IN-SITU OBSERVATIONS OF CALCIUM CARBONATE PHASE HETEROGENEITY
DURING MINERAL PRECIPITATION: IMPLICATIONS FOR THE
INTERPRETATION OF CALCIUM CARBONATE BASED PROXIES

A Thesis in

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by

Matthew S Gonzales

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The thesis of Matthew S. Gonzales was reviewed and approved* by the following:

Matthew S. Fantle  
Assistant Professor of Geosciences  
Thesis Advisor

Michael Arthur  
Professor of Geosciences

Peter Heaney  
Professor of Geoscience

James Kubicki  
Professor of Geosciences

Lee Kump  
Professor of Geosciences  
Head of the Department of Geosciences

*Signatures are on file with the Graduate School.
Abstract

The isotopic and trace element composition of CaCO₃ minerals are powerful proxies for global weathering rates, changes in ocean chemistry, and global and local climate. The use of CaCO₃ based proxies is complicated by isotopic and elemental fractionation during precipitation, the formation of multiple polymorphs under Earth surface conditions, and the observation that isotopic and elemental partitioning is a function of the mineralogy of the solid phase. While calcite is the most stable polymorph, and thus the dominant mineralogy found in the rock record, the pathway by which calcite form remains a topic of intense research.

In this study, the influence of solution chemistry on the formation of CaCO₃ polymorphs was investigated. Unseeded precipitation was initiated in small volume (0.1 mL) flow-through reactors, and mineral growth observed in-situ using optical microscopy and Raman spectroscopy. Solutions containing CaCl₂ and Na₂CO₃ were mixed in the reactor from separate reservoirs using syringe pumps to control flow rate (0.005–0.1 mL/min). Initial saturation indices (with respect to calcite) ranged from 1.1 to 2.3, while starting pH varied from 8.3 to 10.5. A subset of the experimental solution included NaCl, NH₄Cl, Na₂HPO₄, and L-arginine as low level additives. In nearly all experiments, both rhombic calcite and vaterite (in almond or flower morphologies) were observed. Calcite to vaterite ratios varied within reactors between 0.1 and 187 and were dependent mainly on saturation state, though the location of growth within the reactor was also important. Both calcite and vaterite precipitated concurrently and no phase transitions were observed during experiments. The presence of additives such as Na₂HPO₄ and L-arginine and, to a lesser extent, NH₄Cl inhibited the precipitation of vaterite, while NaCl slightly promoted the precipitation of calcite. The results suggest that the interpretation of Ca isotope data from precipitation experiments may be markedly affected by sampling protocol, the effects of additives, and method by which saturation is achieved to initiate precipitation.
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1. Introduction

1.1 Motivation

Calcium carbonate (CaCO$_3$) rocks and minerals are among the most important sources of information about the past available to geologists. It is estimated that as much as 20% of sedimentary rock deposited during the Phanerozoic is composed of CaCO$_3$ minerals, making it one of the most ubiquitous on the Earth’s surface (Morse et al., 2007). During mineral formation, CaCO$_3$ effectively records the solution chemistry in such a manner that the geochemical composition of the mineral can be interpreted in terms of climate, ocean chemistry, and redox state. For instance, the carbon isotopic composition of CaCO$_3$ deposits across the globe have provided insight into such events as global scale glaciation during the Neoproterozoic, which led to the Snowball Earth hypothesis (Hoffman and Schrag, 2002; Halverson et al., 2002). Both carbon and oxygen isotopes in lacustrine CaCO$_3$ have proven valuable tools in the reconstruction of local climates and have provided insight into past temperatures, precipitation patterns, and evaporation rates (Leng and Marshall, 2004). Pairing carbon and oxygen isotopes with Mg/Ca ratios in planktonic tests from deep sea cores has constrained the temperature rise associated with rapid climate change during the Paleocene-Eocene Thermal Maximum (Zachos et al., 2003). The isotopic composition of trace strontium in marine CaCO$_3$ has provided evidence for increased weathering rates during the Cenozoic, supporting the contention that climate is influenced by silicate weathering and, hence, tectonic activity (Richter et al., 1992; Raymo and Ruddiman, 1992). Given the apparent usefulness of CaCO$_3$ as a recorder of the past, it is then critical that the processes by which it forms and records geochemical signals are fully understood. Without sufficient knowledge of these mechanisms, geochemical records could be misinterpreted, leading to uncertain or inaccurate understandings of the Earth system.

Currently, one of the most significant gaps in our knowledge of CaCO$_3$ formation is the effect that precipitation of metastable CaCO$_3$ phases can have on geochemical
proxies. While calcite is the most common CaCO₃ mineral found in the rock record and the most thermodynamically stable of the CaCO₃ phases, there are at least six additional metastable CaCO₃ phases that have been observed to form (Morse et al., 2007; Cartwright et al., 2012) Because the non-calcitic phases are metastable at Earth surface conditions, they will eventually react to form calcite; however, the observed pathways, or series of metastable phases that form prior to calcite, are varied (Ogino et al., 1987; Brečević and Nielsen, 1989; Clarkson et al., 1992, Pouget et al., 2009; Rodriguez-Blanco et al., 2011; Rodriguez-Blanco et al., 2014). There is experimental evidence that suggests that mixtures of calcite and metastable polymorphs often precipitate (Niedermayr et al., 2013) and can transition to calcite within days or hours (Ogino et al., 1987; Rodriguez-Blanco et al., 2011; Nielson and De Yoro, 2014). Given that calcite may initially form as a metastable phase, the process by which geochemical proxies are recorded within CaCO₃ is not dictated solely by the precipitation of calcite as. Instead, metastable phases are responsible for the initial capture materials that make up the precipitated solid and record a geochemical signal. That signal may be or may not be preserved as the solid matures and eventually becomes calcite. Therefore, Intimate knowledge of the formation of metastable phases and their evolution to calcite is required to accurately interpret CaCO₃ based proxies.

The work presented in this study investigates the formation of CaCO₃ phases at conditions relevant to calcium carbonate mineral formation in nature. This is intended to inform discussion on proxy development and, in particular, the Ca isotopic composition of CaCO₃. The Ca isotopic composition of CaCO₃ has become an increasingly utilized proxy for past ocean chemistries, climate, and weathering rates (DePaolo, 2004; Fantle, 2010). However, experimental observations designed to constrain the Ca isotopic fractionation factor during the precipitation of calcite contradict each other (Lemarchand et al., 2004; Tang et al., 2008b; Tang et al 2012). Herein, it is hypothesized that the coprecipitation of multiple CaCO₃ phases may be the root cause of the experimental disagreement. The experiments discussed here will test to what extent phase
heterogeneities could exist in previous CaCO₃ precipitation experiments and how such heterogeneities could influence the interpretation of the Ca isotope fractionation factor.

1.2 Background

1.2.1 The Carbonate System and Saturation Index

The thermodynamic potential for CaCO₃ to precipitate or dissolve in a solution can be determined using the following equation:

$$\Omega_j = \frac{a_{Ca^{2+}} a_{CO_3^{2-}}}{K_{sp,j}}$$  \hspace{1cm} (1)

where $\Omega_j$ is the saturation state with respect to phase $j$, $a_{Ca^{2+}} a_{CO_3^{2-}}$ is the ion activity product (IAP) of the Ca²⁺ and CO₃²⁻ ions, and $K_{sp,j}$ is the solubility product of phase $j$. For the purposes of this thesis, saturation state is described in terms of saturation index (SI): $SI_j = \log \Omega_j$. For simplicity, SI will refer to the saturation index with respect to calcite throughout this thesis (unless otherwise noted). When $SI_j > 0$, the solution is saturated with respect to the phase $j$, and the precipitation of CaCO₃ (Eqn. 2) is energetically favorable:

$$Ca^{2+} + CO_3^{2-} \rightleftharpoons K_{sp} CaCO_3(S)$$  \hspace{1cm} (2)

As CaCO₃ precipitates from an oversaturated solution, the saturation state is reduced as the IAP drops. The loss of carbonate ion is mitigated to some extent by the reequilibration of the aqueous carbonate system (Eqns. 3 and 4):

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \hspace{1cm} K_{a1}$$  \hspace{1cm} (3)

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+ \hspace{1cm} K_{a2}$$  \hspace{1cm} (4)

where $K_{a1}$ and $K_{a2}$ are the acid disassociation constants for $H_2CO_3$ and $HCO_3^-$, respectively.
1.2.2 Description of CaCO₃ Phases

Calcium carbonate can take the form of one of six major phases: calcite, aragonite, vaterite, ikaite, monohydrocalcite, and amorphous calcium carbonate (ACC). All phases, save for calcite, are metastable at Earth surface conditions and will transform into calcite unless the reaction is otherwise inhibited (Ogino et al., 1987; Koga et al., 1998; Rodriguez-Blanco et al., 2011). Critical to this stability is the observation that calcite has the lowest solubility product of all CaCO₃ phases (Table 1). Calcite and aragonite are the most common in nature and generally the most intensively studied of the polymorphs.

Calcite is the most commonly observed CaCO₃ phase in nature and is one of the most ubiquitous minerals on the planet. This is largely due to the solubility of Ca, the biological mediation that overcomes kinetic barriers under Earth surface conditions, and the stability of calcite relative to other CaCO₃ phases. The formation of biogenic calcite is widespread as building material in the shells and hard parts of nearly all the biologic kingdoms (Lowenstam, 1981; Addadi et al., 2003 Cartwright et al., 2012). Most notably, calcite is the primary building material for most foraminiferal tests (Todd and Blackmon, 1956; Erez, 2003), making it the primary mineral found in pelagic carbonates at the seafloor and a valuable proxy archive.

Aragonite is the second most thermodynamically stable of the CaCO₃ phases and commonly forms as the result of biologic processes, especially in settings where the Mg/Ca ratio is above 1 (Morse et al., 1997). The presence of Mg²⁺ is known to favor the formation of aragonite compared to calcite (Berner, 1975, Mucci and Morse, 1983; Bots et al., 2011). Relative to aragonite, calcite surfaces will adsorb about an order of magnitude more Mg onto its surface causing growth to be inhibited (Morse et al., 2007). Additionally, less MgCO₃ coprecipitation occurs with aragonite compared to calcite such that aragonite tends to be more pure, less soluble, and the favored polymorph compared to calcite in a high Mg environment (Katz, 1973; Morse and Mackenzie, 1990). As the
molar Mg/Ca ratio in solution approaches 1 at 25 ºC, aragonite becomes dominant (Morse et al., 1997, Niedermayer et al., 2013). The precipitation of calcite in an ocean setting requires the active removal of Mg$^{2+}$ from solution, so it has become energetically favorable for organisms to form aragonite rather than calcite for their tests and structural components throughout various periods in Earth’s history (Lowenstam, 1981). Perhaps the most notable of these are corals, many of which precipitate aragonitic skeletons (Erez, 2003).

Vaterite is the third most thermodynamically stable form of CaCO$_3$, but is rarely observed in nature. This is primarily due to its instability; in experiments, it has been observed to transition to calcite over time scales of hours (Ogino et al., 1987; Rodriguez-Blanco et al, 2011). Despite this instability, vaterite has been found in the shells of mollusks and arthropods (Lowenstam, 1981; Addadi et al., 2006; Hasse et al., 2000; Furuhashi et al., 2009) and in soils (Rodriguez-Navarro et al., 2007). Because of its transience, vaterite is often undetected in experiments that are not specifically designed to either stabilize it or observe on short time scales. Often vaterite is seen as a precursor of calcite; however, the transition between vaterite and calcite has been suggested to occur via dissolution-reprecipitation, which leaves no clear evidence of vaterite’s presence in the final solid (Ogino et al., 1987; Rodriguez-Blanco et al., 2011). Interestingly, the crystal structure of vaterite is not fully explored. It is thought to be composed of at least two coexisting structures: a major hexagonal structure and multiple minor structures that have yet to be fully characterized and confined to nanodomains (Demichelis et al., 2013; Kabalah-Amitai et al., 2013).

The hydrated forms of CaCO$_3$, ikaite and monohydrocalcite, are the least observed of the CaCO$_3$ phases in nature, though may be favored to form under specific environmental conditions. The formation of ikaite, for instance, appears to be favored at low temperatures (<4 ºC; Clarkson et al., 1992; Gussone et al., 2011) and ikaite has been observed in polar sea ice (Dieckmann et al, 2008; Dieckmann et al., 2010). Under experimental conditions, monohydrocalcite has been observed as a precursor to aragonite.
as it also tends to form in Mg\(^{+2}\) rich waters (Kralj and Brečević 1995; Zhang et al., 2012; Rodriguez-Blanco et al., 2014). In nature, monohydrocalcite has been found in lake sediments or as crusts along lake shorelines where the molar Mg/Ca ratio of lake water was \(\sim 7\), about 1.4 times higher than that of the modern ocean (Taylor, 1975; Bird et al 1995).

Amorphous calcium carbonate (ACC) is the least thermodynamically stable of the CaCO\(_3\) phases, but plays a critical role as a precursor phase to the crystalline polymorphs. Amorphous calcium carbonate is actually a group of at least twelve different possible structures of CaCO\(_3\) with short range order relative to calcite, aragonite, and vaterite that serve as a precursor to the stable CaCO\(_3\) polymorphs (Clarkson et al., 1992; Addadi et al., 2003; Weiner et al., 2005; Rodriguez-Blanco et al., 2011; Cartwright et al., 2012). Defining these structures remains an area of active debate, but there is evidence that at least some forms of ACC exhibit distinct short range structures that can be related to the various crystalline CaCO\(_3\) polymorphs (Cartwright et al., 2012). The transition from ACC to crystalline CaCO\(_3\) has been reported to occur as quickly as 20 minutes (Rodriguez-Blanco et al., 2011) making it difficult to capture in experiments or freely in nature. Despite being metastable, biogenic ACC has been recognized within many organisms (Weiner et al., 2003). For example, it is a structural component in sponge spicules and serves as a reservoir for future availability of CaCO\(_3\) in earthworms and arthropods (Cartwright et al., 2012).

### 1.2.3 Experimental Precipitation of CaCO\(_3\) and Observations of Phase Transitions

Researchers have employed a wide variety of techniques to precipitate CaCO\(_3\) and observe phase transitions. Primarily, the technique employed is dependent first on which CaCO\(_3\) phase or phases are desired and secondly on what parameters need to be controlled or tested. Most experiments are designed to precipitate CaCO\(_3\) for some time and then remove it from the growth reactor for analysis while the chemical properties of the parent solution are monitored through time.
The precipitation of calcite and aragonite are mostly dependent on controlling the saturation state of the parent solution such that other phases do not become thermodynamically favored. A popular method is to slowly introduce CO₂ by gas diffusion into an undersaturated, Ca²⁺-rich solution such that CO₃²⁻ will form and the solution become saturated. This technique has its roots in studies by Holland et al. (1964) and Gruzenski (1967). Holland et al. (1964) precipitated calcite using a bomb reactor in which CO₂ was pressurized in the headspace above a CaCl₂ solution up to 60 atm at temperatures as high as 100°C. Gruzenki (1967) used the decomposition of ammonium carbonate crystals to create a CO₂ atmosphere within a sealed vessel containing an undersaturated Ca²⁺ solution, an approach adopted by both Paquette and Reeder (1990) to observe crystal zoning and Lemarchand et al. (2004) to determine the Ca isotopic fractionation factor associated with calcite precipitation. Variants on the slow CO₂ dissolution technique include CO₂ diffusion through a polyethylene membrane into an undersaturated Ca²⁺ solution (Dietzel and Usdowski, 1996; Dietzel et al., 2004; Tang et al., 2008a; Tang et al., 2012) and bubbling N₂-CO₂ gas mixtures through the Ca²⁺ solution (Watkins et al., 2013). Another popular method has been the use of titrators that slowly add Ca²⁺ and CO₃²⁻ solutions to a reacting parent solution (White, 1977; Mucci and Morse, 1983; Lorens 1981). These experiments had the advantage of controlling solution chemistry to maintain such parameters as pH and saturation state. To precipitate aragonite, researchers have added Mg²⁺ to synthetic growth solutions or used natural solutions already rich in Mg²⁺, such as seawater (Kinsman and Holland, 1969; Berner, 1975; Gussone et al., 2003; Gussone et al., 2005; Zhang et al., 2012, Niedermayr, 2013).

Experimental studies that have precipitated vaterite, ikaite, and monohydrocalcite, have done so at high saturation states to produce the desired phase. For vaterite, these high saturation states are generally induced through high speed addition of gaseous CO₂ into an undersaturated Ca²⁺-rich solution or mixing of CaCl₂ and Na₂CO₃ or NaHCO₃ solutions (Turnbull, 1973; Gussone et al., 2011; Rodriguez-Blanco et al., 2011). Ikaite has been produced by mixing concentrated (> 0.2 M) Ca²⁺ and CO₃²⁻ while keeping the temperature of the mixture below 4°C (Clarkson et al., 1990; Gussone et al., 2011).
Recently, a study by Rodriguez-Ruiz et al. (2014) produced ikaite at 20°C by stabilizing precipitation within picoliter and smaller volumes and indicating that ikaite might be a more common precursor than previously thought. Finally, monohydrocalcite is precipitated in solution with molar Mg/Ca ratios greater than 0.5 as a precursor to aragonite (Kralj and Brečević 1995; Zhang et al., 2012; Rodriguez-Blanco et al., 2014).

Typically, inorganic ACC is observed only in experiments, particularly in those conducted at high saturation states (SI > 2; Ogino et al., 1987; Brečević and Nielsen, 1989; Clarkson et al., 1992, Pouget et al., 2009; Rodriguez-Blanco et al., 2011; Rodriguez-Blanco et al., 2014). In all cases, ACC is extremely transient, often transitioning to other phases within minutes of formation (Ogino et al., 1987; Brečević and Nielsen, 1989; Clarkson et al., 1992, Pouget et al., 2009; Rodriguez-Blanco et al., 2011; Rodriguez-Blanco et al., 2014), requiring special methods of observation such as time resolved XRD, rapid filtration, or cryo-TEM. Though ACC forms readily at high saturation states, it is hypothesized that ACC is a precursor to more crystalline CaCO₃ phases such as vaterite and aragonite at lower saturation states (Pouget et al, 2009; Cartwright et al., 2012; Nielsen and De Yoreo, 2014).

Complicating our understanding of CaCO₃ formation is the fact that the pathway by which calcite is precipitated remains unconstrained. While there is a general understanding of what CaCO₃ phases can form as precursors to calcite from a given solution (Fig. 1), the abundances of the more metastable phases like vaterite, ikaite, and monohydrocalcite as well as their longevity is poorly constrained. This is especially a problem in understanding CaCO₃ formation in nature and experiments where SI < 2 as many of the experiments that observe phase transitions occur at higher SI (Fig. 2). This is largely due to the experimental need to produce observable material quickly and in large quantities to meet analytical requirements. Typically, experiments that precipitate CaCO₃ at low SI have had considerable durations (48 hours) and often produce only a few milligrams of material, precluding adequate material characterization (Lemarchand et al., 2004; Tang et al., 2008a.; Niedermayr et al., 2013).
1.2.3 Relevance of CaCO₃ Mineralogy to Development of the Ca Isotope Proxy

The Ca isotopic composition of carbonates has been utilized as a unique proxy over the past decade. In particular, Ca isotopes in marine carbonates have been invoked as a proxy for global weathering fluxes and changes in ocean chemistry over million-year timescales (De La Rocha and DePaolo, 2000; Fantle and DePaolo, 2005; Farkaš et al., 2007; Fantle, 2010, Blättler et al., 2011), as well as a paleo-temperature proxy (Nägler et al., 2000). In the former instance, the Ca isotopic composition of marine carbonates has been used to infer changes in the Ca concentration of the ocean over time that constrains sedimentation relative to weathering mass fluxes and climate on a global scale. Additionally, Ca isotopes have been utilized to constrain diagenetic recrystallization rates of seafloor sediments (Fantle and DePaolo, 2007; Fantle et al., 2010).

The Ca isotopic composition of measured materials is typically reported as $\delta^{44}$Ca (Eqn. 5):

$$
\delta^{44}Ca = \left( \frac{^{44}Ca}{^{40}Ca} \right)_{\text{sample}} \cdot \left( \frac{^{40}Ca}{^{44}Ca} \right)_{\text{standard}} - 1 \cdot 1000
$$

where $^{44}Ca/^{40}Ca$ is the Ca isotopic ratio of either the sample or reference standard. On this relative scale, delta values are reported in units of permil (‰). In the current study, the isotopic fractionation of Ca during mineral formation will be reported as $\Delta^{44}$Ca according to :

$$
\Delta^{44}Ca_{s-f} = \delta^{44}Ca_{\text{solid}} - \delta^{44}Ca_{\text{fluid}}
$$

The reported $\Delta^{44}Ca_{s-f}$ values describe the difference in Ca isotopic composition between a precipitated solid and the solution from which it precipitated. The $\Delta^{44}Ca_{s-f}$ notation approximates the fractionation factor associated with a given reaction in permil units.
\[ \Delta^{44}Ca_{s-f} \approx 10^3 \ln \alpha_{solid-fluid} \] (7)

where \( \alpha_{solid-fluid} \) is defined as:

\[ \alpha_{solid-fluid} = \left( \frac{^{44}Ca}{^{40}Ca}_{solid} \right) / \left( \frac{^{44}Ca}{^{40}Ca}_{fluid} \right) \] (8)

Critical to the use of the Ca isotope proxy is an understanding of isotopic fractionation during the initial precipitation of the proxy material. While there have been numerous studies examining the controls of Ca isotope fractionation during CaCO\(_3\) formation, a clear consensus has yet to be reached. The two studies that laid the foundation for this work are Lemarchand et al. (2004) and Tang et al. (2008b). Both studies report the Ca isotopic composition of calcite precipitated at SI < 2.1 and note a relationship between the precipitation rate of calcite and the Ca isotopic fractionation (Fig. 3a). However, the two studies observed opposite trends such that Lemarchand et al. (2004) saw fractionation decrease (move closer to zero) as precipitation rate increased while Tang et al. (2008b) saw fractionation increase to a maximum of -1.5 ‰ as rate increased. The Tang et al. (2008b) study has since become the favored dataset for constraining kinetic isotope effects during calcite formation and ion-by-ion growth models have provided possible explanations for the observed trend (DePaolo, 2011; Nielsen et al., 2012). Additional precipitation experiments have repeated the outcome at high ionic strength (Tang et al., 2012). The results produced by Lemarchand et al. (2004), however, have yet to be fully explained or replicated, and it is unknown how both studies could produce calcite at similar conditions yet yield completely different results. This apparent contradiction motivates the current study.

Perhaps the largest difference in both the Lemarchand et al. (2004) and Tang et al. (2008b) datasets is the observation that the experiments are conducted at different saturation states. While both sets of experiments document CaCO\(_3\) precipitation at relatively low saturation states (SI < 2), the Tang et al. (2008b) experiments were,
without exception, conducted at SI < 1.25. When the $\Delta^{44}\text{Ca}_{\text{vaterite}}$ values from both studies are plotted relative to saturation state, it becomes clear that the two trends do not overlap to the same extent as they do in precipitation rate space. Instead, the two studies are roughly separated at SI = 1.25 with the Lemarchand et al. (2004) dataset occurring at significantly higher saturation states (Fig. 3b). The resultant question this raises, then, is if the higher saturation states characteristic of the experiments of Lemarchand et al. (2004) supported the precipitation of non-calcite CaCO$_3$ phases. Such a hypothesis is thermodynamically reasonable as a SI$_{\text{vaterite}}$ was between 0.4 and 1.9 during the Lemarchand et al. (2004) experiments. In fact, Lemarchand et al. (2004) state that vaterite was observed in a some of the experimental reactors, though the Ca isotopic composition of these were not measured. However, the observation begs the question: was vaterite was more widespread than previously thought? If so, its presence could have had an important effect on the interpretation of the Ca isotope fractionation factor.

To date, there is little work on Ca isotope fractionation associated with the precipitation of non-calcite CaCO$_3$ phases. The work done to date suggests that the Lemarchand et al. (2004) experiments may have been affected by polymorph precipitation (Gussone et al., 2003; Gussone et al., 2005; Gussone et al. 2011). These data suggest that the Ca isotope fractionation associated with the precipitation of aragonite, vaterite, and ikaite are similar to the Lemarchand et al. (2004) trend in rate space (Fig. 3a). The Gussone et al. (2011) experiments produced a limited array of data for vaterite and ikaite as a function of rate, but clearly demonstrate minimal fractionation ($\Delta^{44}\text{Ca}_{\text{vaterite}} \sim -0.5\%$) at relatively high precipitation rates. While the work on Ca isotopic fractionation during non-calcite CaCO$_3$ precipitation is not extensive, it has been observed that the mixed precipitation of aragonite or vaterite with calcite can alter the measured fractionation factors of C and O isotopes (Tarutani et al., 1969; Romanek et al., 1992). Therefore, it is hypothesized that the magnitude by which Ca isotopes are fractionated could also vary depending on the phase precipitated.
Recent studies suggest that the mechanism by which isotopes are fractionated during vaterite precipitation could be fundamentally different than during calcite precipitation. This idea comes from the notion that calcite and vaterite growth occurs by different mechanisms. In particular, during nucleation it has been observed that crystalline calcite nucleates directly from solutions while vaterite passes through an ACC intermediate (Nielsen and De Yoreo, 2014). As vaterite crystals continue to grow, it has been hypothesized that it does so by aggregating nanoparticles, and not by the ion-by-ion step growth mechanisms associated with calcite precipitation (Hu et al., 2012). Further, vaterite has been shown to be disordered, containing several nanodomains that do not exhibit the typical hexagonal crystal structure typically assigned to the phase (Demichelis et al., 2013; Kabalah-Amitai et al., 2013). It is therefore possible that the nanoparticle aggregation pathway isotopically fractionates Ca differently compared to the ion-by-ion growth mechanism associated with calcite. Given the results from Gussone et al. (2011), aggregation should be less fractionating than ion-by-ion growth at high precipitation rates.

1.3 Objectives

The main goal of this work is to provide a bridge between our current understanding of CaCO₃ phase formation and controls on Ca isotopic fractionation during CaCO₃ precipitation. There is a considerable gap between experiments whose aim is to explore metastable CaCO₃ phases, which are conducted at high saturation states (SI > 2.5), and experiments whose aim is to constrain Ca isotopic fractionation, which are conducted at relatively low saturation states (SI > 2.5). This work fills this gap by exploring the precipitation of CaCO₃ at saturation states relevant to those of previous Ca isotope experiments and seeks to answer the following questions:

1) Do non-calcite CaCO₃ phases readily form at low saturation states (SI < 2.5)?
a. What controls the formation of the various CaCO₃ phases in a low saturation state setting?

b. How stable are metastable CaCO₃ phases in low saturation settings?

2) How can the formation of non-calcite CaCO₃ phases at low saturation states affect our understanding of the Ca isotopic fractionation factor?

To address these questions, CaCO₃ precipitation experiments are conducted using a new flow-through reactor system which allows for *in-situ* observations of precipitation occurring at the micrometer scale. These reactors provide an opportunity to detect metastable phase that could transition to calcite before an experiment is over as well as quantification of phase abundance throughout the reactor. As a flow through system is employed, any chemical composition or saturation state can be achieved within an experiment and maintained in time.

**2. Methods**

**2.1 CaCO₃ Precipitation Method**

Calcium carbonate precipitates were formed by mixing Ca²⁺ rich and CO₃²⁻ rich solutions together in small volume flow-through reactors. These solutions were made gravimetrically by dissolving a measured mass of ACS grade anhydrous CaCl₂ and ACS grade anhydrous Na₂CO₃ salts (both 99.5% pure) within the appropriate mass of deionized water (18.2 MΩ·cm) to achieve the desired concentrations. Calcium chloride solutions contained ~300, 150, or 20 mmol Ca per kg of solution, while Na₂CO₃²⁻ solutions contained ~300 μmol CO₃²⁻ per kg solution. Target CO₃²⁻ concentrations were achieved by matching the DIC needed at a given pH, as calculated by the equilibrium thermodynamic program PHREEQC (version 2.18.3.5570). In some experiments, ACS grade NaCl or ACS grade NH₄Cl salts (99.5%) were added gravimetrically to the CaCl solution. Pre-diluted solutions comprised of ACS grade Na₂HPO₄ (99.5% pure) or L-arginine (98% pure) salts were added to CO₃²⁻ solutions. Both the CaCl and Na₂CO₃ (± additives) solutions were then adjusted to the target pH by adding 0.5 N NaOH or 0.5N
HCl, respectively. All pH values were determined using a Mettler Toledo Easy-Five Benchtop pH meter. The resulting solutions were then continuously injected into the flow-through reactor at equal flow velocities (Table 1).

2.2 Reactor Design

A major objective of this study was to conduct experiments in which the distribution of CaCO$_3$ phases could be observed in time without halting the precipitation reaction. This required a closed, transparent, and portable reactor capable of being observed over time, as opposed to the batch reactors used previously (e.g., Dietzel and Usdowski 1996; Lemarchand et al., 2004; Tang et al., 2008a; Watkins et al., 2013). Accordingly, a flow-through reactor was constructed in which the two mother solutions could be continuously mixed (Fig. 4). The reactor is sized so that it is compatible with both optical microscopic and RAMAN spectroscopic instrumentation, which permit the identification and characterization of crystals larger than 1 μm..

Reactors were constructed from 1/16 inch (~1.6 mm) thick clear impact resistant polycarbonate, cut into into 25 x 75 mm strips (about the size of a standard glass microscope slide). A 3.175 x 22 mm channel was carved lengthwise through the strip to form the reactor cell. Using a 5/64 inch drill bit, three diagonal cuts were then made into the channel ends, one on the left parallel to the channel and two on the right approximately 30° from the channel. These cuts were angled to form ramps leading into and out of the channel; the two on the right served as inlets and the single ramp on the left served as an outlet. The reactor was sanded smooth using 600 grit wet sandpaper, covered with a microscope cover slide (0.17 to 0.25 mm thick) on the top and bottom of the channel, and sealed with paraffin wax. The bottom was covered using a 22 x 40 mm glass slide while the top was covered with a 22 x 22 mm glass slide. PTFE microbore tubing with a 0.066 inch outer diameter was inserted into the reactor along the ramps on the top and all remaining openings sealed with paraffin wax. Each input tube was
connected to a 10 mL syringe using a 19 gauge 1 inch long single use needle while the output tube was connected to a waste container. Finally, the syringes were connected to a syringe pump capable of controlling the flow rates into the reactor from each syringe.

### 2.3 Reactor Operation

Prior to all experiments, newly constructed reactors, new cover glasses, and new BD Luer-Lok tip syringes were rinsed using ethanol then sonicated in deionized water (18.2 MΩ·cm) for at least 30 minutes. After sonication, the reactors and cover glass were allowed to dry overnight. Three lengths of PTFE microbore tubing (0.042"ID x 0.066"OD) were cleaned by pushing through at least 20 mL of deionized water. Excess water was allowed to slowly drain from tubing so that no water droplets remained. In all but one experiments, two 10 mL syringes were used to deliver mother solutions to the reactor (typical runtimes ~#). In the case of experiment 7, two 60 mL syringes were used to accommodate the 72 hour runtime.

Reactors were constructed from the clean and dried materials, as described above, and connected to the syringe pump. Syringes were filled with the mother CaCl₂ and Na₂CO₃ solutions and placed on a Harvard Apparatus PHD 2000 syringe pump. One-inch, 19-gauge BD PrecisionGlide disposable needles were connected to the syringes and the needle connected to the reactor; 19-gauge needles have a nominal outer diameter of 0.042”, which fit tightly into the tubing with a 0.044 inch inner diameter. The output tubing was secured to a waste container using tape. Finally, the seals between the syringes, needles, and tubing were coated with paraffin wax to prevent air bubbles from forming within the tubing. Flow rates were set by inputting the syringe inner diameter into the pump.

Experiments were initiated by activating the syringe pump. Before the mother solutions reached the reactor, the pump was stopped and the flow rate adjusted to 0.1 mL/min. The pump was then restarted and the reactor held vertically to prevent bubble formation. Once the cell was filled and the effluent began to be collected, the reactor was
laid flat. After five minutes of operation, the delivery rate from the pump was adjusted to either 0.005 mL/min or 0.01 mL/min and experiments run for 14 to 72 hours (typical runtimes: ~24 hours). Runtimes were chosen to achieve sufficient mass to precipitate, which allowed for visual precipitate identification and sufficient time to observe growth patterns and phase changes (if present). Experiments were not temperature controlled, but temperature was monitored and ranged from 20 to 25°C.

After an experiment was completed the tubing was removed from the reactor by cutting through it and the wax just outside of the mixing cell. The output tube was always removed prior to the input tubes to prevent solution movement due to both ends of the reactor being open. Immediately after a tube was removed the exposed hole was coated with paraffin wax. With all three tubes removed, the reactor was analyzed by Raman spectroscopy or imaging using an optical microscope and/or a scanning electron microscope (SEM). In some cases, the reactor cover glass was removed to allow for more precise Raman analysis or imaging by SEM. First the cell area was traced using a permanent marker on the outside of the cover glass. The glass was then removed by peeling away the wax seal with a razor blade, rinsed with ethanol, then dried with N₂ gas. Care was always taken to not touch or disturb deposited solids by slowly spraying the ethanol onto areas of the glass that did not directly cover the reaction cell and allowing the rinse to slowly move over the glass.

2.4 Optical Microscopy

To observe CaCO₃ precipitation in-situ, all experiments were observed using a Nikon Eclipse TE2000 – U inverted optical microscope. Fully constructed reactors fitted to the syringe pump were placed on the microscope stage using a plate designed to hold standard microscope slides by the edges (Fig. 5). The reactor was manipulated with a mechanical stage that allowed for precise horizontal movement in the X and Y directions via a joystick. Movement along the Z axis was controlled by changing the focus of the objective. Still images and video were captured using a digital camera that allowed
images up to a resolution of 2080 x 1552 while viewing an area of approximately 1 mm² at 4x magnification. Typically, mineral precipitation was recorded at 0.5 frames per second in grayscale with a lowered resolution of 960 x 540 at 4x magnification. This allowed for a balance between visible details, wide field of view, and manageable file sizes for post-experiment analysis. Still images were captured at the maximum resolution using a range of magnifications from 4x up to 20x. At 4x magnification, particles 1 μm in diameter were visible through the camera. At higher magnifications the ability to observe particles smaller than 1 μm did not significantly increase and the wider field of view and larger range of focus provided by the 4x magnification was preferred.

2.5 Raman Spectroscopic Analysis

The mineralogy of experimental solids was determined using a WITec CRM 200 confocal Raman spectrometer. Individual crystals were targeted using the optical microscope, and then excited with an Ar 630 nm laser with power output ranging from 5 to 10 mW. Scattering data were collected over the course of 15 seconds to 1 minute depending on the sample and laser output. Before collecting scattering data, the instrument was calibrated with a Si standard. Generally, crystals were analyzed through the cover glass amid the trapped solution of a sealed reactor, but in cases in which crystals were difficult to focus on, the cover glass was removed. Most Raman analyses were conducted within 4 hours of the completion of a given experiment, although experiment 5 had been analyzed 3 months after it was conducted. Collected spectra were compared to previous work on Raman analysis of CaCO₃ polymorphs by Kontoyannis and Vagenas (2000) and Gopi et al. (2013) in order to identify mineralogy.

2.6 Scanning Electron Microscopic Imaging

Detailed images of precipitated solids were captured using a FEI Quanta 200 Environmental SEM instrument (ESEM), with beam voltages of 10 to 15 kV at pressures of 60 to 80 Pa. In all cases, the reactor was dismantled so the solids precipitated on the cover glass could be imaged directly. As the glass was susceptible to surface charging
effects, partial vacuum was used to mitigate some of the charging. Both the secondary electron and backscatter detectors were utilized depending on the imaging target. Images were generally collected within 6 hours of an experiment’s completion save for experiment 5, which was 3 months old prior to images.

2.7 Quantifying Phase Heterogeneity

Phase heterogeneities were quantified during and after experiments with the aid of still images of solids deposited on the upper and lower cover glasses. Nearly all visible areas of the top and bottom cover glass were imaged such that they could be combined into a large photo mosaic using photo editing software that showed the entire area in a single image (see appendix A). These images are approximately 1 gigapixel in size and retained all details from the original images. Once the mosaic was created the images were overlaid with a 7 x 44 grid. Using information gathered by Raman spectroscopy as described above to link observed morphologies to CaCO₃ phases, modal percentages were estimated within these grid spaces. This was accomplished by first determining the percent of glass coverage by each CaCO₃ phase using the area select tools in photo editing software and charts for estimating percent compositions (Terry and Chilingar, 1955). Areas that were obscured by more than 50 percent were ignored. Total coverage was calculated by adding the coverage in each grid and dividing by the total visible area across the grid. Finally, ratios of observed phases were determined by first calculating the percentage of coverage for each phase across the grid and then dividing those percentages by each other.

2.8 Computation Fluid Dynamics Model

A computational fluid dynamics model of solutions moving through the reactor was created using OpenFOAM. OpenFOAM (Open Field Operation and Manipulation) is an open source computational fluid dynamics package that includes a wide array of solvers, mesh creation tools, and post-processing applications. OpenFOAM was used to create a 3D mesh of the cell portion of the reactor where most of the solution mixing and
mineral precipitation occurs. The mesh was created on 1:1 scale with an idealized reactor cell using tetrahedra approximately 0.03 mm$^3$ in volume with a portion of the input and output tubes (Fig. 6). Flow within the reactor is generated through a fixed flux of 0.05 mL/min coming from the ends of the input tubes. Both transient and steady-state solvers for incompressible flow were used to model the movement of solution into and out of the reactor. The transient solver modeled 10 seconds of real time data while the steady-state solver achieved stability within 0.05 seconds. No significant difference was observed between the end results.

2.9 Modeling Effect of Phase Heterogeneities on Ca Isotope Fractionation Factor

To simulate the evolution of an experimental CaCO$_3$ precipitation system, a time dependent box model was created. Evolution of the Ca isotopic composition for a reservoir, $i$, was governed by the following general form equations:

$$\frac{dr_i}{dt} = \sum_{\text{input}} \frac{F_{\text{input}}}{N_i} \left( r_j - r_i \right) + \sum_{\text{output}} r_i \frac{F_{\text{output}}}{N_i} \left( 1 - \alpha_{\text{output}} - i \right) \tag{10}$$

and the mass evolution of reservoir $i$:

$$\frac{dN_i}{dt} = \sum_{\text{input}} F_{\text{input}} - \sum_{\text{output}} F_{\text{output}} \tag{11}$$

where $i$ refers to the reservoir of concern (parent solution, bulk precipitated solid), $N$ is the size of the reservoir (in moles), $j$ is another reservoir in communication with $i$ via flux, $F_{\text{input}}$ (in moles per time), $F_{\text{output}}$ is the flux leaving reservoir $i$, $r$ is the $^{44}\text{Ca}/^{40}\text{Ca}$ ratio and $\alpha_{\text{flux-reservoir}}$ is the fractionation factor between a flux and a reservoir. The model was built such that Ca was removed from a parent solution to form either calcite or vaterite (Fig. 7). The specific equation for the isotopic evolution of the parent solution:

$$\frac{dr_{\text{soln}}}{dt} = r_{\text{soln}} \frac{F_{\text{vaterite}}}{N_{\text{soln}}} (1 - \alpha_{\text{vaterite}}) + r_{\text{soln}} \frac{F_{\text{calcite}}}{N_{\text{soln}}} (1 - \alpha_{\text{calcite}}) \tag{12}$$
the isotopic evolution of the precipitating bulk vaterite:

\[
\frac{d r_{\text{vaterite}}}{d t} = \frac{F_{\text{vaterite}}}{N_{\text{vaterite}}} (r_{\text{solution}} \alpha_{\text{vaterite}} - r_{\text{vaterite}})
\]  

(13)

and the isotopic evolution of precipitating bulk calcite:

\[
\frac{d r_{\text{calcite}}}{d t} = \frac{F_{\text{vaterite}}}{N_{\text{calcite}}} (r_{\text{solution}} \alpha_{\text{calcite}} - r_{\text{calcite}})
\]  

(14)

Fractionation factors were chosen to reflect observed fractionation from the highest rate or most saturated experiments. For calcite, \( \alpha_{\text{calcite}} = 0.9985 \) (\( \Delta^{44}\text{Ca}_{s-f} = -1.5\‰ \)) was based on the results of Tang et al. (2008b and 2012). For vaterite, \( \alpha_{\text{vaterite}} = 0.9995 \) (\( \Delta^{44}\text{Ca}_{s-f} = -0.5\‰ \)) was based on the results of Gussone et al., (2011). Fluxes were variable within the model and represented the precipitation rate of both calcite and vaterite. Fluxes were chosen such that:

\[
F_{\text{calcite}} = F_{\text{vaterite}} \left( \frac{N_{\text{calcite}}}{N_{\text{vaterite}}} \right)
\]  

(15)

where \( N_i \) is the mass of the phase \( i \), in moles, and the ratio of the two phases held constant over the course of a simulation. The initial \( N_{\text{soln}} \) was chosen to be 0.15 moles. While the model worked in isotopic ratios, solutions are converted to and reported here as \( \delta^{44}\text{Ca} \). All \( \Delta^{44}\text{Ca}_{s-f} \) values were calculated relative to the initial Ca isotope ratio of the solution. The model was solved using Euler’s method within Stella.
3. Results

3.1 Identified Calcium Carbonate Morphologies and Phases

In all experiments conducted, a heterogeneous mixture of calcite and vaterite precipitated throughout the reactor cell (Fig. 8). While these phases took on a variety of morphologies, they had several consistent features that made them readily identifiable from optical microscope images.

Calcite observed was typically limited to rhombic morphologies (Fig. 9). Deviations from the ideal rhombohedron calcite included the presence of additional steps on one or more crystal edges, twinning or fused crystals. The surfaces of most calcite particles imaged contained pits or lines that could indicate zoning or possible dissolution features, although no dissolution was visually observed in calcite during experiments. Calcite precipitated with additives such as Na$_2$HPO$_4$ or L-arginine tended to be more pitted and deviate from the simple rhombic shape; however it was difficult to quantify this using the optical microscope. However distorted the shape of calcite, it always retained straight edges and an overall rhombic appearance. Calcite grew as large as 100 μm from edge to edge. All morphological variants of calcite yielded a typical calcite Raman spectra, with the major peak at 1091 cm$^{-1}$ and three minor peaks at 163, 288, and 719 cm$^{-1}$ (Fig. 10). This characteristic spectra is similar to those observed by Kontoyannis and Vagenas (2000) and Gopi et al. (2013), though shifted to slightly higher wavenumbers (~1 to 5 cm$^{-1}$).

The morphologies of vaterite precipitates were more varied. In general, vaterite had characteristic almond, shell, flower, or spherical morphologies (Fig. 11). Often all four morphologies were visible within proximity of each other. While all morphologies were present in nearly all experiments, almonds and shells were more dominant in the high pH (10.5) experiments and flowers were more dominant in the low pH (8.3) experiments. Spheres were most common in experiments where the SI was greater than 2. Vaterite particle sizes were also quite varied. Almonds and shells grew as large as 50 μm,
flowers could grow as large as 150 μm, and spheres were observed to be larger than 5 μm. All variations of vaterite morphologies yielded similar Raman spectra (Fig. 10). The distinguishing feature of the vaterite spectra is a split peak centered around 288 cm\(^{-1}\) with additional peaks at 270 and 304 cm\(^{-1}\). Otherwise, vaterite shares the major peak at 1091 cm\(^{-1}\) with calcite and minor peaks appear at 216 and 748 cm\(^{-1}\). Often these minor peaks are obscured as the baseline of vaterite spectra tends to be elevated. The observed vaterite spectra are in close agreement with previous studies (Kontoyannis and Vagenas, 2000; Gopi et al. 2013).

3.2 Spatial Distribution of Phases in Reactors

As saturation state increased in the experiments, vaterite became the dominant phase precipitated (Fig. 12a). In experiments where the initial pH was 8.3, the calcite to vaterite ratio decreased from >10 to <0.1 as SI increased from 1.65 to 2.32. While pH 10.5 experiments also became more vateriteric with increasing SI, this shift occurred at much lower saturation states compared to experiments conducted at pH 8.3. At pH 10.5, the calcite to vaterite ratio decreased from >5 to <0.1 as SI increased from 1.05 to 1.3. As with vaterite abundance, the total coverage of the reactor surfaces by precipitates also increased with saturation state (Fig. 12b). At pH 8.3, coverage increased from nearly zero at SI = 1.7 to >40% at SI = 2.35. Again, there is a marked difference between pH 10.5 and 8.3 experiments, where the total coverage at comparable saturation states (~1.5 ± 0.2) was ~15% greater at high pH relative to low pH.

Low calcite to vaterite ratios in experiments with SI > 2 can be attributed to the formation of vaterite spheres. In these experiments, a large mass of small (1 – 3 μm) vaterite spheres coated the bottom cover glass of the reactors. These spheres were observed flowing through the center reactor before settling to the bottom glass. Their formation was never observed directly as their size made them difficult to focus on as they moved freely through the solution, although they were observed flowing from the solution inputs. This mass of sphere contributed greatly to the vaterite count on the
bottom glass of experiments higher with SI greater than 2 (Fig. 13b). Vaterite spheres were not observed in high pH experiments.

Within a single experiment, the largest disparity in phase distribution occurred between the top and bottom cover glasses (Fig. 13). The bottom glass was typically more vateritic and generally decreased the calcite to vaterite ratio by about an order of magnitude in experiments conducted at pH = 8.3. Additionally, coverage increased by as much as 30% on the bottom glass relative to the top. In terms of morphologies, the bottom glass was usually dominated by vaterite spheres at SI > 2, although spheres could be present in low abundances at lower saturation states. At the same time, more vaterite flowers and calcite rhombs precipitated on the top glass that were often larger by 20 – 30 \( \mu m \) relative to similar morphologies found on the bottom glass. In experiments conducted at pH 10.5, the calcite to vaterite tended to favor vaterite more than the lower pH experiments and the bottoms always had more coverage than the top.

Phase distribution changed across a reactor within a single experiment such that calcite to vaterite ratios generally increased moving from the input towards the output (Fig. 14). Along the top, coverage usually increased quickly moving from the input, peaked about 1/5 of the way through the reactor, and then trailed off moving towards the output. Along the bottom glass, coverage tended to increase moving from the input towards the output.

In experiments with no additives, the calcite to vaterite ratio tends to be in favor of vaterite (Fig. 14). Even at the highest saturation states (SI = 2.3), the top glass retains a calcite to vaterite ratio of 0.1 indicating the calcite occupies 10% of the precipitates and easily distinguished from vaterite. At the lowest saturation state (SI = 1.65), calcite dominates vaterite two to one on the top glass and vaterite remains a significant portion of the precipitated solid. On the bottom glass, the appearance of calcite becomes rare as the calcite to vaterite ratio can drop below 0.05 at SI > 2. Muddling this result is the high percentage of calcite viewed on the bottom glass of experiment 1 (SI = 2.35). Despite the
same starting conditions and longer duration, the bottom glass of experiment 1 only had 35% coverage compared to 61% in experiment 2. It should be noted that the settled vaterite spheres were more tightly pack within the center of the reactor and did not obscure precipitated calcite at the edges.

A higher initial pH (10.5) can lead to a greater dominance by vaterite and coverage (Fig. 15). Despite a lower saturation state (SI = 1.33 compared to 1.7), experiment 5 yielded at least 6 times more coverage on the top glass relative to experiment 4 while producing more vaterite relative to calcite. This is very similar to experiment 1, which had a saturation index of 2.35. The bottom glass of high pH experiments tends to be very different from those experiments with a starting pH of 8.3. High pH experiments have the lowest coverage on the bottom glass of all experiments and show almost no coverage by vaterite spheres. Usually precipitation is focused near the inputs, but quickly dissipates moving towards the output.

Adding NaCl to experiments resulted in a redistribution of phases spatially and a twofold increase in coverage across the entire reactor (Fig. 15). Compared to an equivalent experiment with the nearly the same saturation state and duration (SI = 1.33, 24 hours), the addition of NaCl resulted in a top glass that increased in coverage from 13 to 28% and a calcite to vaterite ratio that dropped from 0.1 to less than 0.05. This increase in vaterite was offset by the bottom glass where coverage dropped from 4 to 2% and the calcite to vaterite ratio increased from 0.1 to 19.6. While this may make calcite appear more dominant in an experiment, when taken as a whole the calcite to vaterite ratio was unchanged from 0.1 and coverage increased from 9 to 15%.

In general, the addition of additives such as NH₄Cl, L-arginine, and Na₂HPO₄ results in less coverage and higher calcite to vaterite ratios (Figs. 16, 17, and 18). At almost all saturation states explored, these additives resulted in precipitation that covered less of the total reactor area and more calcite versus vaterite. As calcite to vaterite ratios increased it was more at the expense of lost vaterite mass rather than calcite replacing
vaterite coverage. Additives were most effective at low saturation states (SI = 1.7) as the increase in calcite dominance and decrease in coverage was not always clear at higher saturation states (SI = 2.35). For example, experiment 8 resulted in an increase in coverage from 17 to 20% on the top glass and a decrease in the calcite to vaterite ratio on the bottom glass relative to experiment 1, which was equivalent save for NH₄Cl being added to experiment 8 (Fig. 16). This is ignoring the issues with experiment 1 previously discussed while the narrow confinement of vaterite spheres may have caused the calcite to vaterite ratio to appear larger than it is in reality. If the relative strengths of the inhibitors were to be judged by the decrease in coverage and increase in calcite to vaterite ratios, Na₂HPO₄ is the strongest, followed by L-arginine, and NH₄Cl is last. In the case of Na₂HPO₄, precipitation of CaCO₃ at SI = 1.69 resulted in calcite dominating vaterite by more than 100 to 1 on both the top and bottom glass (Fig. 18).

3.3 Temporal Precipitation Dynamics and Phase Stability

Nucleation of CaCO₃ occurred rapidly upon initiating an experiment. At high SI, homogeneous nucleation of vaterite, in the form of the spheres, become visible within the first minutes of mixing. Within 20 to 60 minutes, depending on the saturation state of the solution, the first visible calcite rhombs and vaterite shells, almonds, and flowers were visible on the top and bottom glass. New precipitation on the glass was first observable near the input; within the first two hours at all saturation states explored, precipitates were observed across the entire reactor. Typically, if no precipitates were observed by 1 hour on a given surface, no solids would form in that location at any point in the experiment. Rarely, vaterite almonds or calcite rhombs precipitated more than 6 hours after initiation on a previously uncovered surface, but this occurred only at experiments with SI greater than 2 and was not pervasively observed.

Once calcite and vaterite shells, almonds, and flowers became large to detect by optical microscopy no changes in phase or morphology were observed. In such cases, precipitation would continue to growth throughout an experiment as a result of particle
enlargement (Fig. 19). Particles morphology remained constant and no evidence of phase transitions or dissolution were observed. There was never evidence of vaterite shells, almonds, or flowers transitioning to calcite during the course of an experiment.

Throughout an experiment, new vaterite spheres continually formed and displayed evidence of phase and morphological transitions depending on their location relative to other precipitates. At a flow rate of 0.005 mL/min, vaterite spheres formed in solution typically settle at the far end of the reactor. Coverage increased with time by the addition of new spheres to the bottom glass surface. Within the first hour, a small fraction of the settled spheres would begin to grow in size as calcite rhombs. This suggests that a phase transition from vaterite to calcite occurs on the bottom glass early in the experiment (Fig. 20). Over time, these calcite rhombs grow without morphological changes, while new spheres continue to settle onto the glass. There is some evidence that spheres that settle near (within 5 μm) or onto the pre-existing calcite rhombs dissolve, which appears as a halo of open space surrounding the calcite. In areas of dense vaterite spheres coverage sphere did not appear to grow in size. However, in areas where vaterite spheres are less dense (~10 μm between each sphere) many sphere would transition to a different morphology and could grow large than 5 μm (i.e., almonds or shells; Fig. 21).

Precipitate morphology is robust in time. For instance, vaterite almonds formed in experiment 5 (SI = 1.33, pH_i = 10.5) retained their morphology in solution for at least three months after formation. Over the three-month period, the almonds showed minimal signs of dissolution in the form of rounding. After two and three months of the termination of the experiment, Raman analysis revealed that the almonds remained vaterite. All other experiments also showed similar resistance to changes in both morphology and mineralogy weeks after termination. Vaterite that was washed with ethanol and dried did not visibly alter over the course of 4 months, even upon exposure to atmosphere.
3.3 Computational Fluid Dynamics Model Results

The results of the computational fluid dynamics model revealed that the solution velocity within the reactor varied with proximity to the cell walls (Fig. 22). Most of the solution moving through the center had a speed of about $4 \times 10^{-5}$ m/s that allowed it to travel through a cell in about 9 minutes. Closer to the sides and cover glass, solution traveled at about $1 \times 10^{-5} – 2 \times 10^{-5}$ m/s such it required 20 – 40 minutes to travel through the cell. As solution left the reactor, center portions speed up to $6 \times 10^{-5}$ m/s while the outer portions near the walls slowed by several orders of magnitude.
4. Discussion

4.1 Spatial Phase Distribution in Experiments

Within single experiments multiple modes of precipitation can occur leading to spatial variability and potential sampling bias. Homogeneous nucleation appears to favor vaterite, which is evident in the vaterite to calcite ratios on the top and bottom glass. Many of the solids formed in the center of the reactor eventually settled on the bottom as vaterite spheres cover glass leading to a large vaterite to calcite ratio. New mass is added to the surface by the formation of new spheres. Heterogeneous nucleation favors the precipitation of particles that nucleate early in an experiment, some distance from each other (30 – 50 μm), and grow in size over time such that new mass is added by growth on existing particles. Both modes of precipitation are evident throughout experiments in varying degrees, although the top glass is dominated by particles nucleated heterogeneously while the bottom glass is dominated by particles nucleated homogeneously. Such spatial variability is critical, as experiments seeking to constrain isotopic fractionation or element partitioning could preferentially sample solids in solution or on surfaces, biasing results.

Varying reactor conditions with respect to saturation state during experimental initiation or termination could bias results. For example, the results of this work suggest that a slow increase in saturation state during initiation of a batch experiment promotes calcite precipitation along reactor walls. When the saturation state reaches its final value, ostensibly near a SI of 2, vaterite precipitation is favored. Yet, due to the lack of sufficient space for nucleation, vaterite would only precipitate in solution. Thus, if only the calcite along the walls is sampled, as it typically done, the vaterite would be unaccounted for.

Vaterite that is unaccounted for in experiments has the potential to alter the interpretation of isotope fractionation and trace element partitioning during CaCO$_3$ formation. Data on trace element partitioning and isotopic fractionation in vaterite
relative to calcite is currently limited, but based on the previous work two phases are usually chemically distinct (Tarutani et al., 1969; Frenzel and Harper, 2011; Gussone et al., 2011). The presence of vaterite in experiments, especially in large masses, can alter the parent solution composition such that it becomes depleted isotopically or in a trace element of interest. Reservoir effects then become a substantial problem as the composition of precipitated calcite also reflects the depletion of the solution.

4.2 Effect of Phase Heterogeneities on the Interpretation of the Ca Isotopic Fractionation Factor

Our experiments indicate that at SI < 2.5 vaterite is present at levels that can affect the interpretation of the Ca isotopic fractionation factor by altering the composition of the bulk precipitated solid. To illustrate this, we apply a box model. Assuming characteristic fractionation factors for calcite (-1.5‰) and vaterite (-0.5‰) and a 1:1 vaterite:calcite ratio, vaterite alters the calculated \( \Delta^{44}\text{Ca}_{s-f} \) of the precipitated solids from -1.5‰ to -1.0‰ (10% Ca removed from solution; Fig. 23a). This effect becomes more dramatic as the vaterite to calcite ratio increases, reaching a \( \Delta^{44}\text{Ca}_{s-f} \) value of 0.5‰ at a vaterite:calcite ratio of 10 and. The range of vaterite to calcite ratios observed in this study was 0.1 to 2.1 in experiments without additives resulting in apparent fractionations of -1.3‰ and -0.8‰ from the bulk solid (SI 1.7 and 2.35, pH 8.3, 10% Ca removed), both of which are marked different from the expected -1.5‰ of pure calcite. As vaterite precipitation becomes more substantial at SI > 1.7 and pH 8.3, then it is reasonable to expect the Ca isotopic composition of bulk CaCO\(_3\) precipitated solid to be measurably affected by the coprecipitation of vaterite.

It should be noted that the Ca isotope fractionation factor for vaterite remains poorly constrained. If the fractionation factor assigned to vaterite formation is closer to zero, then the final \( \Delta^{44}\text{Ca}_{s-f} \) of the bulk solid would also be closer to zero at higher saturation states where vaterite becomes more dominant relative to calcite (0‰ when vaterite:calcite = 10). Given that calcite was precipitated along with vaterite at all
saturation states observed, the reported fractionation by Gussone et al., (2011) could also be affected by a small amount of mixing with calcite. As the kinetic endmember of Ca isotope fractionation results in calcite with Δ$^{44}$Ca$_{w-f}$ = -1.5‰ (Tang et al., 2008b, Tang et al., 2012), the presence of even a small amount of calcite would cause a mostly vateritic sample to appear measurably lighter. More work is required to constrain the fractionation of Ca isotopes in pure vaterite samples so that predictions of mixing results can be more accurate.

Should the vaterite to calcite ratio be controlled by SI over the range of 1.7 to 2.35 at pH = 8.3, then is it possible that the results of Lemarchand et al. (2004) were affected by the precipitation of vaterite. The bulk of the Lemarchand et al. (2004) experiments took place from SI = 1.25 to 2.00 suggesting that vaterite could account for at least 50% of precipitation in some experiments. While Lemarchand et al. (2004) observed small amounts of vaterite in some experiments; it is unlikely that an equal portion of vaterite to calcite would go unnoticed if both were sampled.

While the Lemarchand et al. (2004) dataset shows Δ$^{44}$Ca$_{w-f}$ moving closer to 0‰ as saturation state increases, the results of this study cannot fully explain the Lemarchand et al. (2004) data. The results of this study suggest that at SI = 2 the vaterite to calcite ratio should be 1, which results in a Δ$^{44}$Ca$_{w-f}$ of -1.0‰. At SI ≈ 2, Lemarchand et al. (2004) observed CaCO$_3$ such that Δ$^{44}$Ca$_{w-f}$ = 0‰. This could be partially offset by a higher percentage of Ca removal than reported that would drive the Δ$^{44}$Ca$_{w-f}$ of the bulk solid closer to zero with a smaller vaterite to calcite ratio. This would require precipitates to go unnoticed as Lemarchand et al. (2004) reported no more than 10% Ca removal in experiments. If precipitates did go unnoticed, the parent solution would be enriched with heavy isotopes driving both the solution and newly precipitated CaCO$_3$ heavier (Fig. 23; Panel b).

Given that the results from this study show that vaterite should have influenced the Lemarchand et al. (2004) dataset, the question of why vaterite was not readily
observed by Lemarchand et al. (2004) becomes a critical one. As previously mentioned, Lemarchand et al. (2004) observed the precipitation of vaterite in some experiments but did not include those in their Ca isotope fractionation data. Since Lemarchand et al. (2004) were actively looking for vaterite, they were at least aware of the potential for vaterite. Perhaps critical to this question is the sampling procedure by which precipitated solids were removed from their reactor. In particular, only solids that precipitated on the reactor walls were saved for analysis. Additionally, after the solution was poured out of the reactor the solids were washed with DI water. This gives rise to several possible scenarios.

The first possibility is that vaterite precipitation in the Lemarchand et al. (2004) experiments was limited to the bulk solution and subsequently lost when the reactor was drained. Lemarchand et al. (2004) noted a slow increase of saturation state over the first 15 hours of an experiment that would have allowed calcite to nucleate on the reactor walls before a saturation state more suitable to vaterite precipitation was achieved. Further nucleation of vaterite on the reactor walls would be inhibited by the calcite, although vaterite would continue to precipitate in the bulk solution. In this case, only calcite would be observed although its Ca isotopic composition would be affected by the precipitation of vaterite in the bulk solution such that $\Delta^{44}\text{Ca}_{s-f}$ measured would be closer to zero.

A second set of possibilities for vaterite precipitation in the Lemarchand et al. (2004) experiments is that vaterite did precipitate on the walls but was lost or transitioned to calcite before analysis. Transitioning to calcite could have occurred during an experiment if the saturation state began to drop, perhaps due to Ca removal and a decrease in pH over the course of the experiment. With vaterite possibly moving out of its range of stability, it could have dissolved and reformed as calcite. Whether the isotopic composition of the vaterite was retained or not would be open to question, but remaining calcite would still have an isotopic composition influenced by the precipitate of vaterite. Transitions to calcite could have also occurred after the reactor was drained.
and washed with DI water. If the reactor was dried at air temperature, there may have been sufficient water adhered to the reactor walls to allow for the vaterite transitioning to calcite and retaining its isotopic composition before any analysis is performed. Given that the vaterite to calcite transition can occur within hours, this seems a distinct possibility. Finally, vaterite could have been lost during the rinsing of the reactor after an experiment. Here, the DI water could easily dissolve the metastable vaterite depending on how rigorous the wash was. In this case, the calcite would remain and retain an isotopic composition affected by the precipitation of vaterite.

If the bulk phase composition of precipitation is a major control on the Ca isotope fractionation factor, it suggests that the mechanisms by which Ca isotopes are fractionated during calcite and vaterite precipitation are significantly different. Given the hypothesis that vaterite grows by aggregation of nanoparticles, the idea that Ca isotope fractionation during vaterite growth is controlled by different processes compared to calcite is not implausible. The Tang et al, (2008 and 2011) data can be replicated using ion-by-ion growth models where the rates of ion attachment and detachment from the calcite surface result in the observed fractionation trends. Since this model cannot explain data from the Lemarchand et al. (2004) experiments where vaterite was likely present, then it suggest that Ca isotope fractionation cannot be explained by ion-by-ion growth. Given the results from Gussone et al. (2011) where vaterite was fractionated by -0.5‰ relative to its parent solution, it could be argued that the formation of nanoparticles is an inherently less fractionating process compared to ion-by-ion growth.

4.3 Implications of Non-Calcite CaCO₃ Precipitation at Low Saturation States

The abundance of vaterite even at low saturation states (SI ≈ 1) observed in these experiments could indicate that vaterite is even more common than previously thought. In particular, vaterite might be the major CaCO₃ mineral formed in many settings where Mg²⁺ is not in abundance. For example, Aloisi (2008) reported that the saturation index of cyanobacterial mats through history was about 1.6, with respect to calcite. This suggests
that the CaCO₃ precipitation that cements the mats forming stromatolites initially may have initially formed as vaterite. Additionally, the formation of foraminiferal CaCO₃ tests could occur in vacuole with SI as high as 2.5, if not higher, assuming the starting solution is seawater and precipitation is induced through the use of H pumps to increase the pH (Erez, 2003). Should that be the case, than the tests of foraminifera initially form as vaterite before transitioning to calcite. One major caveat is that formation of biogenic CaCO₃ is often templated off an existing structure within or on a biomineralizing organism. In such situations, the template may play a larger role than saturation state on the phase of CaCO₃ precipitated. However, if vaterite is the dominate pathway to the stable calcite generally observed in nature, then it is also possible that the isotopic fractionation factors and trace element distribution coefficients of calcite based proxies are heavily influenced by vaterite precipitation. For Ca isotopes, this could mean that the formation of the solid CaCO₃ fractionates less than previously thought.

The precipitation of vaterite can be inhibited by the presence of additives such as NH₄Cl, Na₂HPO₄, and L-arginine as indicated by the results of this study. Their presence could play a critical role in the CaCO₃ in many organisms that promotes the precipitation of calcite over vaterite. Given that calcite is more thermodynamically stable and less soluble, it serves as a more effective material for building tests and other hard parts. Even if vaterite precipitates in large proportions and transitions to calcite during biomineralization, the presence of an amino acid like L-arginine or nutrient like PO₄³⁻ could make that process easier by increasing the percentage of calcite in the resulting solid. As there is more calcite, the resulting solid is stronger and less resistant to dissolution prior to the transition of vaterite to calcite.

4.4 Future Work

The most critical piece of information required to make further use of the observations made in this study is further constraints on the Ca isotopic fractionation factor for vaterite relative to saturation state. The experimental apparatus used here could
put to use in such an endeavor as individual vaterite crystals could be separated from calcite after an experiment. The Ca isotopic composition of a pure vaterite sample could be measured and compared to saturation states, which are well constrained within the flow reactors compared to larger batch reactors dependent on CO₂ dissolution techniques. The fractionation factors obtained in such a study would allow bulk measurements of CaCO₃ mixtures to be deconvolved with the assumption of various calcite to vaterite ratios such that more interpretations about the environment in which solids precipitated could be made. Additionally, better knowledge of fractionation factors inherent to vaterite allows for some arguments to be made as to the process by which vaterite precipitates. If it is shown that vaterite is less fractionating than calcite, it could be argued that vaterite is grows by an aggregation model as described above while calcite growth fits something similar to an ion-by-ion model.

5. Conclusions

In this study it was observed that vaterite was a major constituent of CaCO₃ precipitation from solutions with saturation states relevant to both nature and experiments designed to determine the portioning of trace elements and isotopes. Not only is the precipitation of vaterite probable at saturation states in which it was previously considered unlikely, it can precipitate in large quantities relative to calcite. Complicating the understanding of experiments in which vaterite might precipitate is that the precipitation of CaCO₃ phases is heterogeneous in space and time. These heterogeneities make it more difficult to accurately characterize growth within a reactor and could lead to bias sampling in characterization. Whereas, this work focused on the effect of vaterite precipitation in determining the Ca isotopic fractionation factor, the observations made could also be applied to any number of proxies that depend on an isotopic fractionation factor or trace element distribution coefficient which might vary by CaCO₃ phase. With better characterization of isotope fractionation and trace element distribution during the formation of vaterite, interpretations of CaCO₃ based proxies can become more accurate leading to a better understanding of geologic processes.
Appendix A – Tables

Table 1: Key properties of CaCO₃ polymorphs

<table>
<thead>
<tr>
<th>Phase</th>
<th>Chemical Formula</th>
<th>Crystal Structure</th>
<th>Log Ksp*</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Calcite</td>
<td>CaCO₃</td>
<td>Trigonal</td>
<td>-8.48</td>
<td>Plummer and Busenberg, 1982</td>
</tr>
<tr>
<td>Aragonite</td>
<td>CaCO₃</td>
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<td>-8.34</td>
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<td>Vaterite</td>
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<td>Plummer and Busenberg, 1982</td>
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<td>Ikaite</td>
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<td>Monoclinic</td>
<td>-7.46</td>
<td>Brečević and Nielsen 1993</td>
</tr>
<tr>
<td>Monohydrocalcite</td>
<td>CaCO₃ · H₂O</td>
<td>Trigonal</td>
<td>-7.15</td>
<td>Kralj and Brečević, 1995</td>
</tr>
<tr>
<td>ACC</td>
<td>CaCO₃ · XH₂O</td>
<td>Amorphous</td>
<td>-6.40</td>
<td>Brečević and Nielsen 1989</td>
</tr>
</tbody>
</table>

* Calculated Ksp at 25 °C in a low ionic strength solution using information from the provided references
† The crystal structure of vaterite remains unclear at present. See text for more details
Table 2: Summary of experiments

<table>
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<tr>
<th>No.</th>
<th>T(hrs)</th>
<th>[Ca]*</th>
<th>DIC*</th>
<th>Additive</th>
<th>[Add]*</th>
<th>pH_{ini}</th>
<th>pH_{final}</th>
<th>Flow†</th>
<th>SI‡</th>
<th>Calcite: Vaterite Top</th>
<th>Calcite: Vaterite Bottom</th>
<th>Calcite: Vaterite Total</th>
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<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>132.9</td>
<td>9.5</td>
<td>-</td>
<td>-</td>
<td>8.3</td>
<td>7.75</td>
<td>0.005</td>
<td>2.32±0.24</td>
<td>0.1</td>
<td>0.8</td>
<td>0.5</td>
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<td>2</td>
<td>14</td>
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<td>-</td>
<td>-</td>
<td>8.3</td>
<td>7.75</td>
<td>0.005</td>
<td>2.32±0.24</td>
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<td>&lt; 0.05</td>
<td>0.1</td>
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<td>-</td>
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<td>7.75</td>
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<td>2.19±0.24</td>
<td>0.6</td>
<td>&lt; 0.05</td>
<td>0.1</td>
</tr>
<tr>
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<td>22</td>
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<td>-</td>
<td>-</td>
<td>8.3</td>
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<td>0.005</td>
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<td>2.1</td>
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<td>-</td>
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<td>-</td>
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<td>4.0</td>
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<tr>
<td>8</td>
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<tr>
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<td>9.4</td>
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<td>13</td>
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<td>0.005</td>
<td>1.65±0.26</td>
<td>554.0</td>
<td>141.4</td>
<td>187.2</td>
</tr>
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* Concentrations in mmol / kg soln
† Flow rate in mL / min from each syringe
‡ Saturation index with respect to calcite as determined by PHREEQC
Appendix B – Figures

Figure 1: Flow chart showing the development of CaCO₃ as it is currently understood. Question marks indicate areas that remain uncertain.
Figure 2: Observed saturation indices in the natural world and experiments. In many cases, the specific chemistry leading to biogenic CaCO_3 remain unclear and it is currently unknown what the upper limit is. References: 1) Easley et al., 2013. 2 & 3) Aloisi, 2008. 4) Erez, 2003. 5) Lemarchand et al., 2004; Tang et al., 2008a; Tang et al., 2012; Niedermyer et al., 2013. 6) Koga et al., 1998; Pontoni et al., 2003, Rieger et al., 2007; Pouget et al., 2009. 7) Pontoni et al., 2003; Pouget et al., 2009; Rodriguez-Blanco et al., 2011; Rodriguez-Blanco et al., 2014.

Figure 3: Summary of Ca isotopic fractionation data from previous studies. $\Delta^{44/40}{\text{Ca}}$ is the isotopic difference between the bulk solid formed during an experiment and the original parent solution. a) Relationship between $\Delta^{44/40}{\text{Ca}}$ and growth rate of the precipitate. b) Relationship between $\Delta^{44/40}{\text{Ca}}$ and the reported saturation index of parent solutions with respect to calcite.
Figure 4: Schematic representation of flow-through reactor.

Figure 5: Experimental setup for viewing and recording mineral precipitation \textit{in-situ}. The reactor is placed on the microscope stage while the syringe pump provides fresh solutions from the right. The stage and camera are controlled by the joystick and computer on the right, respectively.
Figure 6: 3D mesh of cell portion of the reactor used in CFD modeling.

Figure 7: Schematic of the box model used to model the evolution of CaCO₃ precipitation experiments.
Figure 8: Representative images from experiments showing both calcite and vaterite precipitating together. a) High saturation state experiment. SI = 2.35, 22 hrs. b) Low saturation state experiment. SI = 1.69, 22 hrs.

Figure 9: Representative calcite morphologies observed in this study. a) Images obtained from optical microscopy. Scale is 100 μm. b) Images obtained from ESEM. Scale is 50 μm.
Figure 10: Observed Raman spectra of calcite and vaterite from experiments.

Figure 11: Representative calcite morphologies observed in this study. a) Images obtained from optical microscopy. Scale is 100 μm. b) Images obtained from ESEM. Scale is 50 μm for all but the spheres where the scale is 10 μm.
Figure 12: Summary of results from experiments. Data are divided up to show results from the top and bottom cover glass along with the summary total results. a) Relationship between the final calcite:vaterite ratio with saturation index with respect to calcite. b) Relationship between the final percent of area covered with precipitates and the saturation index with respect to calcite.

Figure 13: Representative images of dichotomy between top and bottom cover glasses. SI = 2.35, 22 hrs, NH₄Cl added. a) Top cover glass. b) Bottom cover glass.
Figure 14: Growth patterns from experiments using no additives with a starting pH = 8.3. The sizes of the blocks show the percent coverage within their grids such that a filled grid had 100% coverage. The colors of blocks reflect the calcite to vaterite ratio such that areas with cooler colors are dominated by calcite and the warmer colors are dominated by vaterite.
<table>
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<td>pH $= 10.5$</td>
<td>pH $= 10.5$, NaCl added</td>
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<tr>
<td><strong>Top cover glass</strong></td>
<td><strong>Top cover glass</strong></td>
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<td>Coverage = 13%, calcite:vaterite = 0.1</td>
<td>Coverage = 28%, calcite:vaterite &lt; 0.05</td>
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<td><strong>Bottom cover glass</strong></td>
<td><strong>Bottom cover glass</strong></td>
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<td>Coverage = 4%; calcite:vaterite = 0.1</td>
<td>Coverage = 2%; calcite:vaterite = 19.6</td>
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<table>
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<tr>
<th>Experiment 7: SI = 1.07, 73 hours</th>
<th>Experiment 4: SI = 1.69, 22 hours</th>
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<tr>
<td>pH $= 10.5$</td>
<td>pH $= 8.3$</td>
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<tr>
<td><strong>Top cover glass</strong></td>
<td><strong>Top cover glass</strong></td>
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<tr>
<td>Coverage = 1%, calcite:vaterite = 5.2</td>
<td>Coverage = 2%, calcite:vaterite = 2.1</td>
</tr>
<tr>
<td><strong>Bottom cover glass</strong></td>
<td><strong>Bottom cover glass</strong></td>
</tr>
<tr>
<td>Coverage = &lt;1%; calcite:vaterite = 1.6</td>
<td>Coverage = 6%, calcite:vaterite = 0.3</td>
</tr>
</tbody>
</table>

Figure 15: Growth patterns from experiments starting at a high pH (10.5) compared to those either adding NaCl or starting at a low pH (8.3). The sizes of the blocks show the percent coverage within their grids such that a filled grid had 100% coverage. The colors of blocks reflect the calcite to vaterite ratio such that areas with cooler colors are dominated by calcite and the warmer colors are dominated by vaterite.
Figure 16: Growth patterns from experiments containing NH₄Cl compared to those with no additives at a low pH (8.3). The sizes of the blocks show the percent coverage within their grids such that a filled grid had 100% coverage. The colors of blocks reflect the calcite to vaterite ratio such that areas with cooler colors are dominated by calcite and the warmer colors are dominated by vaterite.
Figure 17: Growth patterns from experiments containing L-arginine compared to those with no additives at a low pH (8.3). The sizes of the blocks show the percent coverage within their grids such that a filled grid had 100% coverage. The colors of blocks reflect the calcite to vaterite ratio such that areas with cooler colors are dominated by calcite and the warmer colors are dominated by vaterite.
Figure 18: Growth patterns from experiments containing Na₂HPO₄ compared to those with no additives at a low pH (8.3). The sizes of the blocks show the percent coverage within their grids such that a filled grid had 100% coverage. The colors of blocks reflect the calcite to vaterite ratio such that areas with cooler colors are dominated by calcite and the warmer colors are dominated by vaterite.
Figure 19: Representative evolution of CaCO$_3$ precipitation on top glass of all experiments or bottom glass of low saturation state (SI < 2) experiments.
Figure 20: Representative evolution of CaCO$_3$ precipitation on the bottom glass of an experiment with a saturation state (SI $> 2$).
Figure 21: Example of vaterite spheres that have transitioned into almond and vaterite morphology.
Figure 22: Results from computational fluid dynamics model that shows flow velocities through the cell portion of the reactor. All colors reflect the magnitude of the flow velocities. A) Flow vectors as viewed from above. B) Flow vectors as viewed from the side. C) Colormap showing the magnitude of the flow velocities in a horizontal slice through the center of the cell. D) Colormap showing the magnitude of the flow velocities in a vertical slice through the center of the cell.
Figure 23: Results from the box model of experiment evolution. a) The change in the bulk $\delta^{44}$Ca value of precipitated solid in relation to the vaterite to calcite ratio. Different lines show the amount of Ca removed during the course of the experiment. b) Change in the instantaneous precipitated solid and bulk solution with respect to the amount of Ca removed over the course of an experiment.
Appendix C – Detailed Views of Experimental Results

Figure 24: Mosaic image of the top cover glass from experiment 1.
Figure 25: Mosaic image of the top cover glass from experiment 1 after removal from the reactor.
Figure 26: Mosaic image of the bottom cover glass from experiment 1.
Figure 27: Mosaic image of the top cover glass from experiment 2.
Figure 28: Mosaic image of the top cover glass from experiment 2 after removal from the reactor.
Figure 29: Mosaic image of the bottom cover glass from experiment 2.
Figure 30: Mosaic image of the top cover glass from experiment 3.
Figure 31: Mosaic image of the top cover glass from experiment 3 after removal from the reactor.
Figure 32: Mosaic image of the bottom cover glass from experiment 3.
Figure 33: Mosaic image of the top cover glass from experiment 4.
Figure 34: Mosaic image of the bottom cover glass from experiment 4.
Figure 35: Mosaic image of the top cover glass from experiment 5.
Figure 36: Mosaic image of the bottom cover glass from experiment 5.
Figure 37: Mosaic image of the top cover glass from experiment 6
Figure 38: Mosaic image of the bottom cover glass from experiment 6.
Figure 39: Mosaic image of the top cover glass from experiment 7
Figure 40: Mosaic image of the bottom cover glass from experiment 7.
Figure 41: Mosaic image of the op cover glass from experiment 8.
Figure 42: Mosaic image of the bottom cover glass from experiment 8.
Figure 43: Mosaic image of the top cover glass from experiment 9.
Figure 44: Mosaic image of the bottom cover glass from experiment 9.
Figure 45: Mosaic image of the top cover glass from experiment 10.
Figure 46: Mosaic image of the top cover glass from experiment 10 after removal from the reactor
Figure 47: Mosaic image of the bottom cover glass from experiment 10.
Figure 48: Mosaic image of the top cover glass from experiment 11.
Figure 49: Mosaic image of the bottom cover glass from experiment 11.
Figure 50: Mosaic image of the top cover glass from experiment 12.
Figure 51: Mosaic image of the bottom cover glass from experiment 12.
Figure 52: Mosaic image of the top cover glass from experiment 13.
Figure 53: Mosaic image of the bottom cover glass from experiment 13.
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