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APPLICATION NOTE

DESI Spray Stability in the Negative Ion Mode Is Dependent on Relative Humidity

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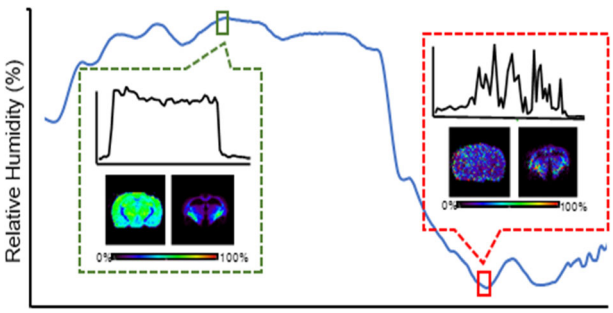

Abstract. Ambient ionization mass spectrometry (MS) techniques, such as desorption electrospray ionization (DESI), have been increasingly used due to their simplicity, minimal sample preparation requirements, and potential applications in the field and the clinic. However, due to their intrinsic nature, the performance of these methods is susceptible to variations in ambient conditions. Here, we present data that suggests DESI-MS analysis becomes inconsistent below a relative humidity (RH) level of ~ 35%. At low RH, we hypothesize that the DESI spray is subjected to frequent electrical discharges, resulting in unstable ionization and atypical mass spectra. Consequentially, poor image quality is observed when used for tissue imaging. Our results suggest that RH control should be considered in DESI-MS experiments to assure data quality.

Keywords: Desorption electrospray ionization, Mass spectrometry imaging, Humidity, Data quality

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Introduction

The field of ambient ionization MS was introduced over a decade ago with the development of desorption electrospray ionization (DESI) [1], and direct analysis in real time (DART) [2] techniques, opening opportunities for MS to be used in settings where complex sample preparation and in vacuum analysis are not desired. Since then, tens of ambient ionization MS techniques have been established and applied in various research fields including drug development [3], disease analysis [4–6], plant biology [7], forensics [8], and others. A fundamental feature of ambient ionization MS is an open atmosphere analysis with minimal sample preparation requirements [9]. In DESI, a spray of charged microdroplets is directed towards a sample surface to desorb, ionize, and transfer molecules from the sample surface to a mass spectrometer for analysis [1]. When DESI-MS is performed in the imaging mode, two-dimensional maps of the chemical constituents are obtained, displaying the intensity and distribution of the ions throughout the surface analyzed [10]. DESI-MS imaging has been applied to a variety of samples [11, 12], with exciting applications to biological tissue section imaging and disease diagnosis [13]. As such, the effects of sample handling and experimental protocols on the quality of the data obtained from biological tissue sections have been previously investigated [14]. Nevertheless, operation in an ambient environment comes with the cost of potential susceptibility to changes in environmental conditions. In particular, relative humidity (RH) has been shown to impact ionization stability and efficacy in several MS techniques [15, 16]. For example, the effect of RH on the mass spectra obtained using plasma-based ambient ionization sources, such as DART and flowing atmospheric pressure afterglow (FAPA), has been reported [17, 18]. Newsome et al. suggested that small variations in RH that occur in a laboratory environment can lead to large variations in DART and FAPA mass spectra due to altered ion fragmentation during ionization [19]. Several studies have investigated how variations in RH alter the Taylor cone formation and ionization in electrospray ionization (ESI) sources [20, 21]. Throughout the last year, our

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group observed substantial variations in spray stability during DESI-MS imaging experiments performed in the negative ion mode in our laboratory. After many attempts in source optimization followed by a systematic investigation of the potential factors leading to instability, we determined RH to be a crucial yet often overlooked factor influencing data quality in DESI-MS imaging. Here, we describe how RH affects DESI spray stability in the negative ion mode, and briefly discuss why this instability due to RH should be avoided in DESI-MS imaging.

Experimental

DESI-MS analyses were performed in the negative and positive ion modes on an LTQ-Orbitrap Elite and a Q Exactive mass spectrometers (Thermo Fisher Scientific) at 60,000 resolving power, using lab-built DESI sprayers, a commercial DESI sprayer (Prosolia Inc., Indianapolis, IN), and a commercial 2D moving stage (Prosolia Inc., Indianapolis, IN). Ion images were assembled using BioMap software (Novartis). The solvent system used was 1:1 acetonitrile:dimethylformamide at a flow rate of 1.2 µl/min, unless otherwise stated. Mouse brain tissues were sectioned at a 16-µm thickness, mounted onto glass slides, and stored at – 80 °C until analysis. The Pierce LTQ ESI negative ion calibration solution was used for spray stability analysis (Thermo Fisher Scientific, Waltham, MA). RH was monitored using a TP425 Dickson data logger (Addison, IL). Metabolite and lipid species were tentatively identified using high mass accuracy measurements, collision-induced dissociation, and high-energy collision-induced dissociation tandem MS analyses, performed using the Orbitrap as the mass analyzer of the LTQ-Orbitrap Elite mass spectrometer.

Results and Discussion

The DESI-MS imaging experiments of mouse brain tissue sections described here were performed in the negative ion mode from October 2017 to April 2018 using optimized experimental parameters [22]. During this period, unexpected scan-to-scan variability was observed in the total ion current (TIC) chromatograms obtained on different days of analysis, as shown in Figure 1 for a period of 9 days from February 28 to March 8. In conjunction with TIC instability, the mass spectra obtained were inconsistent from scan-to-scan (Figure S1), with atypical relative abundances of metabolites and lipids species commonly detected in DESI mass spectra. Under optimized experimental conditions, the relative standard deviation (RSD) of the TIC obtained when analyzing across a single line of a mouse brain tissue section was 21.3%, resulting in chromatograms presenting a smooth pattern. The RSD calculated for the TIC under unstable conditions were significantly higher (68.6%), indicating an issue in our experiments. Furthermore, we observed that the spray stability often changed day-to-day or even hour-to-hour, despite no change in the DESI-MS source or instrument parameters. To investigate the potential

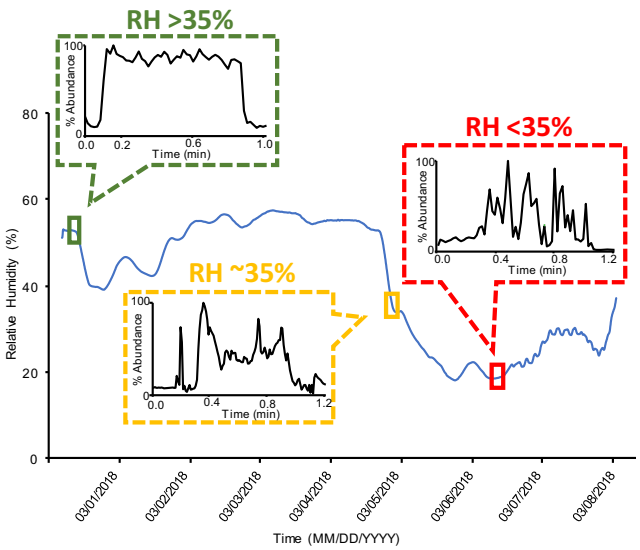

Figure 1. TIC plots obtained from a single line scan DESI-MS analysis of mice brain tissue sections at various RH levels. At RH > 35% (green outline), the TIC is stable and rapidly increasing and decreasing when moving on and off the tissue sample, respectively. At RH ~ 35% (yellow outline), the TIC begins to destabilize, showing seemingly random fluctuations. At RH < 35% (red outline), the TIC plot shows large variations throughout the tissue that are indicative of DESI spray instability

causes for the inadequate DESI-MS performance, we systematically evaluated hardware and experimental conditions including DESI source optimization and mass spectrometers (details described in the Supporting Information and Figures S2–S7), all of which led to the hypothesis that the spray instability was rather related to an external or environmental factor. A noteworthy observation was that decreasing the voltage applied from 5 kV to 0 kV (Figures S2 and S3) during a single DESI experiment appeared to alleviate spray instability, suggesting that the issue could be related to corona discharges occurring at the spray tip. Corona discharge is a common phenomenon in both negative and positive ion mode ESI, although corona discharge often onsets at higher voltages in the positive ion mode (> 5 kV) than in the negative ion mode [23]. This hypothesis is also corroborated by the alterations observed in the mass spectra, as shown in Figure 2a. Typical mass spectra acquired from mouse brain tissue sections using optimized DESI-MS conditions are commonly reproducible [14, 24, 25]. In the negative ion mode, deprotonated lipid species including *m/z* 834.528 (glycerophosphoserine (PS) 18:0_22:6) and *m/z* 885.549 (glycerophosphoinositol (PI) 18:0_20:4) are commonly observed at high relative abundances in the gray matter region of the brain, while *m/z* 888.623 (sulfatide (ST) C24:1) is commonly observed at the high relative abundance in the white matter [14, 24, 25]. On the other hand, atypical mass spectra obtained during the period of experimental instability presented a higher relative abundance of chloride adducts of lipid species, specifically ceramide (Cer) d36:1 at *m/z* 600.513. The increase in chloride adducts suggests that electrical discharge may be occurring at the DESI spray tip, as chloride adduct formation is more common in plasma-based

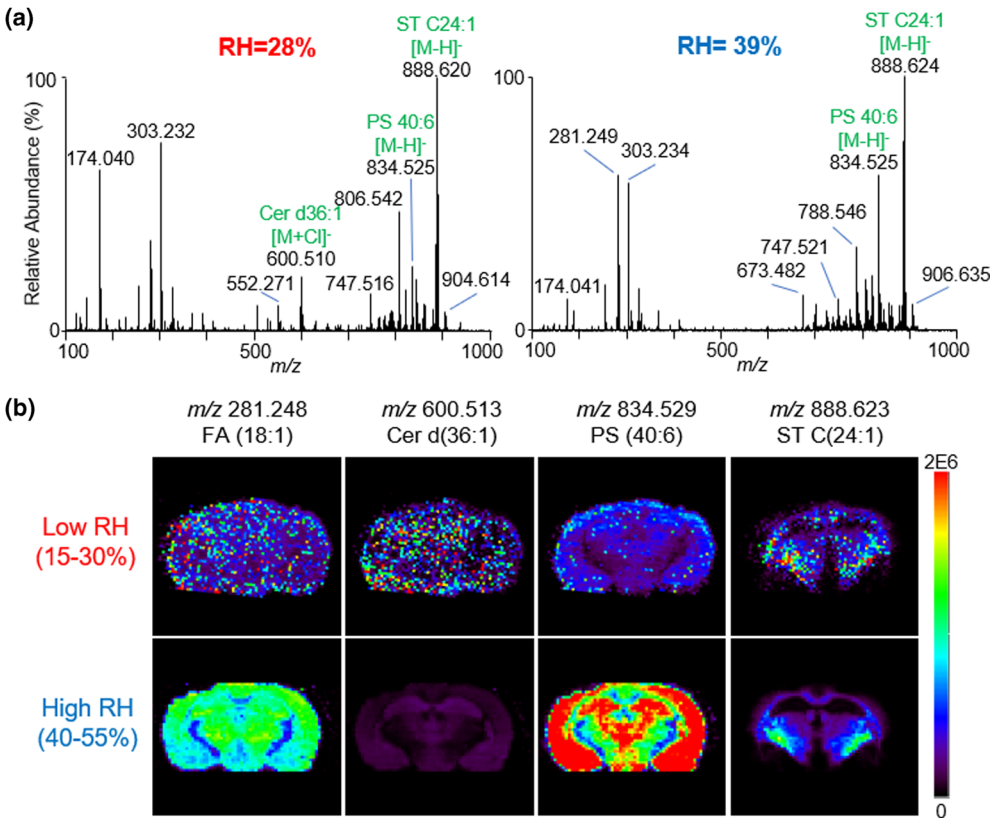

Figure 2. (**a**) Representative mass spectra from the white matter of a mouse brain tissue section at high (39%) and low (28%) RH. Each spectrum is an average of 10 scans. (**b**) Representative DESI-MS ion images collected at low and high RH levels. Low humidity results in images with erratic ion distributions that are not reflective of the true molecular distribution. High humidity results in images that are consistent with what has been published in the literature

than spray-based ionization mechanisms [26]. Thus, our observations suggested that an external factor was contributing to a discharge increase, causing spray instability. Previous work has suggested that the maximum voltage that can be applied to an atmospheric pressure ESI source without inducing corona discharge is higher in summer months when atmospheric conditions are presumably more humid [16]. Therefore, we explored the possibility that the external factor affecting DESI spray stability was the RH in our laboratory.

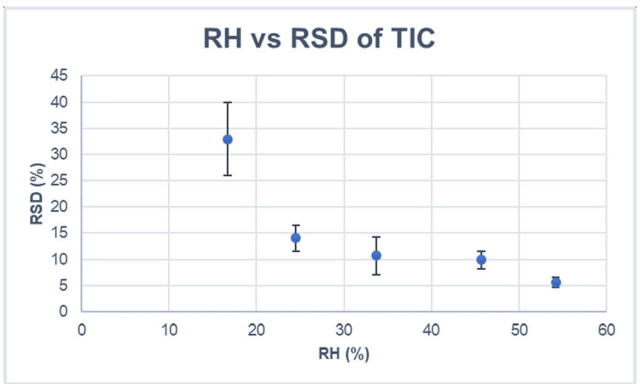

Figure 3. RH% vs. RSD% of the TIC at five RH% ranges: 10–20% ($n = 4$), 20–30% ($n = 6$), 30–40% ($n = 9$), 40–50% (n = 4), and 50–60% ($n = 10$). Both the average RSD and the RSD variance for the 10–20% and 50–60% ranges were significantly different from each other ($p < 0.05$), suggesting there are observable and noteworthy differences in the stability of DESI depending on RH%. An average of eight scans were used for each recorded RH value. *T* tests were performed using unequal variance assumptions

Upon investigation of DESI-MS spray stability during times of low (~ 15–30%) and high (~ 40–55%) RH, we found a startling difference in the steadiness of the overall TIC (Figure 1) and the mass spectra (Figure 2a) depending on RH. Figure 2b shows the DESI-MS imaging results of two serial mouse brain tissue sections analyzed only 20 hrs apart at low and high RH levels, using the same mass spectrometer and nearly identical DESI source conditions and spatial resolution. As expected from the large variations in the TIC and mass spectra patterns in low-RH conditions, the DESI ion images acquired at low-RH conditions were not in agreement with the lipid distributions commonly seen in mouse brain tissue, which have been extensively reported in the literature using various ionization methods [25, 27]. At low RH, the intensity of the molecular ions spiked sporadically, resulting in spotty ion images and a higher relative intensity of the chloride adduct of (Cer)

d36:1 at *m/z* 600.513. An atypical reduction in the relative abundance and total intensity for a variety of phospholipid species, including PS 18:0_22:6 and PI 18:0_20:4, was also observed. Under higher RH levels, smooth TIC as well as typical mass spectra and ion spatial distributions were observed in the DESI-MS imaging data for the mouse brain tissue, in agreement with what has been extensively reported in the literature [10, 25].

Further experiments were performed to more confidently determine that RH significantly contributes to DESI spray instability. A semi-enclosed DESI-MS chamber connected to a humidifier and a nitrogen gas source was used to control RH levels during DESI-MS imaging experiments at constant temperature ($T = 23$ °C). Figure S8 shows the data acquired in a DESI-MS experiment within the same mouse brain tissue section, with the first TIC chromatogram taken at ~ 60% RH followed by a purge of the humid air with N_2 gas to acquire the second TIC chromatogram at ~ 16% RH. The RSD in TIC of the scans acquired at 60% RH was 3.3%, while an RSD of 23.6% was calculated from the data collected at 16% RH. Note that these RSD values are lower than those reported when the DESI system is not enclosed, suggesting that there is a difference in running these experiments in an enclosed system compared to the open environment. However, these results still suggest an increase in DESI spray instability due to RH levels. To further evaluate the relationship between DESI signal stability and RH, we plotted the RSD between TIC values collected at five RH ranges, 10–20%, 20–30%, 30–40%, 40–50%, and 50–60%, for the same regions of mouse brain tissue sections (Figure 3) using the semi-enclosed chamber. These data show that the RSD of the TIC is higher and more variable at lower RH than at higher RH levels, and the difference was statistically significant between the lowest and highest RH ranges investigated ($p < 0.05$). Similar experiments were conducted in the positive ion mode and as previously noted RH did not appear to have a significant impact on DESI spray stability or the acquired spectra (Figure S9). Note that further work is needed to evaluate the effects of even higher RH (> 70%) on DESI spray stability, as this range could not be achieved and thus investigated in our study due to the limitations in our laboratory and experimental conditions.

Conclusions

We have performed a study to evaluate the causes of signal instability in optimized DESI-MS experiments and have determined that DESI spray stability is dependent on RH levels in the negative ion mode under the experimental conditions evaluated. Under low-humidity conditions (~ 15–30%), negative ion mode DESI mass spectra were prone to spray artifacts resulting in atypical ion abundances and inaccurate spatial distributions, while stable and typical mass spectra were obtained in RH levels of 35–60%. Our data suggest that signal instability could be due to corona discharges occurring at the DESI spray under low-humidity conditions, although this hypothesis is not fully confirmed (Figure S10). As a primary goal of DESI-MS imaging is to obtain reproducible and spatially accurate mass spectral data from tissue sections, the reduction in data quality associated with low-RH conditions is noteworthy. Further, as DESI-MS imaging is often used to qualitatively evaluate trends in mass spectra patterns that allow disease diagnosis and statistical classification [13], attention to this matter is important. Special consideration should be given in winter months when RH is typically lower, which was the case in our study, as buildings may not have advanced humidity control infrastructure to adjust for drastic local weather fluctuations. Thus, based on the data presented here, we suggest that RH conditions should be monitored and maintained at levels of 35–60% RH to favor the acquisition of high quality and reliable negative ion mode DESI-MS data.

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