

# The Co-operative Influence of Gel Solid Content and Reagent Solution Concentrations on Gel Occlusion and Crystal Micro-organization in Biomimetic Calcite-Agar Composites

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## INTRODUCTION

Carbonate biominerals are composite materials which consist of a compliant organic polymeric matrix and a stiff mineral component (calcite or aragonite ( $\text{CaCO}_3$ )). The polymeric matrix is arranged in nanometer thick membranes and fibrils. The membranes compartmentalize the space and envelope the mineral component, which appears as aggregations of nanoparticles. The fibrils infiltrate the space between these nanoparticles, affecting their degree of co-alignment. The organic polymeric matrix becomes occluded within the mineral component during the biomineralization process through mechanisms that are not fully understood yet.

Carbonate biominerals form in gelatinous environments that share numerous features with hydrogel systems. Experiments using hydrogels to support calcite crystallization have shown that the gel polymeric network becomes occluded within calcite crystals during growth (Asenath-Smith et al. 2012; Nindiyasari et al. 2014a, 2014b). Furthermore, it has been demonstrated that this occlusion significantly influences the internal micro-structuring of the mineral component in these calcite-gel composites. Since hydrogels share numerous features with biomineralization environments and their characteristics regarding composition, diffusivity and strength can be finely tuned, calcite crystallization in gel experiments are a most suitable tool

to mimic biogenic calcite formation and investigate the factors that may control the incorporation of organic polymeric matrices within calcite biominerals.

In a previous work (Nindiyasari et al. 2014a), we used gelatin hydrogels with different solid contents to study the influence of the gel porosity in the crystallization of  $\text{CaCO}_3$  meso- and single crystals. We observed a correlation between the increase in solid content in the hydrogel and higher delays in the beginning of crystallization. Furthermore, we observed that calcite crystals grown in denser gels incorporated higher amounts of gel matrix. This higher incorporation in turn promoted resulted in higher degrees of internal structuring of the mineral component. Thus, the calcite-gelatin composites grown in the gelatin gel with the highest gel content showed portions that were mesocrystals, while in other portions calcite was in a mosaic arrangement.

We later augmented our investigation to calcite-gel composites formed in other types of hydrogels (silica and agar) (Nindiyasari et al. 2015). We observed that the occlusion of silica gel had the less important influence in the organization of calcite-gel composites, which were single crystals with calcite almost as highly co-oriented as in calcite grown from solution. The micro-structuring of the mineral component in calcite-agar composites showed characteristics which were intermediate between those found in composites

formed in silica and gelatin hydrogels.

In this work, we conduct calcite crystallization experiments using agar gels with two different solid contents and two different concentrations of the counter-diffusing reagents. With the combination of both, we define scenarios with distinct gel strengths and supersaturation conditions during calcite growth. Our aim is to understand the influence of the combination of both parameters, gel strength and growth rate, on the characteristics of the occlusion of agar polymeric matrix in calcite crystals and how this occlusion affects the internal structuring of the mineral component in the calcite-agar composites.

## METHODOLOGY

In our experiments, we apply the double-diffusion method to crystallize calcium carbonate using agar hydrogels. The hydrogels are prepared with two different agar solid contents (0.5 wt% and 2 wt%). We use two concentrations for the counter-diffusing reactants (0.1M  $\text{CaCl}_2$  - 0.1M  $\text{Na}_2\text{CO}_3$ ; 0.5M  $\text{CaCl}_2$  - 0.5M  $\text{Na}_2\text{CO}_3$ ). We use FE-SEM to visualize on microtome polished, etched and critical point dried sample surfaces, the distribution of gel within the composites and the characteristics of the gel-mineral interlinkage. With high-resolution electron backscatter diffraction (EBSD) we determine the microstructures of the composites and their degree of crystal co-alignment.

**palabras clave:** Cristalización Biomimética, Composites Calcita-Gel, Difracción de Electrones Retrodispersados (EBSD).

**key words:** Biomimetic Crystallization, Calcite-Gel Composites, Electron Backscatter Diffraction (EBSD).

## RESULTS

The combination of low agar solid content (0.5 wt%) and high reagent solution concentrations (0.5M) gives composites with the highest calcite crystal co-orientation, whereas composites from experiments with the combination of 2 wt% agar and 0.1M reagent solutions show the lowest crystal co-orientation. The former aggregates are almost single crystals, with a MUD value of 717. In contrast, the later aggregates are polycrystals (overall MUD: 316), although they consist of highly co-oriented subunits. Aggregates obtained from experiments with a combination of 0.5 wt% agar and 0.1M concentration of reagent solutions have MUD values of 665, comparable to the MUD values (624) of aggregates formed in gels with 2 wt% agar and reagent solutions with a 0.5M concentration.

A high amount of gel was occluded in calcite-gel composites that were grown within 2 wt% agar and with 0.5M reagent solutions concentration. The overall misorientation of calcite within these composites is five degrees, which is significantly higher than observed in crystals grown under low gel strength conditions. The combination of lighter agar gel (0.5 wt%) and less concentrated reagent solutions (0.1M) leads to the incorporation of smaller amounts of occluded gel. The maximum overall misorientation of these composites is three degrees. Further we found, that aggregates obtained in the presence of 2 wt% agar and 0.1M concentrated reagents show gel occlusions which define concentric oscillatory patterns.

## DISCUSSION

The observed patterns of gel incorporation into calcite are in good agreement with the influence of gel strength and growth rate as established by Asenath-Smith et al. (2012). These authors demonstrated that high values of both, growth rate and the gel strength result in the occlusion of most of the gel matrix within the crystal during growth. In contrast, the combination of a low growth rate and a weak gel should lead to the formation of crystals with only small amounts of gel occluded or that are virtually free of gel. Growth rate directly relates to the supersaturation. In gel systems, where mass transfer mainly occurs by diffusion, supersaturation builds up upon time

and, in principle, higher supersaturation values are to be expected during crystal growth the higher the concentration of the counter-diffusing reagents is (Henisch 1988; Prieto et al. 1991).

We find that the highest amount of occluded gel corresponds to calcite-agar composites formed in experiments with 2 wt% agar, the thicker and stronger gel, and with the more highly concentrated (0.5M) reagent solutions, that guarantee a higher supersaturation. Similarly, the low amount of occluded gel found in the composites formed in lighter agar gel (0.5 wt%) using less concentrated reagent solutions (0.1M) can be explained as the consequence of the weakness of the gel network combined with a slow growth rate under moderate to low supersaturation conditions. It is worthwhile to note that in these composites local gel accumulations are present. These accumulations can be interpreted as arising from fibres of the weak gel which are pushed ahead during crystal growth. This leads to a local increase in gel density and, therefore, gel strength.

The concentric pattern defined by the oscillation in the amount of occluded gel, which is observed in composites grown in 2 wt% agar from 0.1M reagent solutions, is the consequence of oscillatory changes in growth rate that lead to changes in gel incorporation. These changes can be explained considering that supersaturation will change in a gel system as crystal growth proceeds. This supersaturation change results from the balance between mass transfer from the reagent deposits to the growing crystal and the mass consumption by the crystal (Greiner et al. 2017). The higher density and lower porosity of the gel used in this experiment, together to the low concentration of the reagents, determines that mass transfer occurs a slower rate, preventing that it counterbalances the mass consumption by the crystal (Prieto et al. 1991). Therefore, supersaturation and growth rate will progressively drop and, eventually of the crystal growth will stop. Because mass transfer will still be progressing, supersaturation will slowly build up again until a supersaturation barrier is overcome and crystal growth restarts. The succession of similar growth cycles will be recorded within the crystal by changes in the amount of gel polymeric matrix that becomes incorporated, resulting in the observed

concentric oscillatory patterns.

Finally, the correlation that we observe between higher amounts of gel becoming occluded and higher degrees of misorientation in the calcite-gel composites highlights the relevant role of the polymeric matrix in the development of hierarchical orientation patterns in calcite.

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