EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Probing mineralisation mechanism in Calcium Phosphate using coherent X-ray diffraction imaging	**Experiment number**: MA-4673
Beamline: ID 10C	**Date of experiment**: from: 25-Feb-2021 to: 05-March-2021	**Date of report**: 11-09-2023
Shifts: 18	**Local contact(s)**: Yuriy Chushkin	*Received at ESRF:*

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Report:

Coherent X-Ray Diffraction Imaging (CXDI) was performed on calcium carbonate ($CaCO_3$) microparticles to study effect of temperature, concentration and time on crystalline phases and morphology of chemically precipitated micro-particles. In the original proposal the model system to study was identified as calcium phosphate. The choice of calcium carbonate was based on in-house experiments on crystallisation which allowed us to optimise the experimental condition e.g., temperature and concentration, to produce particles with 2-5µm diameter. For precipitation of calcium phosphate, it was not possible to obtain reproducible particles of the above-mentioned sizes which are necessary for the ID10 setup at ESRF. The long-term goal of the project is to understand the mechanism of biomineralization and establish CXDI as a powerful methodology to study such phenomenon. CaCO3 is one of the most abundant natural minerals besides being presence in skeletons / shells of several organisms. Hence the choice of $CaCO_3$ was relevant for the project. The experiments were done as part of the ICONIC project (funded by the Research Council of Norway) which is coordinated by NTNU, Norway and ESRF is a central partner.

The $CaCO_3$ microparticles were prepared in house at NTNU by mixing 0.05l 10 mM $CaCl_2$ with 0.5l of 2 mM Na_2CO_3 containing 50mM NaCl bringing the total solution volume up to 0.55l. The process was repeated for 3 different temperatures 25º, 35º and 45º C. The reactants were under constant stirring at 700 rpm for 5 minutes and then the reaction was stopped by pouring the solution into vacuum filter with a 0.22 µm Millipore membrane. The particles on the filter were washed with ethanol to get rid of any ions residue ions and wetting solution left around and were dried at 40ºC for 24 hours to halt any further change in particles after the reaction. The whole procedure was identically repeated for all 3 temperatures. $CaCO_3$ microparticles were removed from the solution during different times of the experiments, 2, 5 and 30 minutes, and under different conditions of supersaturations. So, we had a series of samples as a function of temperature, time and supersaturation. The particles were then mounted on Si_3N_4 membranes to be studied with CXDI.

The experimental setup at ID10C beamline of ESRF allows measurement of wide-angle diffraction (WAXD) together with CXDI. A highly coherent and monochromatic beam of intensity $7.6e^{+11}$ ph/s and energy 7.24 keV was used to illuminate sample with a pencil shaped well collimated beam from the new first of a kind, high energy, and low emittance Extremely Brilliant Source (EBS). The beam size at FWHM was selected to be 10x10 um using rollerblade slits. forward scattering patterns from the sample were collected on a two-dimensional detector Eiger-4M which is a Hybrid Photon Counting detector with a pixel size of 75µm square with a total pixel array format of 2070 x 2167 pixels. Sample to detector distance in the measurements was kept at 7.15m, which, coupled with the 75µm detector pixel size can give a theoretical real space reconstruction down to 16 x 16 x 16 nm of voxel size. The measurements were taken by mounting a subject particle on Si_3N_4 membranes fixed on goniometer which rotated in angle w roughly from -80º to +80º. The increment in measurement angle was constant of 0.2 degrees each time with an exposure of 2 to 3 seconds with one background measurement each time and an auto alignment every 40 to 50 steps. WAXD data were collected on a 1D Mythen detector mounted next to the sample simultaneously with the CXDI measurements. LaB_6 powder with its well-known brag peak locations, was used to calibrate the Mythen detector. The geometry of the setup allowed us to measure $0.75A^{-1}$ to $3.39A^{-1}$ of q range in WAXD measurements.

During the beamtime, there were in total 21 measurements. WAXD collected in tandem with the CXDI measurements allowed us to identify the crystal form of $CaCO_3$ present in the microparticles. Note that this experiment was one of the first CXDI measurements done after the EBS upgrade. We encountered change in morphology of the microparticles due to the high flux of the EBS. The observed change is demonstrated in Figure 1.

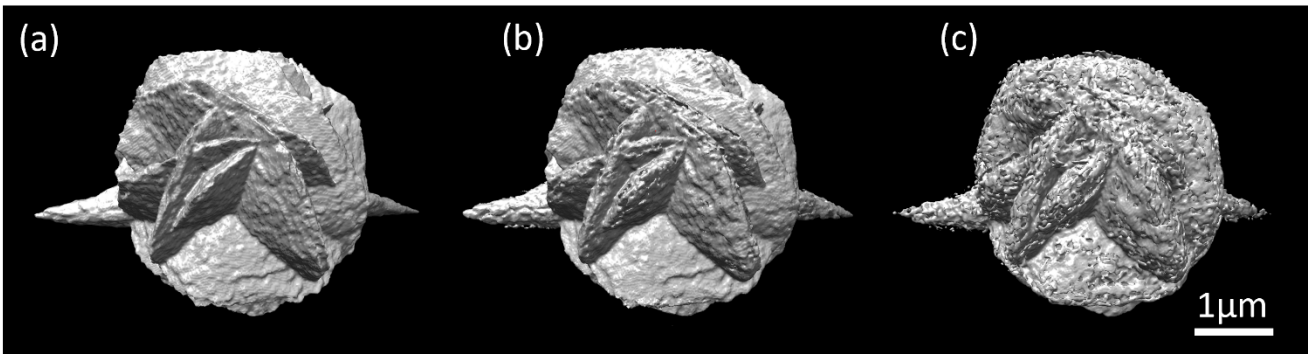

Figure 1. *Deformation in outer morphology of vaterite microparticle as it is exposed to the EBS beam. (a) Particle 162s exposure showing no damage. Particle exhibiting surface deformation after (b) 324s exposure and (c) 486s.*

More measurements were done with the other fabricated microparticles while ensuring that the dose is limited to avoid any sample damage. The exposure time and steps were adapted accordingly. Given in Figure 2 are the changes in morphology and as well as crystalline phase when precipitation was done at temperatures of 25°C, 35 °C and 45 °C. The particles undergoes change of morphology from nested plates to mix phase of nested plates and needles and 35 °C and then to a fully needle like morphology whereas its crystalline phase also changes from vaterite to mix of vaterite and aragonite and finally to a hundred percent aragonite at 45 °C. In sample 2, we can visually appreciate the presence of both vaterite, and aragonite based on their grey values. Note the density of vaterite is 2.65g/cm^3 and that of aragonite is 2.95g/cm^3.

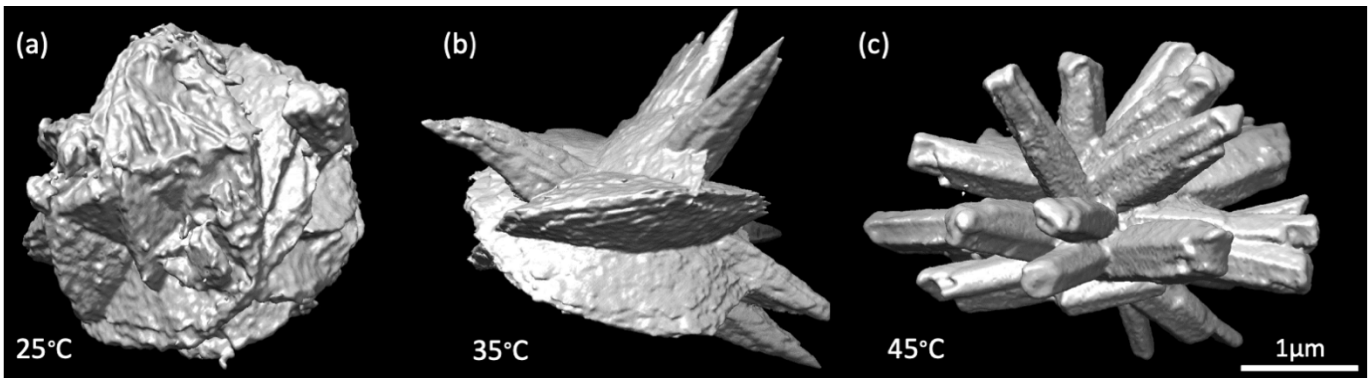

Figure 2. *Results from ex-situ CXDI experiments for proposal no MA 4673. 3D Iso surface rendering of reconstructed $CaCO_3$ microparticles precipitated under different temperatures showing the external morphology of (a) vaterite at 25°C, (b) vaterite + aragonite at 35°C and (c) aragonite at 45°C.*

Another interesting set of studies carried out during the beamtime is controlled growth over vaterite seed particles under constant supersaturation as shown in Figure 3. The seed (Fig. 3a) and seeded growth (Fig.3b) particles show two very different growth patterns and morphology. The seeded particle in (Fig. 3a) shows a porous growth pattern and has a very rough surface without any obvious preference on growth direction. The growth particle in (Fig. 3b) however shows an obvious preferred orientation along one axis and all the growth plates appear to be well oriented along vertical axis. Furthermore, growth particle in Fig. 3b is no more porous while the seed is well preserved inside without undergoing any crystal dissolution and recrystallization. From the WAXD data it is ascertained that both particles are pure vaterite.

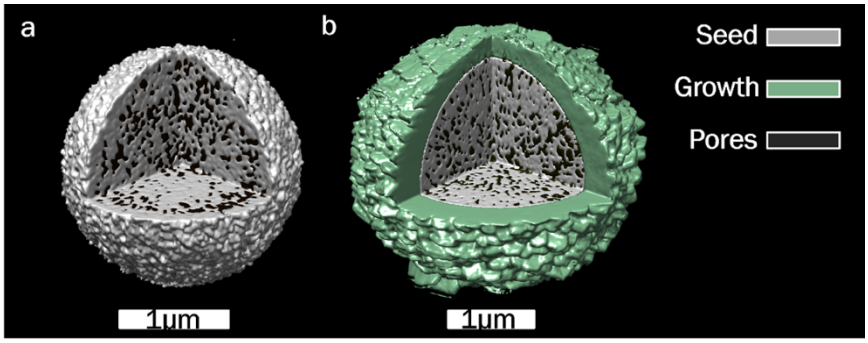

Figure 3. *Cutaway view of 3D CXDI images of CaCO3 microparticles. a) Seed particle, b) seed with additional growth layer.*

The manuscripts based on the studies shown above are under preparation and we expect to submit them soon.

1. Younas D. *et al* "*Nanoscopy of temperature controlled $CaCO_3$ precipitation monitored by Coherent X-ray Diffraction Imaging*", In preparation.
2. Wang L. *et al* "*Morphology Development of Vaterite Hierarchical Structures*", In preparation.
3. Younas D. *et al* "*Radiation induced morphology change in vaterite microparticles at the ESRF-EBS*", in preparation.

The results have also been presented in multiple conferences as both oral and poster presentations:

1. Younas, D. *et al.* (**2021**) "Coherent X-ray diffraction imaging to investigate structure and morphological evolution of calcium carbonate microparticles." *International Union of Crystallography* 25th Congress and General Assembly of the International Union of Crystallography, Prague 2021-08-14 - 2021-08-22. *Poster.* Acta Cryst. (**2021**). A77, C918.
2. Younas, D. et al. (**2022**) "X-Ray Nanoscopy Reveals Oriented Growth of Seeded Calcium Carbonate Microparticles." *NTNU* TNNN Conference 2022, Trondheim 2022-11-30 - 2022-12-02. *Oral presentation*.
3. Younas, D. et al. (**2022**) "3D nanoscopy of $CaCO_3$ microparticles using coherent X-rays." *International Solvay Institutes* SOLVAY WORKSHOP ON "Nucleation: multiple pathways multiple outcomes", Brussels 2022-12-07 - 2022-12-09. *Poster.*

Beamline Configuration:

Beam height: 10 µm
Beam width: 10 µm
Energy: 7.24 keV
Wavelength: 1.7124 Å
Detector: Eiger-4M. 2070 x 2167 pixels (horizontal × vertical), with a square pixel size of 75 µm.
Sample-detector distance: 7.15 m