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Thesis Paper

# Heterogeneous Nucleation of Calcium Carbonate: Effects of Substrate Chemistry

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Investigating heterogeneous calcium carbonate nucleation is essential in the pursuit to the barriers of nucleation. However, heterogeneous calcium carbonate systems are difficult to characterize due to the influence of homogeneous nucleation. Homogeneous nucleation must be removed in order to reliably report the saturation indices used in the experiments. In this paper, homogeneous nucleation and heterogeneous nucleation are experimentally examined with atomic absorption spectroscopy, optical microscopy and ultraviolet-visible spectroscopy. The impact of homogeneous nucleation on concentration and the correlation between concentration and induction time are investigated. We document a significant impact on concentration suggesting data from current literature is compromised. Based on data, we propose a concentration range of 0.7 mM – 0.9 mM for obtaining barriers to nucleation using optical microscopy. Learning how surfaces influence the barriers and kinetics of $CaCO_3$ nucleation can increase our understanding of biomineralization and improve carbon sequestration.

## 1 Introduction

Calcium carbonates ($CaCO_3$) are found both dissolved in water and as naturally occurring minerals in the subsurface. Following classical nucleation theory $CaCO_3$ is considered to precipitate in two ways[1]: in the presence of a surface, known as heterogeneous nucleation, and free in solution, known as homogeneous nucleation. Homogeneous nucleation occurs when the saturation index (SI) is high enough and either calcite forms directly or more commonly, through the precursor phases amorphous calcium carbonate (ACC) and vaterite. Both are unstable intermediates. ACC has water in the structure and generally transforms to vaterite in seconds. Vaterite can be stable for minutes to several hours before transforming to calcite[2]. Heterogeneous nucleation dominates at lower saturation indices and happens because the presence of a substrate (e.g. mineral surface or polymer) decrease the interfacial free energy and hence lowers the barriers to nucleation. Barriers to nucleation can be obtained using classical nucleation theory and by counting heterogeneously precipitated nuclei pr area over time at different saturation levels[3 4 5].

Previous work investigated the difference in interfacial free energy for $CaCO_3$ and different substrates, resulting in different nucleations per second. Giuffre *et al.*[4] and Hamm *et al.*[5] found that surface charge and structure can control the heterogeneous nucleation rate.

The initial aim of this study was to test how different substrates could alter the interfacial free energy and promote heterogeneous nucleation. Replicating the results by Hu *et al.*[3] and Hamm *et al.*[5] resulted in an unexpected amount of homogeneous nucleation, as seen in figure 1.

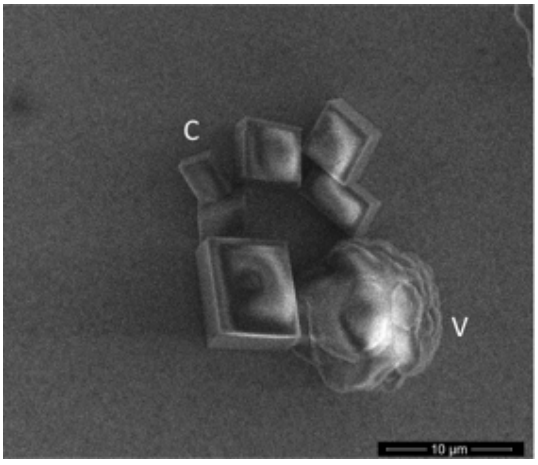


**Fig. 1** Scanning electron microscope image, showing both calcite, marked with c, and vaterite, marked with v, on the substrate. The sample were made at a SI of 1.71 on an amin-functionalized substrate.

If we have homogeneous nucleation the SI is expected to change significantly and we have no control over the SI, hence we cannot calculate the true barriers. We therefore

modified our aim to removing homogeneous nucleation from the experiments. We used self-assembled monolayers[6] as substrates: A 11-Mercaptoundecanoic acid monolayer result in a carboxyl-functionalized surface and a 11-Amino-1-undecanethiol monolayer giving an aminfuntionalized surface, at the pH conditions of this study that gives a negative and a positive surface charge respectively. An optical microscope was used to examine a substrate's effect on heterogeneous nucleation. The SI's used are shown in Table 1.

**Table 1** Concentrations and saturation indices

| Concentration of Ca and $CO_3$ [mM] | Saturation index |
|---|---|
| 2.5 | 2.28 |
| 1.3 | 1.88 |
| 1.25 | 1.86 |
| 0.8 | 1.56 |
| 0.6 | 1.35 |
| 0.4 | 1.04 |
| 0.2 | 0.45 |

The concentration of Ca and $CO_3$ are assumed to be equal.
Saturation indices used in this study, in log scale calculated with PHREEQC.

The SI's have been calculated by the following equation:

$$SI = log_{10}\left(\frac{IAP}{K_{sp}}\right) \quad (1)$$

where the ionic activity product, $IAP$, is the product of $a_{Ca^{2+}}$ and $a_{CO_3^{2-}}$. The solubility constant, $K_{sp}$, is the value of how soluble $CaCO_3$ is in water.

We investigate homogeneous nucleation with Ultraviolet-visible spectroscopy (UV-Vis) and Atomic absorption spectroscopy (AAS) to find a concentration window where homogeneous nucleation is avoided. AAS was used to find the concentration of a specific ion in solution, this is used to track the impact on concentration due to homogeneous nucleation. UV-Vis follows the onset and progress of homogeneous nucleation live, by monitoring an increase in absorption due to particles forming in the solution. The Avrami equation is fitted to the UV-Vis data to obtain three parameters, the rate constant, the order of nucleation, and the induction time. The latter parameter is particularly interesting because it provides a time frame where heterogeneous nucleation can be studied without interference from homogeneous precipitation.

By obtaining a fundamental kinetic and thermodynamic understanding of $CaCO_3$ mineral formation barriers and stability in the presence of a range of biological and mineral substrates, we can predict and engineer the fate of trapped $CO_2$ to a much greater extent than what is possible with current knowledge.

## 2 Experimental setup

In this section, the materials used and the experimental setups will be covered. Further information is reported in supporting information.

### 2.1 Materials

Solutions used for calcite nucleation experiments contain $Na_2CO_3$ (Merck ACS reagent $\geq 99$ %) and $CaCl_2$ (Sigma-Aldrich ACS reagent $> 99$ %), which provides equal concentrations of $Ca^{2+}$ and $CO_3^{2-}$ when mixed. The pH of the mixed solution was 11.3. The water used for dilution and equipment cleaning was MilliQ water with a resistivity of $> 18$ Mcm. For AAS the samples were diluted with 2 % $HNO_3$ and 0.1 % KCl. All materials used were reagent grade.

Substrates were prepared using the self-assembled monolayers (SAMs) technique[6] on template stripped gold surfaces from platypus®.

### 2.2 Experiments

We collected scanning electron microscopy (SEM) images of selected substrates using a Quanta 3D FEG 200/600 SEM, in high vacuum ($5 \cdot 10^{-}4$ Pa), with an acceleration voltage of 2.00 kV and a beam current of 16.6 pA.

For SI calculations, the software PHREEQC with the phreeqc.dat database, was used. PHREEQC calculates the SI by equation 1 using the ionic activity for each ion.

For nucleation experiments a flow cell within an optical microscopy was used. The microscope was a Zeiss optical microscope set at 10x zoom and bright field. The experiment was conducted under the assumption that all nuclei will grow, since the initial nucleation is the energy barrier, therefore, 10x zoom will be sufficient to see all crystals. The setup consists of two beakers containing $Na_2CO_3$ in one and $CaCl_2$ in the other. The liquids are pumped into a mixer and from there flow into the flow cell which contains the substrate. The flow rate should be above 1 ml/min to ensure enough flow to have a diffusion controlled environment[5]. The flow cell setup is illustrated in Figure 2 and is inspired by the setup used by Hu *et al.*[3] and Hamm *et al.*[5]. One significant difference between our setup and the one e.g. Hamm *et al.*[5] used is that they placed the substrate on the top of the flow cell, facing down, to avoid fall-down of homogeneous nucleation, where we placed it in the bottom of the cell facing up. Having the substrate facing down in the cell makes homogeneous nucleation difficult to detect and they therefore assumed that all crystals on the substrate were from heterogeneous nucleation. In contrast having

the substrate in the bottom means that the crystals on the substrate are a combination of homogeneous and heterogeneous nucleated crystals, making homogeneous nucleation visible, therefore, experiments for this study were set up to investigate the effect of homogeneous nucleation.

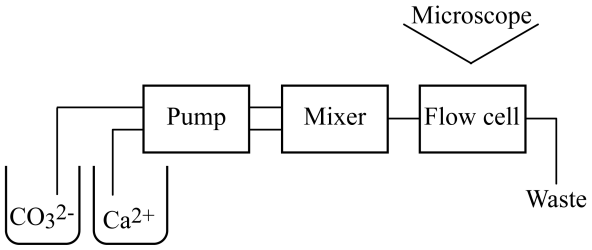


**Fig. 2** Experimental setup for the flow cell experiment. The induction time is the time between the mixer and the flow cell. The experiment time is started once the solution covers the entire substrate.

The flow cell setup was rinsed with Milli-Q water and 0.5 mM HCl after every experiment.

AAS was carried out on Perkin Elmer precisely AAnalyst 800 using AS 93plus for sample management and WinLab32 for AA as software. The AAS measure the concentration of $Ca^{2+}$-ions in a sample at 422.7 nm. One sample (a) was made for each concentration before mixing $CaCl_2$ and $Na_2CO_3$. The second sample (b) was made by mixing equal amounts of the two solutions and allowed to equilibrate for a few days. The sample was filtered to remove any crystals and acid were added to stop any further nucleation.

UV-Vis experiment was carried out on Avantes AvaLight-DH-S-BAL using OceanView as software at 450 nm wavelength. The experiment measure absorption, if homogeneous nucleation are present, the absorption will increase. In the cuvettes $CO_3^{-2}$ was added first and then $Ca^{2+}$. To ensure proper mixing a magnetic stirring rod was inside the cuvette during the experiment. To analyze the UV-Vis data, the Avrami equation was fitted. The Avrami equation is given by:

$$\alpha = 1 - e^{-(k\cdot(x-i))^n} \tag{2}$$

The Avrami equation encompasses three parameters: the rate constant (k), the order of reaction (n) and the induction time (i) prior to nucleation. In the top right corner in Figure 4 is a fitting shown for 2.5 mM.

The rate constant is the rate at which nucleation occurs.

The order of reaction is determined by several qualities of the reaction. It follows the given equation:

$$n = N_{dim} \cdot g + B \tag{3}$$

The order of reaction encompasses three different components, two are related to growth, that is $N_{dim}$, the dimensions of growth, and g, the nature of the growth limiting reaction, being 1 for surface controlled reactions and 0.5 for diffusion controlled reactions. The last component is B, the rate of nucleation, being 0 if all nuclei are present initially and 1 if nuclei are formed at a constant rate. For homogeneous nucleation B should be 1 since the nucleation rate is constant until the concentration drops too low.

The induction time is the time from the beginning of the experiment till nucleation starts.

AAS and UV-Vis were conducted multiple times to improve statistics. Sample (a) were taken before mixing and sample (b) were taken after mixed during UV-Vis and then measured with AAS afterwards, thereby ensuring that the two experiments can be compared. In this paper one experiment is followed, but all experiments are used for calculating averages. Each concentration have been color coded for all experiments.

## 3 Results and discussion

To observe heterogeneous nucleation on a substrate the flow cell setup is used. Replicating the SI reported in literature[5], showed homogeneous nucleation in the flow cell. The highest SI reported is 2.28 and the lowest is 1.88. Homogeneous nucleation was observed in both. The optical microscopy experiments at SI 1.86 in Figure 3 show homogeneous nucleation.

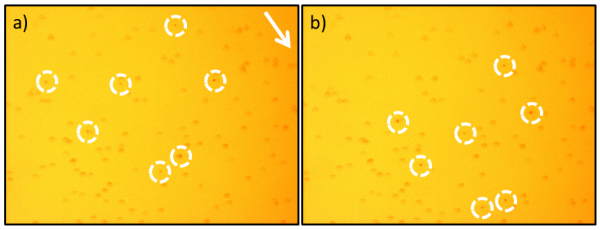


**Fig. 3** Optical microscopy at SI 1.86, 1 ml/min flow rate, on a carboxyl-functionalized substrate. a) is after 136 s and b) is after 138 s. Focus in both pictures are above the substrate. All 7 crystals move down right from picture a) to picture b). All the circles show homogeneous nucleated crystals flowing freely in the water.

Each homogeneous nucleation is marked with a white circle. The two pictures are taken in the same location with a 2 second time interval. All the crystals move in the flow direction at almost the same speed indicating that they flow freely in solution as homogeneous nucleated crystals. In the background the substrate can be seen, it is not in focus, because the crystals in focus are above the substrate. The crystals are thus not in contact with the substrate and that indicate that the crystals are indeed homogeneously nucleated.

The homogeneous nucleations fell down on the substrate and in addition to changing the chemistry of the reacting solution the particles made it impossible to differentiate between heterogeneous and homogeneous nucleation using optical microscopy. To avoid homogeneous nucleation we needed lower concentrations. Changes in the concentration due to homogeneous nucleation were investigated using AAS

### Atomic absorption spectroscopy

Two samples were measured for each concentration. Sample (a) contained $CaCl_2$ and sample (b) was a mixture of $CaCl_2$ and $Na_2CO_3$. The measured value of sample (a) should be close to the expected value, since no nucleation could occur. The measured value of sample (b) should, without any nucleation, be half that of sample (a) due to dilution.

**Table 2** AAS results

| | Exp. [mM] | Meas. [mM] | Meas./Exp. | % loss |
|---|---|---|---|---|
| | (a) 5.0 | 4.835 | 96.7 % | 96.2 |
| | (b) 2.5 | 0.01 | 0.5 % | |
| | (a) 2.6 | 1.419 | 54.6 % | 52.8 |
| | (b) 1.3 | 0.024 | 1.8 % | |
| | (a) 1.6 | 0.895 | 55.9 % | 53.5 |
| | (b) 0.8 | 0.020 | 2.4 % | |
| | (a) 1.2 | 0.608 | 50.7 % | 46.6 |
| | (b) 0.6 | 0.024 | 4.1 % | |
| | (a) 0.8 | 0.556 | 69.5 % | 65.6 |
| | (b) 0.4 | 0.016 | 3.9 % | |
| | (a) 0.4 | 0.211 | 52.7 % | -8.5 |
| | (b) 0.2 | 0.122 | 61.2 % | |

(a) is the sample before mixing and (b) is after mixing.

Table 2 show the intended and measured calcium content before and after UV-Vis experiments (as shown in Figure 4. Any calcium loss between before and after the experiment is indicativve of homogeneous precipitation. The first column shows the color coding for each concentration. The second column show the expected concentration in mili molar of each sample. Here (a) is the sample before mixing and (b) is after mixing. The third column show the measured concentration in mili molar. The fourth column show how close the measured $Ca^{2+}$ concentration is to the expected concentration. The fifth column show how much further the b-sample is from the expected value than the a-sample is. A high number means a large impact on the concentration due to homogeneous nucleation.

As seen in Table 2 the measured concentration of the (b) sample is much lower than half that of sample (a). The closer the measured percentage is between sample (a) and (b), the less homogeneous nucleation has occurred. Most of the samples show large discrepancy between (a) and (b), confirming that homogeneous nucleation does have a large impact on the concentration. Only the lowest concentration has no sign of homogeneous nucleation. The large impact on the concentration means that homogeneous nucleation will disrupt the concentration of available $Ca^{2+}$ and $CO_3^{-2}$ and the reported SI will be wrong. Using a too low concentration can lead to no nucleation at all, therefore UV-Vis were used to determine induction time, to use as high a concentration as possible without homogeneous nucleation.

### UV-Vis

The UV-Vis setup will show the induction time and if there is any difference in the pathway for the homogeneous nucleation. The induction time is determined for all the concentrations of $CaCO_3$ used in AAS, to investigate the increase in induction time, due to decrease in concentration, furthermore, the nucleation path at different concentrations were also examined by the Avrami parameters.

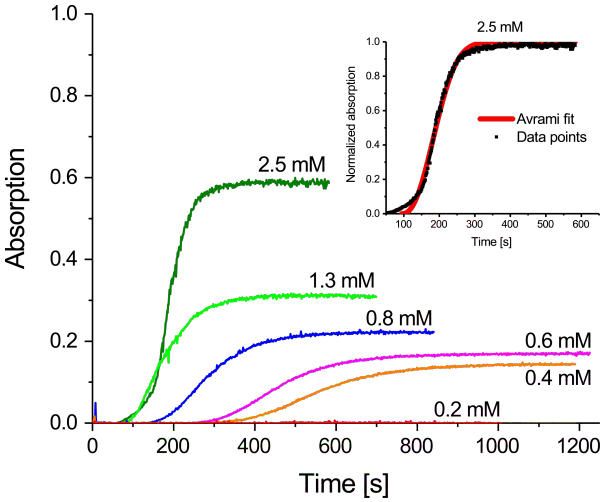


**Fig. 4** UV-Vis experiment showing absorption for 2.5 mM (dark green), 1.3 mM (light green), 0.8 mM (blue), 0.6 mM (mangan), 0.4 mM (orange), and 0.2 mM (red) as a function of time in seconds. In the top right corner is a graph showing the fitting profile of an Avrami fitting to the 2.5 mM curve. In the Avrami plot the y-axis is normalized absorption and the x-axis is time in seconds. Data from the fitting for all concentrations is shown in Table 3.

Figure 4 show, that homogeneous nucleation follows a s-formed curve. The 2.5 mM concentration show the shortest induction time, the steepest slope, and the highest absorption maximum. The trend for all the lower concentrations are longer induction time, lower slope, and a lower absorption maximum. The 0.2 mM sample is the only con-

centration which has no homogeneous nucleation in the duration of the experiment. The trend is as expected since these phenomena are diffusion controlled[1] and therefore directly related to the concentration. To make a mathematical foundation for the data analysis, an Avrami fitting is used[7]. The results from the Avrami analysis can be seen in Table 3.

**Table 3** Avrami data

| | Conc. [mM] | k | n | i [s] |
|---|---|---|---|---|
| | 2.5 | 0.0089 | 2.36 | 94.3 |
| | 2.5 *avg.* | 0.0092 | 1.65 | 95.3 |
| | 1.3 | 0.0091 | 1.31 | 90.6 |
| | 1.3 *avg.* | 0.0093 | 1.35 | 92.0 |
| | 0.8 | 0.0065 | 1.23 | 168 |
| | 0.8 *avg.* | 0.0055 | 1.44 | 218 |
| | 0.6 | 0.0047 | 1.31 | 291 |
| | 0.6 *avg.* | 0.0040 | 1.33 | 387 |
| | 0.4 | 0.0035 | 1.31 | 323 |
| | 0.4 *avg.* | 0.0043 | 1.54 | 265 |

Mathematical analysis of the UV-Vis absorption curves shown in Figure 4 and averages from multiple identical experiments. Conc. is the $CaCO_3$ concentration in mM. k is the rate constant, n, is the order of reaction, and i, is the induction time.

The first column in Table 3 show the color corresponding to the UV-Vis figure. The second column show the concentration of each sample. The third, fourth, and fifth column show the three fitted Avrami parameters, the rate constant, k, the order of reaction, n, and the induction time, i. The average values have been calculated for: 2.5 mM, 3 experiments; 1.3 mM, 4 experiments; 0.8 mM, 5 experiments; 0.6 mM, 3 experiments; and 0.4 mM, 2 experiments.

The Avrami parameters shows that the rate constant decrease with decreasing concentration. That was as expected due to it being diffusion controlled, since there was no surface present and no limiting steps in mixing the solutions. In contrast the average order of reaction, for each concentration, is stable and only varies between 1.33 and 1.65. The similar order of reaction between the different concentrations suggest that nucleation and growth mechanisms are similar in the majority of the experiments. To explain the approximately order of reaction to be 1.5, equation 3 is used with three-dimensional growth, diffusion controlled as the limiting factor and that there is nucleation sites present.

$$1.5 = 3 \cdot 0.5 + 0 \tag{4}$$

The presence of nucleation sites could explain why there is homogeneous nucleation in the flow cell experiment.

The induction time increases with decreasing concentration. The induction time can provide a window in which pure heterogeneous nucleation experiments can be conducted. The discrepancy between the shown experiment and the average, indicates an instability at low concentrations.

The flow time between the mixer and the substrate in the flow cell setup is 60 seconds. The mixing in the flow cell was not identical to the mixing in the UV-Vis experiment, therefore, was the actual induction time not the same, though the trend to increase time with reducing concentration remains. At 0.8 mM the measured induction time is more than double the flow time and is therefore chosen as the best candidate for the flow cell setup.

### Flow cell

At 0.8 mM $CaCO_3$ there should be no homogeneous nucleation and pure heterogeneous nucleation was expected.

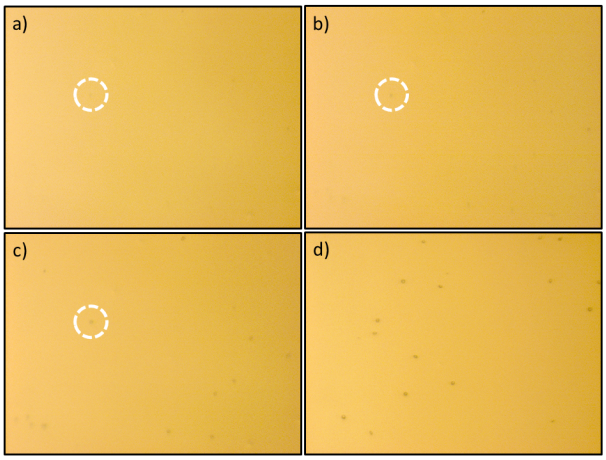


**Fig. 5** Optical microscopy at 3 ml/min, 0.8 mM on a carboxyl-functionalized gold substrate. a) is after 210 s, b) is after 352 s, and c) is after 581 s. a), b), and c) are all taken in the same location, showing signs of steady nuclear growth over time. d) is taken after 613 s in a different location showing nucleation forming uniformly across the substrate.

Figure 5 show a pure heterogeneous nucleation, because there are no crystals floating in the solution and there is growth over time. From picture a trough b to c there is a steady growth of the nuclei, highlighted by the one inside the white circle. Picture d is taken in another location showing heterogeneous nucleation forming uniformly across the surface. The result is exactly what was expected.

To study the nucleation behavior at 0.8 mM concentration, an amin-functionalized substrate was used. The interfacial free energy between this substrate and $CaCO_3$ is different. It was expected to see pure heterogeneous nucleation though at a different nucleation rate than the

carboxyl-functionalized substrate. The difference in nucleation rate was expected based on the results by Hamm *et al.*[5]. Here they found that the surface charge effects the nucleation rate.

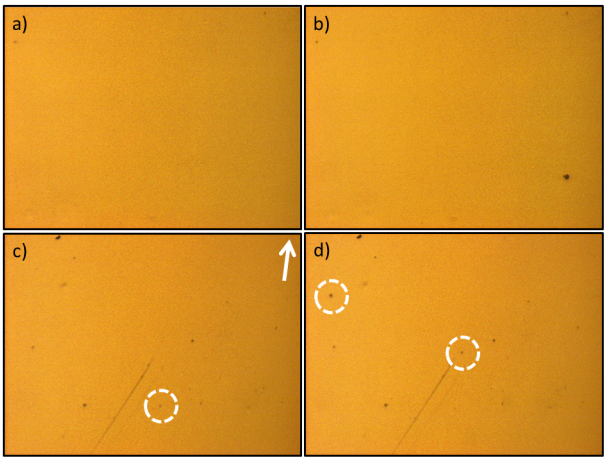


**Fig. 6** Optical microscopy at 3 ml/min, 0.8 mM on an amin-functionalized gold substrate. Here a) and b) are from the same experiment, and c) and d) are from a second experiment. a) is after 251 s and b) is after 905 s. The impurity in the bottom right grows but no new nucleations occur. c) is after 324 s and d) is after 326 s. The white circles indicate homogeneous nucleation. The white circle in c) move up to according to the arrow shown ending in the right circle in d).

The top of Figure 6 shows no nucleation at 0.8 mM concentration while the bottom show homogeneous nucleation. All three experiments at 0.8 mM should yield similar results. The inconsistency in whether homogeneous nucleation was produced can be due to external disruption e.g. crystals from previous experiment. The flow cell is a closed system and the setup was washed with water and acid before each experiment to reduce unwanted influence. However, it is found that the change in substrate affects the heterogeneous nucleation. Extreme care should be taken when cleaning the flow cell system between experiments, to prevent any residue to build up. More research is needed to develop a better understanding of the system and obtain consistent results.

## 4 Conclusion

These studies show that AAS and UV-Vis provides knowledge of induction time and homogeneous nucleation's impact on concentration. If homogeneous nucleation is present during a flow cell experiment the initial specific saturation index should not be reported, since it will not be representative for the SI in the actual reaction. Conducting the experiment at a concentration range of 0.7 mM – 0.9 mM are proposed as a suitable range for pure heterogeneous nucleation. Once heterogeneous nucleation is obtained further research in inhibiting and promoting substrates could be conducted. Discovering how substrates interact with $CaCO_3$ during heterogeneous nucleation could prove significant in the development of carbon sequestration approaches and further our understanding of biomineralization processes.

## 5 Acknowledgements

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