

Beauty and complexity of calcium carbonate precipitation: optical microscopy and *in situ* Raman microspectroscopy characterization

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The formation of biogenic CaCO₃ has fascinated scientists over decades. A specific polymorph can be selected by living organisms and used to assemble complex morphology for biological functions, as seen in corals, coccoliths, bivalves and others. Many studies focus on how organic matter scaffolds or selects polymorphism [1], while others recreate *in vitro* these morphologies by using organic additives [2, 3]. Nevertheless, it has been reported that complex morphologies similar to biominerals can be obtained within inorganic matrix. These “biomorphs” trigger the question of how much we should trust complex morphology to be the trace of organic matter or even living organisms [4].

Crystal nucleation is a critical remaining question in CaCO₃ crystallization to understand its crystal growth and design. Several crystallization pathways, classical and non-classical nucleation, have been proposed, but no consensus has been reached due to the complexity of the system [5, 6]. CaCO₃ has a low solubility and can precipitate with either carbonate or bicarbonate ions, each species depending on pH. Therefore, slight differences in initial conditions lead to different pathways. Furthermore, the initial concentrations and pH widely vary among literature, as well as the precipitation protocols [5-8]. These variabilities result in contrasting observations and a proposal of divergent crystallization pathway. Another cause is the choice of techniques to study nucleation. In *in situ* Transmission Electron Microscopy (TEM) provides incredible nano-scale details of crystal formation [8]. However, the field of view of the observation is limited. Spectroscopic means (FT-IR, Raman, SAXS, WAXS, neutron scattering etc.) could capture the signatures of various stages of precipitation, but a spectrum is typically an average of ensemble.

Surprisingly, bright field optical microscopy is not used for key insights, despite being well-suited to bridging the length scale gap between TEM and ensemble spectroscopy approaches. This is probably due to the limited spatial resolution and difficulty in assigning polymorphs without a characterization technique. In our study, we combined optical microscopy with *in situ* Raman microspectroscopy as a mesoscale length scale technique [9]. Using this method, we conducted precipitation experiments without stirring over a wide range of supersaturations, varying initial concentrations and pH, and observed the evolution of the mixed solutions up to 24 hours. This allowed us to obtain a landscape of crystallization processes and to categorize the types of precipitates formed for a specific supersaturation. At high supersaturation, a gel precipitated whilst at low supersaturation, calcite crystals formed directly, followed by amorphous calcium carbonate. Morphologies observed with additives in literature, such as calcitic spherulites and aragonite, were observed without additives in our study. Our contribution will emphasize the importance of studying the system at the length scale between nano and bulk scale as well as that of initial conditions so that the debates can converge in near future [9].

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