

Production of calcium oxalate monohydrate, dihydrate or trihydrate

A comparative study

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Summary. Procedures to obtain calcium oxalate monohydrate, dihydrate and trihydrate are presented and discussed. The influence of several additives and conditions in the formation of calcium oxalate dihydrate crystals are comparatively evaluated. It seems that the presence of colloidal phosphate favours the formation of calcium oxalate dihydrate crystals through heterogeneous nucleation.

Key words: Calcium oxalate hydrates – Production

Introduction

Calcium oxalate can crystalize into three forms: as monoclinic monohydrate, tetragonal dihydrate or triclinic trihydrate. Nevertheless only the monohydrate form is thermodynamically stable. Thus, in renal stones, only monohydrate and dihydrate forms were found. To obtain the trihydrate form low temperatures are required. On the other hand, transformation of dihydrate to monohydrate in the solid form has been described and is known as the whewellitezation process [1]. The most problematic process corresponds to the formation of pure dihydrate crystals [10]. Thus, the presence of the so-called inhibitors of calcium oxalate crystallization seems to play an important role in the formation of a concrete crystalline form of such a substance. In this paper, procedures, to obtain the three oxalate crystalline forms are compared, and the influence of some additives is presented. Calcium oxalate monohydrate or dihydrate urinary calculus formation and the probable influence of other urinary parameters is discussed.

Experimental

Solutions of calcium chloride, sodium oxalate, ammonium chloride, sodium phosphate, sodium chloride, sodium citrate, sodium sulfate, magnesium sulfate and potassium chloride were prepared using

Reagent Grade chemicals (PANREAC) with triple distilled deionized water.

The crystals were observed with a Kyowa optical microscope, or a Hitachi S-530 scanning electron microscope, or a Kyowa stereoscopic microscope. All observations were repeated ten times to ensure statistical significance of the results.

Thermogravimetric analyses were carried out on a Perkin Elmer TGS 2 electromicroanalytical balance with a heating rate of 10°C/min in oxygen

Synthesis of calcium oxalate monohydrate

7 litres of distilled water were placed in a crystallizer and heated to 70°C. Solutions of $\text{Na}_2\text{C}_2\text{O}_4$ ($7.5 \cdot 10^{-3}$ M) and CaCl_2 ($7.5 \cdot 10^{-3}$ M) were dropped simultaneously at the same speed (250 ml per hour). The size of the crystals was time dependant. The slurry was filtered and the crystals were washed with water and ethanol and then dried at 50°C with vacuum during for 24 hours.

Synthesis of large calcium oxalate dihydrate crystals

The slurry from COM precipitation was filtered and the solution obtained was placed in a large crystallizer. After 15 days of free evaporation a certain amount of regular COD crystals of an approximated size of 2 mm was produced.

Synthesis of calcium oxalate dihydrate

50 ml of Ca^{2+} 10 g/L were added to 200 ml of solution containing: 1.36 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.437 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 2.7 g of NaCl , 0.23 g of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, 0.97 g of Na_2SO_4 , 0.29 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.928 g of NH_4Cl , 2.42 g of KCl and 0.05 g of $\text{Na}_2\text{C}_2\text{O}_4$ maintained in an ice bath. After 1 hour the slurry was filtered and the crystals were washed and dried.

Synthesis of calcium oxalate trihydrate

Solutions of CaCl_2 0.075 M and Na_2Ox 0.075 M were added dropwise to a 500 ml of solution containing 3 g of NH_4Cl maintained in an ice bath. After 1 hour the slurry was filtered and the crystals were washed and dried.

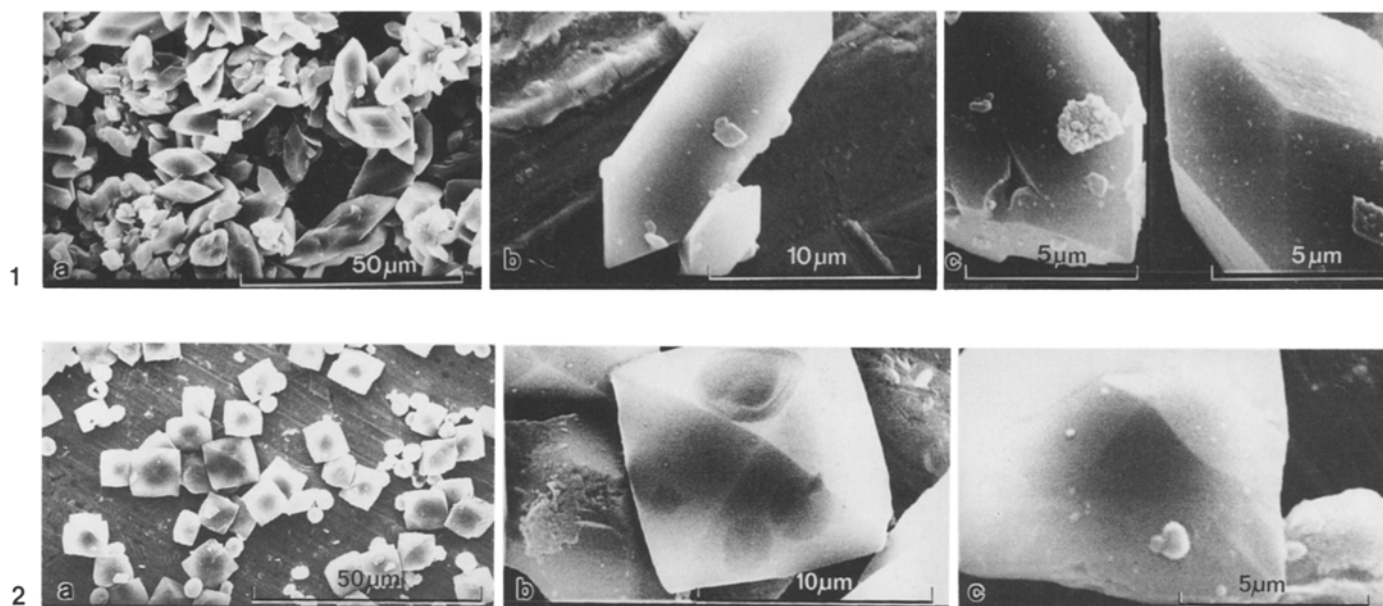


Fig. 1a-c. S.E.M. images of COM crystals obtained according to recommended procedures

Fig. 2a-c. S.E.M. images of COD crystals obtained according to recommended procedures

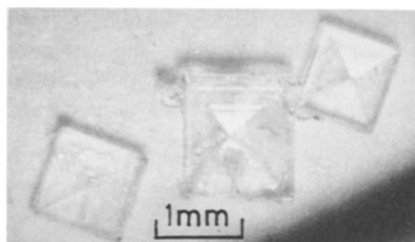


Fig. 3. Stereoscopic microscope images of large COD crystals obtained by evaporation

Results

Synthesis of calcium oxalate monohydrate, dihydrate and trihydrate were assayed and the results are shown in Figs. 1–4. The images are representative of the results obtained, and, as can be seen, visual analysis permits a

clear identification of the hydrated forms of calcium oxalate produced. The production of pure calcium oxalate dihydrate crystals was the most difficult and consequently different procedures were used and evaluated.

As other authors previously noted, the presence of large amounts of citrate favoured the formation of COD crystals. Nevertheless, we found that the presence of phosphate in a colloidal form, greatly enhanced COD production (Figs. 5 and 6). pH effects on this process are shown in Fig. 6. Moreover COD production was attained by evaporation at room temperature of a saturated calcium oxalate solution (Fig. 3).

Thermograms showing the mode of hydration of the different types of precipitates obtained by the first, third, and fourth methods can be seen in Fig. 7. Curve 1 is typical of COM, curve 2 is representative of COD, curve 3 corresponds to COT.

Discussion

The monohydrate was the only thermodynamically stable form of calcium oxalate. Consequently its formation is enhanced at high temperatures. Thus, in these experimental conditions only monohydrate crystals were obtained,

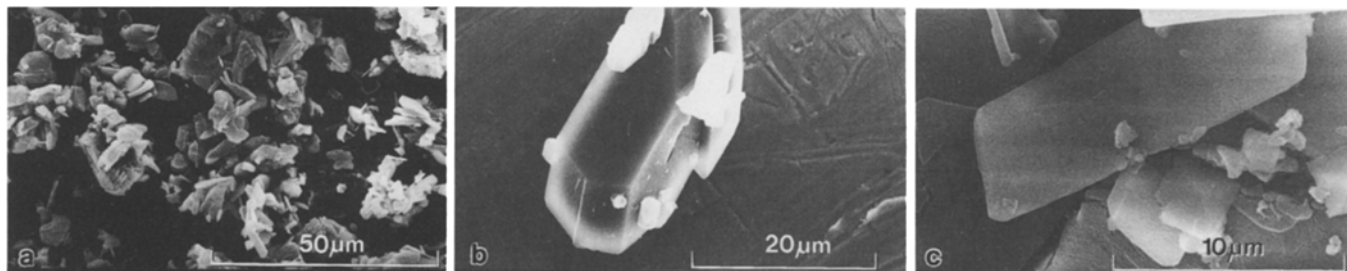


Fig. 4a-c. S.E.M. images of COT crystals obtained according to recommended procedures

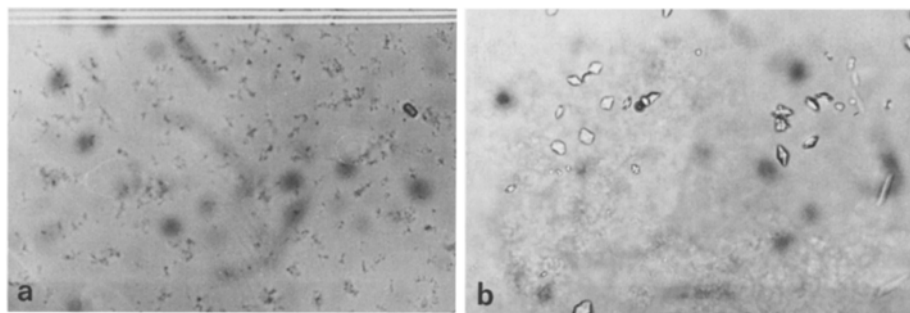


Fig. 5a and b. Optical microscope images of calcium oxalate crystals obtained adding 50 ml of solution 0.75 M CaCl_2 to 200 ml of solution $1.875 \cdot 10^{-3}$ M $\text{Na}_2\text{C}_2\text{O}_4$, $T = 4^\circ\text{C}$, $\text{pH} = 7.25$, 20 minutes after precipitation. **a** without additives, **b** $[\text{PO}_4^{3-}] = 0.133$ M

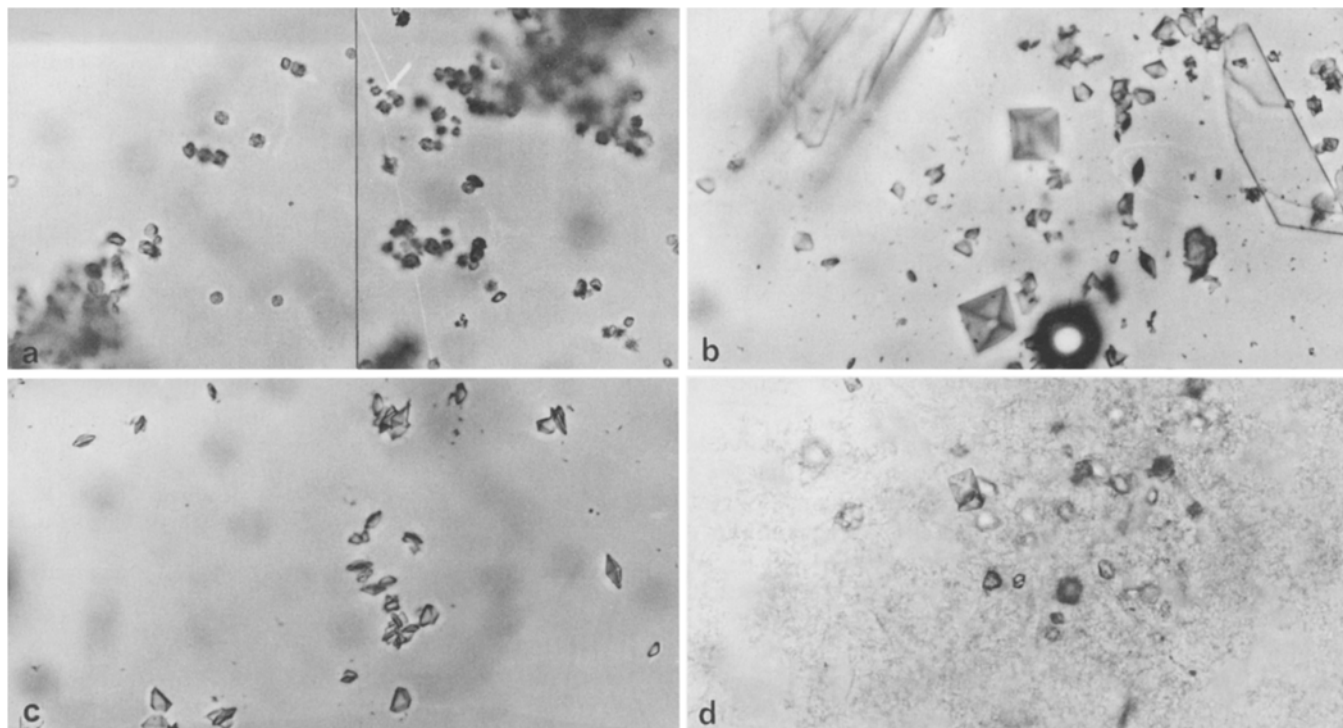


Fig. 6a-d. Optical microscope images of calcium oxalate crystals obtained adding 50 ml of solution 0.75 M CaCl_2 , to 200 ml of solution $1.875 \cdot 10^{-3}$ M $\text{Na}_2\text{C}_2\text{O}_4$, $T = 4^\circ\text{C}$, $[\text{PO}_4^{3-}] = 0.1$ M, 48 hours after precipitation. **a** $\text{pH} = 5$, **b** $\text{pH} = 6.5$, **c** $\text{pH} = 7$, **d** $\text{pH} = 8$

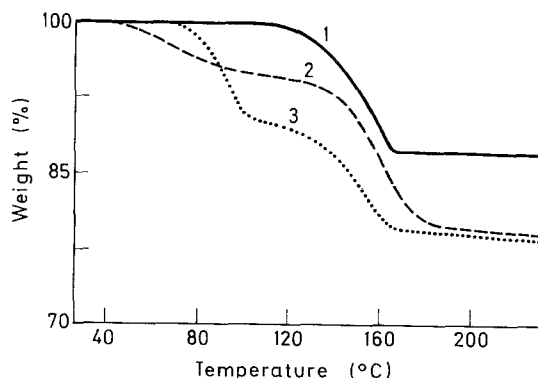


Fig. 7. Thermograms of calcium oxalate precipitates obtained by: 1) COM synthesis method, 2) COD synthesis method, 3) COT synthesis method (Rate: 10 deg/min)

as can be seen in Fig. 1. Moreover, some superficial nucleation was also evident. Aggregate formation was problematic (Fig. 1).

The production of pure bipyramidal calcium oxalate dihydrate, is difficult. As it is postulated by several authors [10], the use of high Ca^{2+} /oxalate ratios (avoiding high supersaturations), high ionic strength, the presence of citrate and low temperatures (4°C), favour production. In Fig. 2 s.e.m. images, corresponding to COD crystals obtained using the ordinary recommended procedure (see experimental part) are shown. Both well defined bipyramidal crystals and spheroidal undefined crystals can be seen, as the presence of high quantities of citrate provoke the formation of COD crystals with a rounded morphology. Moreover we have found that by evaporation of a saturated calcium oxalate solution at room temperature, big bipyramidal COD crystals were obtained (Fig. 3). We have shown that the presence of colloidal phosphate favours the formation of COD crystals, as can be deduced from the results shown in Fig. 5. Thus the presence of phosphate as heterogenous nucleant probably enhances the formation of COD crystals. The study of the pH influence on the formation of COD crystals in the

presence of phosphate, demonstrated that the production of larger crystals was accomplished at pH 7.2. At higher pH values COD crystals appeared with deformities and at lower pH values they were smaller and an increasing number of COM crystals were detected on optical microscopy (Fig. 6). The particle size of phosphate colloid decreased with pH.

Finally, the production of calcium oxalate trihydrate crystals was fulfilled following the procedure stated in the experimental part, and was favoured using equimolecular calcium and oxalate quantities, ionic strength and at low temperatures. The results obtained are shown in Fig. 4. As can be seen, crystals of diverse and irregular sizes were obtained. Cracks in the big crystals can also be observed and this can be attributed to dehydration processes due to its thermodynamic instability.

Thermograms showing the mode of hydration of the different crystals obtained by the proposed procedures are shown in the Fig. 7. In accordance with the literature, the hydration grade of the three known forms of calcium oxalate is not stoichiometric but are as follows:

- COM; monoclinic monohydrate: $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$.
- COD; tetragonal dihydrate: $\text{CaC}_2\text{O}_4 \cdot (2 + X)\text{H}_2\text{O}$, $X = 0.5$
- COT; triclinic trihydrate: $\text{CaC}_2\text{O}_4 \cdot (3 - X)\text{H}_2\text{O}$, $X < 0.5$.

Consequently the similarity between the final values corresponding to COD and COT is to be expected but, the thermograms obtained by us for the three mentioned hydrates are similar to those presented by other authors [4].

Thus, it can be deduced that the detection of a calcium oxalate monohydrate calculus probably will be related to a citrate deficit (inhibitory deficit) and to the presence of normocalciuria, this coinciding with our clinical experience. On the other hand, the calcium oxalate dihydrate calculus can be probably assigned to hypercalciuric patients, with normal levels of citraturia and with probable favoured phosphate precipitation. These data con-

firm the importance of determining the different forms of oxalate present in a calculus.

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