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Recent developments in pre-treatment and analytical techniques for synthetic polymers by MALDI-TOF mass spectrometry

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A great deal of effort has been expended to develop accurate means of determining the properties of synthetic polymers using matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS). Many studies have focused on the importance of sample pre-treatment to obtain accurate analysis results. This review discusses the history of synthetic polymer characterization and highlights several applications of MALDI-TOF MS that recognize the importance of pre-treatment technologies. The subject area is of significance in the field of analytical chemistry, especially for users of the MALDI technique. Since the 2000s, many such technologies have been developed that feature improved methods and conditions, including solvent-free systems. In addition, the recent diversification of matrix types and the development of carbon-based matrix materials are described herein together with the current status and future directions of MALDI-TOF MS hardware and software development. We provide a summary of processes used for obtaining the best analytical results with synthetic polymeric materials using MALDI-TOF MS.

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1. Introduction

Polymers are found almost everywhere in daily life, including packaging materials, automotive parts, medical supplies, and countless more examples.[1,2] Polymer manufacturers improve or tailor the characteristics of their product by adding antioxidants, dyes, waxes, and other materials. Characterizing the chemical aspects of these complex polymer systems is vital, and numerous analytical methods[3,4] have been developed toward this end. Thermal methods, either directly coupled with mass spectrometry (MS) or uses for desorption/decomposition studies with gas chromatography (GC)-MS, have been used to study additives and polymer degradation products.[5–8] Liquid chromatography (LC) and various liquid extraction methods combined with LC-MS have been used to analyze additives and contaminants.[9,10] Matrix-assisted laser desorption/ionization (MALDI) MS and electron spray ionization (ESI) have been used to characterize specific systems,[11–14] including insoluble polymers using a solvent-free method.[15] Recently, polymer identification has been demonstrated using direct atmospheric pressure chemical ionization (APCI).[16,17] However, the vast majority of these methods require multiple time-consuming steps, including extraction and chromatographic separation, that limit high-throughput analyses and often require a regulatory laboratory. A single, rapid method such as MALDI MS eliminates many of these drawbacks, and can be used to identify quickly the additives in a polymer sample and/or identify and characterize the polymer structure.

MS was first introduced by physicists in the 1880s. In 1889, the physicist Eugen Goldstein discovered a new form of radiation composed of positive ions. In 1898, Wilhelm Wien demonstrated that overlapping electrical and magnetic fields can be used to deflect positive ions.[18,19] Inspired by their findings, Joseph John Thomson, a professor of physics at the University of Cambridge, invented the first mass spectrometer.[20] In the 1940s, commercial mass spectrometers able to identify organic substances began to appear in response to the demands of the petroleum industry.[18,19] Gas chromatography, invented in 1952, was used to separate thermally stable biological compounds[21] and in 1957 the first gas chromatography mass spectrometer was developed by Holmes and Morrell.[22] Chemical ionization (CI)[23] and plasma desorption (PD)[24] methods were introduced in 1966 and 1974, respectively. Other mass analysis methods were developed soon after, such as fast atomic impact fast atom bombardment (FAB)[25] and liquid

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secondary ion mass spectrometry (LSIMS).[26] Electrospray ionization (ESI)[27,28] and matrix-assisted laser desorption ionization (MALDI)[29,30] were developed as ionization methods and dramatically revolutionized the use of mass spectrometers.

MALDI time-of-flight (TOF) MS[31–34] is a state-of-the-art technique for characterizing chemical structures of macromolecules, including synthetic polymeric materials (organic or inorganic).[35–40] Unlike ESI, in which molecules are separated according to their state of charge, MALDI provides a simple mass spectrum containing a peak corresponding to a singly charged molecule and configurational information (end groups and structures) that is difficult to obtain by IR or NMR spectroscopies.[41] In addition, MALDI-based methods boast ease of analysis, speed, and high mass accuracy. Since the first report of MALDI for synthetic polymer analysis,[42,43] significant advances have been made in the development of sample preparation protocols and instruments. MALDI MS has been used to obtain important data on monomer reactivity ratios,[44,45] end groups,[46–48] telomer repeat units, mechanisms of polymerization,[49–51] and average molecular weights. Numerous attempts, with various degrees of success, have been undertaken to obtain weight average molecular weight (M_w), number average molecular weight (M_n), polydispersity (PD), and repeat unit and end group mass using this technique.[52–55] MALDI was developed to handle a wide variety of polymeric materials containing a wide variety of chemical and molecular weight ranges.

The role of the matrix in MALDI MS is to facilitate smooth desorption and ionization processes, assuming that the analyte is homogeneously embedded in the pre-structured system of the matrix.[56,57] Accordingly, sample pre-treatment is important for accurate measurements of polymer characteristics by MALDI MS. Pre-treatment conditions, including the choice of matrix, cationization agent, deposition volume, and deposition technique, are often tailored for a specific sample type. Although a wide variety of sample preparation methods have been developed, determining an optimal method for a new sample remains challenging. Thus, the quality of the mass spectral information is often compromised due to suboptimal methods.

Many studies have positioned MALDI as a representative ionization method for mass spectrometry. Methods for analyzing end groups, including acetyl groups,[58] terephthalates,[59] oligosaccharides,[60] have been developed and compiled in a review paper.[61] Methods for bio-polymer analysis[62–66] have been the topic of several review papers.[67,68] MALDI results have been examined *via* image analysis,[69,70] with the findings being described in review papers.[71,72] Research on sample pre-treatment, an important part of the MALDI analytical process, is also underway. Studies have been conducted on the dried droplet method,[73] matrix selection,[74] the standard-addition method,[75] MALDI sample preparation,[76] and solvent-free methods.[77–83] However, to date, a thorough summary of solvent-free methods, which account for much of the current MALDI sample pre-treatment research, is lacking.

Polymer science has developed rapidly since the development of MALDI-TOF MS.[84–86] Carbon is the most widely distributed element on Earth and the analysis of carbon-based materials, such as carbon nanotubes[87–89] and graphene,[90–93] is an emerging area of interest. These relatively new materials are being explored in various fields and ongoing studies have examined them as matrix materials in MALDI analyses.[94–97] To date, however, a review summarizing the findings of these studies has not been published. This review combines and correlates recent research to provide the reader with a comprehensive overview of solvent-free methods in MALDI sample pre-treatment processes and the use of carbon nanotubes and graphene as MALDI matrix materials.

2. Sample preparation

The 'solvent-free method'[98–102] was established in the early 20th century as a new sample preparation method for MALDI mass spectrometry. Solvent-free methods have been applied in numerous studies for the analysis of many types of analytes, including synthetic polymers. Conventional solvent-based methods[101,103] often suffer from problems with solubility, miscibility, and phase separation that can occur during crystallization due to polarity differences between the analyte and the matrix. Solvent-free MALDI MS was developed to overcome these problems and significantly simplifies MALDI data acquisition and improves analyses. These advantages were demonstrated in a recent study based on the ball-mill homogenization/loose powder delivery method. However, the most striking advantage of solvent-free methods is that they can be used to characterize insoluble compounds such as polyvinylpyrrolidone and polyfluorene.[104] As a result, solvent-free methods are frequently used to characterize large polycyclic aromatic hydrocarbons.[105,106] The lack of a solvent also simplifies data analysis and eliminates problems associated with analyte–solvent interactions. In general, solvent-free methods provide a more homogeneous analyte/matrix mixture than do solvent-based methods, resulting in higher analytical reproducibility. Accordingly, fragmentation[103,104] is reduced because MALDI conditions are attained at lower laser power. This also reduces background signal and thereby improves mass resolution. However, it should be noted that solvent-free methods are usually less efficient for samples that can be accurately measured using solvent-based methods. The primary drawback of solvent-free methods is that they are difficult to implement in high-throughput applications. In many cases, however, this can be partially overcome by using the vortex method[107] or the mini-ball-mill (MBM) method.[108–110]

In general, three methods are used for the homogenization of synthetic polymers prior to MALDI-MS analysis: grinding by mortar and pestle,[98,101] ball-milling,[101,103,104,111] and vortexing.[107] In the latter two methods, analyte, matrix material, and salts are added to a container together with appropriately sized ceramic or metallic balls to aid in the process of homogenization. It has been reported that the efficiency of each of these methods can differ for different polymers.[101,107] After homogenization, two methods are commonly used to deliver the sample powder to the MALDI plate. One method is to compress the sample powder into a pellet and attach it to the MALDI plate with double-sided adhesive tape.[98] In the second method, loose

homogenized sample[101,103,104,111] is gently transferred to the MALDI plate using a spatula and spread as a thin powder film.

These methods provide a means of quickly and easily acquiring mass spectral data from important industrial polymers and can improve the throughput of routine analyses. Solvent-free methods are very important in MALDI analysis because most polymers do not dissolve well in solvents. Additionally, these methods can provide both quantitative and qualitative improvements to mass spectral workflows and are amenable to future automation. This review covered the role of analysis methods without the use of solvents, enabling objective comparison of various MALDI sample preparation protocols.

2.1. Solvent-free methods

Because synthetic polymers possess different functional groups and different chemical unit structures, chain lengths, and polydispersities, it is necessary to develop new complementary sample target preparation protocols to characterize them by MALDI. If solvents are avoided in the sample preparation step, then polymer solubility, pH of the analyte and matrix solutions, crystallization temperature, and secondary solvent effects should not play an important role in determining the quality of the MALDI mass spectrum fingerprint. This might also provide new insights about the applicability of MALDI to synthetic polymer analysis and the multistep mechanism of the desorption/ionization process.[96]

Solvent-based sample preparation methods for MALDI MS consist of multiple important parameters that need to be optimized to ensure reproducibility and accuracy. A more general method with fewer such parameters is a long-standing goal in the MALDI MS community. Research papers describing solvent-less pre-treatment methods for MALDI MS began to appear in the early 21st century. In a previous study, solubility-limited polyamides (<5000 Da) were characterized by mechanically mixing the analyte and matrix by mortar and pestle.[101] The resulting powder was pressed into a solvent-free pellet for MALDI MS analysis.[98] This type of sample preparation has been used to obtain MS data on large, insoluble polycyclic aromatic hydrocarbons (PAHs),[111–113] polyfluorenes,[15] and polydithiathianthrenes.[114] UV-absorbing compounds, such as unstable side-chain-protected synthetic peptides[115] and pigments,[101] and high-molecular-weight polymers; this method has been validated as a suitable sample pre-treatment for MALDI MS analyses.

Solvent-free methods[107] published prior to 2005 yielded excellent mass spectra, although the mortar and pestle method showed a relatively high probability of cross-contamination, requiring significant tool cleaning. Mini ball milling reduces labor and a method was developed using two BBs to mill samples in small glass bottles. The BBs method behaves as a mini-ball mill to effectively grind together the analyte, matrix, and cationization agent.[103] This new method yielded mass spectra of the same quality as those obtained with previous methods, while being much faster, and had the additional advantages of increased productivity, reduced cross-contamination, and suitability for liquid or waxy analytes (Fig. 1 and 2).

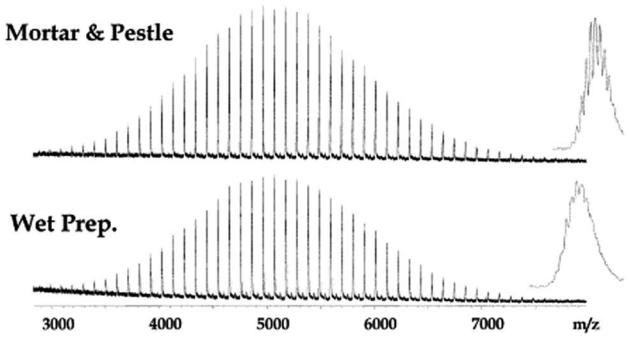

Fig. 1 MALDI mass spectra produced for a sample of PS 5050 using the mortar and pestle dry preparation method (top) and a typical solvent-based sample preparation (bottom). In both cases, the sample was prepared with Ret A as the matrix and AgTFA as the cationization agent. The dry prepared spectrum shows higher signal intensity, improved signal-to-noise, and a flatter baseline. The expanded spectra are from the oligomers at the peaks of the distributions. Reproduced from ref. 103 Hanton *et al.*, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 90–93.

In a previous report,[103] MALDI-MS spectra obtained using solvent-free and solvent-based methods were compared using polyethylene glycol (PEG), polymethyl methacrylate (PMMA), poly(vinylpyrrolidone) (PVP), poly(dimethylsiloxane) (PDMS), poly(butylmethacrylate diole) (BD), polystyrene (PS), and partial glycerides as samples and dihydroxybenzoic acid (DHB), indoleacrylic acid (IAA), dithranol, and tetracyanoquinodimethane (TCNQ) as matrix materials. In the solvent-free method, the analyte/matrix mixture was applied to the MALDI target as a thin film of fine powder. In the solvent-based method, the analyte and matrix were applied by completely dissolving them in a suitable solvent such as dichloromethane or tetrahydrofuran (THF). Compared to the solvent-based method, the solvent-free method yielded a more uniformly distributed analyte/matrix mixture. This result demonstrates that the

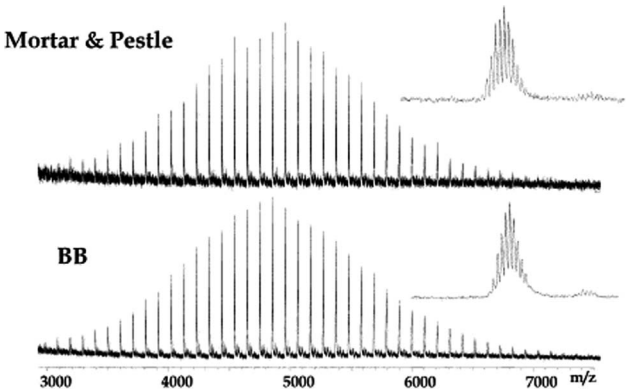

Fig. 2 MALDI mass spectra produced for a sample of PS 5050 using the mortar and pestle dry preparation method (top) and the new BB solvent-free sample preparation (bottom). In both cases, the sample was prepared with DHB as the matrix and AgTFA as the cationization agent. The two spectra are nearly identical. The expanded spectra are from the oligomers at the peaks of the distributions. Reproduced from ref. 103 Hanton *et al.*, *J. Am. Soc. Mass Spectrom.*, 2005; **16**, 90–93.

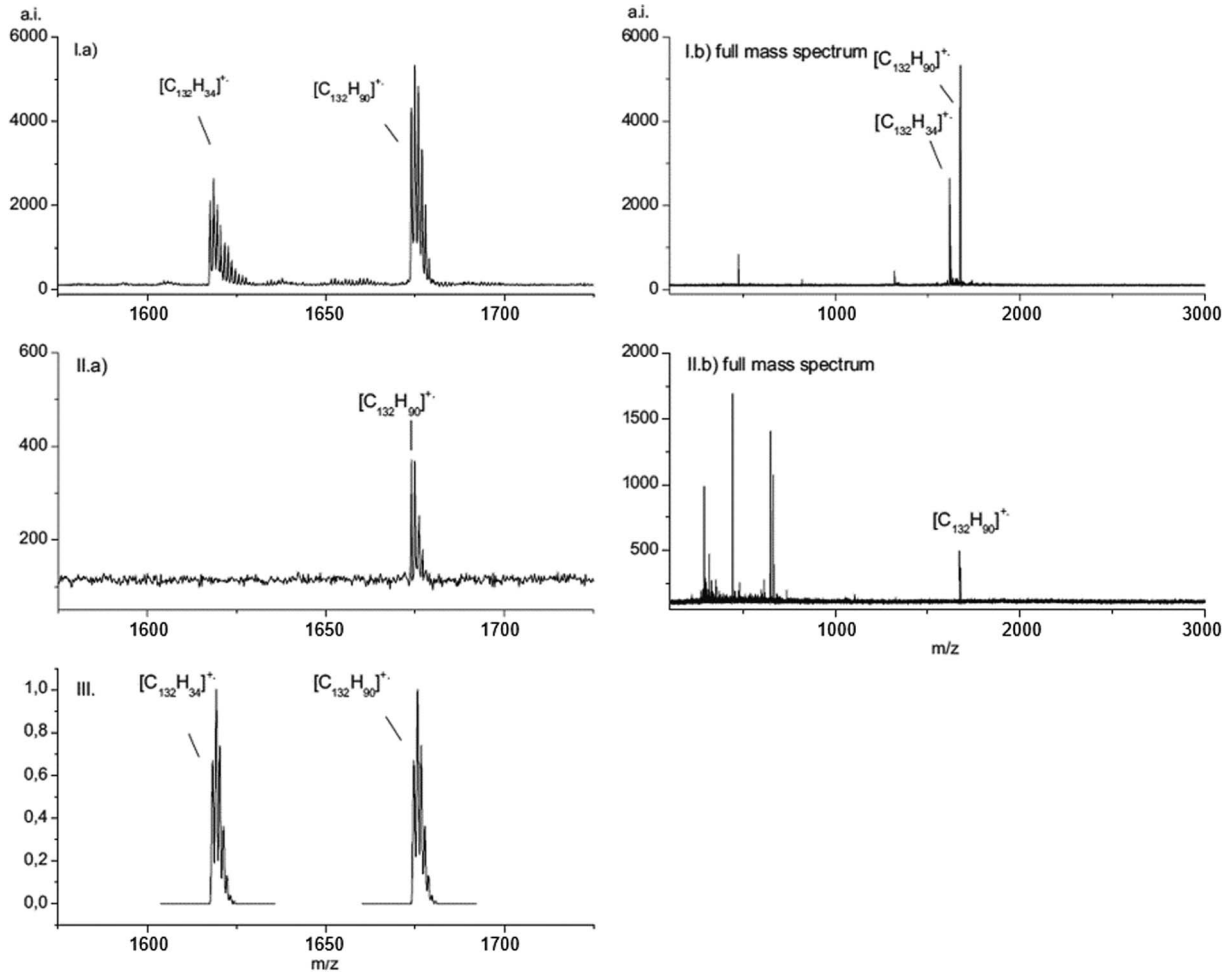

Fig. 3 MALDI mass spectra of a model mixture simulating a 90% reaction yield in the synthesis the insoluble PAH product C132H34 6 from the dendrite precursor C132H90 5: [I] solvent-free sample preparation: (a) inset spectrum, (b) full mass spectrum; [II] conventional solvent-based sample preparation: (a) inset spectrum, (b) full mass spectrum; [III] simulated isotopic distribution of the analyte mixture calculated from the elemental compositions. Reproduced from ref. 99 Trimpin *et al.*, *J. Am. Soc. Mass Spectrom.*, 2006, **17**, 661–671.

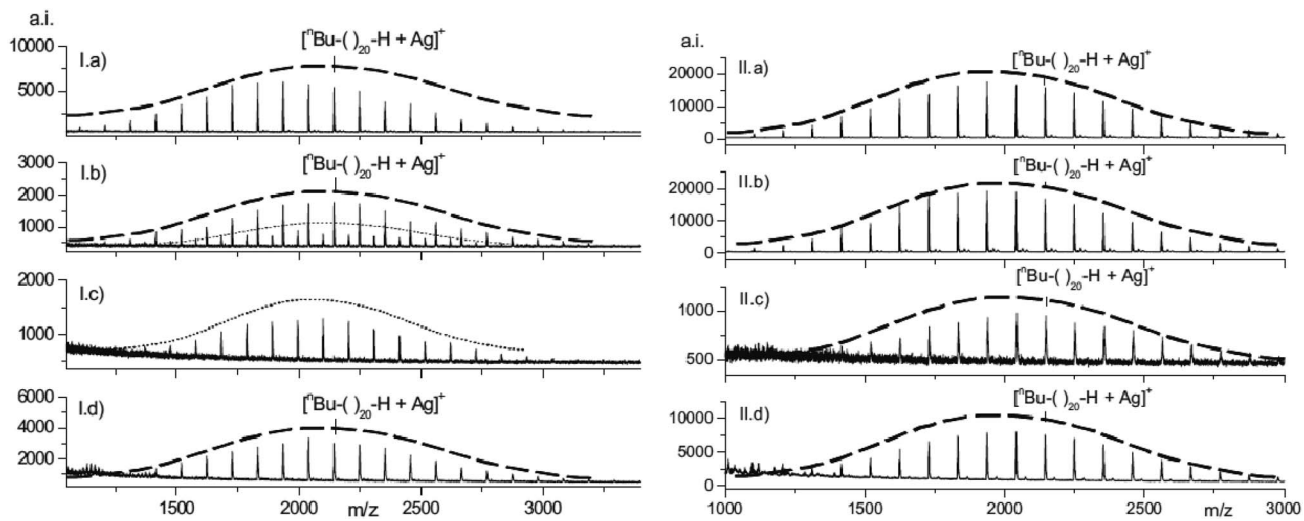

Fig. 4 MALDI mass spectra of PS 7 (2000 Da) as a function of molar ratios of [I] PS : dithranol : silver salt and solvents applying conventional solvent-based MALDI-MS: (a) 1 : 50 : 10 dissolved THF; (b) 1 : 500 : 10 dissolved THF; (c) 1 : 5000 : 10 dissolved in THF; (d) 1 : 5000 : 10 dissolved in dichloromethane; [II] PS : matrix : silver salt and matrices applying solvent-free MALDI-MS: (a) 1 : 50 : 10 and dithranol; (b) 1 : 500 : 10 and dithranol; (c) 1 : 5000 : 10 and dithranol; (d) 1 : 5000 : 10 and IAA. Reproduced from ref. 99 Trimpin *et al.*, *J. Am. Soc. Mass Spectrom.*, 2006, **17**, 661–671.

solvent-free method can be used to solve several long-standing problems associated with phase separation. Solvent-free sample preparation eliminates analyte, matrix, and solvent incompatibility due to crystallization effects and differential solubility and allows analysis of compounds containing strong UV-adsorbing moieties (Fig. 3 and 4).

To help enable high-throughput, solvent-free processes, a single vortex method[107] was multiplexed by incorporating a technique for multi-sampling and an appropriate sample holder ((a) TissueLyser method, (b) vortex method). Trimpin *et al.*[74] explored this strategy by developing a method for delivering a homogeneous mixture of matrix, analyte, and salt onto a MALDI plate. Their approach significantly reduced the effort and time required to prepare multiple samples, making it competitive with solvent-based methods for low-molecular-weight (MW) polymer analyses. PEG and PMMA with narrow polydispersities were used as standards with matrices of dithranol and 2,5-dihydroxybenzoic acid (2,5-DHB). MALDI analyses were performed using transretinoic acid and sodium trifluoroacetate. While the TissueLyser approach and the delivery of homogenized sample using a generally available vortex device were successful, the technique would have been more useful if more samples had been available on a single MALDI plate (Fig. 5 and 6).

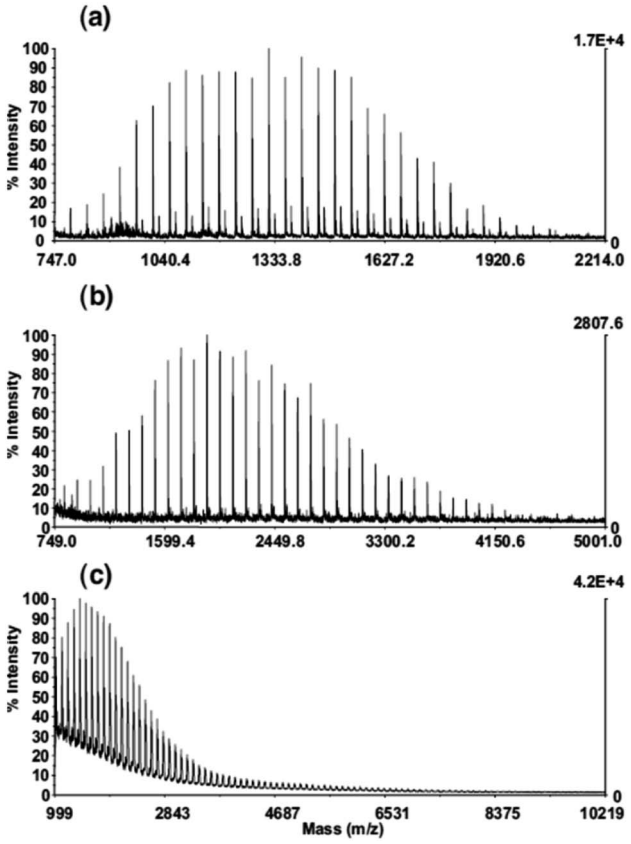

Fig. 5 On-target homogenization/transfer solvent-free MALDI mass spectra using the TissueLyser approach for (a) PEG 1470; (b) PMMA 1830; (c) a mixture of poly(methylmethacrylate) (PMMA) narrow distribution standards including PMMA 1830, 2400, 2990, 3800, 5270, 6950, and 10 300 in an equivalent weight ratio designed to mimic a broad polydisperse polymer. The mass measurements were obtained 5 days after the sample preparation. Reproduced from ref. 74 Trimpin *et al.*, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 377–381.

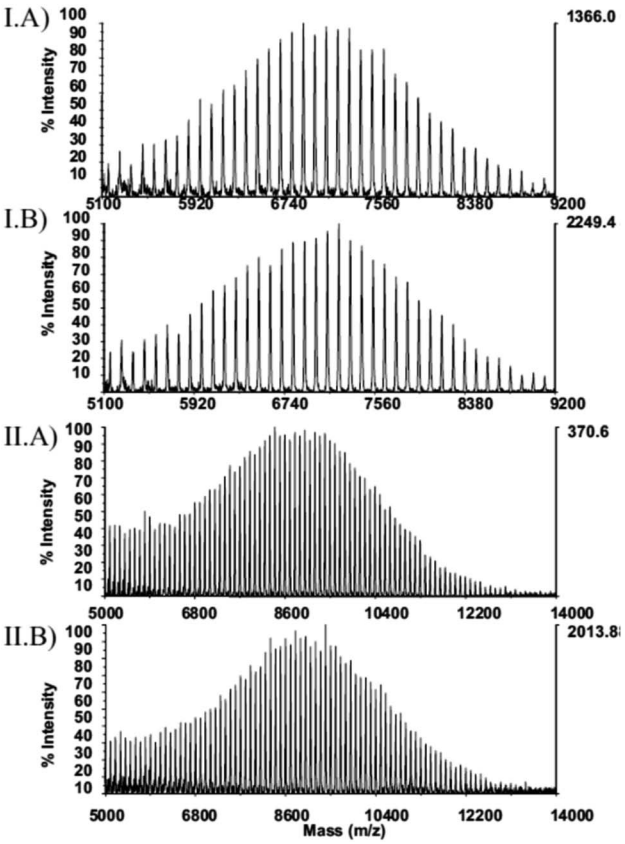

Fig. 6 Mass spectra of PS 7200 (IA and IB) investigating spot-to-spot reproducibility and PMMA 10 300 (IIA), using on-target homogenization/transfer solvent-free MALDI with the vortex device compared to a solvent-based approach (IIB) by adding THF to the PMMA/matrix/salt mixture used to obtain spectra IIA. Reproduced from ref. 74 Trimpin *et al.*, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 377–381.

The vortex method of sample pre-treatment has been examined in several studies. Hanton *et al.*[75] investigated multiple methods for preparing solvent-free samples and those developed by Trimpin and Räder are widely employed.[99–110] They developed a simple means of solvent-free sample preparation, called the current vortex method, which reduces analysis time, makes sample preparation easier, and reduces the risk of cross-contamination (Fig. 7).

The vortex method was shown to be effective with a very short mixing time of only 10 seconds. It should be noted, however, that this study focused on low-mass polymers below 5000 Da and that analyses of larger polymers would benefit from longer mixing times. This pre-treatment method (multi-sample solvent-free) was developed previously[78] and published in a separate report in 2009.[80] Several polymeric materials, including polyethylene oxide, methacrylate, styrene, and siloxane, were analyzed using multiple pre-treatment methods. MALDI analyses were then performed using polyethylene terephthalate (PET) as a standard. The simultaneous, multi-sample,

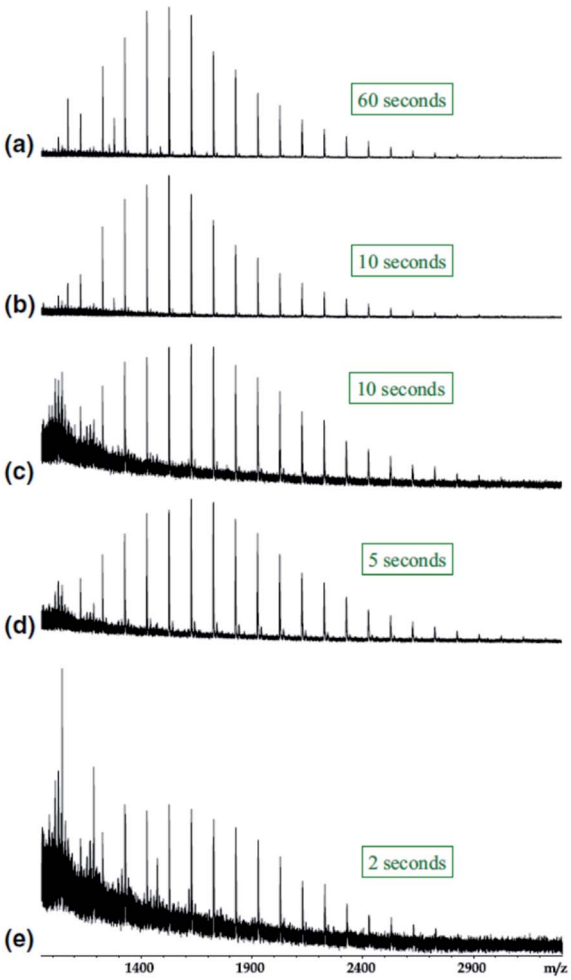

Fig. 7 MALDI mass spectra of samples of PMMA 2000 Da prepared with IAA as the matrix and NaTFA as the cationization agent utilizing 60 (a), 10 (b) and (c), 5 (d), and 2 (e) seconds of vortex mixing. We see excellent mass spectra with S/N _ 100 for spectra from 60–10 s, good mass spectra with S/N _ 50 for spectra from 10–5 s, and meaningful mass spectra with S/N _ 10 for spectra from the ultrashort mixing time of 2 s. Reproduced from ref. 75 Hanton *et al.*, *J. Am. Soc. Mass Spectrom.*, 2009, **20**, 1115–1118.

solvent-free preparation and delivery method[78,116] boasts the advantages of the solvent-free method described above[103] as well as the advantages of high throughput and the use of disposable microtiter plates. However, as mentioned above, methods employing a vortex device to homogenize mixtures of matrix and analyte have a practical mass limit of 10–15 kDa. Additionally, several of the disadvantages of the aforementioned method[78] were evident during homogenization and it was difficult to wash the MALDI plate thoroughly after analysis (Fig. 8).

The properties of the synthetic, low-molecular-weight polyamide (PA11), an insoluble compound, were analyzed by MALDI-TOF MS.[117] It is essential to develop analytical methods for characterizing compounds that are insoluble in common solvents. To a large extent, solvent-based sample preparation, dried-droplet deposition, and thin-layer deposition are optimized by selecting the best-performing matrix and salt. Specific solvents for PA, including trifluoroacetic acid (TFA) and hexafluoro isopropanol (HFIP), were evaluated using a solvent-based approach and 2,5-dihydroxy benzoic acid (2,5-DHB) and sodium iodide (NaI) were used as the optimal matrix and cationic agent, respectively. The solvent-based (thin layer) and solvent-free methods were then evaluated in terms of relative performance. Samples prepared using the dried-droplet method yielded non-uniform deposits, although this was not a problem when using the thin-film method. Overall, the solvent-free method was the easiest and safest preparation method and provided results equivalent to those of solvent-based methods.

Several studies have compared solvent-based methods[81] and the vortex method,[78,107] described above. P4VP polymer samples were prepared in individual methanol solutions of DCTB or 2,5-DHB matrix. Regardless of the matrix material, dried droplets of P4VP sample mixtures did not always produce a MALDI-MS signal. In contrast, protonated P4VP species were reliably generated from solvent-free MALDI samples using the same matrix-to-analyte molar ratio as the smallest P4VP polymer (Fig. 9).

Poly(4-vinylpyridine) (P4VP) was prepared[82] by nitroxide-mediated polymerization (NMP) using the vortex method[78,107] and matrices of *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malonitrile (DCTB), 2,3-dihydroxybenzoic acid (2,3-DHB), 2,5-dihydroxy-benzoic acid (2,5-DHB), 2,5-dihydroxyacetophenone (2,5-DHAP), sinapinic acid (SA), and 1,8-dihydroxy-9(10*H*)-anthracenone (dithranol). Their results demonstrated reactive MALDI when the ionization of P4VP was assisted by an acidic matrix. The high number of nitrogen atoms in each 4VP unit enabled strong hydrogen bonding with molecules in the acidic matrix. This has been shown to weaken the C–ON linkage between the final monomeric unit and the nitroxide end. As a result, the release of nitroxides upon laser irradiation of these MALDI samples yields radical chains that can react with matrix molecules. A new polymer by-product is thereby obtained where one matrix molecule is involved in chain termination. Of note, these matrices and polymer covalent adducts were not observed when using MALDI sources operating under high-vacuum conditions (Fig. 10).

The MALDI mass spectra obtained with 2,5-DHB, 2,5-DHAP, and 2,3-DHB matrices were identical, but differed significantly with SA. This implies that covalent adducts associated with SA or 2,3-DHB are the most vulnerable and most active in the MALDI source, respectively.

Chendo *et al.*[79] reported the formation of double-charged molecules during MALDI analysis of certain small synthetic polymers. This was especially true of PEG when using MALDI sources operating at high pressures (~60 mTorr). High-pressure sources have been shown previously to favor gas-phase interactions that can result in adducts consisting of analyte and matrix fragments.[118] For example, under such conditions, MALDI processes can result in a matrix molecule being integrated into the end of a synthetic polymer chain. It has been shown[82] that the results obtained from these studies are consistent with the combined chemical and physical dynamics model proposed by Knochenmuss,[119,120] which describes the

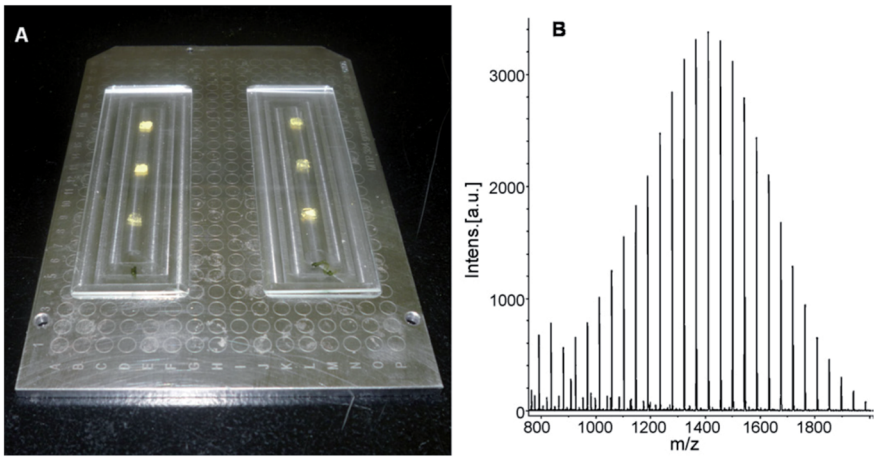

Fig. 8 (A) Bruker Daltonics MALDI plate modified to hold two glass microscope slides. Matrix/polymer are shown that are automatically transferred to the glass slides using a TissueLyser. The glass slides are discarded after use. (B) A mass spectrum of PEG 1000 obtained off of the glass slide shown in (A). Reproduced from ref. 76 Trimpin *et al.*, *Anal. Chim. Acta*, 2009, **654**, 20–25.

enhanced interactions between matrix ions and analyte as due to the high-pressure MALDI plume density. As shown below, they explored various parameters, including sample preparation methods, matrix properties, and choice of cationic agent, to learn about the cationization of synthetic polymers during MALDI[121] (Fig. 11).

In contrast to the results obtained with DCTB, no double-charged species were observed when using a matrix other than dithranol. However, a low abundance of PEG molecules with two lithium cations was detected. Thus, the tendency of the MALDI matrix to favor the development of double-charged PEG correlates with lithium affinity.

2.2. Solvent-based methods

Solvent-based methods have been used and developed continuously since the early days of commercial MALDI availability. A wide range of sample deposition techniques, including dried-droplet,[122,123] electrospray,[124–126] and sublimation,[127–129] were developed with varying degrees of success in attaining sample homogeneity.[124,125,130] In synthetic polymer analyses, deposition patterns such as coffee stains,[54] matrix crystals,[130] and mass-dependent distribution differences[131] can interfere with measurement precision. This is especially true of the dried-droplet method, which is a common technique due to its flexibility and compatibility with almost all liquid samples.

Therefore, researchers tend to rely on methods that have been shown successful in previous studies or make educated guesses based on those studies. For new compounds that have not been previously analyzed by MALDI-TOF MS, it can be exceedingly difficult to identify a suitable sample preparation method and the development process for new pre-treatments is slow. Predictions on the suitability of pre-treatment conditions for MALDI analysis based on the molecular properties of the sample components have been attempted[133–135] with a limited range of results.[133–135] Generally, however, the most common sample preparation methods[37,136] form the basis for new sample formulations.

The reproducibility and quality of MALDI-TOF mass spectra are highly dependent on the type of matrix used, the ionizer, and the sample preparation method. A previous study by X. *et al.* evaluated MALDI-TOF spectra of PEG that had been prepared using a variety of methods (dried droplet, solvent-free, thin layer), matrices (dithranol, dihydroxybenzoic acid, 2-[(2E)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]-malononitrile), and solvents (THF, toluene, dimethylformamide, H_2O). Although very high sensitivity, in terms of the signal-to-noise ratio, could be obtained from certain areas of inhomogeneous samples, they found that uniform deposition and sample homogeneity were more important for obtaining quantitative data and good reproducibility. In fact, none of the above combinations yielded good reproducibility, demonstrating that the choice of solvent and matrix can be as important as the sample preparation method.[137] Thus, for quantification studies, they selected a relatively common combination: dried-droplet deposition using dithranol as the matrix and toluene as the solvent.

MALDI-TOF analysis is considered a suitable method for analyzing fluorotelomer-based acrylate polymers (FTACPs) using the conventional sample preparation method of a dithranol matrix and THF as the solvent. However, the poor solubility of high-MW FTACPs in THF can lead to mass discrimination. FTACPs are side-chain fluorinated polymers composed of urethane and oxetane materials.[138] Since highly fluorinated FTACPs are not highly soluble in non-fluorinated solvents, a fluorinated sample formulation should be used in FTACP analyses. The highly volatile HCFC-225 appears to be the most suitable solvent for such analyses and MALDI-TOF spectra of FTACP in a DCTB matrix prepared in HCFC–methanol yielded optimal results (Fig. 12).

2.3. Recent updates of matrix types

In MALDI-TOF MS, sample preparation, including the selection of the matrix material, greatly affects the quality of the mass spectrum.[140–143] Due to the diversity of polymer analytes, there

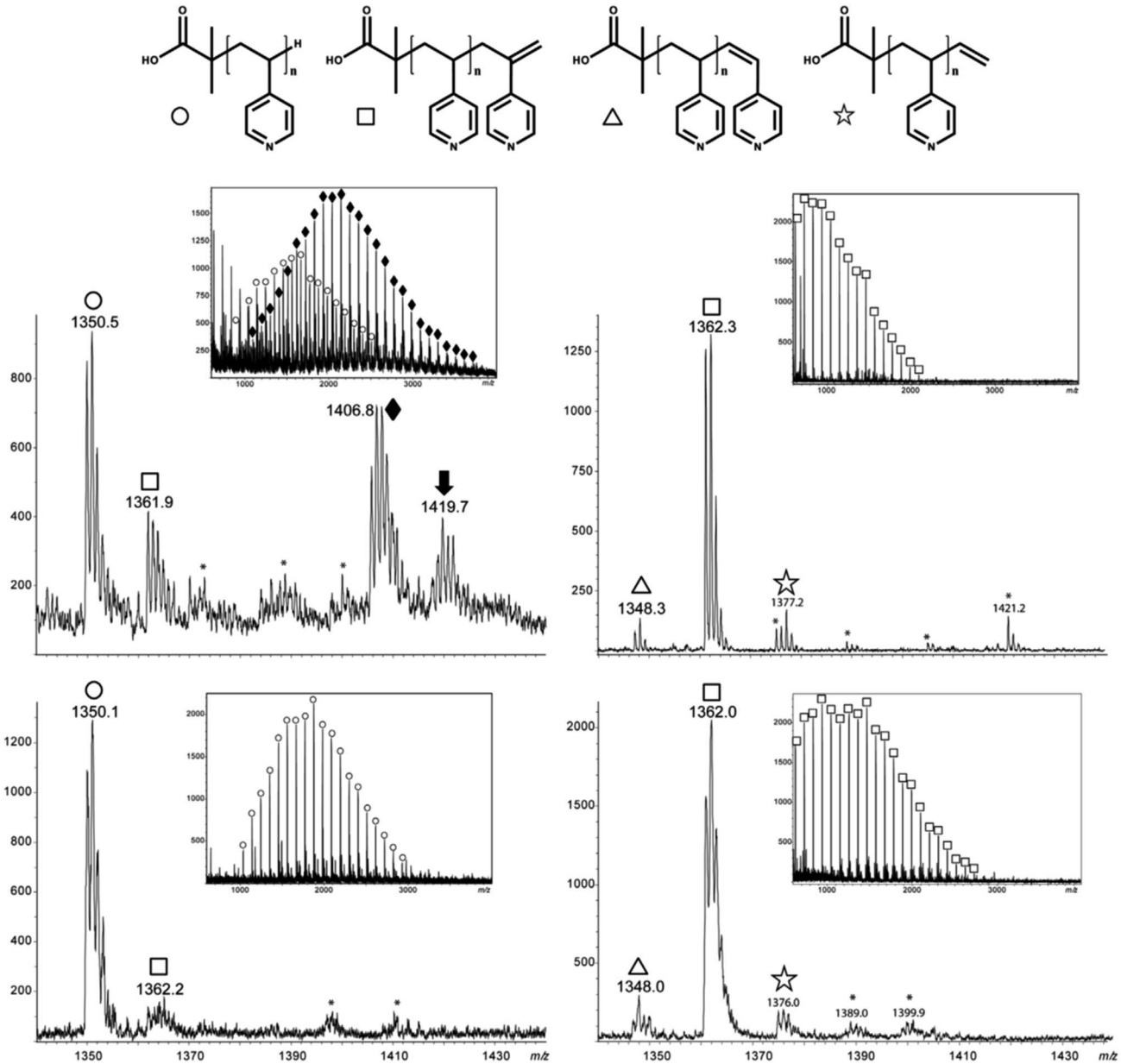

Fig. 9 Expanded range (*m*/*z* 1340–1450) of positive mode MALDI mass spectra obtained for P4VP (M_n 1700 Da) using 2,5-DHB (left panel) or DCTB (right panel) as the matrix, and prepared according to a solvent-based (top) or a solvent-free (bottom) sample preparation. Insets: full MALDI mass spectra on the 600–4000 *m*/*z* range. Open symbols designated protonated P4VP species (with proposed structures shown on top) while matrix-analyte adducts are annotated with filled symbols. Ion abundances (*y*-axes) are in arbitrary units * designates unidentified minor species. Reproduced from ref. 77 Chendo *et al., Int. J. Mass Spectrom.,* 2015, **376**, 90–96.

are still no clear guidelines for matrix selection and new matrix materials are often found only after screening multiple candidate compounds.

Three commercially available polymer guanidine biocides, poly(hexamethylene guanidine)chloride (PHMG), poly(hexamethylene biguanide) (PHMB), poly[2-(2-ethoxy)-ethoxy ethyl]-guanidinium-chloride (PEEG), were used to evaluate matrix materials for MALDI-TOF MS.[139,144] No single matrix material was suitable for all three guanidines. SA and α-cyano-4-hydroxycinnamic acid (CHCA) matrices seemed to be ideal for PHMG and PHMB, but 2-(4-hydroxybenzeneazo) benzoic acid (HABA) and 2,5-dihydroxybenzoic acid (DHB) yielded opposite results. To explain this phenomenon, they explored the proton affinity (PA) of the matrix and analyte and found that, at low guanidine concentrations, matrices with low PA were more sensitive to differences in polymer PA.

A recent report[145] described an evaluation method for identifying optimal matrix conditions. They developed an overall metric of quality based on eight parameters consisting of the most relevant spectral features, including spectral intensity and peak shape, and sample spot homogeneity as measured by spectral changes as the point of analysis was moved across the

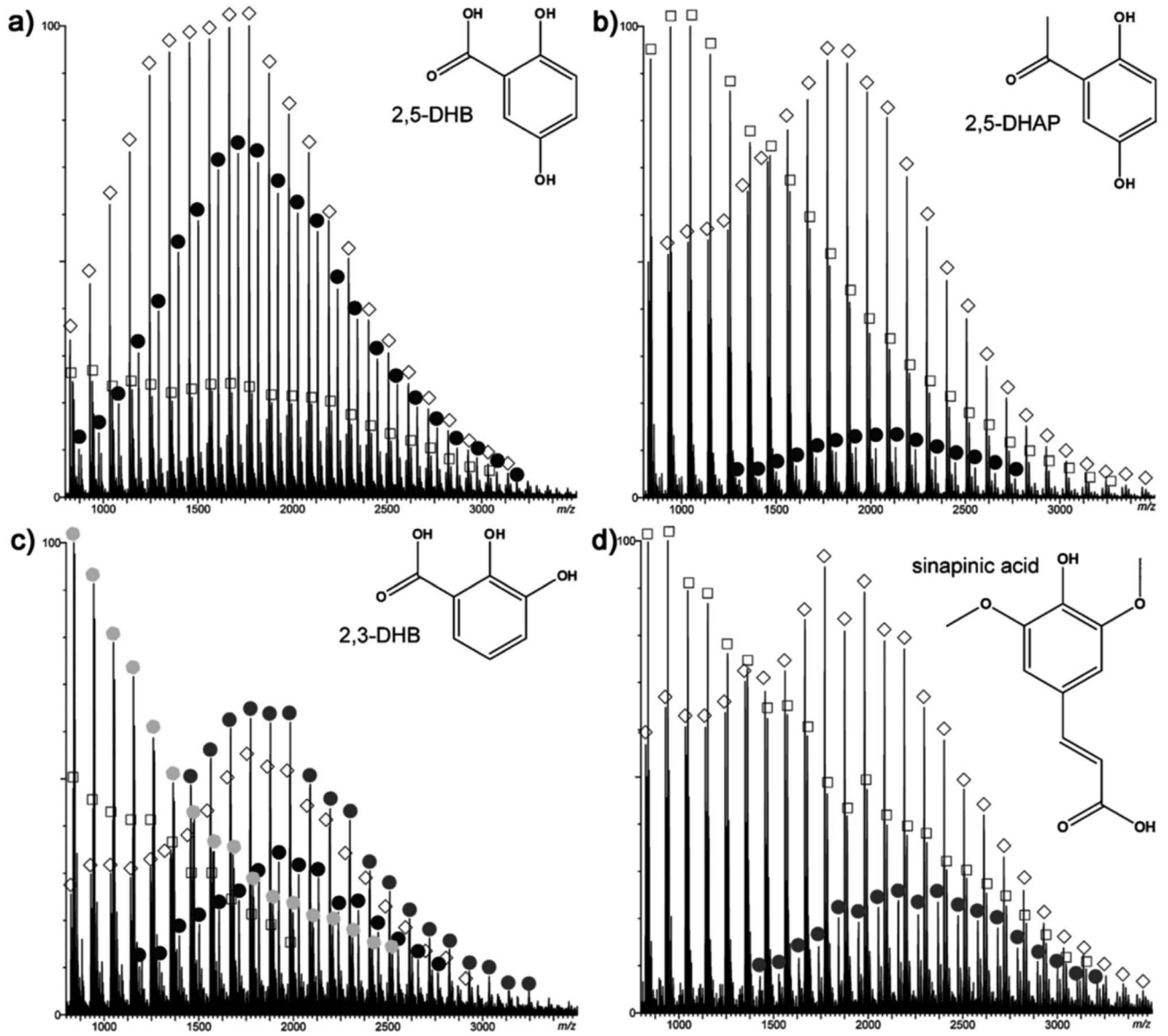

Fig. 10 Solvent-free MALDI mass spectra recorded in the positive ion mode for MAMA-P4VP-SG1 sample prepared with (a) 2,5-DHB, (b) 2,5-DHAP, (c) 2,3-DHB, and (d) sinapinic acid. White diamonds and white squares designate MAMA-P4VP-H (In) and MAMA-P4VP-exo (IIn), respectively. Black circles designate covalent P4VP/matrix adducts while their in-source fragments are annotated with dark and pale grey circles. Reproduced from ref. 78 Chendo *et al.*, *Int. J. Mass Spectrom.*, 2016, **405**, 50–58.

sample spot. This method was used to select the most appropriate deposition method (water droplets, solvent-free, electronic spray), matrix, and solvent. To demonstrate their evaluation method, DCTB and CHCA were used as matrices for PEG analyses. The chosen parameters were shown to be essential for identifying optimal MALDI-MS experimental conditions. Sample solutions containing a PEG standard were dissolved in THF and DCTB or CHCA was added as the matrix material. DCTB and CHCA are frequently used as matrices in synthetic polymer analyses[18,67] and were selected because of their very different precipitation and crystallization patterns (Tables 1 and 2) (Fig. 13).

In a recent study,[146] standard polystyrene (M_n = 4000 g mol^{-1}) was used to determine suitable conditions for MALDI-TOF MS analysis. The best results were obtained with a dithranol matrix and silver trifluoroacetate (AgTFA) as a cationic agent. No analytical signal was obtained with 2,5-DHB or CHCA matrices, emphasizing the importance of matrix selection in MALDI-TOF MS. Other cationization reagents, including copper(II) chloride[212] and other chlorides, and other matrix materials, including transretinoic acid, *trans*-3-indole acrylic acid, and *trans*-1,4-diphenyl-1,3-butadiene were also evaluated. Analyses with sulfate, primary copper chloride or sodium iodide, and sodium iodide or cesium iodide were also attempted.

In MALDI analyses, the organic matrix is essential for promoting the desorption and ionization of analytes under laser irradiation. However, MALDI-TOF MS analyses of low-MW

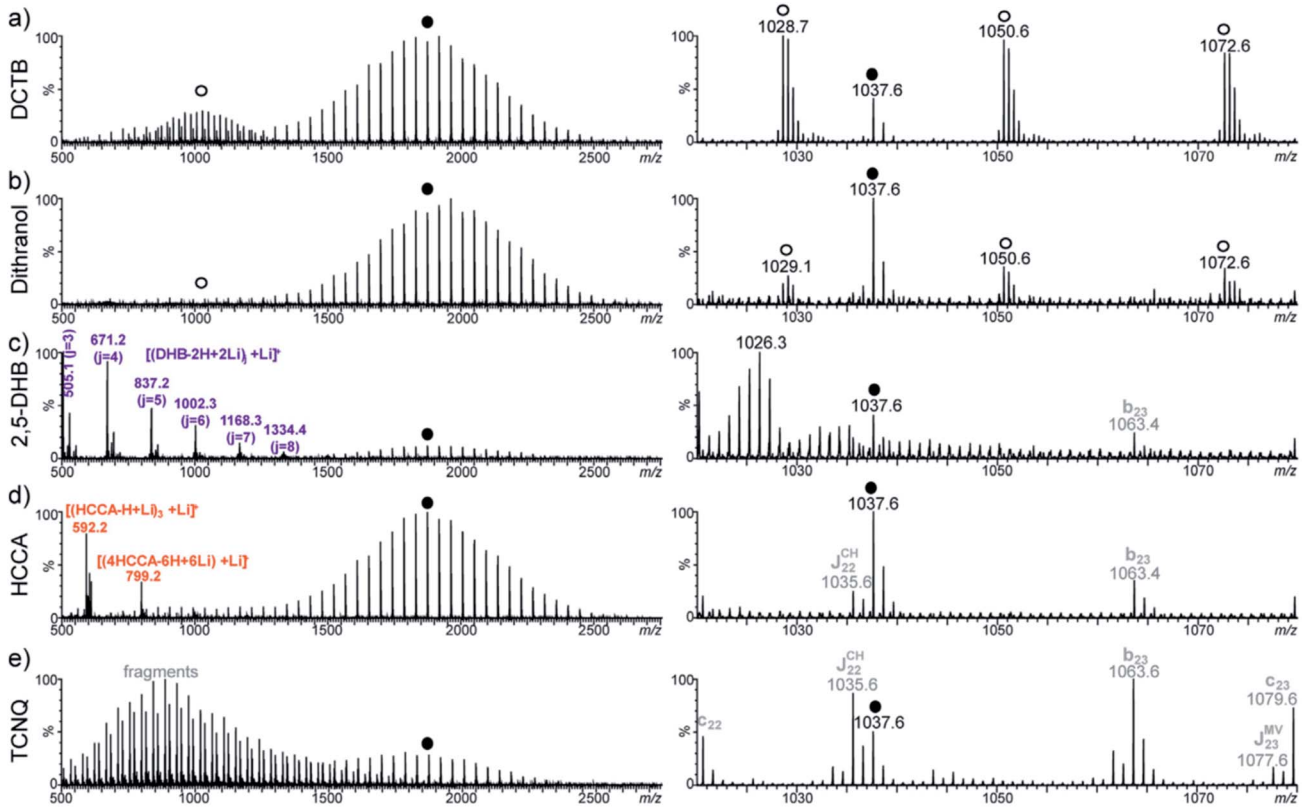

Fig. 11 Full range (left) and expanded *m/z* 1020–1080 range (right) MALDI mass spectra of PEG-2k, recorded with the IMS-MS instrumental configuration with (a) DCTB, (b) dithranol, (c) 2,5-DHB, (d) HCCA, and (e) TCNQ. Lithiated PEG adducts are designated by black ($z = +1$) and white ($z = +2$) circles. Peaks annotated with *m/z* values in grey were assigned to PEG fragments according to the nomenclature established by Wesdemiotis *et al.*[117] Reproduced from ref. 79 Chendo *et al.*, *Int. J. Mass Spectrom.*, 2017, **416**, 46–52.

materials is limited by low sensitivity, low reproducibility, and strong matrix interference.[147–151] To solve these problems, carbon nanotubes,[94] graphite,[152,191,192] metal–organic frameworks (MOFs),[153] covalent organic frameworks (COFs),[154] fullerenes,[155,156] and graphene (G) materials[96,157] have been developed recently as matrices for particle analysis.[147,158,159] Graphene-based materials have shown tremendous potential in multiple fields of study due to their striking properties, including unique two-dimensional nanostructures and excellent optical and electronic properties.[91,160,161] In particular, the light-absorption characteristics and efficient electron–phonon coupling of G make it an excellent MALDI matrix.[95,162] However, in practice, MALDI with G-based matrices can exhibit significantly reduced performance due to cohesion.[163–165] Five types of chemically functionalized G-based materials, non-modified G, G-F, G-NH_2, G-COOH, and GO, were evaluated as matrices in a recent report.[97] The performance of each G-based matrix in MALDI-TOF MS analyses was evaluated using 10 typical low-molecular-weight chemical contaminants as targets in both positive and negative ion modes. They found that the functional group attached to G and the incubation time of targeted matrices significantly affected analytical performance. The next section explores the use of carbon-related matrix materials in MALDI-TOF MS.

3. Application of carbon materials as MALDI matrices

3.1. Carbon nanotubes as MALDI matrices

3.1.1. The discovery of carbon nanotubes as MALDI matrix materials. Since its invention by Karas *et al.*,[29] MALDI-TOF MS has been widely used for analyses of large molecules, such as polymers and proteins, due to the outstanding advantages of speed and simple sample preparation.[147,166–168] However, MALDI analyses of low-molecular-weight compounds is largely limited by low sensitivity, low reproducibility, and matrix interference.[147,148,150]

To solve this problem, several carbon-based materials, including nanotubes[169] and graphene, have been evaluated as MALDI matrices. Since their discovery by Iijima in 1991,[163] carbon nanotubes have been studied extensively for a broad range of applications including nanoprobes, catalyst supports, and hydrogen storage.[170–173]

Carbon nanotubes have also been evaluated as a matrix for MALDI-TOF MS.[94] After grinding a sample of coal and subjecting it to an electrical arc discharge, carbon nanotubes were deposited on the cathode. The nanotubes were collected from the arc reactor and used directly as a matrix material without refining. MALDI spectra of Na-benzoyl-L-arginine ethyl ester hydrochloride (BAEE), collected using a traditional CCA organic

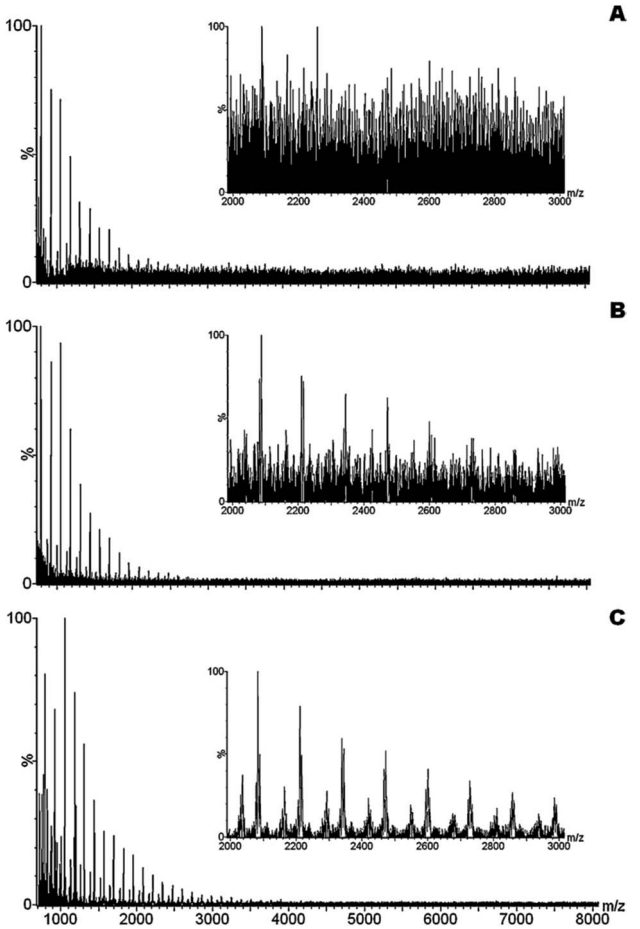

Fig. 12 MALDI-TOF mass spectra of poly(8 : 2 FTAC-*co*-BA) prepared in (A) trifluorotoluene (TFT) using DFAB as the matrix, (B) HCFC-225 using DFAB as the matrix and (C) TFT using DCTB as the matrix. All samples used NaTFA as the cationization agent and a mixing ratio of 10 : 5 : 1. Reproduced from ref. 134 Rankin *et al.*, *Anal. Chim. Acta*, 2014, **808**, 115–123.

matrix and a carbon nanotube matrix, are shown in Fig. 15a and b, respectively. The carbon nanotubes exhibited excellent decontamination/ionization of BAEE. In Fig. 15a, BAEE was desorbed/ionized as $[M - Cl]^+$ but several peaks attributed to the CCA matrix obscured the analyte data in the low-mass regime. The spectrum in Fig. 15b shows that carbon nanotubes were also effective for the desorption/ionization of BAEE. However, interference from matrix ions was completely removed, allowing low-molecular-weight fragment peaks to be observed.

Fig. 16 is a direct comparison of the intensity of analyte ion peaks obtained *via* MALDI analysis of the same amount of BAEE with CCA and carbon nanotube matrices. Compared to the CCA matrix, the carbon nanotube matrix yielded stronger peaks with higher shot-to-shot reproducibility. Thus, MALDI with carbon nanotubes as a matrix may potentially improve quantitative analyses of small molecules.

3.1.2. The development of MALDI matrices incorporating carbon nanotubes. In the above section, we described how carbon nanotubes came to be considered as a MALDI matrix

Table 1 Individual quality parameter values and normalized scores (0–1) for sample preparation quality of CHCA protocols. Reproduced from ref. 189 Kooijman *et al.*, *Anal. Chim. Acta*, 2016, **919**, 1–10

			Spot							
Quality parameter	Unit	Range	1	2	3	4	5	Average	Score (0–1)	SD
Average ASPI	Arb.	Abs.	4.08×10^3	3.94×10^3	4.18×10^3	4.30×10^3	4.63×10^3	4.23×10^3	1.00	232
Spatial variation ASPI	—[a]	0–∞	6.60×10^{-2}	8.67×10^{-2}	7.88×10^{-2}	7.84×10^{-2}	7.32×10^{-2}	7.66×10^{-2}	0.923	6.85×10^{-3}
Average MW ratio (H/L)	Frac.	0–∞	7.63	7.76	6.45	9.05	7.26	7.63	0.131	0.844
Spatial variation MW ratio (H/L)	—[a]	0–∞	0.153	0.151	0.156	0.151	0.174	0.157	0.843	8.64×10^{-3}
Radial signal distribution	—[a]	0–1	4.05×10^{-3}	5.77×10^{-3}	2.53×10^{-3}	4.37×10^{-3}	7.13×10^{-3}	4.77×10^{-3}	0.995	1.57×10^{-3}
Angular signal distribution	—[a]	0–1	2.87×10^{-3}	5.88×10^{-3}	4.15×10^{-3}	1.33×10^{-3}	2.36×10^{-3}	3.32×10^{-3}	0.997	1.57×10^{-3}
Spot filling	Frac.	0–1	0.996	0.990	0.993	0.998	0.999	0.995	0.995	3.54×10^{-3}
Detected peaks	#	Abs.	482	461	602	466	662	535	1.00	82.1
Overall quality score									0.861	

[a] Dimensionless value.

Table 2 Individual quality parameter values and normalized scores parameter values for sample preparation quality of DCTB protocols. Reproduced from ref. 189 Kooijman *et al.*, *Anal. Chim. Acta*, 2016, **919**, 1–10

			Spot							
Quality parameter	Unit	Range	1	2	3	4	5	Average	Score (0–1)	SD
Average ASPI	Arb.	Abs.	3.33×10^3	3.70×10^3	2.89×10^3	2.50×10^3	3.07×10^3	3.10×10^3	0.733	405
Spatial variation ASPI	—[a]	0–∞	0.433	0.352	0.484	0.592	0.413	0.455	0.545	8.06×10^{-2}
Average MW ratio (H/L)	Frac.	0–∞	1.923	1.98	1.72	2.88	2.91	2.28	0.438	0.508
Spatial variation MW ratio (H/L)	—[a]	0–∞	0.768	0.596	0.505	0.347	1.03	0.650	0.350	0.235
Radial signal distribution	—[a]	0–1	8.78×10^{-2}	0.128	7.94×10^{-2}	0.173	0.156	0.125	0.875	3.66×10^{-2}
Angular signal distribution	—[a]	0–1	0.8245	0.796	0.604	0.797	0.701	0.744	0.256	8.19×10^{-2}
Spot filling	Frac.	0–1	0.164	0.146	0.209	7.53×10^{-2}	0.141	0.147	0.147	4.31×10^{-2}
Detected peaks	#	Abs.	163	206	253	131	220	195	0.364	43.0
Overall quality score									**0.464**	

[a] Dimensionless value.

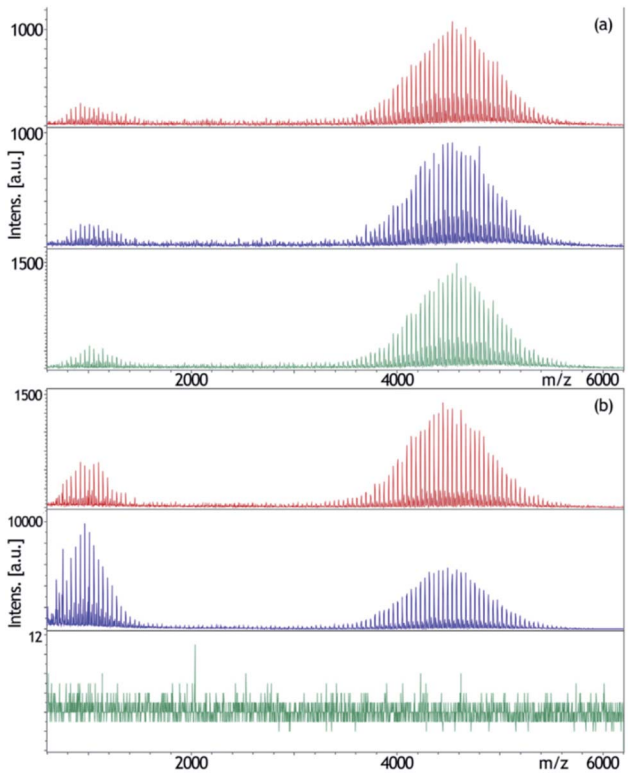

Fig. 13 Mass spectra from PEG-1050 with the CHCA (a) and DCTB (b) protocol illustrative for sample preparation dependent results. Three mass spectra from a single sample spot are shown; sampling positions 100 mm apart, along a single line within the sample deposition region. Reproduced from ref. 189 Kooijman *et al.*, *Anal. Chim. Acta*, 2016, **919**, 1–10.

material and discussed their suitability for analyzing low-molecular-weight compounds. However, due to their low solubility, it is often difficult to deposit carbon nanotubes from water or organic solvents and obtain a uniform, homogeneous film. The oxidation and chemical functionalization of carbon nanotubes has yielded several new materials and methods for MALDI analyses.[174,175,193]

Compared to non-oxygenated carbon nanotubes,[94] carbon oxide nanotubes are highly soluble in water, which greatly simplifies sample preparation. In addition, the efficiency of analyte desorption/ionization from the carbon oxide nanotube surface, as well as the reproducibility of peak intensity across the sample spot, are improved. These improvements enable quantitative analyses.

Oxidized carbon nanotubes are generally shorter than non-oxidized nanotubes and feature thinner exterior walls with rougher surfaces. Fig. 17a and b show TEM micrographs of carbon nanotubes and oxidized carbon nanotubes at 50 000× magnification, respectively.

Fig. 18a compares the solubility of carbon nanotubes in water before and after oxidation. Even after sonicating for several minutes, the carbon nanotubes quickly settled to the bottom of the vial. In contrast, the oxidized carbon nanotubes remained in solution even after centrifuging for 10 minutes at 5000 rpm. The insolubility of carbon nanotubes in water or organic solvents makes it difficult to control the amount of nanotubes deposited onto a MALDI sample target. This problem is eliminated with oxidized carbon nanotubes, which exhibit excellent solubility in water.

Fig. 18b compares layers of matrix material composed of carbon nanotubes or oxidized carbon nanotubes after solvent evaporation under ambient conditions for 5 to 10 minutes. The matrix spots formed by the oxidized carbon nanotubes are conspicuously more homogeneous than those formed by the non-oxidized analogues.

Berberin (337, $[M + H]^+$), jatrorrhizine (339, $[M + H]^+$), and palmatine (353, $[M + H]^+$) were selected as analytes to assess the ability of MALDI-TOF MS to differentiate and quantitate materials with similar molecular structures using oxidized-carbon-nanotube-based matrices. Each dot in Fig. 19 represents the average of five spectra, where each spectrum is the accumulated signal from 20 laser shots at 10 different positions on the sample, *i.e.*, a total of 200 laser shots per sample. The R^2 values of quantitative calibration curves for jatrorrhizine and palmatine were 0.9859 and 0.9898, respectively, and the linearity of the calibration range extended from approximately 1 to 100 ng mL^{-1}.

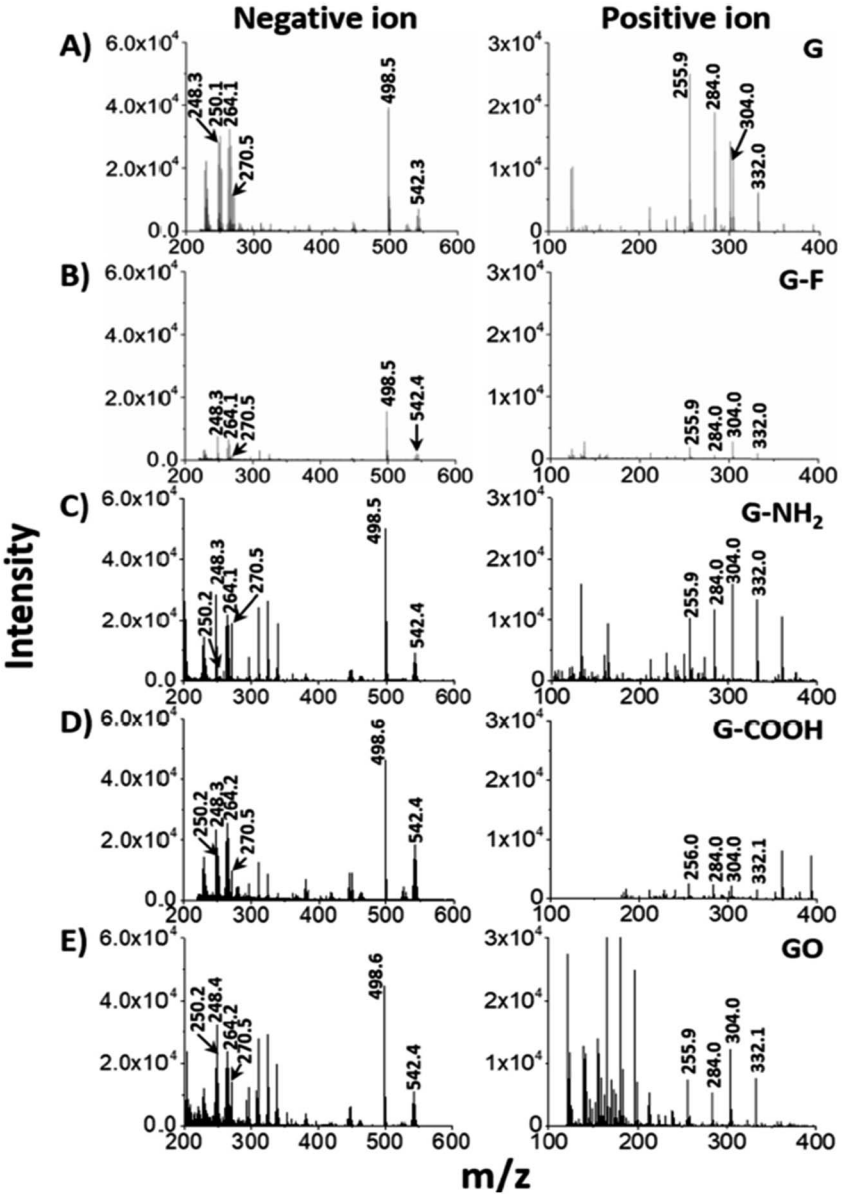

Fig. 14 Comparison of performance of different matrices in MALDI-TOF MS detection of typical chemical contaminates (BPS, BDE-47, PCP, E2, PFOS, TBBPA, TDBAC, DDBAC, CTAB, and TTAB) with an incubation time of 1 h. Matrix: (A) G, (B) G-F, (C) G-NH_2, (D) G-COOH, and (E) GO. The left and right column represent the spectra obtained in negative and positive ion detection mode, respectively. Analyte concentration: BPS, 10 μg mL^{-1}; BDE-47, 5 μg mL^{-1}; PCP, 10 μg mL^{-1}; E2, 50 μg mL^{-1}; PFOS, 1 μg mL^{-1}; TBBPA, 10 μg mL^{-1}; TDBAC, 5 μg mL^{-1}; DDBAC, 5 μg mL^{-1}; CTAB, 1 μg mL^{-1}; and TTAB, 1 μg mL^{-1}. Reproduced from ref. 93 Xiu *et al.*, *Talanta*, 2019, **199**, 532–540.

Fig. 20 shows the quantitative calibration curves obtained using the standard addition method for palmatine and jatrorrhizine. R^2 values for these analytes were 0.9911 and 0.9947, respectively. The line equations derived from the calibration curves correspond to 8.65 ng and 10.4 ng of jatrorrhizine and palmatine, respectively, in 1 mL of the sample solution. The RSD value of each point was less than 10%.

The authors noted a clear advantage to using oxidized carbon nanotubes over unmodified carbon nanotubes in MALDI-TOF MS analyses. The oxidized nanotubes made sample preparation much easier, yielded more uniform and compact matrix layers, and exhibited excellent desorption characteristics, ionization efficiency, and reproducibility.

3.2. Applications of graphene as a matrix in MALDI-TOF MS

3.2.1. The discovery of graphene as a MALDI matrix. Graphene is the isolated atomic surface of graphite. In 2004, Chernogolovka discovered graphene by exfoliating graphite with Scotch tape.[176] Since then, graphene has been researched extensively for a wide variety of applications, including ballistic transistors[176] and transparent conducting electrodes.[177]

Graphene has also been used as a novel matrix material for MALDI-TOF MS analysis of small molecules. Graphene

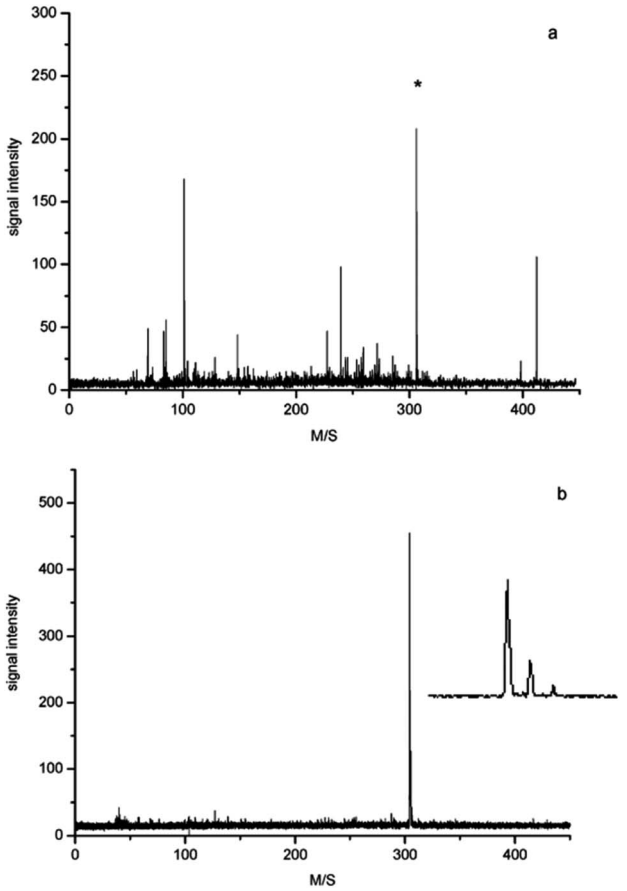

Fig. 15 Mass spectra of NR-benzoyl-L-arginine ethyl ester hydrochloride (3 mg mL^{-1}) at *m/z* 307.87 $[M - Cl]^+$ obtained by using matrixes of (a) CCA and (b) carbon nanotubes. 50 pulsed laser shots were applied under laser power set at 195 μJ. Reproduced from ref. 167 Xu *et al.*, *Anal. Chem.*, 2003, **75**, 6191–6195.

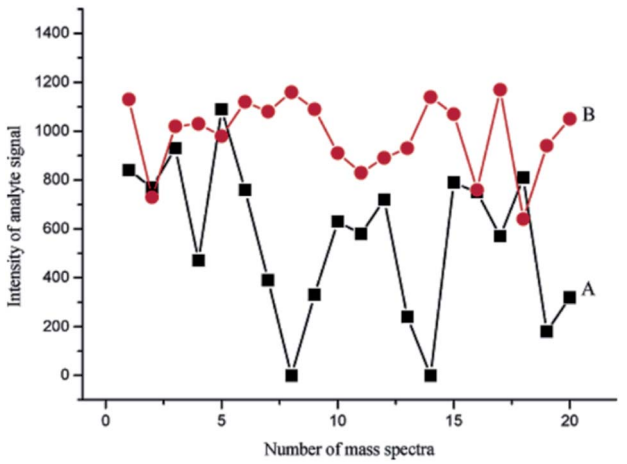

Fig. 16 Comparison of intensity of analyte ion signal in the discrete location of probe well with the matrixes of (A) CCA and (B) carbon nanotubes. 20 pulsed laser shots were applied under laser power set at 205 μJ. Equal concentration of analyte BAEE was used for MS experiments in both cases. Reproduced from ref. 167 Xu *et al.*, *Anal. Chem.*, 2003, **75**, 6191–6195.

functions as an energy receptor for laser radiation and can act as a locking agent for analyte molecules. The large surface area of graphene nanosheets allows them to adhere tightly to sample targets. As a result, graphene does not separate from the sample target under vacuum, avoiding contamination of the ion source. In addition, the structure and unique electronic properties of a graphene matrix can improve desorption/ionization efficiency. Graphene has been shown to simplify spectral interpretation greatly by eliminating interference from background matrix ions.

Using graphene as a matrix, amino acids were analyzed by MALDI-TOF MS as representative small molecules. Fig. 21 shows the resulting mass spectra, obtained in LDI mode, from a mixture of amino acids consisting of Glu, His, and Trp. Spectra were collected in the absence of a matrix (Fig. 21a) and with CHCA (Fig. 21b) or graphene (Fig. 21c) matrices. No analyte signals were obtained without a matrix. While all analytes were detected as $[M + H]^+$ ions (Glu, *m/z* 147; His, *m/z* 156; Trp, *m/z* 205) using the CHCA matrix, strong background interference greatly obscured the detection of low-molecular-weight compounds. All three amino acids were detected as strong $[M + Na]^+$ ion peaks using a graphene matrix. In addition, very few background peaks were produced, implying that graphene is a "soft" matrix for MALDI-TOF MS analyses. This allows for analyses of separation/ionization phenomena of small molecules using low laser power. The structural characteristic of graphene promote strong adsorption and efficient energy transfer to analytes.

Furthermore, the analyte solution is simply deposited onto the graphene matrix layer on the MALDI plate. Thus, the use of graphene as a matrix eliminates the need to optimize solvent conditions for different analytes and simplifies sample preparation.

3.2.2. Analysis of nonpolar compounds. Another example of the benefits of a graphene matrix is the analysis of cholesterol and squalene, a precursor in cholesterol biosynthesis. Both compounds are exceedingly hydrophobic and are not easily protonated and ionized. Cholesterol has been analyzed previously using traditional matrices such as 2,5-DHB, but matrix ions significantly interfered with detection.[178] The use of graphene as a matrix, however, allowed both compounds to desorb and ionize simultaneously, providing high S/N ratios in the resulting spectrum. Fig. 22b shows intense peaks corresponding to the $[M + Na]^+$ ions and a clean background due to the absence of matrix interference. For comparison, a CHCA matrix was also used to analyze these two compounds. The peak assigned to cholesterol (*m/z* 369) showed the loss of H_2O, and the peaks at *m/z* at 378 and 338 were attributed to matrix-related ions (Fig. 22a). However, with a graphene matrix (see above), cholesterol was detected as a complete sodium-added molecular ion (*m/z* 409), indicating that graphene is a soft matrix for MALDI MS. Of note, under the same experimental conditions, squalene was not detected when using CHCA as a matrix. This implies that ionization of non-polar compounds was not achieved with a CHCA matrix.[179]

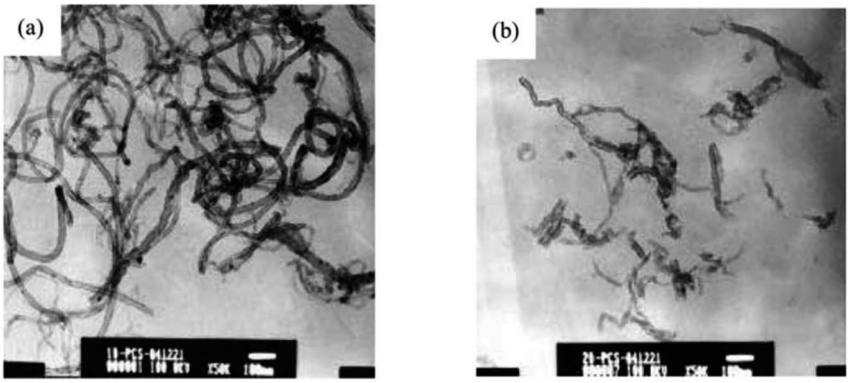

Fig. 17 TEM images (_50 000-fold) of (a) carbon nanotubes and (b) oxidized carbon nanotubes. Reproduced from ref. 91 Pan *et al.*, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 883–892.

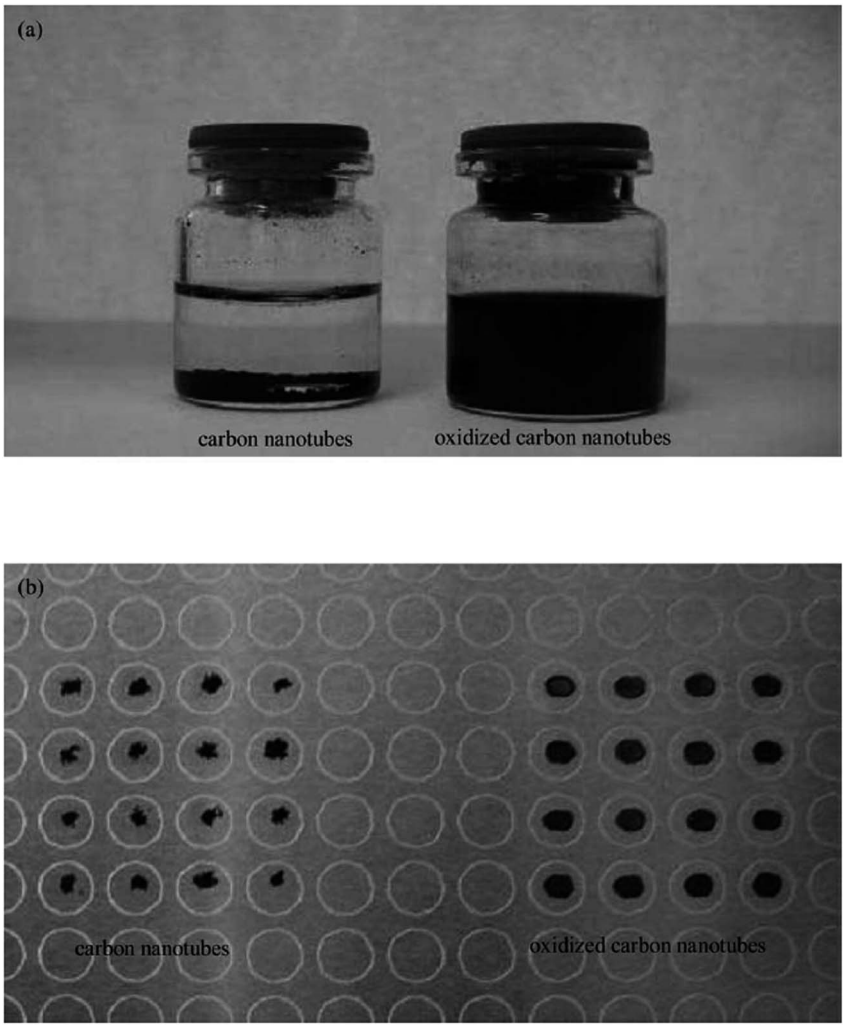

Fig. 18 Comparison of (a) the solubility of carbon nanotubes and oxidized carbon nanotubes in water, and (b) the matrix layers of carbon nanotubes and oxidized carbon nanotubes. All matrix layers were formed after evaporating solvent by pipetting 1 uL of matrix solution onto the sample target. Reproduced from ref. 91 Pan *et al.*, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 883–892.

Graphene is an electron-rich, hydrophobic material with a large surface area, making it suitable for solid-phase extraction (SPE). To test this hypothesis, graphene was used as the SPE sorbent in the extraction of squalene from a 20 μM solution. After enrichment, direct analysis of the graphene using MALDI-TOF MS yielded a good signal (Fig. 22c). Squalene was even

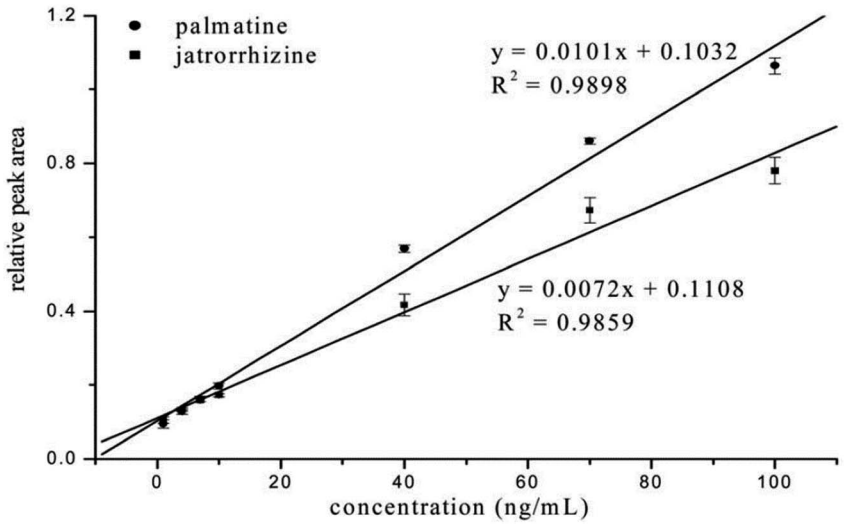

Fig. 19 Quantitative calibration curves between the relative area ($A_{analytes}/A_{IS}$) of analyte peaks to IS peak and concentrations of analytes. Every dot in Fig. 20 is obtained with the average of 5 spectra and each spectrum is accumulated from 20 laser shots at 10 different laser spots. Reproduced from ref. 91 Pan *et al.*, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 883–892.

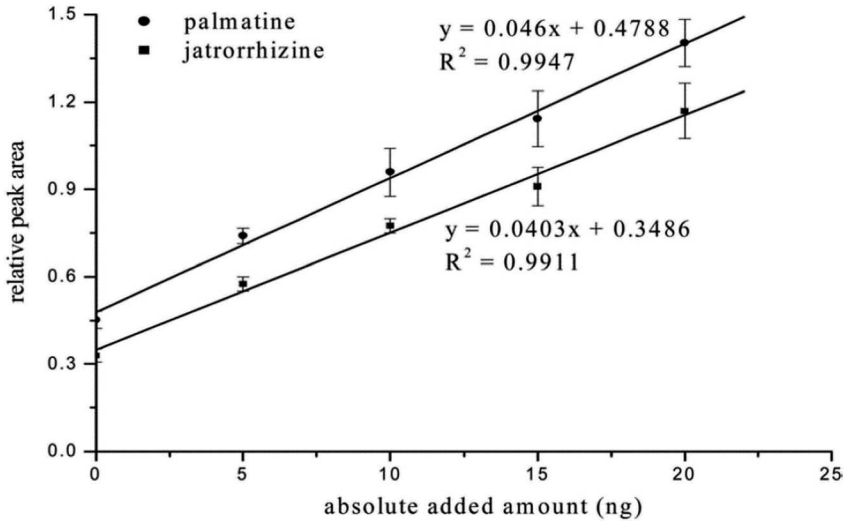

Fig. 20 Quantitative calibration curves between the relative area ($A_{analytes}/A_{IS}$) of analyte peaks to IS peak and the absolute added amount of analytes. Every dot is the average of 5 spectra and each spectrum is accumulated from 20 laser shots at 10 different laser spots. Reproduced from ref. 91 Pan *et al.*, *J. Am. Soc. Mass Spectrom.*, 2005, **16**, 883–892.

detected after graphene SPE from a 0.2 μM solution (Fig. 22c and d). Thus, using graphene as a matrix for MALDI-TOF MS allows detection of various small molecules while simplifying sample preparation.

Graphene matrices can be used reliably for analyses of small molecules such as amino acids and steroids and exhibit distinct advantages when analyzing non-polar compounds. Graphene facilitates analyte desorption/ionization at relatively low laser energies and yields strong peaks for molecular ions. Matrix background ions are largely eliminated, greatly simplifying sample preparation while improving the reproducibility between shots. However, measurement sensitivity with a graphene matrix is not sufficiently high. Future studies focusing on the chemical derivatization of graphene matrices will undoubtedly yield better performance in MALDI-TOF MS analyses.

3.2.3. The development of graphene-based MALDI matrices. Representative chemical derivatives of graphene, such as sulfonated graphene (G-S), graphene oxide (GO), and fluorinated graphene (G-F), have been used as MALDI matrices.[180,181,194] However, there is a lack of comparative research on the various types of graphene and the mechanisms governing graphene performance in MALDI-TOF MS are still poorly understood.

The studies introduced in this section imply two independent approaches to enhancing the performance of graphene

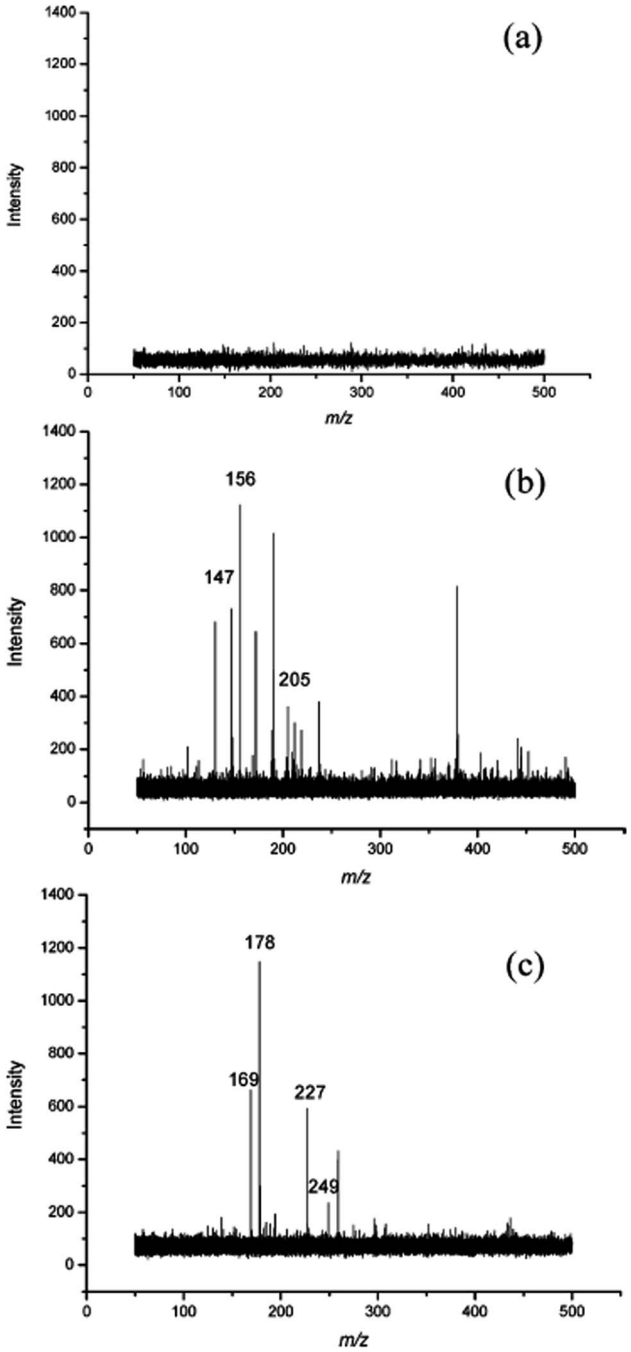

Fig. 21 MALDI-TOF mass spectra of a solution containing Glu (*m*/*z* 147, $[M + H]^+$; *m*/*z* 169, $[M + Na]^+$), His (*m*/*z* 156, $[M + H]^+$; *m*/*z* 178, $[M + Na]^+$), and Trp (*m*/*z* 205, $[M + H]^+$; *m*/*z* 227, $[M + Na]^+$; *m*/*z* 249, $[M + 2Na-H]^+$) in the LDI mode without a matrix (a) and with a matrix of CHCA (b) or graphene (c). A 0.5 μL sample solution was deposited on the target spots, and the concentrations of all analytes were at 0.5 mM each. Reproduced from ref. 92 Dong *et al.*, *Anal. Chem.*, 2010, **82**, 6208–6214.

(G)-based matrices in dual-ion-mode MALDI-TOF-MS: chemical functionalization of G and the incubation of G with target analytes (Fig. 23). Five types of chemically modified graphene were selected as matrix materials: non-strained G, G-F, amide graphene (G-NH_2), carboxylated graphene (G-COOH), and GO.

Experiments were conducted using an incubation time of 1 hour. Fig. 23 and Table 3 show that all five types of G-based materials were adequate MALDI matrices, including GNH_2 and G-COOH, which had not been previously reported as MALDI matrices. However, each of the different G-based materials produced different MS patterns, indicating that functional groups on the matrix have a significant impact on the LDI process. When considering dual-ion mode, G and G-NH_2 showed the best performance, with all analytes being detected at high intensity (see Fig. 14a and e). For analytes detected primarily in cation mode, such as halogenated surfactants, non-strained G was the most suitable MALDI matrix. The researchers also examined all five G-based substances as surface-enhanced laser desorption/ionization (SELDI) probes. Due to differences in the ionization mechanisms of MALDI and SELDI, they found that the MALDI- and SELDI-TOF MS methods require separate optimization even when using the same matrix or probe materials.

4. Hardware & software

4.1. Instrumentation for MALDI-TOF MS

Matrix-assisted laser desorption/ionization is a powerful ionization method for characterizing synthetic polymers by mass spectrometry.[182] It is a 'soft ionization' method that results in less fragmentation than most other methods. UV lasers, particularly nitrogen (N_2) gas lasers and solid-state lasers, are the most commonly used light sources in MALDI. More recently developed MALDI-TOF MS systems utilize solid-state lasers due to the limitations of N_2 lasers, which include low life cycle, low repetition frequency, unstable output, and poor beam profile characteristics.

Species ionized by MALDI are most commonly separated using a time-of-flight (TOF) mass analyzer. In theory, ions with the same mass-to-charge ratio (*m*/*z*) should have the same flight time and arrive at the detector at the same time. In practice, however, ions with the same *m*/*z* often exhibit significantly different kinetic energies. This phenomenon results in the broadening of mass peaks and reduces resolution. There are, however, two commonly employed solutions to this problem: delayed extraction and reflector time-of-flight (RTOF) analyzers.

Delayed extraction, also known as time-lag focusing or pulsed ion extraction, is often used to enhance mass resolution in MALDI-TOF MS. After initial separation, ions at a particular *m*/*z* will exhibit an initial kinetic energy spread for several hundred nanoseconds (called the "delay time"). After the delay time, the acceleration voltage is turned on with a fast pulse. This reduces the initial kinetic energy spread by providing less acceleration to ions with higher initial kinetic energy. Wetzel *et al.*[177] emphasized that one of the most significant factors affecting signal-to-noise in MALDI-TOF polymer analyses is the choice of delay time.

RTOF instruments were first designed by Mamyrin in 1994.[178] In RTOF, ion mirrors use electric fields to repulse ions back to the opposite side of the detector. Ions with higher kinetic energy fly deeper into the deceleration field and require a longer time to arrive back at the detector. This configuration effectively corrects

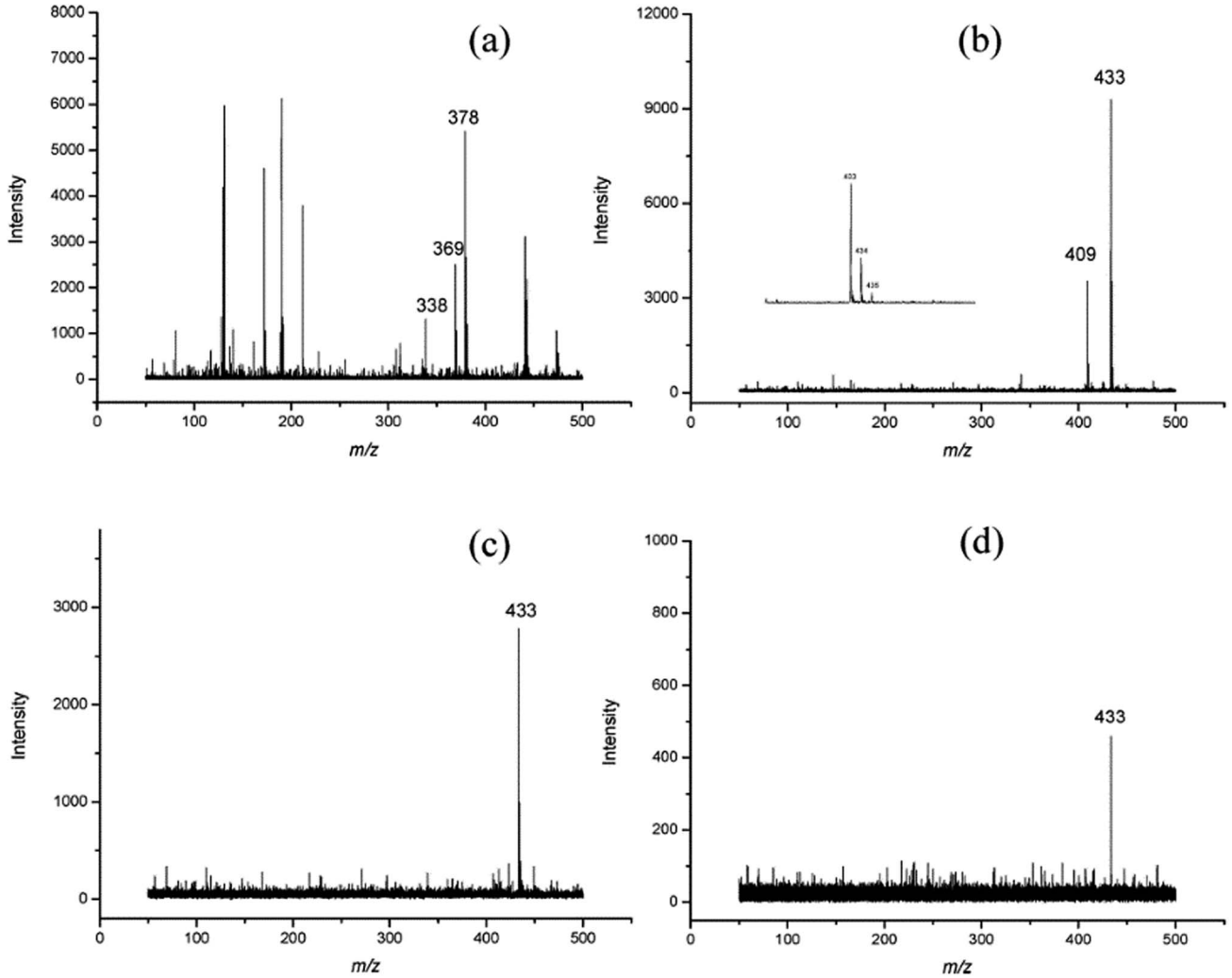

Fig. 22 MALDI-TOF mass spectra of (a) cholesterol (*m/z* 369, $[M-H_2O + H]^+$) and squalene (*m/z* 433, $[M + Na]^+$) with the use of CHCA as the matrix, (b) cholesterol (*m/z* 409, $[M + Na]^+$) and squalene (*m/z* 433, $[M + Na]^+$) with the use of graphene as the matrix, (c) graphene as the adsorbent for SPE of squalene at 20 μM, and (d) graphene as the matrix for SPE of squalene at 0.2 μM. Reproduced from ref. 92 Dong *et al.*, *Anal. Chem.*, 2010, **82**, 6208–6214.

for the initial energy spread of ions and substantially improves the resolution of the resulting mass spectra.

MALDI-TOF MS can provide detailed structural information about polymer chains.[184] However, a limitation referred to as "mass discrimination" is frequently reported in polymer analyses using MALDI-TOF MS.[185,186] Mass discrimination is the phenomenon wherein masses obtained from MALDI-TOF analyses are lower relative to those obtained using other MS methods.[187] This is due to several factors, including sample preparation, ionization procedures, ion transmission, and detection. Size-exclusive chromatography (SEC),[188] and collision-induced dissociation (CID)[189,190] have been coupled to MALDI-TOF MS to reduce mass discrimination.

At the time of writing this review, commercial MALDI-TOF instruments are produced by three companies worldwide: Shimadzu Corp. (Japan), Bruker Daltonic GmbH (Germany), and ASTA Inc (South Korea). Table 4 shows detailed specifications for each of these instruments optimized for polymer analyses. Although there are several parameters that determine the performance of a MALDI-TOF instrument, all three provide for MS/MS capabilities, which are especially useful in characterizing polymer chain and end-group structures. Additionally, Shimadzu and Bruker currently provide unique polymer analysis software. Analogous software from ASTA is currently under development (Fig. 24).

4.2. Software of MALDI-TOF for polymer analysis

As previously mentioned, MALDI-TOF has been used as an important tool to characterize the chain-end and structural fidelity of polymers prepared *via* controlled/living radical polymerization and other living polymerizations.[190–193] When applied to homopolymers, it can be used to further confirm certain structural information, such as end groups[204] and post-polymerization backbone modifications,[205] as well as differentiating between linear and cyclic polymers.[206] However for

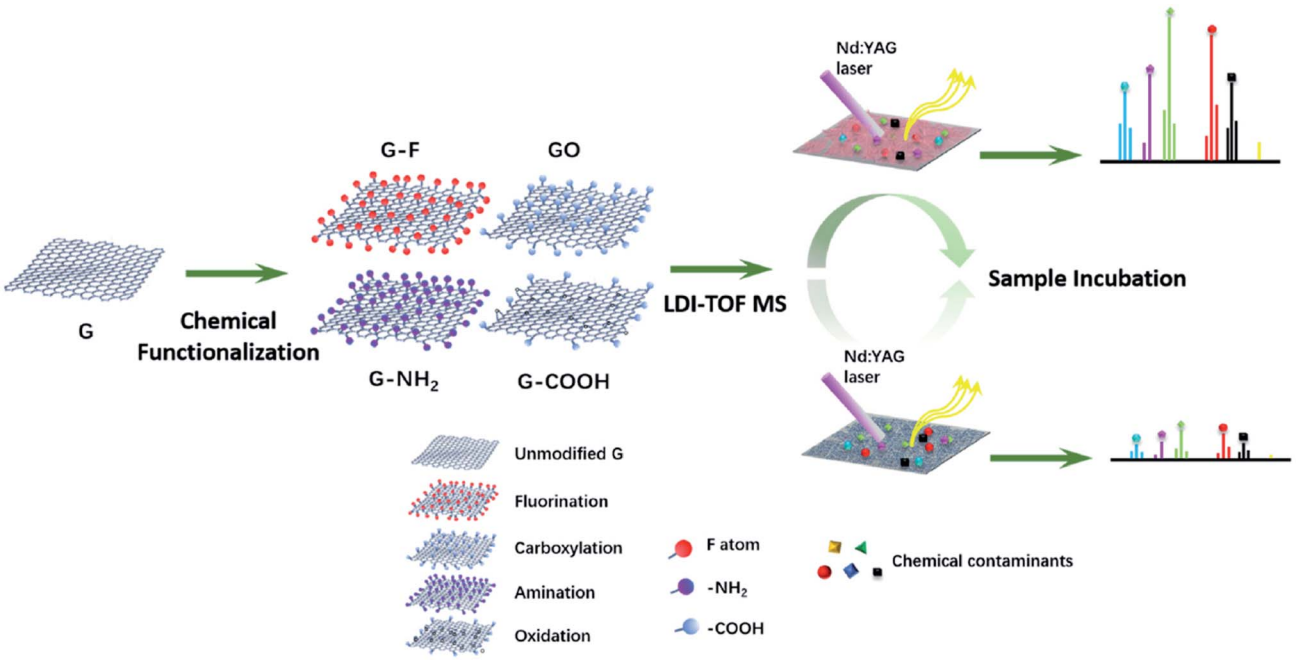

Fig. 23 Scheme showing the procedures for LDI-TOF MS analysis by using graphene-based materials as MALDI matrices or SELDI probes. Reproduced from ref. 93 Xiu *et al.*, *Talanta*, 2019, **199**, 532–540.

Table 3 The performance of different G materials as matrices in detection of typical chemical contaminant by MALDI-TOF MS[a]. Reproduced from ref. 93 Xiu *et al.*, *Talanta*, 2019, **199**, 532–540

Compound	After 1 h incubation								After 48 h incubation							
BPS	G	>	$G-NH_2$	>	GO	>	G-COOH	>	G-F	$G-NH_2$	>	G-F	>	GO	>	G-COOH	>	G
BDE-47	G	>	G-COOH	>	GO	>	$G-NH_2$	>	G-F	G-F	>	$G-NH_2$	>	GO	>	G	>	G-COOH
PCP	$G-NH_2$	>	G	>	G-COOH	>	GO	>	G-F	$G-NH_2$	>	G-F	>	G-COOH	>	G	>	GO
E2	G	>	$G-NH_2$	>	GO	>	G-COOH	>	G-F	$G-NH_2$	>	G-F	>	G	>	GO	>	G-COOH
PFOS	$G-NH_2$	>	G-COOH	>	GO	>	G	>	G-F	G-F	>	$G-NH_2$	>	GO	>	G-COOH	>	G
TBBPA	G-COOH	>	G	>	$G-NH_2$	>	GO	>	G-F	$G-NH_2$	>	G-F	>	GO	>	G-COOH	>	G
TTAB	G	>	$G-NH_2$	>	GO	>	G-COOH	>	G-F	G-F	>	$G-NH_2$	>	G	>	GO	>	G-COOH
CTAB	G	>	$G-NH_2$	>	GO	>	G-COOH	>	G-F	G-F	>	$G-NH_2$	>	G	>	GO	>	G-COOH
DDBAC	$G-NH_2$	>	G	>	GO	>	G-COOH	>	G-F	G-F	>	$G-NH_2$	>	GO	>	G	>	G-COOH
TDBAC	$G-NH_2$	>	GO	>	G	>	G-COOH	>	G-F	G-F	>	$G-NH_2$	>	GO	>	G	>	G-COOH

[a] The ">" means that the feature peak intensity of analyte obtained on the left matrix was higher than that on the right one.

Table 4 Detailed specification of commercial MALDI-TOF MS instruments

	Shimadzu AXIMA performance	Bruker Daltonic Autoflex maX	ASTA microIDSys RT
Laser	N_2 laser	smartbeam™ II Nd:YAG laser	Yb:YAG DPSS laser
Beam size	100 μm	10 μm (adjustable)	5 μm (adjustable)
Wavelength	337 nm	355 nm	343 nm
Repetition rate	Up to 50 Hz	Up to 2 kHz	Up to 1 kHz
Mass resolution	20 000 in reflector mode	26 000 in reflector mode	25 000 in reflector mode
Mass sensitivity	250 amol in reflector mode	250 amol in reflector mode	250 amol in reflector mode
MS/MS	Collision-induced dissociation (CID)	LIFT™	Post-source decay (PSD)
Polymer analysis software	PolymerAnalysis™	PolyTools™	ASTA polymer analysis (developing)

copolymers, it has mostly been applied to block copolymers,[183,207–211] usually to determine the length of the blocks.[191]

In the following figures, the bromine ω-terminal short-term instability (Fig. 25), fragmentation pathways (Fig. 26), and checked end-groups are shown through the MALDI-TOF spectrum of the polymer. The highest intensity series of peaks in most of the spectrum was found to correspond to bromine-terminated poly(methyl acrylate) as an Na^+ adduct. It has been

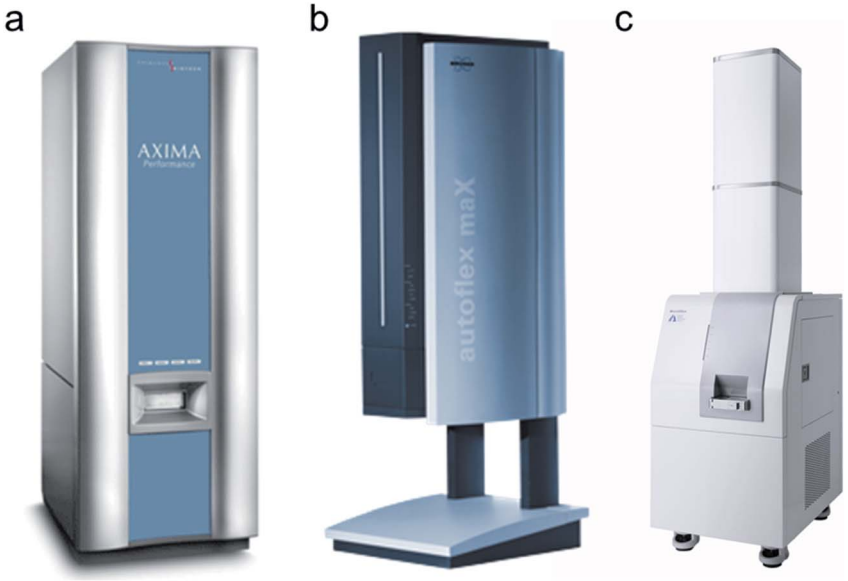

Fig. 24 Commercial MALDI TOF instruments for polymer analysis. (a) AXIMA performance (Shimadzu), (b) Autoflex maX (Bruker Daltonic), (c) microIDSys RT(ASTA).

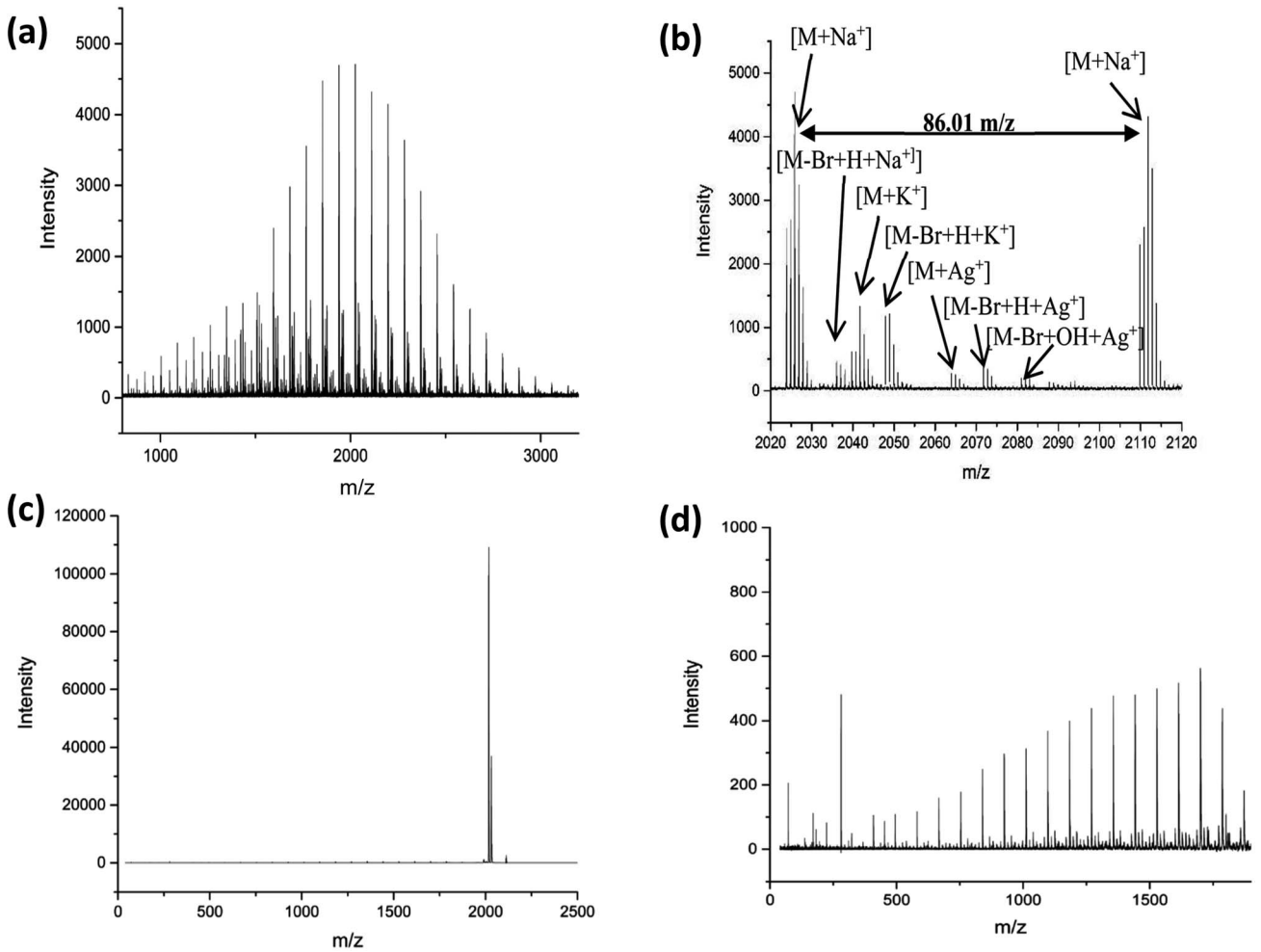

Fig. 25 (a) (Top left): full MALDI-TOF spectra of poly(methyl acrylate) homopolymer. (b) (Top right) expanded image of the MALDI-TOF results in the 2020–2120 *m/z* range, showing the issues with end group fidelity and K^+ adducts. (c) (Bottom left) full spectra of the MALDI-LID-TOF. The large peak shows the loss of the end group, but little else. (d) (Bottom right) expanded image of the spectra, excluding the large peak for the loss of the end group. Reproduced from ref. 191 James S. Town *et al.*, *Polym. Chem.*, 2018, **9**, 4631–4641.

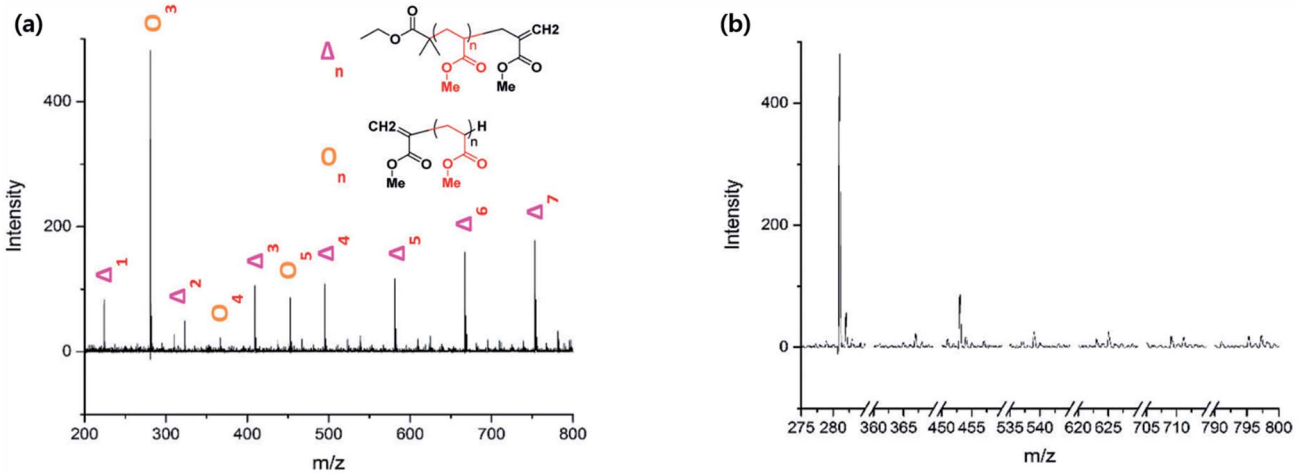

Fig. 26 (a) Assignment of the 200–800 *m/z* range of the poly(methyl acrylate) homopolymer. Beyond this point the peaks continue as a major series of Δ fragments of increasing repeat units. (b) Comparison of all the O fragment chains. Reproduced from ref. 191 James S. Town *et al.*, *Polym. Chem.*, 2018, **9**, 4631–4641.

Enabled	Label	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Adduct Charge	Loss	Low Mass	High Mass
☑	PEG	H	OCH2CH2	OH	1	Na	1		1000	1500
☐	PET		C10H8O4		1		0		1000	1500
☐	Polyvinylpyrr		C6H9NO		1		0		1000	1500
☐	Poly(acrylic a		C3H3NaO2		1		0		1000	1500
☐	PP (Polyprop		CH2CH(CH3)		1		0		1000	1500
☐	PE (Polyethy	H	CH2CH2	H	1		0		1000	1500

Fig. 27 Example of a homopolymer analysis screen.

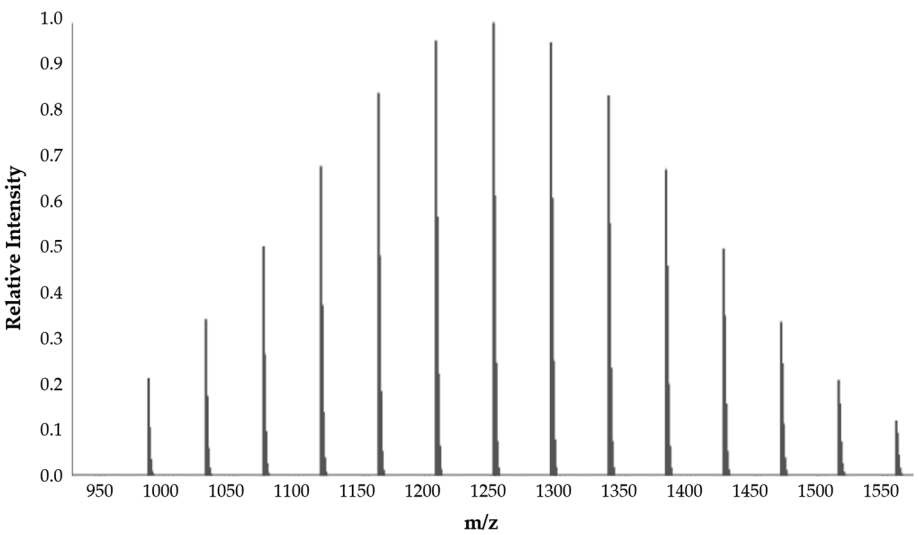

Fig. 28 MALDI-TOF spectrum of PEG.

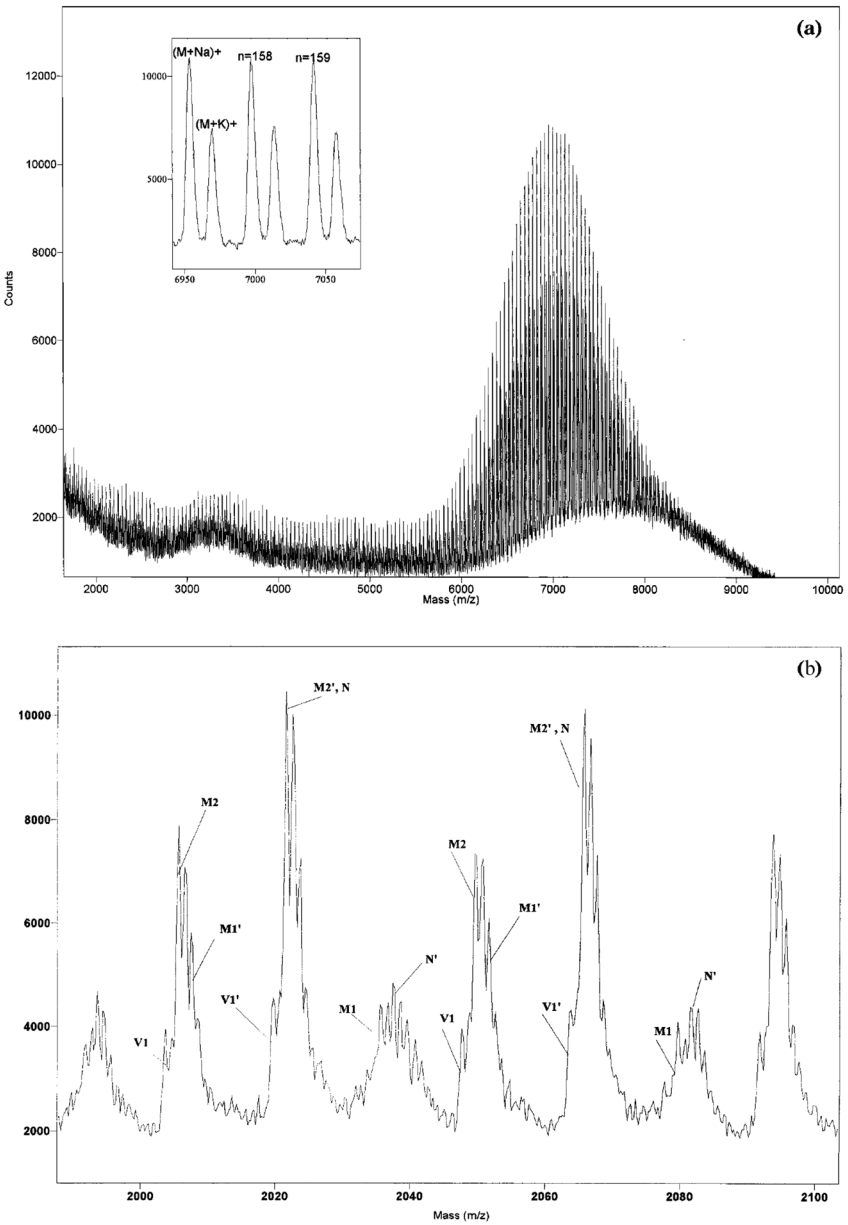

Fig. 29 MALDI-TOF spectra of poly(ethylene glycol) 6000 obtained by (a) dried droplet sample target preparation with dithranol as the matrix and dichloromethane solvent. (b) Pyrolysis products of PEG in the *m*/*z* 2000 and *m*/*z* 2100 mass range (see text for explanation). Reproduced from ref. 96 A. Marie *et al.*, *Anal. Chem.*, 2000, **72**(20), 5106–5114.

previously found that conventional MALDI-TOF MS does not affect unstable end groups, and many spectra of polymers prepared by polymerization have been reported, with similar end groups that are stable under these conditions.[213–221]

The main functions of polymer analysis software are homopolymer and copolymer analyses. Homopolymers consist of only one kind of monomer while copolymers consist of more than one kind of monomer. In homopolymer analysis mode, the user can select or input the molecular formulae of the repeat unit, end group, adduct, and mass range (Fig. 27).

Measured MALDI-TOF spectra are compared with theoretical peaks based on the molecular formulae. Each peak in the spectrum can be assigned to a theoretical peak if the mass error is within a user-specified range. Isotope clusters should be considered during matching. Finally, matching statistics are calculated automatically (Fig. 28).

Fig. 29a shows the MALDI mass spectrum of PEG 6000 co-crystallized with a dithranol matrix using a dichloromethane solvent system (preparing dried droplets). The MALDI mass spectrum reveals the molecular weight distribution (MWD) centered around 7000 u. Fig. 29c shows the distribution of single isotope peaks in the minor series and the major series separated by 2 Th.

In addition to the many conventional polymers (*e.g.*, PEG, PMMA, PVP, PDMS, PS) mentioned above, MALDI-TOF has also been found to be useful in analyzing other characteristic

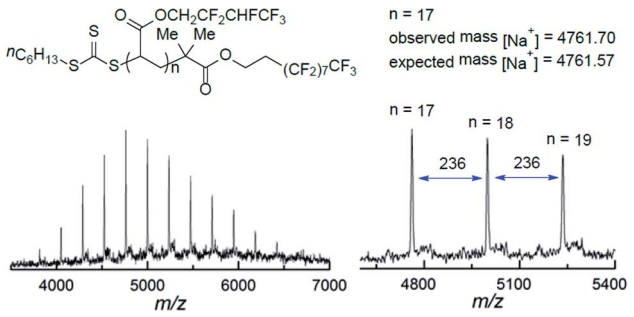

Fig. 30 MALDI-TOF MS analysis of PHFBA prepared from TTC-2F. Reproduced from ref. 190 A Honghong Gong *et al.*, *Angew. Chem., Int. Ed.*, 2018, **57**, 333–337.

polymers, *e.g.*, fluoropolymers,[194] S-containing polymers, and Se-containing polymers.[195,196] Fluoropolymers[203] are industrially and commercially important due to their special chemical and physical properties including thermal stability, chemical inertness, and strong oil and water repellence, and their valuable electrical properties. They have important applications in paints and coatings, bio-materials, high-performance resins, functional materials for photo resists, and optical fibers and thermostable polymers for aerospace applications.[96] The development of synthetic methods to prepare semi-fluorinated polymers is attracting much attention because they often have interesting properties such as high hydrophobicity, tunable lipophilicity, and improved thermal and chemical stability. Therefore, the fluorinated polymer structure can be verified by MALDI-TOF mass spectrometry of the prepared samples. In the following example, the polymer structure represents the analysis results of a purified sample (PHFBA) prepared from semi-fluorinated trithiocarbonate (TTC) or perfluoroalkyl iodide. As expected, all spectra (see Fig. 30) had a single set of peaks and each peak was separated by the molar mass of the corresponding monomer unit, but it has been reported that the absolute m/z value is consistent with the expected M_n with chain ends.[190]

Fig. 31 shows that the structure of the fluorinated polymer (high chain end fidelity) was confirmed by MALDI-TOF MS. Isolated samples were prepared *via* organic catalyzed CRP in CTA. As shown in Fig. 31A and B, the MALDI-TOF spectrum showed a single set of peaks, with each peak separated by 318 m/z, corresponding to a single monomer unit. The MALDI-TOF results also confirmed high chain end fidelity and good molar mass dispersion, supporting the proposed macro-CTA structure and low $Đ$ values.[192]

MALDI-TOF MS is used to identify Se-contained polymers[201–203] in addition to fluoropolymers. The retention times of the available standards do not usually match those of the peaks found in sample chromatograms, so molecule-specific techniques such as electrospray or MALDI MS are the only way to identify selenium species. The inadequacy of electrospray MS, especially when a triple quadrupole mass spectrometer is used, is likely to be due to the poor detection limits obtained in the presence of complex matrixes (either native or resulting from the use of chromatographic buffers) and the formation of multiply charged ions of selenopeptides that make recognition of the selenium isotopic pattern in a mass spectrum difficult. These drawbacks were reported to be overcome by the use of MALDI-TOF MS. Matrix-assisted laser-desorption ionization is much less vulnerable to matrix suppression than electrospray[132] and produces mostly singly charged ions, with little fragmentation.[197–199] These features make it an attractive technique for the identification of target ions for a structural study (amino acid sequencing) by electrospray tandem MS that would otherwise remain undetected in the electrospray MS background noise. MALDI-TOF MS therefore offers a more efficient approach for the detection of selenocompounds eluted in the chromatography of biological extracts than does electrospray MS, as already indicated elsewhere.[199,200]

Fig. 32 shows a set of MALDI-TOF mass spectra for the five chromatographically pure seleno-containing fractions. The identified peaks are summarized in Table 5. The mass spectra allowed the identification of a selenium-containing compound in each of the fractions. They confirmed the presence of three DDMNMDMGMGHDQSEGGMK peptides, in which three (Mr 2228), four (Mr 2275), and five (Mr 2322) methionine residues were replaced by selenomethionine *via* MALDI MS (Table 6).

For MALDI-TOF MS, isotopic resolution can be used in a polymer mixture to obtain a clear spectrum of a single

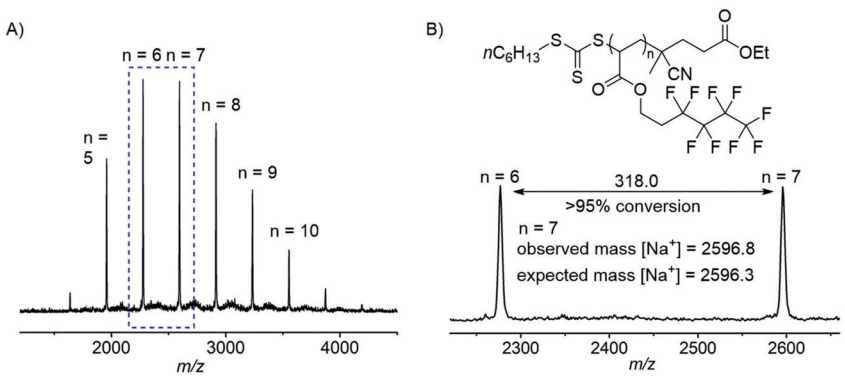

Fig. 31 MALDI-TOF MS analysis. (A) PNFHA prepared *via* the photoredox polymerization method. (B) Magnified region. Reproduced from ref. 192 Qinzhi Quan *et al.*, *Polym. Chem.*, 2018, **9**, 4161–4171.

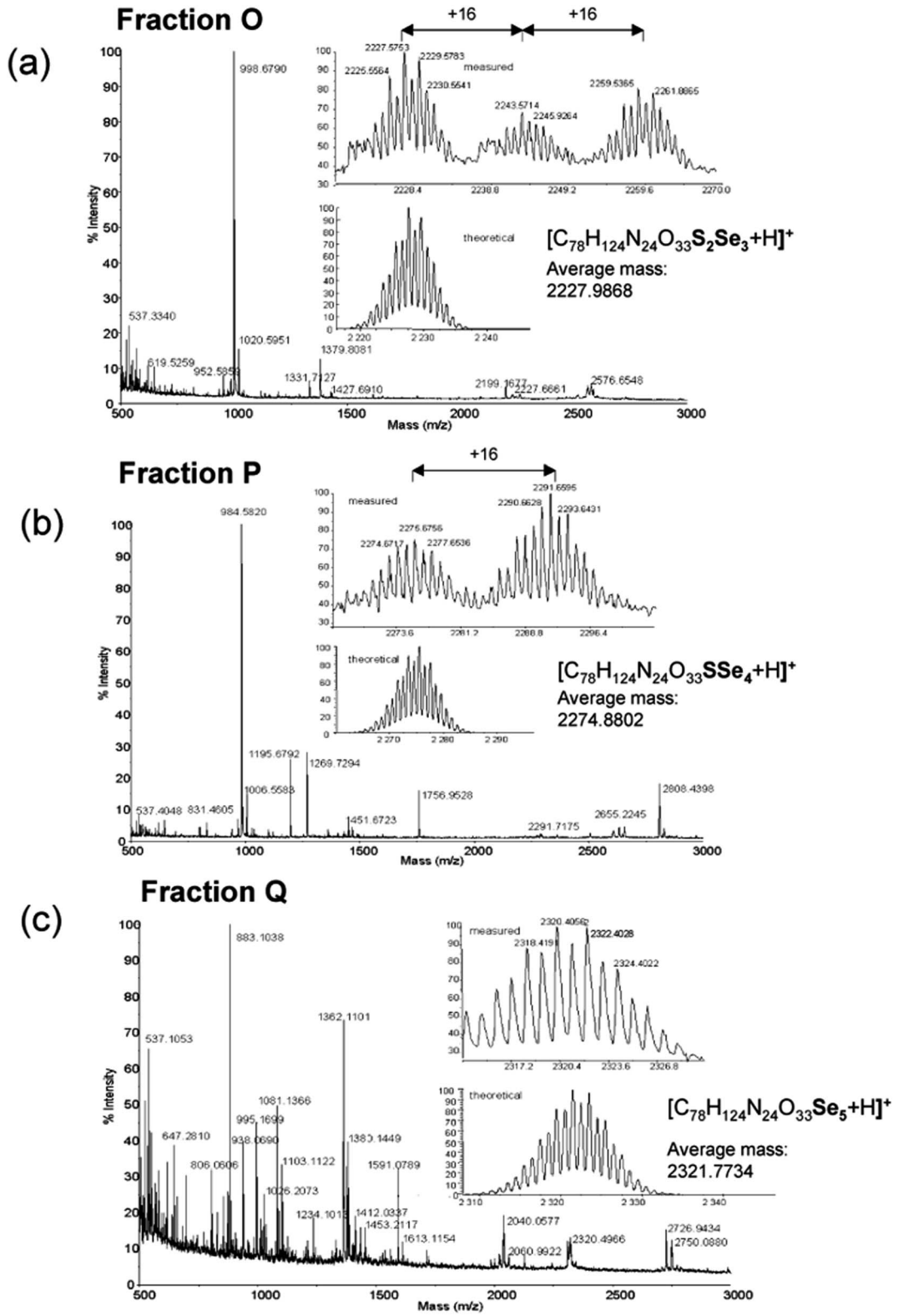

Fig. 32 MALDI-TOF mass spectra of the DDMNMDMGMGHDQSEGGMK peptide in which (a) three, (b) four, and (c) five methionine residues were replaced by selenomethionine. The insets show closeups of the selenopeptide peak (plus its oxidized forms) and the theoretically calculated isotopic molecular patterns. Reproduced from ref. 195 Jorge Ruiz Encinar *et al.*, *Anal. Chem.*, 2003, **75**(15), 3765–3774.

oligomer. However, copolymers often present even more important challenges, with some difficulties (presented below) that require a more complex analysis to overcome.[219,220] The overlapping peaks and different ionization schemes between the co-monomers[221] determine the orientation and position of the monomers along the backbone, and thus the type of

Table 5 Comparison of measured and calculated masses of the observed Se peptides using MALDI-TOF. Reproduced from ref. 195 Jorge Ruiz Encinar *et al., Anal. Chem.* 2003, **75**(15), 3765–3774[a]

Fraction	Meas. $(M + H)^+$/Th	Se peptide sequence	Theor. $(M + H)^+$/Th	Mass difference (ppm)	% of total Se
A	1008.3787	XGHDQSGTK	1008.3653	−13	6.3
B	596.1918	XNAGT	596.2059	24	6.8
C	961.4170	TYENXKK	961.3898	−28	1.9
E	833.2226	TYENXK	833.2948	87	7.8
F	803.3396	DYXGAAK	803.2845	−69	1.8
G	814.2939	Ac-SNSMNK	814.2672	−33	6.2
I	862.2497	Ac-SNXXNK	862.2116	−44	12.1
L	2466.403	ERDDXNXDMGMGHDQSEGGMK	2466.3944	−3	3.6
	2512.946	ERDDXNXDXDXGMGHDQSEGGMK	2513.2877	136	
M	2466.542	ERDDXNXDMGMGHDQSEGGMK	2466.3944	−60	5.1
	2513.529	ERDDXNXDXGMGHDQSEGGMK	2513.2877	−96	
N	2513.453	ERDDXNXDXGMGHDQSEGGMK	2513.2877	−66	10.0
O	2560.289	ERDDXNXDXGXGHDQSEGGMK	2560.1810	−42	14.7
	2228.066	DDXNXDXGMGHDQSEGGMK	2227.9868	−36	
P	2607.152	ERDDXNXDXGXGHDQSEGGXK	2607.0743	−30	13.2
	2275.049	DDXNXDXGXGHDQSEGGMK	2274.8802	−74	
Q	2321.666	DDXNXDXGXGHDQSEGGXK	2321.7734	46	5.7
Total identified					95.2

[a] X corresponds to SeMet: masses corresponding to fractions A–I and to fractions L–Q are monoisotopic and averaged masses, respectively.

Table 6 Polymer analysis statistics

M_n	Molecular weight (number average)	$\frac{\sum(M_i n_i)}{\sum n_i}$
M_w	Molecular weight (weight average)	$\frac{\sum(M_i^2 n_i)}{\sum(M_i n_i)}$
M_z	Molecular weight (Z average)	$\frac{\sum(M_i^3 n_i)}{\sum(M_i^2 n_i)}$
PD	Polydispersity	$\frac{M_w}{M_n}$
DP_n	Degree of polymerization (number average)	$\frac{M_n}{R}$
DP_w	Degree of polymerization (weight average)	$\frac{M_w}{R}$
DP_z	Degree of polymerization (Z average)	$\frac{M_z}{R}$

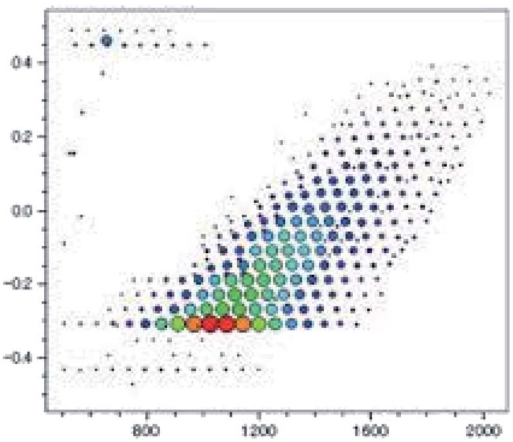

Fig. 33 Kendrick analysis plot. Reproduced from ref. 11 C. N. McEwen *et al., Anal. Chem.* 2002; **74**; 2743–2748.

copolymer.[222] In copolymer analysis mode, the distribution analysis of multiple repeat units results in a multi-dimensional grid. Kendrick mass analysis (KMD) uses fractional parts, which contain information about elemental composition, of accurate m/z values as a second dimension in a mass spectrum. Typically, a mass spectrometer with very high resolution and mass accuracy is required to produce a well-resolved and aligned plot (Fig. 33).

Software tools can also apply a Fourier transform to mass spectral data to identify periodic peaks and help with analyzing unknown polymers. The major software products available for MALDI-TOF polymer analysis are Polymerix (by Sierra Analytics), Polytools (by Bruker), and Polymer Analysis Software in AXIMA Control Software (by Shimadzu).

5. Conclusions

This review describes the importance and development of pre-treatment techniques for obtaining optimal results when analyzing synthetic polymers using MALDI-TOF mass spectrometry. MALDI-TOF mass spectrometry features high mass accuracy and is widely recognized as being easier and faster than other mass-spectrometry techniques for analyzing polymers and for identifying end groups and structures. However, proper sample preparation is crucial to obtaining accurate data using MALDI.

We described herein the history and developmental processes of various MALDI sample pre-treatment methods and technologies, starting from solvent-based methods and concluding with solvent-free methods developed in the 2000s. In solvent-based sample preparation, differential solubility, miscibility, and crystallization effects often stand in the way of achieving the desired results. Although many of these problems

are eliminated with solvent-free methods, solvent-based methods are more flexible and can be tailored more easily to the wide variety of polymer materials that may be encountered.

This review also examined carbon-based matrix materials as an emerging technology. In recent studies, small molecules were successfully analyzed by MALDI-TOF MS using graphene as the matrix. Matrices composed of carbon nanotubes can simplify sample preparation, eliminate interference from matrix ions, and improve shot-to-shot reproducibility. Oxidized carbon nanotubes form a relatively uniform and compact matrix layer and improve analyte desorption/ionization. Compared with conventional matrices like CHCA, graphene offers simple sample pre-treatment, highly efficient analyte desorption/ionization, and improved reproducibility. Graphene matrices have been successfully applied to analyses of amino acids, polyamine, steroids, and anti-cancer drugs, boasting significant advantages with non-polar analytes. The optoelectronic properties of graphene allow desorption/ionization to occur at relatively low laser power, enabling higher signals from small molecules. In addition, chemical functionalization of graphene and sample incubation can be used to further optimize matrix performance in dual-ion-mode MALDI-TOF MS analyses.

The development of new polymer materials is an ongoing goal of modern research. Thus, new analytical methods, including MALDI MS techniques, must be developed concurrently to allow for complete polymer characterization. With constant advances in MALDI-TOF MS hardware and software, we will examine polymer structures at new levels of detail. In addition, MALDI MS spectra will enable identification of unknown polymers through innovative approaches to sample analysis, including molecular simulations and advanced software techniques. This review paper provides a summary of current research to be used as the basis for research into more advanced matrix materials and pre-treatment techniques for MALDI MS.

In the future, mass analysis methods will be further developed, especially analysis methods using MALDI. Additionally, as the development of polymeric materials progresses, corresponding analysis methods using MALDI are expected to be developed. Polymeric materials have a large molecular weight and various analysis methods are required depending on the chain-end, which makes them difficult to analyze. Analysis methods using MALDI, which began in 1985 with Franz Hillenkamp, Michael Karas, and their colleagues, have been developing rapidly. Recently, studies have been conducted to compensate for the shortcomings of MALDI, such as low molecular weight analyses and quantification research using carbon nanotube and graphene as a matrix, as well as detailed and diverse analysis methods through the development of solvent-free methods and updated matrix materials. Given this trend, MALDI MS is expected to be widely used for both quantitative and qualitative analysis.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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