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Local distortion and octahedral tilting in BaCexTi_{1-x}O₃ perovskite

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Investigating the Growth of Polar and Ionic Crystals under Electric Fields

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ABSTRACT:

Ionic and polar crystals are important in industries where properties such as shape, alignment and quality define their function. These properties are influenced by a number of factors. This study investigates the effect of electric fields on controlling crystal growth. Micro Lysozyme and Alanine crystals were grown using hanging-drop vapour diffusion under an electric field of 200N/C. Macro MgSO_4 and $\text{H}_2\text{C}_2\text{O}_4$ crystals were grown from crystal nuclei using slow evaporation under an electric field of 12000 N/C. Electric fields were generated with parallel plate capacitors. Under electric field, lysozyme crystals demonstrated up to a 115% increase in length compared to control. Alanine crystallization under electric fields exhibited a variety of results with high uncertainty which require further study. Under an electric field, both $\text{H}_2\text{C}_2\text{O}_4$ and MgSO_4 demonstrated greater crystallization towards the positive terminal. Additionally, MgSO_4 showed greater alignment in crystals. These results are useful in the semiconductor and jewellery industries where controlling growth locations of polar crystals, and reducing imperfections/increasing alignment in ionic crystals are important. Additionally, affecting protein crystallization rates is important in pharmaceuticals.

INTRODUCTION:

BACKGROUND
Electric fields exist throughout the world in which we live. They are used in the semi-conductor industry, medical equipment and can be important tools in the manipulation of charged objects. An electric field will exert a force on charged substances and this can be used to alter velocity, position and orientation of an object, for example, a crystal. Crystals, which are made up of repeating units, can have associated functional groups that contribute to polar and ionic character.

CRYSTAL CHEMICALS

- Oxalic acid ($\text{H}_2\text{C}_2\text{O}_4$) is a dipolar molecule containing to two negatively charged carboxyl groups (1).
- Magnesium Sulfate (MgSO_4) is ionic and is highly soluble, it readily dissociates into ions (1).
- Alanine is an amino acid with a negatively charged carboxyl group, positive amine group and polar character (1).
- Lysozyme is a relatively large protein molecule with some positive charge (5).

OBJECTIVES AND HYPOTHESIS:

OBJECTIVES

- Observe the effect of an external electric field on growth and structure of two macro and two micro crystals containing different molecular structures with different polar/ionic properties.
- Through observation of relative crystal growths we aimed to infer the effect of polar/ionic character on crystal growth under an electric field. Properties like size, number and position (in the solution) of the crystals will be measured.

HYPOTHESIS

- MgSO_4 crystals will be most affected by electric fields where alignment and larger size crystals are likely to be observed. This effect would be greater than for oxalic acid which is a neutral molecule. $\text{H}_2\text{C}_2\text{O}_4$ crystal growth would be affected due to the polar nature of the molecule.
- Lysozyme is a large protein molecule with a large mass-to-charge ratio and as such won't be significantly affected by electric fields.
- Alanine's polar structure means that it should be affected by electric fields and so should demonstrate some alignment with an electric field.

METHODS:

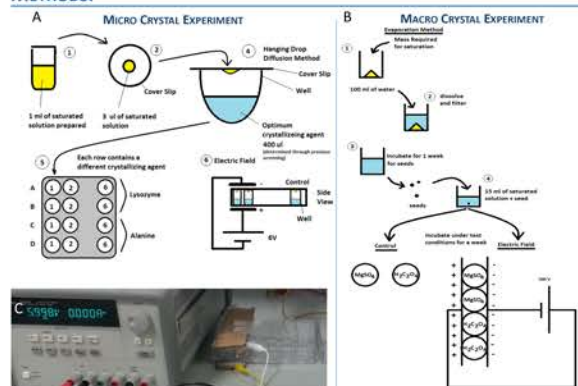


Figure 1: A schematic of the procedure followed with regards to the micro and macro crystals. A shows the experimental set up for the micro crystals. B shows the experimental set up for the macro crystals. C shows experimental set up of macro crystal set up.

RESULTS:

MACRO CRYSTALS:

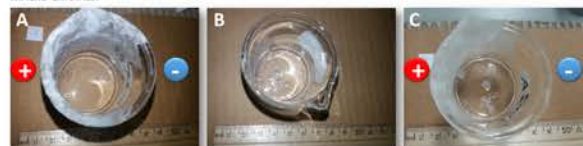


Figure 2: Comparison of Oxalic acid's crystallization under electric field to control condition. Both A and C were grown under electric field of 12000N/C and displayed major crystallization on the positive side of the beaker whereas B was left under no electric field.



Figure 3: Comparison of Magnesium Sulfate's crystallization under an electric field to control condition. Both A and C were grown under an electric field of 12000N/C and displayed a solid mesh. A showed a greater build up of mass on the positive side. C showed an aligned crystal perpendicular to the plates.

MICRO CRYSTALS:

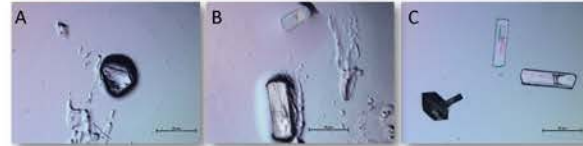


Figure 4: Comparison of Alanine's crystallization in row A under an electric field to control condition. Both A and C were grown under an electric field of 200N/C and grew no crystals whereas B was left under no electric field and showed several small crystals.

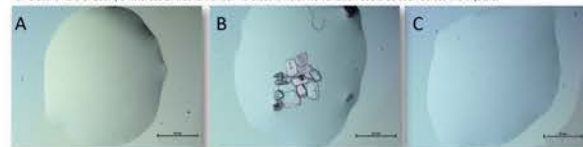


Figure 5: Comparison of Alanine's crystallization in row B under an electric field to control condition. Both A and C were grown under an electric field of 200N/C and grew no crystals whereas B was left under no electric field and showed several small crystals.



Figure 6: Comparison of Lysozyme's crystallization in row C under an electric field to control condition. Both A and C were grown under an electric field of 200N/C and displayed on average larger crystals than B. B was left under no electric field.



Figure 7: Comparison of Lysozyme's crystallization in row D under an electric field to control condition. Both A and C were grown under an electric field of 200N/C and showed random, small crystal growth. A showed crystals with less imperfections and bigger average size than the control. C did not crystallize.

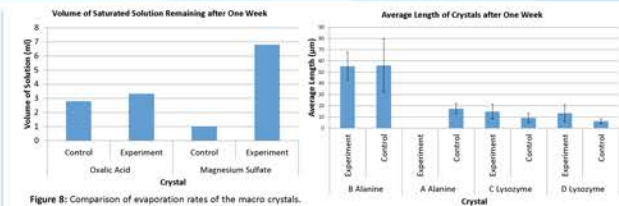


Figure 8: Comparison of evaporation rates of the macro crystals. More solution remaining implies slower evaporation rate.

Figure 9: Comparison of average length of crystal of micro crystals

DISCUSSION:

DISSOCIATION AND OXALIC ACID

- Oxalic acid has pKa's of 1.25 and 4.14 (1) and dissociates into $\text{C}_2\text{O}_4^{2-}$ and HC_2O_4^- .
- The negatively charged ions migrate towards the positive side of the beaker as a result of the electric force exerted by the electric field. In turn, concentration of the oxalate ions on the positive side increase, speeding up crystallization.
- This study suggests that dissociation and migration of ions are responsible for the crystallization on the positive side.

EVAPORATION RATES AND MAGNESIUM SULFATE

- Reduced evaporation rates of MgSO_4 solutions under electric field supported results of a study that concluded boiling point of water was increased under electric field(2).
- Having studied dipolar orientations and carried out an energetics tests, the study suggests that water molecules align in the direction of the electric field as a result of their dipolar nature. This was reported to a lesser extent in the vapor phase implying the liquid phase is more energetically stable. This supports lower evaporation rates in the evaporation of MgSO_4 under an electric field.
- However, another study reported higher drying rates under an electric field (3).
- It is important to note that evaporation rates did not significantly differ in $\text{H}_2\text{C}_2\text{O}_4$ solutions (Figure 8).
- Nevertheless, reduced evaporation rates are known to produce higher quality crystals with less imperfections (4) and this was what was generally observed with MgSO_4 .

CHARGE DOMAINS AND LYSOZYME

- Greater length lysozyme crystals have been reported in a similarly conducted study (5).
- The study attributed the results to the large positive charge of +12 associated with the lysozyme protein.
- Molecules are attracted to the negative side under an electric field increasing the concentration in that area.
- This in turn accelerates crystallization and larger sized crystals are observed.
- The results obtained in this study are statistically inconclusive, however, on average, crystal sizes are larger. This may support the presence of charged/polar domains in the protein and interaction with electric fields.

OTHER OBSERVATIONS

- The alignment of the crystal in Figure 3.C could have been influenced by the alignment of the water molecules but this could not be verified.
- Alanine's results carried large uncertainty and yielded no conclusion.



Figure 10: A. Schematic of oxalate ion towards the positive side. B. Schematic of water molecules aligning in direction of electric field. C. Schematic of lysozyme concentration increasing on the negative side.

CONCLUSION:

Oxalic acid crystallized on regions of opposite charge. This suggested similar behavior for other polar acids that dissociate into ionic species in solution. Magnesium Sulfate showed greater alignment parallel with electric fields and its solution showed greater evaporation rates under an electric field. Larger size crystals were observed under an electric field. This study suggests that this behavior may be present with other ionic species where greater alignment may be achieved. Lysozyme showed no statistical difference in size. However, on average, crystals were larger in the presence of electrical fields. Alanine showed too much variation for a conclusion to be made.

FUTURE DIRECTIONS:

- Repeat experiment with greater sample size and a variety of polar/ionic molecules to reduce error.
- Verifying mechanism proposed in our discussion.

REFERENCES:

1. Zumdahl SS & DeCoste DJ (2011) Chemical principles (Cengage Learning).
2. Maerzke KA & Siepmann J (2010) Effects of an applied electric field on the vapor-liquid equilibria of water, methanol, and dimethyl ether. The Journal of physical chemistry. B 114(12):4261-4270.
3. Cao W, Nishiyama Y, Koide S, & Lu ZH (2004) Drying Enhancement of Rough Rice by an Electric Field. Biosystems Engineering 87(4):445-451.
4. Röhner SC, Wedin P, & Bergström L (2002) Effect of Electrolyte and Evaporation Rate on the Structural Features of Dried Silica Monolayer Films. Langmuir 18(24):9327-9333
5. Nanev CN & Penkova A (2001) Nucleation of lysozyme crystals under external electric and ultrasonic fields. Journal of Crystal Growth 232(1-4):285-293.

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The Effect of pH on Macro- and Microcrystal Growth of Potassium Ferricyanide

Foundations of Science 4, Spring 2015

Yumi Gambrill & Anna-Lisa Hennig

M3

INTRODUCTION

Crystal Formation

- Crystals form as a solution exceeds its solubility capacity¹
- The solution can't hold any more dissolved solute, so some of it comes out of solution and forms the crystal¹

Crystal Structure

- Crystals are ordered solids with a lattice structure.²
- Potassium ferricyanide forms both monoclinic (space group P2₁/c) and bipyramidal base-centered orthorhombic (space group Pnma) structures.^{1,2}

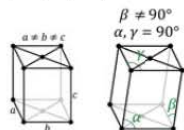


Fig. 1. Base-centered orthorhombic (II) and base-centered monoclinic (I) structure of potassium ferricyanide.^{2,4}

Effect of pH on Crystal Growth

- Should have no effect on crystal structure itself - any change in structure is due to an actual chemical reaction
- KOH and HCl used because low concentrations do not react with potassium ferricyanide
- Solutions with higher pH should evaporate faster, increasing rate of crystal growth. However, the resulting crystals, though numerous, are not very large.
- If the concentration of HCl is too high, it reacts with the potassium ferricyanide to form cyanic acid, iron (III) chloride, and potassium chloride.⁵

Applications of Potassium Ferricyanide in Industry

- Potassium ferricyanide + KOH → Murakami's reagent, used in steel etching⁶
- Dot etching in photography⁶
- Fe(III)+Potassium ferricyanide → Prussian Blue pigment. One of the first synthetic pigments. Used in blueprints and paints.⁷
- Iron removal in wine - prevents oxidation of the alcohol.⁸

OBJECTIVES

- Examine the difference in growth of macrocrystals as pH of the solution is varied.
- Examine the difference in crystal growth of microcrystals as pH of the solution is varied, and as the crystal screen used is varied
- Compare growth patterns of micro and macro crystals under different pH conditions

METHODS

MACROCRYSTALS

Seed Crystals

- A saturated solution of K₃Fe(CN)₆ was prepared.
- The solution was left to fully evaporate in a half-covered petri dish and the resulting crystals were collected.

Moderate pH Solutions

- Five saturated solutions of K₃Fe(CN)₆ were prepared.
- The following pHs were created: 4.3, 5.5, 6.5 for the control (no HCl or KOH added), 7.5, and 8.4. This was done by adding certain amount of 0.1M KOH or HCl to neutral solutions.
- Seed crystals were suspended in the solutions, and were left to evaporate in beakers over two weeks.

Extreme pH Solutions

- Five saturated solutions of K₃Fe(CN)₆ were prepared.
- The solutions were manipulated as previously mentioned to create pHs of 1.5, 1.8, 11.8, and 12.1.
- The solutions were left to evaporate in half-covered petri dishes for two weeks.



Fig. 2. Moderate pH Macrocrystal Experimental Setup

MICROCRYSTALS

- Two 24-well microcrystal growth plates were prepared for hanging-drop vapor diffusion with 48 different crystal screen solutions.
- 3µL of each K₃Fe(CN)₆ solution of moderate pH were dropped onto a coverslip and placed over each well.
- The microcrystals were left to grow for two weeks.

MACROCRYSTAL RESULTS

Moderate pH Solutions

Crystal structure itself did not seem to be affected by the basic solutions; all crystals were red and of similar size and shape

The following data on crystal size was collected:

Table 1. Mass of crystals produced from solutions of different pH

| pH of Solution | Mass of Crystals Produced (g) |
|----------------|-------------------------------|
| 4.3 | 0.7876 |
| 5.5 | 1.0771 |
| 6.5 | 0.5863 |
| 7.5 | 0.4750 |
| 8.4 | 0.7132 |



Fig. 3. Macrocrystals grown from seed crystals suspended in solution

Extreme pH Solutions

- Acidic solutions changed from light brown to nearly black with a blue precipitate
- Basic solutions didn't change colour but formed an orange precipitate
- Changed suggest that the concentrations of the KOH and HCl used were too high, causing a reaction. The reactions were likely the following:
 $K_3Fe(CN)_6 + KOH + H_2O \rightarrow K_3Fe(CN)_6 \cdot KOH \cdot H_2O$
 $6 HCl + K_3Fe(CN)_6 \rightarrow 6 HCN + FeCl_3 + 3 KCl$
- Crystals that formed through quick evaporation were small and needle-like; those that formed over a longer period of time created longer rod-like structures.

The following results were collected on the crystals:

Table 2. Observations of crystal growth from solutions with extreme pH

| | KOH pH 1.5 | KOH pH 1.8 | KOH pH 11.8 | KOH pH 12.1 |
|--------|--|---|---|---|
| Week 1 | - Long thin, needle-like crystals forming as form a splendable structure - Crystals emerged in some solution | - Thicker needle-like structures - Very little solution evaporated | - One large, red crystal with some smaller ones - Very little evaporation | - One large, red crystal, one medium sized and one tiny one - Most of the solution still present |
| Week 2 | - Solution fully evaporated - Thick, needle-like crystals - Crystals are dark brown but covered in gold - Some crystals growing up one side of the petri dish in spoked, branch-like structures | - Solution fully evaporated - Thicker needle-like crystals - Dark brown crystals covered in gold precipitate - Spiked and branched crystals growing up and protruding from the sides of the petri dish | - Some solution left, but less than in solutions of pH 11.8 - Two very large, red crystals - One very large and one medium sized red crystal surrounded by many smaller ones (more than in pH 11.8) | - Some solution left, but less than in solutions of pH 11.8 - One very large and one medium sized red crystal surrounded by many smaller ones (more than in pH 11.8) |

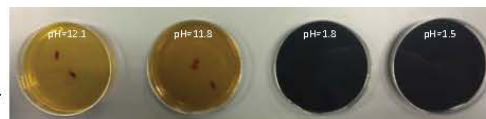


Fig. 5. Initial solutions of extreme pH

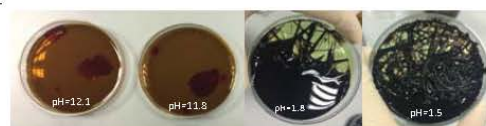


Fig. 6. Solutions after 1 week of evaporation



Fig. 7. Solutions after 2 weeks of evaporation



Fig. 4. Seed crystals used to grow macrocrystals



Fig. 8. Detail of crystal growth in pH 11.8 solution



Fig. 9. 20x magnification of surface growth in pH 11.8 solution

MICROCRYSTAL RESULTS

- Of the 48 wells tested, crystals on grew in wells number 4, 5, 7, 13, 16, 21, 23, 32, 33, 34, 38, 39, 47
- The solutions in some wells (18, 24, 30, 31, 37, 42, 43, 45, 46) changed color from amber to green. Of these, only well 34 produced crystals.
- In general, all drops under one condition showed same changes.



Fig. 11. Wells after preparation

Fig. 12. Wells after 1 week of evaporation

Crystal Screen 13: 0.2M Sodium citrate tribasic dihydrate with 0.1M TRIS hydrochloride pH 8.5, 30% v/v Polyethylene glycol 400

- One small, orthorhombic crystal created in each drop
- All five crystals are of very similar size, shape and thickness

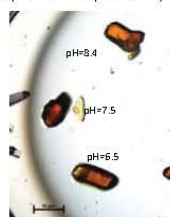


Fig. 14. Crystal growth in well number 13

Crystal Screen 34: 0.1M TRIS hydrochloride pH 8.5 with 2.0M Ammonium Sulfate

- Small clusters of red crystals formed in each drop
- The amount and size of crystals were roughly the same for all solutions

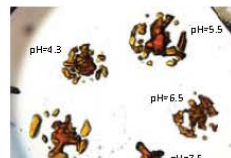


Fig. 13. Crystal growth in well number 4

Crystal Screen 34: 0.2M Sodium acetate trihydrate pH 4.6 with 2.0M Sodium formate

- Dark red, almost black crystals of similar size and shape in each drop
- The drop containing solutions of pH 6.5, 7.5 and 8.4 show small needle-like structures protruding from the crystals

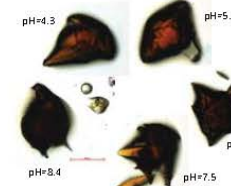


Fig. 15. Crystal growth in well number 34

CONCLUSION

- pH has no apparent effect on growth of potassium ferricyanide macrocrystals or microcrystals
- The different masses of macrocrystals likely due to random growth the different sizes of seed crystals used
- High concentrations of HCl can react with the potassium ferricyanide, changing the type of crystal. This crystal is probably iron (III) chloride.

FUTURE EXPERIMENTATION

General Applicability of Results

- Replicate experiment with a different crystal
- Repeat experiment using different acid and base
- Repeat macrocrystal analysis using supersaturation method

Further Testing of the solutions of Extreme pH

- Investigate concentration of HCl needed to produce new type of crystal
- Use chemical analysis to determine identity and composition of precipitates that formed from high concentrations of HCl/KOH

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REFERENCES

- Chen, C. H., Shiao, A., & Hsiao, B. S. Inorganic Chemistry and Materials Science. Academic Press (1998). Vol. 8, 1-17.
- Agar, S., Datta, S., & Hsiao, B. S. Crystal structure of the base-centered orthorhombic and base-centered monoclinic structures of potassium ferricyanide. Australian Journal of Chemistry 1979, 31 (6), 1185-1189.
- Mayer, P., Base-Centered Orthorhombic Crystal Structure. Chemical Reviews, 2007, Vol. 704, 1024-1025.
- Mayer, P., Base-Centered Monoclinic Crystal Structure. Chemical Reviews, 2007, Vol. 704, 1026-1027.
- Shiao, A., Liang, L., & Hsiao, B. S. The Crystal Structure of the Base-Centered Orthorhombic and Base-Centered Monoclinic Structures of Potassium Ferricyanide. Australian Journal of Chemistry 1979, 31 (6), 1185-1189.
- Shiao, A., Liang, L., & Hsiao, B. S. The Crystal Structure of the Base-Centered Orthorhombic and Base-Centered Monoclinic Structures of Potassium Ferricyanide. Australian Journal of Chemistry 1979, 31 (6), 1185-1189.
- Shiao, A., Liang, L., & Hsiao, B. S. The Crystal Structure of the Base-Centered Orthorhombic and Base-Centered Monoclinic Structures of Potassium Ferricyanide. Australian Journal of Chemistry 1979, 31 (6), 1185-1189.
- Shiao, A., Liang, L., & Hsiao, B. S. The Crystal Structure of the Base-Centered Orthorhombic and Base-Centered Monoclinic Structures of Potassium Ferricyanide. Australian Journal of Chemistry 1979, 31 (6), 1185-1189.

The effect of acidic impurities and different surfaces on the growth of copper (II) sulfate pentahydrate crystals

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A

Abstract

The main purpose of this study was to determine the effects of acidic impurities and different surfaces on the growth of copper (II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) crystals. The way different surfaces and acidic impurities affect the formation of macro- and micro- crystals were investigated in a series of evaporation, supersaturation and hanging drop vapor diffusion experiments (1). Copper (II) sulfate pentahydrate macrocrystals were grown on four different types of surfaces: ceramic, plastic, glass and metal. A direct correlation was concluded between the static friction and adhesive forces of the surface and the number of nucleation sites formed. An evident inverse relation was found between the forces and the average size of the crystals grown. In terms of the microcrystals, the existence of organic (citric) and inorganic (hydrochloric acid) impurities showed a complete inhibition of copper (II) sulfate pentahydrate crystal formation in many crystallizing conditions. However, it was also concluded that the HCl impurity had no significant effect on crystal size, while the citric acid impurity enhanced the rate of the crystal growth.

Introduction

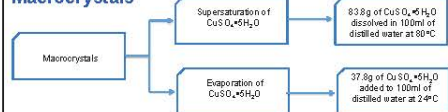
- Crystals constitute of atoms, molecules, or ions that are arranged in an orderly repeating pattern extending in all three dimensions (2).
- Crystallization is kinetically hindered and a crystal can grow only from saturated solutions (3).
- Surfaces provide nucleation sites, where molecules stick to surfaces and start growing into a crystal. Crystals could practically form on any rough surface available, but the extent to which the formation might occur can differ.
- Crystal growth can also be affected by different acidic impurities, which, depending on their chemical nature, suppress the growth of crystals (4).
- The degree of inhibition might be independent from the strength of acid, but dependent on whether the acid is inorganic or organic, with the organic acid being a better inhibitor.
- Two common methods (5) to grow **macrocrystals** is supersaturation and evaporation. For **microcrystals**, on the other hand, the best methodological approach is a hanging drop vapour diffusion method.

Objectives

- Growing copper (II) sulfate pentahydrate **macrocrystals** on different material surfaces namely: glass, plastic, metal and ceramic and observing how different surfaces affect crystal formation.
- Examining how acidic impurities can inhibit the growth of **microcrystals** and investigating, whether inorganic (HCl) or organic acid (citric acid) is a better inhibitor in a crystal formation process.

Methods

Macrocrystals



- Samples both kind of solutions were distributed to metal, ceramic, glass and plastic dishes for evaporation and supersaturation techniques at two different temperatures (16 experimental samples in total).
- Four samples on different surfaces for each technique were kept in room temperature (25° C), while the other four samples on different surfaces for each method were placed in a fridge (4° C).

Microcrystals



- The experiment was repeated twice. The first time growing crystals for one week and the second time growing crystals for two days.

Results

Macrocrystals

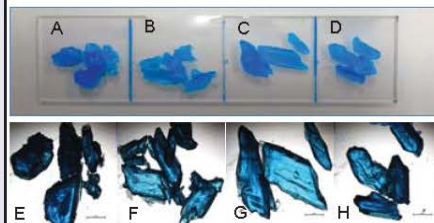


Figure 1. Copper (II) sulfate pentahydrate crystals grown in ceramic (A,E), plastic (B,F), glass (C,G) and metal (D,H) dishes using supersaturation technique in 25° C. A-D images taken using Samsung photo camera and E-F images taken using SMZ Nikon 1500 Stereomicroscope (Magnification 20x).

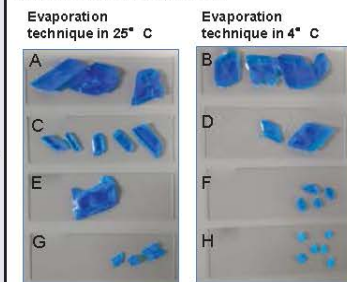


Figure 3. Copper (II) sulfate pentahydrate crystals grown in ceramic (A,B), plastic (C,D), glass (E,F) and metal (G,H) dishes using evaporation technique in 25° C and 4° C respectively.

Microcrystals

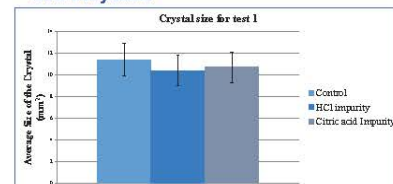


Figure 5. Comparison of the average size of crystal between the control, HCl and citric acid impurity solutions in test 1 (crystallized for one week).



Figure 7. The effect of acidic impurities to copper (II) sulfate pentahydrate microcrystal growth: citric acid (A), hydrochloric acid (B) and the control (C) samples.

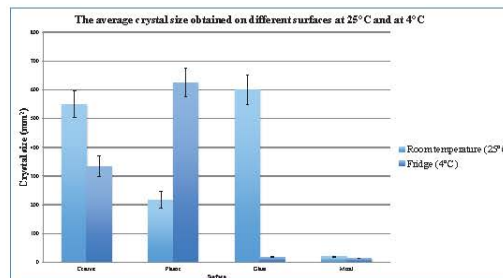


Figure 4. Bar chart illustrating how different surfaces affect the size of copper (II) sulfate pentahydrate crystals.

Discussion

Macrocrystals

Table 1. Physical and chemical properties of surfaces used in the experiment.

| Surface | Static friction coefficient (μ) | Chemical bonds of surfaces | Adhesive forces |
|-------------------|---------------------------------|----------------------------|-----------------|
| Ceramics | 0.37 | polar bonds | strong |
| Plastic | 0.35 | non-polar bonds | very weak |
| Glass | 0.95 | polar bonds | strong |
| metal (aluminium) | 1.20 | metallic (electrostatic) | very strong |

- Supersaturation of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ yielded approximately the same results on a macro-scale for experimental samples kept at 25° C and at 4° C.
- There is a positive relation between the static friction coefficients as well as the chemical bonds of the surface and the number of nucleation sites formed on different materials, namely: metal, plastic and ceramic surfaces.
- A direct relationship between the high abundance of nucleation sites and the small size of crystals was noticed only when investigating the experimental samples grown in a fridge.

Microcrystals

Statistical analysis using the ANOVA test:

Table 2. ANOVA test values evaluating the significance of the results

| Test | | | | |
|------|--|--|--|--|
| | | | | |
| | | | | |
| | | | | |

- The ANOVA test demonstrated that crystals in test 1 reached a growth limit, so only the results in test 2 showed the effect of acidic impurity on the rate of crystal growth.

The main observations:

- The inhibition effect of acid can be evaluated by the number of crystals produced - in some conditions the acidic impurity halted the growth of microcrystals.
- The citric acid impurity enhanced the actual average size of crystals: 10mm² in the control sample and 12.4mm² in the citric acid impurity sample.
- The hygroscopic property (6) of citric acid allowed it to hold and attract water. Due to this property, citric acid brought the dissolved copper and sulfate ions closer together effectively increasing the concentration of these ions in the region and thus increasing the crystallization rate.

Conclusion

- Different surfaces had different chemical and physical properties (static friction coefficients and adhesive forces) which affected the accumulation and attachment processes of macrocrystal particles to the particular surface as well as influenced the size of crystals formed.
- Acidic impurities inhibited microcrystal formation in many crystal screening conditions. However, provided that crystals formed with some crystallizing agents present, citric acid had an enhancing effect on the rate of crystal growth.

Bibliography

- Luft JR, et al. (2003) A deliberate approach to screening for initial crystallization conditions of biological macromolecules. *J Struct Biol* 142(1):170-179.
- Altman J, Black AJ, & Whitelides OH (1999) Oriented growth of calcite controlled by self-assembled monolayers of functionalized alkanethiols supported on gold and silver. *J Am Chem Soc* 121(9):4000-4009.
- Frezza A, Landgraf F, Sedler M, & Giulietti M (1999) The influence of magnetic field on crystallization from solutions. *14th International Symposium on Industrial Crystallization*, University of Cambridge, pp 12-16.
- Nagata T, Sakane K, & Wada H (1997) Effect of Acids on Crystallization of Lithium Borate Films. *Journal of Sol-Gel Science and Technology* 8(1-3):431-435.
- Rau KS, Indian Institute of Mineral Engineers, Goa Mineral Ore Exporters' Association, & Indian Bureau of Mines. (2003) *Proceedings of the International Seminar on Mineral Processing Technology: IMPT-2003*, February, 6-8, 2003, Goa, India (Allied Publishers, Mumbai) pp viii, 466.
- Peng CO, Chow AHL, & Chan CK (2001) Hygroscopic study of glucose, citric acid, and sorbitol using an electrodynamic balance. Comparison with UNIFAC predictions. *Analyst* 126(3):353-358.

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Macro Crystal Growth in Superhydrophobic Systems And pH Effects on Lysozyme Micro Crystal Growth

Foundations of Science 4, Spring 2016

Daniel B. Carelli

Liam Kirwan

Keerthana Prakash

Introduction: The aim of the macro crystal experiment was to limit crystal growth on specific locations of the system by using superhydrophobic paints. The goal was to prevent crystallization on these surfaces to focus it on desired locations. Essentially, the ultimate goal of the project was to develop more predictable methods in which crystallization will occur. The goal behind the micro crystal project was to observe the effects of NaOH base and HCl acid on the growth of lysozyme micro crystals. We used the hanging drop method to crystallize lysozyme from the whites of chicken eggs over wells containing various crystal screens. In changing the pH of each microcrystal we were expecting the protein with added acid to become denatured, thus altering/preventing the crystallization process.

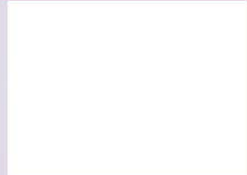
Macro Crystal Experiment

What does it mean to be Superhydrophobic?

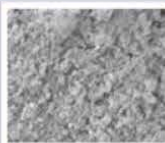
- Contact angle $> 120^\circ$
- Droplets to reduce surface energy
- Repels water & water based substances



The Nature of our Surface: A Sub-100 nm Nanostructure



SEM Picture of Beaker Surface



Drop on Sample Surface

Anticipated Results: Reduced Nucleation on Superhydrophobic Surfaces



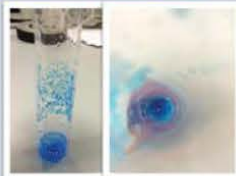
Figure on Left

Inner Rectangle:
No Coating

Outer Area:
Superhydrophobic

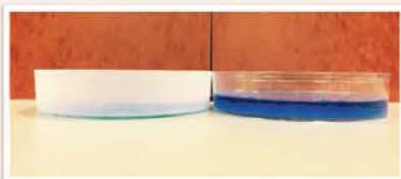


Superhydrophobic Test Tubes



Control Test Tubes

Unanticipated Results: Accelerated Evaporation



Liquid levels comparison

Test Tubes & Petri Dishes:

Left: Superhydrophobic
Right: Control

Micro Crystal Experiment

Protein used: Lysozyme

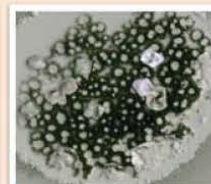
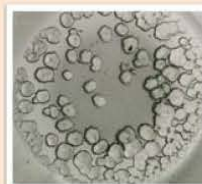
- Egg whites (chicken)
- Optimal pH range: 6.0-9.0
- Molecular mass: 14,307 Da

Control Microcrystals: Extensive Predictable Growth

- Normal Growth According to literature research



Microcrystals Under Acidic Conditions: 0.25 M HCl Added



- Very limited growth. Acidic conditions appear to inhibit nucleation

Microcrystals Under Basic Conditions: 0.25 NaOH Added

No Growth – Basic conditions completely denature protein

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Alkalization of urea, oxalate and struvite crystals grown in vitro as a potential treatment and prevention of bladder stones

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&

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Abstract

While researchers use biologically-extracted bladder stones for experimental endeavors, we attempted to investigate in vitro growth of urinary stones, something that has not been studied extensively. We investigated the growth of oxalate and struvite microcrystals in different pHs using the simple diffusion technique in agar gel. This was done to test the potential preventive effects of alkalization on stone formation. Our results suggest that oxalate and struvite grow best in acidic media. After growing urea macrocrystals, using both supersaturation and evaporation methods, we tested the critical concentration of sodium bicarbonate (NaHCO_3) that would cause crystal dissolution. Our results demonstrate slow dissolution of urea crystals in NaHCO_3 at 20 mM, the maximum physiological concentration in the urinary tract. We infer that increasing the NaHCO_3 concentration would enhance dissolution or prevent formation of additional bladder stones. Herein, we additionally report initial findings suggesting that iodine stain does not accomplish full staining of a crystal, implying that an X-ray would not provide an accurate representation of crystal size.

Introduction

Urolithiasis is one of the leading health issues in canine and feline animals. They result from the supersaturation of minerals, such as calcium or magnesium, in concentrated urine. Chemical identities of the most common urinary calculi are given in Table 1. While some calculi have a stone morphology, they can also exhibit regular crystalline structures, examples of which are shown in Figure 1.

Bladder stones are diagnosed through palpatory examination, ultrasound or X-ray imaging. In the latter technique, an iodine based contrast medium is required for radiolucent crystals. The obstructions are removed surgically and smaller stones are treated by diet and antibiotics. The drugs, such as sodium bicarbonate tablets, are directed to alkalinize the urine and dissolve the crystals and/or prevent their growth, since many of them are potentiated at lower pH levels, as shown in Table 2.

In our experiments on alkalization, dissolution and staining we have made the following hypotheses:

- Struvite grows best in alkaline conditions; oxalate and urea grow best in acidic conditions
- Urea crystals will be readily soluble in alkaline conditions
- Iodine staining will show the entire surface of the crystal

Table 1. Bladder stone composition listed by occurrence.

| Urolithiasis Composition and Occurrence | |
|---|-------|
| Oxalate | 70% |
| Whewellite (monohydrate) | |
| Weddellite (dihydrate) | |
| Calcium phosphate | 10% |
| Hydroxy-apatite | |
| Carbonate-apatite | |
| Calcium hydrogen phosphate | |
| (Brushite) | |
| Tri-calcium phosphate (Whitlockite) | 5-10% |
| Triple phosphates (Struvite) | <5% |
| Uric acid | 1% |
| Cystine | |

Table 2. Common crystals found in urine categorized by optimal pH growth condition

| Common Crystals Found in Urine | |
|--------------------------------|------------------|
| Alkaline pH | Acid pH |
| Amorphous phosphates | Amorphous urates |
| Triple phosphates | Uric acid |
| Ammonium bicarbonates | Calcium oxalates |
| Calcium phosphates | Cystine |
| Calcium carbonates | |

Figure 1. Ideal crystal of oxalate, struvite

Objectives

- Investigated the growth of oxalate and struvite microcrystals in different pH media using the simple diffusion technique in agar gel
- Tested the technique of dissolution of urea macrocrystals by alkalization with sodium bicarbonate as a possible treatment of bladder stones
- Studied the stain patterns from iodine based contrast medium

Materials and Methods

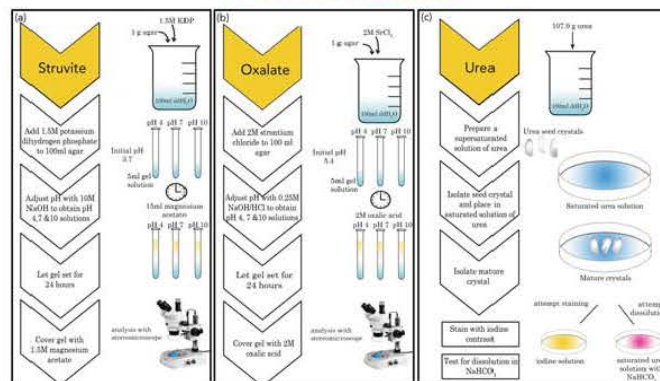


Figure 2. (a) After growing struvite microcrystals, using the methodology established by V.B. Suryawanshi, we attempted to test the growth at different pH by alkalizing the gel with concentrated NaOH. Mature crystals were observed with a stereomicroscope. (b) After growing oxalate microcrystals, following the procedure described in a study by P.V. Dalal, we attempted to test the growth in different media by adjusting the pH using HCl and NaOH. The mature crystals were observed with a stereomicroscope. (c) After obtaining a seed crystal from a supersaturated solution of urea, the crystal was isolated and placed in a saturated solution of urea to grow a mature crystal by evaporation. The mature crystal was then used to test for dissolution in NaHCO_3 and stained with iodine. The staining pattern was observed under a stereomicroscope.

Results

The oxalate microcrystal control group was imaged under a stereomicroscope, as shown in Figures 3 and 4. Similarly, the mature urea crystals were isolated and imaged in the same manner (see Figure 6). However, we were unsuccessful in our attempt to grow struvite microcrystals and instead discovered struvite stones in our gels. An image of the isolated struvite stones is seen in Figure 5.



Figure 3. Oxalate microcrystal



Figure 4. Oxalate microcrystal exhibiting regular bipyramidal shape



Figure 5. Struvite stones



Figure 6. Urea macrocrystal

Urea macrocrystals demonstrated rapid dissolution at 1M, 0.5M and 0.25M concentrations of sodium bicarbonate. For concentrations 0.125M and 0.02M, crystals completely dissolved after 2 days. After another 2 days we observed formation of large single, highly ordered urea crystals in the resulting solution. Crystal growth was greater at the lower 0.125M concentration.



Figure 7. Mature urea crystals



Figure 8. Urea crystal growth in NaHCO_3

Oxalate microcrystals showed the same growth levels and rates in all three media. The struvite stones, which grew only in acidic pH, were smaller in size compared to our control group.



Figure 9. Oxalate control

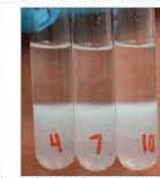


Figure 10. Oxalate grown at pH 4, 7 and 10, respectively



Figure 11. Struvite control



Figure 12. Struvite grown at pH 4, 7 and 10, respectively

Urea crystals showed signs of dissolution in the iodine solution and unclear staining pattern, although suggestive of affecting the whole surface. Struvite stones were stained after remaining in iodine solution for 2 days.



Figure 13. Urea stained with iodine



Figure 14. Stained (left) and unstained (right) struvite stone

Discussion and Conclusions

- Oxalate crystals grew at all pH conditions, supporting our hypothesis that oxalate crystals grow well in acidic conditions. Additionally, considering that the pH of the urinary tract generally ranges from pH 4-7, our findings support the fact that oxalate crystals are the most commonly occurring bladder stone.
- While struvite stones have demonstrated good growth levels in acidic media, due to the inconclusiveness of the alkaline growth, we cannot confirm our hypothesis. Experiments could be repeated with a 2% ammonia solution instead of NaOH.
- While NaHCO_3 assists in reducing urine acidity levels, thereby dissolving and/or preventing the crystals potentiated in acidic conditions, it may cause higher ordering of alkaline crystals such as urea. Investigating its effects on struvite, also more readily formed at higher pH, offers ground for possible further research.
- While the pattern of iodine staining on urea crystals was inconclusive, our results for struvite stones suggest that the contrast medium might not affect the entire stone surface. We can therefore induce that an X-ray image would not be a good representation of the calculi's true size and could potentially be a medical risk for the animal.

Acknowledgements

We would like to take this opportunity to thank our lab instructors, Joseph Koussa and Jamie Whelan, and Wael Rabeh for their support, guidance and encouragement.

Literature Cited

1. Dalal, P.V.; Suresh, K.R. Growth of struvite crystals in agar-gel. *Bulletin of Materials Science*. 2011, 34(2), 177-182.
2. International Year of Crystallography 2014. "How to Grow Crystals."
3. Khan, S.R. Animal Models of Calcium Oxalate Kidney Stone Formation. In *Animal Models for the Study of Human Diseases*; Elsevier Inc.: 2011; 461-496.
4. Narayana, Kulkarni, S.; Varshney, V.S.; Kulkarni, M.; Ramprasad, P. Crystallization of urea acid. *Journal of Crystal Growth*. 1988, 85(1-2), 429-439.
5. Smith, C.L.; St. Peter, J.V. The effect of traditional risk factors for stone disease on calcium oxalate crystal adherence in the rat bladder. *Urological Research*. 2007, 35, 183-186.
6. Hargreaves, K. Dietary management of canine urolithiasis. In *Practical*. 2009, 31, 290-312.
7. Sun, W.; Wang, J.; Zhang, K.; Wang, X. Study on precipitation of struvite and struvite-K₂ crystals during onset of urolithiasis. *Research in Veterinary Science*. 2012, 93, 481-484.
8. Suryawanshi, V.B.; Chaudhary, R.T. Synthesis and Characterization of Struvite Crystals by Agar Gel. *Journal of Crystallization Process and Technology*. 2014, 4, 111-124.
9. Stone, J.M. Stones in cats and dogs: What can be learnt from them? *Arab Journal of Urology*. 2012, 10, 230-239.
10. Yonaguchi, S.; Watanabe, J.H.; Hasegawa, A.T.; Itagaki, K.Y.; Masahashi, G.; Masahashi, N.S. Study of a rat model for calcium oxalate crystal formation without severe renal damage in electrodialysis. *International Journal of Urology*. 2004, 11, 290-298.

THE EFFECT OF VIBRATIONS AND VARYING CONCENTRATIONS ON L-CYSTINE CRYSTAL GROWTH: A POTENTIAL APPROACH FOR ALTERNATIVE KIDNEY STONE TREATMENTS?

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F

ABSTRACT

In this experiment, crystals were grown in a variety of conditions to investigate whether the final structure could be disrupted, these methods could then be adapted to provide new approaches to kidney stone treatments. The effect of vibrations on the macrocrystal formation of urea, sodium nitrate, and L-cystine was tested using buzzers. In addition, varying concentrations of L-cystine were tested to see how the growth of microcrystals would be affected. Evaporation produced higher-quality, larger crystals in comparison to supersaturation and microcrystals were grown in multiple conditions using the hanging drop method. Powderly solids of urea and sodium nitrate formed when vibrations were applied during evaporation and fracturing was observed when the vibrations were applied to formed crystals. Microcrystals of L-cystine increased in size as the concentration of the prepared solution increased. Our results indicated that vibrations are ineffective against the growth of L-cystine crystals, which make up ten percent kidney stones. However, reducing the cystine intake in one's diet may potentially decrease the chance of crystals forming.

INTRODUCTION

- Crystals are present in the body, both beneficially and as an illness. One of the most well-known examples of this is kidney stones – predominantly made of calcium and sodium compounds (1). As biology students, we were thus interested in studying crystals that grow inside our bodies.
- The main chemicals present in the kidneys and in kidney stones that concerned us were urea, sugars (glucose), sodium, and amino acid L-cystine.
- Present kidney stone treatments range from open surgery to diet change. Stones either pass out through the urine or must be attended to medically (2). We tested the possibilities of using physical vibrations and varying L-cystine concentration on crystal growth. It was predicted that decreasing solution concentration – larger urine volumes – would decrease crystal growth and that exposing the crystals to vibrations will disrupt their structural formation making it easier to break them apart after they have been formed or more difficult to grow from solution.

OBJECTIVES:

- To grow macro-crystals of urea and sodium nitrate and micro-crystals of urea, D-glucose, and L-cystine.
- Examine the effect of vibrations on the macrocrystals and their structural formation.
- Study the effect of varying concentration on L-cystine crystal growth.

MATERIALS AND METHODS

Table 1: List of chemicals used to make crystals and their solubility/concentration

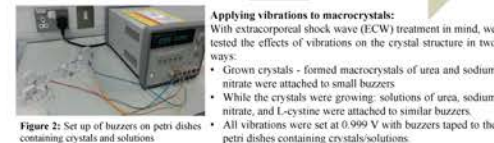
| Materials used: | Solubility and concentrations: |
|-------------------|--------------------------------|
| Urea | 1080g/L at 20°C |
| D(+) Glucose | 909g/L at 25°C |
| Sodium nitrate | 912g/L at 25°C |
| L-cystine | 50g/L |
| Hydrochloric acid | 6M (diluted to 1M) |

MACROCRYSTALS:

Supersaturation: crystals of Sodium nitrate and Urea



Evaporation to produce crystals of Sodium nitrate, Urea and L-cystine



MICROCRYSTALS:

- 10.82 g of urea and 9.14 g of D-glucose were dissolved separately in 20 mL of water each, 1.255 g of L-cystine were dissolved in a 25 mL, 1 M HCl solution.
- 3 μ L drops of the urea and D-glucose solutions were placed onto 96 different biological conditions on 4 screens.
- 3 μ L drops of calcium oxalate were added to 48 of the conditions and 3 μ L drops of L-cystine to the other 48 conditions.

RESULTS

MACROCRYSTALS

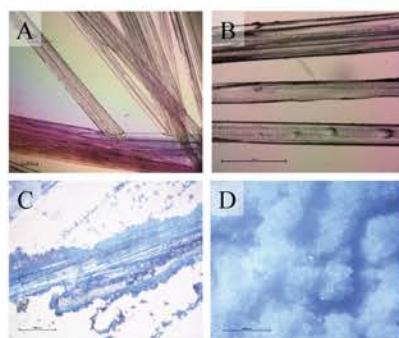


Figure 3: Macrocrystals of Urea. A and B show the long, needle-like structures formed in control conditions by evaporation. C is the result of a formed crystal being exposed to vibrations - the linearity of the structure has been disrupted to form rough edges. D is the result of urea solution being exposed to vibrations as the crystals are growing - crystals do not form properly, instead a powdery solid is the product.

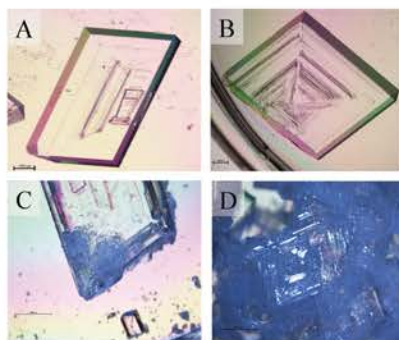


Figure 4: Macrocrystals of Sodium Nitrate. A and B clearly show the cubic structure of the Sodium nitrate grown in control conditions by evaporation. C shows the effects of vibrations on the Sodium nitrate crystal as seen in B. The fracturing around the edges can be clearly seen. D is the result of sodium nitrate solution as it was exposed to vibrations while the crystals were forming.

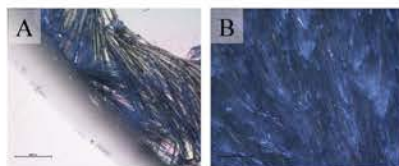


Figure 5: Macrocrystals of L-Cystine. A (at 0.04 g/mL of HCl) and B (at 0.05 g/mL of HCl) show the fine, crossed crystal needle structure of the crystals that form by evaporation.

MICROCRYSTALS

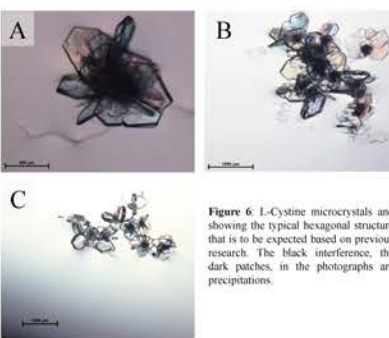


Figure 6: L-Cystine microcrystals and showing the typical hexagonal structure that is to be expected based on previous research. The black interference, the dark patches, in the photographs are precipitations.

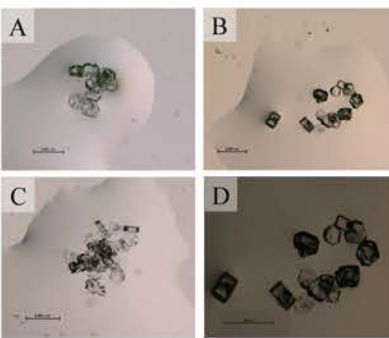


Figure 7: Microcrystals of L-cystine grown in condition 4 (0.1 M Bis-Tris, pH 6.5, 2M ammonium sulfate) at concentration of 0.04 g/mL of HCl.

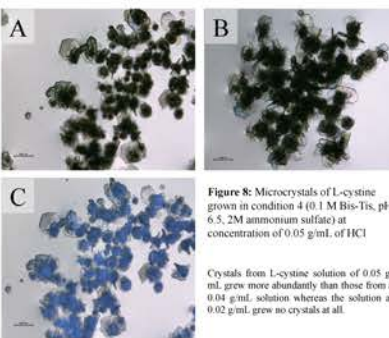


Figure 8: Microcrystals of L-cystine grown in condition 4 (0.1 M Bis-Tris, pH 6.5, 2M ammonium sulfate) at concentration of 0.05 g/mL of HCl.

Crystals from L-cystine solution of 0.05 g/mL grew more abundantly than those from a 0.04 g/mL solution whereas the solution at 0.02 g/mL grew no crystals at all.

DISCUSSION

MACROCRYSTALS

- When vibrations were applied to formed crystals, there was some fracturing of the crystal structure that caused rough edges to happen.
- When vibrations were applied to solutions as evaporation occurred, crystal growth was disrupted.
- The motional disturbance caused Urea to form a powder rather than a crystal, whilst Sodium nitrate formed smaller, less distinct crystals.
- The formation of L-Cystine crystals was unaffected by the vibrations applied during the evaporation process.
- The evaporation method produced higher quality crystals than when the supersaturation method was carried out. Supersaturation, the "sealed jar method", produced many fine crystals but none suitable for seed crystals. The evaporation method allows for a slower rate of crystal formation and so the crystals may grow larger in the petri dishes.
- In Figure 3 B, interesting node formations were observed on the needle-like crystal structures of the Urea crystal.

MICROCRYSTALS

- L-Cystine crystals formed in only 6 of the 48 screened conditions, these conditions were then used for following tests.
- L-Cystine forms microcrystals with a hexagonal structure.
- The glucose present in the first run mixed with the L-Cystine and contaminated the samples. This caused mould to form as glucose is a biological food source.
- The greater the concentration of L-Cystine, the larger the total microcrystal formed. The microcrystal is made up of multiple hexagonal structures together.
- Seed crystals suspended in saturated solution were able to grow larger as they act as a homogenous nucleus for further crystalline deposits to form.
- L-Cystine microcrystals grew in crystal screen conditions that were acidic, at pH 6.5 and 3.5. This hints at alkalization as a treatment for cystine-based kidney stones.
- Crystals grown from 0.05g/ml solution had green participate, due to the supersaturation of the sample being too high. The crystals formed stacks rather than clearly defined hexagonal shapes.

SUMMARY

Crystals exposed to vibrations had only minor fractures, which means this method would not be effective against formed kidney stones, and ESWL is still the best treatment for kidney stones, as the noise is of a higher energy. Constant motional disturbance for patients susceptible to recurring kidney stone formation would also be ineffective as L-cystine crystals would still form.

A concentration based approach may have the potential for alternative treatments to ESWL and surgery.

Areas of interest for further research would be whether an increase in the energy of the vibrations, by increasing the voltage through the buzzers, would cause greater fracturing and become a viable option for treatments. Based upon the conditions that L-cystine microcrystals grew under, analysis of the effects of pH would be of interest to see how physiological conditions play a part in the formation of such kidney stones.

MEDICAL APPLICATION

The ability to fracture and prevent crystals by motional disturbance could be a treatment to combat other crystal-based conditions, such as Gout – the build up of uric acid in the joints.

There are already reports on how changing one's diet can reduce kidney stones and our results concur that a reduced L-cystine concentration reduces crystal formation. A reduced concentration can be reached by increased urinary volume, by drinking more water, or by reducing the consumption of L-cystine by dietary management.

By identifying the optimised conditions for L-cystine crystal growth, this data may be used for improved diagnosis of Cystinuria, which can be identified in patients by the presence of L-cystine in their urine.

BIBLIOGRAPHY

- D Vilamathi, Leela Abraham, and S Gunasekaran. (2009) Growth of calcium oxalate monohydrate crystal by gel method and its spectroscopic analysis, *Indian Journal of Pure and Applied Physics* 46: 36-38.
- Worcester, Elaine M., and Frederic L. Cox (2010) Calcium Kidney Stones, *N Engl J Med* 363: 954-963.
- Daniel M. Asplin and John R. Asplin (2013) The Interaction of Thiol Drugs and Urine pH in the Treatment of Cystinuria, *The Journal of Urology* 6189: 2147-2151.
- Hampton Research Solutions for Crystal Growth. *HRC-144*: 1-4

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