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Research Article

GROWTH AND CHARACTERISATION OF COD, COM AND HYDROXYAPATITE CRYSTALS BY GEL METHOD.

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Abstract

Calcium oxalate monohydrate(COM), Calcium oxalate dihydrate (COD) and Hydroxy apatite (HA) were prepared by using a single and double diffusion technique at different concentration and pH. The harvested crystals were investigated the composition and phase purity by Fourier transform infrared spectroscopy and X-ray diffraction.

Keywords: COM, COD, HA, Single diffusion , Double diffusion,FT-IR,XRD

Introduction

Crystals play a major role in biology also. Most of the living beings form crystals. There are many minerals present in the dissolved form in the human body [1]. The body fluids contain minerals at various levels of saturation. When the body fluids get supersaturated with minerals, crystallization takes place. These crystals have both beneficial as well as pathological effects on humans. The major beneficial roles of mineralization are the formation of bones and teeth, which consist of oriented micro crystals of hydroxyapatite. Our sense of balance and acceleration is dependent upon small calcite crystal present in the inner ear. The pathological effects result in the crystal deposition disease. Crystal deposition disease may be defined as a pathological condition associated with the presence of crystals which contributes to the tissue damage and cause pain and suffering. Bone disease and caries formation; involve dissolution of hydroxyapatite under acidic condition. Atherosclerosis involves the crystallization of cholesterol and hydroxyapatite in blood vessel walls. Gallstones consist mainly of cholesterol with small quantities of calcium phosphates and calcium carbonates. Clusters of small crystals formed in the urinary systems give rise to urinary calculi which consist primarily of calcium oxalates, calcium phosphates , uric acid, urates etc.,. The three major mineral phases associated with joint disease are monosodium urate monohydrate, calcium pyrophosphate dihydrate and hydroxyapatite. The increasing incidence of crystal deposition disease are the result of a complex sequence of events that give rise to disease through simple mechanical effects such as blocking ducts or by hardening or weakening of the flexible tissues. Crystal deposition occurs in a variety of tissues including kidneys, eyes, thyroid glands and bone marrow.

Carr (1953) and Iwata et al (1986) had shown that the urinary stones grow in a gel like medium, which is probably one of the reasons for the radially striated growth of crystals found in urinary calculi. Achilles et al (1995) suggested that crystallization experiments which are intended to understand urinary stone formation

should be carried out in a gelatinous medium rather than in solutions or suspensions. Thus gel growth seems to be an ideal medium to study the crystallization of urinary stone components (biomolecules) as its viscous nature provides simulation of biological fluids in which it grows.

A large number of people in this world are suffering from problems due to urinary stones [2]. There are many areas of high incidence of urinary calculi which include British Isles, Scandinavin Countries [3], northern Australia, central Europe, northern India, Pakistan and Mediterranean Countries. It has also an economic impact on the society [4].

Calcium-containing stones are the most common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate(5%) and a mixture of both (45%). Calcium Oxalate stones are found in two different varieties, Calcium Oxalate Monohydrate(COM) or Whewellite, and Calcium Oxalate Dihydrate (COD) or Weddellite [3]. The Chemical compositions of various calcium phosphates are roughly equivalent to that of the inorganic matrix of the human bone and they are found to be the most suitable as implant materials [5]. The major phase found in bone is hydroxyapatite (HA) or basic calcium phosphate(BCP) and the other commonly known phases are octacalcium phosphate, tricalcium phosphate, dicalcium phosphate dihydrate and dicalcium phosphate [6]. Hydroxyapatite has been used to study the hard tissue calcifications such as bone, teeth and many undesirable cases of pathological mineralization of the articular cartilage [7,8], cardiac valves [9,10] and kidney stones [11].

The present investigation deals with the growth of Calcium oxalate and Hydroxyapatite crystals by gel method. The slow and controlled diffusion of nutrients to the growing crystals in the gel medium is very useful to study the growth and inhibition of Calcium Oxalate crystals in vitro. Hydroxyapatite crystals are grown in the silica gel medium in the Lieseganag rings. These crystals were characterized by various experimental techniques.

Materials and Methods

The Chemicals used for the experiments were of analar grade. Single and double diffusion growth techniques were employed for the growth of calcium oxalate monohydrate crystals.

In a single diffusion method, gel was set by mixing sodium meta silicate solution of density 1.03gcm^{-3} was adjusted to a pH of 6.3 by adding 5% acetic acid [12]. Calcium chloride, one of the reactants was incorporated inside the gel. After the gel was set, 0.5 M oxalic acid solution was slowly added over the gel as the supernatant solution and the experiments were conducted at room temperature. Within a day, a white column of tiny crystals of microscopic sizes were formed. The depth of the column was found to increase with increase in time and there was no change in the size of the crystals.

The double diffusion gel growth technique was used to grow calcium oxalate crystals. The crystallization apparatus used was glass U-tubes with 2.5cm internal diameter, 16mm limb lengths and the separation between two limbs approximately 12cm. The nucleation of COM crystals was due to the calcium chloride and oxalic acid in the silica hydro-gel medium. The neutral gel was prepared by hydrated sodium meta-silicate solution of specific gravity 1.03gcm^{-3} was adjusted to a pH of 6.3 by adding 5% acetic acid. It was then allowed to gel in U-tubes. It took about 48h to became as gel. After gelling, equal volume of 1M Calcium chloride solution was added to one limb of the U-tube and 0.5M Oxalic acid was added to the other limb. The tubes were kept at room temperature. Within 24hrs, a disc of tiny single and twinned prismatic crystals appeared exactly at the centre of the gel column and in due course, the thickness of the disc increased. In some tubes the number of crystals formed in the disc was found to be high and in some other tubes it was found to be less.

Calcium oxalate crystallization-initial process

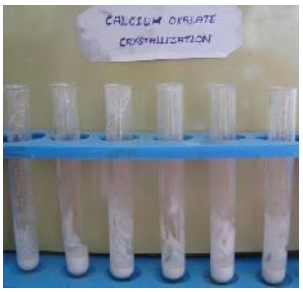

Fig.1 Single Diffusion - Calcium Oxalate Crystals

Fig. 2 Calcium Oxalate Crystals

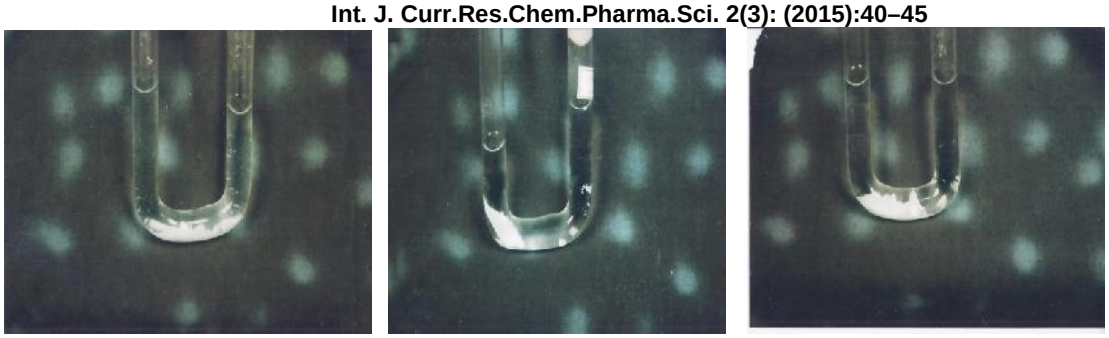

Fig.3 Double Diffusion – Calcium Oxalate

Table. 1 Growth of Calcium Oxalate Crystals at different pH and different concentration.

S.No	Volume of Oxalic acid (ml)	Volume of Calcium Chloride (ml)	pH	Duration of Crystal formed	Quality and Nature of the Crystal
1.	0.5	0.5	5.5	24hr.	Many tiny crystals
			6.0	24hr.	Many tiny crystals
			6.3	24hr.	Large size crystals
			6.5	24hr.	White column of tiny crystals
			6.7	24hr.	White column of tiny crystals
			7.0	24hr.	White column of tiny crystals
2.	1.0	1.0	5.5	24hr.	Many tiny crystals
			6.0	24hr.	Many tiny crystals
			6.3	24hr.	Large size crystals
			6.5	24hr.	White column of tiny crystals
			6.7	24hr.	White column of tiny crystals
			7.0	24hr.	White column of tiny crystals
3.	1.5	1.5	5.5	24hr.	Many tiny crystals
			6.0	24hr.	Many tiny crystals
			6.3	24hr.	Large size crystals
			6.5	24hr.	White column of tiny crystals
			6.7	24hr.	White column of tiny crystals
			7.0	24hr.	White column of tiny crystals
4.	2.0	2.0.	5.5	24hr.	Many tiny crystals
			6.0	24hr.	Many tiny crystals
			6.3	24hr.	Large size crystals
			6.5	24hr.	White column of tiny crystals
			6.7	24hr.	White column of tiny crystals
			7.0	24hr.	White column of tiny crystals
5.	2.5	2.5	5.5	24hr.	Many tiny crystals
			6.0	24hr.	Many tiny crystals
			6.3	24hr.	Large size crystals
			6.5	24hr.	White column of tiny crystals
			6.7	24hr.	White column of tiny crystals
			7.0	24hr.	White column of tiny crystals
6.	3.0	3.0	5.5	24hr.	Many tiny crystals
			6.0	24hr.	Many tiny crystals
			6.3	24hr.	Large size crystals
			6.5	24hr.	White column of tiny crystals
			5.7	24hr.	White column of tiny crystals
			7.0	24hr.	White column of tiny crystals

Fig.4 Double Diffusion – Hydroxyapatite.

Table. 2 Growth of Hydroxy Apatite Crystals at different pH and different concentration.

S.No	Volume of Oxalic acid (ml)	Volume of o-Phosphoric Acid (ml)	pH	Duration of Crystal formed	Quality and Nature of the Crystal
1.	0.5	5	5.5	24hr.	Indistinguishable Liesegang rings are formed.
		5.8	6.0	24hr.	Indistinguishable Liesegang rings are formed.
		6.5	6.3	24hr.	Distinguishable Liesegang rings are appeared
		7.2	6.5	24hr.	Coagulant appearance
		7.8	6.7	24hr.	Coagulant appearance
		8.3	7.0	24hr.	Coagulant appearance
2.	1.0	5	5.5	24hr.	Indistinguishable Liesegang rings are formed.
		5.8	6.0	24hr.	Indistinguishable Liesegang rings are formed.
		6.5	6.3	24hr.	Distinguishable Liesegang rings are appeared
		7.2	6.5	24hr.	Coagulant appearance
		7.8	6.7	24hr.	Coagulant appearance
		8.3	7.0	24hr.	Coagulant appearance
3.	1.5	5	5.5	24hr.	Indistinguishable Liesegang rings are formed.
		5.8	6.0	24hr.	Indistinguishable Liesegang rings are formed.
		6.5	6.3	24hr.	Distinguishable Liesegang rings are appeared
		7.2	6.5	24hr.	Coagulant appearance
		7.8	6.7	24hr.	Coagulant appearance
		8.3	7.0	24hr.	Coagulant appearance
4.	2.0	5	5.5	24hr.	Indistinguishable Liesegang rings are formed.
		5.8	6.0	24hr.	Indistinguishable Liesegang rings are formed.
		6.5	6.3	24hr.	Distinguishable Liesegang rings are appeared
		7.2	6.5	24hr.	Coagulant appearance
		7.8	6.7	24hr.	Coagulant appearance
		8.3	7.0	24hr.	Coagulant appearance
5.	2.5	5	5.5	24hr.	Indistinguishable Liesegang rings are formed.
		5.8	6.0	24hr.	Indistinguishable Liesegang rings are formed.
		6.5	6.3	24hr.	Distinguishable Liesegang rings are appeared
		7.2	6.5	24hr.	Coagulant appearance
		7.8	6.7	24hr.	Coagulant appearance
		8.3	7.0	24hr.	Coagulant appearance
6.	3.0	5	5.5	24hr.	Indistinguishable Liesegang rings are formed.
		5.8	6.0	24hr.	Indistinguishable Liesegang rings are formed.
		6.5	6.3	24hr.	Distinguishable Liesegang rings are appeared
		7.2	6.5	24hr.	Coagulant appearance
		7.8	5.7	24hr.	Coagulant appearance
		8.3	7.0	24hr.	Coagulant appearance

The hydroxyapatite crystals were grown by single diffusion technique. Sodium Meta silicate solution of specific gravity 1.03 was acidified by 1N orthophosphoric acid so that the pH of the mixture could be set within 6-6.5[13]. This mixture was transferred into different test tubes and allowed to set into the gel form. After setting the gel, 1M Calcium chloride solution was poured gently on the set gel. Good quality and very small hydroxyapatite crystals were grown in the form of Liesegang ring. The crystals in the Liesegang rings are of micrometer size and hence are not observasble in the photograph as separate crystals. In a spencer method we can also prepare the hydroxyapatite crystals. These experiments were repeated for different densities 1.03, 1.04 and 1.05 and pH 5.5 -7.

Calcium oxalate crystals were obtained for the conditions of gel density 1.03 with pH 6.3. The hydroxyapatite crystals were appeared as Liesegang rings in silica hydrogel density 1.06 with pH 6.3. The harvested crystals are subjected to FT-IR, XRD and TGA.

Results and Discussion

In order to confirm the grown crystals to be Calcium oxalate monohydrate , Dihydrate and Hydroxyapatite, the crystals were characterized by Fourier Transform Infra-Red Spectroscopy and X-Ray Diffraction. Thermogravimetric analysis can be carried out for monohydrate crystals.

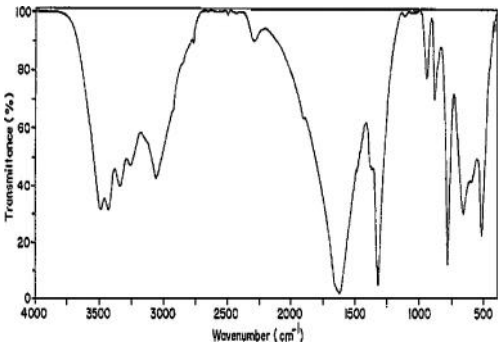

Fig.5 IR for COM

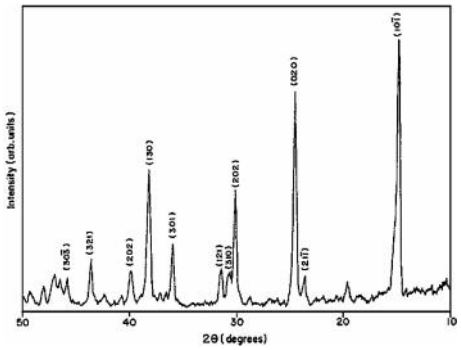

Fig.6 XRD for COM

A bruker IFS 66v FTIR spectrometer was used to record the IR spectrum using KBr pellets and the spectrum is shown Fig.5. The peak at 519cm^{-1} arised due to O-C-O in plane bending. The metal – carboxylate strectch appears at 1316cm^{-1}. The water molecules coordinated with the calcium oxalate molecules produce characteristic peaks corresponding to the bending modes at 1621cm^{-1}. The peaks for asymmetric and symmetric stretch of the coordinate water molecule are shown by the broad spectrum of peaks above 3000cm^{-1}.

The XRD pattern of the grown crystal was obtained from a diffractometer instrument using CuKα radiation was shown in Fig.6. The following diffraction peaks (2θ) 14.95,24.39,30.12 and 38.13 which can be correlated to the indices (101),(020), (202) and (130) of Calcium Oxalate Monohydrate phase.

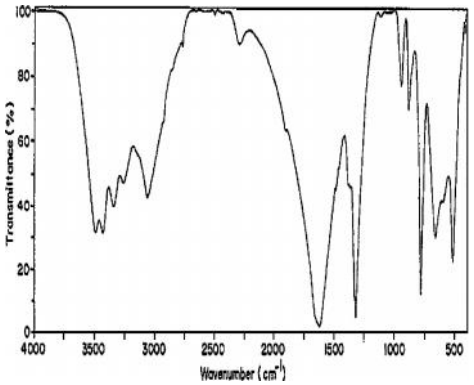

Fig.7 IR for COD

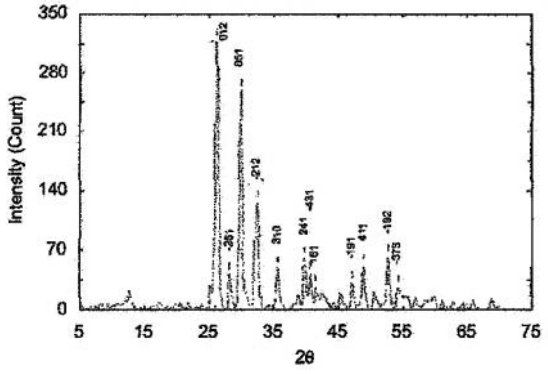

Fig.8 XRD for COD

A bruker IFS 66v FTIR spectrometer was used to record the IR spectrum using KBr pellets and the spectrum is shown Fig.7. The two peaks are at 915 and 615cm^{-1} arised due to O-C-O in plane bending. The metal – carboxylate strectch appears at 1318cm^{-1}. The water molecules coordinated with the calcium oxalate molecules produce characteristic peaks corresponding to the bending modes at 1652cm^{-1}.

The XRD pattern of the grown crystal was obtained from a diffractometer instrument using CuKα radiation was shown in Fig.8. The following diffraction peaks (2θ) 14.26, 20.01, 24.15, 32.17, 37.21 and 40.00 which can be correlated to the indices (200),(211), (002), (411), (103) and (213) of Calcium Oxalate dihydrate phase.

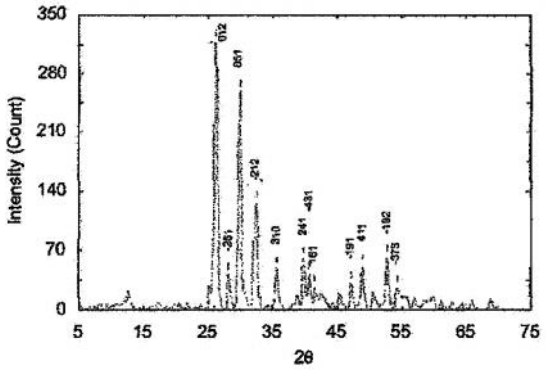

Fig.9 XRD for HA

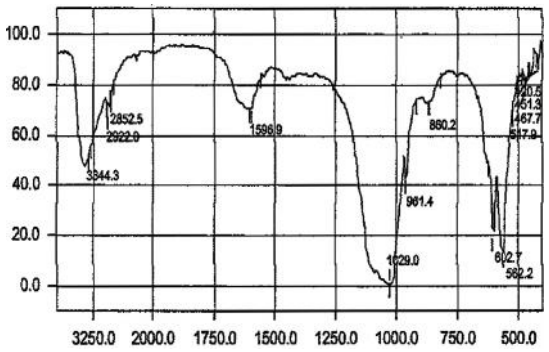

Fig.10 IR for HA

The XRD pattern of the grown crystal was obtained from a diffractometer instrument using CuKα radiation was shown in Fig.9. The following diffraction peaks (2θ) 25.00, 27.13, 32.19, 34.25,35.09, 47.87 and 54.00 which can be correlated to the indices (012),(251),(051), (212),(310),(191) and (102) of Hydroxyapatite phase.

A bruker IFS 66v FTIR spectrometer was used to record the IR spectrum using KBr pellets and the spectrum is shown Fig.10. The absorptions occurring at 3344.3cm^{-1} and 1596.9cm^{-1} are due to O-H stretching and O-H plane bending vibrations. The PO_4^{3-} bands were appeared at 467cm^{-1},562.2 cm^{-1}, 602.7 cm^{-1} , 961.4 cm^{-1} , and 1029 cm^{-1}.

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Conclusion

The Oxalate crystals are very good yield at 6.3 pH with density 1.03 and Phosphate rings density 1.06 with 6.5 pH at low concentration. The grown crystals are characterized and found that it was pure.

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