

Bio-Molecules Growth, Characterization Studies, and Nucleation Reduction Mechanism (SeCaHPO₄) in Agar-Agar Gel Medium at Different Environments

J. Kishore Kumar¹, P. Sundaramoorthi²

¹Department of Physics, Arignar Anna Govt. Arts College, Cheiyar–604407, India

²Department of Physics, Thiruvalluvar Govt. Arts College, Rasipuram, Namakkal–637401, India

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Abstract. Exposures of the biologic materials – well-organized many functional structural systems – are found to be attracting and interesting in scientists' work in many areas. The researchers direct their attention towards features, characteristics, and growth of many naturally-occurring bio-materials, and it is called *in-vitro* approach. According to the literature vital features of bio-mineralization in artificial and controlled crystallization or growth of inorganic, organic and semi-organic materials are complications. To identify the influences of single, poly-mineral formations on the kinetics of crystal growth, a thorough study has been taken. The thermodynamically most stable calcium with mixed minerals phases are grown at different pH values at 32°C. Experimental data support the mechanism of inhibition through molecular adsorption of poly-minerals on the surface of growing crystals. The grown crystals are characterized and reported in details.

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1 Introduction

Bio-mineralization is the process by which organisms form minerals and it is important in a wide range of biological events ranging from bone and tooth formation and resumptions to pathological mineralization, such as renal stone and salivary stone formation. It is a true multidisciplinary field that spans the inorganic, organic and semi organic. The formation of calcium phosphates carbonates with hydroxyl and oxalates in supersaturated biological fluids such as

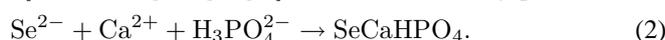
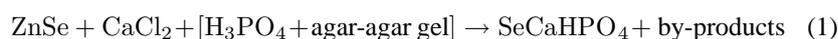
serum, saliva and urine. Biologists focus primarily on the compositions, structures and morphology of the matrices while chemists have been mainly concerned with parameters, such as solution composition, ionic strength, ionic activity, dissociation constants, pH and temperature only [1-2]. However, all other factors are usually ignored in the discussions on induced or controlled crystallization of mineral phases and the connection between chemical processes and biological events is the interfacial free energy at the fluid interfaces. This includes the fluid or nucleus, fluid or matrix and nucleus or matrices. In order to make a quantitative study of kinetic and interfacial tension involved in the crystallization and dissolution reactions, it is essential to use experimental methods which could reveal the interfacial reactions. In the present study, the interfacial tensions between water and the solid surfaces are considered using surface tension component theory. Values for the calcium phosphate phases, such as dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), hydroxyapatite (HAP) are used to elucidate the individual growth and dissolution mechanisms. The ability of the surface to nucleate mineral phases as a function of interfacial tension is also discussed [1].

The growth of SeHP (Selenium hydrogen phosphate) and BHP (Barium hydrogen phosphate) crystals in silica gel medium at room temperature is reported [1-2]. The next approach is to grow the mixed crystal in agar-agar gel medium at different environments, which contains two major elements (Calcium, Phosphate) and one minor or trace element (Selenium). Authors have done a series of experiments in biological crystals growth at different pH values ranging from 5.5 to 11 and have proved that one can obtain the periodic precipitation, Liesegang rings of biological crystals [3-5]. Selenium is a trace mineral that is essential to good health but it is required in small amounts. Selenium is an important antioxidant enzyme which prevents cellular damage of human body from free radicals. Plant foods are the major dietary sources of Selenium. The RDA (Recommended Dietary Allowances) for adults is 55 μg per day. Selenium deficiency in human is rare but it is seen in countries where soil concentration of Selenium is low. Selenium deficiency may contribute to the development of heart disease, hypothyroidism, and a weakened immune system. High blood levels of Selenium give the result in a condition called selenosis. If the mineral level of the body fluid increases, the automatic mixed mineral deposition starts and eventually leads to the kidney or renal stones formation.

2 Materials and Methods

SeCaHP crystals are grown in three different growth environments (room temperature, sunlight and laser exposed medium). The dissociation of ortho phosphoric acid (OPA) system can be represented by three-dissociation equilibrium and the presence of various ions at various pH values has been reported [6]. The crystallization apparatus employed is glass test tube of 25 mm diameter

and 150 mm length for single diffusion method (SD). The chemicals used are Excelar-Qualigens (E-Q) AR grade ZnSe, CaCl₂ and OPA (Sp.gr.1.75). The agar gel is prepared as per the literature [7]. One of the reactant OPA is mixed with agar gel at a desired gel densities and elevated temperatures. After the gel set, the supernatant (ZnSe+CaCl₂) at a required mole solution is slowly added along the walls of the growth columns (test tubes) over the set gels and the tubes are tightly closed to prevent evaporation during the growth period. The growth systems are allowed to react within the gel medium and the following chemical reaction takes place:



3 Result and Discussion

The SeCaHPO₄ crystals are grown in three different growth faces by applying various growth parameters. The growth of SeCaHPO₄ crystals in different environments and the harvested crystals are shown in Figures 1–5. The growth parameters of SeCaHPO₄ crystals (SDP) are observed and the optimum growth parameters are identified. Among them, the laser exposed growth medium (Semiconductor laser light, wave length is 7200 Å, 20 mW of power, continuous apply of laser light of ten months with SMPS arrangement) shows better nucleation reduction and there are no crystals formed because of the inability to attain super saturation. In the sun light exposed medium partial nucleation is observed, since exposure of sunlight to the growth medium is only in day time that is nine hours per day and the growth period is ten months.

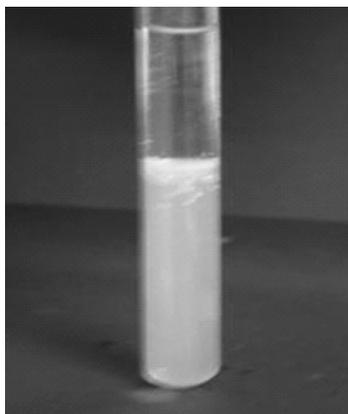


Figure 1. Growth of SeCaHP crystals within laboratory environment.



Figure 2. Growth of SeCaHP crystals in sun light exposed medium.



Figure 3. Growth of SeCaHP crystals in laser exposed medium.



Figure 4. Harvested SeCaHP crystal.

3.1 FTIR spectral analysis of SeCaHP crystal

FTIR Spectrometer having KBr pellets sample holder and KBr detector is used for the analysis. The KBr pellet samples are used and the absorption frequencies range from 600 to 4000 cm^{-1} . The absorption bands, absorption frequencies and percentages of transmittance of SeCaHP crystal are recorded and compared with the reported values. The important spectral characteristics of SeCaHP are grouping of five bands from 3477 cm^{-1} to 2807 cm^{-1} which is due to symmetric and asymmetric O-H stretch. The band at 692.02 cm^{-1} may be due to O-H out of plane vibration. The phosphate group usually has absorption frequencies from 1000 cm^{-1} to 1100 cm^{-1} . These values confirm the presence of grown crystal constituents [8-9].

3.2 Thermogravimetric (TGA and DTA) analysis of SeCaHP crystal

The TGA and DTA of SeCaHP crystals are carried out by STA 11500-PLTS instrument. The SeCaHP crystal of 10.349 mg sample is taken to the TGA process. The TGA is started from room temperature to 900 °C by heating at a constant rate. The weight percentage of the sample present in SeCaHP at a particular temperature is tabulated in Table 1 [10]. In the differential analysis five exothermic and four endothermic peaks are observed. Figure 5 shows the TGA/DTA spectrum of SeCaHP crystal.

Table 1. Thermal analysis of SeCaHP crystal

Points	TGA		
	Temperature [°C]	% of SeCaHP crystal present	Remaining sample [mg]
1	28	100	10.349
2	95.96	99.706	10.318
3	126.32	95.690	9.903
4	181.86	86.855	8.989
5	205.23	81.017	8.384
6	425.87	77.675	8.039
7	481.89	74.453	7.705
8	850	74	7.658

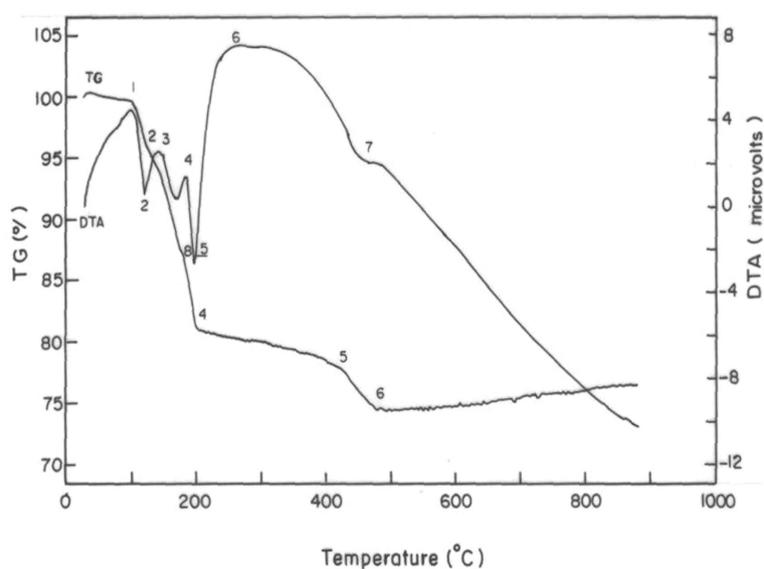
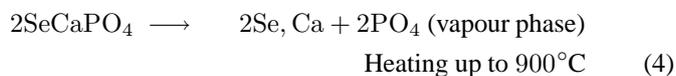
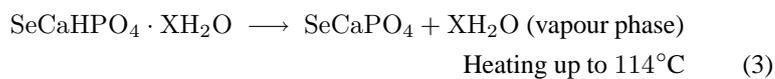


Figure 5. TGA/DTA spectrum of SeCaHP crystal.

The expected chemical reactions are



Se and Ca are stable compounds with respect to the temperature up to 1089 °C. About 26% of SeCaHP crystals are decomposed due to hydroxyl with phosphates and 74% (7.658 mg) of the sample remain stable.

3.3 Etching study of SeCaHP crystal

A well-grown SeCaHP crystal is immersed in HCl solution at a desired concentration. The dissolution of SeCaHP crystal depends upon the etchant concentration, temperature, crystal morphology, etching time, *etc.* [1-3,13-16]. The etch pits are shown in Figure 6. The etch pits are observed as rough sharp pits, stem pits and step pits.

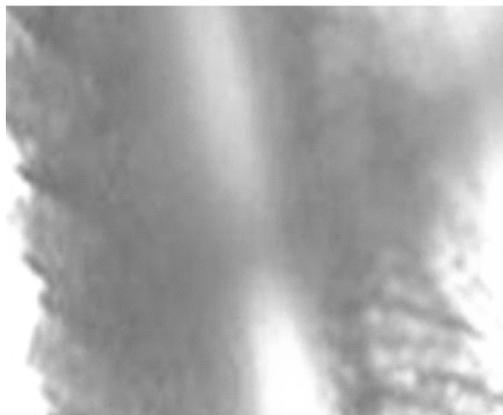


Figure 6. Chemical etch pit of SeCaHP crystal at room temperature.

3.4 X-ray diffraction of SeCaHP crystal

Single crystal XRD results revealed the crystalline property of the grown crystal. The XRD diffraction indices of SeCaHP crystal are recorded and calculated the lattice parameters of SeCaHP crystal. The lattice parameters are $a = 10.01 \text{ \AA}$, $b = 10.22 \text{ \AA}$, $c = 10.59 \text{ \AA}$, $\alpha = 90.19^\circ$, $\beta = 90.01^\circ$, $\gamma = 90.06^\circ$. The volume of the unit cell is 1082.8 \AA^3 . From this data, it is confirmed that grown SeCaHP crystal system is **triclinic** [1-3,21-22].

4 Conclusion

The crystal structure, growth morphology, chemical constituents, surface morphology and TGA/DTA analysis of selenium calcium hydrogen phosphate (SeCaHP) crystals have been investigated. The SeCaHP crystals are grown in three different growth faces by applying various growth parameters. Among them, the laser exposed growth medium shows better nucleation reduction and no crystals are formed because of the inability to attain required super saturation. FTIR spectrum is recorded and the functional group frequencies of SeCaHP crystal are analyzed, which confirms the presence of SeCaHP chemical constituents.

Chemical etching studies are carried out at room temperature and the etch pits are noted which shows the grown crystal defects. The decomposition temperature and percentage of weight loss of grown crystal are recorded by TGA/DTA analysis. Single crystal XRD data identified the SeCaHP crystal system as triclinic structure.

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