Impact of Citrate Ions on the Nucleation and Growth of Anhydrous CaCO₃

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ABSTRACT: Citrate has been shown to inhibit CaCO₃ growth and it is also found in biogenic calcite, but full understanding about how citrate controls CaCO₃ formation, and hence CaCO₃ polymorph stability and crystal morphology, is still lacking. We investigated the effect of citrate (CIT) on CaCO₃ crystallization by monitoring crystallization rates and crystal polymorph, size, and morphology as a function of increasing mol % CIT/Ca ([Ca²⁺] = [CO₃²⁻] = 4 mM). At CIT/Ca ≤ 50%, both vaterite and calcite formed, but nucleation and growth were retarded as CIT/Ca was increased, demonstrated by the increase in induction time and decrease in the reaction rate constant, fitted using the Johnson-Mehl-Avrami-Kolmogorov model. This is partly explained by increased CIT-Ca complexation with higher [CIT], thus reducing saturation state with respect to vaterite and calcite. Simultaneously, CIT sorption inhibited crystal growth, demonstrated by smaller vaterite spheres and more elongated calcite crystals as CIT/Ca increased. At CIT/Ca ≥ 75%, vaterite formation was completely inhibited, with only elongated calcite forming and growth rate decreased further as CIT/Ca was increased. Overall, citrate inhibited growth rates most at CIT/Ca ≤ 50%, while nucleation was less affected. In contrast, at CIT/Ca > 50%, citrate strongly inhibited nucleation but had less effect on the growth rate. The results illustrate the spectrum of impacts that complexing compounds can have on CaCO₃ crystallization, by forming complexes in solution, affecting CaCO₃ saturation state, and also by interacting with the crystal surfaces, thereby controlling nucleation and growth. The extent and the type of effect depend on concentration regimes.

1. INTRODUCTION

Calcium carbonate is one of the most ubiquitous minerals in nature and is a key component in the global carbon cycle. It is formed inorganically through precipitation from CaCO₃ supersaturated waters and biogenically through the activity of organisms. In biogenic CaCO₃ mineralization, the presence of organic molecules leads to modification of the crystal structure and morphology, often leading to crystals with higher stability and resilience.6−9 Understanding the CaCO₃ crystallization mechanisms and pathways in the presence of organic molecules is a key to understanding its growth in nature and for optimizing CaCO₃ products for commercial applications, such as production of antiscalants and products for manufacture of paint, ceramics, paper, and abrasives.

Citric acid, mainly found in its deprotonated form, citrate (C₃H₅O(COO)₃⁻; CIT), in natural conditions, is a well-known complexing agent for dissolved Ca and has been shown to inhibit CaCO₃ growth. It is an intermediate in metabolic pathways and is found associated with CaCO₃ biominerals. Its functional groups, namely, hydroxyl and carboxyl, are also the main functional groups in the complex polysaccharides used by organisms to control biomineral growth and in the polar compounds present in oil and organic groundwater contaminants. Thus, improved understanding about how citrate controls CaCO₃ formation, and hence CaCO₃ polymorph stability and crystal morphology, would improve our ability to understand nature and to innovate better processes and products, such as scale inhibitors and surfactants.

Previous laboratory studies have shown that citrate strongly inhibits CaCO₃ growth.5−9 For example, in seeded experiments, both calcite and aragonite growth rates drastically decreased in the presence of citrate but aragonite was more affected than calcite.6 We recently showed that citrate strongly affects calcite crystallization from an amorphous precursor, leading to calcite crystals with modified morphologies and sizes. An observation shared by previous studies is that if citrate is present, vaterite is not formed during CaCO₃ crystallization. Calcite forms either directly from solution5−7 or following the breakdown of the amorphous precursor phase.5,9 Literature reports that vaterite formation has only been observed in the presence of citrate
when combined with dodecyl benzenesulfonate and where CaCO₃ growth was induced by CO₂ gas diffusion. Overall, it is well accepted that citrate interacts strongly with dissolved calcium, through complexation, as well as with the CaCO₃ phases that form from supersaturated solutions. The exact mechanism of these interactions and how these impact kinetics, crystal type, morphology, size, and stability is, however, still not fully understood, particularly in systems where CaCO₃ crystals nucleate and grow directly from solution (i.e., in the absence of seeds or a poorly ordered precursor phase).

In this study, the effects of CIT on CaCO₃ crystallization kinetics were investigated in solution, as a function of varying CIT/Ca content (0–100 mol %). For this, CaCO₃ nucleation and growth were monitored using time-resolved UV–vis spectrophotometry and the data were fitted using the Johnson-Mehl-Avrami-Kolmogorov (JMAK) particle nucleation model to obtain growth rate constants. Data from X-ray diffraction (XRD), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and thermogravimetric analysis (TGA) provided information about CaCO₃ polymorph selection, size, morphology, and composition at specific times during the crystallization experiments.

2. METHODS

2.1. CaCO₃ Synthesis and Crystallization. CaCO₃ crystallization experiments were performed at 22 °C by mixing equal volumes of a 4 mM CaCl₂ solution and a 4 mM Na₂CO₃ solution containing varying citrate (CIT) concentrations ([CIT] = 0 to 4 mM, i.e., 0–100% CIT/Ca). Solutions were prepared using reagent grade chemicals and ultrapure deionized water (Milli-Q, resitivity >18 MΩ cm). To ensure identical pH in all CIT containing Na₂CO₃ solutions, a 100 mM CIT stock solution was prepared first and then titrated to pH 11.2 using 2 M NaOH, to match the pH of the pure Na₂CO₃ solution. Na₂CO₃ solutions were stored in tightly closed plastic bottles to avoid CO₂ absorption from the air.

UV–vis spectrophotometry was used to investigate the effect of CIT on CaCO₃ crystallization rates by monitoring the time dependent change in solution absorbance, i.e., turbidity, following methods detailed in Tobler et al. For this, 1 mL of CaCl₂ solution was added to 1 mL of Na₂CO₃ solution (±CIT) inside a plastic cuvette placed in the spectrophotometer (STS-UV, OceanOptics). The solution was continuously stirred and the turbidity was measured at a wavelength of 450 nm (cuvettes were open to the atmosphere). The measurements were stopped once solution turbidity reached a plateau, i.e., the turbidity did not increase further. At this point, the cuvette was removed from the setup and the suspension was vacuum filtered. The solids retained on the filter paper were quickly rinsed with isopropanol, to remove remaining water, and then gently dried by blowing air (25 °C) onto the filter paper. The solids were stored for later SEM and XRD analysis. Four to six replicate experiments were performed for each tested condition. In the following description, these experiments are referred to as turbidity experiments.

Large batch experiments (500 mL total volume, covered with paraffin) were set up with identical concentrations and conditions as above and run for 2 days to characterize the final crystallization products with XPS, SEM, TGA, and XRD (referred to as large batch experiments in the following text). The solids were separated from solution and rinsed with isopropanol as described above. All surfaces exposed to air and solution attract some degree of organic carbon contamination. We assumed that the contamination on the crystal surfaces derived from the air, the aqueous solutions, the isopropanol mix, and the compressed air used for driving off the water was the same for all samples so the differences in the XPS and TGA data reflect only the changes in composition resulting from the presence of CIT.

CaCO₃ saturation indices (SI) for the mixture of CaCl₂ and Na₂CO₃ (±CIT) solutions were calculated with the hydrogeochemical code PHREEQC using the minteq v4 database and the solubility products of calcite and vaterite. SI is defined here as

\[ SI = \log K_{sp} / IAP \]

where IAP represents the ion activity product and \( K_{sp} \) the solubility product. At the concentrations used here, amorphous calcium carbonate is undersaturated, confirmed by its absence in our experiments.

2.2. CaCO₃ Characterization. XRD (Brucker D8, Cu Ko 1.2 radiation, 0.02° step size from 10 to 70° 2θ, 1 min⁻¹) was used to identify the crystalline CaCO₃ polymorphs, while SEM (Quanta 3D, 5 kV, high vacuum) was applied to image changes in particle morphology and size as a function of added CIT. SEM samples were prepared by placing the dry solids on sticky carbon tape attached to SEM stubs.

XPS was used to quantify the sorption of CIT within the top 10 nm of the collected CaCO₃ solids. The analyses were performed with a Kratos Axis Ultra system, operated with a monochromated Al Kα X-ray source (power = 150 W, ka = 1486.6 eV), using pass energies of 20 or 160 eV for high resolution and wide scans. Data were analyzed using the CasaXPS software and the binding energy calibration was done using the C 1s peak from carbonate at 290.1 eV for energy calibration.

The total amount of CIT incorporated into the CaCO₃ precipitates was determined by TGA (Netzsch TG 209 F1 Libra). Samples were heated from 30 to 900 °C at a rate of 10 °C/min and under N₂ atmosphere.

2.3. Kinetic Data Analysis. The turbidity data were normalized using the maximum turbidity value measured for that experiment and then fitted to the Johnson-Mehl-Avrami-Kolmogorov (JMAK) particle nucleation model, which is based on the Avrami equation.

\[ n = 1 - e^{-\left(k_{1} + t_{0}\right)^α} \]

where \( α \) represents the degree of the reaction (normalized turbidity data), \( t_{0} \) the induction time, which corresponds to the time lapse between solution mixing (\( t_{0} \)) and onset of particle nucleation, \( k \) represents the reaction constant, and \( n \), the Avrami constant. This constant can be expressed as \( n = d + 1 \), where \( d \) is the growth dimensionality. For example, vaterite grows spherically (nucleation controlled growth), most often in 3 dimensions, which results in \( n = 4 \) (\( d = 3 \)). In contrast, when the crystallization rate decreases as a result of decreasing supersaturation, \( n = 1 \) and large, single calcite crystals form. The parameters, \( k \) and \( t_{0} \), were determined from the best linear fit using Origin 9.1, with the Levenberg–Marquardt iteration algorithm.

3. RESULTS AND DISCUSSION

3.1. CaCO₃ Crystallization Experiments. Representative profiles from time-resolved UV–vis spectrophotometry of CaCO₃ crystallization for each tested CIT concentration are shown in Figure 1. In these plots, \( t = 0 \) denotes the time when the calcium and carbonate solutions were mixed (\( t_{0} \)) and the onset of CaCO₃ formation is identified by an increase in solution turbidity. At all tested conditions, CaCO₃ crystallization was delayed by an induction period, \( t_{ind} \) and this time increased with an increase in CIT/Ca ratio (Figure 1). For CIT/Ca = 0% (pure system), the onset of CaCO₃ crystallization was observed ~1 min after solution mixing, while in experiments with 50% and 100% CIT/Ca, crystallization was delayed by ~5 and 30 min, which is about 5 and 30 times longer than for the pure system. This increase in \( t_{ind} \) with increasing CIT/Ca coincided with a decrease in crystallization rate. This is interpreted from the decrease in the slope of the absorbance profile with increasing CIT content.

XRD and SEM analyses of CaCO₃ precipitates collected at peak crystallization (i.e., when the turbidity reached a plateau)
Figure 1. Crystallization experiments monitored with UV–vis spectrophotometry. Normalized turbidity is plotted as a function of time and % CIT/Ca, with the dashed lines showing the fits of the JMAK model. For clarity, only experiments with 0%, 25%, 50%, and 75% CIT/Ca are shown. Inset: close-up of the initial 60 min of the crystallization reaction, which more clearly shows the increase in induction time with % CIT/Ca.

Figure 2. SEM images of CaCO3 formed in the various turbidity experiments, when turbidity reached a plateau, with (A) 0%, (B) 25%, (C) 50%, and (D) 100% CIT/Ca. Vaterite spheres decreased in size with increasing CIT/Ca, while calcite crystals became more elongated, with roughened crystal faces and edges. The scale bar is 3 μm.

Table 1. Data Collected from Turbidity Experiments

<table>
<thead>
<tr>
<th>CIT/Ca (%)</th>
<th>JMAK model fit</th>
<th>Vaterite diameter (μm)</th>
<th>Saturation index, SI (log IAP/Ksp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.8 ± 842</td>
<td>1.9 ± 0.9</td>
<td>calcite 2.2 vaterite 1.6</td>
</tr>
<tr>
<td>2.5</td>
<td>1.3 ± 702</td>
<td>1.5 ± 0.6</td>
<td>calcite 2.2 vaterite 1.6</td>
</tr>
<tr>
<td>5</td>
<td>1.0 ± 587</td>
<td>1.3 ± 0.4</td>
<td>calcite 2.2 vaterite 1.6</td>
</tr>
<tr>
<td>10</td>
<td>1.5 ± 413</td>
<td>1.1 ± 0.3</td>
<td>calcite 2.1 vaterite 1.6</td>
</tr>
<tr>
<td>20</td>
<td>2.2 ± 333</td>
<td>0.8 ± 0.3</td>
<td>calcite 2.1 vaterite 1.5</td>
</tr>
<tr>
<td>25</td>
<td>2.2 ± 227</td>
<td>0.7 ± 0.2</td>
<td>calcite 2.1 vaterite 1.5</td>
</tr>
<tr>
<td>50</td>
<td>3.9 ± 101</td>
<td>0.5 ± 0.1</td>
<td>calcite 2.0 vaterite 1.4</td>
</tr>
<tr>
<td>75</td>
<td>10.0 ± 44</td>
<td></td>
<td>calcite 1.8 vaterite 1.2</td>
</tr>
<tr>
<td>100</td>
<td>27.0 ± 16</td>
<td></td>
<td>calcite 1.5 vaterite 1.0</td>
</tr>
</tbody>
</table>

*Extracted from JMAK n = 4 model fit. **Extracted from JMAK n = 1 model fit. *Between 33 and 94 vaterite spheres (69 on average) were measured to derive the means listed. **XRD data showed no vaterite for the 75% CIT/Ca experiment (SEM data could not be obtained). *Only calcite present. Calculated for initial solution composition, prior to CaCO3 precipitation.

and the number of step edges increased. Moreover, the formation of elongated crystals showed preferential growth in one direction, which became more pronounced with increasing CIT/Ca (Figure 2).

To get a more accurate measure of the changes in tind and CaCO3 crystallization rate with CIT/Ca, the normalized turbidity profiles were fit to the JMAK model (eq 2). The data could be fairly well modeled using a first order reaction rate (R2 values >0.98 for n = 1). However, closer inspection of the data obtained during the onset of crystallization showed that it was possible to fit the early reaction stages to a JMAK model with n = 4. This can be explained by spherulitic growth of vaterite at the beginning of the reaction, when supersaturation is highest. Some initial calcite may have also formed via a spherulitic growth mechanism, but this could not be clearly identified in SEM images. The fact that large, single calcite crystal formed in all experiments indicates that the nucleation and growth mechanism must have changed during the crystallization reaction, explaining the improved fit to n = 1, particularly for the later crystallization stages. In order to show the evolution of the crystallization mechanisms, the first part of the turbidity profiles (α = 0–0.3) was fit to a JMAK model with n = 4, while the later part was fit with n = 1 (α = 0.3–1; Figure 1). The fitted kinetic parameters (tind and k; Table 1) nicely show the increase in tind and decrease in crystallization rate constant, k, with increasing CIT/Ca (Figure 1). Overall, the fitted parameters clearly demonstrate that the presence of CIT retards nucleation and growth of CaCO3.

Previous studies have shown that CIT retards CaCO3 crystallization and this has been explained by CIT forming solution complexes with calcium ions, decreasing the activity of free calcium ions in solution and thus decreasing CaCO3 saturation levels. This was verified by PHREEQC modeling of saturation index (SI) for the conditions tested here (Table 1). SI for calcite and vaterite decreased substantially as CIT/Ca increased from 0% to 100%. For calcite, SI changed from 2.2 to 1.6, and for vaterite, from 1.6 to 1.0. No vaterite was observed in the XRD data at 75% and 100% CIT/Ca although the PHREEQC modeling suggests that the experimental solution is still supersaturated with respect to vaterite (SI ≥ 1.0 under all CIT/Ca conditions, Table 1). The absence of vaterite is likely a...
consequence of the supersaturation not being high enough to overcome the nucleation barrier imposed by the presence of citrate, an observation also made in previous CaCO₃ crystallization studies. The PHREEQC calculations were made assuming a pure vaterite phase but if CIT is adsorbed on or incorporated into the vaterite, its solubility product, thus the calculated saturation index, would be different.

To mimic only the impact of SI reduction by Ca-citrate complexation on CaCO₃ crystallization, a set of pure CaCO₃ crystallization experiments with lower Ca concentration, i.e., lower SI values, were performed (Figure S1) and the turbidity data were fit to the JMAK model as detailed above (Table S2). These showed that in the absence of citrate, a reduction of SI from 2.2 to 1.6 leads to about a 10-fold lower impact on CaCO₃ crystallization, compared with experiments where citrate is present (Table S2). Also, vaterite forms under all tested SI values with little change in particle size (Figure S3). Thus, the effect of the reduced SI resulting from Ca-citrate complexation on CaCO₃ crystallization is minimal compared to the impact that citrate sorption has on CaCO₃ nucleation and growth.

### 3.2. Calcite Morphology, Size, and Composition.
Following crystallization for 2 days, only calcite crystals remained, as verified by XRD and SEM of solids collected for all tested CIT/Ca conditions. Overall, the calcite crystal sizes and morphologies observed in samples crystallized for 2 days did not differ from those formed within the first 1–2 h of the turbidity experiments (Figures 2 and 3). Calcite crystals developed irregular, rough faces (Figure 3A–C) and became more elongated with increasing CIT/Ca (illustrated by the measured aspect ratio in Figure 3D). The elongation of the calcite crystals is along the c-axis, which means that the smooth top surfaces could be identified as {1014} faces and the irregular surfaces as {1010} faces. Vicinal faces are formed from a series of {1014} steps that are offset by one, two, three, or more CaCO₃ molecule units. Thus, in calcite crystallizing experiments with added CIT, CIT adsorption at {1014} steps results in step growth inhibition, which then leads to the formation of {1010} planes. The volume of single crystals, however, did not change much with CIT.

The observed calcite morphologies are consistent with those observed by Meldrum, who reported CIT influence on the size and morphology of calcite grown during CO₂ degassing of saturated Ca(HCO₃)₂ solutions with CIT/Ca varying from 1.75% to 270%. Citrate led to crystals elongated along the c axis with an aspect ratio as much as 7 for a solution with CIT/Ca = 81%. The crystals typically had relatively smooth {1014} faces and rough, irregular faces with stepped edges, identified as {1010} faces, such as those shown in Figure 3B and C. Similar morphological control on calcite growth, favoring formation of particular vicinal faces, has been shown in other studies on CaCO₃ crystallization with CIT as well as with other carboxylates (e.g., malate, aspartate) and more complex organic molecules, such as polysaccharides, peptoids, and peptides.

XPS was used to quantify the amount of CIT sorbed in the outer 10 nm of calcite crystal surfaces (crystallized for 2 days). The presence of CIT was identified by the C 1s peak with binding energy of 288.6 eV, which represents carbon in O=C=O bonds. The atomic ratio between C 1s from the CIT carboxylic groups and Ca 2p from the calcite increases with % CIT/Ca: 0.03 in the pure system (where C is probably adventitious carbon), 0.08 at 25% CIT/Ca and 0.10 at 50% and 100% CIT/Ca. The proportion of CIT adsorbed and incorporated within 10 nm of the surface increased with increased CIT/Ca in solution, but at CIT/Ca ~ 50%, the Ca sites became saturated, so there was no further increase in CIT surface sorption for CIT/Ca > 50%.

In a second step, CIT sorption on calcite was quantified using thermogravimetric analyses (TGA). The results are shown in Figure 4. In the pure system (0% CIT/Ca), one major weight loss occurred between 250 and 530 °C (more clearly shown in the inset B) correlates with adsorbed and incorporated citrate (H₂O). Weight loss increases with CIT/Ca. Total % weight loss is 0, 1.6, 1.9, and 2.9 for the 0%, 25%, 50%, and 100% CIT/Ca samples.
presence of CIT showed a similar CO₂ loss but slightly shifted to a lower temperature range, 570 to 770 °C. In addition, there were other events already at lower temperatures. Specifically, a two-step weight loss occurred between 250 and 530 °C (Figure 4A), which increased for higher CIT/Ca conditions (Figure 4). This additional weight loss must be directly linked to CIT that was adsorbed and incorporated during calcite growth because there were no other additives in the system. Pure citric acid monohydrate, analyzed using identical conditions, lost ∼80% of its mass between 180 and 260 °C. The higher temperatures required to remove it from the calcite samples suggests that the citrate is strongly bound on or in the calcite. Lee et al. performed TGA on calcite overgrowths formed in the presence of citrate in seeded experiments (with SI ∼1.1, CIT/Ca = 1.2%) and observed a weight loss of about 1% between 375 and 550 °C, which they linked to the decomposition of CIT. This temperature range is similar to that observed for our samples. The slight differences can be explained by differences in the calcite formation conditions, i.e., crystallization rate, calcite morphology, size, and structure, as well as possible differences in the TGA protocol. Noteworthy, FT-IR and NMR analyses of these CIT rich calcite overgrowths produced by Lee et al. and by Phillips et al. revealed incorporated water molecules (0.4 wt %) within the bulk calcite. Fluid inclusions are common in calcite, especially accompanying included organic molecules so this is not a surprise. Lee et al. and Phillips et al. explained water incorporation by electrostatic interactions and H bonding by carboxylic and hydroxyl groups to water molecules and free ions, which permits their incorporation into the mineral. This water is then released along with CIT during heating to 600 °C. Overall, the incorporation of citrate along with structural water decreases the thermal stability of calcite ever so slightly. Its decomposition to CaO started at lower temperature than for pure calcite.

The solution composition in this study was chosen to exclude the formation of amorphous calcium carbonate and to assess CIT impact on the nucleation and growth of crystalline CaCO₃. It was expected that vaterite formation would be inhibited in these experiments because previous studies, that tested similar or higher CaCO₃ saturation indices, only observed calcite crystallization from CaCO₃ supersaturated systems when citrate was present. In studies where an amorphous CaCO₃ precursor phase (ACC) preceded crystalline CaCO₃ formation, vaterite was also absent, even though under those conditions, i.e., when ACC transforms to crystalline phases, SI can be as high as 2–3 during crystallization. What has to be considered in these studies, however, is that CIT presence affected ACC structure, composition, and stability, which likely also influenced its direct transformation pathways. In summary, despite the fairly high CaCO₃ saturation state (as much as 3), vaterite was not observed in previous studies, but it was clearly observed here, in experiments with SIvaterite > 1.2 and vaterite sphere size decreases with increasing CIT/Ca, i.e., decreasing CaCO₃ supersaturation.

Vaterite might not have been observed in previous studies because samples were taken after several hours or days or they were dried at high temperature so if it had initially been present, it had transformed to calcite prior to analysis. Unfortunately, some articles provide little information about sample collection and treatment. From our results, there is no doubt that CIT inhibits vaterite nucleation. We could not identify it in experiments at high CIT concentration. At SIvaterite > 1.2, supersaturation is sufficiently high to overcome CIT inhibition of vaterite nucleation but particle size is affected.

Figure 5. Dependence of induction time, t_{ind}, and JMAK reaction constant, k, on the CIT/Ca ratio. (A) t_{ind} and k plotted versus CIT/Ca, (B) t_{ind} and k plotted versus (CIT/Ca)^2, (C) t_{ind} and k plotted versus log CIT/Ca, and (D) the ratio t_{ind}/k plotted versus CIT/Ca.
strongly correlated with increasing CIT/Ca, confirming CIT influence. To determine which process was most inhibited by CIT, nucleation or growth, we examined dependence of induction time ($t_{ind}$) and the JM Ak rate constant ($k$) on the CIT/Ca ratio. Figure S shows that induction time has an overall linear dependence with (CIT/Ca)$^2$ (Figure SB), with $t_{ind}$ increasing only slightly at low CIT/Ca ratio but drastically more when CIT/Ca > 25%. In contrast, the JM Ak rate constant strongly decreases at low CIT/Ca ratio but as CIT/Ca increases, the decrease in $k$ becomes smaller. Overall, $k$ is linearly dependent on log CIT/Ca (Figure SC). In summary, a slight increase in CIT/Ca at low CIT concentrations considerably reduces growth rate but does not affect the onset of nucleation, whereas at high CIT concentrations, nucleation inhibition exponentially increases with CIT/Ca but the effect on growth rate is minimal. The fact that both vaterite and calcite form at CIT/Ca ratios ≤ 75% (Figure SAD), somewhat complicates interpretation of the trends discussed above. Nevertheless, it is fair to say that while vaterite and calcite form at CIT/Ca ratio ≤ 75%, citrate inhibited growth more strongly than it inhibited nucleation, which is also consistent with the observed decrease in the size of the vaterite spheres. In contrast, at CIT/Ca ≥ 75% (i.e., $S_{\text{vaterite}}$ < 1.2), nucleation was strongly inhibited, particularly for vaterite, so only calcite formed (Figure SD).

Independent of the formation or the absence of an intermediate vaterite phase, calcite morphology was increasingly affected by increasing CIT/Ca. Particularly at CIT/Ca > 25%, the aspect ratio of calcite crystals increased more strongly, leading to more elongated crystals (Figure 3D). This may be interpreted by a larger number of crystals, with lower aspect ratio, forming at low CIT/Ca (regime where nucleation is minimally inhibited), while a smaller number of crystals, with higher aspect ratio, form at higher CIT/Ca (regime where nucleation is strongly inhibited). Under all circumstances, CIT deactivates steps which would produce {1014} faces, and leads to the formation of rough and irregular {1010} vicinal faces. From the data at hand, it is not possible to determine the specific calcite surface sites at which citrate adsorbs and in what configuration. Molecular scale techniques such as in situ atomic force microscopy and computational modeling could provide insights into such mechanistics, an approach that should be considered for the calcite-citrate system in future studies.

4. CONCLUSIONS

This study demonstrates that CIT simultaneously affects calcium carbonate nucleation and growth, by complexing calcium ions in solution and by sorbing on crystal surfaces. Although CIT-Ca complexation reduces CaCO$_3$ saturation levels by a considerable amount, the observed changes in CaCO$_3$ formation kinetics, polymorph stability, and crystal morphology result mainly from citrate interactions with the forming CaCO$_3$ phases. Composition data, rates of reaction, and the character of the final crystals formed showed that CIT affected CaCO$_3$ crystallization (1) by inhibiting CaCO$_3$ crystal nucleation, more so for vaterite (and aragonite) than calcite, (2) by controlling crystal growth through specific CIT-surface interactions with {1014} steps, and (3) by changing crystal solubility and thermal stability through CIT adsorption and incorporation. Citrate, C$_3$H$_5$O(COO$_3$)$^{3-}$, with its hydroxyl and carboxyl groups, has the same functional groups as are common in the complex polysaccharides that organisms produce to control biogenic calcite growth. This new understanding about the effects of citrate thus provides insights into the complex and not univocal effect of organic molecules on calcium carbonate crystallization and the mechanisms used by organisms to design fit-for-purpose biomaterials.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.cgd.7b00796.

Additional figures that show turbidity measurements (S1) and SEM images (S2) from pure CaCO$_3$ crystallization experiments, where saturation indices were decreased by reducing Ca concentration to mimic reduced SI in CIT containing experiments; and a table (S3) that compares JM Ak fits for pure and CIT containing experiments with identical SI (PDF).

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**Notes**
The authors declare no competing financial interest.

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