Journal of Scientific and Technical Research
9 (1), June 2019, 11-18
http://www.publishingindia.com/jstr

Effect of pH and Sugar on the Morphology, Bulk Transparency and Thermal Characteristics of Kidney Stones

N. B. Singh[1*], Ching-Hua Su[2], Fow-Sen Choa[1], Brian Cullum[1], Bradley Arnold[1], Lisa Kelly[1], Neelesh Agarwal[1], Stacey Sova[1] and Christopher Cooper[1]
[1]Department of Chemistry and Biochemistry and Computer Science and Electrical Engineering
University of Maryland Baltimore, USA
[2]EM31, NASA/Marshall Space Flight Center, Huntsville, USA
*Corresponding author: singna@umbc.edu

Abstract: Kidney stone issues in humans have doubled since the early seventies. A variety of morphologies have been observed during stone removal from the kidney. The formation of these morphologies depends on various factors such as pH, temperature, impurities and composition of the nutrients remaining in the kidney after filtration. Growth kinetics, rate and morphology of stones depend strongly on the local concentration of insoluble species and nucleation surface in the kidney. In this paper, we have investigated the effect of pH, sugar ($C_6H_{12}O_6$) and magnesium as an excess impurity on the kidney stone growth. At lower pH of the solution fat prism crystals are observed. Whereas at higher pH (>7), long fat needle crystals with large aspect ratio (length to width) are observed. The coarsening experiments showed further growth in different shapes. The impurity enhanced the colorization. The re-melting experiments were performed to understand the dissolution process of stones, revealing that during dissolution the joining material breaks first leaving the large faceted crystals undissolved. In addition, it was also found that the effect of excess sugar caused significant variations in the morphology and produced brown colored stones. Crystals containing sugar also retained significantly higher quantities of potassium compared to undoped stones. Finally, the effect of additional impurities e.g. magnesium on stone formation and morphology were studied demonstrating the ability to produce mushroom and cauliflower type morphologies. Thermogravimetric analysis revealed that characteristics of stones with sugar were very different, with dehydration of stones taking continuously as function of temperature.

Keywords: Coarsening, Crystal, Growth, Impurity, Kidney, Needles, pH, Remelting.

I. Introduction

Studies by Harvard Medical School have shown [1] that kidney stone in human has been diagnosed in Egyptian mummies, and in the United States problem of kidney stones increased from 3.2% in the mid-'70s to 5.2% in the mid-'90s, and the rates are continuing to rise. In the past fifty years, growth of kidney stones in stomach and operations to take stones out of body have doubled in the world's population. Although there are no single reasons for the formation of kidney stones [1-4], several factors including increasing exposure to antibiotics and antacids increased the risks. A normal kidney is bean-shaped organ one on each side of the backbone and represent approximately 0.3%-0.6 % of the total weight of the body and each kidney contains more than million nephrons which are the microscopic structural and functional unit of the kidney. The nephron makes urine by filtering the blood and removes small molecules and reclaims useful materials. The waste molecules and ions flow out as urine. Although it will be very difficult to observe a study to correlate nanomorphology of nephron and performance will be of great importance to increase efficiency of kidney. There are various studies performed on the content of urine. However, it is estimated that in twenty-four hours, kidneys process large amount of sodium chloride, bicarbonate and glucose through several liters of water that entered the tubules. This causes water, small molecules and ions to filter through the capillary and is referred as nephric filtrate. At this stage some of the salt, glucose and active gradients are reabsorbed and some are removed with the water as urine. The resulting gradients of uric acid, urea, glucose, inorganic salts and amino acid vary on the intake of salt, glucose and water of the person as well as to their exposure to antibiotics. During the filtering process several less soluble salts and metallic impurities in the form of oxides, oxalates, and carbonates are left behind, with kidney reclaiming hundreds of grams (range of 200 g to 450g) of various compounds including sodium chloride and sodium bicarbonate a day. Because the pH range of 6.8-7.4, a typical kidney does not facilitate fast dissolution or decomposition of oxides and kidney stones can readily formed. In some instances, reduction of the kidney pH through the introduction of fluids

(e.g. lemon juice, etc.) has demonstrated temporary relief from the kidney stone formation. In addition to stone formation and its size, other important factors in understanding the variability of kidney stone discomfort depends on the morphology of the stone, its composition, size and effect on body. Typical values of the constituents of kidney stones are given in Table I. The typical morphologies reported by the Herring Lab are shown in Fig. 1. In this work, we have characterized the effects of pH, sugar and MgO on the formation and morphology of stones.

Table I: Typical Composition of Stones Obtained from Human Kidney

Major Constituting Material	*Approximate Composition (%)*
Calcium oxalate (carbonate)	75
Calcium phosphate	5
Mixed oxides	5
Uric acid	<2-10
Oxidized polymers (dimers) of amino acids	0.1 -1.0
Cystine ($C_6H_{12}N_2O_4S_2$)	0.1 -1.5
Miscellaneous metal salts and oxides	<1.0
Sugar (Glucose)	0-0.01

These values differ from person to person depending on the medications of the person [2, 3].

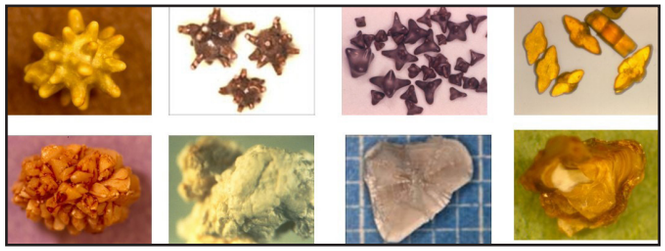


Fig. 1: Morphologies of Kidney Stones Reported by Herring Lab ([2] and www.Herringlab.Com, with their permission)

These structures were reported by Herring laboratory lab from real kidney stones.

II. Experimental Methods and Results

The details of preparation of mixtures and solution growth process were similar to that reported [5-7] previously. However, unlike the growth of vanillin, we did not use any organic solvents in the present study. We have performed two sets of experiments using the calcium salts as major component. In the first case we used calcium carbonate and in the second set we used calcium oxalate as the base material to evaluate effect of carbonates and oxalates.

A. Material Preparation, Purification and Reaction

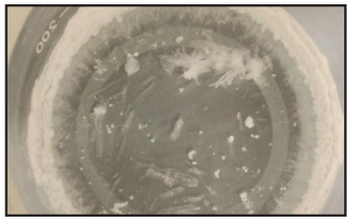
Calcium carbonate ($CaCO_3$) 99.9% and glucose 98% (sugar) purchased from Sigma Aldrich were used in this study. No further purification was performed. All these parent components were mixed and dissolve in dilute acetic acid. Except urea, there was no additional organic added in solution. The pH was controlled by adding solution of sodium hydroxide (NaOH). During the early stage of the reaction, 2 mL-5 mL of nitric acid was added to enhance the dissolution of calcium salt. Sodium from the sodium hydroxide was used as the nutrient for sodium during synthesis.

B. Growth from Solution

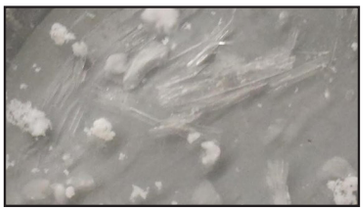
Solution growth method was used in this study. The solution was prepared in the water-acetic acid solvent. The original solution contained a 3:1 ratio of water to acetic acid. The solution was prepared using weight percentage of $CaCO_3$, Urea, and sodium salt in 70:20:10 ratios. The amount of sugar was small (10 mg). This amount was dissolved in 200 mL solvent. Finally, NaOH concentration and acetic acid were added to change the pH. During the preparation of solutions, white oxide residue precipitated in some cases and nitric acid was added to dissolve these oxide residues. The solution constituting material was heated to 80 °C to completely dissolve all constituents and to achieve transparent solution before starting the growth. The temperature was lowered to 45 ^oC and kept for few hours to achieve homogeneity.

C. Effect of pH on Growth and Morphology

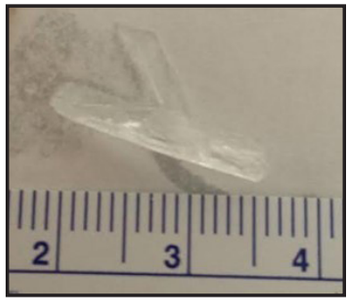
It is well established [6-13] that the quality of crystals, morphology and segregation in crystals depend on several growth factors including composition of the constituents in the solution, cooling rates, thermal and solutal flows during growth and impurities. Effect of pH on morphology was studied by adding acetic acid in growth solution. The volume and physical dimension of the all test solutions were kept identical to avoid the differences due to thermal and diffusive flow in the solution. The solubility is the most important parameter for the growth of crystals from solution. The solubility of calcium carbonate in water-acetic acid is known, however, addition of urea, sugar, NaOH and other impurities significantly affect it. Therefore, it was not possible to use solubility data of pure carbonate for recrystallization. When self-cooling of the solution from 45 °C, to room temperature was allowed to occur, uncontrolled nucleation and growth with trapped white precipitates between needle crystals was observed. These recrystallization materials were bundles of needles attached to polycrystalline precipitates of very small sizes Fig. 2 (a) Fig. 2 (b) and Fig. 2 (c) shows morphology of two crystals harvested from solution. Due to fast growth rate and added impurities, striations were observed in the bulk and material was not completely transparent.



(a)



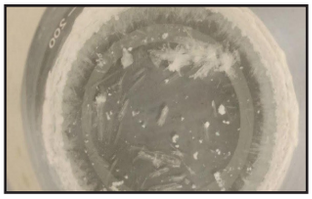
(b)



(c)

Fig. 2: (a) Nucleation Started in the Solution (Diameter of Container 8.5 cm), (b) Entrapment of Multiple Crystals During Uncontrolled Cooling in the Solution and (c) Morphology of a cm Size Crystals Harvested from Uncontrolled Growth from the Solution

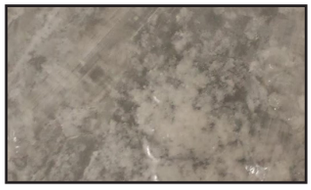
Several growth experiments were performed at pH values of 1, 3, 5, 6 and 8. In these studies, the temperature of the solution was raised to 45 °C before being lowered to 35 °C at the rate of 1K/3hours for the growth. The seeded crystal growth was performed by adding a small seed harvested from earlier uncontrolled growth experiment. Since the fluid flow in the solution has pronounced effect on the morphology, solution was not stirred. Although, some stagnant layers formed at the growth interface due to lack of stirring, the volume of the solution was large enough to provide nutrient during cooling of the solution. Fig. 4 shows morphology of crystals grown at several pH values. The experimentally observed data revealed that hard precipitates were formed at lower pH (pH=1). This solution was very acidic causing dissolution of some of the nuclei, with solid aggregates of small crystals growing while neighboring crystallites disappeared. Nucleation and growth at the pH = 3 showed the transition from hard multi-crystalline morphology to dendritic morphology, indicating free nucleation in front of the growth interface of main grain. These two morphologies are very different than growth morphologies of pure $CaCO_3$ crystals. In addition, observations at higher magnifications revealed that solid crystal layers of dendritic arrays.



(a)

(b)

(c)



(d)

(e)

Fig. 3: Morphology of Crystals Grown at (a) pH=1, (b) pH=3, (c) pH=5, (d) pH=6 and (e) pH=8 Showing Different Type of Morphologies in Solution Indicating that pH Affects Nucleation and Shape

As the pH was raised to 5, there is a transition from dendritic morphology to faceted morphology. Crystals grown at higher

pH (pH5 and pH6) showed plate morphology. For crystals grown at these high pHs, it was observed that even at low saturation, smaller grains (plates) dissolved and larger grains kept growing similar to coarsening phenomena.

D. Bulk Transparency of Crystals

Crystals grown at high pH (>7.5-8.5), showed large aspect ratio were observed. These crystals are transparent and good quality. These crystals (Fig. 4) were free from gross defects such as bubbles, voids, precipitates and cracks and demonstrated low gross defects such as surface roughness and line defects. The observed morphologies have similarity to that reported by Herring Laboratory for real kidney stones. Similar to that of vanillin morphology [6], pH has significant effect on the shapes and morphologies. To evaluate the effect of aging on the optical quality and morphology, we left the growth solutions at 30 °C for a period of 15 days. The sizes in this case grew to larger crystal. However, the morphology of the crystal did not change.

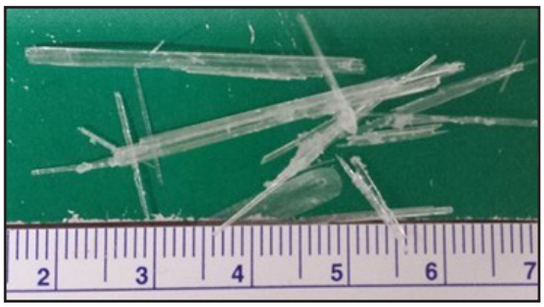
Fig. 4: Different Size of Transparent High-Quality Crystals Grew at pH=8 in the Solution. These Crystals were Harvested for Characterization

(a)

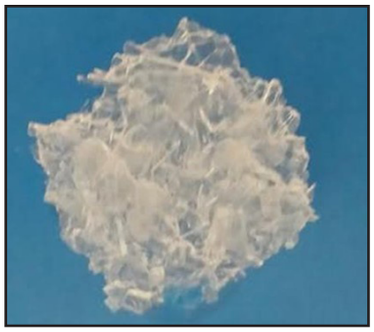
(b)

Fig. 5: Re-Melting Characteristics of Grown Crystals

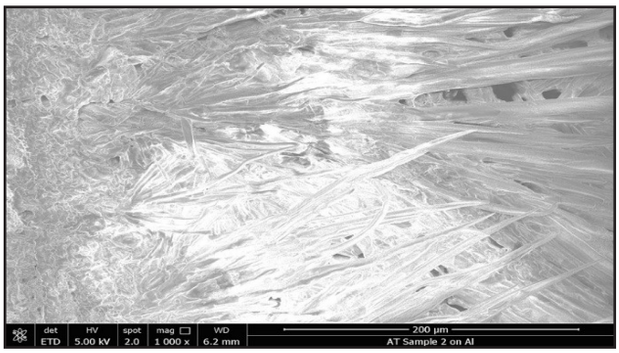
We performed re-melting experiments by raising the temperature to evaluate effect of heat on the transparency and morphology. Fig. 5 shows the re-melting characteristics. It was observed that small polycrystalline translucent material which crystallized between needles melts first and large needles break in the part of melting. This clearly indicates that during dissolution of kidney stones the large facets break into small crystallites. There is no sign of layered growth or growth steps in large needles. The crystal growth of calcium urate [7] indicated needle morphology as shown in Fig. 6 (d). To compare the results with experiments of carbonate based solution, we prepared a solution where calcium carbonate was replaced by calcium oxalate keeping all other parameters identical. The oxalate salt has higher solubility than carbonate which helps in doping. The pH was kept in the range of 4. The nucleation and growth is shown in Fig. 6. As shown in Fig. 6 (a) the nucleation started very similar to that in Fig. 2 (a). However, growth was very anisotropic and long needles are observed. Fig. 6 (b) is a coarsened bundle of needles shown in Fig. 6 (c). Needles observed were very similar to that [8]. Based on reported morphology of kidney stones [2-4], it appears that the aspect ratio for the crystal is very large and unrealistic for human kidney stones. Also, as reported [8], this was observed for the high purity calcium urate. In a real situation, it is expected that calcium urate will be contaminated with varieties of metallic and nonmetallic impurities in a kidney. Since impurities modify kinetics and morphology, needles can easily change into plates or dendrites during the formation or coarsening. As a result, the morphology of kidney stones does not remain needles with very large aspect ratio.



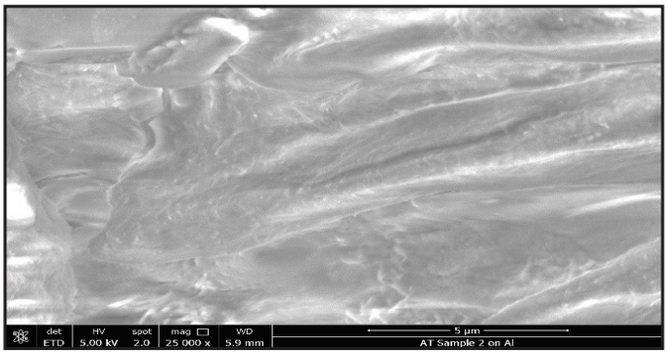
(a)



(b)



(c)



(d)

Fig. 6: (a) Nucleation of Large Aspect Ratio Needles was Observed in Solution, (b) Needles Coarsened Bundle of Semi-transparent Hopper Crystals (c) Morphology of Entangled Needles to Make Bundles of Needles and (d) Morphology of Needles at High Magnification

E. Effect of Excess Magnesium Impurity

Impurities have been shown to exhibit pronounced effects on the morphology of organic crystals [9-13] by decreasing the growth rate in melt and modify the nucleation as well as the micro morphology. In addition, impurity driven hardening is very common. Since numerous impurities are present in the real system, the magnesium ions being most abundant, effect of Mg impurity on stone morphology was investigated. Doping of MgO impurities in the range of 5% has changed the nucleation, growth and morphology. The typical morphology of Mg ion doped crystal is shown in Fig. 7. This cauliflower morphology has similarity with reported morphology of struvite. The mechanism of the modification is very complex since it affects the growth interface, segregation and hence magnesium changed the nucleation and growth morphology. The preliminary results indicate that growth starts with nucleation forming spherulytes (mushrooms). As shown in Fig. 7 the morphology consists of many small identical mushrooms forming cauliflower type structures. Also, these structures are very hard compared to undoped material.

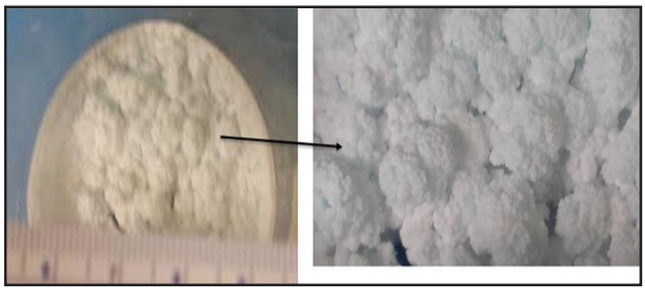


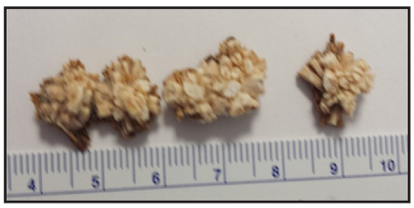
Fig.7: Effect of MgO Addition in the Morphology at pH=6-6.5. MgO Addition Produced Cauliflower Type Morphology. These Structures were Hard

F. Effect of Sugar

Experiments were performed to study effect of sugar ($C_6H_{12}O_6$) on kidney stones by preparing the stoichiometric composition listed in Table II. The amounts listed in Table II were dissolved in 15 ml acetic acid and 40 ml water by heating up to a temperature 60 °C. When we heated sample for few minutes, the solvent containing beaker started turning slightly brown. We reduced the temperature slowly to cooldown to room temperature. Since the goal was to grow crystals at room temperature, the solution was placed at room temperature for a period of 150 hours.

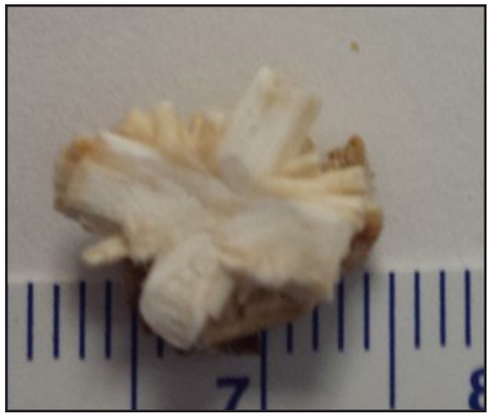
TABLE II: COMPOSITION OF THE CONSTITUENTS USED IN PREPARING STONES TO EVALUATE EFFECT OF SUGAR

Major Constituting Material	*Amount (g)*
Calcium carbonate	3.05
Trisodium phosphate	0.31
Urea	1.20
Sucrose	0.24
Magnesium Oxide	0.27



(a)

(b)



(c)

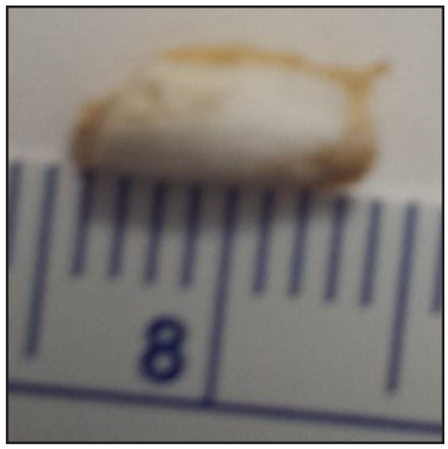
Fig. 8: (a) A Thin Rough Layer of Impurities in the Bottom Act Like Nucleus Followed by Growth of Crystallites Bunched Together, (b) Growth Morphology is Similar to That of Contaminated Uric Acid with Calcium Phosphate at Early Stage (c) Coarsened Structure of Uric Acid where Small Grains Disappear to form Larger Grains

The Morphology was Similar to Real Kidney Stones Reported [2].

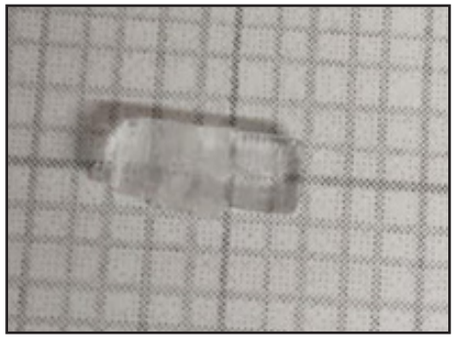
Fig. 8 (a) shows that reaction product after heating to 60 ^{o}C and cooling to room temperature. The calcium acetate produced by the reaction of calcium carbonate and acetic acid reacts with sodium phosphate solution. The temperature of the solution was lowered to a temperature of 35 ^{o}C and kept for 2 hours. During this period when small crystallites were formed. When we cooled down the solution to 25 ^{o}C in a period of 5 hours (cooling rate 3 ^{o}C/hr) small crystallites were observed. Morphology indicates the formation of a rough layer of nucleus in the bottom followed by multiple grains which grow on the top of each other. As shown in Fig. 8 (b) some crystals grew longer than cm size. Crystals demonstrated anisotropic growth behavior. These grew faster in one crystallographic direction compared to other. Also, as crystals grew longer, small crystallites merged into larger grains and tend to form structure like Fig. 8 (c).

G. Micromorphology of Un-Doped and Sugar Doped Materials

We harvested large grains from a sugar doped solution for comparison with un-doped crystal. As shown in Fig. 9, there is huge difference in transparency, color and overall quality. Effect of sugar is responsible for the colorization and thin fibrous layer on the surface growth on the surface Fig. 9 (a). Un-doped crystal and Fig. 9 (b) was transparent and free from gross defects such as bubbles and voids. Fig. 10 shows surface morphology at high magnification. Un-doped crystal showed some ridges between the planes. These small ridges disappear after the crystal was left in the solution and transparent fat crystal. As shown in Fig. 10 (b) sugar doped crystals had interface breakdown during the growth. This is common in presence of impurities which segregate at the growth interface. Also, in between layers there were small crystallites oriented randomly. Fig. 10 shows 10 shows irregular micromorphology of crystal at high magnification indicating presence of small structures in between layers.



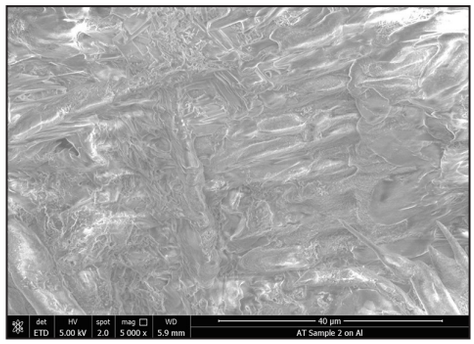
(a)



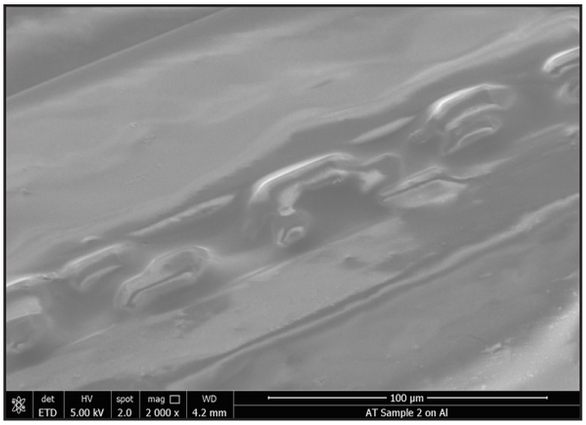
(b)

Fig. 9: Bulk Transparency of (a) Doped and (b) Undoped Crystals

The doping destroyed the transparency and quality of the crystal due to entrapment and composition of the stones



(a)



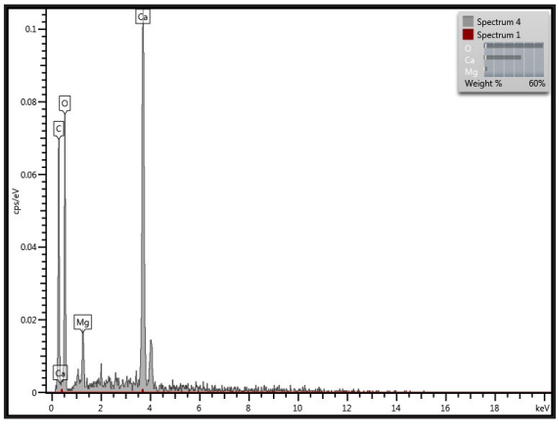
(b)

Fig. 10: Micromorphology of Sugar Doped (Shown in Fig. 9(a)), and Undoped Crystals (Shown in Fig. 9 (b)) at High Magnification

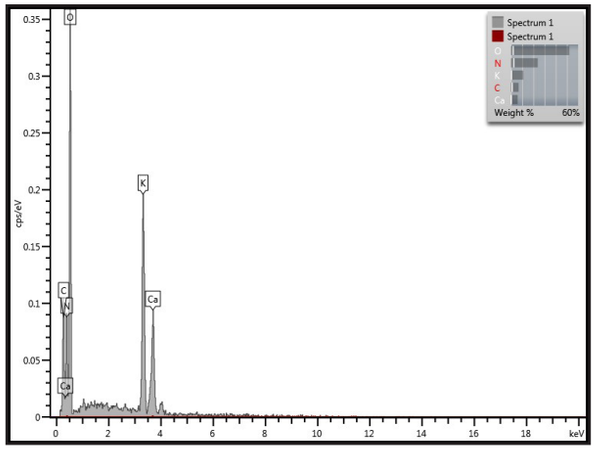
Sugar doping caused surface breakdown causing multiple grains and misorientations. This caused deterioration in quality.

H. Compositional Analysis (xps) Analysis

Both pure and sugar doped crystallized stones were studied for compositional analysis. The sample surface was exposed to monochromatic X-rays (10 KeV) and the ejected electrons' kinetic energies and intensities were measured simultaneously. Electrons are emitted from the top 10 nm layer. Because the incident X-ray photon energy and ejected electron kinetic energy are known, binding energy of the electron is estimated. The elements were identified from the binding energy values. The measurements were used for sample constituents, and their abundances were determined. The technique is suitable if the dopant concentration is 0.1% or higher. Experimental results of compositional analysis are shown in Fig. 11. Results of Fig. 11(b) showed that crystal doped with sugar had retained higher concentration of potassium compared to pure sample which revealed no sodium or potassium in the crystal. It appears that in presence of sugar higher concentration of potassium segregated in the stone and did not completely dissolve in water.



(a)

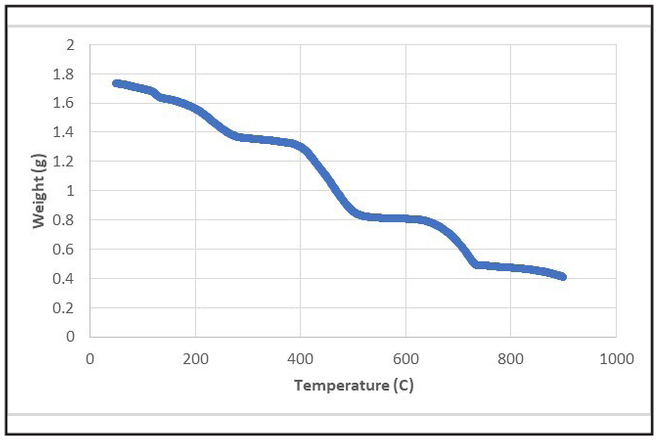


(b)

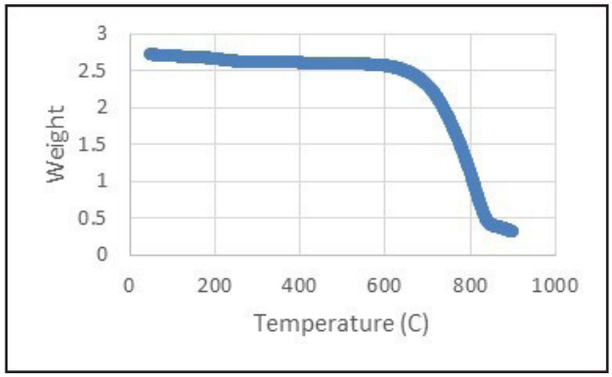
Fig. 11: Compositional Analysis of Crystals Grown from the Nutrient (a) Without and (b) with Sugar in the Solution. Sugar Addition Showed Higher Content of Carbon and Decrease in Calcium

I. Thermal Characteristics

Thermogravimetric analysis of the samples with and without sugar was also performed to understand the decomposition of kidney stones. Experiments were performed using 1.7 g and 2.7 g samples. Samples were purged with Helium. The temperature range investigated was 50 °C to 900 °C with a rate of heating 50 °C minute. Results are shown in Fig. 12 for both samples. The sugar containing sample started decomposing at low temperature. It showed some steps in the decomposition curves. For example, it decomposed when temperature was raised to 275 °C. It showed stability from 275 °C to 400 °C, and then 500 °C to 650 °C temperature range. This step decrease indicates that sugar ($C_6H_{12}O_6$) may cause formation of low temperature decomposition products in the crystals. As shown in Fig. 12 (b) the un-doped sample did not show any significant decomposition up to 650 °C. It started decomposing at approximately 650 °C. Both samples completely decomposed above 700 °C.



(a)



(b)

Fig. 12: Decomposition Characteristics of Samples (a) Doped with Sugar and (b) Undoped Sample

The sugar doped crystal showed continuous decrease weight loss as function of temperature while as pure crystal showed sharp melting.

III. Conclusion

Effect of pH, sugar and impurities on the morphology, thermal and optical characteristics of kidney stones was studied. We observed cm size faceted plates, long needles and dendrites as a function of pH. We have performed experiments using carbonate, oxides and urea to simulate and understand the morphologies of the residue filtered and coarsened in different conditions. It was observed that morphologies are affected by impurities significantly. The remelting studies indicate that during dissolution of kidney stones the joining polycrystalline material breaks first and faceted crystals dissolve in the later part of dissolution. These crystals slowly dissolve into small crystallites. It is very much dependent on the acidity of the fluids. This pH variation affects the content and amount of filtering residue and its morphology. Concentration measurements showed that that crystal doped with sugar had retained higher concentration of potassium compared to the pure sample which did not show sodium or potassium in the crystal. Sugar doped sample started decomposing at very low temperature and showed steps wise decomposition. We observed that different of morphologies of kidney stones can be explained based on acidity and impurities. Crystals with sugar showed continuous decomposition starting at low temperature.

Acknowledgements

The authors would like to acknowledge the supports of Space Life and Physical Sciences Division, Human Exploration and Operations Mission Directorate, NASA Headquarter. The authors are also grateful to Northrop Grumman for providing the chemicals used in this project.

Disclosure

The authors have no relevant financial interests in this article and no potential conflicts of interest to disclose.

References

[1] R. H. Shmerling, *Kidney Stones are on the Rise*, Harvard Health Publishing, Harvard Medical School, February 12, 2016.

[2] Glenn Austin, *Integrated Approach to Kidneystone Analysis*, Louis C. Herring Corporation (Private Communication), July 2018.

[3] S. D. Rosa, M. Antonelli, and C. Ronco, "Hypothermia and kidney: A focus on ischaemia–reperfusion injury," *Nephrology Dialysis Transplantation*, vol. 32, no. 2, pp. 241-247, 2017.

[4] H. E. Möller, A. Gaupp, K. Dietl, B. Buchholz, and T. Vestring, "Tissue pH in human kidney transplants during hypothermic ischemia," *MagnReson Imaging*, vol. 18, no. 6, pp. 743-751, 2000.

[5] N. Agarwal, S. Sova, N. B. Singh, B. Arnold, F. S. Choa, B. Cullum, and C. H. Su, "Effect of pH on the morphology of kidney stones," *Proc. SPIE* 9863, *Smart Biomedical and Physiological Sensor Technology XIII,* 986303, 13 May 2016.

[6] O. P. Singh, N. Singh, Y. P. Singh, and N. B. Singh, "Growth of vanillin crystals for second harmonic generation applications in near-IR wavelength region," *J. Crystal Growth*, vol. 225, no. 2, pp. 470-473, 2001.

[7] N. B. Singh, T. Henningsen, E. P. A. Metz, R. Hamacher, E. Cumberledge, R. H. Hopkins, and R. Mazelsky, "Solution growth of vanillin, crystals," *Materials Letter*, vol. 12, no. 4, pp. 270-275, 1991.

[8] J. B. Presores, K. E. Cromer, C. Capacci-Daniel, and J. A. Swift, "Calcium urate hexahydrate," Cryst. Growth Des., vol. 13, no. 12, pp. 5162-5164, 2013.

[9] N. B. Singh, "Solidification behavior of eutectic; naphthalene-p. chloronitrobenzene system," *J. Crystal Growth,* vol. 43, no. 4, pp. 637-640, 1978.

[10] R. P. Rastogi, N. B. Singh, and N. B. Singh, "Chemistry of organic eutectics: II. The naphthalene-p-chloronitrobenzene eutectic system," *J. Crystal Growth,* vol. 37, no. 3, pp. 329-334, 1977.

[11] R. P. Rastogi, D. P. Singh, N. Singh, and N. B. Singh, "Chemistry of binary and ternary eutectics," *Mol. Cryst. & Liq. Cryst*., vol. 73, no. 1-2, pp. 7-34, 1981.

[12] N. B. Singh, and M. E. Glicksman, "Effect of anisotropy on dendritic morphology," *J. Crystal Growth,* vol. 98, p. 277, 1989.

[13] U. S. Rai, O. P. Singh, and N. B. Singh, "Slidification behavior of organic eutectic," *J. Chim. Phys.*, vol. 84, pp. 483-487, 1987.