

Characterization of growth inhibition studies of Struvite Crystals

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Abstract: *The formation of Urinary or kidney stones is a serious debilitating problem throughout the world. It has been rectified by Ayurveda, herbal extracts and juices are commonly recommended for urinary stone treatments and some of them have been evaluated for their role as inhibition for urinary crystals. In the present study Tribulu sterrertris herbal extracts are used to inhibit the growth of struvite crystals using single diffusion gel method. The inhibition growth of struvite crystals can be characterized by using Fourier Transform Infrared Spectroscopy, X-ray diffraction and Scanning Electron microscope.*

Key Words: *Gel method, struvite crystals, Tribulu sterrestris, herbal extracts, FT-IR, XRD and SEM-EDX.*

1. INTRODUCTION:

Urolithiasis is a very common and highly recurring painful disease both develop and developed nations due to the change in life style and food habits. Among the phosphates, magnesium phosphates namely ammonium magnesium phosphate hexahydrate-[$\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$], mineralogically known as struvite and magnesium hydrogen phosphate trihydrate, i.e., new beryite were reported to occur as constituents in urinary calculi not only in adults but also in children. Struvite is also known as 'urine sand', 'triple phosphate stone', 'phosphate stone', 'infection stone' or 'urease stone'. Struvite is the main component of the infectious urinary stones and is associated with the chronic urinary tract infections (UTI) with ureolithmic micro organisms, which split urea into ammonium and cause persistently alkaline urine, which further combines with phosphate and magnesium. Struvite stones are among the most difficult and dangerous problems in stone disease because of the potential of life threatening complications from infection. Struvite stones may grow rapidly over a period of weeks to months and, if not adequately treated, can develop into a staghorn or branched calculus that involves the entire renal pelvis and calyces. Patients with infected staghorn calculi who receive no treatment have about a 50% chance of losing kidney. Therefore, it is very much necessary to study the growth-inhibition of struvite crystals. In the present investigation, struvite crystals growth were grown by single diffusion gel growth technique and the study of the growth inhibition effect on the struvite crystals in the presence of the herbal extract of one of the medicinal plants Tribulus terrestris Linn. was carried out.

2. MATERIALS:

Sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) and ammonium dihydrogen phosphate (Na_2HPO_4), Tribulus terrestris extract, acetic acid

3. METHOD:

3.1. Crystal growth:

Sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) and ammonium dihydrogen phosphate (Na_2HPO_4) was used for preparing the gel and glacial acetic acid was used for adjusting the pH value. All the reagents used in this experiment are of analar grade. Crystallizations were conducted in the gel densities between 1.03 and 1.06 g/cc and pH values from 4 to 6.5. Optimum values of gel density and pH values for the crystallization were found to be 1.03 g/cc and 6 respectively. Magnesium acetate and 20ml herbal extracts Tribulus terrestris Linn were gently poured on the set gel in test tubes were used to determine the growth inhibition of Struvite crystals. The growth was completed within a period of 21 days. The inhibition growth of struvite crystal in herbal extract as shown in fig 1. Harvested crystal as shown in fig 2 and 3.



Figure 1. Struvite crystal grown in Tribulus terrestris extract

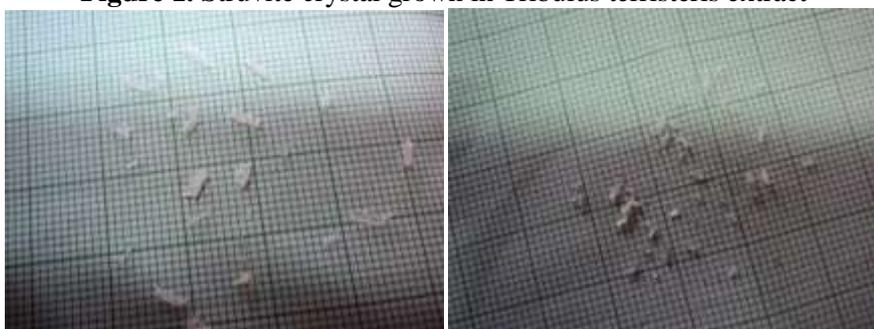


Figure 2. Harvested crystal without herbal

Figure 3. Harvested crystal with herbal extract

3.2 Herbal extracts of Tribulus terrestris Linn:

In the Ayurveda, many herbal medicines have been recommended for the treatment of urinary stone. In the present growth inhibition study used one of the herbal extracts Tribulus terrestris Linn. In the plant root and seed in known to have medicinal properties and are used as an Ayurvedic medicine in India for the treatment of kidney stone problem. Different parameters such as concentration of reactants , PH of gel, impurities in the solvent, gel setting time, etc have considerable effect on growth rates.

Table.1. The optimum condition for the growth of Struvite crystals

S:No	Parameter	Optimum Condition
1	Density of SMS solution	1.03 g/cm ³
2	Percentage of gel	30
3	Concentration of ADP	0.1M
4	Concentration of magnesium acetate	1M
5	Ratio	1:1
6	Gel setting period	3 days
7	Gel ageing	2 months
8	PH range	4-6
9	Temperature	Room temperature

4. RESULT AND DISCUSSION:

FT-IR spectra were recorded using Nicolet IS5 Model FT-IR spectrometer. FT-IR spectrometer is available in the department of chemistry, Annamalai University. Powder X-ray diffraction of the sample is carried out by BRUKER ECOD8 ADVANCE with $\text{CuK}\alpha$ radiation ($\lambda=1.5401\text{\AA}$) is available at Department of physics, Kalasalingam university, Krishnankoil. The morphology of the crystals are studied by SEM is available at Centralized Instrumentation and Service Laboratory (CISL), Department of Physics, Annamalai University, Tamil Nadu, South India and element present in the crystals are identified by EDX which is present in Gandhigram rural institute (Deemed university) GandhigramDindugal, Tamilnadu, South India. The inhibition growth of struvite crystals using Tribulus terrestris herbal extract by single diffusion gel method and the harvested crystals are analysed by FT-IR, powder X-ray diffraction and SEM-EDX analysis. Fig.1 shows Struvite crystals grown in Tribulus terrestris herbal extract. In the presence of the aqueous Tribulus terrestris herbal extract, nucleation was delayed, reduced size and morphology changed Dendritic X shaped in to needle shaped crystals were observed as shown in fig 2 and fig 3.

4.1. FT-IR Analysis:

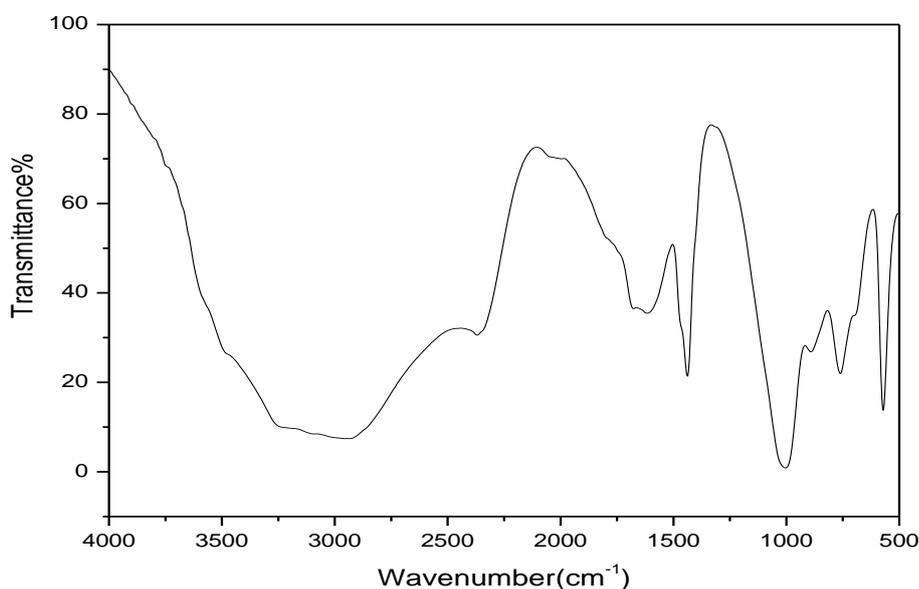


Figure 4. FT-IR analysis of Struvite crystals

FT-IR spectrum depicting the absorptions due to water of crystallization in struvite which are closely matched to the previously reported peaks in several inorganic hydrated compounds (Miller and Wilkins, 1952). FTIR spectra due to its general position in the crystal, which correspond to the previously reported peaks in several inorganic NH_4^+ containing compounds. Vibration modes of tetrahedral PO_3^- anions are also in accordance with reported values. The absorption occurring at 3266, 3266, 3264, 3254 cm^{-1} are due to O-H and N-H stretching vibrations. The absorption occurring at 2956, 2933, 2924, 2924, 2923, 2916, 2853, 1676 cm^{-1} is due to NH_4^+ ion. The absorptions at 2368 cm^{-1} due to H-O-H stretching vibration. The absorptions occurring at 1629, 1618, 1611 cm^{-1} are caused by H-O-H bending vibrations. On the other hand, the absorptions taking place at 1102 cm^{-1} are due to asymmetric stretching vibration of ionic phosphate. The absorption at 891, 872 cm^{-1} due to deformation of OH linked. Wagging modes of vibration appeared at the frequency of 770, 764, 761, 759, 758 cm^{-1} . The absorption at 514, 507 cm^{-1} is due to asymmetric bending vibration of P-O modes. The absorption at 491, 489, 483, 482, 460 cm^{-1} are due to metal-oxygen bonds. The absorptions at 458, 446, 438, 423 cm^{-1} maybe due to symmetric bending vibration of ionic phosphate. Thus the FT-IR spectra proves the presence of water of hydration, N-H bond, P-O bond, NH_4^+ ion and PO_4^{3-} ion and metal oxygen bond.

4.2 Powder XRD Analysis:

The powder XRD pattern was recorded using diffractometer with $\text{CuK}\alpha$ radiation ($\lambda=1.5406$). The powder XRD analysis of the Struvite crystals grown in herbal extracts and the reported values are matched with JCPDS file (89-4211, 77-2303, 70-069, 38-0203). The indexed powder data for the struvite crystals are presented in Table.2. From this XRD data struvite crystals belong to Orthorhombic system.

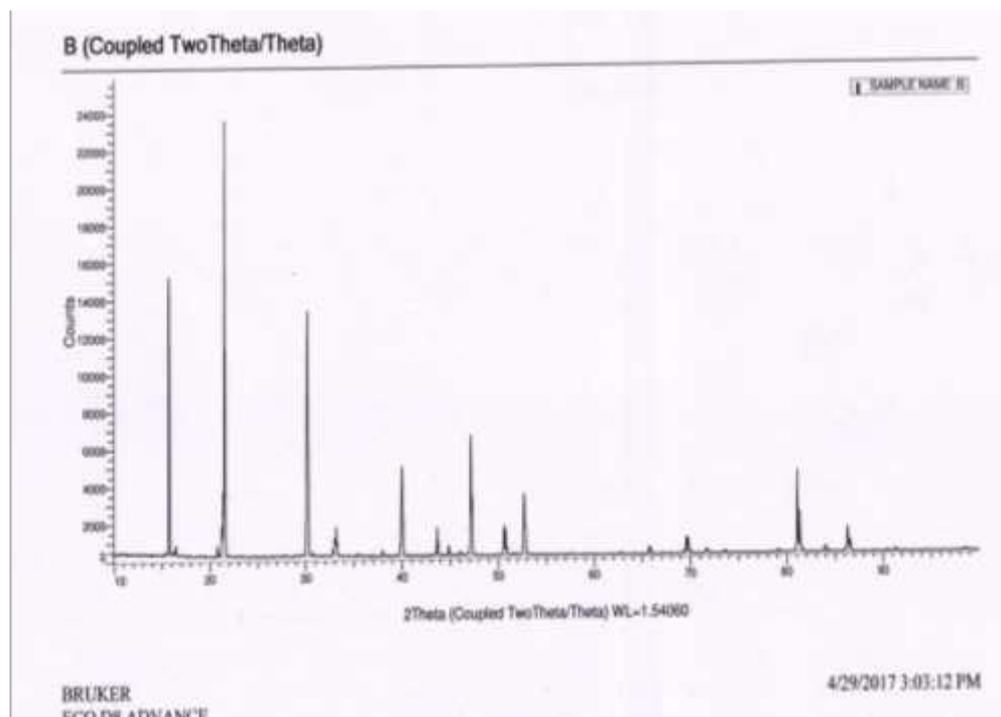


Figure 5. XRD analysis of struvite crystal

Table.2. Powder diffraction data of struvite crystal

Observed value		Standard value		Observed value	Standard value	h k l value
2θ	I/I ₀	2θ	I/I ₀	d spacing [Å]	d spacing [Å]	
15.661	1.0	15.814	446	5.64	5.70	0 0 2
16.236	44.3	16.458	159	5.45	5.44	0 1 1
21.015	83.0	21.437	244	5.38	5.38	0 1 1
28.883	41.6	28.237	999	4.26	4.25	1 1 1
29.228	1.2	29.242	451	4.18	4.19	0 1 2
29.966	1.1	29.921	342	4.12	4.14	0 1 2
30.045	5.1	30.023	14	3.20	3.19	0 1 3
33.032	7.1	33.233	47	2.95	2.94	1 1 3
35.073	8.2	35.024	282	2.90	2.90	1 1 2

The crystalline phases and d-values obtained from the XRD pattern have been compared with those in the JCPDS data. The XRD pattern indicating that the sample consisted of struvite crystalline. From the above results that the Tribulusterrestris extract affected the nucleation and growth of struvite crystals .

4.3 SEM Analysis:

The morphological analysis of Magnesium-ammonium phosphate hexahydrate crystals. Magnesium-ammonium phosphate crystal appeared in pyramid shaped morphology. From EDX analysis presence of magnesium and phosphate is confirmed.



Figure 6. SEM analysis of Struvite crystals

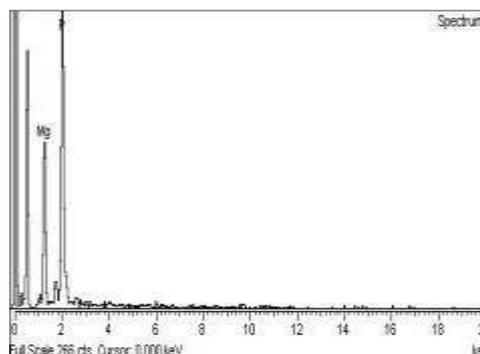


Figure 7. EDX analysis of Struvite crystals

5. CONCLUSION:

In the addition of herbal extract *Tribulus terrestris* in the supernatant solution, the number of grown crystals in the silica gel medium was decreased. The size of the struvite crystals was reduced due to inhibition produced by *Tribulus terrestris* Linn and the morphology of dendritic X shaped struvite crystal in to needle shaped can be observed. From FT-IR the XRD confirms the crystalline nature of the crystal, FT-IR confirms the presence of organic compounds and morphology is identified by SEM –EDX.

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