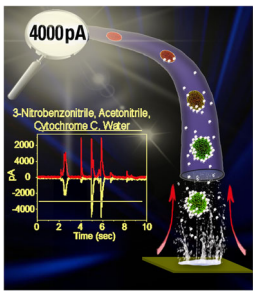
J. Am. Soc. Mass Spectrom. (2018) 29:304–315
DOI: 10.1007/s13361-017-1788-7

FOCUS: 32nd ASILOMAR CONFERENCE,
NOVEL INSTRUMENTATION IN MS AND ION MOBILITY: RESEARCH ARTICLE

Spontaneous Charge Separation and Sublimation Processes are Ubiquitous in Nature and in Ionization Processes in Mass Spectrometry

Sarah Trimpin,[1,2] I-Chung Lu,[1] Stephan Rauschenbach,[3] Khoa Hoang,[4] Beixi Wang,[1] Nicholas D. Chubatyi,[4] Wen-Jing Zhang,[1] Ellen D. Inutan,[1,2] Milan Pophristic,[2,4] Alexander Sidorenko,[4] Charles N. McEwen[2,4]

[1]Department of Chemistry, Wayne State University, Detroit, MI 48202, USA
[2]MSTM, LLC, Newark, DE 19711, USA
[3]Electrospray Ion Beam Deposition Laboratory, Nanoscale Science Department, Max-Planck-Institute for Solid State Research, DE-70569, Stuttgart, Germany
[4]Department of Chemistry and Biochemistry, University of the Sciences, Philadelphia, PA 19104, USA



Abstract. Ionization processes have been discovered by which small and large as well as volatile and nonvolatile compounds are converted to gas-phase ions when associated with a matrix and exposed to sub-atmospheric pressure. Here, we discuss experiments further defining these simple and unexpected processes. Charge separation is found to be a common process for small molecule chemicals, solids and liquids, passed through an inlet tube from a higher to a lower pressure region, with and without heat applied. This charge separation process produces positively- and negatively-charged particles with widely different efficiencies depending on the compound and its physical state. Circumstantial evidence is presented suggesting that in the new ionization process, charged particles carry analyte into the gas phase, and desolvation of these particles produce the bare ions similar to electrospray ionization, except that solid particles appear likely to be involved. This mechanistic proposition is in agreement with previous theoretical work related to ion emission from ice.

Keywords: Sublimation/evaporation, Spontaneous charge separation, Temperature/pressure relationship, Charged particles, Microscopy

Received: 2 July 2017/Revised: 8 August 2017/Accepted: 14 August 2017/Published Online: 27 October 2017

Introduction

In matrix assisted ionization (MAI) mass spectrometry (MS) [1], typically analyte is mixed in low concentration with a high concentration solution of a specific matrix compound such as 3-nitrobenzonitrile (3-NBN) [2], or one of the more than 40 other matrix compounds reported [3, 4]. The solution is allowed to dry and then exposed to the sub-atmospheric pressure of the mass spectrometer, although drying is not a requirement as it will occur when the sample is introduced to sub-atmospheric pressure. Without the use of additional energy input such as a laser, high voltages, or heat, and without the need for nebulizing or desolvation gases, as little as a few femtomoles of analyte are sufficient to obtain excellent quality mass spectra with detection limits of a few attomoles [5–7]. All MAI matrix compounds visually sublime under the conditions of a successful experiment [3, 4]. When introduced directly from atmospheric pressure, the ionization event is observed for several seconds, with increasing time being associated with colder conditions. At intermediate and low pressure (high vacuum) at room temperature, the duration increases to minutes, and under some condition to more than 30 min, with increasing time associated with lower pressure and minimal gas flow across the sample, or use of more matrix [3, 8–10].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13361-017-1788-7) contains supplementary material, which is available to authorized users.

Correspondence to: Sarah Trimpin; *e-mail:* strimpin@chem.wayne.edu

Regardless of the method of sample introduction, when a solid or solution matrix is employed, the charge states of analyte ions closely match those obtained by electrospray ionization (ESI) [11, 12]. The terminologies *inlet* ionization and *vacuum* ionization, without implying differences in mechanism, are used to differentiate whether the sample is introduced into the inlet directly from atmospheric pressure or inserted through a vacuum lock directly into the mass spectrometer.

It has been shown that a matrix can be either a solvent or a small molecule compound that is a solid at room temperature [13, 14]. More matrix compounds produce analyte ions when introduced to an inlet aperture from atmospheric pressure compared with direct insertion into intermediate pressure, even when both methods are at or near room temperature, which is believed to be the result of higher gas flow in the former [3, 4, 15]. Interestingly, it was shown that introducing neat sample into a heated inlet tube produces protonated molecular ions of the sample with minimal fragmentation without addition of a matrix [16]. In this way, singly-charged ions of the drug levaquin and multiply-charged ions of the protein myoglobin were observed. Freezing water or methanol, and mixtures thereof, containing analyte within a cold inlet tube, or just outside using dry ice, also produces analyte ions with ESI-like charge states [4, 15, 17]. Analogously, organic small molecules that are liquids at room temperature produced ions after being cooled to the solid state [4].

A critical step in any ionization method is separating positive from negative charge carriers. The exceptional sensitivity observed with some of the matrix compounds from which spontaneous ionization is achieved [7] strongly suggests an efficient non-statistical charge separation process. One such non-statistical process results in luminescence upon surface fracturing and is referred to as triboluminescence or fractoluminescence [18, 19]. These processes are also known to occur with fluids, so called sonoluminsecence and hydroluminescence, sub-categories of triboluminescence [20, 21], and of a variety of other means and conditions [22–31]. The light observed is due to a discharge across oppositely charged surfaces. A significant fraction of compounds (over 50%) are predicted to show triboluminescence [19]. Both frozen water and frozen methanol are known triboluminescence compounds [32, 33]. Triboluminescence has been well studied at and above atmospheric pressure [18, 34, 35], but fewer studies describe this charge separation process at sub-atmospheric pressure [36–38]. An overarching consensus in the literature is that although charge separation is natural and simply produced, understanding the processes involved is limited due to its complexity of influencing parameters.

In the absence of a neutralizing discharge, crystal fracturing is a potential mechanism for charge separation in MAI. The first, and still the best, MAI matrix to produce analyte ions without application of thermal energy is 3-NBN. This and other matrices [3, 4] are known to produce a triboluminescence discharge when fractured [39]. With 3-NBN, a pure dinitrogen discharge is observed in air, which is nearly identical to the spectrum observed from lightening. This result is achieved because the absorption of 3-NBN crystals in the spectral region of a dinitrogen discharge (337 nm) is poor, making photon driven ionization of the matrix, as has been suggested for MALDI [40], highly unlikely. Further, thermal desorption of bare ions from a surface requires substantial energy to overcome the image charge attraction [41]. A logical mechanistic proposition for MAI is that crystal fracturing produces both the necessary charge separation and the gas-phase particles from which bare ions are produced [1, 16]. An advantage of considering a fracturing process is that the energy necessary to break the crystal also potentially provides a physical means to propel the charged particles into the gas phase.

If particles are the carriers of analyte into the gas phase as ions, we need to ask what is the evidence for particles, how are gas-phase charged particles produced simply by exposing the matrix to sub-atmospheric pressure, are the particles composed of matrix molecules or residual solvent, and what is the mechanism for analyte ion release from the particles. Herein we discuss efforts to understand the processes whereby even proteins, at least as large as bovine serum albumin (BSA), 66 kDa [2, 42], are transported from the solid phase into the gas phase highly charged without application of external energy. Answering more detailed questions may provide a path to even higher sensitivity and utility of these new ionization processes developed into methods for MS, as recently shown [43–45], but also because these processes, previously unknown, have potential impact beyond MS.

Experimental

Materials

All chemicals including the analytes BSA and angiotensin I, the matrices 3-nitrobenzonitrile (3-NBN) and 2-methyl-2-nitro-1,3-propanediol (MNP) (Scheme 1), and the solvents acetonitrile (ACN) and HPLC water were obtained from Sigma-Aldrich. The mass spectrometers used were Thermo Scientific Orbitrap Exactive and LTQ Velos, and Waters SYNAPT G2 and G2S. The atomic force microscopy (AFM) instrument was a Bruker Veeco diInnova. The Quartz Crystal Microbalance was from Gamry (eQCM 10M). The microscope used was a Nikon TiE (Eclipse) confocal microscope equipped with a CSU-X spinning disk confocal scan head (Yokogawa).

Sublimation Experiments

A mixture of leucine enkephalin, angiotensin I, angiotensin II, and bovine insulin at ca. 10 μM in a concentrated 3-NBN solution in 1:1 ACN:H_2O was dried in a Chemglass (Chemglass Life Sciences, Vineland, NJ, USA) CG-3034-02A sublimation apparatus overnight. The bottom of the apparatus was immersed in an oil bath heated to 95 °C to the upper level of the matrix after it was connected to the condensation tube and pumped to a pressure of ca. 0.18 Torr with a rotary pump. Matrix crystals were noted to form on the walls above the matrix. The crystals observed using a Nikon microscope range

1–20 μm and were similar in appearance to crystals observed to fracture in microscopy experiments described below. Crystals were removed from different levels above the matrix and inserted into the skimmer inlet of a Waters SYNAPT G2 mass spectrometer to collect mass spectra. In a second experiment, a concentrated solution of 3-NBN (ca. 1 gram) in 7:3 ACN:water was added to the bottom of a test tube and allowed to dry overnight similar to the above experiment. A syringe needle was suspended ca. 1 cm above the matrix and the test tube with syringe needle was placed in a sand bath heated to 70 °C and the entire setup was placed in a vacuum desiccator pumped by a rotary pump. Matrix collected on the syringe was analyzed by MAI using an Orbitrap Exactive mass spectrometer.

In another experiment, a Corning glass Pasteur pipette (Sigma-Aldrich) was connected to a rotary pump, and 3 μL of a saturated solution of 3-NBN in 1:1 ACN:H_2O was allowed to dry for 1 min on the tip of a 10 μL syringe before being inserted into the small opening of the pipette. A laboratory microscope was used to monitor at 10× what occurs when the matrix is placed inside the narrow tube.

Microscopy

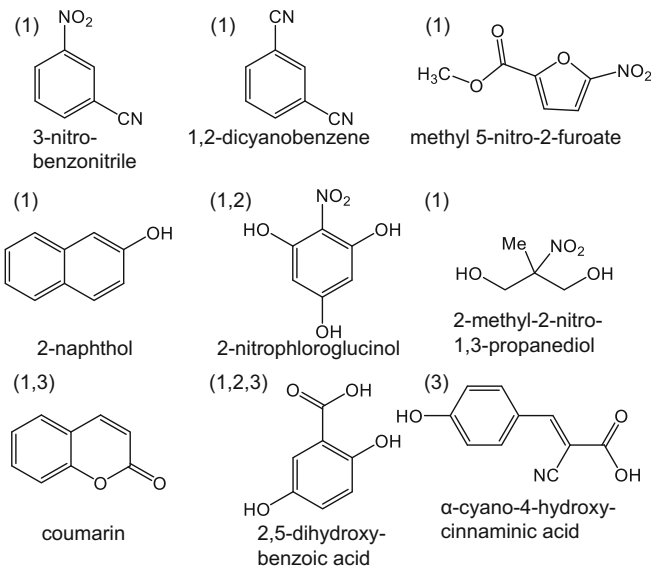
Two different optical microscope experiments were performed. In one, matrix with bovine insulin (10 pm μL^{-1}) was dried on a microscope slide and observed over 28 min. In the second, matrix solution in 7:3 ACN:H_2O was dried on a microscope slide that was placed above a second microscope slide with a 2 mm spacing. The top slide was gently heated with a laboratory heat gun while the bottom slide was cooled with ice. When crystals formed on the bottom slide, they were placed under a microscope and pictures taken every 10 s for 3 min. In both cases, the crystals were observed to spontaneously fracture, and occasionally crystal particles landed in the frame of view.

In the AFM experiment, the 3-NBN matrix was either dissolved into a 220 μM solution of BSA in 7:3 ACN:H_2O and 3 μL was placed on a silicon wafer to dry, or 3 μL of a 200 mg mL^{-1} ACN solution of 3-NBN was placed on a silicon wafer. The scanned image size of a selected crystal was 7 × 9 μm and the time to raster in each direction was 8.5 min for a total time of 120 min.

Charge Separation Inlet Tube Detection Devices

Two different instruments were used to detect transmission of charged particles of neutral compounds tapped into an inlet tube that separates atmospheric pressure from a vacuum stage. Both instruments are equipped with identical inlet tubes which were previously built for high efficiency transmission and collection of ions produced by ESI [46]. Instrument 1 is a mass spectrometer (Supplementary Scheme S1) and was used to determine that with MAI matrices, analyte ions are observed as previously reported, while Instrument 2 (Supplementary Scheme S2) is a simplified version of the source region of Instrument 1. In Instrument 2, charge was measured using circuits that connect ground and the inlet tube, as well as a detector plate placed a few centimeters downstream from the inlet exit as shown in Supplementary Scheme S3 along with a drawing of the transfer tube and raw data. Less than 1 mg of various small molecule compounds were then tapped into the inlet tube and the current readings on the tube and detector measured. Typically just the matrix powder was used straight out of the bottle and a pipette tip used to move the material to the inlet aperture. In some cases, a grounded metal spatula or a glass plate was used as material support to explore any differences between substrates. Matrix samples were also dissolved in ACN or ACN:water, and in some cases combined with an analyte. Data was acquired with the inlet tube at room temperature or 160 °C. The compound categories studied include known triboluminescence compounds, compounds with known tendency to sublime, well and poorly performing MAI and SAI matrices, MALDI and ESI matrices, as well as compounds known not to be MALDI or MAI matrices. Key structures are depicted in Scheme 1 and the complete list of structures is included in Supplementary Table S1.

Another study using Instrument 2 collected material onto a clean graphite sheet that replaced the detector. 3-NBN matrix solution was combined with BSA using typical MAI conditions. The sample was placed on a glass plate using a pipette and, while still rather wet, was affixed to the flared entrance to the heated inlet tube by the pressure differential. After the material was desorbed from the glass plate, the experiment was stopped and the instrument disassembled to collect the graphite sheet assembly from the vacuum chamber to be observed by AFM. More detailed information regarding materials



Scheme 1. Structures of some of the matrices studied. Annotations indicate compounds produce analyte ions by (1) MAI without additional energy supplied, (2) laserspray ionization (LSI) with additional thermal energy supplied using a heated inlet tube or a laser, (3) MALDI. A complete list of matrices used in this study can be found in Supplementary Table S1

used, sample introduction, and experimental parameters can be found in the Supporting Information.

Computational Method

The Gaussion09 [47] program package was used for all the calculations. The structures of charged and neutral matrix molecules were optimized with density functional theory (DFT) employing the long-range corrected hybrid functional LC-ωPBE. The standard Pople type 6-311++G(d, p) basis set was used for all the atoms. Harmonic vibrational frequencies were calculated to ensure that the optimized structures correspond to real local minima.

Results and Discussion

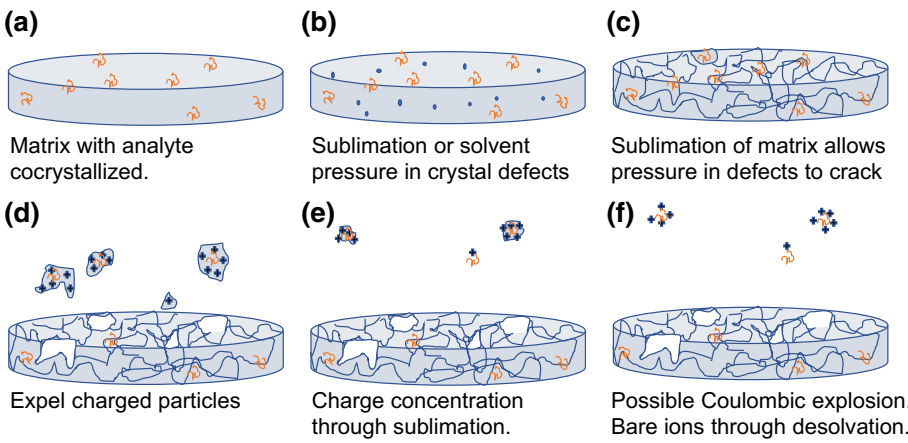
Charged Particles as Precursors to Bare Ions

An assumption has been made that pressure within defects in the matrix crystals, caused either by matrix sublimation or expansion of included solvent, overcomes the crystal structural integrity, expelling matrix particles into the gas phase [1]. The process can be visualized as sublimation of the crystal causing surface thinning of the crystal near a defect until it catastrophically disintegrates. In this scenario, the rapid expansion of gaseous matrix or solvent into vacuum propels charged matrix particles into the gas phase; a cartoon representation is provided in Scheme 2.

The physical force provided by vapor pressure would need to be able to overcome van der Waals forces, and for aromatic matrices, also possibly Pi–Pi interactions, and, most importantly, the electrostatic attraction between the sum of the charges in the exiting particle and the image charge on the original surface. In the case of a charged particle, the electrostatic attraction is greatly decreased relative to a bare ion because of the charge separation caused by the matrix solvating the charges and increasing the distance between opposite charges. Additionally, the surface area-to-charge ratio is much larger in the particle than in a bare ion, substantially decreasing the force per unit area necessary to expel the particle from the surface. Release of bare gas-phase ions from the gas-phase particles would then occur through sublimation or evaporation of the matrix, depending if the matrix is a solid or liquid [1, 16], similar to the ESI charge residue model [11, 12].

Experiments were conducted to determine if crystal fracturing can be visualized using both optical and AFM. Studies in which needle-like 3-NBN crystals on a glass microscope slide were observed over a time period of 3 min taking a photograph every 10 s using optical microscopy at 60× magnification show crystal cracking and bits of crystal appearing in the field of view and subliming away (Figure 1, Supplementary Movie S1). A similar optical microscopy experiment with similar results was obtained over a 3 h period (Supplementary Figure S1, Supplementary Movie S2). In both experiments, the samples at atmospheric pressure were being gently heated by the microscope lamp, and the results of matrix sublimation were visible in the microscope image. While the material pieces that were not present in one image but appeared in the next are much larger than expected in the ionization process, they demonstrated that sufficient energy is contained within the crystals to spontaneously cause particles to fly a distance from their source. An interesting observation is that the original crystals appear rather stable until visual loss of matrix is observed and then at that location loss of matrix becomes more rapid, possibly suggesting a cascade effect.

So far, particle emission has not been visualized, possibly suggesting that they are nanometer in size and below the diffraction limit of visible light. A quartz crystal microbalance was therefore used to detect weight changes of matrix crystals under sub-atmospheric pressure conditions. If particles are formed slowly enough and are large enough, instead of a smooth weight loss expected from sublimation, step changes in weight loss might be observed as particles leave the surface. While we indeed detected weight loss, using typical MAI conditions and 3-NBN as matrix, step changes are not observed (Supplementary Figure S2). The particles may be too small to be observed above the noise, or they exit the surface



Scheme 2. A representation of a possible process for producing gaseous charged analyte ions using MAI matrices

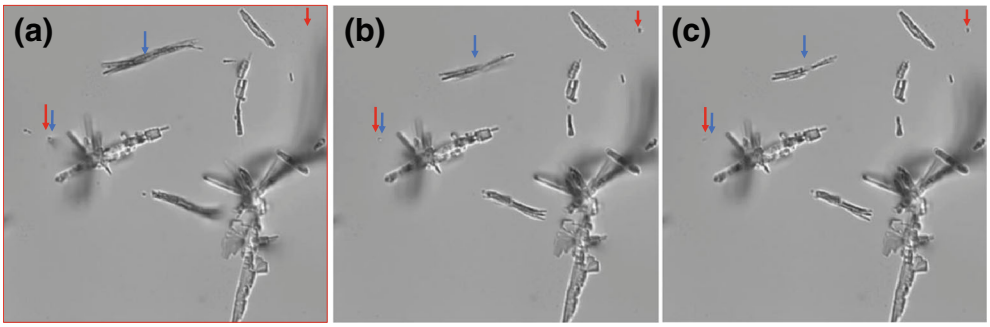


Figure 1. 3-NBN matrix observed under a microscope at 60× magnification showing areas where matrix appears (red arrow) and area of lost matrix (blue arrow). **(a)** Time 60 s, **(b)** time 100 s, **(c)** 130 s relative to deposition of crystals on microscope slide. Sublimation is a dynamic process in which micron sized particles are seen to appear on the slide; see Supplementary Movie S1 and S2

sufficiently rapidly, similar to droplets leaving the emitter tip of sonic spray [48] or ESI, to give the appearance of a continuous process, as is also observed in the continuous and prolonged signal from the mass spectrometer detector when the matrix sample is inserted into an intermediate pressure vacuum enclosure [3, 9, 43]. Of course, another explanation would be that under the conditions of the experiment, particles are not expelled from the surface. The microbalance and mass spectral results suggest that in MAI, the large matrix particles that have been observed with optical microscopy to migrate over a distance are not observed in the microbalance experiment, or in pulses of ion current in the mass spectrometer. The best explanation for these observations is that similar to ESI, ionization is a continuous process, but unlike ESI, the MAI sample is limited and ionization ceases when the matrix is depleted even though most of the analyte may remain [43].

An AFM experiment was used to explore if evidence could be obtained on loss of nanometer-sized particles associated with the 3-NBN matrix with and without incorporated BSA protein. With pure 3-NBN matrix crystallized from an ACN solution by drying, only a smooth decrease in the crystal size was observed, which is explained by sublimation. Further, the crystal surface and the surface of the freshly peeled silicon substrate were smooth. However, crystals obtained by drying a 1:1 ACN:water solution containing 220 μM BSA protein showed drastically different results (Figure 2 and Supplementary Figure S3). On the silicon wafer and on the crystal surface, there were what appeared to be particles that were somewhat larger in height and width than expected for BSA and might represent BSA encased in matrix or an artifact of the sampling. Only uniform thinning of the surface was noted in the initial scans, but then a small crack and a missing piece of the crystal appeared. In subsequent scans, this area enlarged with rough edges and missing matrix until finally only the side walls of the crystal remained with indication that little BSA was left behind where the crystal had been located. These observations suggest

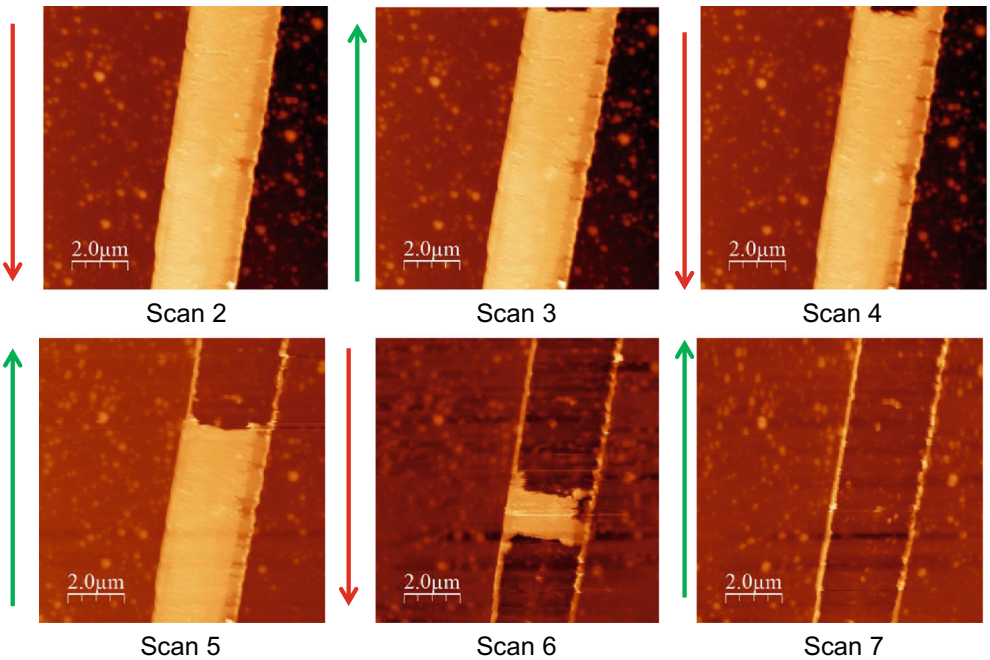


Figure 2. AFM image of BSA/3-NBN crystal at various time intervals after deposition: (Scan 1) 0 min, (Scan 2) 34–51 min, (Scan 3) 51–68 min, (Scan 4) 68–85 min, (Scan 5) 85–102 min, and (Scan 6) 102–119 min

that either BSA is not incorporated into the matrix in the area scanned or that the BSA was removed by some process during sublimation. Although not direct evidence, these results are consistent with particle emission rather than sublimation. Why the walls remained intact is unknown, and potentially provides a sharp edge from which field emission might occur and therefore needs further investigation.

A number of experiments were carried out in which the 3-NBN matrix containing a mixture of bovine insulin, angiotensin I, angiotensin II, and leucine enkephalin was sublimed. In one set of experiments, a common sublimation apparatus was used (Supplementary Figure S4). Matrix was collected on the walls just above the oil bath, as well as further away from the heat source. All of the collected crystals, when exposed to the inlet of a mass spectrometer (LTQ Velos), gave multiply-charged signals for the analytes. However, these crystals were micron sized, similar to what is observed in the microscope study described above and likely not the particles from which gas-phase ions are directly produced. A similar experiment using a heated sand bath and a vacuum chamber with a syringe needle suspended about 1 cm from the matrix:analyte mixture was used. The syringe needle was observed through a microscope and no visual matrix particles were observed to be collected. The sublimate that collected on the syringe needle (Supplementary Figure S5) was scraped off and analyzed using MAI on an Orbitrap Exactive mass spectrometer. The peptide, angiotensin I, was in the initial 3-NBN solution at 100 μM concentration, an amount ca. 1000× that needed to obtain MAI mass spectra in which the peptide ions are the most abundant. The doubly- and triply-charged ions for the peptide were observable using MAI, but the representative signals were within the chemical noise in the mass spectra. This experiment suggests that the amount of analyte relative to matrix that transfers to the cold finger is reduced by >1000×, an indication that sublimation is dominant and the ion formation process leaves most of the analyte behind. However, this experiment is not definitive because particles containing the analyte would need to travel more than a centimeter against gravity, and it also cannot be ruled out that crystal cracking, as observed in the microscope studies, might propel crystals of matrix with analyte sufficient distance to stick to the needle, without visual detection, and produce the observed results. After the sublimation of matrix, the analyte was readily detected from the bottom of the flask. These results relate well with MAI probe experiments where completely subliming the 3-NBN matrix containing analyte and re-applying just matrix solution produced analyte ions through 20 repeats of this procedure [43], and is reminiscent of MALDI experiments showing significant analyte remains on the MALDI plate after complete laser ablation of the matrix [49]. This is also consistent with MALDI experiments that show between 1:1000 and 1:1,000,000 analyte molecules are converted to detectable ions [50]. These results suggest that in MAI and MALDI, there is considerable room for increasing sensitivity.

An interesting observation was made during sublimation of solid matrix freshly crystallized from ACN:water onto the tip of a syringe needle. Inserting the syringe needle with matrix into a Corning glass Pasteur pipette and pumping from the larger end of the pipette with a rotary pump resulted in initial observation, using a microscope, of a clear liquid collecting on the inside of the glass near the syringe needle tip (Supplementary Figure S6). The liquid quickly pumped away but suggests the possibility that solvent droplets are the source of analyte ions, which would readily explain the similarity to ESI. This observation is consistent with the hypothesis that the included solvent is involved in the MAI process. However, a number of experiments suggest charged liquid droplets are not the source of observed analyte ions. For example, the observation of collected clear liquid is from freshly prepared matrix and analyte, yet analyte ions are observed from well-dried matrix, including freeze drying, and 120 min under vacuum conditions (Supplementary Figure S7). Further, the observation of liquid is momentary, yet inserting matrix on the tip of a syringe needle into the heated (70 °C) inlet tube of the Orbitrap Exactive produces a burst of ions that diminish upon leaving the matrix in the inlet for over 1 min. The conditions of flowing gas and temperature of 70 °C are expected to remove solvent capable of producing droplets unless incorporated within the matrix crystals, yet upon removing the syringe barrel with matrix:analyte from the inlet, an even larger analyte signal is observed (Supplementary Figure S8). Pockets of water within the clear 3-NBN crystals are not observed by optical microscopy. Thus, it is not obvious how charged solvent droplets would be produced in the quantity to produce the prolonged ion current that has been observed. However, a protic solvent, such as water or methanol, is critical for successful ionization of non-volatile analyte using MAI. One logical explanation is that the analyte retains its solution charge state in the crystal, implying that it also retains its solvent shell. This has also been suggested for MALDI matrices, and using electron microscopy, a high density of cavities were observed in 2,5- and 2,6-dihydroxybenzoic acid crystals, which was speculated to be the result of incorporated matrix with associated solvent [51, 52].

Further evidence against charged liquid droplets comes from experiments using identical solvent conditions but different MAI matrices. Under these conditions, the matrix determines the ionization efficiency [3, 4, 15]. Also, various MAI matrices ionized proteins out of basic solution by protonation, and best ion abundances were typically observed without the addition of acid or base [4]. Additionally, ionization in MAI is primarily by protonation [1], even under high salt conditions such as proteins from whole blood or drugs in urine [53, 54], whereas in ESI, ionization by Na^+ adduction under these conditions is the dominant process. Another interesting experiment that gives strong evidence that included water is not necessary for ionization is one in which 3-NBN is crystallized from ACN onto a syringe needle and, once dry, dipped into a 0.5 μM water solution of angiotensin II. When dry, the matrix is inserted into

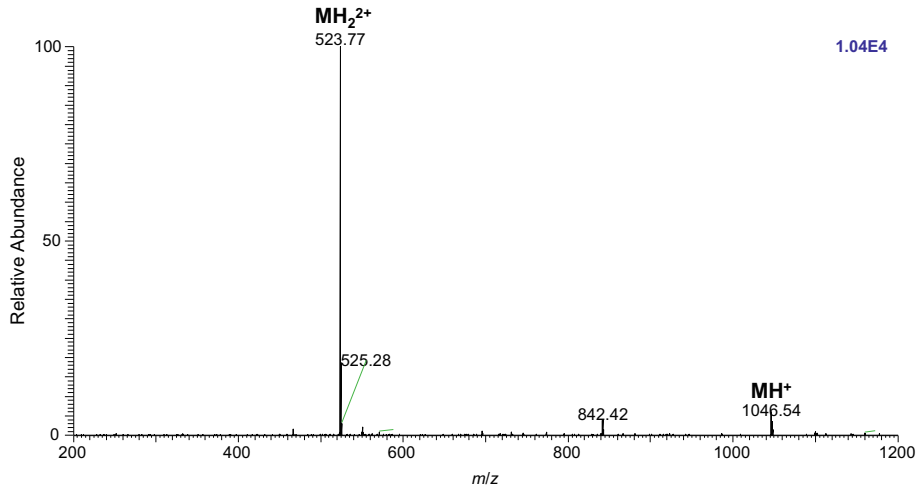


Figure 3. Mass spectrum obtained by dipping a syringe needle containing 1 μL of an acetonitrile solution of 3-NBN (100 mg mL^{-1}) that had been allowed to dry for 30 s into a 500 nM solution of angiotensin II in water and removed, allowed to dry for 1 min, and inserted into the inlet of a Thermo Orbitrap Exactive using the MS manual platform. The 3-NBN matrix is insoluble in water, demonstrating that incorporation of the analyte into the bulk of the matrix is not required

the inlet of an Orbitrap Exactive and produces a mass spectrum with little background even though 3-NBN is insoluble in water (Figure 3). These and other experiments indicate that liquid droplets are not the physical means by which the analyte ions are transferred to the gas phase in MAI. It is documented that as little as the solvent used for recrystallization will determine the morphology and, consequently, the ability to triboluminesce and to produce analyte ions [4, 15, 55]. While one can speculate about the role of solvent inclusion, the differences observed are more likely to arise from the differences in crystal morphology.

Even though direct observation of particles has not been obtained, there is reasonably strong circumstantial evidence that charged particles carry analyte into the gas phase and upon matrix sublimation release the ionized gas-phase analyte ions. The evidence is especially strong with less volatile matrices that require high inlet tube temperature to observe bare ions. Early work using commercial sources together with less volatile MAI and LSI matrices showed results indicating that final desolvation of the analyte ions occurs as late as in the mass analyzer [15, 16, 56]. It is important to note that using the more volatile 3-NBN matrix with the commercial atmospheric pressure (ESI) or intermediate pressure (MALDI) sources provided comparable MS/MS results using collision induced dissociation (CID) and electron transfer dissociation (ETD) to those obtained with ESI, presumably indicating the charged clusters of this matrix, which more readily sublimes, are desolvated in time to use the full capabilities of the commercial mass spectrometer [57].

Further evidence for charged particles is exemplified using the MAI matrix, MNP [4], especially when a heated inlet tube is not used for sample introduction. For example, using the commercial intermediate pressure vacuum MALDI source of a Waters SYNAPT G2S mass spectrometer requires that ions or charged particles produced by laser ablation or MAI travel through an extraction lens and 10 cm long hexapole ion transmission rods before reaching the aperture to the mass spectrometer. Both processes benefit from gas leaked to the enclosure, which may aid the sublimation process to enhance charged particle formation as well as desolvation. Even though analyte ions are observed for ubiquitin using MNP with 10% 3-NBN matrix, the IMS dataset shown in (Figure 4a) is drastically different from that using the more volatile MAI matrix, 3-NBN (Figure 4b). A plausible explanation for these results is that with the predominantly MNP matrix, desolvation of the charged clusters mostly does not occur before the IMS TriWave region. These and other similar results demonstrate that the MNP matrix compound is capable of lifting proteins into the gas phase, but that removal of the hydroxyl-containing matrix molecules from the charged clusters is slow relative to this process using pure 3-NBN matrix.

This is similar to producing ions by freezing water:methanol mixtures in which a common feature is the inability to readily fragment ions in the 'in-source' fragmentation region, or to select ions for fragmentation without first providing thermal energy or collisions with surfaces, or gases, presumably to remove matrix molecules from the bare ions [17]. This is not surprising because as early as in the 1960s, Latham and Stow calculated that ion evaporation from an ice surface agrees with experimental results when the ion is removed in a particle [58]. With less volatile matrix compounds, the charged clusters can travel long distances before they fully desolvate, often with the aid of collisions and electric fields [15, 16, 56, 59–61].

Charge Separation by Traversing Particles Through an Inlet Tube

In order to further study the charge separation processes, an inlet tube, previously built for high efficiency transmission and collection of ions produced by ESI [46], was employed. One of the objectives was to quantify charge separation when particles of various compounds are passed through an inlet tube, and to

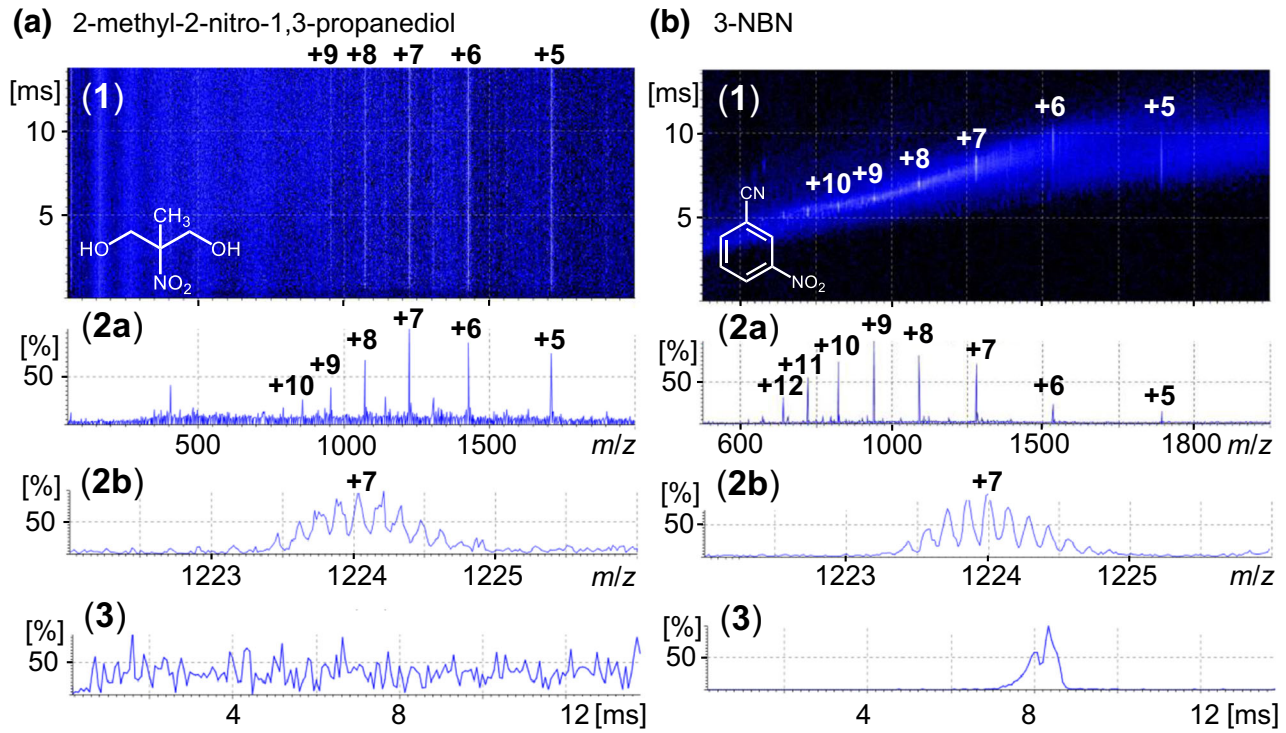


Figure 4. MAI-IMS-MS of ubiquitin on an intermediate pressure vacuum MALDI source without use of a laser: **(a)** MNP /ca. 10% 3-NBN (90% water:10% ACN), and **(b)** 3-NBN (90% water:10% ACN). (**1**) IMS-MS display of drift time versus *m/z*; (**2**) mass spectra **(a)** full and **(b)** inset; (**3**) drift time distributions. Annotations of the charge states of the protein ions are provided in bold letters. Only sufficiently volatile matrices are effectively removed from the charged clusters to obtain the bare analyte ions prior to entering the IMS region

determine which compound properties might contribute to improved charge separation.

To relate the generation and separation of charge in the inlet region to analyte ions detected in the mass spectrometer, a preparative mass spectrometry instrument was used (Instrument 1, Supplementary Scheme S1). While the preparative capability was not used, this instrument was particularly useful because it is capable of measuring absolute ion currents midway through the instrument at several positions (e.g., after leaving the rf-ion guides or before entering the TOF-mass spectrometer), and obtaining the corresponding mass spectra. For MAI experiments, the ESI ion source is operated without voltages applied to the capillary. In this mode, only the flowing gas can transport ions (and other particles) to the ion optics.

Using the inlet tube and 3-NBN as matrix, mass spectra similar to those previously reported were obtained for both positive and negative ions. Upon introduction of the matrix:analyte mixture, several short pulses (less than 1 s) of ion current are detected, which coincide with the detection of ion intensity in the mass spectrometer. The currents generated using a pipette tip or spatula under various solution condition are in the range of 10–100 picoamp (Supplementary Figures S9–S11), considerably lower than ESI currents of the same substances, which are detected at nanoamp intensity. Part of the reason for higher ion abundance in ESI is because of higher background signal.

To complement the MS measurements with charge separation data, the same ion source design was used in a setup where the ion currents flowing in the ion source chamber were detected (Instrument 2, Supplementary Scheme S2). Ions or charged particles impinging either the inlet tube or the detector plate are detected separately. The electrically isolated transfer capillary and a detector plate, about 3 cm downstream from the transfer capillary exit and in line with the inlet tube, were separately connected to electrometers.

Matrix powder of 3-NBN and a long list of other potential matrix and reference compounds were introduced dry from spatulas and pipette tips. The current readings on both the inlet tube and the detector were found to be independent of the device used. Since the pipette tips produced more reproducible results, partially because of better control of the matrix amount used, these tips were used for all following experiments. For reference, the apparatus was also tested with a low flow ESI source in positive ion mode. When positive ions are created by ESI and injected into the transfer capillary, both the transfer capillary and detector register a positive current, meaning electrons flow from ground to the transfer capillary and the detector to compensate the impinging positive ions. The results of these experiments are demonstrated in Figure 5 and summarized in Supplementary Table S1 (see also Supplementary Figures S12–S20 for details). All of the tested compounds, solids and liquids, produce detectable ion currents even with a room temperature inlet.

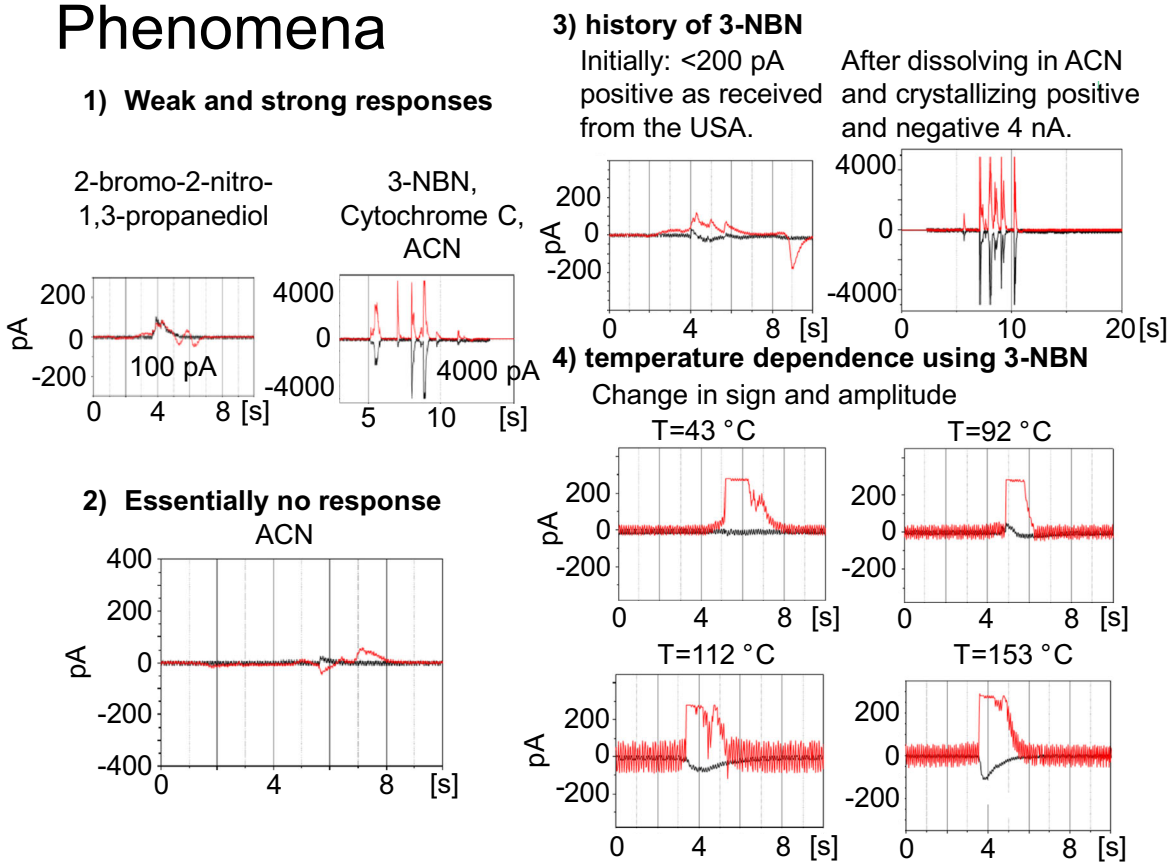


Figure 5. Current measurements for different compounds, solids and liquids, passing through the transfer tube of Instrument 2. A detailed list of results is included in Supplementary Table S1 and in the graphical abstract

The matrix 3-NBN created one of the strongest signals of all tested compounds. When crystals or powder from the original container are inserted using a pipette tip, a signal of several nanoampere is observed. The signal is positive on the detector but negative on the capillary, an indication that the charge separation takes place inside the capillary. Most compounds show signals on both capillary and detector with different intensity. While all signals are individual in shape because of the undefined nature of the injected powders/droplets, the magnitude of the signals is rather reproducible. Heating the inlet and/or dissolving the compound in ACN:water followed by drying, identical to sample preparation protocols in MAI, increases the detected current for most compounds studied (Supplementary Table S1). The detected signals varied greatly between compounds, with only a few compounds producing strong signals at room temperature, whereas most require a heated inlet to produce a significant signal.

Depending on the compound, and sometimes conditions employed, the current readings at the transfer tube and downstream detector plate were mostly of opposite polarity, e.g., positive/negative or negative/positive, but interestingly some compounds produced positive/positive or negative/negative readings on the transfer tube and detector, respectively. The readings of the same sign on both the inlet and the detector may suggest that particles of these compounds enter the inlet from the pipette tip with a net charge much larger than the current generated by charge separation, which leads to a reading similar to positive ion ESI. The majority of typical MALDI matrices showed positive/positive signs on the detectors (Supplementary Table S1).

The intensity detected for most compounds was in the order of 100 pA, with a few compounds such as 3-NBN reaching significantly higher currents in the nanoampere range. A particularly interesting finding was that the injection of 3-NBN powder from secondary vials, shipped from the USA to the lab in Germany, resulted in very low current readings on both detectors, whereas the 3-NBN available in the lab, purchased from the same vendor and used directly from the bottle, gave high current readings (Figure 5). Dissolving the matrices that produced low current in ACN:water and drying in room air for several min before passing through the inlet produced some of the strongest current reading observed in the study. We speculate on two possible reasons for this outcome: one was that the repackaged matrix shipped by airfreight lost water content during shipment, or that crystal morphology changed due to high temperature differences during the transport. Whatever the correct reason here, other results have shown that morphology, at least for 3-NBN, is important for the ionization process [3, 4, 15, 18, 19, 39]. What is clear from these results is that charge separation is a feature common in particles passing through an

inlet tube from a higher to a lower pressure region and that heating the inlet tube is usually associated with increased charge separation. Other than the increased heat, dipole moments appear to correlate with successful charge separation, at least at low inlet tube temperature (Supplementary Table S1). This relates well with triboluminescence [18, 19].

Using Instrument 2, it was possible to observe the sublimation of a macroscopic 3-NBN deposit whereby particles are expelled from the crystal into the gas phase. Glass plates with various matrices including 3-NBN and typical MALDI matrices (Supplementary Figure S15), applied from solution and fixed to the conical shaped inlet tube entrance by the pressure differential, were used to visually observe small changes in the matrix while simultaneously recording the current collected on the inlet tube and detector. Small particles of matrix on the glass plate sublime over a duration of minutes and slowly disappeared without detection of current (Supplementary Figure S13). Only when a piece of matrix material is observed to dislodge from the glass surface is a signal detected. Warming the atmospheric pressure side of the glass plate with a heat gun increased the rate of sublimation and the rate at which pieces of matrix could be seen leaving the glass plate, both visually observed and recorded as a current signal. These processes are displayed in Supplementary Movies S3 and S4 for BSA with 3-NBN and 2,5-DHB matrix, respectively. While the sublimation of the matrix or evaporation of solvent at the glass/matrix interface might provide the pressure to crack the matrix material and propel particles into the inlet tube, the generation of the detected ions appears to predominantly take place inside the transfer capillary.

In addition to solid compounds, solvents traversing through the pressure differential within the inlet tube create charge separation as measured on the electrodes. Acetonitrile gave the lowest reading (Figure 5). These findings using liquids relate most closely to solvent-assisted ionization (SAI) [13, 62, 63], and work from the groups of Jarrold and Ewing reporting increased charge upon passing droplets produced by e.g., sonic spray through an inlet tube into vacuum [64]. These authors measured an average charge of 12,000 e on droplets having a radius of 2–3 μm. Such droplets would need to be about 1/10th this diameter to reach the Rayleigh limit. In SAI, increasing inlet tube temperature increases the analyte ion abundance, which directly relates to the increase in current measured on the inlet tube and detector in the experiments conducted here when the inlet tube was heated to 160 °C. SAI is preferred for some compounds even at low inlet tube temperatures relative to MAI using 3-NBN, as demonstrated for 1,5-diaminonapthalene (Supplementary Figure S21). Horan and Johnston recently reported achieving exceptional sensitivity for aerosol droplets passed through an inlet tube heated as high as 950 °C [65].

Although previous results have shown that at high inlet tube temperatures [13–16, 61–63] many of the compounds studied here assist in producing bare analyte ions as measured by MS, few of the compounds that show charge separation near room temperature produce analyte ions that are observed with the mass spectrometer. One likely factor is the ability to desolvate the charged particles to release the bare ions, but other factors are also important. In order to observe monomeric analyte ions, it is necessary that the final particle before release of the gas-phase ion be small enough to carry only a single analyte molecule/ion. Another factor might be the ability to transfer a proton to or from the analyte in the presence of the matrix compound. These and other studies show that a multitude of other factors are important, such as temperature, crystal morphology, solvent, additives, gas flow, collisions, and pressure on either side of the transfer tube. Understanding this simple to perform and sensitive ionization process, a challenge theoretically, may require thinking beyond what has been previously modeled for ionization processes [11, 12, 66–70], but there is room for great improvement in sensitivity with better knowledge of the processes involved in transferring solid-phase molecules to gas-phase ions without application of external energy input.

In summary (Overview 1), relative to the mechanism underlying observation of gas-phase ions by MS upon exposing the matrix:analyte sample to sub-atmospheric pressure, certain small molecule matrix compounds containing minute quantities of analyte, by theory and experimentation, appear to originate with expulsion of charged particles of matrix:analyte from the solid crystal surface. Such a process was proposed for ion emission from ice [41, 71], and freezing water containing analyte has been shown to produce gas-phase ions of the analyte by MS [17]. Experimental results point to expulsion of solid particles rather than charged solvent droplets from solid MAI matrices. Expansion of trapped solvent has not been ruled out as playing a role in particle expulsion from the solid crystals. Observations also suggests that the initial process upon exposure of the matrix crystals to sub-atmospheric pressure is sublimation, but once a defect is produced, the process becomes faster as might be expected if pressure buildup in defects cause ‘micro explosions’, as these would increase the surface area, providing an avalanche effect rather than an increase in sublimation due to a greater surface area. In this scenario, the expelled particles could be charged during the fracturing as occurs in producing triboluminescence (this implies that the charge is not dissipated by the discharge) [18]. Clearly, experiments conducted here and elsewhere, as well as natural events such as lightening and static electricity, show that charging is a common phenomenon in nature. Even if the analyte is precharged, there must at least be sufficient charge to neutralize the counter ions. The final particles must be sufficiently small to carry only a single analyte ion, and this must occur either with the original particle formation or through a mechanism analogous to producing smaller droplets through Taylor cone formation in ESI, possibly a so called Coulomb explosion. Release of the bare analyte ion requires sufficient energy to remove the

matrix from the gas-phase particle, and the energy necessary is a function of the volatility of the matrix.

Overview 1: Key Findings Relative to MAI Mechanism
- Gas-phase ion production is inefficient relative to sublimation
- Gas-phase ion molecule reactions cannot produce highly-charged ions
- Energy is not available to remove multiply-charged ion from matrix surface
- Alternative is removal of charged droplets or particles
- Freshly crystallized matrix contains sufficient solvent to form liquid droplets
- Protic solvent is necessary for ionization of nonvolatile compounds
- Analyte ions are produced from dried matrix with exceptional sensitivity
- Ionization selectivity determined by the matrix and not the solvent
- Ionization is predominately by protonation even in salt solutions
- Particles have not been directly observed
- Means for producing matrix particles sufficiently small to contain a single analyte molecule
- Circumstantial evidence for charged particles as analytecarrier is presented

Concluding Remarks

One concern from the beginning of the discovery of MAI was why, with all the sublimation experiments conducted over the years, was the phenomena in which large molecules are transported into the gas phase not previously reported as this would not be conducive to purification. The answer appears to lie in the inefficiency of particle emission, which leaves the great majority of nonvolatile compounds behind after complete sublimation, and yet the MAI method has sensitivity comparable to ESI and MALDI [2, 5–7]. There are great opportunities for improving this technology ahead.

Acknowledgements

This material is based in part upon work supported by the National Science Foundation under Grant NSF CHE-1411376 to S.T., and NSF Phase II 1556043 to MSTM, LLC. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

References

1. Trimpin, S.: “Magic” ionization mass spectrometry. J. Am. Soc. Mass Spectrom. **27**, 4–21 (2016)
2. Inutan, E.D., Trimpin, S.: Matrix assisted ionization vacuum, a new ionization method for biological materials analysis using mass spectrometry. Mol. Cell. Proteom. **12**, 792–796 (2013)
3. Trimpin, S., Inutan, E.D.: Matrix assisted ionization in vacuum, a sensitive and widely applicable ionization method for mass spectrometry. J. Am. Soc. Mass Spectrom. **24**, 722–732 (2013)
4. Trimpin, S., Lutomski, C.A., El-Baba, T.J., Woodall, D.W., Foley, C.D., Manly, C.D., Wang, B., Liu, C.W., Harless, B.M., Kumar, R., Imperial, L.F., Inutan, E.D.: Magic matrices for ionization in mass spectrometry. Int. J. Mass Spectrom. **377**, 532–545 (2015)
5. Chubatyi, N.D., McEwen, C.N.: Improving the sensitivity of matrix-assisted ionization (MAI) mass spectrometry using ammonium salts. J. Am. Soc. Mass Spectrom. **26**, 1649–1656 (2015)
6. Trimpin, S., Thawoos, S., Foley, C.D., Woodall, D.W., Li, J., Inutan, E.D., Stemmer, P.M.: Rapid high mass resolution mass spectrometry using matrix-assisted ionization. Methods. **104**, 63–68 (2016)
7. Hoang, K., Pophristic, M., Horan, A.J., Johnston, M.V., McEwen, C.N.: High sensitivity analysis of volatile and nonvolatile compounds using matrix-assisted ionization (MAI) mass spectrometry. J. Am. Soc. Mass Spectrom. **27**, 1590–1596 (2016)
8. Trimpin, S., Inutan, E.D.: New ionization method for rapid analysis on atmospheric pressure ionization mass spectrometers requiring only vacuum and matrix assistance. Anal. Chem. **85**, 2005–2009 (2013)
9. Wang, B., Akhmetova, E., Trimpin, S., Wilkins, C.L.: Matrix-assisted ionization vacuum for high-resolution fourier transform ion cyclotron resonance mass spectrometers. Anal. Chem. **86**, 6792–6796 (2014)
10. Woodall, D.W., Wang, B., Inutan, E.D., Narayan, S.B., Trimpin, S.: High-throughput characterization of small and large molecules using only a matrix and the vacuum of a mass spectrometer. Anal. Chem. **87**, 4667–4674 (2015)
11. Whitehouse, C.M., Dreyer, R.N., Fenn, J.B.: Electrospray interface for liquid chromatographs and mass spectrometers. Anal. Chem. **57**, 675–679 (1985)
12. Fenn, J.B.: Electrospray wings for molecular elephants. Angew. Chem. Int. Ed. **42**, 3871–3894 (2003)
13. Pagnotti, V.S., Chubatyi, N.D., McEwen, C.N.: Solvent assisted inlet ionization: an ultrasensitive new liquid introduction ionization method for mass spectrometry. Anal. Chem. **83**, 3981–3985 (2011)
14. McEwen, C.N., Pagnotti, V., Inutan, E.D., Trimpin, S.: New paradigm in ionization: multiply charged ion formation from a solid matrix without a laser or voltage. Anal. Chem. **82**, 9164–9168 (2010)
15. Li, J., Inutan, E.D., Wang, B., Lietz, C.B., Green, D.R., Manly, C.D., Richards, A.L., Marshall, D.D., Lingenfelter, S., Ren, Y., Trimpin, S.: Matrix assisted ionization: new aromatic and non aromatic matrix compounds producing multiply charged lipid, peptide, and protein ions in the positive and negative mode observed directly from surfaces. J. Am. Soc. Mass Spectrom. **23**, 1625–1643 (2012)
16. Trimpin, S., Wang, B., Inutan, E.D., Li, J., Lietz, C.B., Pagnotti, V.S., Harron, A.F., Sardelis, D., McEwen, C.N.: A mechanism for ionization of nonvolatile compounds in mass spectrometry: considerations from maldi and inlet ionization. J. Am. Soc. Mass Spectrom. **23**, 1644–1660 (2012)
17. Pagnotti, V.S., Chakrabarty, S., Wang, B., Trimpin, S., McEwen, C.N.: Gas-phase ions produced by freezing water or methanol for analysis using mass spectrometry. Anal. Chem. **86**, 7343–7350 (2014)
18. Olawale, D.O., Okoli, O.O.I., Fontenot, R.S., Hollerman, W.A. (Eds.): Triboluminescence theory, synthesis, and application. Springer: (2016)
19. Jha, P., Chandra, B.P.: Survey of the literature on mechanoluminescence from 1605 to 2013. Luminescence. **29**, 977–993 (2014)
20. Biryukov, D.A., Vlasova, M.I., Gerasimov, D.N., Sinkevich, O.A.: Light emitted from a liquid that flows in a narrow channel as triboluminescence. Optics Spectrosc. **114**, 704–708 (2013)
21. Dawson, T.L.: Changing colors: now you see them, now you don’t. Color Technol. **126**, 177–188 (2010)
22. Hardy, G.E., Kaska, W.C., Chandra, B.P., Zink, J.I.: Triboluminescence–structure relationships in polymorphs of hexaphenylcarbodiphsohorane and anthranilic acid, molecular crystals, salts. J. Am. Chem. Soc. **103**, 1074–1079 (1981)
23. Guardiola, J., Rojo, V., Ramos, G.: Influence of particle size, fluidization velocity, and relative humidity on fluidized bed electrostatics. J. Electrostatics. **37**, 1–20 (1996)
24. Itakura, T., Masuda, H., Ohtsuka, C., Matsusaka, S.: The contact potential difference of powder and the tribo-charge. J. Electrostatics. **38**, 213–226 (1996)
25. Matsusaka, S., Umemoto, H., Nishitani, M., Masuda, H.: Electrostatic charge distribution of particles in gas–solids pipe flow. J. Electrostatics. **55**, 81–96 (2002)
26. Briscoe, W.H., Horn, R.G.: Direct measurement of surface forces due to charging of solids immersed in a nonpolar liquid. Langmuir. **18**, 3945–3956 (2002)
27. Watanabe, H., Ghadiri, M., Matsuyama, T., Ding, Y.L., Pitt, K.G., Maruyama, H., Matsusaka, S., Masuda, H.: Triboelectrification of pharmaceutical powders by particle impact. Int. J. Pharmaceutics. **334**, 149–155 (2007)
28. Gouveia, R.F., Galembeck, F.: Electrostatic charging of hydrophilic particles due to water adsorption. J. Am. Chem. Soc. **131**, 11381–11386 (2009)
29. Forward, K.M., Lacks, D.J., Sankaran, R.M.: Triboelectric charging of granular insulator mixtures due solely to particle-particle interactions. Ind. Eng. Chem. Res. **48**, 2309–2314 (2009)

30. Matsusaka, S., Maruyama, H., Matsuyama, T., Ghadiri, M.: Triboelectric charging of powders: a review. Chem. Eng. Sci. **65**, 5781–5807 (2010)
31. Singh, S.V., Kusano, Y., Morgen, P., Michelsen, P.K.: Surface charging, discharging, and chemical modification at a sliding contact. J. Appl. Phys. **111**, 083501 (2012)
32. Quickenden, T.I., Selby, B.J., Freeman, C.G.: Ice triboluminescence. J. Phys. Chem. A. **102**, 6713–6715 (1998)
33. Trouti, G.J., Moorez, D.E., Hawke, J.G.: Triboluminescence in Solid Methanol. Nature Phys. Sci. **235**, 174–175 (1972)
34. Leyrer, E., Zimmermann, F., Zink, J.I., Gliemann, G.: Triboluminescence, photoluminescence, and high-pressure spectroscopy of tetracyanoplatinate salts. Determination of the pressure at triboluminescent sites. Inorg. Chem. **24**, 102–106 (1985)
35. Gordon, E.H., Zink, J.I.: Triboluminescence and pressure dependence of the photoluminescence of tetrahedral manganese(i1). complexes. Inorg. Chem. **15**, 3061–3065 (1976)
36. Stoecker, H., Ruehl, M., Heinrich, A., Mehner, E., Meyer, D.C.: Generation of hard X-ray radiation using the triboelectric effect by peeling adhesive tape. J. Electrostatics. **71**, 905–909 (2013)
37. Nevshupa, R.A.: Effect of gas pressure on the triboluminescence and contact electrification under mutual sliding of insulating materials. J. Phys. D: Appl. Phys. **46**, 185501 (2013)
38. Nevshupa, R.A., Conte, M., Igartua, A., Roman, E., de Segovia, J.L.: Ultrahigh vacuum system for advanced tribology studies: design principles and applications. Tribology Int. **86**, 28–35 (2015)
39. Sweeting, L.M., Cashel, M.L., Dott, M., Gingerich, J.M., Guido, J.L., Kling, J.A., Pippin, R.F., Rosenblatt, M.M., Rutter, R.A., Spence, R.A.: Spectroscopy and mechanism in triboluminescence. Mol. Cryst. Liq. Cryst. **211**, 389–396 (1992)
40. Knochenmuss, R.: A quantitative model of ultraviolet matrix-assisted laser desorption/ionization including analyte ion generation. Anal. Chem. **75**, 2199–2207 (2003)
41. Latham, J., Stow, C.D.: Electrification associated with the evaporation of ice. J. Atmos. Sci. **22**, 320–324 (1965)
42. Lietz, C.B., Richards, A.L., Ren, Y., Trimpin, S.: Inlet ionization: protein analyses from the solid state without the use of a voltage or a laser producing up to 67 charges on the 66 kDa BSA protein. Rapid Commun. Mass Spectrom. **25**, 3453–3456 (2011)
43. Lu, I-C., Pophristic, M., Inutan, E.D., McKay, R.G., McEwen, C.N., Trimpin, S.: Simplifying the ion source for mass spectrometry. Rapid Commun. Mass Spectrom. **30**, 2568–2572 (2016)
44. Lu, I-C., Elia, E.A., Zhang, W.-J., Pophristic, M., Inutan, E.D., McEwen, C.N., Trimpin, S.: Development of an easily adaptable, high sensitivity source for inlet ionization. Anal. Methods. **9**, 4971–4978 (2017)
45. Trimpin, S., Elia, E.A., Lu, I.-C., Meher, A.K., Inutan, E.D., El-Baba, T.J., Zhang, W.J., Tapa, B., Lutomski, C.A., Fischer, J.L., Foley, C.D., Halvorsen, T.G., Schlegel, H.B., Stemmer, P.M., Weidner, S.M.: Mechanistic insights and practical utility of highly-charged protein ions on vacuum source mass spectrometers. 65th ASMS Conference on Mass Spectrometry and Allied Topics, Indianapolis (2017)
46. Pauly, M., Sroka, M., Reiss, J., Rinke, G., Albarghash, A., Vogelgesang, R., Hahne, H., Kuster, B., Sesterhenn, J., Kern, K., Rauschenbach, S.: A hydrodynamically optimized nano-electrospray ionization source and vacuum interface. Analyst. **139**, 1856–1867 (2014)
47. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery Jr., J.A., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, Ö., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D.J.: Gaussian 09, Revision D.01. Gaussian, Inc., Wallingford (2009)
48. Hirabayashi, A., Hirabayashi, Y., Sakairi, M., Hideaki, K.: Multiply-charged ion formation by sonic spray. Rapid Commun. Mass Spectrom. **10**, 1703–1705 (1996)
49. Page, J.S., Sweedler, J.V.: Sample depletion of the matrix-assisted laser desorption process monitored using radionuclide detection. Anal. Chem. **74**, 6200–6204 (2002)
50. Lu, I-C., Chu, K.Y., Lin, C.Y., Wu, S.Y., Dyakov, Yuri, A., Chen, J.L., Gray-Weale, A., Lee, Y.T., Ni, C.K.: Ion-to-neutral ratios and thermal proton transfer in matrix-assisted laser desorption/ionization. J. Am. Soc. Mass Spectrom. **26**, 1242–1251 (2015)
51. Figueroa, I.D., Torres, O., Russell, D.H.: Effects of the water content in the sample preparation for MALDI on the mass spectra. Anal. Chem. **70**, 4527–4533 (1998)
52. Laboy, J.L., Murray, K.K.: Characterization of infrared matrix-assisted laser desorption ionization samples by Fourier transform infrared attenuated total reflection spectroscopy. Appl. Spectrosc. **58**, 451–456 (2004)
53. Inutan, E.D., Wager-Miller, J., Narayan, S.B., Mackie, K., Trimpin, S.: The potential for clinical applications using a new ionization method combined with ion mobility spectrometry-mass spectrometry. Int. J. Ion Mobility Spectrom. **16**, 145–159 (2013)
54. Chakrabarty, S., DeLeeuw, J.L., Woodall, D.W., Jooss, K., Narayan, S.B., Trimpin, S.: Reproducibility and quantitation of illicit drugs using matrix-assisted ionization (MAI) mass spectrometry. Anal. Chem. **87**, 8301–8306 (2015)
55. Franco, O., Reck, G., Orgzall, I., Schulz, B.W., Schulz, B.: Characterization of a new non-centrosymmetric polymorph of diphenyl-1,3,4-oxadiazole. J. Mol. Struct. **649**, 219–230 (2003)
56. Inutan, E.D., Wang, B., Trimpin, S.: Commercial intermediate pressure MALDI ion mobility spectrometry mass spectrometer capable of producing highly charged laserspray ionization ions. Anal. Chem. **83**, 678–684 (2010)
57. Marshall, D.D., Inutan, E.D., Wang, B., Liu, C.W., Thawoos, S., Wager-Miller, J., Mackie, K., Trimpin, S.: A broad-based study on hyphenating new ionization technologies with MS/MS for PTMs and tissue characterization. Proteomics. **16**, 1695–1706 (2016)
58. Latham, J., Stow, C.D.: The mechanism of charge transfer associated with evaporation of ice. J. Atm. Sci. **23**, 245–247 (1966)
59. Trimpin, S., Inutan, E.D., Herath, T.N., McEwen, C.N.: Laserspray ionization – a new AP-MALDI method for producing highly charged gas-phase ions of peptides and proteins directly from solid solutions. Mol. Cell. Proteom. **9**, 362–367 (2010)
60. Inutan, E.D., Trimpin, S.: Laserspray ionization (LSI) ion mobility spectrometry (IMS) mass spectrometry. J. Am. Soc. Mass Spectrom. **21**, 1260–1264 (2010)
61. Inutan, E.D., Trimpin, S.: Laserspray ionization-ion mobility spectrometry-mass spectrometry: baseline separation of isomeric amyloids without the use of solvents desorbed and ionized directly from a surface. J. Proteome Res. **9**, 6077–6081 (2010)
62. Pagnotti, V.S., Inutan, E.D., Marshall, D.D., McEwen, C.N., Trimpin, S.: Inlet ionization: a new highly sensitive approach for liquid chromatography-mass spectrometry of small and large molecules. Anal. Chem. **83**, 7591–7594 (2011)
63. Wang, B., Trimpin, S.: High throughput solvent assisted ionization inlet (saii) for use in mass spectrometry. Anal. Chem. **86**, 1000–1006 (2014)
64. Zilch, L.W., Maze, J.T., Smith, J.W., Ewing, G.F., Jarrold, M.F.: Charge separation in the aerodynamic breakup of micrometer-sized water droplets. J. Phys. Chem. A. **112**, 13352–13363 (2008)
65. Horan, A.J., Apsokardu, M.J., Johnston, M.V.: Droplet assisted inlet ionization for online analysis of airborne nanoparticles. Anal. Chem. **89**, 1059–1062 (2017)
66. Consta, S., Oh, M.I., Soltani, S.: Advances in the theoretical and. molecular simulation studies of the ion chemistry in droplets. Int. J. Mass Spectrom. **377**, 557–567 (2015)
67. Zhigilei, L.V., Garrison, B.J.: Microscopic mechanism of laser ablation of organic solids in the thermal and stress confinement irradiation regimes. J. Appl. Phys. **88**, 1281–1298 (2000)
68. Knochenmuss, R.: MALDI and related methods: a solved problem or still mystery? Mass Spectrom. Soc. Japan. **2**, 1–8 (2013)
69. Karas, M., Kruger, R.: Ion formation in MALDI: the cluster ionization mechanism. Chem. Rev. **103**, 427–439 (2003)
70. Lu, I-C., Lee, C.H., Lee, Y.-T., Ni, C.-K.: Ionization mechanism of matrix-assisted laser desorption/ionization. Ann. Rev. Anal. Chem. **8**, 21–39 (2015)
71. Gay, M.J., Latham, J.: The freezing behavior of supercooled water drops. J. Glaciology. **17**, 99–109 (1976)