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Growth and study of micro-crystalline calcium oxalate monohydrate crystals by agar gel system

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ABSTRACT

Various crystals are found in human urine. Of those, the calcium oxalate is one of the principle components of urinary stone. It is found in three different hydrate forms, Calcium oxalate monohydrate (COM), calcium oxalate dihydrates (COD) and calcium oxalate trihydrates (COT).Calcium oxalate monohydrate ($CaC_2O_4.H_2O$) is most thermodynamically stable form of calcium oxalate and occupies the biggest proportion of all the urinary stones.The growth of calcium oxalate monohydrate crystals is achieved in agar-agar gel by single diffusion and double diffusion gel techniques. Result shows the white microcrystalline precipitation was formed in both techniques.The grown crystal were characterized by optical microscopy, Fourier Transform Infrared Spectroscopy (FTIR), powder X-ray diffraction (XRD) and thermogravimetric analysis (TGA). Morphology of COM crystals were reveled by Optical microscopy, whereas functional groups were confirmed by FTIR analysis. Crystalline nature was confirmed by XRD. In third stage of decomposition, sample was converted as a residue of calcium oxide after releasing of CO_2 which confirmed the structure of COM.

Keywords: Calcium oxalate crystal, Gel growth, Agar-agar, FTIR, XRD, TGA.

INTRODUCTION

Urinary disease is one of the oldest and common afflictions of humans. All over the world, 12% of the world population was suffering from such urinary stone problem due to global warming, life style and dietary habits [1]. Not only humans, but animals and birds are also suffering from this urinary stone problem [2].The urinary stone formation is a biological process that involves crystallization and comprises by several urinary minerals [3]. These urinary stones were developed, when the minerals in the urine clump together and grow instead of being diluted and passed out of the body.

Mostly, calcium stones are the common urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) or a mixture of both (45%) [4].Calcium oxalate has low solubility in water and crystallizes in three hydrates forms, calcium oxalate monohydrate (COM), calcium oxalate dihydrates (COD) and calcium oxalate trihydrates (COT) [5-7]. Among these, COD and COT are difficult to form urinary stone because they are unstable and easy to eject from body along with the urine [8]. However, calcium oxalate monohydrate ($CaC_2O_4.H_2O$) is the most thermodynamically stable form of calcium oxalate and occupies the biggest proportion of all the urinary stones [9-11].

Gel method is the most versatile and simple technique for growing urinary crystals [12].In this method, gel acts as an ideal and viscous medium for the growth of these crystals [13-14]. Also the structure of the gel controls the whole process of diffusion and growth rate.

Literature reports on the growth of calcium oxalates in aqueous solution, diluted urine [15], undiluted urine [16-17] or either in artificial urines [18]. The growth of calcium oxalates crystals was observed in silica gel and carried out

the surface topographical studies and Knoop microhardness studies [19-20]. Also, the growing method was reported in gelatin gel [21], but was not reported in agar- agar gel.

In the present work attempts have been made to grow COM crystals in agar-agar gel at room temperature and characterized by optical microscopy, Fourier transform infrared (FTIR) spectroscopy, powder X-ray diffraction (XRD) and thermogravimetric analysis (TGA).

MATERIALS AND METHODS

Experimental

The calcium oxalate monohydrate crystals were grown by single and double diffusion techniques [22].In these methods glass test tubes of size 15 cm in length and 1.8 cm in diameter and U-tube of size 25 cm in length and 2.5 cm in diameter were used as crystallization vessels. Agar gel (Himedia) solution was prepared by mixing (1.0- 2.0 gm) of agar powder in 100 ml double distilled water at boiling temperature. Oxalic acid (Merck) of concentration (0.25-1.0M) and calcium chloride (Qualigens) of concentration (0.25-1.0M) were used as reactants.

In single diffusion techniques, the agar solution was mixed with the desired concentration and appropriate volume of calcium chloride. After setting the gel, an aqueous solution of oxalic acid was slowly poured over it. Immediately, a white microcrystalline precipitation was observed in the gel column. Within a few days, the length of this precipitation was found to be increased.

The same method was repeated by reversing the reactants. After pouring supernatant solution, precipitation in the gel column was delayed by few hours.

In double diffusion techniques, gel solution was poured in U tube up to appropriate height. After setting the gel, an aqueous solution of oxalic acid was poured in one limb, while an aqueous solution of calcium chloride was poured in other limb. When both the solutions diffused in the gel, a week later white microcrystalline precipitation layer was observed in one limb. The length of precipitation was increased with time. After two month, the product was washed three times by double distilled water and the residue was characterized by optical microscope, FTIR, TGA and XRD.

RESULTS AND DISCUSSION

Like silica gel and gelatin gel, agar-agar gel provided a favorable growth supporting medium on which the slowly diffusing calcium and oxalate ions nucleated. Thus, in the present work, when the upper reactant ($H_2C_2O_4$) was in contact with the agar-agar gel, precharged with lower reactant ($CaCl_2$), a thin white microcrystalline precipitation was immediately formed. This may be due to the fact that large numbers of calcium ions (Ca^{2+}) were gathered near the interstitial of the gel during the period of setting by diffusion of ions.When the supernatant is poured, oxalate ions ($C_2O_4^{2-}$) present in it get to react with calcium ions, as a result spurious nucleation are formed, which in turns into a precipitation band [23].With the passage of time, this precipitate was proceeds to diffuse in the gel column as shown in **Figure 1(a)**. After few days, in a small transparent zone left at the bottom very tiny crystals were observed as shown in **Figure 1(b)**. About 30 days of pouring of the upper reactant, the precipitate had reached almost to the end of gel column at the bottom of quite transparent.

The experiment was carried out by changing the position of reactants, no white microcrystalline precipitation was observed in gel, however the precipitation was delayed byone hour of diffusion.This may be due to fact that, the rate of diffusion of Ca^{2+} ions in agar gel is less than the $C_2O_4^{2-}$ions. **Figure 1(c)** shows no precipitation was observed in gel column by changing the position of reactants.

In double diffusion, initially the microcrystalline precipitation layer observed in chloride side, but was not found in the middle of U tube.This may be due to the change in rate of diffusion of Ca^{2+} and $C_2O_4^{2-}$ions and difference in molar masses of precipitating ions.The molar mass of Ca^{2+} ions is less than the $C_2O_4^{2-}$ ions, so Ca^{2+} ions diffuse more slowly than $C_2O_4^{2-}$ions from the microporus capillaries formed in agar- agar gel. The length of precipitation was further increased towards the center of U tube as shown in **Figure 2**. After washing precipitation, a quite shiny microcrystalline residue was observed as seen in **Figure 3**.

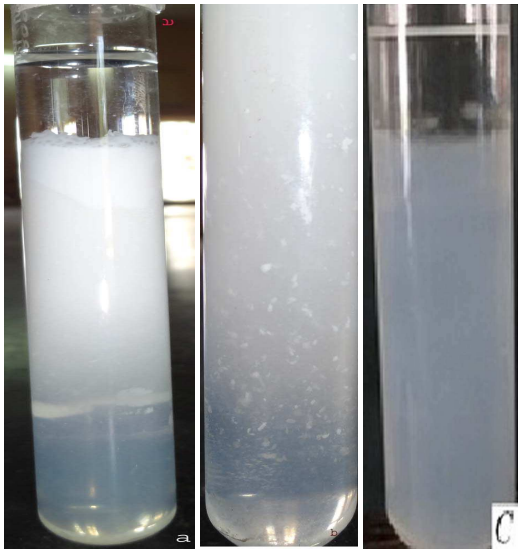

Figure 1: Growth of COM crystal in single diffusion.

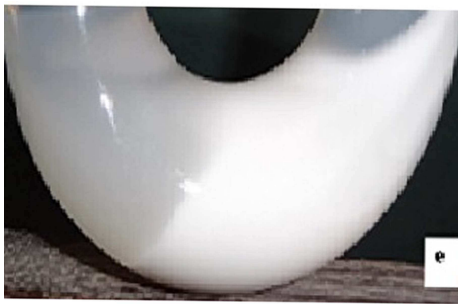

Figure 2: Growth of COM crystal in double diffusion.

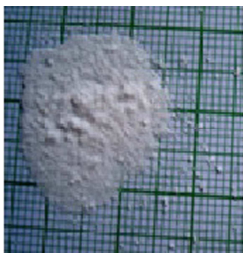

Figure 3: Microcrystalline Calcium Oxalate Monohydrate crystals.

Optical Microscopy

The microcrystalline morphology of the grown crystals was studied using a "LABOME, Ix300" microscope with magnification of 4x, 10x and 40x using a camera eyepiece. Pictures were recorded by the software programme Future winjoe Images. **Figure 4** shows the different morphology of the COM crystals. These images were interpreted by matching with typical morphologies [24]. These includes monoclinic prismatic, six sided platy habit, agglomerates and dendrites of COM crystals.

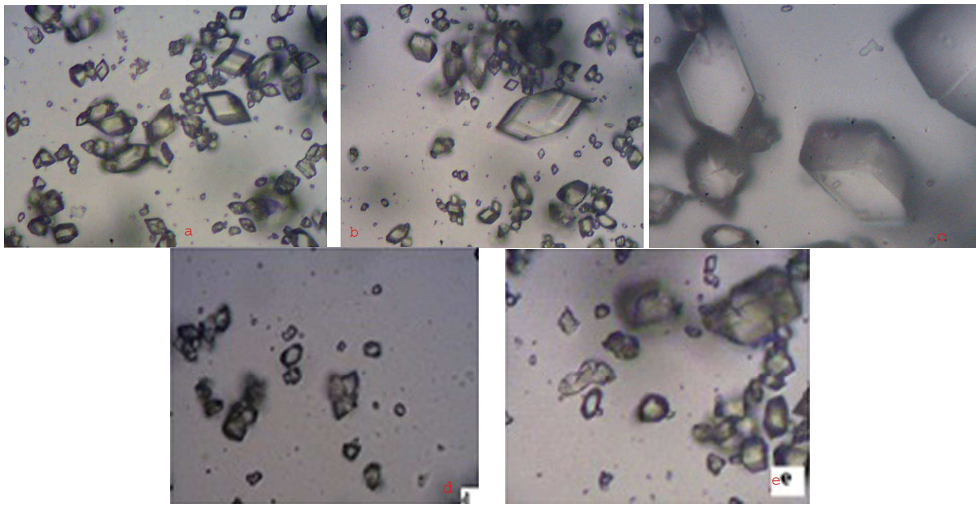

Figure 4: Morphology of COM crystals grown in agar-agar gel. (a) Prismatic (b) monoclinic prismatic (c) tetragonal and six sided platy habit (d) agglomerates (e) dendrites.

FTIR analysis

Figure 5 shows the recorded FTIR spectra of COM crystals.The spectrum shows various frequencies of vibrational modes. The observed absorption frequencies were compared with the reported data [7,10, 25-27].

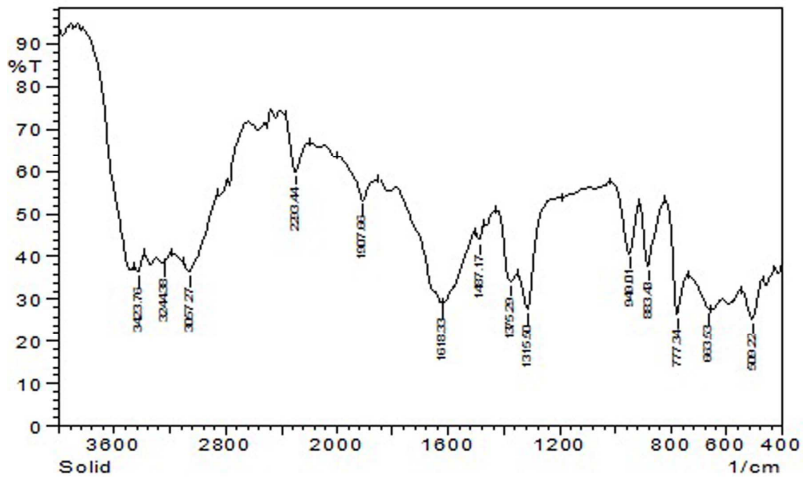

Figure5: FTIR spectra of COM crystals.

Table1.Assignment of FTIR wave numbers of COM crystals.

wave number in cm^{-1}	Assignment of peaks/ bands
3423.76	Asym. OH stretch
3057.27	Sym. OH stretch
3244.38	Inter mole. Hydrogen bonded OH stretch
1618.33	Asym. C=O stretch
1315.50	Sym. C=O stretch
1020.00	Asym. C-O stretch
949.01	Sym. C-O stretch
883.43	C-C stretch
777.34	O-C=O
663.53	OH wagging
509.22	M-O

In the spectrum a strong band at 3423.76 cm^{-1} and 3057.27 cm^{-1} are due to asymmetric and symmetric OH stretching, while a band at 3244.38cm^{-1} shows intermolecular hydrogen bonded OH stretch[28]. Intense absorption band at 1618.33 cm^{-1} and 1315.50 cm^{-1}can be assigned to asymmetric and symmetric C=O stretching bands specific to the COM[29]. The sharp band at 883.43 cm^{-1} is due to C-C stretching vibrations which confirm the existence of oxalate group in COM. The sharp peak at 777.34 cm^{-1} are due to O-C=O and the widebands at 663.53 cm^{-1}can be assigned to the bending and wagging modes of the water molecule. However, the peak at 509.22 cm^{-1} is assigned to the

presence of metal oxygen bond [25-26]. Thus the FTIR spectroscopy confirmed the growth of COM crystals are due to the presence of water of crystallization, C=O, C-C, O-C=O and M-O bonds. The observed vibrational frequencies and their tentative assignments are listed in **Table 1.**

Powder X-ray diffraction (XRD)

The X-ray diffractogram of gel grown COM crystals is seen in **Figure 6**. This diffractogram was processed using in built PANalyticalX'pert High Score software to examine the peak positions and its corresponding intensity. The grown crystals were identified as COM by XRD and compared with that of the powder diffraction JCPDS file [30-31]. All intensity peaks of the XRD patterns were matched with the structural data of the COM described in the standards. **Table 2** gives the position, d-values and matching peaks of the COM crystals. **Figure 7** shows plot of identified phases of COM crystals.The occurrence of resolved peaks at specific angles indicates that the material is a well crystalline nature.

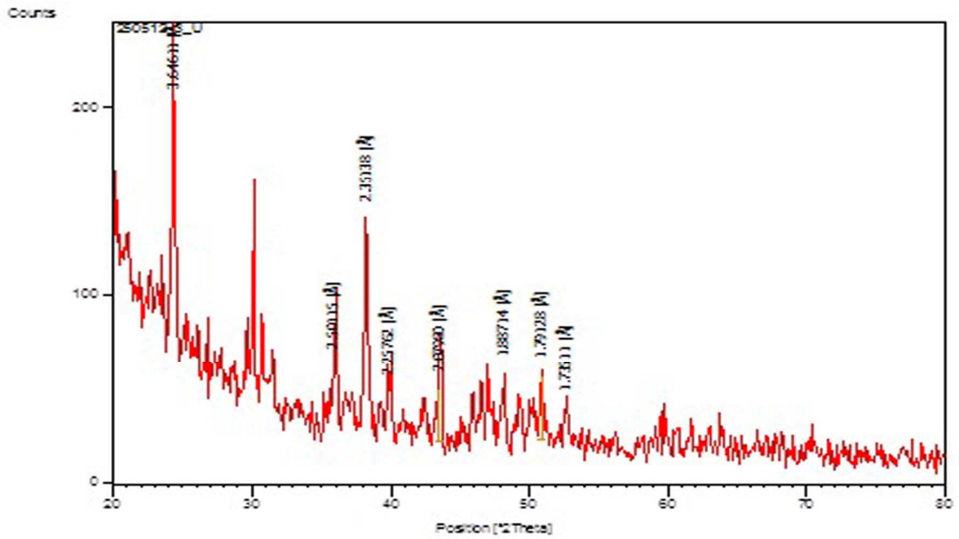

Figure 6: Powder X-ray diffraction pattern of COM crystals.

Table 2: X-ray diffraction analysis of COM crystals.

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Tip width [°2Th.]	Matched by
24.3670	319.29	0.2952	3.65298	95.15	0.3542	00-013-0601; 00-014-0789
30.1072	335.56	0.1968	2.96831	100.00	0.2362	00-013-0601; 00-014-0789
35.9757	137.06	0.2952	2.49644	40.85	0.3542	00-013-0601; 00-014-0789
38.2547	152.59	0.2952	2.35279	45.47	0.3542	00-013-0601; 00-014-0789
39.8972	65.31	0.2952	2.25964	19.46	0.3542	00-013-0601; 00-014-0789
43.5989	107.02	0.2952	2.07599	31.89	0.3542	00-013-0601; 00-014-0789
48.1206	24.84	0.3936	1.89095	7.40	0.4723	00-014-0789

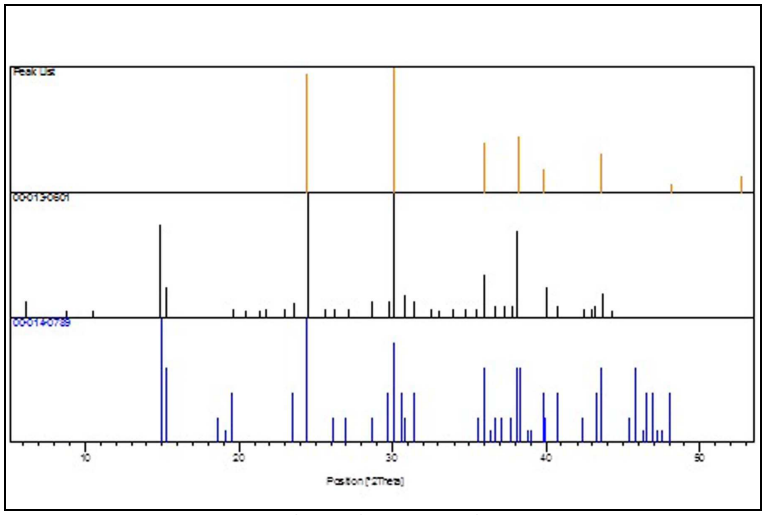

Figure 7: Plot of identified phases of COM crystals.

Thermogravimetry

To study the thermal transformations of the COM crystals, the sample was heated at the temperature 50-850^{0}C. **Figure 8** shows the thermogram of a sample $CaC_2O_4.H_2O$.

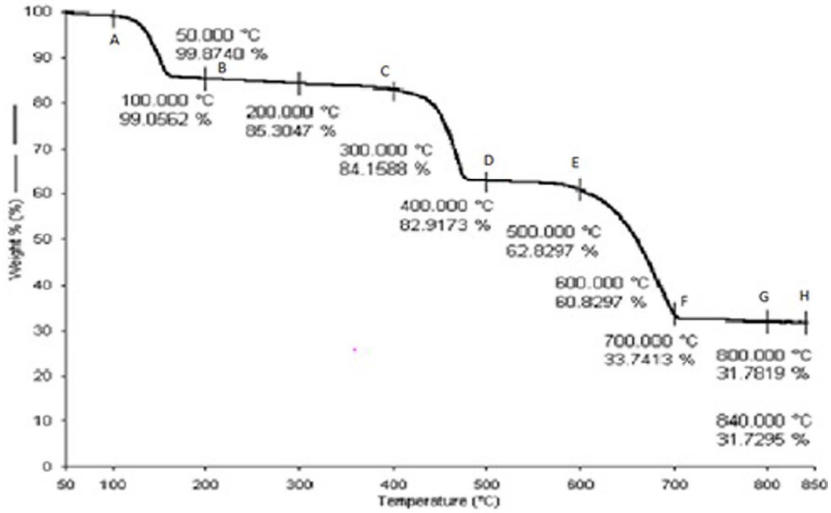

Figure8: TGA curve of COM crystals.

The curve shows that the calcium oxalate monohydrates lose its weight in three well-defined steps [32]. The loss of water crystallization in first step, carbon monoxide in second step, while carbon dioxide in third step [4,33-36]. These steps are;

$$CaC_2O_4.H_2O \rightarrow CaC_2O_4 \rightarrow CaCO_3 \rightarrow CaO$$

Stage I: $CaC_2O_4.H_2O(s) \rightarrow CaC_2O_4(s) + H_2O\ (g)$
Stage II: $CaC_2O_4\ (s) \rightarrow CaCO_3\ (s) + CO\ (g)$
Stage III: $CaCO_3(s) \rightarrow CaO\ (s) + CO_2\ (g)$

In the study of thermal transformations of the COM crystals, several intervals were considered, characterizing the mass losses [37]. As seen in **Figure 8,** the curve from ambient temperature (about 50^{0}C) to A (about 100 ^{0}C) is due to humidity; whereas the peak from A to B (about 200^{0}C) is attributable to the loss of water crystallization, started from 110^{0}C and end at 200^{0}C, corresponding to dehydration of sample in first stage. The peak from B (200^{0}C) to C (about 400^{0}C) is due to oxidation of organic compounds present in the sample. However starting from C (400^{0}C) and finished at point D (about 500^{0}C) is the decomposition of the calcium oxalate. In this step, some carbon monoxide is evolved from calcium oxalate to formed crystalline calcium carbonate. Finally in last step, the peak from D (about 500^{0}C) to G (about 800^{0}C), is the decomposition of calcium carbonate into calcium oxide with releasing of carbon dioxide. Further decomposition of the sample was not observed up to 840^{0}C.

The observed and theoretical mass loss of COM crystals was given in **Table 3**.

Table 3: Mass loss percentages of COM crystals.

Mass loss in the interval	A-B Bounded H_2O/	C-D CO/	D-G CO_2/
Observed	13.75%	20.08%	31.04%
Theoretical	12.33%	19.17%	30.12%

The result shows the good agreements to the theoretical value [37]. Whereas a very small difference in observed and theoretical values was may be due to that, the quality of thermogravimetric measurement of calcium oxalate monohydrate is affected by the three common experimental variables, heating rate, shape of sample container and size of sample [38]. Also the variation in observed temperature may be attributed to the way in which the experiment was undertaken [39].Thus, the three well-defined decomposition stages [40] and observed mass losses from TGA confirmed the existence of COM crystals.

CONCLUSION

- Gel growth techniques are suitable for to growing urinary crystals.
- The dendrite, prismatic, agglomerates, tetragonal and six sided platy habit microcrystalline COM crystals were found to be grown in agar-agar gel by single and double diffusion techniques.
- FTIR spectrums of COM crystals confirmed the presence of functional groups.
- The crystalline nature was identified by XRD measurement.
- The three oxalate decomposition stages- the dehydration (splitting water), decarbonylation (splitting carbon monoxide) and decarbonation (splitting carbon dioxide) was found in TG analysis.
- The stepwise decomposition of sample confirmed the structure mechanism of COM crystals.

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REFERENCES

[1] K.J. Diana, K. V. George, *J. Cryst. Growth.*, **2013**, 363**,**164.
[2] C. K. Chauhan, M. J. Joshi, A.B.Vaidya, *Ameri J Infec. Disease*., **2009**,5,3, 170.
[3] D. J. Kok, S.E. Papapoulos, L.J.M.J. Blomen, O.L.M. Bijvoet, *Kidney International*., **1988**,34 346.
[4] V.S.Joshi, B.B. Parekh, M.J. Joshi, A.B.Vaidya, *J. Cryst. Growth.*,**2005,** 275**,** 1403.
[5] C.Bouropoulos, N. Vagenas, P. Klepetsanis,N. Stavropoulos,N. Bouropoulos, *Cryst. Res. Technol.*, **2004**, 39, 699.
[6] J.M.Ouyang, F. Deng, L. Duan, *Collids & surface A: Physicochemi. Engg. Aspects.*, **2005**,257,58, 215.
[7] P. Sayan, S.T. Sargut, B. Kiran, *Cryst. Res. Technol.*, **2009**, 44, 807.
[8] Y.Zhang, J. Tao, N. Feng, X. Han, *Cryst. Res. Technol.*, **2008,** 43, 931.
[9] Y.Shen, S. Li, A. Xie, W. Xu, L. Quu, H.Yao, X. Yu, Z. Chen, Collids& surface B:Biointerfaces., **2007**, 58**,** 298.
[10] A.J.Xie, L. Zhang, J. Zhu, Y.H. Shen, Z. Xu, J.M. Zhu, C.H. Li, L. Chen, L.B. Yang, *Colliids & Sur. Phyic. Eng.Aspec*., **2009**, 332, 192.
[11] P.Thanasekaran, C.M. Liu, J.F. Cho, K.L. Lu, *Inorg. Chem. Communi.*, **2012, 17, 84**.
[12] A.R.Patel, A.V. Rao, *Bull Mater. Sci*.,**1982**, 4**,** 527.
[13] J.M.Ouyang, Y.H. Tan, Y.H. Shen, Acta *Physico-Chimca Sinica.*, **2003**, 19,4, 368.
[14] J.Ouyang, S. Deng, X. Li, Y.Tan, T. Bernd, *Sci. in China Ser. B Chem.*,**2004**, 47, 311.
[15] T.Bretherton, A. Rodgers, *J. Crystal Growth.*, **1998**, 192**,** 448.
[16] P.K.Grover, R.L. Ryall, V.R. Marshall, *Kidney International*., **1992**,41**,**149.
[17] N.P.Buchholz, D.S. Kim, P.K. Grover, C.J. Dawson, R.L. Ryall, *J. Bone and Mineral Res.*,**1999**,14**,**1003.
[18] N.Laube, B. Mohr, A. Hesse, *J. Cryst.Growth.*, **2001,233**, 367.
[19] M.Deepa K.R.Babu, V.K.Vaidyan, *J. Mater. Sci. Lett.*, **1995, 14**, 1321.
[20] E.K.Girija, S.C.Latha, S.N. Kalkura, P.Ramasamy, *Mat. Chem. Phy.*, **1998, 52**, 253.
[21] S.Bisaillon, R. Tawashi, J. *Pharmaceutical Sci*., **2006**, 64, 458.
[22] H.K.Henisch,*Crystals in Gels and Liesegang Rings*. Cambridge University Press, Cambridge **1988.**
[23] P.V.Dalal, *Indian J Materi. Sci.*, **2013.**
[24] V.Thongboonkerd, T. Semangoen, S. Chutipongtanate, *Clinica Chimica Acta.*, **2006**, 367, 120.
[25] P.Bhatt, P. Paul, *J. Chem. Sci.*, **2008**, 120**,** 267.
[26] B.B.Parekh, S.R.Vasant, K.P.Tank, *America J Infec. Disease*., **2009**, 5,3, 232.
[27] D.Valarmathi, L. Abraham, S. Gunasekaran, *Indian J. Pure & Appl. Phys*., **2010**, 48, 36.
[28] R.SaiSathish, B. Ranjit, K.M. Ganesh, *Curr Sci*., **2008**, 941, 104.
[29] A.Thomas, *pdf Thesis*, **2009**.
[30] Joint Committee for Powder Diffraction Standards Reference Card Number 14-0789
[31] Joint Committee for Powder Diffraction Standards Reference Card Number 13-0601
[32] S.N.Basahel, A.Y.Obaid, E.H.M.Diefallah, Radiat. Phy. Chem., **1987**, 29, 447.
[33] K.J.Kociba, P.Gallagher, *Thermochimica Acta.*, **1996**, 282, 277.
[34] N.Kutaish, P.Aggarwal, D. Dollimore, *Thermochimica Acta.*, **1997**, 297, 131.
[35] C.Paluszkiewicz, J. Sciesinski, M.Galka, *Mikrochima. Acta*,, **1998**, I,45.
[36] L.Viaev, N. Nedelchev, K.Gyurova, Zgorcheva, *J. Anal. Appl. Pyrolysis.*, **2008**, 81, 253.
[37] J.Kaloustian, A.M. Paul, G.Pieroni, H. Portugal, *J. Therm. Analy. & Calori.*, **2002**, 70, 959.
[38] E.L.Simons, A.E. Newkirk, *Talanta*., **1964,**11**,** 549.
[39] R.L.Frost, M.L. Weier, *ThermochimaActa.*, **2004**,409, 79.
[40] C.G.R.Nair, K.N. Ninan, *ThermochimaActa.*, **1978,**23, 161.