correia, O.V. Nemirovskiy, M.L. Gross, J. Am. Soc. Mass Spectrom. 10 (1999) 217.
[28] E. Stimson, O. Truong, W.J. Richter, M.D. Waterfield, A.L. Burlingame, Int. J. Mass Spectom. Ion Process. 169/170 (1997) 321.
[29] J.H. Scrivens, A.T. Jackson, H.T. Yates, M.R. Green, G. Critchley, J. Brown, R.H. Bateman, M.T. Bowers, J. Gidden, Int. J. Mass Spectrom. Ion Process. 165/166 (1997) 363.
[30] R. Murgasova, D.M. Hercules, J. Mass Spectrom. 36 (2001) 1098.
[31] A.T. Jackson, T.H. Yates, J.H. Scrivens, M.R. Green, R.H. Bateman, J. Am. Mass Spectrom. 9 (1988) 269.
[32] A.T. Jackson, T.H. Yates, J.H. Scrivens, M.R. Green, R.H. Bateman, J. Am. Soc. Mass Spectrom. 9 (1987) 1206.
[33] R. Murgasova, D.M. Hercules, unpublished results.
[34] J. Franzen, R. Frey, A. Holle, K.-O. Kräuter, Int. J. Mass Spectrom. 206 (2001) 275.
[35] S.J. Pastor, C.L. Wilkins, Int. J. Mass Spectrom. Ion Process. 175 (1998) 81.
[36] G. Montaudo, D. Garozzo, M.S. Montaudo, C. Puglisi, F. Samperi, Macromolecules 28 (1995) 7983.
[37] G. Montaudo, E. Scamporrino, D. Vitalini, P. Mineo, Rapid Commun. Mass Spectrom. 10 (1996) 1551.
[38] F. Dubois, R. Knochenmuss, R. Zenobi, Rapid Commun. Mass Spectrom. 13 (1999) 1958.
[39] F. Dubois, R. Knochenmuss, R. Zenobi, A. Brunelle, C. Deprun, Y. LeBeyec, Rapid Commun. Mass Spectrom. 13 (1999) 786.
[40] G. Montaudo, M.S. Montaudo, C. Puglisi, F. Samperi, Rapid Commun. Mass Spectrom. 9 (1995) 1158.
[41] J. Liu, R.S. Leowe, R.D. McCullough, Macromolecules 32 (1999) 5777.
[42] C.E. Kasses, J.M. DeSimone, R.W. Linton, E.E. Ramsen, G.W. Lange, R.M. Friedman, Rapid Commun. Mass Spectrom. 11 (1997) 1134.
[43] S.D. Hanton, M.X. Liu, Anal. Chem. 72 (2000) 4550.
[44] L. Li, A.P.L. Wang, L.D. Coulson, Anal. Chem. 65 (1993) 493.
[45] K.K. Murray, D.H. Russell, Anal. Chem. 65 (1993) 2534.
[46] X. Fei, K.K. Murray, Anal. Chem. 68 (1996) 355.
[47] Q. Zhan, A. Gusev, D.M. Hercules, Rapid Commun. Mass Spectrom. 13 (1999) 2278.
[48] A.I. Gusev, Fr. J. Anal. Chem. 366 (2000) 691.

[49] K.K. Murray, Mass Spectrom. Rev. 16 (1998) 283.
[50] M.S. Montaudo, G. Montaudo, Macromolecules 32 (1999) 7015.
[51] M.S. Montaudo, Macromolecules 34 (2001) 2792.
[52] H. Yun, S. Olesik, E.H. Marti, J. Microcolumn Sep. 11 (1999) 53.
[53] H. Lee, T. Chang, D. Lee, M.S. Shim, H. Ji, W.K. Nonidez, J.W. Mays, Anal. Chem. 73 (2001) 1726.
[54] Y. Mendernik, R. Peters, C.G. deKoster, S. Van der Wal, H.A. Claessen, C.A. Cramers, J. Chromatogr. A 914 (2001) 131.
[55] C. Keil, E. Esser, H. Pash, Macromol. Mater. Eng. 286 (2001) 161.
[56] P.O. Danis, A.D. Saucy, F.J. Huby, Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.) 37 (1996) 311.
[57] V. Coessens, K. Matyjaszewski, J. Macromol. Sci. Pure Appl. Chem. A36 (1999) 667.
[58] M.-A. Dourges, B. Charleux, J.-P. Vairon, J.-C. Blais, G. Bolbach, J.-C. Tabet, Macromolecules 32 (1999) 2495.
[59] O. Laine, H. Osterholm, H. Jarvinen, K. Wickstrom, P. Vainiotalo, Rapid Commun. Mass Spectrom. 14 (2000) 482.
[60] J.P. Barry, W.J. Carton, K.M. Pesci, R.T. Anselmo, D.R. Radtke, J.V. Evans, Rapid Commun. Mass Spectrom. 11 (1997) 437.
[61] J.T. Mehl, R. Murgasova, X. Dong, D.M. Hercules, H. Nefzger, Anal. Chem. 72 (2000) 2490.
[62] J. Wei, J.M. Buriak, G. Siuzdak, Nature 399 (1999) 243.
[63] Z. Shen, J.J. Thomas, C. Averbuj, K.M. Broo, M. Engelhard, J.E. Crowell, M.G. Finn, G. Siuzdak, Anal. Chem. 73 (2001) 612.
[64] M. Han, J. Sunner, J. Am. Soc. Mass Spectrom. 11 (2000) 644.
[65] H.-J. Kim, J.-K. Lee, S.-J. Park, H.W. Ro, D.Y. Yoo, D.Y. Yoon, Anal. Chem. 72 (2000) 5673.