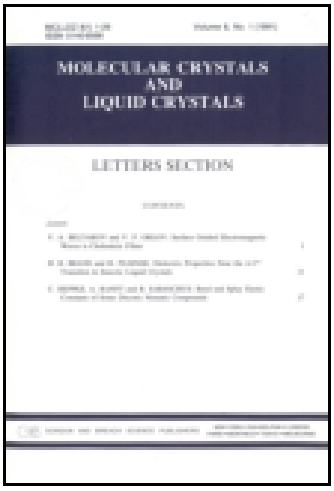

Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Control of Phase and Morphology of Calcium Oxalate Crystals by Natural Polysaccharide, Gum Arabic

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Published online: 08 Apr 2014.

To cite this article: Kranthi Kumar Gangu, Govinda Rao Tammineni, Anima S. Dadhich & Saratchandra Babu Mukkamala (2014) Control of Phase and Morphology of Calcium Oxalate Crystals by Natural Polysaccharide, Gum Arabic, Molecular Crystals and Liquid Crystals, 591:1, 114-122, DOI: 10.1080/15421406.2013.836880

To link to this article: http://dx.doi.org/10.1080/15421406.2013.836880

Mol. Cryst. Liq. Cryst., Vol. 591: pp. 114–122, 2014

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421406.2013.836880

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Control of Phase and Morphology of Calcium Oxalate Crystals by Natural Polysaccharide, Gum Arabic

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Controlled crystal growth of calcium oxalate was examined under the influence of Gum Arabic (GA) and urea at pH 4.0, 7.0, and 9.0. GA when alone directed the formation of calcium oxalate dihydrate (COD) up to 2.0% w/v concentration, whereas urea influenced the formation of calcium oxalate monohydrate (COM). The combination of urea and GA in mixed proportions (2.0% w/v each) led to the formation of nanocrystallites of COD. The present study on crystallization behavior of calcium oxalate under the influence of natural polysaccharides and urea may prove to be important in understanding the control of kidney stone formation.

Keywords Additive; calcium oxalate; crystal morphology; kidney stones

Introduction

Calcium oxalate is an abundant biomineral found in plants and animals [1]. It occurs in three different hydrated forms: calcium oxalate monohydrate (COM, $CaC_2O_4 \cdot H_2O$), calcium oxalate dihydrate (COD, $CaC_2O_4 \cdot 2H_2O$), and calcium oxalate trihydrate (COT, $CaC_2O_4 \cdot 3H_2O$). The monoclinic COM is the thermodynamically most stable phase, followed by the triclinic COT and the tetragonal COD. Calcium oxalate is the principal crystalline constituent in human kidney stones [2–5] especially COM phase with plate like morphology is the major component of most urinary calculi [6,7]. COM has been known to be a possible source of urinary and kidney stones because of its strong affinity for renal tubule cell membranes and its difficulty in ejection along with urine as compared to COD and COT [8,9]. The urinary proteins play an important role in transforming COM phase to COD, which is not developed into urinary and kidney stones. The same result was observed by in vitro testing of proteins isolated from human urine [10–12].

In nature, biological macromolecules play a key role in controlling the inorganic crystal growth [13]. A previous study by one of the authors also showed that organic carboxylate molecules direct the formation of calcium carbonate biominerals [14]. Regarding calcium

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oxalate, the formation of COD with controlled crystal morphology rather than COM is an important approach to reduce the risks of urolithiasis. Many studies have identified the role of organic molecules as additives in controlling the phase and morphology of calcium oxalate crystals in vitro [15–17]. Organic macromolecules such as oligopeptides are able to stabilize the COD with tetragonal morphology [18] and poly(sodium 4-styrene) sulphonates direct the COD with various crystal morphologies such as plates, leaves, bipyramids, and cylinders [19]. Sulphated polysaccharides isolated from marine algae [20] were reported to not only influence the size of crystals but also promote the formation of tetragonal bipyrimidal COD phase. Similarly polysaccharide-based biopolymers directed the formation of COD phase with tetragonal prismatic morphology [21].

Another important biomolecule, urea also plays an important role in human kidney stone formation. Human urine contains about 9.3 g l^{-1} of urea and influences the aggregation of calcium oxalate crystals of COM phases in kidneys. Urine like solutions could direct the formation of COM when used as additive [22].

There are a very few reports about cooperative influence of polysaccharides and urea on crystal growth morphology and promotion of COD phase, mimicking the kidney stone formation. Gum Arabic (GA) is a natural polysaccharide obtained from Acacia trees. It contains higher fraction of glucoronic acid terminated composite polysaccharides and smaller fraction of glycoproteins [23]. GA dietary supplement was reported to have beneficial effects on renal diseases via increased production of serum butylate and short-chain fatty acids in human colon [24,25]. Influence of natural polysaccharide, GA as a crystal growth modifier in presence of urea on phase and crystal morphology of calcium oxalate has been reported in this paper.

Experimental

GA (a natural polysaccharide) was obtained from Sigma-Aldrich (USA). All the other chemicals used were of analytical grade without further purification. The precipitation reaction of calcium oxalate (CaC_2O_4) was conducted in a glass beaker at room temperature. In a typical synthesis, a solution of 5.0 mmol sodium oxalate (0.2 M, 25.0 ml) was transferred to an aqueous solution of 20 ml GA (1.0% or 2.0% w/v in total 50 ml) and was thoroughly stirred for a few minutes to get a homogenous solution. The pH of solution was adjusted to 4.0/7.0/9.0, respectively, using diluted NaOH (0.1 M) and HCl (0.1 M). Then a solution of 5.0 mmol calcium chloride (1.0 M, 5.0 ml) was added quickly into the above solution and stirred for a minute using a magnetic stirrer. The solution was covered with a parafilm and was kept for crystallization. After 24 hr, the crystals were filtered, washed several times with distilled water, and dried at ambient temperature.

Calcium oxalate crystals obtained in the above experiments were characterized for identification of phase, morphology, and stability. An Xpert Pro, PANalytical (Netherlands) powder diffraction system operating with monochromated Cu radiation and Fourier transform infrared spectroscopy (FT-IR; Spectrum 2, PerkinElmer, UK) were used for phase characterization. The morphology of products was examined by scanning electron microscopy (FEI Quanta 200 FEG with EDS, Netherlands). Thermogravimetric analysis (TGA1, Metler Toledo, Switzerland) was used to analyze the decomposition products and stability of calcium oxalates. Size of the calcium oxalate particles was measured using Dynamic Light Scattering technique (SZ100, Nanopartica, Horiba Scientific, Japan).

Results and Discussion

The crystal growth process of calcium oxalates was monitored, under the influence of crystal growth modifier, GA, at three different pH values 4.0, 7.0, and 9.0. The effect of variable concentrations of GA (1.0% w/v and 2.0% w/v) on growth process was also carried out at each pH value. The results showed that the concentration of GA and pH play an important role in controlling the morphology of calcium oxalate.

In control experiments, in the absence of GA or urea, COM was the dominant phase at pH 4.0, 7.0, and 9.0 (Fig. 1). The hexagonal plates like crystals of COM phase with variable sizes ranging from 300 nm to 1.0 μm were obtained at pH 4.0. Hexagonal edges became rounded on increasing the growth solution pH to 9.0. No considerable change in the size of the crystals was observed. X-ray Diffraction (XRD) revealed that the trace amounts of COD crystal were also produced along with the predominant phase of COM.

Influence of urea (1.0% and 2.0% w/v) was studied under different experimental conditions. The formation of truncated hexagonal plate-like morphology of COM phase with aggregated state was observed under the influence of urea at pH 4.0, 7.0, and 9.0

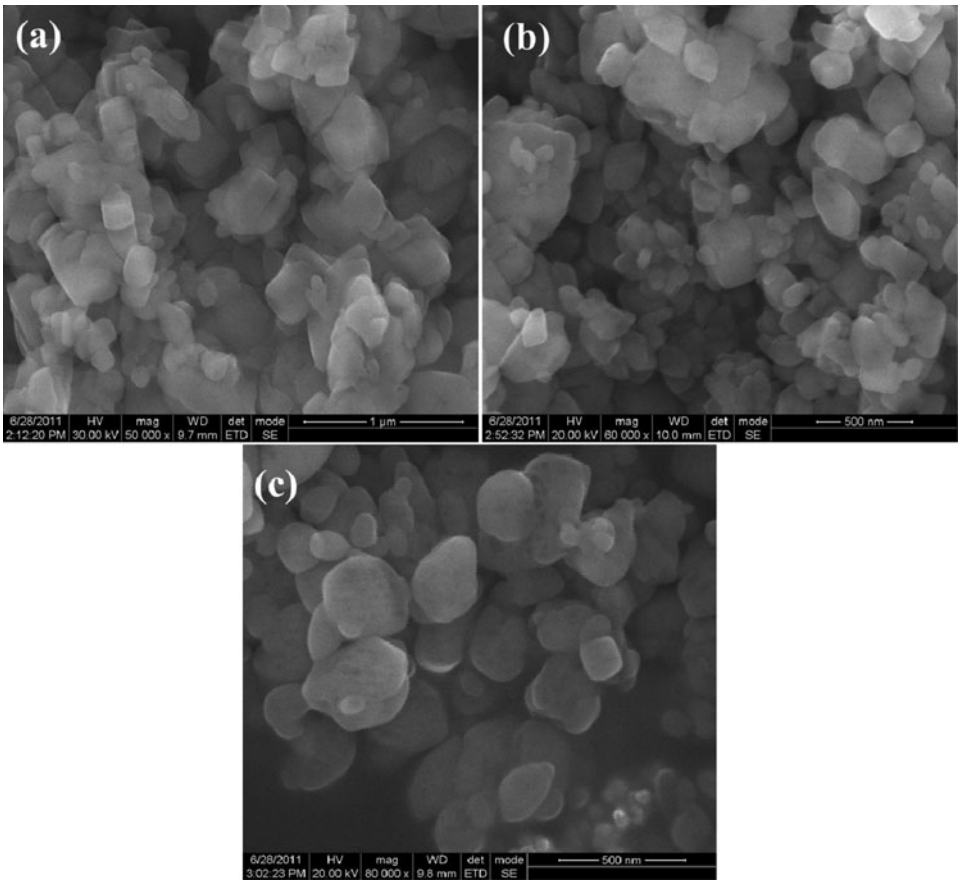

Figure 1. SEM images of calcium oxalate crystals produced at room temperature in control experiments at pH 4.0 (a), 7.0 (b), and 9.0 (c).

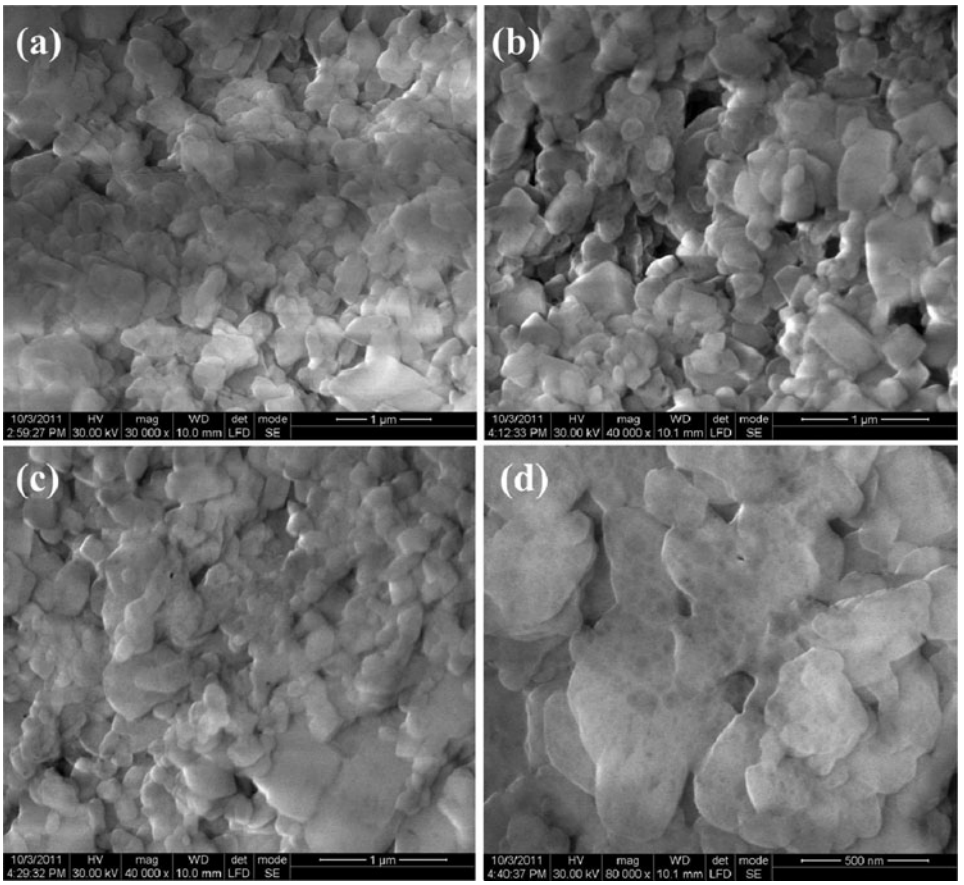

Figure 2. SEM images of calcium oxalate crystals produced at room temperature in the presence of 1.0% urea at pH 4.0 (a), 2.0% urea at pH 4.0 (b), 7.0 (c), and 9.0 (d).

(Fig. 2). No considerable change in the COM crystal morphology was observed with either 1.0% or 2.0% w/v urea at pH 4.0 (Figs. 2(a) and (b)). Due to rapid aggregation, the hexagonal shape of COM slowly disappeared on increasing the pH of the growth solution to 9.0 (Fig. 2(d)). Rapid aggregation of COM crystals in the kidneys has profound influence not only on crystal size but also on initial kidney stone development [22].

Figure 3 shows the formation of calcium oxalate crystals with the influence of GA (1.0% and 2.0% w/v) at pH 4.0, 7.0, and 9.0. COD phase with hexagonal plate-like morphology was observed using 1.0% w/v GA at pH 4.0 (Fig. 3(a)). No considerable change in the crystal morphology of COD produced at pH 7.0 and 9.0. Similar hexagonal plate-like morphology was observed in the presence of 2.0% GA at pH 4.0, 7.0, and 9.0 (Figs. 3(b) and 2(c) and (d)). Hexagonal plate-like morphology with size ranging from 500 nm to 1.0 μm was observed in all the experiments. XRD results revealed that the trace amount of COM was obtained along with predominant phase of COD. These results clearly indicated that at any given concentration of GA, COD is the dominant phase. Further, cooperative influence of GA and urea (1.0% w/v each or 2.0% w/v each) was examined at pH 4.0, 7.0, and 9.0 to identify the phase and morphology of calcium oxalate. Figure 4(a)

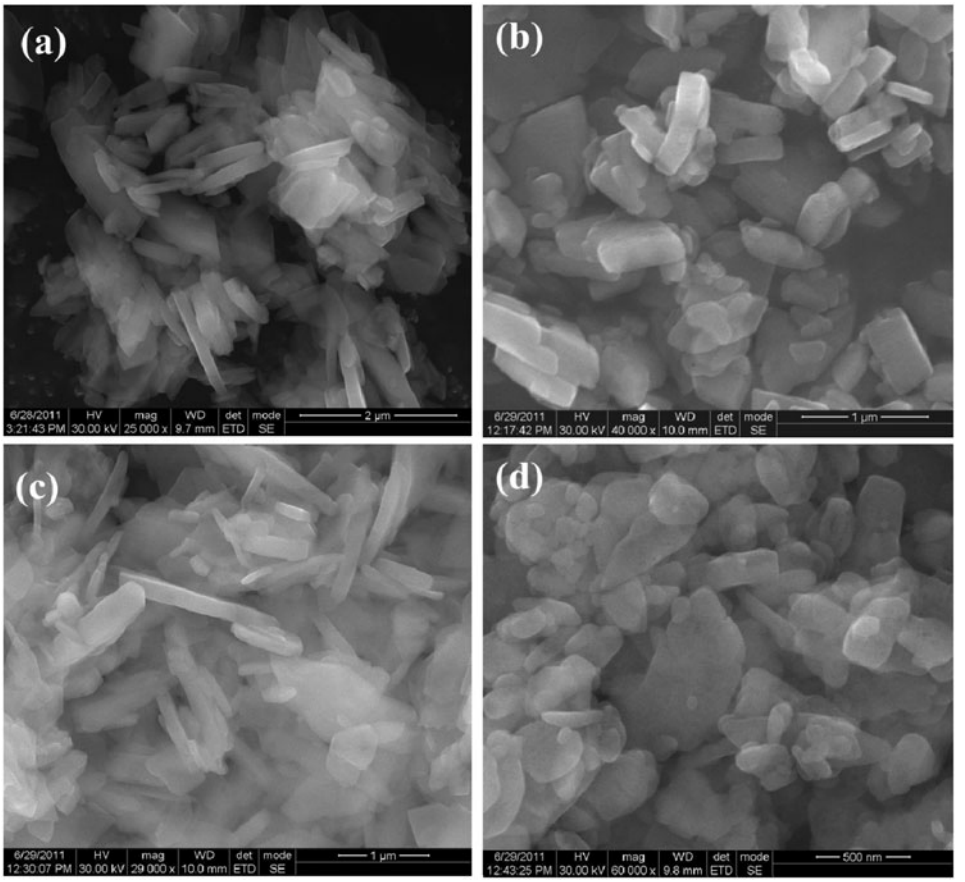

Figure 3. SEM images of calcium oxalate crystals produced at room temperature in the presence of 1.0% GA at pH 4.0 (a), 2.0% GA at pH 4.0 (b), 7.0 (c), and 9.0 (d).

shows the COD phase with truncated hexagonal-shaped particles ranging from 200 nm to 400 nm obtained with the cooperative influence of 1.0% w/v each of GA and urea at pH 4.0. Surprisingly nano-sized crystallites of COD with size 100–200 nm were resulted in all experiments conducted at 2.0% w/v each of GA and urea at pH 4.0, 7.0, and 9.0 (Figs. 4(b)–(d)).

In order to examine the phase and the thermodynamic stability, the crystals produced in all the reactions were analyzed with XRD and TGA. Figure 5 shows the XRD patterns of calcium oxalates obtained from different experimental conditions and compared with JCPDS card No. 20–231 (COM phase) and JCPDS Card No. 17–541 (COD phase). Diffraction peaks projected at 15.11, 24.60, 30.24, 38.35 support the COM phase and 14.40, 20.17, 32.27, 40.32 support the formation of COD phase. FT-IR spectra revealed the information of further characterization of calcium oxalate crystals (Fig. 6). The asymmetric and symmetric carbonyl stretching bands were observed at 1617 cm^{-1} and 1317 cm^{-1}, respectively. Further, five discrete peaks above 3000 cm^{-1} represented the formation of COM. Whereas the absorption peaks noticed at 1646, 1325 cm^{-1} for C=O stretching and a strong peak at 3461 cm^{-1} for –OH stretching vibration of water instead of five weak bands at above

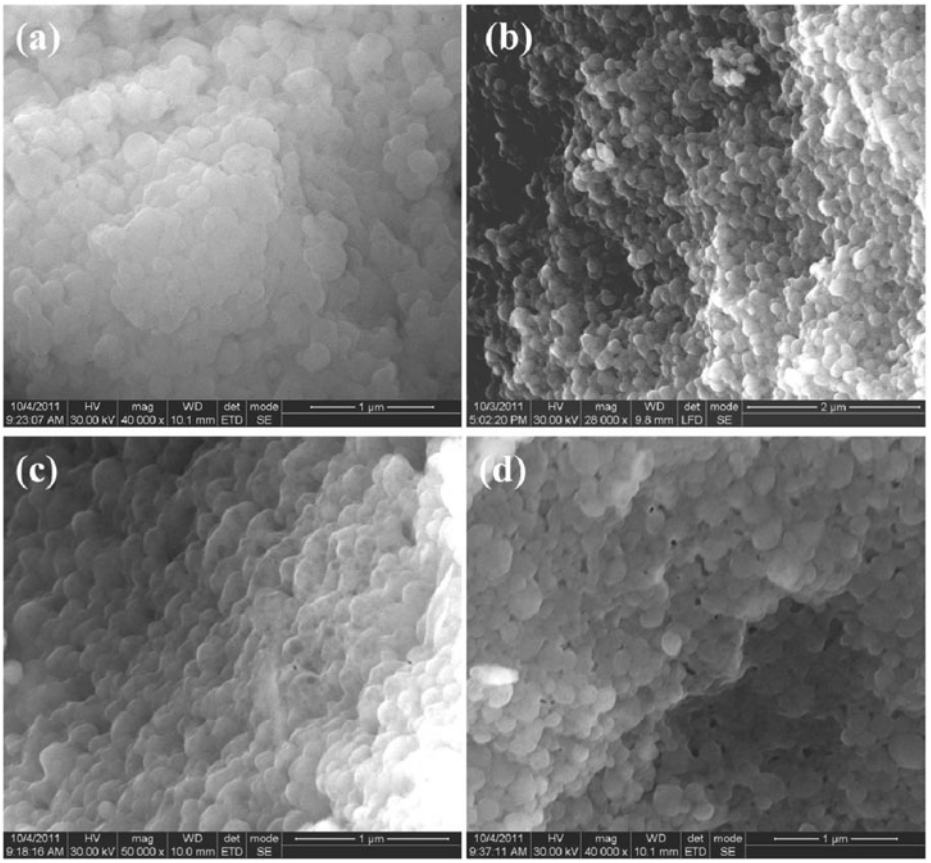

Figure 4. SEM images of calcium oxalate crystals produced at room temperature in the presence of a mixture of GA and urea 1.0% each at pH 4.0 (a), 2.0% each at pH 4.0 (b), 7.0 (c), and 9.0 (d).

3000 cm^{-1} as in the COM showed the formation of COD (Fig. 6(b)). A C–O–C stretching peak at 1071 cm^{-1} of polysaccharide in GA was also observed in COD phase (Figs. 6(b) and (c)). Furthermore, TGA was carried out for crystals produced from the above reactions to identify the phases of decomposition process. As seen in Fig. 7(a), TGA curves of COM showed a first decomposition step (loss of 12.3% Wt.) under 400°C corresponding to the conversion of COM to anhydrous calcium oxalate. TGA curves of COD, produced under cooperative influence of GA and urea at 2.0% w/v each, exhibited two decomposition steps under 400°C, the first one corresponding to the conversion of COD to anhydrous calcium oxalate (loss of 21.9% Wt.), and the second one corresponded to the decomposition of polysaccharide from GA (loss of 6.9% Wt.). These above results indicated the presence of a thin layer of GA around the COD crystals. Figure 8 shows the particle size distribution of COD crystals examined under Dynamic Light Scattering technique produced after 24 hr reaction with influence of GA and urea (2.0% w/v each).

Several approaches indicate that the organic molecules can control the size, phase, and morphology of inorganic crystals [26]. These organic molecules may bind Ca^{2+} ions through the functional groups of carboxylate or sulfate or phosphate and could control crystal nucleation and growth. However, the growth inhibition is most likely to be caused

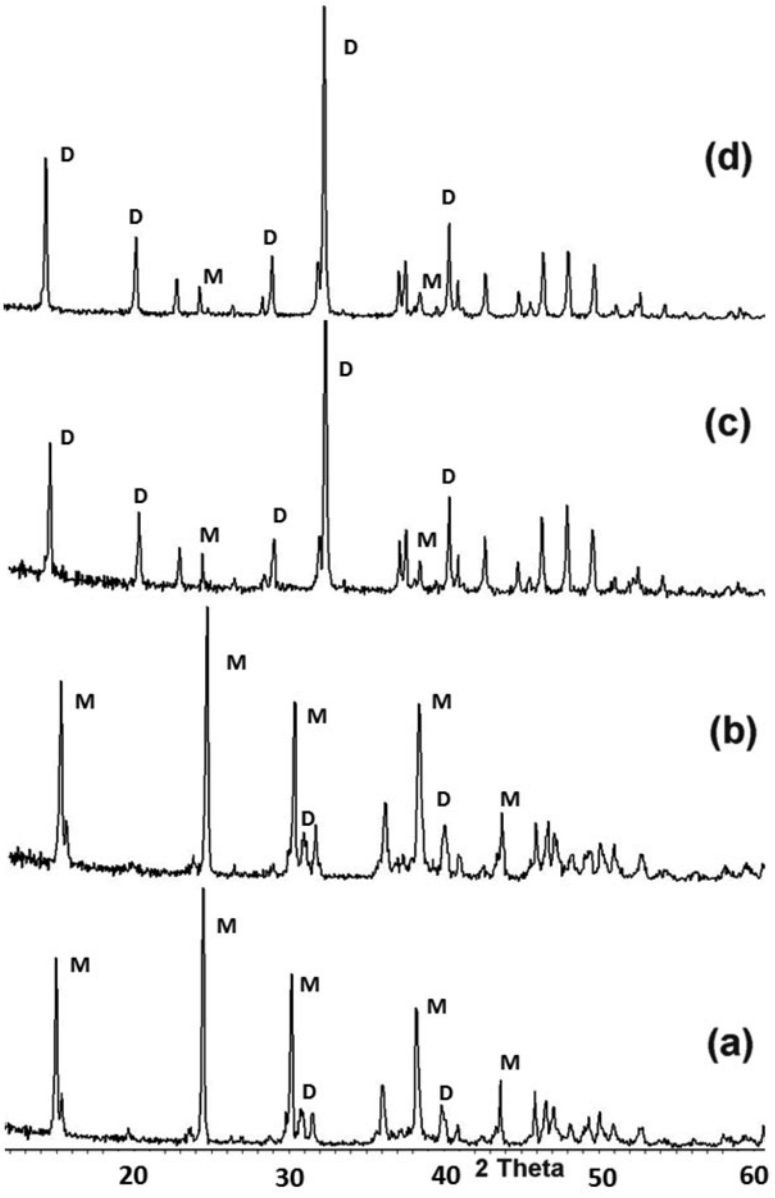

Figure 5. Powder X-ray diffraction patters of calcium oxalate crystals produced in control experiments (a), in the presence of 2.0% urea (b), 2.0% GA (c), mixture of GA and urea 2.0% each (d). (M = COM, D = COD).

by the adsorption of polymers on crystal surface rather than by the binding of Ca^{2+} ions because macromolecule size is much larger than the carboxylate ions [21]. A similar mechanism was proposed by Kok et al. [27] for the control of calcium oxalate crystal growth and morphology through a monolayer type of adsorption of polysaccharides extracted from coccoliths of marine algae. The TGA results obtained support the assumption that the

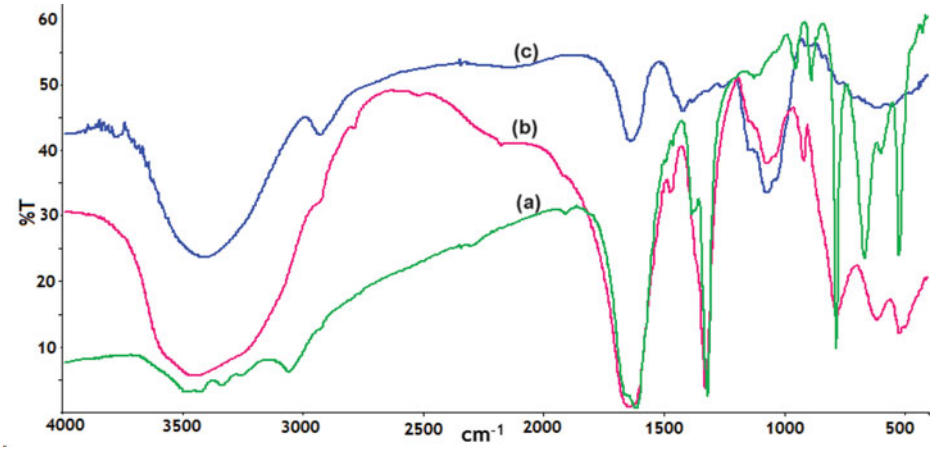

Figure 6. FT-IR spectra of calcium oxalate crystals. (a) COM phase without GA, (b) COD phase with GA, and (c) GA.

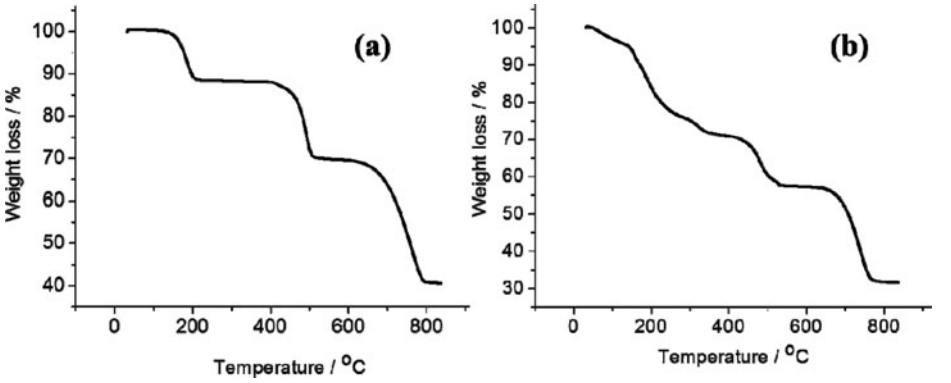

Figure 7. TGA curve of calcium oxalate (COM) produced in control experiments (a), TGA curve of calcium oxalate (COD) produced in the presence of a mixture of GA and urea 2% each.

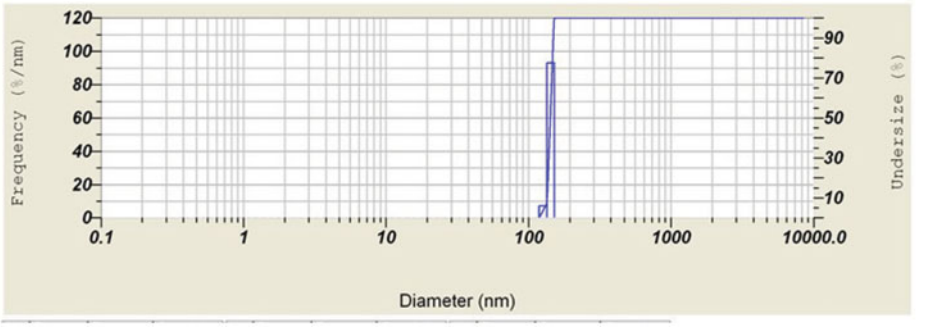

Figure 8. Particle size distribution of COD by Dynamic Light Scattering technique.

polysaccharide molecules were probably adsorbed on the surface of growing crystal and promoted the formation of COD phase with nanocrystalline morphology by inhibiting the COM growth.

Conclusions

GA was found to be an important additive to control the phase and morphology of calcium oxalate. Various crystal morphologies, such as hexagonal plates and nano-sized crystallites, were obtained by simple biomimetic route. COM was the predominant phase not only in control experiments but also in the presence of urea. GA favored the formation of COD phase with hexagonal morphology whereas in presence of urea it could direct the formation of COD with nanodimensional crystallites. This work may provide new insights into the transformation of COM to COD phase as well as the control of crystal morphology.

Acknowledgments

The authors are grateful to SAIF, IIT, Madras for SEM analysis. This work was supported by GITAM University, Visakhapatnam, Andhra Pradesh, India.

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