

How to make 'stable' ACC: protocol and preliminary structural characterization

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ABSTRACT

A reproducible and simple protocol to synthesize and stabilize the metastable $\text{CaCO}_3 \cdot n\text{H}_2\text{O}$ phase termed amorphous calcium carbonate (ACC) was developed in order to allow the characterization of its structure at the nanoscale using high-resolution microscopy combined with Raman spectroscopy and X-ray diffraction. 'Stable' ACC consists of relatively smooth spherical particles, 50–200 nm in size, that have XRD and Raman patterns with no intense peaks or sharp bands, as expected from amorphous material. Furthermore, high-resolution imaging also supports this finding but in addition, beam-damage induced crystallization and the concomitant formation of locally ordered domains in the ACC spheres are discussed.

Introduction

AMORPHOUS calcium carbonate (ACC) is a metastable hydrated phase which can form as a precursor to crystalline CaCO_3 phases such as vaterite, calcite or aragonite (e.g. Faatz *et al.*, 2005; and references therein). ACC is known to form during biomineralization and low-*PT* aqueous geochemical processes within natural environments (Reeder, 1983; Addadi *et al.*, 2003; Weiner *et al.*, 2003). Based on powder diffraction data, ACC is thought to be amorphous or poorly ordered, as seen by the lack of distinct peaks within its pattern. Recently, Di Tommaso and De Leeuw (2008) and Michel *et al.* (2008) have used molecular modelling and scattering techniques to show that ACC has local order out to $\sim 15 \text{ \AA}$. ACC rapidly transforms to crystalline CaCO_3 phases (calcite formation within 1 min at 25°C , Ogino *et al.*, 1987). The instability and rapid transformation kinetics of ACC makes in depth characterization of its structure difficult. Here we present a simple and reproducible protocol for the synthesis and preservation for ACC for up to 3 days and

show preliminary data for its structural characterization that support its truly amorphous nature.

Experimental

ACC was synthesized from a CaCO_3 supersaturated aqueous solution, which was produced by mixing two pre-cooled (to 1°C) equivolometric 10 mM CaCl_2 and Na_2CO_3 aqueous solutions in a Teflon reactor equilibrated in a thermostatic bath at 1°C . Experiments were carried out at 1°C in order to retard the transformation of ACC into crystalline CaCO_3 (Ogino *et al.*, 1987). Upon mixing, a white precipitate formed immediately and after 1 min of reaction, the suspension was vacuum-filtered to remove the aqueous solution using a glass filtration unit ($0.2 \mu\text{m}$ Cyclopore filter). Following this the solids were quenched/washed ($<30 \text{ s}$) with isopropanol. The resulting material was dried using flowing air and stored in a sealed container. All characterizations were carried out in the dry state and experiments were repeated a minimum of five times to validate the method and results.

The resulting solids were characterized using powder X-ray diffraction ($\text{Cu-K}\alpha = 1.54 \text{ \AA}$) with patterns recorded at a scan speed of $0.08^\circ/\text{min}$ with a 2θ range from 5 to 60° . In addition the

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samples were analysed with a Renishaw 2000 Raman spectrometer (using a 633 nm He-Ne laser) at a 1 cm^{-1} spectral resolution. Finally, the solids were imaged using Field Emission Gun Scanning Electron Microscope (FEG-SEM, LEO 1530 Gemini at 3keV, with solids deposited on an Al stub and coated with 3 nm of Pt) and High-Resolution FEG-Transmission Electron Microscopy (HR-FEG-TEM, Oxford Instruments Philips CM200 with Supertwin Objective lens, a Gatan Imaging Filter and selected area diffraction (SAED) capabilities). Samples for FEG-TEM were prepared by dispersing the solids in isopropanol in an ultrasonic bath and depositing an aliquot onto a standard holey carbon TEM grid and imaged at 197 keV.

Results and discussion

In all experiments, a white, fluffy precipitate formed within milliseconds–seconds after mixing. X-ray analysis of the product (Fig. 1a) showed only broad reflections (from 25° to 38° 2θ and from 40° to 54° 2θ), identical to those reported by Faatz *et al.* (2004) for ACC. The stabilized dry ACC samples were rescanned after 6, 12, 24, 36, 48 and 72 h and no crystalline peaks were observed. In one sample, after 72 h, minor Bragg peaks associated with vaterite (2θ of 24.85° , 27.08° and 32.77°) and calcite (2θ of 29.45° , 39.49° , 43.03° and 47.30°) were observed, but these could have resulted from dehydration and consequent crystallization of the samples due to inappropriate storage. Furthermore, the Raman spectra of the precipitates (Fig. 1b lower pattern) showed no sharp bands. The main Raman band at 1085 cm^{-1} (carbonate symmetric stretching peak ν_1) was substantially broadened relative to the crystalline counterparts (Fig. 1b upper pattern; see also Addadi *et al.*, 2003; Weiner *et al.*, 2003) revealing the poorly ordered nature of the ACC.

Scanning electron microscopic observations (Fig. 1c) revealed the occurrence of relatively

smooth spheres with an average diameter of $\sim 125\text{ nm}$ (200 particles measured) with a moderate degree of polydispersity. HR-TEM images revealed no order in the majority of the observed particles, which was confirmed by the diffuse rings in the selected area electron diffraction patterns (Fig. 2 upper right, inset). However, upon exposure of the ACC spheres to the high voltage electron beam in the TEM, ordered domains (2–5 nm at 1 s, Fig. 2, upper right image, black arrows) gradually formed within the large ACC particles. Upon longer exposure (90 and 180 s) larger crystalline domains (5–20 nm with clear lattice fringes; middle and lower right high-resolution images) formed within the spherical particles. This indicates that the electron beam induced the transformation of the amorphous ACC to a more ordered structure. This process started within the interior of the spherical particles (Fig. 2) and was probably a result of dehydrating the initially porous and highly hydrated ‘amorphous’ ACC (can contain up to 19% water, Huang *et al.*, 2007) and its gradual ordering to a more stable CaCO_3 phase. Whether these more ordered domains correspond to a ‘proto-vaterite’ or ‘proto-calcite’ precursor is unclear, but the appearance of nanodomains within the ACC particles could have major implications for understanding the stability and transformation pathways of ACC to crystalline calcium carbonate phases. Such crystallization reactions induced by electron beam damage have been previously documented for other inorganic and organic samples (e.g. graphite or glycine; Egerton *et al.*, 2004 and references therein). Although, additional work is needed to determine which nanocrystalline CaCO_3 phase(s) are present within these domains, the results presented here show that high electron-dosages and long exposure times have to be avoided when imaging poorly-ordered ACC. This study has also shown that with careful defocusing and imaging at lower dosage, ACC imaging is feasible and we are testing a cryo-TEM mode using a plunge-freeze system to establish whether

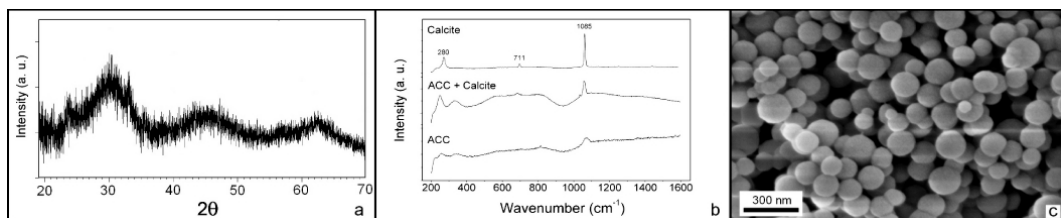


FIG. 1. Bulk characterization of ACC. (a) X-ray diffraction pattern; (b) Raman spectra; and (c) FEG-SEM image.

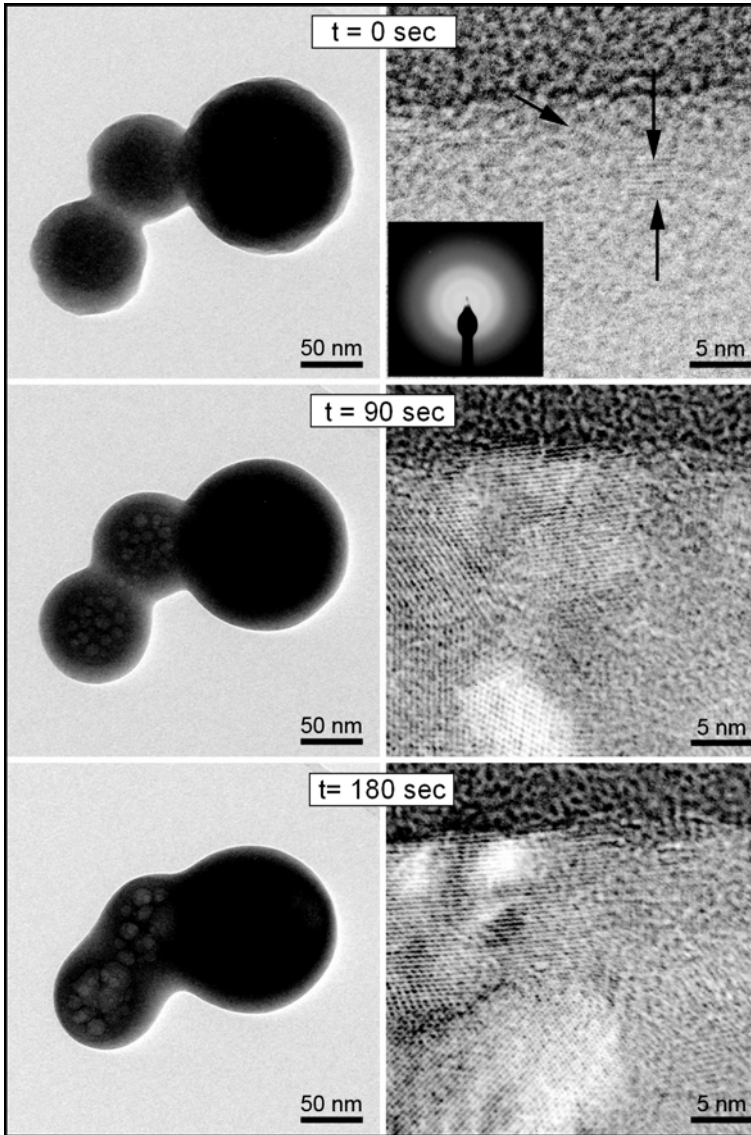


FIG. 2. TEM images of the crystallization of ACC as a consequence of beam damage. From top to bottom, pictures show the ACC morphology and internal structure at ~ 1 , 90 and 180 s. Insert in upper right image shows a selected area electron diffraction pattern of ACC, revealing only diffuse rings related to poorly ordered materials.

such a cryo-approach is more suitable for the high-resolution imaging and structural characterization of ACC with no beam damage. The results presented here however, support the recent results of Michel *et al.* (2008) who, based on wide-angle scattering and pair distribution function analyses revealed that ACC only exhibits local order at the Å scale.

The occurrence of ordered domains in the ACC particles (Fig. 3) at the initiation of its dehydration/crystallization to stable CaCO_3 phases opens the question about the role of these domains in controlling the pathways and mechanisms of the crystallization process. Whether these domains have similar or equivalent nanostructure characteristics to a crystalline CaCO_3 polymorph

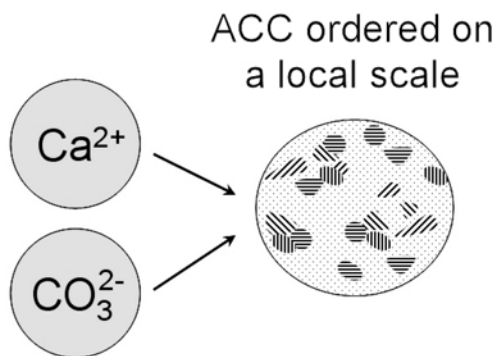


FIG. 3. Hypothetical evolution from a CaCO_3 supersaturated aqueous solution to an amorphous calcium carbonate with low-range ordered domains.

would not only provide a new perspective into the nature and bulk characteristics of ACC, but may also shed light on the possibility of ‘engineering’ and thus controlling the crystallization pathways of amorphous ACC to calcite or vaterite.

Conclusions

A simple method to obtain ‘stable’ (<3 days) pure ACC from aqueous solutions at low temperature has been developed, allowing the preliminary characterization of this compound. The nanoscale characteristics and the crystallinity of this material corroborate the true amorphous nature of ACC.

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