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Surface Topographical Study of Manganese Doped Urinary Struvite-K Crystals Grown in Agar Gel

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Abstract: The impact of manganese on the growth of urinary struvite-K crystals was studied using the spectroscopic techniques, which included optical microscopy and SEM. It was found that, manganese ions transform the morphology of struvite-K crystals. The different morphologies such as translucent twins, x- shape, dumb-bell shape multiple faces dendratic as well as reddish color spherulites were observed under the stereo microscope. The morphologies seen in the SEM images were significantly affected due to dopant reactant transforming from rectangular bar as well as needle shaped crystals to layer by layer growth pattern. The change in initial habit of crystals was due to impact of additive on struvite-k crystals.

Index Terms **– Urinary Crystals, Doped Struvite-K, Agar Gel, Single diffusion.**

1. INTRODUCTION

Crystals occur in urine when there are too many minerals in the urine and not enough liquid. This tiny piece of masses in urine is called crystalluria. Some crystals don't cause problems. Others can get big and form stones that get stuck in parts of your urinary tract and cause blockages. Blockages can cause serious problems, like acute kidney injury (AKI), which is also called acute renal failure [1] Such urinary crystals are deposited in the urinary system, which belong the pair of kidney, ureters, a bladder, and urethra [2]. 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 [3]. Struvite ($MgNH_4PO_4.6H_2O$) is also one of the renal and vesical calculi which is common constituents of magnesium phosphates. A series of orthorhombic struvite analogues crystals were also reported in the literature. One of analogous compound of Struvite is potassium magnesium phosphate hexahydrate ($KMgPO_4.6H_2O$), known as Struvite-K crystals is natural minerals as well as found in animal urinary calculi [4-6]

Agar gel method is used as the growth media. The preset paper is the continuation studies of struvite-K crystal [6-8], 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 [9-12]. However, the impact of manganese 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 and hence the effect of manganese on the growth of struvite-K is studied. So the present studies focus the impact of manganese ions on the surface morphology of struvite-K, those are grown in agar gel by single diffusion techniques 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 [6]. 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 Mn^{2+} in the form of chloride solution in supernatant solution. 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 dihydrogen 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 the tubes were tightly closed by cotton plugs and kept undisturbed for crystallization. After crystallization of forty five days, the struvite-K crystals grown in Mn^{2+} were found to be in grown in agar gel. The growth parameters of manganese doped struvite-K crystals was shows in **Table 1**.

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

Set No.	Concentration of $Mg(CH_3COO)_2.4H_2O$ I Reactant (M)	Concentration of KH_2PO_4 II Reactant (M)	Concentration of $MnCl_2$ (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

3. RESULT AND DISCUSSION

Struvite-K crystals were grown in manganese chloride additive solution by agar-agar gel method. There has been a significant change in the growth pattern as compared to the similar crystals those are grown with zinc doped[8] and without doping manganese[6]. In case of manganese added experiment, the manganese ions did not influence the selective nucleations, as that was found in zinc doped, but did have a pronounced effects on the growth habits of crystals. The colorless spherulite crystals those are found in calcium [7] and zinc doped were not observed in this manganese doped process. In such experiment, the reddish irregular spherulites were noticed. The Liesegang ring phenomenon was observed in higher concentration of manganese ions. Because the formation of Liesegang rings it is very sensitive to various factors like concentration, pH and ageing of the gel medium, concentrations of the reactants, the presence of additives, temperature, light etc. [13]. It was also observed that the manganese doped struvite-K crystals was in different morphology such as translucent twins, x- shape, and dumbbell shape multiple faces dendratic. The shape and size of doped struvite-K crystal was indirectly affected by concentration and volume of the dopant manganese ions. When the concentration and volume of Mn^{2+} additive impurities were increased, then the size of grown crystals were found to be increased. For the surface topographical study, the grown Mn^{2+} doped struvite-K were characterized by Optical microscopy and scanning electron microscopy (SEM) techniques.

4. CHARACTERIZATION

Optical Microscopy

The optical microscopic of the crystals is the first step study and characterization of the crystals morphology. It uses visible light and a system of lenses to magnify images of small sample. 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 1 s**hows the crystalline morphology of Mn^{2+} doped struvite-K crystals.

The different morphologies such as translucent twins, x- shape, dumb-bell shape multiple faces dendratic as well as reddish color spherulites were observed under the stereo microscope. When the struvite-K crystals images of Mn^{2+} doped compared to undoped sample [6], it was found that the manganese ions resemble the structure and found to affect the morphological growth of struvite-K crystals. This may be due to fact that the additive can affect the crystal growth rate by adsorbing onto the surface of a growing crystal or by altering solution properties [14].

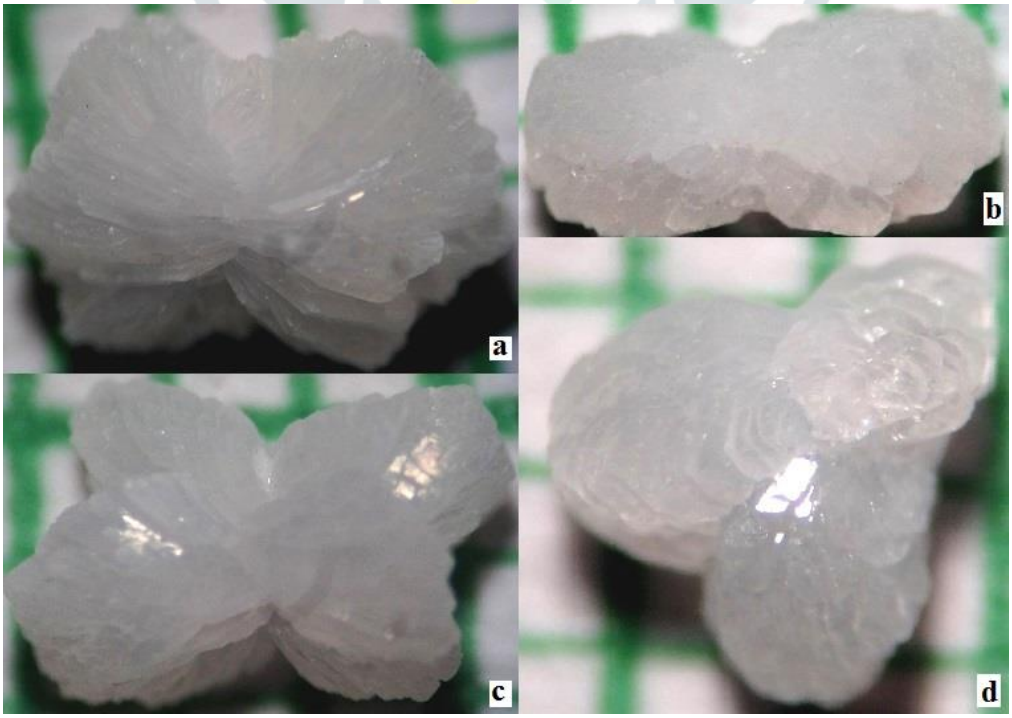

Figure 1: (a-d) Harvested Struvite-K crystals grown in manganese chloride.

As seen in **Figure 1: (a-d)**, in doped cases, the crystals grown have a different morphology with a good shining on its faces. All of the grown crystals were found to be grown by layer deposition. The dumb-bell shape crystals were shown in **Figure 1** (a & b), very few thin growth layers was seen on such crystals. The growth layers have velocity in the longer direction. However the X shape morphological crystal was seen in **Figure 1** (c), in such crystal, its faces and edges are also grown by layer deposition. The growth was radiating from the center to all direction. In **figure 1**(d), the growth is a group of radial step by step semi rounded crystal having well-defined faces on the top was observed. The initial habit of the crystal may be modified due to impurity.

However in **Figure 2**, the different size of roughly spherulites crystals were observed. They are formed due to single nucleus either by an impurity of doping ions. As the local concentration surrounding of this nucleus is large, the growth takes place resulting in the formation of large spherical growth.

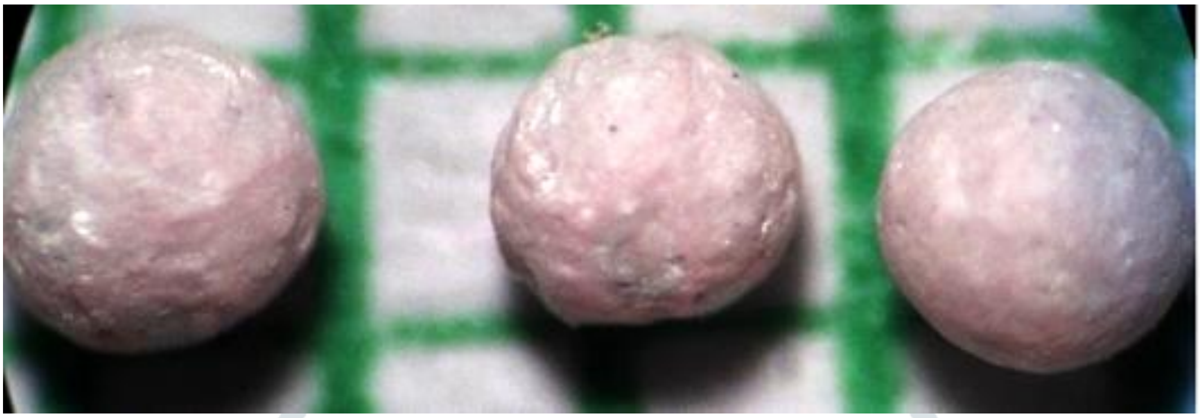

Figure 2: Reddish spherulite crystals of Struvite-K grown in manganese chloride.

SEM analysis:

In SEM, sample surfaces can be examined at depth of field greater than that of optical microscope. **Figure 3** (a,b,c) shows the images of the struvite-K crystals obtained in manganese doped reaction. The morphologies seen in the SEM images were significantly affected due to dopant reactant transforming from rectangular bar as well as needle shaped crystals to layer by layer growth pattern. As seen in **Figure 3 (a)**, the spiral, stair-stepped structure of struvite-K crystals is the result of a higher growth rate around the bottom edges than on the upper edges pattern observed in the scale bar of 1.00 mm.

In **Figure 3 (b)** whose scale bar is 500.0 μm, layer by layer growth having with pyramidical tips was seen. The observed layer is very sharp in different size. The top of such layer was flat and rough, while some foreign particles were deposited in such region.

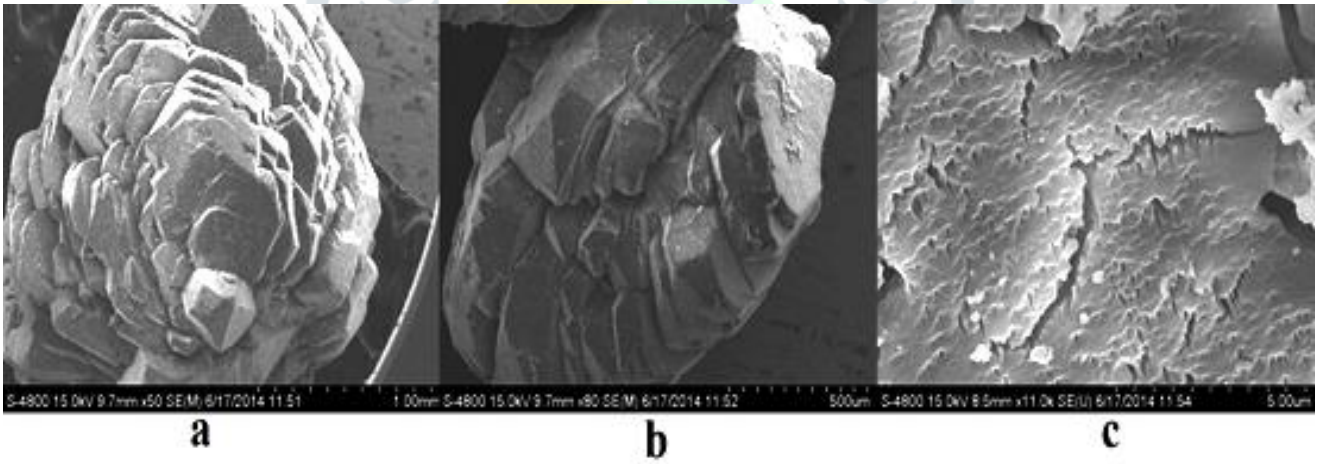

Figure 3: (a-c) SEM images of Struvite-K crystals grown in manganese chloride.

However in **Figure 3(c)** whose scale bar is 5.0 μm, the highly cracked laminar habit layer was observed. A similar microstructure has been previously reported by Gardner et al [15]. However, the micro-sized perforated holes were also appeared on the crystal morphology. Such observed morphologies suggest that the small impurity of manganese ions change the crystal habit, because an impurity atom attached itself to a higher energy site thereby interrupting the chain like sequence upon which normal growth depends[16]. If the impurities having different size are incorporated in the crystal changes the crystal habit. Thus the incorporation of impurities changes the morphology of struvite-K crystals growth in agar gel.

5. CONCLUSIONS

The Struvite-K crystals are successfully grown by Mn^{2+} doped in agar- agar gel by single diffusion techniques. It was found that the manganese doped struvite-K crystals were relatively in different morphology such as translucent twins, x- shape, and dumbbell shape multiple faces dendratic. The reddish irregular shape spherulite crystals were grown due to manganese doping. Increasing the concentration and volume of Mn^{2+} impurity, it apparently increases the size of grown struvite-K crystals. Optical morphology focused on the layer deposition growth of Mn^{2+} doped struvite-K crystals. SEM images show that Mn^{2+} ions transforms rectangular bar and needle shaped undoped struvite-K crystals to layer by layer growth with pyramidical tips. The highly cracked laminar habit layer and micro-sized perforated holes were found in higher magnification. The incorporation of impurities was changes the morphology of struvite-K crystals growth in agar gel.

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