Asian Journal of Chemistry; Vol. 23, No. 1 (2011), 319-322

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Influence of Different Kinds of Polysaccharides on Crystal Growth of Calcium Oxalate in Microemulsion

WENJIN YUE[1,2,*], GUANGJUN NIE[1,2], ZHENXING CHEN[1], YANBIN LI[1] and QIANQIAN XU[1]

[1]Department of Biochemical Engineering, Anhui University of Technology and Science, Wuhu 241000, P.R. China
[2]Institute of Plasma Physics, Chinese Academy of Sciences, Hefei 230031, P.R. China

*Corresponding author: Tel: +86 553 5688311; E-mail: yuewenjin_79@163.com

(*Received*: 10 March 2010; *Accepted*: 27 August 2010) AJC-9048

Crystal growth of calcium oxalate in bulk aqueous solution, reverse microemulsion of *p*-octyl polyethylene glycol phenylether (OP)/iso-octyl alcohol (octyl alcohol)/cyclohexane/water and above microemulsion containing different kinds of polysaccharides, such as β-cyclodextrin, glucan, carboxyl methyl chitosan was studied. Calcium oxalate crystals were characterized by Fourier transform infrared spectroscopy, X-ray diffraction spectrum and scanning electron microscopy. The results indicated that different from manifold irregular crystal morphologies of calcium oxalate formed in bulk aqueous solution, more regular crystals such as calcium oxalate monohydrate, calcium oxalate dihydrate, calcium oxalate trihydrate existed in reverse microemulsion. The additive of β-cyclodextrin promoted crystal growth of calcium oxalate monohydrate to parallel with $(\bar{1}01)$ face, while crystal growth of CaC_2O_4 preferentially oriented with the (020) face of calcium oxalate monohydrate after the addition of glucan. Carboxyl methyl chitosan promoted the formation of calcium oxalate trihydrate and prohibited the formation of calcium oxalate monohydrate, calcium oxalate dihydrate strongly. These facts suggested that different kinds of polysaccharides promote crystal growth of different calcium oxalate hydrate selectively. Furthermore, it could be concluded that carboxyl methyl chitosan may be the depressor of urinary stone in future.

Key Words: Calcium oxalate, Crystal growth, β-Cyclodextrin, Glucan, Carboxyl methyl chitosan.

INTRODUCTION

Urinary stones afflict patients worldwide. Stones can be deposits of calcium phosphates, uric acid, struvite or even calcium carbonate, but by far the most common are those that contain calcium oxalate as the principal inorganic component[1]. Calcium oxalate crystallizes with different crystallization kinetics, *e.g.*, monoclinic monohydrate ($CaC_2O_4 \cdot H_2O$), tetragonal dihydrate ($CaC_2O_4 \cdot (2 + x)H_2O$, $x < 0.5$, calcium oxalate dihydrate) and triclinic trihydrate ($CaC_2O_4 \cdot (3-x)H_2O$, $x < 0.5$, calcium oxalate trihydrate)[2-11]. However, calcium oxalate occurs in stones either as the calcium oxalate monohydrate or calcium oxalate dihydrate form or as a mixture of the two species. In comparison with them, calcium oxalate trihydrate is the thermodynamically least stable phase and much easier to be ejected out along urine.

The other important component of urinary stone is the organic matrix that accounts for about 2-3 % of the total mass and is comprised of proteins, carbohydrates, lipids and other cellular components. It plays important role in the formation of the urinary stone. For example, Talham *et al.*[1] explored the influence of the lipid monolayer on crystal growth of calcium oxalate, Liu *et al.*[12] observed the role of bovine serum albumin on the urinary stone formation, Shen *et al.*[13] studied different concentration of human serum albumin absorbed by octadecanoic acid/octadecylamine monolayer inducing different crystal morphologies of CaC_2O_4. In those systems, polysaccharides have little been considered.

Another necessary factor in studying urinary stone formation is system selecting, including monolayer, Langmuir-Blodgett film, microemulsion, micelle[14-16]. Reverse microemulsion consisted of micron-size water pools dispersed within a continuous oil phase, the water solubilized in reverse microemulsion, in any respects, is similar to the interfacial water presented near the biological membranes. Reverse microemulsion was chosen to induce crystal growth of calcium oxalate in present study and different kinds of polysaccharides, *i.e.*, β-cyclodextrin, glucan, carboxyl methyl chitosan were added to microemulsion system to observe the role of matrix.

EXPERIMENTAL

Sodium oxalate ($Na_2C_2O_4$), anhydrous calcium chloride ($CaCl_2$), acetone, cyclohexane and anhydrous alcohol were all of analytical purity. *p*-Octyl polyethylene glycol phenylether and octyl alcohol were both of chemical purity. β-Cyclodextrin,

glucan, carboxyl methyl chitosan were biochemical reagents. They were produced by Aldrich Chemical Company and used without further purification. All solutions were prepared with double deionized water.

Crystal growth of calcium oxalate in different microemulsion systems: 0.01 mol/L $CaCl_2$ was dropped in 0.01 mol/L $Na_2C_2O_4$ aqueous solution by the speed of 6 drop/min to mix. After the reaction finished, the precipitate obtained from centrifugation was washed with distilled water and absolute ethanol at least five times in order to remove the residual reactants and byproducts. The products were dried in vacuum over for 24 h until a constant weight was achieved, which was called crystal (**I**). The same concentration of $CaCl_2$ or $Na_2C_2O_4$ aqueous solutions were added into the organic phases consisted of 26.7 wt % *p*-octyl polyethylene glycol phenylether, 17.8 wt % octyl alcohol and 55.6 wt % cyclohexane to form two types of reverse microemulsions and stirred slowly for 0.5 h to prepare microemulsion containing CaC_2O_4. The microemulsion was dropped double deionized water slowly to make the microemulsion destroyed. The precipitate was washed and dried as above procedure, which was called crystal (**II**). For the preparation of crystals (**III**)-(**V**), same procedures were operated except that $CaCl_2$ solution was replaced by $CaCl_2$ solutions containing certain concentration (wt: 0.1 %) of β-cyclodextrin, glucan, carboxyl methyl chitosan, respectively. Reverse microemulsion and reverse microemulsion containing different polysaccharides named microemuslin (I) to microemulsion (IV), respectively.

Characterization: Fourier transform infrared (FT-IR) measurements were conducted on a Nexus 870 Fourier transform infrared spectroscopy. The XRD measurements were performed by a MAP18XAHF x-ray diffractometer at a scanning rate of 4°/min, using a monochromatized CuK_α radiation (λ = 0.154 nm). The sizes and morphologies of CaC_2O_4 crystals were performed using a S-450 scanning electron microscope at an accelerating voltage of 20 KV (made in Japan).

RESULTS AND DISCUSSION

FT-IR spectra: Infrared spectra of CaC_2O_4 crystals produced in different systems were shown in Fig. 1. It could be seen from infrared spectrum of CaC_2O_4 crystals gained from bulk aqueous solution (Fig. 1a) that the main antisymmetric carbonyl stretching band ν_{as}(COO-) specific to the oxalate family occurs at 1618 cm^{-1}. The secondary carbonyl stretching bands, the metal carboxylate stretch, $\nu_s(COO^-)$ is located at 1318 cm^{-1}. In the fingerprint region, two characteristic absorptions are located at 780 and 665 cm^{-1} and also the five peaks in between 3000 and 3500 cm^{-1} correspond to the asymmetric and symmetric stretch of the water molecules coordinated with the calcium oxalate molecules[17]. Fig. 1(b-e) showed infrared spectra of crystal (**II**)-(**V**), respectively. It could be found that the intensity of antisymmetric oxalate C=O bond became weaker and the full width at medium height of symmetrical and antisymmetric oxalate C=O bond became wider. The change may be attributed to the complex hydrogen bond, such as the interaction among carboxyl group of $C_2O_4^{2-}$, β-cyclodextrin, glucan or carboxyl methyl chitosan. The changes of FT-IR spectra suggested that

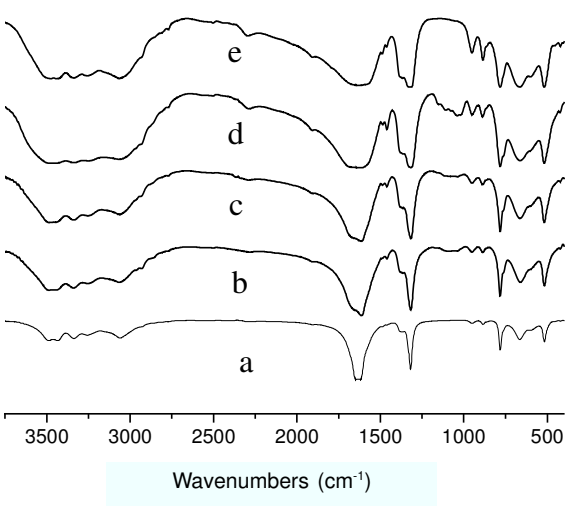


Fig. 1. FT-IR spectra of different CaC_2O_4 crystals. (a-e) Crystal (I)-(V), respectively

these polysaccharide molecules had important influences on the microstructures of CaC_2O_4 crystals.

XRD: Fig. 2 shows the XRD spectra of CaC_2O_4 formed in different systems. XRD patterns of CaC_2O_4 crystals grown in bulk aqueous solution showed many peaks (shown in Fig. 2a). Diffraction peaks corresponding to the index of the reflection planes for $(\bar{1}01)$, (020), $(20\bar{2})$, (130), (100), (001) and (110) planes could be found, which indicated the presence of calcium oxalate monohydrate, calcium oxalate dihydrate and calcium oxalate trihydrate. In comparison with Fig. 2a, 2b, displayed different results, crystal orientation growth became more obvious. Diffraction peaks became weak except the peaks corresponding to the index of the reflection planes for $(\bar{1}01)$ planes. In Fig. 2c, the corresponding main diffraction peaks are located at 0.593 and 0.297 nm, which could be assigned to the $(\bar{1}01)$, $(20\bar{2})$ planes of the calcium oxalate monohydrate crystal. When glucan was added into the reverse microemulsion, the XRD patterns of CaC_2O_4 was shown in Fig. 2d. Compared with Fig. 2b, the corresponding main diffraction peaks assigned to the (020) planes of the calcium oxalate monohydrate crystal became stronger, while that diffraction peaks assigned to the $(\bar{1}01)$, $(20\bar{2})$ planes of the calcium oxalate monohydrate crystal became weaker and the diffraction peak assigned to (100) plane of calcium oxalate dihydrate crystal disappeared. In comparison with it, Fig. 2e showed largely different. Stronger peaks assigned to (001), (110) planes of the calcium oxalate trihydrate crystal and weaker peaks assigned to $(\bar{1}01)$, (020), $(20\bar{2})$ planes of the calcium oxalate monohydrate crystal could be observed. It may be seen that crystal growth of calcium oxalate monohydrate was strongly inhibited. The different adsorption affinities exhibited by the three CaC_2O_4 hydrates may be associated with electrostatic accumulation and structural similarity based on the concept of molecular recognition[15]. Owing to the induction of surfactant *p*-octyl polyethylene glycol phenylether and cosurfactant octyl alcohol in reverse microemulsion, it could decrease the special activation energy for CaC_2O_4 crystal nucleation at water/oil interface, which resulted in orientation growth of CaC_2O_4. Transformation of calcium oxalate monohydrate into calcium oxalate trihydrate

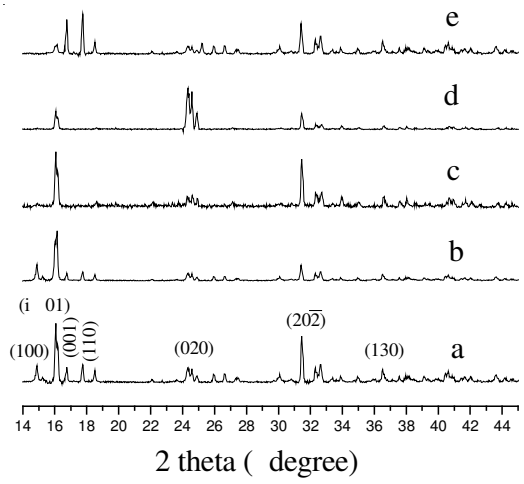


Fig. 2. XRD patterns of different CaC_2O_4 crystals. (a-e) Crystal (I)-(V), respectively

in the CaO_x-organic additive crystallization systems was strongly driven by variations in the ionic characters of the different crystal phases. Structural fit between the lattice parameters of polymeric additives and ionic spacings on major CaC_2O_4 crystal faces may be involved in the higher specific adsorption of organic macromolecules for this hydrate form[16]. For example, the addition of β-cyclodextrin was beneficial to CaC_2O_4 oriented along Ca^{2+}-rich $(\bar{1}01)$ face (Fig. 3a), glucan could promote CaC_2O_4 orientation growth along $C_2O_4^{2-}$-rich (020) face (Fig. 3b), carboxyl methyl chitosan could attract Ca^{2+} to form calcium oxalate trihydrate owing to the interaction of carboxyl groups. Accordingly, the general idea that the formation of calcium oxalate monohydrate and calcium oxalate trihydrate selectively induced by different kinds of polysaccharide molecules may be explained by specific geometric relationship between active functional groups of polysaccharide molecules and interatomic distances on the crystal faces of calcium oxalate trihydrate or calcium oxalate monohydrate.

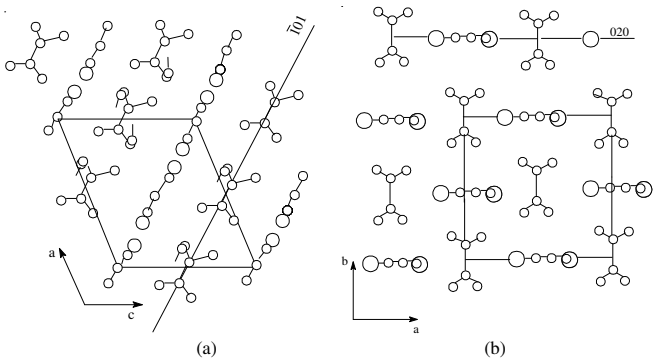
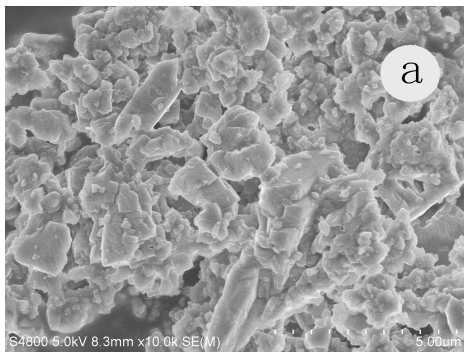
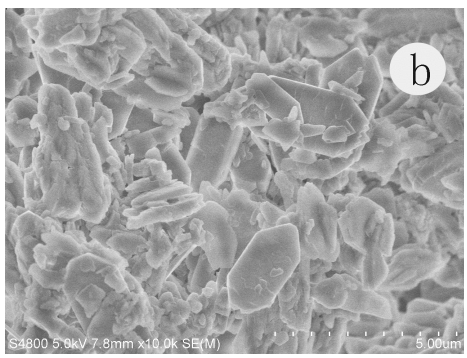


Fig. 3. Different orientation face of calcium oxalate monohydrate (a) $(\bar{1}01)$ face (b) (020) face

Scanning electron microscopy: The morphologies of CaC_2O_4 crystals occurred in different systems were shown in Fig. 4. Fig. 4a was the crystal morphologies of calcium oxalate grown in bulk aqueous solution. Most of crystals were irregular, only a few hexagonal cross-section calcium oxalate monohydrate crystals could be found[17]. It can be concluded that crystal morphologies were irregular without matrix induction. In reverse microemulsion system, crystal morphologies were more regular than that in bulk aqueous solution (Fig. 4b), three morphologies of CaC_2O_4, *i.e.*, cross-section hexagonal calcium oxalate monohydrate, rhombic calcium oxalate dihydrate and stylolitic calcium oxalate trihydrate could be observed[17]. It showed that *p*-octyl polyethylene glycol phenylether and octyl alcohol as the surfactant and cosurfactant in reverse microemulsion could afford template to induce CaC_2O_4 crystal orientation growth along special face. Compared with Fig. 4b, the crystal (III) morphologies became more single. The electron micrographs of CaC_2O_4 (Fig. 4c) showed that the precipitates obtained from microemulsion (II) contained almost aggregates of monoclinic hexagon calcium oxalate monohydrate crystals. The crystal size became twice larger than that grown in microemulsion (I). It indicated that β-cyclodextrin could promote crystal growth and make CaC_2O_4 orient along $(\bar{1}01)$ face. Fig. 4d showed that the presence of glucan promoted the formation of quadrangular calcium oxalate monohydrate, which oriented along (020) face. After the addition of carboxyl methyl chitosan, the crystal morphology was also different (Fig. 4e). It was seen that most of them were stylolitic crystals. The fact suggested that the additive of carboxyl methyl chitosan was beneficial to the formation of calcium oxalate trihydrate with calcium oxalate monohydrate crystals barely visible, no matter hexagon or quadrangular crystals, consistent with the XRD spectra. calcium oxalate trihydrate and calcium oxalate monohydrate crystals differ in their ability to adhere to the wall of the kidney and/or renal tubule. As a consequence calcium oxalate trihydrate can be expelled out by the urine more easily than calcium oxalate monohydrate can and calcium oxalate monohydrate is retained in the urinary system and induces the formation of urine stones. Therefore, calcium oxalate trihydrate is less harmful to the human body than calcium oxalate monohydrate and the presence of carboxyl methyl chitosan could reduce the danger of urine stone formation.





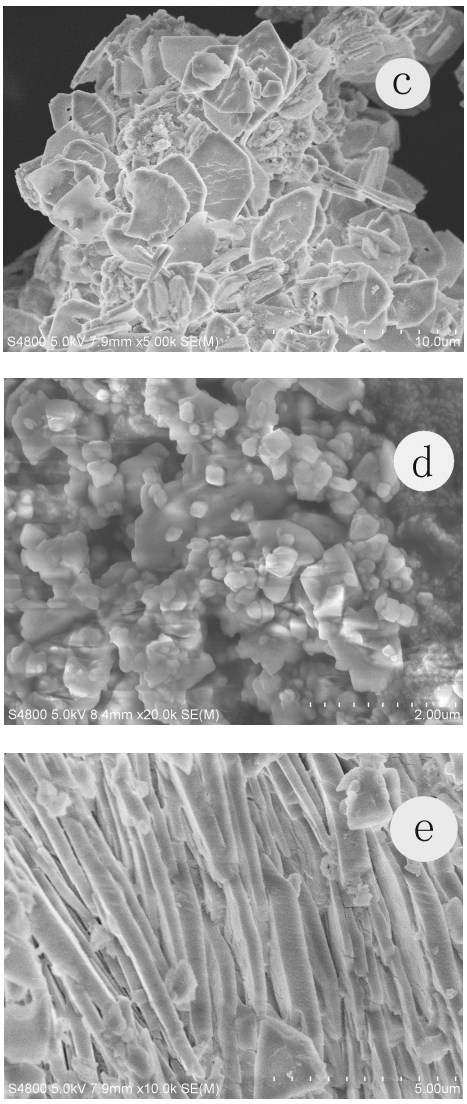


Fig. 4. Morphologies of different CaC_2O_4 crystals. (a-e) Crystal (I)-(V), respectively

Conclusion

Different form of crystal growth of CaC_2O_4 in bulk aqueous solution, reverse microemulsion and that containing β-cyclodextrin,glucan, carboxyl methyl chitosan could decrease free energy of some special lattice planes and make orientation growth of CaC_2O_4 crystal paralleled with different lattice planes, which promoted different calcium oxalate hydrates to form. For example, β-cyclodextrin promoted orientation growth along $(\bar{1}01)$ face of calcium oxalate monohydrate, glucan facilitated crystal growth preferentially oriented with the (020) face of calcium oxalate monohydrate and the addition of carboxyl methyl chitosan make crystal growth of calcium oxalate trihydrate form. Owing to calcium oxalate trihydrate is the thermodynamically least stable phase and much easier to be ejected out along urine, it can be concluded that carboxyl methyl chitosan may be the depressor of urinary stone in future.

ACKNOWLEDGEMENTS

This work is supported by Excellent Project for Talents of Universities of Anhui Province under contract No. 2009SQRZ093 and Key Project of Wuhu Science and Technology Bureau under contract No. 2008508.

REFERENCES

1. D.R. Talham, R. Backov, I.O. Benitez, D.M. Sharbaugh, S. Whipps and S.R. Khan, *Langmuir*, **22**, 2450 (2006).
2. V. Thongboonkerd, S. Chutipongtanate, T. Semangoen and P. Malasit, *J. Urol*., **179**, 1615 (2008).
3. A.J. Xie, L. Zhang, J. Zhu, Y.H. Shen, Z. Xu, J.M. Zhu, C.H. Li, L. Chen and L.B. Yang, *Colloid. Surface. A: Physicochem. Eng. Asp*., **332**, 192 (2009).
4. I.O. Benitez and D.R. Talham, *J. Am. Chem. Soc.*, **127**, 2814 (2005).
5. W.O.S. Doherty, *Ind. Eng. Chem. Res*., **45**, 642 (2006).
6. J.M. Ouyang, N. Zhou, L. Duan and B. Tieke, *Colloid. Surface. A: Physicochem. Eng. Asp.*, **245**, 153 (2004).
7. H. Yu, R. Sheikholeslami and W.O.S. Doherty, *Powder Tech*., **160**, 2 (2005).
8. R.C. Walton, J.P. Kavanagh, B.R. Heywood and P.N. Rao, *J. Crys. Growth*, **284**, 517 (2005).
9. A.M. Ali, N.A.N. Raj, S. Kalainathan and P. Palanichamy, *Mater. Lett.*, **62**, 2351 (2008).
10. S. Kato, H. Unuma and M. Takahashi, *Adv. Powder Tech*., **12**, 49 (2001).
11. X. Xu, J.T. Han and K. Cho, *Langmuir*, **21**, 4801 (2005).
12. J. Liu, H. Jiang and X.Y. Liu, *J. Phys. Chem. B*, **110**, 9085 (2006).
13. Y.H. Shen, W.J. Yue, A.J. Xie, Z.Q. Lin and F.Z. Huang, *Colloid. Surface. A: Physicochem. Eng. Asp.*, **234**, 35 (2004).
14. R. Backov, C.M. Lee and S.R. Khan, *Langmuir*, **16**, 6013 (2000).
15. P. Sriboonlue, S. Suwantrai and V. Prasongwatana, *Clin. Chim. Acta*, **273**, 59 (1998).
16. T.A. Viel, C.D. Domingos and A.P.D.S. Monteriro, *J. Ethnopharmacol.*, **66**, 193 (1999).
17. M.S. Liang, Y. Bai, L.L. Huang, W.J. Zhang and J. Liu, *Colloid. Surfac. A: Biointerface*., **74**, 366 (2009).