Calcium carbonate crystallization in tailored constrained environments

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Synthesis of inorganic particles using routes inspired by biomineralization is a goal of growing interest. Recently it was demonstrated that the size and geometry of crystallization sites are as important as the structure of charged templating surfaces to obtain particles with controlled features. Most biominerals are formed inside restricted, constrained or confined spaces where at least parts of the boundaries are cell membranes containing phospholipids. In this study, we used a gas diffusion method to determine the effect of different lecithin media on the crystallization of CaCO\textsubscript{3} and to evaluate the influence of the spatial arrangement of lecithin molecules on templating CaCO\textsubscript{3} crystal formation. By using inorganic synthesis, Raman spectroscopy, dynamic light scattering, electrochemical methods and scanning electron microscopy, we showed that the occurrence of surface-modified calcite crystals and diverse textured vaterite crystals reflects the geometry and spatial distribution of aqueous constrained spaces due to the lecithin assembly controlled by lecithin concentration in an ionized calcium chloride solution under a continuous CO\textsubscript{2} diffusion atmosphere. This research shows that by tailoring the assembly of lecithin molecules, as micelles or reversed micelles, it is possible to modulate the texture, polymorphism, size and shape of calcium carbonate crystals.

Introduction

Biomineralization refers to the process by which organisms precipitate inorganic minerals on an organic template.\textsuperscript{1-3} Bio-minerals present unique morphologies and hierarchical structures which strongly determines their functions and mechanical properties. Several studies have demonstrated that organic templates, such as proteins, carbohydrates or synthetic polymers, are able to control inorganic crystal growth.\textsuperscript{4-10} Complementarity to the inorganic phase surface structure, charge and geometrical configuration, and binding energies are important aspects of this influence.\textsuperscript{11,12} Most biominerals are formed inside confined spaces where at least parts of the boundaries are cell membranes containing phospholipids. Among known biominerals, calcium carbonate (CaCO\textsubscript{3}) is one of the most studied minerals and presents different structural forms referred to as calcite, aragonite, vaterite, monohydrocalcite, hexahydrocalcite and amorphous calcium carbonates (ACC).\textsuperscript{13} The occurrence of these forms depends on the kinetics and thermodynamics of the reaction of CaCO\textsubscript{3} formation,\textsuperscript{14} which is particularly influenced by the interaction of the mineral with the organic moiety.\textsuperscript{15} To study the effect of confinement on biomineralization, different model systems have been developed focusing on the precipitation of ACC. Several groups have reported an increased lifetime for ACC when confined in pores,\textsuperscript{16} between crossed cylinders,\textsuperscript{17} in picoliter droplets,\textsuperscript{18} within a silica coating,\textsuperscript{19} or in liposomes.\textsuperscript{20} Other studies using confined aqueous nano- or picovolume droplets in oil have shown that precipitation of CaCO\textsubscript{3} occurs via hydrated forms instead of ACC.\textsuperscript{21} However, in porous media such as gel systems, which are not completely confined, pore size strongly influences nucleation.\textsuperscript{22}

In biological systems, most likely a combination of all of these phenomena occurs in such a way that a comparative analysis of a variety of biomineralized systems has led to the following general principles:\textsuperscript{2,23}

1. Biomineralization occurs within specific confined microenvironments, which implies crystal production at certain functional sites and inhibition or prevention of the process at other sites.
2. A specific mineral is produced with a defined crystal size and orientation.
3. Macroscopic growth is accomplished by packing many incremental units together; this results in unique composites with layered microarchitectures that impart peculiar properties.
The main compartments in biology are cells, tissues or extracellular matrices, meaning that confinement for mineral formation occurs in an environment which is partly surrounded by cellular membranes, that is, lipid bilayers.

Phospholipids like dipalmitoylphosphatidylcholine (DPPC), commonly known as lecithin, are the principal components of the cell membrane. The polar part of the lecithin molecule has two spatially separated and oppositely charged moieties: the positive choline group and the negative phosphate group. In an aqueous medium, lecithin molecules form micelles, wherein the hydrophobic groups are arranged inward while the hydrophilic groups are arranged outwards. In previous studies it has been found that when the soluble medium contains calcium ions (Ca$^{2+}$), an interaction occurs between the ions and the surface of the lipid vesicles. This is the basis for intermicellar CaCO$_3$ crystallization.

Although there are several studies regarding the effect of phospholipid molecules on the in vitro biomineralization process, there has been no analysis of the effect of micelle spatial arrangement on the crystallization of CaCO$_3$. In this study we used a gas diffusion method to determine the effect of different lecithin media on the crystallization of CaCO$_3$ and to evaluate the influence of the spatial arrangement of lecithin molecules on templating CaCO$_3$ crystal formation.

**Experimental**

To determine the effect of lecithin on CaCO$_3$ crystal formation, a gas diffusion method was used (Fig. 1).

Briefly, it consists of a Petri dish of 85 mm diameter with a 10 mm hole in its base to allow communication with a cylindrical glass vessel of 50 mm diameter and 30 mm height that is attached to the Petri dish (Fig. 1). The base plate was divided into ten equal circular sectors, where polystyrene microwells (microbridges; Hampton Res, Aliso Viejo, CA) were placed. In each microwell 35 μl of 200 mM CaCl$_2$ in a Tris buffer, pH 9, with or without lecithin at different concentrations was added.

Four lecithin stock solutions were prepared at concentrations of 1.6 mg ml$^{-1}$, 10 mg ml$^{-1}$, 150 mg ml$^{-1}$ and 300 mg ml$^{-1}$. All solutions were prepared with lecithin Vital Nature®, which was dissolved in deionized water. To obtain a homogeneous emulsion, the solutions were sonicated with a Branson Digital Sonifier (Merek) for 20 minutes in continuous mode at 10% amplitude. Sonication was done using ice to avoid an increase in solution temperature. Different mass ratios of CaCl$_2$/lecithin solutions were added to the microwells as described in Table 1.

Dynamic light scattering was used for determination of the lecithin particle size distributions employing a Malvern Nano ZS instrument with a 633 nm laser diode. Experiments were carried out at 25 °C using a quartz cuvette of 1 cm optical path length. The conductivity was measured with a conductometric cell (Metrohm no. 6.0910.120) connected via a conductivity module (856, Metrohm no. 2.856.0010) to a computer. For crystal formation, 3 ml of NH$_4$HCO$_3$ solution was placed in a cylindrical vessel, and the Petri dish was covered with a glass lid, sealed with Parafilm® and allowed to stand for 24 hours at room temperature. Then the microwells were washed 2 times with distilled water, then with 50%, 80% and 100% ethanol solutions, and finally washed 2 times with 5% sodium hypochlorite solution. Washing with 5% sodium hypochlorite was necessary to observe crystals in the conditions where lecithin formed a highly viscous sol. The 5% sodium hypochlorite solution acted as an oxidizing agent to remove the lecithin and did not affect the shape and morphology of the crystals already formed.

A DeltaNu Advantage Systems Raman spectrometer was used to obtain the Raman spectra of the precipitates that formed. Analysis of the Raman spectra of the precipitates was done to determine the polymorphism of the crystals that formed.

Microwells were coated with gold by using the electron microscopy science EMS-550 equipment (Automated Sputter Coated). Microwells were observed by scanning electron microscopy (SEM) with a Tesla BS-343A instrument at 15 kV and with a FEG SEM Hitachi 6400 instrument. The average crystal size was obtained by measuring crystals from four pictures of different fields. The values were statistically analyzed by the R Studio program to obtain the mean values and standard deviation for each population of crystals.

**Results**

The aggregation of lecithin in the 200 mM CaCl$_2$ solution was analyzed by dynamic light scattering. In the stock solutions obtained by dissolution of lecithin in water, the hydrodynamic radii of lecithin aggregates were 29.1 ± 5.6 nm, 22.4 ± 2.3 nm, 23.7 ± 4.1 nm and 20.0 ± 3.6 nm at concentrations of 1.6 mg ml$^{-1}$, 10 mg ml$^{-1}$, 150 mg ml$^{-1}$ and 300 mg ml$^{-1}$,
respectively. When different aliquots of a lecithin stock solution were diluted in 100 ml of 200 mM CaCl₂ solution a large increase was observed in the hydrodynamic radius of the lecithin particles. The translucent liquid solutions obtained in the less concentrated lecithin samples were increasingly transformed into more opaque highly viscous sols when lecithin concentration was increased. Therefore, the hydrodynamic radius was around 100 nm when low concentrations of lecithin were used, and the radius was not determined in the more highly concentrated viscous sols. The conductivities

<table>
<thead>
<tr>
<th>Microwell code</th>
<th>Lecithin solution added to 100 μl of 0.2 M CaCl₂</th>
<th>Lecithin quantity in the microwell (μg/35 μL of solution)</th>
<th>Hydrodynamic radius (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>0</td>
<td>0.56</td>
<td>88.9 ± 4.9</td>
</tr>
<tr>
<td>M1</td>
<td>1 μL of 1.6 mg ml⁻¹</td>
<td>2.8</td>
<td>98.3 ± 2.7</td>
</tr>
<tr>
<td>M2</td>
<td>5 μL of 1.6 mg ml⁻¹</td>
<td>5.6</td>
<td>104.0 ± 4.3</td>
</tr>
<tr>
<td>M3</td>
<td>10 μL of 1.6 mg ml⁻¹</td>
<td>3.5</td>
<td>91.8 ± 8.9</td>
</tr>
<tr>
<td>M4</td>
<td>1 μL of 10 mg ml⁻¹</td>
<td>17.5</td>
<td>125.0 ± 2.6</td>
</tr>
<tr>
<td>M5</td>
<td>5 μL of 10 mg ml⁻¹</td>
<td>35</td>
<td>124.1 ± 7.6</td>
</tr>
<tr>
<td>M6</td>
<td>10 μL of 10 mg ml⁻¹</td>
<td>52.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>M7</td>
<td>5 μL of 150 mg ml⁻¹</td>
<td>262.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>M8</td>
<td>10 μL of 150 mg ml⁻¹</td>
<td>525</td>
<td>n.d.</td>
</tr>
<tr>
<td>M9</td>
<td>1 μL of 150 mg ml⁻¹</td>
<td>105</td>
<td>n.d.</td>
</tr>
<tr>
<td>M10</td>
<td>1 μL of 300 mg ml⁻¹</td>
<td>525</td>
<td>n.d.</td>
</tr>
<tr>
<td>M11</td>
<td>5 μL of 300 mg ml⁻¹</td>
<td>1050</td>
<td>n.d.</td>
</tr>
<tr>
<td>M12</td>
<td>10 μL of 300 mg ml⁻¹</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* n.d., not determined.

**Table 1** Microwell codes of lecithin quantity (μg) in 35 μL of Tris/CaCl₂ solution. The hydrodynamic radius of lecithin particles determined by a measure of dynamic light scattering is also reported: the standard error is indicated.

**Fig. 2** M0 and M10 polystyrene microwell Raman spectra obtained using a laser of 785 nm on the DeltaNu Advantage Systems equipment.
for 1.6 mg ml\(^{-1}\) and 300 mg ml\(^{-1}\) lecithin in Milli-Q water were 0.099 \pm 0.02 mS cm\(^{-1}\) and 0.57 \pm 0.06 mS cm\(^{-1}\), respectively. However, in 200 mM CaCl\(_2\) in a Tris buffer, pH 9, the conductivity for 1.6 mg ml\(^{-1}\) lecithin was 27 mS cm\(^{-1}\), while the conductivity for 300 mg ml\(^{-1}\) lecithin was 7.7 mS cm\(^{-1}\). After 24 h on the bench, the conductivity decreased to 20.3 mS cm\(^{-1}\) and 6.6 mS cm\(^{-1}\), respectively.

To determine the CaCO\(_3\) polymorphism, Raman spectra were analyzed by comparison with calcite, aragonite and vaterite standard spectra reported by Wehrmeister et al.\(^{34}\) Raman spectra were measured in the microwells without any solution or glass, and the Raman spectra for M0 and M10 microwells are shown in Fig. 2.

The Raman spectrum for M0 microwells corresponds to bands of calcite crystal standard described by Wehrmeister et al.\(^{34}\) with characteristic peaks observed at 154, 275, 712, 1085 and 1435 cm\(^{-1}\), while the Raman spectrum for M10 microwells is characteristic of vaterite.\(^{25}\) In M1 to M6 microwells both calcite and vaterite were present, and in M7 to M12 microwells only vaterite was detected.

SEM of M0 microwells showed a massive precipitation of rhombohedral crystals of calcite 20 to 25 \(\mu\)m in size, exhibiting regular \{104\} faces (Fig. 3).

The presence of lecithin has a strong effect on the morphology of the crystals formed. In M1 microwells calcite crystals were obtained together with a few vaterite spherical particles. The shape of the calcite crystals appeared modified and showed cavities and curvatures (Fig. 4). In M2 and M3 microwells the particles of calcite appeared to be formed by the assembly of rhombohedral units of micrometer and submicrometer sizes. This gave the final calcite particles a stepped and complex shape. These particles showed surfaces with cavities having micrometer size curvatures (dotted curved lines in Fig. 4B) that formed sculpted architectures. Also, the vaterite particles changed their shape with respect to those in M1 microwells, becoming less regular spherulites and having as building units irregular plate-like particles. In M4, M5 and M6 microwells only a few aggregates of calcite (Fig. 5F) were observed, similar to those observed in M2 and M3 microwells, and big spherulitic vaterite crystals were also observed. The spherulitic crystals of vaterite changed their texture quite drastically from M4 to M6 microwells. Indeed the plate-like crystals forming the spherulites almost disappeared and spheroidal grains appeared (Fig. 5C, E, I).

In M7 to M12 microwells only vaterite was detected (Fig. 6 and 7). The particles appeared as irregular spheres formed by the assembly of nanoparticles that changed the structure from one microwell to another. In M7 (Fig. 6) and M10 (Fig. 7) quite regular rhombohedral grains were observed with a size around 100 nm (Fig. 7C). In M8 (Fig. 6) and M11 microwells (Fig. 7) the presence of vaterite formed by irregularly aggregated leaf-like layers a few nanometers in thickness was observed. In M9 and M12 microwells (Fig. 6 and 7) the presence of almost shapeless particles was observed. These particles locally appeared as compact materials in which randomly distributed pores were present (black regions in the SEM pictures in Fig. 6I and 7I).

The distribution of CaCO\(_3\) polymorph type and size varied for the crystals according to lecithin concentration (Fig. 8).

Variability in the size of calcite crystals was observed among different types of microwells. However, in each particular microwell, calcite crystal size was fairly uniform except when 17.5 \(\mu\)l of lecithin was used (M3 microwell), where a much greater size distribution was observed (Table 2).

Except for M1 microwells, vaterite particles (73.66 \pm 31.39 \(\mu\)m) were always bigger than calcite crystals (14.95 \pm 17.22 \(\mu\)m). At intermediate concentrations the crystals were more homogeneous in size than in extreme concentrations, especially for crystals obtained with the 300 mg ml\(^{-1}\) lecithin solution. Calcite crystals are formed only below 35 \(\mu\)g of lecithin in 35 \(\mu\)L of buffered CaCl\(_2\) solution. In higher concentrations of lecithin only vaterite was formed. When Tables 1 and 2 are compared it was observed that calcite crystal size decreased with the increase in lecithin concentration.

**Discussion**

Different CaCO\(_3\) polymorphs (calcite, aragonite and vaterite) can be formed depending on the conditions of crystallization. Calcite is the most thermodynamically stable polymorph, but vaterite and aragonite can form under specific kinetic conditions and be precursors of calcite. Other studies of CaCO\(_3\) nucleation and the influence of proteins on CaCO\(_3\) formation involved formation of CaCO\(_3\) by vapor diffusion with set-ups similar to that used in the present study.\(^{35,36}\) In these studies it was shown that not only the concentration and nature of the additive influence nucleation and the specific polymorph(s) formed, but also the way in which the experimental system becomes supersaturated influences such variables.\(^{35,36}\) In fact, Gomez-Morales et al.\(^{36}\) have shown that under the conditions of their experimental set-up, calcite, aragonite and vaterite precipitated in the absence of any
additive. However, with the same chamber we have demonstrated that, using buffered solutions, it is possible to select only calcite or a mixture of two or three polymorphs, depending on the diameter of the central hole (controlled gas
diffusion) or by altering the time of incubation or the incubation temperature.\textsuperscript{37}

The analysis of the distribution of polymorph gives information on the mechanism of precipitation. Raman
spectroscopy is an appropriate method for monitoring the formation and evolution of CaCO₃ polymorphs. These analyses showed the presence of calcite and vaterite in the microwells when lecithin was used as an additive.

The results of dynamic light scattering analysis show that lecithin assembly is not strongly affected by its concentration until a highly viscous sol forms (Table 1). Different crystal morphologies were obtained when lecithin concentration was increased, confirming the strong interaction of the CaCO₃ crystals with the lecithin molecular assemblies. In fact, it has been proposed that calcite crystal growth is guided by lipid templates, described as intermicellar growth, where the surface of the micelles or liposomes has a strong interaction with calcium ions causing crystallization between the micelles. Indeed, divalent cations have strong propensity to bind with the phosphocholine headgroups of lecithin and induce a transition from spherical to long cylindrical micelles (also called wormlike micelles or worms), reducing the electrostatic repulsion between the charged surfactant headgroups (in effect decreasing the Debye screening length), which facilitates micellization and the assembly into cylindrical structures. This can be observed in crystals obtained in the presence of a small amount of lecithin (until 5.6 μg, M3 microwell), where calcite crystals of uniform size but with different degrees of surface modification were observed. Interestingly, at this small concentration of the lipid we also observed some vaterite crystals.

The characteristics of micelles are dependent on the emulsion in which they are formed. There are different types of emulsions, such as oil-in-water emulsions, wherein traditional micelles are formed with polar heads outward and hydrophobic tails inward. However, in water-in-oil emulsions reversed micelles can also be formed in which the polar heads of the micelles are arranged inward and the hydrophobic tails outward. Such micelles are formed to achieve a thermodynamic equilibrium, and it has been found that reversed micelles constitute stable compartments that serve as crystallization templates. In addition, in a CaCO₃ crystallization study conducted by Kang et al., calcite crystals were obtained when low amounts of micelles were formed. However, by increasing the amount of surfactants, reversed micelles were formed and vaterite crystals were obtained. Normally lecithin reversed spherical or ellipsoidal micelles are obtained when additional surfactants, nonpolar organic solvents or oils are added, in the absence of bivalent ions or buffered solutions. However, it has been shown that inorganic salts can modulate the self-assembly of phospholipids like lecithin in organic solvents by binding with the phosphocholine headgroups of lecithin and induce a transition from spherical reversed micelles to cylindrical fibrils. However, it is important to keep in mind that lecithin is a zwitterionic surfactant that in our experimental conditions (i.e., in the presence of 200 mM CaCl₂ in a Tris buffer solution, pH 9) has highly ionized negatively charged phosphate groups in the polar region of the molecule where a strong interaction with calcium ions is expected to occur. In fact, such calcium concentration-dependent interaction has been recently reported. In addition, it has been reported previously that the binding of Ca²⁺ and other multivalent metal ions causes a small conformational change of the phosphocholine polar group. Moreover, in the case of bilayered phosphocholine, the binding of Ca²⁺ appears to involve the formation of a well-defined chemical complex in which one Ca²⁺ coordinates with two phosphocholine molecules.

### Table 2: Morphological features and size of calcite and vaterite crystals obtained in each microwell (mean ± SD)

<table>
<thead>
<tr>
<th>Microwell code</th>
<th>Calcite shape/texture</th>
<th>Calcite size (μm)</th>
<th>Vaterite size (μm)</th>
<th>Calcite shape/texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>Rhomb. (10.4) single crystals</td>
<td>20.00 ± 4.08</td>
<td>—</td>
<td>Vaterite/plate-like crystals</td>
</tr>
<tr>
<td>M1</td>
<td>Modified rhomb./[10.4] single crystals</td>
<td>29.00 ± 8.87</td>
<td>32.75 ± 12.62</td>
<td>Spherulites/plate-like crystals</td>
</tr>
<tr>
<td>M2</td>
<td>Rhomb. (10.4) faced/polycryst. assemblies</td>
<td>30.00 ± 7.43</td>
<td>100.25 ± 8.96</td>
<td>Spherulites/polycryst. assemblies</td>
</tr>
<tr>
<td>M3</td>
<td>Rhomb. (10.4) faced/polycryst. assemblies</td>
<td>24.50 ± 6.04</td>
<td>71.25 ± 7.41</td>
<td>Spherulites/polycryst. assemblies</td>
</tr>
<tr>
<td>M4</td>
<td>Rhomb. (10.4) faced/polycryst. assemblies</td>
<td>39.50 ± 7.12</td>
<td>100.75 ± 9.36</td>
<td>Spherulites/polycryst. assemblies</td>
</tr>
<tr>
<td>M5</td>
<td>Rhomb. (10.4) faced/polycryst. assemblies</td>
<td>20.75 ± 10.31</td>
<td>100.00 ± 15.73</td>
<td>Spherulites/polycryst. assemblies</td>
</tr>
<tr>
<td>M6</td>
<td>Rhomb. (10.4) faced/polycryst. assemblies</td>
<td>10.15 ± 3.47</td>
<td>100.50 ± 15.04</td>
<td>Spherulites/polycryst. assemblies</td>
</tr>
<tr>
<td>M7</td>
<td>—</td>
<td>99.5 ± 15.04</td>
<td>Spherulites/compact spheroidal grains</td>
<td></td>
</tr>
<tr>
<td>M8</td>
<td>—</td>
<td>79.00 ± 7.71</td>
<td>Spherulites/compact spheroidal grains</td>
<td></td>
</tr>
<tr>
<td>M9</td>
<td>—</td>
<td>70.00 ± 7.75</td>
<td>Spherulites/pored spheroidal grains</td>
<td></td>
</tr>
<tr>
<td>M10</td>
<td>—</td>
<td>70.00 ± 15.98</td>
<td>Spherulites/compact spheroidal grains</td>
<td></td>
</tr>
<tr>
<td>M11</td>
<td>—</td>
<td>70.00 ± 22</td>
<td>Spherulites/thin platelike crystals</td>
<td></td>
</tr>
<tr>
<td>M12</td>
<td>—</td>
<td>61.50 ± 15.31</td>
<td>Spherulites/shapless grains</td>
<td></td>
</tr>
</tbody>
</table>
In the present study, we showed that by increasing lecithin concentration in Tris-buffered CaCl$_2$ solution, a change from hydrophilic to hydrophobic environment occurs, in which conductivity is drastically reduced, determining not only the type of CaCO$_3$ polymorph obtained, but also where the crystal is formed. In very dilute lecithin solutions (0.56 μg) vaterite formation occurred, although calcite was predominant. This could reflect the ability of lecithin molecules to nucleate and stabilize vaterite crystals, which are inherently unstable in aqueous solution. The presence of some worm-like aggregates of two to eight vaterite crystals reported herein correlates with a statement by Eastoe et al. that the reversed micelles, formed in the presence of additional surfactants, restrict random movements, which can facilitate contact between two or more micelles. The more hydrophobic environment obtained under these conditions as a function of the amount of lecithin should correspond to the formation of a complex highly viscous sol network. Therefore, this indicates that the vaterite crystals reported herein were formed inside ionized aqueous confined spaces which are specified by the geometry of the micelles and which lie between the hydrophilic oily tails of the lecithin molecules, while the calcite crystals with surface modification preferentially form in the intermicellar spaces of regular micelles. This is consistent with our observation that calcite disappeared and vaterite became the only polymorph when lecithin concentration was increased. The diverse texture of the vaterite crystals obtained in this study (i.e., grains and leaf-like layers) is an indication that higher lecithin concentrations produce an environment consisting of a complex network, which contains confined aqueous charged spaces that act as templates for crystallization of vaterite and in which the occurrence of reversed micelles cannot be disregarded. This could be related to the observation reported by Kang et al., that similar vaterite crystals were favored when the surfactant content (sodium dodecyl sulfate) was increased in the reversed micelles. These authors demonstrated that there is an effective supersaturation induced in the micelles, which is much higher than that in the bulk aqueous solution. In fact, it has recently been demonstrated that under high supersaturation the growth of pumpkin-shaped vaterite, such as that reported here, is favored.\textsuperscript{14} Occurrence of vaterite has been reported in reversed micelles of the cetyl trimethylammonium bromide surfactant at 5 °C.\textsuperscript{50} Under our experimental conditions, the formation of vaterite when lecithin concentration was increased requires conditions of high supersaturation. The aspect ratio of the confined continuous or discontinuous aqueous spaces containing 200 mM CaCl$_2$ in Tris buffer solution, pH 9, present in the viscous sol at high lecithin concentrations, determines the conditions of the required supersaturation. This is because there is an increase in the water that is bound to the charged surface and is also due to a preferential solubility and diffusivity of the lipophilic CO$_2$ through the lipid matrix, as has been demonstrated elsewhere.\textsuperscript{51,52}

The morphology of the crystals changes with lecithin concentration and also with the procedure for preparing the lecithin media. Indeed, morphological differences were observed between crystals that were obtained from different lecithin media with the same final lecithin concentration. An example is given by the crystals obtained in M9 and M11 microwells, in which both have a final lecithin concentration of 525 μg. However, the M9 microwell was prepared using a stock solution of 150 mg ml$^{-1}$ and the M11 microwell was prepared with a stock solution of 300 mg ml$^{-1}$. This indicates that the geometry or spatial distribution of the continuous or discontinuous ionic confined aqueous spaces in the viscous sol is already determined at an early stage of the lecithin molecule assembly.

Controlling polymorphism has great technological significance especially because it can determine specific mechanical or optical properties of materials. In addition polymorphism is important in many fields like pharmaceuticals, agrochemicals, pigments and foods where dissolution rates depend on polymorphism. Here, we demonstrated that it is possible to get different proportions of calcium carbonate polymorphs depending on the lecithin concentration in the crystallization medium.

Conclusions

In summary, herein we demonstrated that the occurrence of both surface-modified calcite crystals and diverse textured vaterite crystals that are formed in an ionized calcium chloride solution under a continuous CO$_2$ diffusion atmosphere reflects the geometry and spatial distribution of ionized aqueous confined spaces due to the specifics of the lecithin assembly, which are largely controlled by lecithin concentration. The results obtained, in conjunction with the related discussion, suggest that as a function of lecithin concentration the precipitation of CaCO$_3$ occurs inter-micelle, producing sculpted crystals of calcite, or intra-reversed micelle, producing different assemblies of vaterite crystals. To demonstrate these precipitation paths, which has implications for biominalization as well as in materials science, further studies will be needed to determine the exact type and characteristics of the arrangement of lecithin molecules (i.e., micelles, reversed micelles, layers, worm-like tubes, etc.) in each lecithin medium. However, the presence of different polymorphs and surface modifications observed in this study is compatible with the occurrence and effect of traditional and reversed micelles determining special confinement spaces where crystallization of CaCO$_3$ occurs in different lecithin concentration media.

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Notes and references