

## Crystallization of Calcium Carbonate Observed In-situ by Combined Small- and Wide-angle X-ray Scattering

Diego Pontoni,<sup>\*,§</sup> Joerg Bolze,<sup>#</sup> Nico Dingenouts,<sup>#</sup> Theyencheri Narayanan,<sup>§</sup> and Matthias Ballauff<sup>\*,#</sup>

Polymer-Institut, Universität Karlsruhe, Kaiserstrasse 12, 76128 Karlsruhe, Germany, and European Synchrotron Radiation Facility, B.P. 220, F-38043, Grenoble, France

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The transformation of amorphous colloidal calcium carbonate into single microcrystals was observed in supersaturated solutions in situ. The observations are done by simultaneous time-resolved small-angle and wide-angle X-ray scattering experiments (TR-SAXS/WAXS) at a third generation synchrotron source. TR-SAXS/WAXS demonstrates that the particles generated by reaction of  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  ions are amorphous. The transformation of these amorphous  $\text{CaCO}_3$  particles proceeds via dissolution and subsequent heterogeneous nucleation of the crystalline modification on the walls of the quartz capillary containing the reacting mixture. The crystalline modifications thus generated could be identified. No solid–solid transition is observed. TR-SAXS/WAXS is therefore well-suited to follow the mineralization from aqueous solution in great detail.

The formation of calcium carbonate from supersaturated solutions has been studied for more than a century and it has received renewed interest, especially in the field of biomineralization.<sup>1</sup> The metastable forms of calcium carbonate can be investigated more precisely nowadays thanks to the improvement of the experimental techniques.<sup>2</sup> In particular *amorphous* calcium carbonate (ACC) has emerged as a precursor to the formation of more stable crystalline forms.<sup>3</sup> A topic of intense research activity is presently the detailed mechanism of phase transformation leading from the initial amorphous material to the final, thermodynamically stable, crystalline modification (calcite). Different mechanisms are being discussed, as, e.g., a solid/solid phase transition and a dissolution/crystallization process. This process also depends strongly on reagent concentrations and reaction conditions.<sup>4</sup>

The transition from the amorphous state into a stable, crystalline modification is difficult to be studied in a time-resolved experiment. A number of experimental techniques have been applied to the study of the calcium carbonate formation, including optical, electron, and X-ray microscopies, dynamic light scattering, turbidimetry, and conductivity methods.<sup>2,5</sup> These methods, however, do not give the structural information as a function of time. On the other hand, the application of X-ray scattering methods is particularly well suited because it allows in-situ measurements of the size, morphology, and interactions of colloidal particles growing in solution.<sup>6,7</sup> Hence, scattering experiments probing directly the crystal structure of a given population of particles would be helpful to investigate the mineralization leading to a stable crystalline form.

Recently, we studied the nucleation and growth of colloidal spheres made of calcium carbonate ( $\text{CaCO}_3$ ) by using time-resolved small-angle X-ray scattering (TR-SAXS).<sup>6</sup> This method allows us to follow the growth of the particles from  $\sim 20$  ms after mixing the reagent solutions to their final size with a time

resolution down to  $\sim 0.1$  s. In particular, we showed that the evolving particles consist of amorphous calcium carbonate. This could be deduced from the low mass density of the growing particles. Clearly, a combination of these SAXS measurements with wide-angle X-ray scattering (WAXS) is necessary to analyze the evolving particles directly and to monitor the subsequent transition of the ACC into the stable crystalline forms.

The present communication reports time-resolved investigations of the phase transformation of ACC spheres into more stable crystalline modifications (aragonite, vaterite, calcite). We use the combination of the stopped-flow technique with time-resolved, *simultaneous* small-angle and wide-angle X-ray scattering experiments (TR-SAXS/WAXS) at the ID2 beamline<sup>8</sup> of the European Synchrotron Radiation Facility (ESRF) in Grenoble (France). Such an experimental approach allows in situ investigation of the formation and transformation of calcium carbonate with unprecedented precision and sensitivity.

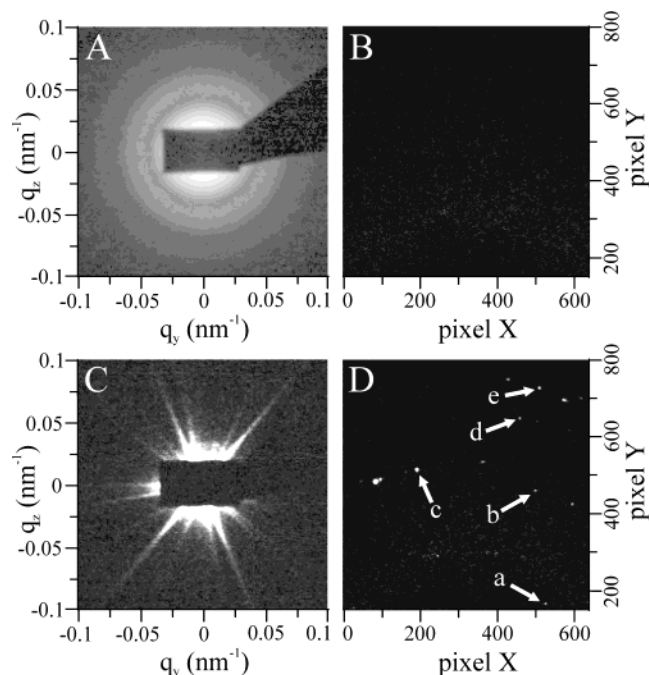
TR-SAXS/WAXS proved to be useful in particular in the field of polymer crystallization.<sup>9,10</sup> The process of mineralization of calcium carbonate was recently studied by Heeley et al. using TR-SAXS/WAXS.<sup>10</sup> These experiments were set up to mimic the crystallization in an industrial process. The present experiment is done differently: two equimolar aqueous solutions of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) are mixed rapidly by means of a stopped-flow apparatus, which is coupled to a thin-walled quartz capillary flow-through cell. After the transfer, the supersaturated solution is left undisturbed in a steady condition and its evolution is followed by TR-SAXS/WAXS. Hence, the mineralization is observed in a confined volume and the experimental setup can monitor directly the formation of particles. Moreover, possible transitions in the same population of particles can be followed by this method.

First of all, the present results validate our previous findings concerning the amorphous character of the initially formed colloidal particles.<sup>6</sup> This is apparent in Figure 1A, where the isotropic and modulated SAXS signal indicates the formation

\* Corresponding author. E-mail: Matthias.Ballauff@chemie.uni-karlsruhe.de.

<sup>#</sup> Polymer-Institut, Universität Karlsruhe.

<sup>§</sup> ESRF, Grenoble.

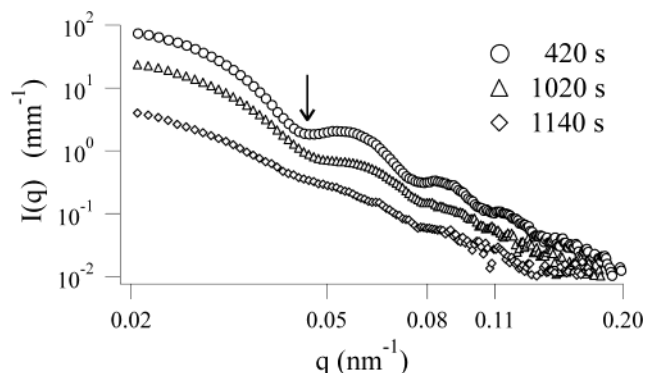


**Figure 1.** Simultaneous SAXS (A) and WAXS (B) 2d images acquired 30 s after mixing 9 mM solutions of  $\text{Na}_2\text{CO}_3$  and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ . The pH value was adjusted to 11. The typical modulation of the isotropic SAXS intensity indicates the formation of colloidal spheres with a diameter of ca. 200 nm. The absence of Bragg diffraction spots in the WAXS data confirms that these spheres are made of *amorphous* calcium carbonate. Simultaneous SAXS (C) and WAXS (D) 2d images acquired 1 h after mixing 50 mM reagent solutions. The streaks in the SAXS pattern indicate the presence of microcrystallites nucleating on the capillary wall. The corresponding WAXS image shows diffraction spots that can be assigned to the various crystalline modifications of calcium carbonate. The spots indicated by arrows correspond to the following Bragg reflections: (a) calcite (012); (b) vaterite (004); (c) aragonite (032); (d) aragonite (310); (e) aragonite (302).

of colloidal spheres in solution,<sup>6</sup> whereas the absence of diffraction spots in the WAXS image (Figure 1B) confirms that they consist of ACC. Because the concentration of reactants is the same as in the previous experiment (9 mM), the present results fully support our previous findings.<sup>6</sup>

By a careful analysis of the SAXS images it is possible to extract quantitative information on the size, polydispersity and number, and mass densities of the amorphous particles.<sup>6</sup> It is also possible to detect the onset of sedimentation by an overall decrease of the scattered intensity, and of particle dissolution by changes in the frequency of the oscillations in the scattered intensity typical of the spherical form factor, as indicated in Figure 2.

As the process of dissolution/sedimentation proceeds, the intense isotropic SAXS signal disappears and sharp intense streaks appear in the SAXS pattern (Figure 1C). Such a peculiar SAXS pattern indicates microcrystals precipitating presumably on the wall of the capillary.<sup>11</sup> This is confirmed by the WAXS images acquired simultaneously with the SAXS data. Figure 1D shows a typical WAXS image corrected for background scattering, which was obtained at the late stage of a reaction using 50 mM initial reagent concentrations. The white spots correspond to Bragg reflections by single crystals of calcium carbonate. This can be seen from the fact that only a few isolated spots are observed, which are detectable at least for several minutes. Moreover, the microcrystals grow at fixed positions and conserve well-defined orientations. It can therefore be concluded that these microcrystals do not form in solution but



**Figure 2.** Radially averaged SAXS scattering profiles at different times after mixing 9 mM reagent solutions. The arrow indicates the first minimum of the spherical form factor. Its shift toward larger scattering angles ( $t = 1020$  s) indicates dissolution of the amorphous particles. The particles become more polydisperse, as suggested by the less pronounced oscillations in the scattering curve. The overall intensity decrease is due to particle dissolution and sedimentation. The last profile corresponds to a 2d SAXS image starting to show an anisotropic character similar to that of Figure 1C, thus indicating nucleation of microcrystals on the capillary wall.

nucleate by heterogeneous nucleation directly at the wall of the capillary. Similar results have been obtained at lower concentrations but the number of crystallites was lower in this case. The SAXS intensity along the direction of the streaks in Figure 1C shows a  $q^{-3}$  type behavior, suggesting that they are due to interface scattering.<sup>12</sup>

It is also found that the observation of intense streaks in the SAXS pattern is always accompanied by diffraction spots in the WAXS images. Conversely, the earliest Bragg spots are detected when the SAXS images still show the intense isotropic scattering due to the spheres of amorphous calcium carbonate. However, a careful inspection of these SAXS images reveals the presence of faint radial streaks camouflaged by the intense isotropic scattering. Such streaks become progressively better visible as the dissolution/sedimentation of the amorphous spheres proceeds, leading to the final characteristic anisotropic pattern (Figure 1C). These observations indicate that the reaction proceeds through an intermediate stage of coexistence of amorphous spheres in suspension and crystalline material nucleating on the walls of the capillary.

The indexing of the diffraction spots allows distinction of the various crystalline modifications being formed. The Bragg spots depicted in Figure 1D demonstrate that, at 50 mM initial reagent concentrations, at least one crystallite was detected for each of the three known anhydrous crystalline forms of calcium carbonate in a single experiment. By repeating experiments of this kind, it is possible to investigate the relation between the initial reagent concentrations and the relative abundance of the different crystalline modifications.

The time evolution of the diffraction spots gives insight into the kinetics of the phase transformation. In experiments at 15 mM initial reagent concentrations, it was observed that Bragg spots can appear at random times but no diffraction is observed earlier than 250 s after mixing. This time corresponds to the onset of the dissolution of the amorphous spheres, as indicated by the SAXS data, which agrees with the obvious fact that the heterogeneous nucleation of crystallites can start only when dissolution provides the material needed. In this case all the Bragg spots detected pertained to the trigonal structure of calcite. Therefore one can conclude that the phase transformation follows the path of dissolution/crystallization rather than that

of a solid/solid-phase transition. Further studies on the formation of crystals as a function of the reactant concentration are under way.

In conclusion, we report here the first in situ online observation of the formation of single calcium carbonate microcrystals as a function of time. We have shown that TR-SAXS/WAXS is a highly sensitive tool to analyze in situ and in real time the phase transformation pathway of  $\text{CaCO}_3$  from the initial supersaturated solution into the final product. The present data strongly suggest that the mineralization of  $\text{CaCO}_3$  proceeds in two steps: First amorphous particles are formed by homogeneous nucleation. Slow heterogeneous nucleation at the walls of the capillary then leads to the formation of single crystals of calcite, vaterite, or aragonite, depending on the initial reagent concentration. At this stage the amorphous particles slowly dissolve again and  $\text{CaCO}_3$  is turned into the more stable crystalline modifications. No solid–solid transformation takes place. We plan to pursue these studies in the presence of (bio)-polymer additives that are of particular interest in the field of biomineralization.

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