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**POST-DEPOSITIONAL ALTERATION OF ORGANIC MATERIAL:
IMPLICATIONS FOR INTERPRETING MOLECULAR BIOSIGNATURES**

A Dissertation in

Geosciences

by

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ABSTRACT

Interpretations of biomarkers' molecular and isotopic composition have been critical to understand the evolution of life and its environment on Earth. As a result, the identification of biomarkers in planetary and space environments is a top priority in the search for life beyond Earth. Both terrestrial and extraterrestrial organic materials are subject to extensive degradation and alteration after deposition, which may obscure primary molecular and isotopic signals. In order to identify biomarkers and interpret their biological- and environmental-specific characteristics, the effects of post-depositional processes in terrestrials and extraterrestrial environments must be constrained. In this dissertation, the alteration of molecular and isotopic signatures is evaluated for two post-depositional processes, degradation by exposure to ionizing radiation and preservation through organic-mineral interactions.

Exposure to ionizing radiation has the capacity to completely destroy organic material or drive reactions between organic and inorganic species that lead to secondary radiolysis products. In environments that are not adequately shielded from exogenous radiation by a magnetic field or thick atmosphere, high doses of radiation will destroy organics on the planet surface, while higher energy radiation can alter and potentially destroy organic material at depth in a soil or regolith. To understand how subsurface organics are altered on Mars, I exposed meteorite-relevant macromolecular organics in fused silica or an iron mineral mixture to high-energy radiation for doses equivalent to millions of years on the surface. Organic acids were the main radiolysis product in all samples, independent of mineral matrix or organic starting material. The presence of organic acids in fused silica samples suggests that previously proposed Fenton reactions, which require a redox sensitive mineral, were not the only driver of organic acid formation. I proposed the illumination of semi-conductor surfaces by high energy radiation leads to radical species that can break down macromolecular organics. The ubiquity of organic acids as a radiolysis product in

this work and others indicates that high-radiation environments do not preserve primary molecular signals.

Organic material can be shielded from ionizing radiation, and other degradation processes such as microbial attack or chemical oxidation, by organic-mineral interactions. Intermolecular forces between organics and mineral surfaces control the strength of these interactions and thus influence organic material preservation in the geologic record. The isotopic properties of the organic molecule can influence the strength of intermolecular interactions, suggesting primary isotopic signals may not be preserved. I investigated the isotopic effects of two sorption interactions, *n*-octane sorbed to kaolinite, which is dominated by van der Waals forces, and amino acids sorbed to ice, which is dominated by hydrogen bonds. For *n*-octane/kaolinite sorption, I developed new computational methods that incorporated the effects of anharmonicity to predict sorption-driven H-isotope effects that were confirmed by surface-sensitive IR techniques. Although the global H-isotope fractionation was minor ($< 2 \text{ ‰}$), we confirmed that anharmonic contributions cannot be ignored for predicting non-covalent isotope effects. In contrast, the stronger interaction involving hydrogen bonding between amino acids and ice surfaces caused significant position-specific C isotope fractionation, up to 8 ‰ . The position and magnitude of C isotope fractionation was driven by the orientation of the amino acid on the surface. The results of both sorption studies presented in this dissertation indicate that mineral interactions do not influence global isotope compositions significantly, but can mask position-specific primary isotope signals.

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Chapter 1

Introduction

1.1 Introduction

Molecular biosignatures, or biomarkers, are organic molecules preserved in the geologic record that indicate the presence of past life. The molecular and isotopic composition of biomarkers reflect biological- and environmental-specific characteristics that can be interpreted to understand past ecologies and climates (Freeman et al., 1990; Hayes et al., 1990; Sauer et al., 2001). Biomarkers are some of the most compelling evidence for the origin and evolution of life on early Earth (Eigenbrode, 2008), which make them attractive targets for detection in extraterrestrial environments. Organic molecules have been detected on the surface of Mars (Freissinet et al., 2015; Eigenbrode et al., 2018), in meteorites (Botta and Bada, 2002), and as a component of interstellar dust (Busemann et al., 2006), suggesting organic molecules are abiotically produced throughout the solar system. Measurable attributes of biomarkers distinguish them from abiogenic organic compounds, including isotopic ordering or composition, enantiomeric excess, structural isomer preference, and uneven distribution patterns of related homologues (Summons et al., 2008).

Reliance on the attributes of organic molecules to distinguish biomarkers and interpret their ecological or environmental signatures requires they retain their molecular and isotopic characteristics during preservation. Biomarkers experience *in situ* biological, chemical, and thermal reactions in sediments after deposition, which have the potential to alter their molecular structures and isotopic compositions (Eigenbrode, 2008). The exposure of biomarkers to different

chemical and thermal reactions is highly dependent on their environment. While alteration on Earth may be primarily driven by microorganisms, different planetary environments may be dominated by exposure to radiation or chemical oxidants. *Our ability to identify biomarkers and interpret their biological and environmental characteristics is dependent on constraining post-depositional alteration of preserved organic material in these diverse environments.* This dissertation investigates the effects of post-depositional processes on the isotopic and molecular composition of organic material with the ultimate goal of providing a framework to interpret primary signals that acknowledge post-depositional alteration in both terrestrial and extraterrestrial environments.

1.2 Drivers of Organic Material Degradation

1.2.1 Microorganisms

Organic material degradation on Earth primarily results from the combined effort of billions of individual microorganisms in marine and terrestrial sediments. The extent of organic degradation is heavily controlled by the environment, which influences the types and ecology of microorganisms, along with their metabolic pathways, respiration rates, and mobility (Schmidt et al., 2011; Dungait et al., 2012; Arndt et al., 2013). While the specific mechanisms that control microbially mediated oxidation and transformation of organic material are debated, evidence for the influence of microorganisms on surviving organic material is apparent in soil and water column depth profiles. For example, soil organic material (SOM) shows an increase of ^{13}C abundance with increasing depth that is strongly correlated to organic carbon concentration, consistent with a model Rayleigh distillation of ^{13}C due to kinetic fractionation during microbial respiration in a closed system (Torn et al., 2002; Wynn et al., 2006). Isotopic shifts are also

observed in marine environments. Unlike soils, marine environments generally show a decrease in ^{13}C abundance at depth, which is generally attributed selective removal via degradation of isotopically enriched biochemicals, such as proteins, carbohydrates, or lignin (Meyers, 1994; Lehmann et al., 2002).

Microorganisms recycle primary organic material into new microbial products and biomass, primarily through uptake and use of low molecular weight carbon substrates, such as acetate, liberated from breakdown of biopolymers during oxidation and fermentation processes (Blair and Carter, 1992; Almendros et al., 2000; Feng et al., 2011). But recycled materials can also potentially include larger compounds such as lipids incorporated more-or-less intact into cellular membranes (Takano et al., 2010; Dippold and Kuzyakov, 2016). Decreasing C/N ratios, increasing aryl and carbonyl C, and decreasing alkyl C with increasing depth in soil are all evidence of increased microbial input (Torn et al., 2002; Eusterhues et al., 2007; Kögel-Knabner, 2017). In lake and ocean waters, between 96 and 99% of organic material is recycled by microorganisms before reaching the bottom (Meyers and Eadie, 1993), resulting in an increase of specific and presumably less reactive molecular structures at greater depths (Hatcher et al., 1983; Wakeham et al., 1984; Hamilton and Hedges, 1988).

1.2.2 Ionizing Radiation

From exogenous cosmic rays to mineral radioactivity, ionizing radiation is prevalent throughout our solar system. The influence of ionizing radiation on organic material on the surface of a given planet or solar system object is dependent on the extent of shielding by atmospheric gases or magnetic fields. Earth's thick atmosphere and strong magnetic field block a significant portion of exogenous ionizing radiation from reaching its surface, which minimizes radiation-driven organic oxidation (Dartnell, 2011). In contrast, celestial bodies with thin or no

atmosphere experience high levels of radiation on the surface that can alter or destroy organic material. For example, the lack of a magnetic field and thin atmosphere on Mars leads high doses of ionizing radiation that alter organic material up to 2 m below the surface (Patel et al., 2002; Dartnell et al., 2007; Pavlov et al., 2012; Hassler et al., 2014).

Exposure to high energy radiation will completely destroy most organic materials, but interactions are much less destructive when they involve lower-energy radiation or indirect interactions involving chemical oxidants or radical species. Previous work has found that interactions between ionizing radiation and water, atmospheric gases, and minerals form radical species and oxidants that can alter organic material (Mao et al., 1991; Zhang et al., 1997; Yen et al., 2000; Woon, 2002). Radical species (such as $\text{OH}^\bullet$, $\text{O}_3^\bullet$, etc.) and oxidants break down more complex organics into smaller organic species, that subsequently can either be further degraded or react to form new organic species (Gebicki and Gebicki, 1993; Zacheis et al., 1999; Poch et al., 2013; Chu et al., 2018).

1.2.3 Chemical Oxidants

Chemical oxidants formed independently of microorganisms and ionizing radiation can facilitate alteration and destruction of organic material. In both marine and terrestrial environments, redox-active minerals facilitate electron transfer reactions that can oxidize organic material. These mineral phases contain redox-sensitive elements (i.e., iron, manganese, sulfur) that act as electron receptors to organic electron donors (Sander et al., 2015). In aqueous environments, chemical oxidants are formed by interactions between inorganic species in the environment and ionizing radiation, or via dissolution of minerals. Dissolved species that can act as oxidants in soil waters and in oceans and sediment porewaters include alkyl halides (Keppler et al., 2000), metal oxides (permanganate, perchlorate, etc.) (Bohlke et al., 2005; Lasne et al., 2016)

and peroxide species. Similar to the consequences of ionization exposure, interactions with chemical oxidants often break down more complex organic molecules into smaller, oxygen-rich compounds such as carboxylic acids.

1.2.4 Thermal Alteration

Organic material that survives extensive biotic and abiotic oxidation during sedimentation and diagenesis is then subject to alteration that occurs with rising temperatures and pressures during burial in geologic basins (Fig 1-1). During early diagenesis ($< 50^{\circ}\text{C}$), random polymerization and condensation reactions form macromolecular organic structures, which are called humin in soils, or more generally known as kerogen. This remaining organic material has experienced substantial loss of carbon via microbial respiration, and the residual polymer has lost nitrogen and oxygen functional groups via hydrolysis, deamination, and decarboxylation (Hedges and Prahl, 1993; Vandenbroucke and Largeau, 2007).

With burial, thermal breakdown of kerogen begins to occur during catagenesis ($50^{\circ} - 150^{\circ}\text{C}$). During this stage, organic material undergoes isotopic, structural, and molecular changes. The elevated temperatures can foster H exchange in C-H bonds in buried organic material. This can lead to a progressive increase or decrease in $\delta^2\text{H}$ values as exchangeable hydrogen equilibrates with available basin waters. This effect disproportionately affects different compound classes (Pedentchouk et al., 2006; Schimmelmann et al., 2006).

Trends in $\delta^{13}\text{C}$ values vary for different compound classes. Generally, kerogen $\delta^{13}\text{C}$ values remain relatively constant with increasing temperatures during catagenesis, while bitumen and pyrolysate fractions show a gradual ^{13}C enrichment related to the kinetic effects associated with breaking ^{13}C - ^{13}C bonds versus ^{12}C - ^{12}C bonds (Lewan, 1983). Bond breaking causes molecular and structural changes that include loss of heteroatom-containing compounds, loss of

biological chain length preferences with increasing depths, and selective loss of thermally unstable isomers, (Philip and Lewis, 1987; Peters and Moldowan, 1991; Mibbach et al., 2016). The breakdown of macromolecules into simpler hydrocarbons (known as “thermal cracking”) leads to fluids such as oil and gas, which migrate away due to buoyant forces. The residual kerogen becomes increasingly C-rich and H-poor with increasing maturity. At even higher temperatures during the metagenesis stage ($> 150^{\circ}\text{C}$), gas production ensues, and the remaining kerogen becomes increasingly ordered, eventually approaching the structure of graphite (Rullkötter, 1993).

1.3 Drivers of Organic Material Preservation

Organic material preservation is driven by properties of the organic material and its environment. In most cases, various protection processes occur simultaneously, making it difficult to determine the relative importance of a given mechanism. Mechanisms that rely on the intrinsic properties of an organic compound (chemical recalcitrance) are likely important for short-term preservation, while physical and chemical protection mechanisms (i.e., sorption, aggregation, etc.) are more important for long-term preservation (Fig 1-2) (Keil and Mayer, 2013).

1.3.1 Chemical Recalcitrance

Observations of the preferential degradation of specific compounds by microorganisms gave rise to the importance of chemical recalcitrance as a major driver of organic material preservation. Certain properties of an organic compounds including elemental composition, presence of specific functional groups, and molecular conformations contribute to chemical

recalcitrance, or resistance to degradation (Marschner et al., 2008). In terrestrial settings, chemical recalcitrance is tied to aqueous solubility, which limits its bioavailability, and to molecular structures (i.e., trans vs. cis conformation or alkylation of aromatic rings) that inhibit enzyme activity. For example, the biodegradation of petroleum hydrocarbons shows an initial preference for short-chain hydrocarbons ($< C_{25}$) over long-chain hydrocarbons, due to their greater aqueous solubility (Wang et al., 2011). Laboratory and field studies in soils and marine sediments have similarly observed that microorganisms will decompose certain labile molecular structures (simple sugars, amino acids, etc.) before more recalcitrant compounds (PAHs, lignin, etc.) (Hatcher et al., 1983; Hedges and Prahl, 1993; Lichtfouse et al., 1998; Santruckova et al., 2000; Biasi et al., 2005; Zonneveld et al., 2010; Kothawala et al., 2012; Knicker et al., 2013).

However, the role of chemical recalcitrance in long-term organic material preservation is contentious. ^{14}C -dating of classically recalcitrant compounds, such as lignin or aromatic structures, revealed they do not persist in soils beyond tens of years (Fig 1-3) (Marschner et al., 2008; Schmidt et al., 2011; Dungait et al., 2012). Similarly, bulk organic composition in marine water columns shows little change with depth, despite extensive biodegradation (Hedges and Keil, 1995; Hedges et al., 2001; Estes et al., 2019). As a result, the role of chemical recalcitrance is likely important in organic material preservation on short timescales, days to years, but is not a main driver of preservation on geologic timescales.

1.3.2 Accessibility

The accessibility of organic material to microorganisms and their enzymes exerts a major control on its preservation. Soil and sediment matrices are often compartmentalized, limiting the transfer of enzymes, substrates, water, oxygen and microorganisms. In soils, organic material can become physically inaccessible to microorganisms through aggregation, encapsulation in

macromolecules, and intercalation in phyllosilicates (Philip and Lewis, 1987; Lützow et al., 2006). Further, in sediment porewaters and saturated soils, the relatively long timescales of chemical diffusion can restrict the supply and types of electron acceptors and thereby limit microbial communities and respiration rates. When there are physical perturbations in soils or sediments, such as erosion, agricultural tilling, or infauna burrows, the physical protections for organic material are disturbed and there is an increase in organic material degradation (Dieckow et al., 2009; Aller and Cochran, 2019; de Nijs and Cammeraat, 2020).

The relative importance of accessibility as driver of organic material preservation is difficult to disentangle from the role of organic-mineral interactions, as mineral sorption plays a key role in the formation of aggregates (Schmidt et al., 2011; Keil and Mayer, 2013). The strong correlation between mineral surface area, organic carbon burial efficiency, and oxygen exposure time in marine environments suggests both physical protections and factors that limit the transport of oxygen are important for preservation (Hartnett et al., 1998).

1.3.3 Organic-Mineral Interactions

Over the majority of the Earth's surface, organic material concentrations in sediments are tightly correlated with the abundance of fine-grained minerals, indicating the importance of organic-mineral interactions for preservation. Depending on the properties of the organic molecule and mineral surface, organic-mineral interactions can include ligand exchange, ion exchange, cation bridging, van der Waals interactions, hydrogen bonds, and hydrophobic effects (Keil and Mayer, 2013). A combination of these sorption interactions can lead to the self-organization of an organic bilayer on mineral surfaces, in which the polar portions of amphiphilic molecules shield an inner hydrophobic zone from the aqueous phase (Fig 1-4) (Kleber et al., 2007; Carrington et al., 2012; Dümig et al., 2012).

The strong association between organic material and mineral surfaces protect organic material by leading to the formation of aggregates or limiting the movement microbes and enzymes via sorption (Keil and Mayer, 2013). Often a large portion of organic material cannot be physically separated from the mineral matrix without the use of aggressive methods, like HF dissolution. The mineral-bound fraction tends to have a lower ^{14}C activity than free organic material, indicating this fraction is older and has been preserved longer (Hedges and Keil, 1995; Torn et al., 1997; Kaiser and Guggenberger, 2000; Eusterhues et al., 2007).

1.4 The Role of Preservation and Degradation Processes in Extraterrestrial Environments

The majority of previous work evaluating the role of various degradation and preservation processes in altering organic material has focused on terrestrial environments. As current and future work studies the origins and evolution of organic material in extraterrestrial environments, it is critical to reevaluate how alteration occurs in different planetary and space environments.

1.4.1 Organic Material in Extraterrestrial Environments

Overwhelmingly, studies of organic material from extraterrestrial environments have focused on organics found in meteorites. Meteoritic organic material primarily exists as insoluble macromolecular material, similar to kerogen. Also present is a soluble fraction that includes a diverse set of compounds (amino acids, carboxylic acids, aromatic and aliphatic hydrocarbons, etc.), which can be separated and analyzed to understand how organic material was produced and altered throughout the solar system (Pizzarello et al., 2006; Alexander et al., 2017). More critical to the detection of extraterrestrial biomarkers is whether organic material is present in planetary

and space environments capable of sustaining life currently or in the past. Exploration of Mars has led to the direct observation of small aromatic, aliphatic, and S- or Cl-bearing hydrocarbons (Freissinet et al., 2015; Eigenbrode et al., 2018), as well as indirect evidence for more complex organic material in the form of CO and CO₂ gases observed during evolved gas analysis of Martian sediments (Ming et al., 2014). Future missions seek to observe organic material predicted to be present on various ocean worlds (National Research Council, 2011), including amino acids and other small organic compounds predicted on Europa (Levy et al., 2000) and Enceladus (Steel et al., 2017). Each of these environments has its own set of chemical processes that can degrade and preserve organic material to different degrees. Their potential influence needs to be considered to identify whether extraterrestrial biomarkers may be present and to determine their potential for retaining unaltered signatures that reflect their origins.

1.4.2 Alteration in Planetary and Space Environments

On Earth, the preservation of organic material is primarily governed by its physical and chemical availability to microorganisms. For planetary and space environments, microorganisms are either non-existent or incredibly rare, thus preservation is more likely a function of exposure to ionizing radiation. Unlike Earth, most celestial bodies do not have a strong magnetic field or thick atmosphere that shield them from high doses of ionizing electromagnetic (ultraviolet [UV] and γ) and particle (i.e., proton, neutron, and higher mass atoms) radiation (Dartnell et al., 2007; Dartnell, 2011). To interpret the molecular and isotopic signals of organic material, we must understand how the preservation mechanisms observed on Earth operate to protect organic material in these high-radiation environments.

Similar to terrestrial environments, both intrinsic and extrinsic factors influence the preservation of organic material in space and planetary environments. Computational results

predict that radiolysis of larger organic molecules (< 300 amu) occurs at a faster rate than smaller molecules when exposed to γ and solar radiation, suggesting that in planetary environments chemical recalcitrance is primarily a function of molecular weight (Pavlov et al., 2012). Likely the best protection from ionizing radiation is physical shielding by minerals, as the energy of radiation dissipates as it moves through sediment. For example, the penetration depths of UV, solar, and γ radiation in Martian sediments is limited to 2-3 mm, 5 cm, and 2 m, respectively (Cockell and Raven, 2004; Dartnell et al., 2007; Pavlov et al., 2012). However, ionizing radiation can interact with chemical species present on the surface or in the atmosphere to form radical species and chemical oxidants that can potentially diffuse to greater depths and degrade subsurface organic material (Zent and McKay, 1994; Yen et al., 2000; Quinn et al., 2013). Organic mineral interactions can potentially protect organic material from ionizing radiation and chemical oxidants, as evidenced by the persistence of mineral-bound organic material relative to free organics when exposed to UV and γ radiation in laboratory settings (Ertem et al., 2017; Fornaro et al., 2018). However, in some cases, minerals can act to catalyze radiolysis reactions, increasing organic material degradation (dos Santos et al., 2016).

1.5 Research Questions and Organization of Dissertation

The chapters in this dissertation encompass my research at the Pennsylvania State University, the University of Nantes, and NASA Goddard Space Flight Center from 2016 to 2020. The overarching goal of this research is to understand how primary isotopic and molecular signatures could be altered by post-depositional processes in different environments. My dissertation focuses on the following research questions:

Chapter 2: Exogenous and possibly endogenous macromolecular organic material present on the surface of Mars interacts with UV, solar and γ radiation to decompose into CO_2 or

form secondary radiolysis products. UV-driven organic radiolysis has been extensively studied, but reactions driven by high energy radiation have not been described. This knowledge gap impedes interpretation of organic compounds buried at depth on Mars that are vulnerable to γ radiation but shielded from UV and solar radiation. *What secondary products are formed by interactions with γ radiation? Do the composition of secondary products depend on the starting organic material or mineral matrix? Do certain minerals protect organic material from γ radiolysis better than others?*

Chapter 3: Interactions between clay surfaces and non-polar organic material are crucial for their preservation. While sorption between non-polar organics and mineral surfaces involves relative weak intermolecular forces, computational modeling suggests these interactions could impart a measurable H isotope effect. Quantifying sorption-driven isotope effects is crucial for interpreting primary isotope signals of organic material preserved in the geologic record. *What is the magnitude of isotope effects for sorption processes? What intermolecular forces drive sorption isotope effects? Can traditional density functional theory tools predict the direction and magnitude of sorption-driven isotope effects?*

Chapter 4: Sorption to mineral surfaces is a key process that protects amino acids from oxidation and aids their polymerization into more complex biomolecules. Both the strength of the intermolecular interaction and the orientation of an amino acid on a mineral surface influence its preservation and efficiency of polymerization. Understanding the factors that control this interaction are vital to understanding how organic-mineral interactions played a role in the evolution of life on early Earth and elsewhere. *Do the distribution of isotopomers between free and sorbed phases reflect the strength of organic-mineral interactions? Can position-specific isotope analysis describe the orientation of an amino acid on surface? What factors control the orientation of amino acid on a mineral surface?*

1.6 Anticipated Publications

Each chapter in this dissertation is intended to be a publishable unit of research.

Anticipated publications include:

Chapter 2: “Radiolysis of Macromolecular Organic Material in Mars-Relevant Mineral Matrices”

was published in 2019 in the *Journal of Geophysical Research: Planets* with co-authors

J. Eigenbrode and K. H. Freeman

Chapter 3: “Density Functional Theory Predictions of Non-covalent Hydrogen Isotope Effects

During Octane Sorption to a Kaolinite Surface” will be submitted to *ACS Earth and*

Space Chemistry with co-authors J. D. Boettger, J. D. Kubicki, and K. H. Freeman.

Chapter 4: “Position-Specific Isotope Fractionation in Amino Acids Sorbed to Ice: Implications

for Amino Acid-Mineral Interactions” will be submitted to the *Proceedings of National*

Academies of Sciences with co-authors E. Martineau, G. Remaud and K. H. Freeman.

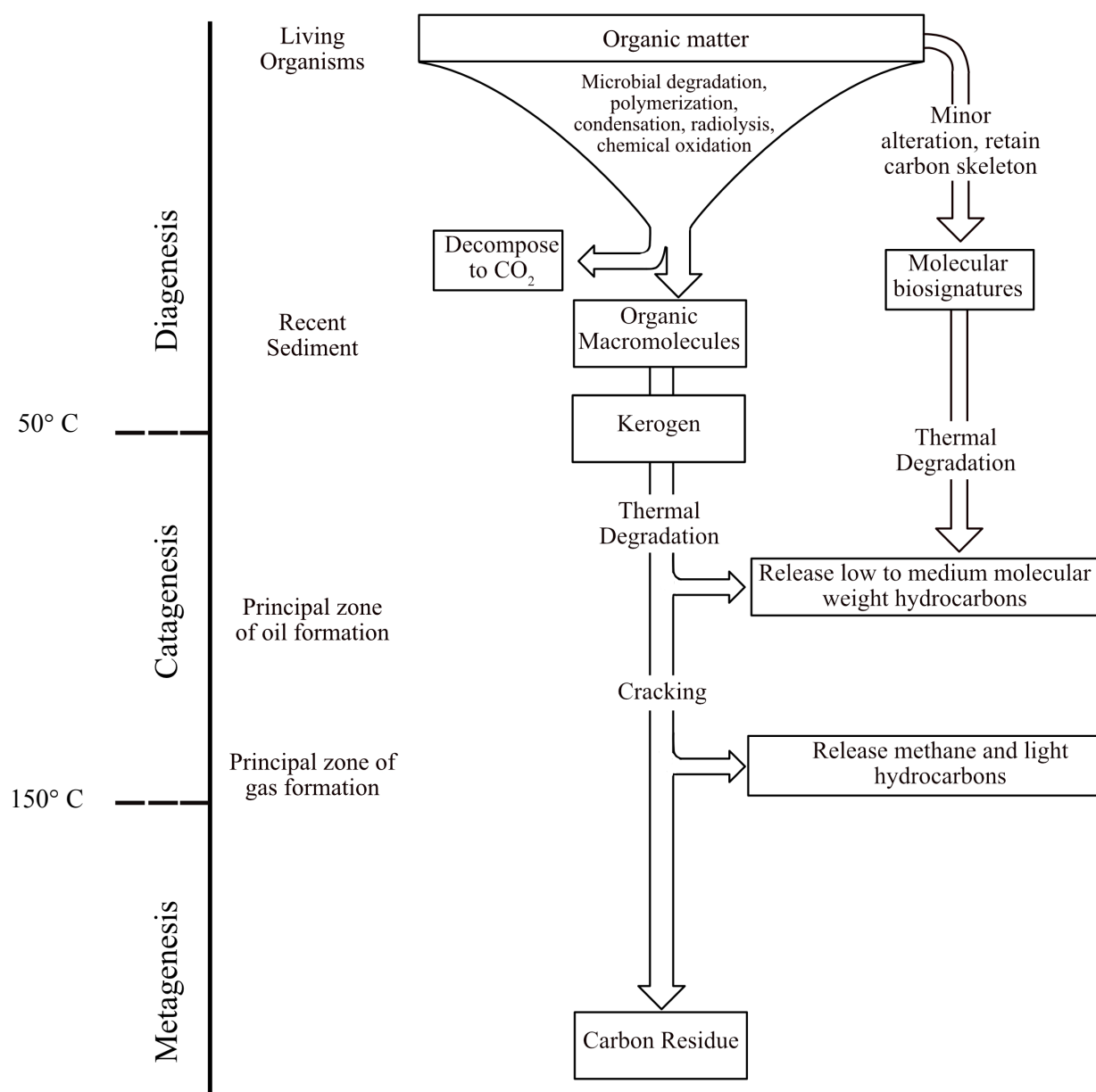


Figure 1-1: Degradation and alteration processes during diagenesis, catagenesis and metagenesis. Split arrows indicate compounds that are released from degradation processes.

This figure was adapted from Rullkötter (1993).

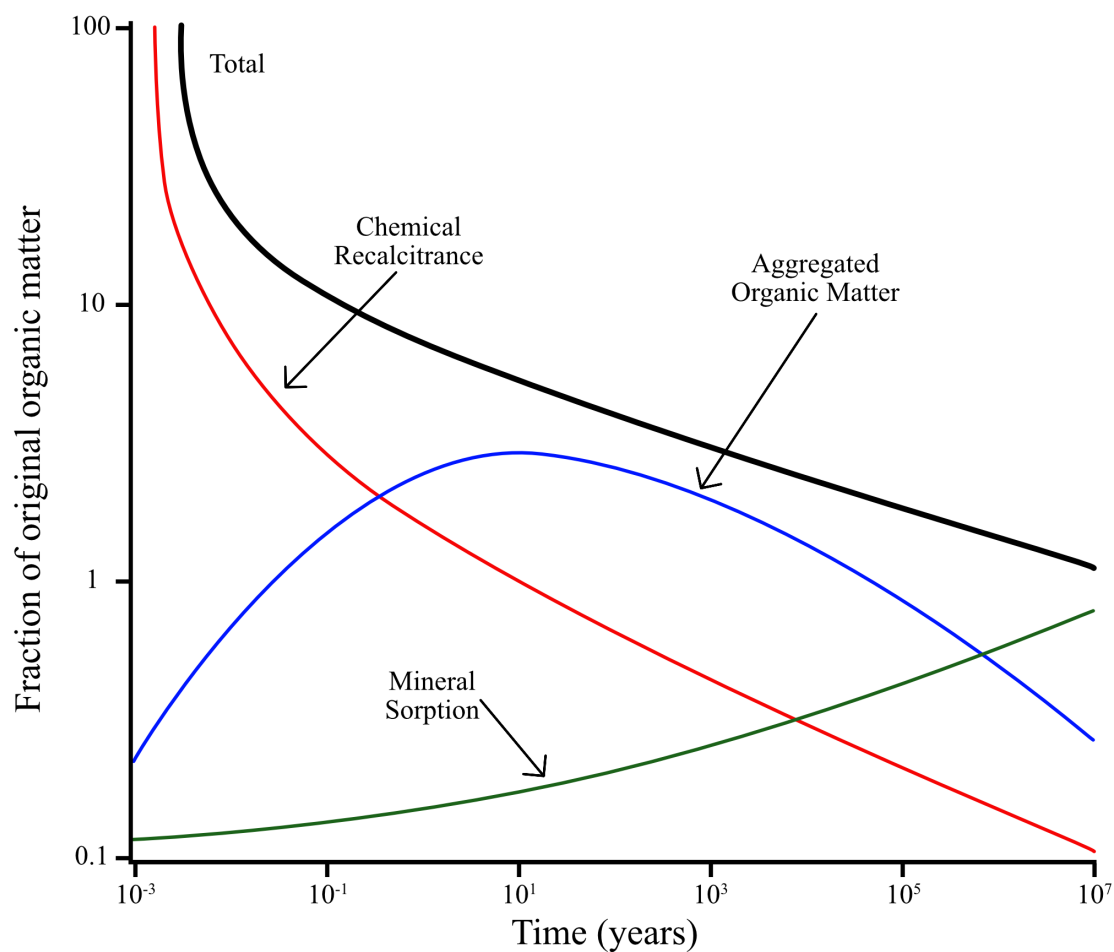


Figure 1-2: Idealized diagram displaying the temporal importance of various protective mechanisms for organic material in sediments. Selective preservation of chemically recalcitrant molecules is initially important, but over time processes such as aggregation and mineral sorption become the dominant driver of preservation.

This figure was adapted from Keil and Mayer (2013).

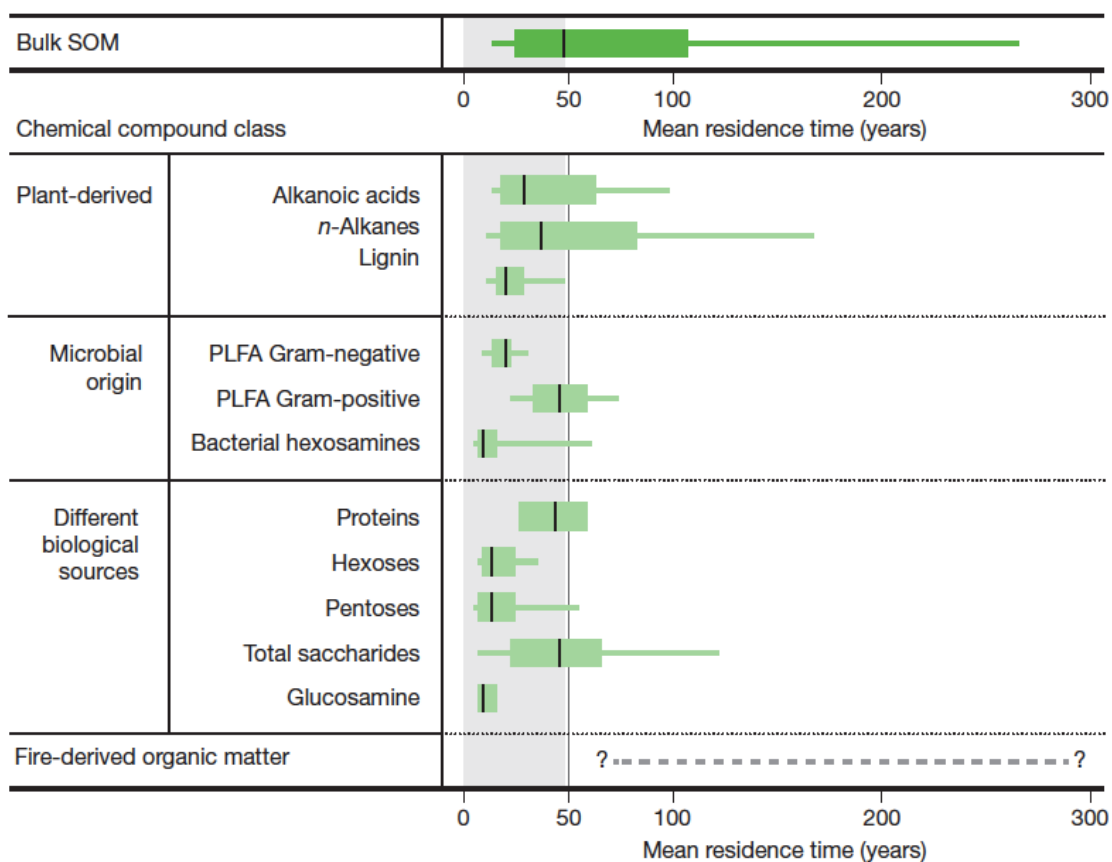


Figure 1-3: Classically recalcitrant, plant-derived molecules appear to have similar turnover times to the chemically labile proteins and saccharides. Most compounds, regardless of origin or molecular structure, have similar turnover times to bulk soil organic material indicating chemical recalcitrance does not influence long-term preservation.

Figure from Schmidt *et al.* (2011).

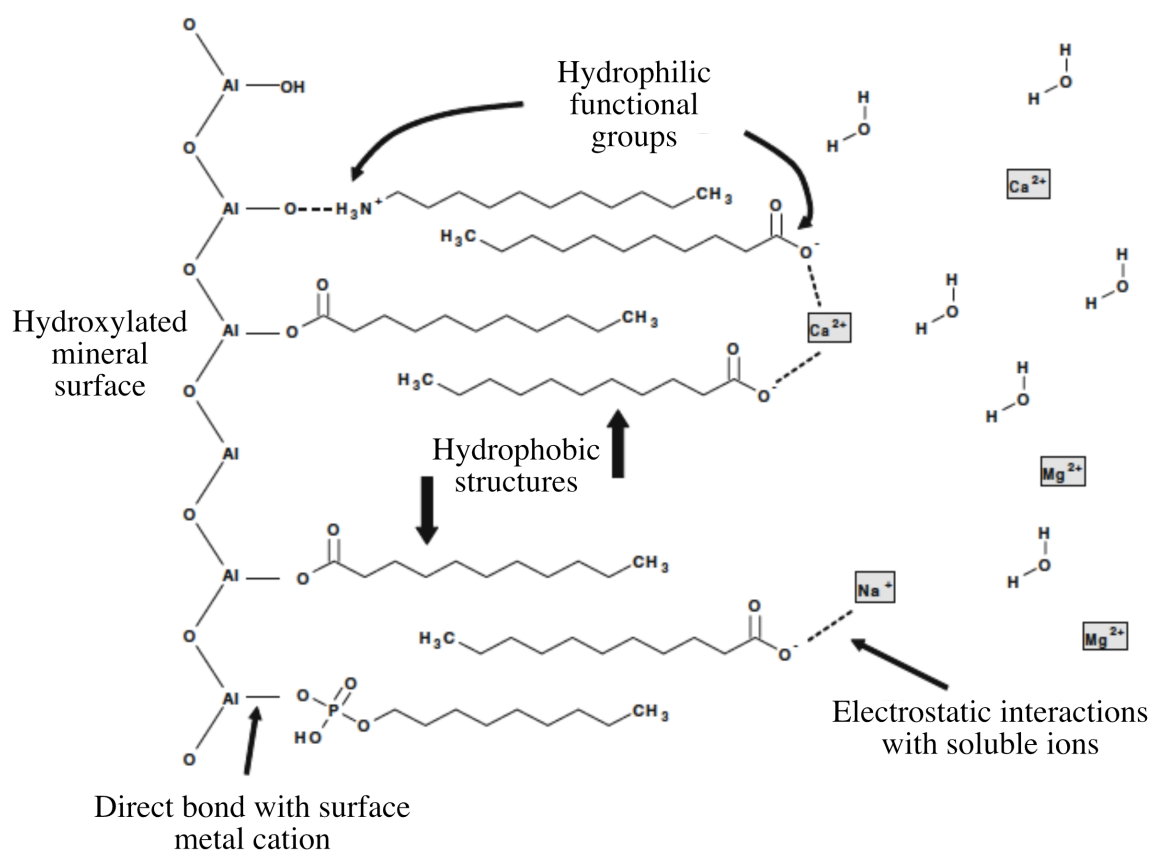


Figure 1-4: Conceptual model of the organic bilayer formed on mineral surfaces. Polar (hydrophilic) portions of amphiphilic molecules interact with a hydroxylated mineral surface via direct bonds, electrostatic interactions or hydrogen bonding. The non-polar (hydrophobic) portions of the surface molecules are shielded by another layer of amphiphilic molecules, forming the bilayer.

This figure was adapted from Kleber et al. (2007).

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Chapter 2

Radiolysis of Macromolecular Organic Material in Mars-Relevant Mineral Matrices

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2.1 Abstract

The fate of organic material on Mars after deposition is crucial to interpreting the source of these molecules. Previous work has addressed how various organic compounds at millimeter depths in sediments respond to ultraviolet radiation. In contrast, this study addressed how high-energy particle radiation (200-MeV protons, simulating the effect of galactic cosmic rays and solar wind at depths of $< 4\text{--}5$ cm) influences organic macromolecules in sediments. Specifically, we report the generation of organic-acid radiolysis products after exposure to radiation doses equivalent to geological time scales (1–7 Myr). We found that formate and oxalate were produced from a variety of organic starting materials and mineral matrices. Unlike ultraviolet-driven reactions that can invoke Fenton chemistry to produce organic acids, our work suggests that irradiation of semiconductor surfaces, such as TiO_2 or possible clay minerals found on Mars, forms oxygen and hydroxyl radical species, which can break down macromolecules into organic acids. We also investigated the metastability of benzoate in multiple mineral matrices. Benzoate was added to samples prior to irradiation and persisted up to 500 kGys of exposure. Our findings suggest that organic acids are likely a major component of organic material buried at depth on Mars.

2.2 Introduction

2.2.1 Organic Material on Mars

The preservation and detection of organic material on Mars is critical to our understanding of Mars' ability to host life throughout time. Chemical signatures encoded in organic material provide information on ancient ecologies if life existed in the past and, in the absence of life, provide clues about the environmental and thermal history of Mars. Detecting organic material is a central goal of the Mars Exploration Program (MEPAG, 2015), but this goal is complicated by evidence for oxidizing Martian soils (Oyama and Berdahl, 1977; Zent and McKay, 1994). Various oxidizing species have been proposed to explain the high soil reactivity, including superoxide ions (Yen et al., 2000), hydroxyl and oxygen radicals (Benner et al., 2000), and perchlorate intermediates (Quinn et al., 2013; Carrier and Kounaves, 2015). These reactive species likely form by interactions with ionizing radiation. The lack of a magnetic field and thin atmosphere exposes the Martian surface to high doses of ionizing electromagnetic (e.g., ultraviolet [UV] and γ) and particle (i.e., proton, neutron, and higher mass atoms) radiation that can oxidize organic matter directly or through reactions with secondary oxidants (Patel et al., 2002; Hassler et al., 2014).

Despite a hostile environment, there is evidence for organic material at several locations on Mars. The Sample Analysis at Mars instrument onboard the Mars Science Laboratory (MSL) Curiosity Rover detected chlorinated hydrocarbons indigenous to the ~3.5-Ga lake sediments at Yellowknife Bay, Gale crater (Freissinet et al., 2015). Other aliphatic, aromatic, and sulfur-bearing hydrocarbons that were detected in lake sediments studied at Pahrump Hills are thought to be derived from larger macromolecular structures that aided their preservation (Eigenbrode et al., 2018). Other potential indications of indigenous organic matter are indirect and are observed

as thermal evolution of CO₂ and CO observed during Sample Analysis at Mars' evolved gas analysis and are in part attributed to decarboxylation and decarbonylation of organics in samples from diverse rocks and aeolian sediments in Gale crater (Ming et al., 2014; Sutter et al., 2016), which may also explain CO₂ observed by the Viking GCMS (Biemann et al., 1976).

Chloromethanes (Navarro-González et al., 2010) and chlorobenzene (Guzman et al., 2018) detected in Viking data may also be Martian carbon signals, but terrestrial sources have not been definitively excluded. Additionally, reduced carbon phases including graphite, polycyclic aromatic hydrocarbons, and macromolecular phases with diverse synthesis mechanisms have been detected in Martian meteorites (Steele et al., 2016). These observations together indicate that Martian organic matter is present in different preservation states but the sources and alteration processes affecting these organics are not well constrained.

2.2.2 Alteration of Organic Material by Ionizing Radiation

Unlike Earth, Mars lacks a magnetic field and is therefore bombarded by ionizing radiation that can alter organics. Previous work has employed a range of UV, γ rays, and X-rays to study the effect of ionizing radiation on organic material. Of these, the majority of studies concerning organic matter preserved on Mars have focused on the effect of UV radiation. Studies on the survivability of amino acids (ten Kate et al., 2005; Stalport et al., 2009; Poch et al., 2013), carboxylic acids (Stalport et al., 2009), PAHs, and urea (Poch et al., 2014) exposed to varying doses of UV radiation revealed that long-term exposure to UV radiation (< 10 years on Martian surface) completely degraded organic material. Stalport et al. (2009) also found that exposure to UV led to the formation of metastable compounds that survived on longer time scales than the initial compounds.

Stalport and colleagues' work supports an idea originally introduced by Benner et al. (2000) to explain the lack of organics detected by the Viking missions. Benner et al. predicted that UV-driven, Fenton reactions of meteorite-delivered organics would produce organic acids as a metastable intermediate. Although traditionally an aqueous reaction, Fenton reactions degrade organic material by producing OH radicals from H₂O₂ in the presence of an iron catalyst. Hydroxyl radicals then react with organic material, likely via H abstraction, to form new degradation products (Pignatello et al., 2006). The predicted organic acids are largely nonvolatile and were likely invisible to the Viking gas chromatograph-mass spectrometer. Laboratory studies have shown that the rate of formation of organic acids outpaces their destruction by a factor of 10^3 , suggesting these molecules can persist on long time scales (Lamrini et al., 1998; Benner et al., 2000). Based on an influx of 2.4×10^8 g/year of reduced carbon and 1-m mixing depth, Benner et al. predicted organic acid concentrations of ≈ 500 ppm. While Benner et al.'s estimate did not account for processes that remove organic acids, to fall below Viking detection limits (approximately tens of ppb) over 99% of organic acids would have to be destroyed. Benner's hypothesis was further supported by more recent work that suggests solid oxalic acid is stable on the surface of Mars and may be present in reinterpreted Viking, Phoenix, and MSL data (Applin et al., 2015).

Benner *et al.* invokes Fenton chemistry that requires exposure to UV radiation (3–100 eV), which can only penetrate the first several millimeters of Martian soil (Cockell and Raven, 2004). In contrast to UV, other forms of ionizing radiation, such as galactic cosmic and solar rays, are predicted to penetrate up to 2 m of Martian soil (Pavlov et al., 2012; Hassler et al., 2014). Understanding how organic matter in rocks, regolith, and aeolian sediments is altered by ionizing radiation below the Martian surface (2–200 cm deep) is crucial to interpreting data from current and future missions. *In situ* samples analyzed by the MSL and Viking missions were collected at

depths of 5–10 cm, and the ExoMars rover will have the ability to drill up to 2 m (Oyama and Berdahl, 1977; Vago et al., 2006; Grotzinger et al., 2012).

At depths relevant to MSL (~5 cm), contributions from solar rays are minimal (Pavlov et al., 2012; Hassler et al., 2014). Galactic cosmic rays are primarily composed of high-energy protons (87%) that span energies from 10 to 1,000 MeV (Benton and Benton, 2001). Their high energy allows them to cleave bonds indiscriminately within the Martian soil profile creating a highly reactive environment. Studies investigating the effects of γ radiation on macromolecular organic material have found destruction of organic material via cross-linking, oxidation, and bond scission accompanied by increases in aromaticity of remaining organic material (Brown and Weiss, 2003; Court et al., 2006; Schäfer et al., 2009). Similar results were reported for nucleobases that were destroyed after exposure to γ radiation (Ertem et al., 2017). From these results, it is unclear if metastable species can form or persist below the Martian surface (2–200 cm) or if organic material would be completely destroyed.

Here, we report metastable products formed from radiolysis of macromolecular organic species exposed to high-energy protons (200 MeV). We irradiated mixtures of recalcitrant organic material and Mars-relevant minerals for cumulative doses up to 500 kGy, representing ~6.6 million years at the surface of Mars. The focus of this study was to determine if organic acids are produced due to interactions with high-energy protons. Using these data, we aim to determine the likely metastable products below the Martian surface and constrain how long these products persist in different mineral matrices.

2.3 Materials and Methods

2.3.1 Samples

Mineral matrices included fused silica (SiO_2), CaSO_4 , or a synthetic analog mixture. The analog included 50% olivine sand ($(\text{Fe}, \text{Mg})_2\text{SiO}_4$) and 50% nontronite ($\text{Na}_{0.3}\text{Fe}_2(\text{Si}, \text{Al})_4\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O}$, Nau-1; Clay Mineral Society). The olivine sand was acid washed, rinsed profusely to remove the acid, and dried. Organics in the sand and fused silica were ashed (i.e., combusted in air at 550 °C for 8 hr). The sample was then powdered in an ashed stainless steel ball mill and sieved to $< 250 \mu\text{m}$. The nontronite was pre-extracted with a series of solvents to remove preexisting organic matter. A subset of samples contained 1 wt% of inorganic salts, either CaSO_4 , CaCl_2O_8 , or NaCl . Minerals were chosen based on observations from the Mars Reconnaissance Orbiter CRISM instrument (Mustard et al., 2008).

Organic material included either 5 wt% of an Archean kerogen isolate described in detail in Eigenbrode and Freeman (2006), Pony Lake fulvic acid, or 50 ppm of a standard solution referred to as organic mix. The organic mix consisted of 50 ppm of pristene, phytane, sterane, *n*-heptadecane, stigmasterol, pyrene, phenanthrene, C14:0 fatty acid, C18:1 fatty acid, L -alanine, L -serine, glycine, mellitic acid, benzoic acid, and adipic acid. These organic materials were chosen to represent different types and different chemical states of sedimentary organic material common in the terrestrial geologic record. All solid materials including any solid organics were mixed at low speed in a ball mill. In some cases, water or organic solvents were used to evenly distribute organic material in the mineral matrix. Homogenization was achieved by mixing samples in a rotary evaporator while drying the samples at 50 °C, 10 mbar.

Organic removal from minerals was not complete, and some molecules were detected in control samples that did not have added organic material. Specifically, formate and oxalate were

detected in the synthetic analog and fused silica control samples, and benzoate was detected in fused silica control samples. Nonirradiated control samples had concentrations near detection limits (~ 100 ppb), and irradiated control samples had significantly higher concentrations. Deionized water samples run during extraction and analysis were free of these compounds, and this suggests the acids were present in the synthetic analog and fused silica matrices. The increase in background organic acid signal in control samples after irradiation indicates that the source material of the organic acids was altered by high-energy proton exposure (Fig. 2-1 and 2-2).

2.3.2 Radiation by High-Energy Protons

After mixing, samples were packed to 1.6 ± 0.1 g/cm³ density in an ashed quartz-glass cuvette, then purged under 99.999% Ar and covered with ashed aluminum foil held in place by Kapton tape with acrylic adhesive on the outside of the vessel. Samples were irradiated using 200 MeV protons at the Indiana University Cyclotron Facility. Proton energy was chosen based on the predicted energy profile for inbound protons to Mars (Heynderickx et al., 2004) and is in reasonable agreement with Radiation Assessment Detector (RAD) measurements on MSL (Ehresmann et al., 2014). Samples received a cumulative dose of either 0, 63, 125, 250, or 500 kGys. The RAD instrument measured the galactic cosmic ray dose rate at 76 mGy/year (Hassler et al., 2014), so these experiments represent up to $\sim 6.6 \times 10^6$ years on the Martian surface. This exposure age estimate assumes that the single 1.25-mm quartz glass cuvette wall that the proton beam penetrates has negligible effects on the exposure age estimate for the sample and that proton cumulative dose for the experiments is comparable to the long-term effects of the full spectrum of radiation that make up the dose rate monitored by RAD.

2.3.2 Organic Acid Extraction and IC-MS Analysis

Samples were extracted for low-molecular-weight organic acids and analyzed using ion chromatography mass spectrometry (IC-MS). One gram of each sample was mixed with 5 ml of deionized, ultrapure water (pH = 6.5) and agitated for 2 hr. The liquid extract was separated from the sample by centrifugation for 15 min at 5,500 RPM. This procedure was repeated with 95 °C DI water, to remove larger organic acids, for a total extraction volume of 10 mL. Previous work reports between 85% and 95% recovery for the reported organic acids extracted from clayey-loamey soils (Mimmo et al., 2008). The extracts were concentrated to 5 ml and run on a Dionex ICS-2100 MSQ+. Column choice, eluent concentration, and MSQ parameters were chosen to detect acetate, formate, benzoate, oxalate, phthalate, and citrate, which represent a range of common organic acids and products predicted by Benner et al. (2000). Compounds were separated on a 2 mm AS11 guard and analytical column with a multistep gradient of KOH (0.5 mM 0–6 min, 5 mM 6–18 min, and 38.3 mM 18–22 min). Control samples without added organic material were run on a Dionex ICS-5000+ Q Exactive with the same analytical column and eluent concentration gradient. No organic acids were detected in calcium sulfate-containing samples. This is likely the result of the extraction method, where hydration of calcium sulfate produced sulfuric acid, which oxidized organic material. Therefore, calcium sulfate results are not reported.

Positive identification of target compounds met three criteria. First, peaks eluted ± 1 min of the average retention time for the given organic acid standard. IC-MS analysis produced both an ion chromatogram and a mass spectrum. For the second criterion, there must be a peak in both the ion chromatogram and mass spectra of the target mass. Third, the ratio of peak areas between the ion chromatogram and mass spectra had to be in the same range as the standards. Due to mass interferences from silica at $m/z = 60$, acetate could not be identified and therefore is not reported. IC-MS detection limits for the target compounds was 100 ppb. Each analysis set was bracketed

by standards. The values for formate and benzoate standards fell within $\pm 20\%$ for each analysis set. Uncertainty for each sample was determined by the standard deviation of two runs of and fell within $\pm 20\%$ for formate and benzoate.

2.4 Results

Of the target compounds, only formate and oxalate were detected in irradiated samples. Concentrations of formate are reported in Fig. 2-1. Formate was detected in two samples, fused silica and organic mix and synthetic analog and kerogen. In the case of the fused silica and organic mix samples, a small amount of formate was detected in the nonirradiated control sample. However, irradiated samples showed an order of magnitude increase of formate, suggesting radiation led to additional formate production. There was no detection of formate in the synthetic analog and kerogen control sample, consistent with the breakdown of kerogen to formate. The detection of formate in irradiated control samples without added organic material suggests that this formate may be the product of an unknown contaminant, rather than kerogen or organic mix. However, formate was not detected in any other samples (i.e., fulvic acid, Green River shale) nor samples containing added additional inorganic salts, indicating these materials either promoted the destruction or inhibited the production of formate.

Due to coeluting peaks and low concentrations, oxalate abundance could not be reliably quantified, a presence/absence chart is given in Fig. 2-2. Oxalate was detected in all synthetic analog samples, including the nonirradiated control samples and a subset of samples with added inorganic salts. Therefore, it cannot be assumed that oxalate was produced from radiation-driven mechanisms. However, oxalate was not detected in the fused silica and kerogen nonirradiated control sample but was detected in the irradiated fused silica and kerogen samples. This indicates that oxalate can be produced by radiation-driven processes. It is possible that oxalate was

produced in other samples by radiation-driven processes, but subsequent work that can quantify oxalate in these samples is needed to test this theory.

Benzoate was detected in samples containing the organic mix, of which benzoate was a component. The addition of 10 ppm of benzoate prior to irradiation in these samples allows a comparison of different matrices to protect organic acids from radiolysis. Fig. 2-3 shows benzoate concentrations in analog and fused silica samples at different cumulative doses. Extraction of benzoate from the nonirradiated control samples shows that extraction is more efficient in fused silica, 77%, than synthetic analog samples, 58%. Fig. 2-4 shows the relationship between benzoate concentration and radiation dose. More benzoate persisted in fused silica samples compared to analog samples.

2.5 Discussion

The presence of formate and oxalate coupled with the loss of benzoate suggests that organic acids are simultaneously created and destroyed by processes driven by high-energy protons. Mechanisms of production and destruction rely on interactions between mineral surfaces and high-energy protons to produce radicals that can both break down larger organic matter and combine to form small organic molecules.

2.5.1 Organic Acid Formation

Organic acids were detected in irradiated samples that were not detected prior to exposure to high-energy protons. Therefore, interactions between macromolecular organics, the mineral matrix, and high-energy protons formed formate and oxalate. Previous authors have reported similar results. Mao *et al.* (1991) reported organic acids were produced from irradiation

of chloroethanes on TiO₂ semiconductor surfaces. Formate and oxalate were produced when pyridine and quinoline were irradiated with TiO₂ nanoparticles (Chu et al., 2018). To predict where organic acid production is likely on Mars, we must understand the mechanism. Studies of UV-driven organic acid formation invoked Fenton chemistry as the dominant mechanism. Generally, Fenton reactions occur when photons interact with iron (or other redox sensitive elements) and hydroxyl species to form hydroxyl and oxygen radicals (Pignatello et al., 2006). This reaction is unlikely in fused silica samples because they lack mentionable iron. The only possible iron source would be from powdering in a stainless-steel ball mill. Any iron contribution would likely be small and should not contribute significantly, suggesting Fenton reactions are not the primary driver of radical production in fused silica samples.

Alternatively, organic acid formation could be driven by the irradiation of semiconductor surfaces. Prior work has observed that silica particles exposed to γ radiation produced hydrogen atoms, which are attributed to radiolytic homolysis of hydroxyl impurities on the silica surface via Reaction 2-1.



In reaction 2-1, high-energy particles excite electrons to higher energy levels in the conduction band, leaving behind positive “holes” in the valence band. The decoupled electrons and holes get trapped at surface defects. Adsorbed species can then either scavenge trapped electrons or donate an electron to a hole (Zhang et al., 1997). Interactions with trapped electrons or holes result in either radical formation or oxidation (Zacheis et al., 1999; Thomas, 2005).

We propose a similar mechanism for formate and oxalate production in fused silica samples, illustrated in Fig. 2-5. High-energy protons excite electrons in the valence band of the silica surface splitting an electron-hole pair. The electron (e⁻) and positive hole (h⁺) become trapped at surface defects in semiconductors. On Mars, possible semiconductors include oxide

minerals such as TiO_2 or Al_2O_3 (Baird et al., 1976) and clay minerals (Bish et al., 2013).

Hydroxyl and oxygen species present in the Martian soil due to the radiation driven breakdown of hydrated clays (Vaniman et al., 2014), oxide minerals (Gebicki and Gebicki, 1993), or salts such as perchlorate (Hecht et al., 2009; Quinn et al., 2013) can form radicals by interacting with trapped species, which then break down macromolecular organics to produce organic acids. The diversity of starting organic materials both in previous studies and reported in this study suggest that many oxidation reactions can lead to organic acid production.

Synthetic analog samples contain iron-bearing minerals. The presence of redox-sensitive elements can facilitate Fenton reactions, which may be the dominant mechanism for organic acid production in the analog samples. However, semiconductor surface interactions may also be present because nontronite can behave as a semiconductor. Clay minerals have a smaller band gap between their valence and conduction band, allowing electrons to be excited to the surface more easily. As a result, nontronite is potentially a more effective semiconductor surface than fused silica that yields increased radicals relative to fused silica (Thomas, 2005). It is not possible to determine which mechanism, either Fenton reactions or irradiation of semiconductor surfaces, is primarily responsible for organic acid production in synthetic analog samples from this data set. Future work could determine the role of surface-mediated reactions by measuring changes in organic acid production with nontronite surface area.

Alternatively, radicals can be produced by the breakdown of oxides exposed to ionizing electromagnetic radiation. Gamma radiation can generate reactive oxygen species that can break down proteins in aqueous solutions (Gebicki and Gebicki, 1993) and is likely responsible for perchlorate production in chlorinated ices or aqueous oxide solutions (Schuttlefield et al., 2011; Kim et al., 2013). While we cannot rule out this mechanism contributing to radical production in fused silica and analog samples, previous work has shown that radical production on SiO_2 is a

function of particle's surface area. The dependence on surface area suggests radicals are produced by surface-mediated reactions rather than the breakdown of SiO_2 (Zhang et al., 1997).

2.5.2 Metastability of Organic Acids in Different Matrices

Benner et al. (2000) suggested the rate of organic acid formation was significantly faster than their rate of destruction by UV radiation, allowing them to exist on the surface of Mars. It is unclear if similar formation and destruction rates can be expected for organic acid synthesis driven by high-energy protons. This work provides a direct test of benzoate's survival to bombardment with high-energy protons for a range of radiation doses.

In analog and fused silica matrices, exposure to radiation caused benzoate to be lost (Fig. 2-3), potentially degraded to CO_2 , or incorporated into new organic phases. Benzoate decreased linearly ($R^2 = 0.93$) with increased radiation exposure in fused silica samples. This is consistent with increased radical production at higher cumulative radiation dose, which led to higher benzoate loss (Fig. 2-4). Analog samples do not show a strong linear relationship to radiation dose. The reason for the lack of trend in these data is unclear. Possible explanations include (1) multiple formation and destruction mechanisms competed (i.e., Fenton chemistry and surface reactions), (2) differences in the surface area of nontronite grains caused inconsistent production, or (3) experimental uncertainty masked a trend.

Fused silica samples had higher benzoate concentrations at all radiation doses. This could be the result of a less-efficient extraction of benzoate in synthetic analog samples, evidenced by the poor recovery in the nonirradiated synthetic analog and organic mix sample. Extraction in the fused silica and organic mix control sample had a recovery of 77%, consistent with previous reporting. Alternatively, the higher benzoate concentrations and lower formate concentrations could be due to fewer radicals produced in fused silica samples. These observations further

support that analog samples experienced both Fenton reactions and semiconductor surfaces interactions to produce radicals. As a result, both rates of production and destruction of organic acids are increased in synthetic analog samples.

Despite enhanced losses, benzoate persisted in most matrices and radiation doses. Measurements from the RAD on the MSL Curiosity rover show that 500 kGys is roughly equivalent to 6.6 Myrs at the Martian surface (Hassler et al., 2014). Future work should further investigate the relative rates of the production and destruction of organic acids demonstrated here in order to determine if organic acids are likely metastable products beneath the Martian surface that have been exposed to high-energy protons for millions of years.

2.6 Conclusions

We found bombardment of macromolecular organics with high-energy protons common to the Martian cosmic ray spectrum produced organic acids in fused silica and analog matrices. Our results are consistent with previous UV radiolysis studies that found organic acids to be a major radiolysis product. We proposed an alternate mechanism of formation for radical production that does not invoke Fenton chemistry and therefore does not require redox sensitive elements. Taken together, these studies indicate that organic acids are likely a major component of present-day Martian organics at and below the surface. Furthermore, we demonstrated that organic acid products were not dependent on starting material or mineral matrix, suggesting they cannot record information about the source organic material. Based on this work, samples will have to be taken at depths that are not exposed to high-energy protons in order to learn more about the Martian organic sedimentary record that are not altered by ionizing radiation.

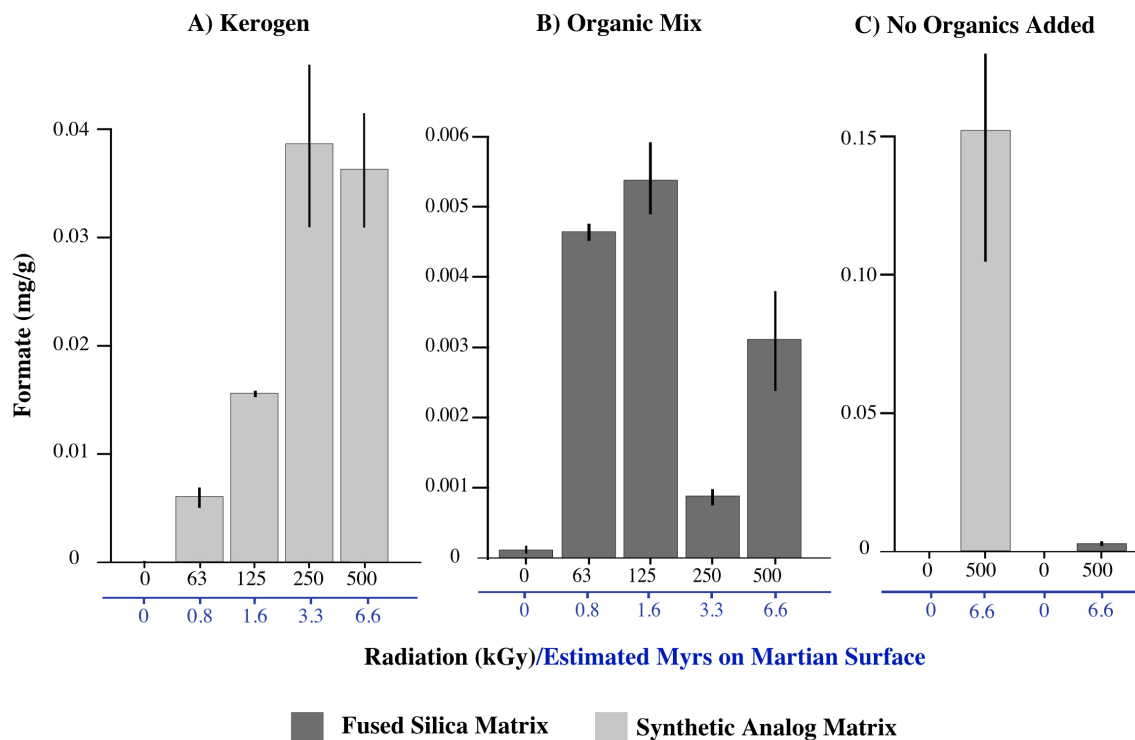


Figure 2-1: Formate concentration in all sample combinations where it was detected: (A) synthetic analog and Archean kerogen isolate, (B) fused silica and organic mix, and (C) control samples for synthetic analog and fused silica.

A) Fused Silica		Radiation (kGy)				
Organic Material		0	63	125	250	500
	Control Samples	—				✓
	Organic Mix	✓	✓	✓	✓	✓
	Fulvic Acid	✓	✓	✓	✓	✓
	Kerogen	—	✓		✓	✓

B) Synthetic Analog		Radiation (kGy)				
Organic Material		0	63	125	250	500
	Control Samples	✓				✓
	Organic Mix	✓	✓	✓	✓	✓
	Fulvic Acid	✓	✓	✓	✓	✓
	Kerogen	✓	✓	✓	✓	✓

Figure 2-2: Oxalate presence and absence charts for (A) fused silica and (B) synthetic analog samples. Check marks indicate positive detection, minus symbols indicate no detection, and gray boxes indicate those conditions were not tested.

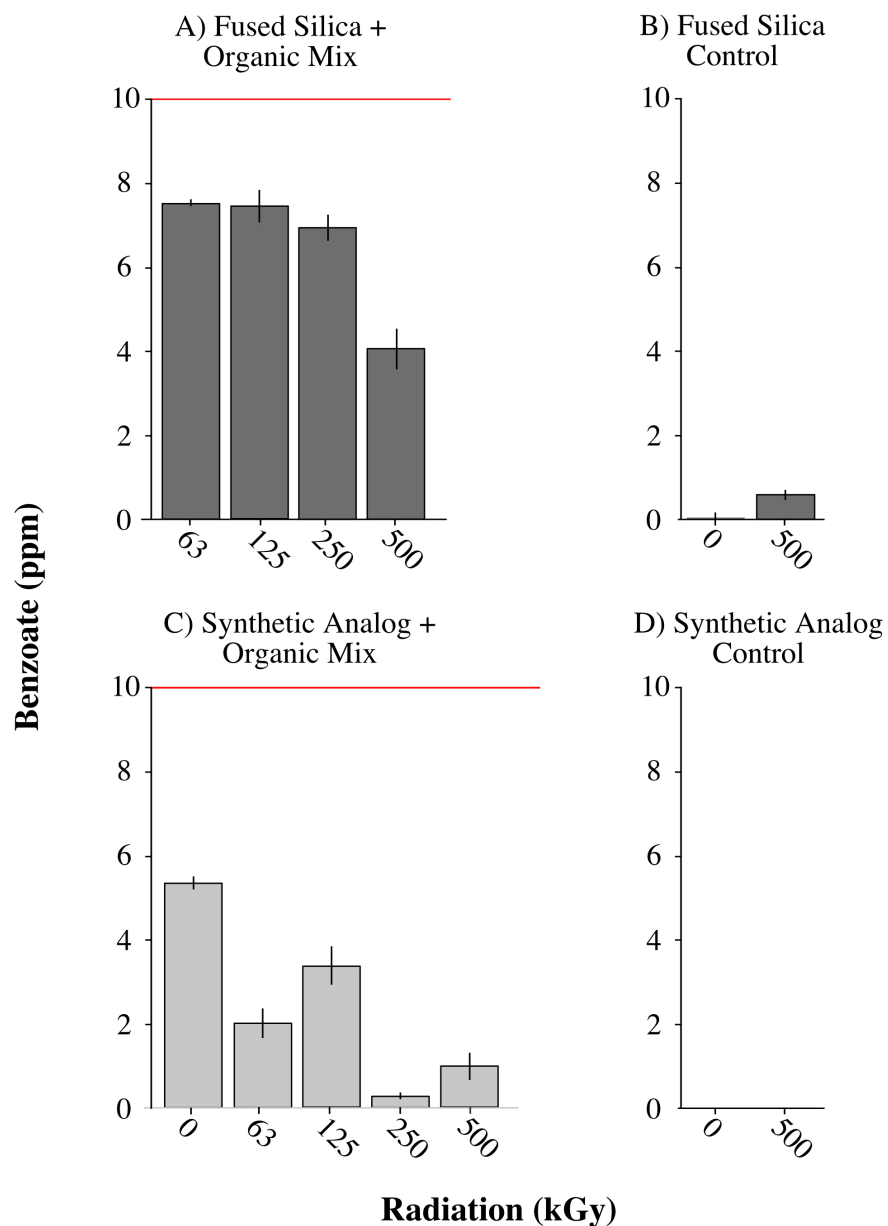


Figure 2-3: Benzoate concentration in (A) fused silica and organic mix, (B) fused silica control, (C) synthetic analog and organic mix, and (D) synthetic analog control. Red lines on panels a and c indicate the starting concentration of benzoate prior to irradiation. Control samples did not have any benzoate added prior to irradiation; no benzoate was detected in the synthetic analog control. Error bars show the standard deviation of two analyses.

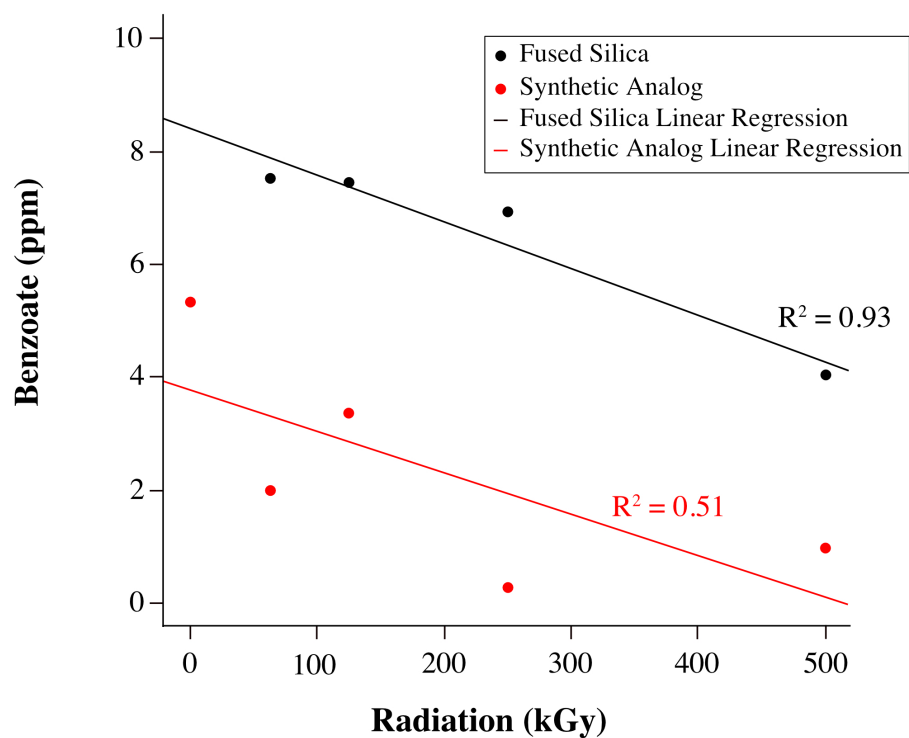


Figure 2-4: Benzoate concentration as a function of radiation dose for organic mix samples. Fused silica and organic mix samples are shown in black, and synthetic analog and organic mix samples are shown in red. Linear regressions were performed for both matrices, and R² values are displayed on the chart.

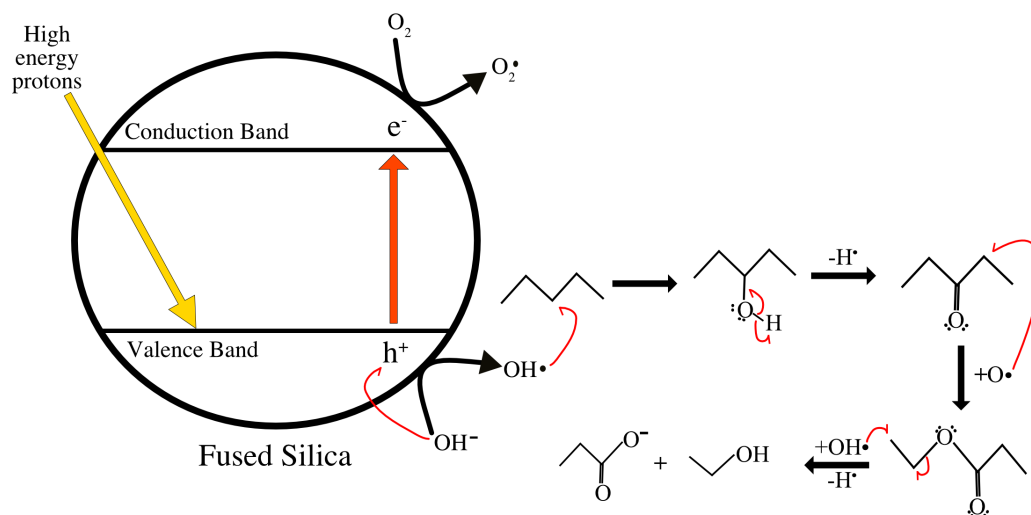


Figure 2-5: Proposed formation mechanism for organic acids in samples containing fused silica. High-energy protons excite valence band electrons to separate electron-hole pairs. These electrons and positive holes (h^+) get trapped at surface defects and can interact with oxygen and hydroxyl species to form radicals. Radicals can then form organic acids via a general oxidative pathway, shown here for pentane interacting with a hydroxyl radical.

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Chapter 3

Density Functional Theory Predictions of Non-covalent Hydrogen Isotope Effects During Octane Sorption to a Kaolinite Surface

3.1 Abstract

Interactions with mineral surfaces are crucial to the short- and long-term survival of organic compounds in the natural environment. The weak interactions that drive sorption of organic phases to mineral surfaces have the potential to impart H isotope effects that could complicate the interpretation of $^2\text{H}/^1\text{H}$ signatures in modern and ancient organic material. The influence of hydrophobic interactions on H-isotope signatures of *n*-octane during sorption to a kaolinite surface were studied using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). These experimental results were compared to both harmonic and anharmonic vibrational frequencies calculated with density functional theory (DFT) to determine the role of anharmonicity in predicting isotope effects during non-covalent interactions of the molecule and mineral surface. Experimental results showed a relatively minor ($< 2 \text{ ‰}$) ^2H depletion of the sorbed octane, indicating that hydrophobic interactions do not impart significant isotope effects during sorption. Comparisons of experimental and computational results highlighted the importance of anharmonic contributions to the direction of non-covalent isotope effects. Calculations that incorporated anharmonicity predicted a 3.5 ‰ depletion of sorbed octane, whereas purely harmonic calculations predicted a 0.9 ‰ enrichment. Differences in C- ^1H and C- ^2H bond lengths influence the molecular polarizability, surface area, and molar volume. The longer C- ^1H bonds are more hydrophobic and interact with the kaolinite surface more strongly, leading to a slight ^2H depletion in sorbed octane. Harmonic approximations cannot account for

differences in bond length, and are insufficient for predicting non-covalent isotope effects caused by hydrophobic intermolecular interactions.

3.2 Introduction

Stable H isotope compositions of organic compounds are sensitive indicators of molecular origins, environmental history, and fates of modern and ancient organic matter. In modern applications, $\delta^2\text{H}$ of organic pollutants are used to determine the parties liable for contamination and monitor the chemical and biochemical transformation of the compound in the environment (Mancini et al., 2002; Elsner et al., 2005; Thullner et al., 2012). Similarly, $\delta^2\text{H}$ of ancient organic material records information about the source of organic biomarkers (i.e., paleoecology) and past environmental conditions, such as paleoclimate and paleoaltimetry (Estep and Hoering, 1980; Smith et al., 1983; Schimmelmann and DeNiro, 1984; Sessions et al., 1999; Sauer et al., 2001; Polissar et al., 2009; Polissar and Freeman, 2010; Tipple et al., 2015). Interpreting $\delta^2\text{H}$ signatures of modern and ancient organic material assumes that other physical processes that occur during the preservation and migration do not significantly alter isotopic composition.

A particularly important physical process for both modern and ancient organic matter is sorption to mineral surfaces. Although specific interactions between individual organic molecules and mineral surfaces depend on the chemical properties of each, most organic pollutants and biomarkers are relatively hydrophobic. As a result, they interact with mineral surfaces through weak intermolecular forces, including van der Waals and hydrophobic interactions (Lützow et al., 2006; Moyo et al., 2014). Although relatively weak, these interactions are a driving force behind the preservation of organic material on short, i.e., days to weeks, and geologic, i.e., thousands to millions of years, timescales (Schmidt et al., 2011; Keil and Mayer, 2013).

Theoretically, even weak intermolecular interactions between hydrophobic organics and mineral surfaces can cause $^2\text{H}/^1\text{H}$ fractionation that could complicate the interpretation of their isotopic signatures. Differences in the physical properties of C- ^1H and C- ^2H bonds affect a molecule's bond polarizability, its surface area, and its molar volume, and each can potentially influence the strength of noncovalent interactions (Wade, 1999). As a result, for a population of molecular isotopologues, a specific isotopic form potentially has a slightly different tendency to be preserved relative to others in natural environments. Direct evidence of these effects in geologically relevant systems are limited. Schüth *et al.* (2003) found small ($< 9\text{‰}$) H-isotope fractionations for halogenated hydrocarbons sorbed to coal-like solids but no consistent trend in direction. In contrast, Höhener and Yu (2012) found that hydrocarbons sorbed to soil matrices were depleted $\sim 9.2\text{‰}$ relative to free hydrocarbons. These studies suggest that sorption-driven isotope effects are minor. However, both sets of authors determined fractionation factors by comparing initial $\delta^2\text{H}$ values of the compound to the $\delta^2\text{H}$ of the compound present in the headspace of the water-solid interface (Schüth *et al.*, 2003; Höhener and Yu, 2012). Isotopic fractionation associated with liquid-to-vapor transitions are well documented for hydrophobic compounds (Jancso and Van Hook, 1974; Wang and Huang, 2001; Muhammad *et al.*, 2015), so it is unclear if the observed fractionations were due to sorption or vaporization.

Subsequent authors have circumvented this issue by directly measuring compounds sorbed to or partitioned into an organic phase. Imfeld *et al.* (2014) measured hydrogen isotope fractionation for benzene and toluene as a result of successive batch phase partitioning between water and a variety of organic solvents. They reported depletions between 6 and 20‰ for compounds partitioned into the organic phases. In one case, for benzene partitioning into 1-octanol, they reported an 88‰ depletion, but this value was not confirmed by later work (Kopinke *et al.*, 2017). Imfeld and coworkers also reported a 14‰ depletion in their control sample, comprised of only the aqueous phase, which they attributed to evaporation loss (Imfeld

et al., 2014). Alternatively, the compounds could have experienced sorption to the glass reaction vessel. The high hydrophobicity of the studied compounds is known to drive sorption to glass and Teflon surfaces (Ackerman and Hurtubise, 2000; Qian et al., 2011), which could mask isotopic fractionation caused by partitioning into the organic phase.

Although computational predictions of isotope fractionation during sorption are limited by the high computational costs of modeling complex organo-mineral interactions, available studies provide valuable insights. Wang et al. (2009) demonstrated the importance of field inductive effects that affect the polarization of bonds, which influence intramolecular $^2\text{H}/^1\text{H}$ equilibrium values of neighboring hydrogens in different compound classes, i.e., alcohols, carboxylic acids, etc. They found electron-donating or withdrawing groups changed intramolecular $\delta^2\text{H}$ values by as much as 40‰ (Wang et al., 2009). Their work highlights the importance of long-distance dispersion forces, which are relevant to weak intermolecular forces involved to sorption. However, these results are not strictly comparable to fractionation during sorption, as the model represented $^2\text{H}/^1\text{H}$ exchange, which does not occur during sorption. In a subsequent modelling study, benzene sorbed to a graphite surface was slightly enriched in ^2H (Pokora and Paneth, 2018). This finding is not supported by experimental results, which have consistently reported an inverse H isotope effect (i.e., ^2H depletion in the sorbed phase).

Discrepancies between models and experimental results could be a consequence of the contribution of anharmonicity, which is not included in common density functional theory (DFT) approaches. Previous work has noted that the large frequency shifts associated with H isotope substitutions contain large contributions from anharmonicity (Bigeleisen and Mayer, 1947; Liu et al., 2010). Further, modeling studies that incorporated anharmonic contributions have had better success in recreating experimental isotope effects (Marklanda and Berneb, 2012; Pinilla et al., 2014; Webb and Miller, 2014; Liu and Liu, 2016), suggesting that future modeling efforts cannot ignore anharmonicity.

Alternatively, inconsistencies between existing models and experiments could be due to the differences between experimental setups and modeled interactions. Most experiments measured fractionation of a molecule partitioned between an aqueous phase and an organic phase. In these experiments, isotope fractionation is potentially driven by the energy required to remove different isotopologues from the aqueous phase, which is influenced by differences in their molar volume (Kopinke et al., 2017). Further, experiments typically use various glass and Teflon reaction vessels that can strongly sorb the non-polar target compounds (Ackerman and Hurtubise, 2000). In contrast, modeling efforts often exclude an aqueous phase to minimize computational costs and do not consider sorption to other phases. As a result, both available experimental and computational studies may not fully describe the isotopic effects caused by sorption to a mineral surface.

This study addresses differences in weak intermolecular interactions between *n*-octane ($C_8^1H_{18}$) and perdeuterated *n*-octane ($C_8^2H_{18}$) with a kaolinite surface. Unlike previous work, the sorption interaction was quantified using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), which measures the vibrational frequencies of sorbed versus gaseous octane. By examining differences in DRIFTS spectra of protiated octane and deuterated octane on the surface, fractionation factors are solely due to molecule interactions with the kaolinite surface, and not the aqueous phase or reaction vessel. Bond energy changes during sorption determined from DRIFTS spectra were then compared to harmonic and anharmonic approximations of sorption-driven H-isotope effects in octane. Comparisons of these two different model results with observed data allowed us to determine the importance of anharmonicity in predicting H isotope effects during non-covalent interactions with the mineral surface.

3.3 Methods

3.2.1 Chemicals

Sorption experiments were performed with both protiated *n*-octane, referred to as octane- $^2\text{H}_0$ (Sigma Aldrich), and perdeuterated *n*-octane, referred to as octane- $^2\text{H}_{18}$ (Sigma Aldrich). Low-defect kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$) was obtained from the Clay Mineral Society (KGa-1b) and pre-extracted 3 times sequentially with methanol, dichloromethane, and hexane prior to sorption experiments. After extraction, kaolinite was dried at 50°C for 24 hours.

3.2.2 DRIFT Spectroscopy

Spectra were collected on a Vertex 70 spectrometer (Bruker Optics, Bellerica MA) equipped with a wide band mercury cadmium telluride detector. The sample was contained in a praying mantis accessory (Harrick Scientific, Pleasantville, NJ) fitted with a high temperature *in-situ* cell to allow DRIFTS measurements. Each spectrum was an average of 100 scans at 40 kHz scan velocity at a 6 cm^{-1} resolution over the range $4000\text{--}600\text{ cm}^{-1}$. The praying mantis accessory was constantly purged with N_2 . The *in-situ* cell where sorption occurred was continuously purged with ultrahigh-purity (UHP) N_2 with injector flow controller set at 140 mm. The sample was introduced by means of a gas chromatography (GC) inlet that was heated and maintained at 150°C.

3.2.3 Sorption Experiments

Spectra of ‘gaseous’ octane- $^2\text{H}_0$ and octane- $^2\text{H}_{18}$ were collected on powdered KBr to obtain vibrational frequencies of *n*-octane on a non-interacting surface. Sorbed octane- $^2\text{H}_0$ and

octane- $^2\text{H}_{18}$ were collected on powdered kaolinite. Prior to injection, the KBr and kaolinite were heated to 300°C and maintained at that temperature for 30 minutes to remove surface H_2O . Peaks associated with C-H vibrations decreased but were not completely eliminated during pre-baking of the kaolinite samples, indicating additional organics were removed by heating that were not removed during pre-extraction with organic solvents. The temperature was then returned to 40°C and allowed to equilibrate, reference spectra were collected throughout this process. During analyses, KBr or kaolinite were dosed with $1\ \mu\text{L}$ of either octane- $^2\text{H}_0$ and octane- $^2\text{H}_{18}$. Each octane isotopologue passed through the cell, where it was exposed to the KBr or kaolinite (40°C) for 30 min, during which time spectra were collected every 2 minutes.

3.2.4 Harmonic and Anharmonic Approximations of Octane-Kaolinite Sorption

For the harmonic approximation, GAUSSIAN 09 DFT energy-minimization calculations were performed for octane with and without a kaolinite (001) model cluster at 300 K (Fig. **3-1**). Models were initially energy minimized using the B3LYP/6-31G(d,p) level of theory and underwent subsequent energy-minimization calculations at the B3LYP/6-311G(d,p) level of theory to improve the precision of their calculated hydrophobic interaction energies. The second energy minimization step was performed using Tight optimization criteria (maximum/RMS atomic displacement change per step 0.00006/0.00004 Bohr, maximum/RMS force 0.000015/0.00001 Hartrees/Bohr or Hartrees/Radian), with an Ultrafine integration grid mesh. Harmonic frequencies were then obtained for both octane- $^2\text{H}_0$ and octane- $^2\text{H}_{18}$ isotopologues. Models reached a local energy minimum, as indicated by a lack of imaginary frequencies in the harmonic results.

Anharmonic calculations including vibrational-rotational coupling were performed on subsets of the model vibrations in GAUSSIAN using the freq=(Anharm,VibRot) keywords. These

keywords request a form of second-order vibrational perturbation theory for evaluation of force constants beyond quadratic terms (Barone and Minichino, 1995; Barone, 2005). For the gas-phase models, anharmonic calculations were performed on all vibrational modes, on the subset of 18 vibrational modes consisting of mostly C-H stretching motion, and on selected individual C-H stretching modes, for both octane-²H₀ and octane-²H₁₈. For the sorbed models, anharmonic calculations were performed only on selected individual C-H stretching modes. For the sake of comparison, the same selected C-H vibrational modes were also calculated using the harmonic approximation. Anharmonic calculations on the sorbed octane models were infeasible for the full set of all 381 modes, the subset of 78 modes consisting of mostly octane motion, and the subset of 18 modes consisting of mostly C-H stretching motion.

3.2.5 Definitions and Equations

Experimental and computational vibrational frequencies were collected for octane-²H₀, octane-²H₁₈, octane-²H₀ on kaolinite surface, and octane-²H₁₈ on a kaolinite surface. Harmonic frequencies can be used to calculate the partition functions needed to predict hydrogen-isotope fraction. The reduced partition function ratio (RPFR, or β) can be calculated for both sorbed and free octane by Eq. 3-1.

$$(Eq. 3-1) \quad \beta_{(free,sorbed)} = \left(\frac{Q_h}{Q_l} \right) = \prod \frac{u_h}{u_l} \left(\frac{e^{-\frac{u}{2}}}{1 - e^{-u}} \right)_h \left(\frac{1 - e^{-u}}{e^{-\frac{u}{2}}} \right)_l$$

where $u_{(h,l)} = hc\nu_{(h,l)}/kT$. The $\nu_{(h,l)}$ are the frequencies (usually in cm^{-1}) for the heavy (h, ²H) and light (l, ¹H) isotopes for free and sorbed octane. Isotopic fractionation between free and sorbed values can be described by the fractionation factor, α , which is related to β by Eq. 3-2.

$$(Eq. 3-2) \quad \alpha_{sorbed/free} = \frac{\beta_{sorbed}}{\beta_{free}}$$

In cases where $\alpha_{\text{sorbed/free}} > 1$, the sorbed phase is enriched in the heavy isotope. In the present work, $\alpha_{\text{sorbed/free}}$ describes the isotopic fractionation between octane isotopologues that have either only protium or only deuterium (i.e., octane- $^2\text{H}_0$ and octane- $^2\text{H}_{18}$). In natural environments where ^2H is rare, fractionation would likely occur between octane- $^2\text{H}_0$ and octane- $^2\text{H}_1$. For α values near 1, the translational formula given in Eq. 3-3 can be applied to predict fractionation factors at natural abundance (NA) (Höhener and Yu, 2012).

(Eq. 3-3)
$$\alpha_{\text{sorbed/free}}^{\text{NA}} = (\alpha_{\text{sorbed/free}})^{1/n}$$

where n is the number of deuterium substitutions, which in this case, is 18.

3.4 Results

3.3.1 DRIFTS

In order to directly compare octane vibrations on KBr and kaolinite surfaces, only regions greater than 2000 cm^{-1} were considered due to interfering vibrational frequencies from the kaolinite surface at wavenumbers $< 2000\text{ cm}^{-1}$. On the KBr surface, a spectral window showing peaks from octane- $^2\text{H}_0$ and octane- $^2\text{H}_{18}$ can be observed in 3000 to 2800 cm^{-1} and 2250 to 2050 cm^{-1} regions, respectively. The octane- $^2\text{H}_0$ -KBr spectrum shows 3 major peaks at 2968 , 2932 , and 2867 cm^{-1} with two shoulders at ~ 2880 and $\sim 2885\text{ cm}^{-1}$. The octane- $^2\text{H}_{18}$ -KBr spectra shows two major peaks at 2222 with a shoulder at $\sim 2205\text{ cm}^{-1}$ and 2104 with a possible shoulder at $\sim 2075\text{ cm}^{-1}$ (Fig 3-2). These peaks completely disappeared within 5 minutes of the initial dose.

On the kaolinite surface, peaks can be seen in the same spectral region, however for octane- $^2\text{H}_0$ and octane- $^2\text{H}_{18}$ peak positions have shifted to lower wavenumbers (i.e. redshift). The octane- $^2\text{H}_0$ -kaolinite spectrum shows 3 major peaks at 2960 , 2929 , and 2858 cm^{-1} with more

prominent shoulder at $\sim 2875\text{ cm}^{-1}$. The octane- $^2\text{H}_{18}$ -kaolinite spectrum shows two major peaks at 2216 with a shoulder at ~ 2200 and 2098 with a possible shoulder at $\sim 2070\text{ cm}^{-1}$ (Fig 3-3). In both experiments, octane- $^2\text{H}_0$ and octane- $^2\text{H}_{18}$ peaks persisted approximately 30 minutes after the initial dose (Fig 3-4).

In the octane- $^2\text{H}_0$ /kaolinite spectrum, the three major peaks shift by 8, 4 and 9 cm^{-1} , respectively, and in the octane- $^2\text{H}_{18}$ /kaolinite spectrum, the two major peaks both shift by 6 cm^{-1} . The $\alpha_{\text{sorbed/free}}$ can be estimated using the vibrational frequencies of the major peaks. This is not an exact calculation of $\alpha_{\text{sorbed/free}}$ because the experimental frequencies are anharmonic. These calculations also do not include vibrational frequencies $< 2000\text{ cm}^{-1}$. Using Eq. 1 and 2, $\alpha_{\text{sorbed/free}} = 0.974$ and at natural abundance, $\alpha^{\text{NA}}_{\text{sorbed/free}} = 0.998$, which indicates a 2‰ ^2H -depletion of sorbed octane relative to gaseous octane.

3.3.1 Computational Results

A complete list of vibrational frequencies from the B3LYP harmonic calculations are given in Table B-2. The C-H stretch of octane is described by 18 vibrational modes that encompass the symmetric and asymmetric stretch of CH_2 and CH_3 groups. Each mode involves the stretching of several C-H bonds simultaneously. The calculated harmonic vibrational frequencies are higher than observed vibrational frequencies. Previous work has noted the overestimation of vibrational frequencies by harmonic approximations and usually applies a scaling factor (Pople et al., 1981). However, as these frequencies were used to calculate a fractionation factor rather than directly compared to experimental values, we report the uncorrected frequencies.

Individual C-H stretch vibrational modes show both red shifts and blue shifts associated with sorption, indicating there are competing isotopic effects at individual positions within

octane. Specifically, vibrations associated with asymmetric CH₂ stretches shifted to higher frequencies during sorption while symmetric CH₂/CH₃ and asymmetric CH₃ shifted to lower frequencies. Averaged together, the harmonic models predict an overall blue shift in C-H vibrational frequencies (i.e. frequencies shift to higher wavenumbers), which is inconsistent with the experimental results. Using the calculated frequencies and Eqns **3-1**, **3-2** and **3-3**, the model predicts $\alpha_{\text{sorbed/free}} = 1.044$, corrected to the expected fractionation at natural abundance, $\alpha_{\text{sorbed/free}}^{\text{NA}} = 1.0025$, corresponding to a 2.5‰ ²H-enrichment of sorbed octane, when all vibrational modes are modeled harmonically.

Vibrational frequencies for gaseous octane calculated by the anharmonic model are reported in Table **B-3**. Calculated frequencies for gaseous octane are in reasonable agreement with experimental frequencies and are lower than the harmonic calculated frequencies (Fig **3-2**). For octane-kaolinite models it was not feasible to calculate vibrational frequencies for all modes; instead, anharmonic approximations were performed on a single mode consisting of asymmetric CH₂ stretches. This specific mode was chosen because its molecular motion was similar in both the gaseous and sorbed model, and because the harmonic frequency for this mode was blue shifted upon sorption. To verify that using a singular anharmonic mode was sufficient for the octane-kaolinite models, the chosen vibrational mode was compared for results of three gaseous octane models using: a) all harmonic modes, b) one anharmonic and seventeen harmonic modes, and c) all anharmonic modes. Versions b and c (one-anharmonic and all-anharmonic models) gave similar results for the selected mode (within 10 cm⁻¹), but these results were substantially lower than the all-harmonic model result by more than 100 cm⁻¹ (Fig **3-5**).

Based on these results, the anharmonic octane-kaolinite model was performed with one anharmonic mode. Gaseous octane-²H₀ shifted from 2891 to 2850 cm⁻¹ and gaseous octane-²H₁₈ shifted from 2167 to 2156 cm⁻¹ when sorbed to a kaolinite surface. Using Eqns **3-1**, **3-2**, and **3-3** to estimate the fractionation factor, anharmonic models predict $\alpha_{\text{sorbed/free}}^{\text{NA}} = 0.9965$, or a 3.5 ‰

depletion of sorbed octane relative to gaseous octane. The red shift to lower vibrational frequencies is driven lengthening of C-¹H and C-²H bonds when octane interacts with the kaolinite surface. The C-¹H bond length increases by 0.0042 Å when sorbed, compared to a 0.00041 Å increase C-²H. In contrast, the harmonic approximation predicts a similar decrease in both C-¹H and C-²H bond lengths of 0.0018 Å when sorbed, which corresponds to a $\alpha^{\text{NA}}_{\text{sorbed/free}} = 1.0009$ or a 0.9 ‰ enrichment of sorbed octane when modeling the selected mode.

3.5 Discussion

3.4.1 Driving Forces for Non-Covalent Isotope Effects

Fig. **3-3** compares spectra of octane-²H₀ and octane-²H₁₈ on KBr vs kaolinite surfaces. The short residence time of octane-²H₀ and octane-²H₁₈ on KBr (< 5 min) indicates there was minimal interaction with the surface. In contrast, octane-²H₀ and octane-²H₁₈ persisted on the kaolinite surface for ~30 min under a constant N₂ stream (Fig **3-4**). Kaolinite spectra also show a change in peak shape and relative intensity, consistent with an interaction with the surface. Furthermore, Fig. **3-3** shows a small redshift for octane-²H₀ and octane-²H₁₈ sorbed on the kaolinite compared to KBr.

Small shifts to lower wavenumbers (< 10 cm⁻¹) have been observed in other spectral studies of organo-mineral interactions, primarily between amino acids and clays (Johnston et al., 2002; Garcia et al., 2007; Carneiro et al., 2011). Here, the redshift is likely caused by a lengthening, and thus weakening, of C-¹H/C-²H bonds due to attractions between ²H and ¹H atoms and the kaolinite surface. The magnitude of this redshift is larger for octane-²H₀ compared to octane-²H₁₈, indicating a stronger non-covalent interaction between the protiated octane and

kaolinite. Overall this is a relatively small isotopic effect ($< 2\text{‰}$) and would not affect interpretations of $\delta^2\text{H}$ in most geologic applications.

Kopinke et al. (2017) concluded that this minor fractionation is largely driven by interactions between the aqueous phase and non-polar compound based on similar fractionations when partitioning benzene between gas/aqueous phases and organic/aqueous phases. However, we observed a slight ^2H -depletion in sorbed octane in the absence of an aqueous phase. Alternatively, Turowski et al. (2003) attributed fractionation to primarily enthalpic effects controlled by the interaction between the solute and organic phase (i.e., specific hydrophobic interactions). They found between 0.5 and 4‰ depletion of sorbed nonpolar compounds during reverse-phase chromatographic separation, similar to the $\sim 2\text{‰}$ depletion reported here. The magnitude of fractionation correlated to the van der Waals volume, which is a calculated property that incorporates aspects of a molecule's surface area and bond polarizability (Turowski et al., 2003).

Changes in the physical properties (i.e. polarizability) of octane due to ^2H substitution result from differences in $\text{C}-^1\text{H}$ and $\text{C}-^2\text{H}$ bond lengths. $\text{C}-^2\text{H}$ are shorter and stiffer than $\text{C}-^1\text{H}$ bonds, and as a result, ^2H -containing compounds are smaller in size and have an increased ability to donate electron density via inductive effects. This leads to a slight increase in aqueous solubility (i.e., decreased hydrophobicity) for deuterated compounds and can result in ^2H -depletion in sorbed compounds when sorption is driven by hydrophobic effects (Wade, 1999). Consistent with this, in this work, kaolinite's hydrophobic siloxane surfaces (Jaynes and Boyd, 1991) interacted with protiated octane more strongly than perdeuterated octane, and such interactions would cause slight isotopic depletion at natural abundance for sorbed octane.

3.4.2 Comparison of Experimental and Computational Results

Fig 3-3 shows the shifts of a CH₂ vibration due to sorption for the harmonic and anharmonic approximations compared to the DRIFTS spectra. The harmonic approximation predicts that the vibration will shift to a higher frequency (blue shift) due to the shortening and strengthening of C-H bonds oriented towards the surface. Stronger bonds tend to concentrate heavy isotopes, so the harmonic model predicts that deuterated octane is preferentially sorbed. In contrast, both the anharmonic approximation and the experimental data show a red shift associated with sorption and the magnitude of this shift is higher for octane-²H₀ compared to octane-²H₁₈, consistent with longer C-H bonds and an isotopic depletion in the sorbed phase. Previous modeling work has shown that anharmonic corrections more accurately predict equilibrium isotope distributions for ¹H-²H exchange reactions (Liu et al., 2010). These contributions are likely even more important when predicting non-covalent isotope effects, as no bonds are broken or formed.

Differences between the vibrational frequencies of ²H and ¹H are substantially larger than for isotope systems of heavier elements, such as ¹³C/¹²C. Experimental evidence shows that differences in bonds lengths between hydrogen and heavier elements (e.g., C-¹H and C-²H) are greater than differences between two heavier elements upon isotope substitution (e.g., ¹²C-¹²C, ¹²C-¹³C, or ¹³C-¹³C). Because of the small energy differences involved, proper treatment of changes in their vibrations requires methods that address mechanistic origins for the different bond lengths. The harmonic approximation fails to consider these differences. It assumes the concavity of the potential energy curve is constant, and that different bond lengths from electrostatic interactions are represented only at the bottom of the energy well. As a result, these approximations treat C-²H and C-¹H bonds as the same length, and predict equal shortening in the deuterated and protiated models (Fig 3-6). Because heavier isotopes partition into the shorter,

stronger bonds and thus lower potential energy, the harmonic model predicts that isotopologues with ^2H substitutions will preferentially sorb to kaolinite.

In contrast, anharmonic models consider how these electrostatic attractions/repulsions change away from the bottom of well, which allows them to incorporate differences in $\text{C-}^2\text{H}$ and $\text{C-}^1\text{H}$ bond length. In this work, the anharmonic model predicts longer $\text{C-}^1\text{H}$ bonds compared to $\text{C-}^2\text{H}$ bonds during sorption. This is because interactions between protiated octane and the kaolinite surface lower the potential energy more than interactions between the perdeuterated octane. Importantly, during sorption, the potential energy curve flattens and thus becomes increasingly anharmonic due to electrostatic interactions between octane and the kaolinite surface. This causes a greater decrease in potential energy for protiated octane relative to perdeuterated octane (Fig 3-7). Therefore, the anharmonic model predicts that isotopologues depleted in ^2H are more likely to sorb to a kaolinite surface, consistent with the experimental data. Further, frequencies predicted from anharmonic modeling for gaseous octane were more consistent with the DRIFTS spectra than the harmonic approximations. Our experimental and model comparisons, like previous studies, suggest the anharmonic corrections better represent hydrogen isotope effects during sorption by weak attractive forces.

3.6 Conclusion

The influence of hydrophobic interactions on H isotope fractionation in *n*-octane during sorption was investigated using DRIFTS and DFT calculations. Observed experimental depletions in the sorbed octane were minor, less than 2 ‰. In most geologic applications, H isotope fractionations less than 10 ‰ are considered within analytical error, indicating that H isotope effects from sorption during preservation will not influence interpretations of H isotope signatures. However, comparison to model results suggested the important role of anharmonicity

in predicting non-covalent isotope effects. Here, H isotope effects were caused by differences in C-¹H and C-²H bond lengths, that cause protiated octane to have a larger volume/surface area and sorb more strongly to the kaolinite surface. Harmonic approximations fail to capture these bond length differences and thus do not accurately predict the depletion of the sorbed octane. From this work we can conclude that sorption-driven isotope effects are relatively minor with substantial anharmonic contributions that should be incorporated into future models.

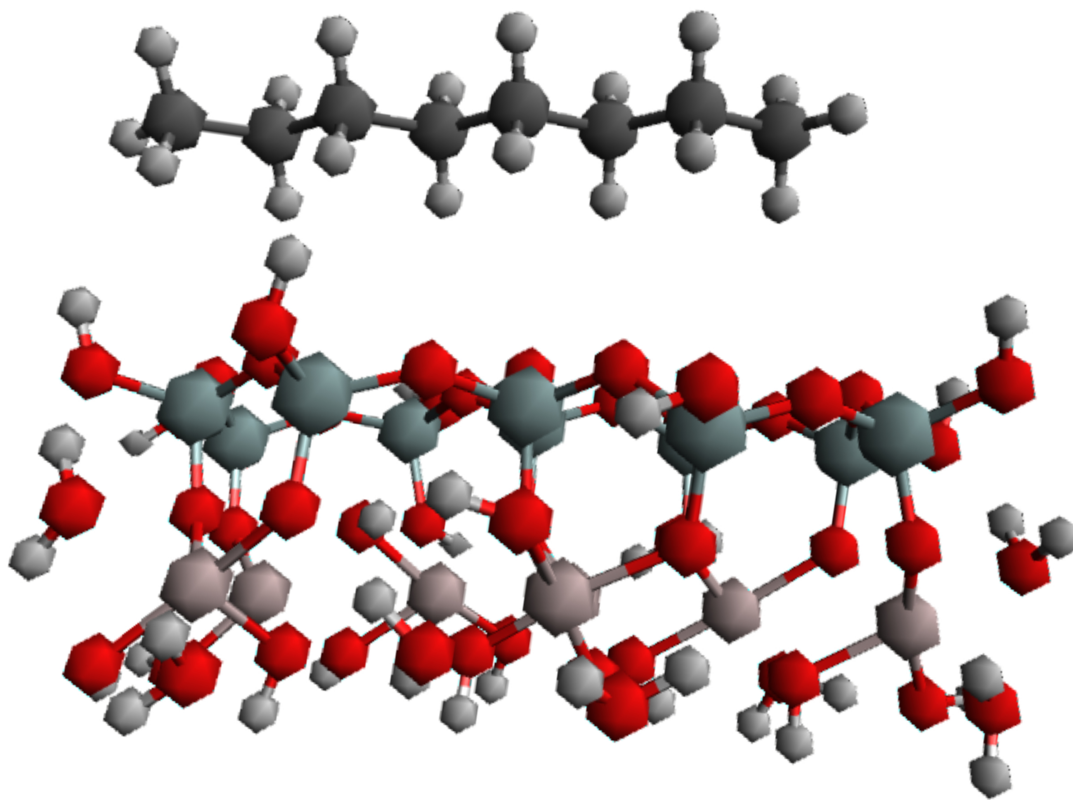


Figure 3-1: Kaolinite-octane model cluster; carbon (black), hydrogen (white), oxygen (red), silicon (gray), and aluminum (pink).

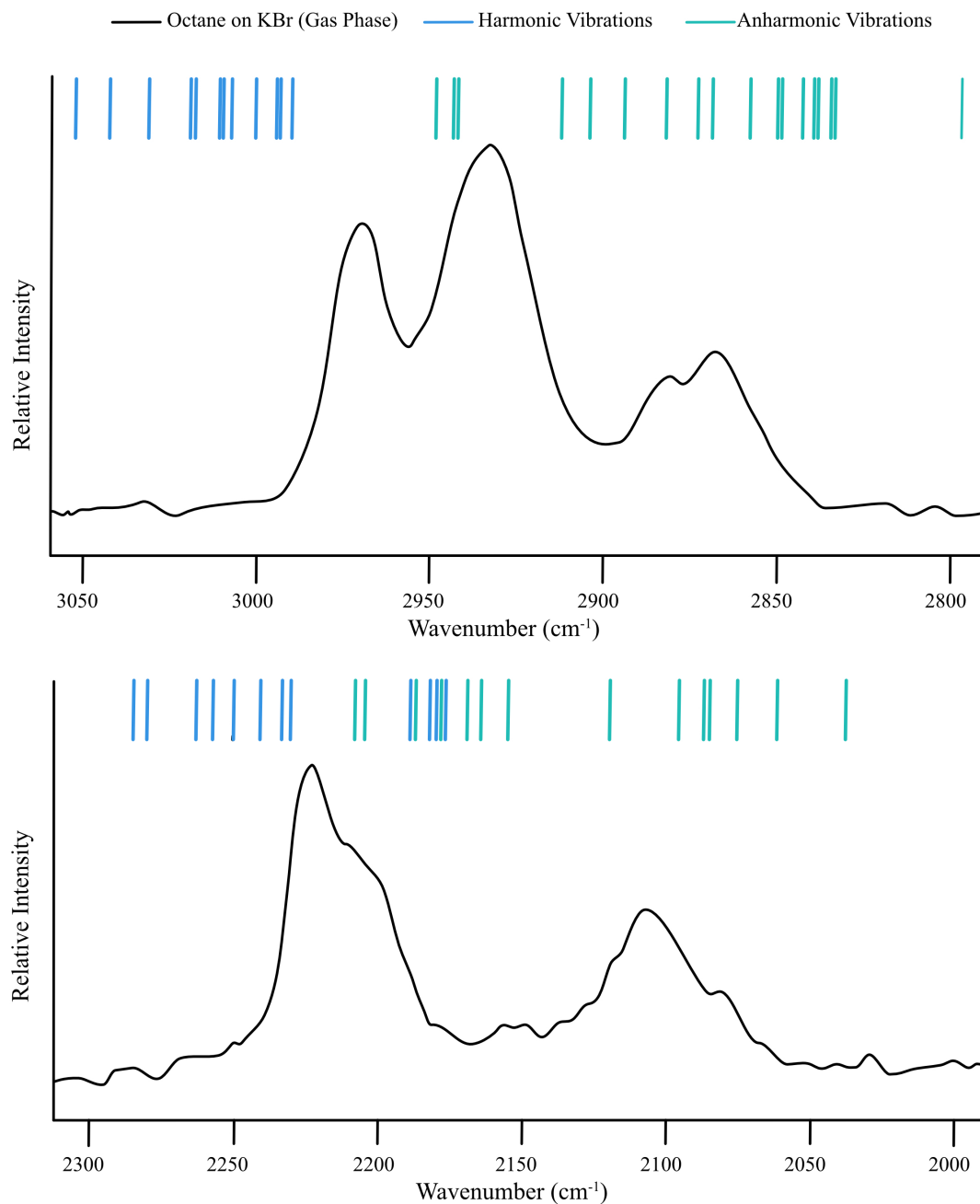


Figure 3-2: DRIFTS spectra for protiated (top) and deuterated (bottom) gaseous octane on KBr compared to the calculated vibrational frequencies from the harmonic (blue lines) and anharmonic (green lines) models. Values reported for model results in Table B-3.

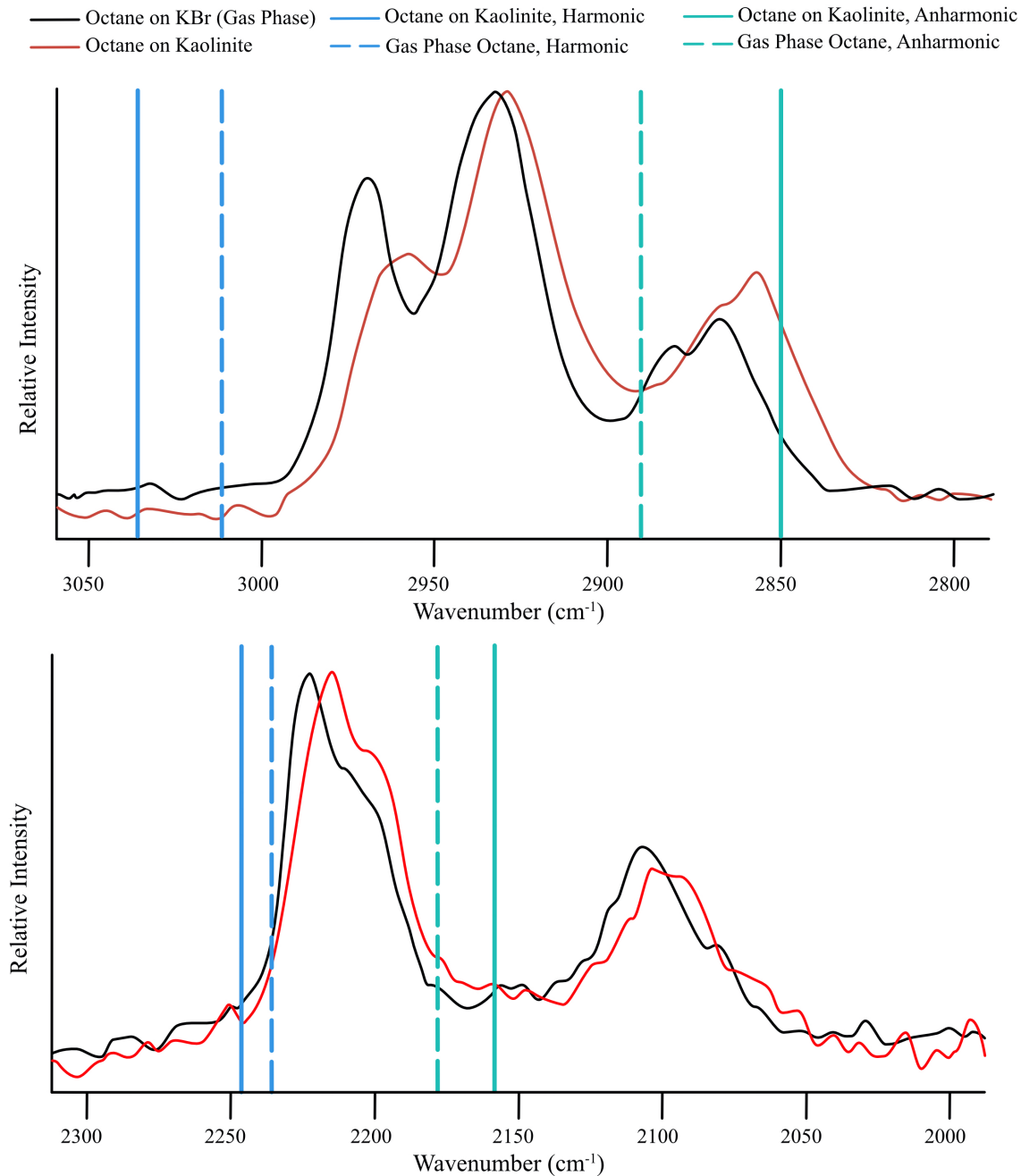


Figure 3-3: DRIFTS spectra for protiated (top) and deuterated (bottom) octane sorbed to a kaolinite surface (red curve) compared to 'gaseous' octane on KBr (black curve). Calculated vibrational frequencies from one CH_2 vibrational mode are shown as vertical lines for the harmonic and anharmonic models.

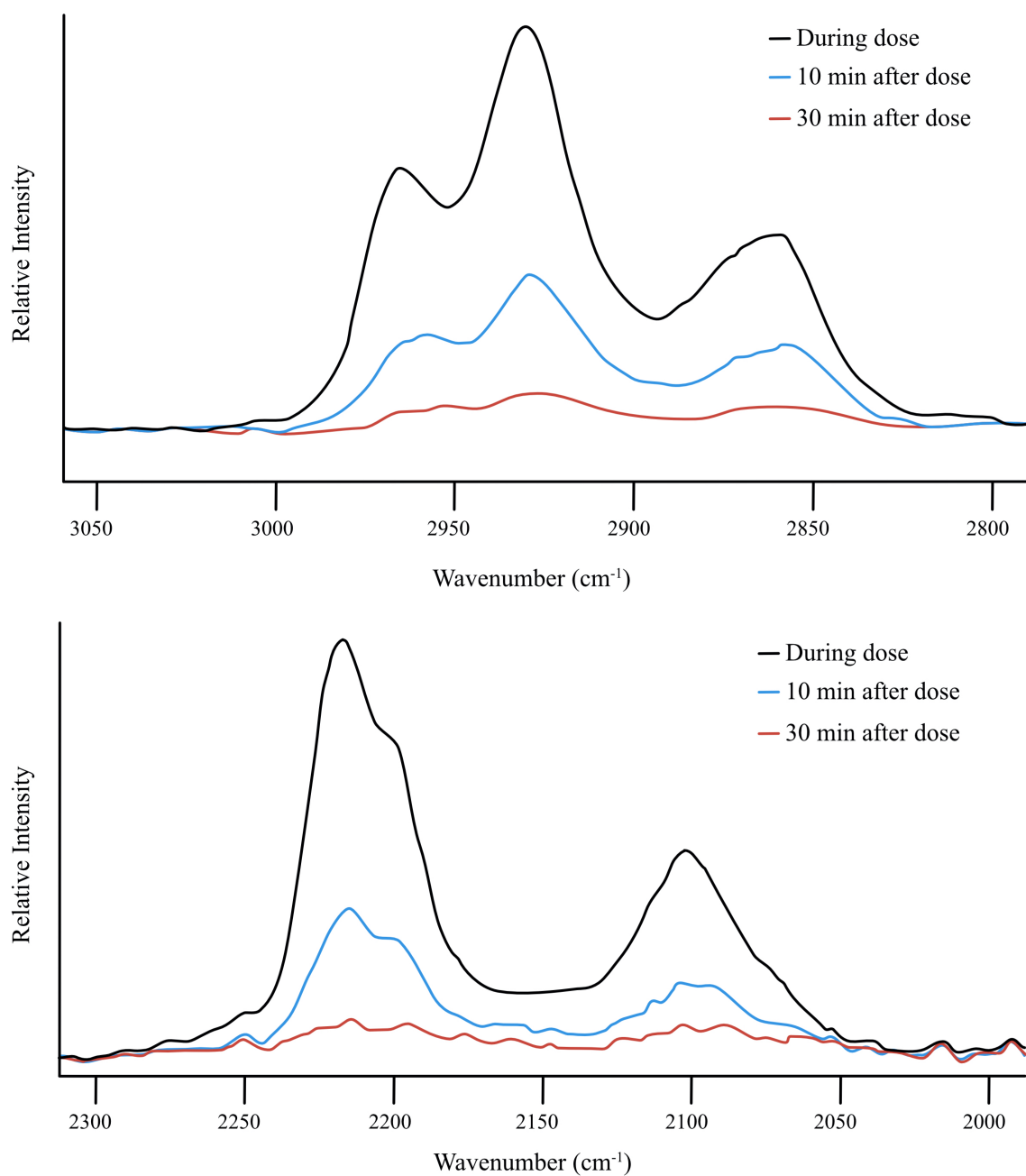


Figure 3-4: DRIFTS spectra of protiated (top) and deuterated (bottom) octane on a kaolinite surface during dosing (black curve), 10 min after dosing (blue curve), and 30 min after dosing (red curve). Peaks associated with C-H stretching in octane are still visible 30 min after dosing.

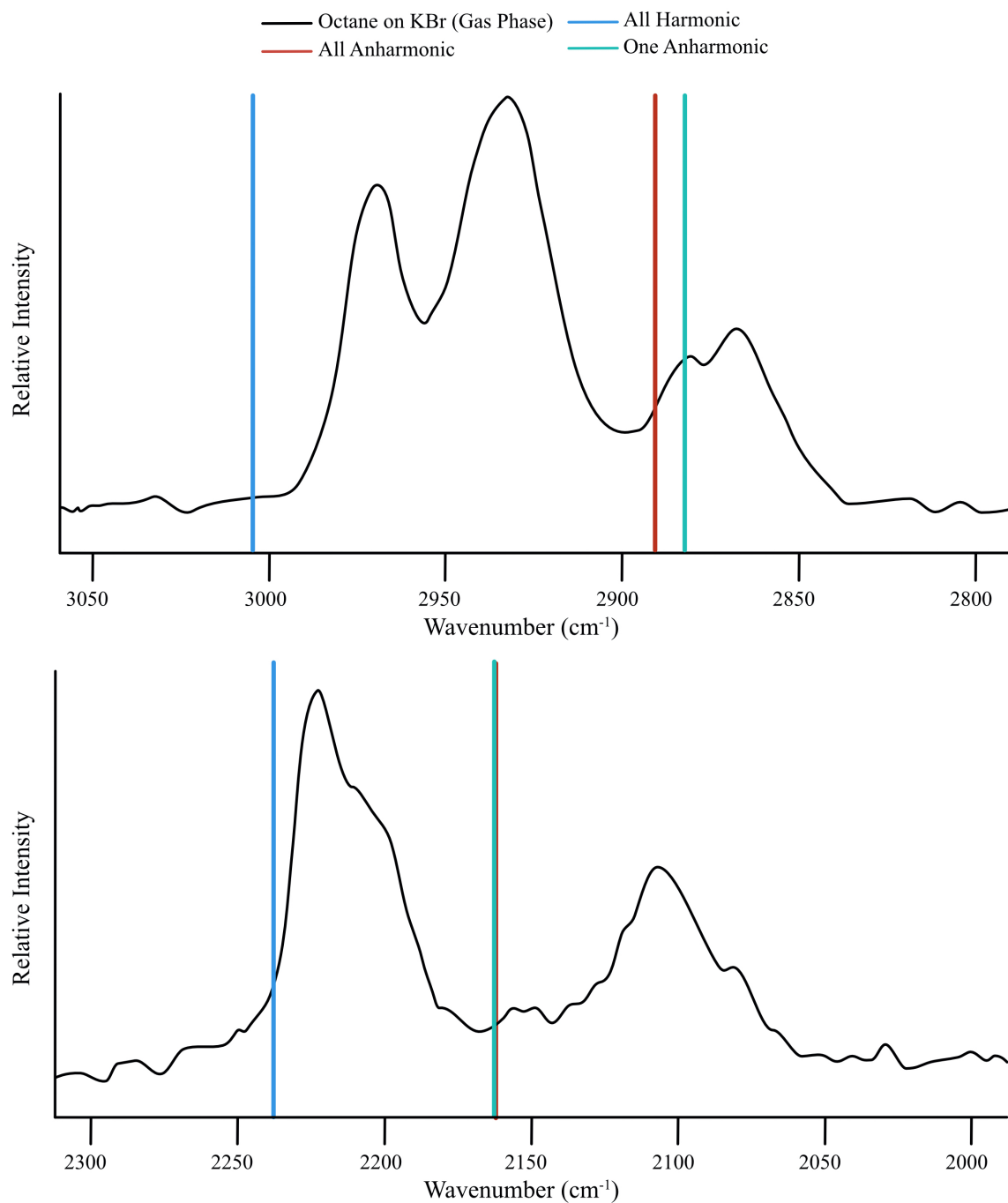


Figure 3-5: DRIFTS spectra of protiated (top) and deuterated (bottom) gaseous octane on KBr. Calculated vibrational frequencies from one CH_2 vibrational mode are shown as vertical lines for the all harmonic vibrations (blue), one anharmonic vibration (green), and all anharmonic vibrations (red).

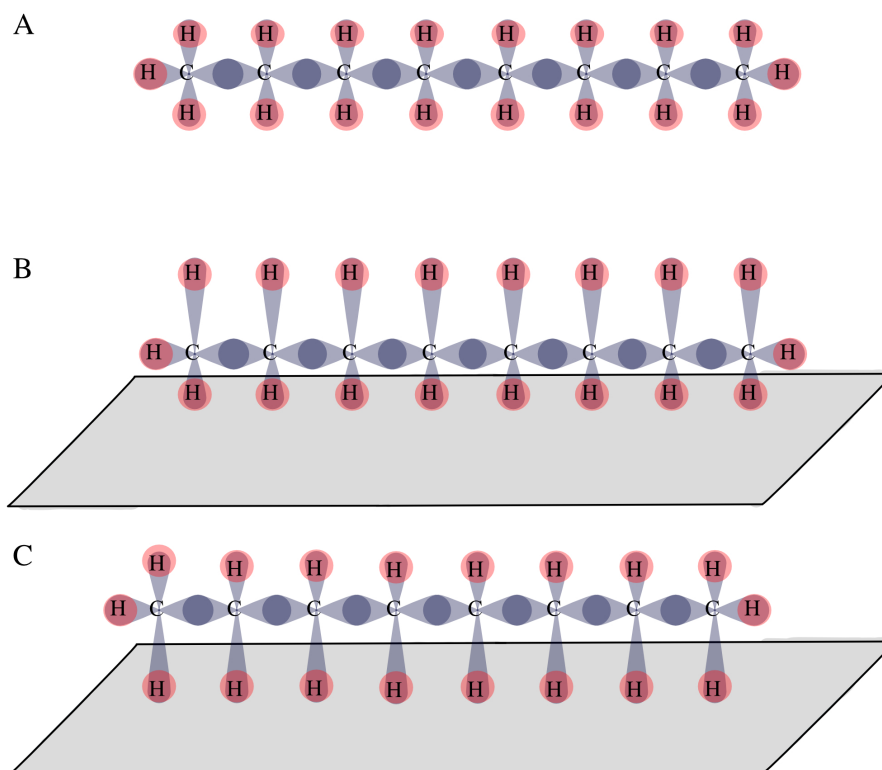


Figure 3-6: The electron distribution of octane on a kaolinite surface during sorption for the (A) gas phase model (harmonic and anharmonic models were identical), (B) sorbed phase harmonic model and (C) sorbed phase anharmonic model. In the harmonic model, C and H atoms are attracted to the electron-rich kaolinite surface, causing C-H bonds to shorten near the surface and, to a lesser degree, lengthen away from the surface. In the anharmonic model, H atoms are attracted to the surface more strongly than C atoms, which lengthens C-H bonds.

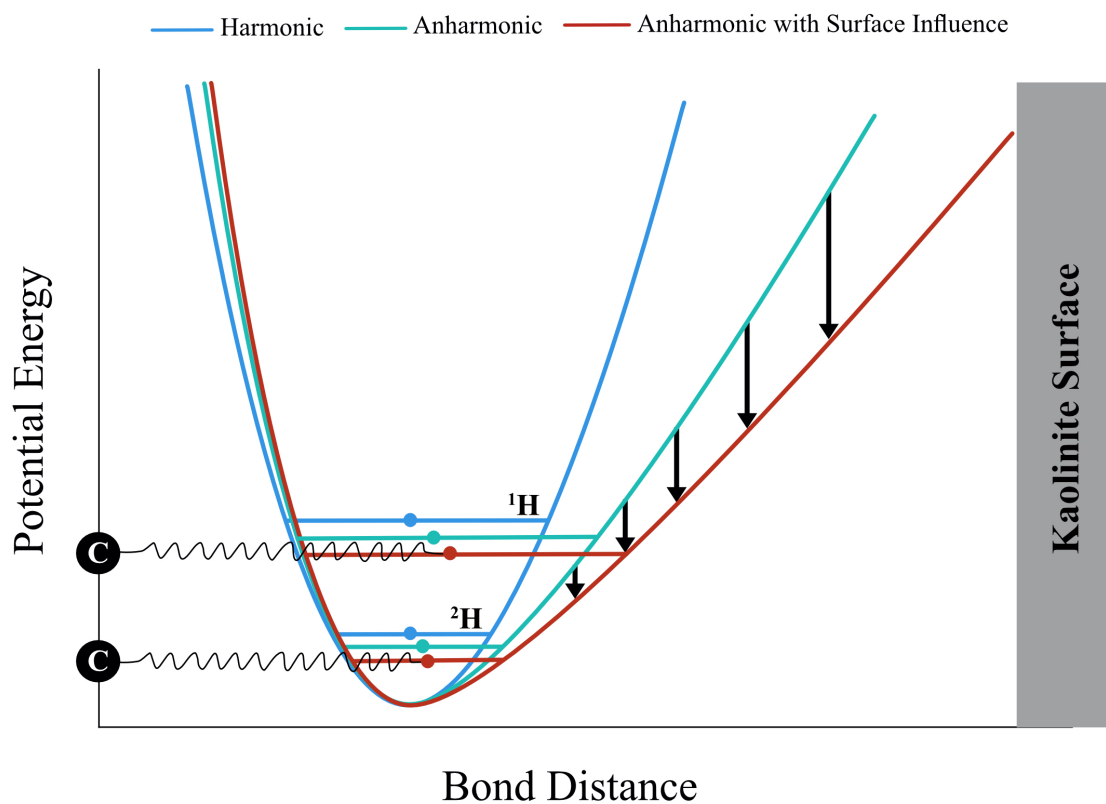


Figure 3-7: The potential energy diagram for the harmonic model (blue), anharmonic model (green) and anharmonic model with the influence of the kaolinite surface (red). In the harmonic model, C- ^1H and C- ^2H bond lengths are equivalent. In the anharmonic model, C- ^1H bonds are longer C- ^2H . When the electron-rich kaolinite surface is added to the anharmonic model, the anharmonicity increases and there are larger potential energy decreases for C- ^1H bonds than C- ^2H bonds.

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Chapter 4

Position-Specific Isotope Fractionation in Amino Acids Sorbed to Ice: Implications for Amino Acid-Mineral Interactions and Preservation of Isotopologue Biosignatures

4.1 Abstract

Sorption to mineral surfaces is a key process that protects amino acids from oxidation and aids their polymerization into more complex biomolecules. Sorption of neutral amino acids is driven by a combination of intermolecular forces, commonly through hydrogen bonds with surface functional groups. Substitutions of heavy isotopes are known to influence the strength of intermolecular interactions, but global (whole-molecule) C isotope fractionation associated with sorption is small ($< 1\text{ ‰}$). However, larger fractionation of C isotopes ($> 2\text{ ‰}$) was observed for specific positions within a molecule during chromatographic separation, indicating that fractionation during sorption is likely more significant at positions that interact with the surface. We used quantitative isotopic ^{13}C NMR to measure position-specific C isotopic distributions within glycine, L-alanine, L-serine, L-leucine, and L-phenylalanine sorbed to an ice surface from an aqueous solution. Isotopic differences up to 8 ‰ at functional sites were observed between sorbed and free amino acids, suggesting that sorption can alter primary isotopic patterns associated with their synthesis. Further, in sorbed amino acids with non-polar side chains, we observed a depletion of ^{13}C in the carboxyl carbon, consistent with hydrogen bonding between the carboxyl group and hydroxyl groups on the ice surface. In contrast, the ^{13}C depletion was observed at another site within serine, which has a polar side chain. Hydrogen bonding at the carboxyl carbon lessens its electron density and promotes peptide bond formation via

nucleophilic attack by another amino acid, which could explain the dominance of amino acids with non-polar side chains in modern proteins.

4.2 Introduction

Interactions between organic molecules and mineral surfaces likely helped to direct the chemical evolution of prebiotic compounds and protected early biotic substrates and products. In the case of amino acids, mineral-mediated reactions potentially facilitated their initial polymerization into proteins. Self-assembly of amino acids into proteins is a vital step for the evolution of life, but polymerization in aqueous solutions is thermodynamically unfavorable (Lambert, 2008). Mineral-mediated reactions have been proposed as a solution to these unfavorable thermodynamics. Laboratory experiments have shown that the addition of a mineral phase can lead to polymerization of amino acids in high pressure and temperature environments (Bu et al., 2019; Takahagi et al., 2019), after successive wetting and drying cycles (Lahav et al., 1978; Yanagawa et al., 1990; Kitadai et al., 2017), and in combination with other activating agents (McKee et al., 2018). The majority of these experiments found that polymerization was either impossible or resulted in very low yields without the presence of a mineral phase, highlighting the importance of mineral surfaces in these reactions. In modern environments, mineral surfaces help protect amino acids from photochemical and biological oxidation. Associations with certain minerals can decrease the rate of radiolysis of amino acids exposed to UV radiation (dos Santos et al., 2016; Fornaro et al., 2018) and organo-mineral interactions are widely thought to promote preservation in terrestrial systems (Schmidt et al., 2011; Keil and Mayer, 2013).

The strength of amino acid-mineral interactions are governed by the surface charge and the molecular structure and charge of the amino acid (Lützow et al., 2006). Early work found the highest sorption for basic (positively charged) amino acids on negatively charged silica surfaces, which interact via strong electrostatic forces (Hedges and Hare, 1987). More relevant to the evolution of life is the sorption of neutral amino acids, as they compose the majority of proteins today (< 40%) (Zaia, 2004), which is driven by hydrogen bonding between neutral amino acids and silica surfaces at circumneutral pH (Garcia et al., 2007; Carneiro et al., 2011; Trachta et al., 2019). The location of the hydrogen bond influences surface-mediated polymerization reactions. Notably, hydrogen bonding at the carboxyl group makes a sorbed amino acid more susceptible to nucleophilic attack by the amine group of another amino acid, promoting polymerization (Kitadai et al., 2017). Knowledge of factors that influence intermolecular forces between amino acids and mineral surfaces will improve our ability to predict how amino acids persisted and polymerized on the early Earth and, potentially, in other planetary and space environments.

Assessing the strength of intermolecular forces, such as hydrogen bonds, in complex systems is difficult because they cannot be directly measured in the same way as covalent bonds. However, recent work investigating position-specific isotopic fractionation during distillation of organic solvents found a possible link between the magnitude of fractionation and the strength of intermolecular interactions (Julien et al., 2017). While further work is needed to definitively attribute isotope effects to intermolecular forces, modeling and experimental studies show isotopic substitutions can influence the strength of intermolecular interactions. An extreme example of this influence is illustrated by the chromatographic separation of perdeuterated compounds from their protiated counterparts. Perdeuterated hydrocarbons are less retained on non-polar columns and elute significantly earlier than non-substituted forms. This indicates ^2H substitutions weaken attractive forces between the compound and the stationary phase. Attempts to observe sorption isotope effects at natural abundance for a variety of compounds and sorbents

have found relatively small C and H isotope effects, less than 1.5 and 8 ‰, respectively (Van Hook, 1969; Freeman et al., 1994; Dias and Freeman, 1997; Schüth et al., 2003; Höhener and Yu, 2012; Imfeld et al., 2014). These global isotope studies would suggest that isotopic substitution at natural abundance has a relatively minor effect on the strength of intermolecular interactions.

However, a recent study measuring position-specific C isotope fractionation during normal-phase liquid-liquid chromatography found that individual isotopomers of vanillin and similar compounds had different affinities to the stationary phase (Botosoa et al., 2009). Previous studies were limited to measuring isotope effects based on the difference in ^{13}C abundances for sorbed and free whole molecules, i.e., global isotope measurements. Global isotope abundances are traditionally measured by isotope ratio mass spectrometry (IRMS) techniques that convert compounds to CO_2 and measure the total $^{13}\text{C}/^{12}\text{C}$ abundance ratio relative to a standard. As a result, conventional compound-specific isotope measurements represent the average ^{13}C contribution of all isotopologues and cannot provide information about specific isotopomers. In contrast, quantitative isotopic ^{13}C NMR measures the ^{13}C contribution of each position within a molecule, giving information on the distribution of isotopomers within the mixture of isotopologues (see Fig. 4-1 for a more detailed explanation).

Using quantitative isotopic ^{13}C NMR, Botosoa et al. (2009) observed site-specific fractionation up to 9 ‰ between eluted and retained vanillin, despite a global fractionation of < 1‰. They found the largest magnitude of fractionation at carbon positions directly sorbed to the stationary phase, i.e., at the polar functional groups, although all positions displayed some degree of fractionation. This indicates that intermolecular forces are influenced by isotope substitutions at different positions to different degrees. Botosoa et al. also found that position-specific fractionation was highly dependent on the stationary phase polarity, which provides additional evidence for the important influence of intermolecular forces involved in molecule-surface interactions, as originally described by Van Hook (1969).

Associations between amino acids and ice surfaces rely on the similar intermolecular forces to those that drive chromatographic separation, and thus likely also impart position-specific isotope effects. Mineral and ice surfaces concentrate and protect amino acids throughout the solar system. As a consequence of such widespread interactions with minerals, sorption-driven isotope effects need to be characterized and quantified before intramolecular isotope patterns can be used to discern amino acid origins. Here, we report position-specific C isotope fractionation for five neutral amino acids sorbed to an ice surface. Ice has surface hydroxyl groups capable of forming hydrogen bonds with amino acids, comparable to clays or other silicate mineral surfaces. Similar to previous work, the magnitude and position of fractionation likely reflect both the orientation and strength of the intermolecular interaction between the surface and the amino acid in an aqueous solution. The potential connection between the magnitude of fractionation and strength of intermolecular interaction provides insights about how different types of amino acids sorb to surfaces, and thus which are more likely to be preserved by organo-mineral interactions. Additionally, insights about the orientation of different amino acids on the ice surface enhance our understanding of their potential efficiency of mineral-mediated polymerization.

4.3 Methods

4.3.1 Chemicals

High purity (<99.9%) amino acid standards were purchased from Sigma Aldrich and used without further purification. Amino acids included glycine (Lot #S2BE0900V) referred to as Gly, L-alanine (Ala; Lot #BCBV9832), L-leucine (Leu; Lot #BCBL9296V), L-serine (Ser; Lot #SLCC6622), and L-phenylalanine (Phe; Lot #BCBX4042).

4.3.2 Sorption Experiments

Approximately 300 mg of individual amino acids were dissolved in 50 mL of 18.2 MΩ H₂O in an Erlenmeyer flask with a stir bar. Amino acid solutions were then placed in an ice bath maintained at $0 \pm 5^\circ \text{C}$. Five 10 g ice cubes of 18.2 MΩ H₂O (total ice approximately 50 g) were added to the solution. Amino acid-ice solutions were agitated by stirring for one hour. After agitation, the liquid and ice fractions were separated into clean beakers and evaporated to dryness at $95 \pm 5^\circ \text{C}$ under an N₂ stream.

4.3.3 IRMS Analysis

Two replicates of approximately 1.0 mg of a given amino acid was sealed in a tin capsule and carbon and nitrogen isotope ratio measurements were performed using a Delta-V Advantage isotope ratio mass spectrometer coupled to an NA2100 elemental analyzer (Thermo Scientific). Global ¹³C/¹²C and ¹⁵N/¹⁴N ratios measured for each amino acid are expressed in the delta notation, $\delta^{13}\text{C}_g$ and $\delta^{15}\text{N}_g$, relative to the Vienna Pee Dee Belemnite (VPDB) Standard and atmospheric air, respectively. $\delta^{13}\text{C}_g$ and $\delta^{15}\text{N}_g$ are calculated by the Eq. 4-1:

$$\text{(Eq. 4-1)} \quad \delta_g (\text{‰}) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right]$$

where R represents the isotopic ratio, i.e., the ratio of the rare isotope (either ¹³C or ¹⁵N) to the common isotope (either ¹²C or ¹⁴N) in the sample or standard. The isotopic ratio, R, is related to the mole fraction, x, by the formula $R = x/(1-x)$. $\delta^{13}\text{C}_g$ and $\delta^{15}\text{N}_g$ were determined by reference to a working standard of glutamic acid standardized against calibrated using international reference materials (IAEA-N1, Ammonium Sulfate for ¹⁵N and IAEA-CH-6, Sucrose for ¹³C (IAEA, Vienna, Austria)). Errors are reported as the standard deviation of two

runs, which was less than 0.3 ‰ for all samples. Global isotopic fractionation factors are defined by Eq. 4-2:

$$\text{(Eq 4-2)} \quad (\Delta^*X_{\text{liquid-ice}}) = \delta^*X_{\text{liquid}} - \delta^*X_{\text{ice}}$$

A positive $\Delta^*X_{\text{liquid-ice}}$ indicates that the liquid phase is enriched in the rare isotope relative to the ice phase. Isotopic differences reported here closely approximate fractionation factors expressed in alpha notation: $\Delta^*X \cong 1000\ln(\alpha)$.

4.3.4 NMR Analysis and Processing

Approximately 30-50 mg of amino acid was dissolved in 600 μL solution of acetic acid- d_2 (EURIOSTOP) and 18.2 M Ω H_2O . Each sample was homogenized and inserted into a 5 mm NMR tube (WILMAD) before analysis. Quantitative ^{13}C NMR spectra were performed at 303 K on a Bruker Avance HD 700 spectrometer (Bruker Biospin), at a frequency of 176.09 MHz with a cryogenic $^1\text{H}/^{13}\text{C}/^2\text{H}/^{19}\text{F}$ probe. All the adjustments were done manually (tuning, matching, 90° hard pulse calibration – PW90 (^{13}C) was initially set at 10.8 μs at 180 W then slightly adjusted for each sample and shimming was automatically applied. ^{13}C spectra were recorded under quantitative conditions, with inverse-gated decoupling for ^1H by using an adiabatic scheme (Tenailleau and Akoka, 2007). To determine the decoupling attenuation, power level was optimized to achieve a pulse length of 14.1 s for each individual sample but did not exceed 6.98 W, per spectrometer recommendations. This pulse sequence was applied to avoid nuclear Overhauser effect, and the repetition delay between each 90° was set to $10 \times T_{1\text{max}}$ for each analysed amino acid to achieve quantitative relaxation of the magnetization. The spectra were recorded with TD = 81516 and SW = 231.4602 ppm. The central frequencies for ^{13}C and ^1H decoupling were individually adjusted for each amino acid. Five spectra per measurement were

recorded and number of scans was adjusted to ensure a signal to noise ratio greater than 500 (Caytan et al., 2007). The T_1 of each amino acid were measured by applying an inversion-recovering pulse sequence: for Ala, between 3.77 and 4.50 s; for Gly, 3.2 s; for Leu, between 1.07 and 1.37; for Phe, 2.13 s; and for Ser, 2.0 s.

Processed spectra were obtained by applying an apodization function (exponential function with LB = 1.5 Hz) prior to Fourier transformation, with zero-filling (SI = 262144). A baseline correction was applied with a specific correction for sub-parts of the spectra. After baseline correction, a curve fitting was carried out with a Lorentizan mathematical model using the rnmrfit R package (Sokolenko et al., 2019) (See Section C.2 for R script).

4.3.5 Calculations

Intramolecular $\delta^{13}\text{C}$ values for each carbon in a given amino acid were calculated using Eq. 4-3 (Jézéquel et al., 2017):

$$\text{(Eq 4-3)} \quad x_i = x_g(f_i / F_i)$$

where x_i and x_g are the mole fraction of the rare isotope over the sum of the rare and common isotopes for the position i and whole molecule (global), respectively. The ratio of the area of peak i to the total area of all peaks in the molecule, f_i , is determined by NMR in which peak area reflects the abundance of ^{13}C at a given position. F_i is the statistical molecular fraction, or molar fraction for the carbon position i in case of a homogeneous ^{13}C distribution within the molecule (i.e. $F_i = 1/6$ for leucine). Errors are reported as the standard deviation of five runs.

Isotopic differences between amino acids free in solution versus those sorbed to ice surfaces are reported as $\Delta_{\text{liquid-ice}}$, which is the permil difference between the liquid and ice $\delta^{13}\text{C}$ values (Eq. 4-4):

$$\text{(Eq 4-4)} \quad (\Delta^{13}\text{C}_{\text{liquid-ice}})_i = \delta^{13}\text{C}_{\text{liquid},i} - \delta^{13}\text{C}_{\text{ice},i}$$

And as a fractionation factor ($\alpha_{\text{liquid/ice}}$) given in Eq. 4-5:

$$\text{(Eq 4-5)} \quad (\alpha_{\text{liquid/ice}})_i = (R_i)_{\text{liquid}} / (R_i)_{\text{ice}}$$

Where R is the isotopic ratio of $^{13}\text{C}/^{12}\text{C}$ for a given position, i. Positive $\Delta_{\text{liquid-ice}}$ values or $\alpha_{\text{liquid/ice}}$ values greater than 1 indicates that isotopologues with ^{13}C -enrichments at that position are more abundant in solution rather than sorbed to the ice surface, relative to other isotope forms.

4.4 Results

4.4.1 Sorption and Recovery

For all sorption experiments, recoveries exceeded 94%, for liquid and solid phases combined. Molecules were recovered from ice that had been separated and melted, which ensured that amino acid loss was not due to inefficient extraction from the solid phase. As a result, the minor loss (< 6%) of amino acids is attributed to loss of water product (i.e., by drips or minor physical spilling) during the experiment, which is unlikely to have caused isotopic fractionation. Between 6 and 17% of the amino acids in solution sorbed to the ice phase, consistent with the findings of Hedges and Hare (1986), who reported 10-15% sorption for amino acids. Percentages of compounds sorbed were as follows: Phe, 6.93%; Leu, 10.19%, Ala, 12.06%; Ser, 15.83%; and Gly, 16.59%.

4.4.2 Global $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ Fractionation

Individual global $\delta^{13}\text{C}_{\text{VPDB}}$ and $\delta^{15}\text{N}_{\text{AIR}}$ values for each amino acid standard and for each standard compound partitioned into the liquid and ice samples are reported in Table C-1. Global

C and N isotope fractionation factors were negligible for all sorption experiments. $\Delta^{13}\text{C}_{\text{liquid-ice}}$ and $\Delta^{15}\text{N}_{\text{liquid-ice}}$ values for all samples were less than 0.1 and 0.4 ‰, respectively, $\Delta_{\text{liquid-ice}} < 0.5\text{‰}$ are considered insignificant given the precision of IRMS measurements.

4.4.3 Position-Specific $^{13}\text{C}/^{12}\text{C}$ Fractionation

Figure 4-2 and Table 4-1 summarize the $\Delta^{13}\text{C}_{\text{liquid-ice}}$ and $\alpha_{\text{liquid/ice}}$, respectively, for all positions for each amino acid. With the exception of Ser and Ala, all amino acids show significant ^{13}C -enrichment in the carboxyl carbon in the liquid phase relative to the ice phase (i.e., a positive value for $\Delta^{13}\text{C}_{\text{liquid-ice}}$ and $\alpha_{\text{liquid/ice}} > 1.0$). Ala does not show any significant fractionation within error, and Ser exhibited its largest enrichment at the alcohol carbon position. Leu had the highest isotopic differences among carbon positions within the molecule, followed by Phe and then Gly.

Table 4-1: Position-specific $\alpha_{\text{liquid/ice}}$ for amino acids.

Amino Acid	$\alpha_{\text{liquid/ice}}$						
Carbon Number	1	2	3	4	5	6	7
Glycine	1.003 ± 0.002	0.997 ± 0.002	-	-	-	-	-
L-Alanine	1.000 ± 0.003	1.000 ± 0.004	0.999 ± 0.004	-	-	-	-
L-Serine	1.001 ± 0.004	0.997 ± 0.005	1.003 ± 0.004	-	-	-	-
L-Leucine	1.009 ± 0.004	1.004 ± 0.004	0.997 ± 0.003	0.998 ± 0.005	0.996 ± 0.003	0.996 ± 0.004	-
L-Phenylalanine	1.005 ± 0.004	1.004 ± 0.005	0.999 ± 0.002	0.998 ± 0.002	0.996 ± 0.004	1.000 ± 0.005	0.999 ± 0.005

4.5 Discussion

Intramolecular isotope patterns reflect the origins of a molecule, and thus are an attractive tool to distinguish abiogenic from biogenic organic matter, as well as formation substrates and pathways. For example, the tendency for the rare isotope (in this case, ^{13}C) to occupy the central versus terminal carbon position in propane, referred to as the site preference, reflects its synthetic pathway. Gilbert *et al.* (2019) used site preference to distinguish propane in thermally mature seeps produced by anaerobic oxidation vs. thermal cracking, despite indistinguishable global $\delta^{13}\text{C}$ values (Gilbert *et al.*, 2019).

Intramolecular isotope patterns that represent molecular origins and potential biosignatures can be overprinted by post-depositional processes. The present work expands our understanding of these influences by demonstrating how intermolecular interactions can lead to the preferential sorption of certain isotopomers. At some positions, these isotope effects are large, up to 8 ‰, and potentially sufficient to mask intramolecular isotope patterns associated with biochemical reactions. In the case of amino acids, intramolecular differences at the carboxyl carbon in leucine produced by different photosynthetic microorganisms can vary between 1 and 11 ‰ (Abelson and Hoering, 1961). The intramolecular isotope patterns that distinguish these biochemical pathways can be overshadowed by many of the sorption-isotope fractionation factors that we observed in this study. Notably, isotope fractionation was greatest at positions that most strongly interact with surfaces. Thus, positions less involved in surface sorption interactions are more likely to retain their primary isotope signals.

Depending on the amino acid – mineral pair, intermolecular interactions can include hydrogen bonding, hydrophobic interactions, electrostatic attraction, and van der Waals forces (Basiuk, 2002). At circumneutral pH, neutral amino acids exist as zwitterions with a positively charged amine group and negatively charged carboxyl group. Previous spectroscopic work

confirms that for many mineral surfaces, zwitterionic amino acids sorb primarily through hydrogen bonds. Zwitterionic amino acids can form hydrogen bonds through both protons and unbonded electron pairs, respectively in their amine and carboxyl groups, in addition to any R groups that contain hydrogen bond acceptors or donors. Garcia *et al.* (2007) found alanine sorbed to alumina through hydrogen bonding between the carboxyl group and surface hydroxyl groups at low and neutral pH, but at high pH hydrogen bonds formed between the amine group and surface (Garcia *et al.*, 2007). A similar trend was reported for glycine and serine on TiO₂ nanoparticles (Ustunol *et al.*, 2019). In contrast, on zeolite surfaces hydrogen bonds form with the amine group at neutral pH, likely due to zeolites' negative surface charge. In the same study they also found that more hydrophobic zeolites had higher sorption capacities, indicating that hydrogen bonds are not the only bonding mechanism (Carneiro *et al.*, 2011).

In the present work, ice was chosen to avoid any isotopic effects associated with extraction of amino acids. In addition, ice is chemically less complex than clay minerals, and offers the advantage that interactions between amino acids and ice surfaces are limited to hydrogen bonding with hydroxyl groups and van der Waals interactions. Isotopic effects associated with these weak intermolecular are small, but generally researchers have found that ¹³C-enriched compounds sorb less strongly. For example, Dias and Freeman (1997) observed a minor enrichment in polar compounds remaining in solution during sorption to a solid phase microextraction fiber from an aqueous solution (Dias and Freeman, 1997). Additionally, Botosoa *et al.* (2009) found the earliest eluting vanillin isotopomers in a normal phase silica gel column were enriched at position proximal to polar groups relative to later eluting vanillin isotopomers. Taken together, previous work and the data presented here suggest that enrichment at a specific site of amino acids in solution is consistent with hydrogen bonds forming between that site and the ice surface (i.e., the highest depleted site in the sorbed phase is most directly interacting with the ice surface). With the exception of serine, amino acids in solution show the highest

enrichment in ^{13}C relative to sorbed amino acids at position 1, the carboxyl carbon (Fig. 4-2), indicating they primarily sorb through hydrogen bonds between the carboxyl group and ice surface. For serine, the highest enrichment was observed at position 3, suggesting that hydrogen bonds primarily form through the alcohol group rather than at the carboxyl position.

Isotopic fractionation during phase partitioning is a net consequence of the relative strengths of intermolecular attractive forces between the amino acid isotopologues and the ice and the surface environment, including vicinal water, and between the isotopologues and bulk water (liquid phase). Interactions with the liquid phase are highly sensitive to the energy needed to displace water molecules from each other in order to make space for the amino acid. The ionic strength, temperature, pH and presence of cosolvents can act to weaken or strengthen interactions between amino acids and liquid water molecules (Schwarzenbach et al., 1993). Of these, pH likely exerts the greatest influence on amino acid partitioning because it controls the extent of amino acid dissociation into different ionic forms. In this work, experiments were performed at pH = 6, so the amino acids were predominantly in their zwitterionic form. In alkaline or acidic environments amino acids exist as charged ions, which will significantly affect its intermolecular interactions with the liquid water phase and ice surface environment. As a result, sorption-driven isotope effects in other environments are likely to be different than those reported here, especially in hyper-saline or non-neutral pH environments such as brines on Mars (Chevrier et al., 2009; Javier Martín-Torres et al., 2015) or Europa (McCord et al., 1999; Levy et al., 2000; Ozgurel et al., 2018) that could potentially host amino acids.

Julien et al. (2017) hypothesized that the degree of position-specific isotopic fractionation during distillation of organic solvents reflects the strength of intermolecular interactions. In this work, the largest magnitude $\Delta^{13}\text{C}_{\text{liquid-ice}}$ is observed for leucine, with decreasing $\Delta^{13}\text{C}_{\text{liquid-ice}}$ values for phenylalanine, serine, glycine, and alanine. Here, the magnitude of $\Delta^{13}\text{C}_{\text{liquid-ice}}$ does not appear to be driven by the strength of intermolecular forces. Basiuk and Gromovoy (1996)

measured the free energy of sorption between amino acids and silica, which is dominated by hydrogen bonds with surface silanol groups, using HPLC retention data. They found ΔG (J/mol) of -1480 for serine, -1370 for glycine, -1140 for alanine, -170 for leucine, and -130 for phenylalanine. Their free energies agree with the percent sorbed observed in this work, indicating similar free energies of sorption to ice surfaces, but are inconsistent with our measured $\Delta^{13}\text{C}_{\text{liquid-ice}}$ values.

Rather, we suggest the magnitude of $\Delta^{13}\text{C}_{\text{liquid-ice}}$ reflects the primary orientation of the amino acid on the ice surface and the participation of other position within the molecule in intermolecular interactions. All of the amino acids except serine display the largest $\Delta^{13}\text{C}_{\text{liquid-ice}}$ at the carboxyl position, indicating they primarily sorb through hydrogen bonds at the carboxyl position. But other positions display non-zero $\Delta^{13}\text{C}_{\text{liquid-ice}}$ values, indicating they are also affected by intermolecular forces during sorption. The data here suggest that participation of other positions in intermolecular forces during sorption lessens the magnitude of $\Delta^{13}\text{C}_{\text{liquid-ice}}$ at the carboxyl position. In the case of leucine, $\Delta^{13}\text{C}_{\text{liquid-ice}}$ is large relative to the other amino acids because its large aliphatic side chain would have minimal interaction with an ice surface. In contrast, phenylalanine and serine have aromatic and polar side chains, respectively, that are capable of interacting with an ice surface and thus display lower $\Delta^{13}\text{C}_{\text{liquid-ice}}$ values at the carboxyl position. If correct, this indicates that different mineral surfaces, such as kaolinite or montmorillonite, that have surface sites capable of interacting with other sites within the amino acid (i.e., hydrophobic siloxane surfaces, etc.) and may show different intramolecular isotope fractionation patterns than the ice surface. observed in alanine at neutral pH (Garcia et al., 2007). These intramolecular hydrogen bonds could disrupt intermolecular hydrogen bonds, thus lessening $\Delta^{13}\text{C}_{\text{liquid-ice}}$ at the carboxyl position.

An amino acid's preferred orientation on a surface potentially influences how readily it undergoes polymerization. Zaia (2007) pointed out that while charged amino acids sorb in higher proportion than neutral amino acids, they make up a smaller proportion of those within modern proteins. Subsequent work attempted to reconcile this discrepancy by identifying other mineral surfaces that preferentially sorb neutral amino acids. Our study potentially offers an alternative explanation, that neutral amino acids are more likely to sorb in an orientation conducive to polymerization than amino acids with polar side chains. During sorption of neutral amino acids, the carboxyl group participates in the hydrogen bond which lessens the carboxyl C's electron density. This makes it more susceptible to nucleophilic attack from another amino acid and, thus, more likely to polymerize (Fig 4-3). While this reaction is unlikely on an ice surface, due to low temperatures, other hydroxylated mineral surfaces could facilitate this reaction. In contrast, serine has a polar side chain that appears to shift the interaction with the ice surface to the alcohol-bound carbon (position 3), which makes the carboxyl site less likely to polymerize with a nucleophile. These effects will be more pronounced for amino acids with charged or polar functional sites on their side chain. We suggest this could be a factor in why the majority of proteins today are composed of neutral amino acids with non-polar side chains.

4.6 Conclusion

We presented $\Delta^{13}\text{C}_{\text{liquid-ice}}$ for amino acids sorbed to ice surfaces at neutral pH. Sorption caused significant C isotope effects in leucine, phenylalanine, serine, and glycine. Individual carbon positions demonstrated isotope fractionation up to 8 ‰, which is large enough to mask some primary intramolecular isotope signals associated with synthetic pathways. The largest fractionations were concentrated at the carboxyl position, consistent with its significant role in hydrogen bonding during sorption. The magnitude of fractionation at the carboxyl group was

dependent on whether functional groups on the side chain were involved in sorption to the ice surface. In the case of serine, the side-chain alcohol group had greater ^{13}C -enrichment at the alcohol group position than the carboxyl site, indicating diminished participation of the carboxyl group in hydrogen bonding. The data presented here suggest polar side chains that affect amino acid orientation on mineral surfaces will also decrease its tendency to polymerize. Neutral amino acids with non-polar side chains sorb primarily via hydrogen bonds at the carboxyl position, which promotes polymerization.

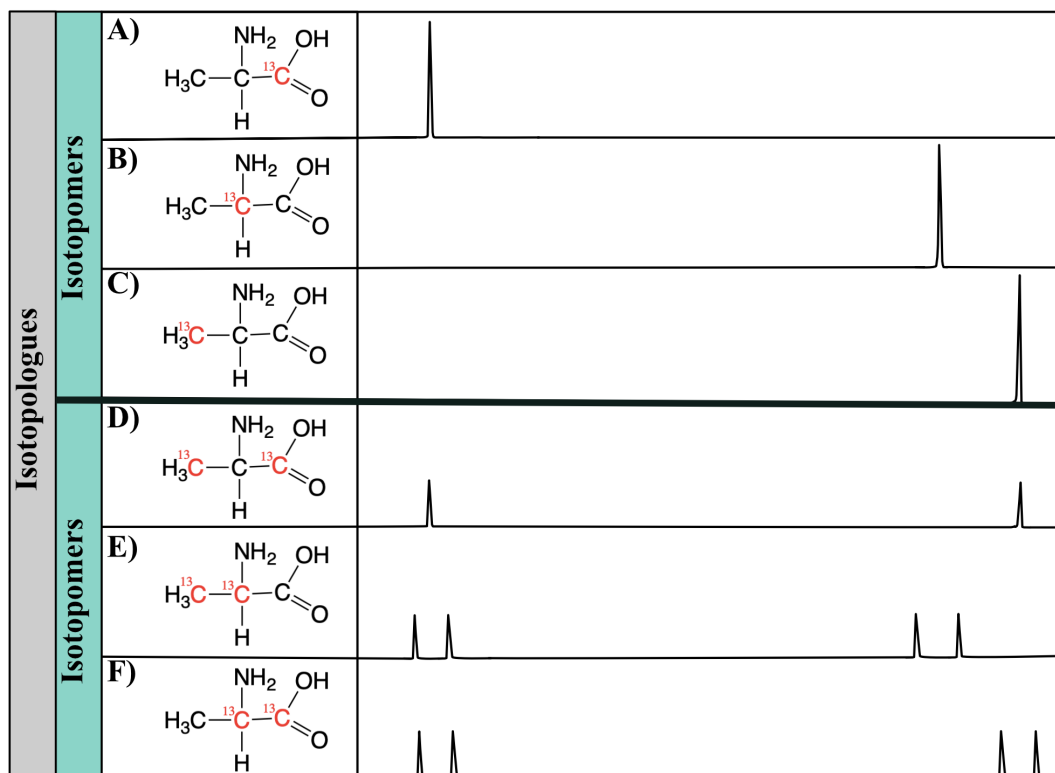


Figure 4-1: Isotopologue contributions to individual ^{13}C NMR peaks for alanine. There are two groups of isotopomers: A, B, and C and D, E, and F. Isotopologue D contributes approximately 1% to the main peak signals, assuming no or a very small long-range coupling between ^{13}C atoms. Isotopologues E and F have vicinal ^{13}C atoms and instead are satellites that do not contribute to main peak signals. Theoretically, a fully substituted alanine isotopologue ($^{13}\text{C}_3\text{H}_7\text{NO}_2$) would also contribute to observed peaks, but are too low abundance to detect via NMR.

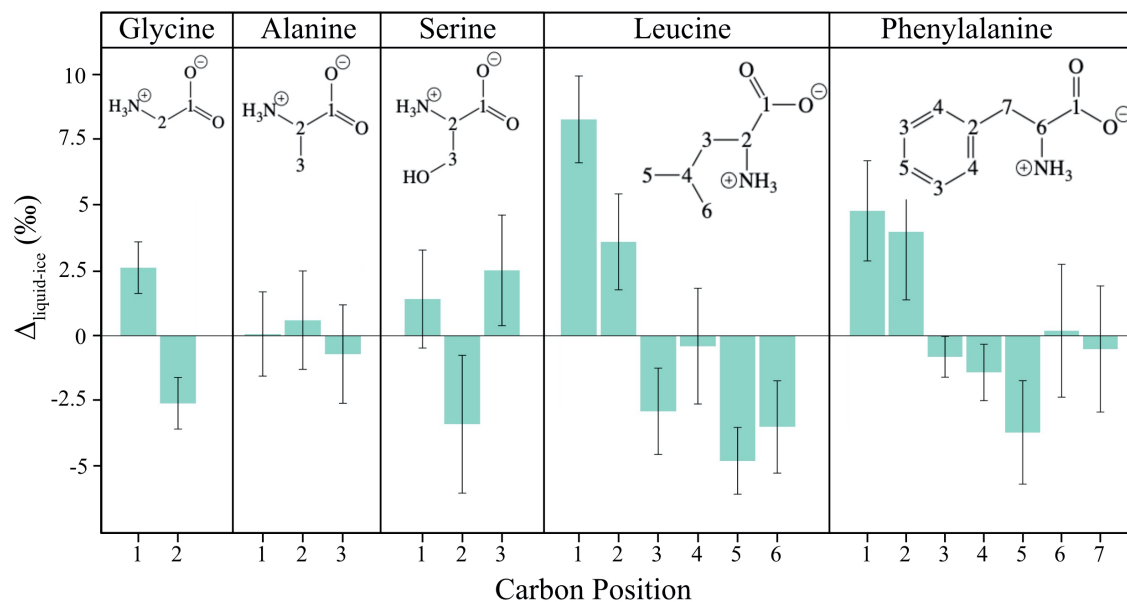


Figure 4-2: $\Delta^{13}\text{C}_{\text{liquid-ice}}$ for each position with carbon numbering and structure of each amino acid given above. Error bars calculated as the standard deviation of 5 analyses. Exact values given in Table C-2.

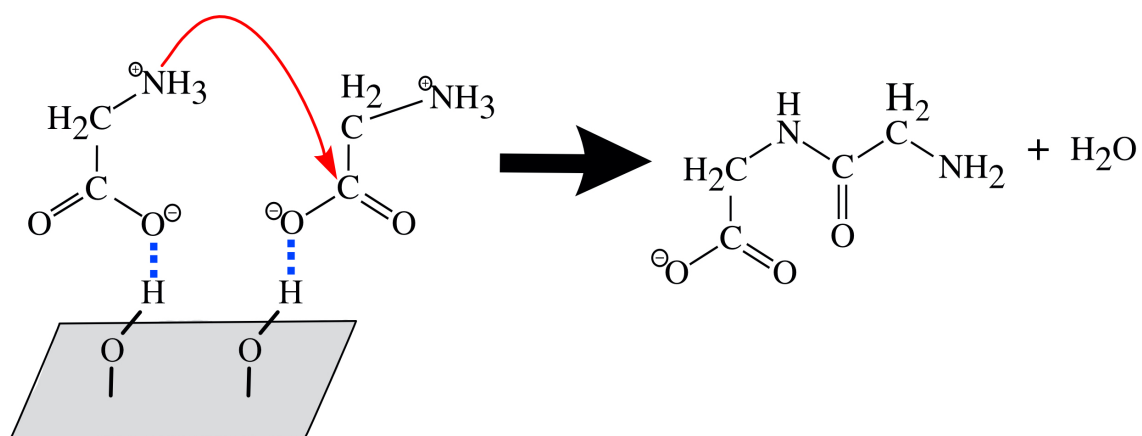


Figure 4-3: Possible surface-mediated polymerization reaction for glycine on a hydroxylated surface. Hydrogen bonds between the surface and carboxyl group weaken the carboxyl carbon making it more susceptible to nucleophilic attack by another neutral glycine, promoting polymerization. While amino acids are unlikely to polymerize on an ice surface due to low temperatures, amino acids will likely sorb in the same orientation on other hydroxylated mineral surfaces that are conducive to polymerization reaction.

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Chapter 5

Summary and Future Work

5.1 Research Summary

The isotopic and molecular attributes of organic matter preserved in the geologic record provide information about past ecologies and environments. Post-depositional processes associated with organic matter degradation and preservation can alter these attributes, complicating the interpretation of its isotopic and molecular composition. This dissertation constrains the effects of two post-depositional processes, degradation by exposure to γ radiation and preservation through organic-mineral interactions. The results of this work can be incorporated into interpretations of biomarkers in environments where these processes are prevalent.

Chapter 2 described the γ radiolysis products of macromolecular organic compounds in Mars-relevant mineral matrices. Our analyses revealed that formate and oxalate were the dominant products, and that their formation was independent of the type of starting organic material, or the mineral matrix. UV-driven organic acid formation has been attributed to Fenton chemistry in redox-sensitive minerals form radical species that break down organic molecules (Benner et al., 2000; Pignatello et al., 2006). Our results showed that organic acid formation took place, both with and without redox-sensitive species, indicating another mechanism for radical production was involved. We proposed that illuminating semiconductors with high energy radiation created electron ‘holes’ in mineral surfaces that interacted with sorbed gases to create radical species. The radicals then broke down larger organic compounds and polymers into small organic acids. Both Fenton chemistry and our proposed semiconductor mechanism likely

contributed to the break-down of macromolecular organic material. These reactions also have the potential to destroy the produced small organic acids. We therefore tested the stability of benzoate in a fused silica matrix and a Mars analogue matrix exposed to γ radiation. More benzoate was destroyed in the Mars analogue sample, but benzoate persisted within both mineral mixtures even after exposure to radiation doses equivalent to 6.6 Myrs on the surface of Mars. As a result, small acids produced from organic matter in the top 2 m of soil on the surface of Mars have the potential to persist for millions of years. Future missions to Mars that seek to study organic carbon should prioritize samples buried below 2 m or are otherwise shielded from radiation by geologic structures, as organic material that is exposed to γ radiation is not likely to retain its primary molecular characteristics.

Chapter 3 provided an experimental and density functional theory-based modeling study of isotope fractionation during non-covalent sorption between organic compounds and mineral surfaces. Organic-mineral interactions foster molecular preservation, and constraining their potential influence on the isotopic composition of preserved organics can aid and refine estimates of primary isotopic signals. In order to investigate H isotope effects associated with the sorption of *n*-octane to a kaolinite surface, we calculated the vibrational frequencies of sorbed and free octane using models that did and did not include anharmonic contributions. Calculations that incorporated anharmonicity predicted a 3.5 ‰ ^2H -depletion of sorbed octane, whereas purely harmonic calculations predicted an enrichment of 0.9 ‰. These results were compared with diffuse reflectance infrared Fourier transform spectra that indicated bond energies shifts which translate to a ^2H -depletion of 2 ‰ for sorbed octane, consistent with the anharmonic model. While both computational and experimental results indicate that isotopic effects associated with organic-mineral interaction are likely too minor to obscure significant primary isotope variations, they do show that non-substituted octane has a slightly stronger interaction with kaolinite than ^2H -enriched octane. Under the model and laboratory conditions in this study, interactions between

octane and the kaolinite surface were driven by van der Waals forces that are sensitive to bond polarizability, molecule surface area, and molecule volume. C-¹H bonds are longer than C-²H, which makes them more polarizable and can increase molecular volumes and surface areas, all of which promote stronger van der Waals interactions (Wade, 1999; Turowski et al., 2003).

Harmonic models do not account for differences in C-¹H and C-²H bond lengths, and thus they cannot accurately predict isotope effects driven by van der Waals forces. While the observed and predicted isotope fractionation associated with non-polar and therefore weak intermolecular forces were no more than a few permil, stronger intermolecular interactions have the potential to impart far more significant isotopic differences.

Chapter 4 investigated isotope fractionation during strong intermolecular interactions by measuring position-specific C isotope fractionation in neutral amino acids sorbed to an ice surface from an aqueous solution. Interactions between amino acids and minerals help protect amino acids and can promote polymerization reactions between amino acids (Hedges and Hare, 1987; Zaia, 2004). At circumneutral pH, neutral amino acids are in their zwitterionic form in aqueous solution, and tend to sorb to the ice surface primarily through hydrogen bonds (Carneiro et al., 2011). Using quantitative isotopic ¹³C NMR, we found that glycine, L-leucine, and L-phenylalanine showed the highest magnitude ¹³C-depletion at the carboxyl position, consistent with hydrogen bonds between the carboxyl group and ice surface. Of these, L-leucine had the highest magnitude of fractionation (8 ‰), which appeared to be driven by the participation of other sites within the molecule in the sorption interaction. Leucine has a long aliphatic chain that would not interact strongly with the surface hydroxyl groups on ice, as a result, the interaction was likely concentrated at the carboxyl group. In contrast, L-serine, the only amino acid with a polar side chain, had the highest ¹³C-depletion at the hydroxyl carbon, indicating hydrogen bonds preferentially form between its alcohol group and the ice surface. The orientation of amino acids on a mineral surface affect the efficiency of surface-mediated mineral interactions (Kitadai et al.,

2017). Sorption at the carboxyl group of an amino acid makes it more susceptible to nucleophilic attack, thus more likely to polymerize. Our work shows that neutral amino acids with non-polar side chains are more likely to sorb in this orientation than those with polar side chains, which could explain their dominance in modern proteins. Furthermore, the isotopic fractionation observed at some positions is large enough to mask primary isotope signals, indicating that organic-mineral interactions should be considered when interpreting intramolecular isotope signatures.

5.2 Future Work

The fluxes and fates of organic carbon, both in specific environments and at global scales, depends on processes that stabilize C and protect it from degradation (Conant et al., 2011). While it is generally accepted that chemical and physical protection mechanisms are vital to organic matter preservation, it is unclear how they influence each other (Schulten and Leinweber, 2000; Keil and Mayer, 2013). For example, a major mechanism of physical protection is aggregation, where soil components form large complexes that are inaccessible to microorganisms. While various properties contribute to soil's propensity to aggregate, organic-mineral interactions are central in aggregate formation (Elliott and Cambardella, 1991; Lützow et al., 2006). Even so, how specific organic-mineral interactions might aid aggregate formation is not well constrained, which makes it difficult to predict the potential for isotope and molecular alterations associated with sorption to influence organic matter preserved in the geologic record. Future work should address the prevalence and isotopic imprint of different organic-mineral interactions that form aggregates.

In Chapter 4, the largest ^{13}C depletion were observed at the carboxyl position in most of the studied amino acids. This functional group can form hydrogen bonds with hydroxyl groups on

an ice surface. Other positions also displayed ^{13}C depletions and enrichments although at lower magnitudes than the carboxyl site. Some of these positions are not capable of forming hydrogen bonds, indicating that weaker intermolecular forces involved in sorption (i.e., hydrophobic interactions, van der Waals forces) are also capable of imparting position-specific isotope effects. The direction and magnitude of isotope effects for non-covalent intermolecular forces have not been constrained. Previous work hypothesized that the magnitudes of such isotope effects would be controlled by the strength of intermolecular interactions (Julien et al., 2017). However, in this and the available published studies, it is difficult to discern a relationship between the magnitude of fractionation and strength of interaction because most sorbent-sorbate pairs can facilitate multiple types of interactions. Future work should combine position-specific isotope analysis with thermal desorption measurements to compare fractionation with the energy required to liberate sorbed molecules. Ideally, these experiments would be performed on simple systems with well-defined surfaces that limit the possible interactions between sorbates and sorbents.

Chapter 4 measured position specific C isotope fractionation caused by sorption to ice surfaces, which has similar properties to hydroxylated clay surfaces. Clay minerals were hypothesized to play a major role in the evolution of amino acids to form complex biomolecules on early Earth (Lahav et al., 1978; Erastova et al., 2017), thus understanding the properties of amino acids that influence these reactions is important to understanding the evolution of life. Unlike ice, clays and other mineral surfaces have many unique surface sites that are capable of interacting with amino acids via different intermolecular forces. Future work should expand sorption studies to minerals that were important on early Earth (i.e., montmorillonite and kaolinite) (Hazen, 2006; Lambert, 2008), and minerals that prevalent in planetary environments that were once habitable, such as iron oxide and iron clay minerals abundant on Mars (Bish et al., 2013; Vaniman et al., 2014). Furthermore, studying position-specific non-covalent isotope effects in other organic-mineral interactions relevant in interstellar dust and meteorites, is critical to

using position-specific isotope analysis to interpret organic synthetic pathways. Possible organic targets could include polycyclic aromatic hydrocarbons, small aliphatic hydrocarbons, and ketones, all of which have been detected in interstellar dust (Allamandola et al., 1987; Sandford et al., 2006) and meteorites (Botta and Bada, 2002).

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Appendix A

Supplemental Text and Tables for Chapter 2

A.1. Extended Discussion

A.1.1 The role of inorganic salts in organic acid destruction and formation

Chapter 2 investigated organic acid formation using different starting materials and mineral matrices. The role of inorganic salts in the formation or destruction of organic acids were also investigated by adding 5 wt % of either $\text{Ca}(\text{ClO}_4)_2$, NaCl , or CaSO_4 to select samples. The identification of organic acids in these samples were not possible because the anions overloaded the IC-MS column, causing retention time and peak shape issues. Dionex OnGuard II Ag and Ba Cartridges (Thermo) were used to remove chloride and sulfate ions, respectively, from NaCl - or CaSO_4 -containing samples. $\text{Ca}(\text{ClO}_4)_2$ -containing samples were not analyzed further as perchlorate anions were not easily removed. Organic acids were not detected in any of the treated samples. Several possibilities could explain the lack of organic acids: (1) cartridges did not completely remove all anions and the remaining peaks could overlap and obscure organic acid peaks such that organic acids were not detected, (2) the presence of salt inhibit the formation of organic acid and instead lead to the formation of alternate radiolysis products not visible to IC-MS analysis, and (3) the additional salts formed more radical species that degraded organic acids at a faster rate than they are formed.

Previous work has observed the formation of radicals from various inorganic salts, including perchlorate (Quinn et al., 2013) and oxides (Zacheis et al., 1999), supporting the latter hypothesis. However, interpretation of the lack of organic acids in CaSO_4 -containing samples is

complicated by a loss of benzoate in organic mixture control samples that were not exposed to radiation. The organic mixture contained 10 ppm of benzoate and between 5 and 8 ppm of benzoate was measured in equivalent salt-free samples, indicating the lack of benzoate is not due to inefficient extraction. Instead, benzoate was likely degraded or altered during the aqueous extraction phase. Sulfuric acid in the atmosphere is known to react with organic material to form new compounds (Hawkins et al., 2010; Wang et al., 2016) and it is often a component of wet oxidation methods for organic matter removal (Baldwin, 1996; Brändli et al., 2009). Taken together, the data presented here is not sufficient to interpret the role of salts in organic acid formation or degradation.

Future work can address this knowledge gap by using alternative extraction procedures and analytical techniques. Extraction procedures that completely remove salts from solution (i.e., column chromatography) will ensure that organic acid peaks are not obscured by high salt concentrations. Using analytical techniques in addition to IC-MS analysis will allow the identification of any alternative radiolysis products that are produced in the presence of salts.

Table A-1: Concentration and Standard Deviation for Organic Acids in Irradiated Samples.

This dataset in Excel format and raw IC chromatograms are available for download at:
<https://doi.org/10.26208/sgav-f021>

Dataset abbreviations are as follows:

BLANK – No organic matter added
 OM – Organic mixture of standards (defined in Chapter 2)
 KER – Archean Kerogen
 GB – Guaymas Basin sediment
 FA – Pony Lake fulvic acid
 GRS – Green River Shale
 FS – Fused silica
 A1 – Analog mineral mixture (defined in Chapter 2)
 CP – Calcium perchlorate
 CS – Calcium sulfate
 SC – Sodium chloride
 WET – Water present in sample
 DRY – No water present

Numbers following samples indicate the total dose of γ radiation in kGy. All concentrations are reported in ppm.

	Benzoate	Citrate	Formate	Oxalate	Phthalate
<i>BLANK 8-15</i>	0	0	0.37505	N/A	0
<i>A1XXXXRY500</i>	0	0	22.45215	N/A	0
<i>A1XXXXWET000</i>	0	0	0	N/A	0
<i>A1KERXXRY500</i>	0.0221	0	10.40495	N/A	0
<i>A1KERXXRY250</i>	0.02155	0	11.9737	N/A	0
<i>A1KERXXRY125</i>	0.0289	0	5.367	N/A	0
<i>A1KERXXRY063</i>	0.0379	0	2.15615	N/A	0
<i>A1KERXXWET250</i>	0.0305	0	34.75355	N/A	0
<i>FSGBSCWET250</i>	0	0	0	N/A	0
<i>BLANK 8-16</i>	0	0	0.4915	N/A	0
<i>FSGBCPWET250</i>	0	0	0	N/A	0
<i>FSGBSCRY000</i>	0	0	0	N/A	0
<i>FSGBXXRY000</i>	0	0	0	N/A	0
<i>FSGBCPRY000</i>	0	0	0	N/A	0

<i>FSOMXXDRY063</i>	3.0705	0	1.9168	N/A	0
<i>FSOMXXDRY125</i>	3.0354	0	2.0969	N/A	0
<i>FSXXXXDRY500</i>	0.28365	0	1.31915	N/A	0
<i>FSPYOMDRY250</i>	1.32055	0	0	N/A	0
<i>FSOMXXDRY500</i>	1.67995	0	1.4199	N/A	0
<i>A1OMXXWET250</i>	0	0	0	N/A	0
<i>A1OMXXDRY000</i>	2.19595	0	0	N/A	0
<i>FSPYOMDRY000</i>	1.1204	0	0	N/A	0
<i>FSOMXXDRY250</i>	2.8326	0	0.7636	N/A	0
<i>A1CSXXDRY000</i>	0	0	0	N/A	0
<i>A1CPXXWET000</i>	0	0	0	N/A	0
<i>A1CPXXDRY000</i>	0	0	0	N/A	0
<i>BLANK 8-17</i>	0.05925	0	0.61545	N/A	0
<i>FSFAXXDRY063</i>	0	0	0	N/A	0
<i>FSFAXXDRY500</i>	0	0	0	N/A	0
<i>FSKERXXDRY500</i>	0	0	0	N/A	0
<i>FSXXXXDRY000</i>	0.06935	0	0.06305	N/A	0
<i>FSFAXXDRY125</i>	0	0	0	N/A	0
<i>FSFAXXDRY250</i>	0	0	0	N/A	0
<i>FSKERXXDRY250</i>	0	0	0	N/A	0
<i>FSKERXXDRY063</i>	0	0	0	N/A	0
<i>FSFAXXDRY000</i>	0	0	0	N/A	0
<i>A1OMXXDRY063</i>	0.8614	0	0	N/A	0
<i>A1OMXXDRY250</i>	0.17425	0	0	N/A	0
<i>A1OMXXDRY125</i>	1.40915	0	0	N/A	0
<i>A1OMXXDRY500</i>	0.45375	0	0	N/A	0
<i>A1OMSCWET000</i>	0	0	0	N/A	0
<i>A1FAXXDRY500</i>	0	0	0	N/A	0
<i>A1FAXXDRY125</i>	0.2674	0	0	N/A	0
<i>A1GRSCPDRY000</i>	0	0	0	N/A	0
<i>A1GRSXXWET250</i>	0	0	0	N/A	0
<i>A1GRSCPDRY250</i>	0	0	0	N/A	0
<i>A1GRSCPWET250</i>	0	0	0	N/A	0
<i>A1FACPDY000</i>	0	0	0	N/A	0

<i>A1KERCPDRY250</i>	0	0	0	N/A	0
<i>A1FASCDRY000</i>	1.967	0	0	N/A	0
<i>A1KERSCWET250</i>	0	0	0	N/A	0
<i>BLANK 9-7</i>	0	0	0	N/A	0
<i>A1KERSCDRY250</i>	0	0	0	N/A	0
<i>A1KERCSDRY250</i>	0	0	0	N/A	0
<i>A1SCXXDRY250</i>	0	0	0	N/A	0
<i>A1FACSDRY250</i>	0	0	0	N/A	0
<i>A1FACPCDRY250</i>	0	0	0	N/A	0
<i>CSXXXXDRY000</i>	0	0	0	N/A	0
<i>A1FASCDRY250</i>	0	0	0	N/A	0
<i>CSXXXXWET000</i>	0	0	0	N/A	0
<i>CSOMXXDRY000</i>	0	0	0	N/A	0
<i>CSOMXXWET000</i>	0	0	0	N/A	0
<i>A1GRSXXDRY000</i>	0	0	0	N/A	0
<i>A1OMCPDRY250</i>	0	0	0	N/A	0
<i>A1SCXXWET000</i>	0	0	0	N/A	0
<i>A1FAXXDRY250</i>	0	0	0	N/A	0
<i>A1GRSXXDRY250</i>	0	0	0	N/A	0
<i>A1KERCPDRY000</i>	0	0	0	N/A	0
<i>A1GRSSCWET250</i>	0	0	0	N/A	0
<i>CSSCXXDRY125</i>	0	0	0	N/A	0
<i>CSSCOMDRY000</i>	0	0	0	N/A	0
<i>CSSCOMWET125</i>	0	0	0	N/A	0
<i>A1GRSSCDRY250</i>	0	0	0	N/A	0
<i>CSOMXXDRY250</i>	0	0	0	N/A	0
<i>CSSCXXDRY250</i>	0	0	0	N/A	0
<i>CSOMXXDRY125</i>	0	0	0	N/A	0
<i>CSSCOMWET250</i>	0	0	0	N/A	0
<i>CSOMXXWET125</i>	0	0	0	N/A	0
<i>CSSCOMWET000</i>	0	0	0	N/A	0
<i>A1FACPWET250</i>	0	0	0	N/A	0
<i>A1FAXXDRY063</i>	0	0	0	N/A	0

<i>A1OMCPWET00</i>	0.6807	0	0	N/A	0
<i>A1OMSCDRY000</i>	0.4181	0	0	N/A	0
<i>A1OMSCWET250</i>	0	0	0	N/A	0
<i>A1OMCPDRY000</i>	1.20225	0	0	N/A	0
<i>A1GRSSCDRY000</i>	0	0	0	N/A	0
<i>A1OMCPWET250</i>	0	0	0	N/A	0
<i>A1KERCPWET250</i>	0	0	0	N/A	0
<i>A1FAXXWET250</i>	0	0	0	N/A	0
<i>A1CSXXWET000</i>	0	0	0	N/A	0
<i>A1SCOMDRY250</i>	0	0	0	N/A	0
<i>A1FAXXDRY000</i>	N/A	0	0.2955	N/A	0
<i>FSFAXXDRY000</i>	N/A	0	0.0105	N/A	0
<i>A1KERXXDRY000</i>	N/A	0	0	N/A	0
<i>FSKERXXDRY000</i>	N/A	0	0	N/A	0
<i>A1OMXXDRY000</i>	N/A	0	0.2766	N/A	0
<i>FSOMXXDRY000</i>	N/A	0	0.0842	N/A	0

	Benzoate Standard Deviation	Citrate Standard Deviation	Formate Standard Deviation	Oxalate Standard Deviation	Phthalate Standard Deviation
<i>BLANK 8-15</i>	0	0	0.01732412	N/A	0
<i>A1XXXXDRY500</i>	0	0	3.40874966	N/A	0
<i>A1XXXXWET000</i>	0	0	0	N/A	0
<i>A1KERXXDRY500</i>	0.00127279	0	0.71764267	N/A	0
<i>A1KERXXDRY250</i>	0.00459619	0	1.12132993	N/A	0
<i>A1KERXXDRY125</i>	0	0	0	N/A	0
<i>A1KERXXDRY063</i>	0.00240416	0	0.11448059	N/A	0
<i>A1KERXXWET250</i>	0.00494975	0	4.12717015	N/A	0
<i>FSGBSCWET250</i>	0	0	0	N/A	0
<i>BLANK 8-16</i>	0	0	0.00919239	N/A	0
<i>FSGBCPWET250</i>	0	0	0	N/A	0
<i>FSGBSCDRY000</i>	0	0	0	N/A	0
<i>FSGBXXDRY000</i>	0	0	0	N/A	0
<i>FSGBCPDRY000</i>	0	0	0	N/A	0
<i>FSOMXXDRY063</i>	0.00749533	0	0.01329361	N/A	0

<i>FSOMXXDRY125</i>	0.06844794	0	0.06873078	N/A	0
<i>FSXXXXDRY500</i>	0.01152584	0	0.01491995	N/A	0
<i>FSPYOMDRY250</i>	0.01619275	0	0	N/A	0
<i>FSOMXXDRY500</i>	0.09086322	0	0.10238906	N/A	0
<i>A1OMXXWET250</i>	0	0	0	N/A	0
<i>A1OMXXDRY000</i>	0.02679935	0	0	N/A	0
<i>FSPYOMDRY000</i>	0.00183848	0	0	N/A	0
<i>FSOMXXDRY250</i>	0.05275017	0	0.01414214	N/A	0
<i>A1CSXXDRY000</i>	0	0	0	N/A	0
<i>A1CPXXWET000</i>	0	0	0	N/A	0
<i>A1CPXXDRY000</i>	0	0	0	N/A	0
<i>BLANK 8-17</i>	0.00275772	0	0.07827672	N/A	0
<i>FSFAXXDRY063</i>	0	0	0	N/A	0
<i>FSFAXXDRY500</i>	0	0	0	N/A	0
<i>FSKERXXDRY500</i>	0	0	0	N/A	0
<i>FSXXXXDRY000</i>	0.01195011	0	0.00120208	N/A	0
<i>FSFAXXDRY125</i>	0	0	0	N/A	0
<i>FSFAXXDRY250</i>	0	0	0	N/A	0
<i>FSKERXXDRY250</i>	0	0	0	N/A	0
<i>FSKERXXDRY063</i>	0	0	0	N/A	0
<i>FSFAXXDRY000</i>	0	0	0	N/A	0
<i>A1OMXXDRY063</i>	0.06519525	0	0	N/A	0
<i>A1OMXXDRY250</i>	0.00940452	0	0	N/A	0
<i>A1OMXXDRY125</i>	0.08704485	0	0	N/A	0
<i>A1OMXXDRY500</i>	0.0596091	0	0	N/A	0
<i>A1OMSCWET000</i>	0	0	0	N/A	0
<i>A1FAXXDRY500</i>	0	0	0	N/A	0
<i>A1FAXXDRY125</i>	0.00282843	0	0	N/A	0
<i>A1GRSCPDRY000</i>	0	0	0	N/A	0
<i>A1GRSXXWET250</i>	0	0	0	N/A	0
<i>A1GRSCPDRY250</i>	0	0	0	N/A	0
<i>A1GRSCPWET250</i>	0	0	0	N/A	0
<i>A1FACPDRY000</i>	0	0	0	N/A	0
<i>A1KERCPDRY250</i>	0	0	0	N/A	0
<i>A1FASCDRY000</i>	0.07297342	0	0	N/A	0

<i>A1KERSCWET250</i>	0	0	0	N/A	0
<i>BLANK 9-7</i>	0	0	0	N/A	0
<i>A1KERSCDRY250</i>	0	0	0	N/A	0
<i>A1KERCSDRY250</i>	0	0	0	N/A	0
<i>A1SCXXDRY250</i>	0	0	0	N/A	0
<i>A1FACSDRY250</i>	0	0	0	N/A	0
<i>A1FACPDRY250</i>	0	0	0	N/A	0
<i>CSXXXXDRY000</i>	0	0	0	N/A	0
<i>A1FASCDRY250</i>	0	0	0	N/A	0
<i>CSXXXXWET000</i>	0	0	0	N/A	0
<i>CSOMXXDRY000</i>	0	0	0	N/A	0
<i>CSOMXXWET000</i>	0	0	0	N/A	0
<i>A1GRSXXDRY000</i>	0	0	0	N/A	0
<i>A1OMCPDRY250</i>	0	0	0	N/A	0
<i>A1SCXXWET000</i>	0	0	0	N/A	0
<i>A1FAXXDRY250</i>	0	0	0	N/A	0
<i>A1GRSXXDRY250</i>	0	0	0	N/A	0
<i>A1KERCPDRY000</i>	0	0	0	N/A	0
<i>A1GRSSCWET250</i>	0	0	0	N/A	0
<i>CSSCXXDRY125</i>	0	0	0	N/A	0
<i>CSSCOMDRY000</i>	0	0	0	N/A	0
<i>CSSCOMWET125</i>	0	0	0	N/A	0
<i>A1GRSSCDRY250</i>	0	0	0	N/A	0
<i>CSOMXXDRY250</i>	0	0	0	N/A	0
<i>CSSCXXDRY250</i>	0	0	0	N/A	0
<i>CSOMXXDRY125</i>	0	0	0	N/A	0
<i>CSSCOMWET250</i>	0	0	0	N/A	0
<i>CSOMXXWET125</i>	0	0	0	N/A	0
<i>CSSCOMWET000</i>	0	0	0	N/A	0
<i>A1FACPWET250</i>	0	0	0	N/A	0
<i>A1FAXXDRY063</i>	0	0	0	N/A	0
<i>A1OMCPWET000</i>	0.02361737	0	0	N/A	0
<i>A1OMSCDRY000</i>	0.04200214	0	0	N/A	0
<i>A1OMSCWET250</i>	0	0	0	N/A	0
<i>A1OMCPDRY000</i>	0.11179358	0	0	N/A	0
<i>A1GRSSCDRY000</i>	0	0	0	N/A	0

<i>A1OMCPWET250</i>	0	0	0	N/A	0
<i>A1KERCPWET250</i>	0	0	0	N/A	0
<i>A1FAXXWET250</i>	0	0	0	N/A	0
<i>A1CSXXWET000</i>	0	0	0	N/A	0
<i>A1SCOMDRY250</i>	0	0	0	N/A	0
<i>A1FAXXDRY000</i>	N/A	N/A	N/A	N/A	N/A
<i>FSFAXXDRY000</i>	N/A	N/A	N/A	N/A	N/A
<i>A1KERXXDRY000</i>	N/A	N/A	N/A	N/A	N/A
<i>FSKERXXDRY000</i>	N/A	N/A	N/A	N/A	N/A
<i>A1OMXXDRY000</i>	N/A	N/A	N/A	N/A	N/A
<i>FSOMXXDRY000</i>	N/A	N/A	N/A	N/A	N/A

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Appendix B

Supplemental Text and Tables for Chapter 3

B.1. Extended Discussion

B.1.1. Modeling position-specific H isotope fractionation during *n*-octane sorption to kaolinite

Chapter 3 presents the global H isotope fractionation when *n*-octane sorbs to a kaolinite surface. The computational and experimental results displayed relatively minor isotopic fractionation, less than 3.5 ‰ difference between sorbed and free *n*-octane. However, the global $\Delta^2\text{H}_{\text{sorbed/free}}$ values presented in Chapter 3 are an average of individual $\Delta^2\text{H}_{\text{sorbed/free}}$ at each H atom position within octane. Individual $\Delta^2\text{H}_{\text{sorbed/free}}$ values can be significant (for H isotope analysis, > 10 ‰), even in cases where the global $\Delta^2\text{H}_{\text{sorbed/free}}$ is small. We calculated the position-specific isotope fractionation for each H atom within *n*-octane using the harmonic frequencies. Results are given in Figure **B-1** and Table **B-1**. The $\Delta^2\text{H}_{\text{sorbed/free}}$ values range from -19.95 to 39.92 ‰ at individual H atoms within octane, despite a global $\Delta^2\text{H}_{\text{sorbed/free}}$ values of 2.47 ‰.

Global $\Delta^2\text{H}_{\text{sorbed/free}}$ values calculated using harmonic vibrational frequencies did not correctly predict the direction of sorption-driven isotope effects, because harmonic approximations cannot account for differences in C-²H and C-¹H bond lengths. Generally, the harmonic approximation shows a ²H-enrichment in positions oriented towards the surface and a ²H-depletion in those oriented away from the surface. Based on the anharmonic model and experimental results of Chapter 3, it is more likely that individual positions oriented towards the surface would be depleted in ²H. While future work should determine position-specific

$\Delta^2\text{H}_{\text{sorbed/free}}$ values using anharmonic corrections, the harmonic approximation reasonably predicts the magnitude of global $\Delta^2\text{H}_{\text{sorbed/free}}$ values and therefore may provide a reasonable estimate of position-specific $\Delta^2\text{H}_{\text{sorbed/free}}$ values. The large position-specific $\Delta^2\text{H}_{\text{sorbed/free}}$ values predicted by the harmonic approximation indicate that intramolecular H isotope interpretations cannot ignore fractionation caused by van der Waals interactions between organics and minerals.

Previous work observed position-specific carbon isotope fractionation up to 3 ‰ during the distillation of heptane (Julien et al., 2017). Similar to sorption, isotopic fractionation associated with distillation is driven by van der Waals forces between molecules in the liquid phase, where isotopomers with weaker intermolecular interactions will vaporize first (Jancso and Van Hook, 1974). Octane sorption to siloxane surfaces on kaolinite is also driven primarily by van der Waals interactions (Jaynes et al., 1991; Lützow et al., 2006) and will likely also display position-specific isotope fractionation. In the case of position-specific H isotope fractionation, the larger relative mass difference and closer proximity to the kaolinite surface would lead to much larger magnitude H isotope effects than C isotope effects observed in heptane distillation (up to 3 ‰), consistent with the modeling results presented here. While the experimental data in Julien et al. (2017) indirectly support the modeled results that van der Waals interactions can impart large position-specific H isotope effects, direct experimental evidence is needed. Future work could use chiral oriented solvents to measure position-specific $^2\text{H}/^1\text{H}$ abundances of sorbed compounds using quantitative isotopic ^1H NMR to verify modeling results (Lesot et al., 2008).

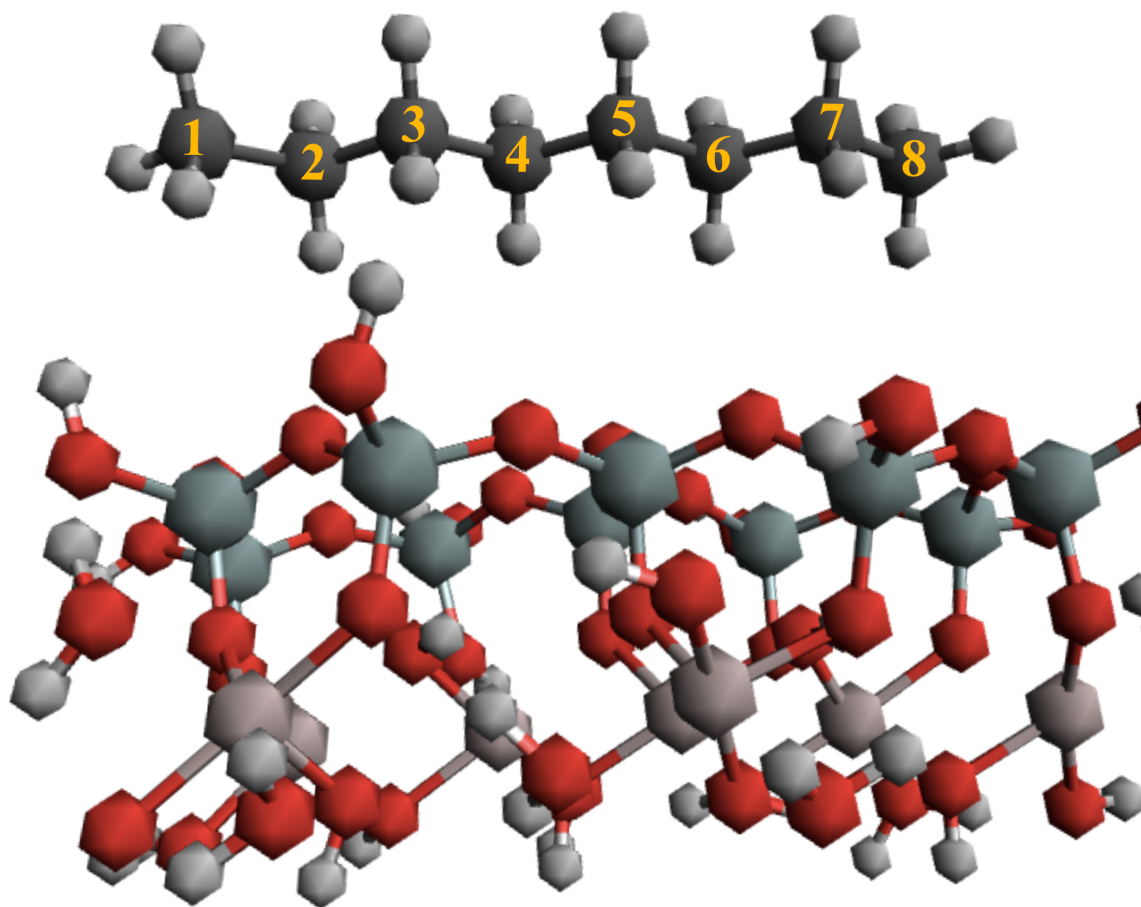


Figure **B-1**: Carbon numbering scheme for n-octane on kaolinite, carbon (black), hydrogen (white), oxygen (red), silicon (gray), and aluminum (pink). $\Delta^2H_{\text{sorbed/free}}$ values correspond to the carbon atom they are bonded to and are either oriented towards or away from the surface.

Table B-1: Position-specific $\Delta^2H_{\text{sorbed/free}}$ of n-octane during sorption to an (001) kaolinite surface calculated by DFT-calculated harmonic vibrations.

Carbon Number	1	2	3	4	5	6	7	8
<i>Oriented Toward the Kaolinite Surface</i>	-1.10, -11.41	39.92	-17.36	32.94	15.91	27.29	19.96	20.75
<i>Oriented Away from the Kaolinite Surface</i>	-4.98	-4.51	-7.07	-8.94	-9.57	-10.69	-7.21	-19.95, -9.51

Table B-2: Harmonic vibrational frequencies for sorbed and gaseous deuterated and protiated n-octane with corresponding molecular motion.

Protiated Gaseous	Deuterated Gaseous	Protiated Sorbed	Deuterated Sorbed	Molecular Motion
3083.83	2284.08	3070.2887	2274.0276	CH ₃ stretch, asymmetric, single CH ₃ , some coupling to adjacent CH ₂ stretches. CH ₃ 1 is slightly more positioned over edge sites vs "bulk surface". Terminal C-H is asymmetric with both other CH ₃ C-H
3083.81	2284.09	3067.7292	2271.1461	CH ₃ stretch, asymmetric, single methyl, some coupling to adjacent CH ₂ stretches. CH ₃ is slightly more positioned over "bulk surface" vs edge sites. Terminal C-H is asymmetric with both other CH ₃ C-H
3079.43	2280.57	3083.8624	2284.1222	CH ₃ stretch, asymmetric, single CH ₃ , some coupling to adjacent CH ₂ stretches. CH ₃ is slightly more positioned over "bulk surface" vs edge sites. Terminal C-H is symmetric with another CH ₃ C-H
3079.16	2280.46	3078.787	2279.4828	CH ₃ stretch, asymmetric, single CH ₃ , some coupling to adjacent CH ₂ stretches. Methyl is slightly more positioned over edge sites vs "bulk surface". Terminal C-H is symmetric with another CH ₃ C-H

3052.48	2261.94	3059.0146	2266.2463	CH ₂ stretch, asymmetric within. 0 nodes between CH ₂ groups
3042.53	2256.45	3054.1712	2263.2581	CH ₂ stretch, asymmetric within. 1 node between CH ₂ groups
3031.35	2249.03	3046.9276	2258.4068	CH ₂ stretch, asymmetric within. 2 nodes between CH ₂ groups
3019.38	2239.94	3034.9532	2247.4052	CH ₂ stretch, asymmetric within. 3 nodes between CH ₂ groups
3018.44	2168.13	3010.7233	2162.463	CH ₃ stretch, symmetric, mostly single CH ₃ , some symmetric coupling to other CH ₃ . Most active CH ₃ is slightly more positioned over "bulk surface" vs edge sites.
3018.37	2168.04	3009.2149	2161.0711	CH ₃ stretch, symmetric, mostly single CH ₃ , some asymmetric coupling to other CH ₃ . Most active CH ₃ is slightly more positioned over edge sites vs "bulk surface".
3010.39	2232.71	3018.9214	2231.8787	CH ₂ stretch, combination asymmetric and symmetric within. Either 4 nodes (lower H) or 2 nodes (upper H) between CH ₂ groups
3010.29	2230.14	3025.3593	2237.6177	CH ₂ stretch, combination asymmetric and symmetric within. Either 5 nodes (lower H) or 3 nodes (upper H) between CH ₂ groups
3007.24	2187.93	3006.6156	2195.4659	CH ₂ stretch, combination asymmetric and symmetric within. Either 3 nodes (lower H) or 2 nodes (upper H) nodes between CH ₂ groups.
3007.04	2188.59	3002.622	2188.9596	CH ₂ stretch, combination asymmetric and symmetric within. Either 0 nodes (lower H) or 4 nodes (upper H) nodes between CH ₂ groups.
3000.35	2182	2997.4762	2184.5011	CH ₂ stretch, combination asymmetric and symmetric within. Either 1 node (lower H) or 3 nodes (upper H) nodes between CH ₂ groups

2993.71	2179.8	2990.574	2178.5952	CH ₂ stretch, mostly symmetric within. Maybe 2 nodes between CH ₂ groups
2991.99	2176.04	2991.8614	2177.9181	CH ₂ stretch, symmetric within. 2 nodes between CH ₂ groups
2990.27	2175.33	2986.896	2172.3737	CH ₂ stretch, symmetric within. 1 node between CH ₂ groups

Table B-3: Harmonic and Anharmonic Gaseous Vibrational Modes for n-octane.

Protiated <i>n</i> -octane		Deuterated <i>n</i> -octane	
Harmonic Mode	Corresponding Anharmonic Mode	Harmonic Mode	Corresponding Anharmonic Mode
3083.831	2947.418	2284.084	2206.781
3079.432	2941.956	2280.571	2203.948
3052.482	2910.709	2261.934	2186.929
3031.36	2892.427	2249.041	2164.233
3018.438	2831.319	2232.713	2154.705
3010.394	2871.032	2187.928	2095.71
3007.246	2856.017	2179.796	2084.725
2993.717	2794.514	2176.05	2075.341
2991.996	2836.719	2168.13	2037.955
1512.516	1472.45	1262.169	1223.976
1501.399	1460.32	1216.366	1184.144
1499.422	1458.297	1159.457	1136.367
1489.752	1446.695	1126.873	1099.32
1486.384	1445.527	1112.226	1088.656
1415.112	1385.782	1099.2	1080.313
1400.985	1369.298	1081.762	1061.406
1385.836	1357.119	1080.327	1058.416
1338.056	1308.206	1032.245	1015.277
1317.535	1292.582	1028.992	1012.1
1303.619	1281.428	1004.495	986.661
1234.727	1208.06	992.081	973.987
1153.634	1128.188	963.191	945.245
1066.451	1041.065	892.784	878.555
1051.108	1025.94	869.499	856.774

1038.895	1025.101	839.913	829.368
1003.021	981.326	748.526	741.065
904.683	888.149	722.531	715.543
881.365	874.002	657.577	653.806
756.436	753.09	556.782	554.604
732.258	729.956	529.902	527.035
470.85	469.831	406.712	405.599
272.782	270.734	249.4	244.67
243.34	222.851	177.887	167.165
194.493	191.497	171.053	166.779
158.034	161.014	131.155	130.661
66.02	64.075	51.769	49.84
46.186	46.828	40.725	34.659
3083.813	2947.331	2284.089	2207.34
3079.161	2941.767	2280.456	2204.176
3042.536	2902.692	2256.449	2177.635
3019.397	2880.775	2239.955	2168.702
3018.366	2880.247	2230.138	2119.495
3010.292	2847.347	2188.592	2087.138
3007.04	2867.169	2182.011	2075.39
3000.357	2841.112	2175.334	2086.144
2990.278	2832.852	2168.042	2061.418
1516.835	1476.423	1250.053	1211.798
1506.254	1466.522	1165.351	1138.756
1501.382	1461.377	1132.8	1104.5
1493.08	1450.452	1117.008	1094.575
1485.663	1440.199	1109.474	1087.945
1414.306	1384.782	1081.795	1060.138
1401.94	1369.346	1081.794	1061.151
1350.439	1324.571	1077.373	1057.971
1338.186	1308.891	1036.372	1017.507
1327.212	1300.567	1018.573	1000.174
1280.157	1252.38	987.856	971.654
1251.889	1230.276	985.715	969.684
1204.043	1176.171	943.371	922.501
1099.189	1078.472	910.589	896.98
1063.748	1038.576	879.54	865.23
1010.531	986.749	855.339	844.102
963.84	954.737	716.988	710.221
889.617	874.429	711.335	706.101

806.954	803.993	600.73	598.793
735.798	733.367	535.487	532.739
468.5	463.866	415.507	411.084
337.813	339.68	293.029	294.33
246.236	226.78	181.167	170.724
133.906	133.059	107.872	106.154
108.721	111.715	92.073	89.239
77.796	76.89	69.445	61.681

B.2 References

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Appendix C

Supplemental Text and Tables for Chapter 4

C.1 Density Functional Theory Modeling of Intramolecular Isotope Fractionation during Sorption

C.1.1 Methods

Gaussian 09 density functional theory (DFT) energy-minimization calculations were used to determine how the equilibrium distribution of ^{13}C in the neutral and zwitterionic forms of glycine, L-alanine, and L-leucine were affected by its interaction with an ice surface. Models were initially energy minimized using the B3LYP-D/6-31G(d,p) level of theory and underwent subsequent energy-minimization calculations at the B3LYP/6-311++G(d,p) level of theory to improve the precision of their calculated intermolecular interaction energies. The DFT-calculated vibrational frequencies and a statistical mechanics partition function (Fig C-1) were used to determine the $\Delta^{13}\text{C}_{\text{Liquid-Ice}}$ at each carbon position. Position-specific $\Delta^{13}\text{C}_{\text{Liquid-Ice}}$ were averaged over each molecule to calculate global $\Delta^{13}\text{C}_{\text{Liquid-Ice}}$.

C.1.2. Results and Discussion

Computational results predict a ^{13}C -depletion in all amino acids in the liquid phase relative to the sorbed phase. In contrast, the experimental results presented in Chapter 4 and previous experimental results (Dias and Freeman, 1997; Schüth et al., 2003; Botosoa et al., 2007) displayed a ^{13}C -enrichment in the liquid phase relative to the sorbed phase. In Chapter 3, the similar discrepancy between computational and experimental results was attributed to the inability of harmonic approximations to account for differences in C- ^1H and C- ^2H bonds. The

longer C-¹H bonds in *n*-octane were more hydrophobic (Wade, 1999) and thus interacted with the kaolinite surface via van der Waals forces more strongly than the perdeuterated *n*-octane. Bond length differences between ¹³C- and ¹²C-compounds are significantly smaller than bond length differences between ²H- and ¹H-compounds, but may still be significant in imparting non-covalent isotope effects.

The smaller molar volume and bond length of ¹³C-compounds decreases its polarizability compared to ¹²C-compounds. While the strength of a hydrogen bond is mainly driven by the electronegativity of the heteroatom (i.e., F, N, O, etc.), computational work has found that polarizability can influence its strength as well. Increased polarizability strengthened hydrogen bonds (Xu et al., 2002), suggesting that more polarizable ¹²C-compounds may form stronger hydrogen bonds with an ice surface. Furthermore, the polarizability of a molecule is sensitive to the presence of intramolecular hydrogen bonds, which could act to weaken intermolecular hydrogen bonds (Dong et al., 2007; Yang et al., 2010). Further investigation is needed to determine if the difference in polarizability of amino acid isotopomers is responsible for the observed isotope effects during sorption to ice surfaces. However, the inability of the harmonic model to accurately predict the sorption isotope effects supports that these effects are in part caused by differences in bond length and molar volume between ¹²C- and ¹³C-compounds influencing the strength of intermolecular interactions.

C.2 R Script for Peak Intergration using rnmrfit Package

This is a sample R script to integrate peaks for a ¹³C NMR spectra of alanine.

```
# R script to calculate peak areas from NMR spectra

# This script uses the R package 'rnmrfit' documentation can be
found at
# https://rdr.io/github/ssokolen/rnmrfit/man/
```

```
library(rnmrfit)
setwd("/opt/topspin4.0.8/examdata/20200224_ACIRE/27")

# Load raw data file from topspin

nmrdata <-
nmrdata_1d('/opt/topspin4.0.8/examdata/20200224_ACIRE/27')

# Set range for individual peak

range <- filter_1d(nmrdata, 174.25, 175)

# Identify peak center within the given range

peaks <- ('174.75 s')

# Make scaffold, including phase correction and a baseline
correction with degree 2

scaffold <- nmrscaffold_1d(peaks, range, include.phase = TRUE,
baseline.degree =2)
fit1 <- nmrfit_1d(scaffold)
area1 <- calc_area(fit1)

# Repeat for subsequent peaks

range <- filter_1d(nmrdata, 49.84,50.84)
peaks <- ('50.34 s')
scaffold <- nmrscaffold_1d(peaks, range, include.phase = TRUE,
baseline.degree =2)
fit2 <- nmrfit_1d(scaffold)
area2 <- calc_area(fit2)

range <- filter_1d(nmrdata, 15.4,16.4)
peaks <- ('15.9 s')
scaffold <- nmrscaffold_1d(peaks, range, include.phase = TRUE,
baseline.degree =2)
fit3 <- nmrfit_1d(scaffold)
area3 <- calc_area(fit3)
```

The partition function (β) is calculated by the relationship:

$$\beta = \prod_{i=1}^{3N-6} \left(\frac{\nu_i^a}{\nu_i^b} \right) \left(\frac{\exp[h\nu_i^a/2k_B T]}{\exp[h\nu_i^b/2k_B T]} \right) \left(\frac{1 - \exp[h\nu_i^b/k_B T]}{1 - \exp[h\nu_i^a/k_B T]} \right)$$

Where ν is the frequency in Hertz (s^{-1}), calculated from each $\tilde{\nu}$ (cm^{-1} , calculated by the model) using the relationship, $\nu = \tilde{\nu}c$, where c is the speed of light ($3 \times 10^{10} \text{ cm} \cdot \text{sec}^{-1}$). T is 298K, h is the Planck constant ($1.520 \times 10^{-16} \text{ Hartree} \cdot \text{sec}^{-1}$), and k_B is the Boltzmann constant ($3.167 \times 10^{-6} \text{ Hartrees} \cdot \text{K}^{-1}$). The superscripts “a” and “b” on ν refer to the non-substituted and substituted forms of the models, respectively. After β has been calculated (a unitless quantity) for the sorbed and unsorbed molecule, the fractionation factor (α) can be calculated by:

$$\ln(\alpha_{\text{free/sorbed}}) = \ln(\beta_{\text{free}}) - \ln(\beta_{\text{sorbed}})$$

In this equation, negative $\ln(\alpha)$ indicate that the sorbed molecule is enriched relative to the free.

Figure C-1: Statistical Mechanics Partition Function used to calculate isotopic fractionation in sorbed versus free amino acids.

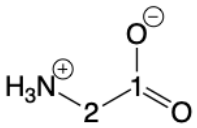
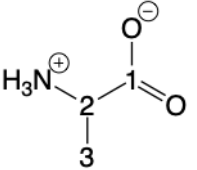
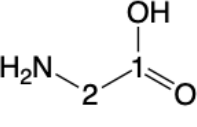
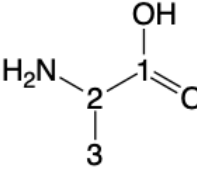
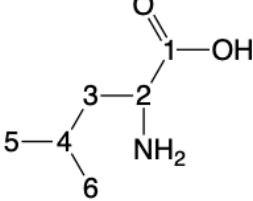
Table C-1: Bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for amino acids. All values given in ‰, errors calculated as the standard deviation of two analyses.

Sample	$\delta^{13}\text{C}_{\text{VPDB}}$ (in ‰)	$\delta^{15}\text{N}_{\text{AIR}}$
Glycine Standard	-25.31 ± 0.08	0.79 ± 0.12
Glycine Liquid	-25.27 ± 0.01	0.60 ± 0.05
Glycine Ice	-25.31 ± 0.07	0.60 ± 0.28
Alanine Standard	-18.88 ± 0.01	-2.98 ± 0.00
Alanine Liquid	-18.89 ± 0.02	-3.11 ± 0.03
Alanine Ice	-18.90 ± 0.02	-2.95 ± 0.15
Leucine Standard	-13.72 ± 0.01	1.38 ± 0.10
Leucine Liquid	-13.76 ± 0.01	1.38 ± 0.17
Leucine Ice	-13.78 ± 0.04	1.43 ± 0.09
Leucine Standard 2	-13.80 ± 0.02	1.81 ± 0.03
Leucine Liquid 2	-13.84 ± 0.03	1.81 ± 0.04
Leucine Ice 2	-13.89 ± 0.07	1.73 ± 0.11
Serine Standard	-8.27 ± 0.17	-3.02 ± 0.04
Serine Liquid	-8.07 ± 0.08	-2.95 ± 0.00
Serine Ice	-8.23 ± 0.09	-3.06 ± 0.01
Phenylalanine Standard	-11.94 ± 0.03	1.74 ± 0.06
Phenylalanine Liquid	-12.00 ± 0.00	1.75 ± 0.04
Phenylalanine Ice	-12.05 ± 0.03	1.89 ± 0.00

Table C-2: Position-specific $\Delta^{13}\text{C}$ for amino acids. All values given in ‰, errors calculated as the standard deviation of five analyses.

Amino Acid	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7
<i>Gly</i>	2.6 ± 1.0	-2.6 ± 1.0	-	-	-	-	-
<i>L-Ala</i>	0.1 ± 1.6	0.6 ± 1.9	-0.7 ± 1.9	-	-	-	-
<i>L-Ser</i>	1.4 ± 1.9	-3.4 ± 2.6	2.5 ± 2.1	-	-	-	-
<i>L-Leu</i>	8.3 ± 1.7	3.6 ± 1.8	-2.9 ± 1.7	-0.4 ± 2.2	-4.8 ± 1.3	-3.5 ± 1.8	-
<i>L-Phe</i>	4.8 ± 1.9	4.0 ± 2.6	-0.8 ± 0.8	-1.4 ± 1.1	-3.7 ± 2.0	0.2 ± 2.5	-0.5 ± 2.4

Table C-3: DFT predictions of global and position-specific $\Delta^{13}\text{C}_{\text{Liquid-Ice}}$ (in ‰) for neutral and zwitterion amino acids sorbed to an ice surface.

Amino Acid	1	2	3	4	5	6	Global $\Delta^{13}\text{C}_{\text{Liquid-Ice}}$
 Glycine Zwitterion	-1.69	-3.27	-	-	-	-	-2.48
 Alanine Zwitterion	-4.50	-4.62	+0.61	-	-	-	-2.84
 Glycine Neutral	-2.18	+0.68	-	-	-	-	-0.75
 Alanine Neutral	-2.76	-0.87	-2.87	-	-	-	-2.17
 Leucine Neutral	-1.71	-0.60	-0.02	+0.76	-0.07	-0.33	-0.33

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