

Growth, spectral, structural and mechanical properties of struvite crystal grown in presence of sodium fluoride

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Abstract. Struvite or magnesium ammonium phosphate hexahydrate (MAP) is one of the components of urinary stone. Struvite stones are commonly found in women. It forms in human beings as a result of urinary tract infection with urea splitting organisms. These stones can grow rapidly forming “staghorn-calculi”, which is a painful urological disorder. Therefore, it is of prime importance to study the growth and inhibition of struvite crystals. The growth inhibition effect of struvite crystals in sodium metasilicate (SMS) gel in the presence of sodium fluoride has been carried out. Crystals obtained have been analysed by powder and single crystal XRD, SEM–EDX, FTIR and TG–DTA. The results show that the presence of fluoride significantly affects struvite crystal growth and the characteristics of the crystallites produced. The mechanical property of the grown crystals has been investigated by Vickers microhardness testing. Work hardening coefficient was found to be >1.6 for both pure and doped samples which suggests that the crystal belongs to the family of soft material. Presence of sodium fluoride further softened the crystal.

Keywords. Struvite; crystallization; SEM; EDX; FTIR; microhardness.

1. Introduction

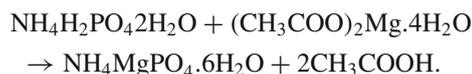
The formation of urinary stones in human body is a serious clinical problem that affects up to 20% of the population (Benramdane *et al* 2008) with the recurrence after treatment at a level of 50%. Urinary stones are located in the kidneys, and only a small percentage is lodged in the urinary bladder and urethra. Urinary stones are formed in the course of many physical and chemical processes. General conditions that contribute to stone formation include a high concentration of salts in urine, retention of these salts and crystals, pH, infection, and a decrease in the body's natural inhibitors of crystal formation. Calculi are often heterogeneous, containing mainly oxalate, phosphate and uric acid crystals. A separate kind of urinary stones is the so-called infectious stones related to urinary tract infection (Bichler *et al* 2002). Struvite stones are (NH₄MgPO₄·6H₂O) also known as infection stones, urease or triple-phosphate stones. About 10–15% of urinary calculi consist of struvite stones (Lerner *et al* 1989).

Fluorine is widely dispersed in nature and is estimated to be the 13th most abundant element on our planet. Fluoride ion in drinking water is known for both beneficial and detrimental effects on health. Fluorides are essentially required for the healthy growth of bones and teeth of human beings. Fluoride concentrations above 1.5 mg/l may lead to dental mottling (fluorosis). At higher concentrations, fluorides are known to inhibit the formation of biological materials such

as brushite and HAP (Sekar *et al* 2009; Kanchana and Sekar 2010). In the present work, investigations have been carried out to study the role of sodium fluoride addition on the crystallization and mechanical properties of struvite.

2. Experimental

Single diffusion gel technique was adopted for the growth of struvite crystals under the physiological pH 7.2. Sodium meta silicate gel (SMS) of specific gravity, 1.03 g/cc, was used as the crystallizing medium into which 1.5 M ammonium dihydrogen phosphate was added as inner reactant. After gelation, 1.5 M of magnesium acetate was placed over as a supernatant solution. The following reaction is expected to occur in the gel between the two reactants (Chauhan and Joshi 2008)



Tiny crystals started appearing at the interface between the gel and supernatant solution two days after the initiation of growth.

In case of fluoride addition, magnesium acetate was mixed with various concentrations of sodium fluoride (0.1–0.5 M) and the crystal growth experiments at room temperature were conducted as described above. At higher concentrations (0.3–0.5 M), crystallization got delayed and tiny crystal appeared 5 days after pouring the supernatant solution. After

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three weeks, the grown crystals were harvested by decanting the glass test tubes and the gel could be removed by dissolving in water.

Growth features of MAP crystals were studied using scanning electron microscope (SEM) and the elemental analyses were done using the OXFORD INCA energy dispersive X-ray spectrometer. Powder X-ray diffraction (XRD) pattern was recorded on Bruker advanced diffractometer within the range 10–80 degree. FTIR spectra of the grown crystals were recorded using Perkin Elmer, Spectrum Rx1 detector and KBr beam splitter. Thermal analysis was performed using simultaneous TG instrument with thermal solution version 1.2J controller software. Data analysis was carried out using TA Instrument Universal Analyser version 2.3C software. Microhardness measurements were carried out on the grown crystals using Shimadzu tester.

3. Results and discussion

3.1 Crystal growth

Gel method is used to grow struvite because the gel acts as a 'three dimensional crucible' which supports the crystals and at the same time yield to its growth without exerting major forces upon it. This relative freedom from constriction is believed to be an important factor in the achievement of high structural perfection. Urinary stones grow in a gel-like medium which provides simulation of synovial cartilage and other biological fluids (Kalkura *et al* 1993).

In the present work, struvite crystals have been grown from pure and fluoride added supersaturated solutions at a constant pH value of 7.2 at room temperature. In pure system, tiny crystals appeared at the gel–liquid interface two days after adding the supernatant solution. These crystals were found to be dendritic in nature. In addition to this, small envelope and rectangular bar shaped crystals grew in the gel medium after about eight days (figure 1a). The formation of acetic acid is maximum at the gel–liquid interface due to high concentration gradients and faster reactions that take place. But as one goes towards the bottom of the test tubes, diffusion of reactants is comparatively less and the amount of acetic acid produced is also less, which does not dissolve the growing crystals and hence a steady growth of crystals is observed. As the depth of the gel column increases from the gel–liquid interface, the sizes of the grown crystals became gradually smaller. The crystals were harvested after three weeks. As confirmed by powder XRD results, all the three types of crystals with dendritic, envelope and rectangular like shapes were found to be struvite. Morphology of the crystals changed along the test tube from top to bottom depending on the availability of the nutrients and its diffusivity in the gel medium.

Impurities in solution from which a compound may precipitate can affect the growth rate of crystals due to blocking of active growth sites inhibiting the increase of crystal size (Jones 2002). In case of struvite crystallization foreign



Figure 1. MAP crystals in tubes a. pure and b. 0.3 M NaF.

compounds such as sodium fluoride could be adsorbed on the crystal surface and retard struvite formation.

At lower concentrations, (0.1 and 0.2 M NaF) there was no visible change in the crystallization behaviour. Further increase in fluoride content (0.3–0.5 M) delayed the crystal growth and tiny crystals started appearing only after 4–5 days. The envelope shaped crystals appeared after tenth day. Number of such crystals increased in the case of 0.3 M NaF and the size decreased as shown in figure 1b. The results indicate that the presence of NaF affects the nucleation and further growth of struvite crystals resulting in different morphologies and poor yield.

3.2 Morphological studies

Among different types of crystals grown in each test tube, rectangular bar shaped crystals (figures 2a–c) were chosen for morphological and elemental analyses. Surface of the as grown pure crystal appeared clean and free from major defects. There has been a significant change in the surface morphology of the grown crystals due to the presence of sodium fluoride in the growth environment. Optical microscope picture of the pure and fluoride doped struvite crystals are shown in figure 3. It can be noticed that there is a systematic decrease in the surface area of the (101) plane with an increase in the additive concentration and this plane finally disappears at a concentration of 0.5 M NaF. Based on the computer model of the struvite crystal morphology and experimental findings, the authors have concluded that the additive phosphocitrate is not only having a strong affinity for the selective growing faces but also an ability to get incorporated into the crystal lattice (Wierzbicki *et al* 1997). They also reported that the adsorption of phosphocitrate on

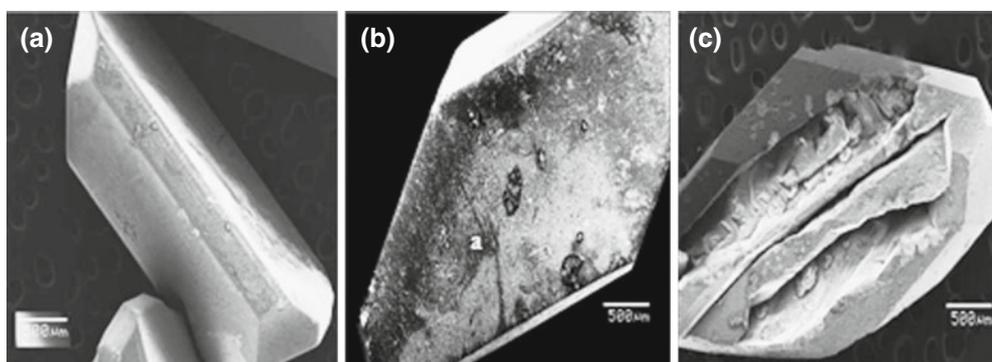


Figure 2. SEM pictures of MAP crystals **a.** pure, **b.** 0.4 M and **c.** 0.5 M NaF.

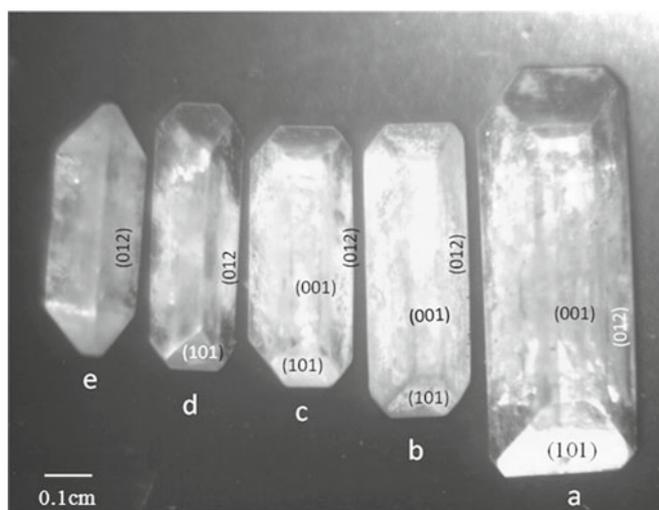


Figure 3. Optical micrographs of MAP crystals: **a.** pure, **b.** 0.1 M, **c.** 0.2 M, **d.** 0.3 M and **e.** 0.4 M NaF.

(101) plane of struvite is of primary importance to its strong inhibitory activity. Similar results have been obtained for the addition of NaF.

EDX measurements were made at different points on the surface of the crystals and the average value was chosen as the final composition of the individual elements. Figures 4a and b show EDX spectrum of pure and (0.3 M) fluoride doped struvite crystal. The results are compiled in table 1.

Magnesium content in all the doped crystals was less than the stoichiometric value and there is a simultaneous increase in the phosphorous content. The free fluoride ions may react with magnesium forming magnesium fluoride (MgF_2) (Ryu *et al* 2008). The fluoride content in the crystals increases with the increase in NaF concentration at the growth environment. It can be noticed that there is a small amount of sodium in the struvite crystals grown in the presence of NaF.

3.3 X-ray diffraction studies

The powder XRD pattern of pure and doped MAP crystals are shown in figures 5a–d. In general, all the XRD patterns agree well with the JCPDS (71-2089) value for struvite. The results confirm the phase purity and crystalline nature of the samples (figure 5a). However, intensities of many peaks of the doped samples showed significant shifts as compared to that of pure samples (figures 5b–d). These discrepancies in the peak intensities could be attributed to changes in preferred growth direction due to the presence of excessive fluoride.

MAP crystals are known to crystallize in the orthorhombic system (space group, $Pm2_1n$) with the lattice parameters, $a = 6.941 \text{ \AA}$, $b = 6.137 \text{ \AA}$ and $c = 11.199 \text{ \AA}$. We have determined the lattice parameters from the single-crystal X-ray diffraction data obtained with a four-circle Nonius CAD4 MACH3 diffractometer ($\text{MoK}\alpha$, $\lambda = 0.71073 \text{ \AA}$). The

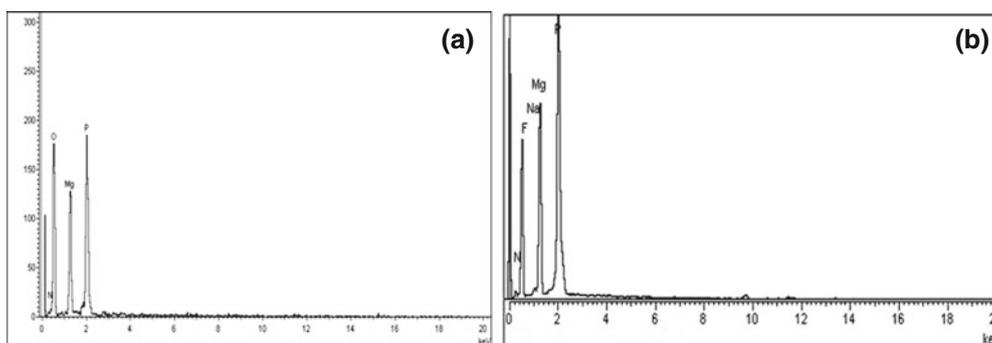


Figure 4. EDX spectra of NaF doped MAP crystals: **a.** pure and **b.** 0.3 M NaF.

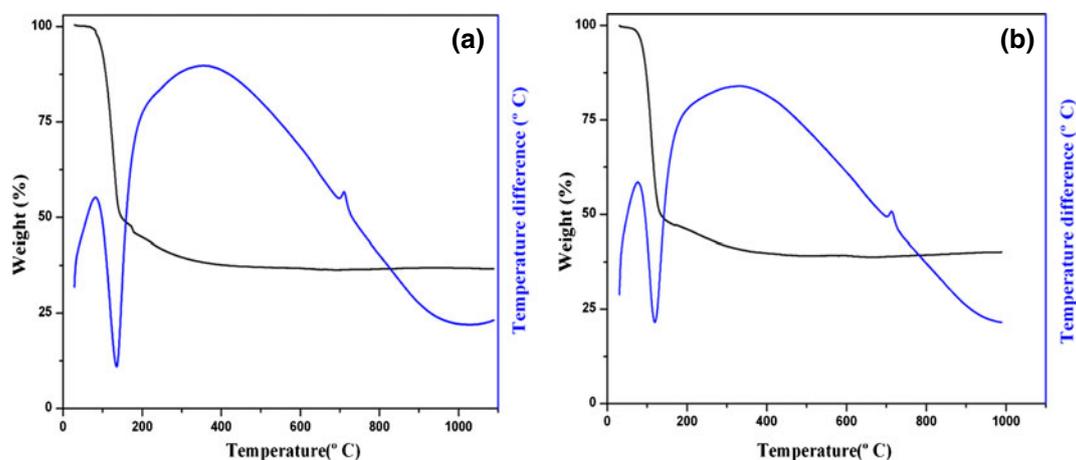
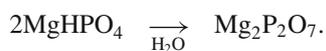


Figure 7. TG – DTA curves of a. pure and b. 0.3 M NaF added crystal.

highest concentration of 0.5 M NaF, the band at 887 cm^{-1} due to N–H rocking disappeared. Thus the FTIR result indirectly confirms the doping of fluoride into struvite.

3.5 TG–DTA studies

Figures 7a and b illustrate thermal behaviour of pure and fluoride doped MAP samples recorded in the temperature range between 30 and 1200°C at the rate of $20^\circ\text{C}/\text{min}$ in nitrogen atmosphere. In pure sample the weight loss occurs in two stages. The major weight loss of about 53% occurs between 79°C and 179°C which indicates the elimination of six water molecules and an ammonia molecule. DTA shows strong endothermic peak at 136°C corresponding to the weight loss. In the high temperature region ($179\text{--}730^\circ\text{C}$), two molecules of MgHPO_4 combine and result in the elimination of a water molecule leading to the formation of magnesium pyrophosphate and nearly 36% of the sample is stable. The following chemical reactions are expected to occur during dehydration and decomposition stages (Frost *et al* 2004).



Nearly similar thermal behaviour occurred in fluoride doped MAP also. The major weight loss of about 51% occurs between 73°C and 173°C in the crystals grown with NaF (0.5 M) addition. The mass loss corresponds well with the DTA results by the appearance of an endothermic peak at 121°C . Thus there is a decrease in the peak temperature which indicates a reduced thermal stability of MAP due to fluoride doping. In the second stage, nearly 7% weight loss occurs and the rest of the sample (40%) was stable. The exothermic peak around 710°C in both pure and doped sample was due to the loss of one water molecule resulting in the transition from the ‘amorphous’ product to γ -crystalline form (Kaloustian *et al* 2002).

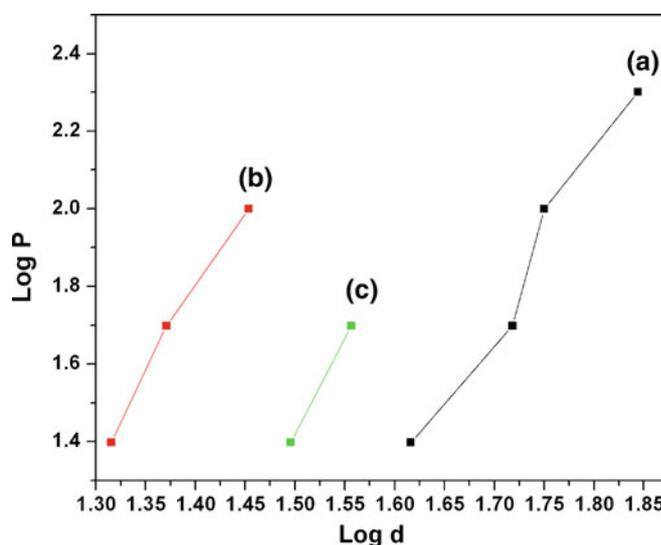


Figure 8. Variation of microhardness with load: a. pure, b. 0.1 M and c. 0.2 M NaF.

3.6 Vickers microhardness studies

Microhardness of a crystal is its capacity to resist indentation. Physically hardness is the resistance offered by a material to the localized deformation by scratching or by indentations. The study of mechanical properties of urinary calculi in real conditions (*in vivo*) is difficult and possible only to a limited extent. So, study of the mechanical properties of urinary calculi *in vitro* will be useful in designing and optimizing the parameters to develop a technique for the urinary stone fragmentation. Vickers hardness indentations are made on the flat face of the crystals (001 plane) at room temperature for loads 25, 50, 100 and 200 g using Vickers hardness tester. The average value of the diagonal lengths of the indentation mark for each load is used to calculate the hardness. The Vickers microhardness number is determined from the relation

$$H_v = 1.8544 P/d^2 \text{ kg/mm}^2,$$

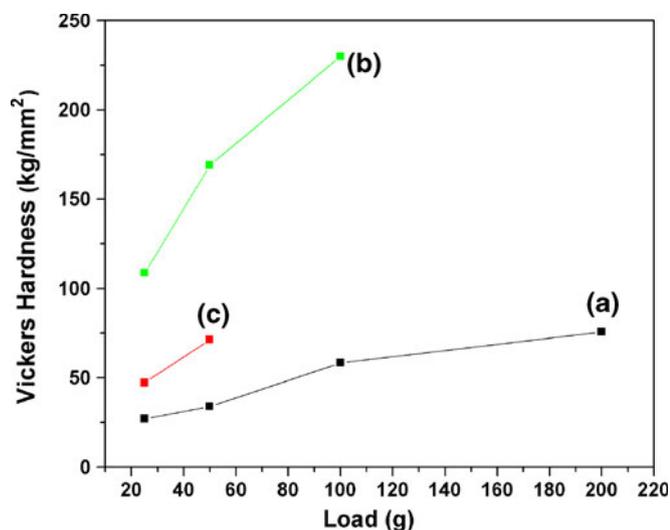


Figure 9. Variation of $\log d$ with $\log P$: a. pure, b. 0.1 M and c. 0.2 M NaF.

where P is the load, d the length of the diagonal of the indentation impression and H_v the Vickers hardness.

The variation of H_v with load is shown in figure 8. It was observed that in all the cases, the microhardness increased with increase in load. The pure crystal withstands loads up to 200 g but in the case of fluoride doped samples crack initiation and materials chipping become significant beyond 100 and 50 g for 0.1 M and 0.2 M NaF doped crystals, respectively. Hardness test could not be carried out above this load. The plot $\log P$ vs $\log d$ is shown in figure 9. According to Meyer's relation, the work hardening coefficient is related to the diagonal length by

$$P = Kd^n,$$

where K is an arbitrary constant and n the work hardening coefficient. The value of n is determined from the slope of $\log P$ vs $\log d$ plot. H_v increases with increase in P and ' n ' is estimated as 3.1987 for pure, 3.644 for 0.1 M and 4.923 for 0.2 M NaF doped MAP crystals, respectively. According to Onitsch (1998), the Meyer's index in the range of $1.0 \leq n \leq 1.6$ is for hard materials and $n > 1.6$ for soft materials. Hence, it is inferred that MAP belongs to the soft category of materials and agrees well with the reported result (Cohen and Whitfield 1993) i.e. the struvite is the softest naturally occurring stone. The addition of fluoride further softened the struvite crystal.

4. Conclusions

The role of NaF on struvite crystallization in gel has been investigated. At higher concentrations, the fluoride inhibited

the nucleation and growth resulting in less number of smaller crystals. Further, the development of certain crystal planes are inhibited due to the restricted access of ionic species to the growing face of the crystal. This resulted in a significant reduction in the number and size of the crystals. Optical microscope picture reveals that (101) plane's surface area decreases with increase in fluoride concentration and finally disappears. The adsorption of fluoride on (101) plane of struvite crystal seem to have strong inhibition effect. Elemental analyses by EDX revealed that the magnesium content in the crystals grown in presence of fluoride are less than the stoichiometric value and that the quantum of phosphorous increases in these crystals. Vickers microhardness studies reveal that the addition of fluoride made the struvite crystal softer. These results suggest that the presence of fluoride exhibits adverse effect on crystallization of struvite.

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