Humidity Affects Relative Ion Abundance in Direct Analysis in Real Time Mass Spectrometry of Hexamethylene Triperoxide Diamine

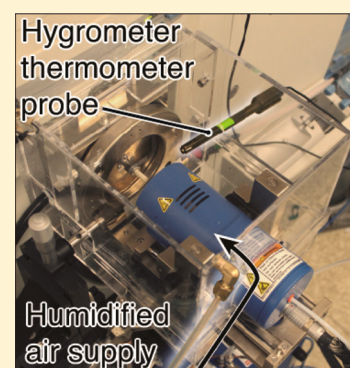G. Asher Newsome,*,[†] Luke K. Ackerman,[‡] and Kevin J. Johnson[§]

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S Supporting Information

ABSTRACT: Unstable explosive hexamethylene triperoxide diamine (HMTD) is danger- ous in quantity and benefits from the minimal sampling handling associated with atmospheric pressure chemical ionization for mass spectral analysis. Seasonal variation observed in HMTD mass spectra suggested a humidity dependence. Therefore, direct analysis in real time (DART) ionization mass spectra were acquired at a range of humidity values. An enclosure was designed to fit around the ion source and mass spectrometer inlet at atmospheric pressure. The enclosure was supplied with controlled amounts of humidified air from a test atmosphere generator to create programmable conditions for ambient analysis. The relative abundance and fragmentation of analyte ions were observed to change reliably with changing humidity values and, to a lesser degree, temperature. Humidity at such plasma-based ion sources should be regulated to avoid ~90% shifts in relative ion abundance and provide stability and reproducibility of HMTD analysis.

Cyclic peroxide explosives have seen widespread use in terrorist acts and are of interest to law enforcement and forensic research communities. Compounds like hexamethylene triperoxide diamine (HMTD) are sensitive to impact, friction, heat, and static electricity. Hazards associated with handling this substance increase with larger quantities, and the difficulty of transporting such explosives precludes military and commercial use. HMTD in particular has been little studied, as it decomposes at room temperature[1,2] and has a low vapor pressure.[3,4] Mass spectrometric detection is desirable to accurately identify and characterize trace amounts of HMTD. Ambient ionization techniques like atmospheric pressure chemical ionization (APCI) can be safely used for rapid, real- time analysis of HMTD with no sample pretreatment and minimal handling.[5,6] APCI techniques also minimize fragmen- tation of HMTD to produce abundant signal from an intact molecular adduct ion.[7]

A variety of plasma-based APCI techniques operating on similar principles have been developed in recent years.[8−13] The present work makes use of the only commercialized post- plasma APCI method, direct analysis in real time (DART).[14] DART is thought to sample analytes on surfaces by thermal desorption, and among the various discharge gases tested, helium has produced the greatest sensitivity for a range of analytes.[15] Metastable helium produced by a plasma discharge is proposed to ionize analytes directly by Penning ionization[14] and to ionize atmospheric gases like nitrogen and water vapor to form reagent ions. Penning ionization and charge transfer reagent ions (protonated water) produce analyte ions and a

residual background of protonated water clusters.[15−17] Intact analyte molecular adduct ions like the protonated molecule (MH$^+$) are commonly observed, but radical cations and deprotonated molecules can also be produced for some analytes.[17]

The effects of humidity on DART are rarely acknowledged in ambient ionization literature, apart from a minimum necessity for water vapor as a proton source.[18−20] The implication has been that humidity above some excess of thermally desorbed analyte causes no change in mass spectra. This is widely assumed in part because the literature refers to reaction with water vapor as rapid[14] and because the role of water vapor concentration on DART mass spectra has never been explored. Indoor humidity is directly dependent on the operation of building temperature control, air handling, and the source of the air. In outdoor settings, ambient humidity is inherently unstable over time and uncontrollable. As a consequence, the concentration of reagent water vapor in ambient air at the ion source may vary widely over time. As development of post- plasma techniques such as DART has become a competitive field, applications move from qualitative analysis toward quantitation, and as ambient ionization is performed in field analysis, practical effects on reproducibility must be addressed. The present work represents the first published systematic
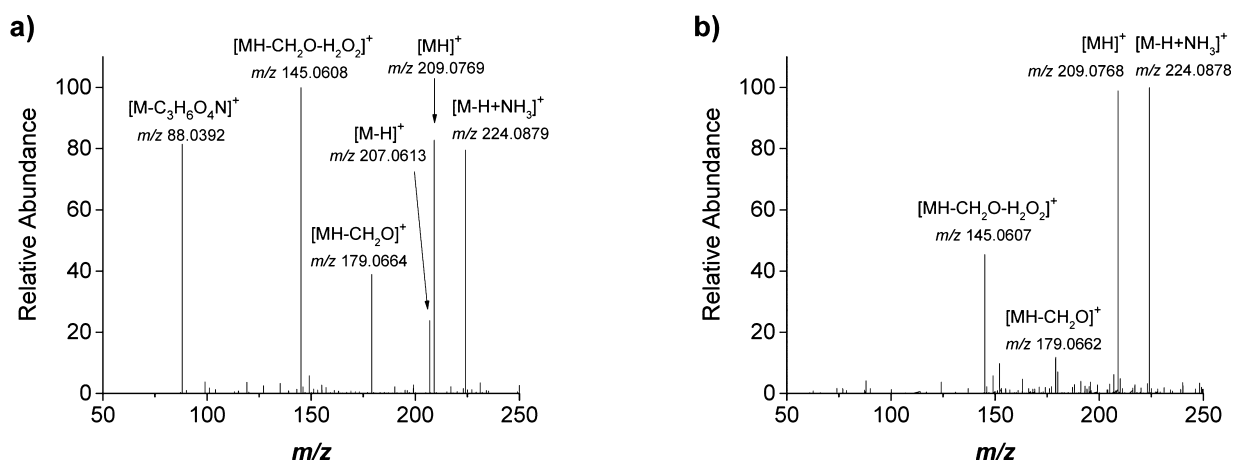
Figure 1. DART mass spectra of HMTD from surface deposit in an enclosure at (a) 5.0 g/m^3 absolute humidity and 30.8 °C and (b) 12.8 g/m^3 absolute humidity and 30.5 °C, background-subtracted.

study of humidity effects on DART mass spectra of an analyte. An enclosure around the ion source and MS inlet was used to supply and regulate humidity. Data is presented in terms of signal ratios between observed ions and as percent fragmentation,

$$\% \text{ fragmentation} = 100 \times \frac{\sum_i F_i}{M + \sum_i F_i}$$

where M is intact molecular adduct ion abundance and F is the abundance of each fragment ion originating from the intact molecular adduct ion.

■ METHODS

HMTD was obtained from the FBI Explosives Unit (Quantico, VA) in static-free vials containing a maximum of 0.5 g of solids within an MK663 container (Camtech Precision Manufacturing, Inc., Auburn, NY). With laboratory surfaces and personnel electrically grounded and with no lightning within 30 nautical miles of the laboratory presently or within the prior 2 h, HMTD was diluted to 80 ppm in acetonitrile behind a blast shield certified to a net explosives weight of 1.5 g. Bulk solution was spotted onto laboratory tissue and allowed to dry, leaving analyte deposits on the order of nanograms/mm^2. Solids and solutions were stored at −40 °C.

A DART-SVP with Vapur interface (IonSense, Saugus, MA) was used with an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA) for analysis. Ultra high purity helium (5.0 grade, Airgas, Radnor, PA) was used as DART discharge gas. The DART SVP source was on-axis with the MS and Vapur inlets and ∼2 cm from the Vapur's 1 cm i.d. ceramic transfer tube. The DART SVP was operated in positive mode with the helium discharge heated to 150−450 °C, controlled by software. The differential membrane pump for the Vapur interface was adjusted until the LTQ ion gauge was at or below 2.4 × 10^{-5} Torr. The MS inlet capillary was operated at 200 °C and 38 V with a 100 V tube lens. Sample deposits on paper were mounted in front of the ion source orthogonally to the flow and positioned by an *xyz* translation stage so that the edge of the paper intersected the flow path. Orbitrap mass spectra were collected with 25 ms maximum inject time. Data were extracted from .raw files for integration of ion signals collected in profile mode.

A humidity control enclosure was designed to meet to the instrument housing around the ion inlet and fabricated from 3.2 mm-thick acrylic sheets (California Quality Plastics). The design consisted of a 20 cm cube with one open face joined to a frame in contact with the instrument housing. The enclosure interface to the instrument housing was not sealed but reinforced with tape at select points where the housing had a degree of curve. The enclosure was supported from below by table mounts. The upper portion of the enclosure was removable to allow placement of sample surfaces and to allow installation of the IonMax ESI source for periodic instrument calibration. Prior to use with DART, the enclosure was allowed to outgas from materials used in its construction and mounting.

The enclosure contained feedthroughs for atmosphere sensors and was cut to allow the rear of the DART source and stage to extend out of the cubic chamber. A test atmosphere generator (Miller-Nelson HCS-501-50, Assay Technology, Livermore, CA) humidified air by passing filtered air over a water reservoir that was heated according to an internal feedback relative humidity measurement of outgoing test atmosphere. Humidified air at a flow rate of 23 L/min was supplied by a 0.22 m long Teflon tubing to an elbow fitting in a corner of the enclosure opposite the ion inlet. A 10 × 7 cm baffle (removed in graphical abstract) was positioned 11 cm from the humidity supply port to aid in atmospheric mixing within the enclosure. The enclosure was not vacuum-sealed, and humidity-controlled air escaped at the edges of the MS housing interface and the feedthroughs. Ambient pressure was maintained, as measured by a pressure transducer (Omegadyne model PX309-015GV, Sunbury, OH) when the test atmosphere flow was on. A thermohygrometer probe (Testo model 625, Sparta, NJ) was mounted inside the enclosure 5 cm lateral to the ceramic ion inlet of the Vapur interface. Relative humidity and temperature measured inside the enclosure were used as feedback to the test atmosphere generator settings and were recorded during every analyte spectrum acquisition.

The recommended operating conditions for DART are 30−70% relative humidity at 10−32.2 °C,[20] a total absolute humidity range of 2.8−24.0 g/m^3. All analyses using the enclosure were performed within the recommended absolute humidity range and near the upper temperature limit. Analyses performed with the enclosure below ∼10 g/m^3 were below the recommended relative humidity range but modeled the
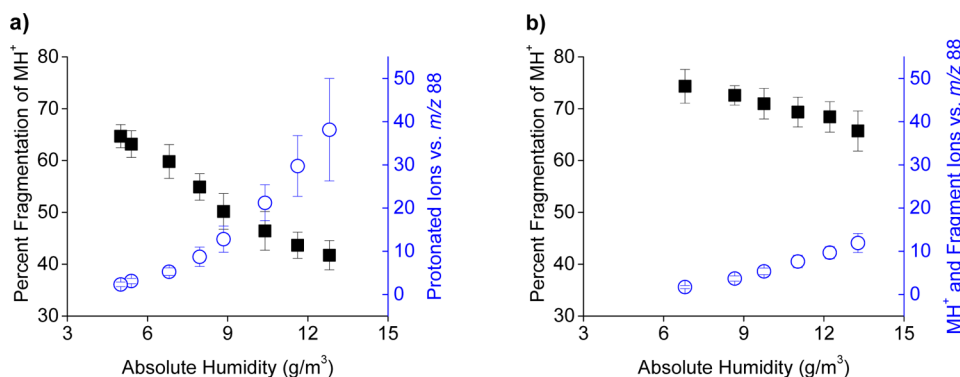
Figure 2. DART signal from HMTD with variation of enclosure humidity at (a) 150 °C discharge gas temperature and 30−31 °C enclosure temperature and (b) 350 °C discharge gas temperature and 34−35 °C enclosure, shown as percent fragmentation of protonated molecule (■) and ratio of protonated molecule plus fragment ions to $M^{+\bullet}$ radical fragment ion m/z 88 (blue ○) (±one standard deviation).

frequent occurrence of ambient laboratory air having less than the recommended 30% relative humidity. Over the course of one year in the laboratory where the present work was performed, relative humidity levels were recorded from 7% to 56% at 21−29 °C, an absolute humidity range of 1.3−16.0 g/m^3. With variations in local weather, laboratory relative humidity changed up to ±15% between consecutive days and up to ±5% over the course of a single day.

DART is unique among post-plasma sources in its ability to control discharge gas temperature. Regardless of the temperature setting, the source necessarily creates a gradient of temperature and humidity in air around the heated discharge gas. Thermohygrometer readings were recorded at a constant position within this gradient and were used as representative of the ionization region. The humidity was varied at both 150 and 350 °C discharge gas temperatures, and discharge temperature was varied at constant humidity. Humidity in the enclosure was adjusted by changing the settings of the test atmosphere generator and allowing thermohygrometer readings to stabilize for several minutes before analysis. Operation of the DART source caused a small temperature increase within the enclosure relative to the room. Mass spectra at various enclosure humidity values were acquired consecutively within 1 °C of each other. Pressure in the enclosure did not increase detectably above atmospheric pressure.

■ RESULTS AND DISCUSSION

Changing the humidity dramatically altered the DART-mass spectrum of HMTD, with major ions reproducibly changing by as much as 90% in relative abundance at commonly observed room humidity conditions. The effects of changing humidity were studied at approximately constant enclosure temperature. The major ions in DART mass spectra of HMTD were the previously observed MH^+ at m/z 209 and two fragment ions of MH^+ at m/z 179 and 145;[21,7] $[M − H]^+$ at m/z 207;[22−24] $[M − H + NH_3]^+$ at m/z 224;[25] as well as a radical fragment ion at m/z 88[26] (Figure 1). The latter radical fragment ion, identified by accurate mass as $[M − C_3H_6O_4N]^+$ ($[C_3H_6O_2N]^+$), was not observed from separate collision induced dissociation experiments on the intact molecular adduct ions or other ions, whereas protonated fragment ions were (not shown). An intact molecular ion corresponding to the radical fragment ion was also not observed. Depending on the humidity, the radical fragment ion at m/z 88 was observed near base peak abundance or less than 5%.

At 150 °C, MH^+ fragmentation decreased with increasing humidity, reducing the abundance of m/z 145 from base peak to less than half of MH^+ (Figure 2a). In the same mass spectra, protonated molecule and fragment ion abundance changed inversely with radical fragment ion abundance as a function of humidity. The inverse trend suggests competitive ion mechanisms between protonation and radical formation. The ratio of $[M − H]^+$ to $[M − H + NH_3]^+$ was decreased by increasing humidity, and the abundance of deprotonated molecule adduct ions remained constant relative to protonated molecule adduct ions (Supporting Information Figure S-1). Unlike the competition between protonation and radical fragmentation, there was no observed competition between proton and ammonia addition. These trends were also observed at discharge gas temperatures of 350 °C (Figure 2b), where MH^+ fragmentation and the abundance of the m/z 88 radical fragment ion decreased with increasing humidity, although the slopes changed.

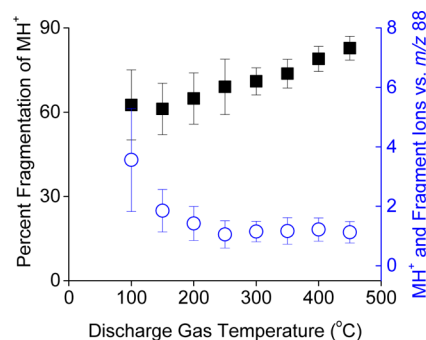The increasing DART-MS discharge temperature produced similar effects to decreasing humidity (Figure 3). MH^+

Figure 3. DART signal from HMTD with variation of discharge gas temperature at ~5.7 g/m^3 ambient absolute humidity and 24.7 °C, shown as percent fragmentation of protonated molecule (■) and ratio of protonated molecule plus fragment ions to $M^{+\bullet}$ radical fragment ion m/z 88 (blue ○) (±one standard deviation).

fragmentation increased linearly with temperature above 150 °C, while the ratio of MH^+ and fragment ions to the radical fragment ion did not change above 150 °C. The relative abundance of the m/z 88 radical fragment ion increased with discharge gas temperature to make it the base peak above 200 °C. Heating the discharge gas likely has a drying effect on the ionization region, and fragmentation also increased from excess

thermal energy. Although the results depicted in Figures 3 and 2a are observed at lower ambient temperatures than those in Figure 2b and have larger error bars, the trends are consistent and significant. The degree of uncertainty also decreases as discharge gas temperature increases. It is likely that, at higher temperatures, a more uniform humidity and temperature gradient in the ionization region causes a decrease in the ambient fluctuations that affect signal.

■ CONCLUSIONS

DART mass spectra of HMTD were shown to change significantly with variations in humidity around the ion source. Mass spectral response was shown to depend on humidity at approximately constant temperature. DART control of discharge gas temperature has a stabilizing effect on humidity. Increasing the DART discharge gas temperature lowers the effect of humidity on the spectrum but also causes more fragmentation. The humidity range commonly observed in ambient laboratory air changes which intact or fragment ion adduct is the base peak in the HMTD DART mass spectrum.

In the absence of a source enclosure or sweep gas, a commercialized APCI system like DART may specify an operating range for humidity. However, as with the effects of background gas-phase contaminants familiar to ambient ionization users, circumstances frequently prevent control of ambient humidity. Laboratory air in poorly and well-regulated facilities alike is susceptible to humidity variation, particularly with seasonal changes. As results with HMTD show, even small variations in humidity may cause significant mass spectral variations. Such significant changes in signal abundance especially impair trace detection and quantitation. Other molecules forming protonated molecule and radical ion forms by DART are likely to exhibit similar humidity effects to those observed with HMTD. The effects of humidity on post-plasma APCI mass spectra will be further explored with additional analytes and ion sources in future work.

■ ASSOCIATED CONTENT

ⓢ Supporting Information
Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Author Contributions
All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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