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Formation and Microanalysis Of Struvite Urinary Calculi

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ABSTRACT

Magnesium ammonium phosphate hexahydrate, also known as Struvite is a major component of urinary calculi. The crystals are grown by the single diffusion gel growth technique. The effect of herbal drugs on the crystallization of struvite in sodium metasilicate gel has been studied at room temperature. Addition of various herbal drugs has caused significant changes on the formation of the struvite crystals. The elemental composition, microstructure and properties of the grown crystals were analyzed using Fourier transform infrared spectroscopy, SEM-EDAX and thermal analysis.

Keywords - Biomineralization , Crystallization, Nephrolithiasis, Struvite, Urinary calculi

1. INTRODUCTION

The formation of urinary calculi is known as nephrolithiasis. Urinary Calculi is one of the most hazardous, painful and existing urological disorder causing threat to the global population since ancient times [1,2] and an estimated recurrence rate of 50 % [3,4]. Hence nephrolithiasis poses a significant health problem. Pathological mineral deposition automatically occurs when the mineral level of the body fluid increases [5]. Struvite also known as infection stone, triple phosphate stone, urase stone is a biological crystal composed of Magnesium Ammonium Phosphate with the chemical formula $MgNH_4PO_4.6H_2O$ [6, 7].This type of kidney stones occur for only 10-20% of all kidney stones [8]. Struvite nephrolithiasis form 'StaghornCalculi' [6,9] and if untreated can lead to end-stage renal disease. Patient treatment might be improved and recurrence prevented if information were more often available on stone formation and composition in individual patients [10]. Hence our attention is given to this type of crystals. In the present study, Struvite crystals were grown by single diffusion gel growth technique and the growth influencing study of the Struvite crystals in the presence of different drugs were carried out. We have choosen gel method because of the viscous nature of gel and they provide simulation of the growth of various biominerals in environments similar to natural biomineralization [11, 12]. This method also facilitates observance of growth of the crystal at all stages. Epidemiological studies report an association between nephrolithiasis and diabetes [13]. Drug associated nephrolithiasis contributes to approximately 1-2 % of the incidence of renal calculi [14]. Several studies have been designed to investigate the correlation between diabetes drugs and kidney stones [15]. The present study is focused on whether the influence of herbal drugs mainly used by Indians for the treatment of diabetes could influence risk of struvite stone formation and their microstructure analysis since no report on this studies were carried out earlier. The mineralogical changes undergone by struvite were monitored throughout the experiment.

2. Experimental

The single diffusion gel growth method was employed to study the growth and promoting or inhibiting behavior of struvite crystals using different drugs .Sodium metasilicate (SMS) $\{Na_2SiO_3_ 9H_2O\}$solution of specific gravity 1.03 was used to prepare the gel. This technique consists of incorporating one reagent in the gelling mixture and later allowing another reagent to diffuse into the gel, leading to high supersaturation to initiate nucleation and crystal growth [l6]. 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 7.2 could be set for the mixture. 20 mL of the prepared gel solution was transferred into the test tubes. Silica gel has the advantage that it remains stable and does not react with the reacting solutions or with the product crystal formed. After gelation, 10 ml supernatant solution of pure 1.0 M magnesium acetate$\{C_4H_6MgO_4 \ 4H_2O\}$ was added. 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:

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

Crystals started growing at the gel- solution interface and also inside the gel medium as shown in Fig.1.In the same way 1.0 M magnesium acetate prepared with 0.005g of the dried, powdered, herbal extract namely fruit of amla (A_1) were gently poured

over the gel medium in test tube without disturbing the latter to study the influence of added extract on the growth of Struvite crystals. Again herbal extracts namely dried, powdered seed of amla , leaf of amla and rose apple seed (A_2, A_3, A_4) of 0.005 g were added along with 1.0 M magnesium acetate separately. The growths of the crystals without herbal extracts and with herbal extracts were monitored throughout the experiment. Struvite crystals of rectangular platelet and prismatic morphology 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 spectrums of the grown samples were recorded. SEM with Energy dispersive X-ray analysis and Thermogravimetric analysis were carried out.

Fig. 1 Formation of struvite crystals in gel medium

3 . Results and Discussion

It was observed that presence of additives namely A_1, A_2, A_3 has caused an increase in the number of grown struvite crystals and their average size, where as drug A_4 added struvite crystals showed a decrease in the crystal size .

3.1. FTIR studies

Fig. 2 shows the FTIR spectra of pure and various drug added 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 sharp but weak band corresponding to 1445 cm^{-1} is caused by N-O asymmetric stretching vibration. 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 confirm the Struvite crystal constituents. The spectra of drug added struvite crystals shows small shift in the peak values and is due to the incorporation of drug which has modified the vibrational absorptions.

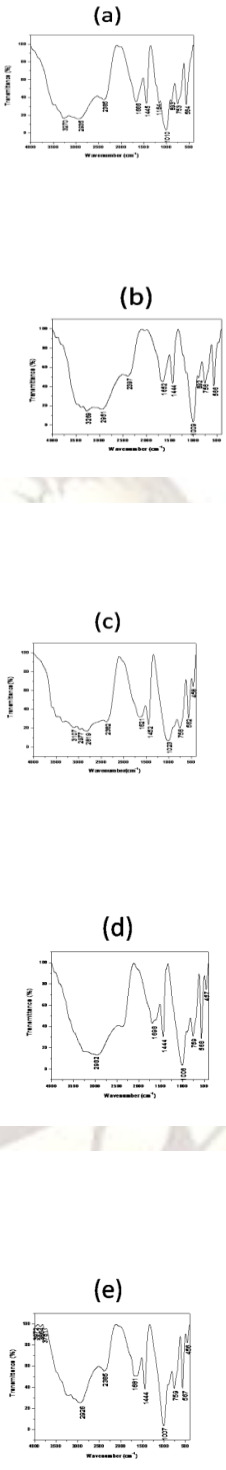


Fig. 2 FTIR spectra of (a) pure struvite, (b) 0.005 g drug (A_1) added sample , (c) 0.005 g drug (A_2) added sample, (d) 0.005 g drug (A_3) added sample, (e) 0.005 g drug (A_4) added sample

Table 1 FT IR Spectral assignments of functional groups[1]

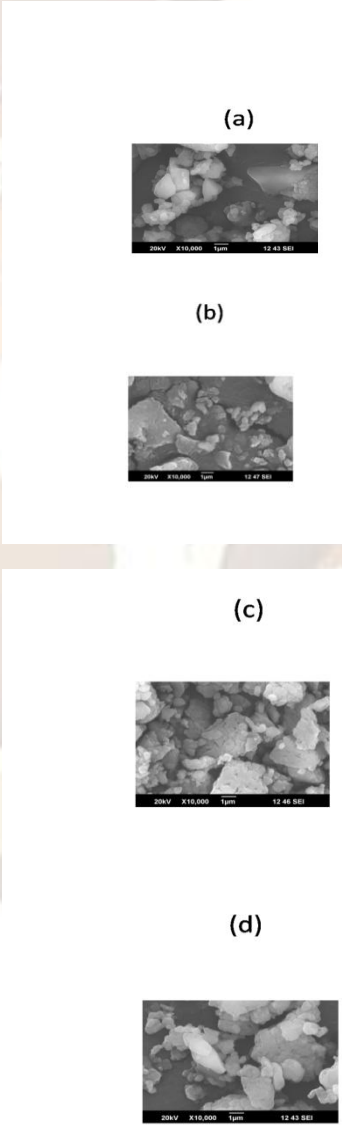
Functional group assignments	Reported IR wavenumber (cm^{-1}) [17,18]	Observed IR wavenumber (cm^{-1}) of pure struvite
H-O-H stretching vibrations of water of crystallization	3280-3550	3270
H-O-H stretching vibrations of cluster of water molecules	2060-2460	2385
H-O-H bending modes of vibrations	1590-1650	1445
Wagging modes of vibration of coordinated water	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
N-H asymmetric stretching Vibration in NH4+ units Ionic phosphate	1390-1640	1010

[1] the elemental composition of the struvite crystals can be understood from the table

3.2 SEM and EDS

Fig. 3 shows the SEM images of pure struvite and various herbal extracts (A_1, A_2, A_3, A_4). 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 1.0µm to 1.7 µm. The variation in size is attributed to the substitution of drugs to the struvite sample, altering the growth of struvite crystals.

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.4. 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.



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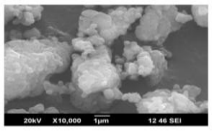
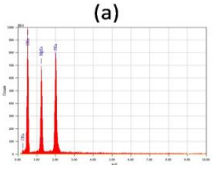
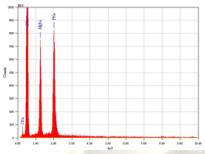


Fig. 3 SEM image – magnification 10000X of (a) pure struvite, (b) 0.005 g of drug (A_1) added sample,

(a)



(b)



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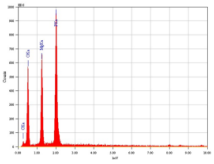

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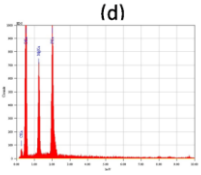

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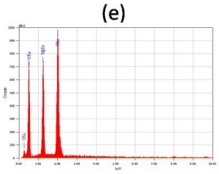


Fig. 4 EDS spectrum of (a) pure struvite , (b) 0.005 g drug (A_1) added struvite, (c) 0.005 g drug (A_2) added struvite, (d) 0.005 g drug (A_3) added struvite, (e) 0.005 g drug (A_4) added struvite

Table 2 EDAX data of pure and drug added struvite crystals[2]

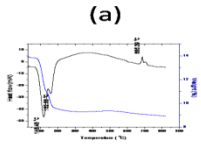
Element	Pure Struvite (atm %)	Drug added struvite 0.005g of			
		A_1	A_2	A_3	A_4
C K	1.71	1.64	4.24	6.4	7.86
O K	53.26	66.91	37.59	59.64	40.83
Mg K	18.63	13.49	21.16	12.79	20.19
P K	26.39	17.96	37	21.17	31.12

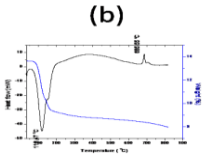
3.3 TGA/DTA studies

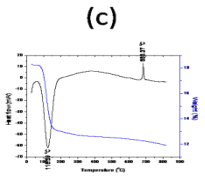
The thermal properties of the sample are understood by conducting TGA. Fig. 5 shows the thermogram of pure struvite crystal and drug added samples.The thermal behavior of the pure struvite crystal were studied in the temperature range 30-900^{o}C at a heating rate of 10 ^{o}C/min in the nitrogen atmosphere. In the case of pure struvite it was observed from the TGA curve that the decomposition of struvite crystal started just above the room temperature and finally at 821 ^{o}C it became 63.8% of the original weight. Total mass loss was found to be 36.2 % which 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

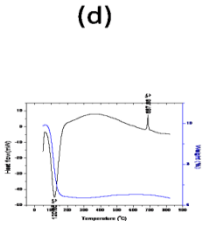
[2] table shows the atomic % of elemental composition of pure struvite and drug added struvite samples

118.48 ^{o}C is assigned to the decomposition, at this stage weight loss has been noticed which may be due to the release of crystalline water along with ammonia. Before decomposition there is no characteristic exothermic or endothermic peaks which suggests the absence of any isomorphic transformation below its decomposition point. 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 curves of various drug added Struvite crystals shows a small shift in the decomposition point, which indicates the influence of drugs on the sample.









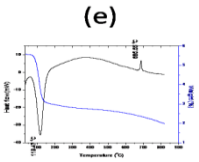


Fig. 5 TGA-DTA curve of (a) pure Struvite, (b)0.005 g of A_1 drug added struvite, (c)0.005 g of A_2 drug added struvite, (d)0.005 g of A_3 drug added struvite, (e)0.005 g of A_4 drug added struvite

4 . Conclusion

The Struvite crystals are successfully grown by single diffusion gel growth technique. The functional groups present in the samples were confirmed using FTIR 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 and below its decomposition point no phase transformations occurs. It also shows that the struvite crystals are thermally unstable. It was observed that the presence of herbal drugs (A_1, A_2, A_3)in the growth environment has caused a promoting effect on the formation of the struvite crystals, whereas the herbal drug A_4 has caused a retarding effect on the formation of the struvite crystal. Hence the herbal extract A_4 may be useful to overcome the major drawback of surgical procedures which is recurrence of stones. Further invivo studies are necessary to better evaluate the potential effect of herbal extracts on struvite formation.

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