# Chapter IX

## Conclusions

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9.1 Introduction

Biomineralization or Biocrystallization is the formation of minerals by living organisms, which is quite common in the world of living beings and occurs in single-celled organisms to complex multi-cellular plants and animals. Biomineralization can be defined as the process by which mineral crystals are deposited in an organized fashion in the matrix, either cellular or extracellular, of living organisms [1]. In Biomineralization, the formation of inorganic crystalline structures takes place in association with biological macromolecules. Biomineralization is a hot topic in the area of materials science, which can be described as the widespread and fascinating process by which living organisms produce inorganic phases like carbonates, phosphates, oxalates, silicates, sulfates, oxides, etc. [2] In this process, a living organism provides a chemical and physical environment that controls the nucleation and growth of a unique mineral phase. Since biomineralization seems to be an important phenomenon in topics ranging from space science to medicine, from biology to geology, and every aspect of life, additional focused research is required. Biomineralization in humans is a physiological process regulated by interactions of minerals and organic extracellular molecules. The study of synthetic minerals in simplified solution lays the foundation for an understanding of mineralization processes in more complex environments found in the body. Mineral analysis using various modern techniques provides insights into the mechanism of biomineralization [3].

Biomineralization is helpful to living organisms when it occurs in the correct location in the body, but often this process occurs in the wrong place at the wrong time (bad biomineralization..!). Humans need biomineralization
for the formation of bone, teeth, otoconia (also known as otoliths - tiny calcium carbonate crystals within the utricle and saccule of the inner ear that are critical for the perception of gravity, linear acceleration and necessary to maintain normal balance) etc., but unfortunately, the body also produces various harmful biominerals (as listed in chapter II) responsible for kidney stones in the urinary system; basic calcium phosphate (BCP), calcium pyrophosphate dihydrate (CPPD), monosodium urate (MSU) crystals responsible for rheumatic diseases like arthritis, uric acid responsible for gout etc.

The better and higher quality of life of individual as well as the society depends on the physical health of the individual, which requires rigorous and extensive research work related to the various diseases, their cost effective medical management as well as precautionary measures. Urolithiasis is one of such diseases, which requires better attention. For a better understanding of formation of urinary type crystals, its effective medical management including diagnosis, treatment and its possible prevention, a more systematic physical chemical study of urinary type crystals is needed. Inhibitors of biocrystallization are of interest in drug design efforts against urolithiasis.

In order to contribute to the present knowledge regarding urinary type struvite crystals, the present author have put his humble efforts to study some of the aspects related to struvite crystals as it has been already discussed in the present thesis.
9.2 General Conclusions

The summary of the study carried out by the present author on the growth and characterization studies of struvite and related struvite family crystals are as follows:

1. Struvite crystals can be grown by single diffusion gel growth technique. Growth conditions played important role in the growth of crystals.

2. The crystal morphology of struvite was strongly dependent on growth parameters. By changing the growth parameters, struvite crystals with different morphologies like prismatic type, pyramidal type, star type, rectangular platelet type, elongated platelet type, needle type, coffin-lid shaped and dendritic type can be grown.

3. The grown struvite crystals had transparent, translucent and opaque diaphaneity, depending upon the location and the growth conditions. Fine transparent prismatic type struvite crystals with optimum apparent size were observed only for the growth parameters as SMS of SG 1.04, 1.0 M ADP, 1.0 M SS with 7.0 pH value of the gel.

4. The powder XRD studies confirmed the structural identity of the grown struvite crystals. It was found that struvite crystallized in the orthorhombic Pmn2₁ space group with unit cell parameters as a = 6.954 Å, b = 6.140 Å, c = 11.216 Å and α = β = γ = 90°, which are closely matching with previously reported values. Every unit cell comprises of two molecules.

5. FTIR spectrum of the struvite crystals revealed the presence of functional groups. The spectrum confirmed the presence of water of hydration, N – H bond, P – O bond, NH₄⁺ ion and PO₄³⁻ ion and metal-oxygen bond.
6. The struvite crystals were found to be thermally unstable from thermal studies. From the TGA, the number of water molecules associated with the crystal was estimated to be 5.

7. In the DTA curve of struvite two remarkable peaks were observed. A very strong endothermic peak observed at 192.1 °C attributed to release of crystalline water along with ammonia. A medium exothermic peak observed at 674.7 °C attributed to high temperature phase transition.

8. The DSC curve of struvite exhibited peaks at the same temperatures as peaks were obtained in DTA curve.

9. By applying the Coats and Redfern relation to the dehydration along with decomposition stage in the thermogram of struvite, the values of kinetic parameters were calculated.

10. The thermodynamic parameters for the dehydration and decomposition process of struvite were also evaluated. The process is non-spontaneous and endothermic. Struvite is thermodynamically unstable.

11. For struvite, the dielectric constant as well as dielectric loss was found to be dependent on the frequency of applied field at room temperature. It was noticed both the dielectric constant and dielectric loss were found to be decreased with the increasing value of frequency of applied field. It was found that a.c. conductivity increased whereas the a.c. resistivity decreased with the increasing value of frequency of applied field.

12. In the *in vitro* growth inhibition studies of struvite crystals, comparative lower values of growth rate, reductions in the growth rates with the increasing concentrations of extracts as well as the reduction in the growth rates with the time proved the inhibitory effect of all the three evaluated
extracts, i.e. *Boerhaavia diffusa* Linn, *Commiphora wightii* and *Rotula aquatica* Lour.

13. Significant percentage of inhibition of growing struvite crystals at gel – liquid interface at the end of first day was noticed in each of the herbal extracts, clearly demonstrating the inhibitory effect of all the three extracts used for the investigation. The maximum percentage of inhibition 46.16% was observed for 1.0% *R. aquatica* extract, whereas minimum 13.33% of inhibition was observed for 0.5% of *B. diffusa* extract.

14. Dissolution rates of struvite crystals for all the cases with the herbal extracts were found remarkably higher than that of control solution.

15. The dendritic type struvite crystals grown at the gel – liquid interface were found to be dissolved completely within 21 to 40 days in all the cases with herbal extract. Minimum 21 days were required for the complete dissolution for 1.0% *B. diffusa*.

16. From the analysis of growth and dissolution rates of struvite crystal, it was found that extracts of *R. aquatica* have retarded the growth rate from the very beginning of the crystal growth, whereas extracts of *B. diffusa* have speed up the dissolution rates once the growth of the crystals took place.

17. All the extracts impeded the diffusion process of reactants occurring in the gel column for the nucleation and, subsequently, the growth of struvite crystals. The reduction in depths of growth indicated the inhibition offered by all the three herbal extracts.

18. Growth rates as well as the apparent size of the struvite crystals grown at the higher depths were lower for all the cases with herbal extracts.
19. The remarkable phenomenon of fragmentation of the grown struvite crystals was observed for all the three tested herbal extracts. The average length of crystals after fragmentation was found even less than 1 mm.

20. Both the total mass and volume of the grown struvite crystals were considerably lower for the cases with extracts depicting the inhibitory effect of all the evaluated herbal extracts. The least mass and volume observed for 1.0 % C. wightii.

21. As the concentration of the juice of Citrus medica Linn was increased in the SS, the number of grown struvite crystals in the gel medium decreased and also average size of the struvite crystals decreased.

22. Lower values of growth rate in comparison to control case, reductions in dimension as well as the growth rates with the increasing concentrations of juice, and the reduction in the growth rates with the time evidently proved the inhibitory effectiveness of the juice on struvite.

23. Struvite crystals could not either nucleate or grow at the gel–liquid interface for the higher concentration of juice in the SS, which significantly proved the inhibitory potency of the Citrus medica Linn juice.

24. Enhanced dissolution rates for struvite crystals were observed for all the concentration with the Citrus medica Linn juice.

25. All the struvite crystals grown in the gel were found to be dissolved completely within 15 to 55 days in all the cases with juice. Moreover, reduction in the number of days required for the complete dissolution with the increasing concentration of juice undoubtedly proved the potency of inhibition of struvite crystals.
26. From this *in vitro* growth inhibition study of struvite crystals, it can be concluded that all the investigated herbal extracts i.e. *B. diffusa*, *C. wightii* and *R. aquatica*, and the juice of *Citrus medica* Linn are found to be potent inhibitors for struvite crystals.

27. The Vickers micro-hardness number $H_V$ was found to be load dependent and phenomenon of reverse ISE was observed for struvite crystals.

28. The measured Vickers micro-hardness number $H_V$ for struvite was seen to vary in increasing order from 7.33 kg/mm$^2$ (71.91 MPa) to 45.40 kg/mm$^2$ (445.23 MPa), with certain variations, over the increasing indenting load range from 0.005 to 0.125 kg.

29. Analysis of the experimental data on hardness as a function of indentation size from the standpoint of Kick's Law, Hays-Kendall's model and the proportional specimen resistance model revealed that these models satisfactorily explain the reverse ISE phenomena observed in the *in vitro* gel grown urinary type struvite crystals.

30. The values of load independent Vickers micro-hardness $H_0$ for struvite were obtained as 40.18 kg/mm$^2$ ($\approx$ 394.05 MPa) and 51.72 kg/mm$^2$ ($\approx$ 507.22 MPa) according to Hays-Kendall’s model and the PSR model, respectively. The difference in the values may be due to different approaches as well as mathematical treatments in these models.

31. This study of Vickers micro-hardness and related mechanical properties for gel grown struvite crystals may provide useful information for the fragmentation of the struvite type urinary calculi by using appropriate parameters in ESWL management without causing any damage to the kidney and urinary tract.
32. Citric acid as an etchant produced triangular etch pits on as grown (1 1 1) surface of the prismatic type struvite crystals.

33. The values kinetic parameters - activation energy of the reaction $E$ and frequency factor $A$ were found as 30.267 kJ Mol$^{-1}$ and 0.4972, respectively for etching process on struvite crystals.

34. The values of standard enthalpy of activation $\Delta^±H^o$ and standard internal energy of activation $\Delta^±U^o$ during the etching process were found to be positive and have decreasing nature with the increasing etching temperature. The positive value of $\Delta^±H^o$ explained etching process as an endothermic process, whereas negative values of $\Delta^±S^o$ proved that the etching process was non-spontaneous.

35. Both the struvite analogs struvite-K and struvite-Na can be grown by using single diffusion gel growth technique. Growth conditions played important role in the growth of struvite-K as well as struvite-Na crystals.

36. The crystal morphology of both the struvite analogs was strongly dependent on growth parameters. By changing the growth parameters, struvite-K crystals with different morphologies like prismatic type, star type, rectangular platelet type, elongated platelet type, coffin-lid shaped and dendritic type can be grown. Whereas struvite-Na crystals of prismatic type, star type and dendritic type can be grown.

37. The grown struvite-K and struvite-Na crystals had transparent, translucent and opaque diaphaneity, depending upon the location and the growth conditions.

38. The phenomenon of the formation of Liesegang rings were observed in the gel growth experiments of both the struvite analogs. The numbers of
formation of Liesegang rings were increased with the increasing value of
the gel pH. The thickness as well as spacing between the Liesegang rings
in the gel column increased with the depth.

39. The powder XRD studies confirmed the structural similarity of the grown
struvite-K and struvite-Na crystals with struvite. It was found that both the
struvite analogs crystallized in the orthorhombic Pmn2₁ space group with
unit cell parameters as follows

Struvite-K :  \[ a = 6.893 \, \text{Å}, b = 6.141 \, \text{Å}, c = 11.222 \, \text{Å}, \alpha = \beta = \gamma = 90^\circ \]
Struvite-Na :  \[ a = 6.893 \, \text{Å}, b = 6.124 \, \text{Å}, c = 11.150 \, \text{Å}, \alpha = \beta = \gamma = 90^\circ \].

40. FTIR spectra of both the struvite analog crystals revealed the presence of
functional groups. The spectra confirmed the presence of water of
hydration, P – O bond and \( \text{PO}_4^{3-} \) ion and metal -oxygen bond.

41. Both the struvite-K and struvite-Na crystals were found to be thermally
unstable. Mass loss in a TGA analysis at temperatures above 100 °C
proved the association of water molecules with these crystals. From the
TGA, the numbers of water molecules associated with both the crystal
were estimated to be 5.

42. In the DTA curve of struvite-K two remarkable peaks were observed. A
very strong endothermic peak observed at 180 °C attributed to release of
crystalline water. A medium exothermic peak observed at 677.8 °C
attributed to high temperature phase transition. Similarly in the DTA curve
of struvite-Na two remarkable peaks were observed. A very strong
endothermic peak observed at 183.4 °C attributed to release of crystalline
water. A medium exothermic peak observed at 674 °C attributed to high
temperature phase transition.
43. By applying the Coats-Redfern relation to the dehydration along with decomposition stage in the thermograms of respective struvite analogs, the values of kinetic parameters were calculated.

44. The thermodynamic parameters for the dehydration and decomposition process were also evaluated for both struvite-K and struvite-Na. For both the struvite analog crystals the values of standard enthalpy of activation $\Delta^\ddagger H^\circ$ are positive, which show that the enthalpy is increasing during the process and such process is an endothermic process. Positive values of $\Delta^\ddagger G^\circ$ demonstrate that both the struvite-K and struvite-Na are thermodynamically unstable.

45. For both struvite-K and struvite-Na, the dielectric constant as well as dielectric loss was found to be dependent on the frequency of applied field at room temperature. It was noticed for both the struvite analog crystals that the dielectric constant decreased with the increasing value of frequency of applied field. For struvite-K the variation of dielectric loss increased up to 2 kHz and followed by decreasing nature with higher frequency. Such increase of dielectric loss at lower frequencies may be attributed to oscillation of dipoles as well as the matching of hopping frequency and the frequency of the externally applied electric field. In case of struvite-Na the value of dielectric loss decreased with the increasing value of frequency of applied field.

46. It was found that for the struvite-K a.c. conductivity increased and consequently the a.c. resistivity decreased with the increasing value of frequency of applied field. The frequency dependence of a.c. conductivity of struvite-K follows the Jonscher's universal power law. Whereas for
struvite-Na initially a.c. conductivity increased with the increasing frequency but it was reduced after 40 kHz frequency, which may be due to the mismatch of dipole frequency and applied field frequency. The a.c. resistivity of struvite-Na decreased up to 40 kHz of applied frequency followed by increasing nature.

9.3 Scope of the Future Work

The future work in continuation to the present work may be pursued as follows:

1. It will be exciting to carry out the studies to grow micro and nano crystalline form of struvite and related crystals.

2. In the continuation of growth inhibition studies, it is also required to apply the results of the growth inhibition studies of all the investigated herbal extracts i.e. B. diffusa, C. wightii and R. aquatica on animal model and check the in vivo inhibitory potency of extracts.

3. It is necessary to go for design effective “green antilithic drug” based on medicinal herbal plants.

4. It will be intriguing to go for multi-technique spectroscopic investigations by Raman, infrared absorption, X-ray photoelectron spectroscopy (XPS), and photoluminescence to have insight on the effects of the investigated herbal extracts on the growth inhibition of struvite crystals.

5. It will be fascinating to explore the micro crystal growth technique to struvite crystals and apply it to in vitro the growth inhibition study.

6. It will be also interesting to compare the properties of pure synthetic struvite crystals grown in laboratory, struvite crystals grown in nature and the struvite stones removed surgically from the patients.

7. One can study for micro-hardness studies of struvite-K and struvite-Na.
8. It may be also enthralling to carry out growth and characterization studies of other struvite related crystals.

9. It will be also interesting to study the surface of struvite crystals using AFM after removal from growth inhibition experiments using various herbal extracts.

10. To develop a dynamical model for crystal growth, where the continuous flow of nutrients is available alike the urinary system.
References

