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Influence of Drugs on the Formation of Struvite Urinary Calculi

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Abstract- Nephrolithiasis is a global problem affecting mankind for several centuries. Struvite stones may cause 'staghorn calculi' and if untreated could eventually damage kidneys. Recent findings showed that medication has caused an increase in the formation of urinary calculi. In view of this , an attempt is made to grow struvite crystals using gel method and to find the influence of drugs on the formation of urinary calculi. The chemical composition, thermal properties and morphology of the grown crystals with and without the addition of drugs were carried out using FTIR, thermogravimetric analysis and SEM-EDAX.

Index Terms- Gel method, struvite, urinary calculi, urolithiasis

I. INTRODUCTION

Urolithiasis has been documented since prehistoric period. Urinary calculi is the most painful and prevalent urological disorder affecting mankind for many centuries. Urinary calculi are more prevalent in men than in women (Suma Vupputuri *et al*., 2003). Struvite stones composed of magnesium ammonium phosphate (MgNH4PO4.6H2O) accounts for 10-20% of all renal calculi (Steven J Scheinman 2003). Worldwide they make up to 30 % of all stones (C.K.Chauhan *et al*., 2009). Struvite form in humans as a result of urinary tract infection.Struvite stones may form staghorn calculi which may damage the entire renal pelvis (Steven J Scheinman *et al*., 2003). It has been already documented that medical conditions and certain drugs has been considered as one of the pathogenic factors for nephrolithiasis (John A. Sayer *et al*., 2010). Hence it is essential to study the pathogenesis of struvite stone formation induced by drugs. Among the various drugs that are used for the treatment of stone disease, we have choosen calcury and zyloric due to their antibiotic property. We have made an attempt to grow struvite crystals invitro and study the influence of drugs on their formation. We have choosen gel method to grow struvite crystals as it a suitable method owing to their viscous nature, which provide simulation of body fluid (T.K. Anee *et al*., 2003) and also gels have proven to be the best method to mimic the growth of various biominerals in environments similar to natural biomineralization. The grown crystals were characterized using FT-IR, Thermogravimetry analysis and SEM-EDAX study.

II. MATERIALS AND METHODS

The single diffusion gel growth method was employed to study the growth and promoting or inhibiting behavior of struvite crystals using different drugs. Reports suggest that gel method is the preferred technique to grow struvite crystals as it provides the simplified invitro model of the highly complex invivo growth of urinary calculi (C.K. Chauhan *et al*., 2009). In this technique of gel growth, the gel acts as a three dimensional crucible which supports the crystals without exerting forces on the crystal, thus leading to high structural perfection in its formation. Sodium metasilicate (SMS) $\{Na_2SiO_3 \_ 9H_2O\}$ solution of specific gravity 1.03 was used to prepare the gel. Silica gel has the advantage that it remains stable and does not react with the reacting solutions or with the product crystal formed. In this method one of the reagent is incorporated in the gelling mixture and later allowing another reagent to diffuse into the gel, leading to high supersaturation to initiate nucleation and crystal growth. All the chemicals used for this technique are AR grade.

Glass test tubes of 25 mm diameter and 140 mm length were used as the crystallization apparatus. Aqueous solution of ammonium dihydrogen phosphate (ADP)—$\{NH_4H_2PO_4 \_ 2H_2O\}$ of 1M concentration was mixed with the SMS solution in appropriate amount so that the pH value 7.0 could be set for the mixture. 20 mL of the prepared gel solution was transferred into the test tubes. After gelation, 10 mL supernatant solution of pure 1.0 M magnesium acetate—$\{C_4H_6MgO_4 \_ 4H_2O\}$ (without drug) and 1.0 M magnesium acetate prepared with 0.005g of the drug calcury and 0.005 g of the drug zyloric were gently poured over the gel medium in test tube without disturbing the latter to study the influence of added drugs on the growth of struvite crystals. After pouring supernatant solution, the test tubes were capped with airtight stopples. Utmost care is taken to avoid microbial contaminations. The experiment was conducted at the room temperature. The following reaction is expected to occur in the gel between the two reactants.

$$NH_4H_2PO_4 \,.\, 2H_2O + CH_3(COO)^2Mg.\, 4H_2O \rightarrow NH_4MgPO_4 \,.\, 6H_2O + 2CH_3COOH$$

Crystals started growing at the gel- solution interface and also inside the gel medium. The growth of the crystals without drugs and with drugs was monitored throughout the experiment. Struvite crystals of different morphologies such as rectangular platelet and prismatic type were obtained. The grown samples were harvested after 3 weeks by decanting the test tubes and the gel was removed and subsequently washed with distilled water and dried. The FTIR spectrum of the grown samples was recorded. SEM with Energy dispersive X-ray analysis and Thermogravimetric analysis were carried out.

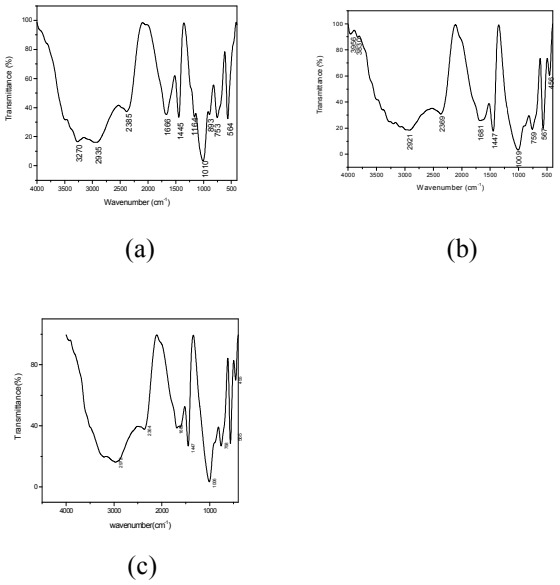
III. RESULTS AND DISCUSSION

It was observed that presence of additives namely calcury and zyloric has caused a decrease in the number of grown struvite crystals and their average size.

FTIR studies: Fig 1 – shows the FTIR spectra of pure struvite and drugs namely calcury and zyloric substitued struvite samples. The FTIR spectra were recorded at room temperature using Perkin-Elmer Spectrophotometer using KBr pellet technique in the wave number range between 400 and 4000 cm^{-1} to analyze the sample qualitatively. The absorption bonds, absorption frequencies were recorded and compared with the reported values. The values are tabulated in Table 1. In pure Struvite the broad envelope occurring at 3270 cm^{-1} corresponds to the O-H and N-H stretching vibrations. The absorption band at 2935 cm^{-1} is due to NH4 + ion. The absorption band corresponding to 1666 cm-1 is assigned to N-H bending vibrations. The absorption band occurring at 1010 cm-1 is assigned to ionic phosphate. Absorption occurring at 564 cm-1 is assigned to metal-oxygen bond. The frequency value of functional groups confirms the Struvite crystal constituents. The small shift in the peak values of drug substituted samples is due to the incorporation of drug which has modified the vibrational absorptions.

Table 1: Assignments of functional groups

Functional group Assignments	Reported IR frequencies wavenumber(cm-1) (Chetan K Chauhan *et al*., 2008)	Observed IR frequencies wavenumber(cm-1) for pure struvite
H-O-H and N/-H stretching vibrations	3280-3550	3270
Rocking of N/-H modes of vibration	808	893
N-H symmetric stretching vibrations in NH4+ units	2800-3000	2935
N-H asymmetric stretching vibration	3280-3550	3270
N-H symmetric stretching vibration in NH4+ units	2800-3000	2935
N-H bending vibration	1630-1750	1666
Ionic phosphate	1390-1640	1010
Metal-Oxygen bond	400-650	564

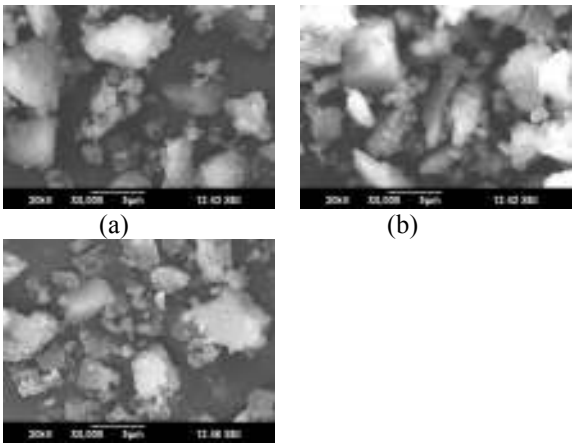


(a) (b)

(c)

Figure.1: FTIR spectra of (a) pure struvite, (b) 0.005 g drug (calcury) added sample, (c) 0.005 g drug (zyloric) added sample.

SEM and EDS: Fig.2 shows the SEM images of pure struvite and drugs substituted samples. The SEM was taken at magnification values 1500 X, 5000X and 10000X. The micrographs show that the samples have granular structure. The average size of the pure struvite crystallite is 1.4µm. However drug added struvite size ranges between 0.9 µm to 1.05 µm. The decrease in size is attributed to the substitution of drugs to the struvite sample, altering the growth of struvite crystals.



(a) (b)

(c)

Figure.2. SEM image – magnification 5000X of (a) pure struvite, (b) 0.005 g of drug (calcury) added sample, (c) 0.005g of drug (zyloric) added sample

The elemental composition of the sample is identified using Energy dispersive X-ray analysis. The EDS spectrum of pure struvite and drug added sample is shown in fig.3. The higher peak of Mg, P and O shows that the more concentrated the

element is in the specimen. Table 2 shows the EDAX data of pure and drug added specimens.

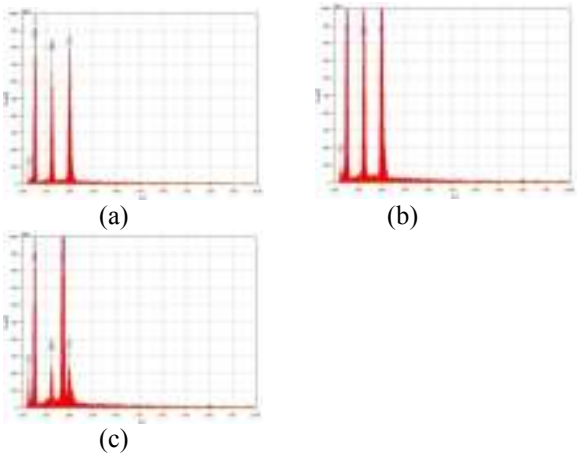


Figure.3: EDS spectrum of (a) pure struvite , (b) 0.005 g drug (calcury) added struvite, (c) 0.005 g drug (zyloric) added struvite

Table 2: EDAX data of pure and drug added struvite samples

Element	Pure Struvite (atm%)	Drug added struvite 0.005g of	
		Calcury	zyloric
C K	1.71	20.22	5.93
O K	53.26	39.22	48.49
Mg K	18.63	3.78	18.56
Si K	-	32.27	-
P K	26.39	4.51	27.02

TGA/DTA studies: The thermal property of the sample is understood by conducting TGA. Fig.4 shows the thermogram of pure struvite crystal and drug added samples. The thermal behavior of the pure struvite crystal were studied in the temperature range 30-820 ^{o}C at a heating rate of 10 ^{o}C/min in the nitrogen atmosphere.It was observed from the TGA curve that the decomposition of struvite crystal started just above the room temperature may be due to the loss of ammonia and water of crystallization, hence the struvite crystal is thermodynamically unstable. In the DTA analysis two sharp endothermic peaks were observed at 118.48 ^{o}C and 162.66 ^{o}C. The peak at 118.48 ^{o}C is assigned to the decomposition point. Before decomposition there are no characteristic exothermic or endothermic peaks. Sharpness of the endothermic peaks observed in DTA indicates good degree of crystallinity of the sample. An exothermic peak was also observed at 685.39 ^{o}C, which might be due to the high temperature phase transition. The TGA- DTA curve of drug added struvite crystals shows a small shift in the decomposition point.

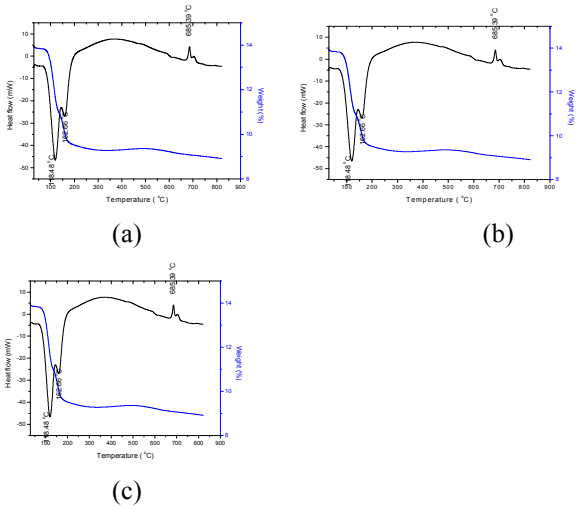


Figure.4: TGA-DTA curve of (a) pure Struvite, (b) 0.005 g of drug (calcury) added struvite, (c) 0.005 g of drug (zyloric) added struvite

IV

IV. CONCLUSION

The struvite crystals with and without inclusion of drugs were grown gel media. The presence of water of crystallization, N-H bonds, PO_4 bonds, NH_4 and metal oxygen bond were confirmed using FT-IR analysis. The SEM-EDS microanalysis gives precise information about the samples and their compositions. The presence of Phosphorous, Magnesium, Oxygen and Carbon were detected. Thermal analysis confirms the decomposition point of the grown crystal. It was found that the pure struvite crystal decomposes at 118 ^{o}C. It also shows that the struvite crystals are thermally unstable. It was observed that the presence of drugs in the growth environment has caused a retarding effect on the formation of the struvite crystals. Further invivo studies are necessary to better evaluate the potential effect of drugs on struvite formation.

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