
Silver Cluster Interferences In Matrix-Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry Of Nonpolar Polymers

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Potential difficulties associated with background silver salt clusters during matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) of nonpolar polymers are reported. Silver salt cluster ions were observed from m/z 1500 to 7000 when acidic, polar matrices, such as 2,5-dihydroxybenzoic acid (DHB), *all-trans*-retinoic acid (RTA) or 2-(4-hydroxyphenylazo)benzoic acid (HABA), were used for the analysis of nonpolar polymers. These background signals could be greatly reduced or eliminated by the use of nonpolar matrices such as anthracene or pyrene. Representative examples of these background interferences are demonstrated during the analysis of low molecular weight nonpolar polymers including polybutadiene and polystyrene. Nonpolar polymers analyzed with acidic, polar matrices (e.g., RTA) and silver cationization reagents can yield lower quality mass spectral results when interferences due to silver clusters are present. Replacing the polar matrices with nonpolar matrices or the silver salts with copper salts substantially improved the quality of the analytical results. In addition, it was found that silver contamination cannot be completely removed from standard stainless steel sample plates, although the presence of silver contamination was greatly reduced after thorough cleaning of the sample plate with aluminum oxide grit. Carry-over silver may cationize polymer samples and complicate the interpretation of data obtained using nonpolar matrices in the absence of added cationization reagents. (J Am Soc Mass Spectrom 2001, 12, 732–743) © 2001 American Society for Mass Spectrometry

The analysis of hydrocarbon polymeric materials has been more challenging than polar polymeric materials. In addition to conventional analytical techniques, such as vapor phase osmometry and gel permeation chromatography, various mass spectrometric techniques have been employed to analyze nonpolar polymers including polystyrene, polybutadiene, polyisoprene and hydrocarbon dendrimers. Among such techniques, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has been of interest [1]. A variety of MALDI analytical protocols have been developed for nonpolar polymers of different repeat units and molecular weight [2–7]. Without exception, each MALDI nonpolar polymer analysis protocol includes a

particular matrix to assist in desorption and a metal salt to facilitate ionization.

While the search for new matrices is still ongoing, at present, only a few matrices, such as 2,5-dihydroxybenzoic acid (DHB), *all-trans*-retinoic acid (RTA), *trans*-3-indoleacrylic acid (IAA) and dithranol, are widely used for the commonly analyzed nonpolar polymers: polystyrene, polyisoprene and polybutadiene. Although these matrices are polar compounds, they have been found to work relatively well for nonpolar analytes. The relative polarity of these matrices is used to help choose the most appropriate matrix for a given analysis [8]. Recently, there have been some reports about the successful application of nonpolar organic compounds as MALDI matrices for nonpolar analytes, including nonpolar polymeric materials [9–13].

A typical MALDI sample preparation for a nonpolar analyte uses a suitable solvent and combines the matrix with salts of soft Lewis acid metal ions, mainly Ag^+ and Cu^{2+} . The metal ions act as cationization reagents by

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interacting with the soft Lewis bases, such as olefinic double bonds and phenyl rings, present in these analytes. These interactions facilitate the generation of the charged species from the neutral analyte molecules in the gas phase. When metal salts are used as cationization reagents for nonpolar polymer analytes, pseudo-molecular ions $[M + \text{Cation}]^+$ of the analyte are produced in the ionization process. In many cases, metal ions have been found to form clusters with the metal salts or the matrix [2, 5, 10, 14, 15]. The clusters can interfere with the low mass oligomer signals of the polymers depending on the molecular weight of the polymer being analyzed.

Three silver salts are commonly used as cationization reagents for nonpolar analytes in MALDI analysis: silver trifluoroacetate (AgTFA), silver nitrate (AgNO₃) and silver acetylacetonate [Ag(acac)]. Under different experimental conditions, these salts have been used with a variety of matrices to obtain MALDI signals from most nonpolar polymers, with the exception of saturated hydrocarbon polymers such as polyethylene and polypropylene. The most likely explanation for the failure to generate MALDI signals from polyethylene and polypropylene is that saturated hydrocarbon polymers do not have a favorable site for cation attachment [10].

During our studies of nonpolar polymers, we have noted the presence of silver clusters at higher masses that appear to be associated with the silver salts and the polar matrices. We noticed that whenever silver salts [AgTFA, AgNO₃ or Ag(acac)] were used as cationization reagents, in combination with polar matrices such as RTA or DHB, a background signal with a mass distribution ranging from around m/z 1500 to 7000 was formed. Rashidzadeh and Guo have also noted that polar matrices can be used to generate high mass silver clusters directly during analysis of silver salts [15].

This background signal observed was not found when a Cu²⁺ salt [(Cu(NO₃)₂)] was used in place of Ag⁺ salts. Interestingly, this polymer-like background signal was not present when Ag⁺ salts were used with nonpolar matrices such as anthracene, acenaphthene or pyrene. The observed signal not only can interfere with the analyte oligomers of similar mass, but in experiments with insufficient mass resolution, can also be falsely considered as positive qualitative results particularly when analyzing some unknown polymeric materials.

Here we have conducted a number of experiments to explore the silver-related clusters formed within the acidic matrix environment in MALDI. Different polar and nonpolar matrices were used in combination with silver and copper salts for verification and comparison of the obtained cluster signals. Nonpolar polymer analytes of molecular weights which fall within the m/z range of the observed cluster signals were analyzed at experimental conditions favoring cluster formation to see the extent to which the MALDI results of the polymers are affected by these clusters. In addition, we

have found that carry-over silver can cationize polymer samples, even those samples prepared without additional cationization reagents.

Experimental

Materials

The matrix compounds *all-trans*-retinoic acid (RTA), 2,5-hydroxybenzoic acid (DHB), dithranol, 2-(4-hydroxyphenylazo)benzoic acid (HABA), anthracene, acenaphthene and pyrene were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). AgTFA, Ag(acac), AgNO₃ and Cu(NO₃)₂ also were purchased from Aldrich. HPLC grade tetrahydrofuran (THF) was obtained from EM Science (Gibbstown, NJ, USA). Polybutadiene 2940 was a gift from Dr. Phil Savickas at Dow Chemical (GPC values: $M_n = 2827$; $M_w = 2940$). Polystyrene 1940 and 2557 were obtained from Scientific Polymer Products, Inc. (Ontario, NY, USA) (GPC values: $M_n = 1690$; $M_w = 1940$ and $M_n = 2500$; $M_w = 2557$, respectively). All compounds and solvents were used as received.

Sample Preparation

Solutions of the matrices and the metal salts were prepared using similar preparation procedures commonly reported in the literature. Typically, RTA was prepared at a concentration of 0.10 M to 0.15 M. DHB and HABA were prepared at a concentration of 0.1 M. Dithranol and the nonpolar matrices were prepared as saturated solutions. AgTFA and Cu(NO₃)₂ were prepared at a concentration of 0.1 M. The preceding solutions were prepared in THF. AgNO₃ was prepared in ethanol as a saturated solution. The polymer solutions were prepared at a concentration of 1×10^{-4} M in THF. For a typical metal salt analysis, 10 μL of matrix was mixed with a minimum of 1 μL of the metal salt. After a brief mixing, 1 to 2 μL of that mixture was spotted on the MALDI plate, air dried then analyzed. The polymer sample analysis included mixing together 10 μL of the matrix, 2 μL of the polymer and 1 or 2 μL of the metal salt solution, from which 1 μL was spotted for analysis.

Instrumentation

All mass spectral data were acquired using a PerSeptive Biosystems (Framingham, MA, USA) Voyager linear time-of-flight mass spectrometer. The instrument is equipped with a nitrogen laser ($\lambda = 337$ nm). The acceleration voltage was held at its maximum value which was about 28 kV. Calibration was performed externally using a two-point calibration with anthracene (m/z 178) and C₆₀ (m/z 720). Data were collected with the laser power set just above the ionization threshold of the matrix. For the data presented in this paper, the mass spectrometer was operated with the

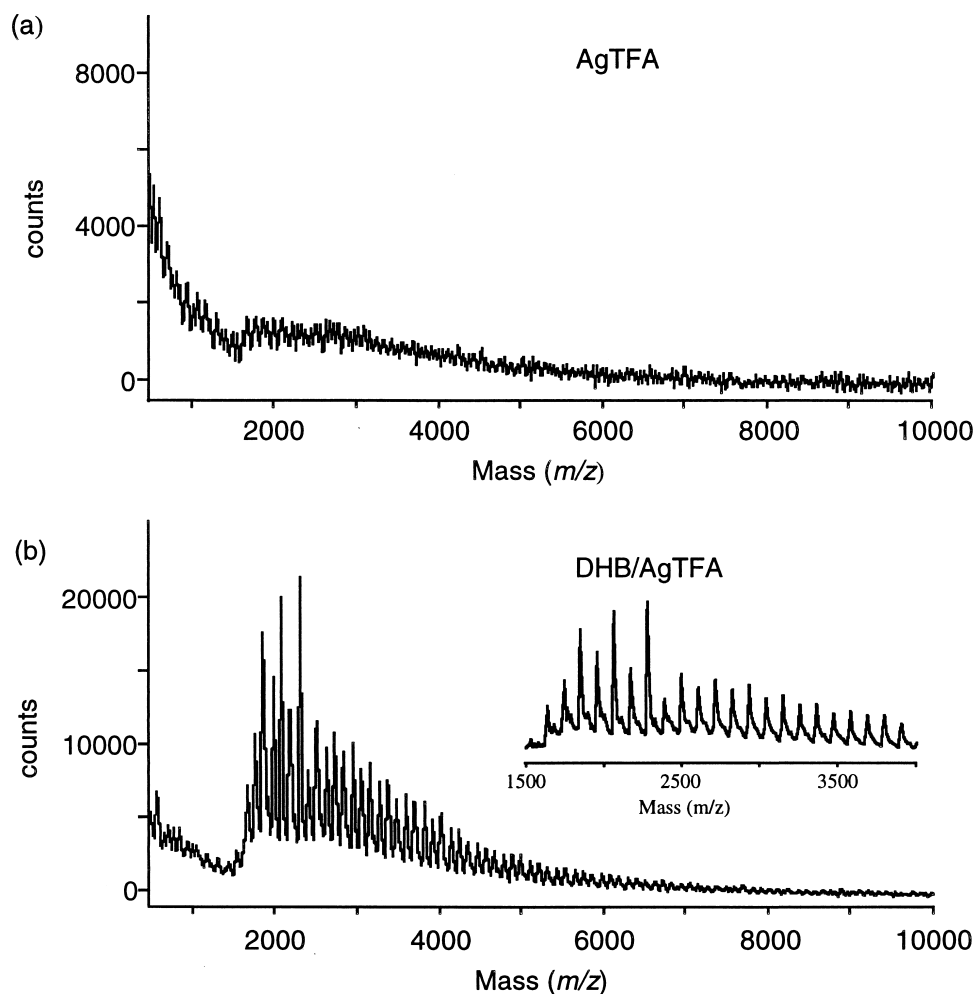


Figure 1. (a) LDI-TOF mass spectrum of AgTFA and (b) MALDI-TOF mass spectrum of a mixture of DHB and AgTFA. Silver-related cluster ions are enhanced when DHB is utilized as a matrix. The inset in Figure 1b shows the polymer-like ion distribution where the mass difference between ions is approximately 108 u.

low mass gate on (i.e., low mass ions are deflected away from the detector). With the low mass gate on, the intensity of the observed silver cluster signals increased significantly. Typically 16 scans were averaged for each mass spectrum obtained. Under conventional experimental conditions, this instrument can achieve mass resolution of about 500.

Results and Discussion

Silver Cluster Formation and Polar Matrices

The effectiveness of Cu^{2+} or Ag^+ salts as cationization reagents for nonpolar analytes in MALDI has been compared by a number of researchers [5, 10, 14, 16, 17]. Unfortunately, there is no consensus about which of the two metal salts is more effective for a particular analysis. The lack of consensus is most likely due to availability of multiple successful sample preparation techniques. For the cases where Ag^+ salts are found to be inferior to Cu^{2+} salts, one possible explanation could be

that silver clusters form preferentially, as opposed to the formation of silver-adducted oligomer ions. These clusters are not the clusters typically observed at lower mass (<1,000 u), but, rather, are clusters in the mass range of about m/z 1500 to 7000.

To confirm that polar matrices facilitate the generation of higher mass silver-related cluster ions, mixtures of silver salts were directly analyzed with several typical MALDI matrices. Figure 1a is the laser desorption mass spectrum of AgTFA (0.1 M) in THF. As seen in this mass spectrum, silver-related cluster ions are obtained at the lower m/z values examined, although the signal is of very low intensity. To ensure that the metal salt or matrix solutions had not been contaminated within the laboratory, new reagents were acquired and prepared as described above. No differences in mass spectral features were found upon use of new metal salts or matrices.

Once the silver salt is mixed with a polar MALDI matrix, formation of silver-related cluster ions is dra-

matically enhanced. The mass spectrum in Figure 1b was obtained by mixing 10 μL of 0.1 M DHB with 2 μL of 0.1 M AgTFA each prepared in THF. A reproducible signal ranging from around m/z 1500 to 7000 was obtained. When the ratio of matrix to salt solutions was varied from 5:1 (v:v), as in the case presented in Figure 1b, to 150:1 (v:v) (data not shown) a similar, although less abundant, distribution was detected. This signal does not arise from the matrix alone as the laser desorption analysis of a 0.1 M DHB solution did not yield signal in the higher mass range (data not shown). In addition, the laser fluence can affect the ion abundance.

To determine if the enhanced formation of silver-related cluster ions was particular to AgTFA, other silver salts were investigated. When AgNO_3 or $\text{Ag}(\text{acac})$ were used in place of AgTFA, similar results were obtained using DHB as the matrix (data not shown). Among the polar matrices, varying the matrix also had little effect on the mass spectral results. For example, Figure 2 contains representative mass spectra obtained from the analysis of AgTFA in dithranol (Figure 2a), RTA (Figure 2b) and HABA (Figure 2c). Dithranol produced a much weaker signal with AgTFA as compared to the other matrices, but in all cases ion signal is detected in the range of m/z 1,500 to 7,000. While dithranol is polar, it is a much weaker acid than DHB, RTA, or HABA. These results confirm the previous report of Rashidzadeh and Guo [15], who generated silver cluster ions from AgTFA and RTA, and extend the matrix:salt combinations from which silver cluster ions can be produced.

These ion signals are due exclusively to silver cluster ions irrespective of the matrix. Linear regression analysis of the data obtained in Figure 1b and Figures 2a–c are presented in Table 1. In all cases, the repeat mass is that for silver and the intercept is zero within the experimental accuracy of our instrument. The identity of the metal salt has no effect on the cluster ions detected. In addition, the experimentally obtained cluster ions agree well with that predicted theoretically based on the natural abundances of the two silver isotopes (Figure 3).

To observe the effect of the metal on these high mass clusters, experiments were conducted using the same matrices and similar experimental conditions as in Figure 2 but with a copper salt [$\text{Cu}(\text{NO}_3)_2$] instead of silver salts. As seen in Figure 4c for RTA, a substantially different background signal is obtained (cf.: Figure 2b). Although lower mass ions are present when AgTFA (Figure 2b) and $\text{Cu}(\text{NO}_3)_2$ (Figure 4c) are analyzed with RTA, the higher mass signal is of very low abundance and individual ions cannot be resolved. Similar results were obtained when polar matrices other than RTA were examined (see Figures 4a,b,d). Thus, it appears that the addition of silver salts to an acidic matrix solution results in the production of silver-related ions of relatively high mass while copper salts analyzed in the same matrices do not efficiently form copper-related ions of similar mass.

Silver Cluster Formation and Nonpolar Matrices

Based on the results obtained in the previous experiments utilizing polar matrices, we were interested in determining whether the nonpolar matrices introduced for nonpolar polymer analysis [13] exhibited similar behavior with silver cationization reagents. To determine the behavior of these matrices, similar studies were performed using nonpolar matrices such as anthracene, acenaphthene and pyrene under similar experimental conditions to those utilized for the polar matrices.

Similar to the results for the polar matrices, no signal in the mass range of interest was detected for any of the nonpolar matrices analyzed directly (data not shown). Figures 5a and 5b are representative results obtained for the analysis of AgTFA with acenaphthene and anthracene, respectively. A low abundance elevated background from m/z 1,000–5,000 was detected in the presence of acenaphthene, although no specific background ions of high abundance were detected unlike the situation found when polar matrices were used (cf. Figs 1 and 2). Anthracene, unlike any of the other matrices investigated, does not result in the production of higher mass silver-related cluster ions. Thus, it appears that silver-cluster related ion formation may be due to the type of matrix utilized, with nonpolar matrices yielding substantially lower or non-existent silver-related background signals.

Silver Cluster Ion Effects on Nonpolar Polymer Analysis

To investigate the extent to which these silver-related cluster ions affect the MALDI analysis of nonpolar polymer analytes, polybutadiene 2940 and polystyrene 2557 were characterized under a variety of sample preparation conditions. As noted in the introduction, polybutadienes and polystyrenes have been analyzed in a variety of ways by MALDI-MS, and these two analytes are relatively easy to characterize at low molecular weight. These particular polymers were chosen because their expected oligomer distribution should fall within a similar mass range as that found for the silver-related cluster ions. These low molecular weight nonpolar polymers are also very forgiving of the MALDI sample preparation method. Acceptable oligomer distributions can be readily produced with a variety of matrices, even the more hydrophilic polar matrices, such as DHB. Higher molecular weight nonpolar polymers will require more hydrophobic matrices to produce successful MALDI experiments.

We report here that, as a general observation, for any matrix/analyte/metal salt combination that produces a reasonable MALDI-MS signal, the signal quality deteriorates as the metal salt concentration in the mixture is increased significantly. It has been pointed out by Schriemer et al. that MALDI-MS signals can be obtained if the metal salt concentration in the sample is in excess

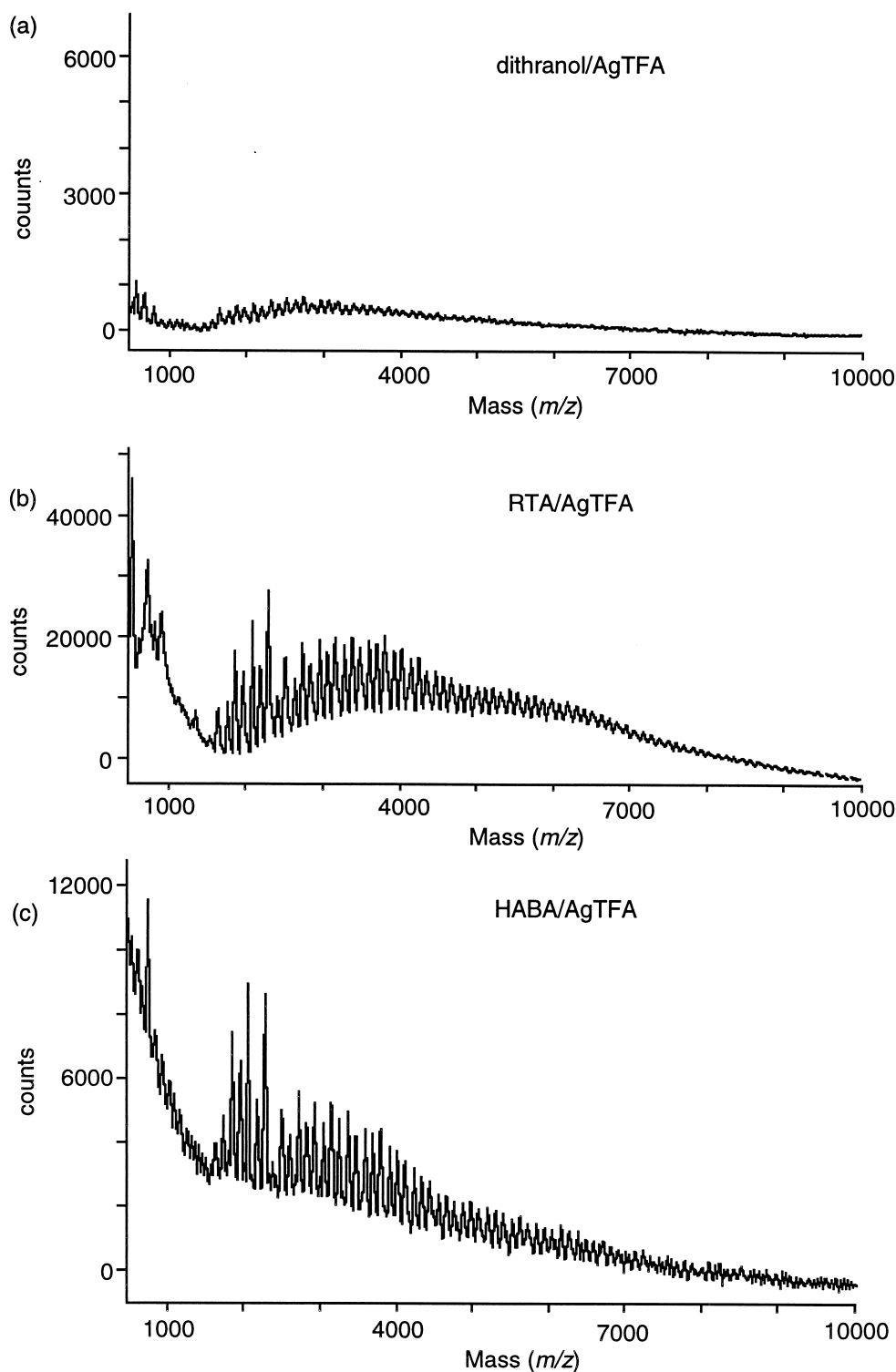


Figure 2. MALDI-TOF mass spectra of (a) dithranol and AgTFA; (b) RTA and AgTFA; and (c) HABA and AgTFA. Dithranol, the weakest acid among the three matrices, yields the lowest production of silver-related cluster ions.

of the molar amount of the analyte but not in excess of the matrix [18].

Representative MALDI mass spectra obtained for the polybutadiene sample are presented in Figure 6. In

Figure 6a, the polar matrix DHB was used as the matrix and AgTFA was used as a cationization reagent. Figure 6a looks very similar to Figure 1c. Due to the complexity of the silver cluster ion spectrum, the extraction of the

Table 1. Silver cluster ions found during MALDI-MS of AgTFA and AgNO₃ with various polar matrices. Only silver clusters (Ag)_n were detected. The intercept values are nearly zero within the experimental error of the measurement.

Salt/matrix combination	Linear regression analysis
DHB/AgTFA	$y = 108.2x + 3.2$ ($n = 15\text{--}60$)
Dithranol/AgTFA	$y = 108.3x + 19.0$ ($n = 15\text{--}39$)
RTA/AgTFA	$y = 108.3x + 3.5$ ($n = 15\text{--}60$)
HABA/AgTFA	$y = 107.9x + 7.9$ ($n = 15\text{--}60$)
DHB/AgNO ₃	$y = 108.3x + 12.5$ ($n = 15\text{--}60$)
Dithranol/AgNO ₃	$y = 109.2x + 3.1$ ($n = 15\text{--}39$)

polybutadiene signal is difficult and the number- and weight-averaged molecular masses ($M_n = 2795$, $M_w = 3381$) are substantially different from the GPC-derived values ($M_n = 2827$, $M_w = 2940$). In contrast, when the nonpolar matrix anthracene was used with AgTFA for the analysis of polybutadiene (Figure 6b), high quality

mass spectral results are obtained, although the number- and weight-averaged molecular masses ($M_n = 3013$, $M_w = 3076$) are higher than those obtained from GPC analysis, an effect which has been noted for these matrices [13]. Interestingly, we have found that only silver salts yield increased background with polar matrices. For example, when Cu(NO₃)₂ is utilized with the polar matrix DHB for the analysis of the polybutadiene sample (Figure 6c), higher quality mass spectral data with experimentally determined number and weight averaged molecular masses close to the GPC-derived values are obtained ($M_n = 2919$, $M_w = 3013$). Thus, it appears that silver-related cluster ion formation can have a deleterious effect on the MALDI mass spectral results of polybutadiene when a polar matrix is used but not when a nonpolar matrix is used.

To determine if the results found with polybutadiene also occur for other nonpolar polymers, a low molecular weight polystyrene sample was analyzed in a similar fashion. Figure 7a is a representative MALDI mass spectrum of polystyrene 2557 obtained with the polar matrix DHB and AgTFA as a cationization reagent. Similar to the polybutadiene example presented in Figure 6a, interfering silver-related cluster ions are observed along with a deterioration in the mass spectral quality ($M_n = 2825$, $M_w = 3556$). In this case, the problem of identifying the polymer and metal ions is more difficult than with polybutadiene because the mass differences between the metal clusters and the polystyrene oligomers are more similar. Instruments with higher mass accuracy could be used to distinguish these ions.

When the nonpolar matrix anthracene was used with AgTFA for the analysis of the polystyrene sample (Figure 7b), a higher quality mass spectrum, was obtained ($M_n = 2655$, $M_w = 2761$). Similar to the polybutadiene example, nonpolar matrices are most effective for polystyrene analysis when silver salts are used as cationization reagents [13]. Replacing the silver cationization reagent with a copper salt when the polar matrix DHB is used as the matrix improves the data quality substantially, as was the case for the polybutadiene sample. Figure 7c is a representative MALDI mass spectrum of polystyrene 2557 obtained with DHB as a matrix and Cu(NO₃)₂ as a cationization reagent. The mass difference between individual oligomers in this spectrum is 104 u, characteristic of the repeat mass for polystyrene. As seen in Figure 7c, when Cu(NO₃)₂ is used, the number- and weight-averaged molecular masses more closely agree with the GPC-derived values ($M_n = 2521$, $M_w = 2644$).

A number of published reports have shown that, with polar matrices (DHB, RTA or dithranol), Cu²⁺ is more effective than Ag⁺ for the analysis of nonpolar polymeric materials [5, 14, 19]. Cu²⁺ salts may be more effective when used with these matrices because Cu²⁺ does not form clusters as observed with Ag⁺ salts. Previously, Belu and co-workers have shown that AgTFA works better with dithranol for nonpolar polymer analysis as compared to more acidic matrices such

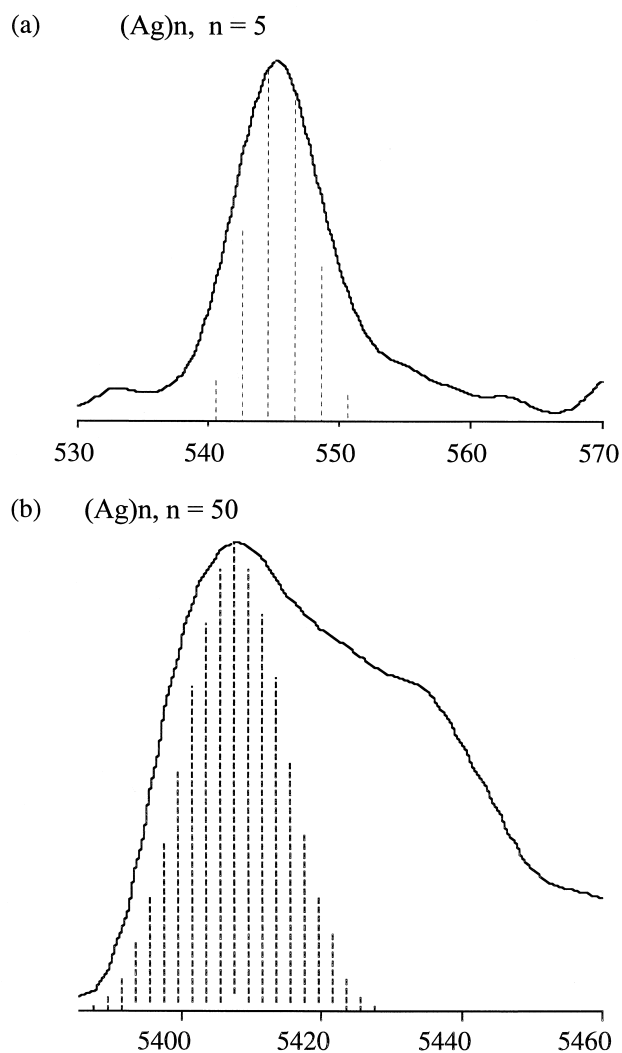


Figure 3. Experimental (solid line) and theoretical (dashed line) mass spectral data for (Ag)_n clusters where (a) $n = 5$ and (b) $n = 50$. Good agreement is found between the experimentally measured and theoretically predicted peak widths.

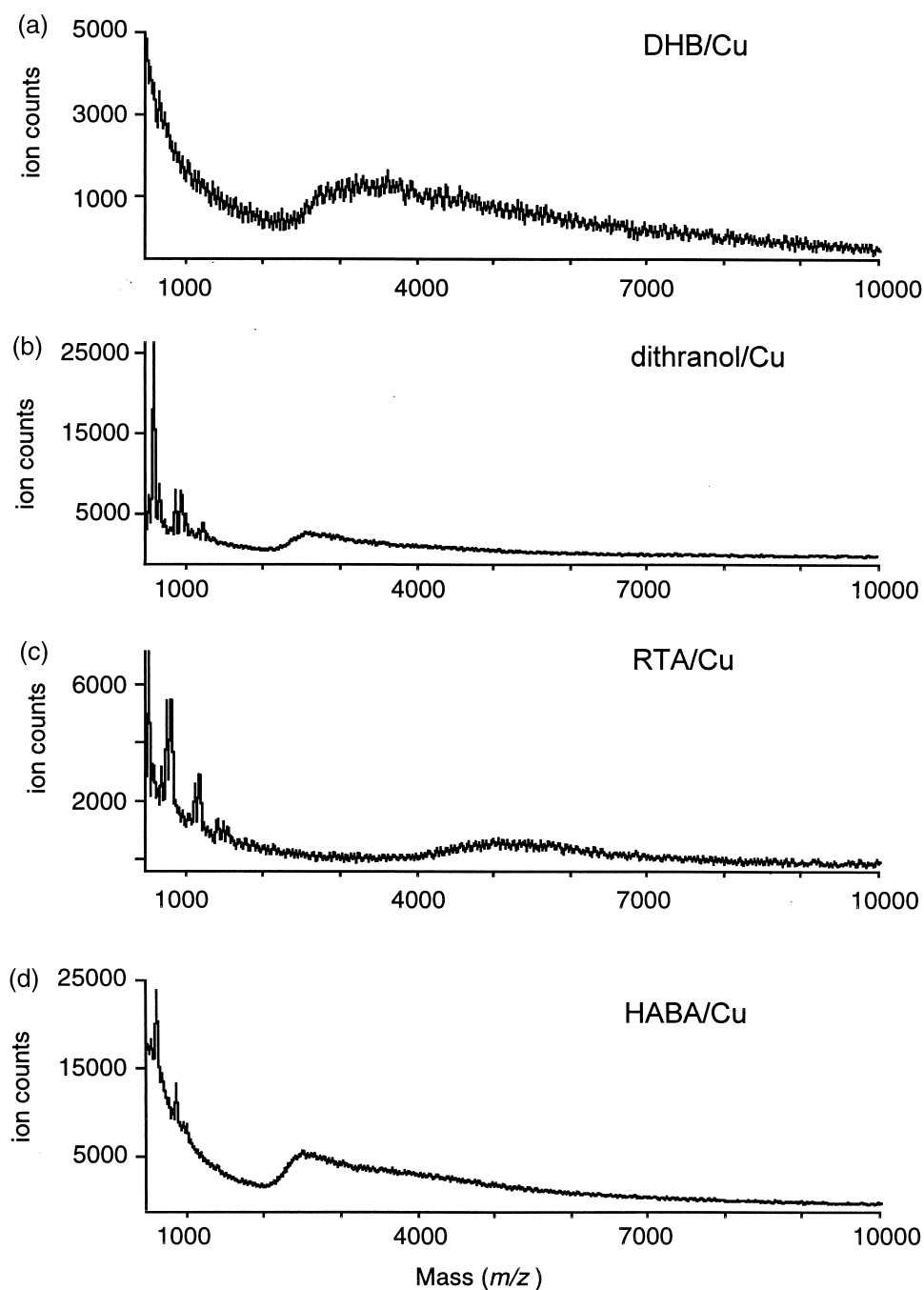


Figure 4. MALDI-TOF mass spectra of (a) DHB and $\text{Cu}(\text{NO}_3)_2$; (b) dithranol and $\text{Cu}(\text{NO}_3)_2$; (c) RTA and $\text{Cu}(\text{NO}_3)_2$; and (d) HABA and $\text{Cu}(\text{NO}_3)_2$. Although lower mass ($< 1,000$) background ions are detected, copper salts do not yield the abundant ion signals found when silver salts are utilized (see Figures 1 and 2).

as HABA and DHB [2]. Our results suggest that the effectiveness of dithranol with Ag^+ salts is due to the reduced formation of silver-related clusters as compared to the more acidic matrices.

Based on the experimental results presented in this work, it appears that Ag^+ salts are the least effective cationization reagents for nonpolar polymers when relatively stronger acids are used as matrices. The optimal matrices for nonpolar polymer analysis with

silver cationization reagents are nonpolar matrices, such as anthracene [13]. Conversely, as demonstrated by others [5, 14, 19], as the relative acidity of the matrix increases, copper cationization reagents become more effective for nonpolar polymer analysis. In any event, caution should be exercised when interpreting mass spectral data obtained at lower m/z values (1,000 to 10,000) when the experimental conditions include an acidic matrix, a nonpolar polymer and silver salts.

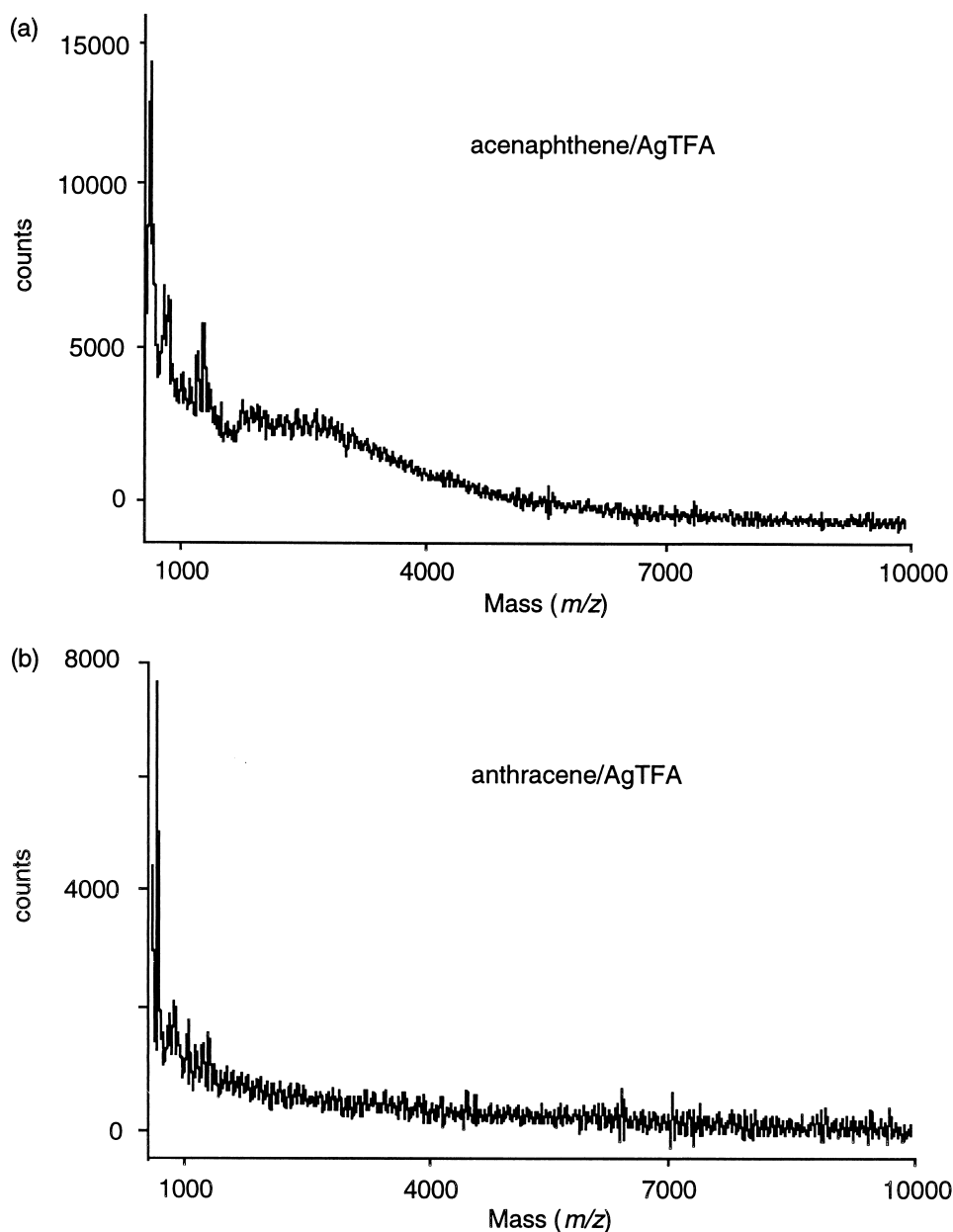


Figure 5. MALDI-TOF mass spectra of (a) acenaphthene and AgTFA and (b) anthracene and AgTFA. Nonpolar matrices do not enhance the production of silver cluster ions.

Nonpolar Matrices and Oligomer Cation Adduction

While the results of the previous experiments suggest that silver salts are to be avoided for nonpolar polymer analysis when relatively acidic, polar matrices are used, caution must be taken when interpreting the results one obtains in the presence of nonpolar matrices, such as anthracene. As mentioned earlier, the analysis of silver salts in the presence of nonpolar matrices, such as anthracene, does not generate a substantial background signal attributable to the silver salt (see Figure 5). However, when nonpolar polymers are analyzed using nonpolar matrices and no additional cationiza-

tion reagent, if the sample surface is not pristine and devoid of residual silver salts, silver-adducted oligomer ions can be detected.

For example, in Figure 8a polystyrene 1940 was analyzed using anthracene as the nonpolar matrix in the absence of any added silver salts. As seen in this mass spectrum, a polymer distribution representative of polystyrene 1940 is detected, albeit at low abundance. However, when this same sample is analyzed under identical conditions except that a pristine sample plate was used, no polymer signal is obtained. We associate the signal in Figure 8a with carry-over from silver compounds previously applied on the MALDI plate.

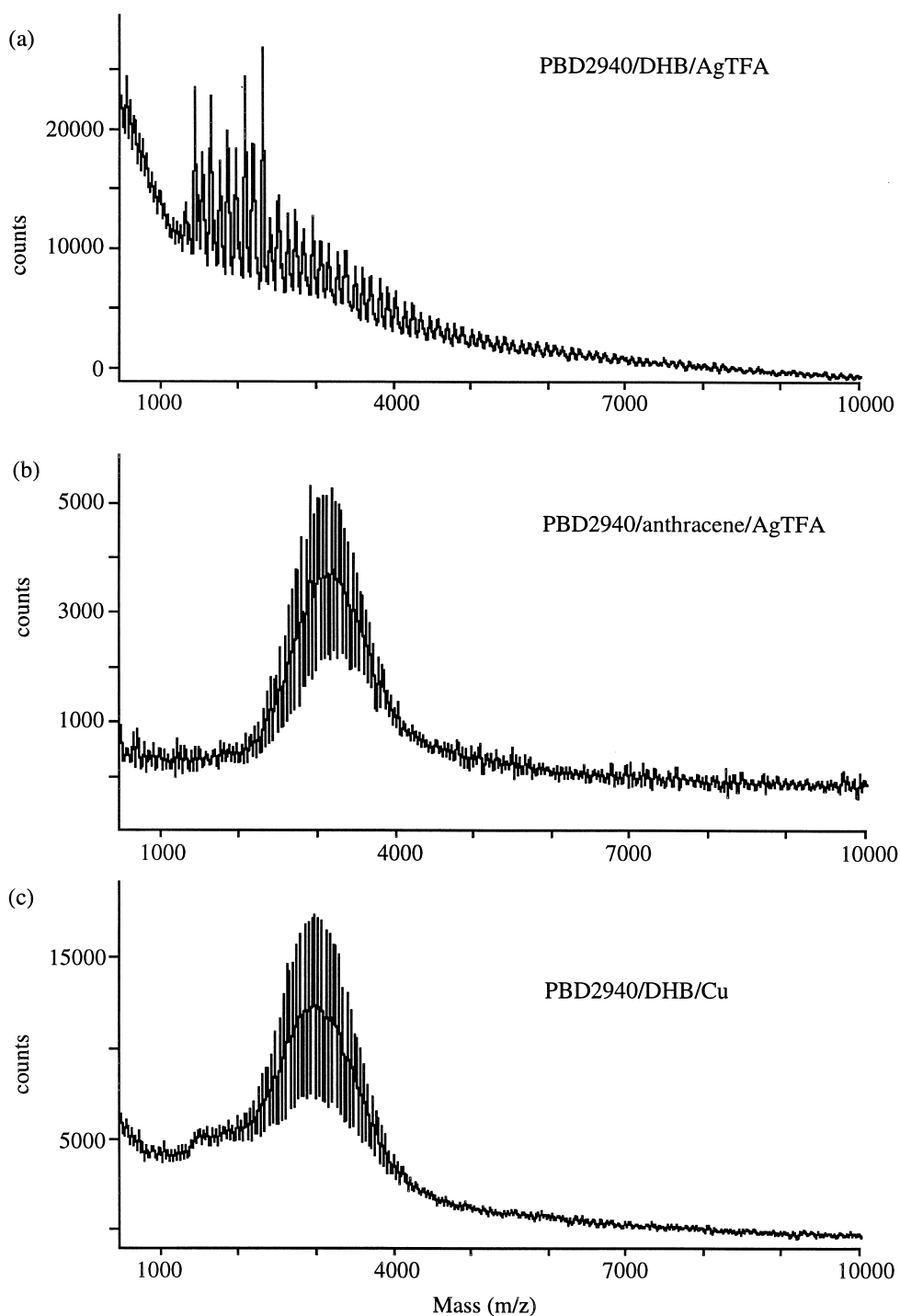


Figure 6. MALDI-TOF mass spectra of polybutadiene 2940 analyzed with (a) DHB and AgTFA; (b) anthracene and AgTFA; and (c) DHB and Cu(NO₃)₂. Copper nitrate is the preferred cationization reagent for acidic matrices due to the interfering silver-related cluster ions found when silver trifluoroacetate is used (see text). Alternatively, interfering silver-related cluster ions are not present when the nonpolar matrix anthracene was used.

These carry-over silver cationized oligomer ions may indicate a competing mechanism for previously published charge transfer results using the nonpolar matrices [11, 12]. Under these experimental conditions, charge-transfer ionization of polybutadiene or polystyrene is not favorable due to the higher ionization

energies of the monomer groups as compared to the ionization energies of the matrices [13]. However, without prior knowledge that residual silver salts could serve as cationization sources in such experiments, and having insufficient mass accuracy (in this case) to distinguish (polystyrene)⁺ from (polystyrene + Ag)⁺

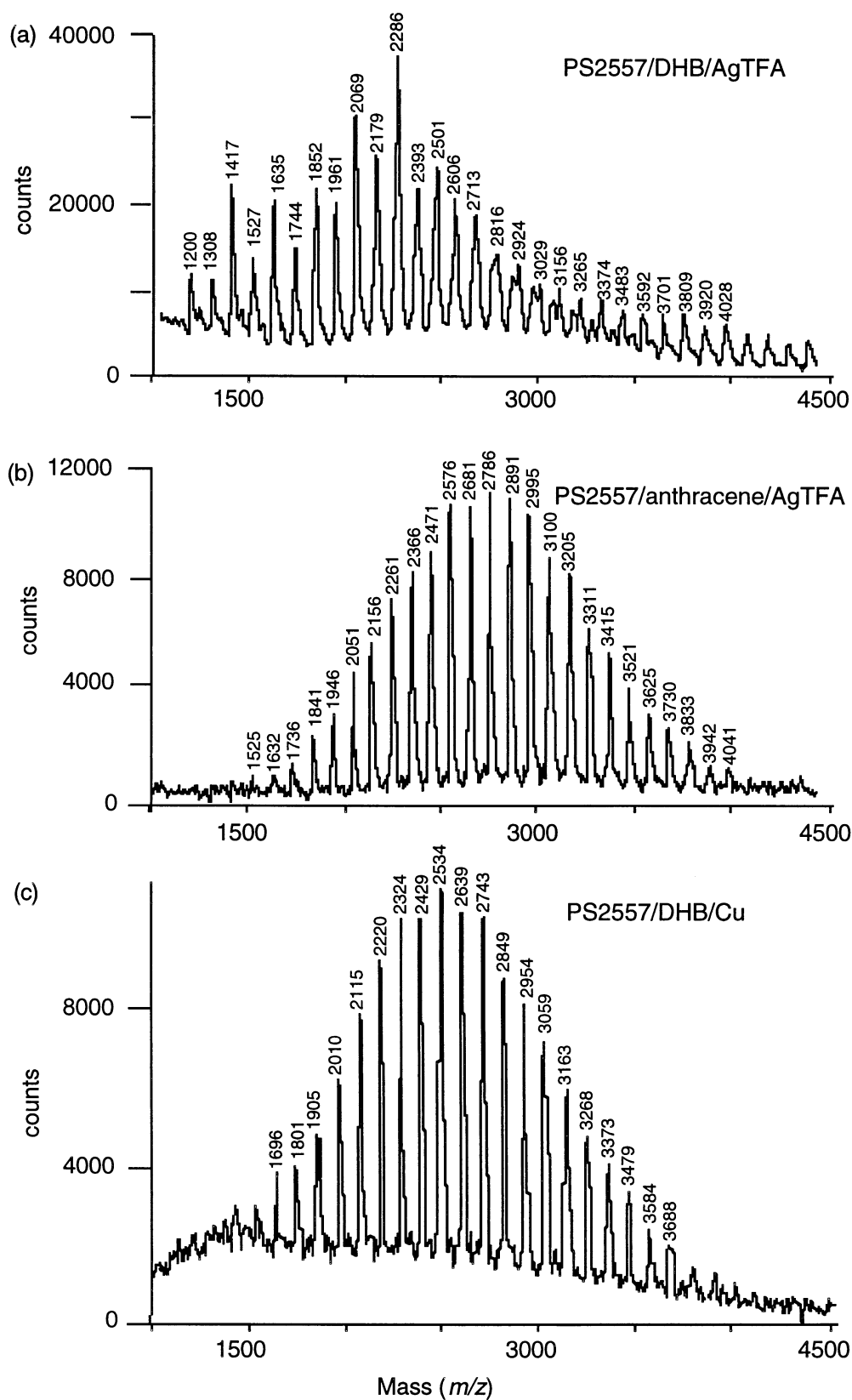


Figure 7. MALDI-TOF mass spectra of polystyrene 2557 analyzed with (a) DHB and AgTFA; (b) anthracene and AgTFA; and (c) DHB and Cu(NO₃)₂. Similar to the results obtained for polybutadiene, copper nitrate is the preferred cationization reagent for acidic matrices while silver trifluoroacetate is preferred for nonpolar matrices due to interfering silver-related cluster ions found for the AgTFA/acidic matrix combination.

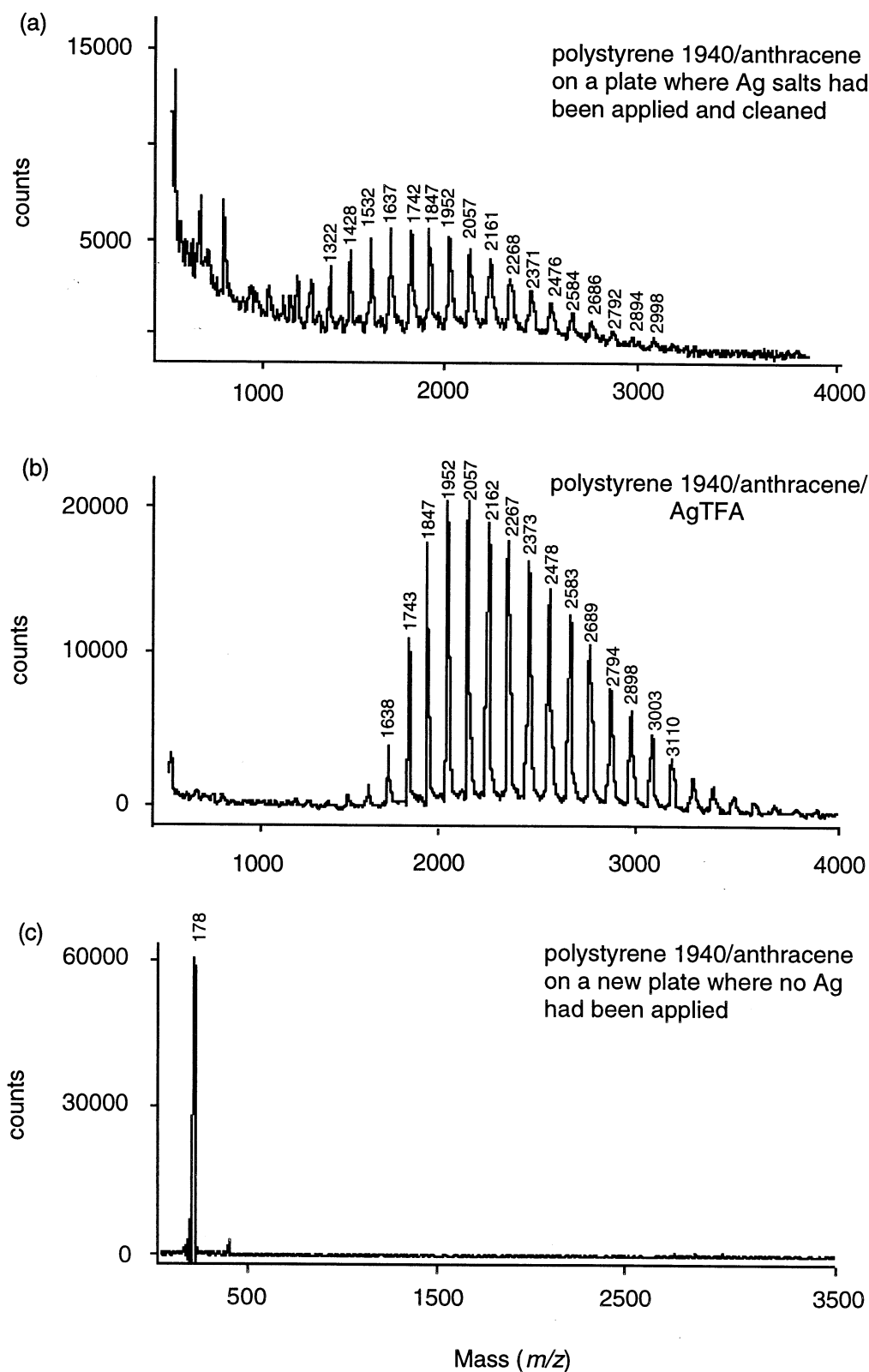


Figure 8. MALDI-TOF mass spectra of polystyrene 1940 in anthracene analyzed by (a) spotting on a thoroughly cleaned sample plate previously exposed to silver salt solutions; (b) addition of AgTFA and analyzed using a thoroughly cleaned sample plate previously exposed to silver salt solutions; and (c) spotting on a new plate not previously exposed to silver salt solutions. All spectra were obtained under similar experimental conditions. As seen by comparing the mass values obtained in Figure 8a and 8b, residual silver salts can serve to ionize nonpolar polymers when nonpolar matrices are utilized.

ions, one could falsely interpret the mass spectrum arising in Figure 8a to be due to charge-transfer ionization as opposed to cationization.

We noticed that the plate on which silver salt solution was spotted continued giving this kind of signal even after thorough sonication. Typically, MALDI target plates are cleaned by rinsing with a good solvent. It is clear from this work that silver contamination remains on used MALDI targets. Even using aluminum oxide paste to clean the plate, the signal was reduced considerably but not completely. The only conditions we have found that completely eliminated the generation of polymer signal in the absence of added cationization reagent was when a sample plate that had not been exposed to silver solutions was used. For laboratories using both polar and nonpolar sample preparation methods, we recommend having a dedicated MALDI target for use with silver cationization.

Conclusion

This work has demonstrated that when acidic matrices, such as DHB, RTA or HABA, are used together with silver salts in MALDI, there is a potential of forming silver-related clusters in the mass range from about m/z 1500 to 7000. These clusters yield a polymer-like distribution, separated by ~ 108 u, the atomic mass of silver. Such clusters decrease in abundance or are not present when less acidic (e.g., dithranol) or nonpolar (e.g., anthracene) matrices are used. Furthermore, cluster formation appears to be specific for silver salts: similar clusters were not observed when the same acidic matrices were used with copper salts. Silver salts were also shown to remain on the MALDI sample plate and can serve to generate silver-adducted oligomers when nonpolar polymers are analyzed with nonpolar matrices having no additional cationization reagents added. It is recommended that silver salts be used only with weakly acidic or nonpolar matrices when analyzing nonpolar polymers, and caution should be exercised when interpreting MALDI mass spectral data of nonpolar polymers analyzed using relatively strong acids as matrices in the presence of silver salts due to potential interferences from silver-related cluster ions.

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