

## **Scholars Research Library**

Archives of Physics Research, 2015, 6 (1):7-12 (http://scholarsresearchlibrary.com/archive.html)



# Effect of neutral gel on nucleation and growth of urinary calcium oxalate monohydrate crystals in 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 oxalate is one of the most common constituents of renal calculi. Large numbers of investigators have designed experiments to study the formation of these crystals and effect of various substances on their growth rate. Gel method is the most versatile and simple technique for growing urinary crystals. Literature reports the growth of calcium oxalate monohydrate crystals in silica gel, in gelatin gel, but did not found in agar gel. Thus, the calcium oxalate monohydrate ( $CaC_2O_4.H_2O$ ) crystals were grown in agar gel using single diffusion method. The effects of neutral gel on the growth of calcium oxalate monohydrate crystals have been studied. The grown crystals were characterized by powder X-ray diffraction, Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy and Energy dispersive X-ray analysis. In the present study it was found that, the neutral gel controls the nucleation density and increases the size of the calcium oxalate monohydrate.

Keywords: Urinary crystals, COM, neutral gel method, XRD, SEM, FTIR, EDXA

## **INTRODUCTION**

Calcium oxalate is one of the most common compounds belonging to urinary crystals[1]. It is found in three hydrates form with different crystallizing morphologies. Calcium oxalate monohydrate (COM,CaC<sub>2</sub>O<sub>4</sub>.H<sub>2</sub>O), dihydrate (COD, CaC<sub>2</sub>O<sub>4</sub>.2H<sub>2</sub>O) and trihydrates (COT, CaC<sub>2</sub>O<sub>4</sub>.3H<sub>2</sub>O) are the typical phases of calcium oxalate crystals. Among these, COM crystals are in monoclinic prismatic, hexagonal or dendratic form, while COD in tetragonal bipyramid or weddellite and COT in triclinic or needle shape [2].The COD and COT crystals were difficult to form urinary stones because they are unstable and easy to eject from body along with the urine [3], while COM is most thermodynamically stable form of calcium oxalate crystals[4].The, COM and COD crystal are commonly found in excreted urine from stone formers as well as individuals with no history of urinary stones [5].

Literature reported that, the calcium oxalate crystals were grown inartificial urine [6], undiluted urine [7], human urine [8] and also in gel[9-10]. While growing the crystals in gel, it acts as an ideal and viscous medium for the growth [4].Gel method is most versatile and simple techniques to grow urinary crystals. Several researchers have attempted to grow COM crystals in silica and gelatin gel, but no more growth information was available in agar gel. Recently, we reported the growth of microcrystalline COM crystals in agar gel by using single diffusion and double diffusion method [11]. The present study deals with the effect of neutral gel on the formation of COM crystals in agar gel by single diffusion method. The grown crystals were examined by powder X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and Energy dispersive X-ray analysis (EDXA).

## MATERIALS AND METHODS

In previous study [11], micro-crystalline growth of COM crystals was reported in agar gel by single diffusion. In that particular study, optimized condition for COM crystals was obtained. The experimental observation of such

## Scholars Research Library

growth was illustrated in **Figure 1(a)**. To control nucleation, this work is further expanded using intermediate neutral gel to grow COM crystals using single diffusion method. A single glass tube of length 20 cm anddiameter2.5 cm were used as crystallizing vessels. Agar gel (Himedia) solution was prepared by mixing (0.5- 1.0 gm) of agar powder in 100 ml double distilled water at boiling temperature. Oxalic acid (Merck) of concentration (1.0M) and calcium chloride (Qualigens) of concentration (1.0M) were used as reactants.

In single diffusion, hot aqueous 1% agar gel and a 5ml solution of calcium chloride solution was mixed and kept for gelation. The period of gelation was found to be 24 hours. After gelation, a 15 ml hot neutral agar gel containing 1% concentration was slowly poured over the acidified set gel and once again kept for setting. This period of setting was again found to be 24 hours. After ensuring firm gel setting, A 20 ml aqueous solution of oxalic acid was carefully added over it. No nucleation was seen, however white microcrystalline precipitation was observed in the gel. This white precipitation band was increased gradually and just below the interfacing of gels, large numbers of small sized crystals were grown as shown in **Figure 1(b)**.

However, the same study was carried out using double neutral gel. For this, a 20 ml hot aqueous agar solution containing 1% concentration was mixed in 5 mlof calcium chloride solution and kept for setting. The period of gelation was found to be 24 hours. After gelation,0.5%, 10 ml neutral agar gel was slowly poured over the acidified set gel and again kept for setting. After setting and aging the gel, once again 1.0%, 20 ml agar gel solution was slowly added over 0.5% set gel and kept for setting. Then on set gel, a 20 ml solution of oxalic acid (1M)was slowly poured over it and kept undisturbed. Immediately, white microcrystalline precipitation was observed in tube. Within a few days, the length of this precipitation was diffused from both the neutral gel as shown in **Figure 1(c)**.

After the crystallization of 45 days, the crystals of COM were harvested from the gel and washed by double distilled water. Further, as grown crystals were characterized by, XRD, SEM, FTIR and EDXA.



Figure 1: Growth of COM crystal in single diffusion,(a) without neutral gel, (b) with single layer neutral gel (c) with double layer neutral gel

## **RESULTS AND DISCUSSION**

## Growth:

In gel growth techniques, gel medium provides a three dimensional structure, which permits the reactants to diffuse slowly at a desirable controlled rate [12].But in previous reported study [11],it was found that the rate of diffusion of reactants was high. Due to high diffusion rate of inner (CaCl<sub>2</sub>) and outer reactant (H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>), immediately white microcrystalline precipitation was observed in gel column. This precipitation was due to gatherings the large numbers of calcium ions near the interstitial of the gel during the period of setting by diffusion of ions. When the oxalic acid is poured, the oxalate ions present in it get reacts with calcium ions as a result in turns into white precipitation band [13].The microscopic morphologies of such growth were as shown in **Figure 2(a)**.

While extending the same work in neutral gel, it was obtained that the neutral gel controlled the diffusion rate of reactants. Initially, white microcrystalline precipitations was seen in gel, but at a later stage this microcrystalline precipitation were starts to nucleate and grows as transparent crystals shown in **Figure 2(b)**.

After pouring the reactant in double neutral gel experiment, white precipitation band was formed in gel. The length of this precipitation was increases gradually due to the diffusion, but at later growth stage no growth morphologies were seen in double neutral gel as those was seen in single neutral gel. It was also found that, the number of grown crystals was decreases in double gel diffusion. This may be due to fact that, the number of nucleation is depends on the specific gravity of gel, aging period and pH of gel as well as thickness of neutral gel. Because of increasing the thickness of neutral gel, it suppresses the diffusion of ions along the gel column [14].**Figure2(c)**shows the growth of dendritic shaped COM crystals grown in double neutral gel diffusion method.



Figure 2: Stereoscope microscopy morphologies of COM crystals (a) growth in without neutral gel (b) growth in single neutral gel (c) ) growth in double neutral gel.

## Characterization:

## X-ray diffraction:

Calcium oxalate monohydrate crystals were powdered and X-ray powder diffraction (XRD) data were collected at room temperature on "Bruker D8 Advanced" X-ray powder diffractometer is shown in **Figure 3**. The sample was scanned from 0<sup>0</sup> to 80<sup>0</sup> in 20. The XRD of the grown COM crystals was matched with the reported database using computer with PAN alytical software and the result was matched with the JCPDs files[15-16]. The collected XRD data are given in **Table 1**. All the reflections were indexed successfully using the program. The lattice parameters were obtained using the program and the refined parameters, a = 8.721(3) Å, b = 8.721(3) Å, c = 9.171(5) Å,  $\alpha = 90[^{\circ}], \beta = 90[^{\circ}], \gamma = 90[^{\circ}]$  and volume = 697.43[Å^3].

Pos. [°2Th.]	FWHM [°2Th.]	h	k	1	Area [cts*°2Th.]	d-spacing [Å]	Rel. Int. [%]
14.0109	0.1476	1	0	1	81.51	6.31581	53.52
14.3839	0.1476	1	1	0	38.26	6.15286	25.12
22.5716	0.3542	2	0	1	19.24	3.93607	5.26
23.5079	0.2066	1	1	2	184.17	3.78136	86.37
24.7538	0.1771	2	1	1	12.24	3.5938	6.7
29.191	0.1771	0	0	3	41.79	3.05682	22.87
29.7946	0.1181	2	1	2	18.11	2.99626	14.87
30.5744	0.1181	3	0	0	41.91	2.92159	34.4
30.7326	0.1181	3	0	0	38.2	2.90691	31.35
35.0818	0.2362	2	2	2	44.42	2.55585	18.23
37.2882	0.2066	2	1	3	95.55	2.40953	44.81
38.279	0.3542	3	2	1	14.03	2.34941	3.84
39.0626	0.2952	0	0	4	29.64	2.30407	9.73
39.8564	0.144	0	0	4	78.79	2.25999	39.23
39.988	0.0886	1	0	4	36.9	2.25285	40.38
42.6827	0.2952	3	0	3	27.14	2.11665	8.91
44.9387	0.2362	3	3	1	11.99	2.01549	4.92
46.0915	0.1476	4	0	2	26.7	1.96773	17.53
47.1856	0.2952	4	1	2	14.09	1.92462	4.63
48.4515	0.2362	3	3	2	19.87	1.87725	8.15
49.3806	0.0886	2	2	4	29.85	1.84409	32.66
50.0022	0.2362	0	0	5	22.81	1.82261	9.36
51.7926	0.2952	1	1	5	13.86	1.76373	4.55
53.2786	0.2362	4	3	1	22.57	1.71799	9.26
57.147	0.108	5	1	2	150.63	1.61054	100
60.8899	0.432	4	3	3	14.05	1.5202	2.33
77.4438	0.72	6	0	4	30.46	1.23141	3.03

Table 1: X-ray	powder	diffraction	data	for	COM	crystal
1 abic 1. 21-1 ay	ponuci	unnaction	uuuu	101	COM	ci youu



Figure 3: X-ray powder diffractogram of grown COM crystal

### **Scanning Electron Microscopy:**

The Morphology of COM crystals were observed by using SEM as shown in Figure 3(a), (b) and (c).



(a)

(b) Figure 3: SEM images of COM crystals

(c)

In **Figure 3**(a) scale bar is 100 $\mu$ m, in such image it seems that the growth of crystals were obtained in different morphologies, such as monoclinic prismatic, hexagonal prismatic and plate like shape etc. However in higher magnification **Figure3(b)** whose scale bar is 10 $\mu$ m, the six sided platy habit as well as ten sided morphologies were observed. The surfaces of such morphologies were seems to be planes and flats. In **Figure 3(c)** whose scale bar is 5 $\mu$ m, a single monoclinic pyramidical morphology was observed. Mechanically it's pyramidical top and sides are flats. The average size of this micro crystal is approximately 5.0X 5.0  $\mu$ m.

## **FTIR Spectroscopic Analysis:**

FTIR spectrum in the range 400cm<sup>-1</sup>to 4000 cm<sup>-1</sup> of the synthesized COM crystal is shown in **Figure 4** and assigned vibrations with observed wave numbers are presented in **Table 2**.

In FTIR spectrum, a strong band at 3489.34cm<sup>-1</sup> and 3064.03cm<sup>-1</sup> is due to asymmetric and symmetric OH stretching, While an intense absorption band at 1580.72 cm<sup>-1</sup> and 1303.92 cm<sup>-1</sup>can be assigned to asymmetric and symmetric C=O stretching bands specific to the COM[17], which is slightly lower than expected values previously[11]. The sharp band at 882.46 cm<sup>-1</sup> is due to C-C stretching vibrations which confirm the existence of oxalate group in COM. The sharp peak at 766.73 cm<sup>-1</sup> is due to O-C=O, however, the peak at 500.54 cm<sup>-1</sup> is assigned to the presence of metal oxygen bond[18-19]. The FTIR spectroscopy detected the growth of COM crystals by the presence of characteristics bands at 3489.34, 3064.03, 949.97, 882.46, 766.73 and 500.54 cm<sup>-1</sup>. Thus FTIR revels that the growth of COM crystals was due to the presence of O-H stretching, C=O, C-C, O-C=O and M-O bonds. The observed vibrational frequencies and their tentative assignments were listed in **Table 2.** 



Figure 4: FTIR spectrum of COM crystal

	Observed wave number in cm <sup>-1</sup>			
<b>Bonds/Vibrational assignments</b>	Without Neutral	With Neutral		
	Gel[11]	Gel		
Asym. OH stretch	3423.76	3489.34		
Sym. OH stretch	3057.27	3064.03		
Asym. C=O stretch	1618.33	1580.72		
Sym. C=O stretch	1315.50	1303.92		
Asym. C-O stretch	1020.00	1140.93		
Sym. C-O stretch	949.01	949.97		
C-C stretch	883.43	882.46		
0-C=0	777.34	766.73		
Metal-Oxygen bond	509.22	500.54		

Table 2: FTIR	wave numbers and	vibrations	assignment	of COM crystals
I HOIC MI I I III	mare mannoers and	vioi actorio	abbiginnente	or com crystais

## Energy dispersive X-ray analysis (EDXA):

The elemental composition of the sample was identified using Energy dispersive X-ray analysis. **Figure 5**shows EDXA spectrum of COM crystal. The higher peak of Ca, O and C shows that these are more concentrated element in the specimen. **Table 3**exhibits the EDXA data of COM crystals.



Figure 5: EDXA spectrum of COM crystal

#### Table 3: EDXA data of COM crystal

El	AN	Series	Unn. C [wt. %]	Norm. C [wt. %]	Atom. C [at.%]	Error [wt.%]
С	6	K- series	23.39	20.04	31.44	3.54
0	8	K- series	57.61	49.36	58.14	7.77
Ca	20	K- series	21.88	18.75	8.82	0.72

#### CONCLUSION

This study concluded that the neutral agar gel controlled the nucleation and increases the size as well as transparency of COM crystals. SEM observations on surface morphologies of COM crystals were shows that the grown crystals are in monoclinic prismatic, hexagonal prismatic, monoclinic pyramid and platy shapes. XRD study confirmed the crystalline nature of grown COM, while FTIR spectrum of COM revealed the presence of various functional groups. It was also found that, the neutral gel does not affect the vibrational frequency of functional groups. EDXA confirms the presence of calcium, carbon and oxygen elements in the grown sample.

#### Acknowledgements

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

### REFERENCES

[1]H.Sindhu, R Gupta, S K Thind, R. Nath, Urol. Res, 1986, 14, 299-303.

[2]V.Thongboonkerd, T. Semangoen, S. Chutipongtanate, ClinicaChimicaActa., 2006, 367, 120.

[3]Y.Zhang, J. Tao, N. Feng, X. Han, Cryst. Res. Technol., 2008, 43, 931.

[4] J.M.Ouyang, Y.H. Tan, Y.H. Shen, ActaPhysico-ChimcaSinica., 2003, 19,4, 368.

[5] R. L. Ryall, M. C. Chauvet and P. K. Grover, *BJU International*, **2005**,96, 654-663.

[6]P.Rajeshwri, G.Rajeshwri, Sk. Jabbirulla, I.V. Vardhan, Int J PharmSci, 2013, 5(3) 599-601.

[7]P.K.Grover, R.L. Ryall, V.R. Marshall, Kidney International., 1992, 41, 149.

[8] R. C.Walton, J. P. Kavanagh, B. R. Heywood, P.N. Rao, J Cryst. Growth, 2005, 284, 517-529.

[9] M.Deepa, K.R.Babu, V.K.Vaidyan, J. Mater. Sci. Lett., 1995, 14, 1321.

[10] E.K.Girija, S.C.Latha, S.N. Kalkura, P.Ramasamy, Mat. Chem. Phy., 1998, 52, 253.

[11]V. B.Suryawanshi, R.T.Chaudhari, Archi. of Phy. Rese. 2014, 5 (2):38-44

[12]S. N.Patil, A. Venkateswara Rao, Cryst.Res. Tech.26,(7),(1991), 847-853

[13]P. V.Dalal, Indian j. Material Science, 2013, ID682950.

[14]S. K.Aarora, V. Patel, A. Kothari, B. Amin, Cryst. Growth & design, 2004, 4(2), 343-349.

[15] Joint Committee for Powder Diffraction Standards Reference Card Number 14-0770

[16] Joint Committee for Powder Diffraction Standards Reference Card Number 14-0789

[17]A.Thomas, pdf Thesis, 2009.

[18] P.Bhatt, P. Paul, J. Chem. Sci., 2008, 120, 267.

[19] B.B.Parekh, S.R.Vasant, K.P.Tank, America J Infec. Disease, 2009, 5,3, 232.