ISSN: 2321-7871 Impact Factor : 2.4210 (UIF) [Yr.2014] Volume - 3 | Issue - 25 | 31th Dec - 2015

Weekly Science Research Journal

EFFECT OF GEL PARAMETERS ON NUCLEATION AND GROWTH OF BRUSHITE CRYSTALS IN AGAR-AGAR GEL

V.B.Suryawanshi and R T Chaudhari
Physics Research Lab, Shri. V. S. Naik Arts, Commerce and Science College, Raver, India.

ABSTRACT

Calcium hydrogen phosphate dihydrate ($CaHPO_4.2H_2O$) also known as Brushite is one of the stable form of calcium phosphate type urinary crystals.Large number of methods, like slow evaporation method, high internal phase emulsion processing method have been adopted by researchers to grow calcium hydrogen phosphate dihydrate crystals. Gel method is the most versatile and simple technique for growing urinary crystals. In this method, gel acts as an inert and viscous medium for the growth of crystals. The main problem in the crystal gel-growth technique is the nucleation control. Literature reports the growth of urinary crystals in silica gel, in gelatin gel but was not more found in agar-agar gel. Thus, the growth study of calcium hydrogen phosphate dihydrate crystals grown in agar-agar gel was done using the diffusion methods. Optical microscopy was focused on various morphologies obtained in the present study, while phosphor is a predominant element in the sample was confirmed by EDXA.

KEYWORD: urinary crystals, brushite, gel method, optical microscopy, agar-agar etc.

1. INTRODUCTION

Urinary diesis is a serious health problem that affects 3-20% of the population, depending on the geographical region [1]. Among the different types of urinary stones, calcium stones are mostly found. The calcium oxalates, calcium phosphates and their hydrates are very common in calcium renal stones. [2], carbonate apatite [3] and calcium hydrogen phosphate dihydrate (CHPD, $CaHPO_4.2H_2O$), also known as Brushite [4-5] are the different phases ofcalcium phosphate found in urinary crystals.Brushiteplay an important role in biological mineralization process as well as setting for orthopedic and dental uses [6].Brushite is more soluble than hydroxyapatite in physiological condition and hence are more completely resorbed after its implantation in animal bodies. Thus the brushite single crystals were use as bone graft [7].

Different methods, like slow evaporation method, high internal phase emulsion processing method [8], and gel method [9] have been used by researchers to grow urinary crystals. Of these, gel method is most versatile and simple technique for growing urinary crystals. In this method, gel acts as an inert and viscous medium for the growth of crystals [10-11].Also the gel medium prevents turbulence and remaining chemically inert, provides a three-dimensional structure, which permits the reagents to diffuse at a desirable controlled rate i.e. the ionic diffusion and velocity are slow down by the soft three-dimensional gel framework. Further, its softness and the uniform nature of constraining force that it exerts upon the growing encourages orderly growth [12]. The main problem in crystal gel growth technique is the nucleation control. Literature reports the growth of urinary Brushite crystals in silica gel [13-14], but was not found in agar-agar gel. So far there are no reported data available regarding the nucleation studied on brushite crystals. Recently we reported the growth of these crystals in agar - agar gel [15].The present paper gives the detailed studies made on nucleation and growth of brushite crystals in agar-agar gel by using different growth parameters such as gel density, aging of gel,volume of reactants and concentration of solutions at an ambient temperature. The grown crystals were characterized by optical microscopy and Energy dispersive X-ray analysis (EDXA).

2. EXPERIMENTALPROCEDURES

The growth of Brushite crystals were carried out by single diffusion techniques.For this method, glass test tubes of size 15cm in length and 1.8 cm in diameter was used as crystallization vessels. Agar gel (Himedia) solution was prepared by mixing (0.25 to1.0 gm) of agar powder in 100 ml double distilled water at boiling temperature. Potassium dihydrogen phosphate (KDP) (Merck) of concentration (0.5-1.0M) and calcium chloride (Qualigens) of concentration (0.5-1.0M) were used as reactants.

The agar solution was mixed with the desired concentration and appropriate volume of calcium chloride and was kept for setting. After setting and againg of gel, an aqueous solution of KDP was poured. Initially no precipitation was observed, however nucleations wereseen at the interstitial of the gel within two to eight days.

The grown crystals were harvested from the gel and washed by double distilled water. After filtering and drying, the crystals were characterized by, Optical microscopy and EDXA.

3. Result and discussion

3.1. Growth Study

In single diffusion techniques, while growing the Brushite crystals using silica gel, according to K. Rajendranet al[16] andMadhurambalet al [17], the growth of these crystals is influenced by the initial Liesegang ring formation, however in the present study, when the upper reactant was in contact with the agar-agargel which is precharged with lower reactant, no precipitation and Liesegang ringswere observed. After one to eight days, the agar- agar gel became impregnated with nucleation of brushite crystals. This nucleation of crystals is a function of gel parameters such as, density of gel,gel aging, volume of reactant andconcentration of reactant. With the passage of time about six week, nucleation was further increased into star, single, twin and needle shape crystals as shown in Figure 1.

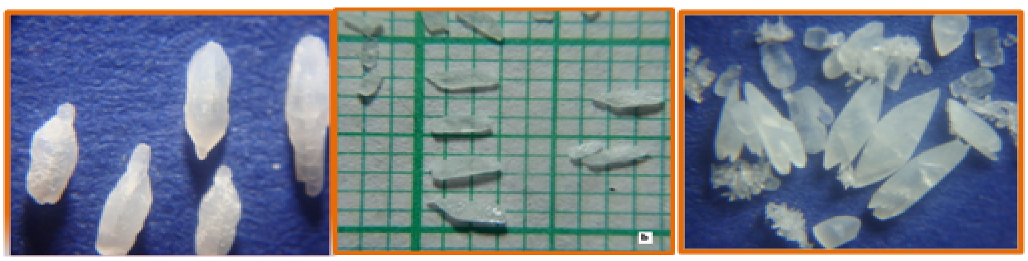

Figure 1: Harvested needle, platy, rectangular, star and prismatic shapeBrushite crystals.

3.1.1. Effect of concentration of gel.

To observed the effect of concentration of gel, experiments were carried out by changing the percentage of agar-agar gel as 0.25%,0.50% and 1.0%.While the other parameters were remain constants such as, volume of first reactant, 4ml and its concentration was 1M, gel aging, 24 hours and lower reactant concentration,1M. In case of 0.25% of gel, below the interface very small size platy shaped crystals were observed. While in 1.0%, comparatively large size crystals were grow at the higher depth from the gel liquid interface. The morphology was same as that of found in 0.25%, but the number of crystals were less. In case of 0.50%, the size, transparency and number of grown crystals were found more than those from 0.25% and 1.0%. The best result was observed in 0.50% agar-agar gel concentration. Thus 0.50% agar-agar gel concentration was used in single diffusion method for all the experiments of crystallization of brushite.

3.1.2. Effect of againg of gel.

To observed the effect of againg of gel on the growth of crystals, experiments were carried out using the gel aging, 24 hours, 48 hours and 72 hours, while keeping the other parameters constants such as, concentration of gel,0.50%, volume of first reactant, 4ml and its concentration was 1M, and lower reactant concentration,1M.

In case of gel aging 24 hours, below the solution gel interface, separated growth of same size platy and rectangular shape crystals were observed. In this test tube no star and twin shaped crystals were observed. While in 48 hours againg, below the interface, more populated platy and rectangular shaped crystals were observed. Due to more growth of crystals in this region, the size of grown crystals was not increased. In case of 72 hours againg, the crystals were grown in the same region of the test tube as that of grown in 48 hours. The number of grown crystals in this region was found to be more than that of in 48 hours, due to this the size of grown crystals was decreased. Best result was observed in case of gel aging 24 hours, Thus 24 hours gel aging was used in all experiment of crystallization of brushite crystals.

3.1.3. Effect of volume of reactant.

The experiments were carried out for first reactant with different volume (2ml-4ml), while the volume (10ml) of second reactant was kept constant. The other parameters such as, gel againg, 24 hours, concentration of gel, 0.50% and concentration of first and second reactant, 1M were remains constant. In case of 2ml volume of calcium chloride solution, the semitransparent platy, needle and prismatic shaped crystals were observed, while in 4ml volume of calcium chloride solution, the size and transparency of grown crystals werefound to be increased. Thus 4ml volume of calcium chloride solution was used in all experiment of crystallization of brushite.

3.1.4. Effect of concentration of reactants.

To observe the effect of upper and lower reactant on the growth of crystals, experiments were carried out by changing the concentration of both reactants. The calcium chloride solution of 0.5 M and 1.0M concentrations were used as lower reactant and KDP solution of 0.5M and 1.0M concentrations were used as upper reactant. While the other growth parameters such as, volume of first reactant, 4ml, gel aging, 24 hours, and concentration of gel, 0.50% were kept constant. From this experiment it was observed that, when the concentrations of upper and lower reactants were 1M, the good quality crystals were grown in the gel. Also it was observed that, when the concentration of upper and lower reactant was (0.5M), no nucleation growth was observed in the gel column.Thus the good quality crystals were found to be grown in the experiment, when the concentration of upper and lower reactant was 1M.

3.2. Characterization

3.2.1Optical Microscopy:

The specific collected samples were observed using a stereoscopic microscopeCZM4 LABOMED. The microscopic photographs of Brushite crystals were as shown in figure 2.The morphology of the crystal is a result of the relative growth of its various faces. In Figure 2(a),it seenthat, the crystal is semi-transparent like a 'flower' shape, elongated as a rod atcenter and was surrounded by petals. However in Figure 2(b) the growth of'snail' shaped morphologywith very small tentacles was observed. The crystals arein semi-transparent nature.

In Figure 2(c), the growth was in dendriticmultilayer form and was observed asthe shape of growth steps. The growth of these crystals was due to entire composition of intergrown crystals.

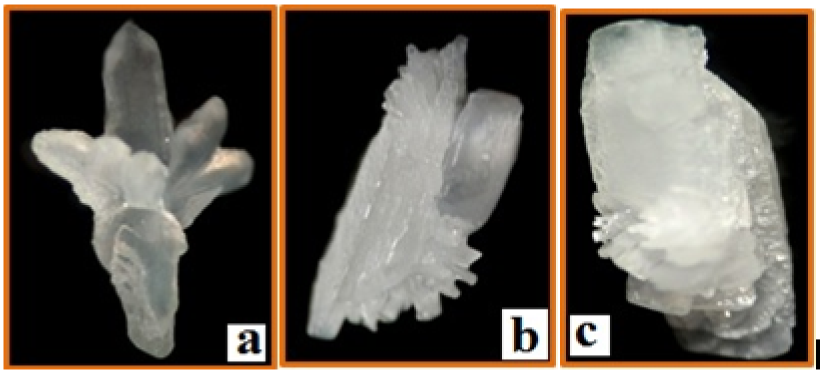

Figure 2:Dendritic growth of Brushite crystals.

3.2.2 Energy dispersive X-ray analysis (EDXA):

The elemental composition of the sample is identified using Energy dispersive X-rayanalysis. Figure 3. Shows the EDX spectrum of Brushite or CHPD crystals. From this EDXA analysis, phosphor was found to be predominant element, while the peak appeared indicates the presence of elementsCa,C and O in the specimen. Table 1 shows the EDX data of brushite crystals.

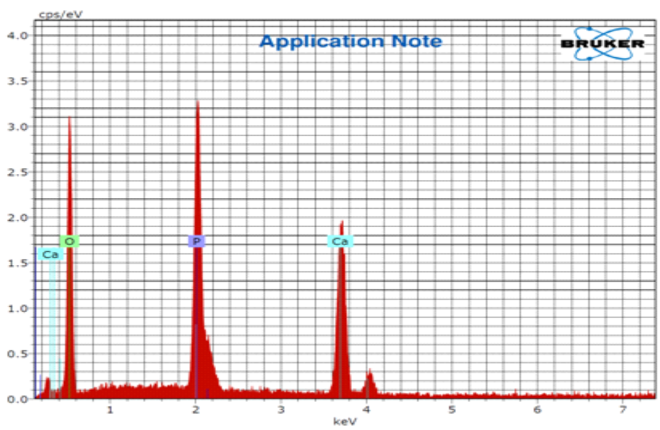

Figure 3: EDX spectrum of Brushite crystal.

Table 1: Values of elemental contents present in Brushite crystal.

No	Element	AN	Experimental Value	
			Wt. %	At. %
1	C	6	3.60	7.42
2	O	8	43.40	67.14
3	P	15	8.73	6.97
4	Ca	20	21.21	13.10

4 CONCLUSIONS.

Brushite crystals can be grown in agar-agar gel.While growing the Brushite crystals in agar-agar gel, the liesegang rings are not form in gel medium.The optimized condition was obtained due to different parameters.It was also found that the growth morphology and size of grown crystals was affected by growth parameters. The morphologies like,star, needle, platy, rectangular and prismatic shape were observed. However in stereo microscope, the different morphologies such as flower shape, and snail shaped dendriticcrystals were obtained.The presence of phosphorus, calciumand carbon in sample was confirmed by EDXA.

ACKNOWLEDGMENTS

The authors are thankful to The Principal, Shri V. S. Naik Arts, Commerce and Science College, Raver (M.S.),to the Director, UDCT, NMU, Jalgaon for providing experimental facilities.

REFERENCES: -

[1] K.J.Diana and K.V.George,J.Cryst. Growth, 363,164-170, (2013)
[2] B. Parekh, M. Joshi, and A. Vaidya, J. of Cryst. Growth, 310, 1749-1753, (2008).
[3]M. Menon, B.G. Parulkar, and G.W. Drach, Campbell's Urology, 3, 2661- 2733, (1998).
[4]K.C.Joseph and M.J. Joshi, Indian Journal of Physics, 76A, 159-163, (2002).
[5]V.S. Joshi and M.J. Joshi, Cryst. Res. Technol., 38, 817-821, (2003).
[6]R.Roop K and M. Wanng, Material letters, 49, 15-19, (2001)
[7]M.P.Binitha and P.P.Pradyumnan,J.Biomaterials and Nanobiotechno.,4,119-122,(2013).
[8]H.N.Lim, A. Kassim, N.M. Huang, P.S. Khiewc, and W.S. Chiu, Colloids and Surfaces A: Physicochemical Engineering Aspects, 345, 211-218, (2009).
[9] B.B.Parekh, M.J. Joshi, and A.D.B. Vaidya,Current Science, 93, 373-378, (2007).
[10] J. Ouyang, S. Deng X. Li, Y. Tan, and T. Bernd, Sci. in China Series B Chemi., 47, 311-319, (2004).
[11]T.Bretherton and A.Rodgers, J.Cryst. Growth, 192, 448-455, (1998).
[12] S.N.Patil and A.Venkateswara Rao,Cryst.Res. Technol., 26,847-853, (1991).
[13] K.C.Joseph, B.B. Parekh, and M.J. Joshi, Current Science, 88(8), 1232-1238, (2005).
[14] B.B.Parekh and M.J. Joshi, Indian J. Pure and Applied Physi, 43, 675-678, (2005).
[15] V. B. Suryawanshi and R. T. Chaudhari, Indian Journal of Mate. Sci., 1-6, (2014).
[16] K.Rajendran and C.D. Keefe, Cryst. Res. and Technol., 45, 939- 945, (2010).
[17] G.Madhurambal, R. Subha, and S.C. Mojumdar, J. Thermal Analysis and Calori., 96(1), 73-76, (2009).