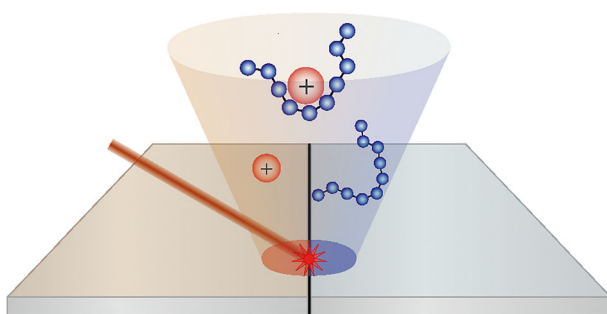**RESEARCH ARTICLE**

Mechanistic Studies on Cationization in MALDI-MS Employing a Split Sample Plate Set-up

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Abstract. In the analysis of polymers by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), a commonly observed ionization pathway is cation-adduct formation, as polymers often lack easily ionizable (basic/acidic) functional groups. The mechanism of this process has been hypothesized to involve gas-phase cation attachment. In previous experiments, a split sample plate set-up has been introduced, enabling separate deposition of the components on individual MALDI plates. The plates are divided by a small gap of a few micrometers, allowing simultaneous laser irradiation from both plates, while precluding the possibility of any other interactions prior to ablation. Here, we extend on these studies by using different polymer-salt combinations to test the generalizability of a gas-phase ionization process. Clear evidence for in-plume ionization is presented for the model polymers poly (methyl methacrylate) and polystyrene. Furthermore, the contribution of in-plume processes to the overall ion formation by cationization is gauged, providing a first estimate for the importance of this pathway.

Keywords: MALDI mechanism, Polymer cationization

Introduction

Synthetic polymers are an important class of analytes accessible by matrix-assisted laser desorption ionization mass spectrometry [1–4]. Since polymers often lack basic or acidic functional groups, the predominant ionization pathway in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is cation-adduct formation [5]. Ionization via cation-adduct formation is governed by the dynamics of both desorption and charge separation. However, neither the time-scales of these processes nor the sequence in which they occur are fully understood. The two main pathways commonly considered to contribute to cationization are a preformation of cation adducts in solution [6–9] and an in-plume cation attachment [7, 10, 11]. In the former case, cation adducts are proposed to be formed in solution, during sample preparation. Upon deposition, the adduct co-crystallizes with the respective counterion. Laser irradiation provides the energy required for counterion separation and release of the adduct [8, 12]. In contrast, within the in-plume hypothesis, it is assumed that the adducts are not preformed in the crystal. Instead, the laser irradiation generates free cations and polymer molecules, which can form adducts upon collision within the dense MALDI plume [7].

Several studies have been presented in favor either mechanism. Lehmann et al. [6, 9] showed that some strongly binding complexes exhibit a striking correlation between their mass spectral patterns and their solution-phase complexation behavior. The feasibility of in-plume cationization, on the other hand, has been addressed by a variety of experimental approaches, for example a layered deposition [6, 13, 14] and solvent free sample preparation [10, 11] to avoid preformation. The most convincing pieces of evidence were given by Belov et al. [15] and Erb et

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[16]. Both studies employed a special experimental set-up, which utilizes separate target plates for polymer and cation generation. The physical separation excludes preformation, while in-plume processes are feasible by mixing of the plumes. The split sample plate developed by Erb et al. [16] is especially convincing as the material is ablated by a single laser shot from two plates separated only by a gap of a few micrometers. These results clearly show that in-plume ionization is possible for some analytes. It is, however, still unclear if this also applies to different polymer classes and to which degree other ionization pathways might contribute. Jaskolla and Karas [17] demonstrated that protonated species can be formed either by preformation or by in-plume proton transfer, which highlights the question whether cationization occurs similarly.

Here, we present additional studies performed on a split-plate set-up, similar to the one employed by Erb et al. [16]. By investigation of different polymers and cationizing agents, we generalize earlier findings and address questions regarding the overall contribution and importance of in-plume processes for cationization in MALDI. A first estimate for the relative contribution of in-plume ionization is provided.

Experimental

Material

Size exclusion chromatography (SEC) standards of polystyrene 2000 (PS, Mw = 1890 Da, Mn = 1790 Da, Mp = 1920 Da, PDI = 1.06) and poly (methyl methacrylate) 2000 (PMMA, Mw = 2130 Da, Mn = 1920 Da, Mp = 2160 Da, PDI = 1.11) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Mass distributions were determined by the manufacturer using size exclusion chromatography.

Furthermore, trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB, ≥ 98%), copper trifluoroacetate, silver trifluoroacetate (99.99 + %), and ethylenediamine diacetate (EDDA, 99%) were acquired from Sigma-Aldrich. Lithium trifluoroacetate (97%) was purchased from Alfa Aesar (Ward Hill, MA, USA). Tetrahydrofuran, methanol, and water were obtained in HPLC grade quality from various vendors. All chemicals were used without further purification. Samples were prepared by pneumatic spray deposition of solutions containing matrix and either polymer or salt. A description of the spraying set-up and procedure is given in the supplementary information.

Instrumentation

Measurements were performed on an Autoflex I MALDI-ToF mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany). The sample plate has been described elsewhere [18]. Briefly, cavities were milled into a commercial MTP 384 target plate (Bruker Daltonics) to hold one set of five small target plates (see Figure SI-1). The target plates are coated separately and joined prior to the measurements. Screws are used to secure the plates and avoid displacement during analysis.

Unless otherwise stated, the same plates were used for all experiments. The gap width between two plates was determined to be 5 ± 2 μm and 14 ± 2 μm without and with the addition of a steel foil (10 μm thickness) spacer, respectively. A spacer was used in all experiments unless otherwise stated, to prevent any transfer across the gap. Each foil was only used for a single experiment. The ablation profile for the set-up was found to be elliptical (111 μm and 57 μm semi-axes). A laser fluence of 0.19 J/cm^2 and an extraction delay time of 280 ns were employed in all experiments; this delay included a constant internal instrumental delay of approximately 80 ns, which was determined previously [18].

Due to the low intensities observed at the junction, a high detector bias voltage was required to obtain sufficient spectral quality. We note that, as a result of these settings, the most abundant matrix signals led to detector saturation. However, this did not have any impact on the formation of cation adducts. This was confirmed by control experiments on a plate containing only salt or polymer, which did not exhibit the adduct signal, despite detector saturation (Figure SI-8 and SI-9).

Results and Discussion

Polystyrene (PS) and poly (methyl methacrylate) (PMMA) were selected as representatives of different polymer classes. PS was chosen as a model compound for non-polar polymers containing unsaturated hydrocarbon chains or aromatic systems. Binding to transition metal cations, such as copper or silver, is facilitated by d–π orbital interactions involving the aromatic ring of the polymer [5]. PMMA was chosen as a second model compound to reflect the binding of alkali cations to polar functional groups of polymers, which is dominated by ion-dipole interactions [19]. As cationizing agents, we chose copper and silver for polystyrene and lithium for poly (methyl methacrylate), due the high ionization efficiency of these combinations [16, 20]. Trifluoroacetate salts were chosen, because of their solubility in organic solvents, making it compatible with the hydrophobic matrix and polymer.

In-Plume Cation-Adduct Formation

Split-plate MALDI experiments (see Figure 1) were performed to assess whether in-plume processes are involved in the formation of polymer–cation adducts. In a split-plate experiment, one plate (hereafter denoted as the "salt plate") was coated with cationizing agent (deposited as its trifluoroacetate salt) and DCTB matrix, whereas the other plate (denoted as the "polymer plate") was coated with polymer and DCTB matrix. Figure 1a–c compare MALDI mass spectra for different polymer–cationizing agent pairs measured by ablation of the polymer plate, far from (gray traces) and in close proximity to (black traces) the junction. Spectra measured by ablation of only the polymer plate show no discernible signals corresponding to the ionized polymer; likewise, no signals corresponding to polymer ions are seen when ablating only the salt plate, as expected (see Figure SI-10). In stark contrast, polymer–cation
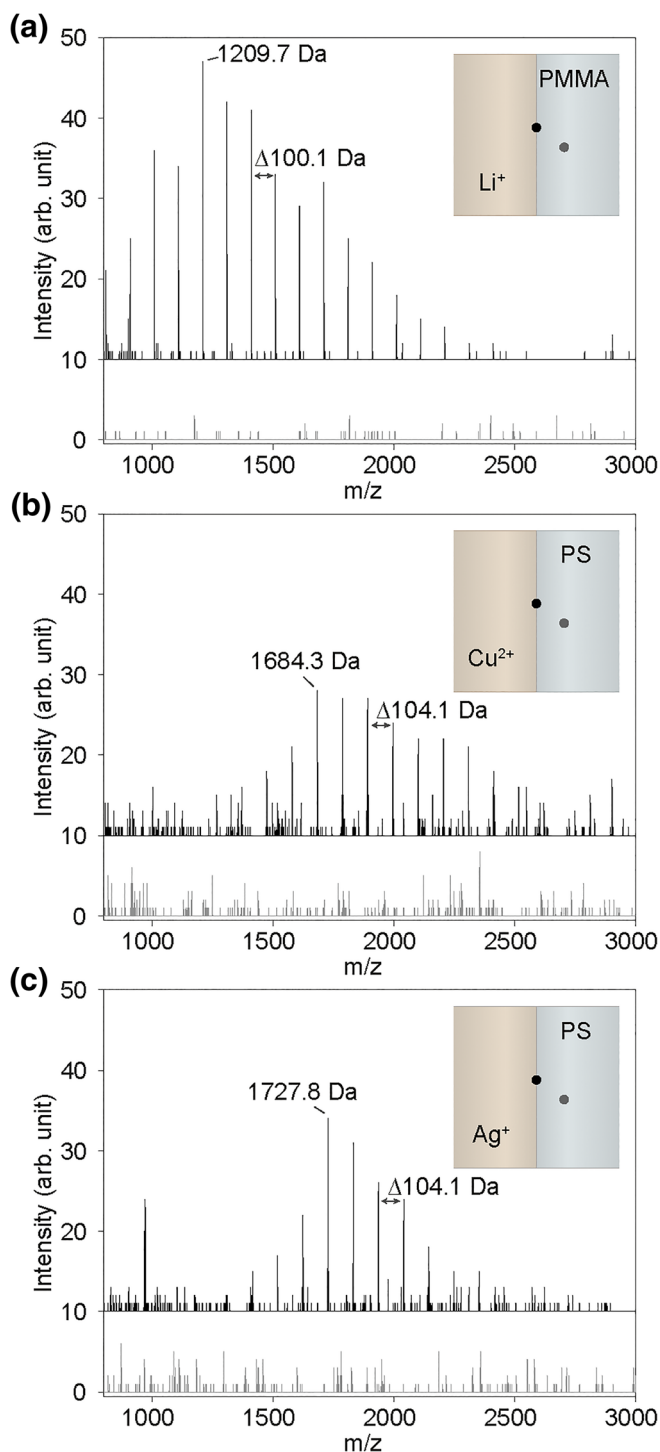
Figure 1. MALDI mass spectra (**a–c**) from split-plate experiments using PMMA-Li (**a**), PS-Cu (**b**), and PS-Ag (**c**). A schematic representation of the split plate and two ablation positions is given in the top-right corner. The black traces are the mass spectra acquired near the junction, whereas the gray traces are those measured by ablating the polymer plate only (sum of 20 shots in both cases). The black traces are offset vertically by 10 counts for ease of comparison. The peaks correspond to singly charged polymer–cation adducts

adducts are clearly visible upon ablation of the region near the junction. This result strongly suggests that ionization requires the simultaneous release of polymer and cationizing agent into the MALDI plume.

To qualify this further, the integrated signal intensity of the most abundant polymer signals was monitored when approaching the junction in small increments (see Figure SI-6 a). A step size of 25 μm was chosen, which is smaller than the laser beam diameter (111 μm diameter perpendicular to the junction axis). Each ablation position was offset by 150 μm along the junction axis to avoid overlapping spots. Representative results of this diagonal scan across the junction are shown in Figure 1. In every experiment, a distinct maximum in the integrated polymer–cation adduct signal is observed within 50 μm of the junction. Signals decrease rapidly with increasing displacement from this position and disappear within 50 μm. Adduct signals are thus observed for only one or two, and rarely three, spots for each experiment. This result is in agreement with the theoretical maximum of four overlapping ablation positions resulting from a gap size of 14 μm and a laser beam diameter of 111 μm perpendicular to the junction axis.

Each diagonal scan shown in Figure 2 was replicated several times to ensure reproducibility of the results (see Figure SI-7). We found that, although each scan shows identical qualitative behavior, there is some variability in the ablation position that gives maximum signal intensity. Namely, across all experiments, the nominal position of maximum intensity occurs at either 0 μm or + 25 μm. We attribute this slight systematic shift of the maximum intensity towards the polymer plate to a misalignment between the intended and actual ablation positions. This seems reasonable, given the limited resolution of the camera used for alignment, and the fact that initial positioning was done by manual alignment, which was necessary since the plate and the motor axes are slightly mismatched for this instrument. However, it should be noted that contributions from differences in ablation efficiencies for the polymer versus cationizing agent cannot be ruled out.

Finally, to exclude carry-over effects from previous experiments as a reason for the observed shift, additional control experiments were performed, in which only a single target plate was coated. Therefore, only polymer or cationizing agent was present at the junction and successful cation-adduct formation would have indicated carry-over of the missing reactant. This was, however, not observed in either case (see Figure SI-8 and SI-9). As the results presented here were obtained by summation of twenty laser shots per ablation position, additional control experiments were required to assess the possibility of redeposition of neutral particles released from the MALDI plume between ablation events. To assess this possibility, single-shot experiments were performed at the junction, thus excluding redeposition. For all polymer/cation pairs examined, polymer–cation adduct signals are clearly present in the single-shot experiments (see Figures SI-11). This
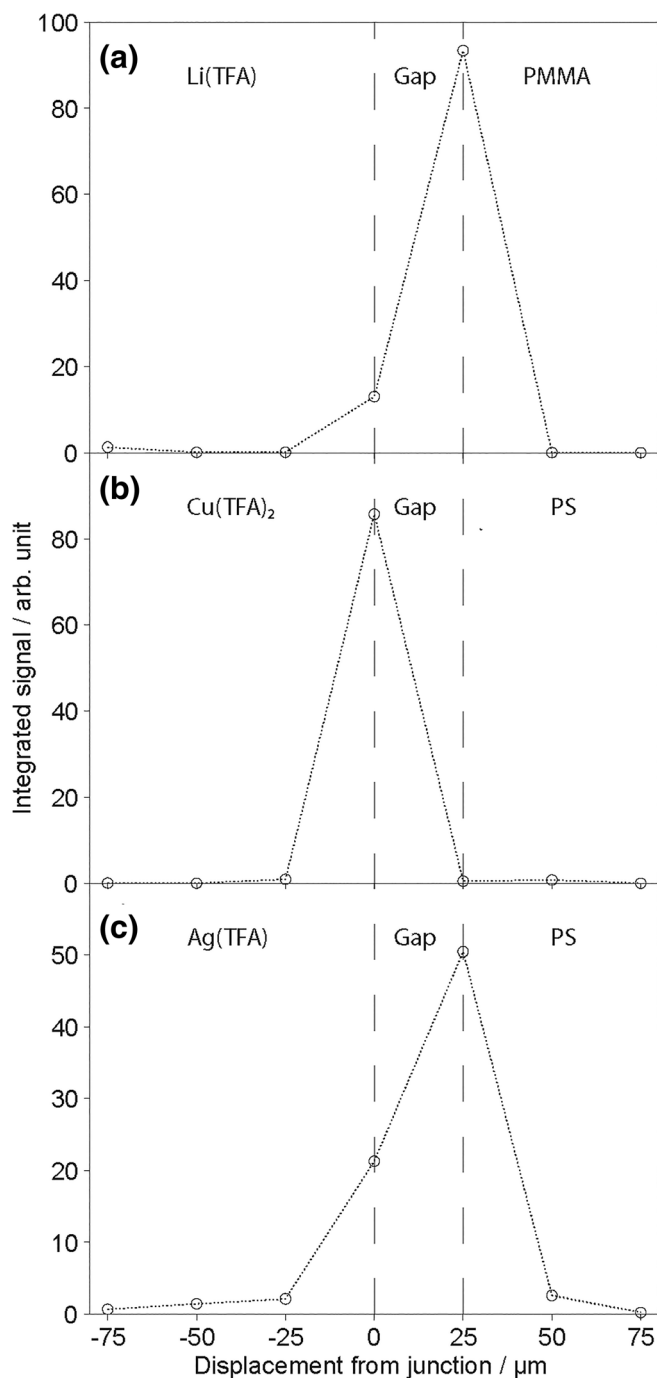
Figure 2. Integrated signal intensities PMMA-Li (**a**), PS-Cu (**b**), and PS-Ag (**c**) adducts as a function of displacement from the junction. Negative values correspond to the plate coated with matrix and metal salt, whereas positive values represent the plate containing polymer and matrix. The estimated positions of the plates and gap are indicated by dashed lines

finding is in agreement with the work performed by Erb et al. [16] and suggests that particle redeposition is not the sole origin of ionization in the experiments presented above. Contributions cannot, however, be excluded, if several laser shots are being acquired for each spot.

Contribution of In-Plume Processes to Cationization

The results presented above provide strong qualitative evidence that in-plume processes take place. However, the degree to which in-plume processes contribute to the overall polymer–cation signals observed remains unclear. As MALDI measurements on polymers are commonly performed by co-deposition, it is still possible that the majority of ions are formed by alternate pathways, most importantly a preformation. To obtain an estimate of the contribution of in-plume cationization, we carried out a comparison of co-deposited and separately deposited (split-plate experiment) polymer and salt samples. Co-deposited samples were prepared on a single MALDI plate, unlike the separate deposition, which required two plates. Thus, the concentrations used for the co-deposited samples were half of those used for the split-plate samples. This was to ensure that the total amount of polymer and cationizing agent deposited relative to the ablated surface area was kept roughly the same for the two experiments. Figure 3a compares a MALDI mass spectrum obtained by ablation of co-deposited PMMA and LiTFA on a single MALDI plate (black trace) with one using the joint plate with separately deposited polymer and cationizing agent (gray trace), as described above. Notably, the spectrum obtained by the split-plate experiment exhibits a clear shift towards lower masses relative to the spectrum measured for a co-deposited sample. Since the split plate increases the distance between the reactants, a mass discrimination based on their velocity may result. Additionally, it must be considered that the distribution of oligomers within the plume might not be homogeneous. In either case, a change of the mass distribution towards higher masses would be expected, if the distance is decreased by the removal of the stainless-steel spacer.

However, this was not observed experimentally (see Figure SI-12). Similarly, no effect of the laser fluence on the mass distribution was found (see Figure SI-14). Therefore, the shift in the apparent mass distribution might indicate a preferential ionization of large oligomers by pathways other than in-plume cationization. This would be in agreement with the findings of Jaskolla and Karas [17], who reported a higher contribution of preformation for protonation of larger molecules, whereas in-plume processes dominated for small molecules. We note, however, that a sample preparation related origin of the mass shift cannot be excluded. Sample preparation has been described previously [21, 22] as an important factor in the determination of mass distributions. To ensure the ablation of equal amounts of each substance for co-deposition and split-plate experiments, different matrix-to-analyte ratios were required. Therefore, the sample preparation was slightly different in terms of concentrations and solvents involved (see SI). Especially the lower matrix-to-analyte ratios could explain the observation, as they have been reported to favor smaller oligomers [21]. Additional split-plate experiments were performed, employing matrix-to-analyte ratios identical to those used in co-deposition experiments. No significant change in the mass distribution was observed towards higher masses (see Figure SI-13).
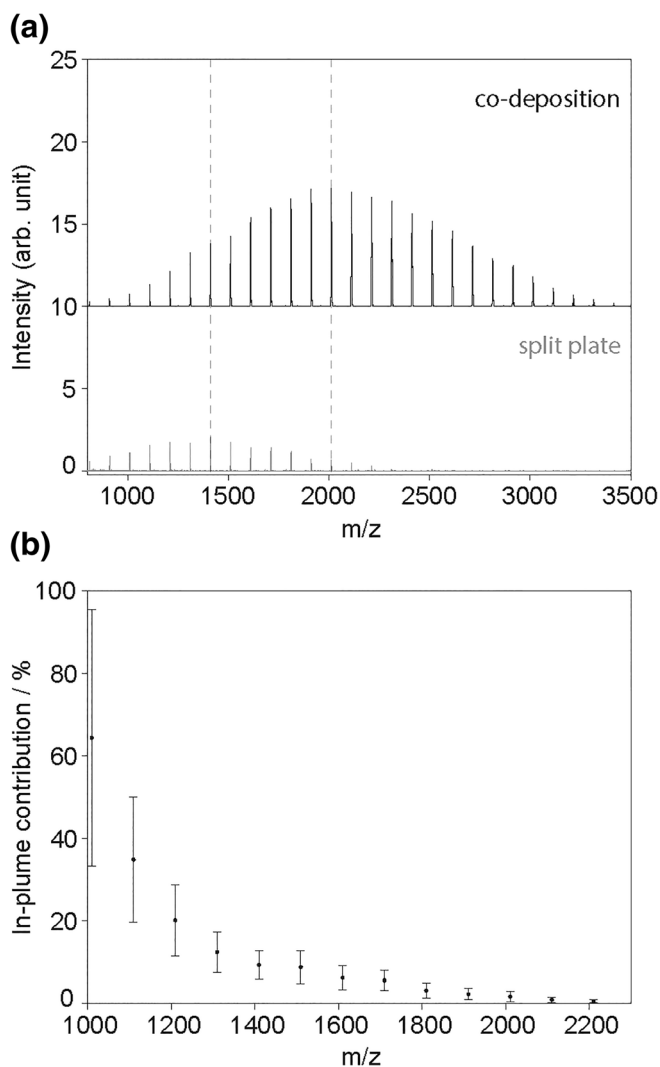
(a)

(b)

Figure 3. MALDI mass spectra (**a**) obtained by co-deposition (black trace) and split-plate experiments (gray trace), using PMMA and Li (TFA). Both spectra represent an average of fifty single-shot spectra obtained on a single plate and at the junction, respectively. The black trace is offset vertically by 10 counts for ease of comparison. Relative contributions of in-plume cationization (**b**) were calculated for each PMMA-Li adduct individually, by division of the integrated signal intensities

Figure 3b compares the integrated peak intensities for each of the individual polymeric species shown in the mass spectra in Figure 3a. The data are shown as the ratio of the absolute abundance measured in the split-plate experiment relative to that measured in the co-deposition experiments, using identical instrument settings. The lower mass PMMA-Li adducts (< 1000 Da) were not considered in the analysis due to their low abundance. These data can be considered as a relative measure of the contribution of in-plume cationization to the total intensity observed in the mass spectrum. However, due to the different mass distributions, contributions were calculated for each species. The relative contribution ranges from 64 to 9% for polymers containing ten to fourteen methyl methacrylate subunits. We emphasize that these values should be only taken

as lower bounds. This is due to the fact that a significant fraction of the ablated material, statistically at least half, will have insufficient radial velocity to merge with the other MALDI plume. Moreover, the split-plate set-up includes a gap between the two MALDI plates, which reduces the total amount of material ablated. In this light, contributions are significant for low mass oligomers, whereas the picture for larger oligomers is less clear due to the unknown origin of the change in mass distribution.

Conclusions

The formation of cation adducts was monitored in the mixed plume at the junction between two MALDI plates, coated with either polymer or cationizing agent. Adducts were exclusively formed upon laser irradiation at or in close proximity to the junction. This demonstrates that polymer–cation adducts, exhibiting different kinds of electrostatic or complexing interactions, can be ionized by an in-plume process. A comparison of the signals obtained on the split-plate set-up with those of a co-deposition experiment reveals a significant contribution of in-plume processes to the ionization of low mass oligomers. However, the relative contribution was found to decrease with increasing polymer mass. This could be due to a preferred ionization by alternate pathways, for example ion preformation, for larger oligomers. Although, a shift in the mass distribution due to the sample preparation conditions cannot be fully excluded either.

Acknowledgements

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