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Morphological control of calcium oxalate particles in the presence of poly-(styrene-alt-maleic acid)

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Abstract

Calcium oxalate (CaOx) particles exhibiting different shapes and phase structures were fabricated by a simple precipitation reaction of sodium oxalate with calcium chloride in the absence and presence of poly-(styrene-alt-maleic acid) (PSMA) as a crystal modifier at room temperature. The as-obtained products were characterized with scanning electron microscopy (SEM) and X-ray diffraction (XRD). The effects of reaction conditions including pH, $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio and concentration of PSMA and CaC_2O_4 on the crystal forms and morphologies of the as-obtained calcium oxalate were investigated. The results show that various crystal morphologies of calcium oxalate, such as parallelograms, plates, spheres, bipyramids etc. can be obtained depending on the experimental conditions. Higher polymer concentration favors formation of the metastable calcium oxalate dihydrate (COD) crystals. Lower pH is beneficial to the formation of plate-like CaOx crystals. Especially, the monodispersed parallelogram-like CaOx crystals can be produced by PSMA as an additive at pH 2. PSMA may act as a good inhibitor for urolithiasis since it induces the formation of COD and reduces the particle size of CaOx. This research may provide new insight into the morphological control of CaOx particles and the prevention of urolithiasis.

Keywords: Calcium oxalate; Precipitation reaction; Morphological control; PSMA (poly-(styrene-alt-maleic acid)); Urolithiasis; Prevention

1. Introduction

In recent years, the controllable synthesis of inorganic particle materials with specific size and morphology has attracted more and more attention in the development of new materials in many fields such as advanced materials, catalysis, medicine, electronics, ceramics, pigments, cosmetics, etc. [1–6]. Compared with particle size control, the morphology control of particles is more difficult to achieve [5–7]. Biological systems, however, use bio-macromolecules as nucleators, cooperative modifiers, and matrixes or molds to exert exquisite control over the processes of biomineralization. This control results in unique inorganic–organic composites (e.g., seashells, bones, teeth, and many others) with various special morphologies and functions [8–11]. The strategy of using organic additives and/or templates to control the nucleation, growth, and alignment of inorganic particles has been universally applied for the biomimetic synthesis of inorganic materials with unusual and complex form [12,13].

The biomimetic synthesis of calcium oxalate (CaOx) has received much attention since it is the most commonly formed biomineral in higher plants and the primary mineral constituent in most human kidney stones [5,14–16]. In nature, CaOx exists in three different kinds of hydrates: the monoclinic calcium oxalate monohydrate (COM, or whewellite), which is the thermodynamically most stable phase at room temperature [14–17], the tetragonal calcium oxalate dihydrate (COD, or weddellite) and the triclinic calcium oxalate trihydrate (COT) [18], which are the thermodynamically metastable phase at room temperature. Monoclinic COM and tetragonal COD are the most common phyto-crystals and are among the main

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constituents of kidney stones whereas triclinic COT was rarely found in kidney stones [5]. In the past decade, an important part about biomimetic synthesis of CaOx crystals is to develop an understanding of the interactions between the stone crystals and the components of the organic matrix. Since biominerals are usually formed within membrane-bound microspaces, and nucleation and growth of the biominerals are regulated by an organic matrix [19], some ordered systems such as Langmuir monolayers [20–22] and vesicle [23] were designed as model systems to mimic the formation of CaOx stones. Furthermore, the morphological and phase changes of CaOx crystals in the presence of surfactants [24], carboxylic acids [25], anionic poly electrolytes [25], and crystal-associated macromolecules [26] have also been investigated. However, up to now, morphological control of CaOx crystals in vitro has been achieved to a very limited extent compared with the morphological control of calcium carbonate crystals [1–3,5–10]. Moreover, the interaction mechanisms between macromolecular additive and inorganic crystal in vitro during the formation of stone are still poorly understood and many questions about the promoting and inhibiting factors for renal stone still remain unanswered.

In the present work, we investigate the crystallization of CaOx crystals from aqueous solutions in the absence and presence of poly-(styrene-alt-maleic acid) (PSMA). The effects of experimental conditions including pH, the $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio and concentration of PSMA and CaC_2O_4 on the crystal forms and morphologies of the as-prepared CaOx crystals have been investigated and discussed.

2. Experimental section

2.1. Preparation

Poly-(styrene-alt-maleic acid) (PSMA) (sodium salt, 30 wt% solution in water, obtained from Aldrich, average molecular weight ~120,000) was used as a crystal modifier. All the other chemicals used were of analytical grade without further purification. The distilled water was used in this experiment. The preparation of CaOx was conducted at room temperature in glass vessels according to the method reported in the literature [27–29]. Aqueous solution of $Na_2C_2O_4$ (0.1 M) and of $CaCl_2$ (0.1 M) was first prepared as stock solution. In a typical (standard) synthesis, a solution of $Na_2C_2O_4$ (0.1 M, 0.8 mL) was injected into an aqueous solution of PSMA (80 mL, 0.1 $g L^{-1}$) and no efforts were made to adjust the pH except for experiments in which a specific variation of pH was designed. Then a solution of $CaCl_2$ (0.1 M, 0.8 mL) was injected quickly into the solution under vigorous stirring by using a magnetic stirrer; this gave a final CaC_2O_4 concentration of 1 mM. The mixture was stirred for 1 min, and then the solution was covered with a glass plate to remain under static conditions for 24 h before the product was collected for characterization. After reaction, the pH of solution was about 7. In the experiments, the concentration of PSMA was varied from 0.05 to 0.25 $g L^{-1}$, and the final concentration of CaC_2O_4 was varied from 0.5 to 2 mM. As reference experiments, the CaOx precipitations were prepared in the absence of PSMA, and all other conditions kept the same.

2.2. Characterization

The resulting CaOx precipitates were characterized by scanning electron microscopy (SEM) (type JSM-5610LV, Japan) with an accelerating voltage of 20 kV. The X-ray diffraction (XRD) patterns obtained on HZG41B-PC X-ray diffractometer using Cu$K\alpha$ radiation at a scan rate of 0.05° $2\theta\,S^{-1}$ were used to determine the identity of crystalline phase. The accelerating voltage and applied current were 35 KV and 20 mA, respectively.

3. Results and discussion

3.1. Effect of the polymer concentration

Fig. 1 shows the influence of the amount of PSMA on the morphologies and sizes of CaOx particles at 25°C under the standard synthesis condition. It can be seen from Fig. 1 that the sizes of CaOx particles obviously decrease with increasing amount of PSMA. This is ascribed to the fact that PSMA, as a negatively charged polymer polyanion, can enrich Ca^{2+} ions at the polymer/water interface, resulting in an increase of the concentration of Ca^{2+} ions in the interface region. According to the nucleation and growth theory [14,30], to form a new nucleator, the activation energy (ΔG_N) must be overcome. ΔG_N can be expressed as [14,30]: $\Delta G_N = 16\pi(\Delta G_1)^3/3(kT\,\text{In}\,S)^2$, where ΔG_1 is the surface energy that is needed to form the new interface and maintain the crystal growth, k the Boltzmann constant, T the temperature, and S the supersaturation of solution. Thus, the decrease of the surface energy ΔG_1 or the increase of the supersaturation S can decrease the activation energy for the nucleation of CaOx. In the presence of PSMA, the nucleation of CaOx often occurs in the chemical microenvironments near the surface of PSMA chains. In these microenvironments, there is an organic–inorganic interface region, and more Ca^{2+} and Ox^{2-} ions were enriched. Therefore, the supersaturation of CaOx in this region is obviously higher than that in the bulk solution. This results in a facile nucleation of CaOx due to a decrease of the activation

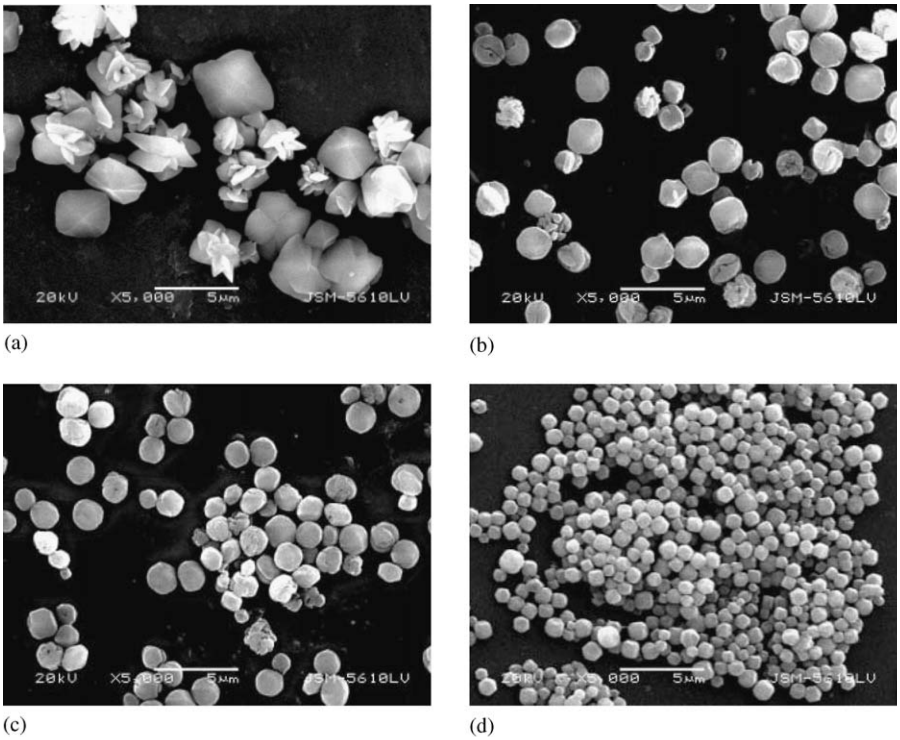


Fig. 1. SEM micrographs of CaOx particles obtained in the absence and presence of PSMA at 25°C. [CaC_2O_4]: 1 mM, pH: 7, [PSMA]: (a) 0, (b) 0.05, (c) 0.1 and (d) 0.25 g/L, the scale bar = 5 μm.

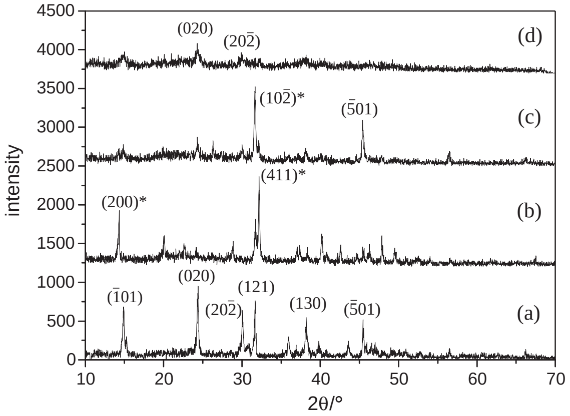


Fig. 2. XRD patterns of CaOx particles obtained in the absence (a) and presence of PSMA (b–d). (b) pH = 7, (c) pH = 2, (d) the [Ca^{2+}]/[$C_2O_4^{2-}$] ratio = 2. The crystal faces with double asterisks show COT, those with asterisks show COD and those without an asterisk show COM. [CaC_2O_4]: 1 mM, [polymer]: 0.25 g/L.

energy ΔG_N. Therefore, the induction time of precipitation decreases and a large amount of CaOx particles with smaller size form in the bulk solution in the presence of PSMA. It was not surprising that the particle size of CaOx decreased with increasing PSMA concentration. Further observation showed that the increase in PSMA concentration greatly influenced the morphologies of CaOx. In the absence of PSMA, irregular-shaped and aggregated particles were obtained (Fig. 1a). The XRD analysis shows that the obtained products mainly consist of COM (as shown in Fig. 2a). When the concentration of PSMA was small ($0.05\,g\,L^{-1}$), many spherical particles, in addition to aggregates, were obtained (Fig. 1b). However, with a further increase in the concentration of PSMA (from 0.05 to $0.1\,g\,L^{-1}$), more spherical particles and less irregular-shaped aggregates were produced (Fig. 1c), typical for the strong interaction between the carboxylic acid groups of PSMA and the crystallizing CaOx crystals [6]. XRD results (not shown here) showed that with increasing PSMA concentration, the amount of COM decreased and the amount of COD increased steadily. When the concentration of PSMA reached $0.25\,g\,L^{-1}$, the obtained crystals were tetragonal bipyramids (Fig. 1d), typical of COD crystals [5,16]. The corresponding XRD pattern also confirmed that the crystal was almost pure COD phase (as shown in Fig. 2b). Fig. 2b also shows that the (200) and (411) peaks are enhanced, suggesting a preferential alignment of the (200) and (411) crystal planes of COD. This is due to the fact that the energy for the nucleation of COD with the (200) and (411) faces are lowered, leading to these faces growing preferentially. It can be concluded from the above results that higher PSMA concentration would inhibit the formation of calcium oxalate monohydrate (COM) phase and meanwhile promote the formation of the calcium oxalate dihydrate (COD) phase. This can be attributed to the following causes.

Usually, there are two kinds of Ca^{2+} ions in an aqueous solution of PSMA. One is the free Ca^{2+} ions, which are the same as those in pure water. The other is the Ca^{2+} ions bound to the interface of polymer chains/water. When sodium oxalate (NaOxa) is added to the PSMA aqueous solution containing $CaCl_2$, it can react with the above two kinds of Ca^{2+} ions at the interface and in the bulk solution. When NaOxa reacts with the free Ca^{2+} ions, the reaction of $CaCl_2$ with NaOxa is the same as that in pure aqueous solution, and the thermodynamically stable COM is the dominant phase. On the contrary, when NaOxa reacts with the bound Ca^{2+} ions at the polymer/water interface, the thermodynamically metastable COD can be formed due to the high supersaturation of CaOx at the interface. This explains why the amount of COD increases with increasing PSMA concentration.

Recently, many researches have shown that COM and COD crystals have significantly different affinities to cell membranes due to the difference of their surface structure. COM exhibits a greater degree of attachment to renal tubule cells in culture compared with COD [31], and theoretical calculations also suggest that COM may have a stronger affinity to cell membranes than COD [32]. Therefore, a preferential adsorption of the cell membrane at the surfaces of nascent COM crystals was the main reason why the crystallization of COD was induced in order to prevent the formation of COM (or urinary stone). That is, COD is easily expelled from the body along with the urine. Thus, if more COD can be induced in urine by a certain additive, this additive may act as a good inhibitor for urinary stones. Hence, it can be inferred from the above results that PSMA may act as a good inhibitor for urolithiasis since it induces the formation of COD and reduces the particle size of CaOx.

3.2. *Effect of pH*

The variation of pH drastically influenced the morphologies of the produced CaOx crystals. Fig. 3 shows SEM micrographs of the products obtained at different pH under the standard condition. It can be seen from Figs. 3a and b that at low pH values (2 or 4), plate-like particles were easily obtained. Especially, monodispersed parallelogram-like particles could be obtained at pH 2 (Fig. 3a), the morphology of parallelogram-like particles is typical for the calcium oxalate trihydrate (COT) [17,33]. This is consistent with the corresponding XRD result (as shown in Fig. 2c), which suggests that the obtained crystals consist predominantly of the COT phase. This may be due to the fact that high supersaturation and low pH favor kinetically the formation of the thermodynamically unstable COT phase [34]. At pH 9, irregular aggregates and plate-like particles were obtained (Fig. 3c) and

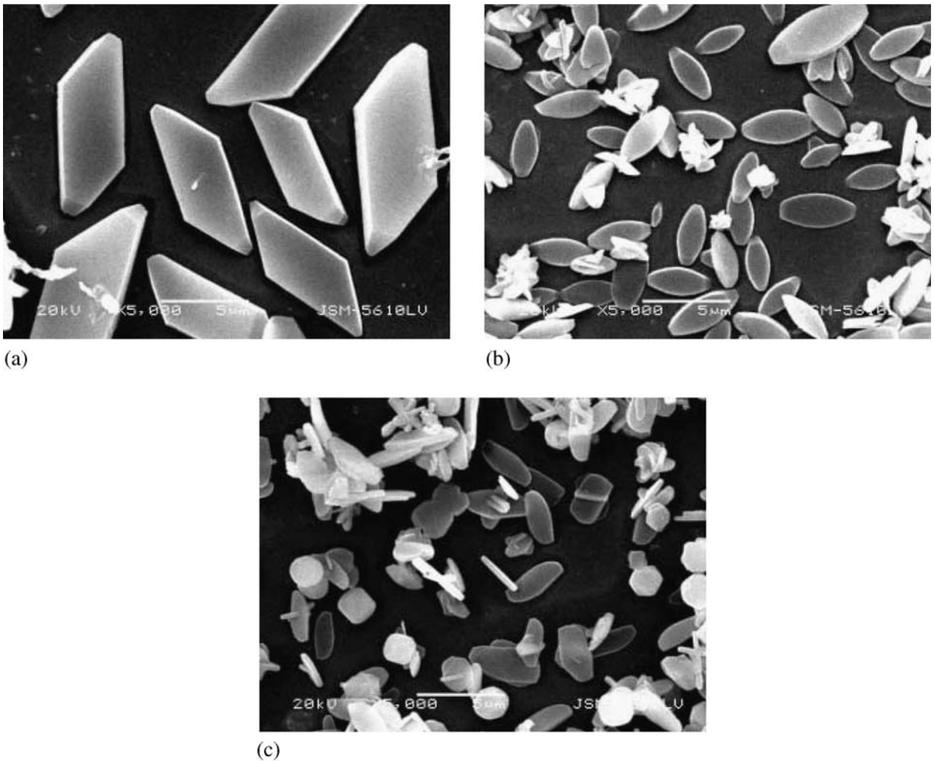


Fig. 3. SEM micrographs of CaOx particles obtained at different pH in the presence of PSMA at 25°C for 24 h. [CaC_2O_4]: 1 mM, [polymer]: 0.25 g/L, pH: (a) 2, (b) 4 and (c) 9, the scale bar = 5 μm.

morphological control almost lost. This may be related to the influence of pH on the protonation degree of the carboxylic acid groups in PSMA. At pH 9, the carboxylic acid groups were completely charged and formed a polyanionic chain [29]. This resulted in a rapid increase of the concentration of Ca^{2+} ions at the polymer/water interface and the solution supersaturation of CaOx increased in the interface region with increasing pH. Therefore, the nucleation rate, at pH 9, was so high that morphology control was already partially lost and the average particle size decreased. This also indicated that the increase of the supersaturation (or high pH) did not favor the morphology control. It can also be seen from Fig. 3 that the particle size is at a maximum at pH 2. That is ascribed to the fact that the nucleation rate is the lowest at pH 2 due to the low protonation degree of the carboxylic acid groups of the polymer.

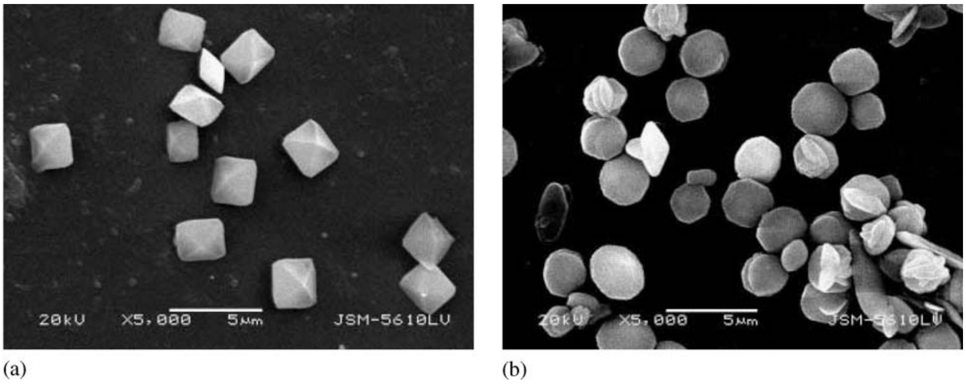
3.3. Effect of the CaC_2O_4 concentration

To investigate the influence of CaC_2O_4 concentration on crystallization, the crystallization experiments were conducted at a fixed polymer concentration ($0.1\,g\,L^{-1}$) but a lower and higher CaC_2O_4 concentration compared with the standard of 1 mM. At the lower CaC_2O_4 concentration of 0.5 mM, the obtained product was bipyramidal crystal (as shown in Fig. 4a), typical for the COD phase [5,16]. These bipyramidal COD crystals were similar to those obtained at a CaC_2O_4 concentration of 1 mM but at a higher polymer concentration of $0.25\,g\,L^{-1}$ (Fig. 1d), although the present products seemed to be more uniform and were much larger. This is due to the fact that with decreasing CaC_2O_4 concentration the supersaturation of CaOx decreases, lower nucleation rate favors the formation of larger CaOx particles [30].

If the CaC_2O_4 concentration was increased to twice the standard concentration; that is, 2 mM, spheroidal particles were obtained in addition to a small amount of irregular particles. This was similar to the sample obtained at the standard CaC_2O_4 concentration but at a low polymer concentration of $0.05\,g\,L^{-1}$ (Fig. 1b). The observed results seemed to be again consistent with the decrease of the ratio of polymer to CaC_2O_4, and the overall concentration seemed to be of secondary importance. Both CaC_2O_4 concentration experiments suggested that the relative proportion between the polymer and CaC_2O_4 concentration was more relevant to determining the morphology of the produced particles.

3.4. Effect of the $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio

Varying the $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio could change the surface electrostatics of CaOx crystals and thus influence the polymer-CaOx crystals interaction, leading to a morphological variation of CaOx crystals. When the PSMA concentration was $0.1\,g\,L^{-1}$ and the oxalate ions concentration was maintained at 1 mM, a mixture of spheroidal and plate-like particles were produced at a $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio of 1/2 (Fig. 5a). This result was similar to the result obtained at the standard experimental condition but at a lower polymer concentration of $0.05\,g\,L^{-1}$. This indicated that the presence of excess oxalate ions in the solution could impair the adsorption of the anionic polymer on the crystal planes, leading to a partial reduction of their ability to control the crystal morphology. On the contrary, at a higher $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio of 2, ellipsoid plate-like particles and a small amount of irregular aggregates were obtained (Fig. 5b). The XRD analysis shows that there are four main XRD diffraction peaks located at 0.593, 0.365, 0.297, and 0.236 nm, corresponding to the $(\bar{1}01)$, (020), $(20\bar{2})$, and (130) planes of COM (Fig. 2d), respectively. It is worth noting that these COM plates generally exhibit rounded surfaces rather than distinct crystal faces of the monoclinic COM hexagonal to rhombic



(a) (b)

Fig. 4. SEM micrographs of CaOx particles obtained at different CaC_2O_4 concentration in the presence of PSMA at 25°C for 24 h. $[CaC_2O_4]$: (a) 0.5 and (b) 2 mM, [polymer]: 0.1 g/L, pH: 7, the scale bar = 5 μm.

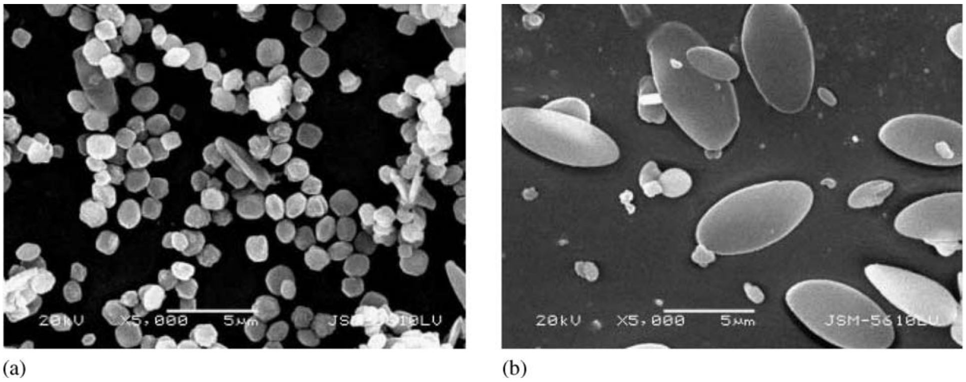


Fig. 5. SEM micrographs of CaOx particles obtained at different $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio in the presence of PSMA at 25°C for 24 h. $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio: (a) 0.5, (b) 2, [polymer]: 0.1 g/L, pH: 7, the scale bar = 5 μm.

[5,14–16]. This result suggested that the presence of excess calcium ions at the surfaces of COM crystals could promote the adsorption of the polymer on COM crystal planes other than the specific planes, resulting in nonspecific inhibition of the COM crystal faces [5].

4. Conclusions

The pH, the $[Ca^{2+}]/[C_2O_4^{2-}]$ ratio and concentration of PSMA and CaC_2O_4 turned out to be important parameters for the control of morphologies and phase structures of CaOx crystals. Various crystal morphologies of CaOx crystals, such as parallelograms, plates, spheres, bi-pyramids etc. could be obtained by changing the experimental conditions. Lower pH was more beneficial to the formation of plate-like CaOx crystals. Especially, the monodispersed parallelogram-like particles were fabricated for the first time with PSMA as an additive at pH 2. Higher polymer concentration favors the formation of the metastable calcium oxalate dihydrate (COD) crystals. PSMA may act as a good inhibitor for urolithiasis since it induces the formation of COD and reduces the particle size of CaOx. This research may provide new insight into the morphological control of CaOx particles and the prevention of urolithiasis.

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References

[1] S. Mann, G.A. Ozin, Nature 382 (1996) 313.
[2] S. Mann, Angew. Chem. Int. Edit. 39 (2000) 3393.
[3] H. Yang, N. Coombs, G.A. Ozin, Nature 386 (1997) 692.
[4] T.S. Ahmadi, Z.L. Wang, T.C. Green, A. Henglein, M.A. ElSayed, Science 272 (1996) 1924.
[5] D.B. Zhang, L.M. Qi, J.M. Ma, H.M. Cheng, Chem. Mater. 14 (2002) 2450.
[6] H. Colfen, L.M. Qi, Chem. Eur. J. 7 (2001) 106.
[7] S. Mann, J. Webb, R.J.P. Williams, Biomineralization, Chemical and Biochemical Perspectives, VCH, Weinheim, 1989.
[8] S. Mann, Nature 365 (1993) 499.
[9] S. Mann, Biomimetic Materials Chemistry, VCH, Weinheim, 1995.
[10] L.M. Qi, J. Li, J.M. Ma, Adv. Mater. 14 (2002) 300.
[11] L.M. Qi, H. Colfen, M. Antonietti, Chem. Mater. 12 (2000) 2392.
[12] G. Falini, S. Albeck, S. Weiner, L. Addadi, Science 271 (1996) 67.
[13] D.B. DeOliveira, R.A. Laursen, J. Am. Chem. Soc. 119 (1997) 10627.
[14] J.M. Ouyang, X.Q. Yao, Z.X. Su, F.Z. Cui, Sci. China Ser. B 46 (2003) 234.
[15] J.M. Ouyang, L. Duan, B. Tieke, Langmuir 19 (2003) 8980.
[16] J.M. Ouyang, S.P. Deng, Dalton T (2003) 2846.
[17] M. Donnet, N. Jongen, J. Lemaitre, P. Bowen, J. Mater. Sci. Lett. 19 (2000) 749.
[18] A. Millan, Cryst. Growth Des. 1 (2001) 245.
[19] P. Calvert, S. Mann, Nature 386 (1997) 127.
[20] R. Backov, C.M. Lee, S.R. Khan, C. Mingotaud, G.E. Fanucci, D.R. Talham, Langmuir 16 (2000) 6013.
[21] R. Backov, S.R. Khan, C. Mingotaud, K. Byer, C.M. Lee, D.R. Talham, J. Am. Soc. Nephrol. 10 (1999) S359.
[22] S. Whipps, S.R. Khan, F.J. O'Palko, R. Backov, D.R. Talham, J. Cryst. Growth 192 (1998) 243.
[23] J.M. Ouyang, L. Duan, J.H. He, B. Tieke, Chem. Lett. 32 (2003) 268.
[24] M.J. Lochhead, L. Touryan, V. Vogel, J. Phys. Chem. B 103 (1999) 3411.
[25] M.A. Webb, Plant Cell 11 (1999) 751.
[26] S.V. Pennisi, D.B. McConnell, L.B. Gower, M.E. Kane, T. Lucansky, New Phytol. 149 (2001) 209.
[27] J.G. Yu, M. Lei, B. Cheng, X.J. Zhao, J. Cryst. Growth 261 (2004) 566.
[28] B. Cheng, M. Lei, J.G. Yu, X.J. Zhao, Mater. Lett. 58 (2004) 1565.

[29] J.G. Yu, M. Lei, B. Cheng, X.J. Zhao, J. Solid State Chem. 177 (2004) 681.
[30] J.W. Mullin, Crystallization, Butterworth-Heinemann, Oxford, Boston, 2001.
[31] J.A. Wesson, E.M. Worcester, J.H. Wiessner, N.S. Mandel, J.G. Kleinman, Kidney Int. 53 (1998) 952.
[32] N. Mandel, J. Am. Soc. Nephrol. 5 (1994) S37.
[33] M. Deepa, K.R. Babu, V.K. Vaidyan, J. Mater. Sci. Lett. 14 (1995) 1321.
[34] T. Bretherton, A. Rodgers, J. Cryst. Growth 192 (1998) 448.