Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry of polymers

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Abstract

Matrix-assisted laser desorption/ionization mass spectrometry has been demonstrated to be a powerful analytical technique for the analysis of polymeric materials. The advantages of this technique for such analyses include low sample consumption, ease of sample preparation, short analysis times, and soft ionization which leads to negligible or no fragmentation of analytes. It provides absolute, fast and accurate molecular masses for polymers with narrow polydispersity as opposed to relative masses provided by other techniques. It provides masses for the entire polymer distribution instead of the average value, hence providing molecular mass information which can be used to obtain the mass of the end-groups, mass of the repeat unit (monomer), and chemical modifications on the polymer if oligomer resolution is attained. This review concentrates on the developments in methodology that have allowed for the increased use of this technique for polymer analysis.

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1. Introduction

There are a number of analytical techniques available for the analysis of hydrocarbon polymeric materials. The most common and useful techniques include pyrolysis, chromatography, thermal analysis, spectroscopy (NMR, IR & Raman) and mass spectrometry. The growth of mass spectrometry for the analysis of synthetic polymers has nearly doubled in eight years as noted by the editor of the Journal of the American Society for Mass Spectrometry [1]. Among the many mass spectrometry techniques available, matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) applications are constantly increasing. The MALDI technique was initially developed for biological molecules, mainly proteins and peptides [2,3], but was eventually utilized for other analytes including synthetic polymers [4].

2. MALDI-MS

MALDI is a soft ionization technique used for the analysis of large nonvolatile molecules such as biopolymers and synthetic polymers. The MALDI process produces intact pseudomolecular ions of the analyte through irradiation by a pulsed laser beam on a solid mixture of a particular analyte dissolved in a suitable matrix compound. Typically, short pulse laser beams that range from 1 to 100 nanoseconds are used. Normally UV lasers are used with the nitrogen laser (λ=337 nm) being the most common [5].

A MALDI experiment involves several steps. The first and most critical step is sample preparation. There are various sample preparation techniques that have been developed depending on the nature of sample to be analyzed. Among the common established techniques include the dried-droplet method [2], fast solvent drying method [**6], two-layer method [7], and most recently, solid/solid compressing method used for hard to dissolve samples [**8,9]. The dried-droplet method is the most common because of its simplicity, although this method is prone to the creation of heterogeneous matrix crystals (hot spots) which result in poor spectral reproducibility [10].

After the desorption/ionization event, the formed ions are introduced into the mass analyzer and separated according to their mass-to-charge (m/z) ratios. The time-of-flight (TOF) mass analyzer is most commonly coupled with a MALDI source for various reasons including cost effectiveness, simplicity in both design and use, high ion transmission, unlimited mass range (theoretically), ability...
to detect all the ion species at the same time, fast analysis, and good compatibility with the pulsed ionization sources [11].

For ion detection in MALDI instruments typically multichannel plate (MCP) detectors are used. These are thin plate-like detectors with many cylindrical channels made of lead-doped glass material with fiber optics properties. The functioning of these detectors depends on the direct ion-to-electron conversion that takes place at the detector surface. The electron signal produced is eventually amplified and collected. Practically the number of emitted secondary electrons depends on the mass and velocity of the impacting analyte ions such that ions of different masses cause different numbers of secondary electrons to be emitted at the detector surface.

Ions of high molecular weight usually fail to produce adequate secondary ions when they impinge upon the detector surface. Hence detector efficiency is low for relatively high molecular weight samples [12]. Also, depending on the amount of ions produced per laser pulse and their velocity, detectors can be saturated and hence give unreliable results. Two types of detector saturation effects have been noted: one is saturation due to a high output ion pulse and the other is due to limited detector dynamic range [13]. The saturation recovery time for a detector is longer than its normal response time; hence the timed events of ion formation and detection under such conditions are overlapped. Therefore, because of these noted detector problems, MCP detectors, though common in MALDI instruments, have not provided reliable results for polydisperse polymers [13,14,15,16,17].

3. Matrices for polymer analysis

MALDI evolved from the early experiments with LD when it was realized that instead of direct laser desorption of the analyte, mixing the analyte with a matrix compound for pulsed laser desorption mass spectrometry not only produces better results but also improves the desorption efficiency of non-volatile analytes into the gas phase [18]. The use of a matrix was found to extend the mass range for thermally labile biomolecules to a range not attainable by other techniques [18,19]. Whether the matrix compound used for analysis is organic as initially proposed by Hillenkamp and Karas [18], or inorganic as initially proposed by Tanaka and co-workers [3], they all have high molar extinction coefficients at the wavelength of the laser used, vacuum stability, and solvent compatibility. The absorption characteristic allows a controlled energy deposition to the analyte that then results in a much softer ionization as compared to the LD technique.

Although the role of the matrix is very crucial in MALDI, very little is known about what makes a particular compound a good matrix. A priori determination of whether a particular chemical compound will function as suitable matrix is not yet possible. To date, matrix compounds are still discovered through trial and error because there has not been enough research to investigate what are the factors that affect the functioning of such compounds in the MALDI process. Apart from absorbing the laser energy at the wavelength used, the matrix dilutes and isolates macromolecules in the sample preparation step, a phenomenon called matrix isolation, and plays a role in the analyte desorption and ionization process [20]. The analyte molecules are evenly distributed within these highly absorbing matrix molecules forming a solid solution of both the matrix and analyte after the solvent has evaporated [21]. In this case, the matrix inhibits potential interactions among the analyte molecules and between the analyte and the target surface [22]. Practically, the ratio of the analyte to the matrix ranges from $1:10^3$ up to $1:10^5$ although for high molecular weight samples such as polystyrene samples from 10 kDa to 900 kDa much higher ratios, up to $1:8 \times 10^9$, have been used [23].

3.1. Polar matrices

The ability of matrix molecules to separate analyte molecules is facilitated by the chemical nature of the interacting molecules. Because of their similar chemical properties, polar matrix molecules (e.g., 2,5-dihydroxybenzoic acid (DHB)) interact well with polar analyte molecules. Through trial and error, some polar matrices such as 2-(4-hydroxyphenylazo)-benzoic acid (HABA), all-trans retinoic acid (RTA), 2,5-DHB and dithranol have been found to work for hydrocarbon polymers. For example, HABA and all-trans-RTA have been applied to polystyrene [24–26,27,28,29]. The matrix all-trans-RTA has also been applied to polybutadiene and polysisoprene [*27]. DHB and trans-indole acrylic acid have also been used for hydrocarbon polymers [24,*30]. The most commonly used matrix for hydrocarbon polymers to date is dithranol [29,*31,32–38].

3.2. Nonpolar matrices

Nonpolar matrices, such as anthracene, pyrene, acenaphthene and terthiophene, are suitable for MALDI-MS analysis of low molecular weight nonpolar analytes [39,40], and have been shown to be effective as matrices for the analysis of low molecular weight hydrocarbon synthetic polymers (Fig. 1) [41,*42]. These matrices have been applied to polybutadienes, polystyrenes and polyisoprenes. Although Tang et al. [43] had previously reported that poly cyclic aromatic hydrocarbons, such as anthracene, benzo[a]pyrene, chrysene, naphthalene, phenanthrene and pyrene, did not function as suitable matrices for the analysis of synthetic polymers, that study focused primarily on polar synthetic polymers, with the exception of a polybutadiene sample.
3.3. Solvent considerations

As mentioned previously, MALDI sample preparation requires a uniform formation of a solid solution between the matrix and the analyte. Juhasz and co-workers [44] suggested that HABA does not work well for polystyrene because it is too polar to form a uniform solid-phase mixture with this nonpolar polymer. Also polystyrene samples mixed with 2,5-DHB when observed under a microscope indicated that the two compounds do crystallize separately [45]. These observations have led to a conclusion that miscibility of the analyte and the matrix in the condensed phase is an essential requirement for a good matrix.

Although it has been shown that water-soluble synthetic polymers can be analyzed by following similar protocols used for the analysis of biopolymers [4,45,46], analytes that are soluble in organic solvents have been difficult to analyze with water-soluble matrices. Danis and Karr [47] started the investigation of the possibility of using organic solvents for both the polymer and matrix as a sample preparation technique. In their work, acidic matrices and some polar polymers were dissolved in acetone instead of water. Since then, several organic solvents have been applied to solvate different synthetic polymers during MALDI sample preparation.

The use of a single solvent system to prepare the polymer and matrix mixture is required for successful
analysis [48]. However, there are cases where the use of a binary solvent system becomes inevitable. In such cases, various problems have been observed. For example, addition of 1–5% water to a PMMA/polar matrix sample prepared in organic solvents resulted in mass discrimination when the samples were analyzed [49]. Yalcin and coworkers [**50] studied extensively the effect of solvent in polymer sample preparation. They showed that binary solvent systems can be used for preparing the samples as long as both solvents are compatible; both the polymer and the matrix must have the same solubility in each of the solvents, and both solvents should have the same volatility. Slight differences in solubility of the polymer and the matrix in the solvents leads to significant differences in the MALDI results. It was concluded from that work that in cases where a common solvent is not available, binary solvents to be used should allow co-crystallization of the polymer and the matrix, or crystallization of the matrix before polymer precipitation.

There are various polymeric materials that are difficult to dissolve in organic solvents under ordinary conditions. For such materials, sample preparation for MALDI analysis is very challenging. Examples of such polymers are polyamides, teflon and polyethylene. Zenobi and co-workers have developed a solvent-free MALDI sample preparation technique for these kinds of samples; a method similar to that used for KBr pellet sample preparation for infrared analysis was proposed (Fig. 2) [**8]. Another group has tried to make this solvent-free method applicable to various kinds of analytes including soluble synthetic polymers and soluble biopolymers [9].

**4. Cationization of polymers in MALDI**

In MALDI-MS the primary means of forming gas-phase ions is by proton or metal ion attachment. Most MALDI amenable analyte molecules contain heteroatoms such as N or O in their molecular structures which provide lone pairs of electrons on which cationization can take place. Similarly, hydrocarbon polymers with unsaturated double bonds have polarizable \( \pi \) bonds on which cationization reagents, mainly metal ions, can be attached. For saturated hydrocarbon polymers like polyethylene, the typical MALDI experiment does not yield reliable results due to the inert nature of such analytes (Fig. 3).

Polar synthetic polymers of more than 200 kDa can be analyzed by following MALDI protocols previously developed for biological analytes [4,45,47]. Examples of such polymers include polyglycols [45], polystyrene sulfonic acid [4], and polymethylmethacrylate (PMMA) [**13,51]. While ionization of biopolymers in MALDI occurs by protonation, the ionization of synthetic polymers is achieved through cationization by metal ions; group I metal salts are good cationization reagents for polar synthetic polymers. Polymers such as polyethyleneglycol (PEG) and PMMA interact well with alkali metal ions [**31,34,35,52–54]. Water-soluble polymers, such as PEG and polypropylene glycol (PPG), have been successfully analyzed with MALDI using 2,5-DHB as a matrix with alkali–metal salt solutions to increase the yield of the cationized species [53].

In general, MALDI-MS has been more successful for the analysis of polar synthetic polymers than for nonpolar synthetic polymers. It has been established that analysis of
molecular weight polystyrene analytes can be cationized using Group I metal salts in MALDI, though not efficiently [36]. It should be recalled here that LD experiments have indicated that some monovalent gas-phase metal ions such as Al^+, Cr^+, Fe^+ and Cu^+ can efficiently cationize both PEG and polystyrene analytes, hence providing alternative cationization to the Na^+, K^+ and Ag^+ ions. However, LD works well only for polymers of less than 10 kDa due to analyte fragmentation at higher masses [**18].

The work done by Reinhold et al. has shed some light on why only specific metal ions work in MALDI [**56]. That work examined the binding energy between the metal ion and the analyte (e.g., oligomer). It was observed that aromatic species, such as polystyrene, and polyolefins, such as polybutadiene and polyisoprene, containing double bonds are ionized less efficiently in MALDI using sodium ions because of the low binding energy between the sodium ion and the oligomer. This observation led to a general conclusion that the binding energy in such systems is not sufficient to prevent dissociation of the metal–oligomer complex during the multiple collisions which take place during the ion extraction in the MALDI process. Other reasons which have been given by some researchers suggest that for a metal ion to be an effective cationization reagent, it should have a strong affinity for unsaturated electrons present on the polymer and also it should be able to sustain an oxidation state of +1 in the MALDI environment [29]. This condition limits the number of transition metals that can be effective cationization reagents.

5. Limitations of MALDI-MS

The main challenges that limit widespread application of MALDI-MS to hydrocarbon analytes include availability of proper matrices for specific analytes, proper cationization reagents, common solvents for both analyte and matrix, as well as challenges in sample preparation techniques. These limitations and various others inherent in MALDI instrumentation such as problems in ion formation (desorption/ionization), ion transmission and detection, and difficulties in reproducing spectra, have rendered this technique applicable for qualitative purposes only. MALDI is generally employed as a tool for obtaining the analyte mass but not the analyte concentration or even its relative amount from the measured ion abundance values. To date, application of MALDI remains an active research area where improved sample preparation methods are developed, new and better performance matrices are explored, and various new applications are discovered.

Information provided by MALDI data for polymer analysis includes polymer average molecular weight, molecular weight distribution, mass of the end-groups, sample purity, and even initiation and polymerization mechanisms [20,57]. It was noted in the early 1990s when MALDI was...
first applied to synthetic polymers that, unlike biomolecules, the total available charge produced during MALDI analysis of synthetic polymers is distributed over a large number of different molecular weights [45]. When MALDI is applied to polymers with broad molecular mass distributions (polydispersity index > 1.2) [15,58], the charge distribution leads to an upper mass limit above which individual oligomers cannot be distinguished from the noise. As a result, MALDI data have been found to underestimate the higher mass polymer distribution hence resulting in noticeably lower average molecular weight values [**16,58,59].

The mass discrimination effect in MALDI is very significant for polymers with polydispersity indices. This effect is thought to be due to a combination of factors including mass discrimination in the ion detection and sample preparation [**13,*14,**16,51,59]. To date, application of MALDI for polymer analysis is still limited to polymers with polydispersity indices < 1.2 [17].

Comparative results have shown that polymer analysis results obtained by MALDI are in good agreement with those determined by conventional methods such as gel permeation chromatography (GPC) only if the polymer has a narrow distribution [15,17,58,60]. For polymers with broad distributions, a combination of GPC and MALDI does provide a useful method for determining molecular weight distributions. In the MALDI-GPC combined method, the polydisperse polymer is first fractionated by GPC, then the collected fractions are analyzed off-line by MALDI which provides data to calibrate the GPC chromatogram [61]. The GPC fractionation of a polydisperse polymer for MALDI analysis requires a separate optimization of the GPC method and determination of the number of fractions and fraction volumes required to provide adequately narrow distributions across the whole GPC chromatogram. Before MALDI analysis, sample concentration might be necessary. Recently a liquid chromatography (LC) interface to the GPC was developed and demonstrated to be a useful GPC-MALDI sample preparation method for fractionated polymers [*62]. The LC interface uses heated sheath gas and a capillary nozzle to remove most of the GPC mobile phase and deposit the required eluents on the precoated matrix on a moving MALDI plate.

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References

Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest;
** of outstanding interest.

[13] Rashidzadeh H, Guo B. Use of MALDI-TOF to measure molecular weight distributions of polydisperse poly(methylmethacrylate). Anal Chem 1998;70:131–5, This paper demonstrates the main problems encountered when analyzing polydisperse polymers using MALDI-MS. Such problems include high mass discrimination and detector saturation. Some solutions are proposed to address these problems.


[21] Ehring H, Hillenkamp F. Role of photoionization and photochemistry in ionization processes of organic molecules and relevance for matrix-assisted laser desorption/ionization mass spectrometry. Rapid Commun Mass Spectrom 1992:27:472–80. Various chemical compounds were investigated as MALDI matrices. This paper demonstrates the general observation that matrices are found only through trial and error.


[27] Yalcin T, Schriemer DC, Li L. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the analysis of polydienes. J Am Soc Mass Spectrom 1997;8:1220–9. The paper presents a good method for analyzing polydienes using acidic matrices and copper salts as cationization reagent. Silver salts were shown to be less effective with the method presented.


[30] Belu AM, DeSimone JM, Linton RW, Lange GW, Friedman RM. Evaluation of matrix-assisted laser desorption ionization mass spectrometry for polymer characterization. J Am Soc Mass Spectrom 1996;7:11–24. The paper demonstrates that dithranol is a good MALDI matrix for hydrocarbon polymers. This particular matrix, unlike many others (more acidic) was found to work better with silver salts as cationization reagent as opposed to copper salts.

[31] Jackson AT, Yates HT, MacDonald WA, Scrivens JH, Critchley G, Brown J, Deer MJ, Jennings KR, Brookes C. Time-lag focusing and cation attachment in the analysis of synthetic polymers by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Am Soc Mass Spectrom 1997:8:132–9. In this paper, the effects of varying the cationization reagent for various polymer analytes is demonstrated. Group I metal salts were shown to work well for polar polymers but not for hydrocarbon polymers; these were shown to work with silver salts.


[40] Macha SF, McCarley TD, Limbach PA. Influence of ionization energy on charge-transfer ionization in matrix-assisted laser desorption/ionization mass spectrometry. Anal Chem Acta 1999;397:235–45. This paper demonstrates among other things, the feasibility of charge transfer reactions in MALDI process, and the required conditions. The ionization potential difference between the analyte and the matrix was demonstrated to be a key issue to be considered for charge transfer reactions in MALDI-MS reactions.


[42] Macha SF, Hanton SD, Owens KG, Limbach PA. Silver cluster interferences in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry of nonpolar polymers. J Am Soc Mass Spectrom 2001;12:732–43. This paper demonstrates formation of silver clusters in the MALDI-MS process. These clusters were found to be formed exclusively in the presence of the acidic matrices. This
work explains why silver salts are not good cationization reagents for hydrocarbon analysis when acidic matrices are used.


[56] Reinhold M, Meier RJ, de Koster CG. How feasible is matrix-assisted laser desorption/ionization time-of flight mass spectrometry analysis of polyolefins? Rapid Commun Mass Spectrom 1998;12:1962–6. This paper presents an ab initio quantum calculation that demonstrates why it is not possible to form charged hydrocarbon polymer oligomers in MALDI-MS with group I metal ions. Metal ion–oligomer binding energy is considered.


