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Prebiotic potential of aerosols

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PREBIOTIC POTENTIAL OF AEROSOLS

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ABSTRACT

A triple quadrupole gas chromatograpy mass spectrometer (GC/MS/QQQ) was used to determine prebiotic molecules of interest in plasma and photochemical aerosols. Glycolic acid was further studied using isotopically doped gases. Plasma and photochemical aerosol analogs were shown to have nucleobases and a few amino acids. Each type of aerosol was also the source of glycolic acid, a precursor to glycine. Using ^{18}O doped CO, it was determined that contamination was not a source of oxygen in the samples. Using different combinations of ^{13}C doped gases in 1:1 combinations of CO and CH_4, it was determined that under present reaction conditions ^{13}C in high concentrations shuts down the photochemistry in the chamber.

DEFINITIONS

Abiotic: a process that is not related to or derived from life

Carboxylic Acid: a compound containing a carboxylic acid functional group

Derivatization: a chemical technique which transforms a compound into a products of similar chemical structure, called a derivative

Ionize: to convert a substance into ions

GC/MS QQQ: a GC/MS uses an ionization source to transform gaseous compounds into ions that hit a detector at different times based on their size. The QQQ function allows different tests to be run at higher concentrations of compound to determine a variety of things

Ions: charged particles

Isotope: different forms of the same element that differ only in number of neutrons, changing their atomic mass but not chemical properties

m/z ratio: distribution of ions by mass

Nucleotide: a compound composed of a nucleoside linked to a phosphate group that forms the basic structural unit of nucleic acids (DNA, RNA)

Organic Compound: a class of compounds in which 1+ atoms of carbon are linked to other elements (most commonly hydrogen, nitrogen, and oxygen)

Photochemical: chemistry that is induced by light

Prebiotic: referring to a time before life

Reactive Species: chemically reactive species containing oxygen

UV radiation: ultraviolet radiation is made up of wavelengths from ~100-400 nanometers

^{13}C: an isotope of carbon, which appears most abundantly in nature as ^{12}C

^{18}O: an isotope of oxygen, which appears most abundantly in nature as ^{16}O

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INTRODUCTION

The prebiotic atmospheres of the early Earth and Titan, Saturn's largest moon, are of current interest to science because of the unique characteristics of their prebiotic hazes. Many reactions take place during the formation of these hazes, but only a few of them are relevant as food sources for early life. There are several common mechanisms by which scientists simulate atmospheric chemistry. The most common, and those examined further in this work, are plasma discharge and UV (ultraviolet) radiation. Other techniques include gamma radiation and soft x-rays, as well as proton or electron beams. Plasma discharge aerosols are created one of two ways, hot plasma discharge and cold plasma discharge. In hot plasma discharge, either sparks or lasers are used to decompose the gas molecules into new compounds. Cold plasma discharge uses electrical excitation of plasma to ionize a small fraction of gas and create reactive species. UV radiation involves using a UV lamp of some variety to excited gas molecules to undergo reactions.[1]

Gas Chromatography/Mass Spectrometry Triple Quadrupoles (GC/MS QQQ) can be used for quantification and determination of compounds present in either volatile or soluble substances such as aerosols. Many different reaction methods can be built to both find unknown compounds and determine the presence of suspected compounds. Isotopically doped (^{13}C) gas mixtures can be used in order to elucidate the mechanism of formation of compounds. If only two gases are potential carbon sources and only one of those gases is ^{13}C doped, then the presence of ^{13}C on any carbon in the resulting molecule indicates that carbon came from the doped gas. The location of the ^{13}C molecule(s) can be determined by the different m/z fragments formed by the different possible substitution patterns of the ^{13}C.Additionally, ^{18}O

carbon monoxide can show that all oxygen present in the molecule comes from intentionally added sources as opposed to contamination.

The aerosols can be analyzed using GC/MS QQQ to determine mechanisms of compound formation as well as to find compounds that could be potential food sources for early life on Earth. Initial research has been performed to investigate compounds that could be potential food sources for early life on Earth, including carboxylic acids, nucleotides, and amino acids. In previous work, some simple compounds have been formed in photochemical aerosols, including glycine, guanidine, and urea.[2] After determination of a few compounds of interest, one compound, glycolic acid, was singled out for further study. The goal of this study is to determine the prebiotic molecules of interest in plasma and photochemical aerosol analogs of the early Earth atmosphere and elucidate the mechanism of formation of glycolic acid using isotopically doped gases in order to further scientific knowledge in the area of photochemical aerosols.

LITERATURE REVIEW

In the 1920s, scientists A. I. Oparin and John Haldane separately speculated that early life must have arisen from abiotically produced organic molecules and in the harsh environment of the early Earth must have survived by consuming these same abiotically produced organic compounds.[3] This hypothesis led to the concept of a "prebiotic soup," or a mixture of gas and liquid that contained the ingredients to sustain the first organisms. At the time, these ideas were just conjecture, but over the years have found support from new data.

Some of the first work on the

atmosphere of the early Earth was performed

by Harold Urey and Stanley Miller, who used a

cycling reaction chamber with a tesla coil to

spark a mixture of ammonia, water vapor, and

methane (Figure 1).[4] Their results show the

formation of 11 out of 20 amino acids among

a host of other prebiotic compounds such as

carboxylic acids and hydroxy acids, and

sparked a wave of research into the
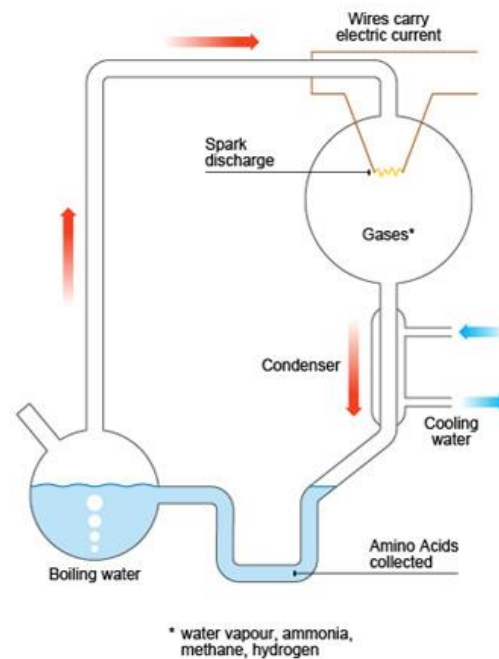
compounds that helped life grow into what it is

today.[5]

Figure 1: Urey-Miller Experimental Set-up[4]

In the years since Urey and Miller's experiment, many things have changed. New

research has improved the gas mixtures used, different chamber types have been created for

atmospheric experiments, and new types of data analysis have become available. Additionally,

new planetary atmospheric environments have come of interest, due to our better

understanding of the early Earth. The atmosphere of the early Earth has been subject to debate

over the years. Current data and speculation suggests that the potential gases present in the

atmosphere prior to oxygenation included CH_4, H_2, H_2S, NH_3, CO, H_2O, CO_2, SO_2, and N_2.[6,7]

One extraterrestrial body of interest is the largest moon of Saturn, Titan. Based on data

from the recent (1997-2017) Cassini-Huygens mission, which sent a probe to take readings of

Saturn and its satellites, the atmosphere of Titan has been determined to be similar to that of

the early Earth. Titan has an organic haze layer that is formed from photochemistry in the upper atmosphere. Laboratory work has shown that using a deuterium lamp as an energy source in a photochemical reaction chamber can produce aerosols that are analogs for the observed haze in Titan's atmosphere.[8] The atmosphere of Titan is known to be N_2 dominated, with data from Cassini-Huygens showing CO, HCN, CH_4, H_2O, and fused-ring polycyclic aromatic hydrocarbons present as well.[9,10]

The different gases present in the atmosphere collide and react with one another via a variety of pathways to create compounds. The current research is interested in the compounds that can be found in photochemical aerosol analogs. Our lab has already examined the compounds that are produced in plasma aerosol analogs.[2] Several amino acids as well as all five nucleotides were detected in plasma aerosol analogs using high resolution mass spectrometry and gas chromatography mass spectrometry.[11] Once a working list of the compounds present in photochemically produced aerosols was gathered, GC/MS QQQ was used with isotopically doped samples to determine the mechanism of formation of glycolic acid.

METHODOLOGY

A photochemical reaction chamber was used to create aerosol analogs of the early Earth and Titan. A diagram of the reaction chamber can be seen in Figure 2. Aerosols are created from different combinations of gases, including CO, CH_4, O_2, and CO_2. For the purposes of this project, CO and CH_4 were mixed in a 1:1 ratio of 0.1% concentration each. To generate the different isotopic ratios, mixtures of 1:1 $CO:CH_4$, $^{13}CO:CH_4$, and $CO:^{13}CH_4$ were created. In order to eliminate contamination as a source of oxygen in the products, a $C^{18}O:CH_4$ mixture was also created.

After the appropriate concentration of the gas mixtures was added to the mixing chamber, N_2 was introduced and turbulently mixed. After at least four hours of mixing, the gas was moved at a constant flow rate through the reaction chamber, where a deuterium lamp was used as an energy source. The particulates were collected on a filter beneath the reaction chamber, and the rest of
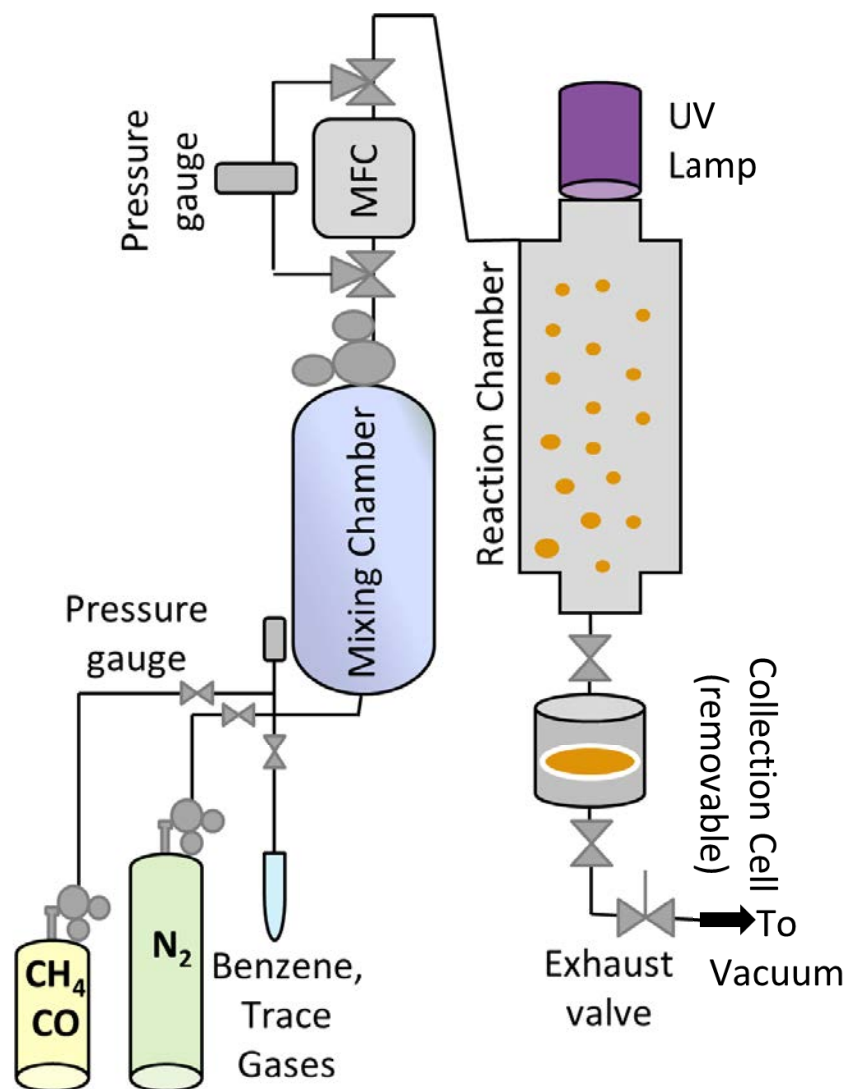

Figure 2: Photochemical reaction chamber. The process starts in the lower left, where the different gases are sent to the mixing chamber. From the mixing chamber, the gases flow through the reaction chamber where they react with UV light. Particulates are collected on a filter and the remainder of the gas goes to the vacuum.

the gas was sent out to vacuum. Samples typically took one week to accumulate, based on a standard pressurization with N_2 to 900 pounds per square inch (psi) and a flow rate of 20 standard cubic centimeters (sccm), which resulted in approximately 12 hours per every 100 psi of pressure.

After the samples were collected, the sample holder was placed under its own vacuum seal and removed from the apparatus. The samples were removed from the holder inside an N_2 purged glove box and stored until analysis.

For analysis, samples were dissolved in acetone and methanol and allowed to dry in an insert-GC vial under N_2 flow in an oven set between 70-80 degrees Celsius. Once the liquid evaporated, the samples were dried with several drops of CH_2Cl_2 in the same fashion. The samples were then derivatized with equal amounts methyl-tertbutyl-silyl-trifluoroacetamide (MTBSTFA) and dimethyl-formaldehyde (DMF) to make them volatile. The derivatization process took 30-45 minutes under N_2 flow in the oven. After derivatization, the samples were immediately run on the GC/MS QQQ.

Three different methods were utilized on the GC/MS QQQ. The first was a mass spectrometry (MS) method, which was used to identify the full mass spectrum of the compounds. The second was a multiple reaction monitoring (MS/MRM) method that selects for specific compounds that have known fragmentation patterns and determines if they are present. The third was a product ion (PI) scan, which examines how molecules are known to fragment from certain parent ions to confirm their identity.

RESULTS AND DISCUSSION

Determination of Compounds Present

In addition to the photochemical aerosols produced for this project, plasma aerosols produced in our collaborator's lab were also analyzed. The plasma aerosols were created using different combinations of gas than the photochemical aerosols- typically they had 5% CH_4 and

varying percentages of CO. Each of these aerosols was analyzed on GC/MS QQQ for compounds of interest, such as amino acids, nucleobases, carboxylic acids, and hydroxy acids. The results of one particular run of the samples on GC/MS QQQ using the MS method is shown in Figure 3.
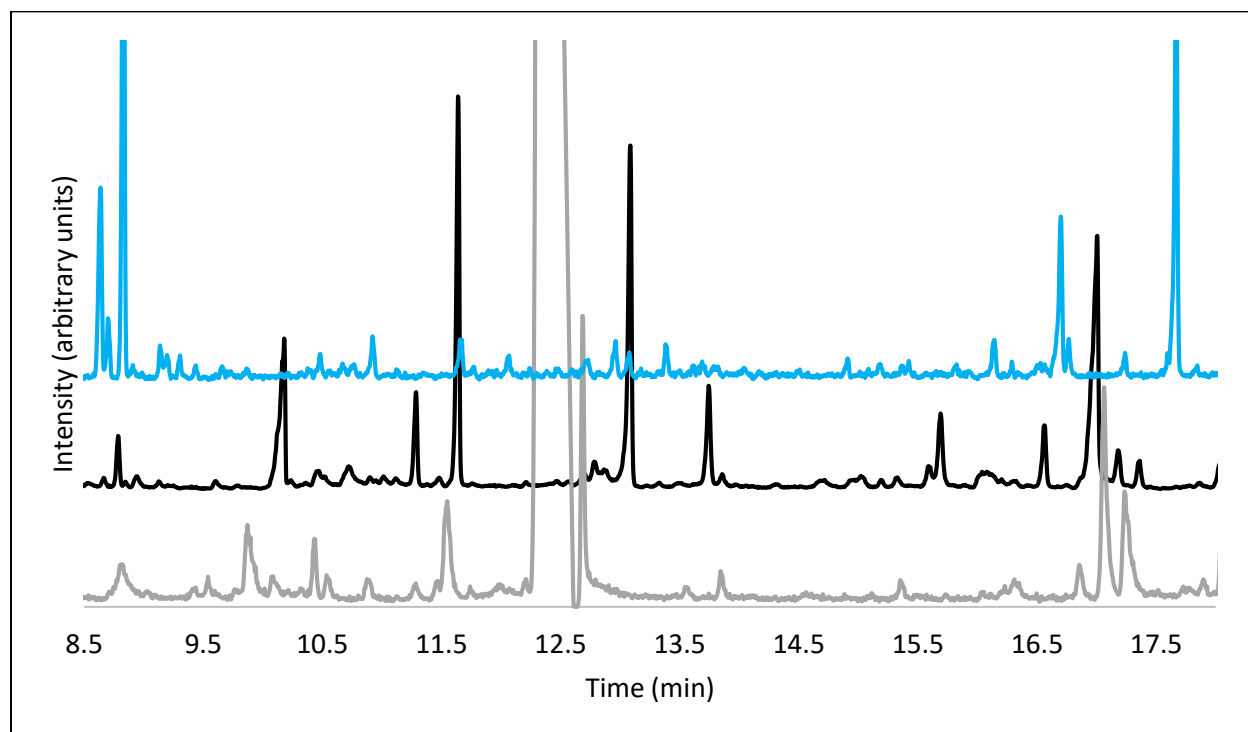

Figure 3: The MS comparison of different runs. In grey is the amino acid standard, in black is the Plasma (5% CH_4, 0.5% CO), and in blue is the UV (1:1 CH_4:CO)

Table 1 shows the relevant peaks and their identities.

Table 1: Identified peaks from sample run shown in Figure 3

Sample	Peak (min)	Identity
Plasma	8.7	Glycolic Acid
	10	Urea
	11.5	Known background
	13	Guanidine
	13.5	Cytosine
	16.5	Adenine
	17	Known background
Photochemical	8.7	Glycolic Acid
	10.5	Urea
	18	Known background

The large peak seen at 12.5 in the amino acid standard is a known intense area where the MS method reads an intense spike and is turned off to avoid destroying the detector. The amino acid standard was used to verify that amino acids were coming off the column at the expected retention times. As the table shows, several compounds of interest are identified in this set of samples, though not all identified compounds are shown here. It can be noted that glycolic acid are urea are observed in both the plasma and photochemical aerosols. An example of an MRM run is shown in Figure 4.
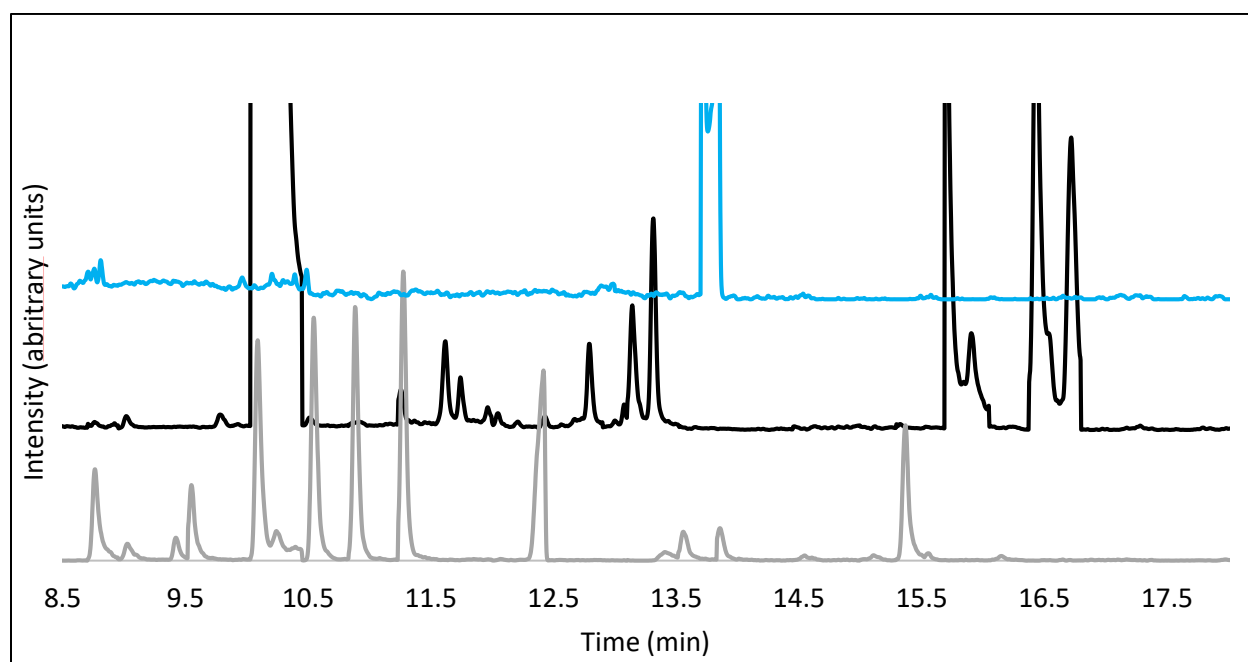

Figure 4: The MRM comparison of different runs. In grey is the amino acid standard, in black is the Plasma (5% CH_4, 0.5% CO), and in blue is the UV (1:1 CH_4:CO)

Table 2 shows the relevant peaks and their identities.

Table 2: Identified peaks from sample run shown in Figure 4

Sample	Peak (min)	Identity
Plasma	9	Ethanolamine
	11.7	Uracil
	12.5	Thymine
	16.5	Adenine
Photochemical	13.7	Methionine spike

The methionine spike seen in the photochemical aerosol is a standard used for

quantitation and as an indication if the peaks begin to shift retention times. The MRM method

was a more useful method for determining whether certain molecules were present in the

samples. Once the initial list of compounds present in each type of sample was determined,

MRM was used to verify their presence in different gas composition samples.

The overall results of the GC/MS QQQ analyses can be seen in Figure 5.
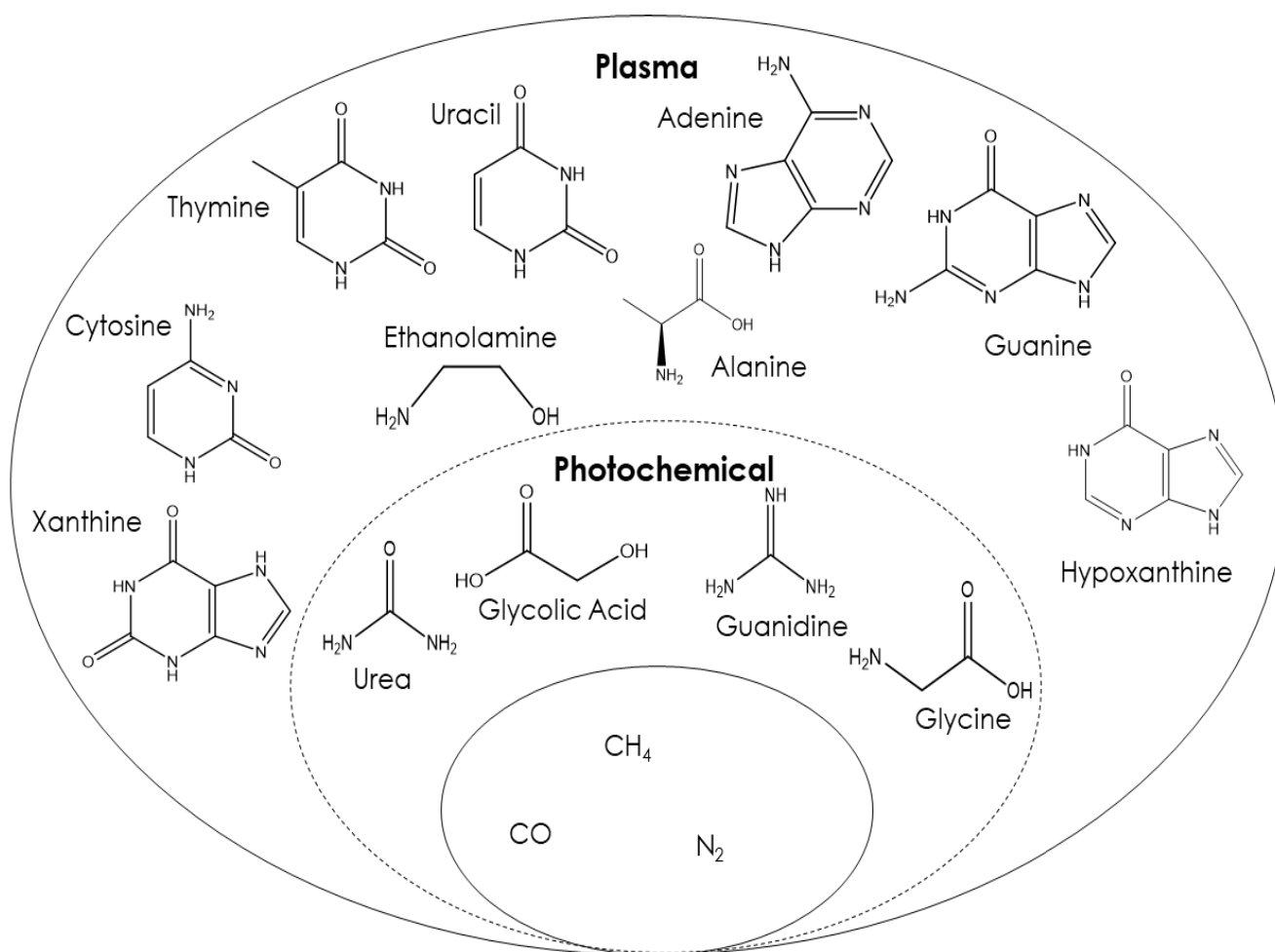

Figure 5: Compounds of interest present in plasma and photochemical aerosols.

As can be seen from Figure 5, more compounds were present in the plasma aerosol

analogs than in the photochemical aerosol analogs. This is due to the incredible strength of

plasma as an energy source. Plasma can shred gas molecules into more radicals than UV radiation and put them back together in a wider range of new compounds. While photochemistry from a UV light source is less powerful, it allows for easier tracking of mechanisms.

Plasma aerosols were seen to produce all five nucleobases, as well as xanthine and hypoxanthine. Xanthine and hypoxanthine are chemical derivatives of adenine and guanine. The presence of these two compounds indicates that the observed nucleobases are coming from abiotic processes inside the chamber as opposed to human contamination. Human contamination could be introduced from a fingerprint on the sample or GC vial, among other sources. If human contamination of the sample had been the origin of the nucleobases, xanthine and hypoxanthine would not be observed. Plasma aerosols were also shown to be the source of valine, while photochemical aerosols produced glycine.

Guanidine, a product found in both types of aerosols, is the functional side chain of the amino acid arginine and is also an oxidative degradation product of guanine. Urea is also found in both types of aerosols and is an important organic compound that helps metabolize nitrogen-containing products. Both of these compounds are important organically, and their presence in the aerosols is promising as potential food sources for early life. Since glycolic acid is a compound found in both plasma and photochemical aerosols, and is an important precursor of glycine, it was chosen for further mechanistic studies.

Isotopic Work

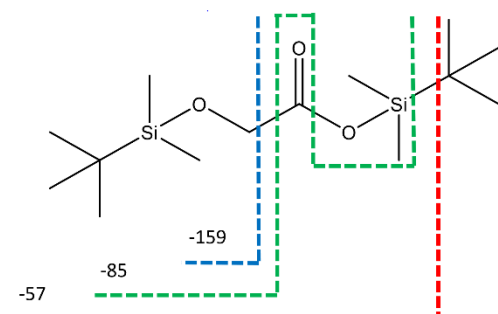Based on previous work done in this lab, the fragmentation patterns of derivatized

molecules is known.[2] Based on these derivatization patterns, molecular weights were calculated for each of the fragments based on how the molecule was substituted with isotopes. This fragmentation pattern is shown in Figure 6.

Figure 6: Known fragmentation pattern of derivatized glycolic acid

For all of the isotopic work, a mix of 1:1 $CH_4:CO$ was used. A regular sample was run with

no isotopes present, as well as $CH_4:C^{18}O$, $^{13}CH_4:CO$, and $CH_4:^{13}CO$. The first goal of the isotopic

work was to determine whether the oxygen present in the samples was coming from the

introduced carbon monoxide or from outside contamination. To

this end, methods were built to check for the known fragments of

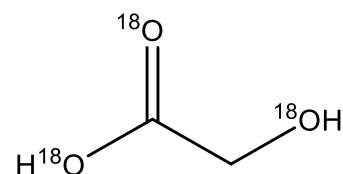fully ^{18}O substituted glycolic acid. This molecule can be seen in

Figure 7.

Figure 7: 18O substituted glycolic acid

To determine lack of oxygen contamination, one particular fragmentation was

examined: the fragmentation of the first fragment (after losing 57 m/z) to the second fragment

(after losing 85 m/z). These fragments can be seen in Figure 8. In the samples run with no
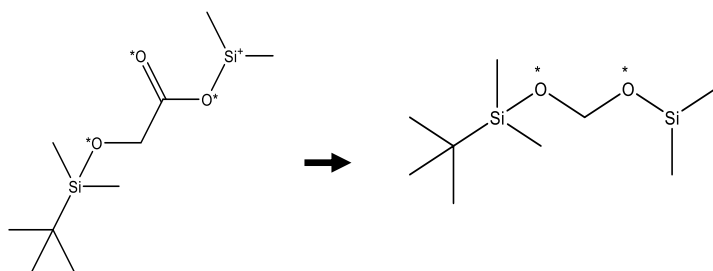

isotopes, the MRM methods built to look for fragments where all ^{16}O had been replaced with ^{18}O returned low abundances, which was expected since
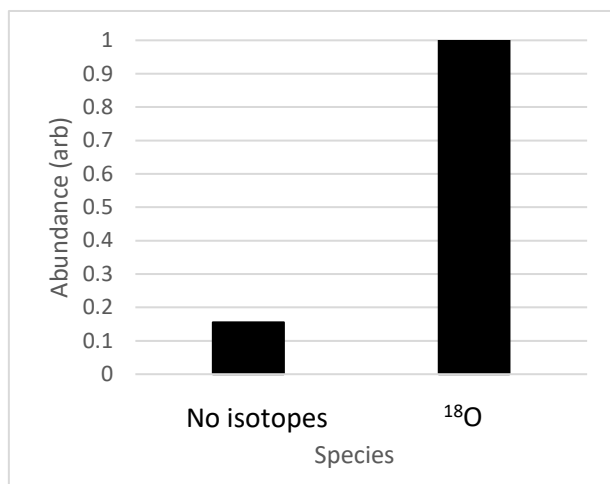
Figure 8: Fragment 1 to fragment 2. *where ^{18}O substitution takes place

Figure 9: normalized abundances of ^{18}O fragments in a non-isotopically doped sample and an ^{18}O doped sample

the natural abundance of ^{18}O is only 0.2%. When the sample created with $C^{18}O$ was run, the MRM methods built to look for ^{18}O-replaced fragments showed statistically significant results, indicating both that the ^{16}O was successfully replaced with the ^{18}O and that contamination is not the source of oxygen in the samples. Figure 9 shows the normalized abundances of the non-isotopic sample versus the ^{18}O doped sample.

The second goal of using isotopically doped gases was to determine the origin of the carbon atoms in glycolic acid. Since glycolic acid is only a two carbon molecule, methods could be built to specifically look at how two different fragmentation patterns change based on which carbons are substituted with ^{13}C. There are three different ways that ^{13}C can be incorporated into the molecule, which are shown in Figure 10.
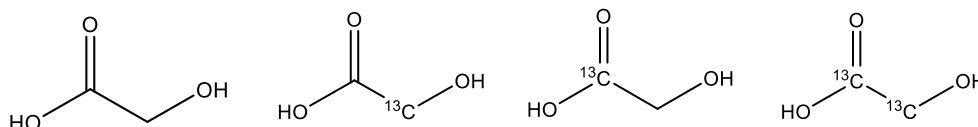

Figure 10: The three substitution patterns of 13C substituted glycolic acid (GA). From left to right: non-isotopic GA, C2 monosubstituted GA, C1 monosubstituted GA, and disubstituted GA

Two different fragmentation patterns were examined to determine which of the substitutions was being observed. The first was the same fragmentation pattern used for the ^{18}O work, that of the first known fragment to the second. The second fragmentation pattern is a known

breakdown of glycolic acid that occurs during GC/MS fragmentation, as determined by previous

work.[2] These fragmentation patterns are seen in Figure 11.

The methods built to examine these two fragmentation patterns took advantage of the fact that GC/MS QQQ work is extremely sensitive, and can be used to determine the difference between two fragments that are only one
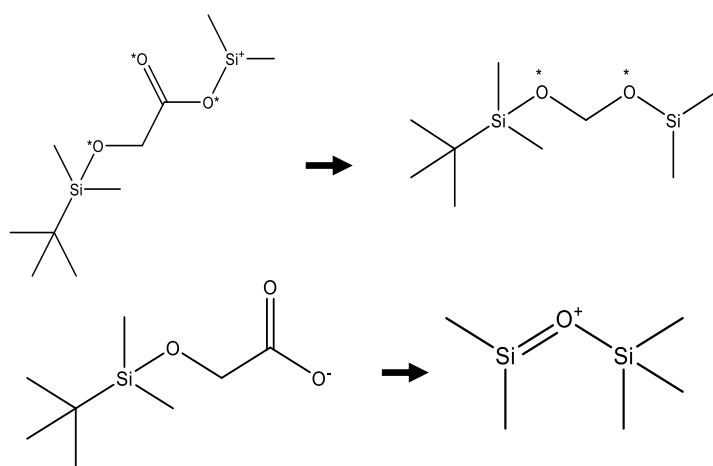
Figure 11: Known fragmentation patterns of derivatized glycolic acid used for determination of ^{13}C substitution. Top is fragment 1 to 2, bottom is characteristic fragmentation of glycolic acid

atomic mass unit (amu) apart in molecular weight. The different patterns that were tested can

be seen in Table 3.

Table 3: The different fragmentation patterns of the ^{13}C substituted variants of glycolic acid

Substitution	Fragmentation	AMU observed
Disubstituted	1 to 2	249 -> 220
	Characteristic	191 -> 147
C1 Monosubstituted	1 to 2	248 -> 219
C2 Monosubstituted	1 to 2	248 -> 220
Either monosubstituted	Characteristic	190 -> 147
Non-isotopic	1 to 2	247 -> 219
	Characteristic	189 -> 147

As can be seen from Table 3, the characteristic fragmentation of glycolic acid cannot be used to

differentiate between the two monosubstituted options, since neither of the carbons remains

in the end fragment and the beginning mass is the same for both molecules. As a result, the

characteristic fragmentation was used to look for the difference between disubstituted and

monosubstituted molecules, as well as a check for the unsubstituted variants. The fragment 1

to fragment 2 methods were used to check for the difference between the two

monosubstituted fragments, the disubstituted fragment, and check for unsubstituted

molecules.

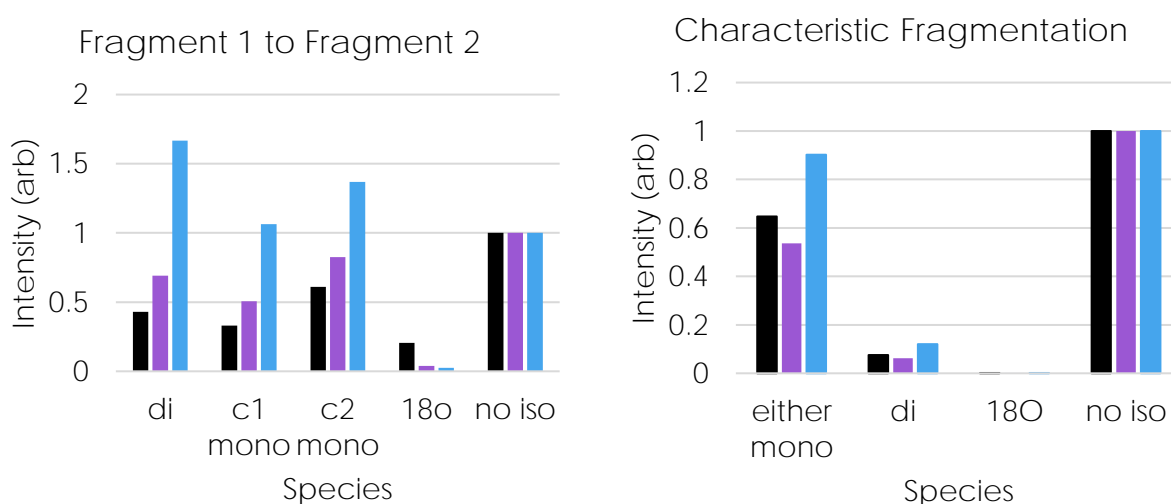The results of the two different fragmentations are shown in Figure 12.

Figure 12: Black = 1:1 CO:$^{13}CH_4$, Purple = 1:1 $^{13}CO:CH_4$, Blue = 1:1 CO:CH$_4$. Results of the two different fragmentation patterns: fragment 1 to fragment 2 and the characteristic fragmentation of glycolic acid; all peaks are normalized to the non-isotopic peak.

The difference in intensities between the different compositions of gas indicate that the

method was successful. Both ^{13}C gas compositions showed lower intensities of substituted

fragments than the non-isotopic gas composition. This indicates that the ^{13}C gas mixtures are

shutting down the photochemistry in the chamber. The deuterium lamp in the chamber has a

specific spectral band at 160 nm that is the highest intensity of light given off by the lamp in the

UV region. Since ^{13}C absorbs slightly differently than ^{12}C, the results indicate that the ^{13}C gases

are moving the region of reactivity of the mixture just slightly off kilter with where the highest

intensity of light given off by the lamp presents. Based on these data, one solution may be to

use a different light source to induce photochemistry.

The ^{13}C gases still show the same intensity relationships between the different substitution patterns as the non-isotopic gas mixture, indicating that the particular 1:1 methane:carbon monoxide mixture does not affect the relationship between the intensity of the peaks in the sample. This shows that the gas favors each of the substitutions in the same way regardless of doping. This result is interesting because it indicates that the integration of carbons into the molecule changes based on the doping of the sample. With the current light source, the results indicate that the most favored substitution position is C_2, regardless of which gas was ^{13}C doped.

CONCLUSIONS

The goals of this study were to determine the prebiotic molecules of interest in plasma and photochemical aerosol analogs of the early Earth atmosphere and elucidate the mechanism of formation of glycolic acid using isotopically doped gases. Both plasma and photochemical aerosol analogs were shown to have prebiotic organic molecules of interest. Plasma aerosols were seen to have all five nucleobases, as well as xanthine and hypoxanthine, which eliminated the chance of human contamination being the source of the nucleobases. Additionally, plasma and photochemical aerosols were seen to be the source of a few amino acids: valine in plasma aerosols and glycine in photochemical aerosols. Each type of aerosol was also the source of glycolic acid, a precursor to glycine, which singled the molecule out for further studies into its mechanism of formation.

Isotopic studies using ^{18}O showed that samples doped with $C^{18}O$ increased production of ^{18}O substituted glycolic acid compared to those that were not doped, indicating that contamination was not a source of oxygen in the samples. Using 1:1 $^{13}CO:CH_4$, $CO:^{13}CH_4$, and

$CO:CH_4$, it was determined that the current light source has a spectral band that is slightly removed from that of the ^{13}C gas mixtures, which causes the photochemistry to shut down when ^{13}C is included in high concentrations. Additionally, the carbon source in the molecule changes based on which gas is isotopically doped. To investigate these phenomena, a different light source can be used to increase the photochemistry while using ^{13}C doped gases. More work can be done to determine the mechanism of formation of glycolic acid. Future mechanistic work of this type can be done on different molecules that are present in these aerosols to further understand the photochemistry that takes place in the atmosphere of the early Earth.

One limitation of the study was the limited number of samples that were created for each gas mixture. Due to time limitations, only one sample of each gas composition was tested, which could have skewed the results if any step of the derivatization was performed incorrectly, chemicals were expired, or the column on the GC/MS/QQQ was contaminated. Additionally, only one mixture of gases was studied and there are a variety of proposed gases that could have been present in the early atmosphere. Theoretically there could be many other reactions that are taking place to form glycolic acid that were not examined in this study. While these are all factors that should be taken into consideration, future exploration into this material can expand scientific knowledge in the fields of photochemistry and prebiotic research.

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