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Evaluation of the Polymer Characterization Capabilities of Matrix-Assisted Laser Desorption/Ionization (Literature Review)

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KEYWORDS: Matrix assisted laser desorption and ionization (MALDI), MALDI-Mass-spectromic imaging (MSI), synthetic polymer analysis

ABSTRACT: Matrix-assisted laser desorption/ionization (MALDI) is becoming a key method in expanding the characterization capabilities of synthetic polymers. While there are still some analytical limitations, technological and methodological improvements have greatly improved this technique for analyzing large molecules. This paper serves as an investigation into MALDI-Time of Flight (TOF) capabilities and potential applications as the Material Science Division at Los Alamos National Laboratory seeks to expand its analytical capabilities. Three MALDI-TOF instrument offerings from Bruker, an industry-leader in MALDI-TOF polymer analysis that will evaluated in further detail.

Matrix-assisted laser desorption/ionization (MALDI) was first developed and coined at the University of Frankfurt 1985.[1] The method has been further refined, over the following decades, to produce instrumentation that has become widely used across many industries that investigate large molecules. This paper will provide a background covering a brief overview and development of the technique, as well as a review of the current instrument capabilities with potential applications that would be beneficial within the Material Science Division at Los Alamos National Lab (LANL).

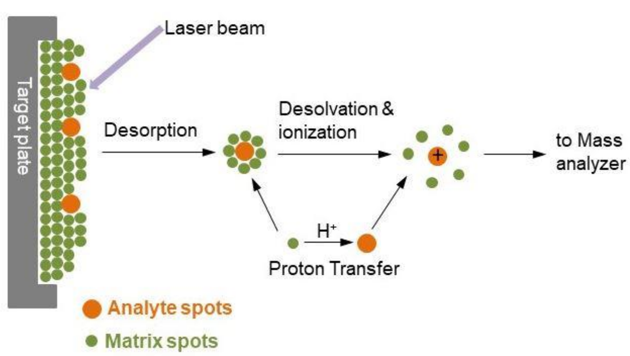

Figure 1. Ionization of analytes by MALDI.[2]

Background. MALDI is soft ionization technique, where a laser strikes an analyte/matrix solution, and the matrix absorbs the energy of the laser which prevents fragmentation and decomposition of the analyte as it enters the gas phase for analysis (Figure 1).[2] The analyte is embedded in a matrix of smaller molecules and deposited onto the surface of a target. This target plate is typically made of a conductive metal. The spot is irradiated by a pulsed laser, providing the necessary heat and vibrational energy required to release the material from the sample plate. As the material is liberated from the surface, it is believed that ionization of the analyte occurs by means of proton transfer with adjacent matrix molecules.[3] Although the mechanism is not fully understood, it is clear that the choice of matrix can have a significant effect on the analytical capabilities of MALDI. This is one of the major areas of deficiency in the general technique, as decisions on sample preparation techniques and the matrices used can be made subjectively, and may have a notable impact on the observed results.[4] In general, like-polarity analytes and matrices work best together. There are many different means of combining the analyte with chosen matrix, from mixing the solutions prior to spotting, to layering, and even solid/solid pressed samples at matrix:analyte ratios ranging from 10:1 to 100,000:1.[2, 5]

Several of the developed mass analysis instruments pair with MALDI. Instruments like ion cyclotron resonance (ICR) and ion trap (IT) work well with MALDI, but have limits in the range of mass-to-charge ratio that can be measured. Time-of-flight (TOF) has become the most common method coupled with MALDI, as it is not limited by the use of a magnetic or electric field to separate ions. This separation of ions based upon molecular velocity in a vacuum gives TOF a near-unlimited mass range. TOF mass limitations are based upon acceleration voltage and the length of the flight tube.

As previously stated, researchers in Frankfurt first discovered that the ionization of aliphatic amino acids, most specifically, alanine, was most effective and reproducible when "matrix-ed" with tryptophan using a pulsed laser at 266 nm. Tryptophan absorbed the laser's energy, allowing for the ionization of the non-absorbing molecule. Technological advancements, coupled with experimentation utilizing different matrices, solvents, and laser wavelengths, has led to a steady increase in analytical capabilities over the past several decades. Coupled with mass spectrometry (MS), and more specifically, TOF, MALDI has been instrumental in the biological and medical fields, as it can rapidly image and characterize large, intact biomolecules, such as proteins, in excess of

100 kDa).[6] This has enabled further understanding in these fields of study concerning biological structure, disease behavior and mechanisms, and drug discovery, as this methodology has become an established tool in clinical biology.[7] MALDI analyses are fast, and produce high-quality data when the correct conditions are used. In Figure 2, a 644,134 pixel MALDI image of a transverse rat brain section was sampled at 20 µm spatial resolution and was acquired in approximately 345 minutes.[8]

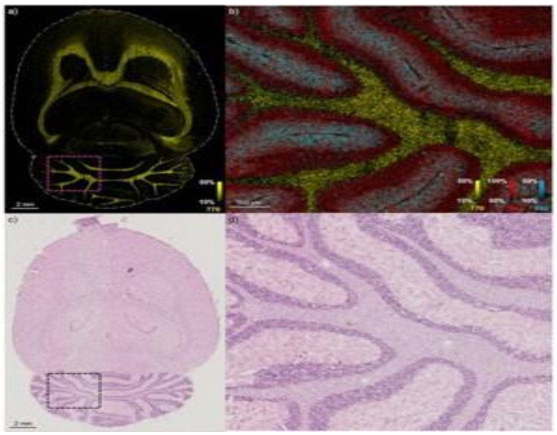

Figure 2. Transverse rat brain section imaged with MALDI-MSI. (a) is an ion image of nominal mass to charge 770 depicts the entire brain, (b) shows images of nominal masses 770, 794, and 885 in an enlarged region of the cerebellum inset from (a).[8]

Utilization of MALDI for polymer analysis has long lagged behind these other fields of study, but the past decade has brought many discoveries and opportunities for expansion into this arena. One of the largest factors in the lack of application has been the difficulty in sample preparation, including matrix and cationization agent selection, as well as the fine-tuning of the sample conditions itself, leading to results that are not quantitatively reproducible. Choosing the wrong matrix and cationization agent combination can result in incorrect results, particularly with fragile polymeric material.[9] Another significant challenge has been the inability to analyze insoluble polymers.[10] This issue may be addressed utilizing solvent-free sample preparation (discussed in technique development). Molecular mass determinations can also vary between MALDI-MS and other analytical techniques. The comparison of MALDI-MS spectra to size exclusion chromatography results can indicate large discrepancies at high polydispersity that are less prevalent than polymers with low polydispersity. Very low polydisperse materials may be overestimated. The factors with greatest impact on results, specific to MALDI polymer analysis, are matrix:analyte ratio, cation concentration, potential multimer formation, and laser energy.

Technique Development. Many researchers have started attacking these shortcomings in the past 10-15 years and, as a result, the methodology has improved. The National Institute of Standards and Technology (NIST) has created a synthetic polymer analysis matrix recipe database from peer-reviewed research that contains the chemicals and matrix preparatory steps, establishing greater consistency in sample preparation, however the database is not all encompassing.[11]

Kooijman et al. have developed a quality scoring statistical approach to determine sample preparation quality in an effort to objectively quantify and qualify sample deposition and optimal matrix composition (Figure 3).[12]

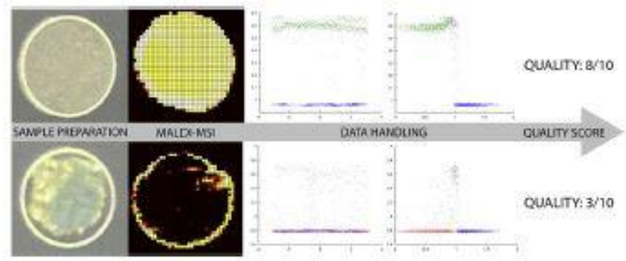

Figure 3. Overview of quality scoring MALDI-MS sample preparations.[12]

Eight quality parameters were evaluated in this approach; Average Analyte Summed Peak Intensity (ASPI), Spatial Variation in ASPI, Average Molecular Weight (MW) Ratio (High:Low), Spatial Variation in MW Ratio (High:Low), Radial Signal Distribution, Angular Signal Distribution, Spot Filling, and Detected Peaks. These parameters were combined to provide an overall quality score to create a high throughput analysis that can serve as a reliable, objective tool to aid in the determination of matrices, solvensts, deposition method, and mixing ratios that produce high-quality results.

Separation techniques can be used to simply complex polymer samples prior to analysis, but is not always necessary. MALDI-MS/MS can be employed to provide molar mass distributions (MMD), end-group information, block composition and length, structures, and copolymer sequences. Tandem MS can also be utilized to determine peak assignments to identify specific end-groups, differentiate isobaric and isomeric species, and analyze the macromolecular structure of polymers based on characteristics of the fragmentation patterns. A robust database of reference spectra is critical for meaningful analysis of fragment distributions, along with a thorough understanding of polymerization methods, as differing end-groups on similar polymer classes will likely exhibit different fragmentation patterns. In a comparison of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and MALDI, it was observed that ESI and APCI better preserved end-group functionality of controlled radical polymerizations via softer ionization, where MALDI would often lose end-group functionality if sample preparation was inadequate.[13] However, MALDI was found superior when obtaining the molar mass information of those same polymers.

Solvent use, or the lack thereof, is an example of a process change that has improved the efficacy of analysis. Trimpin et al. examined the effort to improve shot-to-shot and sample-to-sample reproducibility by creating a more homogenous analyte/matrix mixture.[14] This was achieved utilizing a solvent-free sample preparation method. When the analyte and matrix were mechanically mixed with a mortar and pestle or ball mill and transferred to the target plate as a loose powder, they observed improved sensitivity, a higher mass resolution, and less restrictive matrix:analyte ratios. Insoluble giant polyacrylic aromatic hydrocarbons (>5,700 Da), polyfluorene (<10,000 Da), and polydithiathianthrene (<4,000 Da) were effectively characterized with this technique. This method also allowed for analysis of pyrolysis products, carbonaceous pitches, polyaniline oligomers, UV-absorbing compounds, pigments, and high MW polymers [poly(methyl methacrylate) and polystyrene]. Higher quality mass spectra, with increased levels of detection were observed in solvent-free simulated reaction system when compared to its dissolved counterpart, and spectra were obtainable at lower laser powers, reducing unwanted fragmentation (Figure 4). Comparing Figure 4(I.a) to 4(II.a) reveals a much stronger signal to noise ratio for the solvent-free analysis. The product ion $[C_{132}H_{34}]^+$ was not even detectable in the dissolved/crystallized sample. The report posits that this result is likely due to significant differences in solubility between the product and precursor, as well as segregation on the target plate during crystallization. This result would have led to an extremely incorrect conclusion that a reaction had not occurred when a 90% yield had actually been obtained. Trimpin et al. concluded that solvent-free MALDI-MS was precise and accurate, requiring less preparation time, and produces cleaner spectra, faster. Using MALDI for mass spectrometry imaging (MSI) is a highly valuable technique that allows for surface analysis and mapping without damaging the material. This allows the visualization of the distribution of molecules of the surfaces of material while simultaneously providing compositional analysis.[15] MSI was conducted on two surfaces of a hollow fiber membrane (Figure 5). Figure 5(A, C, E, G, and I) show the abluminal surface of the membrane and (B, D, F, H, J) represent the luminal membrane surface. The mass spectra (C and D) are representative of polysulfone (PS) and polyvinylpyrrolidone (PVP), respectively, indicated differences in composition between the two surfaces. Additionally, the resulting images (E, G, I and F, H, J) provide a visual representation abluminal and luminal surfaces, showing where the two molecules are distributed across the surfaces. The surface images show that PS is more prevalent and more evenly distributed on the abluminal surface, while PVP is more prevalent and distributed more evenly on the luminal surface.

Evaluation of Current Instrument Capabilities. Searching for current MALDI-TOF instrumentation led to three main manufacturers; Bruker, Shimadzu, and JOEL. A vast majority of the research labs contacted were using one of Bruker's three versions of MALDI-TOF instruments. Industry experts and users also echoed the sentiment that Bruker is currently producing the most well-rounded unit, especially with regard to polymer analysis, as their polymer analytical software was very comprehensive. Bruker's instruments come in three tiers; the autofleX maX, the ultrafleXtreme, and the rapifleX TOF-TOF. The autofleX maX (Figure 6) is billed as Bruker's

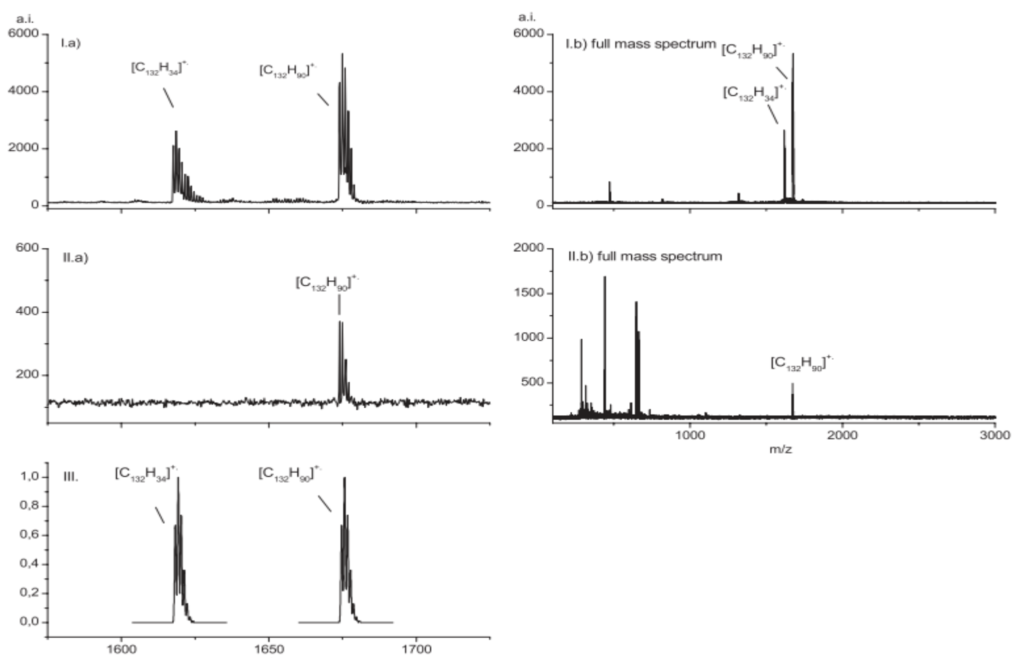

Figure 4. MALDI mass spectra of a model mixture simulating a 90% reaction yield in the synthesis of the insoluble polycyclic aromatic hydrocarbon product $C_{132}H_{34}$ from the dendrite precursor $C_{132}H_{90}$. [I] Solvent-free sample preparation: (a) inset spectrum, (b) full mass spectrum; [II] conventional solvent-based sample preparation: (a) inset spectrum, (b) full mass spectrum; [III] simulated isotopic distribution of the analyte mixture calculated from the elemental compositions.[14]

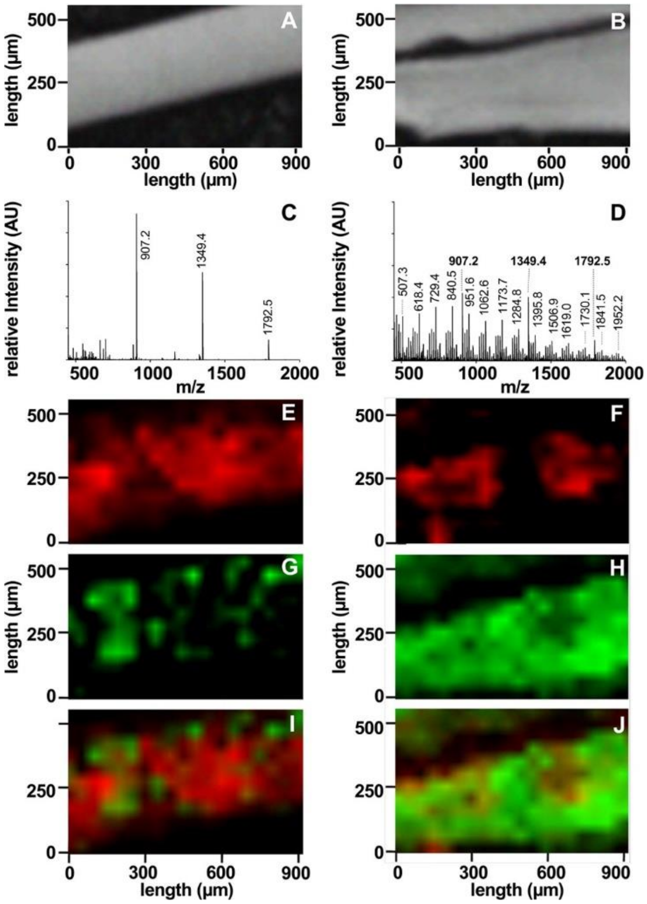

Figure 5. Optical images in (A) abluminal membrane side and (B) luminal membrane surface of hollow fiber membranes. MALDI mass spectra, obtained from (C) abluminal membrane surface and (D) luminal membrane surface at 60 μJ laser intensity and aster raster step size of 50 μm. MALDI images of distribution of PS (red color) in € and (F) and PVP (green color) in (G) and (H), respectively, shown for abluminal and luminal membrane surface. (I, J) The corresponding overlays of both polymers.[15]

most affordable option with polymer characterization abilities. It is a benchtop unit that uses a smartbeam-II solid state laser and operates at speeds of 2 kHz (MS) and 200 Hz (MS/MS)[16]. The autofleX comes equipped with PolyTools for polymer analysis, capable of determining average molecular weights, dispersity, degree of polymerization, and mass of combined end groups. It also comes with SCiLS™ Lab for MALDI imaging. AutofleX has a linear mass range at least up to 300 kDa, with a high mass resolution over a wide mass range.[17] Resolution of 10,000 – 22,000 FWHM across mass/charge range of 900 – 4,500 in a single spectrum in reflectron mode, and >26,000 FWHM in MS mode. Mass accuracy in reflectron mode is ≤ 2 ppm with internal standard.

Figure 6. autofleX maX MALDI-TOF MS[16]

The ultrafleXtreme (Figure 7) is the next model up from autofleX, also utilizing smartbeam-II laser with 2 kHz (TOF) and 1 kHz (TOF-TOF) speeds with a spatial resolution of 10 μM for imaging experiments.[18] UltrafleXtreme has a 1 ppm mass accuracy with a resolving power up to 40,000 FWHM in reflectron mode. In MS mode this unit has a mass to charge range up to 400,000 and in MS/MS up to about 5,000.[19]

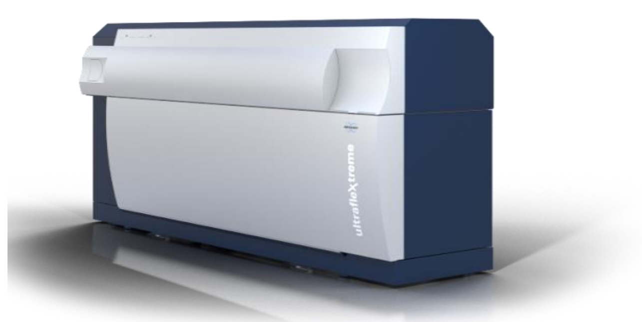

Figure 7. ultrafleXtreme MALDI-TOF/TOF[20]

Finally, the rapifleX TOF/TOF (Figure 8) is Bruker's top of the line unit for polymer analysis. This free-standing unit features a scanning smartbeam 3D laser at a 355 nm wavelength with a variable repetition rate from 1 to 10 kHz, imaging up to five times faster than the ultrafleXtreme.[21] It possesses an optimized laser focus of 5 μm and image at speeds up to 50 pixels/second with a variable squared pixel size from 10 – 100 µm and possesses a flight path up to 300 cm (reflectron mode).

Figure 8. rapifleX MALDI-TOF/TOF[21]

Potential Applications at LANL. MSI would potentially add great value to the laboratory's mission in aging and lifetimes, where surface compositional analysis could be performed on critical polymer components to gain a better understanding the material's longevity and decomposition. Imaging could also be a valuable technique for evaluating thin films and coatings as a means of verifying efficient printing of microstructures onto photoresist layers or determining coating distribution.[22] The TLC-MALDI capability allows TLC plates to be analyzed to further elucidate reaction progress. This could be beneficial during polymer synthesis, tracking polymerization and monitoring byproduct formation. As an established workhorse in pathogen and bio-macromolecule analysis, MALDI-MS/MS analysis could also contribute in the Global Security arena, evaluating biological materials.

Conclusion. While the technique requires further refinement, the literature reviewed for this paper indicate that MALDI-TOF capabilities in the area of polymer characterization are steadily growing. Continued improvements in sample preparation and matrix utilization will increase analysis reliability. The speed, quality, and simplicity of analysis (once the parameters are defined) are key advantages of this methodology. Adding this instrumentation to the laboratory's inventory could potentially expand the capabilities in several key areas of study.

ASSOCIATED CONTENT

N/A

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Author Contributions

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ABBREVIATIONS

MALDI, Matrix-assisted laser desorption/ionization; TOF, time-of-flight; LANL, Los Alamos National Laboratory; ICR, ion cyclone resonance; IT, ion trap; MS, mass spectrometry; NIST, National Institute of Standards and Technology; ASPI, analyte summed peak intensity; MW, molecular weight; mmd, molar mass distribution; ESI, electrospray ionization; APCI, atmospheric pressure chemical ionization; MSI, mass spectrometry imaging; PS, polysulfone; PVP, polyvinylpyrrolidone.

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