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Inhibitory Effect of Aqueous Citrate on the Growth of Calcium Oxalate Crystals

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ABSTRACT

Calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) are frequently found in urinary calculi (stones). Calcium chelation by citric acid can play a major role in chelation therapy of naphrolithiasis. COM crystals were grown in laboratory using gel growth method, and the inhibitory effect of aqueous citrate on the growth of COM crystals was studied. The results indicate that with an increase in the concentration of citrate, the mass and number of the formed crystals was gradually reduced. The crystals were characterized by Fourier transform infrared spectroscopy (FTIR) to confirm the functional groups and powder X-ray diffraction (XRD) analyses to confirm the phases of the COM and COD crystals. Scanning electron microscopy (SEM) confirmed that the morphology of the crystals changed from elongated hexagonal to tetragonal bipyramidal, which is characteristic of a change from COM to COD. This study confirms that the use of citrate can promote the formation of COD crystals and reduce the nucleation rate of COM crystals, a major component of oxalate urinary stones.

Keywords: Calcium Oxalate Monohydrate, Citrate inhibitor, Nucleation, Characterization, Crystal Morphology, X-Ray Diffraction

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INTRODUCTION

Kidney stone disease is a common chronic disorder in humans with the majority of stones being primarily composed of calcium oxalate monohydrate (COM) crystals. Calcium oxalate is generally found in two different varieties, calcium oxalate monohydrate (COM), or Whewellite, and calcium oxalate dihydrate (COD), or Weddellite. It is difficult to form urinary stones from COD because COD crystals are unstable and are easily excreted in the urine of both humans and animals[1]. The formation of COD crystals actually protects against stone disease because of their reduced capacity to form stable aggregates or strong adhesion contacts to renal epithelial cells due to the single micron-sized crystals. Urinary stones are characterized by high recurrence rates and would therefore benefit from a preventive treatment using medicinal plants. In most humans, progression from crystalluria to stone disease is prevented by biologic control mechanisms. Normal urine contains inhibitors that decrease the formation, growth, and aggregation of COM crystals [2-4]. Stone formation is a multi-step process viz. nucleation, crystal growth; aggregation and retention are necessary steps in formation of stones. Deficiency of inhibitors and an abundance of promoters in the urine facilitate stone formation, in addition to recognized dietary factors. Citrate is a potent inhibitor of calcium oxalate stones in particular. In the present study, the effects of aqueous extract of Citrus medica Linn (lemon) on the nucleation and growth of COM crystals using gel is reported for the first time. This study incorporates a multidisciplinary approach for the characterization of COM crystals grown in vitro to facilitate the development of prevention and dissolution strategies aimed at managing urinary stone growth.

MATERIALS AND METHODS

The apparatus used for crystallization of single crystals by gel technique consists of borosilicate glass tubes of length 20cm and diameter 2.5cm. Silica gel was prepared by adding a solution of sodium metasilicate to 1M oxalic acid slowly with continuous stirring to avoid any local ion concentration, which would otherwise cause premature local gelling and make the final solution inhomogeneous. The gel in test tubes was sealed with sheet of plastic to avoid evaporation of the solution and contamination of impurities. Gelation time was two days for the gel with pH 6-7.5, whereas it took 4 days for the gel to set with pH 4-5.5 and two days with pH 8. Over the set gel, an aqueous solution of Calcium chloride (1M) was poured carefully along the walls of the test tube so as to avoid any gel breakage. Citrate was introduced at concentrations in the range of 0.1 to 0.75M. A week later white microcrystalline precipitation layer was observed. The crystals are harvested after 21 days.

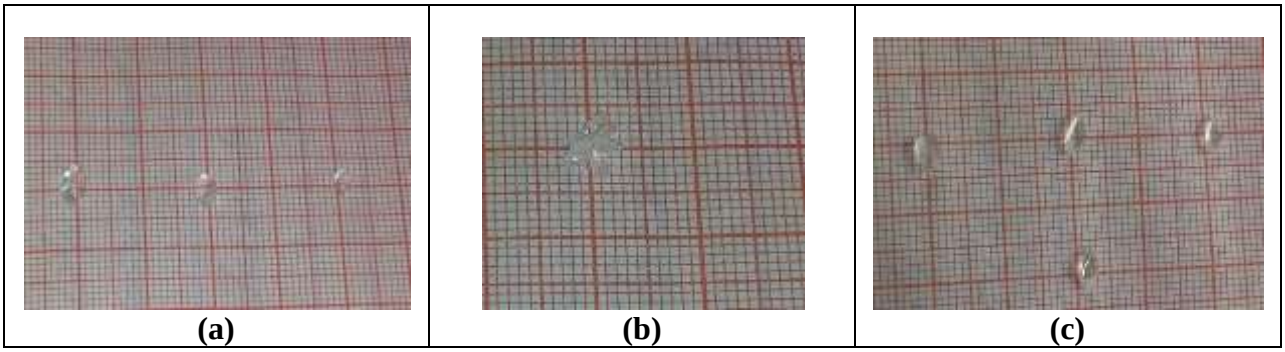

Figure 1: a and b are grown COM crystals and c is citrate added COM crystals

RESULTS AND DISCUSSIONS

Powder X-Ray Diffraction Analysis

Powder X-Ray Diffraction (XRD) of COM and citrate added COM was performed with a XPERT-PRO diffractometer using CuKα radiation. *T*he strongest diffraction peak of whewellite with 2θ value 30° and d-spacing value 2.97 Å (JCPDS PDF file 20-0231) reinforces the conclusion of the spectroscopic analysis, suggesting that calcium oxalate is present in the form of the monohydrate[5,6]. The XRD spectrum of citrate added COM has additional peaks and also the major peak at 30° was shifted. This shows that the incorporation of citrate in crystalline structure of COM, affected the nucleation and growth of COD crystals[7].

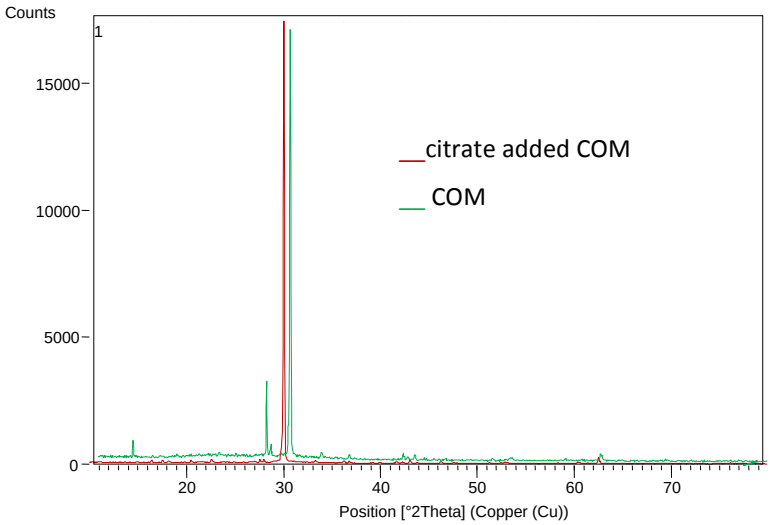

Figure 2: Powder XRD pattern of COM and Citrate added COM crystals

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectra were recorded with a nominal resolution of 4 cm^{-1} and a wave number range from 400 to 4000 cm^{-1} using the KBr pellet technique.

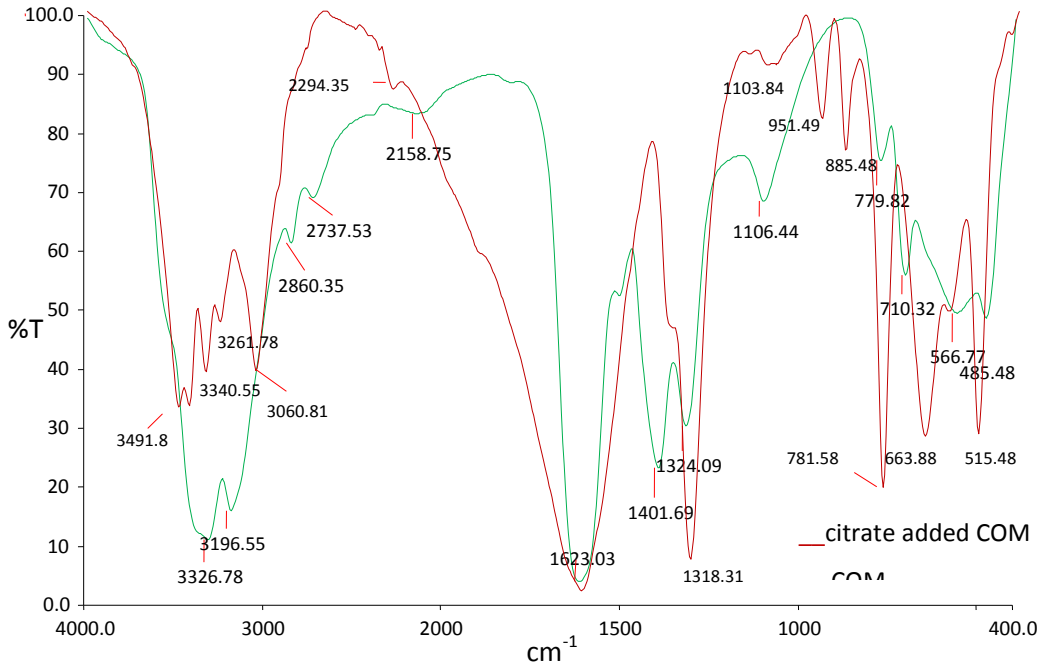

Figure 3: FTIR spectrum of COM and Citrate added COM crystals

The FTIR spectra of COM crystals obtained in the presence and absence of the aqueous extract of citrus are shown in (Figure).The stronger the hydrogen bond the longer O-H bond, the lower the vibrational frequency and the border and more intense the absorption band. For calcium oxalate crystal O-H stretching appears at bands 3500 and 3196 cm^{-1}.Also a band at 3326 cm^{-1} show intermolecular hydrogen bonded O-H stretch. This indicated that water molecule is present in calcium oxalate crystal. The C=O stretching absorption in aliphatic compounds generally occurs 1725-1700 cm^{-1} .The C=O absorption in carboxylic acids appears at 1700 cm^{-1} if carboxylic acid is converted into its soluble salts, then carboxylate anion is formed .The C=O absorption as possible higher wave number for an acid as compared to that in carboxylate anion in calcium oxalate crystal C=O stretching appears at 1623 cm^{-1} and 1324 cm^{-1}. The band at 1106cm^{-1} and 779cm^{-1} shows C-O stretching[8]. This indicates the presence of carboxyl ate anion in calcium oxalate crystal. The bond at 710cm^{-1} specified C-C stretching, which shows the present of two carboxylate anion [9].This confirms the existence of oxalate group in calcium oxalate. In the case of calcium oxalate with citric acid the broad band of around 3491 cm^{-1} to 3060 cm^{-1} with over tones observed, it indicates more accumulation water molecules added citric acid than COM. By chelation of calcium ions, citrate efficiently lowers supersaturation, the driving force for crystallization Citrate in a supersaturation decay system is therefore expected to reduce induction time and rate of nucleation. Also, 1725 cm^{-1}, 1700 cm^{-1} the peaks are disappeared. 1623 cm^{-1}, 1324 cm^{-1} and 1106 cm^{-1} this peaks are shifted to 1621 cm^{-1}, 1318 cm^{-1}

and 1103 cm^{-1}. This indicates carboxylate compound inhibited by citrate compound. The disappearance of the peak at 710cm^{-1} shows the citrate inhibition in COM. Citrate favour the nucleation and or transformation of COM into COD[10].

Morphology Analysis

Scanning Electron Microscopy (SEM) with an accelerating voltage of 20 kV was utilized to analyze the crystal. . SEM studies provide evidence that citrate has two main effects on newly forming COM particles *in vitro*, namely (i) precipitation of significantly smaller crystals and (ii) reduced aggregate formation.

Figure 4: SEM images of COM crystals (elongated hexagonal)

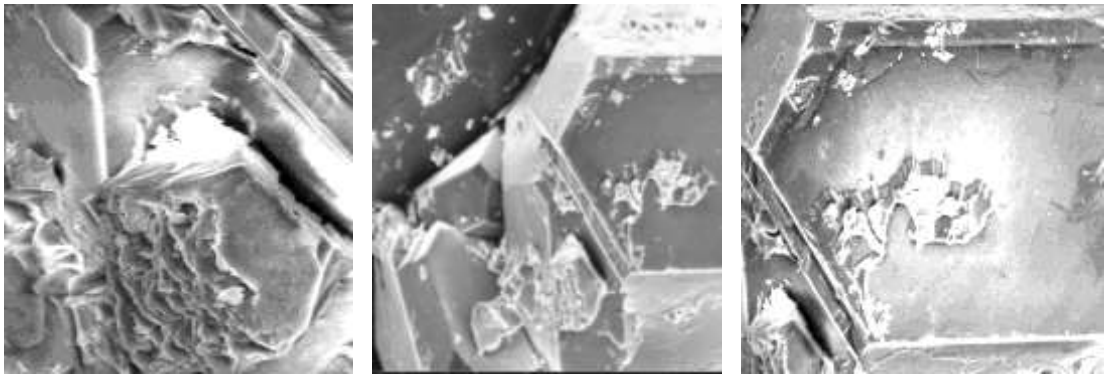

Figure 5: SEM images of citrate inhibited COM crystals (elongated tetragonal bipyramidal)

Morphology of the harvested crystals changed from elongated hexagonal (COM) to tetragonal bipyramidal(COD). COM crystal growth was reduced, and the morphology of the crystals was altered due to the inhibitory effect of aqueous citrate extracts under in vitro conditions by the gel. The presence of citrate improves inhibitory activity, leading to formation of smaller and less aggregated crystals[11]. This was most probably too low for abundant formation of calcium–citrate complexes which bind to specific sites on COM crystal surfaces and are thus crucial for lowering rates of crystal growth and aggregation. Calcium citrate can be easily removed from body while

accumulation of calcium oxalate forms kidney stone. Therefore, formation of significant numbers of calcium–citrate complexes may best explain crystal aggregation inhibition by citrate.

CONCLUSION

Citric acid is an organic and natural component of many fruits. It is also a good chelating agent which produces soluble complex of calcium citrate by reacting with calcium. This chelating property can be used to dissolve kidney stone and prevent the formation of calcium oxalate in the kidney. FTIR and Powder XRD techniques confirmed the functional groups and crystalline phases of the COM and COD crystals. SEM studies confirmed the morphology of the changed crystals. In conclusion of this in-vitro study, citrate is a main determinant of rates of COM nucleation and aggregation as well as of crystal morphology.

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REFERENCES

1. Ansari MS, Gupta NP, Hemal AK, Dogra PN, Seth A, Aron M, et al. Spectrum of stone composition: Structural analysis of 1050 upper urinary tract calculi from northern India. Int J Urol. 2005;12:12–6.
2. Rao PN, Glenn MP, John PK. Urinary Tract Stone Disease; Publisher -Springer–Verlag London Ltd., 2011.
3. Chitme HR, Shashi A, Jain SK, Sabharwal M. Herbal Treatment for Urinary Stones. International Journal of Pharmaceutical Sciences and Research 2010; 1:25-31.
4. Shashi A, Jain SK, Verma A, Kumar M, Sabharwal M. Pathophysiology of Kidney, Gallbladder and Urinary Stones Treatment With Herbal And Allopathic Medicine: A Review. Asian Pacific Journal of Tropical Disease 2013; 3: 496-504
5. Qiu SR, Wierzbicki A, Orme CA, Cody AM, Hoyer JR, Nancollas GH, Zepeda S, De Yorea SS.:Molecular modulation of calcium oxalate crystallization by osteopontin and citrate. Proc. Natl. Acad. Sci.2004; 19: 1811-1815
6. Sheng X, Ward MD, Wesson JA.:Crystal surface adhesion explains the pathological activity of calcium oxalate hydrates in kidney stone formation. J. Am. Soc. Nephrol. 2005; 16: 1904-1908.
7. Anjian X, Yuhua S, Dong M, Fangzhi H, Lingguang Q, Shikuo L, Chen L.:Growth of calcium oxalate crystals induced by complex films containing biomolecules. Cryst. Res.

Technol.2007; 42(7):667-672.

8. yongtai Z, Jiansheng T, Nianping F, Xiangdong H.:Crystal growth of calcium oxalate induced by the extracts of Semen plantaginis and Folium pyrrosiae.Cryst. Res. Technol. 2008; 43: 931-934.
9. Jianming O, Xiugiona Y, Zexuan S, Fuzhai C.:Simulation of calcium oxalate stone in vitro.Science in China Series B-Chemistry. 2003; 46(3): 234-242.
10. Ramegowda Basavaraj D, Shakhar Biyani C, Browning AJ, Cartledge J.:The role of Urinary Kidney Stone inhibitors and promoters in the pathogenesis of calcium containingrenal stones. Eur. Asso. Urol.2007; 5: 26-136.
11. Biyani CS. The Role of Urinary Kidney Stone Inhibitors and Promoters in the Pathogenesis of Calcium Containing Renal Stones. J European Urology. 2007; 5:126–136.