



Growth Study of Calcium Doped Urinary Struvite-K Crystals in Agar Gel

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Abstract: This study observes the effect of foreign ions on the surface of struvite-K crystals. The dopant like calcium in the form of chloride solution was used in supernatant solution. Reported that the impact of calcium on struvite crystals; however the struvite-K is also an analogous to struvite. Due to urinary importance, in the present study, the influence of foreign calcium impurities on the growth of struvite-K was observed. It was found that, calcium ions transform the morphology of struvite-K crystals. The different morphologies such as transparent platy shape, twin shape dendritic, platy shape, polycrystalline shape, translucent spherical as well as multifaceted twinned crystals growth was observed under the stereo microscope. The morphologies seen in stereo microscope images were significantly affected due to dopant reactant transforming from rectangular bar as well as needle shaped crystals to as multifaceted twinned crystals. The initial habit of crystals was changed due to impact of additive on struvite-k crystals.

Index Terms – Urinary Crystals, Doped Struvite-K, Agar Gel, Multifaceted twinned crystals, Single diffusion.

1. INTRODUCTION

Struvite crystals are made up of mixture of magnesium, ammonium, phosphate, and calcium carbonate. These stones are formed due to infection with certain types of bacteria that can produce ammonia. Ammonia acts to increase the pH of the urine, making it alkaline and promoting the formation of struvite. Struvite and struvite-K ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ and $\text{MgKPO}_4 \cdot 6\text{H}_2\text{O}$, respectively), are the components of infectious urinary crystals. Their precipitation occurs when the urinary tract is infected by bacteria. [1] The growth of crystals can be due to, frequently associated with renal tissue damage [2]. In the different cases, it was found that the crystal growth was controlled/inhibited by acting on thermodynamic parameters, such as pH and temperature, the supersaturation level, or by additives [3-5]. Calcium is an important structural and functional element of the body, and has been a part of normal diet. That calcium not use by the body elements can be goes to kidney and flushed out by the urine. Excessive calcium in urine combines with the other waste products to form solid crystals in kidney. Approximately 70-80% of the kidney stones in industrialized countries are calcium oxalate and calcium phosphate [6].

Agar gel method is used as the growth media. The preset paper is the continuation studies of struvite-K crystal [7-9], carried out to observe the effect of different additives on the nucleation, growth and influence on formed crystals. So many investigations have been carried out on the effects of foreign ions on the crystal growth of struvite [9-11]. However, the impact of calcium on the growth of struvite-K was not yet reported. The effect of such foreign ions on the surface of struvite-K is very important in urolithiasis. Hence the present studies focus on the impact of calcium ions on the growth morphology of struvite-k using single diffusion techniques in agar gel by at ambient temperature.

2. EXPERIMENTAL

Optimized condition for the growth of Struvite-K crystals in agar-agar gel by single diffusion technique was discussed in the preceding paper [7]. This study is the extension of such experiment, for to observe the effect of foreign ions on the surface of struvite-K crystals, we add the dopants like calcium in the form of chloride solution in supernatant solution. Kristell et al [12] investigated the impact of calcium on struvite crystals; however the struvite-K is also an analogous to struvite. Due to urinary importance, in the present study, the influence of foreign calcium impurities on the growth of struvite-K was observed. The optimized conditions for the growth of undoped struvite-K crystals as reported in preceding paper [7] were slightly changed. To observe the effect of calcium ions on the growth of struvite-K crystals the calcium in the form of chloride can be incorporated in the supernatant solution. The growth kinetics and morphological effect of calcium chloride on the pure struvite-K crystals were observed by changing different growth parameters. **Table 1** shows different growth parameters of struvite-K crystals grown in calcium chlorides.

Table 1: Growth parameters of struvite-K crystals grown in calcium chloride.

Set No.	Concentration of Mg (CH_3COO) ₂ .4H ₂ O I Reactant (M)	Concentration of KH ₂ PO ₄ II Reactant (M)	Concentration of CaCl ₂ (Additives) (M)	Volume of Additives (ml)	Percentage of Agar-agar gel (%)
1	1.0	1.0	0.25	1.0	1.0
2	1.0	1.0	0.25	2.0	1.0
3	1.0	1.0	0.5	1.0	1.0
4	1.0	1.0	0.5	2.0	1.0
5	1.0	1.0	1.0	1.0	1.0
6	1.0	1.0	1.0	2.0	1.0

For the experiment single glass tubes of 25 mm in diameter and 20 mm in height were used as the crystallization vessels. One gm of agar-agar powder (Himedia) was used in 100 ml double distilled water to prepare gel at concentration of 1.0 %. The prepared hot agar-agar solution was then mixed with an aqueous solution of 1.0M magnesium acetate (Qualigens) in appropriate amount and was allowed to set. Once the gel was set in different test tubes, 20 ml supernatant solution containing 1.0M potassium di hydrogen phosphate (KH_2PO_4 -Merck) and 0.25M, 0.5M and 1.0M manganese chloride (Qualigens) solution in 1 ml and 2 ml volumes was slowly poured over the set gel. Then the ends of tubes were tightly closed by cotton plugs and kept undisturbed for crystallization. After crystallization of forty five days, the struvite-K crystals grown in Ca^{2+} were found in different morphologies

2.1 Effect of Calcium Chloride for volume of 1ml:

Figure 1 shows the growth of struvite-K crystals in 1 ml volume of calcium chloride. In 0.25 M, large numbers of small size crystals were observed in the higher depth of gel-liquid interface. However, in 0.5 M concentration, the growth was also at below the gel liquid interface, while in increasing concentration (1.0M), it was observed that the nucleation growth was from below the gel-liquid interface. Small spherulite crystals were also seen in this doping experiment.

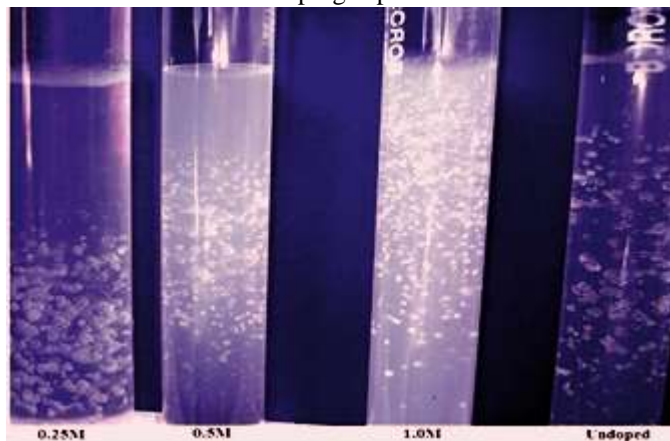


Figure 1: Struvite-K crystals grown in 1ml calcium chloride solution of 0.25M, 0.5M and 1.0M concentration.

2.2 Effect of Calcium Chloride for volume of 2ml:

To observe the effect of additive volume, 2 ml calcium chloride solution was used. **Figure 2** shows the growth of calcium doped struvite-K crystals.

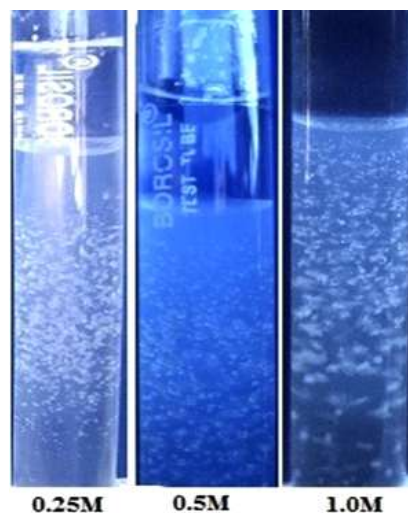


Figure 2: Struvite-K crystals grown in 2ml calcium chloride solution of 0.25M, 0.5M and 1.0M concentration.

In 0.25 M concentration, large numbers of small size crystals were observed at the middle of gel column. However in 0.5 M concentration, the spherulite growth was seen at higher depth of the gel liquid interface. While, in 1.0 M concentration, the growth was started from the gel-liquid interface. The apparent shape and size of the crystals those are grown in depth of gel column was increased. After forty five days of crystallization, calcium doped crystals were harvested from gel and observe under “CZM4 LABOMED” stereo microscope with magnification of 4x, 8x.

3. RESULT AND DISCUSSION

Calcium doped struvite-K crystals were grown in agar- agar gel. As compared to undoped struvite-K crystals, there has been significantly changed in the grown pattern of the similar crystals those are grown by using calcium ions. In case of calcium added experiment, when the impurity of calcium ions is increased from 0.25 M to 1.0 M, the growth of different shaped crystals was started from gel-liquid interface. The shape and size of doped struvite-K crystal was affected by the impurity. It was found that, the volume of additive also influence on the crystallization. When the volume of Ca^{2+} additive was increase, it increases the growth rate of platy shape crystals as well as spherical shape spherulite crystals shown in **Figure 3**. The different morphologies like star shape, polycrystalline, twin shape dendrites, prismatic and spherulitic crystals were observed as shown in **Figure 4**. For better understanding the changes in morphology due to Ca^{2+} doped struvite-K crystals were focused by Optical microscopy.

4. CHARACTERIZATION

Optical Microscopy

The optical microscopy provides a precise reliable method for identifying the morphological, structural and crystalline components [13]. The microscopic morphology of the grown crystals was studied using a “CZM4 LABOMED” stereo microscope with magnification of 4x, 8x and the pictures were recorded by the camera eyepiece. Figure 3 shows the microcoscopic morphology of Ca^{2+} doped struvite-K crystals.

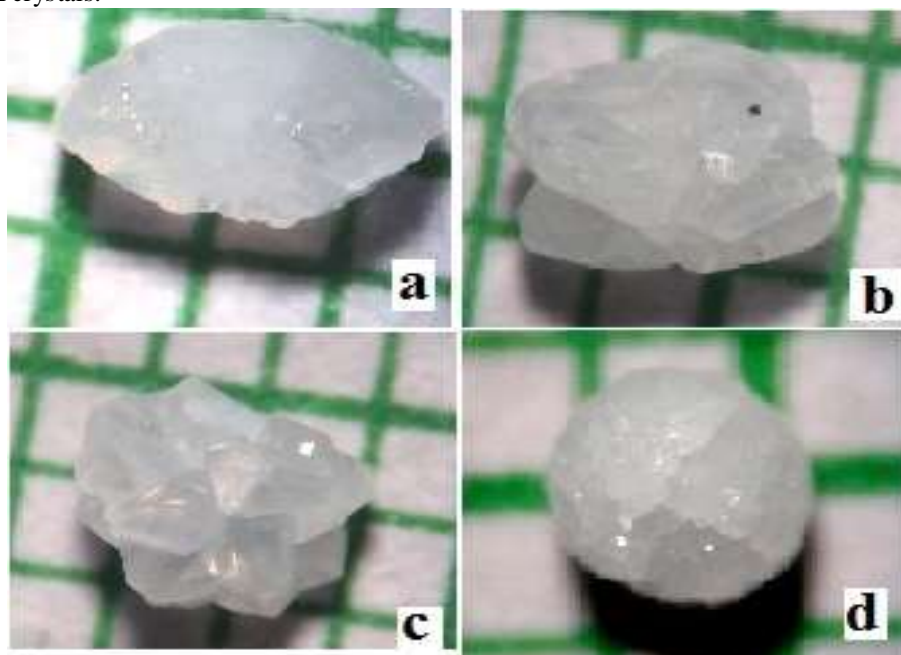


Figure 3: Harvested Struvite-K crystals grown in calcium chloride.

The different morphologies such as transparent platy shape, twin shape dendratic, platy shape, polycrystalline shape as well as transulant spherical growth was observed under the stereo microscope. When the struvite-K images of Ca^{2+} doped compared with undoped sample [7], it was observed that the calcium ions affect the morphological growth of struvite-K crystals. As seen in Figure 3(a), the oval shape was found to be affected by this impurity. Actually, the oval shaped struvite-K crystals grown in the absence of calcium has sharp tips, was changed due to presence of additive. The twin shape, aggregates of platy crystals and the scaly rounded morphology (seen in Figure 3-d) was the result of doping effect of calcium ions. The surface area of spherical crystals was increased due to increasing the concentration and volume of calcium ions. In the twinned shape (Figure 3- b), one platelet elongated in the longer direction grows on the top of other crystals. The edges of such twinned crystals are parallel to each other. However in polycrystalline growth, the crystal edges were found to be radiated from the center as shown in Figure 3(c). The presence of impurities in the solution affects the growth rate of crystals by blocking of active sites and inhibiting the increase of crystal size [14].

The calcium doped struvite-K crystals were also formed as multifaceted twinned crystals as shown in Figure 4.

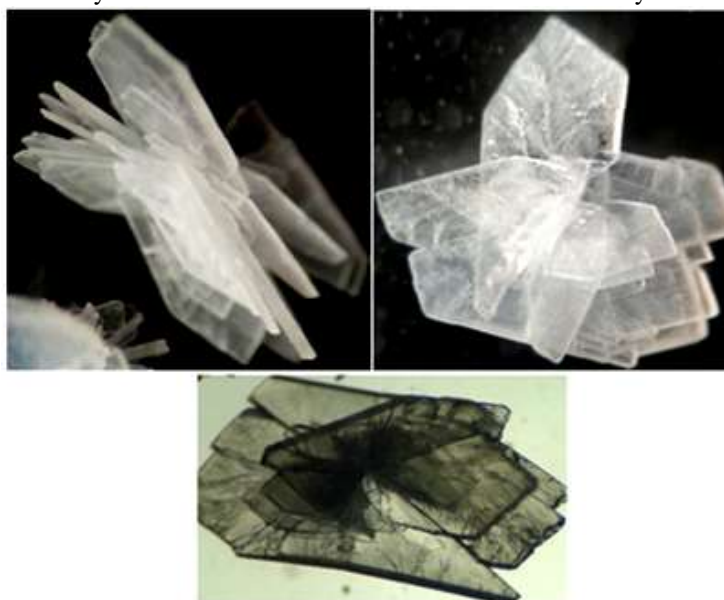


Figure 4: Aggregates of platy Struvite-K crystals grown in calcium chloride.

It was seen that the faces of the crystal was very soft, thin and devoted of any shape. In some crystals, it was observed that the faces were developed in hexagonal form. The reason may be due to the independent nucleation of separate crystals. The platy formation is also due to periodic incorporation of impurities caused by variation in microscopic growth [15-16].

5. CONCLUSIONS

The Struvite-K crystals are successfully grown by calcium doped in agar- agar gel by single diffusion techniques. It was found that the calcium doped struvite-K crystals were relatively in different morphology such as transparent platy shape, twin shape dendratic, platy shape, polycrystalline shape as well as transulant spherical growth was observed under the stereo microscope. The calcium doped struvite-K crystals were also formed as multifaceted twinned crystals. As well as they observed that the faces of the

crystal was very soft, thin and devoted of any shape like in hexagonal form. Thus the incorporation of impurities was changes the morphology of struvite-K crystals growth in agar gel.

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