The role of zinc ions in calcium oxalate monohydrate crystallization

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Abstract

Calcium oxalate monohydrate is a common component of kidney stones and understanding its formation is important. In this work various organic molecules are investigated in combination with zinc ions. Unlike previous studies, this work looked at the impacts at physiological pHs (6-8) and temperature (37 °C). It was found that the number of carboxylate and hydroxyl groups was critical in determining the degree of inhibition. This inhibition was found to be mainly due to the ability of the organics to chelate with calcium ions. Zinc ions were found to chelate with oxalate ions leading to lower nucleation rates, but this is not strictly inhibition but a lowering of the supersaturation due to lowering of the activity of the oxalate. Organics have variable impact depending on whether the zinc ions or calcium ions preferentially chelate with the organic.

Keywords: A1 Biocrystallization; A1 impurities; A2 Growth from solutions; B1 Calcium compounds, B1 Organic compounds

1.0 Introduction

Crystallization is a simple but fascinating process that has been utilised as a purification technique in many industrial processes.1 This process may be modified via the addition of impurities leading to changes in the crystallization pathway (nucleation, growth and/or aggregative processes)2,3. Biomineralization4 (the ability of living organisms to mediate mineral formation) is a hugely important and widespread process that can be seen in everyday materials such as bone,5 teeth,5 skeletal tissues in sponges,6 crustaceans,7 egg shells8, mollusc shells9 and calcium oxalate is a known biomineral that contributes to urolithiasis10. Calcium oxalate makes up approximately 70% of kidney stones while other components are calcium phosphate ~8.9%, uric acid ~10.1%, struvite ~9.3%, various organic materials ~0.8% and cystine ~0.7%.11 Urolithiasis currently affects ~10% of the world’s population per generation12 with an expected increase in the number of carriers for the foreseeable future.

Within kidney stones two calcium oxalate hydrate forms are most common, referred to as whewellite and weddellite. The most thermodynamically stable phase, whewellite, is also known as calcium oxalate monohydrate (COM, CaC₂O₄·H₂O). It has a monoclinic structure. Weddellite is the metastable form at room temperature, also known as calcium oxalate dihydrate (COD, CaC₂O₄·2H₂O), and is tetragonal in structure. A third hydrate form also exists, calcium oxalate trihydrate (COT,
CaC$_2$O$_4$·3H$_2$O) however due to its thermodynamic instability, it is rarely observed within urinary media. COM is often found as a combination of twins; either contact or penetration (see Figure 1). The single crystals of COM are bound by (100), (010), (021), (021), (12$ar{1}$) and (12$ar{1}$) faces (Figure 1a).$^{13}$ The planes for contact and penetration twins are (100) or ($ar{1}$01) shown in Figures 1b&c.$^{13}$

![Figure 1: Calcium oxalate monohydrate habit (a) single crystal and twins bound by labelled faces and directions according to Tazzoli,$^{13}$ (b) penetration twin, (c) contact twin](image_url)

The formation of kidney stones has been heavily studied with multiple factors linked to its cause including dietary, environmental, genetic and urinary infection factors.$^{14}$ The pathogenesis of kidney stones is a multistep process involving nucleation, crystal growth, crystal aggregation and finally adhesion to the lining of the urinary tract. Previous work regarding singly charged alkali metals (specifically Cs$^+$, K$^+$, Na$^+$ and Li$^+$) as growth modifiers found a decrease in crystal growth rates (60 – 30%) and distinct morphology changes depending on the ion present.$^{15}$ Another published study determined that low molecular weight multi-charged metals (Fe$^{3+}$, Cr$^{3+}$ and Al$^{3+}$) had a significant inhibiting ability in the presence of naturally occurring citric acid.$^{16}$ Growth rates of COM were reduced in the presence of metal ions but most prominent at equal ratio concentration of ion and citric acid, with little influence on morphology. The investigation determined that the greatest impact on crystal growth occurred when a mixture of organic-ion complex, free organic molecule and metal ion was present in the COM producing solution. However, the effects varied across the series of metal ions tested, ranging from 80-20% reduction.$^{16}$ It has been suggested that the zinc ion is a moderately good inhibitor within aqueous medium of both calcium oxalate and phosphate crystallization.$^{17}$ The ion has been revealed to have a weak tendency for incorporation within calcium oxalate minerals with preferred formation of a zinc oxalate species.$^{18}$

One of the most important organic molecules studied in urolithiasis is citric acid,$^{19,20}$ due to the natural occurrence within urinary medium. Similar small compounds that are also present within the human body have also been studied, these being mainly amino acids, with varied results.$^{15,21-23}$ The habit of
a crystal can be altered due to the adsorption of these organics on specific faces, for example citric acid was shown to alter the growth in the c direction producing crystal with an apparent round edge.\textsuperscript{24}

In this work, calcium oxalate stone formation experiments in the presence of inhibitors were compared to a known inhibitor, citric acid. Based on the characteristics of citric acid, 8 other organics were chosen. As well as studying the impact of the chosen organic additives on the calcium oxalate crystal system this work also explored the addition of zinc ions. The ion was chosen due to the relatively high content of zinc ions within kidney stone producers compared to non-sufferers,\textsuperscript{25} leading to the suggestion it may promote the growth of calcium oxalate stones. Many studies focus on single growth modifiers; however, few involve the impact of two additives. How the inhibition or growth effects of additives may be promoted or hindered with competition when two additives are present in the calcium oxalate supersaturated solution was of interest. One issue with many previous studies is the temperature or pH range of the experimental conditions\textsuperscript{26-28}. While some do the crystallization within an appropriate pH range, the temperature is sometimes as high as 60 °C.\textsuperscript{26} As kidney stones are formed in the body, the experiments within this study were carried out at physiological conditions, namely at a constant temperature 37 °C and pH 6-8.\textsuperscript{29,30} In order to properly compare and investigate the impact of additives on the formation of calcium oxalate particles the concentration of modifiers relative to that of calcium and oxalate ions was kept constant.

2.0 Materials and Methods

2.1 Calcium Oxalate Crystallisation

A calcium chloride (0.02 M), organic acid (0.02 M) and sodium oxalate stock solution (0.02 M) was prepared in ultrapure water (100 mL). Stock solutions were altered to pH 7 using 0.1 – 1.0 M sodium hydroxide or hydrochloric acid. Clean glass disks (0.5 cm radius) were placed into 20 mL glass vials to act as a transferable surface used for crystal growth. The total crystallization volume was kept constant at 20 mL with the addition of ultrapure water where necessary. The calcium chloride solution (final concentration, 0.50 mM) was equilibrated in a water bath at 37 °C for 1 hour before addition of a stoichiometric amount of sodium oxalate stock solution. When inhibitors were present, addition of the organic additive solution to achieve the desired concentration (20, 10, 1 and 0.1 mM for all organic species) was added to the glass vial before the solution was equilibrated in the water bath for 1 hour. The vial was left in a water bath at 37 °C for 68-76 hours before removing the glass disk, rinsed with ultrapure water and dried, finally it was placed into a labelled sample well. The procedure was run in triplicates and at pH 6 and pH 8 in the presence of zinc chloride with a ratio of 1:1 to the additive
concentration. Each sample was analysed by optical microscopy, Raman spectroscopy and scanning electron microscopy. The organics investigated in this work are listed in Table 1. For the case where sufficient particles could be counted, the microscopy images were also analysed by measuring the length, width and depth of well-separated particles (using ImageJ) the results of which can be found in the supplementary information (STable 2).

<table>
<thead>
<tr>
<th>Name (Abbreviation)</th>
<th>Molecular Weight (g/mol)</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric Acid (CA) C₆O₇H₈</td>
<td>192.12</td>
<td><img src="image_url" alt="Citric Acid" /></td>
</tr>
<tr>
<td>L – Asparagine (ASP) C₄O₃N₂H₈</td>
<td>132.12</td>
<td><img src="image_url" alt="Asparagine" /></td>
</tr>
<tr>
<td>L – Aspartic Acid (AA) C₄O₄NH₇</td>
<td>133.11</td>
<td><img src="image_url" alt="Aspartic Acid" /></td>
</tr>
<tr>
<td>Cis - Maleic Acid (MA) C₄O₄H₄</td>
<td>116.072</td>
<td><img src="image_url" alt="Maleic Acid" /></td>
</tr>
<tr>
<td>L - Tartaric Acid (TA) C₄O₄H₆</td>
<td>150.09</td>
<td><img src="image_url" alt="Tartaric Acid" /></td>
</tr>
<tr>
<td>Trimesic Acid (TMA) C₉O₆H₆</td>
<td>210.14</td>
<td><img src="image_url" alt="Trimesic Acid" /></td>
</tr>
<tr>
<td>Trimethyl Citrate (TMC) C₉O₇H₁₄</td>
<td>276.28</td>
<td><img src="image_url" alt="Trimethyl Citrate" /></td>
</tr>
<tr>
<td>Valeric Acid (VA) C₅O₂H₁₀</td>
<td>102.13</td>
<td><img src="image_url" alt="Valeric Acid" /></td>
</tr>
</tbody>
</table>

### 2.2 Scanning Electron Microscopy (SEM)

The glass slides from samples were placed onto a carbon covered SEM stub and a thin carbon paint layer was applied around the edge to minimise charging effects. Samples were sputter coated with platinum with a thickness of 2 – 20 nm and imaged at Curtin University Electron Microscope Facility using a Zeiss Evo 40XVP SEM with either secondary or back-scattered detectors.
2.3 Dynamic Light Scattering (DLS)

Particle counts were measured using a Malvern Zetasizer Nano-ZS in a plastic cuvette, with a fixed experiment integration time of 10 seconds producing ten readings every three minutes. Measurements were recorded using a DPSS laser with an excitation wavelength 532 nm (green) at 37 °C with the NIBS® technique. Calcium chloride dihydrate (0.8 mM, 5 mL) and sodium oxalate (0.8 mM, 5 mL) solutions were adjusted to pH 7 (using 0.1 – 1.0 M sodium hydroxide and hydrochloric acid), filtered into an enclosed Teflon beaker and constantly stirred (400 rpm) at 37 °C. An aliquot (3 mL) was taken every three minutes, added to a plastic cuvette, placed inside the Zetasizer instrument and analysis was performed recording kilocounts per second (kcp). This represents the average scattering intensity, such that if 1,000,000 photons are recorded over 100 seconds then the Zetasizer records 10 kcp (1,000,000 photons/100 sec). Essentially, high nucleation rates equal more particles present to scatter the light, which produces higher kcp values and vice versa. The procedure was run in triplicate, recorded over 30 minutes with a total of 10 measurements. DLS measurements were also undertaken at pH 6 & 8. The experiment was repeated with additive concentrations 0.8 mM and in the presence of zinc chloride.

2.4 Zeta potential

Zeta potential was measured using a Malvern Zetasizer Nano-ZS in a folded disposable zeta cell. Measurements were recorded at 37 °C with the M3 – PALS® technique (Phase Analysis Light Scattering). Calcium chloride dihydrate (0.8 mM, 5 mL) and sodium oxalate (0.8 mM, 5 mL) solutions were adjusted to pH 7 (using 0.1 – 1.0 M sodium hydroxide and hydrochloric acid) filtered into an enclosed Teflon beaker and constantly stirred (400 rpm) at 37 °C for 30 minutes. After this time, a small sample (~2 mL) was pipetted into the zeta cell and a measurement was recorded. The procedure was repeated in the presence either of organic additive and/or zinc chloride.

3.0 Results and Discussion

3.1 Morphology

The crystal morphology is determined by the relative growth rates of its respective faces. The supersaturation ratio value for these experiments was estimated at ~10, using \( S = \frac{c}{c_e} \) where \( c \) is the concentration and \( c_e \) is the concentration at the equilibrium solubility using the equilibrium solubility product at 37 °C as \( 2.40 \times 10^{-9} \). It is understood that this will be an upper limit to the supersaturation.
since any chelation or complexation that occurs will decrease the activity of the ions. In the absence of any additives (see Figure 2), the most thermodynamically stable form of calcium oxalate, COM, was observed to form from this supersaturated solution. For COM the most common and largest faces observed were the (100) and (010) faces with less prominent faces (12\overline{1}), (02\overline{1}), (1\overline{2}\overline{1}) and (0\overline{2}1) also present along with the usual contact twinning being observed. Where possible, SEM images were analysed and size information obtained (see Supplementary information, STable 2).

![SEM micrographs of calcium oxalate formed in the absence of impurities](image)

**Figure 2:** SEM micrographs of calcium oxalate formed in the absence of impurities

The presence of ASP had very little impact at the pH studied here (pH 7) but the presence of TMA formed much smaller particles (Figure 3a and b respectively). The morphology of COM grown in the presence of CA resulted in more single crystal COM particles as opposed to twinned particles (Figure 3c). The effect of CA has been seen before by Ouyang\(^{24}\), in which rhombus like crystals were produced. Other studies have found significant rounding in the presence of CA, however, those studies were performed at different temperatures and with a large amount of background electrolyte\(^{26}\) and so cannot be directly compared. Unlike CA, the presence of MA produced crystals that appeared longer (Figure 3d), but also smaller than the control.
Aspartic acid (Figure 4a), AA, resulted in much smaller particles, while TMC (Figure 4d) showed aggregation of particles but little changes to the morphology. The presence of VA (Figure 4b) during crystallization appears to have little impact. TA (Figure 4c), appears to form small COM single crystals. Previous results found a mixture of COM and COD was produced\textsuperscript{32}, although the group observed COD at pH 4 which is already known to produce a mixture\textsuperscript{31}.
Figure 4. SEM micrographs of calcium oxalate (0.50 mM) crystal morphology in the presence of additives: (a) AA at 20 mM, (b) VA at 20 mM, (c) TA at 20 mM, (d) TMC at 20mM.

The greatest impact on morphology can be seen in the presence of zinc ions. The particle size does not alter significantly (see STable 2) and twinning was still observed (Figure 5) but the particles were much thinner in the $a$ direction. The reduction in nucleation may be caused by the zinc ion complexing with the oxalate ion changing the supersaturation state (this will be discussed later). The results are in line with Rao’s findings\(^\text{17}\), that zinc heavily inhibits the formation of calcium oxalate.
Organics + Zinc

In the following results the organic and zinc ions are present at the same concentration (1:1), 20mM. In the presence of TMA (Figure 6a) the impact of zinc almost disappears, forming particles very similar to the control but somewhat smaller. A similar effect was observed when TA and zinc ions are present (Figure 6b). Single crystals appeared with CA+Zn, (Figure 6c) and the crystals appeared less than symmetrical when small in size. MA+Zn appeared to form particles that were slightly longer than in the absence of zinc (Figure 6d). Measurements of the crystals showed the MA+Zn crystals were slightly larger on average than the MA only case with an average length of 5.26 µm and 5.00 µm respectively (STable 2).
Figure 6. SEM micrographs of calcium oxalate (0.50 mM) crystal morphology in the presence of additives: (a) TMA+Zn at 20 mM, (b) TA+Zn at 20 mM, (c) CA+Zn at 20 mM, (d) MA+Zn at 20 mM.

The presence of ASP with zinc ions showed similar results to zinc alone (Figure 7a) but thicker crystals were formed. The presence of TMC (Figure 7b) resulted in changes to the aggregation state rather than the morphology when zinc ions were also present.
AA+Zn appeared to form smaller particles that were thicker than those when zinc ions alone are present (compare Figure 7c to Figure 5 and the magnifications used). VA+Zn (Figure 7d) showed similar particles to the case where AA+Zn is present but with larger COM crystals formed (compare Figure 7c to 7d). In all cases the presence of zinc appears to lessen the twinning normally observed for COM.

The impact of these organics when zinc ions are present depends on the competition between the organics to complex with the zinc and calcium ions. For ASP, AA, and CA the complexation constants with zinc ions are slightly higher or similar than zinc ion complexation to the oxalate ion (see STable 1.). These are the samples that show reduced numbers and often smaller COM crystals but with the same features as those with zinc ions alone. For these organics the zinc complexed to the organic and to oxalate ions, leading to small numbers of particles with little twinning observed. Based on the morphology of the COM obtained it would be predicted that TMC would also have high complexation constants with zinc ions, however, no data on this molecule was found. For the organics TMA, TA and
MA what is observed is a morphology reminiscent of the control. Thus, in this case these organics must complex the zinc ions much stronger than the complexation of oxalate ions to zinc ions (thus the oxalate ions are free to react again) and particles like the control are formed. However, the complexation data does not show this trend for MA and TA. Thus, it is unclear why control-like particles of COM are formed in this instance. These molecules may not impact on supersaturation (through complexation) but impact on the surface free energy (and therefore nucleation) or during the growth phase through adsorption effects. Thus, the impact of these organics on nucleation rate was determined through DLS measurements.

3.3 Nucleation Rate
The expected behaviour is shown by the control data where the nucleation of calcium oxalate in the absence of any additives at pH 7 slowly increases to a plateau over time, ~20 minutes (seen in Figure 8). The plateau in particle counts, in essence, shows that nucleation has ceased (but crystal growth can continue).

All organic additives were found to inhibit calcium oxalate nucleation rate, with certain compounds found to be more potent than others. The nucleation rate was reduced most significantly in the presence of CA, TA and TMA (~80% reduction). In comparison, the presence of AA inhibited nucleation of COM by less than ~20%. The organics CA and AA display results consistent with published literature.20, 21 The weaker acid groups ASP, MA and VA all display weak inhibition on the nucleation rate of calcium oxalate monohydrate with a reduction of ~40%. The largest molecule of the tested organic series, TMC, inhibited nucleation of COM the least, with a ~10% reduction in particle number. From the results presented here, changing the functional groups appears to have the greatest impact on nucleation rate. In terms of structure, the number of carboxylic acid groups can be seen to impact the nucleation rate; the three most potent inhibitors all display roughly the same reduction in nucleation rate and these three compounds each contain the highest number of hydroxy and carboxylic acid groups.

The results can be further explained by looking at Equation 133, which expresses the rate of nucleation, \( J \), as number of nuclei formed per unit time per unit volume.

\[
J = Ae^\left(\frac{\Delta G}{kT}\right) = Ae\left[\frac{16\pi\gamma^2v^2}{3k^2T^3(lnS)^2}\right]
\]

Equation 1

where \( \Delta G \) is the Gibbs Free Energy to form a three-dimensional nucleus, supersaturation \( (S) \) and interfacial free energy \( (\gamma) \), \( T \) the temperature, \( k \) is the Boltzmann constant, \( v \) is the molecular volume and \( A \) is the pre-exponential factor. The nucleation experiments are recorded at constant temperature
and $A$ is assumed constant, therefore, the main variables that determine rate of nucleation are supersaturation and interfacial free energy. In order to reduce the nucleation rate either the organic molecules increase the interfacial free energy or the supersaturation might be altering due to complexation and a reduction in the activity of the free ions. As discussed previously, ASP, AA, and CA are expected to complex with Ca or Zn ions to a similar extent as the oxalate. These molecules therefore impact the supersaturation, $S$. Interestingly, despite CA and AA having similar complexation constants for Ca ions, the nucleation inhibition is greatest for CA. This suggests that the carboxylic acid groups are important and may be impacting through cooperative effects. This may also explain the lower nucleation inhibition by MA despite having two carboxylic acids present. Their geometry in the cis configuration may not actually be optimal for interaction with COM. By contrast, TA is not expected to complex significantly. Despite this, tartaric acid shows significant impact on nucleation. Thus, for TA the impact is assumed to be through changes in the surface free energy. TMA is seen to have a significant impact on nucleation – however, data on complexation is not available. For this reason, it is unclear for this organic whether the impact is through supersaturation effects or other effects. The remaining organics do not significantly impact nucleation.

**Figure 8.** DLS data of (A) control and in the presence of organic additives AA, CA, TA and TMA and (B) in the presence of ASP, MA, TMC and VA at 1:1 concentration ratio (calcium oxalate:organic).
Zinc ions were introduced into the calcium oxalate system and nucleation data is shown in Figure 9. At both high and low concentration there is a strong inhibition in particle counts with the addition of zinc ions into the system, which was previously observed in the SEM images. Overall, Zn$^{2+}$ (at 20mM) produced the greatest reduction in nucleation rate out of all the additives tested (~90% nucleation rate reduction).

![Figure 9](image)

**Figure 9.** DLS data of control and in the presence of Zinc chloride concentration ratios 1:1 and 1:20 (zinc ion:calcium oxalate).

Complexation of the zinc to oxalate ion was found to be the main determinant of this behaviour (see supplementary information for complexation data), thus, this is also a supersaturation effect. When organics CA, TA and possibly TMA are present with zinc ions, the number of particles formed is within the error margin of the control (without zinc) system. While the nucleation when AA is present appears similar to that of zinc ions alone. This suggests that the organics CA, TA and possibly TMA interfere with the interaction of zinc with oxalate while AA does not. When the organics TMC, MA and VA are present the nucleation rate is similar to that of the control (without zinc). For ASP the nucleation rate is similar to that of zinc ions alone. In the case of TMC, MA and VA in the presence of zinc ions, this is most likely due to the zinc ions complexing more strongly with these organic molecules than zinc ions with the oxalate. In the case of ASP+Zn it would be assumed that the complexation of zinc ions with oxalate would be stronger than zinc ions with ASP but the complexation constants are actually similar. Similarly, it would be expected that AA would have a lower complexation constant with zinc ions than oxalate, however this is not found (see supplementary information, STable 1).
Figure 10. DLS data of control and in the presence of zinc chloride + organic additives; AA, TMC, VA, MA, ASP, CA, TA and TMA at 20:1:1 concentration ratio (calcium oxalate: organic: zinc)

3.5 Surface Charge

In aqueous solution the calcium oxalate crystal surface was found to be negatively charged and this suggests that the surface has a small excess of negative ions on the surface (most likely oxalate ions) due to the low ionic strength background of this sample. The zeta potential, however, also incorporates the ions within the diffuse double layer and so can be impacted by the ionic strength and potential determining ions. Zeta potential was used to determine the impact of additives alone and how it changes in the presence of zinc ions and organic additives. In general, a large zeta potential value (±30 mV) carried by particles in suspension implies an electrically stabilised system in which particles will repel each other and little coagulation occurs. Alternatively, a low zeta potential value implies little repulsion between particles and possible aggregation.

All zeta potential data is shown in Figure 11. Out of 8 organics only 2 observed any large change in zeta potential in the absence of zinc ions and this change did not necessarily relate to inhibition. The greatest change in zeta potential is shown by TMA and TMC with an ~55% decrease in the absolute value of the zeta potential. This change is possibly due to the size of these organic molecules screening much of the surface charge. The lack of significant change in the zeta potential in the presence of the
other organics could be due to the fact that if adsorption of the organic molecule takes place it may still result in a similar negative charge on the surface.

The presence of Zn ions alone increases (makes more positive) the zeta potential significantly (Figure 11, dashed). The change in zeta potential can be due to both surface adsorption of the positive zinc ions but is also due to ionic strength changes. In all cases the zeta potential of COM+Zn+Organic is more positive than COM+Zn (except for VA), suggesting that the presence of the organics with zinc ions has some impact on the double layer or the surface charge. The largest alteration takes place with AA and ASP, increasing the zeta potential to positive values when in the presence of zinc ions. Both compounds possess amine groups, which may mean the positive end of zwitterion is extending into solution. A smaller increase in zeta potential values is seen when CA, MA and TA are present. Little change is observed in the presence of VA and zinc ions.

**Figure 11.** Zeta potential data of control and in the presence of organics L-Aspartic Acid, L-Asparagine, Citric Acid, Maleic Acid, Tartaric Acid, Trimesic Acid, Trimethyl Citrate and Valeric Acid without (solid) and with zinc (dashed) 20:1:1 concentration ratio (calcium oxalate:organic:zinc).

A small zeta potential would correlate with more aggregation possible. Thus, the presence of zinc ions in the system would generally promote COM aggregation.
4.0 Conclusions

The formation of COM stones biologically is complex and recent literature on the importance of other ions such as zinc require some data to be collected. In this investigation, we show that zinc ions can effectively compete with calcium ions for the oxalate anion and lower the supersaturation of the system. The morphology of the COM formed in the presence of zinc ions is significantly altered, forming thin particles that still show twinning. However, this is an initial study and further work needs to be conducted at a physiological zinc ion level and in a more realistic medium.

The presence of organics can lead to the zinc ions chelating with the organic in preference to the oxalate, this can lead to particles similar to the control COM. For the other organics, a morphology similar to that in the presence of the zinc ions is observed.

These same impacts can be seen in the nucleation rate of the COM. When the organic complexes calcium ions, a significant reduction in nucleation rate is observed. Similarly, nucleation is reduced in the presence of zinc ions due to chelation of zinc ions with oxalate. On the other hand, when zinc ions and organic are present any chelation between them will lead to a nucleation rate closer to the control nucleation rate. The presence of TA was interesting in that significant chelation was not expected and yet significant nucleation reduction was observed. TA appears promising as molecule of interest for further studies.

Finally, the zeta potential for all the cases studied here is low (≤|12mV|) suggesting all of these situations would lead to aggregation of particles. Having said that, the presence of zinc ions leads to even smaller absolute zeta potential values meaning aggregation would be promoted in the presence of zinc ions. The quiescent conditions used here in the morphology experiments, however, are not ideal for studying these effects and further work is required.

5.0 Acknowledgements

We would like to acknowledge the John de Laeter Centre, Curtin University for use of the electron microscope facility and XRD instrument (ARC LE130100053, LE140100150, LE0775551). We would also like to acknowledge the Australian government and Curtin University for Matthew Boon’s Australian postgraduate award. Lastly, we would like to thank the Curtin Institute for Functional Materials and Interfaces (CIFMI) for supporting analytical costs for this project and supporting Timothy Barker.
Supplementary information:

The following information can be found:

- Complexation/chelation reaction scheme (SScheme 1)
- Speciation curves for oxalate, calcium, and zinc interactions (SFig1+2+3)
- Complexation values for both calcium and zinc and the organic compound (STable 1)
- Measurements of CaOx crystals in the presence of additives (STable 2)

6.0 References