University of Colorado, Boulder

Hypervelocity Impact Ionized TOFMS Experiments with Amino Acid Mixtures in Ice Targets

by

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Abstract

of the Thesis of

Michael Voss for Physics Honors

Title: Hypervelocity Impact Ionized TOFMS Experiments with Amino Acid Mixtures in Ice Targets

Time of Flight Mass Spectrometry (TOFMS) has long been an important tool for understanding the molecular composition of substances, and forms the basis of flight instruments in spacecraft exploring the solar system. Spacecraft carrying TOFMS instruments will return a tremendous amount of information about the composition of cosmic dust and ejecta from planets, moons, comets, and asteroids. There are outstanding questions, however, on the details of hypervelocity dust measurements, because the vast majority of laboratory TOFMS experiments thus far have been based on laser or electron ionization rather than hypervelocity impact ionization, which is the mechanism used in flight instruments. This thesis describes a series of experiments which (1) develop methods for creating unique frozen targets from water and water-soluble compounds, and (2) explore the resulting spectra from known mixtures of two amino acids, as a proof-of-principle for extracting mixture data

from planetary samples. The target-preparation methods proved capable of creating a uniform frozen target surfaces of mixed solutions, exceeding the performance of previous approaches. The followup experiments took advantage of these methods to create targets of various mixtures of the amino acids Arginine and Lysine and test their response to hypervelocity impacts using the TOFMS system. It is shown that relative concentrations of these amino acids can be recovered from TOFMS in the low impact velocity regime using a method of line fitting. The target and amino acid mixtures experiments produced promising results and show that by using these methods other experiments could be carried out in the same way to reinforce our understanding of what we may see from spacecraft-mounted TOFMS systems.

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Abbreviations

IMPACT	The Institute for Modeling Plasmas, Atmospheres, and Cosmic DusT
MCP	Micro Channel Plate
RH	Relative Humidity
TOFMS	Time of Flight Mass Spectrometery

1

Introduction

The means to detect bio-signatures from space has long been an important topic in the scientific community. One such tool that can be used for the detection of bio-signatures from materials surrounding planets, moons, comets, or asteroids is time of flight mass spectroscopy (TOFMS) [1]. Through the data captured by TOFMS flight instruments we may be able to detect amino acids entrained in water ice grains released from the surfaces of the moons Europa (Jupiter) or Enceladus (Saturn). The Europa Clipper mission will carry the Surface Dust Analyzer (SUDA), a reflectron type TOFMS, though the ice plumes of Europa in the coming years, in search of such bio-signatures [2].

The data from such a mission may reveal the presence of amino acids and other organic molecules in the ejecta surrounding Europa and the relative concentration and distribution of these molecules may provide the key to identifying bio-signatures [3].

While there is an enormous amount of data describing the behaviour of amino-acids in TOFMS, the vast majority of this data uses electron ionized TOFMS or laser ionized TOFMS [4]. The ability of the aforementioned ionization techniques to adequately mimic data that instruments like SUDA will produce is not well known as SUDA will rely on impact ionization of its sampled particles [2].

Previous studies describing impact-ionized TOFMS of amino acids have only recently been performed, using a single amino acid at a time, and have not examined how various ratios of amino acids react [Zach Ulibarri, paper in preparation]. Amino acids alone do not provide an agnostic bio-signature as they can be produced from abiotic processes [3]. A critical question for future planetary investigations is whether natural mixtures of such complex molecules can be quantitatively measured from the mass spectral data.

There are technical difficulties designing an experiment to test how amino acids in ice will react in impact ionized TOFMS. Current technological limitations rule out the possibility of accelerating amino acid doped ice to high speed and then impacting a surface, as the process will be in flight instruments which intercept ice grains at high speed.

The purpose of this thesis is to address the technical issues with creating a reliable and repeatable impact ionization TOFMS experiment that accurately describes the mass spectra of amino acids and other water soluble compounds suspended in water ice. Using this novel experimental setup, an experiment categorizing the spectra from various ratios of Arginine and Lysine was conducted.

This thesis begins with a description of the experimental setup used at the Institute for Modeling Plasmas, Atmospheres, and Cosmic Dust (IMPACT), including the Colorado Dust Accelerator, at the University of Colorado. The principles of TOFMS are then described. This thesis will cover two main topics. The first is the development of techniques to create a uniform ice target containing solute mixtures for impact-ionized TOFMS. The development of these techniques are described and the results of how these techniques worked in experiments will be discussed. We then describe an experiment to understand the behavior of a mixture of the amino acids Arginine and Lysine in impact-ionized TOFMS. We conclude with a discussion of the experimental results, their ramifications, and their future directions.

2

Experimental Setup

2.1 The Dust Accelerator

The University of Colorado Institute for Modeling Plasmas, Atmospheres, and Cosmic Dust (IMPACT) has developed an accelerator capable of accelerating small dust grains, typically around 1 μm diameter or less, to speeds between 2 km/s and 100 km/s. This is in the hypervelocity regime for most materials where the inertia of a projectile exceeds the strength of the material. This is done using a modified 3 MV Pelletron high-voltage generator equipped with a dust source to create a linear, electrostatic accelerator. This is connected to a beam-line where the speed, mass, and charge of each dust particle can be measured in flight. Dust can also be downselected according to speed, charge, or mass in the beam-line, to give the operator control over the experimental impact conditions. In our experiment the beam-line terminated at the ice target chamber [5]. A schematic of the accelerator is shown in Figure 2.1.

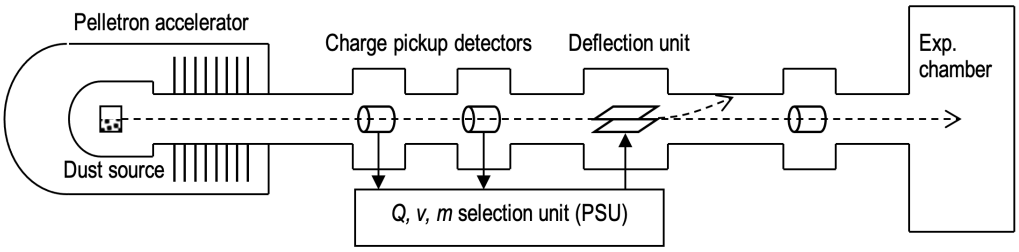


Figure 2.1: Diagram of the Dust Accelerator [https://impact.colorado.edu/]

2.2 The Ice Target Chamber

The ice target chamber consists of a cylindrical vacuum chamber connected to the accelerator beam-line with a vacuum tube and closeable gate valve (Figure 2.2). The chamber is able to achieve a vacuum of 10^{-7} $Torr$. The center of the chamber has a liquid nitrogen cooled cold plate that can be translated vertically and axially for alignment.

The cold plate consists of a lower section where the liquid nitrogen flows and an upper section that is in thermal contact with the lower section but is electrically insulated via a thin layer of Kapton. The upper section is maintained at +4.5 kV in positive ion mode and -4.5 kV in negative ion mode.

Ions which are created during an impact event are accelerated down a drift tube by an electric field created from a grounded grid located 9 mm from the target face. At the end of the drift tube is a microchannel plate (MCP) detector. The target, drift tube, and MCP detector comprise the TOFMS part of the ice target chamber (Figure 2.3).

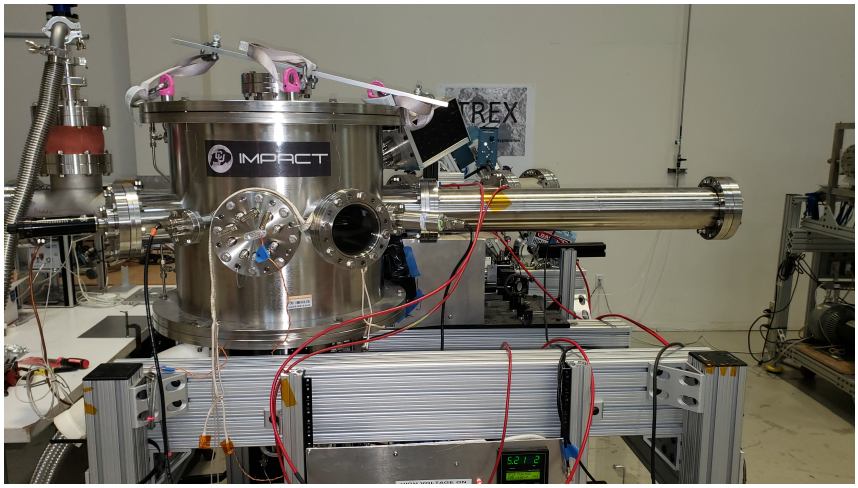


Figure 2.2: Ice target chamber showing the connecting beam-line tube and flange that connects to the beam-line of the dust accelerator on the right.

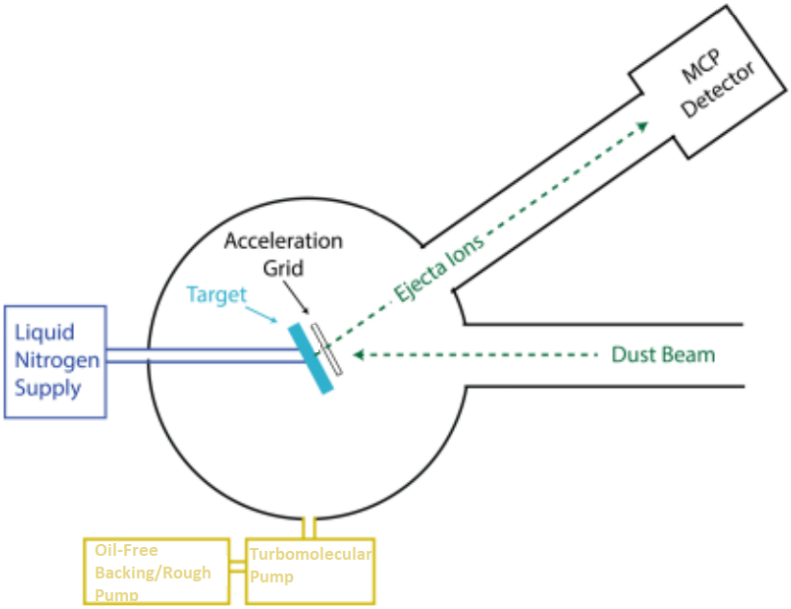


Figure 2.3: Ice Target TOFMS Diagram

2.3 Principles of TOFMS

TOFMS is a tool that can be used to determine the molecular and elemental composition of various compounds. This is done by first ionizing said compounds which are distributed on a target. The ions are then accelerated by an electric field produced by a potential difference between an acceleration grid and the target. After acceleration the ions are allowed to drift without acceleration in a drift tube over a fixed (and known) distance. At the end of the drift tube is a MCP detector that produces a signal as ions arrive (figure 2.4). The heavy ions arrive later than the lighter ions (eqn. 2.4) creating a mass spectrum of ion count vs. time.

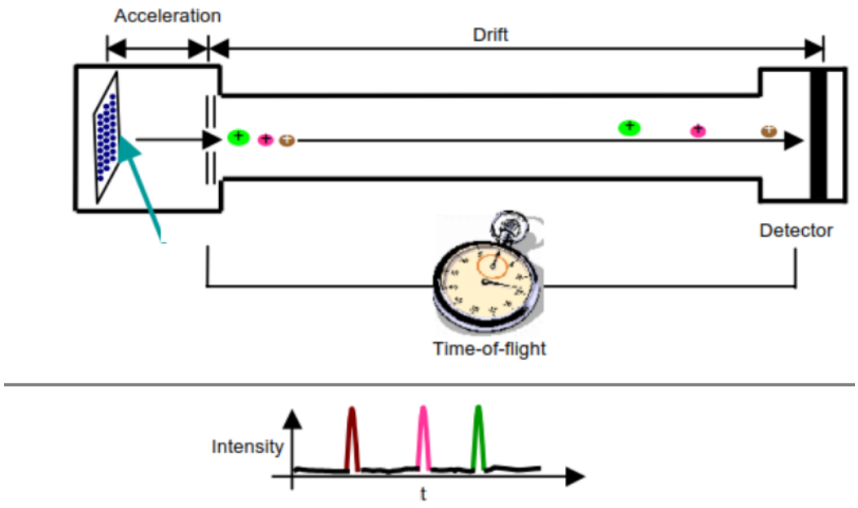


Figure 2.4: Schematic design of a TOFMS system and resulting mass spectrum. [6]

The kinetic energy E of an accelerated ion can be determined by:

$$E = qV \ (= \frac{1}{2}mv^2) \tag{2.1}$$

where q is the charge on the ion, typically $+1e$, V is the voltage difference between the acceleration grid and the target, m is the ion mass, and v is the ion velocity

after acceleration.

From this we can solve for the velocity of the ion to get:

$$v = \sqrt{\frac{2E}{m}} \tag{2.2}$$

and noting that

$$v = \frac{d}{t} \tag{2.3}$$

we can solve for mass as a function of time:

$$m = \frac{2E}{d^2}t^2 \tag{2.4}$$

Since the kinetic energy E is a function of the charge of the ion and the voltage applied to the acceleration grid, and d, the drift distance, is set by the construction of the TOFMS apparatus, we can define the quantity

$$\frac{2E}{d^2} \tag{2.5}$$

as the "stretch parameter", which is intrinsic to the TOFMS device at a given setting of acceleration voltage.

To determine the time of flight of the ions we must know the initial time of the ionization event as well as the time the ions arrive at the detector so the full expression describing the operation of the TOFMS device is:

$$m(t_m, t_o) = \frac{2E}{d^2}(t_m - t_o)^2 \tag{2.6}$$

where t_m is the arrival time of a specific mass ion and t_o is the initial time of the ionization event

Figures 2.5 and 2.6 below show a typical mass spectrum plotted first in the time

domain and then in the mass domain. The mass spectrum used was from pure water, such that peaks corresponding to clusters of H_2O can be seen. The double peaks show nH_2O and nH_3O. Note the even spacing of cluster sequences after the raw time-domain spectrum has been converted to the mass domain.

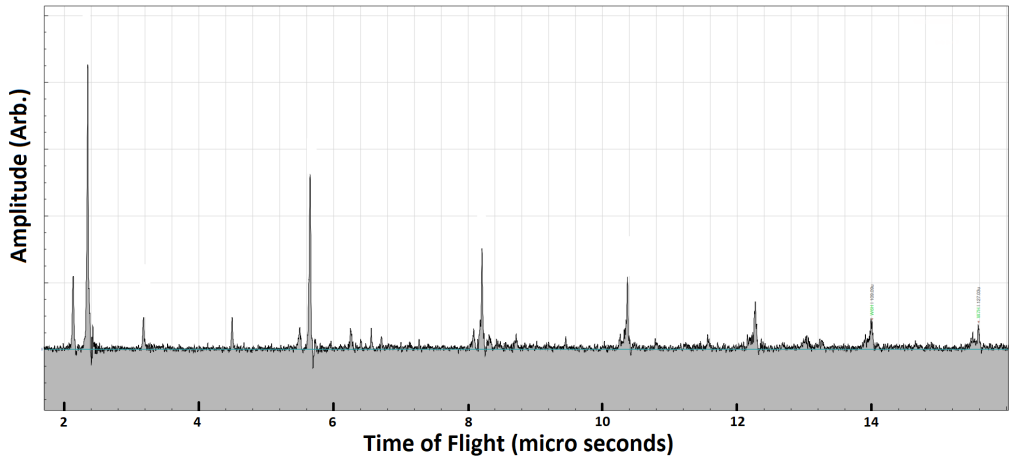


Figure 2.5: Spectrum of water in the time domain. Major peaks from left to right: H_2O, H_3O, Na, C_2OH_2, $2H_2O$, $2H_3O$, $3H_2O$, $3H_3O$, nH_2O, nH_3O,... The distinction between the water and protonated water clusters becomes less distinct farther right.

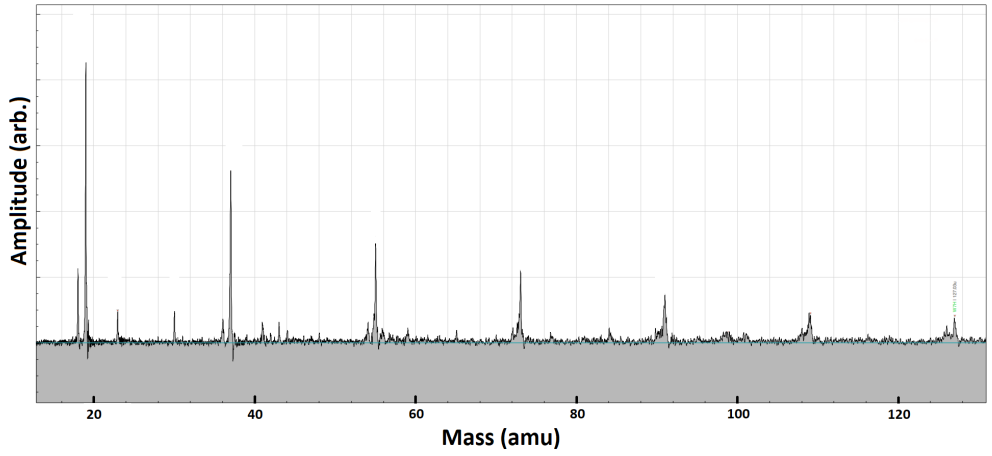


Figure 2.6: Spectrum of water in the mass domain. Major peaks from left to right: H_2O, H_3O, Na, C_2OH_2, $2H_2O$, $2H_3O$, $3H_2O$, $3H_3O$, nH_2O, nH_3O,... The distinction between the water and protonated water clusters becomes less distinct farther right.

3

Airbrush Technique for Creating Uniform Cryogenic Targets

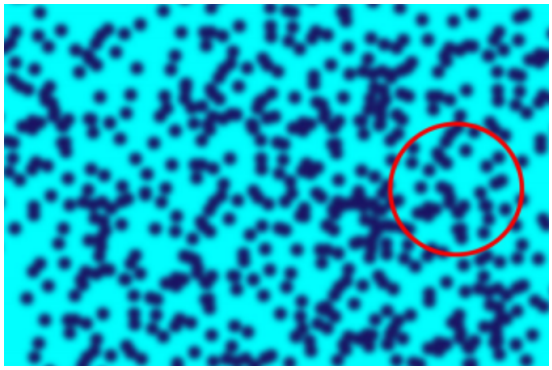
3.1 Background

The means of creating a target consisting of amino acids or other soluble compounds uniformly dispersed in ice was needed so that the impact of hyper-velocity dust would create a partially ionized plasma consisting of water ions and ions of the dissolved compounds. The distribution of the dissolved compounds in the ice needed to be uniform so that the mass spectra would be consistent over repeated impacts.

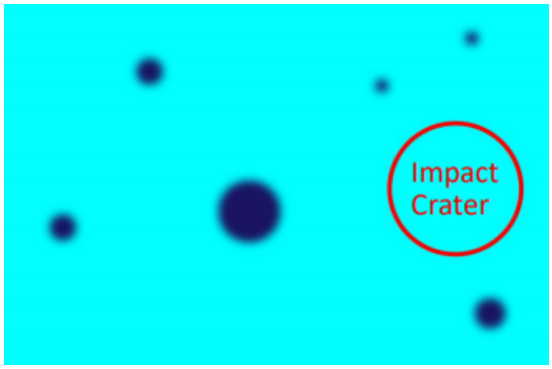
Initial attempts to produce ice targets with impurities made use of a "flash freezing" approach, where the liquid mixture was frozen as quickly as possible to prevent migration of impurities during freezing. These attempts provided inconsistent results, however, possibly because the cooling/freezing process was still slow enough to allow the solute and solvent to separate during freezing. During impact experiments, the dust grains do not strike exactly the same spot every time but instead cover a

"shotgun" pattern with around 6mm diameter [7]. Because of the nonuniformity of the frozen impurity concentrations, this resulted in some mass spectra having only water peaks and other mass spectra having only peaks related to the solute and its breakup products.

A solution to this problem was to use a novel airbrush technique which would spray small droplets of the solution onto an already cold target plate, with the idea that the small thermal mass of the droplets would allow them to freeze in place, such that any migration of impurities would be limited to the scale size of the droplets. A schematic illustration of this idea is shown in Figure 3.1.



(a) Ideal Target Surface



(b) Flash-Freeze Surface

Figure 3.1: Ice Target Surfaces (Light Blue = Solvent, Dark Blue = Solute) [Figures provided by Zach Ulibarri]

3.2 Experimental Setup and Methods

The airbrush that was used was a Paasche Talon which was capable of producing a mist of liquid droplets between 0.010 mm and 0.050 mm. This particle size distribution was important because it was comparable than the iron dust projectile and during impact the projectile should be able to ionize a consistent mixture of solvent and solute over the entire impact area. The airbrush was thoroughly cleaned with water and ethanol between each use.

Contamination of the target plate by condensation of atmospheric moisture was an issue as the plate had to be sprayed with the airbrush after it had been cooled by liquid nitrogen and moisture in the atmosphere would immediately accumulate on the cold target plate. Because of this an acrylic glove box was purchased so that the ice target could be made in an environment of dry nitrogen (Figure 3.2). The glove-box was outfitted with a vacuum pump, valves, and stainless steel plumbing that would allow it to be pumped down to 50 Torr and then back filled with (continuously flowing) dry nitrogen. Dryness of the atmosphere was determined by Sensirion SHT35 high accuracy temperature and humidity sensors that were capable of reading moisture levels in the range of 0-100% relative humidity (RH) with and accuracy of $\pm$ 1.5 % RH. These sensors sampled the atmosphere of the main-chamber of the glove-box and the loading/unloading chamber of the glove-box. A small cooler was outfitted with an internal mechanism that was small enough to fit into the glove-box and facilitated the safe and easy creation of an ice target in the confines of the glove-box. The glove-box was also fitted with 6 check valves that opened at 3 psi of relative pressure. This was done in the event that a spillage of liquid nitrogen resulted in the rapid evolution of gas inside the glove-box, which might otherwise produce an over-pressure condition. The interior of the glove-box had a nitrogen supply line that was regulated at 15 PSI for the airbrush.

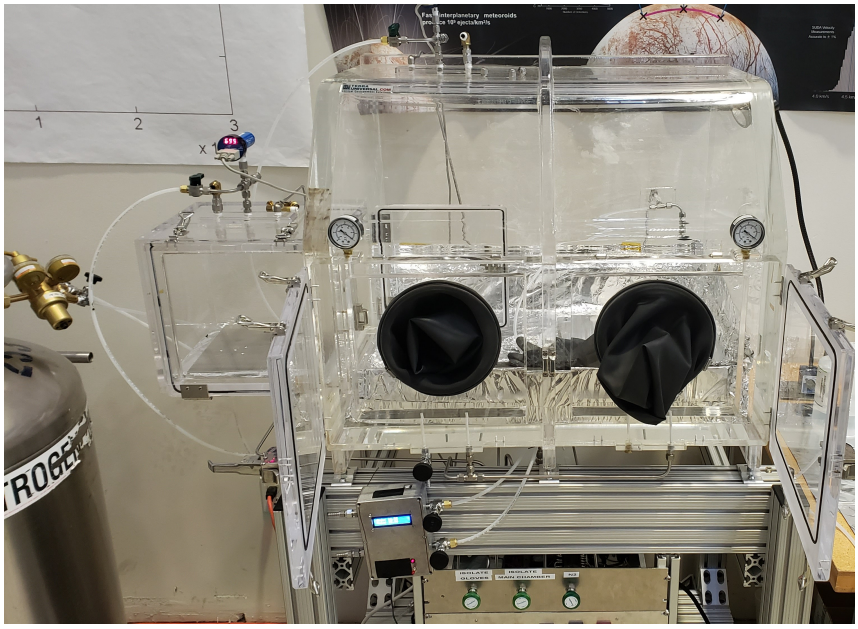


Figure 3.2: Picture of the Glove-box

3.2.1 *Procedures for Making an Ice Target*

Step 1: Cleaning

The first step was to thoroughly clean the airbrush, ice target plate, ice target cooler, and the glove-box if a new solution was being used. Special care must be taken with the cleaning to avoid contaminating the ultra high vacuum environment of the ice target chamber as well as the beam-line. The cooler was wiped down on the outside with de-ionized water and ethanol and the interior mechanism was rinsed with de-ionized water and ethanol and allowed to dry. Cleaning of the ice target consisted of a soap and water wash first, followed by a rinse in de-ionized water. The ice target was then rinsed in acetone and then ethanol in an ultrasonic cleaner, air dried thoroughly, and then placed in the cooler. Special care was taken with the airbrush as it had to be disassembled to clean. Soap was not used due to the small crevices in the airbrush. Components were washed in and ultrasonic cleaner in de-ionized Water first and then ethanol and allowed to dry before re-assembly. Acetone was not used due to incompatibility with rubber gaskets in the airbrush.

The glove-box was wiped down with de-ionized water as any other solvent would damage the acrylic.

Step 2: Dry the Glove-box

The glove-box had to be prepared a few hours ahead of time to ensure a dry environment. First the glove-box was pumped down to 50 $Torr$ and then back-filled with dry nitrogen. Once the glove-box reached the same pressure as the outside atmosphere the sampling valves were opened and nitrogen was allowed to flow until the moisture in glove-box was under 0.5 %RH The sampling valves allowed the humidity sensors to sample the interior moisture of the glove-box without having to be inside the glove-box chambers.

Step 3: Prepare the Solution

While the glove-box was drying, the solution for the airbrush was prepared.

Step 4: Prepare Target and Load the Airbrush.

Once the target plate and sealing slider were thoroughly dried the cooler was filled with liquid nitrogen and the target plate was submerged. While the target plate was cooling down the airbrush was loaded with the solution and tested with an external supply of nitrogen at 15 PSI.

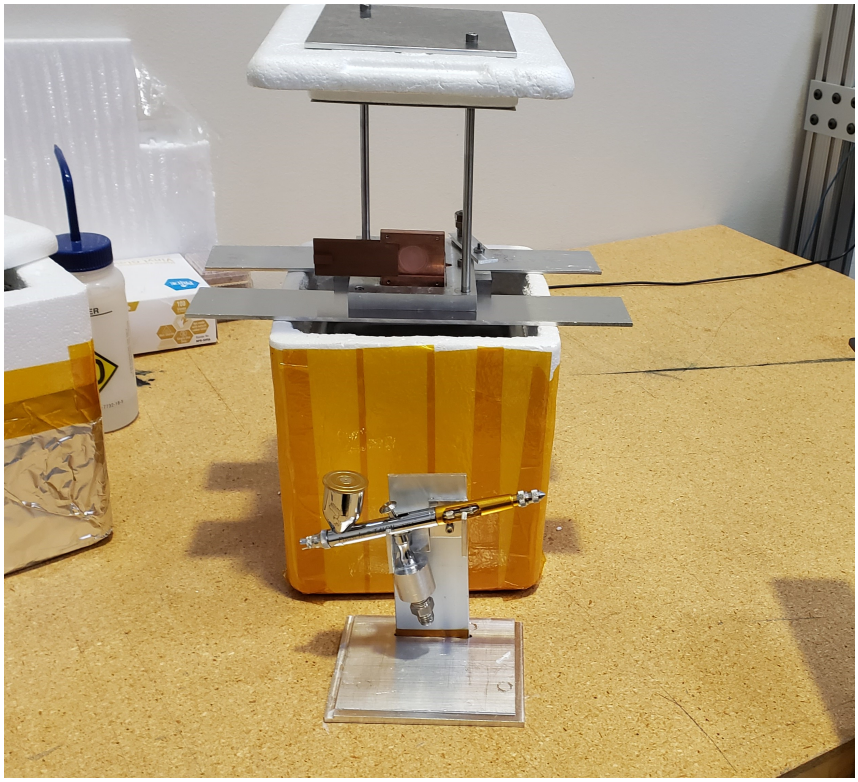


Figure 3.3: Ice target plate with sealing slider open propped on top of the cooler (styrofoam, covered with Kapton tape). Airbrush in front

Step 5: Load the Glove-box

Once the target plate had cooled down enough to stop rapid boiling of the liquid nitrogen the cooler (with the target plate inside) and the airbrush were loaded into the loading/unloading chamber. The loading/unloading chamber was then pumped by the vacuum pump for 4 minutes and then back filled and sampled until it reached 0.5 %RH. The interior door between the main chamber and loading/unloading chamber was then opened and the cooler and airbrush were transferred into the main chamber.

Step 6: Making the Ice Target

After transferring the cooler and airbrush into the main chamber the target plate was lifted out of the cooler via the internal mechanism and propped up on top of

the cooler as in Fig. 3.3. The airbrush was then connected to the internal regulated nitrogen supply. Carefully the target plate window was opened. The airbrush was held at approximately 15 cm from the target plate and operated for about 2-3 seconds until a thin layer of ice was visible. The target plate window was quickly closed and the the target was re-submerged in the liquid nitrogen filled cooler. The cooler was then unloaded without worrying about maintaining the dryness in the glove-box. The liquid nitrogen levels were topped off until the target was ready to be loaded into the ice target chamber.

3.3 Experimental Results from the Airbrush

Initial results from using the airbrush to create an ice target with dissolved solids were conflicting. A pure solution of 1 molar Histadine dissolved in water provided spectra that were mostly water peaks with about 10% of the spectra being pure Histadine peaks and its breakup products. The reason for this was found to be that in the process of making the ice target the airbrush was held too close and the arriving water droplets were not allowed enough time to freeze on the surface of the target plate before more water droplets arrived. This created a transitory water layer on the target plate before freezing which resulted in extrusion of the solute and an non homogeneous target surface. This was verified by conducting an experiment using a solution of 10 ml red dye #40 diluted in 50 ml of water in the airbrush. A micro-graph was then taken of the (still frozen) target surface after activating the airbrush too close to the target plate. Notice that in figure 3.4 there are areas of just water ice and areas of just red dye.

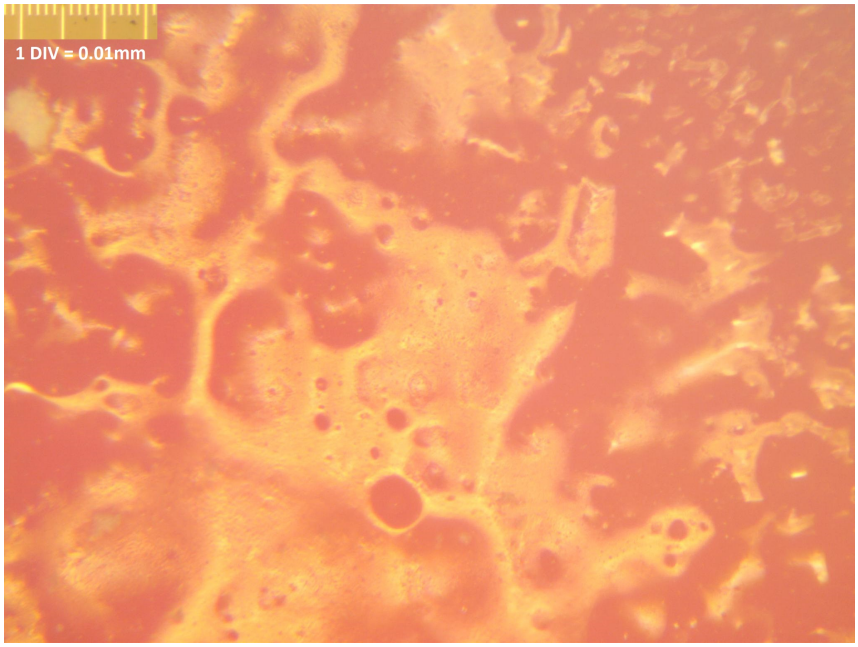


Figure 3.4: Microscope image of target surface produced with dilute red dye mixture and airbrush held too close to cold substrate. Note macroscopic (>0.1 mm) regions of dye/water separation.

Through trial and error a distance of approximately 15 cm was found to be ideal for the creation of a uniform ice target. To verify the surface morphology of the ice target, 10 ml of red dye #40 was diluted in 50 ml of water and an ice target was created. This ice target was observed under 10x magnification and the size and distribution of the particles was as predicted. In Figure 3.5 a micrograph was taken in a dense area of ice accumulation which is characteristic of an actual ice target used in the mass spectrometer. The image in Fig. 3.6 is of a sparse area of ice accumulation just to see the particle size more clearly. These targets were made on a mirror aluminum surface which can be seen as the lighter colored background.

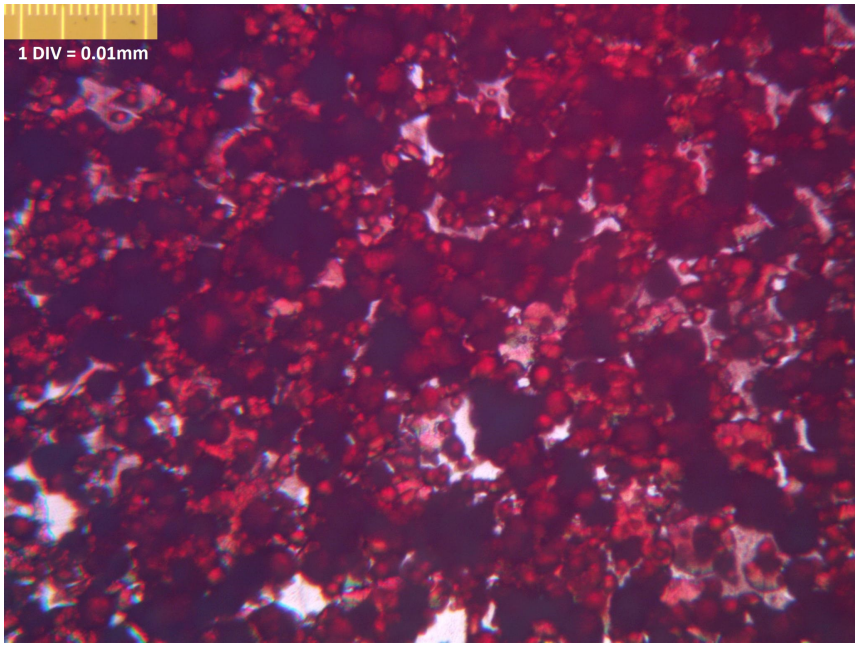


Figure 3.5: Dense ice target

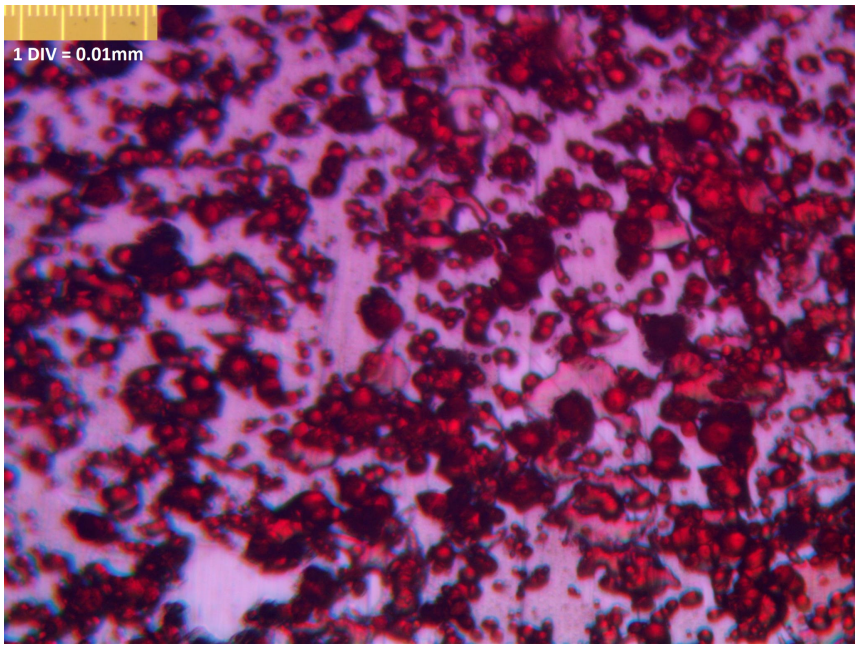


Figure 3.6: Sparse ice target

To see why the flash freezing process yielded such poor results, a flash frozen target was created using the same dye solution as above. This surface was observed under 10x magnification and it was observed that most of the surface of the flash

frozen target consisted of areas of pure, crystalline water ice. This can be observed in fig 3.7. It is difficult to tell from the color of this photograph, but it is suspected that the migration of the impurities during freezing pushed them to beneath the exposed surface, such that they were trapped behind a layer of pure water ice.

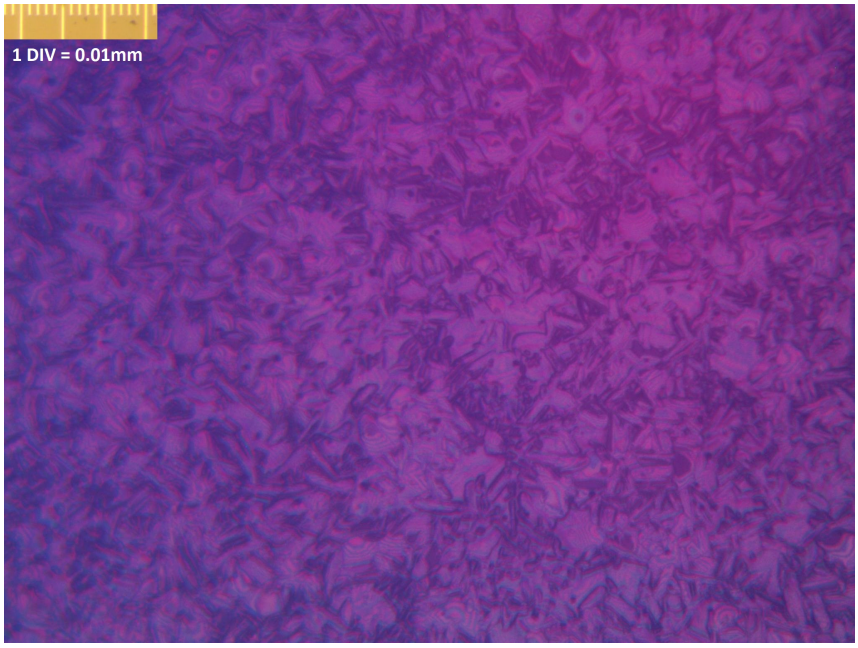


Figure 3.7: Flash frozen ice target

While the ideal airbrush surface yielded more consistent results in the content of the mass spectra, it was observed that when such an ice target was used in an experiment the ratio of spectra collected to impact events drastically decreased. This means that the rate of ionization had decreased when the ideal ice target was used. The plot in figure 3.8 shows the spectra collection efficiency of three types of targets: (a) The true airbrush surface which was made using the above mentioned techniques, (b)a surface that was made as above but then allowed to dry while frozen under vacuum (freeze dried), and (c) a surface that was freeze dried and then had a thin layer of pure ice grown over it using vapor deposition.

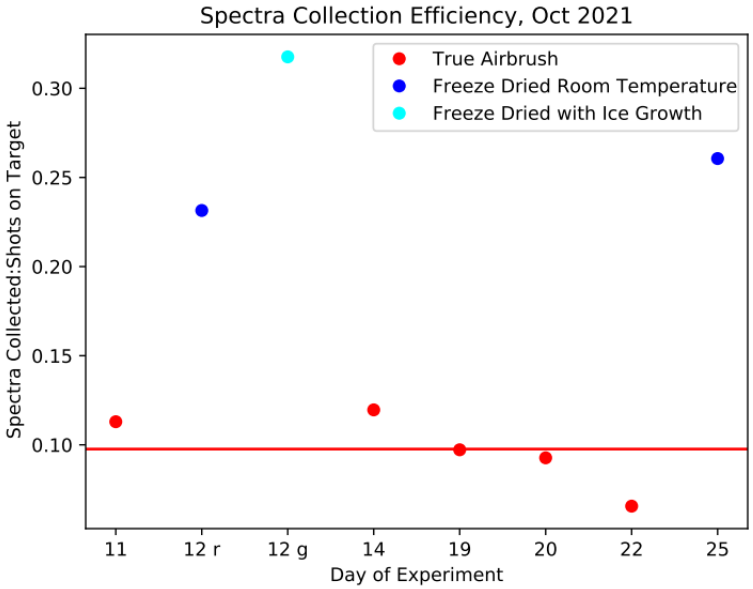


Figure 3.8: Spectra collection efficiency [provided by Zach Ulibarri]

This figure would seem to imply that the roughness of the surface plays an important role in spectra collection efficiency. It can be assumed that the freeze dried surface with vapor deposition would have the smoothest surface since vapor deposition would fill in the spaces between particles. The freeze dried surface would then have the second smoothest surface since the water that formed most of structure of the ice particles had been sublimated away leaving only a fine powder of amino acids. This leaves the true 'ideal' airbrush surface as the roughest.

3.4 Discussion

Why the rough surface decreases spectra collection efficiency is unclear but one hypothesis is that the rough surface could create ejecta plumes that are not directed to the acceleration grid as they would be if the surface was smooth. An example of this would be the glancing blow of a dust particle off a sphere of ice on the target, as can be seen in Fig 3.6. The particle size distribution of the ice is between 10 and 30 μm. The particle size distribution of the iron dust used in the accelerator is between

10 nm and 3 μm. Since the impact dust size is an order of magnitude less than the size of the ice particles it is possible that the dust is cratering the ice particles and not completely obliterating them. The idea of cratering is complemented by work done by Anthony Shu et.al. [8] where it is shown that the dust produced impact craters about 2 μm across and 1.5 μm deep on a surface of polyvinylidene fluoride. How dust from the accelerator craters ice is not precisely known, but could be investigated in future studies.

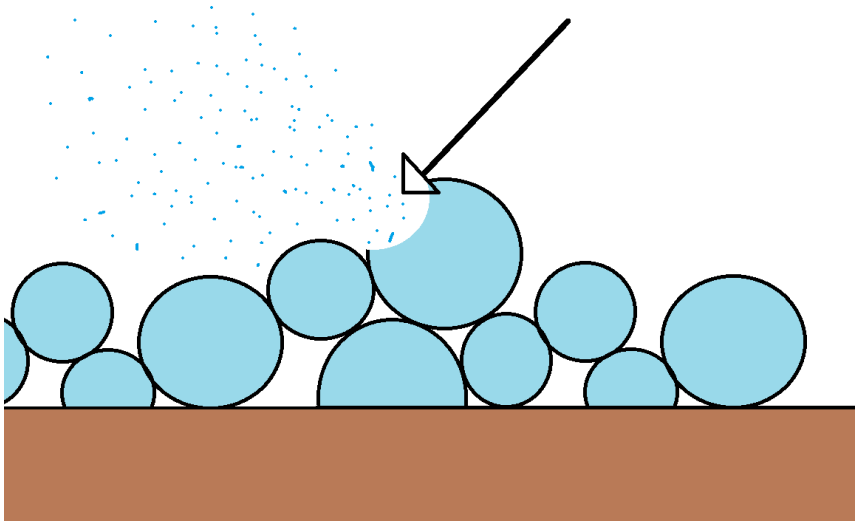


Figure 3.9: Possible misdirection of ejecta due to glancing blow

Overall the ideal ice target provided consistent results, with the caveat that the experiment took longer to run due to the low spectra collection efficiency of the rough surface.

4

Recovery of Arginine to Lysine Ratios Using TOFMS

4.1 Background

Experiments have been done using the novel technique for creating an airbrushed cryotarget as described in the preceding section using single amino acids, and a paper by Zach Ulibarri et.al [in preparation] has explored in depth how the amino acid Histadine behaves when used as a target in impact ionized TOFMS. Mixtures of amino acids have not yet been studied using impact ionized TOFMS. The amino acid ratios experiment was conducted using three ratios of Arginine and Lysine. These amino acids were picked because previous experiments found that the detection limit of Arginine and Lysine were similar, indicating a similar ionization potential, and that they were efficient at producing ions [4]. The ratios used were 10% Arginine and 90% Lysine, 50% Arginine and 50% Lysine, 90% Arginine and 10% Lysine. The experiments were conducted over a number of days as shown in table 4.1. Some of the experiments were repeated due to issues with low ion production.

4.2 Experimental Setup and Methods

To begin an experiment the Lysine and Arginine were measured and dissolved in super de-ionized water. This solution was used to make an ice target as described in section 2.4.3. While that target was being made the cold plate was brought down to 77°K. Once the target was made and the cold plate was at the desired temperature the ice target chamber was brought to atmosphere and the lid was removed. With the lid removed the ice target was quickly secured to the cold plate. The lid was then re-secured to the target chamber and the vacuum pump was started.

Once the target chamber had achieved a vacuum of 10^{-7} Torr the ice target plate window was opened, the gate valves were opened and the accelerator began operation. All velocity ranges of dust were selected for. The accelerator was allowed to operate until a sufficient number of spectra were collected. This typically took many hours. The mass spectra were saved to a LeCroy Waverunner scope and then analyzed later using multiple methods.

4.3 Results from the Arginine-Lysine Ratio Experiment

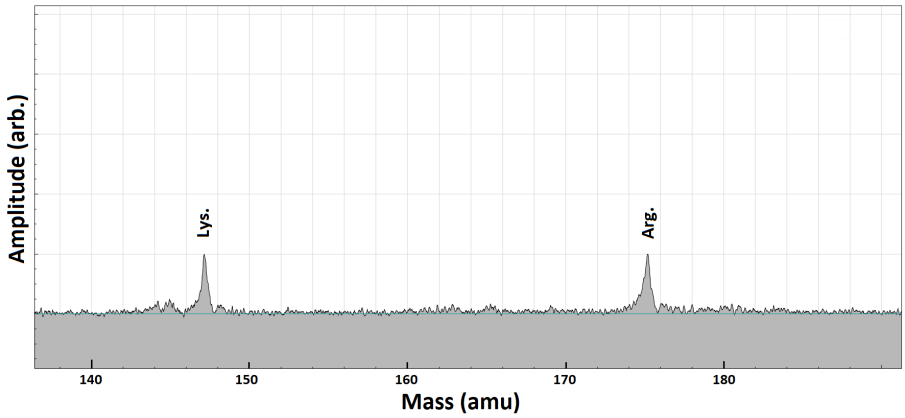
Results from the amino acid ratios experiment show that relative abundances of amino acids can be recovered from TOFMS on an ice target made with two different amino acids. Arginie and Lysine were mixed in three different ratios and an ice target was made from that solution and used in TOFMS. Table 4.1 describes the 5 experimental runs used. The error from mixing the amino acids as well as the number of spectra collected are shown for each run.

Run#	Date	Arg. Amount	Lys. Amount	Number of Spectra
1	10/19/2021	.899 ± 0.004 M	.101 ± 0.003 M	106
2	10/20/2021	.899 ± 0.003 M	.099 ± 0.003 M	753
3	10/11/2021	.501 ± 0.003 M	.499 ± 0.003 M	652
4	10/14/2021	.100 ± 0.003 M	.874 ± 0.004 M	655
5	10/22/2021	.098 ± 0.004 M	.900 ± 0.004 M	418

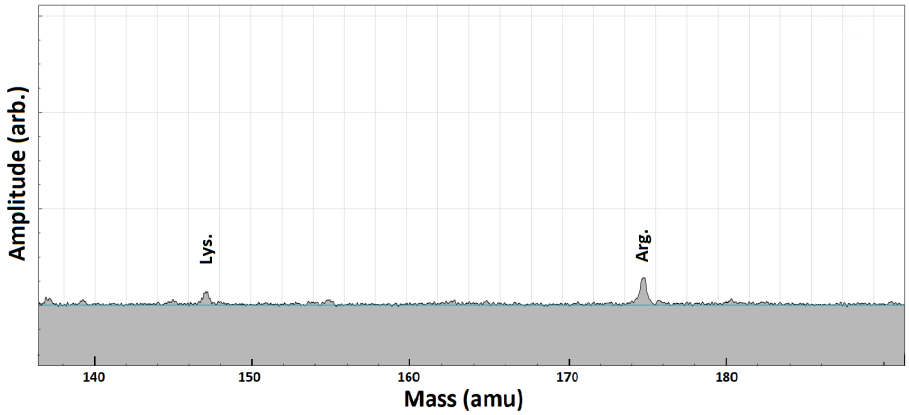
Table 4.1: Ratios Experiment Table

The data from the experiments were saved using a Lecroy Waverunner scope which was triggered when the MCP detector voltage rose above 3.5 mV. Due to the low spectra collection efficiency of the true ice target surface however, the scope trigger level had to be kept lower than usual which allowed electrical noise to trigger the scope. In fact out of all the spectra collected about 50% are just noise.

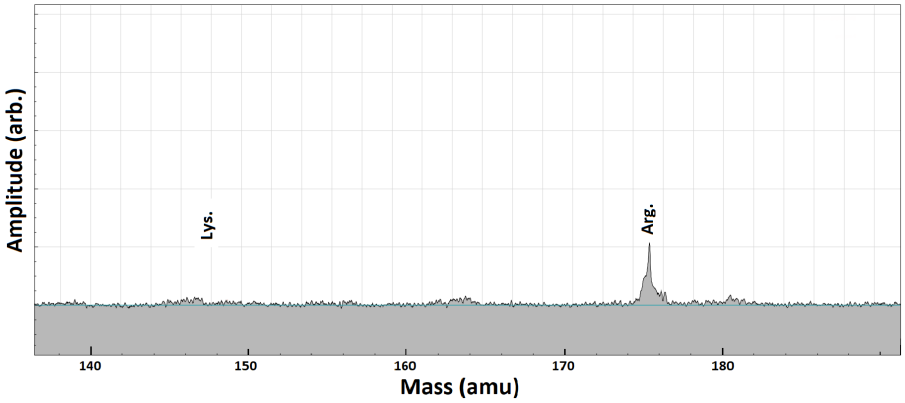
The data from the scope was then analyzed using the proprietary software Spectrum which allowed for setting the stretch parameter and time zero of each individual spectra. Using the Spectrum software, spectra with a velocity between 3 km/s and 10 km/s were selected for and analyzed. Spectra that had visible amino acid peaks were then separated from spectra that just had water peaks. The spectra with amino acids that fell into the aforementioned velocity category were then summed to increase the signal to noise ratio. This allowed for a qualitative assessment of the data (Fig. 4.1).



(a) 10% Arg. 90% Lys. from 10/22/21 n=17



(b) 50% Arg. 50% Lys. from 10/11/21 n=23



(c) 90% Arg. 10% Lys. from 10/20/21 n=18

Figure 4.1: Summed mass spectra from select experimental runs. The number of spectra summed is indicated.

To quantitatively analyze the data and derive a relationship between the actual ratio of amino acids used and the ratio of the ion content for the two corresponding mass peaks in the spectra, the data was exported from Spectrum and imported into a separate Python program which fit a line to the data and integrated that line at each specified mass peak. These integrals were then used to create a ratio. The ratio $\frac{Arg.Content}{Lys.Content}$ was chosen to be used in the analysis. The program was created by Zach Ulibarri and more information can be found in his paper which is in preparation. The data from the program was then used by a separate script (written as part of this work) to create the ratio from the ion content of each amino acid mass peak and determine the error for each ratio (table 4.2).

Run#	Arg./Lys. as Made	Arg./Lys. Recoverd
1	9.00 ± 0.01	7.3± 3
2	9.00 ± 0.01	5.13 ± 2
3	1.00 ± 0.00	1.9 ± 0.5
4	0.11 ± 0.01	0.8 ± 0.2
5	0.11 ± 0.01	1.5 ± 0.5

Table 4.2: Ratios Experiment Results Table

Run# 1 and run # 2 were averaged together and their error propagated as well as run# 4 and run # 5. The data from this was then plotted with the recovered ratio on the y axis and the actual ratio on the x axis. A function was then fitted to the data using the scipy optimize.curve_fit method. (Fig. 4.2).

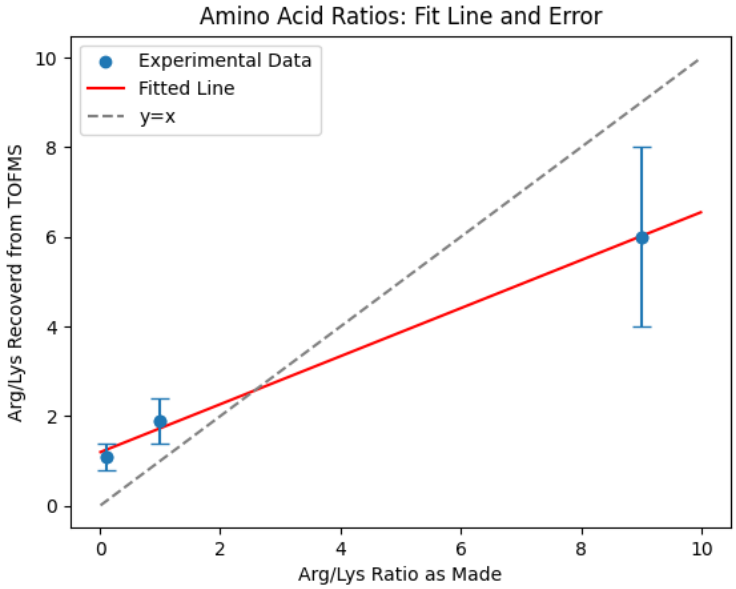


Figure 4.2: Graph of Amino Acid Ratios

The fitted line has the equation:

$$y = (0.5 \pm 0.2)x + (1.19 \pm 0.03) \tag{4.1}$$

Where y is the ratio recovered from the mass spectra and x is the actual ratio of Arginine to Lysine. This equation can easily be rewritten to find the actual ratio of Arginine to Lysine from the recovered ratio of ion content.

$$x = \frac{y - 1.19 \pm 0.03}{0.5 \pm 0.2} \tag{4.2}$$

Using the above equation the actual ratio of Arginine to Lysine can be inferred from TOFMS. It may be unexpected that the slope of this fit line is not one and the intercept is not zero but the experimental results arise due to the difference in the rate of ionization between the two amino acids used. This is due to the different side chains on Arginine and Lysine. These side chains posses properties that make them more or less susceptible to protonation [4].

4.4 Discussion of Results

The above results suggest that the actual relative abundance of Arginine and Lysine can be derived from TOFMS data. It is important to note that the configuration of the TOFMS used in this experiment can only detect positive ions or negative ions at one time so only amino acids that have the same mode of ionization (protonation or deprotonation) can be used. This means that only ratios of amino acids with the same mode of ionization can be found.

To extend these results to data that may be acquired by TOFMS instruments mounted on spacecraft we must assume that impact of iron dust on a target surface of ice ionizes similarly to a particle of ice striking a target surface. Using this assumption we can begin to understand what data from such missions my be able to tell us about the potential for life on icy celestial bodies.

Klenner et.al. describe in their paper *Analog Experiments for the Identification of Trace Biosignatures in Ice Grains from Extraterrestrial Ocean Worlds* [4] that amino acids prefer to form protontated ions. This characteristic is shared with peptides. This means that it likely that both may be able to be detected at the same time in a mass spectra. This is important because in the article *Deciphering Biosignatures in Planetary Contexts* by M.A Chan et.al. [3] it is described that the abundance of organic molecules produced abiotically is inversely proportional to the number of carbon atoms that they are composed of. Further, it is described that "Due to thermodynamic and kinetic constraints on the rate of formation of molecules during abiotic synthesis, a continuous spectrum of molecules, enriched in kinetically allowable low-molecular-weight compounds, is expected." This means that agnostic biosignatures can be detected just by looking at the relative abundance of organic molecules.

As described above, if organic molecules are produced only abiotically, the abun-

dance of each molecule can be known. This can be used to create a library of TOFMS spectra and associated fit functions for different amino acid ratios, and even amino acid to peptide ratios as they would be for abiotic production. With this data on hand it would be easy to see if spectra returned from TOFMS mounted on spacecraft that contained amino acid peaks or other organic molecule peaks matched the abiotic templates or not. If not, the spectra could be used to determine the actual ratios of organic molecules and if it matches what is produced by a biotic system.

One thing to consider is that there may be a dependence on on the velocity of the particle and the recovered ratio as well. This is due to the fact that Lysine and Arginine may break up at different rates when the energy of the collision is increased. This feature of amino acids is discussed in *Revisiting Fragmentation Reactions of Protonated -Amino Acids by High-Resolution Electrospray Ionization Tandem Mass Spectrometry with Collision-Induced Dissociation* by Zhang et. al. [**7**]. In this article, specifically supplemental figures S50 and S51, it is shown that Lysine will tend to break up more than Arginine. This implies that the ratio of Arginine and Lysine will increase with increasing velocity of the projectile. Further investigation is needed to confirm this as this thesis concentrates only on low velocity spectra.

5

Conclusion

A summary of findings is recounted for the novel cyrotarget creation technique followed by future work to improve the technique. This is followed by a summary of findings for the amino acid ratios experiment followed by future research.

5.1 Summary of Findings for Cryotarget Creation

A technique for the creation of a cryotarget for use in impact-ionized TOFMS was developed at IMPACT. The final methods for creating the target resulted in an ice surface with uniform distribution of dissolved solids. The surface was examined under a microscope at various magnifications using red dye which showed that there was indeed a uniform distribution of solute in the accumulated ice on the target. The cryotarget did however result in lower ion production which resulted in less mass spectra being collected compared to other targets. This was attributed to the surface roughness to the cryotarget.

5.2 Future Research for Cryotarget Creation

Determining all the causes for lower ion production of the cryotarget is needed to fully understand what can be done to improve ion production from this surface.

While experiments can still be carried out using this surface, the time it takes to collect enough data to fully understand the behaviour of the target substance could be improved. An experiment has been proposed to temporarily replace the MCP detector with a delay line detector to determine the 2d spatial distribution of the accelerated ions in the drift tube. Information from this could then be used to adjust the voltage on steering plates in the TOFMS to direct more ions to the MCP detector.

5.3 Summary of Findings for The Amino Acid Mixtures Experiment

Using the techniques developed for the creation of a cryotarget for impact ionized TOFMS, an experiment using varying mixtures of Arginine and Lysine was carried out at IMPACT. This experiment showed a relationship between the initial ratio of Arginine and Lysine used and the ratio of ion content of the mass peaks corresponding to each amino acid in TOFMS mass spectra. A linear relationship was shown and a fit line was fitted to the data provided by this experiment. The equation from the fit line could be used to estimate the actual ratio of Arginine to Lysine from the data provided by impact ionized TOFMS mass spectra.

5.4 Future Research from The Amino Acid Mixtures Experiment

While the results from the amino acid mixtures experiment are promising and exciting, only the low velocity regime was focused on in these experiments. The ratio recovered from TOFMS has been predicted to be dependent on the velocity of the projectile used for ionization [4] and confirming this prediction is important to fully understand the behaviour of amino acid mixtures in TOFMS. Further analysis

of the data from the amino acid ratios experiment may show this relationship. Filling in the data points in between the 50% Arginine. and 50% Lysine ratio and the 90% Arginine and 10% Lysine is also important to verify that the relationship between the actual ratio and recovered ratio is indeed linear. Gathering more data for these new points and the existing points will reduce the experimental error and should be done in the future. Other future research includes repeating this experiment with other amino acids and even other organic molecules such as peptides to create a mapping that can be used to analyze data that will be sent back from TOFMS instruments mounted on spacecraft.

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