Report on the formation of CaCO3 particles made with sc-CO2

Experiment SC3114 performed at ID02 – June 2011

Among complex hierarchical structures found in biological systems, calcium carbonate, i.e. $CaCO_3$, is definitely one of the most fascinating one. In biological environment, it forms beautiful architectures that are controlled by the interaction of biomolecules with solutions containing the mineral precursors in a process known as biomineralization. Extensive research has been performed on this material to understand how biomineralization can promote specific functions such as mechanical properties or biological functionalities. Such fascinating investigations have encouraged many researchers to mimic the process of biomineralization. So far, the biomimetization of these composite materials with the high levels of precision found in nature is still out of reach of engineering scientists. Nevertheless new routes to produce $CaCO_3$ particles that are not even stable in nature have been quite successful. Let us recall that calcium carbonate is the most abundant biomineral in nature and occurs in three main crystalline polymorphs (calcite, vaterite and aragonite), two hydrated crystal forms (calcium carbonate monohydrate and calcium carbonate hexahydrate), and also as amorphous material. The most stable polymorph, calcite, has a rhombohedral crystallographic unit cell and appears in the form of rhombohedral crystals. The next abundant form is the less stable aragonite which has an orthorhombic crystal structure and which crystallizes as clustered needles growing along the crystallographic c-axis. Vaterite is the least stable of the anhydrous polymorphs. It has a hexagonal unit cell and forms as hexagonal florets. Vaterite is generally not observed in nature and the reason for which one form is more predominant than others is still under debate. This highlights the fact that the understanding of the formation of $CaCO_3$ is far from being understood. The main reason is the complexity of reactions involved in the process of crystallization. In particular when starting with Ca^{2+} solutions in which the source of carbonates originates from the dissolution of CO_2, many parameters can influence the final state of the product. Among these parameters, one finds of course the pH of the solution, the Ca^{2+} concentration, the nature of biomolecules and the partial pressure of CO_2 in contact with the solution. In this study, we use supercritical CO_2 (Sc-CO_2) to synthetize $CaCO_3$ particles according to a process patented by the group of F. Boury (University of Angers – INSERM U646). Sc-CO_2 is a perfect co-solvent for such experiments since it is not only a solvent but it also participates to the reaction since it is the source of carbonated species. The main drawback of this process is that it is difficult to monitor the synthesis since experiments are carried out at high pressure p>80 bars and at T>35°C. The mechanism of formation of micro

particules is therefore more predictive than conclusive. In order to unravel the mechanism and to derive structural and morphological information *simultaneously*, our approach employs time-resolved *in situ* small angle x-ray scattering (SAXS) combined to wide angle x-ray scattering (WAXS), using a high brilliance synchrotron source (ID02, ESRF Source, Grenoble, France). The experiment consists in co-injecting the Ca^{2+} solution with Sc-CO_2 into silica capillary that is irradiated by a highly brilliant x-ray beam. This allows us to understand and describe the dynamic interplay of the Ca^{2+} solution with sc-CO_2 at any time during the pressurization of the solution. Recent experiments have been complemented by *in-situ* visualization of the solution during the injection of CO_2 at elevated pressures (see Experimental and Figure 1). The difficult task before us is to understand how precisely microparticles consisting of hollow spheres of $CaCO_3$ having the vaterite structure are formed when the Ca^{2+} solution buffered by biomolecules is exposed to Sc-CO_2. The main issue is to answer opened questions such as i) when do particles form, what is the role of CO_2 pressure, what is the role of the initial pH and the one of the biomolecule used in the process. For this we carefully analyze the results of x-ray experiments, which at first seem to be quite erratic and we confirm the mechanism of formation by the analysis of the conditions of precipitation of $CaCO_3$ under pressurized CO_2. The precipitation of $CaCO_3$ is achieved whenever the product of solubility of $CaCO_3$ is reached. As the concentration of bicarbonate ions strongly depends on the pH, which decreases when the pressure of CO_2 increases. Hence, a competition between the precipitation of $CaCO_3$ and its dissolution at elevated pressure takes place. As experiments were performed in Sc-CO_2, the first part of the experiment was dedicated to the understanding of the scattering Sc-CO_2 at small angle. We then evidence that the formation of $CaCO_3$ particles occurs either at low pressure or alternatively during the depressurization of the vessel containing the solution. The signature of WAXS is mainly the one of amorphous particles, which crystallize as a function of time at normal pressure. We also evidence that the precipitation is a highly dynamical process which strongly depends on the pressurization of the cell. Calculation of the precipitation as a function of CO2 pressure is now in progress.

Commentaire [BC1] : Après lecture du rapport, je m'aperçois que l'on ne parle pas de la glycine (elle n'est pas citée). Si vous souhaitez que j'incluse un paragraphe sur la préparation et la composition de la solution calcique, dites le moi.

Experimental

SAXS and WAXS measurements were collected at ID02 beam line at an energy of 12keV. The fluid was located inside a capillary (with a 0.7mm inner diameter) connected to a manual high-pressure set-up purchased from SEPAREX (Champigneulles, France). The capillary has 0.05mm thick walls covered with a polyimide protection. Capillaries were connected to the

high pressure cell via 1/16” metallic pipes. The pressure inside the capillary could be controlled to better than 0.1bar. The capillary was inserted in a cell closed by Kapton windows so as to avoid any thermal fluctuations. The cell was heated by a heating plate located below the capillary. Measurements were made at 22.25°C and 39.16°C with a thermal control better than 0.05°C.

Energy was fixed at 12keV and the sample to detector distance was tunable from 10m to 1m. All measurements were collected at a distance D=2m. The q-range was typically going from $0.05nm^{-1}$ to 40 nm^{-1} with an overlap between SAXS and WAXS measurements. The very high flux used at ID02 combined to high a sensitivity CCD detector allowed fast acquisition in less than 0.1s depending on the contrast of electron density. Pressure could be a tuned from 1 bar to 250 bars with an accuracy better than 0.1 bars.

All measurements were initially collected at low CO_2 pressure so as to obtain a reference curve, which was subsequently subtracted to the other curves collected at higher pressure. The measured signal was therefore only the one of the fluid at high pressure without the capillary contribution and electronic noise.

The experiment was carried out by co-injecting the liquid aqueous solution and pressurized CO_2.

The schematic representation of the set-up is shown in figure 1

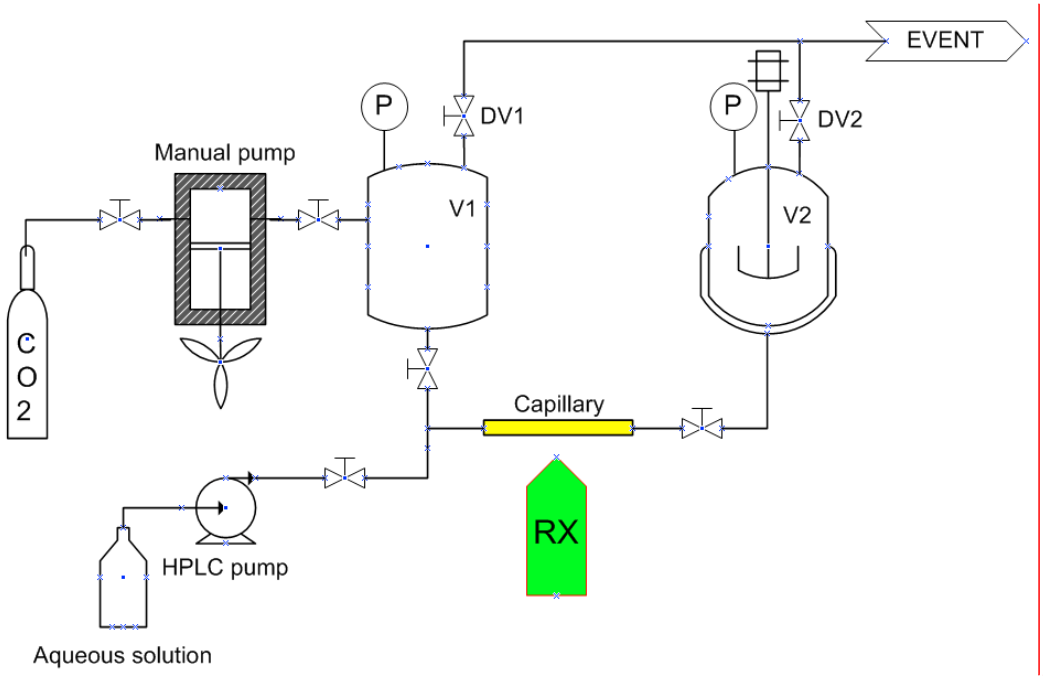

Figure 1: Schematic representation of the experimental set-up in which one can see the co-injection inside the capillary of CO_2 under pressure together with an aqueous phase of Ca^{2+} injected with an HPLC pump. The measurements are made inside the capillary during the flow back and forth of the fluid. First, the fluid flows from vessel V1 to vessel V2 during pressurization. At high pressure, the fluid can flow in the reverse direction by opening the depressurizing valve DV1.

Commentaire [BC2] : Me préciser si l'agita

Commentaire [BC3] : Je n'étais pas là pour cette manip mais si je comprends bien, en fait la pression dans DV1 et DV2 est identique (en équi-pression car il doit y avoir une vanne ouverte à la sortie du capillaire et on augmente la pression en poussant du CO2 dans DV1 ? Je pense qu'il faudrait un peu plus détailler le mode opératoire.

Results an discussion

Gas/liquid and Sc-CO2 experiment

All measurements were initially collected at low CO_2 pressure so as to obtain a reference curve, which was subsequently subtracted to the other curves collected at higher pressure. The measured signal was therefore only the one of the fluid at high pressure cleaned from the capillary contribution and electronic noise.

Gas to liquid state at 22°C

The results concerning the measurements of CO_2 transformation from the gas to the liquid state at 22°C are shown in Figure 2. From the NIST database one expects the gas to liquid phase transition to occur at 60 bars. The onset of the gas to liquid transition is signed by the appearance of both increasing density fluctuations and by a correlation peak around $17nm^{-}$

[1]. The correlation peak is clearly seen in the gas phase at pressure close to the gas-liquid transition pressure. Its intensity increases drastically in the liquid state. The critical fluctuations on the other hand dominate the scattering at low q. The scattering by the fluctuations steadily increases until the transition pressure. They diminish on further compression while the correlation peak continues to increase. As reported several times it is possible to analyze the critical fluctuations by plotting 1/I(q) versus q^2.

The correlation peak can be analyzed by the Fourier transform of the radial intermolecular distribution function.

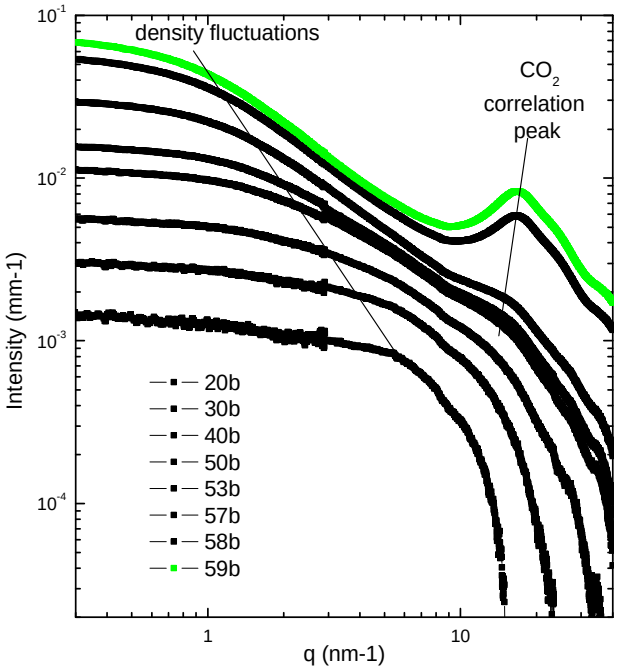

Figure 2 : Evidence for density fluctuations at low q in liquid CO_2 close to the Gas-Liquid line and appearance of a correlation peak due to dense clusters of CO_2 molecules in the liquid state.

Similar measurements were also made in the supercritical domain at 40°C and up to 100 bars. They are basically very similar to the one obtained in the gas state at 22°C and shown in Figure 2.

Formation of $CaCO_3$ particles

The experimental observations are strongly related to the procedure of pressurization and depressurization.

The first step consists in making a circulation of the Ca^{2+} solution inside the capillary at a constant velocity of 3.5ml/min. At a given time, we co-inject simultaneously CO_2 at a tunable pressure with a constant flow of Ca^{2+} solution. CO_2 first passes through vessel V1 before encountering the Ca^{2+} solution in a tee-junction located just before the entrance of the capillary. During this phase x-ray data are collected *in-situ*. The mixture is collected inside the vessel V2 after passing through the capillary. This phase will be labelled FP1 (Flowing phase 1).

The second step consists in depressurizing the whole system by opening either quickly or at a slow rate the Depressurizing Valve (DV1) located at the top of vessel V1. In such a case all the liquid located at the bottom of vessel V2 flows back through the capillary before entering vessel V1. This phase will be further labelled FP2 (Flowing Phase 2).

Commentaire [BC4] : Avant de dépressuriser, avez vous laissé 5 min d'agitation (cf. publi vatérite) ? Dans quel cas, il faudrait le stipuler.

Note that each vessel is equipped with a purging valve enabling the collection of the Ca^{2+} solution after exposure to supercritical CO_2 either in vessel V1 or vessel V2 .

It is therefore possible to monitor the scattering by the fluid flowing inside the capillary either during compression (phase I) or depressurisation (phase II). The final state of the Ca^{2+} solution after exposure to Sc-CO_2 can be monitored by purging vessels V1 or V2 once the system is depressurized. It is worth noting that if depressurization is made in V1 (resp. V2) nothing remains in vessel V2 (resp. V1).

The experimental results are now discussed for the two flowing phases FP1 and FP2.

Phase FP1

At the beginning of FP1, i.e. prior to injecting CO_2, we measure the scattering by the Ca^{2+} solution. The scattering is mainly flat at low angle while a peak typical of intermolecular water molecules is seen at wide angle.

As soon as CO_2 is injected, we observe a strong signal at low angle, which proves that aggregates are forming. This signal vanishes almost immediately and it is replaced by the signal of CO_2, i.e density fluctuations at low q and a peak characteristic of intermolecular CO_2 molecules located around $17nm^{-1}$. This signal evolves with CO_2 pressure.

Commentaire [BC5] : Jusqu'à quelle pression ? 200 ou 250 bars ?

Phase FP2

During this phase, the depressurization with the valve DV1 forces the aqueous solution and CO_2 stored in vessel V2 to flow back by the capillary. We observe immediately after the onset of depressurization, a strong signal coming from big aggregates. This signal is seen for a pressure going from 67 bars to 40 bars after which the CO2 signal is seen again.

Commentaire [BC6] : Au dessus de 67 bars ou en dessous de 40 bars ?

Note that x-ray scattering performed on the collected solution after depressurisation yields a signal identical to the one of the aggregates at the beginning of depressurisation.

The interpretation of these results is not straightforward and several scenarios can be envisaged. The main issue is the observation of the aggregates at low pressure of CO_2 and their disappearance at higher pressure during the FP1 phase. This seems to indicate that aggregates do not exist at high pressure. Nevertheless aggregates are seen once again at high pressure during depressurization before vanishing at lower pressure in the FP2 phase. This observation is in favour of the appearance of aggregates during the depressurisation. Observing that they are not seen at lower pressure can be easily interpreted by the fact that at the beginning of FP2, the liquid phase is at the bottom of vessel V2 because the aqueous phase is denser than the Sc-CO2. When the DV1 valve is opened, it is impossible to observe the CO_2 signal until the aqueous phase has completely transited through the capillary. It is therefore normal to observe first the signal of the aggregates before observing the signal of CO_2. If one comes back to what was seen in the FP1 phase the fact that the signal of aggregates quickly vanishes is puzzling. Either they cannot exist at high pressure of CO_2 (which is contrary to what we observe at depressurisation) or the content of the aqueous phase during the flowing of both the CO_2 phase and the aqueous phase is not high enough to observe the signal of aggregates. This latter explanation is likely the right one and if it is the case this would also rule out the fact that aggregates preferentially form at depressurisation.
This yields to the interrogation of the existence of aggregates at high pressure of CO_2. This issue is again not so easy to sort out because aggregates formation is pH dependant.

It is well known that $CaCO_3$ can precipitate if the pH is high enough to allow the product of Ca^{2+} by CO_3^{2-} concentrations to be bigger than the constant of solubility K_{SO}. At high pH (pH>10.3), the predominant carbonated species in the aqueous phase are CO_3^{2-}. Any adjunction of CO_2 in the initial solution of $CaCl_2$ is bound to favour the precipitation of $CaCO_3$ since the aqueous phase is controlled by a glycine buffer with pH=10. When CO_2 pressure is increased, CO_3^{2-} can become a minority in the aqueous solution because pH is

decreasing with CO_2 pressure. This effect can consequently favour the disappearance of aggregates at high pressure of CO_2.

When the cell is depressurized, most of the CO_2 stored in the aqueous phase is released which in turn favours the increase of the pH. This effect can explain the reappearance of aggregates on depressurization.

Comments on the experiment

This experiment was the first that we have carried out at high pressure in a capillary. We strongly need to complement our results by another run since this experiment was extremely difficult because

1- The capillary broke several time during critical moments of the previous run. To avoid this problem we will use sapphire capillaries in the future instead of quartz
2- The experimental set-up was very demanding to run for the first time and we have now gained a lot of experience on what to do and what to avoid.
3- We have developed a new holder for the capillary so as to avoid many technical problems that we face during the previous run.
4- We have now the necessary equipment at our University to test the experiments before the run at ESRF.

For all these reasons, we are now confident that a second run will bring us much more exciting results with the possibility to include biological molecules inside the solution as proposed in our next proposal.