

Growth of some urinary crystals and studies on inhibitors and promoters. I. Standardisation of parameters for crystal growth and characterization of crystals

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Abstract : Calcium oxalate monohydrate ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$), calcium hydrogen phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) and ammonium magnesium phosphate hexahydrate ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) are known to occur as major constituents of urinary stones. Experiments were carried out for the gel growth of these crystals at various pH values (of the gel) and with different concentrations of the reactants. Best conditions were thus established for the growth of the above crystals. They were characterized using single crystal X-ray diffraction studies, density measurements and IR absorption spectroscopic studies. The best conditions established for the gel growth of these urinary crystals were useful later for studies with some new inhibitors and promoters for crystal growth.

Keywords : Crystal growth, gel technique, urinary crystals

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1. Introduction

More than 99% of the human urinary calculi (stones) are composed of crystalline substances. Most of these are present in nature and known as minerals and consequently the urologists very often indicate them with their mineralogic names. However, they may be divided into two fundamental classes of stones : the first includes organic substances and the second a large percentage of inorganic substances. These substances are : calcium oxalate, calcium hydrogen phosphate, ammonium magnesium phosphate, magnesium phosphate, calcium carbonate, calcium sulfate, uric acid, urates of ammonium, sodium, potassium, calcium and magnesium, hippuric acid, cystine, cholesterol, xanthine and bilirubin. Calcium oxalate (both monohydrate and dihydrate) is the major component

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(about 80%) of the urinary stones. Phosphates account for 15 to 20% and urates, cystine *etc.* form the remaining small percentage (< 5%). Hence, the growth of oxalates and phosphates alone were taken up in our present work.

In India, very little work has been done on the formation and prevention of urinary stones. Systematic studies in crystal growth of the urinary stone components and screening of possible substances which could act as inhibitors or promoters could yield useful results which may become panacea to patients suffering from urinary stones. In the present work, the gel technique has been used for the growth of the crystals. Some earlier reports [1–3] exist on the crystal growth of the above substances. However, a systematic study was necessary since the best conditions obtained for the crystal growth could further be used for the control experiments while carrying out studies with inhibitors or promoters for crystal growth. This is the ultimate aim of the present research project [4].

The best conditions were determined for the gel growth of (i) calcium oxalate monohydrate (COM), (ii) calcium hydrogen phosphate dihydrate (CHP) and (iii) ammonium magnesium phosphate hexahydrate (AMP). The above conditions include the pH of the gel and the concentration of the reactants. Later on, experiments carried out under these best conditions were used as control while carrying out experiments for studying the inhibitory or promotory effects of various substances (to be described in the subsequent parts of this series of papers).

2. Gel growth of crystals

Since the substances taken up for growth are mostly insoluble in water, the usual solution method of crystal growth will not be useful. The gel technique [5] is an useful alternative for such substances. The gel medium prevents turbulence, remain chemically inert and provides a three-dimensional structure which permits the reactants to diffuse at a controlled rate. Also, the softness of gel and the uniform nature of constraining forces that it exerts upon the growing crystals promote orderly growth. Further, the viscous nature of the gel and growth experiments conducted at room temperature *etc.* simulate the human physiological conditions.

In the present work, silica gel prepared from an aqueous solution of sodium metasilicate (specific gravity = 1.06 g/cm^3), was used as growth media. U-tubes (of size : $14.0 \times 1.75 \text{ cm}^2$) and test tubes (of size : $15.0 \times 2.5 \text{ cm}^2$) were used as the crystallization vessels.

The pH of the gel medium and the concentrations of the reactants were varied to arrive at the best conditions for the crystal growth. At these conditions, the number of crystals coming up should neither be very few nor too many. Also, the crystals obtained should be transparent, of reasonable size and have well-defined morphology.

2.1. Growth of calcium oxalate monohydrate :

The pH of the gel solution was reduced by using diluted acetic acid. The solution was transferred to an U-tube and allowed to set. Then, an aqueous solution of calcium chloride

($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was poured into one of the limbs and an aqueous solution of oxalic acid ($\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) in the other limb of the U-tube. These reactants diffuse through the gel medium and form the product, *viz.*, calcium oxalate monohydrate, which appears as crystals at the oxalic acid-gel interface. The crystal growth will be completed in about 20 days. The above experiments were repeated at different pH values (of the gel), in the range 4.5 to 6.5 and at different concentrations of oxalic acid and calcium chloride (0.5 M to 2.0 M). Crystals were obtained in the entire pH range of 4.5 to 6.5. But, transparent rectangular plates of reasonable sizes and good morphology were obtained only at pH of 5.5, while using aqueous solutions of 1 M oxalic acid and 1 M calcium chloride (see Table 1).

Table 1. Summary of the best conditions established for the crystal growth of some urinary stone crystals.

Chemical substance	Apparatus used	pH of the gel	Chemical incorporated in gel	Reactants and their concentration	Transparency, shape and approximate size (mm × mm × mm) of crystals
Calcium oxalate monohydrate	U-tube	5.5	Acetic acid	1 M oxalic acid and 1 M calcium chloride	Transparent plates; 7 × 5 × 1
Calcium hydrogen phosphate dihydrate	Test tube	6.0	Orthophosphoric acid	1 M calcium chloride	Transparent long needles; 10 × 2 × 1
Ammonium magnesium phosphate hexahydrate	Test tube	7.2	1.5 M ammonium dihydrogen phosphate	1.5 M magnesium acetate	Transparent plates, 5 × 3 × 2

2.2. Growth of calcium hydrogen phosphate dihydrate :

A portion of the gel solution was taken in a beaker and its pH reduced to the desired value by adding suitable quantities of diluted orthophosphoric acid. Then, this was transferred to a test tube. After gelation, an aqueous solution of calcium chloride was layered on the top of the gel. In a few days, Liesegang rings were formed and the crystals of CHP started growing. The crystal growth was complete in about 20 days. The above experiments were repeated at different pH values (in the range 4.5 to 7.3) of the gel and at different concentrations of the aqueous calcium chloride solution (0.5 M to 1.5 M). Although crystals were obtained in the pH range of 5.0 to 7.0, optimum nucleation density and hence good transparent long needles were obtained only at a pH of 6.0 while using calcium chloride of concentration : 1 M (see Table 1).

2.3. Growth of ammonium magnesium phosphate hexahydrate :

A portion of the gel solution was taken in a beaker. Its pH was reduced to the desired value by adding suitable quantities of an aqueous solution of ammonium dihydrogen phosphate. Then, the solution was transferred to a test tube. After gelation, an aqueous solution of

magnesium acetate was layered on the top of the gel. This experiment was repeated at different pH values (in the range 5.0 to 8.0) of the gel and using magnesium acetate solutions of various concentrations (0.5 M to 1.5 M). Single crystals of AMP were obtained in the experiments conducted in the pH range 5.5 to 8.0. But, only at a pH of 7.2 and by using aqueous solutions of 1.5 M (both ammonium dihydrogen phosphate and magnesium acetate) transparent plates of AMP of optimum number and of reasonable sizes could be obtained (see Table 1). The crystals exhibit different morphologies such as wedge-shaped, rectangular and prismatic plates. They are colourless but sometimes yellowish or white and chalky. The best conditions established for the crystal growth of COM, CHP and AMP are summarised in Table 1. Photographs of the grown crystals are shown in Figures 1–3.

3. Experimental studies on the grown crystals

The crystals grown must be examined by some experimental techniques to establish their identity and also their single-crystallinity. X-ray diffraction studies on single crystals, measurement of the density of the crystals and IR absorption spectroscopic studies were carried out.

3.1. X-ray studies of the grown crystals :

The crystals grown are of known chemical substances and hence their cell constants are readily available. Hence, it was planned to check atleast any one of the cells of each substance, so that the crystals grown could be characterized. The X-ray oscillation

Table 2. Crystallographic data for the grown single crystals

	Calcium oxalate monohydrate	Calcium hydrogen phosphate dihydrate	Ammonium magnesium phosphate hexahydrate
Chemical formula	$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	$\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$
Molecular Weight	146.12	172.09	245.41
Crystal system	Monoclinic	Monoclinic	Orthorhombic
a (Å)	6.24	5.812 (2)	6.941 (2)
b (Å)	14.58	15.180 (3)	6.137 (2)
c (Å)	9.89	6.239 (2)	11.199 (4)
β	107	116.41 (3)	90.0
Space group	$p2_1/c$	Ia	$\text{Pmn}2_1$
Number of molecules/unit cell	8	4	2
Reference	[7]	[8]	[9]



Figure 1. Photograph of gel grown crystals of calcium oxalate monohydrate.



Figure 2. Photograph of gel grown crystals of calcium hydrogen phosphate dihydrate.

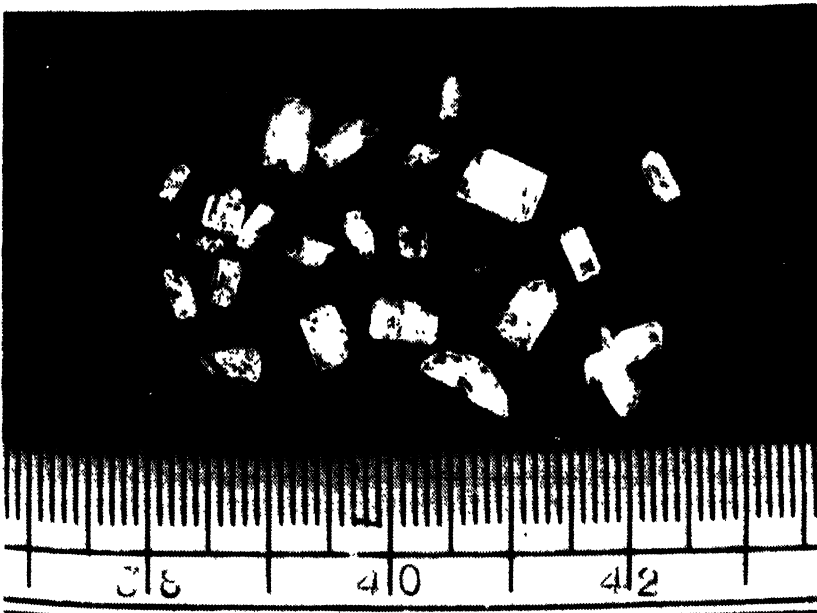


Figure 3. Photograph of gel grown crystals of ammonium magnesium phosphate hexahydrate.

photographs will also help to check the crystalline quality. Therefore, preliminary oscillation photographs were recorded by mounting the crystals along one of the axes. By measuring the layer line separation, the cell constant along the axis of rotation was determined [6]. The values of the cell constants obtained were compared with the available crystallographic data (Table 2) for these substances [7–9], and found to agree well. Thus, the grown crystals were characterized. The X-ray oscillation photographs contained well-defined spots of uniform intensity showing that the crystals are single crystals of good quality [10].

3.2. Measurements of the density of the crystals :

The density of the crystal was determined using the floatation method. Bromoform (density = 2.89 gm/cc) and carbon tetrachloride (density = 1.59 gm/cc) were used for the experiments. The values of the densities determined for the crystals of COM, CHP and AMP are 2.21, 2.29 and 1.69 gm/cc (all values with an esd of 0.02 gm/cc) respectively. The expected values of densities are 2.20, 2.31 and 1.71 gm/cc respectively [11]. There is good agreement between the measured and the standard values, confirming the identity of the substances.

3.3. Infra-red spectra of the grown crystals :

The IR spectra of the grown crystals were recorded in KBr phase in the region 400–4000 cm^{-1} , using a Perkin-Elmer (Model 577) grating infra-red spectrophotometer. The spectra were useful to confirm (i) the identity of the crystals, (ii) the presence of water molecules in the compounds and (iii) the absence of gel inclusion in the crystals. The recorded spectra were compared with the standard spectra of the substances which were already available in the laboratory. The spectra were found to be identical. Hence, the crystals could be characterized as COM, CHP and AMP crystals. Further, it was found that the peaks due to sodium metasilicate were absent when compared with the spectra recorded for the dried gel, indicating the absence of gel inclusion in the grown crystals. In hydrated crystalline salts, the absorption maxima at 3300 cm^{-1} is due to O–H stretching. It is most anharmonic and hence usually broad at room temperature. The absorption maxima at 1700 cm^{-1} is due to O–H deformation, which is generally very weak and hence not usually observed. The absorption maxima in the region 900–500 cm^{-1} is due to H_2O molecular motion (libration) which is also generally weak [12]. The presence of absorption bands at about 3300 cm^{-1} in the IR spectra of all the three compounds confirmed the presence of water molecules in the crystals grown.

4. Studies with inhibitors and promoters

Inhibitors are useful to retard or prevent the growth of the crystals whereas promoters increase the growth rate. Crystal growth studies have been made by incorporating several substances (plant, fruit and cereal extracts, chemicals *etc.*) in the gel medium used for

crystal growth. The results were compared with the control experiment and conclusions derived regarding the effect of the substance incorporated. These details are dealt with in the subsequent parts of this series of papers.

5. Conclusions

From systematic investigations on the gel growth of the above crystals, best conditions have been established to get good crystals of these three substances. The results from the X-ray oscillation photographs, density measurements and IR spectra were used to characterize the crystals grown. The above standardisation of the conditions of the crystal growth were useful in the further work on crystal growth with several inhibitors and promoters.

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References

- [1] Y M F Marickar and P Koshy *Scanning Microscopy* **1** 571 (1987)
- [2] T Irusan, D Arivuoli and P Ramasamy *Cryst. Res. Technol.* **25** K104 (1990)
- [3] T Irusan, D Arivuoli and P Ramasamy *Cryst. Res. Technol.* **29** K71 (1994)
- [4] N Srinivasan *MPhil Dissertation* (Madurai Kamaraj University, India) (1992)
- [5] A R Patel and A V Rao *Bull. Mater. Sci.* **4** 527 (1982)
- [6] C H MacGillavery and G D Reick *International Tables for X-ray Crystallography* Vol. III (Birmingham The Kynoch Press) (1962)
- [7] R W G Wyckoff *Crystal Structures* Vol. V (New York : Interscience) (1966)
- [8] C A Beevers *Acta Cryst.* **11** 273 (1958)
- [9] A Whitaker and J W Jeffery *Acta Cryst.* **B26** 1429 (1970)
- [10] J Auleytner *X-ray Methods in the Study of Defects in Single Crystals* (Oxford : Pergamon) (1967)
- [11] R C Weast *CRC Handbook of Chemistry and Physics 54th edn.* (Ohio : CRC Press) (1973)
- [12] G Fabbri and P Baraldi *Atti. Soc. Nat. Mat. Nodena* **106** 49 (1975)