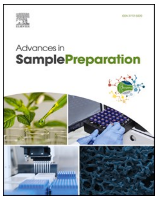
Advances in Sample Preparation 7 (2023) 100088

Contents lists available at ScienceDirect

Advances in Sample Preparation

journal homepage: www.elsevier.com/locate/sampre



Advances in solvent-free sample preparation methods for matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) of polymers and biomolecules

Kayla Williams-Pavlantos, Chrys Wesdemiotis *

Department of Chemistry, The University of Akron, Akron, OH 44325, USA

ARTICLE INFO

Keywords:
MALDI mass spectrometry
Solvent-free sample preparation
Polymer surfaces
Imaging

ABSTRACT

Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) is an essential analytical technique for the molecular characterization of biomolecules and synthetic polymers. Its ability to provide comprehensive compositional information not afforded by other techniques, with high sensitivity and selectivity, is highly influenced by the sample preparation of the material prior to analysis. This review discusses MALDI-MS sample preparation protocols that do not utilize solvents. Such methods provide alternative routes for analyzing insoluble polymeric materials and may also reveal information that is not attainable with solvent-based sample preparation techniques. Additionally, this review will present relevant surface analysis and imaging results only achievable with solvent-free sample preparation methodologies.

Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is widely used for the characterization of natural and synthetic macromolecules due to its ability to desorb intact molecular ions from analytes within a broad range of polarities and molecular weights. Most MALDI ion sources are coupled to time-of flight (ToF) mass analyzers which provide a practical mass range of up to 300 kDa [1]. The sole or predominant formation of singly charged ions in the MALDI process leads to relatively simple mass spectra, allowing for the analysis of complex mixtures not suited for techniques that create a charge distribution, like electrospray ionization (ESI). As a result, MALDI is extensively used for analyzing synthetic polymers to determine repeat units, end groups, and molecular weight, as well as for fingerprinting proteins or other biopolymers, identifying protein modifications, and determining protein interactions [2–9].

Successful MALDI analysis requires both proper sample preparation as well as proper instrument parameters. The sample must be mixed with a chromophoric matrix and, in some cases, an ionization salt if the sample is not easily protonated by matrix ions. This process is typically done by preparing a solution containing the sample, matrix, and cationizing agent, spotting a few microliters of this mixture onto a stainless-steel plate, and allowing the solvent to evaporate [10]. This protocol, often referred to as the dried droplet method, results in the formation of a crystalline mixture containing both analyte and matrix (generally in 1000–10,000 molar excess compared to the analyte amount). While the dried droplet method remains the most common sample preparation procedure, additional methods have been developed for cases where the dried droplet protocol fails to provide homogenous crystals, a requirement for accurate MALDI analysis. Many of the other methods utilize multiple matrix layers to produce more uniform crystals. This can include forming one or two matrix layers on the sample plate before adding the analyte [11,12], or most commonly, implementing the sandwich method which applies one layer of matrix onto the target plate, followed by a layer of the analyte, and then lastly a second layer of matrix [13].

Sample preparation is one of the most important steps in MALDI analysis, as the density and morphology of the sample/matrix (salt) crystals have a direct correlation to the resulting mass accuracy, resolution, and reproducibility of the analysis [14,15]. For accurate and confident results, proper care must be taken when selecting matrix and ionization agents, removing impurities, and preparing the sample. In most cases matrix selection is dependent on mutual solubility with the analyte and absorption at the set laser wavelength. A few of the most frequently used matrices include α-cyano-4-hydroxycinnamic acid (CHCA), trans-2-[3-(4-tert-Butylphenyl)−2-methyl-2-propenylidene]

---

* Corresponding author at: Department of Chemistry, Knight Chemical Laboratory 112, Akron, Ohio 44325-3601, USA.
E-mail address: wesdemiotis@uakron.edu (C. Wesdemiotis).

https://doi.org/10.1016/j.sampre.2023.100088
Received 26 May 2023; Received in revised form 17 July 2023; Accepted 9 August 2023

malononitrile (DCTB), 2,5-dihydroxybenzoic acid (DHB), dithranol (DIT), and (E)−3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (SA).

Proteins and peptides are preferentially mixed with acidic matrices, such as CHCA, presumably because their basic residues (Arg, Lys, His) help to form mixed crystals with the matrix. In contrast, matrix selection for other types of biopolymers and for synthetic polymers is less straightforward. Significant research has been performed to determine which matrices and cationization agents are best suited for polymeric materials; however, since many different polymer categories exist, spanning a wide range of polarities, solubilities, molecular weights, and acid/base or redox chemistries, matrix selection for these substances is still based on trial and error [7,16–20]. For insoluble or sparingly soluble polymers, solvent-free sample preparation approaches are typically used. There are several such variants, but the most common ones involve mechanical mixing or sublimation [15,21–23]. Mechanical mixing creates a blend of matrix and analyte similar to that in the dried droplet and sandwich methods, but is free of solvent restrictions. On the other hand, sublimation directly deposits a layer of matrix and/or ionization agent onto the surface of the analyte. Sublimation and certain mechanical mixing methods can also be used for surface specific analysis, where the absence of solvent is necessary to prevent disruption of the surface environment. This review describes and discusses the various solvent-free approaches developed thus far that enable the successful analysis of insoluble materials as well as the elucidation of surface specific molecular properties which may be different from those of the bulk sample.

Mechanical mixing methods

In traditional solvent-based MALDI-MS preparation techniques, crystallinity and homogeneity of the final analyte/matrix (cationizing salt) mixture are highly dependent on the solvent, matrix, type of cationizing salt, analyte/matrix molar ratio, crystallite thickness, and sample density. Variations in any of these factors can lead to low ionization efficiency, poor reproducibility, and ion suppression, thus introducing bias effects when drawing conclusions from the MALDI-MS data. Several of these factors, however, can be circumvented by using a solvent-free sample preparation approach.

One of the most frequently used solvent-free methods utilizes either a ball mill grinder or mortar and pestle to mix the matrix and analyte into a fine powder. A few micrograms of this powder are then applied to the target plate either by spreading it onto the plate using a spatula or by pressing it into a small pellet and taping it to the target plate [24–26]. Since sample and matrix powder are mixed in their solid forms, the serious limitation of finding a common solvent system for dissolving matrix and analyte is eliminated. Additionally, sample density and thickness can be better controlled by performing mechanical mixing for a longer period to create a more homogenous blend and by having more precise control over the amount of powder deposited onto the plate.

One factor that may still influence the quality of the resulting MALDI-MS spectra is the molar ratio of matrix to analyte. Several solvent-free MALDI-MS studies on synthetic polymers have shown that this ratio has less impact on the resulting spectra than other factors, such as matrix choice and mixture homogeneity, and that spectra with adequate signal-to-noise ratio can be obtained within a broad window of analyte/matrix molar ratios, ranging from 1:50 to 1:5000 [22,27]. Contrary to this finding, a different study found that using much larger analyte/matrix ratios, specifically between 1:1 and 1:5 (w/w), provided the best results in terms of sensitivity and reproducibility for a wide range of both low as well as high molecular weight polymers. Increasing the matrix fraction only resulted in an increase in the matrix signal and no improvement in the polymer distribution intensity [24]. These studies suggest that the optimal analyte/matrix ratio may depend on the type of matrix and sample and should be adjusted for each sample being studied.

Applications to synthetic polymers and peptides/proteins

Several studies have utilized the mortar and pestle preparation method due to its simplicity and the ready availability of the required tools. This sample preparation procedure has been successfully employed for the characterization of insoluble polyamides [24], the assessment of cation binding affinities to polyethers [28], and the minimization of signal suppression effects in the MALDI-MS analysis of peptide mixtures [29]. These studies underscored the benefits of using a solvent-free approach instead of the traditional solvent-based methods.

The continued utilization of solvent-free sample preparation protocols for MALDI-MS analysis has spurred the introduction of more efficient variations of mechanical mixing. It was noted that the mortar and pestle approach was time consuming, preventing quick and high throughput analyses. Additionally, cross contamination was also common with this method if the equipment did not undergo thorough cleaning between samples [26]. To overcome these drawbacks, Hanton and Parees developed a new ball mill mixing method, utilizing a glass vial and small metal balls (commonly called BB balls) for sample and matrix mixing [26]. Since glass vials and BB balls are readily available and inexpensive, they can be disposed of after a single use, thus preventing cross contamination. Additionally, the use of a vortex mixer instead of mixing the powders by hand rapidly decreases the sample preparation time.

A MALDI-MS study on polystyrene (PS) by Hanton and Parees indicated that the two solvent-free methods described give comparable results (cf. Fig. 1a), proving that spectral quality is not diminished by the faster ball milling technique. These spectra are also very similar with the MALDI-MS spectrum acquired using solvent-based sample preparation (Fig. 1a). The ball mill method is beneficial for analyzing samples with various physical properties, ranging from low molecular weight liquids

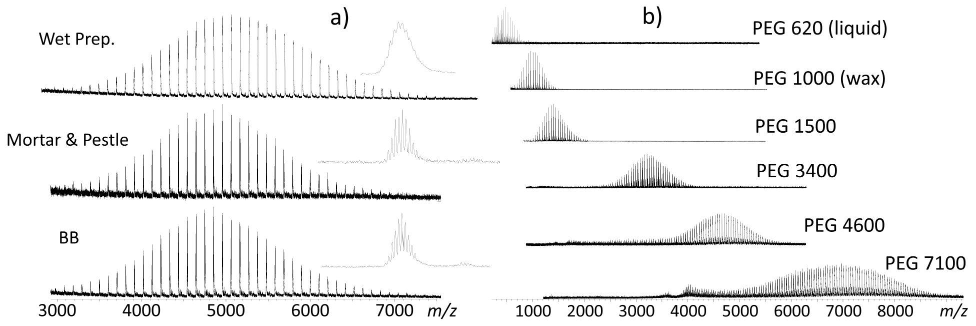


Fig. 1. (a) MALDI-MS spectra of a polystyrene (PS) sample prepared using the traditional solvent-based preparation (top), mortar and pestle (middle), and ball milling methods (bottom). (b) MALDI-MS spectra of poly(ethylene glycol) (PEG) samples of various molecular weights and physical properties, prepared using the ball milling method. Adapted from Hanton and Parees [26] with permission from the American Society for Mass Spectrometry.

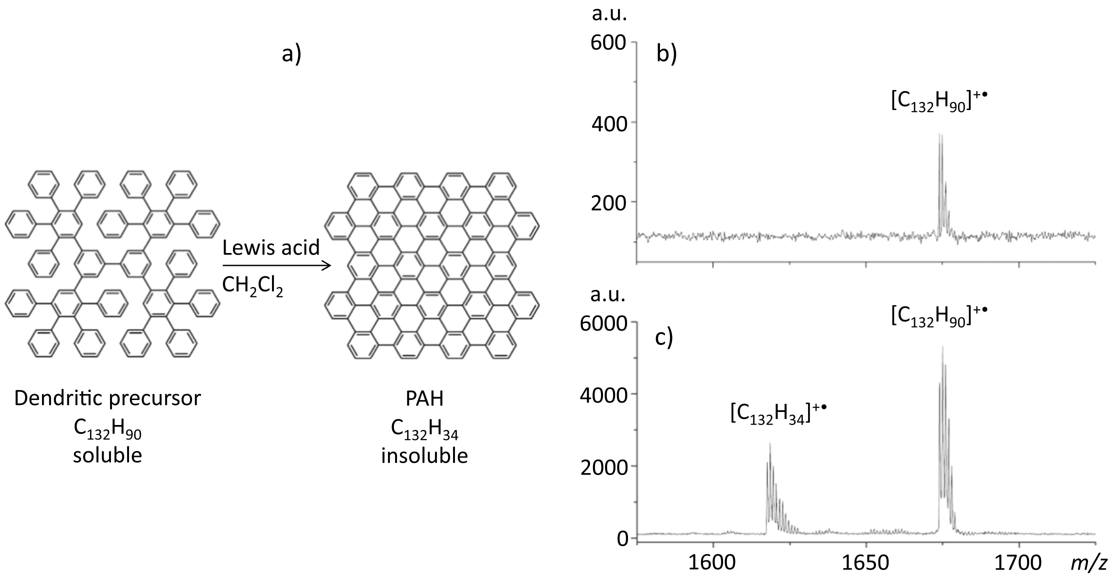


Fig. 2. (a) Synthesis of a polycyclic aromatic hydrocarbon (PAH) from a dendritic precursor. (b,c) MALDI-MS spectra of analyte prepared using (b) solvent-based sample preparation and (c) solvent-free sample preparation. Adapted from Trimpin et al. [27] with permission from the American Society for Mass Spectrometry.

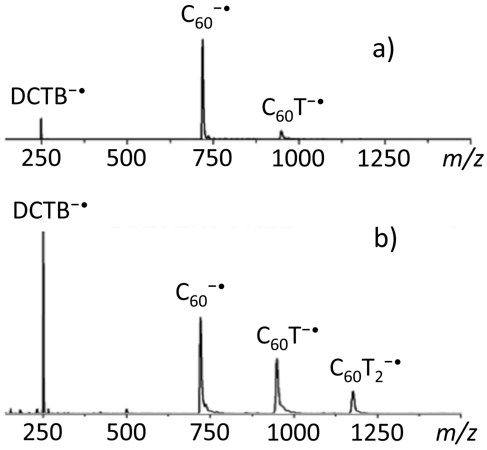


Fig. 3. MALDI-MS spectra of the products formed by [4 + 2] cycloaddition of tetracene (T, a PAH) and C_{60} fullerene, acquired using negative ion mode and either (a) solvent-based or (b) solvent-free sample preparation. The bis adduct $C_{60}T_2$ is clearly detected only with the solvent-free method. Adapted from Kotisiris et al. [34] with permission from SAGE Publishing.

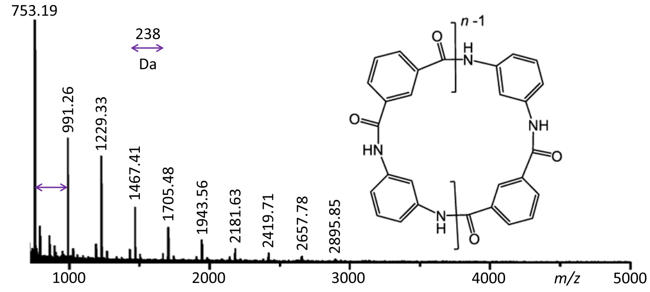


Fig. 4. MALDI-MS spectrum of Nomex, acquired using 3-aminoquinoline as matrix, potassium trifluoroacetate (KTFA) as cationizing salt, and the evaporation-grinding solvent-free sample preparation protocol. The peaks with marked *m/z* values correspond to $[M + K]^+$ ions of macrocycles with the m-phenylene isophthalamide repeat unit ($C_{14}H_{10}N_2O_2$, 238 Da). The minor distributions arise from $[M + H]^+$, $[M – H + 2 K]^+$, and $[M + 3\text{-aminoquinoline} + H]^+$ species. Adapted from Gies and Nonidez [36] with permission from the American Chemical Society.

to high molecular weight solids (cf. Fig. 1b). Since the initial introduction of solvent-free MALDI, this technique has been expanded to multi-sample preparation for high throughput studies [30], and to a variety of analytes, including high molecular weight proteins and polymers [30, 31], insoluble polycyclic aromatic hydrocarbons [25], hydrophobic peptides at femtomolar concentrations [32], and petroleum products [33].

Mechanical mixing of sample and matrix/salt may be necessary for the complete characterization of analyte mixtures. With certain organic and polymer chemistry reactions, precursor and product(s) can have significantly different solubilities (cf. Fig. 2a). In such cases, solvent-based MALDI-MS analysis suppresses the detection of the insoluble compound(s) (cf. Fig. 2b). The solvent-free approach, however, is able to detect both the soluble and insoluble sample constituents, thus providing a more comprehensive and accurate analysis (cf. Fig. 2c).

It is important to note that solvent-free MALDI is applicable in both positive as well as negative ion mode. Fig. 3 illustrates an application involving ionization to negative ions and compares the MALDI mass spectra of fullerene derivatives differing in solubility [34]. Again, the sparingly soluble product is only visible when a solvent-free approach is employed.

Mechanical mixing of analyte, matrix, and ionizing salt can be facilitated by adding a few droplets of an organic solvent, such as tetrahydrofuran (THF), to the mixture being ground to a fine powder. This sample preparation variant, called the evaporation-grinding method, results in a sticky paste that is better homogenized and more easily adheres at the sample holder than a mixture of dry powders [35–37]. The evaporation-grinding method has been successfully applied to insoluble polyamides [36]. Fig. 4 shows the MALDI-MS spectrum obtained from Nomex aramid fibers (poly-m-phenylene-isophthalamide); it includes a series of peaks at *m/z* 753–2896 with a 238-Da distance between adjacent oligomers, validating the m-phenylene isophthalamide ($C_{14}H_{10}N_2O_2$, 238 Da) repeat unit of this polymer. The *m/z* values of the observed ions agree well with potassiated cyclic oligomers with no end groups (cf. Fig. 4).

Aromatic polyamides like Nomex mainly comprise linear chains with high polydispersity and much higher molecular weights than the cyclic oligomers detected in Fig. 4 (M_n > 10 kDa; M_w > 28 kDa) [38]. Cyclization of the growing chains, an unwanted termination side reaction, proceeds most efficiently at low degrees of polymerization, where ring formation is entropically favored [39]. Hence, the observed macrocycles are attributed to a byproduct formed in the early stages of polymerization. MALDI favors desorption and ionization of such low-mass oligomers and discriminates against the much heavier linear chains in polymers of high polydispersity [40,41]. Even though the main constituent of Nomex evades detection, the low-mass fraction detected through solvent-free analysis still provides important compositional insight by identifying the sample's monomer units.

Applications to surface analysis and imaging

The surface layer variant of MALDI (SL-MALDI) was introduced by Wang et al. to characterize the molecular composition of polymer film surfaces [42]. These initial SL-MALDI-MS studies utilized a mechanical mixing method to apply both matrix and cationizing salt to the surface of the film (Fig. 5a). Specifically, fine crystals of matrix and salt were dusted onto the film surface using a spatula. Optical images of the surface indicated a broad size distribution for these crystals (1–10 μm) which, nevertheless, gave rise to adequate mass spectra [42]. The depth profiling of this solvent-free approach was probed with the investigation of a bilayer film comprised of a 1–2 nm poly(methyl methacrylate) (PMMA) layer on top of a 100 nm PS layer. PS was deposited first onto a silicon wafer using spin casting and the PMMA layer was applied on top of the PS film using the Langmuir-Schaefer technique, which deposits single molecular layers. Only peaks corresponding to PMMA were detected in the MALDI-MS analysis (Fig. 5b–d) confirming the probe depth of this method to be less than 2 nm. This unique analysis would be impossible using traditional solvent-based approaches, as the similar solubilities of PS and PMMA would lead to an averaged mass spectrum containing both polymers.

This surface analysis method has since been used to study polymer segregation trends in thin films prepared by spin casting of blends containing polymers with distinct polarities, molecular weights, and architectures (cf. Fig. 6) [43,44]. Consistent with thin film theory, shorter linear chains are entropically driven to the film surface (Fig. 6a) [43], whereas polar chains are enthalpically driven away from the film surface [44]. On the other hand, polymer blends of cyclic and linear PS analyzed via SL-MALDI-MS showed enrichment of the linear chains at the surface (Fig. 6b) [42]; this finding contradicted older theoretical predictions, thus proving the benefits of having analytical methods that can directly investigate surface composition so that theoretical models can be modified accordingly [42].

Similar dry matrix application approaches have been utilized to image tissue samples. Goodwin et al. [45,46] and Puolitaival et al. [47] were among the first groups to report the benefits of using solvent-free sample preparation to image phospholipids and drug segregation in rat brain tissue sections, respectively. Finely ground matrix was added as powder onto the tissue [45,46] or filtered onto the tissue through a 20 μm stainless steel sieve [47]. Fig. 7 compares MALDI-MS imaging (MALDI-MSI) data of sagittal mouse brain sections acquired using either a solvent-free or a spray-coating method [47]. The MALDI-MS images shown reveal the spatial distribution of phosphatidylcholine 36:1 (m/z 826) and phosphatidylcholine 40:6 (m/z 872) in the analyzed tissue section, constructed based on the intensities of these ions at each scanned pixel. As clearly attested by Fig. 7, the dry-coating method produced images with similar ion intensity and phospholipid distribution as those obtained from the spray-coated samples.

Meanwhile, MALDI-MSI analysis of mouse brain sections from both control animals and drug-dosed animals using conventional matrix application via spray coating could detect lipids and cholesterol, but not the drug of interest [45]. The absence of drug signals with solvent-based matrix application was ascribed to analyte spreading and redistribution effects interfering with the analysis [46]. With the dry-matrix method on the other hand, the drug was easily detected in the drug-dosed animal and absent from the control animal [45]. Collectively, these studies [45–47] prove that dry-matrix coating is a simple and efficient alternative to spray coating, providing complementary results and, in some cases, additional information that cannot be achieved by solvent-based sample preparation methods.

Sublimation methods

As has been mentioned, SL-MALDI-MS requires solventless sample preparation to avoid disruption of the chemical environment at the surface. The dry-matrix application discussed above is a convenient and easily implementable procedure, but it can lead to increased noise due to excess matrix/salt powder or uneven matrix/salt distribution on the surface. With sublimation, on the other hand, a more uniform surface coating and improved reproducibility should be achievable. Sublimation devices are typically comprised of a glass outer body and a glass inner body/condenser [48]. The sample is attached to the condenser and oriented with the surface facing the matrix/ionization agent in the flat bottom of the outer body. The pressure in the entire chamber is then reduced to a suitable vacuum level and the bottom of the outer body is heated either using a heating mantle or an oil bath. This causes the matrix and salt molecules to sublime into the gas phase and then deposit onto the cold sample surface [48].

For analytes that are easily protonated, only matrix sublimation is needed to prepare the samples; in such cases, matrix ions formed during the MALDI event act as acids to protonate the analyte via ion-molecule reactions in the MALDI plume [1,10,14]. This scenario does not apply to most polymers, however, which require an additional ionization agent, usually a metal cation, to become charged and detectable by MALDI-MS. This requirement introduces an additional variable in the sublimation process. Sublimation can be done either in one step, where both the matrix and ionizing agent are placed in the apparatus together, or in two individual steps. Sublimation of matrix and ionization salt at the same

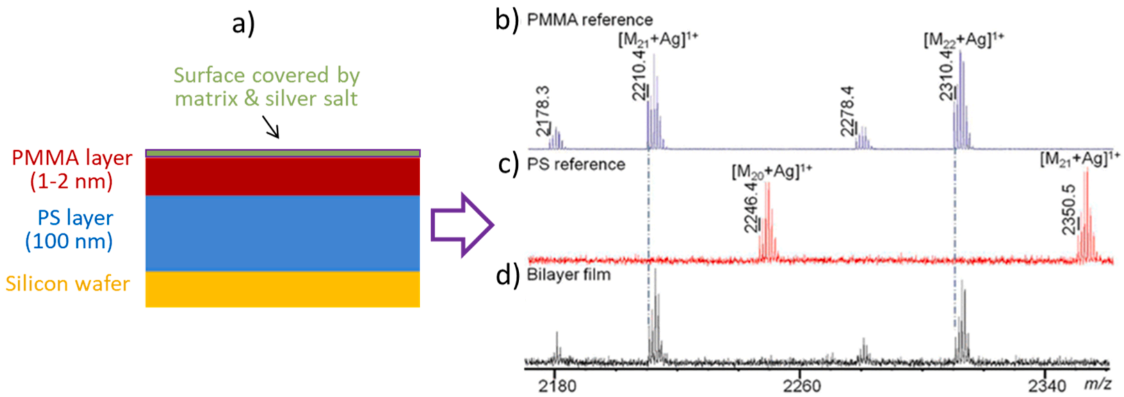


Fig. 5. (a) Schematic of the bilayer polymer film prepared to examine the surface specificity of SL-MALDI-MS. (b,c) MALDI-MS spectra of (b) PMMA and (c) PS standards. (d) SL-MALDI-MS spectrum of the PMMA/PS bilayer film. Adapted from Wang et al. [42] with permission from the American Chemical Society.

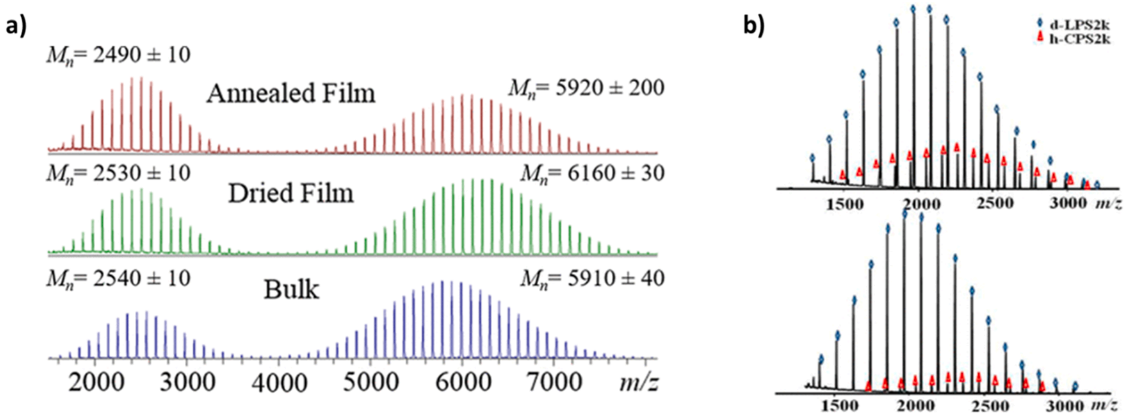


Fig. 6. (a) SL-MALDI-MS spectra of dried and annealed films formed by spin casting a blend of 2.5-kDa (12 wt%) and 6-kDa (88 wt%) polystyrenes, in comparison to the MALDI-MS spectrum of the bulk PS blend. Adapted from Hill et al. [43] with permission from the American Chemical Society. (b) MALDI-MS spectrum (top) of the bulk sample of a blend composed of 20 wt% 2-kDa cyclic polystyrene (CPS; not deuterated) and 80 wt% 2-kDa linear polystyrene (LPS; fully deuterated), and SL-MALDI-MS spectrum (bottom) of the surface of a film prepared by spin casting of this blend. Adapted from Wang et al. [42] with permission from the American Chemical Society.

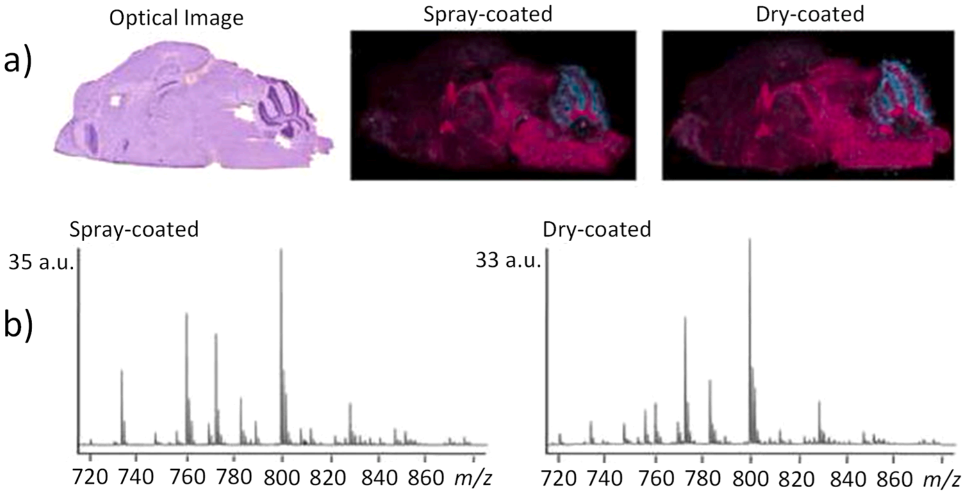


Fig. 7. (a) Optical image of a brain section overlaid with phospholipid ion images of *m/z* 826 (pink) and *m/z* 872 (blue) from the spray-coated and dry-coated experiments. (b) Extracted MALDI-MS spectra from the spray-coated and dry-coated imaging experiments. Adapted from Puolitaival et al. [47] with permission from the American Chemical Society.

time can result in unbalanced concentrations of the two components, as the sublimation temperature for the matrix is often lower than that of the salt, resulting in increased amounts of matrix at the surface [48]. Conversely, a two-step process, where the matrix and ionizing agent are sublimed individually, allows for more control over the concentration of each material deposited on the surface [48].

Sublimation for surface analysis and imaging

MALDI-MSI has been extensively employed to characterize lipid distributions in tissue samples [49,50]. However, the potential for analyte spreading when using spray-based sample preparation techniques is very high due to the solubility of these compounds in commonly used spray solvents. To bypass this problem, Hankin et al. introduced sublimation as a solvent-free approach for matrix application to thereby avoid disruption of the native lipid distribution [23]. Contrary to spray-coating methods, sublimation enhanced the purity of the matrix coating and led to uniform microcrystals on the tissue surface, thus allowing for reproducible and high- resolution images of the lipid profile to be obtained [23].

Since then, sublimation has become a widely used tool for imaging lipids [51–53] and proteins [54] in brain, kidney, liver, and retina tissues. The MALDI-MSI studies of lipids in rat tissues have revealed that 1, 5-diaminonaphthalene (DAN) matrix applied by sublimation is a promising procedure for enhancing the detection of lipids in both positive as well as negative ion mode, cf. Fig. 8 [52,53]. Similarly, MALDI-MSI of the protein composition in chicken livers using tissues of different thickness and varying amounts of matrix (SA) provided insight into the effects of matrix coating. These experiments indicated that the optimal matrix coating is between 0.1 and 0.3 mg cm^{-2} matrix molecules on the surface. Signal intensity was significantly reduced when using a lower amount of matrix, whereas using a thicker coating resulted in an increase in the background noise [54].

Sublimation based MALDI-MS studies on polymer films are scarce, compared to such studies on biological specimen (vide supra). Nevertheless, a recent application of SL-MALDI-MSI to synthetic polymer surfaces has demonstrated the usefulness of this analytical tool for identifying defects on the surface of polymer films due to mechanical alterations and solvent perturbation. Endres et al. utilized SL-MALDI-MSI to characterize defects on PMMA films caused by a PS stamp, obstruction of film formation by tape, scratching/laser etching, and polymer removal by organic solvents [55]. By using a mass filter to only

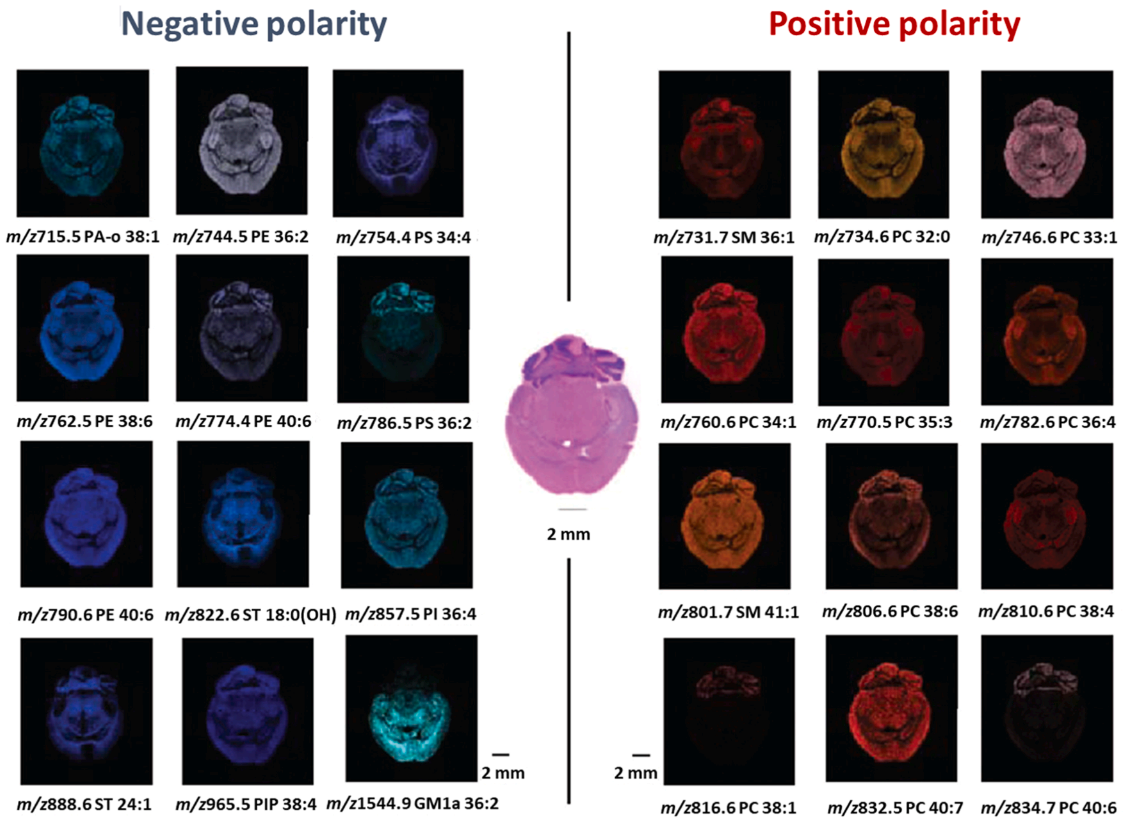


Fig. 8. Consecutive MALDI-MSI analysis of a rat brain tissue in both positive and negative ionization modes after sublimation of DAN matrix. Reproduced from Thomas et al. [52] with permission from the American Chemical Society.

monitor PMMA oligomers, images were obtained that detailed the extent of the defects. Fig. 9 demonstrates the SL-MALDI-MSI analysis of scratched/etched surfaces; matrix and sodium trifluoroacetate cationizing salt were applied onto the damaged surfaces by sublimation. The films shown in Fig. 9a and c were scratched and sample-prepared in the same manner but analyzed using different instruments; even at the lower spatial resolution analysis, the UA logo is clearly discerned [55].

The SL-MALDI-MSI technique with matrix application via sublimation can also be applied to study the distribution of small molecule additives on the surface of polymer thin films. Such analysis has recently been carried out on poly(ester urea) films doped with bupivacaine solutions of varying concentration [48,56,57]. Bupivacaine is a widely used anesthetic drug with the chemical formula $C_{18}H_{28}N_2O$. Bupivacaine signal ($[M + H]^+$ at m/z 289.2) was detected from each surface, and variations in the surface coverage were observed based on the signal intensity measured at each scanned pixel, cf. Fig. 10. At lower drug loads, there is a direct correlation between an increase in the solution concentration and the intensity of the observed bupivacaine signal at the surface, but at higher solution concentrations the signal intensity levels off, indicating surface saturation. These SL-MALDI-MSI results provide useful information about small molecule distributions in polymer films and aid in the development of materials engineered for applications as transdermal patches or biomedical implants.

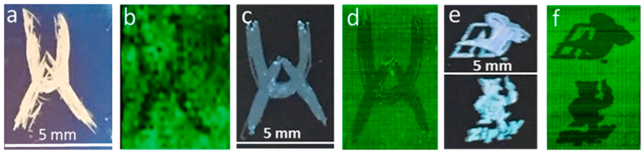


Fig. 9. (a,c) Optical images of UA defect scratched on the surface of a 7-kDa PMMA films and (b,d) corresponding SL-MALDI-MS images acquired with instruments having (b) 200 µm or (d) 35 µm spatial resolution. (e) Optical images of University of Akron logo (top) and mascot (bottom) laser-etched on the surface of a 7-kDa PMMA film and (f) corresponding 35 µm SL-MALDI-MS image. Adapted from Endres et al. [55] with permission from the American Chemical Society.

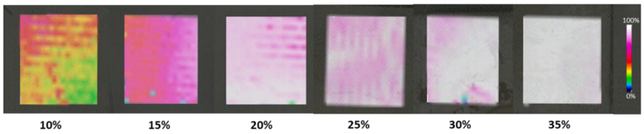


Fig. 10. SL-MALDI images of protonated bupivacaine molecules (m/z 289.2) at the surface of poly(ester urea) films. Adapted from Williams-Pavlantos [48].

Conclusions

This review presented two solvent-free sample preparation methods that can be applied to the MALDI-MS study of both biological materials, such as organ tissues, as well as insoluble polymers and polymeric thin films. The use of SL-MALDI-MS with these solvent-free sample preparation methods can provide information about surface properties and surface chemical composition that cannot be directly obtained with analytical methods that probe the bulk and, thus, are not surface-specific. Furthermore, the SL-MALDI-MS sample preparation method can be a valuable tool for analyzing materials that cannot be ground into a mixture using the basic mechanical grinding approach. The examples covered in this review detailed both the need for, and the current state of solvent-free sample preparation methods for MALDI. The relative ease of use and wide compatibility of the mechanical mixing method make it a valuable tool for studying polymer blend properties, lipid segregation, and protein structures. In cases where high resolution imaging is needed in conjunction with MALDI-MS methods, sublimation serves as the superior method by producing a high purity and uniform matrix (plus cationizing salt) coating.

Solvent-free MALDI has become an essential analytical tool for the analysis of insoluble and sparingly soluble compounds and, thus, increased applications to such samples are anticipated both in materials as well as biological sciences. Similarly, the SL-MALDI-MS technique and its imaging variant have opened the door for gaining molecular structure information on solid surfaces and their coatings. These techniques should benefit the detection and characterization of surface defects and/or drug segregation. Continued advancement of these methods, in combination with orthogonal techniques such as ion mobility spectrometry and tandem mass spectrometry fragmentation, and further development in the design of new sublimators [56–59] will undoubtedly continue to expand the scope and opportunities of compositional and structural characterizations via MALDI-MS and MALDI-MSI.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank the National Science Foundation for generous financial support (CHE-1808115 and DMR-2215940).

References

[1] G. Siuzdak, The Expanding Role of Mass Spectrometry in Biotechnology, 2nd ed., MCC Press, San Diego, CA, 2006. https://masspec.scripps.edu/learn/mass-spectrometry-in-biotechnology-2nd-ed-gary-siuzdak.pdf.

[2] U. Bahr, A. Deppe, M. Karas, F. Hillenkamp, U. Giessmann, Mass spectrometry of synthetic polymers by UV-matrix-assisted laser desorption/ionization, Anal. Chem. 64 (22) (1992) 2866–2869, https://doi.org/10.1021/ac00046a036.

[3] P.O. Danis, D.E. Karr, F. Mayer, A. Holle, C.H. Watson, The analysis of water-soluble polymers by matrix-assisted laser desorption time-of-flight mass spectrometry, Org. Mass Spectrom. 27 (7) (1992) 843–846, https://doi.org/10.1002/OMS.1210270717.

[4] J.S. Cottrell, Protein identification by peptide mass fingerprinting, Pept. Res. 7 (3) (1994) 115–124, https://doi.org/10.1533/9781908818058.185.

[5] T.B. Farmer, R.M. Caprioli, Determination of protein-protein interactions by matrix-assisted laser desorption/ionization mass spectrometry, J. Mass Spectrom. 33 (8) (1998) 697–704, https://doi.org/10.1002/(SICI)1096-9888(199808)33:8<697::AID-JMS711>3.0.CO;2-H.

[6] K. Gevaert, J. Vandekerckhove, Protein identification methods in proteomics, Electrophoresis 21 (6) (2000) 1145–1154, https://doi.org/10.1002/(SICI)1522-2683(20000401)21:6<1145::AID-ELPS1145>3.0.CO;2-Z.

[7] R. Murgasova, D.M. Hercules, MALDI of synthetic polymers—an update, Int. J. Mass Spectrom. 226 (1) (2003) 151–162, https://doi.org/10.1016/S1387-3806(02)00971-5.

[8] G. Montaudo, F. Samperi, M.S. Montaudo, Characterization of synthetic polymers by MALDI-MS, Prog. Polym. Sci. 31 (3) (2006) 277–357, https://doi.org/10.1016/J.PROGPOLYMSCI.2005.12.001.

[9] W. Morelle, J.C. Michalski, Analysis of protein glycosylation by mass spectrometry, Nat. Protoc. 2 (7) (2007) 1585–1602, https://doi.org/10.1038/nprot.2007.227.

[10] J.T. Watson, O.D. Sparkman, Introduction to Mass Spectrometry: Instrumentation, Applications and Strategies for Data Interpretation, 4th ed., John Wiley & Sons Ltd, Chichester, West Sussex, UK, 2008 https://doi.org/10.1002/9780470516898.

[11] Y. Dai, R.M. Whittal, L. Li, Two-layer sample preparation: a method for MALDI-MS analysis of complex peptide and protein mixtures, Anal. Chem. 71 (5) (1999) 1087–1091, https://doi.org/10.1021/AC980684H.

[12] B.O. Keller, L. Li, Three-layer matrix/sample preparation method for MALDI MS analysis of low nanomolar protein samples, J. Am. Soc. Mass Spectrom. 17 (6) (2006) 780–785, https://doi.org/10.1016/J.JASMS.2006.02.012.

[13] M. Kussmann, P. Roepstorff, Sample preparation techniques for peptides and proteins analyzed by MALDI-MS, in: J.R. Chapman (Ed.), Mass Spectrometry of Proteins and Peptides, Humana Press, Totowa, NJ, 2000, pp. 405–424, https://doi.org/10.1385/1-59259-045-4:405.

[14] E. de Hoffmann, V. Stroobant, Mass Spectrometry Principles and Applications, 3rd ed., John Wiley & Sons Ltd, Chichester, West Sussex, UK, 2007. http://www.usp.br/massa/2014/qfl2144/pdf/MassSpectrometry.pdf.

[15] L. Li (Ed.), MALDI Mass Spectrometry For Synthetic Polymers Analysis, John Wiley & Sons Ltd, Hoboken, NJ, 2009. https://mazams.weebly.com/uploads/4/8/2/6/48260335/document.pdf.

[16] M.J. Deery, K.R. Jennings, C.B. Jasieczek, D.M. Haddleton, A.T. Jackson, H. T. Yates, J.H. Scrivens, A study of cation attachment to polystyrene by means of matrix-assisted laser desorption/ionization and electrospray ionization-mass spectrometry, Rapid Commun. Mass Spectrom. 11 (1) (1997) 57–62, https://doi.org/10.1002/(SICI)1097-0231(19970115)11:1<57::AID-RCM772>3.0.CO;2-G.

[17] H. Rashidezadeh, B. Guo, Investigation of metal attachment to polystyrenes in matrix-assisted laser desorption ionization, J. Am. Soc. Mass Spectrom. 9 (7) (1998) 724–730, https://doi.org/10.1016/S1044-0305(98)00038-5.

[18] M.W.F. Nielen, Maldi time-of-flight mass spectrometry of synthetic polymers, Mass Spectrom. Rev. 18 (5) (1999) 309–344, https://doi.org/10.1002/(SICI)1098-2787(1999)18:5<309::AID-MAS2>3.0.CO;2-L.

[19] S.F. Macha, P.A. Limbach, S.D. Hanton, K.G. Owens, Silver cluster interferences in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry of nonpolar polymers, J. Am. Soc. Mass Spectrom. 12 (6) (2001) 732–743, https://doi.org/10.1016/S1044-0305(01)00225-2.

[20] A.J. Hoteling, M.L. Piotrowski, K.G. Owens, The cationization of synthetic polymers in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: investigations of the salt-to-analyte ratio, Rapid Commun. Mass Spectrom. 34 (S2) (2020) e8630, https://doi.org/10.1002/RCM.8630.

[21] A. Marie, F. Fournier, J.C. Tabet, Characterization of synthetic polymers by MALDI-TOF/MS: investigation into new methods of sample target preparation and consequence on mass spectrum finger print, Anal. Chem. 72 (20) (2000) 5106–5114, https://doi.org/10.1021/ac000124u.

[22] S. Trimpin, A. Rouhanipour, R. Az, H.J. Räder, K. Müllen, New aspects in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: a Universal solvent-free sample preparation, Rapid Commun. Mass Spectrom. 15 (15) (2001) 1364–1373, https://doi.org/10.1002/RCM.372.

[23] J.A. Hankin, R.M. Barkley, R.C. Murphy, Sublimation as a method of matrix application for mass spectrometric imaging, J. Am. Soc. Mass Spectrom. 18 (9) (2007) 1646–1652, https://doi.org/10.1016/J.JASMS.2007.06.010.

[24] R. Skelton, F. Dubois, R. Zenobi, A MALDI sample preparation method suitable for insoluble polymers, Anal. Chem. 72 (7) (2000) 1707–1710, https://doi.org/10.1021/AC991181U.

[25] L. Przybilla, J.D. Brand, K. Yoshimura, H.J. Räder, K. Müllen, MALDI-TOF mass spectrometry of insoluble giant polycyclic aromatic hydrocarbons by a new method of sample preparation, Anal. Chem. 72 (919) (2000) 4591–4597, https://doi.org/10.1021/ac000372q.

[26] S.D. Hanton, D.M. Parees, Extending the solvent-free MALDI sample preparation method, J. Am. Soc. Mass Spectrom. 16 (1) (2005) 90–93, https://doi.org/10.1016/J.JASMS.2004.09.019.

[27] S. Trimpin, S. Keune, H.J. Räder, K. Müllen, Solvent-free MALDI-MS: developmental improvements in the reliability and the potential of MALDI in the analysis of synthetic polymers and giant organic molecules, J. Am. Soc. Mass Spectrom. 17 (5) (2006) 661–671, https://doi.org/10.1016/J.JASMS.2006.01.007.

[28] A.R. Hortal, P. Hurtado, B. Martínez-Haya, A. Arregui, L. Bañares, Solvent-free MALDI investigation of the cationization of linear polyethers with alkali metals, J. Phys. Chem. B 112 (29) (2008) 8530–8535, https://doi.org/10.1021/JP802089R.

[29] M.Z. Wang, M.C. Fitzgerald, A solid sample preparation method that reduces signal suppression effects in the MALDI analysis of peptides, Anal. Chem. 73 (3) (2001) 625–631, https://doi.org/10.1021/ac0009090.

[30] S. Trimpin, C.N. McEwen, Multisample preparation methods for the solvent-free MALDI-MS analysis of synthetic polymers, J. Am. Soc. Mass Spectrom. 18 (3) (2007) 377–381, https://doi.org/10.1016/j.jasms.2006.09.006.

[31] S. Trimpin, M.L. Deinzer, Solvent-free MALDI-MS for the analysis of biological samples via a mini-ball mill approach, J. Am. Soc. Mass Spectrom. 16 (4) (2005) 542–547, https://doi.org/10.1016/J.JASMS.2004.12.014.

[32] S. Trimpin, M. Deinzer, Solvent-free mass spectrometry for hydrophobic peptide sequence analysis and protein conformation studies, BioTechniques 39 (6) (2018) 799–805, https://doi.org/10.2144/05396TE01.

[33] S.U. Kulkarni, H.J. Räder, M.C. Thies, The effects of molecular weight distribution and sample preparation on matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis of petroleum macromolecules, Rapid Commun. Mass Spectrom. 25 (19) (2011) 2799–2808, https://doi.org/10.1002/RCM.5166.

[34] S.G. Kotsiris, Y.V. Vasil'ev, A.V. Streletskii, M. Han, L.P. Mark, O.V. Boltalina, N. Chronakis, M. Orfanopoulos, H. Hungerbühlerf, T. Drewello, Application and evaluation of solvent-free matrix-assisted laser desorption/ionization mass spectrometry for the analysis of derivatized fullerenes, Eur. J. Mass Spectrom. 12 (6) (2006) 397–408, https://doi.org/10.1255/ejms.824.

[35] A.P. Gies, W.K. Nonidez, M. Anthamatten, R.C. Cook, J.W. Mays, Characterization of an insoluble polyimide oligomer by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 16 (20) (2002) 1903–1910, https://doi.org/10.1002/rcm.809.

[36] A.P. Gies, W.K. Nonidez, A technique for obtaining matrix-assisted laser desorption/ionization time-of-flight mass spectra of poorly soluble and insoluble aromatic polyamides, Anal. Chem. 76 (7) (2004) 1991–1997, https://doi.org/10.1021/ac035299t.

[37] S.T. Ellison, A.P. Gies, D.M. Hercules, S.L. Morgan, Py−GC/MS and MALDI−TOF/TOF CID study of polysulfone fragmentation reactions, Macromolecules 42 (8) (2009) 3005–3013, https://doi.org/10.1021/ma900161y.

[38] L. Cosimbescu, D. Malhotra, M.R. Pallaka, M.S. Swita, Kevlar-like aramid polymers from mixed PET waste, ACS Omega 7 (36) (2022) 32026–32037, https://doi.org/10.1021/acsomega.2c03059.

[39] V. Martí-Centelles, M.D. Pandey, M.I. Burguete, S.V. Luis, Macrocyclization reactions: the importance of conformational, configurational, and template-induced preorganization, Chem. Rev. 115 (16) (2015) 8736–8834, https://doi.org/10.1021/acs.chemrev.5b00056.

[40] G. Montaudo, R.P. Lattimer (Eds.), Mass Spectrometry of Polymers, CRC Press, Boca Raton, FL, 2001, https://doi.org/10.1201/9780849331275.

[41] H. Pasch, W. Schrepp, MALDI-TOF Mass Spectrometry of Synthetic Polymers, Springer Verlag, Berlin, 2003, https://doi.org/10.1007/978-3-662-05046-0.

[42] S.-F. Wang, X. Li, R.L. Agapov, C. Wesdemiotis, M.D. Foster, Probing surface concentration of cyclic/linear blend films using surface layer MALDI-TOF mass spectrometry, ACS Macro Lett. 1 (8) (2012) 1024–1027, https://doi.org/10.1021/MZ300271W.

[43] J.A. Hill, K.J. Endres, P. Mahmoudi, M.W. Matsen, C. Wesdemiotis, M.D. Foster, Detection of surface enrichment driven by molecular weight disparity in virtually monodisperse polymers, ACS Macro Lett. 7 (4) (2018) 487–492, https://doi.org/10.1021/ACSMACROLETT.7B00993.

[44] J.A. Hill, K.J. Endres, J. Meyerhofer, Q. He, C. Wesdemiotis, M.D. Foster, Subtle end Group functionalization of polymer chains drives surface depletion of entire polymer chains, ACS Macro Lett. 7 (7) (2018) 795–800, https://doi.org/10.1021/acsmacrolett.8b00394.

[45] R.J.A. Goodwin, P. Scullion, L. MacIntyre, D.G. Watson, A.R. Pitt, Use of a solvent-free dry matrix coating for quantitative matrix-assisted laser desorption ionization imaging of 4-bromophenyl-1,4-diazabicyclo(3.2.2) nonane-4-carboxylate in rat brain and quantitative analysis of the drug from laser microdissected tissue regions, Anal. Chem. 82 (9) (2010) 3868–3873, https://doi.org/10.1021/AC100398Y.

[46] R.J. Goodwin, L. MacIntyre, D.G. Watson, S.P. Scullion, A.R. Pitt, A solvent-free matrix application method for matrix-assisted laser desorption/ionization imaging of small molecules, Rapid Commun. Mass Spectrom. 24 (11) (2010) 1682–1686, https://doi.org/10.1002/rcm.4567.

[47] S.M. Puolitaival, K.E. Burnum, D.S. Cornett, R.M. Caprioli, Solvent-free matrix dry-coating for MALDI imaging of phospholipids, J. Am. Soc. Mass Spectrom. 19 (6) (2011) 882–886, https://doi.org/10.1016/J.JASMS.2008.02.013.

[48] K. Williams-Pavlantos, Ph.D. Dissertation, The University of Akron, 2023.

[49] R.C. Murphy, J.A. Hankin, R.M. Barkley, Imaging of lipid species by MALDI mass spectrometry, J. Lipid Res. 50 (2009) S317–S322, https://doi.org/10.1194/jlr.R800051-JLR200.

[50] X. Zhu, T. Xu, C. Peng, S. Wu, Advances in MALDI mass spectrometry imaging single cell and tissues, Front. Chem. 9 (2021), 782432, https://doi.org/10.3389/fchem.2021.782432.

[51] R.C. Murphy, J.A. Hankin, R.M. Barkley, K.A. Zemski Berry, MALDI imaging of lipids after matrix sublimation/deposition, Biochim. Biophys. Acta 1811 (11) (2011) 970–975, https://doi.org/10.1016/J.BBALIP.2011.04.012.

[52] A. Thomas, J.L. Charbonneau, E. Fournaise, P. Chaurand, Sublimation of new matrix candidates for high spatial resolution imaging mass spectrometry of lipids: enhanced information in both positive and negative polarities after 1,5-diamino-napthalene deposition, Anal. Chem. 84 (4) (2012) 2048–2054, https://doi.org/10.1021/AC2033547.

[53] S. Caughlin, D.H. Park, K.K.C. Yeung, D.F. Cechetto, S.N. Whitehead, Sublimation of DAN matrix for the detection and visualization of gangliosides in rat brain tissue for MALDI imaging mass spectrometry, J. Vis. Exp. 121 (121) (2017) e55254, https://doi.org/10.3791/55254.

[54] J. Yang, R.M. Caprioli, Matrix sublimation/recrystallization for imaging proteins by mass spectrometry at high spatial resolution. Anal. Chem. 83 (14) 5728–5734. 10.1021/AC200998A.

[55] K.J. Endres, J.A. Hill, K. Lu, M.D. Foster, C. Wesdemiotis, Surface layer matrix-assisted laser desorption ionization mass spectrometry imaging: a surface imaging technique for the molecular-level analysis of synthetic material surfaces, Anal. Chem. 90 (22) (2018) 13427–13433, https://doi.org/10.1021/ACS.ANALCHEM.8B03238.

[56] K. Williams-Pavlantos, C. Wesdemiotis, Surface layer matrix assisted laser desorption ionization mass spectrometry imaging (SL-MALDI-MSI) of pharmaceutical-loaded polymer films, in: Proceedings of the 69th ASMS Conference on Mass Spectrometry and Allied Topics, Philadelphia, PA, 2021, in: https://www.asms.org/publications/abstracts-and-proceedings.

[57] K. Williams-Pavlantos, N.C. Brigham-Stinson, M.L. Becker, C. Wesdemiotis, Application of surface-layer matrix-assisted laser desorption/ionization mass spectrometry imaging to pharmaceutical-loaded poly(ester urea) films, Anal. Chim. Acta, submitted on (15 May 2023).

[58] R. Fernandez, J. Garate, L. Martin-Saiz, I. Galetich, J.A. Fernandez, Matrix sublimation device for MALDI mass spectrometry imaging, Anal. Chem. 91 (1) (2019) 803–807. https://pubs.acs.org/doi/10.1021/acs.analchem.8b04765.

[59] N. Shanmugaraj, T. Rutten, A. Svatoš, T. Schnurbusch, H.P. Mock, Fast and reproducible matrix deposition for MALDI mass spectrometry imaging with improved glass sublimation setup, J. Am. Soc. Mass Spectrom. 34 (3) (2023) 513–517. https://pubs.acs.org/doi/10.1021/jasms.2c00301.