CHAPTER - 6
Biomimetic growth of flower-like calcite morphology

6.1 Introduction

In Nature, organisms fabricate fascinating structures such as shells, bone, and teeth specifically designed to provide mechanical support and protection via biomineralisation [1]. Biomineralisation is a highly complex phenomenon involving molecular interactions occurring at the interface between the inorganic mineral and the macro molecular organic matrix for fine tuning the deposition of biominerals [2-4]. Much attention has been paid during recent years to adapt the biomineralisation processes to generate new materials with controlled morphologies and unique structural complexity [5-8]. Calcium carbonate crystallization has been widely studied for decades, receiving both scientific and industrial interest. Calcium carbonate is one of the mostly studied biominerals, because of its high abundance and rich polymorphism, and it has been widely used as a model mineral in biomimetic chemistry. In recent years, CaCO₃ has been intensely studied with the aim of understanding how crystal polymorph and structural features can be controlled by organic additives [9, 10]. Calcium carbonate 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. It has been suggested that organisms control crystal morphology by forming crystals within a preformed matrix composed of either macromolecules or lipid membranes, and that the ultimate morphology of the crystal is defined by the form of the matrix [11]. Alternatively, specialized organic macromolecules, which are occluded within the majority of biological CaCO₃ crystals, have been shown to interact with specific crystal faces of growing crystal [12-15]. Applications of calcium carbonate have been focussed on biomedical fields [16,17] such as drug delivery, treatment of dry eye etc in addition to use in coatings, plastics, alloys and catalysts.

In literature, different biomimetic approaches to control the morphology of calcium carbonate using a variety of additives such as amino acids [18] peptides [19], proteins [20], biopolymers [21] carboxylate rich carbonaceous material [22], lecithin
liposome organic template \(^{[23]}\) and biomolecules of lotus root \(^{[24]}\) have been reported. It is now well established that the additives with carboxylate moieties can effectively control the morphology of calcium carbonate by occupying carbonate sites during crystal growth under different reaction conditions \(^{[25-27]}\).

Previously we have reported the synthesis of different polymorphs of calcium carbonate using various carboxylate additives (malonate, succinate, gluterate and adipate etc) by varying the reaction conditions, such as temperature and concentration \(^{[28]}\). Specifically, the use of a polycarboxylate template namely 1, 3-diamino-2-hydroxypropane-\(N, N, N, N\)-tetraacetate has generated calcite microtrumpets \(^{[29]}\) which are mimicking the coccoliths of an unicellular algae, \textit{Discosphaera tubifera}. In the present chapter, we demonstrate the role of \textit{para}-Amino benzoic acid (PABA) and HEIDI as an additive during mineralisation of calcium carbonate. In continuation of our efforts in the process of biomineralisation, we found new flower-like morphology under the influence of PABA.

Nature uses organic molecules to control and direct the crystallization of biominerals with special orientation, texture and morphology under ambient conditions. Furthermore, biominerals are organized from the nano to microscopic scales to form structures with hierarchical long-range order. The carboxylic acids such as para amino benzoic acid (PABA) are the primary active molecules on the organic/inorganic interface of biomineral tissue.

In the present work, the crystal growth of calcium carbonate was investigated in presence of \textit{para}-Aminobenzoic acid (PABA) at different experimental conditions. Calcite phase with new flower-like morphology was obtained biomimetically at room temperature after 24 hrs of crystal growth at pH 8.0 and 0.2mM concentration of PABA. On the other hand complex bundles of plate-like nano subunits were dominant at pH 3.0. PABA played an important role in directing the crystal phase and growth morphology of calcium carbonate. The products were characterized by Scanning Electron Microscopy (SEM) and Powder X-ray diffraction (XRD) techniques.
6.1.1 Structure of para-amino benzoic acid (PABA)

PABA is a naturally occurring compound, found in liver, kidney, wheat germ, bran, and yogurt. Furthermore, PABA is involved in different biochemical reactions in living systems [30]. Hence, we have opted PABA for biomimetic growth of calcium carbonate. Since PABA is consisting of both carboxylate and amino functionalities analogous to aromatic amino acids, a similar function is expected to control the nucleation and growth of calcium carbonate.

![Structure of para-amino benzoic acid](image)

*Figure 6.1: Structure of Para-amino benzoic acid.*

6.2 Experimental Section

The mineralization was carried out by a slow CO\(_2\) gas diffusion technique. 2.0mmol of CaCl\(_2\) (0.294g) in 10ml distilled water was taken along with different proportions of para-aminobenzoic acid (0.04mM, 0.1mM and 0.2mM) in a cell culture dish. The dishes were covered with parafilm and 2 to 3 holes were made, so that the carbon dioxide could be introduced to the solution via vapour diffusion. The dishes were then placed in the desiccator containing ammonium carbonate (CO\(_2\) source) for 24 hours at room temperature (25±1°C). The crystal growth process in the solutions was initiated at three different pH values of 3.0, 6.0 and 8.0. 0.1M NaOH solution was used to maintain the pH. Further, at each pH value the growth process was monitored
by using different concentrations of PABA (0.02 mM to 0.2 mM). After completion of the reaction, the crystals were filtered, washed several times with distilled water and dried at ambient temperature. Powder X-ray diffraction (STOE system operating with monochromated Cu Kα1 radiation) and Scanning Electron Microscopy were used for characterization of products.

6.2.1 Flow chart

Table 6.1 shows the flow chart representation of experimental conditions with para-aminobenzoic acid.

Table 6.1: Flow chart.

6.2.2 Characterization methods

Scanning electron microscopy (FEI Quanta 200 FEG with EDS) was used for morphology assessment of CaCO₃, BaCO₃ and SrCO₃ crystals. The crystals were collected on a round cover glass (1.2 cm), washed with deionized water and dried in a desiccator at room temperature. The cover glass was then mounted on a SEM stub and coated with gold for SEM analysis. X-ray diffraction measurements of CaCO₃, BaCO₃ and SrCO₃ structures were recorded using a Rigaku diffractometer (Cu Kα radiation, λ = 0.1546 nm) running at 40 kV and 40 mA (Tokyo, Japan). FT-IR spectra
of CaCO₃, BaCO₃ and SrCO₃ structures were recorded with a Thermo Nicolet Nexus (Washington, USA) 670 spectrophotometer.

6.3 Results and Discussion

Remarkable effects on the evolved morphologies were observed by varying both the concentration of PABA and pH. Table 6.2 shows the schematic composition and morphology of CaCO₃ at different concentrations and different pH.

Table 6.2: Schematic composition and morphology of CaCO₃ at different concentrations and different pH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>CaCl₂ concentration</th>
<th>PABA concentration</th>
<th>Morphology at pH 3.0</th>
<th>Morphology at pH 6.0</th>
<th>Morphology at pH 8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2mM</td>
<td>0.04mM</td>
<td>Truncated rhombohedral calcite</td>
<td>Mixed phase of calcite &amp; aragonite</td>
<td>Flower shaped calcite</td>
</tr>
<tr>
<td>2</td>
<td>2mM</td>
<td>0.2mM</td>
<td>Bundles of vaterite nano plates</td>
<td>Mixed phase of calcite &amp; aragonite</td>
<td>Calcite flowers</td>
</tr>
</tbody>
</table>

6.3.1 Concentration dependence

Formation of CaCO₃ has been studied at different molar ratios of [Ca²⁺]/[PABA] - 50, 20, 10 at room temperature 21.0 ±1 °C to get the proper phase and interesting effects on the evolved morphologies. At lower concentration of PABA the influence on the CaCO₃ growth morphology was negligible. CaCO₃ crystals produced at higher concentration of PABA i.e., at molar ratio of [Ca²⁺]/[PABA] - 10 has good effect on evolved crystal morphology (Fig. 6.2a,b)

At lower concentrations of PABA (0.04mM), the growth of flower shaped calcite was initiated from elongated truncated calcite crystals as shown in Figure 6.2a,
which however was not completed. Presumably, the concentration is not sufficient to convert the truncated rhombohedral calcite to fully grown flower-like calcite. Whereas at higher concentrations of PABA (0.2 mM), completely grown calcite flowers were seen (Figure 6.2 b & 6.2c). A stepwise growth of petals of calcite flower was also recorded in SEM study and depicted in Figure 6.2d.

**Figure 6.2:** SEM images of various phases of calcium carbonate found at pH 3.0. a) Truncated calcite crystals at PABA concentration 0.04mM. b) vaterite nano plates PABA concentration 0.2mM.

**Figure 6.3:** SEM images of flower-shaped calcite super structures (a, b, c) at pH 8.0 (0.04mM). Stepwise growth on petals of calcite flower at pH 8.0 (0.2mM).
6.3.2 pH dependence

The crystal growth process of calcium carbonate was initiated at three different pH values of 3.0, 6.0 and 8.0 by PABA additive. In addition, at each pH value the growth process was further monitored by using different concentrations of PABA (0.02mM, 0.04mM, 0.1mM and 0.2mM). Three polymorphs of calcium carbonate i.e., calcite, aragonite and vaterite were observed under the above mentioned experimental conditions. However, in the absence of PABA, the thermodynamically most stable regular rhombohedral calcite was the only phase observed. In all the above experiments a reaction period of 24 hrs was found to be appropriate to note the changes in the crystal growth of calcium carbonate. When the solution starting pH was 3.0 and PABA concentration was 0.04mM, truncated rhombohedral calcite morphology (Fig. 6.4a) was obtained. On the other hand, at same pH, when the concentration of PABA was increased to 0.2mM, beautiful bundles of vaterite nano plates along with traces of calcite were produced (Fig. 6.4a,b). Mixed phases of calcite and aragonite were resulted at pH 6.0 with different concentrations of PABA(Fig. 6.5a,b). Interesting transitions in the crystal morphology of calcium carbonate were noted in the experiments carried out at pH 8.0. Flower shaped calcite superstructures are produced at both the concentrations (Fig. 6.6a,b)

In general, organic additives or templates interact with the reacting species either in solution or at the step edge during the crystal growth process and direct the crystal morphology [26, 31]. However, the growth of flower-like calcite structures appears to be a complex phenomenon. The formation process of flower-like calcite could be predicted as per the following reactions:

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \\
\text{HCO}_3^- & \leftrightarrow \text{H}^+ + \text{CO}_3^{2-} \\
\text{Ca}^{2+} + \text{CO}_3^{2-} & \leftrightarrow \text{CaCO}_3
\end{align*}
\]
The ionisation of both carboxylic and amino groups mainly depends on the pH value of the reacting medium. The ionisation constant, pKa of PABA is 4.65. So at higher pH the carboxylate group from PABA was effectively complexed with Ca$^{2+}$ \cite{32}. In a similar way carboxylate group of PABA interacts with calcium and occupy the carbonate sites in the growing calcium carbonate crystals. Presumably, this could be due to the accumulation of PABA molecules from all \{104\} faces and eventually extends the growth outward from all the edges, therefore it gradually grows like a petal with time, leading to the final flower-like crystals. Figure 6.7 shows the Powder XRD patterns of crystalline flower-like calcite phase. The peaks were centered at $2\theta=23.08^\circ$, 29.35$^\circ$, 39.40$^\circ$ and 48.46$^\circ$, respectively. These are characteristic of (hkl) indices of (012), (110), (202), (116) planes, consistent with pure calcite crystals. No impurity peaks were detected, indicating that the powders had high purity.
Figure 6.4: SEM images of calcium carbonate at pH 3.0. a) 0.04mM. b) 0.2mM.
Figure 6.5: SEM images of calcium carbonate at pH 6.0. a) 0.04mM. b) 0.2mM.
Figure 6.6: SEM images of calcium carbonate at pH 8.0. a) 0.04mM, b) 0.2mM.
Figure 6.7: X-ray powder patterns of the calcium carbonate (c=calcite) at pH 8.0 and 0.2mM concentration.
6.4 Conclusion

Formation of CaCO$_3$ has been studied at different molar ratios of [Ca$^{2+}$]/[PABA] - 50, 20, 10 at room temperature 21.0 ±1 °C to get the proper phase and interesting effects on the evolved morphologies. At lower concentration of PABA the influence on the CaCO$_3$ growth morphology was negligible. CaCO$_3$ crystals produced at higher concentration of PABA i.e., at molar ratio of [Ca$^{2+}$] / [PABA] - 10 has good effect on evolved crystal morphology. Since PABA is having carboxylate and amino functionalities, a similar function could be possible during the crystal growth of calcium carbonate. However, further efforts are necessary to better understand the growth of the mechanism mentioned above in the presence of PABA.
6.5 References


