CO₂ HYDRATION AND HYDROXYLATION:  
THE ORIGIN OF CARBONATE KINETIC ISOTOPE EFFECTS

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ABSTRACT

Stable isotope ratios in carbonate minerals record the influence of several climatological and biogeochemical processes. This makes biogenic carbonates valuable inventories of climate proxy records, but also makes them challenging to interpret. Carbonate formation from carbon dioxide via the unequilibrated CO₂ hydration and hydroxylation reactions is one process that may impact stable C, O and clumped C-O isotope ratios in carbonate minerals, particularly carbonate minerals formed by corals. This dissertation reports calculation of the isotopic influence of the CO₂ hydration and hydroxylation reactions using computational chemistry models. Results are compared with observed isotopic trends in corals to determine whether these reactions are consistent with the observed vital effects in corals. Methodological considerations for the application of computational chemistry to the calculation of aqueous isotopic fractionation are reported.

We analyzed several computational chemistry schemes for their ability to predict gas-phase isotope fractionation between CO₂ and H₂O. We also tested their ability to predict several other experimentally observable properties and whether success predicting each property correlated with success predicting isotope fractionation. Only successful prediction of harmonic vibrational frequencies correlated with successful prediction of isotopic fractionation; neither energies nor bond distances are good indications of a model chemistry useful in calculation of isotopic fractionation. B3LYP and X3LYP coupled with Pople triple-zeta basis sets were selected for application to fractionation in aqueous DIC species.

Stable C and O isotope partitioning constants were calculated for both equilibrium fractionation between aqueous CO₂ and H₂CO₃/HCO₃⁻ and for kinetic fractionation during the CO₂ hydration and hydroxylation reactions. Predicted equilibrium fractionation agrees well with experimentally determined values. The CO₂ hydration reaction is predicted to discriminate
against both $^{13}$C and $^{18}$O by 10-11‰, while the CO$_2$ hydroxylation reaction is predicted to discriminate against $^{13}$C by 13-16‰ and against $^{18}$O by 19-21‰. When calculating aqueous fractionation factors, it was necessary to analyze the H-bonding environment and interpolate to the expected H-bond environment experienced by each aqueous species, as individual H-bonds were found to have substantial effects on equilibrium and kinetic isotope fractionations, often of magnitude >1‰ and occasionally of magnitude >3‰ absolute deviation in predicted fractionation factor. H-bonds to hydroxyl groups from H$_2$O always reduced the amount of $^{13}$C and $^{18}$O entering reaction products, while other H-bonds increased the amount of $^{13}$C and $^{18}$O entering reaction products when effects were statistically significant, except for H-bonds from attacking OH$^-$ during CO$_2$ hydroxylation. Fractionation during CO$_2$ hydration and hydroxylation is able to explain most but not all of the isotope disequilibrium observed in the skeletons of shallow-water corals.

Isotope clumping between $^{13}$C and $^{18}$O was calculated during the CO$_2$ hydration and hydroxylation reactions. When accounting for H-bond environment, hydration and hydroxylation increase clumping in product carbonates by 0.10‰ and 0.12‰ respectively. These results are consistent with the increased clumping observed in some shallow-water corals relative to other carbonates. All H-bonds are predicted to decrease the clumping in product carbonates except for H-bonds from water to an attacking OH$^-$ during CO$_2$ hydroxylation. H-bond effects on clumping and single stable isotope fractionation are not consistent with a simple model of stiffer bonds favoring incorporation of both heavy isotopes and clumped isotopologues.

Formation of transient carbonate precursor phases may also affect the isotopic composition of coral skeletons and other biogenic carbonates; however, isotopic compositions of these precursor phases are currently unknown. Synthesized amorphous calcium carbonate (ACC) observed under XRD, FTIR, and Raman bears similar structural features with biogenic stable and transient ACC, depending on concentration of metasilicate stabilizer used. The isotopic
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Chapter 1

Introduction to Aqueous CO₂ Reactions and Coral Paleoclimate Proxies

Introduction

This chapter offers a short introduction to the CO₂ hydration and hydroxylation reactions, which are important in a wide range of geochemical processes. The chapter introduces corals as a potential isotopic proxy record source and describes why they are currently difficult to use, including the possibility that their isotopic compositions are influenced by the CO₂ hydration and hydroxylation reactions. The chapter describes computational chemistry as a useful tool for modeling the expected effects of different processes on isotopic fractionation, including the CO₂ hydration and hydroxylation reactions in the dissolved inorganic carbon (DIC) system which includes H₂CO₃, HCO₃⁻, and CO₃²⁻. The rest of the dissertation then applies computational chemistry to these two reactions to determine the magnitude of their expected isotopic effects.

CO₂ Hydration and Hydroxylation

Aqueous reactions involving carbon dioxide (CO₂) are common at Earth’s surface. The formation of carbonic acid (H₂CO₃) and bicarbonate (HCO₃⁻) proceeds via the CO₂ hydration and hydroxylation reactions:

\[ CO₂ + H₂O \rightarrow H₂CO₃ \rightarrow HCO₃⁻ + H⁺ \] (CO₂ hydration) (1.1)

\[ CO₂ + OH⁻ \rightarrow HCO₃⁻ \] (CO₂ hydroxylation) (1.2)

These reactions are important in various biological, geological, and biogeochemical processes, including carbonate skeleton formation of calcifying organisms like foraminifera and
corals, marine photosynthesis, ocean CO$_2$ uptake, and natural rainwater acidification (Johnson 1982; Cohen and McConnaughey 2003; Bogan et al. 2009). As a result, these reactions have an important impact on the carbon cycle and the composition of biogenic carbonates that serve as sources of proxy records (McCrea 1950; Kump and Arthur 1999). Calcifying organisms build their calcium carbonate skeletons using aqueous bicarbonate and carbonate ions. Isotopic fractionation occurs during formation of bicarbonate by both reaction (1.1) and (1.2), potentially affecting how biogeochemical proxies are interpreted over geologic time.

**Coral Carbonate Proxies and Vital Effects**

Carbonate-based paleoclimate proxies often rely on the assumption that precipitation of carbonate minerals occurs at isotopic equilibrium (Ravelo and Hillaire-Marcel 2007). Many taxa of organisms display apparent isotopic equilibrium in the CaCO$_3$ skeletons they build, including mollusks, brachiopods, and planktonic foraminifera; however, CaCO$_3$ created by corals displays isotopic disequilibrium with surrounding fluids (Weiner and Dove 2003). Coral skeletons tend to favor $^{16}$O and $^{12}$C more than minerals precipitated at equilibrium, with $\delta^{13}$C and $\delta^{18}$O in non-symbiotic corals exhibiting a linear trend (Figure 1-1). Symbiotic corals’ carbonate $\delta^{13}$C values are modified by preferential use of isotopically light CO$_2$ during photosynthesis by symbiotic algae, but they display similar variation in $\delta^{18}$O to non-symbiotic corals. Isotopic disequilibria in biogenic CaCO$_3$ are called “vital effects” because they are caused by biological processes. Inorganically-precipitated minerals formed in laboratory experiments do not display isotopic disequilibrium, unless they are precipitated from concentrated solutions (McConnaughey 1989b; Kim and O’Neil 1997), implying the source of vital effects is related to fast formation of calcium carbonate or another biologically-mediated process.
Vital effects force calibrations of paleoclimate proxies to be empirical in nature, subject to large potential errors in extrapolation to different chemical conditions over long periods of time, and different species over long evolutionary timescales. Most coral studies are species-specific calibrations of corals from one locale grown over ~100 years or less in order to establish a local $\delta^{18}O$ - sea surface temperature (SST) calibration slope, and analyze the local effects of salinity, hydrology, and other variables. This is necessary because calibration slopes vary widely (0.18 - 0.24‰ per °C) between locales, species, and individuals (Guilderson and Schrag 1999; Linsley et al. 1999; Felis et al. 2003; Grottoli and Eakin 2007). There is no commonly accepted physical basis accounting for all the discrepancies between local calibrations, although salinity, SST, and precipitation are known to contribute (Urban et al. 2000) and vital effects are often noted as possible contributing factors. Vital effects in corals have been attributed to pH-dependent kinetic fractionation during hydration and hydroxylation of CO$_2$ (McConnaughey 1989a; 1989b; 2003) or to pH gradients in the calcifying space (Adkins et al. 2003). Crystallization via an amorphous calcium carbonate (ACC) intermediate has not been extensively investigated but may affect the final isotopic signature (Roillon-Bard et al. 2009). Correcting for coral vital effects requires a more complete understanding of the mechanisms that control them.

**Coral Calcification Process**

Research into the mechanism of coral vital effects has focused on creating a model that explains trends among calcifying fluid pH, coral growth rate, and coral skeleton $\delta^{13}C$ and $\delta^{18}O$. Because coral skeletons are intricate and fragile, and because the coral skeletons are precipitated in small spaces isolated from external fluids, it has proven difficult to experimentally investigate the chemical conditions of coral calcification. Thus researchers have been forced to make several assumptions about the process of calcification, notably regarding the membrane fluxes of
different dissolved inorganic carbon (DIC) compounds, and the rates of H\(^+\)-transfer and precipitation reactions.

CaCO\(_3\) in corals precipitates as aragonite in the calcifying space, a thin pocket of fluid between the calicoblastic membrane and the growing coral skeleton (Cohen and McConnaughey 2003; Figure 1-2). As a typical phospholipid bilayer, the calicoblastic membrane is impermeable to small ionic species including HCO\(_3^-\) and CO\(_3^{2-}\), but is permeable to small nonpolar species including CO\(_2\). Precipitation of aragonite is induced primarily by a Ca\(^{2+}\)/H\(^+\) antiporter ATPase, which both raises the concentration of Ca\(^{2+}\) and the pH in the calcifying space (Cohen and McConnaughey 2003). Both processes increase the supersaturation state of the calcifying fluid with respect to CaCO\(_3\), causing precipitation. Growth rate influences aragonite morphology and isotopic composition, partially because the Ca\(^{2+}\) ATPase is up-regulated during symbiont photosynthesis/high light conditions driving growth. Trace compound concentrations and slow growth of morphologically distinct aragonite suggest the coral skeleton continues to interact with seawater after deposition, possibly due in part to vacuole transport (Ip and Krishvaneni 1991).

Nonequilibrium isotope fractionation can occur at multiple points during the coral calcification process. DIC introduced into the calcifying space may be out of isotopic equilibrium with H\(_2\)O and DIC in seawater due to the addition of CO\(_2\) affected by cellular respiration or photosynthesis by symbionts. DIC that ultimately enters the calcifying space may be fractionated by diffusion or by selective introduction of some DIC species over others. Conversion of CO\(_2\) to HCO\(_3^-\) and CO\(_3^{2-}\) may be out of equilibrium due to CO\(_2\) hydration and hydroxylation reactions that have not reached equilibrium. Kinetic fractionation may also occur as a result of fast precipitation that is too fast to re-equilibrate (Weiner and Dove 2003). Given the many possible sources of nonequilibrium fractionation in corals and their importance in interpreting coral paleoclimate proxies, the magnitudes of these sources must be determined.
Carbonic Anhydrase in Corals

Carbonic anhydrase catalyzes the CO$_2$ hydration reaction and thus may be able to reduce or eliminate nonequilibrium kinetic fractionation due to conversion of CO$_2$ to other DIC species. The role of carbonic anhydrase in mediating conversion of CO$_2$ to other DIC species during precipitation is not fully known (Bertucci et al. 2013). Carbonic anhydrase is not known to be present in the calcifying space itself, although it is known to be present in some coral tissues. However, if it were present in high enough quantities, it would rapidly equilibrate O isotopes between CO$_2$(aq) and other DIC species by increasing the rate of the CO$_2$ hydration reaction, leaving only precipitation effects on fractionation. Precipitation of calcium carbonate in the presence of carbonic anhydrase only shows a depletion of 1-2‰ in $^{18}$O as a result of these precipitation effects (Watkins et al. 2013), smaller than the observed ~4‰ difference in $^{18}$O in corals (Figure 1-1). This suggests that the DIC precipitating in corals has not been isotopically equilibrated by carbonic anhydrase. Instead, carbonic anhydrase is thought to promote conversion of DIC to CO$_2$, promoting its diffusion into the calcifying space (Cohen and McConnaughey 2003).

There is some question as to whether the presence of carbonic anhydrase would even result in complete DIC equilibration. Experiments on CO$_2$ $^{18}$O during equilibration with H$_2$O found isotopic equilibration rates increased by only 25-50% upon addition of 19-25 μM carbonic anhydrase (Kelson et al. 2017). In another set of experiments with only 3.7 nM carbonic anhydrase, isotopic equilibration time was nearly halved and equilibration rate doubled (Uchikawa and Zeebe 2012), implying a rate increase of 50-100% due to carbonic anhydrase is about the maximum possible. Equilibrium is achieved more slowly in more saline waters both with and without carbonic anhydrase (Lu 2016; Kelson et al. 2017). Estimates of equilibration
time without carbonic anhydrase range from 3 to 24 hours at physiologically relevant pH and salinity (Uchikawa and Zeebe 2012; Lu 2016).

**Exploration of McConnaughey’s Kinetic Model**

The “kinetic model” (McConnaughey 1989a; 1989b; 2003) of fractionation in corals treats partial equilibration of the CO₂ hydration and hydroxylation as the main source of isotopic disequilibrium. In this model, vital effects are caused by fast and near-complete precipitation of the CaCO₃ before the DIC can equilibrate with CO₂ and H₂O, locking in the kinetic fractionation due to the hydration and hydroxylation reactions while minimizing kinetic fractionation due to the precipitation process (Figure 1-2). For corals without photosynthetic symbionts, δ¹³C and δ¹⁸O of the coral skeleton are linearly correlated. When plotted against each other, coral skeleton δ¹³C and δ¹⁸O values reach equilibrium with respect to DIC and H₂O at slow growth rates; the other endpoint of the line reflects completely kinetic fractionation, with no back-reaction (Figure 1-1). Movement away from the equilibrium point along the line is associated with faster coral growth under laboratory growth conditions; faster growth increases the forward rate of reaction and limits back-reaction by locking the product HCO₃⁻ into CaCO₃.

The kinetic model treats the calcifying space as a system open to CO₂ and H₂O but closed to HCO₃⁻ and CO₃²⁻; this selective membrane permeability is key to the model as it prevents external input of HCO₃⁻ and CO₃²⁻ from external sources. The high pH (≈7.5-9; Al-Horani et al., 2003) of the calcifying space favors CO₃²⁻ and HCO₃⁻ over CO₂, which creates a concentration gradient driving inward CO₂ diffusion. After diffusing in, CO₂ undergoes reactions (1) and (2). The reactions are thought to have different kinetic isotope fractionations (Siegenthaler and Münstich 1981; Marlier and O’Leary 1984; Clark and Lauriol 1992). The magnitudes of the
kinetic isotope effects are poorly constrained (Zeebe 2014), but they both favor the light isotopes in the products.

Marlier and O’Leary (1984) measured the kinetic fractionation of $^{13}\text{CO}_2/^{12}\text{CO}_2$ in the hydration and dehydration reactions using rapid enzymatic product removal and found kinetic discriminations against $^{13}\text{CO}_2$ of 6.9‰ and 14.7‰ for the hydration and hydroxylation reactions respectively (i.e. $\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{DIC}} = 6.9‰$ when the DIC is produced by the unequilibrated hydration reaction). Clark and Lauriol (1992) found a kinetic $^{13}\text{C}$ discrimination of 32‰ for dehydration at 0°C, which when combined with the equilibrium fractionation produces a hydration discrimination of 19.7‰ (Zeebe 2014). No experimental evaluations have been made of the kinetic fractionation of $^{18}\text{O}$ in the hydration and hydroxylation reactions. In general, differences in reaction rate for different isotopologues are difficult to evaluate experimentally because the isotopologues are expensive to separate, and isotopologue reaction rates are too similar to resolve directly by experiment. Additionally, recent evaluations of even the bulk CO$_2$ hydration rate irrespective of isotopologue differ by a factor of ~2 (Wang et al. 2010; Uchikawa and Zeebe 2012), highlighting the difficulty in experimentally evaluating the small differences in reaction rate that drive kinetic fractionation.

At slow growth rates, partial re-equilibration can occur before precipitation occurs via the reverse reactions:

\[ \text{HCO}_3^- + H^+ \rightarrow \text{CO}_2 + H_2O \] (Dehydration) (1.3)

\[ \text{HCO}_3^- \rightarrow \text{CO}_2 + OH^- \] (Dehydroxylation) (1.4)

At fast growth rates, these reactions do not have a chance to occur. In the kinetic model, fast precipitation of CO$_3^-$ prevents (1.3) and (1.4) from occurring at fast coral growth rates, so the fractionation expressed will be kinetic and not equilibrium fractionation. In such kinetically-controlled reactions, any factors affecting reaction rates can influence isotopic fractionation. The
vital effect magnitude is thus controlled by a balance between equilibration of CO$_2$ with DIC and H$_2$O outside the calcifying space, and kinetic fractionation within the calcifying space.

McConnaughey’s kinetic model does not attempt to deconvolute the two effects on carbonate isotopic signatures: (a) the shift from equilibrium to kinetic fractionation with increasing growth rates, and (b) the shift from CO$_2$ hydration to CO$_2$ hydroxylation reactions with increasing pH associated with increasing growth rates. McConnaughey hypothesized that a combination of (a) and (b) is responsible for the linear C-O isotopic trend. This would require close coupling of pH and growth rate, primarily by the Ca$^{2+}$/H$^+$ antiporter ATPase, or deviation from the observed linear trend in C and O isotopes would be expected.

Exploration of Spero and Adkins’ Carbonate Model

A different model for coral vital effects, the “carbonate model” developed by Adkins et al. (2003) for corals and Spero et al. (1997) for foraminifera, suggests vital effects are controlled by carbonic anhydrase-aided equilibration at different calcifying space pH, not by kinetic fractionation and changes in the relative percentages of reactions (1) and (2). Adkins notes deep-sea corals follow the same linear trend as shallow corals (McConnaughey 1989a; 1989b) except for the fastest-growing points, which exhibit steady $\delta^{13}$C with decreasing $\delta^{18}$O (Figure 1-3). These “kinks” in the linear trend cannot be explained by the kinetic model, with two end-members and a linear trend in between.

Adkins et al. proposed the $\delta^{18}$O trend is due to precipitation incorporating varying proportions of HCO$_3^-$ and CO$_3^{2-}$ at different pH. Unlike in the kinetic model, the isotopic compositions are equilibrium ones, and HCO$_3^-$ and CO$_3^{2-}$ are allowed to enter the calcification site directly from seawater via mechanisms such as vacuole transport. At higher pH, proportionally more CO$_3^{2-}$, which is depleted in $^{18}$O relative to HCO$_3^-$, contributes to the skeleton.
At the same time, the carbon source controls the $\delta^{13}$C values. At high pH, inward diffusion of CO$_2$, which is depleted in $^{13}$C relative to HCO$_3^-$ and CO$_3^{2-}$, predominates, while at low pH, more carbon is sourced directly from DIC in seawater, carrying its $^{13}$C enrichment. At the highest precipitation rates, the CO$_2$ is quantitatively precipitated as CaCO$_3$; no CO$_2$ can diffuse outward, and growth rates are fast enough that seawater DIC carrying the equilibrium $\delta^{13}$C of seawater contributes negligibly to the $\delta^{13}$C of the growing CaCO$_3$. This causes $\delta^{13}$C to stabilize while $\delta^{18}$O can continue to change, based on the pH of the fluid. Additionally, the carbonate model allows for some “leakiness” in the membrane, causing HCO$_3^-$ and CO$_3^{2-}$ from seawater to contribute to the final skeleton at slow growth rates.

The carbonate model assumes that DIC can equilibrate with H$_2$O and OH$^-$, even when precipitation is very fast. Consider the reservoirs of carbon-bearing species relevant to calcification at fast growth rates and their fluxes:

$$CO_2(aq)\text{ outside} \Leftrightarrow CO_2(aq)\text{ inside} \Leftrightarrow HCO_3^- \text{ and } CO_3^{2-} \Leftrightarrow CaCO_3 \text{ (1.5)}$$

where “inside” and “outside” refer to species inside and outside the calcifying space in the coral.

In the carbonate model, the flux from HCO$_3^-$ + CO$_3^{2-}$ to CaCO$_3$ is essentially one way, because precipitation is fast and goes to completion with negligible re-dissolution. The method of C isotope discrimination within the carbonate model also implies that flux from CO$_2(aq)$ outside the calcifying space to CO$_2(aq)$ inside the calcifying space is essentially one way. This is because the mechanism requires there to be an isotopic difference between CO$_3^{2-}$ precipitated from CO$_2$ entering the calcifying space and CO$_3^{2-}$ precipitated from HCO$_3^-$ and CO$_3^{2-}$ entering the calcifying space by e.g. vacuole transport directly from seawater. If CO$_2(aq)$ and HCO$_3^-$ + CO$_3^{2-}$ are able to isotopically equilibrate, there can be only very limited flux from CO$_2(aq)$ inside the calcifying space to CO$_2(aq)$ outside within the carbonate model. Otherwise, CO$_2$ outside the calcifying space would be able to equilibrate with CO$_2$ inside the calcifying space and the CO$_3^{2-}$ that it produces, and there would be no difference in C isotopic composition between CO$_3^{2-}$ originating from
CO$_2$(aq) and HCO$_3^-$/CO$_3^{2-}$ from seawater, leaving no distinction between carbon sources and no mechanism for C isotope discrimination.

Additionally, the reactions between CO$_2$(aq) inside the calcifying space and HCO$_3^-$ + CO$_3^{2-}$ must be fast compared to precipitation if they are to equilibrate. This is a fundamentally different assumption than the kinetic model, and probably requires carbonic anhydrase activity in the calcifying space. The carbonate model does not account for why a membrane permeable to CO$_2$ will have essentially no outward flux, nor does it analyze the differences in rates between DIC equilibration and precipitation. The carbonate model also does not specify when the transition to quantitative precipitation must occur, and why this transition does not affect the C-O slope. A more rigorous examination of rates of all processes (membrane diffusion, DIC equilibration, and precipitation) is necessary to differentiate the two models, and to determine the effects of T, pH, and growth rate on coral isotopic composition.

The kinetic and carbonate models do not capture every possible influence on C and O isotopic composition of carbonates in corals. It is assumed that H$_2$O produced by respiration with its unique isotopic composition is a negligible part of the total water in the calcifying space. It is also assumed that diffusion of H$_2$O into the calcifying space is rapid enough to reach diffusion equilibrium. However, this has not been verified, and the isotopic composition of water in the calcifying space may differ from that in seawater as a result. Also, if the product CO$_2$ and H$_2$O of respiration or the leftovers from photosynthesis in symbiotic corals are preferentially introduced to the calcifying space, the isotopic composition of the CaCO$_3$ skeleton would be affected.

This dissertation endeavors to calculate expected isotope fractionations due to the CO$_2$ hydration and hydroxylation reactions. This will enable a better comparison between measured coral isotopic compositions and the kinetic model of coral isotope fractionation. The fractionations are calculated using computational chemistry techniques, as introduced below.
Computational Chemistry

General Overview

Computational chemistry models allow the computation of various properties of molecular systems, including energies, geometries, and vibrational states (Cramer 2008). Computational chemistry involves the separate computation of nuclear positions and electron distributions, employing combinations of different basis functions that themselves approximate solutions to the electronic Schrödinger equation to find the minimum possible energy of a given system. The basis functions have the same shape as the $s$, $p$, $d$, and higher atomic orbitals but decay differently with distance. Linear combinations of basis functions are used to represent molecular orbitals and the distortions that occur in them relative to atomic orbitals due to interactions with multiple nuclei; the set of basis functions employed in a particular calculation is called the “basis set”. Essentially, computational chemistry determines where the nuclei and electrons are by calculating the electrostatic attraction and repulsion between each pair of particles, including the quantum mechanical and relativistic effects that dominate at small and fast scales, as well as the wave-particle nature of matter. Treatment of these complexities is necessary to calculate accurate chemical properties.

Practical application of quantum mechanical models requires the use of several simplifying assumptions to permit efficient use of computer time. Indeed, if some of these assumptions were not used, the calculations could never be completed at all.

First, the calculations must use a finite number of basis functions to represent the electron distribution. Infinite-sized sets of functions are obviously intractable. There is some balancing that needs to be done when picking basis functions: too many will make the computation take far
longer, while too few will give accurate results. Chemical reasoning and comparison to experiment are often used to select a reasonably-sized basis set.

In addition, the form of the basis functions matters, because certain functions take prohibitively long to integrate mathematically. Hydrogen-like orbitals are exponential functions \( e^{-r} \), with negative exponents because the probability of finding an electron decreases with increasing distance from the nucleus. Calculation with these functions in computational chemistry modeling is difficult; mathematical integration is much easier with Gaussian functions, which take the form \( \exp(-r^2) \). (Hence, the name of the popular computational chemistry program Gaussian\textsuperscript{(TM)}). Other functions are used when modeling systems with a periodic structure like minerals.

Second, the mathematical form of wavefunctions makes them difficult to use. A quantum mechanical representation of a chemical system uses wavefunctions to represent the positions of all nuclei and electrons within the system (when a wavefunction is multiplied by its complex conjugate) and all observable properties of the system (when the wavefunction is acted upon by an appropriate mathematical operator). The interactions between those electrons make wavefunctions mathematically difficult to work with. The calculation strategy called density functional theory (DFT) uses electron density instead of wavefunctions to describe electronic positions, and then uses a functional of the electron density to describe the effects of electron interaction - namely the exchange and correlation effects. These functionals cannot be computed exactly, and so introduce some approximation into the calculations based on how they are formulated. DFT is generally the most computationally efficient method to achieve a particular level of accuracy because it depends only on three spatial coordinates instead of three coordinates for each electron (Cramer 2008).

Third, calculation times increase drastically when considering systems of larger and larger size. DFT, one of the most efficient computational schemes, has calculation times that
scale as $N^3$ where $N$ is the number of electrons in the system. Often, it is more feasible to consider systems of a smaller size than to perform calculations on a large system, especially when considering non-periodic (i.e. not repeating in space in an orderly pattern like minerals) condensed phases like aqueous solutions. Careful constructions of small multi-molecular clusters can allow calculation of some condensed-phase properties because long-range intermolecular interactions are much weaker than short-range ones.

**Use of Computational Chemistry in the DIC System**

Computational chemistry can be used to calculate isotopic fractionation factors for reactions that reach equilibrium as well as reactions that lock in a kinetic fractionation (see Chapter 3 Introduction for a definition of equilibrium and kinetic isotopic fractionation factors). Computational chemistry models are useful in the calculation of fractionation factors for variably-protonated aqueous species because dynamic chemical speciation makes interpretation of experimental isotopic fractionation studies difficult. The models can be used to determine the most likely structure of a molecule interacting with water. The structure can then be used to calculate the vibrational frequencies of the fundamental vibrational modes and their dependence on the isotopic composition of the molecule.

Computational chemistry techniques have been used to evaluate equilibrium fractionation in DIC species. Rustad et al. (2008) calculated the equilibrium C fractionation factors between aqueous CO$_2$, HCO$_3^−$, and CO$_3^{2−}$ to within <2‰. Zeebe (2009) calculated the equilibrium O isotope fractionation factor between CO$_3^{2−}$ and H$_2$O to within 0.5‰ (note that this within 0.5‰ of the 24.5‰ difference in equilibrium isotopic composition of H$_2$O and CO$_3^{2−}$ as described in Chapter 3 of this dissertation, or a ~2% relative error). The recent achievement of sub-permil deviations between fractionations measured in aqueous solution and calculated using
computational chemistry renders computational chemistry calculations quantitatively useful in the study of fractionation factors of geochemical importance.

The success of the models in reproducing equilibrium fractionation factors is highly dependent on the basis set and functional used. Rustad et al. (2008) calculated equilibrium C fractionation factors to within 1-1.5‰ with the most accurate methods, while other methods had deviations of >9‰. Notably, basis sets and functionals which produced accurate results when used together did not systematically produce better results when used in other combinations. Zeebe (2009) found differences of <1‰ when using a basis set including diffuse functions that better represent electron density in anions. These calculations also used vibrational frequencies scaled via a basis set/functional-specific scaling factor to agree with experimental values. However these corrections partially introduce anharmonicity to the description of molecular vibrations, which the equations for fractionation factor are derived to use only harmonic vibrational frequencies (Rustad et al. 2008; Zeebe 2009).

Model success is also highly dependent on the description of the solvation environment. Computational chemistry modeling is gradually discovering how to calculate aqueous fractionation factors where the many solute-water interactions have a strong effect on the final fractionation factor. Rustad et al. (2008) found large differences in the hydration state of CO$_2$, HCO$_3^-$, and CO$_3^{2-}$ in a fully-solvated, periodic model. CO$_2$ had at most very weak H-bonds to its O atoms. HCO$_3^-$ accepted 3-4 H-bonds and required 7-8 H$_2$O molecules in its inner solvation shell. CO$_3^{2-}$ accepted 8 H-bonds, with 9-10 H$_2$O molecules present in its first solvation shell. In the cluster models, they compared the effects of using just the inner solvation shell to other solvation models. Their HCO$_3^-$/CO$_2$ fractionation factor differed from the experimental value by 1.1‰ when using the first solvation shell, but only 0.6‰ when including 32 solvating H$_2$O molecules instead of 7. The number of individual H-bonds present in their cluster models was not specified. Use of a polarizable continuum model (PCM) to describe interactions with the 1$^{st}$ shell
instead of explicit H$_2$O molecules substantially increased the deviation, to 5.9‰. Use of no explicit solvating H$_2$O molecules, or only PCM, increased the deviations to 13‰ and 10‰ respectively. Calculated O fractionation factors for CO$_3^{2-}$/H$_2$O from Zeebe (2009) had deviations of 9.5‰ without explicit solvating H$_2$Os, or 0.5‰ with 22 H$_2$Os included, displaying an asymptotic decrease in deviation from the experimental fractionation factor as more H$_2$O molecules were included in the calculation. The largest cluster contained 9 H-bonds to CO$_3^{2-}$; numbers of H bonds for other clusters were not reported.

Accurate calculation of fractionation factors requires use of several solute-solvent conformations to sample the chemical environment experienced by the DIC species. Rustad et al. (2008) used several conformations generated from molecular dynamics calculations in their fractionation calculations. They found a standard error of ~0.5‰ when using a population of ~10 conformations, while individual conformations could differ by 2-3‰.

Clearly, construction of a molecular model capable of replicating experimental fractionation factors in aqueous solution is a challenge. Many careful choices must be made to achieve accurate results that are useful to geochemists.

Computational chemistry techniques have also been used to evaluate reaction kinetics between DIC species. Loerting et al. (2000) found that H$_2$O strongly catalyzed the decomposition of H$_2$CO$_3$ into CO$_2$ and H$_2$O (reaction 3), decreasing activation energy by 80 kJ/mol and increasing the rate constant by 10 orders of magnitude at Earth-surface conditions. Nguyen et al. (2008) found similar catalysis magnitudes during CO$_2$ hydration using cluster calculations involving 1-4 H$_2$O molecules; increasing numbers of H$_2$O molecules increased the catalysis effect further. Activation energies varied from 83 to 113 kJ/mol depending on conformation. This compares favorably with the experimentally-determined value for activation energy of 81 kJ/mol (Wang et al. 2010). Stirling and Pápai (2010) examined CO$_2$ hydration using DFT with periodic boundary conditions to better represent the aqueous phase. They found that HCO$_3^-$ forms first as
an ion pair with $\text{H}_2\text{O}^+$, followed by a discrete protonation step to form $\text{H}_2\text{CO}_3$. This contrasted from cluster calculations in which $\text{H}_2\text{CO}_3$ formed directly from $\text{CO}_2$. The calculated activation energy was 79 kJ/mol. Stirling (2011) studied the hydroxylation of $\text{CO}_2$ and found that the free energy barrier is related primarily to the decrease in entropy required to re-orient and dehydrate the $\text{OH}^-$ ion, rather than the enthalpic barriers of bond breaking and stretching. The free energy of activation energy was calculated to be 58 kJ/mol, similar to the experimental value of 50 kJ/mol (Wang et al. 2010).

One computational study has addressed kinetic fractionation during $\text{CO}_2$ hydration. Zeebe (2014) examined fractionation in clusters including 1-8 $\text{H}_2\text{O}$ molecules, finding discrimination against $^{13}\text{C}$ by 22-32‰ at 25°C, with stronger discrimination when more $\text{H}_2\text{O}$ molecules are present and the reaction switches from direct $\text{H}_2\text{CO}_3$ formation to formation of an $\text{HCO}_3^-/\text{H}_3\text{O}^+$ ion pair. This is broadly consistent with some experimental data (Clark and Lauriol 1992), but a comparison is difficult because of the large discrepancy in experimental values reported by different sources (Marlier and O’Leary 1984; O’Leary et al. 1992). Zeebe (2014) reported a discrimination against $^{18}\text{O}$ of 13-15‰. The calculated activation energy of 91-92 kJ/mol is close to the experimental activation energy of 81 kJ/mol and exactly matches the free energy of activation of 91 kJ/mol (Wang et al. 2010), although this match is serendipitous because activation energy does not include the activation entropy, which is included in the free energy of activation. Use of different basis sets and computation schemes imparted a 1.5-3‰ nonsystematic effect on the kinetic fractionation (Zeebe 2014).

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**Dissertation Layout**

This dissertation focuses on the application of computational chemistry to determination of expected isotope fractionation factors in the DIC-$\text{H}_2\text{O}$ system, in four parts. The dissertation
endeavors to describe both magnitudes of isotope fractionation under different conditions during formation of calcium carbonate minerals and methods by which isotope fractionation can be evaluated using computational chemistry in different aqueous systems. A summary of symbols and abbreviations used throughout the dissertation is provided in Table 1-1.

Chapter 2 focuses on selection of a computational method by which to perform later calculations. The chapter focuses on a subset of DFT functionals that have been used to model aqueous interactions. Evaluating the performance of different computational methods is critical to the assessment of a functional’s appropriateness in a given situation. Many computational method evaluation studies focus on replicating known energies and geometries. Chapter 2 extends evaluation to analysis of harmonic vibrational frequencies and fractionation factors in the CO$_2$-H$_2$O system. The chapter concludes that accurate replication of harmonic vibrational frequencies is a good indicator that fractionation factors will also be accurately predicted, and selects the two best functionals for application in the DIC system with certain basis sets.

Chapter 3 focuses on calculation of O and C fractionation during fractionation in the CO$_2$ hydration and hydroxylation reactions. The chapter demonstrates that the methods selected in Chapter 2 accurately predict reaction energies and equilibrium fractionations for which there are experimental results available. Computational chemistry modeling predicts values for kinetic isotope fractionation in the reactions, parameters for which there is little to no experimental evidence. The chapter highlights the necessity of accurately modeling the hydration environment of aqueous species when predicting isotope fractionation, due to the substantial impact of individual H-bonds on fractionation factors. Analysis by H-bond number and type is new in the field of computational isotope fractionation. The results are applied to isotope trends observed in corals, which suggest that a process other than solely kinetic fractionation must be causing coral vital effects.
Chapter 4 focuses on calculation of clumped isotope signals in the DIC system. Clumped isotope composition in principle depends only on the temperature of formation of a carbonate mineral, if the assumption holds that the mineral formed at equilibrium. Chapter 4 evaluates the extent to which reactions that do not achieve equilibrium may record a kinetic clumped isotope signal. Products of the CO₂ hydration and hydroxylation reaction record substantially more clumping when they are not allowed to equilibrate. The results match trends observed in shallow-water corals and their T dependence, suggesting kinetic fractionation may have an impact on their clumped isotope signal.

Chapter 5 focuses on the isotopic composition of amorphous calcium carbonate, a potential intermediate during carbonate mineral precipitation. Its isotopic composition is found to vary with concentration in a sense opposite that of calcite. The chapter then suggests future work that will be necessary to separate several effects on the isotopic composition of amorphous calcium carbonate.

Chapter 6 summarizes the results from Chapters 2 through 5. The chapter then suggests some future directions for research, both in the general field of computational isotope fractionation and specifically on fractionation in the DIC system of interest to carbonate paleoclimatology.

Publication of Chapters 3 and 4 as individual papers with James Kubicki as coauthor are intended. Publication of Chapter 2 with some additional benchmarking data is also intended with James Kubicki as coauthor.
Figure 1-1. Isotopic disequilibrium displayed by corals. Isotopic composition of non-photosynthetic (open blue squares) and photosynthetic (closed green circles) shallow corals collected from field samples and grown in laboratory experiments. Isotopic equilibrium between DIC species, CO$_2$, and H$_2$O is marked by a red square. Modified from Cohen and McConnaughey (2003).
Figure 1-2. The kinetic model of coral vital effects as developed by McConnaughey (1989a; 1989b; 2003) in the context of the coral calcifying space. The calcifying space (white rectangle) is bounded by the growing CaCO₃ skeleton (white rhombohedron) and the calicoblastic membrane (gray rectangle). The phospholipid bilayer of the calicoblastic membrane is permeable to small neutral species such as CO₂ but not to charged species such as CO₃²⁻ and HCO₃⁻. Ca²⁺ concentration and pH are mediated by a Ca²⁺/H⁺ antiporter ATPase protein (hexagon). CO₂ enters the calcifying space and is reacted to form HCO₃⁻ and eventually CO₃²⁻. The CO₃²⁻ and Ca²⁺ may then precipitate, building the skeleton. The source of vital effects in the kinetic model, the CO₂ hydration/hydroxylation reaction, is marked in red. Modified from Cohen and McConnaughey (2003).
Figure 1-3. Isotopic composition of Desmophyllum cristagalli deep-sea corals sampled from a fjord in Chile. Black squares are samples from trabecular centers, the fastest-growing portions of a coral. These trabecular centers fall off the linear trend produced by the rest of the corals. Modified from Adkins et al. (2003).
Table 1-1. Summary of symbols and abbreviations used in the text of the dissertation.

<table>
<thead>
<tr>
<th>Term</th>
<th>Meaning</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aqueous Carbon Terms</strong></td>
<td></td>
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</tr>
<tr>
<td>DIC</td>
<td>Dissolved inorganic carbon: $\text{H}_2\text{CO}_3$, $\text{HCO}_3^-$, $\text{CO}_3^{2-}$, sometimes $\text{CO}_2(\text{aq})$</td>
<td>1, 65</td>
</tr>
<tr>
<td>hyd</td>
<td>Refers to the $\text{CO}_2$ hydration reaction $\text{CO}_2+\text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3$ or $\text{HCO}_3^-+\text{H}^+$</td>
<td>1</td>
</tr>
<tr>
<td>hydrox</td>
<td>Refers to the $\text{CO}_2$ hydroxylation reaction $\text{CO}_2+\text{OH}^- \rightarrow \text{HCO}_3^-$</td>
<td>1</td>
</tr>
<tr>
<td>Kinetic model</td>
<td>Model of coral vital effects which invokes isotopic disequilibrium in the $\text{CO}_2$ hydration and hydroxylation reactions</td>
<td>6</td>
</tr>
<tr>
<td>Carbonate model</td>
<td>Model of coral vital effects which invokes isotopic equilibrium between DIC species but disequilibrium during precipitation</td>
<td>8</td>
</tr>
<tr>
<td><strong>Computational Chemistry Terms</strong></td>
<td></td>
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</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
<td>12, 27, 68</td>
</tr>
<tr>
<td>Basis set</td>
<td>Spatial representation of electron distributions, similar shape to orbitals</td>
<td>11, 27</td>
</tr>
<tr>
<td>6-311G+(d,p)</td>
<td>Smaller basis set in this dissertation, lacking diffuse functions on H</td>
<td>29</td>
</tr>
<tr>
<td>6-311G++(2d,p)</td>
<td>Larger basis set in this dissertation, including diffuse functions on H</td>
<td>29</td>
</tr>
<tr>
<td>Functional</td>
<td>DFT description of quantum effects based on electron density</td>
<td>27</td>
</tr>
<tr>
<td>LDA</td>
<td>Class of functionals which uses local electron density</td>
<td>27</td>
</tr>
<tr>
<td>GGA</td>
<td>Class of functionals which uses gradients in electron density</td>
<td>27</td>
</tr>
<tr>
<td>PBE0</td>
<td>A GGA functional used in this dissertation, with moderate accuracy</td>
<td>37</td>
</tr>
<tr>
<td>Hybrid</td>
<td>Class of functionals which uses gradients and exact electron exchange</td>
<td>28</td>
</tr>
<tr>
<td>B3LYP</td>
<td>A hybrid functional used in this dissertation, with moderate accuracy</td>
<td>38</td>
</tr>
<tr>
<td>X3LYP</td>
<td>A hybrid functional used in this dissertation, with good accuracy</td>
<td>28, 38</td>
</tr>
<tr>
<td>Model chemistry</td>
<td>A particular combination of basis set and functional</td>
<td>69</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamics calculation, used in this dissertation to mimic aqueous motion, scramble molecules, and make different conformations</td>
<td>70</td>
</tr>
<tr>
<td>Conformation</td>
<td>A particular arrangement of molecules of $\text{H}_2\text{O}$ around a DIC molecule</td>
<td>70</td>
</tr>
<tr>
<td><strong>Isotope Fractionation Terms</strong></td>
<td></td>
<td></td>
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<tr>
<td>$R$</td>
<td>Ratio of heavy to light stable isotopes found in a particular sample</td>
<td>62</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Measurement of the difference in $R$ between a substance and measurement standard, in $%$</td>
<td>63</td>
</tr>
<tr>
<td>$%_\text{p}$</td>
<td>Permil, parts per thousand</td>
<td>63</td>
</tr>
<tr>
<td>$\alpha_{eq}$</td>
<td>Equilibrium isotope fractionation factor; $&gt;1$ indicates concentration of heavy isotopes in the product substance</td>
<td>63</td>
</tr>
<tr>
<td>$\alpha_{kin}$</td>
<td>Kinetic isotope fractionation factor</td>
<td>65</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Representation of $\alpha$ in $%_\text{p}$ units</td>
<td>63</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Reduced partition function ratio used to calculate $\alpha$ from vibrations</td>
<td>64, 160</td>
</tr>
<tr>
<td>Clumped isotopes</td>
<td>When two heavy isotopes clump in the same molecule, e.g. $^{13}\text{C}^{18}\text{O}$</td>
<td>148</td>
</tr>
<tr>
<td>Isotopologue</td>
<td>Molecule with a particular arrangement of isotopes, clumped or not</td>
<td>64, 151</td>
</tr>
<tr>
<td>$\Delta$</td>
<td>Difference in clumped isotopologue abundance between a sample and a stochastic distribution; $\Delta_{47}$ of $\text{CO}_2$ is the relevant quantity in this dissertation</td>
<td>152</td>
</tr>
<tr>
<td><strong>Energetics Terms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZPE</td>
<td>Zero-point energy; minimum energy of a molecule including vibrations</td>
<td>64, 149</td>
</tr>
<tr>
<td>Harmonic frequency</td>
<td>Frequency of a molecular vibration using the harmonic approximation, i.e. in a symmetrical, quadratic potential energy well</td>
<td>30, 34, 64</td>
</tr>
<tr>
<td>$\Delta G^0$</td>
<td>Standard-state Gibbs free energy change of a reaction</td>
<td>66</td>
</tr>
<tr>
<td>TS/TST</td>
<td>Transition state/Transition State Theory</td>
<td>66</td>
</tr>
<tr>
<td>$E_a$</td>
<td>Activation energy</td>
<td>73</td>
</tr>
<tr>
<td><strong>Other Terms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SST</td>
<td>Sea surface temperature</td>
<td>3</td>
</tr>
<tr>
<td>ACC</td>
<td>Amorphous calcium carbonate</td>
<td>3, 219</td>
</tr>
</tbody>
</table>

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Table 1-1. Summary of symbols and abbreviations used in the text of the dissertation.
References


Lu, F.H., 2016. How long is enough: CO$_2$-H$_2$O equilibration for $\delta^{18}$O analysis in saline formation waters? Rapid Communications in Mass Spectrometry, 30(13), 1647-1652.


Chapter 2

Evaluating Computational Chemistry Methods for the CO$_2$-H$_2$O System

Abstract

Computational chemistry methods vary widely in their effectiveness in prediction of various experimental parameters. Most studies on the usefulness of density functionals in these methods focus on predictive accuracy for energetics and geometries. Little is known about the comparative effectiveness of different functionals when predicting isotopic fractionation. In this study, several density functionals are evaluated against experimental dimerization energies, dimerization geometries, harmonic vibrational frequencies, and $^{18}$O/$^{16}$O isotopic fractionation in the CO$_2$-H$_2$O system. Successful prediction of harmonic vibrational frequencies strongly correlates with successful prediction of isotopic fractionation; no energetic or geometric properties are similarly correlated. The B3LYP and X3LYP functionals perform more accurately in evaluation of both harmonic vibrational frequencies and isotopic fractionation factors using the 6-311G+(d,p) and 6-311G++(2d,p) basis sets.

Introduction

Computational chemistry methods vary widely in their ability to accurately predict properties of interest (Cramer 2008). The most accurate calculation methods also take the most computer memory and time, especially as the number of atoms in a system increases. Additionally, there is no guarantee that methods which accurately predict one property also accurately predict others, and new methods are often tailored to replicate a specific property or
describe a specific type of interaction (e.g. Xu and Goddard 2004a; b). Thus it is necessary to evaluate computation methods against experimentally-determined properties in a system of interest to choose the best method for a given situation.

**Density Functional Theory: Functional and Basis Set Evaluation**

Density functional theory (DFT) is a popular class of computational chemistry methods that requires relatively few computational resources for the accuracy it provides (Cramer 2008; Chapter 1, this dissertation). Multiple density functionals have been developed to describe different properties in different systems, and existing functionals are often tested in new systems or on new properties. For example, Zhao and Truhlar (2005) characterized the binding energies predicted by several functionals in non-bonded systems including H-bonded systems. Xu and Goddard (2004b) tailored the X3LYP functional to describe interaction energies in the (H₂O)₂ dimer. Xu and Goddard (2004a) evaluated the ability of several functionals to replicate energies, geometries, and vibrational frequencies in the (H₂O)₂ dimer.

Functionals can be classified by how they use the electron density to calculate exchange and correlation energies. Local density approximation (LDA) functionals compute the exchange and correlation energies at a particular position purely based on the electron density at that position, i.e. energies not depend on nearby electron density or the density gradient at that position. In generalized gradient approximation (GGA) functionals, exchange and correlation densities are taken to depend on the local gradient in electron density, which is in principle still based on local variables, but in practice may involve computation of the gradient from nearby electron densities. Some of these are constructed with empirical parameters designed to mimic the exact exchange energy measured from noble gas atoms. Some use a Taylor expansion-like approach around the local electron density and thus have no empirical parameters. LYP (Lee et
(Becke 1988) is a notable GGA correlation functional which uses four empirical parameters fit to He atoms, and that has seen wide use. Various other methods mix and match various functional expressions for the exchange and correlation energy with exact exchange, weighted by empirical values; these are termed “hybrid” functionals. One of these is called the B3LYP functional (Becke 1993; Lee et al. 1988; Stephens et al. 1994), and is used widely (Cramer 2008). The abbreviation implies: the use of the B GGA functional for exchange energy (Becke 1988), the LYP functional for correlation energy (Lee et al. 1988), and 3 parameters to weight the use of these against local and other energy approximations. Its complexity and lack of a priori foundation are made up for by its close agreement with many experimental measurements across many different systems (Cramer 2008).

The basis sets used to describe electron distributions in a chemical system also influence calculation accuracy and computation time. Two components are of particular interest in H-bonded systems such as aqueous systems: polarization functions and diffuse functions. Polarization functions allow for description of electron distributions that are not spherically symmetric, such as those that occur in both covalently and H-bonded systems. They involve placing functions of higher angular momentum on atoms, such as placing p basis functions on H atoms which would otherwise be described using only s orbitals. Diffuse functions are necessary for describing interactions at large distances from the nucleus. These functions decay slowly with distance from the nucleus. Diffuse functions are especially important when describing anions, where electrons are loosely bound to nuclei, or H-bonded systems (Cramer 2008; Santra 2010).

Functionals are not usually built specifically to predict isotopic fractionation factors in aqueous systems. However, it is a goal of computational geochemistry to use DFT for prediction of isotope fractionation in geochemically-relevant aqueous systems (e.g. Zeebe 2009). This chapter seeks to determine which functionals and basis sets best predict isotopic fractionation in
dissolved inorganic carbon species, and which properties are the best indicator of a functional’s fidelity when predicting isotopic fractionation.

**Methods**

Basis sets and DFT functionals were evaluated using four small systems: a single CO\(_2\) molecule, a single H\(_2\)O molecule, a single CO\(_2\) molecule plus a single H\(_2\)O molecule, and two H\(_2\)O molecules. Starting configurations were built in the program GaussView (Dennington et al. 2009) with bond distances and angles close to available experimental values. Each system underwent a geometry optimization, followed by a harmonic frequency calculation, in the GAUSSIAN™ program (Frisch et al. 2009). Optimization criteria were set to Tight (maximum/root-mean-square (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.

Two basis sets and eleven DFT functionals, plus MP2 perturbation calculations (Head-Gordon et al. 1988), were used for each system. The 6-311G+(d,p) and 6-311G++(2d,p) basis sets were used throughout. The larger 6-311G++(2d,p) basis set includes diffuse functions on H which are thought to be critical in accurately modeling H-bonds (Santra 2010), while the smaller functional only includes diffuse functions on heavy atoms. The eleven functionals selected are as follows: GGA functionals BLYP (Becke 1988; Lee et al. 1988), mPWLYP (Adamo and Barone 1998; Lee et al. 1988), PBE1W (Perdew et al. 1996; Dahlke and Truhlar 2005), and PBEPBE (Perdew et al. 1996); and hybrid functionals BH&HLYP (Becke 1988; Becke 1993; Lee et al. 1988), B3P86 (Becke 1993; Perdew 1986), mPWBT1K (Adamo and Barone 1998; Becke 1996; Zhao and Truhlar 2004), B3LYP (Becke 1993; Lee et al. 1988; Stephens et al. 1994), PBE0
(Adamo and Barone 1999), X3LYP (Xu and Goddard 2004b), and M06-2X (Zhao and Truhlar 2008; 2010).

Each basis set/functional combination, plus each basis set in combination with MP2, was evaluated against available experimental data. Functionals were evaluated using experimental values of CO$_2$-H$_2$O and H$_2$O-H$_2$O dimerization energies, interatomic distances, harmonic vibrational frequencies, isotopic vibrational shifts, and the CO$_2$(g)-H$_2$O(g) equilibrium isotopic fractionation factor to select the most accurate functionals with reasonable computation times (Figs. 2-1 to 2-8). Comparisons with high-level CCSD(T)/CBS calculations, a method with some of the most accurate results for small molecules, were also made when available. Calculated harmonic vibrational frequencies were compared with experimentally-derived harmonic vibrational frequencies which used spectroscopically-derived anharmonicity corrections to determine the harmonic frequency from the fundamental frequency.

The rest of the calculations (Chapters 3 and 4, this dissertation) used the exchange/correlation functionals best representing the experimental parameters, with a preference given to functions that did well in vibration and fractionation factor calculations (X3LYP, B3LYP). Two functionals that did not perform quite as well were also included to analyze the effect of using a poorer-performing functional on results and determine whether differences in functional yield systematic deviations from experimental fractionation factors (PBEPBE, PBE0).

**Results**

**Geometry and Energetics Evaluation**

Evaluation results are presented in Figures 2-1 to 2-8. All graphs are ordered based on the computation time needed to optimize the CO$_2$-H$_2$O dimer (Fig. 2-1). Below, a short overview of
the general success of these methods for each parameter is given, followed by a functional-by-
functional discussion of why it was or was not included in further calculations.

Good agreement is generally achieved for the experimental C(CO$_2$)-O(H$_2$O) distance
(Fig. 2-2), although several functionals differ by more than 0.1 Å from the experimental value
(Peterson and Klemperer 1984) and a few differ by more than 0.2 Å. A high-level CCSD(T)/CBS
calculation (Wheatley and Harvey 2011) differs by 0.053 Å from the experimental value,
suggesting better agreement than this is not to be expected from DFT calculations. Deviations
from the CO$_2$-H$_2$O interaction energy from this high-level calculation are generally off by <4
kJ/mol, and often <2kJ/mol (Fig. 2-3). Optimized structures all achieve the same T-shaped
geometry with no H-bonding that is deduced to be the low-energy structure from experiment
(Peterson and Klemperer 1984).

Poor agreement is achieved with the experimental O(H$_2$O)-O(H$_2$O) distance of 2.976 Å
(Fig. 2-4), which is related closely to H-bonding. All functionals systematically underestimate the
experimental O-O distance (Odutola and Dyke 1980). Even CCSD(T)/CBS calculations
underestimate the O-O distance by 0.064 Å (Klopper et al. 2000). Part of the issue may be the
application of a rigid rotor model to interpret the experimental microwave spectra, which is not a
perfect model due to the “floppiness” of hydrogen-bonded H$_2$O molecules (Xu and Goddard
2004a). Agreement with experimental dimerization energies (Rocher-Casterline et al. 2011) is
better, but there is still a common underestimation of 1-4 kJ/mol (Fig. 2-5) meaning calculations
generally give a stronger interaction between H$_2$O molecules than is experimentally determined.

**Vibrational Frequency Evaluation**

Agreement with harmonic experimental vibrational frequencies is very good with these
methods (Fig. 2-6) without the use of any frequency scaling factor. $R^2$ values are always $>$0.994,
and are often >0.999. The benefit of the larger basis set is strongest in these calculations, with most functionals performing better when the larger 6-311G++(2d,p) basis set is used. For these relatively large basis sets, scaling factors are close to 1; additionally, scaling factors are only significant up to 2 digits (Teixeira et al. 2010). Also, scaling factors are generally only appropriate for converting harmonic frequencies from calculations to anharmonic frequencies observed with spectroscopic methods. Thus, frequency scaling factors are not included in further calculations. Rustad et al. (2008) point out that scaling factors are in part meant to correct for anharmonicity, while the equation involved in calculation of fractionation factors (Equation 3.7; Chapter 3, this dissertation) is based on harmonic vibrational frequencies. Zeebe (2009) found anharmonic corrections to isotopic fractionation factors to be small and very computationally expensive for the CO$_3^{2-}$ - H$_2$O system.

Shifts in vibrational frequency upon isotopic substitution are very important for computing isotopic fractionations, being the dominant control on fractionation factors. Similar root-mean-square (RMS) errors are achieved for most basis setfunctional combinations (Fig. 2-7). Note that both anharmonic and harmonic experimental frequencies are included in this test.

Ultimately, useful functionals should be able to replicate experimental fractionation factors well. The performance of the functionals in computing $^{18/16}\alpha_{eq}(CO_2(g)-H_2O(g))$ (fractionation factor describing equilibrium partitioning of $^{16}O$ relative to $^{18}O$ between gaseous CO$_2$ and gaseous H$_2$O, where values >1 indicate $^{18}O$ enrichment in CO$_2$; see Chapter 3 Introduction for a definition of $\alpha$ and an explanation of the notation) is thus an important tool for evaluation (Fig. 2-8). These calculations were performed using the CO$_2$ and H$_2$O monomers, so the relevant comparison is to H$_2$O(g) instead of H$_2$O(l). Many functionals do relatively poorly in this test, with deviations from experiment >2‰ being common. A few functionals do very well (B3LYP, X3LYP) and thus are selected to calculate fractionation factors in the larger cluster calculations (Chapters 3 and 4, this dissertation).
B3LYP and X3LYP both do well in most tests, and do particularly well in the tests most relevant to the calculation of fractionation factors. Using the 6-311G+(d,p) basis set, their deviations in calculating $^{18/16}\alpha_{eq}(\text{CO}_2(g)\text{-H}_2\text{O}(g))$ are <1‰, and are <0.3‰ when using 6-311G++(2d,p). Both also do very well in the calculation of harmonic vibrational frequencies, again doing slightly better when using 6-311G++(2d,p).

PBE0 does not do particularly well in evaluation tests. PBE0 predicts vibrational frequencies reasonably well (RMS errors of 49.19 cm$^{-1}$ and 41.41 cm$^{-1}$ with the smaller and larger basis set respectively) and has moderate-sized deviations in predicting $^{18/16}\alpha_{eq}(\text{CO}_2(g)\text{-H}_2\text{O}(g))$ (deviations of 2.2‰ and 1.2‰). PBE does a poorer job predicting experimental vibrational frequencies but is not among the worst performers (RMS errors of 83.99 cm$^{-1}$ and 87.06 cm$^{-1}$) and has larger deviations in $^{18/16}\alpha_{eq}(\text{CO}_2(g)\text{-H}_2\text{O}(g))$ (2.9‰ and 3.9‰), with the larger basis set performing worse than the smaller one. These will be tested to examine the effect of using poorer-performing functionals on calculation results. The B3LYP, X3LYP, PBE0, and PBE0PBE functionals along with both basis sets 6-311+G(d,p) and 6-311++G(2d,p) are color-coded throughout this chapter and other chapters in this dissertation; that color coding is summarized in Table 2-1.

Those functionals which do a good job predicting vibrational frequencies also do a good job predicting fractionation factors (Fig. 2-9). Functionals selected for further use and the values of both of these model results are also given in Table 2-1. The only outlier, which predicts vibrational frequencies well but $^{18/16}\alpha_{eq}(\text{CO}_2(g)\text{-H}_2\text{O}(g))$ poorly, is not a functional: it is the MP2 method. Despite the fact that MP2 calculations proceed quickly in this test, they are not used further because of this and their much greater computational requirements with larger systems, scaling as N$^5$ as opposed to the DFT methods scaling as N$^3$ (Cramer 2008). Other researchers are cautioned against using MP2 to predict isotopic fractionation factors. MP2 has seen some use in
the DIC system (e.g. Zeebe 2014) but results using this method are unreliable given the results of this work.

Success at predicting other properties is not associated with success predicting isotopic fractionation (Fig. 2-10 to 2-15). Short or longer calculation times (Fig. 2-10), small deviations from experimental C(CO$_2$)-O(H$_2$O) distance (Fig. 2-11), small deviations from calculated CO$_2$-H$_2$O (Figure 2-12) or experimental H$_2$O-H$_2$O (Figure 2-14) interaction energies, and minimized RMS deviations between observed and calculated shifts in vibrational frequency upon isotopic substitution (Figure 2-15) are not correlated with success predicting $^{18/16}\alpha_{eq}(\text{CO}_2(\text{g})-\text{H}_2\text{O}(\text{g}))$. Success in predicting the measured H$_2$O-H$_2$O distance (Figure 2-13) is negatively correlated with deviation from isotopic fractionation measurements, with models that under-predict the measured H$_2$O-H$_2$O more severely actually predicting CO$_2$-H$_2$O isotopic fractionation better.

**Discussion**

**Best Properties for Prediction of Isotopic Fractionation**

Based on these results, interatomic distance and binding energy comparisons are not particularly useful in selecting suitable functionals for isotope fractionation calculations. Many evaluation studies are performed on these properties (e.g. Xu and Goddard 2004; Zhao and Truhlar 2005; Santra 2010). Any efforts to calculate isotope fractionations would need to evaluate functionals on more than these properties to determine whether the functions are reliable.

If a given combination of basis set and functional accurately predicts experimentally-determined harmonic vibrational frequencies, it will probably also predict isotope fractionation well. This is supported both by theoretical considerations (the source of most energy difference between isotopically-substituted species is in molecular vibrations) and by these model results.
The correlation seen in Fig. 2-9 is particularly strong. However, caution should be taken if using the MP2 method during isotope fractionation calculations, as it is a good predictor of harmonic vibrational frequencies but a poor predictor of isotopic fractionation. This is possibly because of slightly higher error in vibrational shift upon isotopic substitution (Fig. 2-6). In simple systems like CO\(_2\)(g) and H\(_2\)O(g), predicted deviations in isotope fractionation factors can feasibly be reduced below 0.3‰ by selecting a basis set/functional combination in this manner.

The importance of using comparisons to experimental harmonic vibrational frequencies needs to be stressed. Fundamental frequencies as determined by IR and Raman spectroscopy include anharmonicity even in the ground state to first excited state transition that they describe. These experimental fundamental frequencies will tend to be smaller than the frequencies derived under a harmonic oscillator approximation. Vibrational-rotational spectroscopy can aid the translation from fundamental to harmonic frequencies derived from experiment. By fitting observed differences in level spacing obtained from overtone modes that represent transitions to second and higher vibrational states, the effect of anharmonicity can be experimentally determined and subtracted from the fundamental frequencies, leaving an experimental value for the harmonic frequency (e.g. Miller and Ganda-Kesuma 1991). If anharmonicity corrections are not applied to the experimental fundamental frequencies, then they should not be expected to match harmonic frequencies from DFT calculations, and any matches are fortuitous (e.g. Lin et al. 2004). Frequency scaling factors are meant to translate DFT-calculated harmonic frequencies into anharmonic frequencies (Scott and Radom 1996); while they could be applied to compare calculated harmonic frequencies with experimental fundamental frequencies, the scaling factors are approximate and may vary by ~1% across different vibrations using the same functional (e.g. Teixeira et al. 2010). As a result, it is more appropriate to compare calculated harmonic frequencies from DFT with experimental harmonic frequencies using spectroscopically-derived anharmonicity corrections. That is the method carried out here which suggests that agreement
with experimental harmonic vibrational frequencies predicts accurate calculation of fractionation factors (Fig. 2-9).

The B3LYP and X3LYP functionals predict isotope fractionation between CO$_2$(g) and H$_2$O(g) well when paired with the 6-311+G(d,p) and 6-311++G(2d,p) basis sets. These functionals are applied to aqueous DIC species in the rest of this dissertation. Ideally, a calculation for functional evaluation would be performed in the exact system of interest, in this case CO$_2$(aq), H$_2$CO$_3$(aq), and HCO$_3^-$ (aq). However, aqueous systems are complex, involving multiple H-bonds and multiple conformations of H$_2$O molecules around the solvated species. If evaluation were done in these systems, the calculation time would be markedly increased and the benefit of evaluation to select reasonable methods before moving to large systems would be lost. We attempt to avoid this by using vibrational frequency prediction in weakly-bound (CO$_2$-H$_2$O) and H-bonded (H$_2$O-H$_2$O) dimers that will appear in our system. We find that small systems like this can be used to select a few promising basis set/functional calibrations, rather than applying all available methods to large cluster calculations.

**Functional Development and Evaluation in Other Studies**

Many functionals are parameterized to accurately describe intermolecular properties, particularly involving H$_2$O due to its ubiquity. Others that are not parameterized expressly with H$_2$O in mind are evaluated against its properties and often perform well (Santra 2010).

GGA functionals usually outperform LDA functionals in their description of H$_2$O geometries and interactions, but don’t outperform hybrid functionals (Xu and Goddard 2004a). However, they generally have lower computation times relative to hybrid functionals, making them a common choice when describing condensed-phase water (Santra 2010). They also underperform when calculating vibrational frequencies and fractionation factors in this study,
with the best GGA, PBEPBE, underperforming five hybrid functionals, beating only mPW1K and BH&HLYP (Figs. 2-6 and 2-8). This is despite the fidelity of the GGAs studied here in replicating various properties of H$_2$O-H$_2$O interactions. BLYP is the best GGA at predicting frequency shifts in H$_2$O upon dimerization using the aug-cc-pVTZ(-f) basis set (Xu and Goddard 2004a), and is often used to model condensed-phase water despite generally underestimating H-bond energies (Santra 2010); its absolute isotopic fractionation deviations from experimentally-measured values in this study are 4.5‰ and 5.3‰ for the small and large basis set, respectively. Using the 6-311+G(2df,2p) basis set, GGA functional mPWLYP is the best GGA functional for interaction energies in a database of H$_2$O dimers and trimers (Dahlke and Truhlar 2005); its deviations from experimentally-measured values are 4.4‰ and 5.2‰ respectively. The PBE1W functional was developed to outperform mPWLYP in its description of H$_2$O dimer and trimer energetics (Dahlke and Truhlar 2005); its deviations from experimentally-measured values are 3.5‰ and 4.4‰ respectively, beating mPWLYP but not to the extent that it is quantitatively useful. PBEPBE is a simple functional designed to use only fundamental constants for new parameters (Perdew et al. 1996). PBEPBE is the best GGA functional for dimerization energy prediction using the aug-cc-pVTZ(-f) basis set (Xu and Goddard 2004a). The functional tends to overestimate H-bond energies, but is popular for condensed-phase water modeling (Santra 2010). PBEPBE is the most accurate predictor of $^{16}$$\alpha_{eq}$(CO$_2$(g)-H$_2$O(g)) among GGA functionals but still not as accurate as many hybrid functionals, with deviations from experimentally-measured values of 2.9‰ and 3.9‰.

Hybrid functionals generally outperform GGA functionals in structural and energetic tests (Xu and Goddard 2004a) as well as in prediction of harmonic frequencies and fractionation factors. However, not all hybrid functionals perform equally well. BH&HLYP is the only hybrid functional to significantly overestimate H-O-H angle when tested against B3P86, BLYP, B3LYP, and X3LYP using the aug-cc-pVTZ(-f) basis set; it is also outperformed by GGA functionals
PBEPBE and BLYP, probably due to including too much exact exchange (Xu and Goddard 2004a). Its deviations from experimentally-measured values are 5.4‰ and 4.6‰. The mPWB1K functional was developed for reaction kinetics across a wide range of molecules but also performed well for noncovalent interactions (Zhao and Truhlar 2004; 2010). The functional does better than X3LYP and much better than B3LYP in predicting energies of a range of H-bonded systems, using both double- and triple-zeta Pople basis sets (Zhao and Truhlar 2004). Despite this, it performs poorly in fractionation calculations, with 5.4‰ and 4.4‰ deviations. M06-2X was developed to cover a broad range of properties, from barrier heights to aromatic molecule stacking behavior, in main-group elements (Zhao and Truhlar 2008; 2010). In particular, the M06-2X performs well for non-bonded interactions; it is twice as accurate at predicting H-bond energies as B3LYP when the 6-311+G(3d2f,2df,2p) basis set is used. Despite this, its deviations from experimentally-measured values are relatively high, with values of 3.7‰ and 2.6‰. PBE0 was developed as a “parameter-free” functional containing the PBE GGA functional with a component of exact exchange (Adamo and Barone 1999). Despite not being tuned in any way to describe non-bonded interactions, it has lower deviations from experimentally-measured values than many other functionals: 2.2‰ and 1.2‰. The B3P86 functional predicts H$_2$O monomer geometry better than B3LYP and X3LYP, but is not as successful for dimers (Xu and Goddard 2004a). The deviations from experimentally-measured values are acceptable using these basis sets: 1.4‰ and 0.5‰. The B3LYP functional is widely used although it describes metals poorly (Santra 2010). Several studies have highlighted its failures in predicting barrier heights and noncovalent interaction energies such as H-bond energies (Zhao and Truhlar 2004; 2008; 2010; Santra 2010). However its deviations from experimentally-measured fractionation values in this study are minimal, with values of 0.2‰ and 0.6‰. In fact, B3LYP does better than MP2 at predicting harmonic frequencies for a range of small molecules, and is similar to CCSD(T), using the cc-pVTZ basis set (Lin et al. 2004). The X3LYP functional is the best predictor of H$_2$O dimer...
properties when using the aug-cc-pVTZ(-f) basis set (Xu and Goddard 2004a). X3LYP was developed in particular to exceed B3LYP’s descriptions of H-bonded systems (Xu and Goddard 2004b). X3LYP maintains B3LYP’s success in predicting isotopic fractionation, with values of 0.6‰ and 0.2‰.

Despite M06-2X’s success in predicting a range of properties including H-bond energies better than other functionals, it underperforms B3LYP, X3LYP, and PBE0 when calculating both monomer and dimer vibrational frequencies in this study. B3LYP better predicts harmonic vibrational frequencies than M06-2X and most other functionals with several commonly-used triple-zeta basis sets. B3LYP’s scaling factor with basis set 6-311+G(3d2f,2df,2p) is 0.998, much closer to unity than 0.982 for M06-2X and one of the best functionals available in this regard. Lin et al. (2004) found that B3LYP matched the performance of CCSD(T) in calculation of harmonic frequencies using the cc-pVTZ basis set. Success in prediction of geometric and energetic parameters does not guarantee success in prediction of harmonic frequencies.

CO₂ is not nearly as common a target for functional evaluation as H₂O, especially because its interactions with H₂O are comparatively weak. In general, these evaluation tests are not a perfect comparison, because the vibrational comparison involves dimer interactions that do not affect the fractionation factors of gas-phase molecules. In general, studies of fractionation factors would benefit from evaluation of more intermolecular effects on harmonic vibrational frequencies, to allow for comprehensive evaluation, if sub-% accuracy is desired. Future studies may benefit from spectroscopic evaluation of dimers representing important interactions in the system of interest. Studies of CO₂-H₂O dimer harmonic vibrational frequencies were useful in this study, but studies of other aqueous DIC species would benefit from measurement of the e.g. HCO₃⁻-H₂O dimer vibrational spectrum and determination of harmonic frequencies using anharmonic spectroscopic corrections. The B3LYP functional predicts harmonic vibrational
frequencies across a range of different molecules (Lin et al. 2004; Zhao and Truhlar 2008), so it is
a good starting point for calculation evaluation.

When selecting functionals to use in other studies of aqueous isotopic fractionation, there
are four points to keep in mind. First, functionals that perform well with one basis set may
perform very poorly with another, even of similar size. Tests should be performed with any basis
sets that are intended to be used. Second, hybrid functionals are much better suited to
fractionation calculations than GGA functionals or even MP2 calculations. Third, harmonic
vibrational frequencies are a good predictor of fractionation factor accuracy. Fourth, and more
generally, functionals should be tested in the system of interest, not selected on which functional
is the best generalist.

**Summary**

The basis sets 6-311+G(d,p) and 6-311++G(2d,p) are suitable for use to describe
interactions between CO$_2$ and H$_2$O in DFT calculations. Using these basis sets, the B3LYP and
X3LYP functionals do the best job describing harmonic vibrational frequencies and isotopic
fractionations in the CO$_2$-H$_2$O system, with promise for dissolved inorganic carbon species.
Harmonic vibrational frequency comparison is a useful tool to predict suitability for isotope
calculations, much more so than accuracy in energetics or geometries.
Figure 2-1. Evaluation of optimization time of the CO$_2$-H$_2$O dimer, in seconds. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-2. Evaluation of C(CO$_2$)-O(H$_2$O) intermolecular distance in the CO$_2$-H$_2$O dimer. Deviations from the experimental value of 2.836Å (Peterson and Klemperer 1984). The high-level CCSD(T)/CBS calculation of Wheatley and Harvey (2011) is also included. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-3. Evaluation of the interaction energy of CO₂ and H₂O. Deviations from the CCSD(T)/CBS value of 7.38 kJ/mol (Wheatley and Harvey 2011) are plotted. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-4. Evaluation of the O-O distance in the H₂O-H₂O dimer. Deviations relative to the experimental value of 2.976Å without anharmonic correction (Odutola and Dyke 1980) are plotted. The high-level CCSD(T)(FULL)/IO275(extrapolated to CBS limit) calculation of Klopper et al. (2000) is also included. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-5. Evaluation of the H$_2$O-H$_2$O interaction energy. Deviations from the experimental value of 13.2 kJ/mol (Rocher-Casterline et al. 2011) are plotted. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-6. Evaluation of vibrational frequencies. $R^2$ values comparing harmonic experimental vibration frequencies with computed values, for the H$_2$O monomer (Benedict et al. 1956), the CO$_2$-H$_2$O dimer (Andersen et al. 2014), and the (H$_2$O)$_2$ dimer (Fredin et al. 1977). Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-7. Evaluation of vibrational shift upon isotopic substitution. RMS deviations for vibrational shifts upon isotope substitution, scaled by vibrational frequency, compared with experimental values for the CO₂ monomer (Rothman and Young 1991), the H₂O monomer (Tennyson et al. 2013), the CO₂-H₂O dimer (Andersen et al. 2014), and the (H₂O)₂ dimer (Bouteiller et al. 2011). Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-8. Evaluation of $^{18}$O isotopic fractionation between CO$_2$ and H$_2$O(g). Deviations from the experimental value of $^{18/16}_{\text{eq}}$(CO$_2$(g)-H$_2$O(g))= 1.0510 at 25°C (Horita and Wesolowski 1994; Beck et al. 2005) are plotted in ‰. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Table 2-1. Basis sets and functionals selected for further study in this dissertation. Blue/green functionals most accurately predicted \(^{18/16}\alpha_{eq}(\text{CO}_2-\text{H}_2\text{O})\) as well as vibrational frequencies in the \text{CO}_2 and \text{H}_2\text{O} monomers and dimers. Darker colors represent use of the larger basis set. Color scheme is consistent throughout this dissertation.

<table>
<thead>
<tr>
<th>Model/Class and Color</th>
<th>Abbreviation</th>
<th>\text{CO}_2-\text{H}_2\text{O} Vibration RME E&lt;sub&gt;\text{r} \sigma&lt;/sub&gt;</th>
<th>(^{18/16}\alpha_{eq}(\text{CO}_2-\text{H}_2\text{O})) Deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBE0/6-311+G(d,p)</td>
<td>P+</td>
<td>83.50</td>
<td>~2.01</td>
</tr>
<tr>
<td>PBES0/6-311+G(d,p)</td>
<td>P0+</td>
<td>49.10</td>
<td>2.23</td>
</tr>
<tr>
<td>X3LYP/6-311+G(d,p)</td>
<td>X+</td>
<td>38.71</td>
<td>6.63</td>
</tr>
<tr>
<td>M06-2X/6-31++G(2d,2p)</td>
<td>P++</td>
<td>87.06</td>
<td>~3.66</td>
</tr>
<tr>
<td>M06-2X/6-31++G(2d,2p)</td>
<td>P0++</td>
<td>41.41</td>
<td>1.25</td>
</tr>
<tr>
<td>X3LYP/6-31++G(2d,2p)</td>
<td>X++</td>
<td>25.59</td>
<td>~0.18</td>
</tr>
<tr>
<td>M06-2X/6-311+G(d,p)</td>
<td>B+</td>
<td>27.95</td>
<td>6.24</td>
</tr>
<tr>
<td>M06-2X/6-31++G(2d,2p)</td>
<td>B++</td>
<td>25.86</td>
<td>~0.98</td>
</tr>
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Figure 2-9. Absolute values of deviations in calculated $^{18/16}\alpha_{eq}(\text{CO}_2\text{(g)}-\text{H}_2\text{O}(\text{g}))$ (absolute values of data presented in Fig. 2-8) versus RMS errors of harmonic vibration prediction (Fig. 2-6). The major outlier, with low RMS error but $>4\%$ deviation in fractionation factor, is the MP2 method. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-10. Absolute values of deviations in calculated $^{16/16}\alpha_{eq}(\text{CO}_2(\text{g})-\text{H}_2\text{O}(\text{g}))$ (absolute value of Fig. 2-8) versus optimization time of the CO$_2$-H$_2$O dimer, in seconds (Fig. 2-1). Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-11. Absolute values of deviations in calculated $^{18/16}\alpha_{\text{eq}}(\text{CO}_2(\text{g})-\text{H}_2\text{O}(\text{g}))$ (absolute value of Fig. 2-8) versus deviation from the experimental measurement of $\text{C}$(CO$_2$)-O(H$_2$O) intermolecular distance in the CO$_2$-H$_2$O dimer (Peterson and Klemperer 1984; Fig. 2-2). Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-12. Absolute values of deviations in calculated $^{18/16}\alpha_{eq}(\text{CO}_2(\text{g})-\text{H}_2\text{O}(\text{g}))$ (absolute value of Fig. 2-8) versus deviation from the CCSD(T)/CBS interaction energy of CO$_2$ and H$_2$O (Wheatley and Harvey 2011; Fig. 2-3). Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-13. Absolute values of deviations in calculated $\alpha_{eq}(\text{CO}_2(\text{g})-\text{H}_2\text{O}(\text{g}))$ (absolute value of Fig. 2-8) versus deviation from experimental measurement of O-O distance in the H$_2$O-H$_2$O dimer (Odutola and Dyke 1980; Fig. 2-4). Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-14. Absolute values of deviations in calculated $^{16/16}\alpha_{eq}(\text{CO}_2(g)-\text{H}_2\text{O}(g))$ (absolute value of Fig. 2-8) versus deviation from experimental measurements of the H$_2$O-H$_2$O interaction energy (Rocher-Casterline et al. 2011; Fig. 2-5). Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
Figure 2-15. Absolute values of deviations in calculated $^{18/16}\alpha_{eq}(\text{CO}_2(\text{g})-\text{H}_2\text{O}(\text{g}))$ (absolute value of Fig. 2-8) versus root-mean-square (RMS) deviations for vibrational shifts upon isotope substitution, scaled by vibrational frequency, compared with experimental values for the CO$_2$ monomer (Rothman and Young 1991), the H$_2$O monomer (Tennyson et al. 2013), the CO$_2$-H$_2$O dimer (Andersen et al. 2014), and the (H$_2$O)$_2$ dimer (Bouteiller et al. 2011), as represented in Fig. 2-7. Colored dots represent basis sets and functionals chosen for further use: see Table 2-1.
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Chapter 3

Isotopic Fractionation in the CO$_2$ Hydration and Hydroxylation Reactions

Abstract

The formation of H$_2$CO$_3$ and HCO$_3^-$ from CO$_2$ is ubiquitous in aqueous environmental systems and occurs via the CO$_2$ hydration and hydroxylation reactions. The reactions are especially important as the source of CO$_3^{2-}$ for precipitation of CaCO$_3$ minerals, which are analyzed to produce paleoclimate proxy records. The reactions carry with them equilibrium and (as yet unknown) kinetic isotope fractionations that may impact these proxy records. Quantum mechanical calculations of dissolved inorganic carbon species embedded in H$_2$O clusters are analyzed under a transition state theory framework to predict kinetic fractionations associated with the reactions. Experimental measurements of reaction energetics and equilibrium isotope fractionations are well-reproduced by the models. Hydrogen bond counts and, in some cases, C(CO$_2$)-O(H$_2$O or OH$^-$) distances greatly influence the calculated values of isotope fractionation factors. The CO$_2$ hydration reaction is predicted to discriminate against both $^{13}$C and $^{18}$O by 10-12‰. The CO$_2$ hydroxylation reaction is predicted to discriminate against $^{13}$C by 13-16‰ and against $^{18}$O by 19-21‰. Implications for coral carbonate paleothermometry and fractionation in saline environments are discussed.
Introduction

Isotope Fractionation and Aqueous Reactions of CO₂

The CO₂ hydration and hydroxylation reactions (equations 1.1 and 1.2) generate HCO₃⁻ and CO₃²⁻ from aqueous CO₂. The reactions are thus important to the formation of natural carbonates. Equilibrium fractionation due to these reactions is well-known experimentally, but kinetic fractionation has not been extensively evaluated. Kinetic fractionation may be a cause of isotopic disequilibrium in corals, which limits their ease of use as paleoclimate indicators. These reactions and their potential application to coral paleoclimatology are summarized in Chapter 1 of this dissertation. In this chapter, the kinetic fractionation of the CO₂ hydration and hydroxylation reactions is evaluated using computational chemistry, and the results are applied to corals to determine whether kinetic fractionation can explain the isotopic trends in corals.

Equilibrium Isotope Fractionation

Isotopes fractionate because slight mass differences impart slight differences in chemical properties (theory and trends reviewed in Schable 2004). Most fractionation is termed mass-dependent fractionation and scales with the difference in mass of different isotopes, observable when a system has three or more different isotopes. The reason is systematic variations in vibrational, rotational, and translational energies imparted by variations in isotopic mass. At the low temperatures experienced at Earth’s surface, variations in vibrational energies contribute most of the isotopic fractionation.

The isotopic composition of different compounds can be depicted in different ways. Absolute isotopic composition can be described by the R value, which gives the ratio of heavy to light isotopes:
For example, the $^{13}\text{C}$ content of a sample of CO$_2$ can be described relative to $^{12}\text{C}$ content as:

$$R_{sample}^{13/12} = \frac{[^{13}\text{CO}_2]}{[^{12}\text{CO}_2]} \quad (3.2)$$

Often, the light isotope is omitted from the notation, leaving only $^{\text{heavy}}R_{sample}$, but as multi-isotope systems are increasingly analyzed (e.g. the four stable isotopes of S, the three stable isotopes of O), it becomes more important to include the light isotope in the notation to avoid confusion. More common than $R$ notation is delta ($\delta$) notation. This notation makes for easier description of experimental samples relative to measurement standards. For element $E$, $\delta$ notation is given as:

$$\delta^{\text{heavy/light}}_E = \left( \frac{R_{sample}^{\text{heavy/light}}}{R_{standard}^{\text{heavy/light}}} - 1 \right) \times 1000\% \quad (3.3)$$

Delta values of a particular sample will differ based on which measurement standard is used. In contrast, equilibrium fractionation factors ($\alpha_{eq}$) give the fractionation between two compounds and do not depend on standard. Between compounds A and B, $\alpha$ notation is given as:

$$\alpha_{eq}^{\text{heavy/light}}(A - B) = \left( \frac{R_A^{\text{heavy/light}}}{R_B^{\text{heavy/light}}} \right) = \frac{1000 + \delta^{\text{heavy/light}}_A}{1000 + \delta^{\text{heavy/light}}_B} \quad (3.4)$$

The value of $\alpha_{eq}$ is larger when a heavy element concentrates in compound A over compound B. Because fractionations in natural systems are small leaving $\alpha_{eq}$ very close to 1, they are sometimes expressed as epsilon ($\varepsilon$) values:

$$\varepsilon_{eq}^{\text{heavy/light}}(A - B) = \left( \alpha_{eq}^{\text{heavy/light}}(A - B) - 1 \right) \times 1000\% \quad (3.5)$$

The value of $\varepsilon_{eq}$ is the approximate difference, in $\%$, between substance A and B at isotopic equilibrium.
Most fractionation between different compounds concentrates heavier isotopes in compounds with stiffer bonds, whose spring constants and thus vibrational frequencies are larger. This is because most fractionation is controlled by differences in vibrational energy between isotopologues, or compounds with the same chemical composition but different isotopic compositions. The lowest possible (ground-state) vibrational energy is referred to as zero-point energy (ZPE). Isotopologues with lower ZPE are energetically more favorable than those with higher ZPE. The presence of heavier isotopes always lowers the ZPE of a given molecule, meaning all molecules favor heavy isotopes over light ones. However, some molecules favor heavy isotopes more than others, as governed by their specific vibrational frequencies. The ZPE of a molecule with stiffer bonds is lowered more by heavy isotopes than is the ZPE of a molecule with weaker bonds, causing a molecule with stiffer bonds to concentrate more heavy isotopes than a molecule with weaker bonds.

The dependence of $\alpha$ between molecules or molecular clusters with respect to temperature is given by two equations:

$$\alpha_{eq}^{\text{heavy/light}}(A - B) = \frac{\text{heavy/light} \beta_A}{\text{heavy/light} \beta_B} \quad (3.6)$$

$$\beta = \frac{Q_{\text{heavy}}}{Q_{\text{light}}} = \prod_i \left( \frac{\nu_{\text{heavy},i}}{\nu_{\text{light},i}} \frac{e^{-h\nu_{\text{heavy},i}/2k_B T}}{1 - e^{-h\nu_{\text{light},i}/2k_B T}} \right) \quad (3.7)$$

Here, $\beta$ is referred to as the reduced partition function ratio, and is the ratio of the reduced partition functions $Q$ that describe how energy is partitioned among vibrational and rotational modes. $Q$ is calculated from the harmonic vibrational frequencies $\nu$ of the molecule or cluster, which can be derived from infrared and Raman spectroscopic measurements or calculated from computational chemistry models. The frequencies depend on whether the heavy or light isotope is included. The statistical-mechanical derivation of this expression is reviewed by Schauble (2004). Essentially, the $\nu_{\text{heavy}}$ and $\nu_{\text{light}}$ terms arise from translational and rotational energies and their connection to vibrational frequencies via the Redlich-Teller product rule, the $\exp(-h\nu/2k_B T)$ terms
arise from ground-state vibrational energies $h\nu/2$, and the $1-\exp(-h\nu/k_BT)$ terms arise from a series expansion of all excited-state vibrational energies. The mass dependence of isotope fractionation is controlled by the dependence of $\nu$ on mass, with heavier isotopes reducing vibrational frequencies.

In this work, fractionation between CO$_2$ and DIC (either H$_2$CO$_3$ or HCO$_3^-$) is analyzed, for both stable isotopes of C ($^{12}$C and $^{13}$C) and O ($^{16}$O and $^{18}$O, excluding $^{17}$O). The relevant expressions for the hydration reaction at equilibrium with H$_2$CO$_3$ (equation 1.1) are:

\[
\frac{^{13}}{^{12}}\alpha_{eq}(H_2CO_3 - CO_2) = \frac{^{13}/^{12}\beta_{H_2CO_3}}{^{13}/^{12}\beta_{CO_2}} \quad (3.8)
\]

\[
\frac{^{18}}{^{16}}\alpha_{eq}(H_2CO_3 - CO_2) = \frac{^{18}/^{16}\beta_{H_2CO_3}}{^{18}/^{16}\beta_{CO_2}} \quad (3.9)
\]

The relevant expressions for the hydroxylation reaction at equilibrium with HCO$_3^-$ (equation 1.2) are:

\[
\frac{^{13}}{^{12}}\alpha_{eq}(HCO_3^- - CO_2) = \frac{^{13}/^{12}\beta_{HCO_3^-}}{^{13}/^{12}\beta_{CO_2}} \quad (3.10)
\]

\[
\frac{^{18}}{^{16}}\alpha_{eq}(HCO_3^- - CO_2) = \frac{^{18}/^{16}\beta_{HCO_3^-}}{^{18}/^{16}\beta_{CO_2}} \quad (3.11)
\]

**Kinetic Fractionation Factors and Transition State Theory**

Kinetic fractionation refers to fractionation caused by rate differences in a unidirectional process, such as a one-way chemical reaction. Kinetic fractionation can be expressed as a ratio of rates $\alpha_{kin}$:

\[
\frac{\text{heavy}}{\text{light}} \alpha_{kin} = \frac{\text{heavy}k_{rxn}}{\text{light}k_{rxn}} \quad (3.12)
\]

where $k_{rxn}$ refers to an isotope-specific reaction rate constant. The relevant expression for the reactions involving C fractionation during CO$_2$ hydration, for example is:

\[
\frac{^{13}}{^{12}}\alpha_{kin}(hydration) = \frac{^{13}k_{hydration}}{^{12}k_{hydration}} \quad (3.13)
\]
where the values of \( k \) refer to the reaction rate constants of isotopically pure \( \text{CO}_2 \) at a given concentration:

\[
^{13}\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2^{13}\text{CO}_3 \quad (3.14)
\]

\[
^{12}\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2^{12}\text{CO}_3 \quad (3.15)
\]

The equilibrium fractionation factor \( \alpha_{eq} \) is equal to the ratio of the kinetic fractionations in the forward and reverse directions \( \alpha_{\text{kin}}(\text{forward})/\alpha_{\text{kin}}(\text{reverse}) \), in the same way that an equilibrium constant for a chemical reaction is the ratio of the rate constants for the forward and reverse reactions.

Obtaining kinetic isotope effects on elementary reactions from experimental data is problematic because it is difficult to separate out the integrated effects of all steps in a complex reaction mixture that is not isotopically pure. Moreover, it is difficult to prevent reverse reactions to isolate a pure product that has only been affected in a single direction. Computational chemistry methods allow one to model elementary reactions and isotopic fractionations (e.g. Felipe et al. 2001; Kubicki 2008; Zeebe 2014) via computation of the ZPE of different molecules.

In transition state theory (TST), the transition state (TS) is treated as if it were a stable chemical species in computing an equilibrium constant, and an equilibrium expression is set up between the reactant and the TS:

\[
K_{eq(TS)} = \frac{[TS]}{[\text{reactant}]} = e^{-(G_{TS} - G_{\text{reactant}})/RT} \quad (3.16)
\]

Here \( G \) is the Gibbs free energy of the chemical species in question and \( R \) is the ideal gas constant. When the reactant and TS are isotopically pure, \( G_{TS} \) and \( G_{\text{reactant}} \) will have particular values depending on the isotopes in question. Instead of computing heavy \( G \) and light \( G \) separately, Felipe et al. (2001) derive an expression for \( K_{eq(TS)} \) from partition functions \( Q \):

\[
K_{eq(TS)} = \frac{Q_{TS}}{Q_{\text{react}}} e^{-(U_{TS} - U_{\text{reactant}})/RT} \quad (3.17)
\]
Here $Q_{TS}$ and $Q_{reac}$ are analogous to the expressions given in equation 3.7, except that $Q_{TS}$ excludes the imaginary vibrational frequency that represents the transit of the TS complex over the energy saddle point, $U$ is the electronic energy plus the ZPE of the relevant species, and the subscripts TS and reac refer to partition functions and energies of the transition state and reactant species respectively. The imaginary frequency is replaced with an expression representing translation over the saddle $kT/h$. But because we are interested here in ratios between these expressions containing different isotopes and because the translation expression is isotope-independent, those expressions cancel in the final result and can be ignored (Felipe et al. 2001; Kubicki 2008).

The expression for $K_{eq(TS)}$ can be related to $\alpha$ for single-isotope substitutions as described by Schauble (2004), giving the final expression that can be used to calculate $\frac{\alpha_{kin}}{\alpha_{kin}}$:

$$\frac{\alpha_{kin}^{heavy}}{\alpha_{kin}^{light}} = \frac{\alpha_{TS}^{heavy}}{\alpha_{TS}^{light}} \times \frac{\alpha_{reac}^{heavy}}{\alpha_{reac}^{light}} = \left(\frac{Q_{heavy}^{TS}}{Q_{light}^{TS}}\right) \left(\frac{Q_{heavy}^{reac}}{Q_{light}^{reac}}\right) (3.18)$$

where the expression for $Q_{heavy}/Q_{light}$ is given in equation 3.7, and the imaginary frequency is excluded in the case of the TS reduced partition function.

Application of Quantum Mechanical Model Calculations

Isotope fractionation factors could, in principle, be calculated using experimentally observed harmonic vibrational frequencies input into Equation 3.7. However, these are difficult to obtain for several reasons. First, frequencies obtained from vibrational spectroscopy include anharmonicity and must be corrected using extrapolation from different levels of rotational and vibrational excitation to recover harmonic frequencies. Second, spectra involving isotopically pure substances involving both light and heavy isotopes must be available. Third, not all vibrations are infrared-active, but all vibrations need to be obtained for use in Equation 3.7.
Fourth, separating close vibrational frequencies from experimental spectra is difficult, especially in solutions where some vibrations of the solute overlap with that of the solvent.

As a result of these issues, computational chemistry modeling is a more useful method to obtain harmonic vibrational frequencies. These frequencies for motion along normal modes are easily calculated at a potential energy minimum (see Kubicki 2001 for a review of the technique and Ochterski 1999 for a review of its implementation in the GAUSSIAN™ computational chemistry program). In short, calculation of harmonic vibrational frequencies involves calculation of second derivatives of potential energy with respect to atomic motions. These frequencies can then be directly applied in Equation 3.7 if the frequencies are known for species involving both light and heavy isotopes.

Past Computational Chemistry Results on DIC Isotope Fractionation

Previous research has achieved deviations from experimentally-measured fractionation factor values of 0.5-2‰ in the DIC system (Rustad et al. 2008; Zeebe 2009). However, large deviations (>9‰) are encountered using some combinations of basis sets and DFT functionals (Rustad et al. 2008) or when too few solvating H₂O molecules are used (Zeebe 2009). A comparison between harmonic vibrational frequencies from calculations and derived from spectra can be used to select a more accurate combination of basis set and functional, as discussed in Chapter 2 of this dissertation.

Use of single conformations can also impart a 2-3‰ error (Rustad et al. 2008). Some kinetic fractionation factors have been calculated for single conformations of the CO₂ hydration reaction; the calculations found discrimination against ¹³C of 22-32‰ (Zeebe 2014), while experimental evaluations of the same reaction found a discrimination of only 7-20‰ (Marlier and O’Leary 1984; Clark and Lauriol 1992).
Goal

The goal of this work is to accurately predict kinetic fractionation of stable C and O isotopes in the CO$_2$ hydration and hydroxylation reactions. Prior work has predicted a large spread of $\alpha_{\text{kin}}$ values. This work applies multi-conformation modeling and density functionals evaluated for accuracy in isotope fractionation prediction, along with statistical analysis of the solvation environment around reacting species, to achieve a more accurate result.

This work also extends computational methodologies used to calculate isotope fractionation in aqueous systems. Past work has not sought to evaluate the effects of specific intermolecular interactions on fractionation factors. This work suggests a way in which this can be done, applying a limited number of supramolecular cluster conformations to calculate isotopic fractionation in aqueous systems.

Methods

Summary

To model kinetic fractionation in DIC species, the transition states for reactions 1.1 and 1.2 were modeled to determine $E_a$, $^{13/12}\alpha_{\text{kin}}$, and $^{18/16}\alpha_{\text{kin}}$ with various combinations of isotopic substitutions, using TST. Properties were evaluated in molecular clusters of various sizes. Separate reaction rates for different isotopologues were computed. Basis sets and functionals used in the calculations were selected based on evaluation against experimental data on intermolecular interactions, vibrational frequencies with corresponding isotopic shifts, and gas-phase equilibrium fractionation factors (Chapter 2, this dissertation). We will refer to a particular basis set/functionual combination as a model chemistry. Equilibrium properties $\Delta G^0$, $^{13/12}\alpha_{\text{eq}}$, and $^{18/16}\alpha_{\text{eq}}$ were also computed and compared with experimental values. The effects of aqueous solvation
and H-bonding pattern were evaluated by multiple linear regression of H-bond counts against fractionation factors.

**Cluster Generation**

A ~20Å x 20Å x 20Å water ice supercell (i.e. a block of multiple crystallographic unit cells) was constructed in the chemical visualization and modeling suite Materials Studio™. A CO$_2$ molecule was added to the center of the supercell, and a molecular dynamics (MD) calculation was performed for 10 ns with 1 ns equilibration time in the NVT ensemble using the Universal Force Field (Rappé et al. 1992) in the GULP module (Gale 1997). An additional 15 ns MD calculation was performed and a snapshot of the structure was sampled every 1 ns.

For each snapshot, the closest N H$_2$Os, measured by C(CO$_2$)-O(H$_2$O) distance, were selected to build a conformation with a certain number of H$_2$O molecules. Values of N were set at 3, 4, 5, 6, 8, 10, 15, and 25 to cover a range of cluster sizes while also being computationally feasible. Fifteen initial conformations at each of eight cluster sizes were generated, for a total of 120 initial hydration conformations to study reaction equation 1.1. A pre-solvated cluster composed of (H$_2$O)$_8$-OH was attached to each hydration conformation in a random angular position with the O(OH$^-$) oriented towards C(CO$_2$) to generate 120 hydroxylation conformations to study reaction equation 1.2.

Each conformation underwent initial DFT optimization using PBEPBE/6-311+G(d,p) (Ditchfield et al. 1971; Perdew et al. 1996) in the GAUSSIAN™ program (Frisch et al. 2009). Optimization criteria were set to Normal (maximum/RMS atomic displacement change per step 0.0018/0.0012 Bohr, maximum/RMS force 0.00045/0.0003 Hartrees/Bohr or Hartrees/Radian), with a Fine integration grid mesh. The optimized structures served as the starting point for reactant conformation types, consisting of a CO$_2$ molecule, variable numbers of H$_2$O molecules,
and in the case of hydroxylation conformations, a single OH⁻ ion. Most hydroxylation clusters with less than 18 H₂O molecules formed HCO₃⁻ without an activation energy barrier; as a result, all hydroxylation conformations with less than 18 H₂O molecules were discarded.

For each reactant conformation, a scan calculation was carried out using the same criteria as the initial optimization. The C-O distance between the CO₂ and either the closest H₂O molecule (in the case of hydration reactions) or the hydroxide ion (in the case of hydroxylation reactions) was shrunk in increments of 0.2Å until either H₂CO₃ or HCO₃⁻ formed. These product states were then fully optimized. The optimized structures served as the starting point for product conformation types.

Starting points for TS calculations were selected by analyzing the potential energy surface from the scan calculation composed of the completed optimization at each scan step. The potential energy maximum nearest the product state served as the starting point for TS conformation types. In some cases, substantial H₂O rearrangement during the scan process lowered the energy enough that the TS energy was below the original reactant energy. In these cases, a new starting point for the reactant was selected at the potential energy minimum prior to reaching the TS in the scan calculation.

**Cluster Optimization**

Starting points for all three conformation types – reactant, TS, and product – were further optimized in each model chemistry: first PBEPBE/6-311+G(d,p), then each remaining combination of PBEPBE, PBE0, B3LYP, and X3LYP with basis sets 6-311+G(d,p) and 6-311++G(2d,p). X3LYP/6-311++G(2d,p) gave the best results in evaluations (Chapter 2, this dissertation), while X3LYP/6-311+G(d,p) and both B3LYP model chemistries also performed
well: these are referred to as favored model chemistries. PBE0 and PBEPBE performed fairly in evaluations (Chapter 2, this dissertation).

Reactants and products were optimized 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. For some calculations, the Berny optimization algorithm oscillated in energy during optimization without converging, due to the shallow potential energy surface involved in H₂O molecule translation and libration; if this behavior persisted, optimization criteria were changed to Normal. TS optimization began using the synchronous transit-guided quasi-Newton method (Peng and Bernhard Schlegel 1993), using the QST3 keyword in GAUSSIAN™, with the reactant and product states taken from fully-optimized configurations, and the TS guess taken from the TS starting point. In cases where use of redundant internal coordinates caused optimization problems, optimization was continued using a first-order saddle point using the Berny algorithm (GAUSSIAN™ input: opt=(TS,Cartesian)). If the original starting configuration did not optimize to a TS but to a minimum instead, a finer scan calculation with a 0.01Å increment was carried out around the original guess, followed by additional QST3 and (TS,Cartesian) calculations. The calculation was aborted if this process still did not yield a TS.

**Vibrational Frequency Calculation**

Following optimization, each conformation underwent a vibrational frequency calculation (GAUSSIAN™ keyword: freq; see Kubicki (2001) for a general discussion of frequency calculations and Ochterski (1999) for a discussion of the method’s implementation in the program). Reactant and product conformations were checked to ensure they had no imaginary (i.e., “negative” as reported by most programs) frequencies to confirm the structure was in a local
potential energy surface minimum. TS conformations were checked to ensure they had exactly one imaginary frequency corresponding to either H$_2$O or OH attack on C(CO$_2$) for the hydration or hydroxylation reaction, respectively. Frequency calculations were repeated with isotopic substitutions on all atoms of interest: the C (\(^{12}\)C and \(^{13}\)C), the O on the original CO$_2$, and the attacking O from the H$_2$O or OH$^-$ (\(^{16}\)O and \(^{18}\)O) for a total of 16 isotopologues per conformation. The vibrational frequencies under different isotopic substitutions, as well as the ZPE-corrected internal energies, were used to calculate the fractionation factors.

**Thermochemical and Fractionation Factor Analysis: Bulk**

Thermochemical parameters and fractionation factors were calculated both for (1) each individual reactant/TS/product path, and (2) averaged over the bulk ensemble of calculations with the same number of H$_2$O molecules in the clusters. For each individual reaction path, the ZPE-corrected activation energy (\(E_a\)) and Gibbs free energy (\(\Delta G^\circ\)) of the reaction were calculated from the properties of the TS and product, respectively, relative to the reactant, using equation 3.16. For these thermochemical parameters, the properties of the unsubstituted isotopologue were used, which makes up the vast majority of natural DIC compounds. Kinetic and equilibrium fractionation factors were calculated using equations 3.6, 3.7, and 3.18 applied to the product and TS calculations. For the TS calculation, imaginary vibrational frequencies were excluded from calculation of the reduced partition function ratio.

The calculation of fractionation factors is complicated by the use of multiple isotopologues, and by the fact that isotopologues can include both \(^{18}\)O and \(^{16}\)O in different sites. To account for this, the relative abundances of all isotopologues are calculated, and then summed to directly calculate R and thus \(\alpha_{eq}\) by equations 3.1 and 3.4. The total abundance of \(^{16}\)O in CO$_2$, for example, would be \(2\times^{12}\)C\(^{16}\)O$_2 + ^{12}\)C\(^{16}\)O\(^{18}\)O + \(^{12}\)C\(^{18}\)O\(^{16}\)O + \(2\times^{13}\)C\(^{16}\)O$_2 + ^{13}\)C\(^{16}\)O\(^{18}\)O + ^{13}\)C\(^{18}\)O\(^{16}\)O,
thus accounting for all isotopologues containing $^{16}$O, and how much $^{16}$O those isotopologues contain.

To calculate the parameters for the full ensembles with equal numbers of H$_2$O molecules, the same process is carried out, but the relative abundances of all calculations, including the reactants, are weighted according to a Boltzmann distribution $e^{-U/RT}$. This weights more energetically favorable conformations greater than less favorable ones. Weighted standard deviations were computed for each property at each cluster size as a method of error estimation, weighted by this same Boltzmann weight for each conformation.

During model optimization, it was noted that some hydration clusters formed H$_2$CO$_3$ as the final product while others formed an H$_3$O$^+$.HCO$_3^-$ ion pair. The Boltzmann-averaged product-relevant properties ($\Delta G^\circ$, $^{13/12}\alpha_{eq}$, $^{18/16}\alpha_{eq}$) were also computed separately for these two classes of products.

**Model Chemistry Systematics**

Systematic variation in each property for each model chemistry was evaluated, to determine whether use of a different model chemistry had a predictable effect on computation results, and determine whether a computationally less expensive model chemistry could be substituted in future calculations using a correction of constant magnitude. Each conformation using a particular model chemistry was compared to that conformation using every other model chemistry. Property changes were averaged across all cluster sizes, but were averaged separately for hydration and hydroxylation calculations. Error was estimated using the unweighted standard deviation in calculated property changes.
Effect of H-Bonding Pattern and Temperature

The effects of H-bonding pattern on properties of interest were evaluated. Four classes of H-bonds were considered: those donated from H$_2$O to a DIC hydroxyl (H$_\text{wat}$-OH$_\text{dic}$); those donated from H$_2$O to an unprotonated DIC oxygen, such as those participating in the delocalized π bond on HCO$_3^-$ (H$_\text{wat}$-O$_\text{ooc}$); those donated from a DIC hydroxyl to H$_2$O (H$_\text{dic}$-O$_\text{wat}$); and those donated from H$_2$O to H$_2$O (H$_\text{wat}$-O$_\text{wat}$). Cutoffs of <2.4Å O-H distance and >90° O-H-O angle were used to count H-bonds. Properties of individual conformations were compared with the number of each type of H-bond in the cluster.

A multiple linear regression analysis for each model chemistry was carried out to calculate the incremental impact of each type of H-bond on each isotopic fractionation factor. C-O$_\text{attack}$(H$_2$O or OH$^-$) was also included in the regression for calculation of kinetic fractionation factors. This analysis was used with best available information about the true H-bond structure of both the TS and product of hydration and hydroxylation reactions to calculate a best-guess model value for each fractionation factor. The TS H-bonding pattern for both the hydration and hydroxylation reaction was evaluated using a large 40-molecule cluster optimized at a less expensive model chemistry (PBEPBE/6-31+G(d), Normal optimization criteria, Fine integration grid mesh). Where available, experimental data were compared with the best-guess values. The computational fractionation factors were used to calculate expected fully-equilibrated/full-kinetic-fractionation C-O slopes for the hydration and hydroxylation reactions, and these slopes were compared with the observed C-O slope in corals to attempt to falsify the kinetic model of vital effects in corals.

The effects of temperature on fractionation factors were also evaluated. Temperature was adjusted in Equation 3.7 when calculating $^{13/12} \alpha_{\text{eq}}$, $^{18/16} \alpha_{\text{eq}}$, $^{13/12} \alpha_{\text{kin}}$, and $^{18/16} \alpha_{\text{kin}}$ for both hydration and hydroxylation reactions. The H-bond regression was repeated at increments of 1°C between 0
and 100°C, and best-guess values of fractionation factor were calculated using the same H-bond pattern evaluated at 25°C. Results are compared with experimental T-fractionation regressions where available.

Results

Example Reactant and Product Conformations

Example reactant and product conformations for the hydration reaction are given in Figure 3-1. A typical reactant conformation, with relatively complete solvation shell surrounding CO₂, is shown in Figure 3-1a. H₂O molecules typically do not donate H-bonds to O(CO₂), instead preferentially engaging in inter-H₂O H-bonds. This tends to cause H₂O molecules to clump together, and in some cases can result in exsolvation of the CO₂, leaving it on the exterior of a cluster of H₂O molecules.

An example of a product conformation resulting in H₂CO₃ is shown in Figure 3-1b. The H₂CO₃ in this conformation is involved in five H-bonds. One of the H in the attacking H₂O is part of the H₂CO₃, while the second has transferred to another H₂O. In this fashion, H⁺ ions transfer between H₂O molecules in all the reactions; in cases that produce H₂CO₃, the final acceptor is another O on H₂CO₃. Unlike reactant CO₂, the H₂CO₃ molecules actively participate in H-bonds. An example of a product conformation resulting in an HCO₃⁻ + H₃O⁺ ion pair is shown in Figure 3-1c. The HCO₃⁻ engages in seven H-bonds. In these conformations, H⁺ exchange cooperatively along H-bonds until the final acceptor is an H₂O which becomes H₃O⁺.

Example reactant and product conformations for the hydroxylation reaction are given in Figure 3-2. A reactant conformation is shown in Figure 3-2a. The CO₂ is well-solvated and sits at the center of the 33-H₂O cluster. The distance between CO₂ and OH⁻ labeled in green in this
conformation is larger than the CO$_2$-H$_2$O distance in the hydration reaction (Figure 3-1a). These distances tend to be longer in successful conformations; OH$^-$ placed near to the CO$_2$ in the initial cluster tends to bond to CO$_2$ without an energy barrier, especially when there are few H$_2$O surrounding the OH$^-$. A product conformation is shown in Figure 3-2b. Unlike in the hydration reaction, no proton transfer is required to form products in the hydroxylation reaction. The HCO$_3^-$ in this conformation is surrounded well by H$_2$O, but it engages in relatively few H-bonds. Not all species that are surrounded by H$_2$O well engage in H-bonds; if the inter-H$_2$O H-bond network is stable, no bonds may break upon formation of product.

**Example Transition State Conformations**

Example TS conformations from successful calculations are given in Figures 3-3 and 3-4. Those from the hydration reaction are shown in Figure 3-3, and those from the hydroxylation reaction are shown in Figure 3-4.

Figure 3-3a and Figure 3-3b show two TS conformations in the hydration reaction with 25 H$_2$O molecules but with different solvation states. The TS is at the center of the H$_2$O cluster in Figure 3-3a, and the edge of the cluster in Figure 3-3b. An example of a very large cluster is given in Figure 3-3c. This cluster was optimized with a simpler model chemistry to test the H-bonding pattern present when extra H$_2$O molecules are available. Figure 3-3d shows the TS of a very small cluster with only three H$_2$O molecules. Unlike larger clusters, an individual H$_2$O molecule in a small cluster may both donate and accept an H-bond with the TS.

Figure 3-4a and Figure 3-4b show two TS conformations in the hydroxylation reaction with 33 H$_2$O molecules but with different solvation states. Figure 3-4a shows a well-solvated TS with 7 H-bonds and the CO$_2$ and OH$^-$ molecules well-surrounded by H$_2$O molecules. Figure 3-4b
shows a poorly-solvated TS with only 5 H-bonds and a CO$_2$ molecule partially exsolvated from the H$_2$O cluster. Figure 3-4c shows a large hydroxylation cluster with nine H-bonds and 40 H$_2$O molecules, optimized using a simplified model chemistry. No small hydroxylation clusters analogous to Figure 3-3d exist because in these clusters CO$_2$ and OH reacted without a potential energy barrier to form HCO$_3^-$.

**Model Chemistries**

Model chemistries used in this chapter are listed in Table 3-1. The color scheme in this table is important, as it is used throughout this dissertation. Notably, blue and green colors reflect favored model chemistries. Dark green (X3LYP/6-311++G(2d,p)), in particular, performed the best in tests, but all blue and green models perform similarly.

**Boltzmann-Averaged Bulk Results**

Aggregated bulk model results for different model chemistries and cluster sizes are given in Figures 3-5 to 3-16. Aggregated bulk model results demonstrate to what extent large groups of conformations can be expected to replicate experimental parameters and predict unknown parameters without further consideration of intermolecular structure within the clusters. Results for $\Delta G^0$, $E_a$, $^{13/12} \alpha_{eq}$, $^{18/16} \alpha_{eq}$, $^{13/12} \alpha_{kin}$, and $^{18/16} \alpha_{kin}$ are shown for both hydration and hydroxylation calculations. Results are averaged across n=7-15 independent conformations, using the Boltzmann distribution to weight the conformation contributions by relative energy. Error bars represent one weighted standard deviation.
Bulk Gibbs Free Energies and Activation Energies

Model results for $\Delta G^0$ of the hydration and hydroxylation reactions are given in Figure 3-5 and 3-6, respectively. The models generally match the experimental value for CO$_2$ hydration $\Delta G^0$ of $+26$ kJ/mol (Wang et al. 2010), but there is a large spread in calculated values, and there are substantial differences between model chemistries. The weighted standard deviation for each model chemistry ranges from $+5$-$15$ kJ/mol. Favored model chemistries are generally more successful in predicting $\Delta G^0$ as H$_2$O cluster size increases, although this relationship breaks down in the largest clusters.

Results are similar for the calculated value of $\Delta G^0$ of the hydroxylation reaction; the models approximate the experimental $\Delta G^0$ of $-43$ kJ/mol (Wang et al. 2010) but with a large spread between model chemistries, and weighted standard deviations up to $15$ kJ/mol. Favored model chemistries tend to predict $\Delta G^0$ more accurately at large cluster sizes.

$E_a$ for CO$_2$ hydration (Figure 3-7) is well-predicted by model chemistries, reaching within a few kJ/mol of the experimental value of $91$ kJ/mol (Wang et al. 2010) at large cluster sizes, especially with favored model chemistries. Poorer-performing model chemistries predict $E_a$ well for smaller cluster sizes, but this is likely due to fortuitous cancellation of errors; their results worsen as the local chemical environment is more accurately described using more H$_2$O molecules. Weighted standard deviations between model conformations are often $\sim 10$ kJ/mol but can reach $20$ kJ/mol.

$E_a$ for CO$_2$ hydroxylation is somewhat poorly predicted using aggregated bulk model results (Figure 3-8). Most bulk models under-predict the experimental $E_a$ of $50$ kJ/mol (Wang et al. 2010); the only exception is PBEPBE/6-311+G(d,p), a relatively poor model chemistry for prediction of isotopic properties. There is systematic approach to the experimental value with
increasing cluster size. Note that the modeled activation energy excludes the entropic penalty required to place CO\textsubscript{2} and OH\textsuperscript{−} in close association with one another in a cluster.

**Bulk Equilibrium Fractionation Factors**

Equilibrium isotopic fractionation of \(^{13}\text{C}\) (i.e. \(^{13/12}\alpha\text{eq}(\text{product-CO}_2)\)) is described in Figures 3-9 and 3-10 for the hydration and hydroxylation reactions respectively. The product reference state in the hydration \(^{13/12}\alpha\text{eq}\) includes both H\textsubscript{2}CO\textsubscript{3} and the HCO\textsubscript{3}\textsuperscript{−}-H\textsubscript{2}O\textsuperscript{+} ion pair results, whereas the product reference state in the hydroxylation \(^{13/12}\alpha\text{eq}\) consists only of HCO\textsubscript{3}\textsuperscript{−} product. Values are plotted relative to the experimental fractionation factor \(^{13/12}\alpha\text{eq}(\text{HCO}_3^−-\text{CO}_2(\text{aq})) = 1.0090\) (Mook et al. 1974) where values greater than 1 indicate concentration of \(^{13}\text{C}\) in HCO\textsubscript{3}\textsuperscript{−}. No experimental information on \(^{13/12}\alpha\text{eq}(\text{H}_2\text{CO}_3-\text{CO}_2)\) is available for comparison. Hydration results exceed the experimental value by up to 10‰, a large deviation when total experimental fractionation is only 9‰.

The calculated hydroxylation \(^{13/12}\alpha\text{eq}(\text{HCO}_3^−-\text{CO}_2)\) values approach to within 1-5‰ of the experimental value at larger cluster sizes, but generally underestimate the degree to which \(^{13}\text{C}\) concentrates in HCO\textsubscript{3}\textsuperscript{−} (Figure 3-10). The X3LYP and B3LYP calculations are most accurate at intermediate cluster sizes. PBE0 appears to out-perform X3LYP and B3LYP in these bulk-averaged calculations; both PBE0/6-311+G(d,p) and PBE0/6-311++G(2d,p) reach within ~1‰ of the experimental value, while other model chemistries do not.

Equilibrium isotopic fractionation of \(^{18}\text{O}\) is described in Figures 3-11 and 3-12 for the hydration and hydroxylation reactions respectively. Like the experimental \(^{13/12}\alpha\text{eq}\) value, the experimental \(^{18/16}\alpha\text{eq} = 0.9906\) refers to fractionation between HCO\textsubscript{3}\textsuperscript{−} and CO\textsubscript{2} (Beck et al. 2005); no experimental information on \(^{18/16}\alpha\text{eq}(\text{H}_2\text{CO}_3-\text{CO}_2)\) is available. The bulk-averaged hydration calculations again overestimate \(^{18/16}\alpha\text{eq}(\text{HCO}_3^−-\text{CO}_2)\), by up to 10‰; only PBE/PBE model
chemistries get to within 2‰ of the experimental value, and weighted standard deviations can provide up to 3‰ deviation in individual calculations. The hydroxylation calculations, in contrast, underestimate the fractionation factor by ~4‰, and become systematically worse as the cluster size increases.

**Bulk Kinetic Fractionation Factors**

Kinetic isotope fractionation factors $^{13/12}\alpha_{\text{kin}}$ and $^{18/16}\alpha_{\text{kin}}$ are displayed in Figures 3-13 to 3-16. Values are all <1 because heavy isotopes react slower than light isotopes, meaning that heavy isotopes are depleted in products which do not achieve isotopic equilibrium by back-reaction to re-form reactants. Scant experimental evidence is available for $^{13/12}\alpha_{\text{kin}}$, and those studies that have investigated it find widely different results, ±7‰ different than the experimental values used here (O’Leary et al. 1992; Zeebe 2014). As such, any comparison made to experimental kinetic fractionation factors must be made with skepticism.

Figure 3-13 displays the kinetic fractionation results for $^{13}$C during CO$_2$ hydration. There is a large amount of scatter, and there are large, unsystematic differences between results obtained from different model chemistries. Weighted standard deviations range up to 5‰. The calculated values fall near the experimental value quoted here of 0.987 (O’Leary et al. 1992). Using these bulk-averaged calculations, it can only be said that the true $^{13/12}\alpha_{\text{kin}}$ likely falls between 0.98 and 0.995, i.e. a 5-20‰ discrimination against heavy isotopes.

Kinetic fractionation results for $^{13}$C during CO$_2$ hydroxylation are displayed in Figure 3-14. Calculated values for hydroxylation $^{13/12}\alpha_{\text{kin}}$ have slightly less spread than the hydration calculations, with smaller sample standard deviations than the corresponding hydration calculations. Based on the results from larger cluster sizes, the true value for hydroxylation
$^{13/12}_{\text{kin}}$ is probably between 0.983-0.994, with the better B3LYP and X3LYP generally predicting values towards the lower end of that range.

Figure 3-15 displays the kinetic fractionation for $^{18}$O in the hydration reaction. There is slightly less spread to the calculated values for $^{18/16}_{\text{kin}}$ than to the values for $^{13/12}_{\text{kin}}$ (Figure 3-13). Based on these results, the value for $^{18/16}_{\text{kin}}$(hydration) likely falls between 0.980 and 0.993.

Figure 3-16 displays the kinetic fractionation for $^{18}$O in the hydroxylation reaction. The best estimate for this $^{18/16}_{\text{kin}}$(hydroxylation) likely falls between 0.977 and 0.987. No systematic effects due to increasing cluster size are seen in either set of bulk calculations.

**Effect of Different Product Bonding Patterns**

Small hydration reaction clusters usually form H$_2$CO$_3$ as their end product, as the extra proton released during H$_2$O attack is electrostatically attracted to the negative HCO$_3^-$ . However, in larger clusters, the H$^+$ can be transmitted cooperatively along H-bonds away from the new HCO$_3^-$ , forming a stable H$_3$O$^+$-HCO$_3^-$ ion pair. Hydration reactions that produce HCO$_3^-$ (and H$_3$O$^+$) can be tabulated alongside hydroxylation reactions that exclusively produce HCO$_3^-$ to give the total properties of the final HCO$_3^-$ product.

The total $^{13/12}_{\text{eq}}$(HCO$_3^-$-CO$_2$) value for HCO$_3^-$ including both hydration and hydroxylation reactions is reported in Figure 3-19. These aggregated model results cluster around the experimental value of 1.0090 (Mook et al. 1974), but with a spread larger than 5‰ in some cases. Without including hydration calculations that produced HCO$_3^-$, $^{13/12}_{\text{eq}}$(HCO$_3^-$-CO$_2$) was generally underestimated (Figure 3-10).

The total $^{18/16}_{\text{eq}}$(HCO$_3^-$-CO$_2$) value for HCO$_3^-$ including both hydration and hydroxylation reactions is reported in Figure 3-20. These aggregated model results cluster around
the true value of 0.9906 (Beck et al. 2005), albeit with a spread of 4-5‰. When including only hydroxylation results, the true fractionation factor was underestimated by ~4‰ (Figure 3-12).

Together, the results for $^{13/12}$\alpha_{eq} and $^{18/16}$\alpha_{kin} that for isotopic fractionation calculations, aggregated results for similar products should be used when possible, even if paths used to form the products are different, and even if the clusters include different balances of positive and negative ions.

**Model Chemistry Systematics**

Calculated properties could vary systematically by model chemistry. Such systematics would enable the use of more efficient model chemistries without sacrificing accuracy.

Figures 3-17 and 3-18 display the systematic differences between each model chemistry for each property of interest, for both hydration and hydroxylation reactions. Most parameter comparisons have large standard deviations relative to their mean values, indicating different combinations do not behave systematically relative to each other. Also, large systematic differences in one property do not predict similarly large systematic differences in other properties. Kinetic fractionations in particular do not behave in a systematic fashion.

These results suggest that it is inappropriate to calculate properties of interest using a simpler, less costly model chemistry and then apply a scaling factor or a scaling constant. There is substantial variability between model chemistries applied to single conformations. The best model chemistry should be applied that is computationally feasible, where “best” refers to a model chemistry that reproduces relevant properties of interest. For isotopic fractionation studies, unscaled vibrational frequencies of monomers and dimers, and gas-phase isotopic fractionation factors, seem to provide reliable evaluation data (Chapter 2, this dissertation).
Effect of H-Bonding Pattern

Because aggregated bulk model results do not generally replicate experimental fractionation factors, the effect of intermolecular structure needs to be evaluated. In particular, different conformations have different solvation structures around DIC species (Figure 3-3 and 3-4). The effect of H-bonding pattern on fractionation factors was evaluated to determine how much variability could be accounted for by counting different classes of H-bonds, and to extrapolate to a fully-solvated set of results for comparison with aqueous experimental results.

Results of multiple linear regression of isotopic fractionation factors against H-bond type are presented in Tables 3-2 to 3-9. The first four columns present the regression coefficient of that H-bond type (H_{wat}-O_{ohc}, H_{wat}-O_{oc}, and H_{ohc}-O_{wat}) and the regression constant in ‰ notation. For kinetic fractionations, a fifth column is included presenting regression against the C-O_{attack}(H_{2}O or OH) distance in Angstroms. Cells are colored green if the regression coefficient is significant at the P<0.05 level. The next column presents the $R^2$ value, an estimate of the proportion of variation in the isotopic fractionation factor that can be explained by counting different types of H-bonds. The next column presents the average residual magnitude, or the average difference between the regression-fitted isotopic fractionation factor values and the values directly calculated from the vibrational frequencies. The penultimate column presents the number of conformations in the regression.

The final column presents a best-guess for the isotopic fractionation factor, and an estimate of the error taken from the average residual magnitude. The best-guess is calculated using the regression coefficients and estimates of the numbers of H-bonds of different types experienced by the TS or product species in aqueous solution. The hydration $^{13/12}\alpha_{eq}$ and $^{18/16}\alpha_{eq}$ regressions report equilibrium values expected only for models that form $H_{2}CO_{3}$ as the product,
whereas the hydroxylation $^{13/12}\alpha_{eq}$ and $^{18/16}\alpha_{eq}$ regressions report equilibrium values expected for HCO$_3^-$ regardless of whether it was produced by hydration or hydroxylation of CO$_2$.

Estimates of H-bond patterns to product H$_2$CO$_3$ and HCO$_3^-$ are taken from Car-Parrinello molecular dynamics (CPMD) models of H-bond dynamics to H$_2$CO$_3$ and HCO$_3^-$ in aqueous solution (Kumar et al. 2008). They found each hydroxyl group on H$_2$CO$_3$ tends to maintain a H-bond to H$_2$O at all times, for a total of 2 H$_{ohc}$-O$_{wat}$. Each hydroxyl accepted an H-bond only about half the time, for a total of 1 H$_{wat}$-O$_{ohc}$. The C=O carbonyl group accepted 1-2 H-bonds from H$_2$O, for a total of 1.5 H$_{wat}$-O$_{oc}$. Results for the hydration $^{13/12}\alpha_{eq}$ regression are given in Table 3-2. The regression indicates each of these H-bonds has slightly more than a 1‰ effect on the predicted $^{13/12}\alpha_{eq}$, so the best-guess estimate is somewhat sensitive to these assumptions. H-bonds to hydroxyls from H$_2$O lower $^{13/12}\alpha_{eq}$, while H-bonds to the carbonyl from H$_2$O and to H$_2$O from the hydroxyls raise $^{13/12}\alpha_{eq}$. All H-bonds have similar magnitude effects on $^{13/12}\alpha_{eq}$.

Effects on predicted $^{18/16}\alpha_{eq}$ (Table 3-4) are slightly less than 1‰ in general but are still statistically significant. The value of $^{18/16}\alpha_{eq}$ is affected in the same direction as effects from $^{13/12}\alpha_{eq}$; H-bonds to hydroxyls from H$_2$O lower $^{18/16}\alpha_{eq}$, while H-bonds to the carbonyl from H$_2$O and to H$_2$O from the hydroxyls raise $^{18/16}\alpha_{eq}$. All H-bond effects are comparable in magnitude.

Kumar et al. (2008) determined that the hydroxyl on HCO$_3^-$ almost always donates an H-bond despite the species’ overall negative charge, for a total of 1 H$_{ohc}$-O$_{wat}$. They also found that, like in H$_2$CO$_3$, the hydroxyl only accepts an H-bond about half the time, for a total of 0.5 H$_{wat}$-O$_{ohc}$. However, the negative charge on the CO$_2$ moiety accepts a total of 5 H-bonds on average, likely due to its delocalized negative charge, for a total of 5 H$_{wat}$-O$_{oc}$. Both H-bond acceptors exhibit large effects on $^{13/12}\alpha_{eq}$, but in opposite directions (Table 3-3): -3.5‰ per hydroxyl H-bond acceptor, and +2.3‰ per CO$_2$ moiety H-bond acceptor, in the best model chemistries. Thus the best-guess $^{13/12}\alpha_{eq}$ will be very sensitive to the assumed number of H-bonds accepted by HCO$_3^-$.
Hydroxyl H-bond donation does not have a statistically significant effect on \(^{13/12}\)\(\alpha_{\text{eq}}\) as identified by these models.

H-bond effects are closer to \(\sim 1\%\) for the \(^{18/16}\)\(\alpha_{\text{eq}}\) regression (Table 3-5), indicating less sensitivity to H-bond environment on the best-guess estimate of \(^{18/16}\)\(\alpha_{\text{eq}}\) for HCO\(_3^−\). Infrared spectroscopic data are consistent with at least 4 H-bonds accepted by the CO\(_2^−\) moiety in small HCO\(_3^−\)-H\(_2\)O clusters, and one H-bond donation by the hydroxyl in aqueous solution, although H-bond accepting by the hydroxyl is less well-characterized experimentally (Garand et al. 2009). H-bonds from H\(_2\)O to the hydroxyl decrease \(^{18/16}\)\(\alpha_{\text{eq}}\) by slightly more than \(1\%\), while H-bonds from H\(_2\)O to the carbonyl increase \(^{18/16}\)\(\alpha_{\text{eq}}\) by slightly less than \(1\%\). Hydroxyl H-bond donation does not have a statistically significant impact on \(^{18/16}\)\(\alpha_{\text{eq}}\).

Regression results for kinetic fractionations are given in Tables 3-6 to 3-9. Inclusion of C-O\(_{\text{attack}}\) distance was found to substantially increase the fit of most kinetic fractionation regressions, increasing \(R^2\) by 0.1-0.2 in most cases, except for \(^{18/16}\)\(\alpha_{\text{kin}}(\text{hydroxylation})\) where the fit was excellent even without C-O\(_{\text{attack}}\) distances. The H-bond structure of the hydration and hydroxylation TS is difficult to study experimentally, so we use our largest models and chemical intuition to inform our assumptions about best-guess H-bond patterns in evaluation of \(^{13/12}\)\(\alpha_{\text{kin}}\) and \(^{18/16}\)\(\alpha_{\text{kin}}\). In the TS structures of larger clusters, the attacking H\(_2\)O approaches at an oblique angle consistent with lone-pair approach toward the C, allowing for one H-bond from another H\(_2\)O to the attacking O (Figure 3.3c). We call this H-bond H\(_{\text{wat}}\)-O\(_{\text{ohe}}\) in our model as the O H-bond acceptor is in the process of forming a hydroxyl. Across all cluster sizes, the models range from 2-5 H\(_{\text{wat}}\)-O\(_{\text{ac}}\). Garand et al. (2009) found up to four H\(_2\)O interact with the similarly-structured HCO\(_3^−\) in their MP2 optimizations of HCO\(_3^−\)-H\(_2\)O\(_{1-8}\) clusters, and Kumar et al. (2008) found an average of 5 H-bonds to O\(_{\text{ac}}\) during CPMD modeling of HCO\(_3^−\), but both of these are overestimates if anything due to the net negative charge on the CO\(_2^−\) moiety that does not exist in the CO\(_2^−\) hydration TS. Rustad et al. (2008) found 3-4 H-bond interactions on their CPMD model
of HCO$_3^-$, although they did not specify which types of H-bonds they counted. We choose to use 4 H$_{\text{wat}}$-O$_{\text{oc}}$; although 3 might also be appropriate; the model is relatively insensitive to these H-bonds as can be seen by their low (~0.5%) regression coefficients for calculating $^{13/12}$$\alpha_{\text{kin}}$ and $^{18/16}$$\alpha_{\text{kin}}$ (Tables 3-6 and 3-8). The attacking H$_2$O donates one H-bond at the TS to transfer a H$^+$ away, and the other H usually donates an H-bond to H$_2$O as well, even when the H$_2$O is near the cluster surface (Figure 3.3d). We choose 2 H$_{\text{ohc}}$-O$_{\text{wat}}$ although 1.5 might be more appropriate to account for those conformations that don't donate a second H-bond. The model is somewhat sensitive to a choice of 1.5 H-bonds here based on the regression coefficients of ~1.2‰ and 1.5‰. Values of 1 H$_{\text{wat}}$-O$_{\text{ohc}}$, 4 H$_{\text{wat}}$-O$_{\text{oc}}$, and 2 H$_{\text{ohc}}$-O$_{\text{wat}}$ are consistent with a large, 40 H$_2$O hydration TS cluster (Figure 3.3c).

Effects of H-bond pattern on kinetic fractionation in the hydration reaction are generally similar to the patterns in equilibrium fractionation producing H$_2$CO$_3$. H-bonds from the hydroxyl to H$_2$O increase both $^{13/12}$$\alpha_{\text{kin}}$ and $^{18/16}$$\alpha_{\text{kin}}$, and H-bonds to the hydroxyl from H$_2$O decrease both $^{13/12}$$\alpha_{\text{kin}}$ and $^{18/16}$$\alpha_{\text{kin}}$. In contrast with equilibrium fractionation and carbonyl H-bond acceptors, H-bonds to the CO$_2$ moiety do not have a statistically significant effect on either fractionation.

The hydroxylation TS likely accepts more H-bonds than the hydration TS due to its net negative charge, much in the same way that HCO$_3^-$ accepts more H-bonds than H$_2$CO$_3$ in CPMD models of the aqueous ions (Kumar et al. 2008). Given the similarity in the hydroxylation TS to HCO$_3^-$ aside from the long HO-C distance (Figure 3.4), an approximation of 5 H$_{\text{wat}}$-O$_{\text{oc}}$ and 1 H$_{\text{ohc}}$-O$_{\text{wat}}$ seems appropriate. However, there is a difference in the number of H$_{\text{wat}}$-O$_{\text{ohc}}$ H-bonds between the TS and product HCO$_3^-$ that is consistent with both the geometry of the TS and the mechanism of reaction. Hydroxylation clusters with too few H$_2$O molecules formed HCO$_3^-$ without any activation barrier and had to be discarded. However, when OH$^-$ remained well-solvated in larger cluster models, there was an activation barrier, suggesting the breaking of H-bonds provides the barrier seen in the hydroxylation reaction. The experimental $E_a$ of 50 kJ/mol
(Wang et al. 2010) is consistent with breaking 2-3 strong H-bonds. Our TS models almost always have OH coplanar with CO₂, probably because the partial positive charge of the H interacts with the partial negative charge on the O; or because orbital overlap stabilizes the planar structure. In either case, this leaves enough room for three H₄OH-O₂ bonds to form to the OH⁻ even as the OH⁻ approaches the C on CO₂ (Fig 3.4a,c). Partial charge transfer of the negative charge on the OH⁻ may facilitate H-bonding to the CO₂ moiety, so we still include 5 H₄OH-O₂, a configuration we see in our largest model (Figure 3.4c). The regression coefficient for these H-bonds is large for C fractionation (~2.5‰), so this may be a source of error in our estimates. The assumption of 3 H₄OH-O₂, 5 H₄OH-O₂, and 1 OH-OH is consistent with our largest hydroxylation TS model (Figure 3.4c).

There is some similarity between H-bond effects on equilibrium and kinetic fraction in the hydroxylation reaction, albeit with a few notable differences (Tables 3-7 and 3-9). H-bonds from the attacking OH⁻ to H₂O sometimes cause a significant decrease in ¹³/¹₂αₖₐ, while H-bonds from the hydroxyl of HCO₃⁻ had no significant impact. H-bonds to the attacking OH⁻ from H₂O cause a large decrease in ¹⁸/¹₆αₖₐ of >3‰, while H-bonds to the hydroxyl on HCO₃⁻ have a smaller effect of just over 1‰. As with the hydration reaction, no H-bonds to the CO₂ moiety had a statistically significant impact on kinetic fractionation during the hydroxylation reaction.

Effects of H-bonds are similar between H₂CO₃ and HCO₃⁻ products for equilibrium fractionations, and between hydration and hydroxylation TSs for kinetic fractionation. Hydroxyl H-bonds to H₂O only have a significant impact on equilibrium fractionation for H₂CO₃ product, and H-bonds to carbonyl O from H₂O have a somewhat larger impact on HCO₃⁻ ¹³/¹₂αₑ, but those are the only notable differences between equilibrium fractionation effects. H-bonds from the attacking H₂O during the hydration reaction increase ¹³/¹₂αₖₐ, while H-bonds from the attacking OH⁻ during hydroxylation decrease ¹³/¹₂αₖₐ. The effects of H-bonds to the attacking species are greater in the hydroxylation reaction.
Effects of Temperature

Variation of fractionation factor with temperature is demonstrated in Figure 3-21 to Figure 3-28. Results for the hydration reaction are shown in Figure 3-21 to Figure 3-24. For all four fractionation factors, the effect of varying temperature is very similar across all model chemistries. Hydration $^{13/12}\alpha_{eq}$ decreases by $\sim$9‰ from 0-100°C while $^{18/16}\alpha_{eq}$, $^{13/12}\alpha_{kin}$, and $^{18/16}\alpha_{kin}$ vary by <2‰. Results for the hydroxylation reaction are shown in Figure 3-25 to 3-28. Hydroxylation $^{13/12}\alpha_{eq}$ decreases by $\sim$10‰ over the temperature range, and matches the sense of temperature variation while exceeding by 1-3‰ the experimental calibration of Mook et al. (1974) in gray. The temperature calibration of Zhang et al. (1995) does not match these results well. Hydroxylation $^{18/16}\alpha_{eq}$ varies by less than 1‰ over the temperature range including an inversion in temperature effect, and the model results roughly match the experimental calibration of Beck et al. (2005), which was only evaluated from 0-40°C. Both $^{13/12}\alpha_{kin}$ and $^{18/16}\alpha_{kin}$ increase by about 6‰ over the temperature range.

C-O Isotope Slopes

Slopes for change in $\delta^{13}$C vs $\delta^{18}$O from model results are compared to the observed slope taken from corals in Figures 3-29 to 3-30. The slopes represent the change from heavy equilibrium C and O isotope compositions, fixed at the composition of equilibrium corals, to lighter, partial kinetic C and O isotope compositions with a $\delta^{18}$O equivalent to the lightest values found in the corals. Figure 3-29 demonstrates that the modeled trend for just the hydration reaction is similar to the trend observed in corals, but with heavier (less negative) $\delta^{13}$C values by $\sim$2-3‰ for the favored model chemistries. Model chemistries within $\sim$1‰ of the coral value include the PBE0 functional, which was not particularly accurate in predicting $^{18/16}\alpha_{eq}$(CO$_2$-H$_2$O)
(Chapter 2, this dissertation) and which was the least-successful predictor of $^{13/12}\text{ax}_{\text{aq}}\text{(HCO}_3^-\text{-CO}_2^\text{-})$
(Figure 3-25). Figure 3-30 demonstrates that the hydroxylation reaction produces slightly less negative $\delta^{13}\text{C}$ values, with the favored model chemistries predicting 3-4‰ higher values. None of the hydroxylation models are within 2‰ of the measured coral $\delta^{13}\text{C}$ values at the lightest-observed $\delta^{18}\text{O}$ values.

**Discussion**

**Energies**

Broadly, the bulk average values computed for $\Delta G^{\circ}_{\text{rxn}}$ for both hydration and hydroxylation are close to experimentally-measured values (within 15 kJ/mol and centered within a few kJ/mol of the measured value), when looking at the full suite of model chemistries in use in this study. Larger clusters generally do a better job of calculating accurate reaction energies. Given the average H-bond is often cited as having an energy of 4-20 kJ/mol, and the experimentally-determined $\Delta G^{\circ}_{\text{rxn}}$ for hydration and hydroxylation are only +26 kJ/mol and -43 kJ/mol respectively (Wang et al. 2010), it is crucial to have a cluster large enough to solvate the product $\text{H}_2\text{CO}_3$ or $\text{HCO}_3^-$ to correctly model the reaction energy. The measured energies reflect changes to the enthalpy especially due to H-bonding, so smaller clusters with minimal H-bonding can be expected to predict a $\Delta G^{\circ}_{\text{rxn}}$ that is too high, exactly as observed with favored model chemistries in Figures 3-5 and 3-6.

The predicted $E_a$ for the hydration reaction is reasonably accurate as well, with the best-performing model chemistries from evaluations doing the best at large cluster sizes. Here too it is crucial to model the H-bond environment of the TS. Our results compare favorably with other
computational estimates of the hydration reaction $E_a$ (Nguyen et al. 2008; Stirling and Pápai 2010; Zeebe 2014).

Our models underpredict $E_a$ for the hydroxylation reaction (Wang et al. 2010). This is possibly because construction of clusters with CO$_2$ and OH$^-$ already in close proximity bypasses an enthalpic barrier to the close association of CO$_2$ and OH$^-$. Stirling (2011) found entropic barriers to CO$_2$ hydroxylation to be much more influential on the free energy of activation than enthalpic barriers, although the entropic barriers came from OH$^-$ orientation and H-bond breaking, rather than clustering with CO$_2$. The similarity in our calculated $E_a$ to experimental values suggests our TS structures are reasonable.

**Bulk Fractionation Factors**

Bulk-averaged calculations do not give quantitatively useful results for isotopic fractionation. For $\alpha_{eq}$ values, they may come close to the true experimental value, but this is highly dependent on choice of model chemistry, with little insight gained from evaluation with gas-phase fractionation data in a related reaction (Chapter 2, this dissertation). For $\alpha_{kin}$ values, model chemistries can display ranges of 10-15‰ in predicted value, giving little insight when the largest predicted kinetic fractionation is only 25‰.

However, this does not mean that isotopic fractionation calculations are useless. Rather, it means we need a better method to organize and average the calculations, rather than relying on Boltzmann-weighted averages of the fractionation factors. Part of the reason bulk averaging is unsuccessful is the nature of small cluster calculations: they by definition do not represent everything occurring in an aqueous solution of realistic size in the natural world. Thus, some clusters miss important interactions; some more so than others. Figure 3-4b demonstrates that some models are poorly-solvated. CO$_2$ forms only weak H-bonds to H$_2$O, as supported by
molecular dynamics models which find an average of 0.56 H atoms within 2.5 Å of O atoms on CO$_2$ (Lam et al. 2014). The O atoms on CO$_2$ compete with each other to withdraw electrons from C, preventing both from achieving a strong partial negative charge. This explains why a CO$_2$-H$_2$O dimer with the partial negative charge of the H$_2$O point toward the partial positive charge of the C is more energetically favorable than an H-bonded dimer (Peterson and Klemperer 1984). In these calculations, it is more energetically favorable for a CO$_2$ molecule to sit on the outside of the cluster, which can lead the product and TS structures to participate in fewer H-bonds than they would in real solution. Even well-solvated clusters (e.g. Figure 3-2b) may not provide any H-bonds to TS and product structures because the H$_2$O molecules are locked into a rigid structure, or would need to cross an activation barrier themselves to re-orient. Models of a tenable size will have issues with their solvation structures being imperfect.

However, building models large enough to fully solvate the CO$_2$ and keep it solvated are computationally unfeasible, especially when single conformations can have so much variability in their properties. Rustad et al. (2008) report needing ~64 H$_2$O molecules to provide a second solvation shell around DIC, which would require a roughly 8-fold increase in computation time from the largest clusters used here. Deriving information from imperfect models makes more sense than trying to build perfect models. This is the point of constructing a regression against different H-bond types, to approximate their effects when taken together in a well-solvated structure.

Calculated fractionation factor values in this work do not achieve consistent asymptotic or even monotonic approach toward experimental values with increasing cluster size, in contrast with Zeebe (2009) who achieved an asymptotic approach toward the equilibrium fractionation factor $\alpha_{eq}(\text{CO}_2^2\text{-H}_2\text{O})$ by increasing the number of H$_2$O molecules in the cluster. Such behavior seems like an exception to the rule. Zeebe (2009) did not specify how they constructed clusters of different sizes. By adding H$_2$O molecules sequentially and re-optimizing one at a time,
asymptotic approach might be achieved by maintaining the H-bond structure as the cluster size is increased. Possibly, the greater charge on the CO$_3^{2-}$ promotes H-bonding to it rather than to other H$_2$O molecules, which would help maintain this H-bond structure as cluster size is increased. Regardless, aqueous clusters should not be expected to exhibit asymptotic approach to experimental fractionation factor values unless special care is taken with knowledge of the molecule in question and its H-bond pattern.

The Boltzmann weighting technique used to add contributions from clusters of different energies is one approach to weeding out structures that will contribute less to the final property. Rustad et al. (2008) chose to not weight clusters at all in their analysis of equilibrium C fractionation in DIC species, reasoning that the interaction of a cluster with bulk solvent via H-bonding renders any estimate of the cluster energy independent of the bulk unimportant. It is not clear which method is more realistic, given that there is no reason to expect a priori that a low-energy cluster will have higher-energy interactions with the bulk phase and vice-versa. Moreover, most ions reorganize the H$_2$O molecules around themselves in some fashion (e.g. Marcus 2009), and the H$_2$O molecules in these clusters should at least partially reflect that reorganization. What is clear is that Boltzmann weighting is not sufficient to control for all the effects of cluster energy and local chemical environment allowing easy computation of properties of interest.

**Regression Against H-Bond Type**

The regression of isotopic fractionation properties against H-bond pattern was successful in closely replicating experimental measurements of isotopic fractionation between CO$_2$(aq) and HCO$_3^-$ across all model chemistries. The best-guess estimates of $^{13/12}\alpha_{eq}(\text{HCO}_3^--\text{CO}_2)$ range from 1.0104 to 1.0147 (Table 3-3), which compares somewhat favorably with the experimental value of 1.0090 at 25°C given the average residual magnitude of about 2‰. The most accurate model
chemistry is X3LYP/6-311++G(2d,p), also the best performer in evaluations of gas-phase fractionation, although all model chemistries excluding PBE0 are within residual error of one another. The best-guess estimates of $^{18/16} \alpha_{eq}(\text{HCO}_3^- - \text{CO}_2^\cdot)$ range from 0.9896 to 0.9912 (3-5), which compares favorably with the experimental value of 0.9906 at 25°C (Beck et al. 2005); each model chemistry reproduces the experimental value to within 1‰, with average residual magnitudes of about 1‰.

Equilibrium fractionation between H$_2$CO$_3$ and CO$_2$ has not been experimentally determined because H$_2$CO$_3$ is difficult to isolate in aqueous solution. H$_2$CO$_3$ makes up only a small proportion of DIC at any pH (Zeebe and Wolf-Gladrow 2008), meaning its isotopic composition has a relatively small impact on the isotopic composition of total DIC. Our results give reasonable estimates for the equilibrium fractionation that might be expected, given the similarity in magnitude of the experimental $\alpha_{eq}(\text{HCO}_3^- - \text{CO}_3^{2-})$ and calculated $\alpha_{eq}(\text{H}_2\text{CO}_3 - \text{HCO}_3^-)$ values; i.e. the fractionations between H$_2$CO$_3$/HCO$_3^-$ and HCO$_3^-$/CO$_3^{2-}$ are similar. $^{13/12} \alpha_{eq}(\text{HCO}_3^- - \text{CO}_3^{2-})$ has an experimental value of 1.0014 at 25°C (Zhang et al. 1995) while model chemistries using B3LYP and X3LYP give calculated $^{13/12} \alpha_{eq}(\text{H}_2\text{CO}_3 - \text{HCO}_3^-)$ values of 1.0008 to 1.0021.

$^{18/16} \alpha_{eq}(\text{HCO}_3^- - \text{CO}_3^{2-})$ has an experimental value of 1.0068 at 25°C (Beck et al. 2005) while model chemistries using B3LYP and X3LYP give calculated $^{18/16} \alpha_{eq}(\text{H}_2\text{CO}_3 - \text{HCO}_3^-)$ values of 1.0072 to 1.0081.

Best-guess kinetic fractionations calculated via regression are similar across model chemistries. For $^{13/12} \alpha_{kin}(\text{hydration})$, favored model chemistries predict values in the range 0.988-0.989 with average residual magnitudes of 2‰ (Table 3-6). Zeebe (2014) predicts much smaller values for $^{13/12} \alpha_{kin}(\text{hydration})$ of 0.968-0.977. O’Leary et al. (1992) report an experimental value of 0.987, which is closest to our value; other workers report values of 0.981 (Marlier and O’Leary 1984) and 0.993 (Clark and Lauriol 1992). For $^{13/12} \alpha_{kin}(\text{hydroxylation})$, favored model chemistries predict values in the range 0.984-0.987 with average residuals <1‰ (Table 3-7). Discrimination
against $^{13}$C is slightly stronger in the hydroxylation reaction. For $^{18}_{16} \alpha_{\text{kin}}$(hydration), favored model chemistries predict a value of 0.989 with average residuals $\sim$1‰. Zeebe (2014) predicts smaller values of 0.985-0.986. For $^{18}_{16} \alpha_{\text{kin}}$(hydroxylation), favored model chemistries predict values in the range 0.979-0.981. Discrimination against $^{18}$O is stronger in the hydroxylation reaction.

Some of the differences between this work and previous work may be due to differences in calculation scheme. Zeebe (2014) scales the $\beta$ values in Equation 3.17 by the magnitude of the imaginary vibrational frequency, instead of canceling the vibration values when calculating the relative transition frequency. However, alternative derivations (Felipe et al. 2001; Kubicki 2008) cancel the imaginary frequency with the term representing transition state crossing velocity. Different model chemistries are also used; in particular, Zeebe (2014) employs MP2 calculations, which were found to be inaccurate in calculating fractionation factors in Chapter 2 of this dissertation despite predicting harmonic vibrational frequencies well. They also use fewer conformations, employing single conformations in clusters with 1-7 H$_2$O molecules, and two conformations when using 8 H$_2$O molecules. As a result, their calculations do not capture the full range and variability of solvation environments experienced during CO$_2$ hydration.

It should be noted that C-O$_{\text{attack}}$ distance has a substantial effect on some values of $\alpha_{\text{kin}}$, in particular $^{13}_{12} \alpha_{\text{kin}}$ values. Effects can be greater than 20‰ per Å. Modeling results from the literature for the CO$_2$ hydration TS range from $\sim$1.55Å (Nguyen et al. 2008) to $\sim$1.83Å (Zeebe 2014). Our intermediate, best-guess value of 1.72Å, taken from a large cluster calculation (Figure 3-3c), is near the higher end of reported estimates, with many of the models from Zeebe (2014) and Wang and Cao (2013) falling below 1.65Å. However, we observe an increase in C-O$_{\text{attack}}$ as the number of hydrating H$_2$O molecules increases, both in published models (Wang and Cao 2013; Zeebe 2014) and in these models.
Salinity Effects

Salinity is known to impact isotope fractionation in some abiotic systems, although there are no comprehensive theories regarding the mechanism of fractionation. Salinity affects $^{18/16}q_{eq}(CO_2-H_2O)$ in a complex, temperature-dependent way, by up to $\pm 2\%$ (Truesdell 1974). In saline solutions, divalent cations have a much stronger effect than monovalent cations on both O and H fractionation between liquid $H_2O$ and vapor. Effects from monovalent cations are $< 1\%$ from 0-100°C, up to 6 molal; effects from divalent cations are up to 3‰ for Ca$^{2+}$ and up to 6‰ for Mg$^{2+}$ (Horita et al. 1993). Oerter et al. (2014) found that $^{18}$O-enriched $H_2O$ preferentially enters the hydration spheres of Mg$^{2+}$ and Ca$^{2+}$, while K$^+$ concentrates $^{18}$O-depleted $H_2O$. Na$^+$ was found to concentrate neither preferentially with respect to bulk water. Gypsum hydration water $^{18}$O equilibrium composition is not influenced by salinities below 150 g/L, but deuterium preferentially concentrates in gypsum as salinity is increased (Gázquez et al. 2017). Less $^{18}$O partitions into aragonite when Mg$^{2+}$ concentration is increased (Kim et al. 2007), while NaCl has no effect on aragonite isotopic composition (Kim et al. 2014). There is a negligible difference in $\delta^{18}O$ between the Na$^+/HCO_3^-$ ion pair and free $HCO_3^-$, and between the Na$^+/CO_3^{2-}$ ion pair and free $CO_3^{2-}$. NaCl concentration also does not impact the $\delta^{18}O$ of either free $HCO_3^-$ or $CO_3^{2-}$ as salinity is increased (Kim et al. 2014).

Some biotic effects have also been observed. Mussel $\delta^{13}C$ increases as salinity increases. This is likely connected to variations in DIC $\delta^{13}C$ based on salinity (Klein et al. 1996; Hill et al. 2014). Salinity has large impacts on foraminiferal sea surface temperature estimates from $\delta^{18}O$, with disproportionately larger effects at high salinities (Arbuszewski et al. 2010).
**Proposed Mechanisms for Salinity Effects**

Several mechanisms for solute-driven isotope fractionation have been proposed. O’Neil and Truesdell (1991) proposed two mechanisms for $^{18}$O fractionation by solutes that could affect mineral-based paleothermometers: (1) exchange of O between H$_2$O bound in ion solvation shells, bulk water, and CO$_2$ pools; and (2) modification of water structure and H-bond pattern by the presence of ions. Hill et al. (2014) found that salinity can impact CaCO$_3$ isotopic composition by affecting the amount of HCO$_3^-$ vs CO$_3^{2-}$ in the DIC pool. Kim et al. (2007) and Oerter et al. (2014) proposed that cations with large charge/radius ratios have more structurally ordered solvation shells that tend to concentrate H$_2^{18}$O.

**Salinity Effects on H-Bond Structure**

Given the predicted effect of individual H-bonds on fractionation factors, it is possible to speculate on the effect of salinity on fractionation factors. Solutes interact in real solutions as they are not infinitely dilute. This might impact the H-bond structure and thus equilibrium and kinetic isotope fractionation in aqueous DIC. Solutes can ion pair, either in contact, sharing solvation shells, or with their separate solvation shells. Solvent-shared ion pairs in particular may impact isotope fractionation in DIC by modifying the degree of H-bonding. Solutes may also affect the structure of bulk water by affecting the H-bond network past local effects.

Work investigating the effect of solutes on H-bonding in water has focused on description of certain ions as structure makers or breakers which either enhance or reduce the extent of H-bonding in solution respectively, outside the first solvation shell of the ion (reviewed in Marcus 2009). Based on various physical properties, it has been argued that ions affect the structure of water far into solution. Neutron diffraction studies reveal H-bond structure
breakdown in solution due to ion addition resembles changes from applying high pressure, implying effects on H-bonds far from the ions' solvation shell (Leberman and Soper 1995). MD models of alkali halide imply ions decrease number of H-bonds in solution, and water structure is significantly influenced by the presence of ions past the first shell (Chandra 2000; Galamba 2012). H-H coordination numbers determined by neutron diffraction indicate that numbers of H-bonds in CaCl$_2$ solutions are strongly decreased (Megyes et al. 2006), despite Ca$^{2+}$ being classified as a structure maker (Marcus 2009). Dissociation of H$_2$O molecules is suppressed in ionic solutions, implying a large effect on H-bond structure (Jeyachandran et al. 2016). Even solutions as dilute as 10 μM of various electrolytes induce long-range changes in H$_2$O orientation measured by femtosecond elastic second harmonic scattering (Chen et al. 2016). Carbonate ion has at least intermediate-range effects on H-bond pattern measured with 2D IR spectroscopy (Fournier et al. 2016).

Some work has challenged the notion of structure makers and breakers, suggesting the effects of ions on H-bond pattern extend out only one or two solvation shells. MD models indicate water structure is not affected past the second shell around Li$^+$ (Lyubartsev et al. 2001). Ultrafast IR pump-probe measurements indicate only the first hydration shell of an ion is affected in terms of vibrational and rotational relaxation times, implying that reorganization effects of ions on water are localized to the first shell (Omta et al. 2003). Perturbations to the tetrahedral H-bond structure of water do not extend past the first shell in MD models of CaCl$_2$ solutions (Chialvo and Simonson 2004). Perturbations to H-bond structure are weak outside of the first hydration shells around monovalent ions measured by neutron diffraction with D/H substitution (Soper and Weckström 2006). Neutron diffraction studies of H-bonding around monovalent ions can be explained solely by changes immediately around the ion and not extending into solution (Mancinelli et al. 2007). MD models and Raman evaluation of O-H stretches indicate halide ions do not influence H$_2$O molecules past the first shell (Smith et al. 2007). Single-cation effects on H-
bond network are dependent on the nature of the first solvation shell; those ions with an organized first solvation shell do not have effects extending past the first shell, while those with a disorganized first shell have effects extending past the first shell (Migliorati et al. 2011). Ion-induced effects on H-bond pattern are minimal for monovalent ions, but extend over 1 nm for divalent ions (O’Brien and Williams 2012). Statistical-mechanical calculations suggest all cations are associated with a similar number of H-bonds within bulk water past the first shell (Suresh et al. 2012).

There is much disagreement in the literature over effects on H-bond pattern outside the first or second solvation shell. Moreover, many experimental studies use very high solute concentrations which prevents separation of single-ion effects from ion-pair effects (Migliorati et al. 2011). Without extensive MD models and experimental results to describe the particular H-bond environments experienced in different saline solutions by DIC species, speculation regarding solute effects on DIC isotope fractionation is difficult. There is broad agreement that ions alter the structure of water in their first solvation shell, so this discussion will focus on solvent-shared ion pairs between cations and DIC species (i.e. cations and DIC species separated by a single layer of H₂O molecules), and the effects of those cations on the H-bond environment experienced by DIC. Contact ion-pair effects cannot be ruled out, and may also have an impact on isotope fractionation, but such effects have not yet been studied specifically. Ion pairing of different types has been recently reviewed by van der Vegt et al. (2016).

**Salinity and Ion Pairing**

Many cations are known to form contact and solvent-separated ion pairs with HCO₃⁻ and CO₃²⁻ in seawater. About 90% of carbonate is ion-paired, and about 30% of bicarbonate is ion-paired in seawater, including both contact and solvent-separated pairs (Millero 1974). Na⁺, the
most common cation in seawater, does not make contact ion pairs with \( \text{CO}_3^{2-} \), but does make solvent-separated ones; 2-12% of \( \text{Na}^+ \) and \( \text{CO}_3^{2-} \) ions form solvent-separated ion pairs in \( \text{Na}_2\text{CO}_3 \) solutions (Capewell et al. 1999; Kim et al. 2006). About 1% of \( \text{HCO}_3^- \) forms these ion pairs with \( \text{Na}^+ \) in equimolar solutions (Kim et al. 2006). \( \text{Ca}^{2+} \) and \( \text{CO}_3^{2-} \) share significant contact ion pairs, but pairing between \( \text{Ca}^{2+} \) and \( \text{HCO}_3^- \) is at least 3 orders of magnitude lower (Gal et al. 1996).

Stable clusters of up to tens of \( \text{Ca}^{2+} \) and \( \text{CO}_3^{2-} \) units bind together even in undersaturated solutions forming ionic polymers (Gebauer et al. 2008; Kellermeier et al. 2014), and thus their isotopic composition may impact final precipitating carbonate, either by removing some ions from solution or by contributing directly to precipitation. In seawater, all cations are much more prevalent than either DIC species, so the fraction of DIC involved in solvent-separated ion pairs, or contact ion pairs with divalent cations, is substantial (van der Vegt, 2016).

Specific effects of several mono- and divalent ions on H-bond donation and reception in their solvation shells have been studied. Halides cause reorientation of \( \text{H}_2\text{O} \) molecules in their first shells (Smith et al. 2007). H atoms in \( \text{H}_2\text{O} \) orient generally towards the halide, pointing the partial negative charge on the O away and reducing the degree of H-bond donation. The first shell \( \text{H}_2\text{O} \) molecules of high charge/radius ratio halide anions accept more H-bonds than molecules in bulk water, despite the structural rigidity of a solvation shell reducing H-bond interactions. \( \text{F}^- \) presence causes first-shell \( \text{H}_2\text{O} \) molecules to accept 10% more H-bonds, while \( \text{I}^- \) presence causes its first shell to accept \(-5% \) fewer H-bonds. The anion’s electric field makes H-bonds donated from the second shell to the first shell weaker on average. The reverse may happen in cations: \( \text{H}_2\text{O} \) orientation limits H-bond accepting by \( \text{H}_2\text{O} \) molecules around all cations, while cations with high charge/radius ratios enhance H-bond donation by their first shell.

Divalent and trivalent ions are surrounded by \( \text{H}_2\text{O} \) molecules that tend to donate 2 H-bonds instead of only 1 (Näslund et al. 2005). Bulk water consists of 20-80% single donor species (Nilsson and Pettersson 2011). The first shell of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) ions engages in 25% fewer H-
bonds than the first shell of Na\(^+\) or bulk water; most of this effect is due to reduced H-bond acceptance (Suresh et al. 2012). Paired strongly-hydrated ions like Ca\(^{2+}\)/Mg\(^{2+}\) and CO\(_3\)\(^{2-}\) lock much more of the H-bond network than either ion on its own or additively (Tielrooij et al. 2010). As a result, enhancement of H-bond donation to CO\(_3\)\(^{2-}\) should be stronger than that to HCO\(_3\)\(^{-}\) in solutions containing divalent cations. Divalent cations have stronger interactions with H\(_2\)O than monovalent anions as determined with dielectric relaxation spectroscopy, so second solvation shells are likely well-developed and doubly-separated ion pairs may have an impact on H-bond pattern for divalent cations (Buchner and Hefter 2009).

The overall picture of H-bonding to DIC species is dependent on the nature of the cation, which is expected to associate much more closely with the negatively-charged DIC species than the anion. Ca\(^{2+}\) and Mg\(^{2+}\) ions should slightly increase the degree of H-bond donation to DIC and should substantially decrease the degree of H-bond acceptance from DIC. Effects should be stronger on DIC with greater negative charges in the order H\(_2\)CO\(_3\) < HCO\(_3\)\(^{-}\) < CO\(_3\)\(^{2-}\). Effects should also be stronger on the CO\(_2\) hydroxylation TS with its -1 net charge versus the hydration TS. Future work would benefit from a quantitative evaluation on the effect of nearby cations on DIC species’ H-bond patterns.

**Effects of Salinity on H-Bond-Influenced Isotope Fractionation in DIC**

The following statements on salinity effects on DIC isotopic fractionation refer to situations in which divalent cations like Ca\(^{2+}\) and Mg\(^{2+}\) strongly reorient H\(_2\)O molecules and affect H-bond patterns to DIC species. A reduction in H-bond donation by HCO\(_3\)\(^{-}\) will have a negligible impact on both \(^{13/12}\)\(\alpha\)\(_{eq}\) and \(^{18/16}\)\(\alpha\)\(_{eq}\) (Tables 3-3 and 3-5). Increased H-bond donation to the hydroxyl on HCO\(_3\)\(^{-}\) has the potential to decrease \(^{13/12}\)\(\alpha\)\(_{eq}\) by \(~3.6\)% per H-bond and to decrease \(^{18/16}\)\(\alpha\)\(_{eq}\) by \(~1.1\)% per H-bond. The hydroxyl can accept at most 2-H bonds but tends to accept
only about 0.5 when not near cations (Kumar et al. 2008). H-bonding is stronger to the carbonyl O, typically accepting 5 H-bonds with a maximum of about 6 available (Kumar et al. 2008).

Increased H-bond acceptance by carbonyl O could increase $^{13/12} \alpha_{eq}$ by $\sim 2.5\%$ per H-bond and increase $^{18/16} \alpha_{eq}$ by $\sim 0.8\%$ per H-bond. The effects of H-bond donation to different O atoms on HCO$_3^-$ affect isotopic fractionation in opposing directions. Hydroxyl O have a larger capacity for additional H-bonds and a larger magnitude of isotopic effect, but H-bonding appears to be stronger to the carbonyl O with a partial negative charge, especially if these orient towards the cations that drive differences in isotope fractionation. Which effect will win out is difficult to guess. If H-bonding to carbonyl O is greater, both $\delta^{13}C$ and $\delta^{18}O$ of HCO$_3^-$ should increase. If H-bonding to hydroxyl O is greater, both $\delta^{13}C$ and $\delta^{18}O$ of HCO$_3^-$ should decrease.

The CO$_3^{2-}$ ion is somewhat simpler to discuss, given its lack of hydroxyl O. The effects of H-bonding to carbonyl on H$_2$CO$_3$ and HCO$_3^-$ are consistent, so H-bonds to CO$_3^{2-}$ are expected to have the same effect. H-bonds should increase both $\delta^{13}C$ and $\delta^{18}O$ of CO$_3^{2-}$ and thus bring its isotopic composition closer to that of HCO$_3^-$.

There should also be effects on kinetic fractionation if divalent cation concentrations are increased. Increases in H-bond acceptance and decreases in H-bond donation by the attacking O during CO$_2$ hydration should both be associated with a decrease in $^{13/12} \alpha_{kin}$ (Table 3-6) and $^{18/16} \alpha_{kin}$ (Table 3-8), while increased H-bond donation to the CO$_2$ moiety should have little effect. Responses in the CO$_2$ hydroxylation reaction are more complex, with increased H-bond donation to the attacking OH$^-$ decreasing both $^{13/12} \alpha_{kin}$ (Table 3-7) and $^{18/16} \alpha_{kin}$ (Table 3-9), while decreased H-bond donation by the attacking OH$^-$ increasing $^{13/12} \alpha_{kin}$ only. The net effect is that $^{13/12} \alpha_{kin}$ of both reactions should be more similar when there are high concentrations of divalent cations, but
$^{18/16} \alpha_{\text{kin}}$ values may be more distinct, with decreases to $^{18/16} \alpha_{\text{kin}}$ affecting the hydroxylation reaction more strongly than the hydration reaction.

There is limited experimental data to compare with these model results, largely because of the difficulty in separating different components of the DIC in solution. Measurements made of seawater invariably measure total DIC isotopic composition because of the necessity to convert it all to CO$_2$ for analysis (Mook 2000). Differences between freshwater and marine DIC isotopic compositions are largely controlled by balances between photosynthesis and respiration (Boutton 1991). CO$_2$-H$_2$O equilibration experiments with saline reservoir fluids indicate that the salinity effect on $^{18/16} \alpha_{\text{eq}}$(CO$_2$-H$_2$O) is $\sim$1‰ (Becker et al. 2015): The effect on $^{13/12} \alpha_{\text{eq}}$(DIC-CO$_2$) is complex and difficult to analyze, partially because of pH uncertainties in the measurements and the strong effect of pH on DIC speciation over the conditions studied. At most, $^{13/12} \alpha_{\text{eq}}$(DIC-CO$_2$) may be lowered by 1-2‰ as a result of salinity increases. In contrast, oil field brines at 60°C have HCO$_3^-$ with a $\delta^{13}$C value 10.7‰ heavier than CO$_2$, when equilibrium values taken from lab experiments at low salt concentrations should put the difference in DIC at only $\sim$5‰ (Raistrick et al. 2006). Part of this apparent disequilibrium may be due to decomposition of isotopically light oil to CO$_2$, although it is unclear why this would not equilibrate with HCO$_3^-$ in situ. This effect seems somewhat larger than can be produced only by H-bond effects, although there could be additional effects in contact ion pairs that contribute as well.

**Comparison to Corals**

The different fractionations experienced by C and O isotopes during the hydration and hydroxylation reaction can be combined into a single parameter: the slope of $\delta^{13}$C vs $\delta^{18}$O, when transitioning from full equilibrium fractionation given by the $^{13/12} \alpha_{\text{eq}}$ and $^{18/16} \alpha_{\text{eq}}$ values, to full kinetic fractionation values given by $^{13/12} \alpha_{\text{kin}}$ and $^{18/16} \alpha_{\text{kin}}$. Those comparisons are made in Figures
3-29 and 3-30 for the hydration and hydroxylation reactions separately. A more complete
evaluation would connect the increase in growth rate of corals to both a shift from equilibrium to
kinetic fractionation, and a possible increase in pH. However, the simple comparison reveals that
a shift from equilibrium to kinetic fractionation in the hydroxylation reaction alone cannot
explain the full isotope trend observed in corals (Figure 3-30). The isotope trend cause by the
hydration reaction matches more closely with the observed isotope fractionation in corals (Figure
3-29). However, given the high pH of the coral calcifying space, some hydroxylation would be
expected to occur. If the calcifying space pH is suitably high to include sufficient hydroxylation,
the kinetic model of McConnaughey (1989a; 1989b; 2003; Chapter 1, this dissertation) can be
falsified as the sole driver of vital effects in corals. However, the calculated slope is close to the
observed slope, so the CO₂ hydration and hydroxylation reactions may be able to explain a
substantial amount of the observed variability in coral isotopic compositions.

Other effects may impact coral isotopic composition as well as these kinetic effects,
however. If an effect lowers δ¹³C or raises δ¹⁸O preferentially during fast coral growth, or
achieves the opposite during slow coral growth, it could offset the slopes enough to match the
observed coral results. However, it would need to do so in proportion with the growth rate to
maintain the apparently linear relationship observed between δ¹³C and δ¹⁸O. Perhaps, following
the carbonate model of Adkins et al. (2003), respired CO₂ with low δ¹³C values contributes a
greater proportion of the carbonate skeleton at fast growth rates, either because dissolved
seawater CO₂ is slower to diffuse inward, or because fast growth rates are associated with energy
expenditure, driven by respiration, that also increases the activity of Ca²⁺/H⁺ antiporter ATPase
(Cohen and McConnaughey 2003). Further studies of the rates of material transport to/from the
calcifying space would be needed to definitively ascertain the relative contributions of DIC taken
directly from seawater vs respiration and kinetic fractionation.
Summary

Quantum mechanical calculations can reproduce experimentally-determined energetics of aqueous DIC reactions only if care is taken to select model chemistry and construct an accurate solvation environment; increases in H₂O cluster size alone are not enough. Evaluation of isotope fractionation in the DIC system benefits from evaluation of the local H-bonding environment, and the C-O$_{\text{attack}}$ distance in the case of kinetic fractionation. The CO₂ hydration reaction discriminates against both $^{13}$C and $^{18}$O by 10-11‰. The CO₂ hydroxylation reaction discriminates against $^{13}$C by 13-16‰ and against $^{18}$O by 19-21‰. Vital effects in corals cannot be completely explained by kinetic fractionation in the CO₂ hydration and hydroxylation reactions, but the reactions’ predicted effects are close enough to observed trends that they may substantially contribute.
Figure 3-1. Example conformations for the CO\textsubscript{2} hydration reaction, with C in black, O in red, and H in white. (a): Reactant conformation with 25 solvating H\textsubscript{2}O molecules. Species directly involved in the reaction are displayed with larger atomic radii. The C(CO\textsubscript{2})-O(attack of H\textsubscript{2}O) distance is labeled in green. (b,c): Product conformations with 25 solvating H\textsubscript{2}O molecules, reaching either (b) H\textsubscript{2}CO\textsubscript{3} product or (c) HCO\textsubscript{3} \textsuperscript{-} + H\textsubscript{3}O\textsuperscript{+} product. H-bonds involving the product species are shown in green. (b): There are two H-bonds from water to hydroxyl (H\textsubscript{wat}-O\textsubscript{ohc}), one H-bond from water to carbonyl (H\textsubscript{wat}-O\textsubscript{oc}), and two H-bonds from hydroxyl to water (H\textsubscript{ohc}-O\textsubscript{wat}) in this conformation. (c): There is one H\textsubscript{wat}-O\textsubscript{ohc}, five H\textsubscript{wat}-O\textsubscript{oc}, and one H\textsubscript{ohc}-O\textsubscript{wat} H-bonds in this conformation.
Figure 3-2. Example conformations for the CO$_2$ hydroxylation reaction, with C in black, O in red, and H in white. (a): Reactant conformation with 33 solvating H$_2$O molecules and one hydroxyl. Species directly involved in the reaction are displayed with larger atomic radii. The C(CO$_2$)-O(attacking OH) distance is labeled in green. (b): Product conformation with 33 solvating H$_2$O molecules, reaching HCO$_3^-$ product. H-bonds involving the product species are shown in green. There are two H$_{\text{wat}}$-O$_{\text{ohc}}$, one H$_{\text{wat}}$-O$_{\text{oc}}$, and one H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation. See figure 3-1 for a description of H-bond notation.
Figure 3-3. Example TS conformations for the CO$_2$ hydration reaction, with C in black, O in red, and H in white. Species directly involved in the reaction are displayed with larger atomic radii. H-bonds involving the TS are labeled in green. (a,b): Conformations with 25 solvating H$_2$O molecules. (a): Relatively well-solvated conformation, with the CO$_2$ in the center of the cluster. There is one H$_{\text{wat}}$-O$_{\text{ohc}}$, four H$_{\text{wat}}$-O$_{\text{oc}}$, and two H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation. (b): Relatively poorly-solvated conformation, with the CO$_2$ on the edge of the cluster. There is one H$_{\text{wat}}$-O$_{\text{ohc}}$, three H$_{\text{wat}}$-O$_{\text{oc}}$, and two H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation (c): TS conformation with 40 solvating H$_2$O molecules, optimized using a less complex model chemistry. There is one H$_{\text{wat}}$-O$_{\text{ohc}}$, four H$_{\text{wat}}$-O$_{\text{oc}}$, and two H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation. (d): TS conformation with 3 solvating H$_2$O molecules. There are no H$_{\text{wat}}$-O$_{\text{ohc}}$, two H$_{\text{wat}}$-O$_{\text{oc}}$, and two H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation. See figure 3-1 for a description of H-bond notation.
Figure 3-4. Example TS conformations for the CO$_2$ hydroxylation reaction, with C in black, O in red, and H in white. Species directly involved in the reaction are displayed with larger atomic radii. H-bonds involving the TS are labeled in green. (a,b): Conformations with 33 solvating H$_2$O molecules. (a): Relatively well-solvated conformation, with the CO$_2$ in the center of the cluster. There are three H$_{\text{wat}}$-O$_{\text{ohc}}$, three H$_{\text{wat}}$-O$_{\text{oc}}$, and one H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation. (b): Relatively poorly-solvated conformation, with the CO$_2$ on the edge of the cluster. There are two H$_{\text{wat}}$-O$_{\text{ohc}}$, two H$_{\text{wat}}$-O$_{\text{oc}}$, and one H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation (c): TS conformation with 40 solvating H$_2$O molecules, optimized using a less complex model chemistry. There are three H$_{\text{wat}}$-O$_{\text{ohc}}$, five H$_{\text{wat}}$-O$_{\text{oc}}$, and one H$_{\text{ohc}}$-O$_{\text{wat}}$ H-bonds in this conformation. See figure 3-1 for a description of H-bond notation.
Table 3-1. Model chemistries used in this chapter. Color scheme is consistent through the chapter. Favored model chemistries are in blue/green colors; darker colors indicate larger basis sets. Vibration RMS errors and $^{18/16}\alpha_{eq}(CO_2(g)-H_2O(g))$ error from in Chapter 2, this dissertation. Table identical to Table 2-1.

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<th>Model/Chem and Color</th>
<th>Abbreviation</th>
<th>CO$_2$ &amp; H$_2$O Vibration RMS Error</th>
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Figure 3-5. $\Delta G^0$ of hydration reaction, versus number of H$_2$O molecules in the cluster. Multiple ($\geq$7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) from Wang et al. (2010).
Figure 3-6. $\Delta G^0$ of hydroxylation reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) from Wang et al. (2010).
Figure 3-7. $E_a$ of hydration reaction, versus number of H$_2$O molecules in the cluster. Multiple ($\geq 7$) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) from Wang et al. (2010).
Figure 3-8. $E_a$ of hydroxylation reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) from Wang et al. (2010).
Figure 3-9. $^{13/12}\alpha_{eq}$ of the hydration reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) for $^{13/12}\alpha_{eq}(\text{HCO}_3^--\text{CO}_2^2)$ from Mook et al. (1974).
Figure 3-10. $^{13/12}\alpha_{eq}$ of the hydroxylation reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) for $^{13/12}\alpha_{eq}$(HCO$_3$-CO$_2$) from Mook et al. (1974).
Figure 3-11. $^{18/16}\alpha_{eq}$ of the hydration reaction, versus number of H$_2$O molecules in the cluster. Multiple ($\geq 7$) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) for $^{18/16}\alpha_{eq}$(HCO$_3^-$-CO$_2$) from Beck et al. (2005).
Figure 3-12. $^{18/16}\alpha_{eq}$ of hydroxylation reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) for $^{18/16}\alpha_{eq}(\text{HCO}_3^-\text{CO}_2)$ from Beck et al. (2005).
Figure 3-13. $^{13/12}a_{\text{kin}}$ of the hydration reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) for $^{13/12}a_{\text{kin}}$(hydration) from O’Leary et al. (1992); error bars represent the range in experimental values (Marlier and O’Leary 1984; Clark and Lauriol 1992).
Figure 3-14. $^{13/12}\alpha_{\text{kin}}$ of the hydroxylation reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting).
Figure 3-15. $^{18/16} \alpha_{\text{kin}}$ of the hydration reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting).
Figure 3-16. $^{18/16}$α_{kin} of the hydroxylation reaction, versus number of H$_2$O molecules in the cluster. Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting).
Figure 3-17. Systematics by model chemistry in the hydration reaction. Positive values indicate the value for the model chemistry represented by the bar color is larger than the value for the model chemistry abbreviation listed on the X-axis. Error bars reflect 1 standard deviation in differences between model chemistries applied to a single conformation.
Figure 3-18. Systematics by model chemistry in the hydroxylation reaction. Positive values indicate the value for the model chemistry represented by the bar color is larger than the value for the model chemistry abbreviation listed on the X-axis. Error bars reflect 1 standard deviation in differences between model chemistries applied to a single conformation.
Figure 3-19. $^{13/12}\alpha_{eq}(\text{HCO}_3^-\text{-CO}_2)$ when forming HCO$_3^-$ product, either from hydration or hydroxylation calculations, versus number of H$_2$O molecules in the cluster. Multiple ($\geq 7$) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) from Mook et al. (1974).
Figure 3-20. $^{18/16}\alpha_{\text{eq}}$ (HCO$_3^-$:CO$_2$) when forming HCO$_3^-$ product, either from hydration or hydroxylation calculations, versus number of H$_2$O molecules in the cluster. Multiple ($\geq 7$) clusters averaged using Boltzmann weighting of the cluster by electronic and zero-point energies plus thermal contributions. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Experimental value (black) from Beck et al. (2005).
Table 3-2. Multiple linear regression of H-bond effects on $^{13/12}\alpha_{eq}(H_2CO_3-CO_2)$ by model chemistry. Regression coefficients in the first four columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model Chem and Color</th>
<th>$H_{eq}-O_{C2}$</th>
<th>$H_{eq}-O_{eq}$</th>
<th>$H_{eq}-O_{H2}$</th>
<th>Constant</th>
<th>$R^2$</th>
<th>Avg. Resid.</th>
<th>N</th>
<th>Best – guess value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDEPE/6-31+G(d,p)</td>
<td>-2.5</td>
<td>+0.4%</td>
<td>+0.9%</td>
<td>2.4</td>
<td>1006.6</td>
<td>+0.0%</td>
<td>0.62</td>
<td>1.64% 73</td>
</tr>
<tr>
<td></td>
<td>-1.4</td>
<td>+0.3%</td>
<td>+0.9%</td>
<td>0.8</td>
<td>1012.4</td>
<td>+0.0%</td>
<td>0.63</td>
<td>1.30% 72</td>
</tr>
<tr>
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<td>-1.2</td>
<td>+0.3%</td>
<td>+0.9%</td>
<td>0.6</td>
<td>1006.8</td>
<td>+0.0%</td>
<td>0.60</td>
<td>1.36% 73</td>
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<td>-1.5</td>
<td>+0.3%</td>
<td>+0.9%</td>
<td>0.7</td>
<td>1007.0</td>
<td>+0.0%</td>
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<td>1.42% 73</td>
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<tr>
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<td>1.1</td>
<td>+0.3%</td>
<td>+0.9%</td>
<td>0.8</td>
<td>1012.7</td>
<td>+0.0%</td>
<td>0.66</td>
<td>1.33% 72</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>+0.3%</td>
<td>+0.9%</td>
<td>0.8</td>
<td>1010.1</td>
<td>+0.0%</td>
<td>0.64</td>
<td>1.21% 73</td>
</tr>
<tr>
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<td>+0.3%</td>
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<td>1009.3</td>
<td>+0.0%</td>
<td>0.60</td>
<td>1.38% 79</td>
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<tr>
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<td>+0.3%</td>
<td>+0.9%</td>
<td>0.6</td>
<td>1009.7</td>
<td>+0.0%</td>
<td>0.63</td>
<td>1.21% 80</td>
</tr>
</tbody>
</table>

Table 3-3. Multiple linear regression of H-bond effects on $^{13/12}\alpha_{eq}(HCO_3^-\cdot CO_2^-)$ by model chemistry. Regression coefficients in the first four columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model Chem and Color</th>
<th>$H_{eq}-O_{C2}$</th>
<th>$H_{eq}-O_{eq}$</th>
<th>$H_{eq}-O_{H2}$</th>
<th>Constant</th>
<th>$R^2$</th>
<th>Avg. Resid.</th>
<th>N</th>
<th>Best – guess value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDEPE/6-31+G(d,p)</td>
<td>-5.4</td>
<td>+0.5%</td>
<td>+0.5%</td>
<td>1.4</td>
<td>1001.6</td>
<td>+0.0%</td>
<td>0.73</td>
<td>2.16% 97</td>
</tr>
<tr>
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<td>-3.2</td>
<td>+0.4%</td>
<td>+0.5%</td>
<td>0.8</td>
<td>1002.4</td>
<td>+0.0%</td>
<td>0.70</td>
<td>2.00% 52</td>
</tr>
<tr>
<td></td>
<td>-3.7</td>
<td>+0.5%</td>
<td>+0.5%</td>
<td>0.6</td>
<td>999.7</td>
<td>+0.0%</td>
<td>0.71</td>
<td>2.10% 59</td>
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<tr>
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<td>+0.5%</td>
<td>1.6</td>
<td>1002.0</td>
<td>+0.0%</td>
<td>0.67</td>
<td>2.23% 56</td>
</tr>
<tr>
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<td>-2.6</td>
<td>+0.5%</td>
<td>+0.5%</td>
<td>0.4</td>
<td>1003.3</td>
<td>+0.0%</td>
<td>0.68</td>
<td>1.99% 39</td>
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<tr>
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<td>+0.5%</td>
<td>0.5</td>
<td>1000.7</td>
<td>+0.0%</td>
<td>0.73</td>
<td>1.96% 39</td>
</tr>
<tr>
<td></td>
<td>-3.6</td>
<td>+0.5%</td>
<td>+0.5%</td>
<td>0.7</td>
<td>999.5</td>
<td>+0.0%</td>
<td>0.71</td>
<td>2.11% 51</td>
</tr>
<tr>
<td></td>
<td>-3.7</td>
<td>+0.5%</td>
<td>+0.5%</td>
<td>0.4</td>
<td>1000.2</td>
<td>+0.0%</td>
<td>0.74</td>
<td>1.92% 40</td>
</tr>
<tr>
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<td>-3.7</td>
<td>+0.5%</td>
<td>+0.5%</td>
<td>0.4</td>
<td>1000.2</td>
<td>+0.0%</td>
<td>0.74</td>
<td>1.92% 40</td>
</tr>
<tr>
<td></td>
<td>-3.7</td>
<td>+0.5%</td>
<td>+0.5%</td>
<td>0.4</td>
<td>1000.2</td>
<td>+0.0%</td>
<td>0.74</td>
<td>1.92% 40</td>
</tr>
</tbody>
</table>
Table 3-4. Multiple linear regression of H-bond effects on $^{18/16}$q$_{eq}$(H$_2$CO$_3$-CO$_2$) by model chemistry. Regression coefficients in the first four columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model/Chem and Color</th>
<th>H$<em>{eq}$ - O$</em>{CC}$</th>
<th>H$<em>{eq}$ - O$</em>{X}$</th>
<th>H$<em>{eq}$ - O$</em>{AX}$</th>
<th>Constant</th>
<th>$R^2$</th>
<th>Avg. Resid.</th>
<th>N</th>
<th>Best - guess value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEPEB/6 - 311++G(4,p)</td>
<td>-1.2</td>
<td>+/-0.2%</td>
<td>0.4</td>
<td>1.6</td>
<td>994.3</td>
<td>+/-0.4%</td>
<td>0.71</td>
<td>0.74%</td>
</tr>
<tr>
<td>B3LYP/6 - 311++G(4,p)</td>
<td>-0.6</td>
<td>+/-0.2%</td>
<td>0.7</td>
<td>0.8</td>
<td>995.8</td>
<td>+/-0.3%</td>
<td>0.67</td>
<td>0.65%</td>
</tr>
<tr>
<td>PBE0/6 - 311++G(6,p)</td>
<td>-0.5</td>
<td>+/-0.2%</td>
<td>0.8</td>
<td>0.6</td>
<td>995.3</td>
<td>+/-0.3%</td>
<td>0.64</td>
<td>0.69%</td>
</tr>
<tr>
<td>B09/6 - 311++G(6,M,p)</td>
<td>-0.7</td>
<td>+/-0.1%</td>
<td>1.1</td>
<td>1.7</td>
<td>994.5</td>
<td>+/-0.3%</td>
<td>0.76</td>
<td>0.65%</td>
</tr>
<tr>
<td>X3LYP/6 - 311++G(3d,p)</td>
<td>-0.8</td>
<td>+/-0.1%</td>
<td>0.8</td>
<td>1.0</td>
<td>995.3</td>
<td>+/-0.3%</td>
<td>0.70</td>
<td>0.63%</td>
</tr>
<tr>
<td>B3LYP/6 - 311++G(3d,p)</td>
<td>-0.6</td>
<td>+/-0.2%</td>
<td>0.7</td>
<td>1.0</td>
<td>995.3</td>
<td>+/-0.3%</td>
<td>0.69</td>
<td>0.67%</td>
</tr>
<tr>
<td>B3LYP/6 - 311++G(24,p)</td>
<td>0.7</td>
<td>+/-0.1%</td>
<td>0.6</td>
<td>1.0</td>
<td>995.3</td>
<td>+/-0.3%</td>
<td>0.72</td>
<td>0.62%</td>
</tr>
</tbody>
</table>

Table 3-5. Multiple linear regression of H-bond effects on $^{18/16}$q$_{eq}$(HCO$_3$-CO$_2$) by model chemistry. Regression coefficients in the first four columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model/Chem and Color</th>
<th>H$<em>{eq}$ - O$</em>{CC}$</th>
<th>H$<em>{eq}$ - O$</em>{X}$</th>
<th>H$<em>{eq}$ - O$</em>{AX}$</th>
<th>Constant</th>
<th>$R^2$</th>
<th>Avg. Resid.</th>
<th>N</th>
<th>Best - guess value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEPEB/6 - 311++G(4,p)</td>
<td>-2.8</td>
<td>+/-0.4%</td>
<td>0.3</td>
<td>1.1</td>
<td>989.6</td>
<td>+/-1.0%</td>
<td>0.62</td>
<td>1.14%</td>
</tr>
<tr>
<td>PBE0/6 - 311++G(6,p)</td>
<td>-1.3</td>
<td>+/-0.4%</td>
<td>0.8</td>
<td>0.9</td>
<td>986.6</td>
<td>+/-1.1%</td>
<td>0.49</td>
<td>1.09%</td>
</tr>
<tr>
<td>X3LYP/6 - 311++G(4,p)</td>
<td>-1.3</td>
<td>+/-0.3%</td>
<td>0.9</td>
<td>0.7</td>
<td>985.4</td>
<td>+/-1.1%</td>
<td>0.52</td>
<td>1.04%</td>
</tr>
<tr>
<td>PBE0/6 - 311++G(3d,p)</td>
<td>-2.5</td>
<td>+/-0.4%</td>
<td>0.2</td>
<td>1.4</td>
<td>990.1</td>
<td>+/-1.0%</td>
<td>0.54</td>
<td>1.18%</td>
</tr>
<tr>
<td>B3LYP/6 - 311++G(3d,p)</td>
<td>-0.9</td>
<td>+/-0.5%</td>
<td>0.7</td>
<td>1.4</td>
<td>987.0</td>
<td>+/-1.3%</td>
<td>0.43</td>
<td>1.55%</td>
</tr>
<tr>
<td>X3LYP/6 - 311++G(24,p)</td>
<td>1.2</td>
<td>+/-0.4%</td>
<td>0.7</td>
<td>0.6</td>
<td>986.1</td>
<td>+/-1.1%</td>
<td>0.54</td>
<td>1.00%</td>
</tr>
<tr>
<td>B3LYP/6 - 311++G(24,p)</td>
<td>-1.4</td>
<td>+/-0.4%</td>
<td>0.7</td>
<td>0.7</td>
<td>985.4</td>
<td>+/-1.1%</td>
<td>0.51</td>
<td>1.06%</td>
</tr>
<tr>
<td>B3LYP/6 - 311++G(24,p)</td>
<td>-1.2</td>
<td>+/-0.4%</td>
<td>0.7</td>
<td>0.7</td>
<td>986.2</td>
<td>+/-1.0%</td>
<td>0.53</td>
<td>0.96%</td>
</tr>
</tbody>
</table>
Table 3-6. Multiple linear regression of H-bond effects on $^{13/12}\alpha_{kin}$(hydration) by model chemistry. Regression coefficients in the first five columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model Chemistry/Color</th>
<th>$R_{\text{H}} - \alpha_{\text{H}}$</th>
<th>$R_{\text{H}} - \alpha_{\text{O}}$</th>
<th>$H_{\text{H}} - \alpha_{\text{O}}$</th>
<th>$C - \alpha_{\text{O}}$ distance</th>
<th>Coefficient</th>
<th>t</th>
<th>Avg. Bond</th>
<th>N</th>
<th>Beta – generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>-2.5</td>
<td>±2.5</td>
<td>±2.5</td>
<td>±2.5</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>+0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>+0.6</td>
<td>+0.6</td>
<td>+0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>+0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>+0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>+0.6</td>
<td>+0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>+0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Table 3-7. Multiple linear regression of H-bond effects on $^{13/12}\alpha_{kin}$(hydroxylation) by model chemistry. Regression coefficients in the first five columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model Chemistry/Color</th>
<th>$R_{\text{H}} - \alpha_{\text{H}}$</th>
<th>$R_{\text{H}} - \alpha_{\text{O}}$</th>
<th>$H_{\text{H}} - \alpha_{\text{O}}$</th>
<th>$C - \alpha_{\text{O}}$ distance</th>
<th>Coefficient</th>
<th>t</th>
<th>Avg. Bond</th>
<th>N</th>
<th>Beta – generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>-2.5</td>
<td>±2.5</td>
<td>±2.5</td>
<td>±2.5</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>+0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>+0.6</td>
<td>+0.6</td>
<td>+0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>+0.6</td>
<td>+0.6</td>
<td>+0.6</td>
<td>+0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>+0.6</td>
<td>+0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>+0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
<tr>
<td>PBEH/1 – MB1(Gdf,p)</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.6</td>
<td>1002.1</td>
<td>0.48</td>
<td>1.79%</td>
<td>83</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Table 3-8. Multiple linear regression of H-bond effects on $^{18/16}\alpha_{\text{kin}}$(hydration) by model chemistry. Regression coefficients in the first five columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model/Chromatone</th>
<th>$R_{45}$ - $\Delta\phi_{45}$</th>
<th>$R_{45}$ - $\Delta\phi_{45}$</th>
<th>$R_{45}$ - $\Delta\phi_{45}$</th>
<th>C - $\Delta\phi_{45}$</th>
<th>Constant</th>
<th>$R^2$</th>
<th>Avg. Bond</th>
<th>N</th>
<th>Best – generator</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBFPEP - $^{31}_{\text{H}}$+G(p)</td>
<td>-1.4 +/0.3%</td>
<td>+/0.2%</td>
<td>+/0.1%</td>
<td>-4.3 +/1.2%</td>
<td>987.7 +/1.9%</td>
<td>0.75</td>
<td>1.04%</td>
<td>81</td>
<td>0.9987 +/-0.0010</td>
</tr>
<tr>
<td>PBFPEP - $^{31/14}_{\text{H}}$+G(p)</td>
<td>1.2 +/0.2%</td>
<td>+/0.2%</td>
<td>+/0.3%</td>
<td>-4.3 +/1.4%</td>
<td>983.6 +/2.0%</td>
<td>0.78</td>
<td>0.97%</td>
<td>81</td>
<td>0.9903 +/-0.0010</td>
</tr>
<tr>
<td>XLYP - $^{31/14}_{\text{H}}$+G(p)</td>
<td>0.9 +/-0.3%</td>
<td>+/0.2%</td>
<td>+/0.3%</td>
<td>-7.7 +/1.2%</td>
<td>988.1 +/2.5%</td>
<td>0.71</td>
<td>1.10%</td>
<td>81</td>
<td>0.9910 +/-0.0011</td>
</tr>
<tr>
<td>POPEP - $^{31}_{\text{H}}$+G(p)</td>
<td>-1.4 +/0.2%</td>
<td>+/0.2%</td>
<td>+/0.3%</td>
<td>-7.9 +/1.3%</td>
<td>1000.5 +/3.8%</td>
<td>0.83</td>
<td>0.69%</td>
<td>81</td>
<td>0.9989 +/-0.0009</td>
</tr>
<tr>
<td>PBFPEP - $^{31/16}_{\text{H}}$+G(p)</td>
<td>-0.7 +/0.3%</td>
<td>+/0.2%</td>
<td>+/0.3%</td>
<td>-11.9 +/1.5%</td>
<td>1004.1 +/3.7%</td>
<td>0.86</td>
<td>0.62%</td>
<td>81</td>
<td>0.9996 +/-0.0008</td>
</tr>
<tr>
<td>XLYP - $^{31/16}_{\text{H}}$+G(p)</td>
<td>-0.7 +/0.3%</td>
<td>+/0.2%</td>
<td>+/0.3%</td>
<td>-11.1 +/1.5%</td>
<td>998.0 +/3.7%</td>
<td>0.80</td>
<td>1.11%</td>
<td>81</td>
<td>0.9966 +/-0.0011</td>
</tr>
<tr>
<td>REPEP - $^{31/16}_{\text{H}}$+G(p)</td>
<td>-0.6 +/0.3%</td>
<td>+/0.2%</td>
<td>+/0.3%</td>
<td>-8.2 +/1.3%</td>
<td>1002.2 +/3.8%</td>
<td>0.76</td>
<td>0.54%</td>
<td>77</td>
<td>0.9988 +/-0.0009</td>
</tr>
</tbody>
</table>

Table 3-9. Multiple linear regression of H-bond effects on $^{18/16}\alpha_{\text{kin}}$(hydroxylation) by model chemistry. Regression coefficients in the first five columns are colored green if significant at the P<0.05 level. See figure 3-1 for a description of H-bond notation.

<table>
<thead>
<tr>
<th>Model/Chromatone</th>
<th>$R_{45}$ - $\Delta\phi_{45}$</th>
<th>$R_{45}$ - $\Delta\phi_{45}$</th>
<th>$R_{45}$ - $\Delta\phi_{45}$</th>
<th>C - $\Delta\phi_{45}$</th>
<th>Constant</th>
<th>$R^2$</th>
<th>Avg. Bond</th>
<th>N</th>
<th>Best – generator</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBFPEP - $^{31}_{\text{H}}$+G(p)</td>
<td>-4.0 +/0.5%</td>
<td>+/0.8%</td>
<td>+/1.2%</td>
<td>-1.6 +/2.0%</td>
<td>984.3 +/1.7%</td>
<td>0.75</td>
<td>0.92%</td>
<td>23</td>
<td>0.9825 +/-0.0030</td>
</tr>
<tr>
<td>PBFPEP - $^{31/14}_{\text{H}}$+G(p)</td>
<td>-2.7 +/0.7%</td>
<td>+/0.8%</td>
<td>+/1.2%</td>
<td>-2.5 +/2.0%</td>
<td>991.4 +/1.9%</td>
<td>0.55</td>
<td>0.81%</td>
<td>20</td>
<td>0.9868 +/-0.0030</td>
</tr>
<tr>
<td>XLYP - $^{31}_{\text{H}}$+G(p)</td>
<td>3.5 +/0.4%</td>
<td>+/0.7%</td>
<td>+/1.0%</td>
<td>0.5 +/0.3%</td>
<td>984.8 +/0.9%</td>
<td>0.73</td>
<td>0.79%</td>
<td>22</td>
<td>0.9798 +/-0.0008</td>
</tr>
<tr>
<td>PBFPEP - $^{31/16}_{\text{H}}$+G(p)</td>
<td>-3.8 +/0.5%</td>
<td>+/0.7%</td>
<td>+/0.8%</td>
<td>-0.9 +/2.9%</td>
<td>992.0 +/1.3%</td>
<td>0.81</td>
<td>0.81%</td>
<td>23</td>
<td>0.9827 +/-0.0008</td>
</tr>
<tr>
<td>POPEP - $^{31/14}_{\text{H}}$+G(p)</td>
<td>4.6 +/0.5%</td>
<td>+/0.4%</td>
<td>+/0.3%</td>
<td>0.4 +/0.3%</td>
<td>973.3 +/0.9%</td>
<td>0.94</td>
<td>0.35%</td>
<td>11</td>
<td>0.9787 +/-0.0001</td>
</tr>
<tr>
<td>XLYP - $^{31/16}_{\text{H}}$+G(p)</td>
<td>-3.5 +/0.5%</td>
<td>+/0.4%</td>
<td>+/0.6%</td>
<td>0.9 +/0.7%</td>
<td>986.7 +/0.1%</td>
<td>0.98</td>
<td>0.47%</td>
<td>15</td>
<td>0.9787 +/-0.0005</td>
</tr>
<tr>
<td>REPEP - $^{31/16}_{\text{H}}$+G(p)</td>
<td>3.3 +/0.6%</td>
<td>+/0.6%</td>
<td>+/0.3%</td>
<td>0.2 +/0.9%</td>
<td>988.4 +/0.3%</td>
<td>0.70</td>
<td>0.83%</td>
<td>34</td>
<td>0.9766 +/-0.0008</td>
</tr>
<tr>
<td>REPEP - $^{31/16}_{\text{H}}$+G(p)</td>
<td>-3.2 +/0.5%</td>
<td>+/0.5%</td>
<td>+/0.6%</td>
<td>0.8 +/0.7%</td>
<td>986.1 +/0.1%</td>
<td>0.88</td>
<td>0.46%</td>
<td>15</td>
<td>0.9798 +/-0.0005</td>
</tr>
</tbody>
</table>
Figure 3-21. Variation of $^{13/12} \alpha_{eq}(H_2CO_3-CO_2)$ with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment.
Figure 3-22. Variation of $^{18/16} \alpha_{eq}(\text{H}_2\text{CO}_3-\text{CO}_2)$ with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment.
Figure 3-23. Variation of $^{13/12}q_{\text{kin}}$(hydration) with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment.
Figure 3-24. Variation of $^{18/16}q_{\text{kin}}$(hydration) with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment.
Figure 3-25. Variation of $^{13/12}\alpha_{eq}(\text{HCO}_3^-\text{-CO}_2)$ with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment. Two experimental calibrations are shown in gray (with reported regression errors) and black (no errors were reported). Experimental values from Mook et al. (1974; gray) and Zhang et al. (1995; black).
Figure 3-26. Variation of $^{18/16}\alpha_{eq}(\text{HCO}_3^- - \text{CO}_2)$ with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment. Experimental values (black, with error bars) calibrated from 0-40°C from Beck et al. (2005).
Figure 3-27. Variation of $^{13/12}d_{\text{kin}}(\text{hydroxylation})$ with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment.
Figure 3-28. Variation of $^{18}_{16}{\alpha}_{\text{kin}}$(hydroxylation) with temperature. Colors represent calculations done with different model chemistries. Darker colors reflect larger basis sets. Blue/green colors reflect model chemistries which performed better under fractionation evaluation. Calculations were performed using the best-guess H-bond environment.
Figure 3-29. Best-guess slopes for each basis set calculated for the hydration reaction. Slopes connect equilibrated DIC at (-0.2‰, 2.0‰) with DIC undergoing enough kinetic fractionation to fix δ¹⁸O at -3.5‰. Experimental value (black) taken from lab-grown corals; data shown in Figure 1.1.
Figure 3-30. Best-guess slopes for each basis set calculated for the hydroxylation reaction. Slopes connect equilibrated DIC at (-0.2‰, 2.0‰) with DIC undergoing enough kinetic fractionation to fix δ^{18}O at -3.5‰. Experimental value (black) taken from lab-grown corals; data shown in Figure 1.1.
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Chapter 4

Isotopic Clumping during Formation of Dissolved Inorganic Carbon Species

Abstract

Clumped isotope carbonate paleothermometry holds promise for paleotemperature reconstruction because isotopic clumping depends only on formation temperature, in principle. However, it is built on the assumption that carbonates form at isotopic equilibrium. This chapter evaluates the departure from clumping equilibrium that can be expected for carbonates that have incompletely equilibrated via the \( \text{CO}_2 \) hydration and hydroxylation reactions. Quantum mechanical calculations of dissolved inorganic carbon species embedded in \( \text{H}_2\text{O} \) clusters are analyzed under a transition state theory (TST) framework to predict both equilibrium and kinetic clumped isotopic fractionations and resulting compositions expected for dissolved inorganic carbon species. Hydrogen bond pattern and transition state (TS) reaction coordinate position influence calculated clumped isotopic compositions. Values of \( \Delta_{47} \) for dissolved inorganic carbon (DIC) produced by the \( \text{CO}_2 \) hydration reaction without any equilibration exceed equilibrium C-O clumping (\( \Delta_{47} \)) of \( \text{HCO}_3^- \) by \( 0.058\% \) to \( 0.060\% \) and exceed the mineral calibration line by \( \sim0.10\% \). Values of \( \Delta_{47} \) for DIC produced by the \( \text{CO}_2 \) hydroxylation reaction without any equilibration exceed equilibrium \( \Delta_{47} \) of \( \text{HCO}_3^- \) by \( 0.081\% \) to \( 0.083\% \) and exceed the mineral calibration line by \( 0.12\% \). The results approximately match the extent of \( \Delta_{47} \) disequilibrium observed in shallow-water corals. Kinetic fractionation in the \( \text{CO}_2 \) hydration reaction has the potential to greatly affect the \( \Delta_{47} \) of carbonate minerals.
**Introduction**

Clumped isotope carbonate paleothermometry is useful due to its consistency across different carbonate precipitation regimes, including biogenic and abiogenic carbonates, regardless of precipitating fluid composition. While most carbonates are at apparent clumped equilibrium, some carbonates are not, shallow-water corals in particular. Many mechanisms capable of causing apparent clumped isotope disequilibrium to be recorded in carbonates have been investigated, but kinetic fractionations in the CO$_2$ hydration and hydroxylation pathways have not. This chapter evaluates the magnitudes of these kinetic fractionations using computational chemistry and compares them to carbonate records including corals. This chapter also evaluates the conditions necessary for computational chemistry to produce accurate, precise estimates of clumped isotope fractionations.

**Clumped Isotope Geochemistry and Paleothermometry**

Clumped isotope geochemistry, unlike traditional stable isotope geochemistry, is a means of analyzing geochemical records independent of bulk isotopic composition. Traditional stable isotope records like $\delta^{13}$C and $\delta^{18}$O measure the **amount** of a rare isotope, and thus are sensitive to the amount of that rare isotope in the starting material, in addition to recording any fractionations caused by processes of interest. In contrast, clumped stable isotope records reflect the **arrangement** of two rare isotopes within a sample, which avoids compositional dependence.

Heavy isotopes tend to clump together in molecules more often than would be predicted by random mixing of isotopes. This is because a heavy isotope can lower the ZPE of a molecule which already contains another heavy isotope more than it can lower the ZPE of a molecule where all other isotopes are light (Eiler 2007).
The preference for isotopes to clump is greater at low temperatures than at high temperatures, forming the basis for a clumped-isotope paleothermometer. At high temperatures, entropic effects scramble the rare isotopes more than at low temperatures, where the enthalpic effects of reduced ZPE dominate. By comparing a natural sample’s amount of isotopic clumping with a stochastic (isotopically well-mixed) sample, the natural sample’s formation temperature can be determined, by comparison to a clumping-vs-temperature calibration curve.

The main benefit of a clumped-isotope paleothermometer is that it is composition-independent (e.g. Eiler 2007). That is, it does not depend on the absolute amounts of any given isotope that are present in a sample, and thus does not depend on the composition of its source material. This contrasts with paleothermometers such as $\delta^{18}$O whose interpretation in carbonates depends on knowledge of the $\delta^{18}$O of the water and CO$_2$ which produced it.

The main challenges of clumped isotope analysis is the rarity of molecules incorporating two rare isotopes at once, and the small (typically <1‰) variations in their abundance across natural samples (Eiler 2007). Their analysis thus requires sensitive mass spectrometers calibrated to the masses of the molecules including multiple rare isotopes. Analytical difficulties as well as recent developments in the application of computational chemistry techniques to geochemical systems (e.g. Kubicki and Rosso 2016) make computational chemistry a useful tool for comparing the possible mechanisms affecting isotope clumping.

Isotopic clumping, and the processes that affect it, have been reviewed several times in the past fifteen years. Eiler (2007) reviews the theoretical background, analytical procedure, and both equilibrium and kinetic driving factors of early studies of clumped isotopes, focusing in particular on carbonate clumped isotope paleothermometry. Wang et al. (2004) cover the theory and method of calculation of clumped isotope abundances in detail, applying these calculations to common multi-atomic gases. Eiler et al. (2013) describe the sensitive isotope ratio mass spectrometer equipped to separate out very small amounts of clumped isotopic species. Eiler et al.
(2014) discuss the emerging field of clumped isotope geochemistry in the context of other developments in stable isotope geochemistry, with some mention of first-principles computational methods’ role. Eiler (2011) and Affek (2012) review developments in carbonate clumped isotope paleothermometry which is the largest current application of clumped isotope geochemistry, and highlight both the promise of integration with traditional stable isotope records and the perils of non-equilibrium signatures including diagenesis.

**Clumped Isotope Terminology and Notation**

Isotopes are different types of the same element which differ only in the number of neutrons in the nucleus. Different isotopes of an element behave almost identically in a chemical sense, except that the higher mass of heavy isotopes causes them to move more slowly when translating, rotating, or vibrating. These differences cause different isotopes to concentrate, or fractionate, into different substances based on the energies of these motions. The term “isotope” may refer to a single nuclide with a particular number of protons and neutrons, or to all such nuclides as a class.

Molecular substances consist of collections of different isotopologues, or molecules consisting of different isotopes. When two or more rare isotopes exist in an isotopologue, that isotopologue could be called a “rare isotopologue” or a “multiply-substituted isotopologue”. The term “clumped isotopologue” is slightly more specific in that it refers to a multiply-substituted isotopologue in which the two rare isotopes share a bond. For example, consider the stable isotopes of hydrogen, abundant $^1$H (with 0 neutrons) and uncommon $^2$H (with 1 neutron). These isotopes can be combined into three different isotopologues: $^1$H-$^1$H, $^1$H-$^2$H, and $^2$H-$^2$H. The $^2$H-$^2$H isotopologue is a clumped isotopologue because it contains two comparatively rare $^2$H isotopes.
bonded together. Note that $^2\text{H}^1\text{H}$ is equivalent to $^1\text{H}^2\text{H}$ because molecular hydrogen is symmetric: when counting isotopologues, their symmetry needs to be accounted for.

When discussing the abundance of isotopologues, their abundance is often referenced to the abundance that would be expected if all isotopes were stochastically mixed in the sample; that is, if their abundance only depended on the amount of isotopes in the sample and not the different properties of the isotopologues. By referencing to this stochastic distribution, the bulk isotopic composition of the sample can be corrected for. Extending the previous example, if a sample of molecular hydrogen contains 99% $^1\text{H}$ and 1% $^2\text{H}$, the stochastic distribution of $^1\text{H}^1\text{H}$ would be $(0.99)(0.99) = 98.01\%$, $^1\text{H}^2\text{H}$ would be $(2)(0.99)(0.01) = 1.98\%$, and $^2\text{H}^2\text{H}$ would be $(0.01)(0.01) = 0.01\%$. Note again the combination of symmetrical isotopologues, resulting in the factor of 2 for $^1\text{H}^2\text{H}$. In general, clumped isotopologues are more common than would be predicted based on a stochastic distribution, because the ZPE is slightly lower when heavy isotopes are clumped together, versus when they are in separate molecules.

Clumped isotopologue abundances are described in terms of $\Delta$ values which reflect the deviation, in permil units, of the isotopologue’s abundance relative to the stochastic distribution. The value of $\Delta_i$ for isotopologue $i$ is defined as:

$$\Delta_i = \left( \frac{R_i\text{-sample}}{R_i\text{-stochastic}} - 1 \right) \times 1000\% \quad (4.1)$$

where $R_i$ is the ratio of the abundance of isotopologue $i$ relative to the most common, unsubstituted isotopologue (analogous to equations 3.1 and 3.3). Both $R_i\text{-sample}$ and $R_i\text{-stochastic}$ depend on isotope abundances, but in the same manner, so the overall $\Delta_i$ does not depend on isotope abundances.

In the carbonate system, both abundances of $\text{CO}_3^{2-}$ isotopologues and $\text{CO}_2$ isotopologues are considered. The most common target for clumped isotope analysis in carbonate minerals is $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2^{2-}$ which includes one rare $^{13}\text{C}$ isotope and one rare $^{18}\text{O}$ isotope in addition to two
common $^{16}\text{O}$ isotopes. The value $\Delta_{63}$ refers to the abundance of these clumped $\text{CO}_3^{2-}$ isotopologues of mass 63. The most accurate mass spectrometers used for analysis of clumped carbonate isotopes require a gas source, so the carbonate mineral must be acid-digested to form $\text{CO}_2$ gas. When $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2^{2-}$ is digested, it produces some $^{13}\text{C}^{16}\text{O}_2$ and some $^{13}\text{C}^{16}\text{O}^{18}\text{O}$, with similar separation occurring in other isotopologues of $\text{CO}_3^{2-}$ as well. The latter isotopologue of mass 47 is what is analyzed in the mass spectrometer. Typically, carbonates are described by this measured $\Delta_{47}$ value of the evolved $\text{CO}_2$ gas and not the extrapolated $\Delta_{63}$ value of the undigested carbonate.

Examples of isotopologues are diagrammed for $\text{H}_2\text{CO}_3$ in Figure 4-1.

**Carbonate Clumped Isotope Paleothermometry**

The degree to which isotopes clump in carbonate minerals is controlled by the temperature at which they form. Carbonate $\Delta_{47}$ values are approximately 0.5‰ around typical Earth surface temperatures, with a slope of -0.003 to -0.004‰/degree depending on the particular temperature (Eiler 2011). Most calibrations describe the relationship of $\Delta_{47}$ to $T^{-2}$ so some variation occurs in the slope over typical temperature ranges (e.g. Zaarur et al. 2013; Kelson et al. 2017).

Paleothermometry using $\Delta_{47}$ has been applied in carbonates for more than a decade (Ghosh et al. 2006; Eiler 2011; Affek 2012). Calibrations of the $\Delta_{47}$-$T$ slope have produced broadly similar values over a range of biogenic, natural abiogenic, and synthetic carbonates, including foraminifera, coccoliths (Tripati et al. 2010; Grauel et al. 2013), mollusks, brachiopods (Came et al. 2007; Henkes et al. 2013), corals (Thiagarajan et al. 2011), fish otoliths (Ghosh et al. 2007), mammoth bioapatite (Eagle et al. 2010), dinosaur teeth (Eagle et al. 2011), dinosaur eggshells (Eagle et al. 2015), tufas, travertines (Huntington et al. 2010; Kele et al. 2015),
limestones, diagenetic cements (Gallagher et al. 2017), veins (Luetkemeyer et al. 2016), synthetic calcite (Dennis and Schrag 2010), aragonite (Defliese et al. 2015), dolomite (Bonifacie et al. 2017), and siderite (Fernandez et al. 2014). In synthetic carbonates, several parameters are found to have no effect on recorded \( \Delta_{47} \) values, including mineralogy (Defliese et al. 2015), Ca/Mg ratio (Bonifacie et al. 2017), pH, growth rate, and ionic strength (Tang et al. 2014; Kelson et al. 2017). The temperature dependence is consistent in synthetic calcite samples up to 250°C (Kluge et al. 2015).

The consistency of most calibrations has rendered them useful for a large number of applications across the geosciences. These include estimations of animal body temperatures from the Mesozoic (Eagle et al. 2011; 2015) to the Holocene (Eagle et al. 2010), estimates of Paleozoic climate variability and sensitivity to pCO\(_2\) (Came et al. 2007), response of glacier position to sea surface temperature during the last glacial maximum (Tripati et al. 2014), integration with \( \delta^{18}O \) records to determine coeval fluid isotopic composition (Affek 2012), records of diagenetic and hydrothermal alteration history (Gallagher et al. 2017), and estimates of tectonic uplift rates (Huntington et al. 2010) and deep fluid flow (Huntington and Lechler 2015; Luetkemeter et al. 2016). Many of these problems are intractable using traditional stable isotope methods due to the influence of coeval fluid composition (Eiler 2011).

**Carbonate Clumped Isotopes: Remaining Issues**

Although many calibrations of carbonate \( \Delta_{47} \) are consistent, some calibrations have identified differences in calibrated \( \Delta_{47} \)-T slope based on a number of factors. Individual calibrations record a 1.8-fold difference in slope of \( \Delta_{47} \) with response to \( T^{-2} \) and high-T intercept differences of 0.2‰ (Ghosh et al. 2006; Dennis and Schrag 2010; Kelson et al. 2017). Linear T response varies from 0.002-0.006‰/degree (Ghosh et al. 2006; Dennis and Schrag 2010) In
contrast with experimental calibrations, theoretical investigations of mineral ZPE indicate mineralogy can be expected to impart up to a 0.05‰ difference in equilibrium mineral $\Delta_{63}$ and evolved $\Delta_{47}$ at a given temperature (Guo et al. 2009; Hill et al. 2014), imparting an interpreted temperature difference of $>10^\circ$C in the most extreme cases. The interpreted temperature variability is most extreme at low temperatures, but high temperatures also show variability (e.g. Zaarur et al. 2013). Sources of this variability can be grouped into three classes: variation in analytical procedure imparting artefacts on the calibration, differences in carbonate equilibrium $\Delta_{47}$ reflecting real variability in the calibration, and disequilibrium $\Delta_{47}$ recorded in the carbonate reflecting real variability in the calibration (Kelson et al. 2017).

*Analytical Variability in Clumped Isotopes*

Analytical variability has been a subject of extensive investigation, with many attempts to standardize analytical procedures across laboratories and suggest corrections for a variety of factors. These factors include the phosphoric acid digestion process (Guo et al. 2009; Huntington et al. 2009; Henkes et al. 2013; Came et al. 2014; Fernandez et al. 2014; Petrizzo et al. 2014; Wacker et al. 2014; Colman and Olack 2015; Defliese et al. 2015; Müller et al. 2017; Kelson et al. 2017), stochastic reference gas properties (Dennis et al. 2011), CO$_2$ isotopologue scrambling during travel time in the mass spectrometer (Huntington et al. 2009), purification of sample CO$_2$ and removal of contaminant gases (Dennis and Schrag 2010; Bernasconi et al. 2013; Petersen et al. 2016; Kelson et al. 2017), nonlinearity in measured voltages in the mass spectrometer relative to actual isotopologue abundances (Huntington et al. 2009), and data processing including corrections for $\delta^{17}$O interference on $\delta^{13}$C measurement (Kelson et al. 2017). Consistent $^{17}$O corrections remove much of the discrepancies between calibrations made in different laboratories.
There are conflicting reports on whether different carbonate minerals have different equilibrium $\Delta_{47}$ values. Experimental studies show no difference between calcite and aragonite fractionation within measurement error of $\sim$0.015‰ (Defliese et al. 2015; Kelson et al. 2017), but computational methods predict an offset in fractionation factor of 0.015-0.025‰ for a given temperature (Schauble et al. 2006; Guo et al. 2009; Hill et al. 2014; Tripati et al. 2015). Corals grown under similar, constant growth conditions exhibit qualitatively similar offsets between aragonite and high-Mg calcite calcifiers, although with a somewhat larger offset of 0.03-0.05‰ which could be exacerbated by vital effects; high-Mg calcite corals roughly match the theoretical calibration but the aragonite samples show somewhat higher $\Delta_{47}$ values (Kimball et al. 2016). Analysis of different carbonate minerals is complicated by the fact that different carbonates may exhibit different fractionations during the digestion process (Guo et al. 2009).

The carbonate synthesis process may impart disequilibrium $\Delta_{47}$ values on the final carbonate because of disequilibrium conditions imparted by different experimental setups (Affek et al. 2008; Huntington et al. 2009). Internal disequilibrium within the DIC pool may exist if the CO$_2$ hydration and hydroxylation reactions that mediate equilibration are slow compared to the timescale of precipitation (Hill et al. 2014; Tripati et al. 2015), although some experimental evaluations indicate this disequilibrium should be negligible both with and without enzymatic
equilibration by carbonic anhydrase (Kelson et al. 2017). Degassing method may have an impact: passive and active degassing result in different rates of CO₂ removal from solution, potentially imparting kinetic fractionation on the remaining DIC species (Dennis and Schrag 2010). Although attempts to standardize for degassing method within and across laboratories indicate it should not influence Δ47 (Kelson et al. 2017), the departure from typical Δ47-T calibrations in speleothems especially at low temperatures is widely attributed to fractionation imparted by degassing (Daëron et al. 2011; Affek 2012; Kluge and Affek 2012; Kluge et al. 2013; Zaarur et al. 2013; Affek et al. 2014; Affek and Zaarur 2014) which is matched by investigations in some synthetic carbonates (Dennis and Schrag 2010; Fernandez et al. 2014).

**Carbonate Clumped Isotopes and Vital Effects**

Biogenic carbonates are also susceptible to these abiotic disequilibrium processes, and some vital effects unique to biological systems (Eiler 2011). In particular, shallow-water corals (Ghosh et al. 2006; Affek 2012; Saenger et al. 2012; Eiler et al. 2014; Spooner et al. 2016; Saenger and Erez 2016; Figure 4-2), some deep-water corals (Kimball et al. 2016; Saenger et al. 2017), and some bivalves and mollusks (Affek 2012; Eagle et al. 2013) exhibit offsets from the typical calibration line. The offsets tend to be larger under low-temperature conditions (Ghosh et al. 2006). Shallow-water corals exhibit some of the fastest biological carbonate precipitation rates, often in conjunction with photosynthetic symbionts (Affek 2012; Saenger et al. 2012). Processes influencing coral disequilibrium include solution-mineral disequilibrium cause by fast precipitation rates (Affek 2012; Hill et al. 2014; Tripati et al. 2015; Watkins and Hunt 2015), biological mediation of calcifying space chemistry (Saenger et al. 2017), and DIC-DIC disequilibrium mediated by the CO₂ hydration/hydroxylation reactions (Saenger et al. 2012; Saenger and Erez 2016). Deep-water corals also show Δ47 offsets despite a slower growth rate.
than their shallow-water counterparts (Kimball et al. 2016; Saenger et al. 2017). Bivalves and mollusks show $\Delta_{47}$ offsets of somewhat different character, displaying lower-than-expected $\Delta_{47}$ values especially at low T (Affek 2012; Eagle et al. 2013) while both shallow- and deep-water corals display higher-than-expected $\Delta_{47}$ values (e.g. Saenger et al. 2012; Saenger et al. 2017).

Solution-mineral disequilibrium has recently been investigated on the basis of pH influencing incorporation of different amounts of equilibrated $\mathrm{HCO}_3^-$ and $\mathrm{CO}_3^{2-}$, which have different $\Delta_{47}$ values, into fast-growing carbonates (Hill et al. 2014; Tripati et al. 2015). However, the magnitude of $\Delta_{47}$ disequilibrium that could be caused by kinetic fractionation and disequilibrium in the CO$_2$ hydration/hydroxylation reactions has not been quantified. These reactions are responsible for achieving isotopic equilibrium in DIC species, as the agent of O exchange (Affek 2012). Experimental evaluations of the rate of equilibration of both $\Delta_{47}$ and $\delta^{18}$O find that both equilibrate at the same rate, supporting CO$_2$ hydration/hydroxylation as the only mechanism of internal DIC $\Delta_{47}$ equilibration (Affek 2013).

Studies are conflicted on the rate of equilibration of these reactions during formation of natural carbonates. Wang et al. (2010) found these reactions should reach isotopic equilibrium in ~5 hours at 25°C, pH 7. Uchikawa and Zeebe (2012) determined isotopic equilibration should take about 10 hours at pH 8.3, increasing to 18 hours at pH 8.9. Henkes et al. (2013) found days to weeks might be necessary for equilibration, especially at low T. Schmid (2011) found calcite rapidly precipitated at pH 9 recorded a $\Delta_{47}$ signature offset by +0.06‰ versus the typical calibration, indicating the carbonate recorded a disequilibrium signal inconsistent with the composition of CO$_3^{2-}$ (Hill et al. 2014). Falk and Guo (2014) found that rapidly-precipitated calcite records impart the $\Delta_{47}$ signature of the CO$_2$ from which it forms.

Catalysis by carbonic anhydrase could decrease the time required (Kimball et al. 2016), but evaluations of its impact on isotopic equilibration rates also differ. Uchikawa and Zeebe (2012) found that carbonic anhydrase lowered isotope equilibration time from 10h to <4h.
Watkins et al. (2014) found that, even with carbonic anhydrase, laboratory precipitation experiments are unlikely to reach $\delta^{18}O$ equilibrium with coeval solutions. Tripati et al. (2015) found $\Delta_{47}$ of carbonates precipitated with and without carbonic anhydrase were indistinguishable. Kelson et al. (2017) found that carbonic anhydrase increased the permil/min equilibration rate of $\delta^{18}O$ by only 25-50%. Saenger and Erez (2016) suggest that each batch of calcifying fluid is consumed within 6h in corals, somewhat shorter than most timescales of DIC isotopic equilibration.

In corals, disequilibrium in $\Delta_{47}$ has been tentatively attributed to disequilibrium in the CO$_2$ hydration/hydroxylation reactions and their reverse reactions (Saenger and Erez 2016; Spooner et al. 2016). Shallow-water corals have elevated $\Delta_{47}$ values (up to +0.05‰, Figure 4-2) in conjunction with low $\delta^{18}O$ values that could be attributed to kinetic fractionation in these reactions (Saenger et al. 2012; Spooner et al. 2016). In contrast, deep-water bamboo corals exhibit low $\Delta_{47}$ in conjunction with low $\delta^{18}O$, which is more consistent with equilibration of DIC at high pH (Saenger et al. 2017). Not all deep-water corals exhibit the same $\Delta_{47}$-$\delta^{18}O$ response (Kimball et al. 2016), raising the possibility of kinetic fractionation in some deep-water coral species. The reverse reactions involving DIC decomposition to CO$_2$ produce a lower-than-expected $\Delta_{47}$ value in the DIC left behind during speleothem formation (Daëron et al. 2011; Kluge et al. 2013), which would be consistent with a kinetic fractionation in the forward direction producing higher-than-normal $\Delta_{47}$ values (Saenger and Erez 2016). Disequilibrium in these reactions is not the only process that could result in a simultaneous increase in $\Delta_{47}$ and decrease in $\delta^{18}O$; CO$_2$ diffusion processes could also generate such a signal (Eiler and Schauble 2004; Tripati et al. 2010).

In this chapter, the expected $\Delta_{47}$ value of DIC produced by kinetic fractionation of the CO$_2$ hydration and hydroxylation reactions is calculated, including their temperature dependence. This will provide a point of comparison to the magnitude and direction of $\Delta_{47}$ disequilibrium in
corals and evaluate the reactions’ potential to explain observed coral vital effects. It is hypothesized that clumping produced by kinetic fractionation in these two reactions will be able to explain the observed Δ$_{47}$ offset between shallow-water corals and other carbonates precipitated at the same temperature.

**Methods**

**Summary**

To model equilibrium and kinetic fractionation effects on Δ$_{47}$ in DIC, molecular clusters were generated representing reactant CO$_2$, product H$_2$CO$_3$ and HCO$_3^-$, and TSs between the two, using accurate model chemistries (Chapter 3, this dissertation). The abundances of all isotopologues of DIC excluding stable isotopes $^{17}$O and $^2$H (Figure 4-1) were calculated based on vibrational frequencies. The effects of H-bond patterns were identified using multiple linear regression. Temperature sensitivity of Δ$_{47}$ values were calculated by varying temperature terms in the reduced partition function ratios ($\beta$). Model results were compared to experimental trends after adjusting Δ$_{47}$ for the presence of $^{17}$O-containing isotopologues. Effects of pH were evaluated by calculating the relative abundance of H$_2$O and OH$^-$ available to react at a given pH.

**Isotopologue Abundance Calculation**

Isotopologue abundances for reactants, products, and TSs were calculated by first generating equilibrium constants using reduced partition function ratios (Equation 3.7). Ratios of two $\beta$ produce isomerization equilibrium constants that relate isotopologues with the same isotopic composition. Symmetry in the isotopologues is broken by the asymmetric H$_2$O clusters.
Ratios of four $\beta$ generate doubly-substituted, clumped isotopologues from singly-substituted ones, triply-substituted from doubly-substituted, etc. For the sixteen isotopologues investigated, this process generates thirteen equilibrium constants.

The equilibrium constants were then applied to a system including CO$_2$ with a $\delta^{13}$C relative to VPDB of 0‰ and a $\delta^{18}$O relative to VSMOW of 0‰, with H$_2$O $\delta^{18}$O at equilibrium with CO$_2$. Constraining the $\delta^{13}$C of starting CO$_2$ $\delta^{18}$O of starting CO$_2$, and arbitrarily setting the abundance of $^{12}$C$^{16}$O$_2$ provides the final three constraints on the system to calculate abundances of all isotopologues. A similar process is used to calculate the abundance of TS and product isotopologues, except that equilibration with CO$_2$ sets the $\delta^{13}$C and $\delta^{18}$O, and the abundance of H$_2$$^{12}$C$^{16}$O$_3$ and H$^{12}$C$^{16}$O$_3$ is set in proportion to the Gibbs free energy difference relative to CO$_2$ (Equation 3.16).

For an individual cluster calculation, the $\Delta_{47}$ value of CO$_2$ and the $\Delta_{63}$ value of H$_2$CO$_3$, HCO$_3^-$, and TSs are calculated from the abundances of the relevant isotopologues (Figure 4-1 row 4) relative to the abundances expected under a stochastic distribution of isotopologues with identical $\delta^{13}$C and $\delta^{18}$O values (Equation 4.1). To mimic the fractionation that occurs during acid digestion of carbonates during analysis, an acid fractionation factor of +0.22‰ (Guo et al. 2009) is used to convert $\Delta_{63}$ to $\Delta_{47}$.

The theoretical basis for the $\Delta_{47}$ paleothermometer excludes the impact of $^{17}$O both on measured values of $\delta^{13}$C ($^{13}$C$^{16}$O$_2$ and $^{12}$C$^{17}$O$^{16}$O have the same mass) and on measured abundances of mass-47 isotopologues ($^{13}$C$^{18}$O$^{16}$O, $^{12}$C$^{18}$O$^{17}$O, and $^{13}$C$^{17}$O$_2$ all have the same mass). The abundances of $^{17}$O-containing isotopologues are non-negligible when performing measurements of $\Delta_{47}$, and sensitivity to $\delta^{17}$O has the potential to reduce the composition-independence of the $\Delta_{47}$ paleothermometer. However, because carbonate fractionation is mass-dependent fractionation, a relationship between the natural abundances and masses of $^{17}$O and $^{18}$O can be used to correct for impacts of $^{17}$O:
\[
\frac{^{17}\text{O}}{^{16}\text{O}} = K \left( \frac{^{18}\text{O}}{^{16}\text{O}} \right)^{\lambda}
\]

where \( K \) represents the relationship between \(^{17}\text{O} \) and \(^{18}\text{O} \) absolute abundances, and \( \lambda \) represents the mass difference between O isotopes. The parameters \( K=0.01022461, \lambda=0.528 \) (Brand et al. 2010) have been found to minimize discrepancies between \( \Delta_{47} \) calibrations (Schauer et al. 2016). This study does not need to adjust \( \delta^{13}\text{C} \) for \( \delta^{17}\text{O} \) as the calculation scheme already excludes \(^{17}\text{O} \); however, adjustments to mass-47 abundances were made by calculating \(^{12}\text{C}^{18}\text{O}^{17}\text{O} \) abundances using \(^{12}\text{C}^{18}\text{O}^{18}\text{O} \) and \(^{12}\text{C}^{18}\text{O}^{16}\text{O} \) abundances in Equation 4.2. This allows for comparison directly with experimental evaluations of \( \Delta_{47} \) in which \(^{12}\text{C}^{18}\text{O}^{17}\text{O} \) is included. The correction affects the evaluation of equilibrium \( \Delta_{47} \) at 25°C by \( \sim 0.02\%_o \) (Hill et al. 2014).

**Effect of H-Bond Pattern**

The effect of H-bond pattern on isotopic fractionation was evaluated by counting the numbers of different classes of H-bonds in each cluster and regressing these against calculated \( \Delta_{47} \) and \( \Delta_{63} \) values. Four classes of H-bonds were considered: those donated from \( \text{H}_2\text{O} \) to a DIC hydroxyl (\( \text{H}_{\text{wat}}-\text{O}_{\text{ohc}} \)); those donated from a \( \text{H}_2\text{O} \) to an unprotonated DIC oxygen, such as those participating in the delocalized \( \pi \) bond on \( \text{HCO}_3^- (\text{H}_{\text{wat}}-\text{O}_{\text{ohc}}) \); those donated from a DIC hydroxyl to \( \text{H}_2\text{O} \) (\( \text{H}_{\text{ohc}}-\text{O}_{\text{wat}} \)); and those donated from \( \text{H}_2\text{O} \) to \( \text{H}_2\text{O} \) (\( \text{H}_{\text{wat}}-\text{O}_{\text{wat}} \)). Cutoffs of <2.4Å O-H distance and \( >90^\circ \) O-H-O angle were used to count H-bonds. C-O\_attack distance was also included in analyses of \( \Delta_{63} \) of TSs. Best-guess H-bond structures and C-O\_attack distances (Chapter 3, this dissertation) were used to calculate a best-guess value for each \( \Delta \) value at 25°C.
Effect of Temperature

Temperature effects were calculated by adjusting the temperature used in the calculation of $\beta$ (Equation 3.7) and re-performing the multiple linear regression. Best-guess $\Delta$ values were calculated for each degree increment from 0-40°C, and every 5 degrees thereafter until 100°C. A separate single linear regression against $T^{-2}$ was then performed to match the typical form of the equation derived from empirical calibrations of the $\Delta_{47}$-T paleothermometer (e.g. Kelson et al. 2017 and references therein).

Effect of pH

The effect of pH on TS $\Delta_{63}$ was evaluated by calculating the relative abundance of H$_2$O and OH$^-$ available to react during CO$_2$ hydration and hydroxylation, respectively. Concentration of H$_2$O was calculated based on pure water with a density of 1000 g/L, and OH$^-$ concentration was calculated as $10^{\text{pH}-14.0}$. Free energies of activation of 91 kJ/mol and 50 kJ/mol, respectively (Wang et al. 2010) were used in the Arrhenius equation to calculate relative reaction rates at various pH values.

Results

Model Chemistry

Model chemistries used in this study are reported in Table 4-1. The selection method used to pick them is detailed in Chapter 2 of this dissertation. Briefly, those model chemistries were selected which best reproduced measured vibrational frequencies in the CO$_2$-H$_2$O system, as this correlated strongly with the ability to calculate accurate single-isotope fractionation factors.
Model chemistries colored blue and green do the best job replicating these fractionation factors, as discussed in both Chapter 2 and 3 of this dissertation. Thus we expect them to predict Δ values closely as well. Darker colors include slightly larger basis sets which we expect to better describe anion and H-bonding behavior. The model chemistry B3LYP/6-311++G(2d,p) (dark blue) is very similar to the model chemistry used by Hill et al. (2014) and Tripati et al. (2015) to describe equilibrium Δ values in DIC and mineral clusters: B3LYP/6-311++G(2d,2p). Model chemistries in green have a similar density functional (X3LYP vs B3LYP) so we expect their behavior to be similar.

**Bulk Equilibrium Fractionation, 25°C**

Equilibrium clumping compositions averaged using Boltzmann weighting within each cluster size are given in Figures 4-3 to 4-5. CO₂(aq) has a relatively high Δ₁₇ value of 0.9‰ (Figure 4-3) when compared with Δ₁₇ of 0.55-0.77‰ from 0-50°C across acid-digested carbonate mineral samples (Figure 4-2). Within any particular model chemistry, there is ~0.015‰ variability depending on cluster size, but with no consistent trend based on number of H₂O molecules in the cluster. There are consistent differences between model chemistries: those using X3LYP and B3LYP (green and blue) are similar, PBE0 (orange) model chemistries show ~0.025‰ more clumping, and PBEPBE (purple) model chemistries show ~0.04‰ less. Model chemistries with larger basis sets (dark colors) clump ~0.01‰ less than their counterparts.

Equilibrium Δ₆₃ values for H₂CO₃ are ~0.43-0.46‰ (Figure 4-4). The averaged results of a previous set of 10 cluster models of H₂CO₃(aq) are shown with a horizontal black line (Hill et al. 2014). X3LYP and B3LYP models are usually within ~0.02‰ of this prediction The same model chemistry relationships that are true for clumping in CO₂(aq) also hold for Δ₆₃ values of
equilibrium H₂CO₃. Total variability across all models is ~0.08‰, and variability within each model chemistry is ~0.025‰.

Equilibrium Δ⁶³ values for HCO₃⁻ are 0.405-0.43‰ (Figure 4-5). Previous model results (Hill et al. 2014) for HCO₃⁻ (aq) are shown in black, which are ~0.005-0.025‰ lower than these results. The Δ⁶³ of HCO₃⁻ is ~0.02‰ lower than that of H₂CO₃. Model chemistries vary systematically in the same way as equilibrium CO₂(aq) and H₂CO₃. Total variability across all models is ~0.08‰, and variability within each model chemistry is ~0.02‰.

Bulk-averaged kinetic clumping compositions are shown in Figures 4-6 and 4-7. In the CO₂ hydration reaction (Figure 4-6), Δ⁶³ values range from 0.4-0.5‰ with no apparent systematic variation by model chemistry. Equilibrium, not kinetic, fractionation estimates for H₂CO₃ are shown in black (Hill et al. 2014). Kinetic compositions generally, but not always, show more clumping than the equilibrium fractionation line, which makes it difficult to determine using Boltzmann-averaged bulk models whether kinetic fractionation in the CO₂ hydration reaction would produce DIC with more or less clumping than equilibrium DIC.

In the CO₂ hydroxylation reaction (Figure 4-7), Δ⁶³ values range from 0.44-0.57‰ with no apparent systematic variation by model chemistry. Equilibrium, not kinetic, fractionation estimates for HCO₃⁻ are shown in black (Hill et al. 2014). Kinetic fractionations in the CO₂ hydroxylation reaction always, show more clumping than the equilibrium fractionation line, but the magnitude of extra clumping is uncertain using Boltzmann-averaged models.

Regressions to Quantify Local Environment Effects

Multiple linear regression can be used to pick out the incremental effects of various local environment effects, such as H-bond pattern, on fractionation magnitude in single-isotope systems (Chapter 3, this dissertation). The large amount of variation between equilibrium and
especially kinetic fractionation model results could potentially be reduced by evaluation of local chemical effects in a similar manner.

An example scatterplot demonstrating the effect of H-bonding to the lone O on equilibrium $\Delta_{63}$ values of $\text{H}_2\text{CO}_3$ is shown in Figure 4-8. There is a small but discernible decline in $\Delta_{63}$ values from the addition of each H-bond to the lone, double-bonded O on $\text{H}_2\text{CO}_3$ (noted $\text{H}_{\text{wat}}\text{-O}_{\text{oc}}$). Plots of neither H-bonds from $\text{H}_2\text{O}$ to $\text{H}_2\text{CO}_3$ hydroxyls ($\text{H}_{\text{wat}}\text{-O}_{\text{ohc}}$), nor H-bonds from $\text{H}_2\text{CO}_3$ hydroxyls to $\text{H}_2\text{O}$ ($\text{H}_{\text{ohc}}\text{-O}_{\text{wat}}$), showed a discernible trend.

Table 4-2 displays the results of a regression of H-bond counts vs. $\text{H}_2\text{CO}_3$ $\Delta_{63}$ across all cluster sizes for each model chemistry. H-bonds of all types, both to and from $\text{H}_2\text{CO}_3$, decrease the degree of clumping, despite the difficulty in discerning trends from the individual scatterplots. $\text{H}_{\text{wat}}\text{-O}_{\text{oc}}$ H-bonds show the largest decrease in clumping, but $\text{H}_{\text{wat}}\text{-O}_{\text{ohc}}$ H-bonds also display a significant decline in clumping. $\text{H}_{\text{ohc}}\text{-O}_{\text{wat}}$ H-bonds also decrease clumping but the effect is not always significant at the $P<0.05$ level. The coefficients of determination ($R^2$) indicate that counting H-bonds can describe ~80% of the variability in $\Delta_{63}$, leaving an average residual of magnitude 0.002-0.003‰. The best estimate of equilibrium $\Delta_{63}$ derived from the regression is 0.436-0.443‰, slightly higher than the Hill et al. (2014) value of 0.4344‰.

H-bonds from $\text{H}_2\text{O}$ to $\text{HCO}_3^-$ lone O atoms ($\text{H}_{\text{wat}}\text{-O}_{\text{oc}}$) also decrease clumping consistently (Figure 4-9). An $\text{H}_{\text{ohc}}\text{-O}_{\text{wat}}$ from $\text{HCO}_3^-$ also decreases equilibrium $\Delta_{63}$ values (Figure 4-10). As with $\text{H}_2\text{CO}_3$, all H-bonds involving $\text{HCO}_3^-$ decrease equilibrium $\Delta_{63}$ values, but the magnitude of the effect by each type of H-bond is different (Table 4-3). For $\text{HCO}_3^-$ $\text{H}_{\text{ohc}}\text{-O}_{\text{wat}}$ have the largest effect, followed by $\text{H}_{\text{wat}}\text{-O}_{\text{oc}}$, followed by $\text{H}_{\text{wat}}\text{-O}_{\text{ohc}}$ which are not always statistically significant. H-bond regression describes 70-80% of all variability in equilibrium $\text{HCO}_3^-$ $\Delta_{63}$ values, leaving an average residual magnitude of 0.002-0.003‰. The Hill et al. (2014) model result of 0.4033 is  

on the lower end of the best-estimate range of 0.403-0.411 when including preferred model chemistries.
Bulk-averaged values for kinetic fractionation in Δ₆₃ exhibited a much larger range within each model chemistry than did equilibrium Δ₆₃ values. Regressions against H-bond type remove some of that variability, but description of the distance between C on CO₂ and the attacking O, whether from H₂O in the hydration reaction or OH⁻ in the hydroxylation reaction, significantly improves the regression fit. Kinetic clumping in the hydration reaction appears to be slightly increased by H-bonds from water to the attacking H₂O (noted in kinetic fractionation calculations as Hwat-Oohc) (Figure 4-11). Hwat-Oohc also cause a decline in clumping in the CO₂ hydration reaction (Figure 4-12). There is a very strong increase in clumping the further the H₂O is from CO₂ at the TS (Figure 4-13).

The complete regressions (Table 4-4) demonstrate that, despite the appearance of the Hwat-Oohc scatterplot (Figure 4-11), H-bonds to the attacking H₂O actually decrease clumping in most model chemistries. Hohc-Owat H-bonds increase clumping in some cases. The majority of the variability is accounted for by the C-Oattack distance; when it is included, the regression accounts for ~80% of the variability. However, when C-Oattack is excluded from the regression <10% of the variability is accounted for. The complete regression leaves residuals of 0.006-0.008‰ on average.

The best estimate values at 25°C for Δ₆₃ in the hydration reaction are 0.460-0.470‰, clearly higher than the equilibrium values of 0.436-0.443‰ (Table 4-2). The regression values much more clearly show that kinetic fractionation in Δ₆₃ is higher than equilibrium Δ₆₃, whereas the bulk-averaged model was unclear. The best-estimate for C-Oattack distance in this study is placed at 1.72Å, equal to the distance in a well-solvated 25- H₂O cluster (Figure 3-3a). An error of 0.1Å in this estimate would impart additional deviation of ~0.014‰ in the best-estimate result.

Kinetic fractionation in Δ₆₃ for the hydroxylation reaction appears negligibly impacted by H-bonds to the attacking OH⁻ (Hwat-Oohc) (Figure 4-14). Clumping is again strongly increased the further away the attacking OH⁻ is at the TS (Figure 4-15). The complete regression (Table 4-5)
shows that hydroxylation clumping is strongly increased by H$_{\text{wat}}$-O$_{\text{ohc}}$ despite the scatterplot appearance. The full regression explains 95% or more of the variability in hydroxylation $\Delta_{63}$, with leftover residuals of 0.002-0.003‰. Without including C-O$_{\text{attack}}$ distance, the regression explains only ~50% of the variability. The best estimate of 0.486-0.496‰ means clumping in HCO$_3^-$ which experienced only kinetic fractionation is clearly higher than in any other DIC species apart from $\Delta_{47}$ in CO$_2$. The C-O$_{\text{attack}}$ distance is set at 2.12Å, taken from a well-solvated cluster (Figure 3-4a); an error of 0.1Å would impart additional 0.016‰ deviation.

**Temperature Dependence**

The temperature dependences for equilibrium $\Delta_{63}$ values in DIC species are shown for H$_2$CO$_3$ in Figure 4-16 and for HCO$_3^-$ in Figure 4-17. H$_2$CO$_3$ declines from 0.52‰ at 0°C to 0.28‰ at 100°C. HCO$_3^-$ declines from 0.48‰ at 0°C to 0.25‰ at 100°C. Both DIC species display roughly the same temperature dependence, and both display the expected decrease in clumping magnitude at high temperatures. Both curves match well with previous calculations, albeit with slightly more clumping at intermediate temperatures (Hill et al. 2014).

Kinetic T dependence for clumping during CO$_2$ hydration (Figure 4-18) and hydroxylation (Figure 4-19) are roughly equivalent to the equilibrium T dependence values with constant offsets. Hydration varies from 0.54‰ at 0°C to 0.31‰ at 100°C, and hydroxylation varies from 0.57‰ at 0°C to 0.34‰ at 100°C. Notably, hydroxylation $\Delta_{63}$ is higher than hydration $\Delta_{63}$, while equilibrium HCO$_3^-$ $\Delta_{63}$ is lower than H$_2$CO$_3$ equilibrium.
Effect of pH

As pH rises, the contribution of HCO$_3^-$ to the total DIC pool decreases, while the contribution of CO$_3^{2-}$ increases; this results in a decrease in aggregate DIC $\Delta_6$ because CO$_3^{2-}$ exhibits less equilibrium clumping than HCO$_3^-$ (Hill et al. 2014). In a similar fashion, the amount DIC produced by CO$_2$ hydration decreases, while the amount produced by CO$_2$ hydroxylation increases, as pH is increased, based on the relative abundances of H$_2$O and OH$^-$ (Figure 4-20). Applying this relationship to a pool of DIC experiencing only kinetic fractionation yields an estimate of the clumping in excess of what would be expected at equilibrium (Figure 4-21). At low pH, pure kinetic fractionation would produce DIC with $\sim 0.06\%$ more clumping than equilibrium HCO$_3^-$. The kinetic excess clumping increases steeply from pH 7-10 until it reaches a value of $\sim 0.08\%$. Higher temperatures decrease the magnitude of kinetic excess clumping only slightly. Above pH 10, most DIC exists as CO$_3^{2-}$ in solution, which has an equilibrium clumping 0.03$\%$ lower than HCO$_3^-$ (Hill et al. 2014), so the kinetic excess relative to the full DIC pool would be $\sim 0.11\%$ above pH 10. There is a strong impact of pH on the temperature-dependent behavior of the kinetic excess clumping in the form of a constant offset (Figure 4-22).

Comparison to Experimental Regressions

Tables 4-6 to 4-9 compare the temperature regressions of these model results against an experimental regression of various solid carbonates (Kelson et al. 2017) and prior equilibrium DIC calculations (Hill et al. 2014). Comparisons are also summarized graphically for the B3LYP/6-311++G(2d,p) model chemistry in Figure 4-23.

Equilibrium H$_2$CO$_3$ models (Table 4-6) using preferred model chemistries have regression slopes of 0.0400-0.0404$\%$ against (1000/T)$^2$, similar to the temperature dependence of
Hill et al. (2014). The slope is slightly shallower than that of carbonate minerals and just outside the measurement variability, although it would be difficult to distinguish solely based on slope. The regression high-temperature intercept of 0.205-0.208‰ is ~0.06‰ higher than that found in experimental carbonates.

Kinetic CO$_2$ hydration models (Table 4-7) using B3LYP and X3LYP model chemistries have regression slopes of 0.0388-0.0394‰ against (1000/T)$^2$, shallower than the equilibrium H$_2$CO$_3$ temperature dependence and the experimental mineral calibration line. The high-temperature intercepts are also ~0.1‰ higher than equilibrium carbonate minerals, indicating that carbonates formed under conditions of CO$_2$ hydration disequilibrium conditions could be distinguished from equilibrated carbonates.

Equilibrium HCO$_3^-$ models (Table 4-8) using B3LYP and X3LYP model chemistries have regression slopes of 0.0381-0.0386‰ against (1000/T)$^2$, very similar to the temperature dependence of Hill et al. (2014). The slope is slightly shallower than that of carbonate minerals and offset by ~0.05‰. The regression high-temperature intercept of 0.205-0.208‰ is ~0.06‰ higher than that found in experimental carbonates.

Kinetic CO$_2$ hydroxylation models (Table 4-9) using preferred model chemistries have regression slopes of 0.0386-0.0395‰ against (1000/T)$^2$, nearly identical to kinetic fractionation during CO$_2$ hydration. The slope is also shallower than the experimental mineral calibration line. The high-temperature intercepts are also ~0.12‰ higher than equilibrium carbonate minerals, indicating that carbonates formed under conditions of CO$_2$ hydroxylation disequilibrium conditions could be distinguished from equilibrated carbonates.
Discussion

Model Chemistries and Bulk Averages

PBE0 (orange) model chemistries universally predict more clumping than more accurate model chemistries, and PBEPBE (purple) model chemistries universally underpredict clumping. In the bulk-averaged equilibrium models, this translates to a range of 0.08‰ in predictions, and >0.1‰ range in predictions for kinetic clumping. These ranges correspond to a total temperature prediction range >20°C, or >10°C in either direction. This is the uncertainty range on a set of computational chemistry predictions which have not used a procedure to select the most accurate model chemistry for a given situation (Chapter 2, this dissertation).

Even if accurate model chemistries are used, equilibrium Δ\text{63} values can have ranges on the order of 0.025‰, imparting a 6-7°C temperature uncertainty. For example, this study’s bulk averages of equilibrium HCO\text{3}\text− clumping differ from those of a previous study (Hill et al. 2014) by ~0.15‰ corresponding to a 4-5°C difference in temperature prediction using carbonates precipitated quickly from a low-pH DIC pool. In addition, kinetic fractionations do not appear to display systematic variation that might reduce their error by much relative to the ~0.1‰ spread observed here, to the point that they leave uncertainty in whether kinetic fractionation causes increases or decreases in clumping relative to equilibrium DIC. Such results might be useful in a qualitative sense, but the promise of computational chemistry is in providing much more accurate estimates for systems that are not easily investigated by experiment.

H-Bonding and TS Structure

One important lesson from multiple studies is that explicit solvation is necessary when performing isotope fractionation calculations in systems experiencing strong H-bonding (Kubicki
Explicit solvation involves the use of individual H$_2$O molecules instead of describing water using a simpler polarizable continuum approximation. This chapter demonstrates that H-bonds have direct impacts on the degree of clumping in DIC species, and the previous chapter describes their impact on standard isotope fractionation factors, so their presence is necessary in describing the DIC system.

The equilibrium average residuals of 0.002-0.003‰ in this study correspond to errors of <1°C in temperature interpretation, making them quantitatively useful in the study of H$_2$CO$_3$ which is difficult to isolate from solution, and in prediction of kinetic fractionation factors without back-reaction causing partial equilibration in a precipitation experiment. This is only possible by modeling the H-bonding environment, and separating H-bonds by type. The regressions make clear that different types of H-bonds have different effects on clumping in different situations.

TS calculations are also sensitive to H-bond pattern, in addition to sensitivity to the TS’s precise position along the reaction coordinate. The hydration reaction in particular requires an accurate description of the C-O$_{\text{attack}}$ distance. Both reactions would have additional deviations of ~0.015‰ for each 0.1Å difference, which is fairly large. The fact that kinetic clumping excess is 0.06‰ at low pH and 0.08‰ at high pH means that even with an error of >0.2Å, the kinetic fractionation would still result in significantly elevated $\Delta_{63}$ values in the DIC. Errors in H-bond count could also affect $\Delta_{63}$ by 0.01‰ or more, so future studies would benefit from a very careful evaluation of the TS’s structure.

Implicit solvation might be useful in addition to including explicit H$_2$O molecules, but only in conjunction with explicit solvation. Rustad et al. (2008) found that solely including implicit solvation models like PCM made fractionation calculation results less accurate than corresponding gas-phase calculations in the DIC system, and in fact predicted the wrong direction of fractionation between CO$_2$(aq) and HCO$_3^-$/$CO_3^{2-}$. When implicit solvation was used in
conjunction with a first hydration shell, corrections to reduced partition function ratios were small relative to the first hydration shell models without implicit solvation. Additionally, the direction of the correction was opposite that introduced by an explicit second shell of solvation. From this limited information, it seems that implicit solvation may reduce the accuracy of isotope fractionation calculations even when used in conjunction with explicit solvation.

**Kinetic Fractionations Cause Increases in Clumping**

Typically, kinetic fraction factors in standard isotope systems are <1 (e.g. Chapter 3, this dissertation), implying creation of isotopically light product. This is because light isotopes react more quickly due to their lower mass. It might be reasonable to expect that the same effect would promote formation of more product with single isotope substitutions or no isotope substitutions instead of clumped isotopologues, resulting in a product with less clumping than when fractionated at equilibrium. However, in the DIC system, CO$_2$ hydration and hydroxylation produce clumping by kinetic fractionation greater than the equilibrium fractionation in the product.

The causes of this are twofold: first, the nature of the equilibrium is fundamentally different when dealing with isotopic clumping; and second, the reactant CO$_2$ has a very high $\Delta_{47}$ at equilibrium. Because isotopic clumping equilibrium deals with internal equilibrium within a chemical species, it is agnostic as to the reactants and reactions that produce the chemical species. So whatever initial isotopic clumping is imparted on the product H$_2$CO$_3$ or HCO$_3^-$ in this case, equilibration over time will shift the $\Delta_{63}$ to values that reflect the properties of the H$_2$CO$_3$ or HCO$_3^-$ itself. The forward and reverse reactions involving exchange with CO$_2$ and H$_2$O/OH$^-$ never come into play when equilibrium clumping is considered.
Also, the $\Delta_{47}$ of equilibrium CO$_2$ is $\sim0.9\%$. The lighter, non-clumped species can still be rightly expected to react faster, and the H$_2$O or OH$^-$ containing $^{16}$O can be expected to react faster. But that just means the kinetic fractionation should produce DIC with a $\Delta_{63}$ of less than 0.9%. A regime could exist in which the reactant CO$_2$ were not at internal clumping equilibrium, perhaps due to fractionation caused by respiration producing preferentially light $^{12}$CO$_2$ without time to isotopically equilibrate with the H$_2$O. As the $\Delta_{47}$ of the CO$_2$ falls, so would the $\Delta_{63}$ of the first DIC produced. Falk and Guo (2014) reported the inheritance of the original CO$_2$ $\Delta_{47}$ value by rapidly-precipitated carbonates at high pH; our results don’t produce $\Delta_{63}$ values as high as the CO$_2$ $\Delta_{47}$, and in fact they are not able to, but they do record the signature of the original CO$_2$ as modified by kinetic fractionation.

**Mechanism of H-Bond Effects on Traditional Stable Isotopes and Clumping**

The typical explanation for accumulation of both heavy isotopes and clumped isotopes is concentration in strong, stiff bonds with high vibrational frequencies. This trend holds in most singly-substituted compounds at thermodynamic equilibrium (Schauble 2004) and in HCO$_3^-$, CO$_3^{2-}$, CO$_2$, and CO when considering C-O clumping (Schauble et al. 2006). Intermolecular interactions which affect bond stiffness and bond order would thus be expected to affect both equilibrium heavy isotope and clumped isotope concentration in the same direction.

However, these results do not completely follow the expected pattern based on the expected effects of H-bonding. At equilibrium, isotopic clumping is always decreased, but heavy isotope concentration is sometimes increased and sometimes decreased depending on the type of H-bond. Because H-bond donation weakens and elongates both the donor covalent O-H bond and covalent bonds to the acceptor O (Grabowski 2001, Xu and Goddard 2004, Garand et al. 2009, Santra 2010), all types of H-bonds would be expected to decrease heavy isotope concentration in
addition to clumping. Instead, only H-bonds from H$_2$O to the OH group on DIC species decrease $^{13}$C and $^{18}$O concentration.

Spectroscopic studies can help rationalize why heavy isotopes may concentrate in distant covalent bonds due to H-bonding, but they cannot then rationalize why clumping would then simultaneously decrease. Garand et al. (2009) found that H-bond donation from water to bicarbonate carbonyl O in small molecular clusters substantially increases the measured frequency of the distant C-OH bond stretch, indicating stiffening of the C-OH bond. H-bond donation from bicarbonate to water had a similar effect on the C-OH bond stretch in modeled spectra. Single isotope fraction can then be rationalized by the proportionally large effects on C-OH stretch; H-bonds that weaken the C-OH bond (H-bond donation from water to OH) cause decreases in $^{13}$C and $^{18}$O concentration, while H-bonds that weaken distant bonds but strengthen the C-OH bond (H-bond donation from water to carbonyl, H-bond donation from hydroxyl to water) cause increases in $^{13}$C and $^{18}$O concentration. It is unclear why this same effect wouldn't increase equilibrium clumping in those instances as well.

Four empirical rules emerge from these model results on the effect of H-bonds on both equilibrium single-isotope fractionation and equilibrium $^{13}$C-$^{18}$O clumping in DIC species:

1) Hydrogen bonds always decrease clumping in DIC species.

2) Hydrogen bonds affect single-isotope fractionation of both $^{13}$C and $^{18}$O in the same direction for all H-bond types, i.e. a hydrogen bond which increases $\delta^{13}$C will also increase $\delta^{18}$O and vice versa.

3) Magnitudes of H-bond effects on $\delta^{13}$C are about twice as large as those on $\delta^{18}$O, with some variation.

4) Hydrogen bonds that decrease C-OH bond stiffness will decrease $\delta^{13}$C and $\delta^{18}$O. Hydrogen bonds that increase C-OH bond stiffness by decreasing the stiffness of nearby C=O or O-H bonds will increase $\delta^{13}$C and $\delta^{18}$O.
Hydrogen bonds involving transition states can deviate from this pattern, with H-bonds to an attacking OH increasing clumping in the hydroxylation transition state, and H-bonds involving the CO₂ moiety never having a statistically-significant effect on single isotopes. When H-bonds lack a C-OH bond to affect, the pattern seen in equilibrium DIC breaks down.

**Temperature and pH Effects**

The temperature response of Δ₁₈⁴ is roughly the same for CO₂ hydration and hydroxylation kinetic fractionation as well as HCO₃⁻ and CO₃²⁻ (Hill et al. 2014) equilibrium. In the absence of other effects, each of these four impacts would cause relatively constant offsets in Δ₁₇ regardless of temperature. Unfortunately, partial equilibration and variable pH mean these four effects always depend on one another.

Although offsets between equilibrium and kinetic fractionation are roughly independent of temperature, the amount of kinetic clumping occurring at low temperatures could be expected to be greater than at high temperatures, because low temperatures slow the rate of equilibration. Thus offsets toward higher Δ₁₇ in carbonates that are larger in magnitude at lower temperature could be explained by kinetic fractionation in the CO₂ hydration and hydroxylation reactions. This pattern is widely seen in shallow-water corals (Ghosh et al. 2006; Saenger et al. 2012; Saenger and Erez 2016; Spooner et al. 2016; Saenger et al. 2017). In addition, the magnitude of the offset in shallow water corals is up to ~0.05‰; this value is only achievable by equilibrated HCO₃⁻ if the pH is relatively low (<8.5 in saline water, Hill et al. 2014). Otherwise, the presence of CO₃²⁻ would lower the Δ₁₇ offset below 0.05‰. Offsets observed in carbonates rapidly precipitated at pH >9 have delta offsets up to 0.06‰ (Schmid 2011), which match the results of kinetic clumping better than equilibrium clumping within DIC that is at disequilibrium with the growing carbonate mineral.
Kinetic and equilibrium DIC fractionation cause offsets in opposite directions at high pH if the carbonate mineral grows too fast to equilibrate with the solution. At high pH, CO$_2$ hydroxylation dominates, generating an offset at most 0.12‰ above the mineral calibration line if precipitation is fast. In contrast, if the CO$_2$ hydroxylation reaction is allowed to come to equilibrium at high pH, the main DIC species will be CO$_3^{2-}$, which has a negative offset up to 0.025‰ below the calcite equilibrium line (Hill et al. 2014). As pH changes, these systems also experience opposite effects, with $\Delta$$_{63}$ decreasing when kinetic fractionation dominates but increasing if equilibrium DIC dominates. These are fundamentally different responses that can also be used to separate McConnaughey’s kinetic model from Adkins’ carbonate model (Chapter 1, this dissertation). Different classes of corals show each of these responses (Spooner et al. 2016), so it is likely that each model fits a subset of corals.

**Physiological Effects**

If carbonic anhydrase can interact with the DIC pool from which carbonate minerals precipitate, it could change some of these results. The enzyme would more quickly generate DIC with equilibrium $\Delta$$_{47}$ signatures. Even if equilibrium were not achieved, the different chemical environment experienced by the CO$_2$ during catalysis would change the kinetic offset magnitude.

Photosynthetic symbionts preferentially use $^{12}$CO$_2$ and leave behind $^{13}$CO$_2$ (Saenger and Erez 2016). Respiration creates $^{12}$CO$_2$ because organic matter tends to be enriched in $^{12}$C. Both possibilities potentially create a pool of CO$_2$ that is not isotopically equilibrated either internally or with H$_2$O. Both equilibration paths are accomplished in aqueous solution via CO$_2$ hydration and hydroxylation (Affek 2012; 2013). If equilibration is not achieved, the observed magnitude of kinetic clumping enhancement would be different based on the isotope clumping in the disequilibrium CO$_2$ pool.
Salinity Effects

Salinity is known to have impacts on isotope fractionation in some aqueous systems (see discussion in Chapter 3, this dissertation). Salinity may impact isotope fractionation by altering the H-bond pattern in solution or by fractionation between free species and contact ion pairs. This chapter addresses what the effects of H-bonding on isotope fractionation might be as a result of salinity changes, but not contact ion pair effects.

Little experimental work has been done to evaluate the impact of salinity on $\Delta_{47}$ of carbonates. Kluge and John (2015) found cation-specific differences in salinity’s effect on clumping, much like the cation-specific impacts on traditional stable isotope fractionation (e.g. Horita et al. 1993). Increasing concentrations of CaCl$_2$ increased $\Delta_{47}$ in synthetic calcium carbonates by up to 0.03‰, while increasing concentrations of MgCl$_2$ and NaCl had no effect on either calcium or magnesium carbonates. In the context of this dissertation, the difference in effect between Ca$^{2+}$ and Mg$^{2+}$ is surprising, because they have similar effects on H-bond structure in solution; both ions decrease the amount of H-bonds accepted by their hydration shell (Suresh et al. 2012). If H-bond donation from DIC species was eliminated by increasing Ca$^{2+}$ concentration, the effect would only amount to a ~0.006‰ increase in $\Delta_{47}$ value, not nearly enough to explain the results of Kluge and John (2015). More specific modeling of contact ion pair effects and effects occurring at the growing mineral surface might reveal why clumping increases in concentrated CaCl$_2$ solutions only.

Summary

Evaluation of isotope clumping effects in the DIC system benefits from evaluation of the local H-bonding environment’s impact, and the C-O$_{\text{attack}}$ distance in the case of kinetic
fractionation. H-bonds do not affect traditional stable isotope compositions and isotope clumping in the same manner; in DIC species, equilibrium clumped isotopes are always decreased by H-bonds, while only H-bonds that weaken the C-OH bonds decrease equilibrium $\delta^{13}C$ and $\delta^{18}O$. The magnitude of kinetic clumping enhancement from the CO$_2$ hydration and hydroxylation pathway approaches 0.1‰ and 0.12‰ respectively above the experimental calibration line. The $\Delta_{47}$ values of shallow-water corals and their temperature dependence are broadly consistent with the kinetic model of vital effects.
Figure 4-1. Isotopologues of H$_2$CO$_3$ including stable isotopes $^1$H, $^{12}$C, $^{13}$C, $^{16}$O, and $^{18}$O ($^2$H and $^{17}$O omitted). Black: C, red: O, white: H. Light isotopes are depicted as small spheres; heavy isotopes are depicted as large spheres. Rows 1 and 2 are unsubstituted or singly-substituted isotopologues; all others are clumped isotopologues. Calculation of $\Delta_{63}$ involves analysis of carbonate including one $^{13}$C and one $^{18}$O, shown in the fourth row; these are the most common clumped isotopologues of carbonate.
Figure 4-2. $\Delta_{17}$ of biogenic and abiogenic carbonates versus growth temperature. Shallow-water corals represented by green circles, deep-water corals represented by blue circles, and other carbonates represented by gray X’s. Figure adapted from Saenger and Érez (2016), data from Zaarur et al. (2013) and references therein.
Table 4-1. Model chemistries used in this study. Colors are used throughout graphs in this chapter to identify the model chemistry when all are graphed at once. Lighter and darker shades of the same color use identical exchange-correlation functionals; darker colors use slightly larger basis sets. Vibration RMS error values and deviations in fractionation factor $^{18/16} \alpha_{eq}(\text{CO}_2-\text{H}_2\text{O})$ are taken from Chapter 2, this dissertation. Blue and green model chemistries exhibit less error in both values than purple and orange model chemistries. Table identical to Table 2-1.

<table>
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<tr>
<th>Model Chemistries</th>
<th>Abbreviation</th>
<th>$\text{CO}_2-\text{H}_2\text{O}$ Vibration RMS Error</th>
<th>$^{18/16} \alpha_{eq}(\text{CO}_2-\text{H}_2\text{O})$ Deviation (%)</th>
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Figure 4-3. CO₂ Δ₁⁷ at 25°C in clusters versus number of H₂O molecules excluding hydroxide ions. Colors represent different model chemistries (Table 4-1). Multiple (≥7) clusters averaged using Boltzmann weighting of the cluster by electronic and ZPEs plus thermal contributions. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting).
Figure 4-4. Equilibrium $\Delta_{63}$ of H$_2$CO$_3$ at 25°C in clusters versus number of H$_2$O molecules excluding hydroxide ions. Colors represent different model chemistries (Table 4-1). Multiple ($\geq 7$) clusters averaged using Boltzmann weighting of the cluster by electronic and ZPEs plus thermal contributions. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Black line reflects equilibrium $\Delta_{63}$ of H$_2$CO$_3$ calculated by Hill et al. (2014).
Figure 4-5. Equilibrium $\Delta_{63}$ of $\text{HCO}_3^-$ at 25°C in clusters versus number of H$_2$O molecules excluding hydroxide ions. Colors represent different model chemistries (Table 4-1). Multiple ($\geq$7) clusters averaged using Boltzmann weighting of the cluster by electronic and ZPEs plus thermal contributions. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Black line reflects equilibrium $\Delta_{63}$ of $\text{HCO}_3^-$ calculated by Hill et al. (2014).
Figure 4-6. DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydration reaction at 25°C in clusters versus number of H$_2$O molecules excluding hydroxide ions. Colors represent different model chemistries (Table 4-1). Multiple ($\geq$7) clusters averaged using Boltzmann weighting of the cluster by electronic and ZPEs plus thermal contributions. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Black line reflects equilibrium, rather than kinetic, $\Delta_{63}$ of H$_2$CO$_3$ calculated by Hill et al. (2014).
Figure 4-7. DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydroxylation reaction at 25°C in clusters versus number of H$_2$O molecules excluding hydroxide ions. Colors represent different model chemistries (Table 4-1). Multiple ($\geq$7) clusters averaged using Boltzmann weighting of the cluster by electronic and ZPEs plus thermal contributions. Error bars reflect 1 weighted standard deviation (using Boltzmann weighting). Black line at the bottom of the figure reflects equilibrium, rather than kinetic, $\Delta_{63}$ of HCO$_3^-$ calculated by Hill et al. (2014).
Figure 4-8. Equilibrium $\Delta_{63}$ of $\text{H}_2\text{CO}_3$ at 25°C versus number of H-bonds from $\text{H}_2\text{O}$ to a DIC lone oxygen. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium $\Delta_{63}$ of $\text{H}_2\text{CO}_3$ calculated by Hill et al. (2014).
Table 4-2. Multiple linear regression of H-bond effects on equilibrium Δ_{63} of H$_2$CO$_3$ at 25°C by model chemistry. Regression coefficients and constants are colored green if significant at the P<0.05 level. Coefficients of determination (R$^2$) represent the proportion of variability across cluster calculations that is accounted for by the H-bond effects. Average residuals and number of clusters included are also shown. The best-guess value reflects the value of Δ$_{63}$ under the most reasonable H-bonding pattern for H$_2$CO$_3$: 2 H$_{ohc}$-O$_{wat}$, 1 H$_{wat}$-O$_{ohc}$, and 1.5 H$_{wat}$-O$_{oc}$ (Chapter 3, this dissertation).

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<th>H$<em>{wat}$ - O$</em>{ohc}$</th>
<th>H$<em>{ohc}$ - O$</em>{oc}$</th>
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<th>R$^2$</th>
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Figure 4-9. Equilibrium $\Delta_{63}$ of $\text{HCO}_3^-$ at 25°C versus number of H-bonds from $\text{H}_2\text{O}$ to a DIC lone oxygen. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium $\Delta_{63}$ of $\text{HCO}_3^-$ calculated by Hill et al. (2014).
Figure 4-10. Equilibrium $\Delta_{63}$ of HCO$_3^-$ at 25°C versus number of H-bonds from the hydroxyl group hydrogen to a H$_2$O oxygen. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium $\Delta_{63}$ of HCO$_3^-$ calculated by Hill et al. (2014).
Table 4-3. Multiple linear regression of H-bond effects on equilibrium $\Delta_{63}$ of HCO$_3^-$ at 25°C by model chemistry. Regression coefficients and constants are colored green if significant at the P<0.05 level. Coefficients of determination ($R^2$) represent the proportion of variability across cluster calculations that is accounted for by the H-bond effects. Average residuals and number of clusters included are also shown. The best-guess value reflects the value of $\Delta_{63}$ under the most reasonable H-bonding pattern for HCO$_3^-$: 1 H$_{\text{ohc}}$-O$_{\text{wat}}$, 0.5 H$_{\text{wat}}$-O$_{\text{ohc}}$, and 5 H$_{\text{wat}}$-O$_{\text{oc}}$ (Chapter 3, this dissertation).

<table>
<thead>
<tr>
<th>Model/Chem and Color</th>
<th>$H_{\text{ohc}}$ - O$_{\text{ohc}}$</th>
<th>$H_{\text{ohc}}$ - O$_{\text{oc}}$</th>
<th>$H_{\text{ohc}}$ - O$_{\text{wat}}$</th>
<th>Constant</th>
<th>$R^2$</th>
<th>Avg. Resid.</th>
<th>N</th>
<th>Best-guess value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBE0/6-311++G(d,p)</td>
<td>0.0046</td>
<td>-0.0049</td>
<td>-0.0089</td>
<td>0.4085</td>
<td>0.66</td>
<td>0.0041%</td>
<td>57</td>
<td>0.2752</td>
</tr>
<tr>
<td>PBE0/6-311+G(d,p)</td>
<td>-0.0010</td>
<td>-0.0029</td>
<td>-0.0068</td>
<td>0.4581</td>
<td>0.78</td>
<td>0.0021%</td>
<td>52</td>
<td>0.4303</td>
</tr>
<tr>
<td>X3LYP/6-311+G(d,p)</td>
<td>0.0022</td>
<td>-0.0042</td>
<td>-0.0059</td>
<td>0.4378</td>
<td>0.76</td>
<td>0.0024%</td>
<td>50</td>
<td>0.4111</td>
</tr>
<tr>
<td>P3LYP/6-311+G(d,p)</td>
<td>0.0031</td>
<td>-0.0043</td>
<td>-0.0093</td>
<td>0.4008</td>
<td>0.65</td>
<td>0.0039%</td>
<td>56</td>
<td>0.3714</td>
</tr>
<tr>
<td>PBE0/6-311++G(d,p)</td>
<td>-0.0004</td>
<td>-0.0028</td>
<td>-0.0076</td>
<td>0.4532</td>
<td>0.81</td>
<td>0.0022%</td>
<td>39</td>
<td>0.4304</td>
</tr>
<tr>
<td>X3LYP/6-311+G(d,p)</td>
<td>0.0008</td>
<td>-0.0034</td>
<td>-0.0064</td>
<td>0.4302</td>
<td>0.76</td>
<td>0.0026%</td>
<td>39</td>
<td>0.4071</td>
</tr>
<tr>
<td>B3LYP/6-311+G(d,p)</td>
<td>0.0011</td>
<td>-0.0040</td>
<td>-0.0062</td>
<td>0.4330</td>
<td>0.76</td>
<td>0.0024%</td>
<td>51</td>
<td>0.4075</td>
</tr>
<tr>
<td>B3LYP/6-311++G(d,p)</td>
<td>0.0012</td>
<td>-0.0035</td>
<td>-0.0064</td>
<td>0.4267</td>
<td>0.73</td>
<td>0.0028%</td>
<td>40</td>
<td>0.4034</td>
</tr>
</tbody>
</table>
Figure 4-11. DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydration reaction at 25$^\circ$C versus number of H-bonds from another H$_2$O molecule to the attacking H$_2$O molecule. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium, rather than kinetic, $\Delta_{63}$ of H$_2$CO$_3$ calculated by Hill et al. (2014).
Figure 4-12. DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydration reaction at 25°C versus number of H-bonds from H$_2$O to a DIC lone oxygen. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium, rather than kinetic, $\Delta_{63}$ of H$_2$CO$_3$ calculated by Hill et al. (2014).
Figure 4-13. DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydration reaction at 25°C versus distance from C to the attacking H$_2$O O measured in Å. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium, rather than kinetic, $\Delta_{63}$ of H$_2$CO$_3$ calculated by Hill et al. (2014).
Table 4-4. Multiple linear regression of H-bond effects and C-O attack distance in Å on DIC Δ\textsubscript{63} produced by kinetic fractionation in the CO\textsubscript{2} hydration reaction at 25°C by model chemistry. Regression coefficients and constants are colored green if significant at the P<0.05 level. Coefficients of determination (R\textsuperscript{2}) represent the proportion of variability across cluster calculations that is accounted for by the H-bond effects. Average residuals and number of clusters included are also shown. The best-guess value reflects the value of Δ\textsubscript{63} under the most reasonable H-bonding pattern for the CO\textsubscript{2}-H\textsubscript{2}O hydration TS: 2 H\textsubscript{ohc}=O\textsubscript{wat}, 1 H\textsubscript{wat}=O\textsubscript{ohc}, and 4 H\textsubscript{wat}=O\textsubscript{oc} (Chapter 3, this dissertation).
Figure 4-14. DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydroxylation reaction at 25°C versus number of H-bonds from H$_2$O to a DIC hydroxyl. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium, rather than kinetic, $\Delta_{63}$ of HCO$_3^-$ calculated by Hill et al. (2014).
Figure 4-15. DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydroxylation reaction at 25°C versus distance from C to the attacking H$_2$O O measured in Å. Colors represent different model chemistries (Table 4-1). Each cluster calculation is represented by an individual point. Black line reflects equilibrium, rather than kinetic, $\Delta_{63}$ of HCO$_3^-$ calculated by Hill et al. (2014).
Table 4-5. Multiple linear regression of H-bond effects and C-O\textsubscript{attack} distance in Å on DIC $\Delta_63$ produced by kinetic fractionation in the CO\textsubscript{2} hydroxylation reaction at 25°C by model chemistry. Regression coefficients and constants are colored green if significant at the P<0.05 level. Coefficients of determination ($R^2$) represent the proportion of variability across cluster calculations that is accounted for by the H-bond effects. Average residuals and number of clusters included are also shown. The best-guess value reflects the value of $\Delta_63$ under the most reasonable H-bonding pattern for the CO\textsubscript{2}-OH\textsuperscript{-} hydroxylation TS: 1 H\textsubscript{ohc}-O\textsubscript{wat}, 3 H\textsubscript{wat}-O\textsubscript{ohc}, and 5 H\textsubscript{wat}-O\textsubscript{oc} (Chapter 3, this dissertation).

<table>
<thead>
<tr>
<th>Model/Cluster Color</th>
<th>H\textsubscript{ohc} - O\textsubscript{ohc}</th>
<th>H\textsubscript{ohc} - O\textsubscript{wat}</th>
<th>H\textsubscript{ohc} - O\textsubscript{ohc}</th>
<th>C - O\textsubscript{min} Distance</th>
<th>Constant</th>
<th>R$^2$</th>
<th>Avg. Resid.</th>
<th>N</th>
<th>Best - guess value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P808(-H14+G63)</td>
<td>-0.0004 ± 0.0023%</td>
<td>-0.0043 ± 0.0031%</td>
<td>-0.0031 ± 0.0044%</td>
<td>0.1222 ± 0.0166%</td>
<td>0.2228 ± 0.0285%</td>
<td>0.02</td>
<td>0.0033%</td>
<td>23</td>
<td>0.4604 ± 0.0033%</td>
</tr>
<tr>
<td>P808(-H14+G63)</td>
<td>-0.0007 ± 0.0029%</td>
<td>-0.0028 ± 0.0037%</td>
<td>-0.0021 ± 0.0048%</td>
<td>0.1596 ± 0.0166%</td>
<td>0.3986 ± 0.0462%</td>
<td>0.04</td>
<td>0.0031%</td>
<td>20</td>
<td>0.5247 ± 0.0031%</td>
</tr>
<tr>
<td>P808(-H14+G63)</td>
<td>-0.0002 ± 0.0032%</td>
<td>-0.0053 ± 0.0035%</td>
<td>-0.0026 ± 0.0044%</td>
<td>0.1599 ± 0.0166%</td>
<td>0.3813 ± 0.0369%</td>
<td>0.02</td>
<td>0.0029%</td>
<td>22</td>
<td>0.4096 ± 0.0039%</td>
</tr>
<tr>
<td>P808(-H14+G63)</td>
<td>-0.0005 ± 0.0031%</td>
<td>-0.0044 ± 0.0035%</td>
<td>-0.0031 ± 0.0044%</td>
<td>0.1595 ± 0.0166%</td>
<td>0.3811 ± 0.0371%</td>
<td>0.02</td>
<td>0.0030%</td>
<td>23</td>
<td>0.4081 ± 0.0030%</td>
</tr>
<tr>
<td>P808(-H14+G63)</td>
<td>-0.0005 ± 0.0031%</td>
<td>-0.0044 ± 0.0035%</td>
<td>-0.0031 ± 0.0044%</td>
<td>0.1595 ± 0.0166%</td>
<td>0.3811 ± 0.0371%</td>
<td>0.02</td>
<td>0.0030%</td>
<td>23</td>
<td>0.4081 ± 0.0030%</td>
</tr>
</tbody>
</table>

Average residuals and number of clusters included are also shown. The best-guess value reflects the value of $\Delta_63$ under the most reasonable H-bonding pattern for the CO\textsubscript{2}-OH\textsuperscript{-} hydroxylation TS: 1 H\textsubscript{ohc}-O\textsubscript{wat}, 3 H\textsubscript{wat}-O\textsubscript{ohc}, and 5 H\textsubscript{wat}-O\textsubscript{oc} (Chapter 3, this dissertation).
Figure 4-16. Variation in equilibrium $\Delta \alpha_3$ of $\text{H}_2\text{CO}_3$ in response to changes in temperature. Colors represent different model chemistries (Table 4-1). Black curve represents the computational value of $\text{H}_2\text{CO}_3$ equilibrium $\Delta \alpha_3$ from Hill et al. (2014).
Figure 4-17. Variation in equilibrium $\Delta_{63}$ of HCO$_3^-$ in response to changes in temperature. Colors represent different model chemistries (Table 4-1). Black curve represents the computational value of HCO$_3^-$ equilibrium $\Delta_{63}$ from Hill et al. (2014).
Figure 4-18. Variation in DIC $\Delta_{63}$ produced by kinetic fractionation in the CO$_2$ hydration reaction in response to changes in temperature. Colors represent different model chemistries (Table 4-1).
Figure 4-19. Variation in DIC $\Delta_{o3}$ produced by kinetic fractionation in the CO$_2$ hydroxylation reaction in response to changes in temperature. Colors represent different model chemistries (Table 4-1).
Figure 4-20. Fraction of DIC produced by reaction of CO$_2$ from the hydration (red) and hydroxylation (blue) reactions as a function of pH. The crossover pH is 8.25.
Figure 4-21. Elevation in $\Delta_{63}$ produced by kinetic fractionation relative to the $\Delta_{63}$ of pure HCO$_3^-$ as a function of pH. Colors represent different temperatures: 5°C (blue), 15°C (green), 25°C (yellow), and 35°C (red). Calculations use only the B++ model chemistry.
Figure 4-22. Elevation in $\Delta_{\delta3}$ produced by kinetic fractionation relative to the $\Delta_{\delta3}$ of pure HCO$_3^-$ as a function of temperature. Colors represent different pH values: 5 (red), 7 (yellow), 9 (green), and 11 (blue). Calculations use only the B++ model chemistry.
Table 4-6. Regressions of Δ$_{47}$ in CO$_2$ produced by acid digestion of carbonates. Kelson et al. (2017) represents the experimental regression. Hill et al. (2014), and model results listed by model chemistry, represent carbonate formed rapidly by DIC with a Δ$_{63}$ value equal to equilibrium H$_2$CO$_3$, plus an acid fractionation factor of +0.22‰ (Guo et al. 2009).

<table>
<thead>
<tr>
<th>Regression Source</th>
<th>Regression Slope * (1000/T)$^2$ (‰)</th>
<th>Regression Intercept (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelson et al. 2017 minerals</td>
<td>0.0417 (+/- 0.0013)</td>
<td>0.139 (+/- 0.014)</td>
</tr>
<tr>
<td>Hill et al. 2014 eqHbH$_2$CO$_3$</td>
<td>0.0400</td>
<td>0.203</td>
</tr>
<tr>
<td>PBE/PBE/6 – 311+G(d,p)</td>
<td>0.03783 (+/- 0.00012)</td>
<td>0.20029 (+/- 0.00131)</td>
</tr>
<tr>
<td>PBE0/6 – 311+G(d,p)</td>
<td>0.04183 (+/- 0.00015)</td>
<td>0.21358 (+/- 0.00165)</td>
</tr>
<tr>
<td>X3LYP/6 – 311+G(d,p)</td>
<td>0.04037 (+/- 0.00013)</td>
<td>0.20768 (+/- 0.00150)</td>
</tr>
<tr>
<td>PBE/PBE/6 – 311+++G(2d,p)</td>
<td>0.03755 (+/- 0.00011)</td>
<td>0.19819 (+/- 0.00127)</td>
</tr>
<tr>
<td>PBE0/6 – 311+++G(2d,p)</td>
<td>0.04160 (+/- 0.00014)</td>
<td>0.21096 (+/- 0.00161)</td>
</tr>
<tr>
<td>X3LYP/6 – 311+++G(2d,p)</td>
<td>0.04019 (+/- 0.00013)</td>
<td>0.20566 (+/- 0.00147)</td>
</tr>
<tr>
<td>B3LYP/6 – 311+G(d,p)</td>
<td>0.04014 (+/- 0.00013)</td>
<td>0.20681 (+/- 0.00148)</td>
</tr>
<tr>
<td>B3LYP/6 – 311+++G(2d,p)</td>
<td>0.03997 (+/- 0.00013)</td>
<td>0.20468 (+/- 0.00145)</td>
</tr>
</tbody>
</table>
Table 4-7. Regressions of Δ_{47} in CO₂ produced by acid digestion of carbonates. Kelson et al. (2017) represents the experimental regression. Hill et al. (2014) represents carbonate formed rapidly by DIC with a Δ_{63} value equal to equilibrium H₂CO₃, plus an acid fractionation factor of +0.22‰ (Guo et al. 2009). Model results listed by model chemistry represent carbonates produced rapidly from DIC with the maximum kinetic fractionation imparted by CO₂ hydration, plus an acid fractionation factor of +0.22‰.

<table>
<thead>
<tr>
<th>Regression Source</th>
<th>Regression Slope * (1000/T)²(‰)</th>
<th>Regression Intercept(‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelson et al. 2017 minerals</td>
<td>0.0417 (+/- 0.0013)</td>
<td>0.139 (+/- 0.014)</td>
</tr>
<tr>
<td>Hill et al. 2014 equilib H₂CO₃</td>
<td>.0400</td>
<td>.203</td>
</tr>
<tr>
<td>PBEPBE/6 – 311+G(d,p)</td>
<td>0.03739 (+/- 0.00014)</td>
<td>0.23671 (+/- 0.00161)</td>
</tr>
<tr>
<td>PBE0/6 – 311+G(d,p)</td>
<td>0.03949 (+/- 0.00016)</td>
<td>0.25026 (+/- 0.00180)</td>
</tr>
<tr>
<td>X3LYP/6 – 311+G(d,p)</td>
<td>0.03954 (+/- 0.00016)</td>
<td>0.24410 (+/- 0.00173)</td>
</tr>
<tr>
<td>PBEPBE/6 – 311++G(2d,p)</td>
<td>0.03699 (+/- 0.00014)</td>
<td>0.23309 (+/- 0.00156)</td>
</tr>
<tr>
<td>PBE0/6 – 311++G(2d,p)</td>
<td>0.03922 (+/- 0.00016)</td>
<td>0.24867 (+/- 0.00177)</td>
</tr>
<tr>
<td>X3LYP/6 – 311++G(2d,p)</td>
<td>0.03880 (+/- 0.00015)</td>
<td>0.24269 (+/- 0.00172)</td>
</tr>
<tr>
<td>B3LYP/6 – 311+G(d,p)</td>
<td>0.03906 (+/- 0.00015)</td>
<td>0.24336 (+/- 0.00172)</td>
</tr>
<tr>
<td>B3LYP/6 – 311++G(2d,p)</td>
<td>0.03915 (+/- 0.00015)</td>
<td>0.24064 (+/- 0.00169)</td>
</tr>
</tbody>
</table>
Table 4-8. Regressions of $\Delta_{47}$ in CO$_2$ produced by acid digestion of carbonates. Kelson et al. (2017) represents the experimental regression. Hill et al. (2014), and model results listed by model chemistry, represent carbonate formed rapidly by DIC with a $\Delta_{63}$ value equal to equilibrium HCO$_3^-$, plus an acid fractionation factor of +0.22‰ (Guo et al. 2009).

<table>
<thead>
<tr>
<th>Regression Source</th>
<th>Regression Slope x (1000/T)$^2$ (%)</th>
<th>Regression Intercept(‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelson et al. 2017 minerals</td>
<td>0.0417 (+/- 0.0013)</td>
<td>0.139 (+/- 0.014)</td>
</tr>
<tr>
<td>Hill et al. 2014 eqlib HCO$_3^-$</td>
<td>-0.383</td>
<td>0.191</td>
</tr>
<tr>
<td>PBE/PBE, 6-311+G(d,p)</td>
<td>0.03600 (+/- 0.00010)</td>
<td>0.18938 (+/- 0.00110)</td>
</tr>
<tr>
<td>PBE0/6-311+G(d,p)</td>
<td>0.04022 (+/- 0.00013)</td>
<td>0.20268 (+/- 0.00149)</td>
</tr>
<tr>
<td>X3LYP/6-311+G(d,p)</td>
<td>0.03858 (+/- 0.00012)</td>
<td>0.19599 (+/- 0.00131)</td>
</tr>
<tr>
<td>PBE/PBE, 6-311++G(2d,p)</td>
<td>0.03577 (+/- 0.00010)</td>
<td>0.18815 (+/- 0.00107)</td>
</tr>
<tr>
<td>PBE0/6-311++G(2d,p)</td>
<td>0.03991 (+/- 0.00013)</td>
<td>0.20035 (+/- 0.00144)</td>
</tr>
<tr>
<td>X3LYP/6-311++G(2d,p)</td>
<td>0.03838 (+/- 0.00011)</td>
<td>0.19437 (+/- 0.00127)</td>
</tr>
<tr>
<td>B3LYP/6-311+G(d,p)</td>
<td>0.03633 (+/- 0.00012)</td>
<td>0.19530 (+/- 0.00128)</td>
</tr>
<tr>
<td>B3LYP/6-311++G(2d,p)</td>
<td>0.03614 (+/- 0.00011)</td>
<td>0.19330 (+/- 0.00125)</td>
</tr>
</tbody>
</table>
Table 4-9. Regressions of $\Delta_{47}$ in CO$_2$ produced by acid digestion of carbonates. Kelson et al. (2017) represents the experimental regression. Hill et al. (2014) represents carbonate formed rapidly by DIC with a $\Delta_{63}$ value equal to equilibrium HCO$_3^-$, plus an acid fractionation factor of +0.22‰ (Guo et al. 2009). Model results listed by model chemistry represent carbonates produced rapidly from DIC with the maximum kinetic fractionation imparted by CO$_2$ hydroxylation, plus an acid fractionation factor of +0.22‰.

<table>
<thead>
<tr>
<th>Regression Source</th>
<th>Regression Slope x $1000/(T)^2$(‰)</th>
<th>Regression Intercept(‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelson et al. 2017 minerals</td>
<td>0.0417 (+/- 0.0013)</td>
<td>0.139 (+/- 0.014)</td>
</tr>
<tr>
<td>Hill et al. 2014 eqlib HCO$_3^-$</td>
<td>.0383</td>
<td>.191</td>
</tr>
<tr>
<td>PBEPE/6 – 311+G(d, p)</td>
<td>0.03881 (+/- 0.00016)</td>
<td>0.26744 (+/- 0.00183)</td>
</tr>
<tr>
<td>PBE0/6 – 311+G(d, p)</td>
<td>0.04115 (+/- 0.00017)</td>
<td>0.28033 (+/- 0.00194)</td>
</tr>
<tr>
<td>X3LYP/6 – 311+G(d, p)</td>
<td>0.03982 (+/- 0.00016)</td>
<td>0.27064 (+/- 0.00181)</td>
</tr>
<tr>
<td>PBEPE/6 – 311++G(2d, p)</td>
<td>0.03768 (+/- 0.00016)</td>
<td>0.26383 (+/- 0.00178)</td>
</tr>
<tr>
<td>PBE0/6 – 311++G(2d, p)</td>
<td>0.04058 (+/- 0.00017)</td>
<td>0.27618 (+/- 0.00185)</td>
</tr>
<tr>
<td>X3LYP/6 – 311++G(2d, p)</td>
<td>0.03947 (+/- 0.00016)</td>
<td>0.26833 (+/- 0.00177)</td>
</tr>
<tr>
<td>B3LYP/6 – 311+G(d, p)</td>
<td>0.03925 (+/- 0.00017)</td>
<td>0.27315 (+/- 0.00185)</td>
</tr>
<tr>
<td>B3LYP/6 – 311++G(2d, p)</td>
<td>0.03920 (+/- 0.00016)</td>
<td>0.26999 (+/- 0.00175)</td>
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</tbody>
</table>
Figure 4-23. Equilibrium and kinetic $\Delta_{17}$ values from this study (red, blue), an equilibrium value from modeled aragonite clusters (Hill et al. 2014, yellow), and an equilibrium value from experimental measurements of equilibrium carbonates (Kelson et al. 2017, green). Dashed lines represent kinetic fractionations, while solid lines represent equilibrium fractionations. Results from CO$_2$ hydration models and H$_2$CO$_3$ equilibrium in red; results from CO$_2$ hydroxylation models and HCO$_3^-$ equilibrium in blue. Calculations use only the B++ model chemistry.
References


Chapter 5
Isotopic Composition of Amorphous Calcium Carbonate

Abstract

One potential cause of vital effects in corals is formation of an initial amorphous calcium carbonate (ACC) phase. The isotopic composition of ACC is unknown, and there is no knowledge of the effects of parameters such as temperature, precipitation rate, particle size, and solution chemistry on its isotopic composition. This chapter reports analyses the isotopic composition of ACC. Trends in isotopic composition in ACC opposite those found in calcite with increasing saturation state are detailed.

Introduction

Amorphous Calcium Carbonate

Amorphous calcium carbonate (ACC) is an X-ray amorphous nanoparticulate phase with some short-range order under extended X-ray absorption fine structure (EXAFS) measurements (Michel et al. 2008). ACC does not remain stable in solution, but rapidly transforms into a more ordered mineral, such as calcite or aragonite. However, ACC in some living organisms has been found which does not transform into another mineral, even upon removal from the organism. The structure of ACC is difficult to determine, but independent studies (Raiteri and Gale 2010; Goodwin et al. 2010) generated similar atomic structures, which are consistent with X-ray absorption spectroscopy and NMR data (Michel et al. 2008).
ACC occurs in several types with different synthesis conditions. These types vary in their stability, water content, and the polymorph of CaCO₃ mineral that they produce upon transformation to an ordered phase (Günther et al. 2005; Gebauer et al. 2010; Cartwright et al. 2012). Some synthetic and biogenic ACC is unstable in vitro (Brečević and Nielsen 1989; Clarkson et al. 1992). The structural differences that produce different properties in ACC are not yet well understood, though thermogravimetric analysis reveals that stable biogenic forms tend to contain more structural water, and Fourier transform-infrared spectroscopy (FTIR) and Raman spectra can differentiate between stable and unstable forms, finding more short-range order in unstable forms (Addadi et al. 2003).

Because ACC is metastable relative to ordered polymorphs of CaCO₃, transformation to an ordered phase generally occurs unless kinetically inhibited. Chemical stabilizers are generally thought to be responsible for preventing transformation of ACC to stable phases in vivo. In organisms, stabilizers include proteins, organic compounds, and inorganic phosphate. Experimental synthesis of ACC has used these and other stabilizers, including polyacrylate, silica, and Mg²⁺ (Raz et al. 2000; Rieger et al. 2007; Kellermeier et al. 2010). Organisms can stabilize ACC to different degrees using proteins, organic compounds, inorganic phosphate, silica, and Mg²⁺ (Beniash et al. 1997; Raz et al. 2000; Addadi et al. 2003; Akiva-Tal et al. 2011; Gal et al. 2013). These chemical stabilizers can determine which mineral the ACC will eventually form (Rodriguez-Blanco et al. 2012; Zhang et al. 2012).

Classical nucleation theory (Zeldovich 1943; Meldrum and Sear 2008; Sear 2012) has long been used to explain the precipitation of minerals in an aqueous environment. Classical nucleation theory posits that small particles constantly form and dissolve ion-by-ion, only precipitating when particles grow large enough to pass a free energy barrier related to the energy penalty of creating new mineral surface (DeYoreo 2013). However, stable pre-nucleation clusters have been observed during in vitro CaCO₃ precipitation (Gebauer et al. 2008), suggesting both
the ion-by-ion mechanism, and the instability of small particles, may be oversimplifications. The
structure of pre-nucleation clusters is not yet known but has been studied computationally
(Finney and Rodger 2012). Some pre-nucleation clusters may be smaller particles of ACC (0.5-3
nm as opposed to ≥30 nm; Kellermeier et al. 2012). Nonclassical nucleation is reviewed by Van
Driessche et al. (2016).

The difference between the formation conditions of ACC and ordered CaCO₃ phases may
impair differences in their isotopic compositions. However, the isotopic composition of ACC has
not been extensively studied. ACC formed by earthworms has a δ¹³C content 1.2‰ heavier than
coprecipitated calcite (Versteegh et al. 2017) although the discrepancy could be due to
discrimination against heavy DIC during dissolution of the ACC and reprecipitation as calcite.
Speleothem ACC δ¹⁸O values are 2.4‰ lighter than coprecipitated calcite (Demény et al. 2006).
Biological use of ACC to form calcite and aragonite could cause the δ¹⁸O paleothermometer
based on these minerals to depart from apparent equilibrium. In fact, little attention has been
devoted across the geosciences to isotopic fractionation in nanoparticles. ACC could govern the
isotopic signature of biogenic calcium carbonate skeletons if precipitation of the final carbonate
skeleton proceeds via an ACC intermediate, or if dissolution and re-precipitation of the carbonate
begins with ACC. Rollion-Bard et al. (2009) note that neither Rayleigh fractionation nor
equilibration at high pH can explain the low δ¹⁸O at the calcification center of corals, suggesting
that precipitation from ACC may be the cause.

ACC in Corals

In corals, it is not yet known how much of a role ACC plays in skeletal aragonite
precipitation. Meibom et al. (2004) used the microscale Mg distribution of ACC to infer the coral
skeleton was originally built from Mg-rich ACC. The intracellular vesicles in the apical
membrane of the calicoblastic ectoderm in corals may contain organic-stabilized ACC, which contributes to the granular seed crystals found at coral calcification centers (Cohen and McConnaughey 2003). Crystallization via an ACC intermediate has not been investigated, but evidence from the fast-growing centers of coral growth indicates this phase may affect the final isotopic signature (Rollion-Bard et al. 2009).

This chapter presents some analyses of the isotopic composition of ACC and its dependence on saturation state and chemical stabilizer concentration. The chapter then describes some future research that could determine the cause of the observed trends in isotopic composition, and whether ACC could explain the isotopic disequilibrium occurring at the calcification centers of some corals.

Methods

ACC was synthesized using methods described previously (Koga et al. 1998; Huang et al. 2007; Kellermeier et al. 2010). Briefly, a CaCl$_2$ solution of known concentration was mixed with a Na$_2$CO$_3$ solution of known concentration, either rapidly or with syringe-pump titration (Figure 5-1). NaOH was used to adjust the Na$_2$CO$_3$ solution to a desired initial pH, and the pH of the mixed solution was measured at the end of the experiment. Chemical stabilizers were added to the CaCl$_2$ solution prior to mixing, in particular polyacrylate (Rieger et al. 2007; Huang et al. 2007), and silica in the form of a sodium metasilicate solution (Kellermeier et al. 2010; 2012). Reaching a suitably high pH using poly (acrylic acid) was difficult. Additionally, concerns about the ability of polyacrylate to both penetrate the structure of ACC and alter the isotopic composition of degassed CO$_2$ during isotope analysis by decomposition of carboxylic acid groups shifted the focus to metasilicate stabilization. Metasilicate buffers the pH in a much more favorable range than poly(acrylic acid), likely does not contribute oxygen and cannot contribute carbon during
isotope analysis, and is believed to stabilize ACC by surface bonding (Kellermeier et al. 2010) rather than by penetrating ACC and chelating Ca\(^{2+}\). Some runs were performed at identical conditions but without stabilizers to test differences in fractionation between ACC and crystalline carbonates.

ACC was filtered using 0.2 \(\mu\)m filters in a vacuum filtration apparatus. Large amounts of wet precipitate were usually obtained in <10 minutes by filtering only ~15-20 ml solution; after this time, filtration generally slowed and was aborted. Particles were then dried overnight in a 90\(^\circ\)C drying oven.

The ACC particles were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform-infrared spectroscopy (FTIR), Raman spectroscopy, and stable isotope ratio mass spectrometry at the Penn State Laboratory for Isotopes and Metals in the Environment. Isotopic fractionations were compared with supersaturation state.

XRD measurements were made using a PANalytical Empyrean Cu/Mo X-ray diffractometer with a 2θ resolution of 0.0263 degrees at the Penn State Materials Characterization Lab (MCL). Minerals were identified and mineral abundances measured semi-quantitatively to an abundance of 3 wt% with the Jade software (MDI Products).

SEM images were acquired using a Hitachi TM3000 benchtop SEM. Magnifications of 50x-30,000x were used. Samples were placed on an aluminum sample mount.

FTIR measurements were made using a Bruker Vertex 70 FTIR spectrometer fitted with a Harrick MVP-Pro Single Reflection ATR Microsampler at the MCL. Measurements were made in the mid-infrared range (400-4000 cm\(^{-1}\)) with a 0.5 cm\(^{-1}\) resolution. Samples were pressed with KBr prior to analysis.

Raman measurements were made using a WITec CRM 200 confocal Raman spectrometer at the MCL. Samples were targeted using the optical microscope at 100x magnification. Analysis
used a 488nm laser with a diffraction grating spacing of 1200/mm providing a 2000 cm$^{-1}$ range with 2 cm$^{-1}$ resolution and an integration time of 0.5 seconds.

Mass spectrometry was performed on a Thermo Delta V Advantage system at the PSU Laboratory for Isotopes and Metals in the Environment. Carbonate processing was performed using a Combi PAL auto-sampler connected to a Thermo Gas Bench apparatus. Isotope measurements were made relative to VPDB for both $\delta^{13}$C and $\delta^{18}$O, and were measured against NBS-18, NBS-19, and in-house Biogeochem standards.

**Results**

**ACC Characterization**

SEM results comparing two experimental runs with different quantities of sodium metasilicate stabilizer are shown in Figure 5-2. In images without any stabilizer, ~10μm rhombs of calcite are clearly visible. In images including 1500 ppm metasilicate stabilizer, no clear order is visible down to the resolution of the SEM. The only particles present are likely sub-micron sized.

XRD, Raman spectroscopy, and FTIR can all be used to verify the particles produced are indeed amorphous (Addadi et al. 2003; Michel et al. 2008). Raman and FTIR can also be used to distinguish the stability of ACC using peak width and position, and in particular make comparisons to structural features observed in the different varieties of biogenic and synthetic ACC that are currently known (Addadi et al. 2003).

XRD results are shown in Figure 5-3 for the same solutions shown in Figure 5-2. The spectra were taken 3 months after synthesis. Without metasilicate stabilizer, both calcite and vaterite were detected in the XRD spectrum. Only calcite was present in precipitate from solution
containing 1500ppm metasilicate stabilizer. A broad hump between 20 angles 18° and 34° is indicative of the presence of ACC (Michel et al. 2008). Peak areas indicate ~75% of carbonate is stabilized as ACC after 3 months. Whether the partial transformation to calcite in the ACC sample occurred during precipitation, or at some point later after sample storage, is unclear. No calcite rhombs were visible in SEM images (Figure 5-2) taken immediately after synthesis and drying, supporting the idea that transformation occurred later.

Raman spectroscopy results are shown in Figure 5-4. Raman spectra of synthesized ACC share similarities with stable biogenic ACC, including a broad hump above 100 cm⁻¹ and widened peaks at 1090 cm⁻¹ relative to calcite. Peak width for the 1090 cm⁻¹ peak increases as more stabilizer is used; peak width similarly increases in biogenic samples from calcite through transient ACC to stable ACC (Addadi et al. 2003).

FTIR results are shown in Figure 5-5. FTIR spectra of synthetic ACC share similarities with stable biogenic ACC. Notably, the 1425-1488 cm⁻¹ double peak in the 1500ppm metasilicate sample is similar to stable biogenic ACC (Addadi et al. 2003) Single peaks in this region are indicative of calcite or more transient ACC.

It is possible that the drying conditions used to isolate ACC, the high pressure used to produce a pellet for FTIR analysis, or the high vacuum of SEM could impart changes in the ACC structure. However, the fact that FTIR, SEM, and other techniques provide consistent results indicates the structural changes are likely minimal.

ACC Isotopic Composition

IRMS measurements of carbonate isotopic composition are shown in Figure 5-6. Figure 5-6a displays the trends in δ¹³C and δ¹⁸O as metasilicate stabilizer concentration is increased. Calcite formed at 25°C with 0 ppm metasilicate is 8.3‰ heavier in δ¹³C and 1.8‰ heavier in δ¹⁸O
than ACC formed with 3000 ppm metasilicate. The trend does not level out at either low or high metasilicate concentrations.

Figure 5-6b displays the trends in $\delta^{13}C$ and $\delta^{18}O$ as Ca$^{2+}$ and DIC concentrations are changed. While calcite becomes lighter in both $\delta^{13}C$ and $\delta^{18}O$ as Ca$^{2+}$ and DIC concentrations are increased together, ACC exhibits the opposite trend, becoming heavier as concentrations are increased. ACC $\delta^{13}C$ values are always lighter than calcite values formed under the same concentrations, under the conditions studied. However, ACC $\delta^{18}O$ values become heavier than calcite as concentrations of Ca$^{2+}$ and DIC increase. Apparently there is less fractionation between ordered carbonates like calcite and ACC at higher concentrations of reactants, and for some concentrations, ACC-calcite fractionation may favor $^{18}O$ concentration in the ACC.

**Discussion**

**Isotope Trends**

In metasilicate-stabilized ACC nanoparticles, light isotopes are favored at higher metasilicate concentrations but lower calcium and carbonate concentrations (Figure 5-6). Possible explanations include fluid composition effects, Rayleigh fractionation, mixing between ACC and calcite endmembers, pH buffering, kinetic fractionation during precipitation, and particle size effects.

Fluid isotopic composition may have some impact on measured $\delta^{13}C$ and $\delta^{18}O$ by impacting the H$_2$O and DIC isotopic composition from which the carbonate precipitates. Isotopic composition shown in Figure 5-6 gives raw values of $\delta^{13}C$ and $\delta^{18}O$ that have not been corrected for fluid compositions. Measurement of the isotopic compositions of the fluids was difficult; no facilities at Penn State were available to analyze these. Moreover, sample vessels permeable to
CO₂ may allow DIC to leak out over time, changing the isotopic composition if measured later. Because the trends displayed in Figures 5-6a and 5-6b originated from the same stock solutions, it is unlikely that individual measurements are biased by the isotopic composition of H₂O or DIC in the precipitating fluid.

Rayleigh fractionation between the closed pool of DIC and solid carbonate could have an effect on isotopic compositions. As carbonates precipitate from a closed pool, they leave behind DIC of an isotopic composition distinct from the rest of solution. As more of the solution DIC precipitates as carbonate, the final isotopic composition of the carbonate approaches that of the original DIC pool. Some evidence for this may be seen in the smaller discrepancy between calcite and ACC d¹³C at higher Ca²⁺ and DIC concentrations, where proportionally more of the total DIC is precipitated at the end of the experiment. This would imply an equilibrium d¹³C for ACC that is substantially lower than that of calcite, in contrast with results from carbonate precipitated by earthworms (Versteegh et al. 2017). The O pool during precipitation is dominated by H₂O, so d¹⁸O would not show the same trend as d¹³C under Rayleigh fractionation. This may be complicated by the fast kinetics of precipitation in these experiments relative to DIC-H₂O isotope equilibration. Measurement of fluid isotopic composition would be useful in quantifying the extent of Rayleigh fractionation.

Mixing between ACC and calcite endmembers could control the observed isotopic compositions. End-member isotopic mixing between ACC and an ordered mineral such as calcite, where the ACC has a lighter isotopic composition than the ordered mineral, would cause carbonate precipitated with more metasilicate to be lighter, following the trend in Figure 5-6a. However, if this were the case, we would expect to see a plateau in isotopic composition as all the carbonate forms ACC in the higher metasilicate concentration range.

Buffering of pH by alkaline sodium metasilicate could have two different effects on final carbonate isotopic composition. The ACC could precipitate faster at high metasilicate
concentration, which by buffering the system at a higher pH produces more $\text{CO}_3^{2-}$, thus increasing ACC precipitation rate. If this were the main cause of the trend, the trend could be reversed by increasing the pH of the low-metasilicate solutions relative to the high-metasilicate solutions. An increased pH could also mean more $\text{CO}_3^{2-}$ than $\text{HCO}_3^-$ contributes to the final ACC isotopic composition. Equilibrium $\text{CO}_3^{2-}$ is 1.4‰ lighter in $\delta^{13}\text{C}$ and 6.8‰ lighter in $\delta^{18}\text{O}$ than $\text{HCO}_3^-$, so a larger $^{18}\text{O}$ effect than $^{13}\text{C}$ should be observed if this is the primary driver. Instead, the effect of metasilicate concentration is much stronger on $\delta^{13}\text{C}$ than $\delta^{18}\text{O}$.

There could also be a pH-independent influence of metasilicate on ACC precipitation causing kinetic fractionation to be “locked in” sooner at higher metasilicate concentration; perhaps the higher concentration of metasilicate sequesters the growing ACC particles faster, preventing them from isotopically equilibrating with DIC in solution.

Particle size could impact isotopic composition due to differences in fractionation between surface sites interacting with metasilicate stabilizer and $\text{H}_2\text{O}$ molecules and interior sites. Smaller ACC particles form at higher metasilicate stabilizer concentration and lower DIC concentration. Smaller particles have a larger proportion of surface sites. If the surface sites are lighter in isotopic composition than interior sites, it could explain why ACC has a lighter isotopic composition both as metasilicate concentration is increased and as $\text{Ca}^{2+}$ and DIC concentrations are decreased.

More likely, several of these effects are active at once. Future experiments will need to be carefully designed to separate each effect.

**Trends in Natural Carbonates**

The trends observed in this chapter, in which $\delta^{18}\text{O(ACC)}>\delta^{18}\text{O(calcite)}$ but $\delta^{13}\text{C(calcite)}>\delta^{13}\text{C(ACC)}$ generally, are difficult to reconcile with previous work on corals if
ACC controls coral isotope fractionation. Deep-sea corals display decreasing $\delta^{18}O$ with constant $\delta^{13}C$ at their calcification centers (Adkins et al. 2003; Rollion-Bard et al. 2009). At high saturation states, ACC seems to have a heavier $\delta^{18}O$ composition than calcite, counter to the coral observations. At low saturation states, ACC $\delta^{13}C$ is much lighter than calcite, which is also counter to the constant $\delta^{13}C$ observed in deep-sea corals. Given these results, it is likely that ACC is not controlling the isotopic composition of coral calcification centers.

Speleothem ACC $\delta^{18}O$ values are 2.4‰ lighter than coprecipitated calcite at 10°C (Demény et al. 2006). In this chapter, ACC $\delta^{18}O$ values are generally heavier than calcite, except at low saturation states. Because speleothems grow slowly, it makes sense that their precipitation would occur at low saturation states as CO$_2$ is evolved slowly from solution. Thus these results are not necessarily in disagreement with those on speleothems, although the saturation state during formation of ACC is clearly of interest.

**Stabilizers**

This work used metasilicate as a stabilizer because it is cheap and easy to work with and can reversibly stabilize ACC. However, it is likely not a primary stabilizer in organisms, so its use in our experiments may cause a difference in isotopic composition between our synthetic ACC and biogenic ACC. Synthesis of some ACC from solutions of high ionic strength could mimic precipitation from seawater instead. However, the composition of the fluid from which biogenic minerals precipitate does not generally match seawater composition because of biological control over internal fluids (Weiner and Dove 2003), so this cannot be expected to be a perfect representation of true conditions within coral. Moreover, the organic matrix is thought to be important in coral mineralization as a crystallization template or nucleation seed (discussed in Cohen and McConnaughey 2003), so precipitation of ACC in biota likely proceeds in a much
more chemically complex environment than any currently capable of being studied in the laboratory. Stabilizers that use carboxylate groups including amino acids, peptides, and polyacrylate may be a good topic to investigate to better mimic the biogenic stabilization environment.

**Future Work**

Much work remains to be done to interpret the isotopic composition of ACC in light of the many disequilibrium effects that can impact its isotopic composition.

**ACC Synthesis Methods**

A second experimental setup could be employed to determine differences in fractionation when CO₂ hydration and hydroxylation reactions are active. CO₂ bubbled through a solution of Ca(OH)₂ will produce DIC with a kinetic fractionation signature (Günther et al. 2005). Different bubbling rates and Ca(OH)₂ concentrations can be used to change the supersaturation state. By forming ACC using this method, fractionation will be due to the CO₂ hydration and hydroxylation reactions in addition to precipitation. In contrast, ACC generated from a Na₂CO₃ solution exhibits no hydration or hydroxylation fractionation because the DIC is already present as CO₃²⁻. This will provide two things: (1) a direct comparison for the hydration and hydroxylation models (Chapters 3 and 4, this dissertation), when the fractionation in Na₂CO₃-generated ACC is subtracted from CO₂-bubbled ACC; and (2) a means to evaluate whether more fractionation is caused by the hydration and hydroxylation reactions versus fractionation during precipitation when both are active.
Future work should also use more biologically-relevant stabilizers such as amino acids known to associate strongly with ACC (Addadi et al. 2003). Mg$^{2+}$ stabilization still needs to be investigated, as it is a common stabilization method in biota (Raz et al. 2000) including some corals (Meibom et al. 2004), and the presence of Mg$^{2+}$ can induce aragonite to form instead of calcite (Zhang et al. 2012) which comprises many corals.

**ACC Analysis Methods**

In the future, samples of solution isotopic composition should be taken before mixing, after mixing but before filtration, and after filtration for analysis of DIC $\delta^{13}$C and $\delta^{18}$O and water $\delta^{18}$O. These were expected to be analyzed at the PSU LIME facility, where a protocol was in development to analyze $\delta^{18}$O of water, but no protocol had been developed at the time of ACC synthesis.

Some samples of unfiltered ACC could be “frozen” by addition of extra metasilicate after precipitation, to see whether particles stay stable in solution and can be filtered later without altering morphology and isotopic composition. These particles, as well as fresh ACC particles that have not been frozen, should be analyzed by XRD to determine the rate at which ACC transforms once removed from solution and dried.

Analyses could be extended to clumped isotopes. These have the benefit of not requiring knowledge of the precipitating fluid isotopic composition. The magnitudes of kinetic fractionation on clumped isotopes during the CO$_2$ hydration and hydroxylation reactions and during nonequilibrium precipitation have been well-characterized by modeling (Hill et al. 2014 and Chapter 4, this dissertation) which, when combined with the $\delta^{13}$C and $\delta^{18}$O isotope trends, will allow separation of kinetic and equilibrium fractionation effects in the final ACC isotopic signature.
ACC Modeling

Equilibrium C and O isotopic compositions of ACC nanoparticles could be analyzed using density functional theory (DFT) calculations. Clusters of atoms could be sampled from around each ion in the ACC structure, reducing computation time (Figure 5-7). Separate analysis of surface and interior site could inform studies of ACC fractionation changes based on particle size. Pre-developed models of ACC nanoparticles are available on which to base calculations (Raiteri and Gale 2010). Where surface atoms are included, or where \( \text{H}_2\text{O} \) molecules are present in the interior of ACC, solvating \( \text{H}_2\text{O} \) molecules must be incorporated to accurately model vibrations.

The equilibrium fractionation predicted in these models will need to be compared with the fractionation observed in synthesized ACC nanoparticles. This will determine whether synthesized ACC nanoparticles undergo equilibrium fractionation, or whether they lock in kinetic fractionations, and the extent of kinetic fractionation related to supersaturation state and growth rate.

Summary

ACC precipitated abiotically with metasilicate stabilizer has structural similarities to biogenic stable and transient ACC. ACC isotopic composition changes based on the concentration of stabilizers and reactants in a manner not equivalent to behavior in calcite. The isotopic composition of ACC is inconsistent with the composition of the calcification centers in coral.
Figure 5-1. Example experimental setup for ACC synthesis. Solutions of known DIC concentration and pH are injected via syringe at a constant rate (left) into solutions of known Ca$^{2+}$ and stabilizer concentration in a constant temperature bath (right). The solutions are mixed inside closed sample cups. In other experiments, solutions are poured together directly into the closed sample cups without syringe injection. Picture from the Gaetani lab at Woods Hole Oceanographic Institution.
Figure 5-2. SEM images of synthesized carbonates. Samples were synthesized in (a) 0 ppm metasilicate and imaged at 300x resolution; (b) 0 ppm metasilicate at 10000x; (c) 1500 ppm metasilicate at 300x; (d) 1500 ppm metasilicate at 10000x. Calcite rhombs are clearly visible in (a) and (b), and what is likely a spherical vaterite particle is visible in the upper left corner of (b). No clear order is visible in (c). (d) appears to consist of submicron-sized particles below the resolution of the SEM. Scale bar lengths are as indicated in the lower right corner of each panel.
Figure 5-3. XRD spectra of ACC stabilized with 1500ppm metasilicate stabilizer (blue) and carbonate synthesized without stabilizer (red). Peaks in XRD spectra correspond to calcite, with peak heights ~30% of those found in the carbonate synthesized without stabilizer. The broad hump from 18° to 34° is known to be present in ACC spectra and indicates an amorphous phase (Michel et al. 2008). Peaks in un-stabilized carbonate spectrum that do not appear in the ACC spectrum correspond to vaterite.
Figure 5-4. Raman spectra of synthesized carbonates. (blue, top) ACC stabilized at 1800 ppm metasilicate; (green) ACC stabilized at 600ppm metasilicate; (red) calcite synthesized without stabilizer; (dark gray) stable biogenic ACC extracted from Ficus microcarpa leaves; (light gray) transient biogenic ACC extracted from 48 hour old Strongylocentrotus purpuratus spicules; (black, bottom) calcite. Bottom three spectra from Addadi et al. (2003). Peak width in the 1090 rel. cm$^{-1}$ peak increases as more ACC stabilizer is used; peak width similarly increases from calcite through transient ACC to stable ACC. A broad hump from 100-300 rel.cm$^{-1}$ is characteristic of stable biogenic ACC (Ficus microcarpa); a similar hump is seen in synthetic ACC stabilized, although the reason behind the larger width in the synthetic samples is unknown.
Figure 5-5. FTIR spectra of calcium carbonate precipitated with 1500 ppm metasilicate (blue, top); 450 ppm metasilicate (green); and 0 ppm metasilicate (red). Comparison spectra from Addadi et al. (2003) of stable biogenic ACC extracted from *Ficus microcarpa* leaves (dark gray); transient biogenic ACC extracted from 48 hour old *Strongylocentrotus purpuratus* spicules (light gray); and calcite (black, bottom). Peak assignments: (1500 ppm) 1425-1488 cm$^{-1}$ double peak similar to stable biogenic ACC, 1033 cm$^{-1}$ and 448 cm$^{-1}$ peaks related to silica, 870 cm$^{-1}$ peak indicative of carbonate; (450 ppm) similar to 1500 ppm but with single 1386 cm$^{-1}$ peak more like transient ACC or calcite; (0 ppm) 873 cm$^{-1}$ indicative of carbonate, 1395 cm$^{-1}$ and sharp 712 cm$^{-1}$ peaks indicative of calcite.
Figure 5-6. Isotopic compositions of synthesized carbonates, relative to VPDB standard, unadjusted for solution composition. (a) Adjusting [metasilicate] (x-axis) while holding [Ca$^{2+}$] and [DIC] at 10 mM. (b) Adjusting [Ca$^{2+}$] and [DIC] simultaneously while holding [metasilicate] at 0 ppm and 1800 ppm. Squares represent $^{13}$C measurements; circles represent $^{18}$O measurements. In (b), 1800 ppm metasilicate-stabilized ACC is in blue; unstabilized calcite is in red. Error bars represent the average of the measurement standard deviation on 3 different laboratory standards (NBS-18, NBS-19, LIME Biogeochem) (n=16).
Figure 5-7. Cluster model of an ACC nanoparticle. The full ACC particle (adapted from Raiteri and Gale, 2010; left) represents the ACC particle with a solvation shell. The extracted cluster (right) is shown, with Ca in green, C in blue, and O in red, and Pauling bond-strength-conserving atoms in gray (Rustad et al. 2008). Pauling atoms carry a charge of +2/3. The large spherical atoms are free to vibrate, while those represented by sticks are held fixed. H\textsubscript{2}O molecules omitted in the extracted cluster for clarity.
References


Chapter 6

Conclusion and Future Directions

Conclusion

This dissertation endeavors to determine isotopic fractionation in chemical systems relevant to carbonate paleoclimatology. The research focuses on application of DFT calculations to equilibrium and kinetic fractionation on C, O and clumped C-O isotopes. Conclusions are drawn with applicability to carbonate paleoclimatology as well as more generally for methods of isotope fractionation evaluation using computational chemistry.

Basis sets 6-311+G(d,p) and 6-311++G(2d,p) combined with DFT functionals B3LYP and X3LYP are acceptable choices to model DIC isotope fractionation. Using these in a DFT framework, equilibrium and kinetic fractionation factors have been calculated for DIC species and reactions. The value of $^{13/12}\alpha_{eq}(\text{H}_2\text{CO}_3\text{-CO}_2)$ is calculated as $1.0125\pm0.0014$ at 25°C. The value of $^{18/16}\alpha_{eq}(\text{H}_2\text{CO}_3\text{-CO}_2)$ is calculated as $0.9975\pm0.0007$ at 25°C. The value of $^{13/12}\alpha_{kin}(\text{hydration})$ is calculated as $0.989\pm0.002$ at 25°C. The value of $^{13/12}\alpha_{kin}(\text{hydroxylation})$ is calculated as $0.986\pm0.001$ at 25°C. The value of $^{18/16}\alpha_{kin}(\text{hydration})$ is calculated as $0.980\pm0.001$ at 25°C. The value of $^{18/16}\alpha_{kin}(\text{hydroxylation})$ is calculated as $0.980\pm0.001$ at 25°C. DIC with a signature dominated by unequilibrated CO$_2$ hydration will have a Δ$_{47}$ value $\sim$0.10‰ above the carbonate mineral calibration at a given temperature. DIC with a signature dominated by unequilibrated CO$_2$ hydroxylation will have a Δ$_{47}$ value $\sim$0.12‰ above the carbonate mineral calibration at a given temperature. CO$_2$ hydration and hydroxylation can explain most of the C, O, and clumped isotope disequilibrium observed in shallow-water corals better than DIC at isotopic
equilibrium and precipitation disequilibrium. However, another mechanism must be slightly decreasing δ$^{13}$C or increasing δ$^{18}$O in shallow corals at the fastest growth rates as well.

Harmonic vibrational frequencies should be used to evaluate computational chemistry studies of isotope fractionation; computational schemes that perform well at other tests but poorly in calculation of vibrational frequencies also perform poorly for calculation of isotope fractionation. Bulk averages of aqueous cluster results are not suitable for calculation of equilibrium or kinetic fractionation factors, because individual H-bonds have large impacts on fractionation factors. Rather, each cluster should be analyzed in terms of its H-bond environment and extrapolated to the well-hydrated state that should exist in bulk solution. Model chemistries do not behave systematically relative to each other, so the most accurate ones should be applied whenever possible and not extrapolated from simpler model chemistries that may have fortuitously accurate results in some instances.

**Future Directions**

Many research directions on evaluation of isotope fractionation in the carbonate system, and more generally in computational evaluation of aqueous isotope fractionation, are suggested by this dissertation.

A large coral dataset including shallow and deep corals with simultaneous evaluation of δ$^{13}$C, δ$^{18}$O, Δ$_{47}$, and coral growth rate should be constructed. If CO$_2$ hydration and hydroxylation are responsible for most coral vital effects, they should produce lower δ$^{13}$C and δ$^{18}$O while producing higher Δ$_{47}$ as growth rates increase. In contrast, equilibrated DIC which is at precipitation disequilibrium should produce lower Δ$_{47}$ values associated with faster growth rates.

Non-equilibrated CO$_2$ may contribute to the isotope disequilibrium observed in corals. Respired CO$_2$ has a lighter δ$^{13}$C; if it is preferentially incorporated by the coral when the coral is
growing fast and using the most energy, it may be the source of decreased $\delta^{13}\text{C}$ versus what is expected. The CO$_2$ would likely also have a clumped isotope signature that has not been equilibrated. The effects of non-equilibrated CO$_2$ as a starting material on $\Delta_{47}$ of product DIC should also be evaluated.

In the DIC system, the effects of H-bond pattern, C-O$_{\text{attack}}$ distance, and other structural parameters should be evaluated to remove more error in $\alpha_{\text{eq}}$ and $\alpha_{\text{kin}}$ prediction. The number of H$_2$O molecules participating in H$^+$ exchange at the TS may impact fractionation. This and other structural parameters impacting fractionation may be revealed by studying the conformations with the largest residual errors. More stringent attempts should be made to evaluate the precise C-O$_{\text{attack}}$ distance that exists at the hydration and hydroxylation TS. The effects of both H-bonds between nonparticipant H$_2$O molecules and the strengths of all H-bonds on isotope fractionation should be evaluated. Isotopic fractionation in contact and solvent-shared ion pairs between DIC and various cations should be directly calculated. The specific vibrational frequency shifts responsible for isotopic fractionation should be evaluated, and the model predictions should be compared directly with harmonic vibrations of DIC-H$_2$O clusters determined from vibrational-rotational spectroscopy.

ACC should be studied in detail for its isotopic composition. Its relevance to biogenic carbonate precipitation should be analyzed. The isotopic composition of ACC should be carefully studied, with care taken to compare its composition with solution DIC. Different ACC synthesis methods should be tested against each other to check for biases in isotopic composition. ACC surface and interior isotopic composition should be evaluated using cluster models similar to those employed in this dissertation.

Other studies should focus on the methodology of isotope fractionation calculation using DFT. The fidelity of individual H-bond effects should be studied incrementally in small cluster models to determine whether large, computationally expensive models can be avoided altogether.
Effects between a larger set of functionals and basis sets on gas-phase and aqueous isotope fractionation should be evaluated to find the most computationally efficient methods capable of replicating harmonic vibrational frequencies. Isotope effects in other aqueous systems including S- and N-bearing species should be calculated and compared with experimental values, with a focus on the importance of H-bonds to fractionation. Organic molecules, especially proteins that could play a role in calcification, should also be studied, including both whole-molecule and atom-specific isotope fractionation, including the effects of H-bonds. The fidelity of these methods should be tested on H/D fractionation, which would be useful because of the large potential magnitude of fractionation between H and D, but which is rendered more challenging because of quantum effects on H like tunneling which may impact kinetic fractionation in particular.

There is much left to address in the computational study of isotopic fractionation, but this dissertation makes clear that careful evaluation of computational chemistry models may provide quantitatively useful insights into isotope fractionation even in complex aqueous systems.
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