Chemical Controls on the Magnesium Content of Amorphous Calcium Carbonate

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Abstract: Amorphous calcium carbonate (ACC) is a metastable phase that forms in diverse biogeochemical settings. This material can incorporate significant amounts of magnesium and other elements, but the conditions that regulate composition are not established. Using a mixed flow reactor method, we synthesize Mg-free ACC (control) and amorphous magnesium calcium carbonate (ACMC) under controlled chemical conditions to determine the relationship between composition and inorganic solution chemistry. Input solutions contained a constant initial Mg/Ca ratio of 5/1 with variable total carbonate concentration, pH, and supersaturation. Within the reactor, input solution chemistry evolves in proportion to the extent of precipitation whereby the initial Mg/Ca ratio increases to values as high as 14 at steady state conditions. By this approach, we produce reproducible quantities of ACMC with 24 to >70 mole % Mg to give compositions of $\text{Mg}_{(0.24-0.72)}\text{Ca}_{(0.76-0.28)}\text{CO}_3 \cdot 1.42-1.63 \text{ H}_2\text{O}$.

The primary control on ACMC composition is the steady state solution composition that develops in the reactor during precipitation. Analysis of the data shows the Mg content of ACMC is regulated by the interplay of three factors at steady state conditions: 1) Mg/Ca ratio; 2) total carbonate concentration; and 3) solution pH. Using the Henderson-Kracek model to estimate the partition coefficients for the Mg content of ACMC, we find K_D is approximately constant at 0.047 ± 0.003 when steady state pH is less than 9.5, but values of K_D triple as steady state pH increases from 9.5 to 10.3. Our K_D values are lower than previous estimates that are based upon initial solution composition. In contrast, our estimates of K_D are determined from the solution chemistry at steady state conditions and for pH conditions that are less extreme than previous experimental studies. We suggest the approach of using steady state composition to estimate K_D gives a more accurate representation of relationships between ACMC composition and local conditions. The findings demonstrate local pH and total carbonate concentration can be regulated at the time of formation to produce Mg amorphous carbonates of desired composition.

1. INTRODUCTION

Over the last 40 years, the classical terrace-ledge-kink (TLK) model of crystal growth (Burton et al., 1951; Chernov, 1984) has been adopted by the geological community to explain most types of mineralization (Berner, 1980). The process occurs via the ion-by-ion attachment of solutes to the step edges of mineral surfaces, and has provided a foundation for interpreting many studies of calcium carbonate formation. However, the inability of the step growth process to explain a number of observations in biological settings presents long-standing obstacles to fully understanding carbonate mineralization. For example, the ion-by-ion attachment process is too slow to explain the rapid development of skeletal structures that is observed in many calcifying organisms (Politi et al., 2004; Tao et al., 2009). In addition, laboratory studies show the classical crystal growth process is unable to produce calcite with >10–15% Mg (Bischoff et al., 1987), which contrasts with reports of up to 30% Mg in some biogenic calcite (Chave, 1954a). An understanding of biogenic calcification is further complicated by laboratory observations that step growth is fully inhibited at Mg levels far below seawater concentrations (Davis et al., 2000; Stephenson et al., 2008).

Evidence from structural biology shows the limitations imposed by the classical step growth process can be overcome by employing an alternative pathway to skeletal formation. The process begins by accumulating a metastable amorphous calcium carbonate (ACC) that is stored until later transformation to the final crystalline phase (Aizenberg et al., 1996; Nudelman et al., 2007; Politi et al., 2008). For example, the spicules of sea urchin larvae are reported to form via an ACC intermediate (Politi et al., 2004), and ACC is associated with calcite and aragonite in the nacreous layer of some mollusk species (Weiss et al., 2002; Addadi et al., 2006). It has been suggested that the formation and storage of ACC is useful during biomineralization because this

reactive precursor gives organisms greater energetic control over mineralization (Navrotsky, 2004).

Although ACC was recognized over 40 years ago (Towe & Malone, 1970), the local biogeochemical conditions that control composition are not fully understood. Recent characterization studies show this material has a typical composition of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ with unique short- and intermediate-range structures (Michel et al., 2008) and recent neutron diffraction studies demonstrate a homogeneous distribution of Ca and Mg (Cobourne et al., in press). In the absence of impurities, ACC formation is described by the balanced reaction

where the solubility product, K_{sp} , equals $10^{-6.39}$ at 25°C (Brečević & Nielsen, 1989) and the number of hydration waters is reported to vary from zero for some biogenic ACC to unity (Towe & Malone, 1970; Raz et al., 2003; Politi et al., 2006; Politi et al., 2008; Gong et al., 2012). ACC is metastable with respect to calcite with a K_{sp} that is approximately 100 times larger. Anecdotal evidence from recent studies suggests ACC solubility may be considerably lower (Gebauer et al., 2008; Hu et al., 2012)— but to our knowledge Brečević & Nielsen (1989) is the only published value. In the absence of impurities, ACC is stable for only short periods but its lifetime increases significantly with the addition of Mg (Loste et al., 2003; Radha et al., 2010) and in the presence of charged organic molecules (Han & Aizenberg, 2008; Wang et al., 2009).

The Mg content of ACC is of particular interest as a minor element that is frequently found in ACC and the final crystalline products of calcified skeletons (Raz et al., 2000). For example, the ACC of sea urchin spicules can contain up to 5 mole % Mg (Raz et al., 2003) and the shells of benthic foraminifera can contain >12 mole % Mg (Bentov & Erez, 2005). The Mg content of ACC is also reported to regulate the ACC-to-calcite transformation process in

terrestrial crustaceans during molting (Tao et al., 2009). Remarkable quantities of ACC are also produced in the intestinal guts of marine fish (Perry et al., 2011; Woolsey et al., 2012) and recent studies show these deposits can contain as much as 54 mol% Mg (Foran et al., 2013). In the latter case, these carbonates remain amorphous over the lifetime of the animal, presumably due to their high Mg content (Foran et al., 2013). Given that diverse modern (and likely ancient) organisms produce ACC in a wide variety of biological settings, an understanding of chemical controls on the composition of this phase is useful to developing a physical picture of biomineralization processes.

The formation of amorphous calcium, magnesium carbonate (ACMC) can be described by the reaction

where x can exceed 0.88. Values of n are highly variable and both hydrous and anhydrous forms are recognized, but controls on ACC hydration are not yet fully understood. For example, studies of sea urchin spicules indicate n is approximately 1 and declines to zero as the ACC becomes anhydrous and increasingly metastable before transforming to calcite (Levi-Kalisman et al., 2000; Politi et al., 2008; Gong et al., 2012). For synthetic ACC, a range of values is possible from $n = 0.25$ (Schmidt et al., 2014) to 1.80 for very high Mg ACC (Radha et al., 2012). Hydrated forms of ACC are generally recognized as stable while anhydrous ACC readily transforms to crystalline products (Addadi et al., 2003). To our knowledge, the K_{sp} of ACMC has not been quantified.

New findings indicate the chemical signatures and properties of the carbonate polymorphs that transform from an ACC or ACMC pathway may obey a different set of dependencies than those established for growth by the classical process (Bentov & Erez, 2006;

Loste et al., 2003; Rodriguez-Blanco et al., 2012). The question of whether an ACMC intermediate influences the composition of the final skeletal calcites has particular significance for paleoclimate studies that use the Mg content of calcite as a proxy for seawater conditions (Dissard et al., 2010; Hastings et al., 1998; Morse et al., 1997; Rosenthal et al., 1997). Reports of biogenic calcite containing up to 30 mole % MgCO_3 (Chave, 1954a) and high-Mg carbonates in modern sedimentary environments (Raz et al., 2000; Sánchez-Román et al., 2011; Wang et al., 2012) suggest a mineralization pathway involving amorphous intermediates could explain some of the anomalously high Mg calcites that cannot form by step growth processes.

This study establishes the influence of inorganic chemistry on the Mg content of ACC, hereafter referred to as ACMC. To quantify the relationships, we designed a series of experiments that synthesize ACMC under conditions of constant solution chemistry using a mixed flow reactor method. The approach allowed us to produce the amorphous products at steady state conditions in sufficient quantities that can be characterized by multiple methodologies. Advantages of using the mixed flow reactor method for conducting quantitative studies of ACMC include 1) maintaining constant and controlled supersaturation; 2) synthesizing ACMC at known steady state conditions with reproducible compositions; 3) precipitating ACMC at pH values (8.9–10.4) that approximate natural environments better than the previous investigations that synthesized ACC at very high pH conditions. By this approach, we find that ACMC composition is tuned by the interplay of Mg/Ca ratio of the initial solution, total carbonate concentration, and local pH.

2. EXPERIMENTAL METHODS

2.1 Sample synthesis

ACMC and ACC (control) were synthesized for a series of controlled chemical conditions using a mixed flow reactor (MFR) (Blue et al., 2013). The method utilizes a high-precision pump fitted with two syringes. One contains 100 ml of a MgCl_2 and CaCl_2 solution fixed at a 5:1 ratio, while the other contains 100 ml of NaHCO_3 or Na_2CO_3 solution of variable concentration and initial pH (**Table 1**). The reactant solutions were continuously pumped into the reactor while maintaining a constant stirring rate of 1000 rpm to ensure that precipitation was controlled by surface-reactions with minimal mass transport (Rimstidt & Dove, 1986). Previous studies of crystal precipitation show that impurity levels in carbonate and sulfate minerals can exhibit complex relationships between structural constraints and solution composition (Preito et al., 1993; Astilleros et al., 2003). Calcite compositions can also reflect a continuum of mass transport and surface-limited growth processes when rates are varied over many orders of magnitude (DePaolo, 2010; Nielsen et al., 2013). Although our study maintained the rate of ACMC precipitation within a factor of approximately 10X, analysis of the available data did not suggest a relationship between composition and precipitation rate (**Fig. A1, Appendix**).

As the input solutions mixed in the reactor vessel and became supersaturated with respect to ACMC, precipitation proceeded continuously. The average hydraulic residence time (τ) of solution in the reactor was two minutes for all experiments, where

$$\tau = \frac{\text{volume of reactor (ml)}}{\text{flow rate (ml/min)}} . \quad (3)$$

For each experimental condition, the input solutions flowed through the reactor for a minimum of three hydraulic residence times to allow the ACMC products and solutions to reach steady

state conditions within the reactor vessel (Jensen, 2001). The pH of the effluent solution was also monitored to determine the pH at steady state conditions for each experiment (**Table 1**). The time elapsed to steady state and sample collection varied with solution chemistry.

The ACMC-solution suspension exited the reactor through a single effluent tube located at the top of the reactor. At steady state conditions, the exiting solution and solid products were separated by filtration through a 0.20-micron nylon filter fitted onto a vacuum flask. We assume this solution records the solution chemistry inside the reactor at steady state conditions. All ACMC samples were rinsed thoroughly with ethanol and dried in a class II biological safety cabinet for 30 minutes before being stored overnight in a vacuum desiccator. Each sample was weighed the next day to obtain the mass of the dry ACMC and was immediately prepared for the respective mode of analyses. Samples that could not be analyzed were stored at 4°C for no longer than 3 days. Effluent solution samples were immediately prepared for chemical analysis after collection.

This study used three NaHCO₃ concentrations (60 mM, 100 mM, and 200 mM), and two Na₂CO₃ concentrations (60 mM and 100 mM), each with variable pH levels (**Table 1**). The pH of the NaHCO₃ solutions was adjusted by adding small amounts of NaOH immediately before mixing in order to obtain a range of saturation states. The saturation state of a solution (Ω) with respect to pure ACC is expressed by

$$\Omega = \frac{(a_{\text{Ca}^{2+}})(a_{\text{CO}_3^{2-}})}{K_{sp}} \quad (4)$$

where a_i is the activity of species i and $K_{sp} = 10^{-6.39}$ at 25°C (Brečević & Nielsen, 1989). The solubility product of pure ACC was used to calculate Ω because the K_{sp} of an ACMC solid solution has not been quantified. Ion activities were calculated using The Geochemist's

Workbench© for each solution condition in order to quantify saturation state. All experiments were performed at $25\pm 1^\circ\text{C}$.

2.2 Sample Characterization

The composition and morphology of ACC and ACMC products were characterized using a combination of SEM, Raman Spectroscopy, TGA, ICP-OES, and FTIR. A summary of all experimental conditions is given in **Table A1 (Appendix)**.

2.2.1 SEM

All samples were sputter-coated with approximately 10 nm of gold-palladium. Analyses were performed on an FEI Quanta 600 FEG ESEM. Images of the ACMC were acquired using secondary electron (SE) microscopy. Optimal viewing parameters included 10 kV and a 3.5 spot size (instrument specific value). Higher voltages destroyed the samples and could not be used.

All samples were examined to characterize their morphology and particle size.

2.2.2 Raman Spectroscopy

A small amount of sample was removed from the nylon filter and placed on a clean glass slide for analysis by Raman spectroscopy. Raman spectra were obtained on a JY Horiba (800 mm) LabRam HR spectrometer equipped with a 632.81 nm He-Ne laser emitting 15 mW power and focused through an Olympus 50x objective. Three 180-second spectra accumulations were collected and averaged for each of the samples analyzed. The carbonate peak positions were estimated using a summed Gaussian/Lorentzian peak-fitting routine after baseline corrections were made using Jobin Yvon Labspec version 4 software.

2.2.3 Thermogravimetric Analysis (TGA)

Approximately 5 mg of sample was collected from the nylon filter and placed into an aluminum pan for Thermogravimetric analysis. Analyses were performed on a TA Instruments

Hi-Res TGA Q5000 Thermogravimetric Analyzer. Samples were heated in air from 25°C to 700°C at a rate of 10°C per minute. The percentage of water lost during heating was calculated from the difference in the weight percent of the sample between 25°C and 250°C.

2.2.4 Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)

Samples were dissolved from the nylon filters using 0.5 N nitric acid for 20 minutes. Analyses were performed using a Spectro ARCOS SOP. An yttrium internal standard was added to each sample in an internal mixing chamber (Glass Expansion, Inc.) Each sample passed through a cross-flow nebulizer into a modified Scott-style spray chamber. The sample uptake rate was 2 mL/min, with each sample analysis using 5 mL. The Mg and Ca concentrations were determined from calibration curves that were prepared from plasma-grade single element standards (SPEX, Inc.). Ca and Mg standards were analyzed in conjunction with ACCMC and ACC samples to verify the accuracy of the results.

2.2.5 Fourier-Transform Infrared Spectroscopy (FTIR)

Approximately 2 mg of sample was removed from the nylon filter and used for FTIR analysis. Fourier-Transform Infrared Spectroscopy was performed on a Varian 670-IR with a Pike GladiATR attachment with a 4 cm⁻¹ resolution. Samples were analyzed at a standard 32 cm⁻¹ scan rate. All results were background ATR corrected using the instrument's software.

3. RESULTS

3.1 Sample Characterization

The MFR method produces ACC and ACCMC with reproducible compositions and morphology without evidence of secondary phases. For the conditions of this study, ICP-OES measurements found the ACCMC contains 24–70 mole % Mg (**Table A1, Appendix**). All solution compositions produced ACC and ACCMC with the characteristic spherical morphology

reported for synthetic (Schmidt et al., 2014; Long et al., 2011) and biogenic (Foran et al., 2013; Ziegler, 1994) materials. Particle size distributions are similar with diameters of approximately 0.1 μm to 2 μm (average = 1 μm) (**Fig. 1**). ACCMC with the highest Mg content (**Fig. 1c, d**) is predominately comprised of coalesced particles. This texture is consistent with previous studies of high Mg ACC (Ajikumar et al., 2005; Wang et al., 2011).

Raman analyses of the products confirm that all materials are amorphous. In contrast with crystalline carbonates that typically exhibit six Raman vibrational modes, ACC and ACCMC produce only two modes as broadened ν_1 and ν_4 peaks (**Fig. A2a, Appendix**), with the ν_1 peak having the strongest carbonate internal mode (Wang et al., 2011). The position of the ν_1 peak systematically shifts to higher wavenumbers (Wang et al., 2011) with increasing Mg content compared to pure ACC (1080 cm^{-1}). A linear regression fit of the ν_1 peak positions to the measured Mg content of synthetic samples (**Figure A2b, Appendix**) has a slope (0.227 ± 0.030) that is slightly higher than a previous calibration of 0.2017 ± 0.0087 (Wang et al., 2011).

Additional characterization by Fourier-Transform Infrared Spectroscopy (FTIR) confirms that ACC and ACCMC products are amorphous, including the samples that contain up to 70 mole % Mg. Comparisons of FTIR spectra for the ACCMC samples with baseline ACC prepared in the absence of Mg show peaks are present at 1406 cm^{-1} (ν_3), 1073 cm^{-1} (ν_1), and 864 cm^{-1} (ν_2). Peaks due to vibrations of water molecules occur at 1644 cm^{-1} and near 3300 cm^{-1} (**Fig. 2**). These values are consistent with reports of $1455\text{--}1470/1396\text{--}1425\text{ cm}^{-1}$ (ν_3), $1065\text{--}1075\text{ cm}^{-1}$ (ν_1), and $863\text{--}873\text{ cm}^{-1}$ (ν_2) (Aizenberg et al., 1996; Brečević & Nielsen, 1989; Loste et al., 2003). The frequencies shift when Mg is present (**Fig. 2**), but only the ν_1 peaks shift systematically with changes in Mg content (**Table A1, Appendix**).

Thermogravimetric analyses indicate that all ACCMC products contain more than one water molecule per carbonate ion, resulting in compositions of $\text{Mg}_{(0.24-0.72)}\text{Ca}_{(0.76-0.28)}\text{CO}_3 \cdot 1.42-1.63 \text{ H}_2\text{O}$ (**Fig. 3**). The higher water contents are consistent with a previous report of ACCMC formed at very high pH conditions (Radha et al., 2012). The Mg-free ACC prepared as the control in this study produces a TGA spectrum (**Fig. 3**) that is consistent with Schmidt et al., (2014). The materials also suggest three regions similar to those discerned by Schmidt et al., (2014) using a combination of TGA and NMR analysis (Fig 3, inset). They propose the changes in slope (with increasing temperature) represent the abundance of translationally rigid H_2O , restrictedly mobile water, and hydroxyl (**Fig. 3, inset**). By this interpretation, our measurements suggest the ACC and all ACCMC compositions contain qualitatively similar amounts of rigid H_2O as Regime 1. However, the broader temperature span of Regime 2 suggest higher Mg contents may correlate with increasing amounts of restrictedly mobile water.

This interpretation is consistent with the increasing amount of solvation water that would be associated with larger proportions of the charge-dense Mg compared to Ca (e.g. Di Tommaso & de Leeuw, 2010; Rodriguez-Blanco et al., 2011; Cobourne et al., in press). The complexity of the spectra at higher temperatures suggest greater Mg content is correlated with differences in the hydroxyl content and structure. These data suggest a relationship between the Mg and water contents of ACCMC; however, no dependence was measured (**Table A1, Appendix**) for the conditions of this study. Clearly, a detailed structural investigation of ACCMC is needed.

3.2 Controls on Mg Content of ACC

3.2.1 Apparent dependence upon alkalinity

A first group of experiments investigated the effect of alkalinity on the Mg content of ACCMC using constant input Mg/Ca ratio of 5/1 and input solution pH of 9.75. **Figure 4** suggests

a positive correlation between the solution alkalinity and ACMC composition, with a single trend that suggests an increase of 15 mole % Mg. The relationship is misleading, however, because this composition space traverses multiple solution chemistry variables, with increasing Ω (2.23–6.22), total carbonate concentration (60–400 mM), pH (9.1–9.7), and ionic strength (0.093–0.304). Previous studies show ionic strength can also affect the precipitation kinetics of sparingly soluble salts, such as calcite (Zuddas & Mucci, 1998) and barite (Kowacz et al., 2010), but there is no relationship between the ionic strength of the precipitating solution and the Mg content of the ACMC (**Fig. A3**) for the conditions of this study.

3.2.2 Interplay of carbonate concentration, pH, and Mg/Ca ratio

To determine the influence of total carbonate concentration and pH on ACMC composition, additional experiments were conducted to independently vary carbonate concentration and pH (**Table 1**). **Figure 5A** shows the dependence of ACMC composition on the *input* solution saturation state ($\Omega = 4.55\text{--}6.33$). Recall the experimental design maintains a constant initial Ca concentration. Thus, differences in Ω are regulated primarily by adjusting the input CO_3^{2-} concentration. Trends in **Fig. 5A**, however, do not show a simple relationship between input Ω and ACMC composition (**Fig. 5A**). Rather, composition follows contours of the input carbonate concentration. Indeed, the relationship between ACMC composition and the output carbonate ion concentration (**Fig. 5B**) is similar to **Fig. 5A**; reiterating the primary control of CO_3^{2-} concentration on Ω in these experiments. The relationships also show offsets that indicate the greatest Mg contents are associated with the higher pH of the Na_2CO_3 solutions (e.g. **Table 1**).

By comparing ACMC compositions to the steady state pH of output solutions (**Fig. 5C**), we find the data collapse onto one trend whereby higher pH solutions promote a greater Mg

content. Further inspection shows that for a constant pH, small offsets to higher Mg levels are correlated with the higher total carbonate concentrations. Thus, the data demonstrate that solution pH, as regulated by the total carbonate concentration at steady state conditions, has a primary control on ACMC composition. These connections can be understood by recalling that the input pH of the initial carbonate solutions was varied across each of the total carbonate conditions used in this study (**Table 1**). For a given total carbonate concentration, differences in pH account for variations in Ω . Thus, the trends seen in **Figs. 5A** and **5B** are primarily determined by solution pH. This also explains the relationship between alkalinity and ACMC composition (**Fig. 4**).

It is important to remember that the molar Mg/Ca ratio of the steady state solution ($m_{\text{Mg}}/m_{\text{Ca}}$) is the overarching control on composition relations. Although all synthesis experiments began with an initial Mg/Ca ratio of 5/1, experiments with the higher chemical driving forces produced greater extents of reaction within the reactor. Because a greater extent of reaction consumes a larger fraction of Ca^{2+} than Mg^{2+} during ACMC precipitation, each initial solution composition evolves to a higher, condition-specific Mg/Ca ratio (**Fig. 5D**). For examples, those solutions with the greater total carbonate concentrations and pH levels acquired Mg/Ca ratios as high as 14 at steady state conditions.

3.2.3 Partitioning relationships

The dependence of ACMC composition on the interplay of solution variables discussed in **Fig. 5** can be further quantified by applying a simple partitioning model. Assuming that precipitation conditions are in a regime where rates are reaction-limited and mass transport effects are negligible (see **Section 2.1**), the distribution coefficient for Mg partitioning between ACMC precipitates and the reactant solution is expressed by:

$$K_D = \frac{\left(\frac{X_{Mg}}{X_{Ca}}\right)}{\left(\frac{m_{Mg}}{m_{Ca}}\right)} \quad (5)$$

where $\left(\frac{X_{Mg}}{X_{Ca}}\right)$ is the ratio of the Mg/Ca concentration in the ACMC product and $\left(\frac{m_{Mg}}{m_{Ca}}\right)$ is the ratio of the total concentration of cations in the corresponding effluent solutions at steady state conditions. Note that K_D is a consequence of growth and hence, does not reflect equilibrium conditions. This parameter, therefore, normalizes the steady state chemistry of the solution during precipitation with the corresponding composition of the solid that is forming. **Equation (5)**, also known as the Henderson-Kracek or homogeneous distribution coefficient (Henderson & Kracek, 1927), is used because the composition of the solution was maintained constant within the reactor at steady state conditions. **Figure 6** shows that when calculated K_D values are compared to the output pH of the corresponding experiment, the data collapse onto one trend. The average value of K_D is approximately 0.047 ± 0.003 when steady state pH is less than approximately 9.5, and triples from 0.052 to 0.178 between pH 9.5 and 10.3, respectively.

Our estimates of K_D are much lower than the 0.44 ± 0.02 value reported for ACMC with a similar Mg content (Radha et al., 2012). There are two probable explanations for this difference. First, our K_D estimates are based upon the values of Mg/Ca at steady state conditions in contrast with the standard practice of using initial solution Mg/Ca values. **Figure 5D** shows the mole fraction of Mg/Ca in output solutions evolves to significantly higher values in proportion to the extent of supersaturation (extent of precipitation in the reactor). Thus, the larger denominator of **Equation (5)** inevitably produces a lower K_D . This analysis shows the local Mg/Ca values are more representative of the chemical conditions during the time of formation.

A second explanation for our lower K_D values is likely found in the lower pH conditions of this study. To better approximate natural environments, this study was designed to produce

output pH values of 8.9 to 10.4 (e.g. **Fig. 5c** and **Fig. 6**). In contrast, previous studies used reagents of Na_2CO_3 (Koga et al., 1998; Radha et al., 2012; Rodriguez-Blanco et al., 2012) and $(\text{NH}_4)_2\text{CO}_3$ (Aizenberg et al., 1996; Raz et al., 2000; Wang et al., 2009, 2012). Although the *in situ* pH during mineralization is unknown, those experimental conditions are qualitatively associated with very high pH. We attempted additional experiments to extend the pH range upward and make direct comparisons with these higher pH studies, but we encountered an experimental limitation with the precipitation of a different phase, possibly brucite ($\text{Mg}(\text{OH})_2$). We confirmed the appearance of this new phase by SEM observations of rosette features that are morphologically similar to those reported by Hsu and Nacu (2005), the presence of a sharp O-H peak around 3700cm^{-1} in the FTIR spectra (**Fig. A4, Appendix**), and a unique TGA weight loss curve that did not resemble ACMC. Our experimental conditions that produced $\text{Mg}(\text{OH})_2$ concur with previous reports (Hsu and Nacu, 2005).

4. DISCUSSION

By using a synthesis method that allows us to quantify the relationship between ACMC composition and steady state solution chemistry, we find that three inorganic “levers” regulate the Mg content of ACMC. The primary control on composition is the Mg/Ca ratio of the input solution, a conclusion shown previously (Loste et al., 2003; Radha et al., 2012; Wang et al., 2009). A higher initial concentration of Mg^{2+} ions in solution establishes the minimum baseline for total Mg in the resulting ACMC. However, we show the Mg content of ACMC for a *fixed* solution Mg/Ca ratio is regulated by total carbonate concentration, in proportion to the extent of precipitation due to its principal control on supersaturation. While the dependence of Mg content on pH is not fully understood, the data suggest local solution pH influences the amount of Mg in ACMC also through supersaturation and the corresponding extent of reaction

As noted earlier, kinetic effects are known to influence composition under some conditions (**Section 2.1**), and a kinetic explanation is also given in an experimental study of abiogenic aragonite that shows elemental partitioning with similar departures from a thermodynamic model based upon the K_D values of trace elements (Gaetani & Cohen, 2006). In our study, however, precipitation rates were similar within a factor of 10X, suggesting the chemical ‘levers’ proposed herein are more significant in regulating composition for the conditions used in this study (e.g. **Fig. A1, Appendix**).

The quantitative relationships presented in this study raise the point that the controls on APMC composition are most accurately interpreted in conjunction with the local solution composition at the time of formation. As APMC precipitates, solution Mg/Ca ratios evolve to higher values in proportion to the extent of reaction. Thus, conventional estimates of K_D that use the (lower) initial solution Mg/Ca values produce erroneously high values. We show the Mg/Ca ratios of steady state solutions almost triple (from 5 to 14) for some conditions despite the flow through conditions. Previous investigations that used batch (closed) methods, by mixing with $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ solutions or by the ammonium carbonate diffusion technique, show that extreme shifts in Mg/Ca ratios are possible over the course of precipitation with values that can easily exceed 60-100 (Wang et al., 2012).

Our findings suggest the conventional practice of estimating K_D from initial solution chemistry may need to be revisited because this approach does not give meaningful representations of solution conditions at the time of formation. Moreover, the concept of K_D assumes APMC composition is linearly correlated with solution Mg/Ca (**Equation 5**). However, **Figure 6** shows this simple dependence breaks down when K_D is determined by using local solution conditions *at the time of formation*. Although the conventional approach indeed gives a

linear relationship between *initial* solution Mg/Ca ratios and ACMC composition (Blue et al., 2013; Radha et al., 2012; Wang et al., 2012), this is not an accurate representation of solution-solid relationships.

This insight is significant for interpreting the formation of ACMC and perhaps other carbonates in closed and semi-closed biogeochemical environments. As precipitation proceeds, the Mg/Ca ratio of the local chemical environment increases to higher values. Sites of rapid calcification within the semi-closed environment of tissues that produce ACMC provide a natural setting for large increases in local Mg/Ca ratio. Similar processes can also occur in closed basin lakes and some marine porewaters where supersaturations can exceed ACC solubility (Chave, 1954b; Nishiyama et al., 2012; Katz & Nishri, 2013). In these settings, local Mg/Ca ratios are 1) driven to extremely high (and condition-specific) values and 2) evolve over the extent of reaction to complicate estimates of K_D .

It is notable that ACMC can be produced at pH conditions relevant to diverse geological and biogeochemical systems. This suggests ACMC is feasible in environmental settings where waters become highly supersaturated with respect to the crystalline polymorphs for short periods of time. Previous studies that used extreme pH conditions propose that a nonclassical mineralization pathway involving an amorphous intermediate can explain the formation of very high Mg calcite (Long et al., 2011; Wang et al., 2012). The formation of ACMC at milder pH supports this idea and suggests that the carbonate geologic record should be revisited for evidence of calcification by pathways involving this metastable phase.

The findings are consistent with an earlier postulation that the Mg content of calcite minerals is correlated with solution alkalinity (Boyle & Erez, 2003; Given & Wilkinson, 1985). We show, however, this relationship is an apparent dependence that is rooted in the local pH of

the precipitation environment (when total carbonate is held constant). Finally, the findings may have implications for the question of how ocean acidification will affect the formation of carbonate biominerals. For settings where the chemistry of calcification environments closely parallels seawater conditions, APMC may become more difficult to produce and form with a lower Mg content.

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Chemical Controls on the Magnesium Content of Amorphous Calcium Carbonate

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Figure Captions

Fig. 1. SEM images show the similar spherical morphology and particle sizes of all ACMC produced in this study. Particles appear to become more amalgamated with increasing Mg content. (A) pH = 8.9, $\text{NaHCO}_3 = 0.20\text{M}$; (B) pH = 9.5, $\text{NaHCO}_3 = 0.10\text{M}$; (C) pH = 10.0, $\text{NaHCO}_3 = 0.06\text{M}$; (D) pH = 10.3, $\text{Na}_2\text{CO}_3 = 0.06\text{M}$.

Fig. 2. FTIR characterization of the control Mg-free ACC (black) and ACMC with variable Mg contents (colors) shows the presence of Mg increases peak widths. The carbonate vibrational modes of the Mg-bearing samples shift with respect to the pure ACC (e.g. **Table A1** for values). The vibrational modes are given by: ν_3 (anti-symmetric stretch), ν_1 (symmetric stretch), and ν_2 (out of plane bend). The broad peaks around 3300 cm^{-1} and the peaks around 1650 cm^{-1} are due to the vibrational modes of water.

Fig. 3. TGA weight loss curves for six representative ACMC samples. The weight loss curve of the Mg-free ACC (black) estimates a weight loss of 20% at 250°C , giving a stoichiometry of 1.42 water molecules per CaCO_3 . The weight loss curves of ACMC samples (colors) determine a weight loss of 22–24% at 250°C , resulting in 1.37–1.63 water molecules per Mg,CaCO_3 (see Table A1). Findings from Schmidt et al. (2014) (inset) suggest the ACMC produced in this study contain similar amounts of rigid H_2O (regime 1), but greater Mg contents have increasing amounts of restrictedly mobile H_2O (regime 2) and hydroxyl (regime 3).

Fig. 4. The Mg content of ACMC appears to correlate with solution alkalinity but this trend is misleading because pH (9.1–9.7) and supersaturation (0.087–1.82) are variable across alkalinity space. The dashed line provides a visual guide and is not a quantitative fit.

Fig. 5. (a) The mole fraction of Mg in ACMC does not show a simple dependence on the initial supersaturation of the reactant solution (Ω , Eq. 4). The individual trends for each total carbonate solution concentration reflect differences in Ω . (b) The output CO_3^{2-} concentration of the reactant solution has a similar relationship to Mg content, with trends existing for each total carbonate solution concentration. The largest offsets are measured for the higher pH conditions of the Na_2CO_3 solutions. (c) The Mg content of ACMC increases with a combination of higher output pH and total carbonate concentration. (d) In this study, input solutions began with an initial Mg/Ca ratio 5 (red line). As ACMC precipitates, the Mg/Ca ratio evolves to higher values depending upon Ω . The output (steady state) solution Mg/Ca values ($m_{\text{Mg}}/m_{\text{Ca}}$) shown here do not correlate with the Mg/Ca ratios in ACMC ($X_{\text{Mg}}/X_{\text{Ca}}$), thus reiterating our conclusion that additional factors control the partitioning of Mg into ACMC.

Fig. 6. Mg partitioning into ACMC correlates with the steady state pH of the local precipitation environment. Below pH 9.5, K_D is independent of pH, and is primarily controlled by steady state solution Mg/Ca ratio. Above approximately pH 9.5, the K_D is strongly dependent upon pH with values tripling between 9.5 and 10.3.

Table 1. Solution and ACMC compositions for all experiments (25±1°C). The input solutions were combined in the MFR with a second solution containing 0.01 M CaCl₂ and 0.05 M MgCl₂.

Experiments with NaHCO ₃								
Input			Output			ACMC Composition		
[CO ₃ ²⁻] _{Tot} (M)	pH	Ω _(Pure ACC)	pH	[CO ₃ ²⁻] (m)	I. S. (m)	X _{Mg} /X _{Ca}	mole % Mg	K _D (Eq. 5)
0.06	9.93	4.55	9.34	0.00056	0.0420	0.360	26.48	0.054
0.06	10.00	4.67	9.39	0.00058	0.0414	0.351	25.97	0.049
0.06	10.11	4.84	9.59	0.00078	0.0409	0.474	32.16	0.056
0.06	10.19	4.95	9.62	0.00080	0.0406	0.450	30.97	0.055
0.06	10.27	5.05	9.75	0.00098	0.0407	0.569	36.25	0.063
0.06	10.38	5.16	9.80	0.00098	0.0399	0.611	37.94	0.067
0.06	10.78	5.43	10.05	0.00121	0.0390	1.185	54.24	0.115
0.10	9.65	4.94	9.10	0.00068	0.0625	0.332	24.93	0.047
0.10	9.75	5.19	9.22	0.00201	0.0762	0.336	25.17	0.043
0.10	9.79	5.28	9.31	0.00089	0.0608	0.393	28.24	0.046
0.10	9.90	5.50	9.49	0.00117	0.0603	0.455	31.27	0.049
0.10	9.98	5.64	9.56	0.00127	0.0510	0.561	35.95	0.055
0.10	10.12	5.85	9.75	0.00163	0.0596	0.804	44.57	0.068
0.10	10.25	6.05	9.89	0.00370	0.0667	1.098	52.33	0.080
0.20	9.32	4.91	8.98	0.00106	0.1102	0.340	25.39	0.044
0.20	9.43	5.26	9.12	0.00133	0.1097	0.379	27.49	0.044
0.20	9.46	5.35	9.14	0.00140	0.1098	0.401	28.62	0.046
0.20	9.59	5.69	9.33	0.00201	0.1099	0.474	32.15	0.047
0.20	9.72	5.98	9.50	0.00256	0.1096	0.664	39.90	0.056
0.20	9.96	6.33	9.75	0.00721	0.1169	0.836	45.54	0.057
Experiments with Na ₂ CO ₃								
Input			Output			ACMC Composition		
[CO ₃ ²⁻] _{Tot} (M)	pH	Ω _(Pure ACC)	pH	[CO ₃ ²⁻] (m)	I. S. (m)	X _{Mg} /X _{Ca}	mole % Mg	K _D (Eq. 5)
0.06	11.12	5.28	10.13	0.00235	0.0752	1.844	64.84	0.160
0.10	11.24	6.10	10.34	0.00421	0.1130	2.545	71.79	0.178