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Evaluation and optimization of the influence of silver cluster ions on the MALDI-TOF-MS analysis of polystyrene nanoplastic polymers†

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In the analysis of polystyrene nanoplastics (PSNs), a nonpolar polymer (NP), using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), silver salts were used as cationization reagents and simultaneously brought the potential problems of silver clusters that interfered with the PSN signal of MS. To detect PSNs, silver trifluoroacetate (AgTFA) and silver nitrate (AgNO₃) were mixed with five polar matrices, namely 2-(4-hydroxyphenylazo) benzoic acid (HABA), dithranol (DI), sinapic acid (SA), *trans*-3-indoleacrylic acid (IAA), and 2,5-dihydroxybenzoic acid (DHB), and three nonpolar matrices, namely pyrene (PRN), anthracene (ATH) and acenaphthene (ACTH). The results showed that silver salt cluster ions were detected in the range of *m/z* 1000–4000. Five polar matrices with silver salts produced silver clusters, which interfered with the signals in the mass spectrum of PSNs, but the combination of these matrices with copper II chloride (CuCl₂) salt did not produce copper-related clusters. However, the use of nonpolar matrices such as PRN, ATH or ACTH significantly decreased the signals of silver salt cluster ions, and this alteration of matrix types is considered a promising optimization approach for silver cluster ions. The nonpolar matrix conditions were optimized without producing silver cluster ions and the optimal detection conditions were found to be under nonpolar matrices (e.g., pyrene) with silver salts (e.g., AgTFA). The results suggest that when polar matrices, such as HABA, DI, SA, IAA, and DHB, are combined with silver salts in MALDI-TOF-MS analysis, silver-related clusters are detected in the range of *m/z* 1000–4000. Inhibition of the production of silver cluster ions can be achieved by the use of a nonpolar matrix (e.g., PRN) or polar matrix (e.g., DHB) with copper salts.

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1. Introduction

Over the last few decades, plastic pollution has become a major source of concern among scientists, politicians, and the general public.^{1,2} Every year, large amounts of plastic waste leak into the world's oceans.³ This causes larger materials to fragment and break down into microplastics (plastic particles smaller than 5 mm) and, eventually, nanoplastics (sizes ranging from 1 to 100 nm), although the latter have only recently been identified as potentially harmful to the environment and further research is currently underway.^{4,5} Furthermore, these particles may be intentionally manufactured into micro- and nano-sized particles and discarded directly into the environment.⁶ Nanoplastics in the environment easily enter living organisms and are

harmful to them. The use of analytical techniques to analyze micro/nanoplastic samples in the environment is essential.^{7,8} Polystyrene, which is one of the most widely used plastics on the planet, is used in different items such as electrical and transparent products, daily necessities, and packaging.⁹ Polystyrene micro/nanoplastic has been discovered in a variety of environmental media.¹⁰ Polystyrene micro/nanoplastic, a non-polar polymer, has been analyzed using various mass spectrometric methods, such as pyrolysis gas chromatography-mass spectrometry (Pyr-GC-MS), vapor phase osmometry and gel permeation chromatography.^{11–15} Thermal desorption GC-MS (TDS-GC-MS), in combination with thermogravimetry (TGA), has also been used to analyze complex samples of polystyrene using Pyr-GC-MS.¹⁶ However, the mass range of GC-MS is insufficient for analyzing high-molecular-weight substances such as polymer plastics because the mass range is too narrow.¹⁰ Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is considered a powerful tool for synthetic polymer detection, particularly due to its soft ionization and broad mass detection range.¹⁰ However, as

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a hydrocarbon polymeric compound, polystyrene is still more of a challenge to analyze than polar polymeric materials.^{17,18} Meanwhile, for NPs of various molecular weights and repeating units, there are various developed MALDI-TOF-MS methods.^{19,20} The analysis of NPs with MALDI-TOF-MS always needs a cationization reagent to assist in ionization and a matrix to facilitate desorption.²¹ However, there are a small number of polar matrices that are extensively utilized for the most studied nonpolar polymers, including polystyrene. Recently, 2-(4-hydroxyphenylazo)benzoic acid (HABA), dithranol (DI), *trans*-3-indoleacrylic acid (IAA), 2,5-dihydroxybenzoic acid (DHB), and sinapic acid (SA) matrices have been widely utilized in the analysis of synthetic polymers.^{22–26} Despite the fact that these matrices are polar, they have been shown to function effectively with nonpolar analytes.²⁷ Nevertheless, nonpolar matrices work successfully with nonpolar polymers and there are a few studies that have confirmed their suitability with nonpolar polymers.¹⁹ Lately, nonpolar organic compounds such as pyrene, anthracene and acenaphthene have been successfully used as MALDI-TOF-MS matrices for nonpolar polymers.^{28,29} For the analysis of a nonpolar analyte, a common MALDI-TOF-MS sample preparation method involves the combination of metal ions of Lewis acids, primarily Ag^{2+} and Cu^{2+} , and a matrix in a suitable solvent.³⁰ The metal ions serve as cationization reagents by reacting with the soft Lewis bases found in these analytes, such as olefinic double bonds and phenyl rings.^{31,32} It has been reported that in the gas phase, these interactions make it easier as charged species are generated from neutral analyte particles.³³ Nonetheless, when cationization reagents for NP analytes are metal salts, there is a production of the analyte cationized molecule $[\text{M} + \text{cation}]^+$ in the ionization process.^{34,35} In addition, several instances have indicated that matrices and/or metal salts could form clusters with metal ions.^{19,36} Not to mention that clusters can influence the signals of low mass oligomers according to the molecular weight of the polymer being analyzed.^{37,38} In the analysis of NP analytes with MALDI-TOF-MS, salts such as AgTFA , AgNO_3 , and AgCl are frequently employed as cationization reagents and combined with various matrices to produce MALDI-TOF-MS signals from NPs under various experimental requirements.³⁹

In this study, a series of experiments were performed to investigate the silver-related clusters that develop during the MALDI-TOF-MS analysis of polystyrene. There was a strong emphasis on the analysis of nanoplastics in this research, which was based on the background of the silver clusters encountered in the MALDI-TOF-MS method developed to analyse PSNs. Thus, these potential difficulties associated with silver salt clusters are reported, and can be referred to in future experimental research works on synthetic polymers, especially plastic nanoparticles. Therefore, in order to confirm the origin of these clusters, copper and silver salts were mixed with both polar and nonpolar matrices to compare their results. To determine how these clusters influence the MALDI-TOF-MS findings of NPs, PSNs, whose molecular weight is mentioned above and who can favor the formation of clusters, were investigated experimentally. Additionally, the aim of this study is to unveil potential issues related to background silver salt clusters during the

analysis of PSNs using MALDI-TOF-MS. Based on this aim, the following questions are answered: (i) in which mass range can silver salt cluster ions be detected? (ii) Which kind of matrix is able to reduce silver cluster ions so as to prevent interference with PSN MALDI-TOF-MS mass spectra results? (iii) How to optimize the ratio of analyte, matrix and cationization reagent volume to produce PSN mass spectra without/with only a few silver clusters?

2. Materials and methods

2.1. Chemical reagents

PSN standard (50 nm particle size) with a molecular weight of 2740 Da (SEC values: $M_n = 2627$; $M_w = 2740$) and regular spherical beads were used in this study and were purchased from Beijing Dk Nano-Technology Co., Ltd, China. Chemical supply companies of matrices such as 1,8,9-anthracenetriol (dithranol, DI), 2,5-dihydroxybenzoic acid (DHB), sinapic acid (SA), 2-(4-hydroxyphenylazo)benzoic acid (HABA), *trans*-3-indoleacrylic acid (IAA), anthracene (ATH), acenaphthene (ACTH), and pyrene (PRN) are shown in ESI Table S1.† Details of copper(II) chloride (CuCl_2), silver trifluoroacetate (AgTFA), and silver nitrate (AgNO_3) as cationization reagents and solvents such as tetrahydrofuran (THF) and acetonitrile HPLC grade (99.9%) are presented in ESI Table S1.† All commercial chemicals and solvents were used according to the manufacturer's guide.

2.2. Sample preparation

Different matrix solutions (HABA, SA, DHB, DI, IAA, ATH, PRN, and ACTH) were prepared in tetrahydrofuran (THF) at a concentration of 10 g L^{-1} and those of the cationization reagents (AgTFA , AgNO_3) were prepared at a concentration of 0.01 M in THF. PSN solutions were prepared in THF at a concentration of 5 g L^{-1} . For the analysis of a particular metal salt, 5 μL of cationization reagent (C) was combined with 20 μL of the matrix (M) and 4 μL of the analyte (A). Following a short mixing step, the mixture with a CMA ratio of 5 : 20 : 4 (v/v/v) was vortexed (Kylin-Bell®) for 5 min and sonicated for 30 min. Approximately 0.4–1 μL of this combination was then applied to a stainless-steel sample disc, allowing for the collection of several sets of spectra from a single sample without having to remove the probe from the source area. The solvent was allowed to evaporate at room temperature, leaving the solid mixture of the sample for analysis.

2.3. MALDI-TOF-MS instrumentation

An Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a laser working at a wavelength of 337 nm was used to acquire the mass spectra. The positive ion linear mode was employed to record the spectra. For the MS tests, a standard MTP384 polished steel target (Bruker Daltonics) was utilized. For each range, five pulses at 100 distinct locations were collected (representing one pixel). The FlexAnalysis software 3.4 (Bruker Daltonics) was used to analyze the data. The ion intensity plots were

constructed using average ion masses. The mass spectrometer was set to the low mass gate for the results reported in this work. The intensity of the detected silver cluster signals rose substantially when the low mass gate was turned on. The calibration was performed externally using a two-point calibration with anthracene (m/z 178) and C60 (m/z 720).

3. Results and discussions

3.1. Polar matrices

The formation of silver clusters: in order to study the formation of silver clusters delivered from silver salts mixed with polar matrices, first of all, a 0.1 M solution of silver trifluoroacetate in THF was tested, and the laser desorption mass spectrum is shown in Fig. 1. In this case, one can find the range of m/z to be 1000–2000, where the intensities of silver ions are present and appear to be less than 2000 (a.u.). There are peaks that indicate the presence of silver clusters, but to a small extent. On the other hand, it is shown that in this mass spectrum, the signal is extremely weak. In this case, in the absence of a matrix, silver cluster ions with lower m/z (less than m/z 2000) were produced by laser desorption/ionization of AgTFA.

Secondly, to investigate and demonstrate that the matrices, which are polar, mostly promote the formation of clusters that have a relationship with silver, a series of experiments were performed to directly detect silver trifluoroacetate (AgTFA) with polar matrices such as 2,5-dihydroxybenzoic acid (DHB), 2-(4-hydroxyphenylazo)benzoic acid (HABA), sinapic acid (SA), *trans*-3-indoleacrylic acid (IAA), and dithranol (DI), using MALDI-TOF-MS, as shown in Fig. 2. These matrices were chosen specifically because in the MALDI technique, the productivity of silver atom clusters increases when the matrix includes a carboxylic group, as it does in the case of the mentioned matrices. The ratio used for the cationization reagent/matrix combination was 5 : 20 (v/v), and each matrix was mixed with silver trifluoroacetate (AgTFA) for detection. The generation of signals that have a relationship with silver was significantly increased when the silver salt was combined with a polar MALDI-TOF-MS matrix. This shows that the matrix has a distinct influence on the development of silver clusters in the presence of silver salt. A typical example can be explained well with Fig. 2A, which shows the mass spectrum acquired by combining 20 μL 10 g L⁻¹ DHB and 4 μL 0.01 M AgTFA, with

both mixed in THF. Furthermore, experimental investigation was performed where the matrix/cationization ratio was changed from 20 : 2 to 20 : 4 (v/v), as presented in Fig. 2A, but there was no change observed in the obtained mass spectrum. The laser desorption study of 10 g L⁻¹ DHB did not show a peak in the range of m/z 3000–5000, indicating that the signal does not come from the matrix alone. In this case, our results showed that the clusters belonged to silver salts due to two silver isotopes, Ag-107 and Ag-109.³¹ Even though each polar matrix that combined with silver trifluoroacetate (AgTFA) could produce silver clusters, the results showed that their signal intensities and response over silver salt ions are different. This could be clearly explained when DHB and AgTFA were detected in the range of m/z 1000–4000. For less than m/z 2000, the highest peak intensity was observed at more than 8×10^3 a.u. This was a little different for IAA and ATFA, where the highest peak intensity was detected at 7.7×10^3 a.u. (Fig. 2B). Separately, HABA, SA and DI mixed with silver trifluoroacetate (AgTFA) produced silver-related clusters but with low intensity (less than 5×10^3 a.u.). The responses of these matrices are shown in Fig. 2C–E. It is thought that matrix acidity played a significant role in producing these clusters, as mentioned. Even if between m/z 1000–4000, there are signals in the case of all the matrices, when DI was compared with the other matrices, it generated a relatively lower signal with AgTFA.

Dithranol is a polar matrix, although it is a considerably weaker acid compared to HABA, SA, IAA, or DHB.⁴⁰ When comparably stronger acids are employed as matrices, it seems that silver salts are the least efficient cationization reagent, based on the experimental findings reported in this study. Regardless of the matrix, ion signals were attributable only to silver cluster ions. The cluster ions identified are unaffected by the identity of the metal salt. Furthermore, the cluster ions found experimentally match those anticipated theoretically due to the natural abundances of the two silver isotopes.³¹ Additionally, for a mass-to-charge ratio in the range of m/z 2500–4000, in all mass spectra, it was observed that clustering occurred but at a very low level, where the peak intensities observed were less than 4×10^3 a.u. for IAA and DHB, while those for HABA, SA and DI were less than 2.5×10^3 a.u. Previously, a number of studies have evaluated the efficacy of Ag⁺ or Cu²⁺ salts as cationization reagents for nonpolar analytes in MALDI-TOF-MS.^{41,42} However, in MALDI-TOF-MS analysis, consistency, for both metal salts, is still a potential problem as it is hard to find a better choice of metal salt for the analysis.^{12,31}

This lack of consistency is due to the fact that many effective sample preparation methods are available.⁴³ In conditions where silver salt ions are found to be inferior to copper salt ions, a potential reason could be that silver clusters develop favorably over ions of silver-adducted oligomers.⁴⁴ The clusters are often seen with lower mass (less than m/z 1000), although the clusters found were in the range of m/z 1000–4000, as mentioned above. In this study, silver nitrate was also investigated as a silver salt to confirm whether silver was the main cause of silver-related cluster ion production. Similar findings were observed using different matrices such as DI, HABA, SA, IAA, or DHB. As long as both silver trifluoroacetate and silver nitrate could serve the

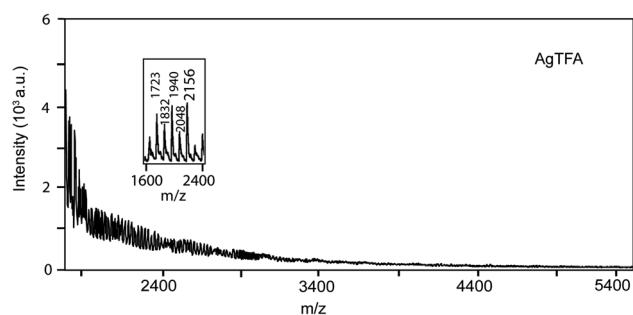


Fig. 1 MALDI-TOF-MS mass spectrum of silver trifluoroacetate (AgTFA).

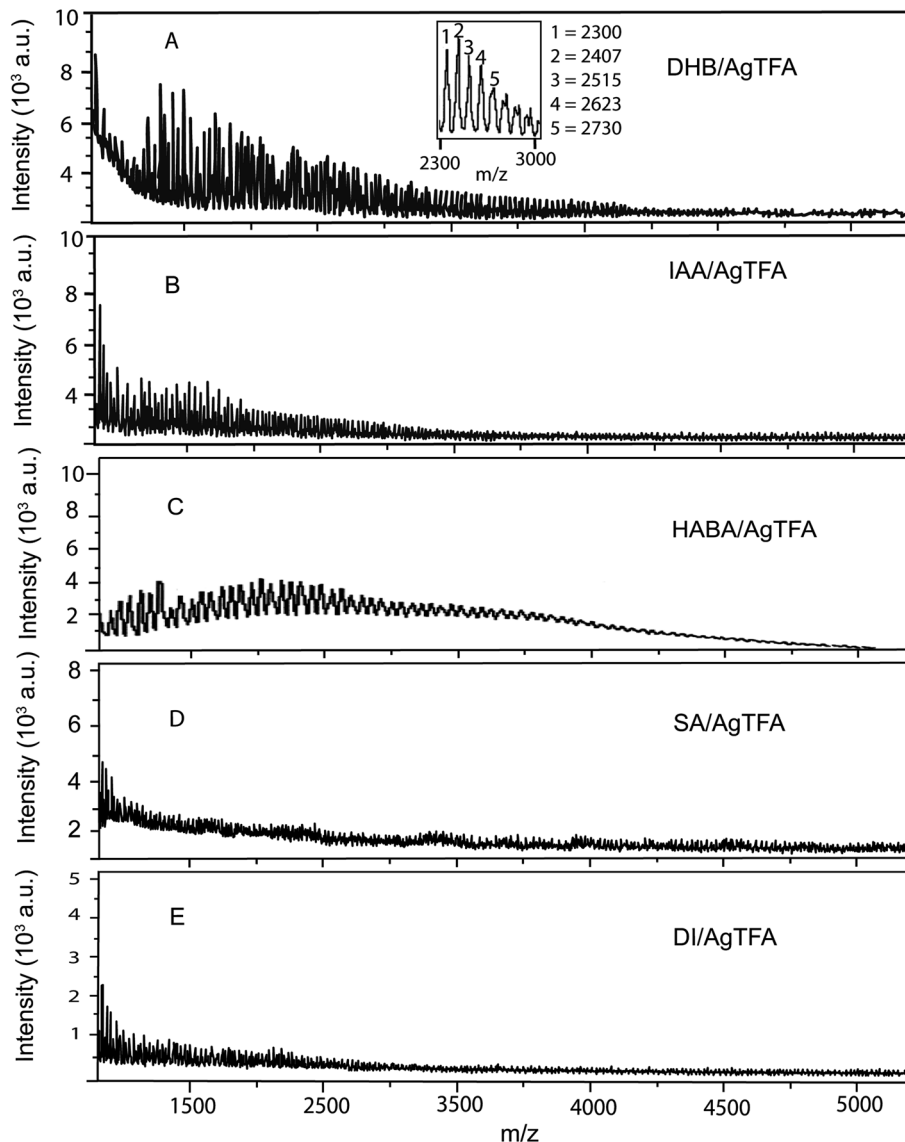


Fig. 2 The MALDI-TOF-MS mass spectrum of (A) 2-(4-hydroxyphenylazo)benzoic acid (HABA) and AgTFA; (B) sinapic acid (SA) and AgTFA; (C) dithranol (DI) and AgTFA; (D) *trans*-3-indoleacrylic acid (IAA) and AgTFA; and (E) 2,5-dihydroxybenzoic acid (DHB) and AgTFA. AgTFA: silver trifluoroacetate.

same purpose, they produced the same results, and according to the experimental conditions used, we utilized and reported silver trifluoroacetate results herein as a commonly used silver salt for the MALDI-TOF-MS analysis of synthetic polymers such as polystyrene nanoplastics. In this case, where a series of experiments series was performed with variation of polar matrices, there was no change in the mass spectral results compared to those obtained using a silver trifluoroacetate salt. A series of experiments were performed under conditions similar to those shown in Fig. 2, where similar matrices were used to investigate how the metal impacts the clusters with high mass. The salt of copper chloride was employed instead of AgTFA, and a different background signal is generated for SA, as shown in Fig. 3B. Lower mass ions are detected, but with extremely low abundance, and high signals in the mass spectrum, including those of single ions, were identified. When polar matrices other

than IAA were investigated, similar findings were found (Fig. 3A–C). Therefore, the combination of silver salts and the solution of acid matrices (polar matrices) generates ions related to silver, which are of comparatively high mass, but when similar matrices were used with salts of copper, the results showed that ions related to copper of comparable mass were not produced.

3.2. Nonpolar matrices

The formation of silver lusters: silver salts are frequently added to the combination of matrix and analyte as cationization reagents in the analysis of various materials.^{45,46} During laser desorption, silver cations are reduced to create silver clusters. This led to the hypothesis that the MALDI-TOF-MS method matrix may influence the creation of silver clusters.⁴⁷ There is research employing silver compounds and organic matrices of

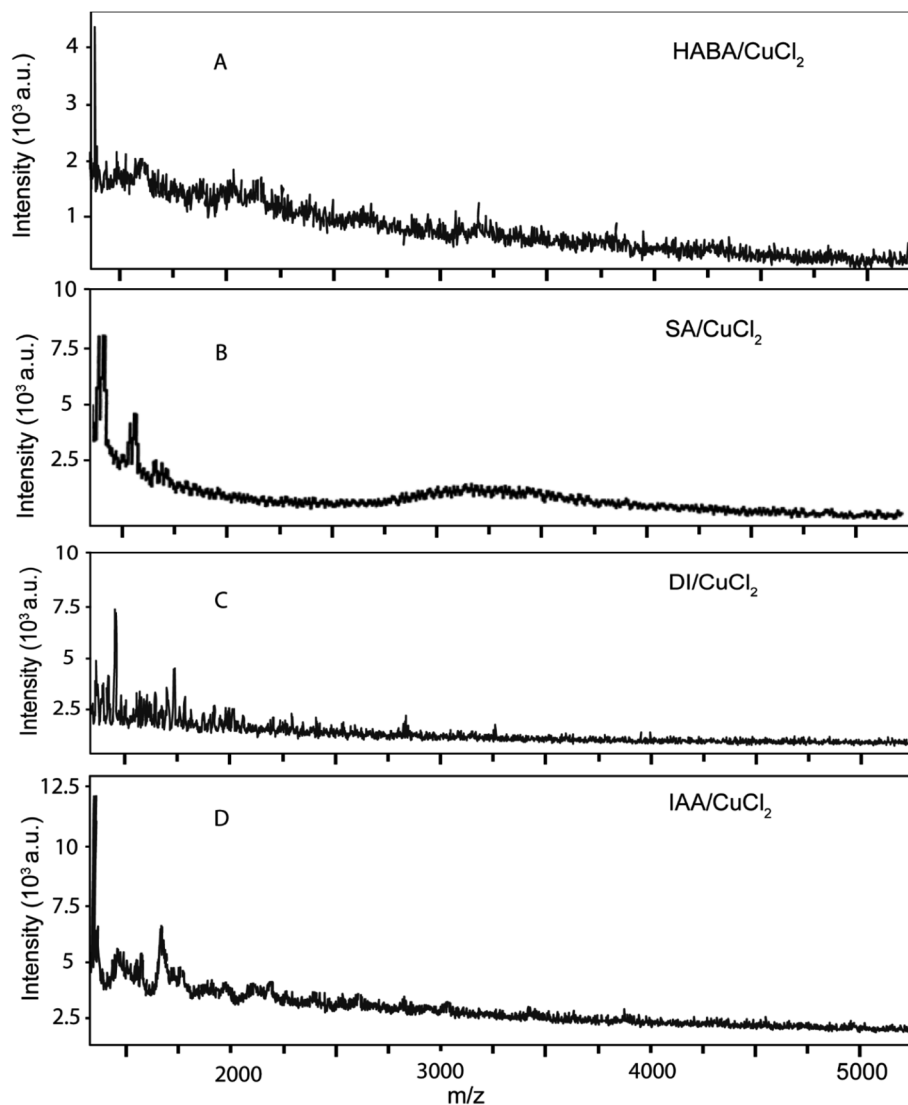


Fig. 3 The MALDI-TOF-MS mass spectrum of (A) 2-(4-hydroxyphenylazo)benzoic acid (HABA) and CuCl_2 , (B) sinapic acid (SA) and CuCl_2 , (C) dithranol (DI) and CuCl_2 , and (D) *trans*-3-indoleacrylic acid (IAA) and CuCl_2 . CuCl_2 : copper II chloride.

diverse chemical natures in the literature.²⁰ Silver trifluoroacetate has been utilized previously, while ATH, ACTH and PRN were utilized in this study. In the analysis of silver clusters using MALDI-TOF-MS, these nonpolar matrices are commonly used. In this study, our main goal was to see if the combination of nonpolar matrices and silver salts as cationization reagents could produce the same findings that were previously found during the analysis of polar matrices. Therefore, nonpolar matrices were used instead of polar matrices to compare the results. ATH, ACTH, and PRN were used as nonpolar matrices and the conditions used in the previous experiments were repeated for further comparative analysis. The results showed that the investigated nonpolar matrices did not produce signals, especially in the mass range of interest. The findings of AgTFA analysis with ATH, PRN and ACTH are shown in Fig. 4A–C, respectively. In the presence of the ACTH matrix, the results showed that in the range of m/z 1200–2500, low abundance was identified, unlike the high abundance

background ions produced previously with the polar matrices. Unlike the other matrices studied, the combination of PRN and ATH matrices with silver trifluoroacetate did not produce silver-related clusters. This shows that the production of silver clusters and their interference were caused by the matrix type used. Thus, the results suggested that the use of nonpolar matrices instead of polar ones could contribute to the production of signals that do not contain silver-related background signals. Therefore, from the obtained results of three nonpolar matrices, PRN and ATH were more suitable for the MALDI-TOF-MS detection of PSNs.

3.3. Comparison and optimization of nonpolar matrix response in the MALDI-TOF-MS analysis

Among the three nonpolar matrices, it was found that PRN and ATH have lower background signals when used as matrices with AgTFA to detect PSNs. Therefore, PRN and ATH were compared and optimized, and a series of experiments were performed to

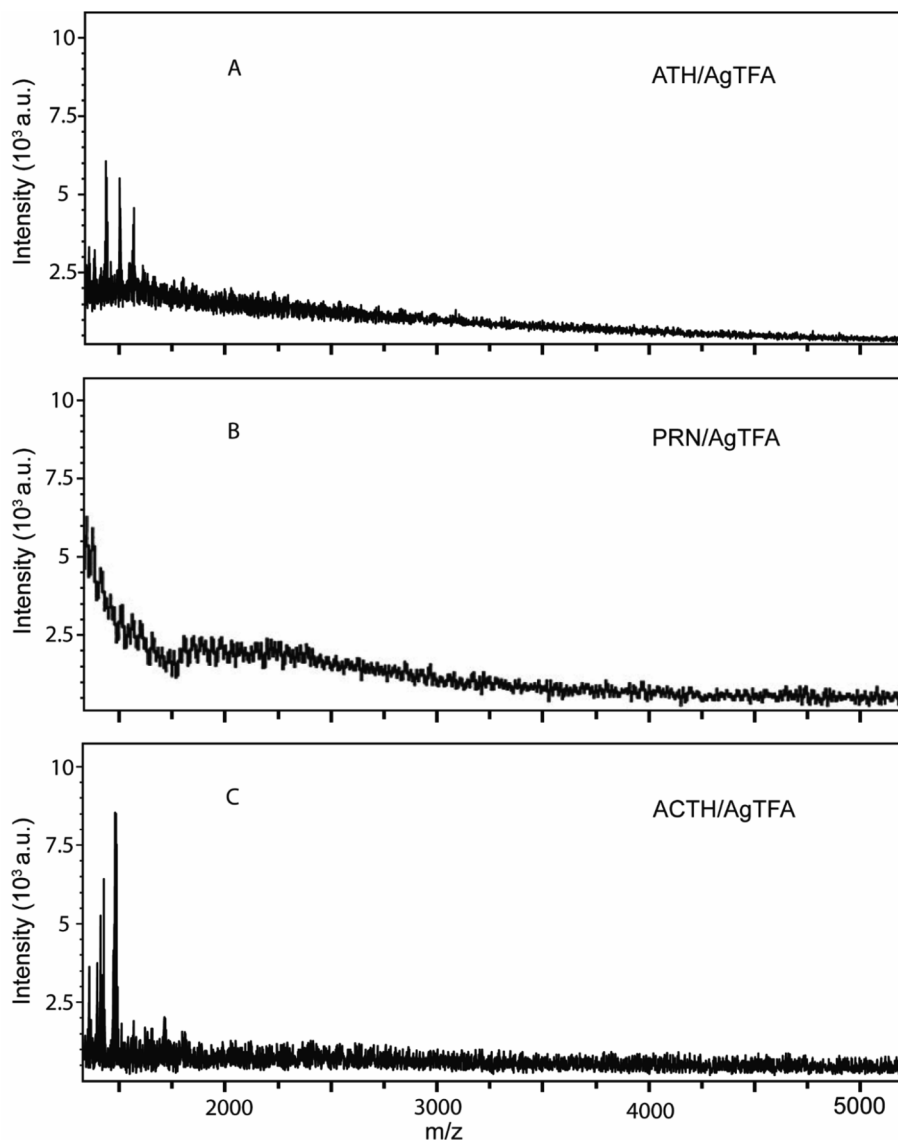


Fig. 4 The MALDI-TOF-MS mass spectrum of (A) anthracene (ATH) and AgTFA (B) pyrene (PRN) and AgTFA; and (C) acenaphthene (ACTH) and AgTFA. AgTFA: silver trifluoroacetate.

ensure that the matrix could provide potential signals with PSNs in their MALDI-TOF-MS analysis. In this case, the relative peak intensities of the PRN and ATH matrices were compared. These intensities were in the range of 1000–9500 (a.u.). The significant peak intensity of PRN was nearly 9000 a.u., which was far better than the ATH peak intensity, and this proved that PRN is a good choice for matrix material in PSN analysis, especially when it is investigated with AgTFA or silver-related salts. The representative example of these facts is shown experimentally in Fig. 5, where the response of PSN with a combination of PRN and silver trifluoroacetate is clearly observed to produce high intensity signals. Similar experiments were performed with copper-related salts, where different concentrations of copper salts, PSN, and matrices were used, but these results were not considered when compared to those obtained when silver salts were used (results are not shown). These results were obtained by optimization of the cationization reagent, matrix, and

analyte ratio, *i.e.*, 5 : 20 : 4 (v/v/v). The relative amounts of the cationization agent–analyte–matrix were systematically changed using a 3 × 3 grid of samples to empirically establish the optimal sample preparation. This was usually accomplished by keeping one of the three variables constant (20 μL of matrix solution) while increasing the amounts of the other two (cationization agent, *y*-axis, and analyte, *x*-axis) by a predetermined factor (2-fold in the example depicted). This kind of variation of the ratio enhanced and supported the optimization of the obtained results to choose the potential matrix. The experimental details of ratio variation are found in the ESI.†

3.4. Impact of silver cluster ions on PSN MALDI-TOF-MS analysis

PSN 2740 (50 nm) was studied as a model under a range of sample preparation conditions to study how silver-related

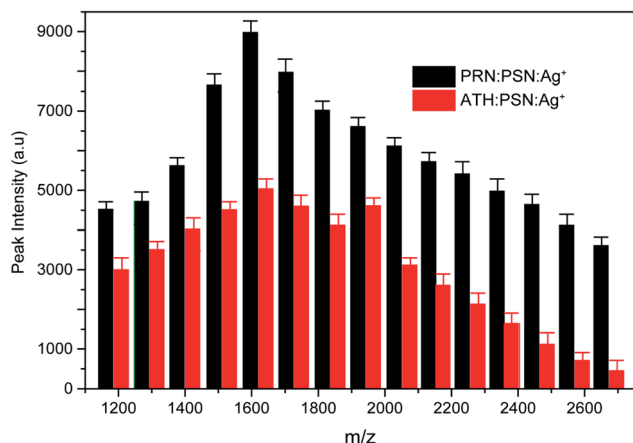


Fig. 5 Comparison of optimized MALDI-TOF-MS m/z and relative intensities of the pyrene/PSN/Ag⁺ and anthracene/PSN/Ag⁺ ratio (v/v).

cluster ions influence the MALDI-TOF-MS detection of nonpolar PSN analytes. As mentioned earlier, MALDI-TOF-MS has been used to analyze polystyrenes in some methods, and this analyte is very simple to describe at a low molecular weight. This polymer was selected since its predicted oligomer distribution is comparable to that of silver-related cluster ions. Such nonpolar polymers with low molecular weight are also tolerant of the MALDI-TOF-MS sample processing technique. Different matrices, including more hydrophilic polar matrices such as DHB, may easily generate acceptable oligomer distributions. To conduct effective MALDI-TOF-MS studies, matrices that are more hydrophobic will be needed for nonpolar polymers of higher molecular weight. This study shows that the signal quality decreased when the metal salt concentration in the mixture is raised substantially for a given combination of matrix, metal salt, and analyte, especially in cases where this mixture can yield acceptable MALDI-TOF-MS signals. However, a previous study revealed that MALDI-TOF-MS signals may be produced when the sample contains a concentration of metal salt that is more than the molar quantity of the analyte but not greater than that of the matrix.⁴⁸ Fig. 6 shows the typical MALDI-TOF-MS mass spectra obtained for the PSN samples. AgTFA and DHB were utilized as a cationization reagent and polar matrix, respectively, as shown in Fig. 6B. Interfering silver-related cluster ions were present, and there was a decrease in mass spectral quality. Since oligomers of polystyrene have masses that are closer to those of the metal clusters, in this situation, distinguishing metal ions from the polymer is a big challenge. As shown in Fig. 6A, the combination of a nonpolar matrix (PRN) and AgTFA with PSNs produced a better-quality mass spectrum. Therefore, when a polar matrix is employed, silver-related cluster ion production seems to influence the MALDI-TOF-MS mass spectrum findings of PSN adversely, but not when a nonpolar matrix is utilized.

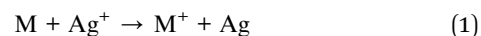
Fig. 6C shows a MALDI-TOF-MS mass spectrum of PSNs using DHB as a matrix and CuCl₂ as a cationization agent. In this mass spectrum, the repeating mass of m/z 104 for styrene is

identified, which confirms the presence of PSNs in the sample. A few studies have demonstrated that the combination of copper salts and any polar matrix, such as HABA, DHB, and DI, yields better results compared to that of silver salts, especially in the analysis of nonpolar synthetic materials.^{36,48,49} In this study, we have experimentally discovered that a nonpolar matrix such as pyrene produces repeatable results when analyzing nonpolar polymers with lower molecular weights such as polystyrene nanoplastics (PSNs).

4. Comparison of the current technique with other MALDI-TOF-MS methods

When the MALDI-TOF-MS technique is compared with other methods, which have been previously used in the identification and/or characterization of synthetic polymers, the literature shows that they differ in the sample preparation techniques, matrices and cationization reagents used. Sample preparation is the most important experimental step.^{50,51} Depending on the type of material to be examined, several sample preparation procedures exist.⁵² The dried droplet method, quick evaporation (fast solvent drying) method, two-layer method, and solid/solid compression method are the most commonly used procedures.⁵³ However, the influence of sample preparation in the formation of clusters (*i.e.*, silver clusters) has not been discussed before.⁵⁴ Sample preparation is considered as the main step to get good or bad results in the MALDI-TOF-MS analysis of synthetic polymers. Successful MALDI-TOF-MS techniques have used different types of matrices and cationization reagents for the same and/or different analytes. Most of these matrices contain carboxylic acid groups, which are believed to form silver clusters once they are mixed with silver-related salts,⁵⁵ and the reduction of Ag⁺ cations to silver atoms is thought to occur first, by one of the following three reactions:

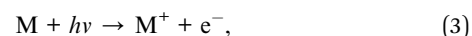
Charge exchange between matrix (M) molecules and silver cations:



Charge exchange between silver cations and excited molecules of the matrix (M):



Two-photon absorption by matrix molecules (M), which is the least likely:



It has been found that the process of clustering is much more efficient if more atoms of silver are captured by the matrix molecules, whereas for organic silver salts, *e.g.*, AgTFA, the separation of acid groups and the addition of the matrix, *e.g.*, DHB, to the ion/atom of silver is more likely, which in turn can

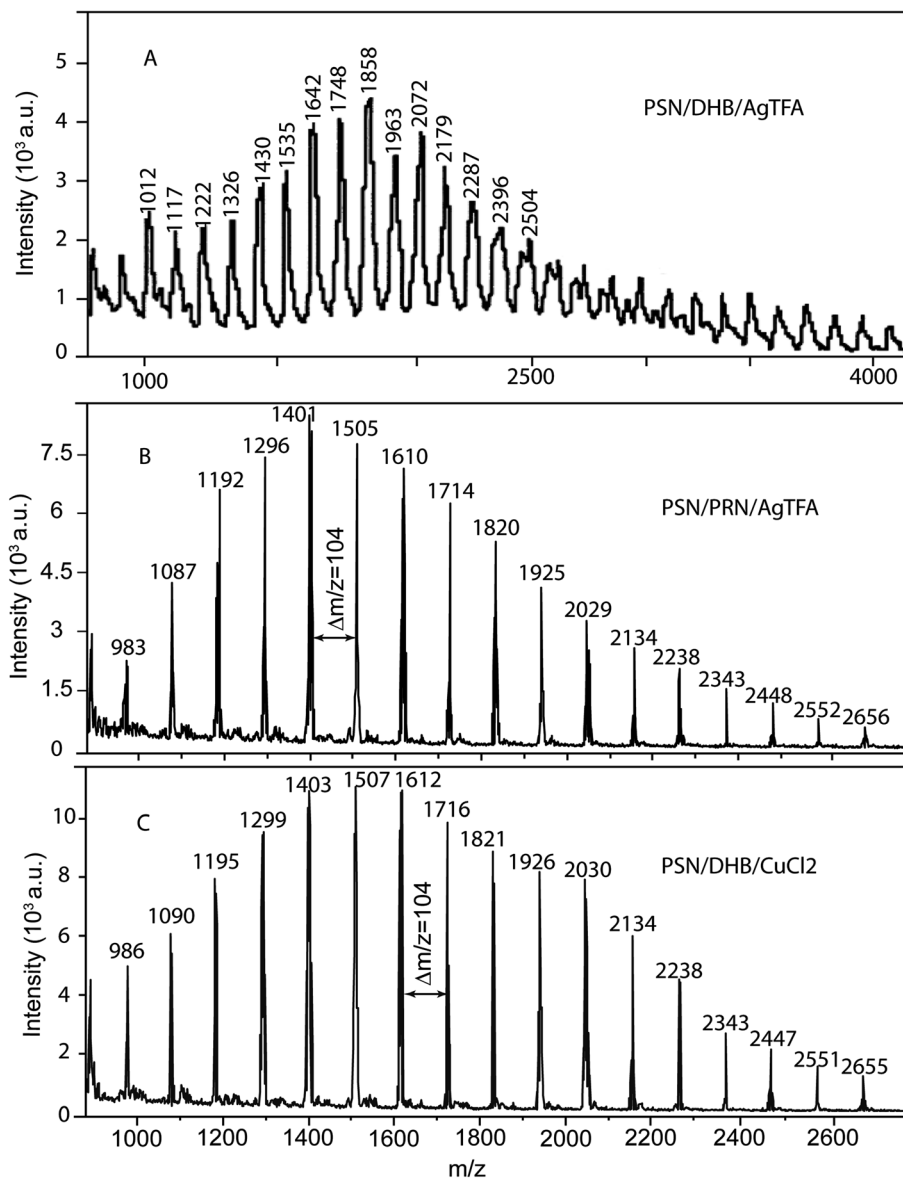


Fig. 6 The MALDI-TOF-MS mass spectrum of PSN examined with (A) dithranol (DI) and AgTFA (B) pyrene (PRN) and AgTFA; and (C) 2,5-dihydroxybenzoic acid (DHB) and CuCl_2 . AgTFA: silver trifluoroacetate; CuCl_2 : copper(II) chloride.

positively influence the process of clustering.^{56,57} Additionally, clustering may possibly be due to the ability of some polar matrices (acidic matrices), *e.g.*, DHB, to absorb laser light with a wavelength of 337 nm.^{58,59} Previously, MALDI-TOF-MS techniques have been used to study synthetic polymers such as micro-(nano-)plastics in the environment and PSNs have been investigated as environmental samples. These PSNs have a molecular weight range that is between low-molecular-weight polystyrene (LM-PS) and high-molecular-weight polystyrene (HM-PS). Pengfei *et al.*, 2020, determined PSN levels of both types, *i.e.*, LM-PS and HM-PS, which were approximately 500 g mol^{-1} and $13\,000 \text{ g mol}^{-1}$, respectively.⁶⁰ Therefore, the PSN used in this analytical method can be found in the range of plastic particles that have been investigated in environmental samples.

5. Conclusion

This work has shown that in MALDI-TOF-MS analysis, the combination of polar matrices such as HABA, DI, SA, IAA, and DHB with silver salts produces silver-related clusters in the range of m/z 1000–4000. The distribution of polymer-like strands for clusters is obtained and separated by silver atomic mass. The use of less acidic and/or nonpolar (*e.g.*, pyrene) matrices does not yield clusters. In our study, the optimization of the volume ratio of pyrene and anthracene with PSN and silver salt showed that pyrene was a good choice as a nonpolar matrix. However, the formation of clusters seems to be unique to the salts of silver. The combination of copper II chloride (CuCl_2) with polar matrices did not yield comparable copper-related clusters. When studying nonpolar polymers, it is

suggested to use nonpolar matrices and silver salts and when polar matrices are used, weakly acidic matrices are better options to reduce significant interference of silver cluster ions. When analysing nonpolar polymers, their mass spectral data should be interpreted carefully, especially in the case where silver salts are combined with moderately strong acids as matrices. Extensive clustering may be observed in the MALDI-TOF-MS mass spectra of sample materials containing transition metal ions and matrices, which may interfere with the signals of analytes having low molecular mass.

Author contributions

Théogène Habumugisha: Investigation, formal analysis, writing – original draft, validation, writing – review & editing. Zixing Zhang: data curation, validation, writing review & editing. Jean Claude Ndayishimiye: formal analysis, writing – original draft, validation, writing – review & editing. François Nkinahamira: data curation, validation, writing – review & editing. Alexis Kayiranga: writing – review & editing. Eric Cyubahiro: writing – review & editing. Rehman Abdul: writing – review & editing. Changzhou Yan: data curation, validation, writing – review & editing. Xian Zhang: conceptualization, methodology, resources, formal analysis, writing – original draft, validation, writing – review & editing.

Conflicts of 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.

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