Early-Earth small molecule reactions: an experimental survey of submarine alkaline hydrothermal vent chemistry

By

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A Thesis Submitted to

Saint Mary's University, Halifax, Nova Scotia

In Partial Fulfillment of the Requirements for

The Degree of Bachelor of Science

with Honours in Environmental Science

July 2017, Halifax, Nova Scotia

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ABSTRACT

Alkaline hydrothermal vents provide a variety of chemical reagents and conditions that may provide insight as to how life emerged on Earth. This study examined small molecule chemistry under conditions found at these vents. Two major experiments were preformed using small molecules that are generated and available at hydrothermal vents. CO_2-switchable ionic liquids were the medium for small molecule reactions to occur. Reactions between cyanamide (CH_2N_2) and glycolaldehyde ($C_2H_4O_2$) produced 2-aminooxazole ($C_3H_4N_2O$) were set up in water, but failed to produce a result in CO_2-switchable ionic liquids. Exploration of the reaction of CS_2 with acetate (CH_3COO^-) in acetonitrile with CO_2 passing over resulted in a product that could not be identified. Interestingly, CO_2 and CS_2 exchanged oxygen and sulfur in the presence of acetate. Thus, suggested that a metal catalyst is not necessary for CO_2/CS_2 scrambling. Observations of this phenomenon demonstrated that CO_2 and CS_2 are more dynamic at low temperature and pressure conditions than previously thought. The Finding of this study show that there is still much to understand about small molecule under pre-biotic conditions.

July 2017

ACKNOWLEDGEMENTS

First, I would like to thank my honours supervisor, Dr. Jason Clyburne for giving me the opportunity to take on this project. I started this project with a very limited background in chemistry, and I can say that I have learned so much more than I could have ever imagined from him. His passion for chemistry, the environment, and students has been inspiring to say the least. On days where I felt completely lost or confused, I could walk into his office in search of support and leave feeling motivated and confident in this project and myself.

I would also like to thank the members of the Clyburne group for their overwhelming support over the duration of this research. Robert Riley, Katrina Turrie, and Ryan McCoy, and Mathew Laprade, for keeping the laboratory full of smiles and positivity, and working as a great team. I would also like to thank Michael Land for committing so much of his own time helping decipher these experiments. His passion and brilliance will no doubt bring him success in life.

Thank you to the staff of the Saint Mary’s University Faculty of Science for assistance in this project. Thank you to Alyssa Doue and Patricia Granados, of the Department of Chemistry, for their help with the technical and analytical parts of this research. Special thanks to Dr. Todd Ventura, of the Department of Geology, for his support, suggestions, and genuine interest in this research.

Lastly, I would like to thank my family and friends for their encouragement throughout this project. Thank you Mary Kate Jollymore, Makeala MacIntyre, and Jeffrey Macdonald, for your support and patience. To my parents who showed me love and support through not just this research, but also my undergraduate degree.

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1. INTRODUCTION AND LITERATURE REVIEW

The origin of life remains a challenging subject for researchers across the world. Ultimately it can be approached from two directions: A top-down, biological approach which examines the phylogenetic tree of life, in an effort to move down the tree toward an organism that represents the first form of life or, alternatively, a bottom-up approach examining prebiotic chemistry and conditions. No matter which approach is chosen, each has inherent experimental biases toward modern biochemistry (Miller & Cleaves, 2006). The earth today is vastly different from conditions 3.7 billion years ago. With this in mind, researchers have debated three theories that could provide the location of life's origin: Extraterrestrial synthesis (panspermia), hydrothermal pools, and lastly hydrothermal vents. Of these theories, hydrothermal vents, or more specifically, alkaline hydrothermal vents provide the necessary chemical feedstocks and conditions to support essential prebiotic chemistry. The known chemistry of early-life molecules is both acidic and basic; however, the connection between these chemical environments is unidentified. The use of CO_2 switchable ionic liquids to bring reactions from basic to acidic chemistry is a possible answer to the chemical bridge within this environment.

1.1 EXTRATERRESTRIAL SYNTHESES

The chemistry required for functions such as metabolism and building membranes requires a source and abundance of biologically-relevant molecules as well as appropriate conditions. The theory of panspermia was first hypothesized by Arrhenius (1908), and described the seeding of planets via extraterrestrial masses such as meteorites (*Figure 1*). The idea in short, is that microorganisms were ejected from a host planet, survive the transit of space, and then survived atmospheric entry to earth. Examination of a Martian meteorite suggests that the shock of planetary ejection is up to 55 GPa and can increase temperature by up to 1000 K (Cockell, 2008). The extreme conditions required to exit have been tested on microorganisms by Mastrapa, et al. (2001), and proven to be non-fatal to some extremophiles. Though survival of ejection is possible, survival of atmospheric entry is less so. The STONE-5 Experiment demonstrated heavy melting in dolerite, sandstone, and impactite, from re-entry of samples into the earth's atmosphere (Brandstatter, et al., 2008). Though each sample survived re-entry, it is likely they microorganisms would not survive the force and heat of impact. Furthermore, the slim-chance of generating a self-replicating system to create life is unlikely (Miller & Cleaves, 2006).

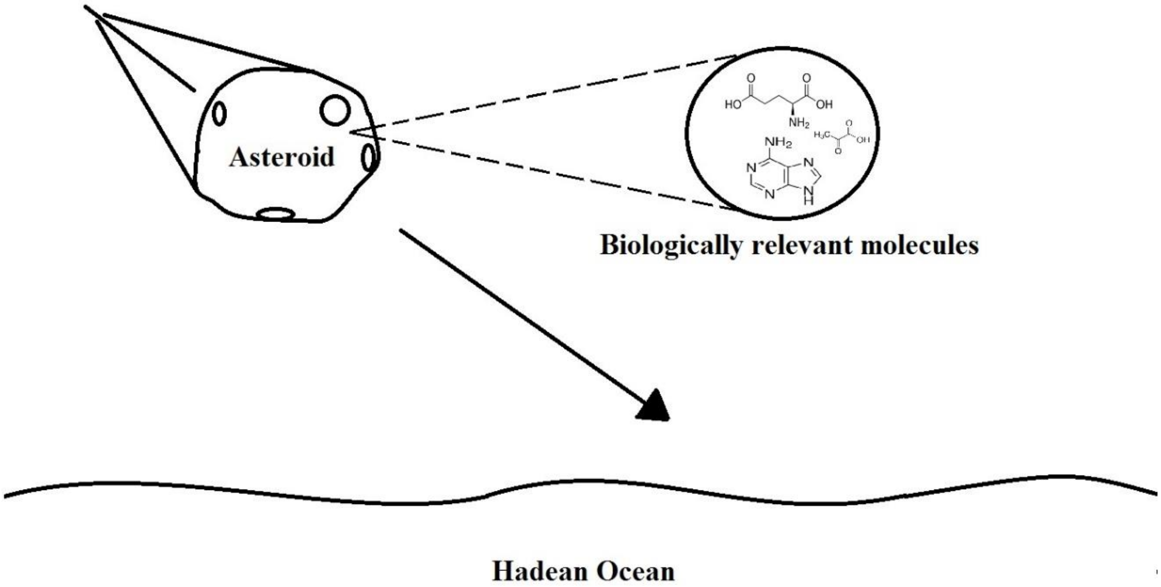


Figure 1. Diagram of extraterrestrial synthesis theory depicting an asteroid carrying organic molecules being deposited into the Earths early ocean.

1.2 HYDROTHERMAL FIELDS

A proposed environment for the origin of life are hydrothermal fields. These are areas of high geothermal activity such as hot springs, geysers, and boiling pools (*Figure 2*). The most interesting effect of hydrothermal fields are their ability to generate a wet-dry cycle. Hydrothermal fluid changes much from high to low volume in a cyclical matter. This allows for hydrous and anhydrous chemistry as well as accumulation of molecules (Deamer & Georgiou, 2015). This accumulation leads to high concentrations of organic compounds along the edge of hydrothermal fields, which then could react (given the temperature on site) and connect monomers into polymers (Deamer D. , 2012). The complexity of molecules was shown to increase through the wet-dry cycle, so further research on possible biomolecule formations within hydrothermal pools is required. In a study by Sydow, et al. (2017), the conditions found in Cinder Pool, Yellowstone National Park were mimicked in a laboratory to provide insight on potential sulfur chemistry in the area. The researchers showed that iron sulfur minerals such as pyrite (FeS) could catalyze initial reactions of a multistep chemical process in which methanethiol (CH_3SH) was formed. Methanethiol could be used as a reaction center for the acetyl-CoA pathway (Sydow, et al., 2017). Evidence of this molecule was not previously documented in hydrothermal fields, and was thought to be produced abiotically in hydrothermal vents (Sydow, et al., 2017). Limitations to hydrothermal pool theories include UV exposure, which can damage molecules, and potential hydrolyzation of ester and peptide bonds caused by low pH. This may lead to difficulties accruing complex polymers (Deamer & Georgiou, 2015).

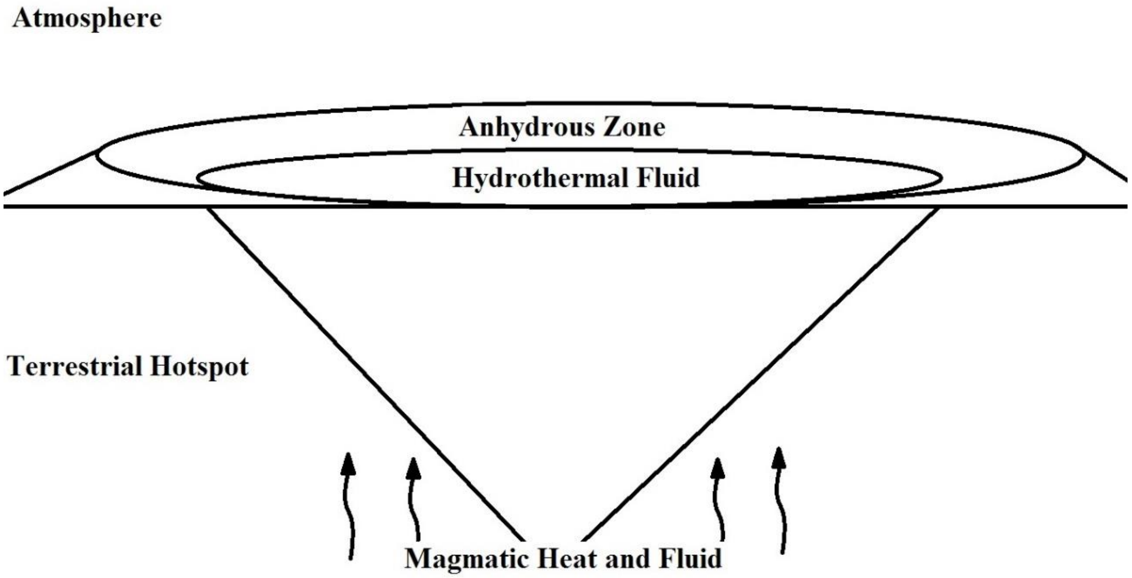


Figure 2. Diagram of terrestrial hydrothermal pools where magmatic fluid cycles through the system.

1.3. SUBMARINE HYDROTHERMAL VENTS

The final, and perhaps most recently theorized, location of the origin of life, is hydrothermal vents (*Figure 3*). The discovery of "black smoker" vents in 1977 (Corliss, et al., 1979) opened the door to new research pertaining to the chemical origin of life. Prior to this discovery, evidence for an autotrophic origin of life was lacking. An article by Günter Wächtershäuser (1992) proposed the theory of an "iron-sulfur world" in which he described the possibility of pyrite (FeS) formation as a feedstock leading to primitive forms of carbon fixation. According to Heredia, et al., (2016), hydrothermal vents provide hot temperatures and geothermal fluids that provide nutrients and chemical gradients. This could allow organic chemical compounds to synthesize at a relatively rapid rate. Furthermore, the quantity of hydrothermal vent systems across the planet is significant.

Although exact numbers are unknown, vents consistently appear at tectonic plate boundaries (Miller & Cleaves, 2006).

It is important to note that there are two types of vents associated with these areas. The first, previously mentioned type, is called a "black smoker" and occur at mid-ocean ridges. They sulfide-rich, and emit a black fluid rich in Fe-Cu-Zn-Pb sulfides. The temperature of the fluid emitted can reach 400°C (Fouquet, 2011). The second type of vent is a "white smoker": also known as alkaline hydrothermal vents. These vents emit alkaline hydrothermal fluid (Kelley, et al., 2001). White smokers are found on older regions of oceanic crust that are further away from spreading centers in mid-ocean ridges. The hydrothermal fluid emitted contains Ba-Ca Sulfates that give it a white color (Arndt, 2011). In addition, the released fluids are cooler than those emitted from black smokers, and may be as low as 40° to 75 °C (Kelley, et al., 2001). This temperature difference is an important distinction because biomolecules and organic compounds in general, are highly thermolabile and can breakdown within minutes after high heat exposure (Miller & Cleaves, 2006). These authors mention the improbability of alkaline hydrothermal vents being a source of essential prebiotic molecules because the heat is still too high However, no mention of the low temperatures found in research by Kelley, et al., (2001) was cited. With this in mind, it is more likely that alkaline hydrothermal vents provided stable prebiotic chemistry than "black smokers".

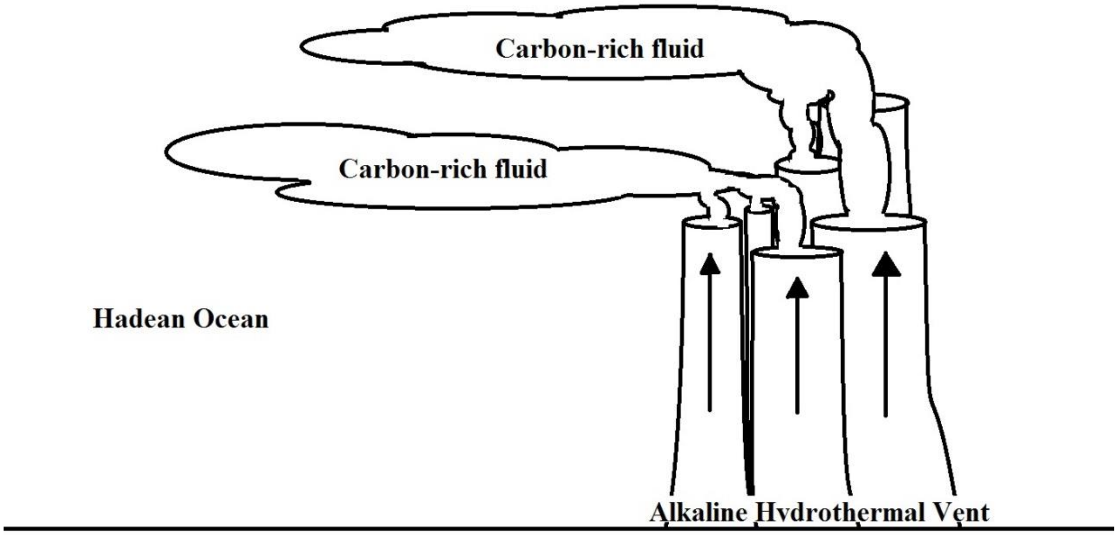


Figure 3. Diagram of submarine hydrothermal vents in an early-earth setting.

1.4. PALLETTE OF CHEMICAL BUILDING BLOCKS WITHIN PREBIOTIC ALKALINE HYDROTHERMAL VENTS

Alkaline hydrothermal vents provide chemistry that is promising for some major theories plausible for prebiotic conditions. The “iron-sulfur world” proposed by Wächtershäuser (1992) has been supported heavily by chemists. Thus, Herschy, et al., (2014) proposed various pre-biotic chemical events are using a simulated alkaline hydrothermal vent reactor. The researchers assumed Hadean oceanic conditions primarily involved Fe(Ni)S minerals that were mentioned in previous studies, and noted that alkaline vents provide excellent feed stocks of H_2 and CO_2. Vent systems like the Lost City, an alkaline hydrothermal system in the north-west of the mid atlantic ridge (Kelley, et al., 2001), are driven by a metamorphic process called serpentinization that produces H_2 in large volumes, which in turn facilitates an electrochemical gradient within vents. Herschy, et al., (2014), state that the transfer of electrons between H_2 and CO_2 is unfavorable under standard conditions (pH 7); however, the electrochemical gradient provided by serpentinization leads to a favorable reduction of CO_2. This reduction permits the formation of organic compounds such as formaldehyde, formate, formic acid, methanol, and methane. A simple diagram depicting the formation of formaldehyde at an alkaline hydrothermal vent wall can be found in *Figure 5*. Herschy, et al., 2014, developed a reactor that mimics plausible prebiotic alkaline hydrothermal vents at various temperatures. In recreating the prebiotic gradient, their system formed a range of sugars, such as ribose, that maintained a stable concentration over several hours (Herschy, et al., 2014). Thus, the abundance of CO_2 and H_2, due to serpentinization within the vents, can lead to the formation of organic molecules within the hydrothermal systems.

1.5 IMPACTS OF METAMORPHOSIS AND GEOLOGY

Serpentinization is an exothermic reaction (*Figure 4)* by which rock is altered by the addition of water into a crystal structure of said rock. Serpentinization provides the H_2 that is today a source of energy for extremophiles living at deep sea vents. However, scientists speculate that this reaction was important for creating important thermochemical conditions and feed stocks in the Hadean era. Serpentinization reactions, as well as active fracturing and topographic forcing, drive fluid flow at the Lost City hydrothermal vent field. The pH of hydrothermal fluids created by serpentinization ranges from 9-11 (Kelley, et al., 2001) but the Hadean ocean is assumed to have been much more acidic (pH = ~5.5) given the evidence for CO_2-rich oceans during this time (Shibuya, Russell, & Takai, 2016). The acidotic conditions, as well as the FeS minerals in vent walls are thought to provide a catalyzed electrochemical gradient from which prebiotic chemistry could take place (*Figure 5*).

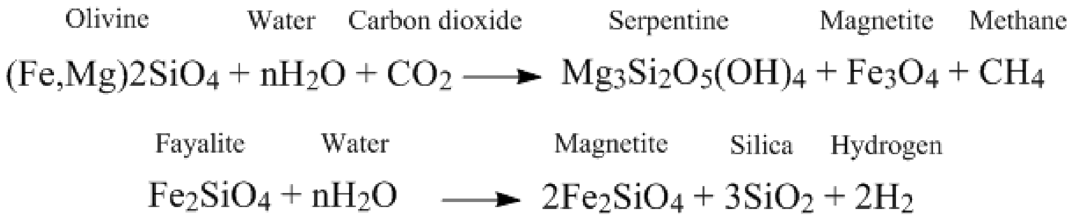


Figure 4. Serpentinization reactions of olivine and fayalite.

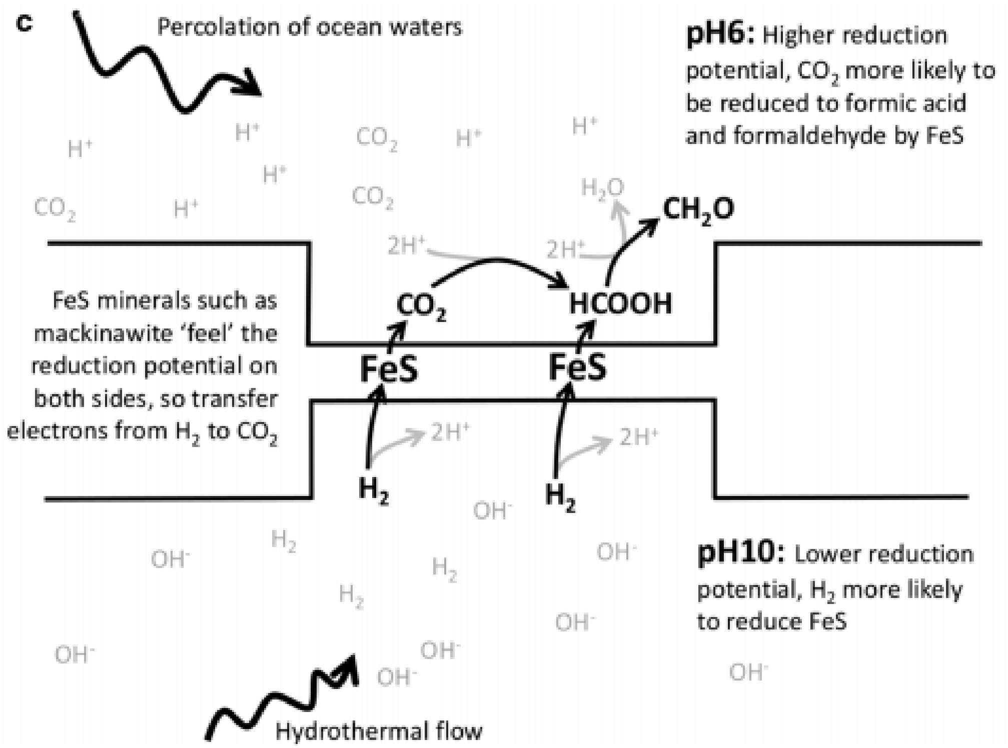


Figure 5. How acid and alkaline fluids could interact inside hydrothermal vents across thin semi-conducting Fe(Ni)S walls, leading to the reduction of CO_2 to formaldehyde via formate. Used with permission by Herschy, et al., (2014).

The reactive and catalytic potential of minerals that form at these vents further increases the list of potential chemical sources available under these conditions. White, et al., (2015) identified mackinawite (Fe(II)S) and greigite ($Fe(II)Fe(III)_2S_4$) as potential catalysts for emergent life. Greigite was observed at 70 °C; however, mackinawite was the primary catalyst due to its higher abundance. Additionally, formaldehyde was seen to inhibit the transformation of Mackinawite into Pyrite, leaving the Greigite formation pathway as the only option. Previous expermiments by Wächtershäuser & Huber (1997) explored carbon fixation pathways within primordial FeS conditions and explained the chemoautotrophic origin of life hypothesis, focusing on the reductive acetyl-CoA pathway. The reaction of CH_3SH and CO in the presence of FeS, CoS, or NiS, lead to the formation of varying concentrations of acetic acid. Furthermore, the formation of amino acids under aqueous FeS conditions has found to be efficient. However, it requires the FeS to be freshly precipitated from $FeSO_4$ as well as the presence of NH_3. The experiments demonstrated yields (highest to lowest yield) of alanine, glutamic acid, phenylalanine, tyrosine, *N*-methyl-phenylalanine and *N*, *N*-dimethyl-phenalanine (Wächtershäuser & Huber, 2003).

In order for complex organic molecules to form in hydrothermal environments, amino acids must persist in the environment. Experiments done by Lee, et al., (2014) demonstrated that the stability of glutamic acid is heavily influenced by the redox state imposed by dissolved H_2. The experiments recreated hydrothermal conditions akin to those in the Lost City Hydrothermal field. The decomposition reaction network of glutamic acid under hydrothermal conditions includes the diacids: Glutamic acid, pyroglutamic acid, glutaconic acid, α-hydroxyglutaric acid, α-ketoglutaric acid, and lastly, succinic acid. Each acid was quantitatively measured in various pressure-temperature conditions, with and without the presence of H_2. The results imply that amino acid stability is possible although initially thought detrimental (Miller & Cleaves, 2006). Additionally, the stability allows for complex molecules to form such as lipids and peptides (Lee, et al., 2014).

1.6 PREBIOTIC SYNTHESIS OF RNA-RELEVANT MOLECULES

Complex molecules such as RNA and proteins require precursor chemicals to form. With such a large pool of potential reagents (see above), complex molecules can form and react with one another. A study by Powner, et al., (2009) synthesized activated pyrimidine ribonucleotides through short sequence reactions. The initial chemical molecules used were the plausible prebiotic feedstock molecules: cyanamide, cyanoacetylene, glycolaldehyde, glyceraldehyde and inorganic phosphate. Reactions between glycolaldehyde and cyanimide using a 1 M phosphate buffer at pH 7.0 lead to a >80% yield of 2-amino-oxazole. Glyceraldehyde was then added to the solution of 2-amino-oxazole to produce varying yields of arabinose, ribose, xylose, lyxose, amino-oxazolines and their elimination products. Ribose and xylose were seen to be very stable in the presence of phosphate at 40°C (Powner, et al., 2009). The issue with this experiment is the gap between glyceraldehyde and glycolaldehyde feedstocks, however the authors mention the possibility of heating and progressive dehydration followed by cooling and rehydration as a mechanism to overcome this problem. In addition, the chemistry used to produce 2-amino-oxazoles via glycolaldehyde and cyanimide is strongly alkaline (Cockerill, et al., 1976). Similar conditions are found within alkaline hydrothermal vent. It is also likely that inorganic phosphate is present within hydrothermal systems. Bryant & Kee (2006) examined H-phosphonates as a potential source of Phosphorus on the early Earth. They identified H-phosphonate as a key product of anoxic hydrolysis of iron phosphides. Photo chemically induced reactions with aqueous alcohol produced organophosphorus compounds. Thus it is plausible that an abundance of phosphorus is present in these systems as well.

1.7 CO_2-SWITCHABLE IONIC LIQUIDS AS A BRIDGE FOR ACID AND BASE CHEMISTRY ON THE EARLY EARTH

The reagents found in early earth alkaline hydrothermal vents, in addition to the chemical conditions, raise questions about naturally occurring switchable ionic liquids. Ionic liquids are used today as solvents in chemical production processes. Organic solvents are typically optimal for specific steps of a chemical reaction, and are removed and replaced with new organic solvents that are more useful after each step. This is hindering both economically and environmentally. Organic solvents are often highly volatile and/or toxic and can threaten animal and plant life (Palos-Pacheco, 2013). An ionic liquid that uses CO_2 as a “switch” to protonate and deprotonate organic molecules could have proved useful in a pre-biotic hydrothermal setting. CO_2 is a known and abundant feedstock released from submarine vents. Thus, there is a possibility that nature has been using environmentally friendly switchable ionic liquids since before the emergence of life.

Many Green Chemists are researching environmentally friendly and reusable solvents. Philip G. Jessop, of Queen’s University in Kingston Ontario, and his team of researchers have provided major contributions to field of green solvents. Developing CO2-switchable ionic liquids. Jessop, et al., (2005) took a non-polar mixture of DBU (1, 8-diazabicyclo-[5, 4, 0]-undec-7-ene) and 1-hexanol then exposed it to gaseous CO_2. This converted the mixture into an ionic liquid, which was then easily converted back into its initial state by bubbling N_2 through the liquid. The entire reaction took place at one atmosphere and standard room temperature. Additionally, the reaction was more rapid at 50°C. Temperatures within this range have been found at the Lost City hydrothermal vent field (Kelley, et al., 2001) and so have molecules akin to DBU. This can be inferred from the research by Heldebrant, et al., (2008). They demonstrated that switchable ionic liquids can be formed by bubbling CO_2 through a mixture of an amidine and amino alcohol. The purpose of this paper was to describe the carbon capture abilities of CO_2 switchable ionic liquids. However, the reactions completed in this experiment are simple in that they require amidines and amino alcohols. Amidines (more specifically carboxamidines) are commonly encountered in organic chemistry. Carboxamidines are daughter molecules to carboxylic acids. These, as well as dicarboxylic acids (Lee, et al., 2014), are known to form in hydrothermal vent systems (Herschy, et al., 2014). The possibility of amidines and amino alcohols being present in prebiotic vents is likely, given that their parent molecules can be formed.

$$R{-}OH + CO_2 \longrightarrow R{-}CO_3H^+$$

Scheme 1: Base to acid switch of dicarboxylic ionic liquids using CO_2.

CO_2-switchable ionic liquids are a relatively new field of research. Fortunately, Cunningham & Jessop (2016) provided a brief introduction to the basic principles of CO_2 switchable polymers and polymer colloids. In this article, they list the various organic reagents and their ability to respond to the addition of CO_2 (Scheme 1). For example, they note the basicity of pyridines and anilines is often too low to respond to a CO_2 stimulus; however, amines, imidazoles, and arylamidines, which have a moderate basicity, will switch to bicarbonate salts very easily at room temperature (Cunningham & Jessop, 2016). Furthermore, the predicted CO_2 pressures required for switching at various temperatures are readily available. The authors also stress the need to consider other components and their effects. For example, the effectiveness of CO_2 switching depends on the equilibrium between acids and bases. Strong acids can cause irreversible switches (Cunningham & Jessop, 2016). This is an important consideration when dealing with prebiotic hydrothermal vents, as there is a multitude of chemical sources, both acidic and basic, in the same area.

1.8 CARBON DISULFIDE AND CARBON DIOXIDE CHEMISTRY

Carbon disulfide and carbon dioxide are known species in hydrothermal vents (Kelley, et al., 2001). Although these molecules are similarly shaped, they have some notable chemical differences. Both molecules are triatomic and linear, and isoelectronic with a central carbon atom and two double-bonded atoms attached (*Figure 6*). The C=O bonds in CO_2 has a length of 1.198Å and C=S bonds in CS_2 have bond lengths of 1.539Å, which is caused by the difference in size between sulfur and oxygen atoms. Both molecules are nonpolar because two C=S or C=O bonds are pulling away from each other, which means there is no dipole moment. It is important to note that C=O bonds are more electronegative than C=S bonds, meaning CO_2 is less volatile than CS_2. The electron density for both molecules causes the carbon atom to be electron deficient, while the oxygen/sulfur bond is electron abundant. This allows the carbon atom to act as a Lewis acid, and the oxygen/sulfur atom to act as a Lewis base in reactions (Paula, 2016).

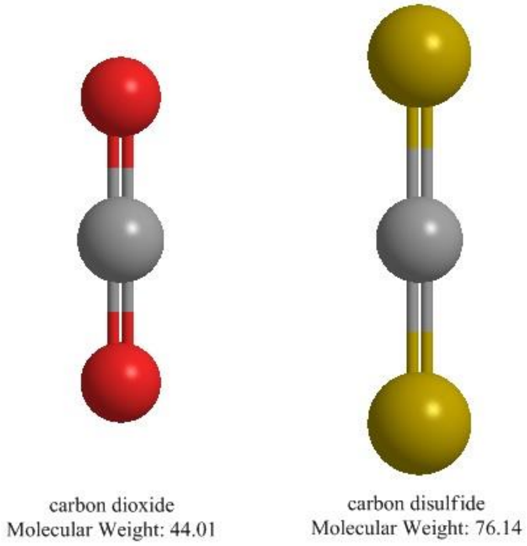


Figure 6. Three dimensional structures and molecular weight of CO_2 and CS_2.

Carbon disulfide is commonly found in both terrestrial and marine hydrothermal settings. Because of this, researchers have studied its significance in the abiotic synthesis of organic compounds. A study by Rushdi, et al., (2005), demonstrated the formation of aliphatic straight-chain and cyclic polysulfides is possible in hydrothermal vents. The results of this study suggest that CS_2 be considered as a carbon feedstock much like CO_2. Activation of these molecules typically requires a metal catalyst (Luther, 2013). Given that vent walls are typically FeS abundant, hydrothermal vent systems are the ideal location for catalysis to occur. Metal ligands can bend CO_2 or CS_2 to make reactions more favorable. CO_2 is naturally an unreactive compound and so catalysis is required for reactions to complete.

2. SIGNIFICANCE AND RELEVANCE OF RESEARCH

The major theories of how life on earth has originated are argued frequently in the scientific community. Panspermia, although providing promising evidence (Brandstatter, et al., 2008) with regards to bacterial survival, is unlikely (Miller & Cleaves, 2006). Hydrothermal fields are have shown recently advances in organic geochemistry at Yellowstone national park (Sydow, Bennett, & Nordstrom, 2017), however Deamer & Georgiou (2015) have pointed out the damaging effects of UV radiation and consistently low pH values. With the discovery of deep sea hydrothermal vents by Corliss, et al., (1979), followed by the findings at the Lost City alkaline hydrothermal vent field (Kelley, et al., 2001), hydrothermal vents, and more specifically alkaline hydrothermal vents, became a hypothesized area from which life originated.

Alkaline hydrothermal vents have unique conditions that give rise to various pre-biotic reagents. The occurrence of serpentization provides heat and feedstocks of H_2 and CO_2 which create an electrochemical gradient at vent walls (Herschy, et al., 2014). The acetyl-coA pathway of carbon fixation is also likely to originate from FeS minerals (Wächtershäuser & Huber, 1997). Furthermore, FeS minerals within the vent walls catalyze reactions to create various carboxylic and dicarboxylic acids as well as their conjugate bases (Wächtershäuser & Huber, 2003). In addition, H_2 appeared to increase persistence of diacids in prebiotic hydrothermal conditions (Lee, et al., 2014). The synthesis of ribose and 2-amino-oxazoles in prebiotic conditions have been outlined by Powner, et al. (2009), justifying the posibility of the occurrence more complex organic structures within alkaline hydrothermal vents.

The possibility of CO_2 switchable ionic liquids occuring within alkaline hydrothermal vents is high considering research done by Jessop, et al. (2005) and Heldebrant, et al., (2008) where they describe a mixture amidines and amino alcohols as reagents in a ionic liquids where CO_2 is the switch. It is possible that a CO_2 switchable ionic liquid when mixed with source chemicals found in alkaline hydrothermal vents, could produce results that give insight to the acid-base chemistry in a pre-biotic world.

Since reactions between CS_2 and acetate have been obvserved under prebiotic conditions (Paula, 2016), and that CS_2 is known to react with CO_2 under metal-catalyzed circumstances (Darensbourg, Wilson, & Yeung, 2013), it is likely they will interact with each other. If a new product forms from these small molecules, it could improve our knowledge of hydrothermal vent chemistry. If the product is relevant to biochemical processes, it may further solidfy hydrothermal vents as the driving theory for the origin of life.

3. RESEARCH HYPOTHESIS

Based on the current understanding of hydrothermal vents and their relation to prebiotic chemistry, two major hypotheses were produced for this project: 1) Mixing reagents present in hydrothermal vents with ionic liquids will produce organic molecules like those thought to occur in prebiotic times, and 2) mixing CS_2, tetrabutylammonium acetate, and CO_2 will produce a molecule relevant to biochemical processes in prebiotic times.

3.1 PRODUCTION OF RELEVANT PREBIOTIC MOLECULES THROUGH CO_2-SWITCHABLE IONIC LIQUIDS

The first objective was to produce an important pre-biotic molecule by mixing reagents found within alkaline hydrothermal vents with a CO_2 switchable ionic liquid that could have occurred within prebiotic conditions. The experiment will be used to study the reactivity of this chemistry; however it is anticipated that by switching the chemistry from very alkaline (pH 9-11) to very acidic (3-5) the reagents will produce an organic molecule relevant to prebiotic conditions. The implication is that CO_2 switchable ionic liquids were present in the hydrothermal vents of the early earth. In addition, findings could extend research beyond the scope of this experiment.

3.2 PRODUCTION OF RELEVANT BIOCHEMICAL MOLECULES THROUGH PREBIOTICALLY VIABLE PRECURSOR MOLECULES

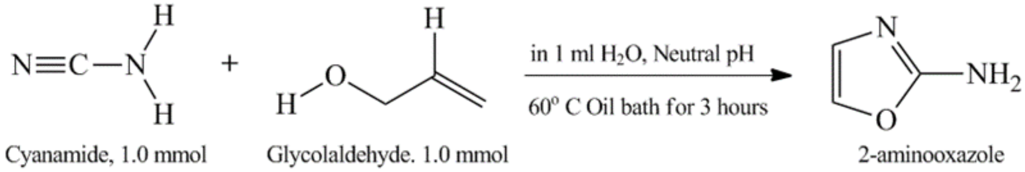
The second objective was is to produce a molecule relevant to biochemical processes using chemistry that simulates alkaline hydrothermal vents. The source molecules selected were CS_2 and tetrabutylammonium acetate because of their known reactivity (Paula, 2016). CO_2 was added to this reaction as a gas passing over, producing a compound relevant to biochemical processes. In doing this, CO_2 could interact with CS_2 in the presence of acetate, giving new insight into the dynamics of these small molecules.

4. RESULTS AND DISCUSSION

4.1 SMALL MOLECULE CHEMISTRY WITHIN CO_2-SWITCHABLE IONIC LIQUIDS

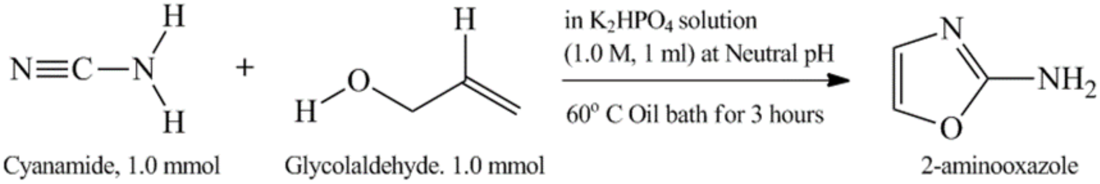
The first objective of this project was to test the capability of CO_2-switchable ionic liquids as a reaction medium for prebiotic reactions. Previous research had shown that 2-aminooxazole was synthesized in a laboratory under prebiotic conditions (Powner, et al. (2009)). This synthesis was chosen as the prebiotic reaction because of the formation of a dicarboxylic acid during an intermediate step. The acid-base chemistry of this reaction was ideal for the ionic liquid solutions. Additionally, the reagents required for this reaction, cyanamide and glycolaldehyde, were inexpensive, because they are common biochemical precursors. These reagents were purchased through Sigma-Aldrich. The disodium phosphate catalyst used in previous experiments (Powner, et al. 2009) was regarded as an unnecessary expense and not purchased. Instead, potassium phosphate (diabasic) was borrowed from Dr. Ylijoki's laboratory at Saint Mary's University.

The first step was to reproduce the synthesis of 2-aminooxazole in order to know that the reaction would complete itself as described in the literature (Powner, et al. 2009). The synthesis of 2-aminoxazole was a simple addition of reagents to solution. This procedure is outlined in Scheme 2.



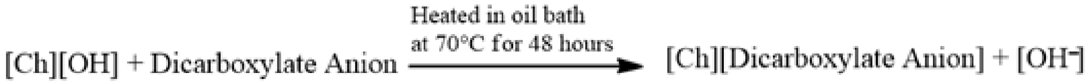
Scheme 2: Synthesis of 2-aminoxazole without a catalyst.

Given the 3-hour length of this procedure, a separate synthesis involving a phosphate catalyst was prepared and completed simultaneously. This procedure is outlined in Scheme 3.



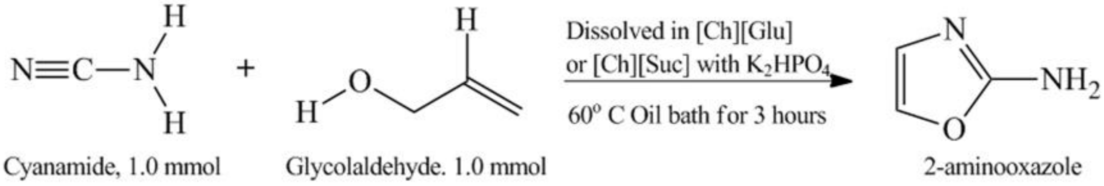
Scheme 3: Synthesis of 2-aminooxazole with a phosphate catalyst.

The resulting liquids were visually different. The reaction that did not involve a catalyst was a colorless transparent solution. The phosphate-catalyzed reaction was a transparent solution with a light yellow color. Isolation of the product was difficult due to the non-volatile nature of the water solution, and so the most effective way to identify 2-aminooxazole was to analyze the mass spectrum. No product was found in the non-catalyzed reaction. This was unsurprising, given reports of little to no product without catalysis by Powner, et al., (2009). The catalysed reaction produced an m/z value at 123.0. This value is higher than the expected value for 2-aminooxazole; however, it was determined that a potassium ion was bound to an unknown location on the 2-aminooxazole structure, which is a common occurrence in ESI-MS analysis.



Scheme 4: Preparation of CO_2-switchable ionic liquids.

With the successful synthesis of 2-aminooxazole, the next step was to produce a CO_2-switchable ionic liquid. The ionic liquids used had cholinium cations and dicarboxylic acid anions. Cholinium hydroxide (in methanol) was readily available in the lab, while glutamic and succinic acid were purchased through Sigma-Aldrich. The preparation of these ionic liquids was a modified procedure based on previous research (Sun, et al., 2014). Cholinium Glutamate ([Ch][Glu]) and Cholinium Succinate ([Ch][Suc]) were prepared over 48 hours with no light exposure. The final products were very similar in color, each being an opaque, dark brown, however [Ch][Glu] was noticeably more viscous than [Ch][Suc] based on lightly stirring the liquids.

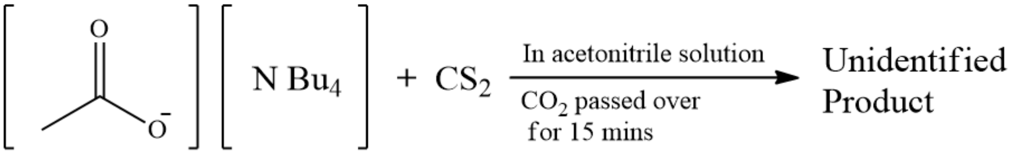


Scheme 5: Synthesis of 2-aminooxazole in CO_2-switchable ionic liquids.

Once the ionic liquids were prepared, the synthesis of 2-aminooxazole (using a phosphate catalyst) was repeated in both [Ch][Glu] and [Ch][Suc] simultaneously. This time however, CO_2 was passed over both reactions for the entire duration in an attempt to switch the environment from a basic setting to an acidic one. After 3 hours, the reagents placed in [Ch][Suc] had fully dissolved and the ionic liquid was less viscous than before. The reagents placed in [Ch][Glu] were still visible in powder form on the surface of the ionic liquid. Although Succinic and glutamic acid are both dicarboxylic acids, they differ stereochemically. Additionally, glutamic acid has an amino group on its third carbon. Because of these factors, it is likely that the reagents were insoluble in glutamate-based ionic liquids. Considering that the abundance of glutamic acid is much smaller than succinic acid in hydrothermal vents (Kelley, et al., 2001), the reaction with glutamic acid was less likely to occur in nature than its sister reaction in [Ch][Suc].

Although the products in the experiment failed to dissolve into solution, both products were analyzed in ESI-MS. The [Ch][Glu] reaction returned no significant results. The analysis for [Ch][Suc] also returned no significant results, however peaks for [Ch^+] at m/z 104.2 and [Suc^-] at m/z 118 were found. Thus, no clear evidence for formation of 2-aminooxazole or other identifiable compound was found in either experiment.

4.2 REACTION OF CARBON DISULFIDE AND ACETATE IN THE PRESENCE OF CARBON DIOXIDE

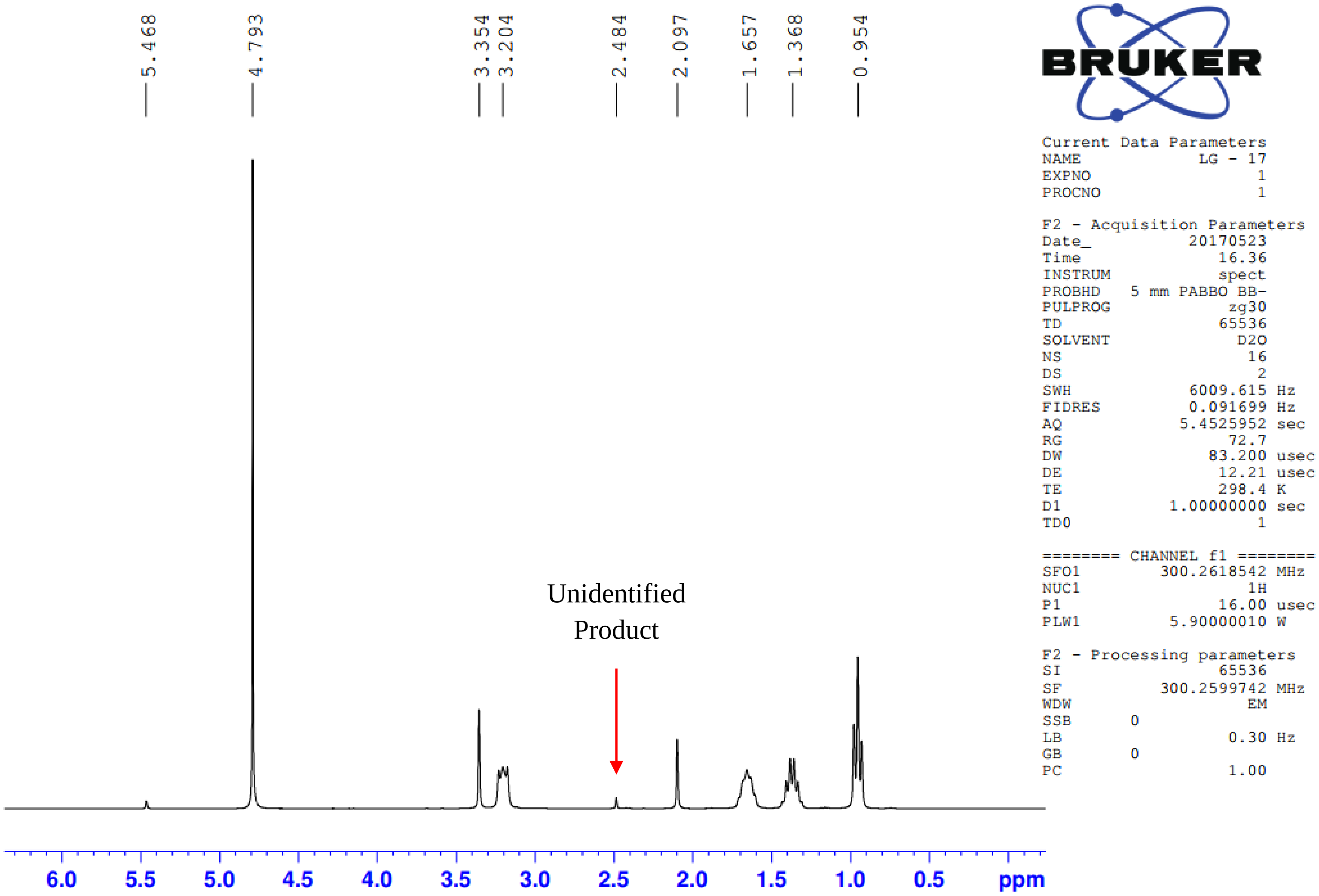


Scheme 6: Reaction between Tetrabutylammonium acetate and CS_2 in the presence of CO_2.

The objective of this experiment was to explore chemistry involving small molecules that are present in alkaline hydrothermal fluid. The Clyburne group had previously explored chemistry involving small carboxylate anions reacting with CS_2 the findings that encouraged further exploration (Paula, 2016). The current objective was to include CO_2 in the reaction and determine its effects. This procedure is outlined in Scheme 6.

The added CO_2 was expected to react with CS_2 and acetate to form a larger molecule such as pyruvic acid. Through ^1H NMR analysis, an unidentified peak was found at 2.484 ppm (*Figure 3*). This was initially thought to be pyruvic acid, as the ^1H chemical shift was similar to those found in previous studies involving the same solvent (Silwood, et al., 1998). However, the study also found other molecules that fell within experimental and analytical uncertainty of the ^1H NMR chemical shift including glutamine, glutamate, succinate and iso-butyrate. The results of ESI-MS showed a peak at m/z 242.4, which was present in the blank run at a much lower intensity. Initially, this peak went unnoticed, as the machine consistently exhibited this peak due to a compound tested by another that had been stuck in the ESI Mass spectrometer at Saint Mary’s University. Therefore, this peak at m/z 242.4 is seen in all ESI-MS analysis to some degree. Nevertheless, the small peak in the blank (methanol) was seen to increase in the experimental compound (sample in methanol). It was then determined that the ESI-MS was measuring the tetrabutylammonium ion, which was a leftover reagent from the experiment. In hindsight, this result was not surprising, as tetrabutylammonium was likely the easiest ionization that the ESI-MS could produce in the sample.

Ultimately, the information provided by ESI-MS analysis was not useful in defining the product from this experimental study. The singlet shown in ^{1}H NMR analysis appeared in repeated experiments but no cause for this was determined.



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TD                 65536
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NS                    16
DS                     2
SWH             6009.615 Hz
FIDRES          0.091699 Hz
AQ             5.4525952 sec
RG                  72.7
DW                83.200 usec
DE                 12.21 usec
TE                 298.4 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         300.2618542 MHz
NUC1                  1H
P1                 16.00 usec
PLW1          5.90000010 W

F2 - Processing parameters
SI                 65536
SF           300.2599742 MHz
WDW                   EM
SSB      0
LB                  0.30 Hz
GB       0
PC                  1.00
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Figure 7. ^{1}H NMR spectrum of reaction between tetrabutylammonium acetate and CS_2 in the presence of CO_2

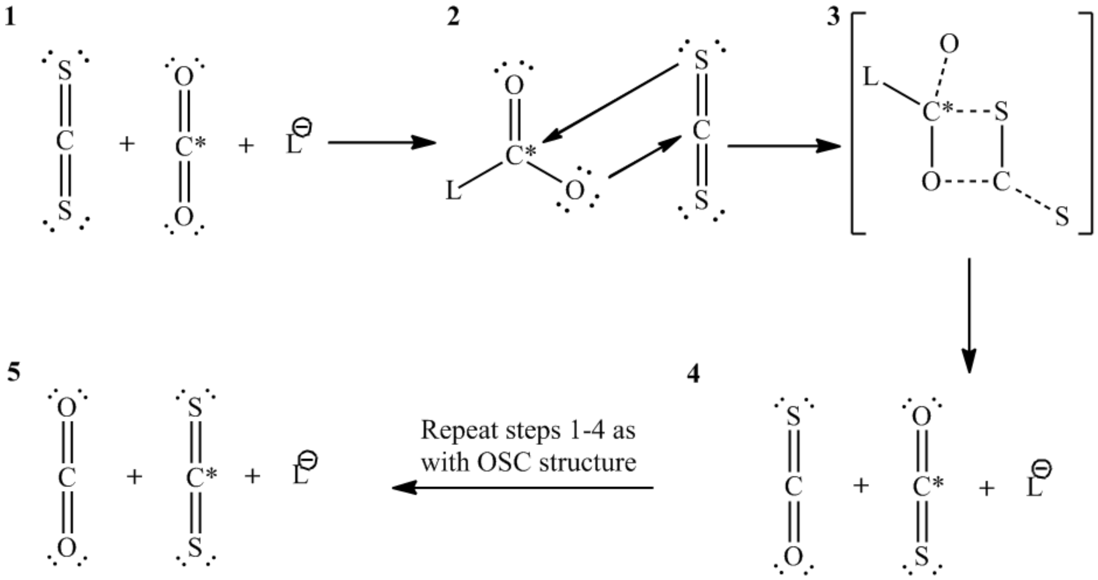
The ESI-MS failed to provide a clear result, and so other analysis was necessary. The experiment was repeated using ^{13}C-labelled CO_2 to understand better the dynamics of the CO_2 carbon atom and ultimately determine where it ended up in the sample. After the reaction was allowed to occur, the sample was left in anhydrous acetonitrile and a ^{13}C NMR analysis was completed. The results of the ^{13}C NMR showed intense peaks occurring at 192.4 ppm and 1.54 ppm, with three very small peaks dispersed between these values (*Figure 8A*). Although the molecule was still undefined, it could not be pyruvic acid. In ^{13}C NMR analysis, pyruvic acid shows three definitive peaks at 193.7 ppm, 161.4 ppm, 25.5 ppm (National Institute of Advanced Industrial Science and Technology, 2017).

4.3. NON-METAL CATALYZED CARBON SCRAMBLING

The chemical shifts found matched very closely with values found for ^{13}C NMR of CS_2 (National Institute of Advanced Industrial Science and Technology, 2017) and given their intensity, there was no doubt the carbon on this molecule originated from the ^{13}C-labelled CO_2. The easiest way to prove that CS_2 had somehow exchanged atoms with CO_2 was to evaporate the solvent from the sample. If CS_2 were still present, it would evaporate with the solvent because of its volatility. After evaporating the solvent, another ^{13}C NMR analysis was completed to see if the peak at 193.7 ppm had disappeared. After evaporation, the peak was no longer there which indicated an atom exchange between CO_2 and CS_2 (*Figure 8B*) had occurred. A comparison of ^{13}C NMR spectra between 1) CS_2 in acetonitrile, and 2) CS_2 and tetrabutylammonium acetate in acetonitrile, was completed to demonstrate CS_2 was not naturally ^{13}C-enriched. Interestingly, CS_2 would not dissolve into acetonitrile by itself, leaving a clear bubble of fluid under the solvent.

^{13}C NMR spectra showed three peaks at 193.51 ppm (CS_2), 118.26 ppm (acetonitrile), and 2.11 ppm (acetonitrile). In this case, the overall intensity for peaks was low. CS_2 was the most intense peak and acetonitrile was slightly lower. ^{13}C NMR results for CS_2 and tetrabutylammonium acetate in acetonitrile showed the expected peaks for CS_2 at 193.86 ppm, acetonitrile at 118.3 ppm and 2.2 ppm, and tetrabutylammonium acetate at 59.7 ppm, 40.2 ppm, 24.9 ppm, 20.9 ppm, and 14.5 ppm. These results showed that CS_2 was much lower in intensity than acetonitrile and tetrabutylammonium acetate. The low intensity of CS_2 in ^{13}C NMR confirmed that it was not naturally enriched with ^{13}C.

This phenomenon observed in this experiment can be described as oxygen/sulfur scrambling. Observations of oxygen/sulfur scrambling between CO_2 and CS_2 have been seen in previous research, although reported examples require a metal catalyst to induce the exchange (Darensbourg, Wilson, & Yeung, 2013). In the reaction completed for this thesis, acetate was acting as a catalyst for CO_2/CS_2 interaction and allowed the exchange to occur. The mechanism (Scheme 7) for this exchange starts with all reagents in solution with a ligand. In this case, the ligand was an acetate ion. The ligand binds to the carbon atom of CO_2 causing a C=O bond to weaken to a C-O bond. The oxygen from this branch then attacks a sulfur atom on one end of CS_2 while the opposite end simultaneously attacks the nucleophilic carbon of the ligand-bound CO_2 molecule. The resulting structure releases an oxygen atom, which then attacks the sulfur tail. The instability of this molecule causes it to divide into two COS molecules. These structures then repeat the cycle as COS molecules and the product is ^{13}C-labelled CS_2 and CO_2.



Scheme 7: Proposed mechanism for the ligand induced exchange of carbon and sulfur. ^{13}C -labelled CO_2 is marked with an asterisk and the ligand is denoted by “L^-”.

These finding have interesting implications for prebiotic hydrothermal vent chemistry. Interactions between CS_2 and CO_2 under low temperature and pressure conditions is more dynamic than expected. Considering that the synthesis of acetate in hydrothermal vent conditions is known to occur (Shock, 1992), the formation of COS and oxygen/sulfur exchange is also probable. More importantly, this reaction can occur away from the FeS vent walls because it does not require a metal catalyst.

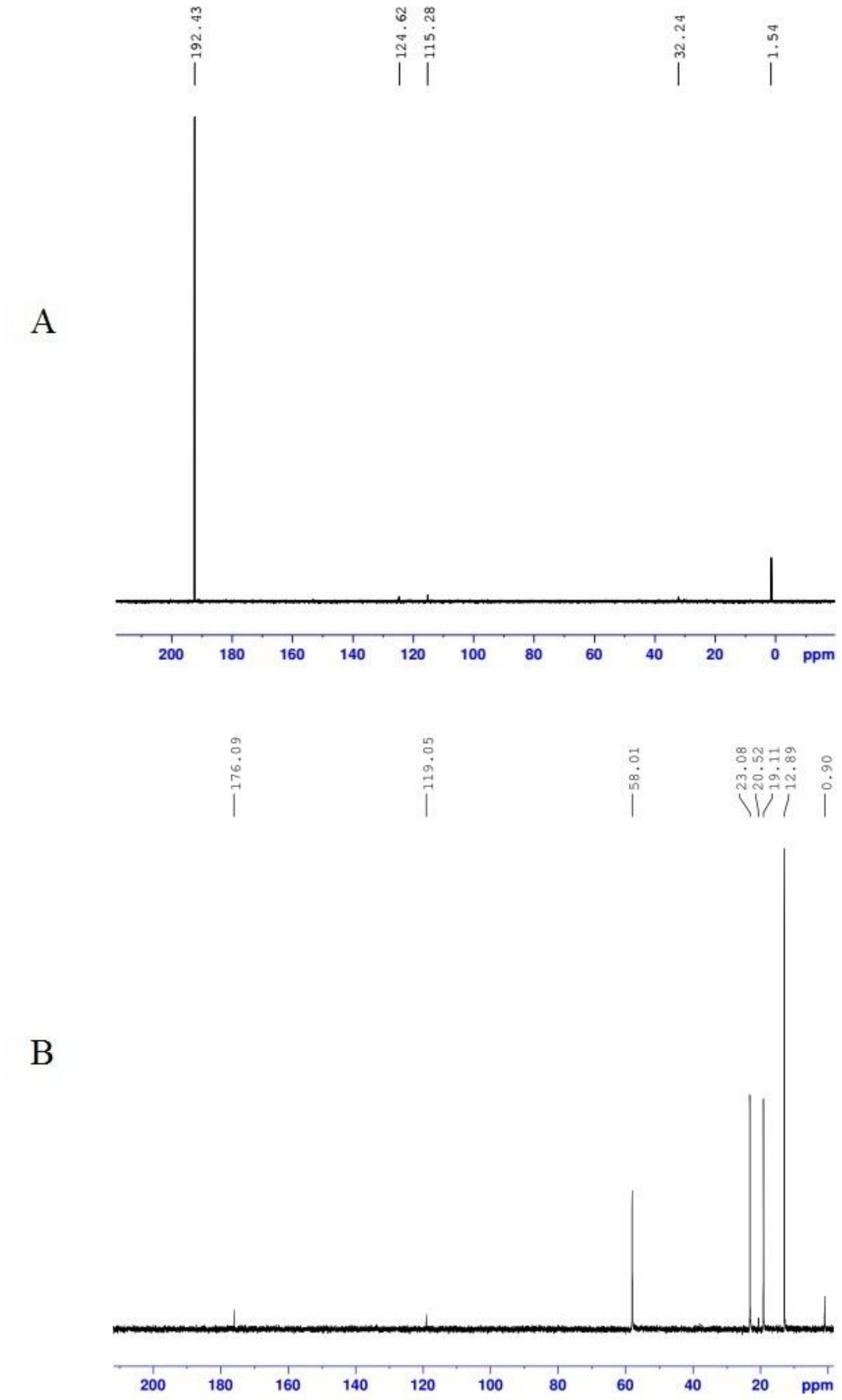


Figure 8. A) ^{13}C NMR from the reaction between tetrabutylammonium acetate and CS_2 with C-13 labelled CO_2 passing over. B) ^{13}C NMR from the reaction between tetrabutylammonium acetate and CS_2 with C-13 labelled CO_2 passing over, with the solvent (acetonitrile) and CS_2 now evaporated from sample.

5. CONCLUSION

The environment in and around alkaline hydrothermal vents is well suited for prebiotic chemistry due to the abundance and diversity of source chemicals and conditions. Reactions between cyanamide and glycolaldehyde under prebiotic conditions, modified from the research by Powner, et al. (2009), were shown to produce 2-aminoxazole in the presence of a phosphate catalyst. However, no product was detected in experiments that excluded a catalyst. Two CO_2-switchable ionic liquids were successfully prepared using cholinium hydroxide the dicarboxylic acids L-glutamic acid and succinic acid, producing [Ch][Glu] and [Ch][Suc]. The synthesis of 2-aminooxazole within [Ch][Glu] and [Ch][Suc] was unsuccessful. [Ch][Suc] dissolved reagents more readily and was less viscous than [Ch][Glu] but still failed to produce an identifiable product.

Previous research within the Clyburne group (Paula, 2016) demonstrated the reactivity of Tetrabutylammonium acetate with CS_2 under prebiotic conditions. In the present study, similar reactions were completed following the addition of CO_2. ^1H NMR spectra revealed a single peak at approximately 2.45 ppm proved difficult to identify within the timeline of this research. In addition to the synthesis of an unknown product, ^{13}C NMR analysis revealed that CO_2 and CS_2 were exchanging sulfur and oxygen atoms. This exchange has been seen previously in reactions with metal catalysts; however, no occurrence of metal-free exchanging has previously been documented. Thus, that the chemistry of CO_2 and CS_2 in low temperature/pressure conditions is more dynamic than previously thought. Reactions between these molecules in the prebiotic world would not, in some cases, have to rely on metal sulfides for atom exchange.

5.2 FUTURE WORK

To further the research of this project, there are two main objectives. First, CO_2-switchable ionic liquids should be studied for their effectiveness based on the dicarboxylic anion used. In the completed experiments, it was noted that [Ch][Glu] was less effective and more difficult to work with than [Ch][Suc]. Experimentation with other dicarboxylic acids would allow a better understanding of switchable ionic liquid chemistry.

For the reaction between CS_2 and tetrabutylammonium acetate in the presence of CO_2, the main objective would be to identify the product of this reaction. It would be best to isolate the product in a column and then complete a chromatographic study with an accompanying mass spectrum. Identification of the reaction product may add to elucidate the biochemistry or precursors to biochemistry in the prebiotic world. To improve the findings that suggest oxygen and sulfur exchange between CS_2 and CO_2 occurrs we propose the following research. First, CS_2 and ^{13}C-labelled CO_2 should be mixed into acetonitrile to see if any exchange occurs without the presence of acetate. Second, CS_2 and acetate mixed into acetonitrile in the presence of CO_2 should be studied and analysed in ^{13}C NMR. This should result in a spectrum that can be deemed the "blank" in which one would expect very low intensity peaks. A repeat of the experiment using CS_2 and acetate in acetonitrile with ^{13}C-labelled CO_2 should be completed and analyzed in ^{13}C NMR, it is necessary to have a fresh sample when comparing to other ^{13}C NMR spectrum with different parameters. A 4-minute ^{13}C NMR analysis (roughly 65 cycles) should be completed for each experiment, to maintain consistency with previously completed research. The thermodynamics of the above reaction remain unknown. A kinetic study should be completed. Upon completion of these experiments and identification of the product, the dynamics of CS_2 and CO_2 in the presence of acetate will be better understood.

6. EXPERIMENTAL

6.1. GENERAL PROCEDURES

All sample preparations were carried out in an MBRAUN glovebox, under an inert nitrogen atmosphere unless otherwise stated. Nitrogen (>99.998%) and carbon dioxide (≥99.998%) were provided by Praxair Inc. Glycolaldehyde dimer (99.8%), succinic acid (BioXtra, ≥99.0%), L-glutamic acid (≥99.0%), potassium phosphate diabasic (≥98%), cyanamide (99%), choline hydroxide (45 wt. % in methanol), tetrabutylammonium acetate (97%), anhydrous acetonitrile (99.8%), carbon disulfide (≥99.9%), carbon-^{13}C dioxide (99 atom % ^{13}C, <3 atom %^{18}O), pyruvic acid (98%), and Deuterium Oxide (99.9 atom % D) were purchased from Sigma-Aldrich. Methanol (≥99.8%) and hydrochloric acid concentrate (36.5-38%, certified grade) were purchased from Fisher Scientific.

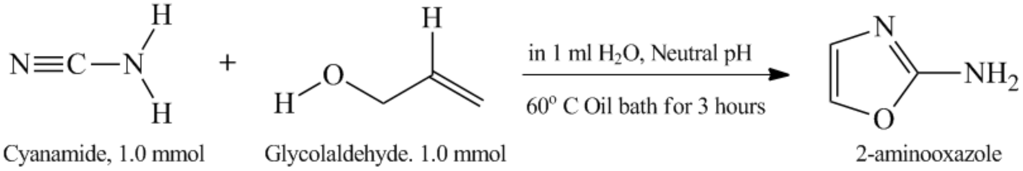
6.2. ANALYTICAL TECHNIQUES

Infrared spectra were collected as KBr pellets with a Bruker Vertex 70 Infrared Spectrometer and the data was processed using the OPUS 6.0 software suite. The ^{1}H NMR spectra were collected using a Bruker Ultrashield 300 MHz NMR spectrometer with a 7.05 magnet. ^{13}C NMR was carried out at 75 MHz, using the same instrument. ^{13}C NMR samples were dissolved in anhydrous acetonitrile (non-deuterated) for analysis. ^{1}H NMR samples were prepared by dissolving a small amount of sample into the minimum amount of deuterium oxide, after quenching the sample with HCl concentrate (36.5-38%, certified grade). ^{1}H NMR spectra were referenced to the residual solvent peaks downfield of deuterium oxide. The data was processed using Bruker TOPSPIN 1.3.

Mass Spectral analyses were performed on a LC/MSD Trap (Agilent 1100). Prior to data acquisition, standard calibration was conducted with solvent blanks produced in the laboratory. Electrospray ionization (ESI) was the atmosphereic pressure source used in all analyses. All mass spectral data acquisition was carried out by Patricia Granados of the Centre for Environmental Analysis and Remediation at Saint Mary's University with the exception of LG-20, which was completed by Xiao Feng of Dalhousie University, Halifax, Nova Scotia, using a Bruker microTOF Focus Mass Spectrometer.

6.3 SYNTHETIC METHODS

6.3.1 SYNTHESIS OF 2-AMINOOXAZOLE IN AQUEOUS SOLUTION IN THE ABSENCE OF PHOSPHATE



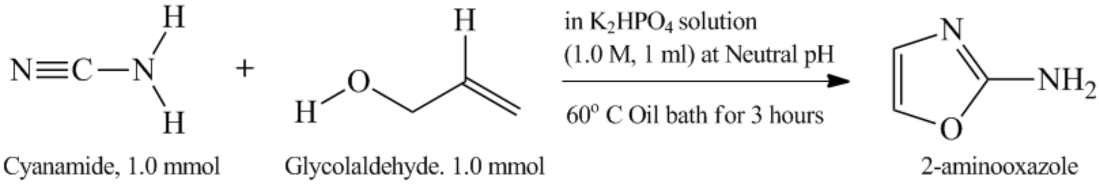
Note: this reaction was on the benchtop, without an inert nitrogen atmosphere.

The following procedure is a modified experiment of Powner, et al. (2009). Glycolaldehyde (60 mg, 1.0 mmol) was dissolved in H_2O (1 ml) at neutral pH. Cyanamide (42 mg, 1.0 mmol) was then added, and the solution was placed in an oil bath at 60°C for 3 hours. The resulting solution was a clear fluid.

Methanol (0.5 ml, 15 mmol) was added to the solution to offset the H_2O in the system, in order to acquire a stronger mass spectrum. A blank was of H_2O (0.5 ml) and methanol (0.5ml) was made to calibrate the LC/MSD Trap. ESI-MS: Positive Mode

Found: background noise with no significant peaks. Negative Mode Found: background noise with no significant peaks. See supplementary info (LG-1).

6.3.2 SYNTHESIS OF 2-AMINOOXAZOLE IN AQUEOUS SOLUTION IN THE PRESENCE OF PHOSPHATE



Note: this reaction was on the benchtop, without an inert nitrogen atmosphere.

The following procedure is a modified experiment based on synthesis by Powner, et al. (2009). Glycolaldehyde (60 mg, 1.0 mmol) was dissolved in K_2HPO_4 solution (1.0 M, 1 ml) at neutral pH. Cyanamide (42 mg, 1.0 mmol) was then added, and the solution was placed in an oil bath at 60°C for 3 hours. The resulting solution was a faint yellow fluid.

Methanol (0.5 ml, 15 mmol) was added to the solution to offset the H_2O in the system, in order to acquire a stronger mass spectrum. A blank was of H_2O (0.5 ml) and methanol (0.5ml) was made to calibrate the LC/MSD Trap. ESI-MS: Positive Mode Found: m/z 123.0[100% $C_3H_4N_2O$ and K^+ complex]; Calc. 123.08 (84.08 + 39.0). Negative Mode Found: m/z 98.5 [100% $C_3H_4N_2O$]; Calc. 84.08. See supplementary information (LG-2).

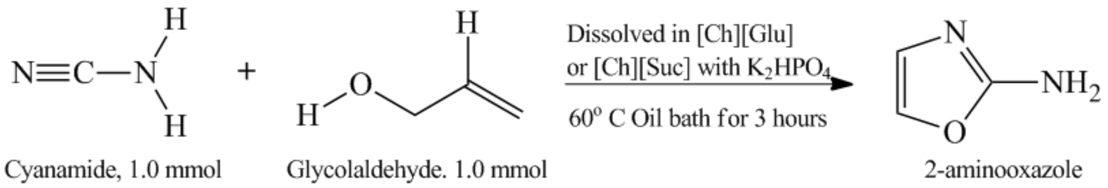
6.3.3 PREPARATION OF IONIC LIQUIDS: CHOLINIUM GLUTAMATE AND CHOLINIUM SUCCINATE

$$[\text{Ch}][\text{OH}] + \text{Dicarboxylate Anion} \xrightarrow{\substack{\text{Heated in oil bath} \\ \text{at 70°C for 48 hours}}} [\text{Ch}][\text{Dicarboxylate Anion}] + [\text{OH}^-]$$

Note: these reactions were completed on the benchtop, without an inert nitrogen atmosphere.

[Ch][Glu] and [Ch][Suc] preparations were based upon [Ch][Lys] synthesized by Sun, et al., (2014). L-Glutamic acid (5.31 g, 30 mmol, 3.6 equivalents) was added to choline hydroxide (9 ml, 33 mmol, 3 equivalents) in methanol at 4°C with the use of an ice bath. In a separate flask of equal volume, Succinic acid (4.275 g, 36 mmol, 3.6 equivalents) was added to choline hydroxide (9 ml, 33 mmol, 3 equivalents) in methanol at 4°C with the use of an ice bath. Each flask was wrapped in aluminium foil to protect the samples from light and then stirred in an oil bath at 70°C for 48 hours. After 48 hours, both [Ch][Suc] and [Ch][Glu] were placed in a rotary evaporator for 30 minutes to remove all methanol. The resulting substances were viscous liquids with a dark brown color. Both ionic liquids were stored in a refrigerator and out of contact with light.

6.3.4. SYNTHESIS OF 2-AMINOOXAZOLE IN [Ch][Glu] AND [Ch][Suc]



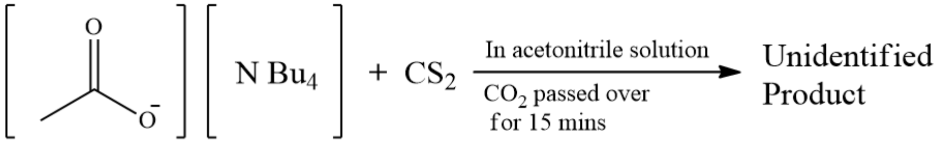
Note: these reactions were completed on the benchtop, without an inert nitrogen atmosphere.

Glycolaldehyde (59.52 mg, 1 mmol) was added with cyanamide (45.41 mg, 1 mmol) and K_2HPO_4 (181.25, 1 mmol) to [Ch][Suc]. Glycolaldehyde (61.00 mg, 1 mmol) was added with cyanamide (42.07 mg, 1 mmol) and K_2HPO_4 (183.44, 1 mmol) to [Ch][Glu].

Both ionic liquid solutions were placed in an oil bath at 60°C for 3 hours with CO_2 passing over each reaction. After 3 hours, the ionic liquids remained viscous and no color change was apparent. Reagent powder was still visible in the [Ch][Glu] but not in [Ch][Suc]. [Ch][Suc] was more viscous than [Ch][Glu].

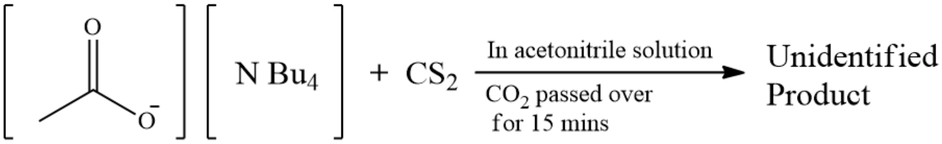
Water (4 ml) added to the ionic liquids to extract products. The extracted solutions were brown and opaque. Methanol (2 ml ml, 15 mmol) was added to each extract to offset the H_2O in the system, in order to acquire a stronger mass spectrum. A blank was of H_2O (4 ml) and methanol (2 ml) was made to calibrate the LC/MSD Trap. [Ch][Suc] ESI-MS: Positive Mode Found: m/z 104.2 [100% $C_5H_{14}NO$]; calc. 85.04. Negative Mode Found: Background noise and ionic liquid ion peaks, but no significant results. [Ch][Glu] ESI-MS: Background noise with ionic liquid ion peaks, but no significant results. See supplementary info for [Ch][Suc] (LG-S) and [Ch][Glu] (LG-G).

6.3.5 REACTION OF TETRABUTYLAMMONIUM ACETATE WITH CARBON DISULFIDE AND CARBON DIOXIDE



Tetrabutylammonium acetate (301 mg, 1 mmol) was weighed in vessel and then deposited into a round bottom flask. Acetonitrile (2 ml, 38.5 mmol) was used to dissolve Tetrabutylammonium acetate into solution by adding to the flask with a 10 ml syringe. The flask was then sealed and brought out of the nitrogen glovebox to added CS_2 (1 ml, 16.6 mmol) to the solution while simultaneously bubbling CO_2 through the system for 15 minutes. Upon addition of CS_2, the solution changed from clear to dark red. After 15 minutes passed, solvent was removed using a rotary evaporator for 15 minutes more. The remaining substance was dark red until exposed to air, where it turned yellow and immediately began to melt. The product was then quenched with 2 drops of HCl concentrate and dissolved in a minimum amount of deuterium oxide. ^{1}H NMR (300 MHz, D_2O, δ ppm) 4.793 (s, D_2O), 3.354 (s, unassigned), 3.204 (t, 8H, $-NC\underline{H}_2CH_2CH_2CH_3$) 2.484 (s, Unassigned), 2.097 (s, CH_3), 1.657 (q, 8H, $-NCH_2C\underline{H}_2CH_2CH_3$), 1.368 (sextet, $-NCH_2CH_2C\underline{H}_2CH_3$), 0.954 (t, $NCH_2CH_2CH_2C\underline{H}_3$). ESI-MS: Positive Mode Found: m/z 242.3 [100% $C_{16}H_{36}N^+$]; calc. 242.4. Negative Mode Found: Background noise. See supplementary information (^{1}H NMR Data: LG-17. ESI-MS data: LG-18)

6.3.6. REACTION OF TETRABUTYLAMMONIUM ACETATE WITH CARBON DISULFIDE AND ^{13}C-LABELLED CARBON DIOXIDE



Tetrabutylammonium acetate (TBAA, 301 mg, 1 mmol) was placed into a tared vessel and then deposited into a round bottom flask. Acetonitrile (2 ml, 38.5 mmol) was used to dissolve Tetrabutylammonium acetate into solution by adding to the flask with a 10 ml syringe. The flask was then sealed and brought out of the nitrogen glovebox to added CS_2 (1 ml, 16.6 mmol) to the solution. A balloon of ^{13}C-labelled CO_2 was attached to the flask via syringe. The flask was then lightly agitated by hand for 15 minutes. Upon addition of CS_2, the solution changed from clear to dark red. After 15 minutes passed, the product was left in solution for ^{13}C NMR analysis. After ^{13}C NMR was completed, the solvent and CS_2 were evaporated to narrow down the location of ^{13}C. The remaining substance was dark red until it was quenched with HCl concentrate and turned yellow and immediately. ^{1}H NMR (300 MHz, D_2O, δ ppm) 4.790 (s, D_2O), 2.934 (t, 8H, -$NC\underline{H}_2CH_2CH_2CH_3$) 2.048 (s, CH_3), 1.968 (s, CH_3), 1.383 (q, 8H, -$NCH_2C\underline{H}_2CH_2CH_3$), 1.112 (sextet, -$NCH_2CH_2C\underline{H}_2CH_3$), 0.695 (t, $NCH_2CH_2CH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, CH_3CN, δ ppm) 192.43 (s, CS_2), 124.62 (s, CO_2) 1.54 (s, CS_2). ESI-MS: Positive Mode Found: No significant data. Negative Mode Found: m/z 197.8 (100%, unassigned)

After ^{13}C NMR analysis, solvent was removed and another ^{13}C NMR was completed with a minimum amount of D_2O. ^{13}C NMR (75 MHz, CH_3CN, δ ppm) 176.09 (s, unknown), 119.05 (s, CH_3CN), 58.01 (s, unassigned), 23.08 (s, unassigned), 20.52 (s, unassigned), 19.11 (s, $C\underline{H}_3CH_2OH$), 12.89 (s, unassigned), 0.90 (s, CH_3CN). See Supplementary information (^1H NMR data: LG-24. ^{13}C NMR data: LG-23 – CH3N, LG-24, LG-28, and LG-30. ESI-MS data: LG-20).

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