

Synthesis-dependant structural variations in amorphous calcium carbonate

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Abstract

- Amorphous calcium carbonate (ACC) was synthesised with the additives magnesium and poly(aspartic acid) (pAsp) and the structure of these ACC samples was investigated using X-Ray Absorption Spectroscopy (XAS), X-Ray Diffraction (XRD) and Infra-Red Spectroscopy (IR).
- Synthetic ACC can be produced with different short-range structures, according to the additives . While the first Mg-ACC precipitates showed short-range structures similar to aragonite, , the initial pAsp-ACC precipitates possessed short-range structures resembling vaterite.
- The influence of these additives on the crystallization of calcium carbonate is apparent in the precipitation of the first amorphous precursor phase, and that the first stages of recrystallization involve the expulsion of water from the structure .

Introduction

- Calcium carbonate is reported to exist in six polymorphic forms.
- The three anhydrous polymorphs, calcite and aragonite are by far the most common in biological and geological samples, while vaterite is rare in biology.
- The two well-defined hydrated crystalline polymorphs, calcium carbonate hexahydrate ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$) and calcium carbonate monohydrate ($\text{CaCO}_3 \cdot \text{H}_2\text{O}$) are typically synthesised at low temperatures in the presence of additives such as polyphosphate and magnesium.
- There are few examples of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ in biology, and none of the hexahydrate.

- Amorphous calcium carbonate (ACC) is quite fascinating as a biomineral when it can vary considerably in terms of stability, composition and short-range structure according its source.
- Inspired by a report that ACC is precipitated as a transient precursor phase to single crystal calcite in sea urchin larvae, the last decade has seen a huge increase of interest in this phase.
- Biogenic ACC can be broadly categorized as one of two forms: stable or transient. While the stable form remains non-crystalline, the transient phase can act as a precursor to either calcite or aragonite.
- Both forms of ACC also contain organic macromolecules and typically significant quantities of magnesium and phosphate, and there is good evidence to suggest that these act in combination to both induce ACC formation and determine its stability.

- Synthetically, ACC can be prepared both in the absence and presence of additives. In the absence of additives, ACC is precipitated as the first phase when the supersaturation of a solution exceeds the solubility product of this phase and it is typically short-lived at room temperature.
- Typically conferring greater stability to the amorphous phase, additives including magnesium and phosphate are also widely used in the synthesis of ACC.
- Providing a biomimetic approach to its formation, organic additives including poly(acrylic acid) and poly(aspartic acid), dendrimers, block copolymers and phytic acid are effective in stabilizing ACC. All of these additives retard the crystallization process such that the ACC becomes stable for periods from hours to months.

- The stability of additive-stabilized ACC, combined with the possibility of controlling the time-frame over which crystallization occurs endows it with great potential as a synthetic phase.
- According to the synthesis method ACC can exhibit liquid-like properties, giving excellent structural reproduction of a fine template structure. The structure and even polymorph of the transformation product can also be controlled according to the chemistry of the crystallization environment.
- A structural investigation of synthetic ACC precipitated in the presence of additives and examine how the rate of crystallization can be controlled according to the quantity of additive used.
- The value of XAS in particular in probing the structure of biogenic ACC is proven, demonstrating that although amorphous to X-rays, this phase typically contains short-range order particular to a given biomineral.
- This order may provide a blue-print for the ultimate transformation product.

- pAsp is widely used to stabilize ACC producing a versatile Polymer Induced Liquid Precursor (PILP) phase, while magnesium provides a contrasting inorganic additive. Both of these additives also have particular relevance to biogenic calcium carbonate samples.
- Soluble organic macromolecules isolated from calcium carbonate biominerals are typically highly acidic, being rich in aspartic and glutamic acids, and pAsp is often considered an excellent synthetic substitute for these molecules.
- Many biogenic ACC and calcitic samples also contain substantial quantities of Mg.
- ACC produced synthetically provides a more versatile system for study than biogenic ACC as it can be precipitated in large quantities under controlled conditions, and effects of systematic changes in the quantities and types of stabilizing additives evaluated.

Experimental

- The structures and crystallisation of amorphous calcium carbonate (ACC) precipitated from solution in the presence of two commonly-used additives, poly-DL-aspartic acid (pAsp) and magnesium ions, were measured.
- In all cases ACC was incubated in solution, before being isolated and examined in a dry state. Isolation from solutions at different times enabled time-resolved evaluation of structural changes in the ACC.
- Analysis of the dry samples over time using IR and XRD demonstrated that no further crystallization occurred over the time frame used in the current experiments.

- The majority of samples were prepared within a week preceding the EXAFS analysis and were stored in sealed vials at 0–4 °C. Selected samples were also prepared and analyzed with XAS to confirm that the spectra obtained were identical to those of the pre-prepared samples.
- The CaCO₃ precipitates were also studied using Infra-Red Spectroscopy (IR), X-Ray Diffraction (XRD), X-Ray Absorption Spectroscopy (XAS) and Thermogravimetric Analysis (TGA) to determine how the structure and crystallization of the ACC samples varied according to the type and quantity of the stabilizing additive used, and the incubation period in solution.

Sample preparation

- **CaCO₃** was precipitated using an ammonium carbonate diffusion method, 15 g of the prepared solutions were transferred to Petri dishes, which were placed in a sealed desiccator containing a vial of solid ammonium carbonate. Upon removal from desiccator, the solutions were filtered through 0.2 μm membrane filters (Millipore), the precipitated solids were rinsed twice with *ca.* 10 ml of absolute ethanol and were then left to air dry for one hour.
- **Poly-DL-aspartic acid (pAsp)**. Calcium chloride dihydrate solutions of concentration 50.0 mM and 20 , 60 and 100 $\mu\text{g}/\text{ml}$ of pAsp (sodium salt, average M_w 3000) were prepared. Samples of each polymer concentration were incubated for periods between 50 min and 1 d.
- **Magnesium ions**. Solutions of 10 mM CaCl₂·2H₂O and 1.00 M magnesium chloride hexahydrate were prepared and mixed in appropriate proportions.

Reference samples

- Aragonite was synthesised by the hydrolysis of a solution of concentration 0.25 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 2.25 M urea at 90 °C in a forced convection oven for 6 h.
- Vaterite was synthesised by adding 250 ml of 5 mM CaCl_2 to the same volume of 5 mM sodium carbonate solution. The pH of the resulting solution was adjusted to pH 10, and the solution was then stirred for 15 min. The product was isolated by filtration, was washed with water and absolute ethanol, and was allowed to air dry..

EXAFS

The solid samples were first ground into a fine powder with cellulose before being pressed into disks. The samples were then mounted into the EXAFS spectrometer and the spectra were collected in transmission mode.

Further analysis of ACC

- Samples for analysis by Infra-Red Spectroscopy (IR) were ground with spectroscopy grade potassium bromide (KBr) and pressed to produce a pellet. Samples were analyzed using a FT-IR spectrometer.
- The morphologies of selected precipitates were determined using Scanning Electron Microscopy (SEM). Glass slides supporting the crystals for analysis were mounted on SEM stubs with adhesive carbon pads and were sputter coated with Pt/Pd using an Agar High Resolution Sputter Coater.
- Powder X-Ray Diffraction (PXRD) was performed in reflection. Samples were gently ground and placed on a piece of silicon wafer. Data for the majority of samples were collected between 20° and 60° in intervals of 0.02° and scan rate of 1° min^{-1} .
- Thermogravimetric Analysis (TGA) was performed, and samples were heated in air from room temperature to 700° C at a rate of $10^\circ \text{ C min}^{-1}$.

Results

- The additives poly(aspartic acid) and magnesium were used to stabilize amorphous calcium carbonate (ACC), and their influence on the structure and solution-mediated crystallization of the ACC was investigated by isolating the precipitates from solution over a range of times.
- The formation of ACC in solution after exposure to ammonium carbonate vapor was indicated by solutions becoming cloudy, and precipitates were first isolated at times when sufficient solid had been produced to enable characterization: 50 min with poly(aspartic acid) and 2–4 h with Mg, depending on the concentration of Mg present.
- The first precipitates produced were analyzed using TGA to determine the composition. The most significant weight loss occurred up to about 200 °C, corresponding to the loss of structural water. Values obtained were weight losses of 21% with both 100 $\mu\text{g ml}^{-1}$ pAsp and 9:1 Mg:Ca, which corresponded to formulae of $\text{CaCO}_3 \cdot 1.5\text{H}_2\text{O}$.

- XRD of the CaCO₃ samples precipitated in the presence of pAsp showed that all samples were fully amorphous at the earliest isolation time (50 min) and that crystallization was slower with higher concentrations of pAsp.
- While crystallization was just commencing after 60 min in samples stabilized with 20 and 60 µg/ml pAsp, Crystallization did not commence until 3–4 h in the 100 µg /mL pAsp sample.
- Vaterite was the initial crystallization product. With continued incubation in solution, a mixture of both calcite and vaterite was produced, and after approximately 5 d samples were principally calcite.

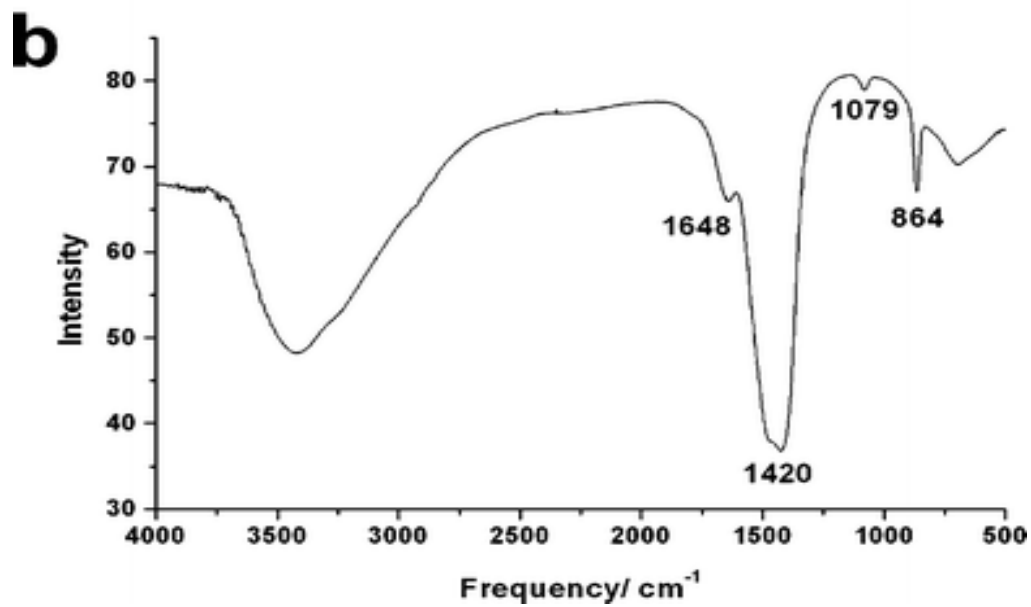
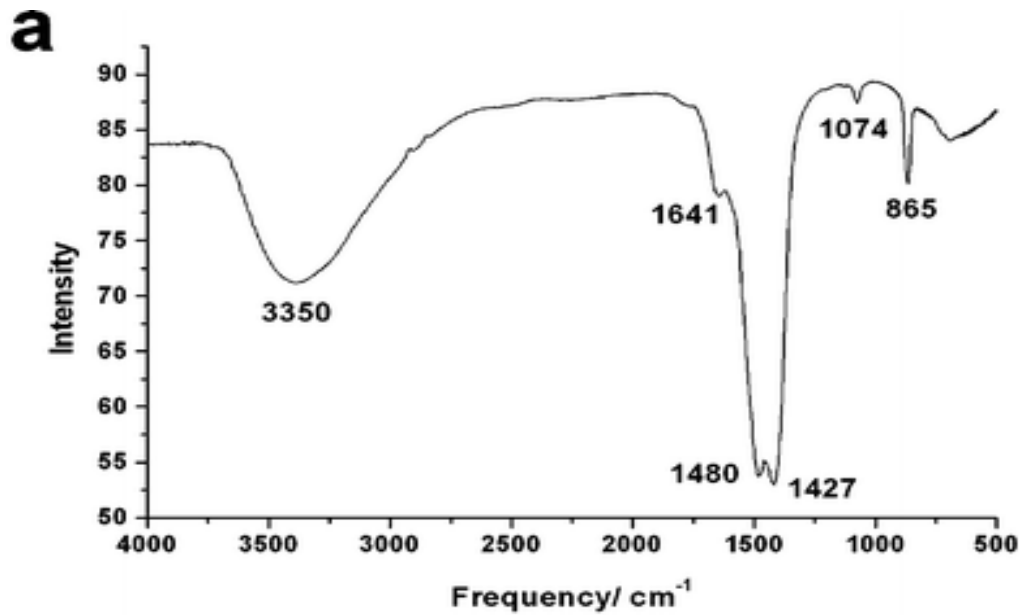


Fig.1. IR spectrum.

- The IR spectrum for the 9:1 Mg:Ca sample at 4 h is representative of these ACC phases and is shown in [Fig.1b](#). Peaks were observed at 1420 cm⁻¹, 1079 cm⁻¹ and 864 cm⁻¹ which are attributable to ACC, together with a peak at 1648 cm⁻¹ and a broad band in the region of 3300 cm⁻¹ due to water molecules. Crystallization was very slow in all samples,

XAS measurements: pre-edge and XANES regions

- [Fig. 2](#) shows spectra for calcite, aragonite and vaterite, comparing them with spectra of ACC samples confirmed to be amorphous using 9:1 Mg:Ca (isolated after 240 min) and 100 $\mu\text{g ml}^{-1}$ pAsp (isolated after 50 min).
- While the edge spectra of the crystalline phases calcite, aragonite and vaterite contain two peaks (A and B), samples amorphous to X-rays show a single large peak at the top of the absorption edge (C) ([Fig. 2](#)).
- The first peak of the crystalline samples appears at a lower energy at about 4049 eV than the amorphous samples, where it appears at 4050 eV. Both the Mg-ACC and pAsp-ACC yield very similar spectra. Some differences are also apparent in the pre-edge region, where a single small peak is apparent in the amorphous and vaterite samples at 4040.5 eV (D).

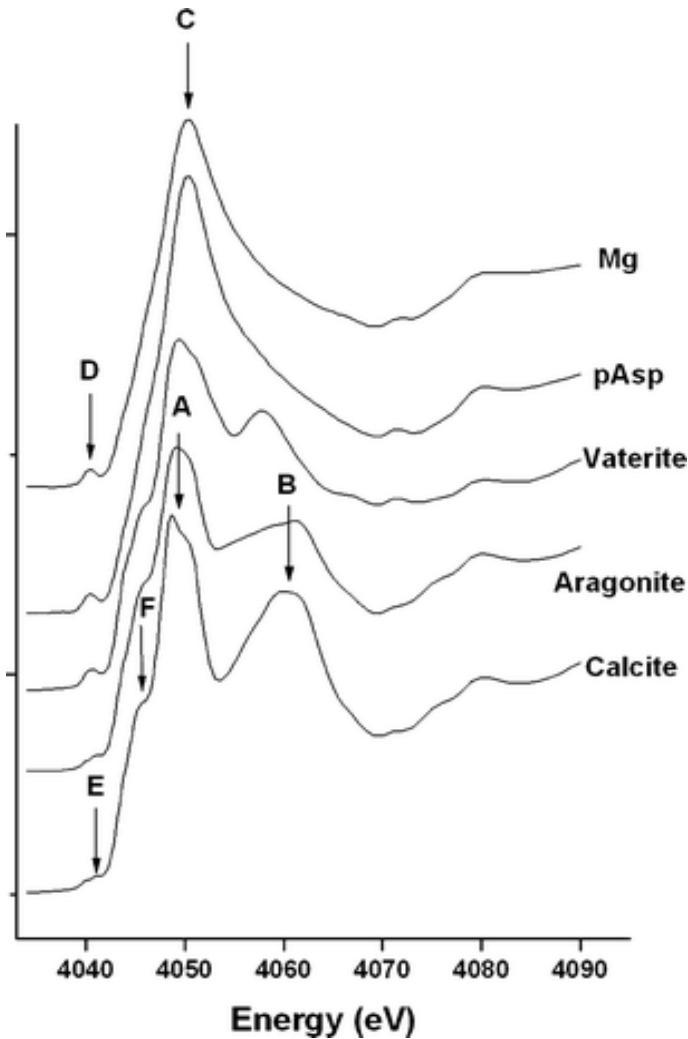


Fig. 2. X-Ray absorption spectra showing pre-edge and XANES regions of calcite, aragonite and vaterite reference samples, and samples of ACC produced using 9:1 Mg:Ca (isolated after 240 min) and 100 $\mu\text{g ml}^{-1}$ pAsp (isolated after 50 min).

Conclusions

- Synthetic ACC can be produced with different short-range structures, according to the solution additives used.
- Many additives are effective in directing the polymorph of the product calcium carbonate. The principal structural changes occurring during solution-mediated recrystallization then involve the expulsion of water from the structure and development of long-range order, while retaining the short-range structure around the calcium ions present in the first-precipitated ACC phase.
- Given that additives such as magnesium, phosphate and organic macromolecules act in combination to stabilize amorphous calcium carbonate *in vivo*, it would be fascinating to investigate the interplay of different additives in stabilizing and directing the crystallization of synthetic ACC.