

Tried Methods of Nanosizing and stabilizing Struvite in a solution

Information in this document is not scientifically validated or reviewed.

Introduction and background

This document describes various attempts at synthesizing and stabilizing nano struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) particles. Synthesis has been attempted using both "top-down" and "bottom-up" approaches, which will be further described.

Stabilization has been explored by using different organic acid anions to coat struvite particles and create a negatively charged surface. This method has proven effective with the somewhat similar phosphate salt, hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$), where citrate was used to create nano hydroxyapatite (nHAP) particles that remain stable in solution. It is hypothesized that citrate binds with the calcium atoms in hydroxyapatite, thereby forming a negatively charged coating.

The idea was to investigate whether the same approach could be applied to struvite, as magnesium, one of the components of struvite, is somewhat similar to calcium, both being divalent positively charged ions.

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Top-Down Method

The "Top-Down" method described in (Szameitat et al. 2021) to create hydroxyapatite nanoparticles has been emulated, with a few tweaks, in the hopes of creating stable nano-struvite particles.

Granulate Crushing to Micro Scale (100-250 μm)

A struvite granulate with an approximate purity of 97% (appendix: ICP Analysis results and calculations) with a size between 2-3 mm, was crushed in portions of approximately 50g by placing it into 250 mL plastic containers with seven 15 mm \O zirconium oxide (ZrO_2) spheres. The mixture was shaken for 9 minutes using a paint shaker. The crushed struvite granulate was then sieved, and the portion with a size between 100-250 μm was set aside for further size reduction.

Attempt at Size Reduction to Nano Scale

In an attempt to produce nanoparticles, the micronized struvite (32.5 g), ammonium citrate at pH 9 (0.5 M, 50 mL), and 0.5 mm ZrO_2 spheres (30 mL) were added into a 125 mL stainless steel grinding jar and placed in the Emax, which shakes the grinding jars at high velocity. Five 5-minute rounds at 1500 Hz were performed. Subsequently, 0.1 mm ZrO_2 spheres (30 mL) were added, and an additional 15 rounds were run at the same settings. The resulting struvite paste was diluted to a concentration of 27 g/L and an ammonium citrate pH 9 concentration of 50 mM. Afterward, the solution was sieved through 200 μm and 50 μm filters to separate the ZrO_2 spheres from the struvite solution.

Results:

The solution settled overnight into two phases. The top part was transparent, while the lower part formed a "fluffy" cloudy white precipitate layer (fig. 1). The solution was sonicated, but it settled in the same way after sonication.

DLS (Dynamic Light Scattering) analysis was conducted on the precipitate, in different dilutions of ammonium citrate, to estimate the particle size. However, no satisfactory analysis results could be obtained. This was likely due to either large particle size, sedimentation of particles, or particle aggregation.

The same procedure was repeated, with the final product diluted to an ammonium citrate pH 9 concentration of 100 mM instead of 50 mM. This did not change the outcome.

Analyzing the precipitate with an ordinary microscope at a 400x magnification showed aggregation of particles (fig. 2)

Furthermore, the same procedure was conducted at pH 5 and 7. The only observed change was a decrease in the thickness of the white bottom layer as the pH decreased. These results indicate that pH may affect either the size of the particles or their surface charge, making them more prone to aggregation at lower pH. However, this effect could also be due to some of the struvite dissolving at lower pH, reducing the proportion of crystallized struvite.

Figure 1: Product of the top down synthesis.

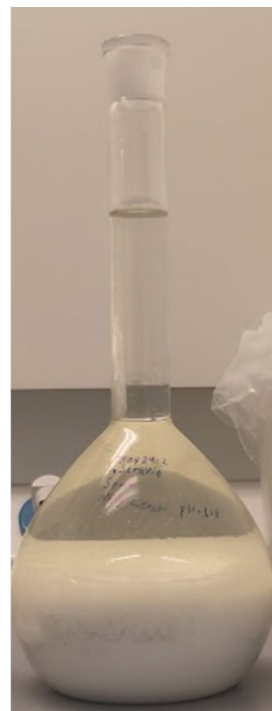
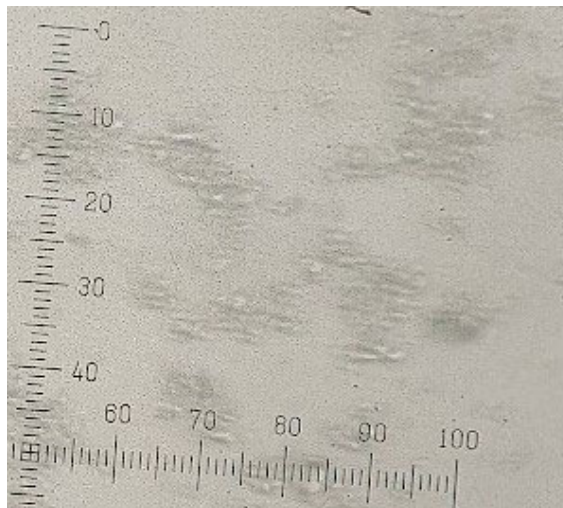


Figure 2: Aggregating Struvite particles created by top down synthesis. 1 length unit $\approx 1.8 \mu\text{m}$



Attempts at producing struvite nano particles via recrystallization

A solution containing 0.2 M struvite (2-3 mm granules) and 0.873 M acetic acid was prepared to dissolve the struvite. The resulting solution was filtered through Whatman 5 filter paper to remove impurities. The pH of the struvite-acetic acid solution (pH 4.37) was then adjusted up using various methods to recrystallize the struvite in as small particles as possible.

Tried recrystallization methods:

Increasing pH slowly

Recrystallization was done by increasing the pH slowly by adding an aqueous ammonia solution with a peristaltic pump, while respectively steering vigorously and sonicating. This procedure resulted in a relatively coarse precipitate. Crystallization was observed at pH 4.6. Using a similar technique a 0.2 M Struvite, 0.3 M Citric acid solution was increased in pH. The results were similar; however, crystallization was first observed at pH 6.

Batch/fast Recrystallization

Puring the struvite solution into an aqueous ammonia solution while respectively steering and sonicating, and reaching different pH levels, by adjusting the amount of ammonia was tried. This procedure resulted in precipitates with differing textures, some being more, and some being less coarse. However, none of the procedures yielded particles that could be analyzed effectively by DLS. It is unknow if the particles were either too large, not stable in solution or both.

Spray-synthesis

The acetic acid struvite solution was sprayed as a fine mist into an aqueous ammonia solution at different pH levels (9-12) while either sonicating or stirring at high speed. The most successful outcome using the misting technique was achieved with the following procedure: 150 ml of Milli-Q water was mixed with 10 ml of 30% aqueous ammonia solution. Into this solution, 5 ml of struvite-acetic acid solution (pH 4.37) was sprayed over five minutes using a 'Camag Laboratory Sprayer 022.6100' while sonicating the solution. The pH of the solution changed from 12 before spraying to 11.35 after spraying. This resulted in slowly settling particles that remained suspended in the solution, appearing as a gray mist even two hours after redistributing the particles by shaking the solution. However, the particles settled completely overnight. Samples taken from the upper part of the settling particles showed non-uniform crystals, ranging in size from indistinguishable to approximately 20 μm wide (fig. 3 and 4). A tendency for the crystals to aggregate was observed (fig. 5). Using the same technique, a lower concentration of the struvite solutions was sprayed into the ammonia solution. This did not lead to any changing results

Figure 3: Microscope photo of non-uniform Struvite crystals created by Spray synthesis. 1 length unit $\approx 1.8 \mu\text{m}$

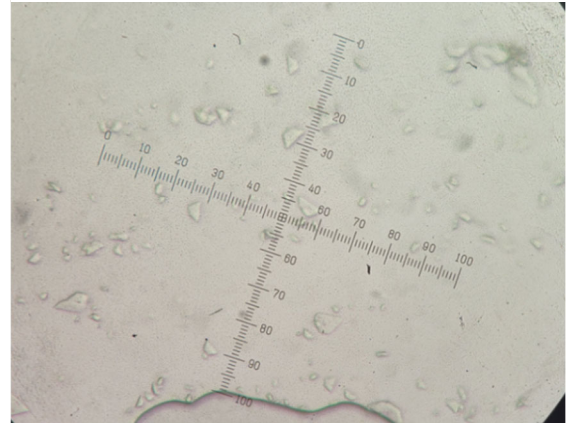


Figure 4: Microscope photo of non-uniform struvite crystals created by Spray synthesis. 1 length unit $\approx 0.8 \mu\text{m}$

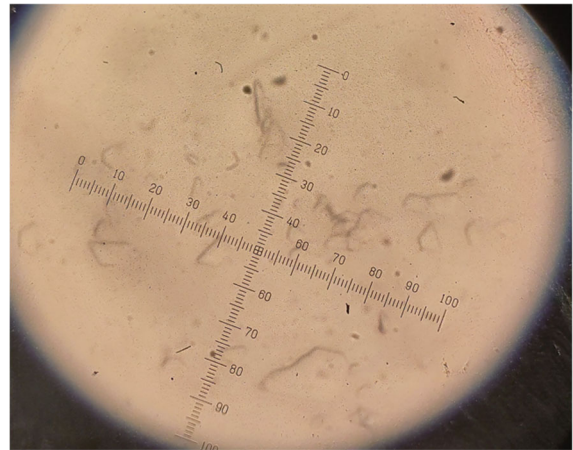


Figure 5: Microscope foto of Aggregated Struvite crystal, created by spray synthesis. Picture taken through a 400x microscope lens.



Attempts at producing struvite nano particles via bottom-up synthesis

Wet chemical precipitation

The following synthesis method was tried:

“wet chemical precipitation technique for the synthesis of struvite nano particles. 0.2 M ammonium dihydrogen phosphate (ADP) solution was taken and 0.2 M magnesium acetate solution was mixed drop wise manner in equal amount under constant stirring at room temperature.” - (Rathod et al. 2015),

Different synthesis approaches were tried due to the poor level of details in the synthesis protocol description in the original paper.

20 ml 0.2 M ammonium dihydrogen phosphate solution and 20 ml 0.2 M magnesium acetate solution of solution was added dropwise in equal amounts with a peristaltic pump, into a 30 ml of Milli-q water under heavy stirring. This created a clear solution with a pH of 5.24. DLS analysis of the solution did not show any indications of nanoparticles. Earlier experiments indicated that a higher pH was needed for formation of struvite crystals, therefore making it likely that the solution was clear, due to no formations of crystals.

A similar experiment was conducted, but instead of dripping the solutions into milli-Q water, the solutions was dripped into 110 ml of an aquas ammonia solution pH 11.1. This procedure resulted in a white precipitate that sank relatively quickly. DLS Analysis indicated no nanoparticles.

Bottom up, fast synthesis.

Inspired by a very successful procedure of synthesizing hydroxyapatite nanoparticles (appendix protocol 1), where a solution consisting of CaCl_2 and ammonium citrate (pH 9) is mixed with a KH_2PO_4 solution (pH 11) under vigorous stirring.

A synthesis was conducted using the previously mentioned protocol, substituting CaCl_2 with MgCl_2 and adjusting the molar ratios so that the ratio between Mg and PO_4 is equal, as in struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$). This resulted in a "fluffy" precipitate (Fig. 6), similar to the product of the top-down synthesis (Fig. 1). Microscope analysis of the precipitate revealed "soft-edged" particles and aggregation of particles at a microscopic level (Figs. 7-8). These particles were quite different from the sharper, crystal-like particles produced by the spray synthesis (Figs. 3-5). This difference could be attributed to a variation in crystal morphology; however, it may also be due to particle aggregation at the nanoscale, forming soft-edged aggregates that appear as particles under microscope observation.

It was investigated whether the concentration of ammonium citrate had an impact on the stabilization of the solution. To test this, 0.75 mL samples were taken from the solution after homogenization by shaking. These samples were mixed with 0.75 mL of ammonium

Figure 6: Product bottom up, fast synthesis method. 300mM Struvite, 100mM citrate.



citrate (pH 10.22) at the following concentrations: 0.25 M, 0.5 M, 1 M, and 2 M. The samples were sonicated and left to mature for a week. Stabilization did not occur, and no differences were observed between the samples.

Figure 7: Microscope photo. Product of bottom up, fast synthesis method. 1 length unit $\approx 1.8 \mu\text{m}$

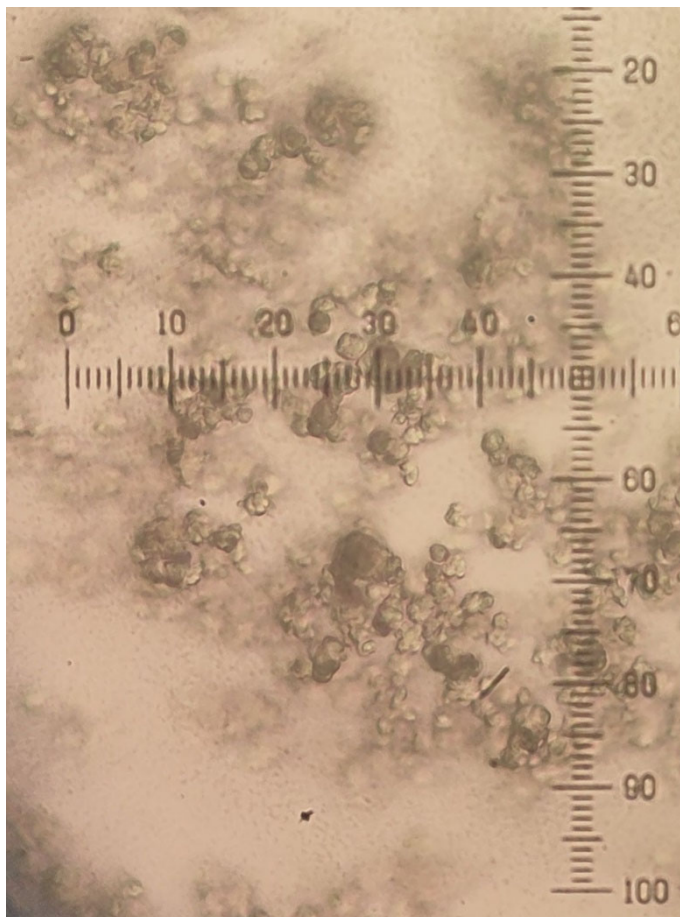
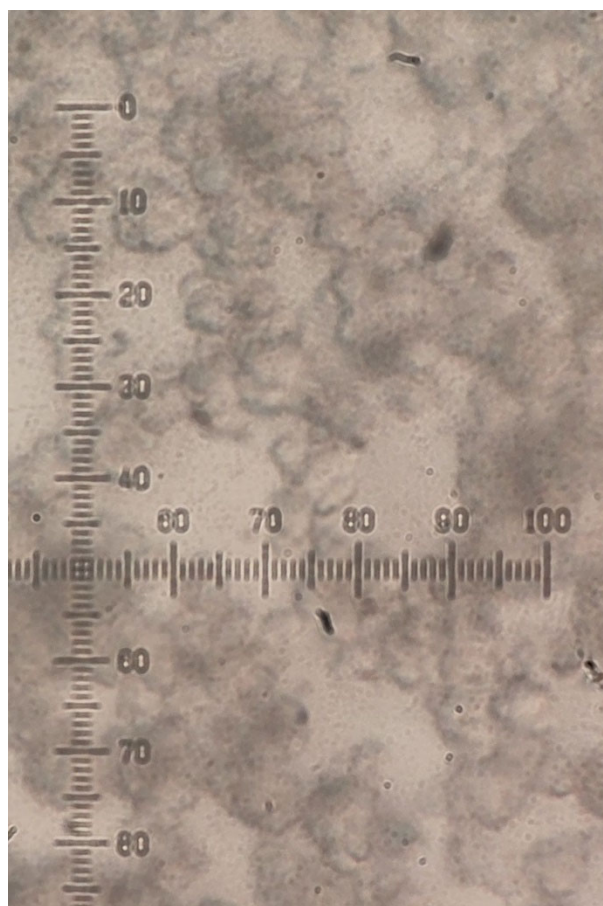


Figure 8: Microscope photo. Product of bottom up, fast synthesis method. 1 length unit $\approx 0.8 \mu\text{m}$



Utilizing maleate, malate and oxalate in bottom-up synthesis

The same bottom-up synthesis approaches were tried out, but utilizing different organic acid anions substituting citrate, in attempt to stabilize the synthesized particles.

The synthesis were conducted at pH 7.25 and 9.2, with different organic acid anions (maleate, malate and oxalate) in different concentrations (50, 100, 200, 250 mM). Utilizing Sodium hydroxide (NaOH) to substitute ammonia to adjust pH was also tried.

All combinations yielded similar results, if not slightly more coarse/aggregated precipitates compared to the product from the synthesis utilizing citrate.

It was observed that crystallization occurred within a few seconds when the synthesis was conducted at pH 7.25, whereas it happened instantly when performed at pH 9.25.

An example of one of the protocols used can be found in Appendix protocol 2.

Magnesium affinity for organic acid anions

During the fast synthesis experiments, it was observed that in the first step of the procedure (Appendix Protocol 2), where the MgCl_2 solution is mixed with the organic anion solution, no precipitate forms except when mixed with an oxalate solution. This behavior contrasts with the reaction observed when synthesizing nHAP, where mixing a CaCl_2 solution with a citrate solution produces a fine white precipitate (Appendix protocol 1). These differing reactions suggest that magnesium has a lower affinity for citrate compared to the strong affinity between calcium and citrate. This is further reflected in the solubilities of Mg-citrate and Ca-citrate in water, which are approximately 50-100 g/L and 0.85-1.5 g/L respectively at 25 °C (Appendix Solubility table).

This suggests that citrate is not effective as a stabilizing agent for struvite nanoparticles because most of the citrate ions remain soluble in the solution rather than binding to the magnesium atoms on the surface of the struvite particles. A similar behavior is observed with malate and maleate, which also do not form a precipitate when mixed with an MgCl_2 solution; however, no solubility constants are available for these compounds.

In contrast, Mg-oxalate does form a precipitate and has a solubility of approximately 0.38 g/L, which is lower than that of Ca-citrate. However, oxalate was not effective in stabilizing the synthesized particles. This could be due to the significantly different chemical structure of oxalate, being divalent, compared to citrate, which is trivalent. These differences might affect the ability of oxalate to create a negative surface charge on the struvite particles.

Conclusion

Attempts to create a stable solution of struvite nanoparticles through various synthesis methods and stabilization with organic acid anions have not been successful with the approaches tested so far. Particles with a size of approximately 1.8 μm have been produced using both “top-down” and “bottom-up” methods. However, it has not been possible to determine whether these particles are single crystals or aggregates of smaller crystals based on analyses conducted with DLS and microscopy. Aggregation has however been seen on a microscopic level on all samples observed with microscope, which indicates that it is likely that aggregation would also happen on nano scale.

The observed aggregation indicates that stabilizing struvite particles with citrate, malate, and maleate is not feasible in the same manner as using citrate to stabilize nano-hydroxyapatite particles. This is likely due to magnesium’s lower affinity for these anions compared to calcium’s strong affinity for citrate. This is reflected in the lower solubility of Mg-citrate, malate, and maleate compared to Ca-citrate.

Oxalate was also tested as a stabilizing agent but proved ineffective, despite oxalate’s strong affinity for magnesium.

To achieve stable struvite particles, alternative stabilizing agents should be explored. Alternative stabilizing agents could be applied to particles produced using either the “top-down” or “bottom-up fast synthesis” approaches, which have been the most successful methods tried so far.

Appendix:

Protocol 1: Non-spiked nHAP, fast synthesis (250 ml batch)

Hydroxyapatite: $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ (MW 502.31 g/mol)

Chemicals:

- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ MW: 147.01 g/mol → Check the hydration state you choose!
- *Or* $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ MW: 219.07568 g/mol Hydrated is easier to dissolve
- KH_2PO_4 MW: 136.086 g/mol
- 2M NH_4 -citrate pH 9 stock
- NH_4OH solution (28-30 %) concentrated

To have 5 g/L P in 0.250 L → 1.25 g P in total → since KH_2PO_4 is 22.76% P → $1.25 / 0.2276 = 5.49$ g KH_2PO_4 required → $5.49 \text{ g} / 136.09 \text{ g/mol} = 0.0404 \text{ mol P}$

Since we want a Ca:P molar ratio = 1.67 → $0.0403 \text{ mol} * 1.67 = 0.0674 \text{ mol}$ → $147.01 \text{ g/mol} * 0.0674 \text{ mol} = 9.9 \text{ g CaCl}_2 \cdot 2\text{H}_2\text{O}$

Stock solutions:

1.348 M $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$

Or 1.348 M $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$

0.806 M KH_2PO_4

27.125 ml conc. NH_4OH (28-30%)

2 M NH_4 -citrate pH 9 stock
synthesis

Amount to scale out:

9.9 g for 50 ml

14.77 g for 50 ml

5.49 g for 50 ml

add 12.5 ml to the Ca-solution for a 250 ml

Procedure:

- 1) Mix 50 ml of the CaCl_2 solution with 12.5 ml 2M NH_4 -citrate pH 9 (in 250 ml final volume = 100 mM citrate).
- 2) Check the pH is 9 and add NH_4OH if necessary (approximately 500 μl NH_4OH extra was added to reach pH 9).
- 3) Adjust the volume to 100 ml with milliQ water. Notice that the solution becomes white and precipitates after a while when NH_4OH is added. Therefore →
- 4) Place the solution on the stirrer at 600 rpm.
- 5) To the 50 ml of KH_2PO_4 you will add 86.44 ml milliQ and 13.56 ml NH_4OH (28-30%). Total volume 150 ml. Check the pH with a paper strip and note down.
- 6) Pour the P-solution into the CaCl_2 solution slowly while stirring at high speed. Make sure the solutions are mixed immediately when in contact. Stir over night at high speed, then remove the bluecap flask from the stirrer. Or leave to stir gently with the lid off if you wish to evaporate some NH_3 .
- 7) After some days at RT, nHAPs will form and there might be a slight separation between a clear phase on top, and a lower phase with nHAPs. Resuspend nHAPs in the whole volume by shaking the flask and sonicating.

8) Measure pH and note down. Dilute at least 20x for DLS size measurement.

Note: The synthesis has been conducted in various volumes ranging from 50 ml to 5 L. The results are the same.

Protocol 2: Nano struvite (nSt), fast synthesis (100 ml batch)

Struvite: $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$ (MW 245.41 g/mol)

Chemicals:

$\text{MgCl}_2 \cdot 4\text{H}_2\text{O}$ MW: 167.27 g/mol

$\text{NH}_4\text{H}_2\text{PO}_4$ MW: 115.03 g/mol

Oxalic acid MW: 90.04 g/mol,

NaCl 2M Stock

Stock solutions:

Amount to scale out:

$\text{MgCl}_2 \cdot 4\text{H}_2\text{O}$ 13.3816 g for 100 ml

0.4 M $\text{NH}_4\text{H}_2\text{PO}_4$ 9.202 g for 200 ml

1 M Na-oxalate 9.004 g for 100 ml

2 M NaOH

All stock solutions pH adjusted to 7.25 with 2 M NaOH

Stock solution Procedures:

100 ml 0.8 M MgCl_2 Solution:

13.3816 $\text{MgCl}_2 \cdot 4\text{H}_2\text{O}$ dissolved in 80 ml milli-Q water, pH adjusted to 7.25 with 2 M NaOH and top off to 100 ml with milli-q water.

200 ml 0.4 M $\text{NH}_4\text{H}_2\text{PO}_4$ Solution:

9.202 g $\text{NH}_4\text{H}_2\text{PO}_4$ dissolved in 150 ml Milli-Q water, pH adjusted to 7.25 with 2 M NaOH and top off to 200 ml with milli-q water.

100 ml 1 M Na-oxalate

9.004 g oxalic acid dissolved in 70 ml milli-Q water, pH adjusted to 7.25 with 2 M NaOH and top off to 100 ml with milli-q water.

- 1) Mix 25 ml of the MgCl₂ solution with 20 ml 1M Na-oxalate pH 7.25 (in 100 ml final volume = 200 mM oxalate). Na-oxalate amount will also be adjusted to a final solution of 50, 100 and 250.
- 2) Check to see that the pH is 7.25 and adjust with 2 M NaOH if not.
- 3) Top off the solution to 50 ml with Milli-Q water
- 4) Place the solution on the stirrer at 600 rpm.
- 5) Prepare 50 ml 0.4 M NH₄H₂PO₄ Solution
- 6) Pour the P-solution into the MgCl₂ solution slowly while stirring at high speed. Make sure the solutions are mixed immediately when in contact. Stir over night at high speed, and let it stir a week at lower speed to mature.
- 7) Measure pH and note down. Dilute at least 20x for DLS size measurement.

List of solubilities (AI generated):

Approximate solubility for calcium and magnesium salts of oxalate, malate, maleate, and citrate. When exact values are not available, general trends are indicated.

Salt	Calcium (Ca) (g/L)	Magnesium (Mg) (g/L)
Oxalate	~0.00067 g/L (very low solubility)	~0.38 g/L (very low solubility)
Malate	~1-5 g/L (moderately soluble)	~10-50 g/L (highly soluble)
Maleate	~50-100 g/L (highly soluble)	~100+ g/L (highly soluble)
Citrate	~0.85-1.5 g/L (sparingly soluble)	~50-100 g/L (highly soluble)

ICP Analysis results and calculations

Theoretical Elemental weight of a struvite molecule ($(\text{NH}_4)_2\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$)

Element	Atomic mass	Number of atoms	Total mass of Element	% of total mass
Mg	24,305	1	24,305	9,90%
N	14,007	1	14,007	5,71%
H	1,008	16	16,128	6,57%
P	30,977	1	30,977	12,62%
O	15,999	10	159,99	65,19%
Sum	-	29	245,407	100,00%

Struvite purity if all P is bound in struvite. 99,08%

Struvite purity if all Mg is bound in struvite. 97,08%

ICP Analysis of solid struvite (100-250 μm)

Theoretical element mass distribution of 1g of pure Struvite					Difference between Theoretical and analysis data						
Element	Mass (ug/g)	% of total mass	Element	Mass (ug/g)	% of total mass	Element	Mass (ug/g)	% of total mass	Element	Mass (ug/g)	% of total mass
Al	4,94	0,00%	Al			Al		4,94	Al	4,94	0,00%
B	0	0,00%	B			B		0,00	B	0,00	0,00%
Ca	1076,12	0,11%	Ca			Ca		1076,12	Ca	1076,12	0,11%
Cu	0,78	0,00%	Cu			Cu		0,78	Cu	0,78	0,00%
Fe	265,12	0,03%	Fe			Fe		265,12	Fe	265,12	0,03%
K	775,37	0,08%	K			K		775,37	K	775,37	0,08%
Mg	96113,72	9,61%	Mg		99039,55	Mg		-2925,83	Mg	-2925,83	-0,29%
Mn	29,05	0,00%	Mn			Mn		29,05	Mn	29,05	0,00%
Na	15,14	0,00%	Na			Na		15,14	Na	15,14	0,00%
P	125017,56	12,50%	P		126227,04	P		-1209,48	P	-1209,48	-0,12%
S	36,71	0,00%	S			S		36,71	S	36,71	0,00%
Sr	0,21	0,00%	Sr			Sr		0,21	Sr	0,21	0,00%
Zn	1,15	0,00%	Zn			Zn		1,15	Zn	1,15	0,00%
C	2300	0,23%	C			C		2300,00	C	2300,00	0,23%
N	56900	5,69%	N		57076,61	N		-176,61	N	-176,61	-0,02%
H		Unknown	H		65719,40	H		-	H	-	-
O		Unknown	O		651937,39	O		-	O	-	-
Unknown	717464,13	71,75%	H+O		717656,79	Unknown and H+O		-192,66	Unknown and H+O	-192,66	-0,02%

References:

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