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THE EFFECT OF CONTROLLED DIFFUSION OF IONS ON THE FORMATION OF HYDRATED CALCIUM OXALATE CRYSTALS

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Abstract

Calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) and calcium oxalate trihydrate (COT) were grown from solutions under controlled release of the reacting ions. The mass transfer kinetics of ions released from an insoluble polyethylene matrix, from an osmotic pump system and from the hydrolysis of diethyloxalate were studied under different experimental conditions. It was possible with simple laboratory techniques to grow well-formed crystals of COM, COD and COT, suitable for single crystal work. Results obtained show that the degree of hydration, size, and morphology of the crystals formed, are controlled by interfacial kinetic factors.

KEY WORDS: Hydrated calcium oxalate crystals, Crystal growth, Controlled diffusion, Osmotic pump, Interfacial crystallization.

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Introduction

In recent years there has been much discussion on the role of different hydrated calcium oxalate crystals in the formation of calcium oxalate stones and the possible transformation between them from the standpoint of urinary calculi formation (Hesse et al. 1976; Hienzsch et al. 1979; Tawashi 1983). Calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) are among the most common constituents of stones (Elliot and Rabinowitz 1980). In 1978, Schafer and Dosch identified calcium oxalate trihydrate in urinary sediments but not in urinary calculi. Blom and coworkers, as well as Deganello and coworkers reported the crystal structure of COT (Blom et al. 1981; Deganello et al. 1981). Recently Heijnen and coworkers were able to demonstrate that COT is not a rare but a common constituent of stones (Heijnen et al. 1985). While pure COM, the most stable form, is easily obtained in vitro from simple supersaturated solutions by precipitation, pure COD and COT are much more difficult to grow from synthetic solutions and methods are not consistently reproducible (Gardner and Doremus 1978; Drach et al. 1978; Werness et al. 1981). The growth of single pure crystals of COD and COT (more than 100μm in size) is still difficult. The growth of such highly perfect crystals is important to the understanding of the phenomenon of adhesion, phase transformation and other surface reactions in urine and in renal stones. This work describes the effect of controlled release of ions on the crystal growth and morphology of COD and COT in aqueous media using controlled release systems for the reacting ions.

Materials and Methods

Three systems were used in this study to obtain a controlled release of the reacting ions, namely the Ca^{++} and the oxalate ions.

Release from polymeric matrix

A compressed disc of polyethylene containing the dispersed particles of the reactant to be released (Figure 1A) was prepared by mixing anhydrous $CaCl_2$ (Fisher Sci. Co., Fair Lawn, N.Y.) with polyethylene powder (for chromatographic analysis, BDH Chemicals, Montreal) followed by fusion then direct compression on a Carver laboratory press (model C, Fred S. Carver Inc., Menomonee Falls, WI). The release characteristics of the soluble dispersed particles are described by the Higuchi equation (Higuchi 1961):

$$Q=[D\epsilon/\tau \cdot (2A-\epsilon C_s)C_s t]^{1/2} \qquad (1)$$

Where:

Q : The quality of substance released per unit area at time t.
D : Coefficient of diffusion.
ϵ : Porosity of the matrix.
Cs: Solubility of the dispersed substance in the release media.
τ : Tortuosity of the matrix.
A : Concentration of substance in the matrix.
t : Time.

The quantity of Ca^{++} released by this system per unit area is directly proportional to $\sqrt{t}$. The matrices were immersed into a 0.9% NaCl solution containing 0.145mM potassium oxalate (Fisher Sci. Co., Fair Lawn, N.Y., Lot N°. 854919) for a period of 24 hours, at 37°C.

Osmotic pump system

The system was originally developed by Theeuwes (1975) and is described in detail in the patent literature (Alza Corp.,Palo Alto Ca.). Recently, it was implanted in rats by Khan et al (1983) to regulate the liberation of oxalate ions, during a study of calcium oxalate nephrolithiasis. In this work the Ca^{++} was delivered at a pumping rate of 0.04mM per hour from a miniosmotic pump (Alzet model 2ML1), at a zero order rate. The osmotic pump released Ca^{++} into a 0.9% NaCl solution containing potassium oxalate 0.145mM over a period of 24 hours and the temperature was maintained at 37°C (see Figure 1B).

Controlled crystallization using interfacial reaction

This system is based primarily on the slow hydrolysis of diethyloxalate (J.T.Baker Chemical Co., Phillipsburg, N.J.) in the presence of bidistilled water at pH 6 and the slow liberation of oxalate at the interface separating the two liquids (Elving and Chao 1949).

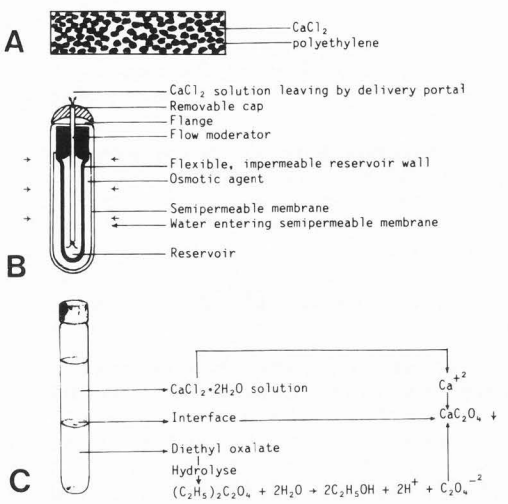

Fig. 1: Schematic representation of three controlled delivery systems used to control release of reacting ions. A, Insoluble plastic matrix. B, Mini osmotic pump. C, Interfacial crystallization.

In these experiments the chemical reaction between the slowly liberated oxalate ion and an aqueous solution containing Ca^{++} ion was used to grow calcium oxalate crystals at the interface. The temperature was controlled at 4°C and crystals were harvested after 3-4 weeks for identification (Figure 1C).

The three controlled delivery systems described above were tested for their release profile and release kinetics under different experimental conditions before starting crystallization. Figure 2A shows the release profile from the insoluble polyethylene matrix and from the osmotic pump system. Figure 2B shows the rate of oxalate production from diethyloxalate. The crystals of calcium oxalate obtained in these experiments were separated and characterized by SEM and X-ray powder diffraction. The SEM analysis was made on a JEOL ISM 840 and X-ray diffraction of powdered material was made on a Philips PN 1130 diffractometer (Lepage and Tawashi, 1982).

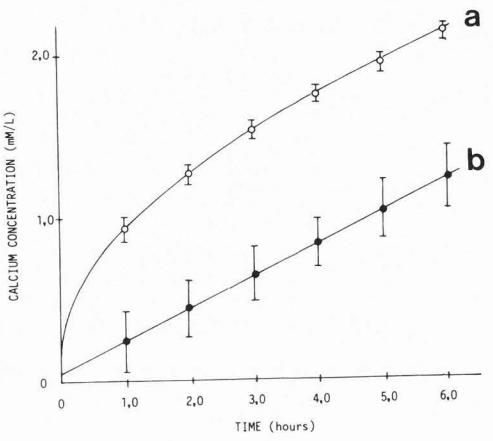

Fig. 2A: Release profile of Ca^{++} from: polyethylene matrix system (a) and osmotic pump system (b), (measured by Nova II. Ca^{++} analyser).

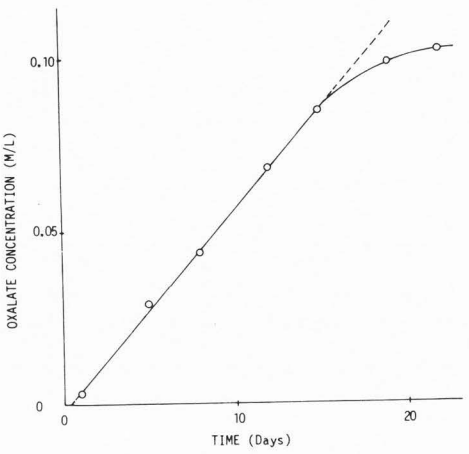

Fig. 2B: Release profile of oxalate ion from diethyloxalate hydrolysis at interface determined indirectly from drop in Ca^{++} concentration in aqueous phase.

Results and Discussion

The growth of different hydrated forms of calcium oxalate crystals under different mass transfer conditions is given in Table 1. The result of controlling the transport conditions of Ca^{++} using an insoluble polyethylene matrix was the formation of well developed COM crystals. Figure 3 shows the surface of the insoluble plastic matrix containing $CaCl_2$ as dispersed phase, before the release experiments and figure 4 shows the surface of a porous polyethylene disc after $CaCl_2$ leaching. The amount of Ca^{++} released in the aqueous media agrees with the Higuchi equation. COM crystals grew on and in the pore structure of the disc. At higher oxalate concentrations, the individual COM crystals obtained were smaller in size than those obtained at lower concentrations (Figures 5 and 6).

In the osmotic pump system the release of Ca^{++} followed a zero order kinetics, independent of agitation (Shaw and Theeuwes 1978). The Ca^{++} pumped at the rate of 0.04mM/h initiated the growth of COT crystals, adhering to the surface of the delivery port (Figures 7-9).

Using diethyloxalate as generator of oxalate ion at 4°C, we were able to grow COD and COT separately by changing the concentration of Ca^{++} in the aqueous medium. Figures 10-12 show the crystals obtained and the conditions of their growth. As regards to the stability of COT crystals, COT was stable at 4°C in tightly closed vials. However, if these crystals were left at room temperature exposed to ambient air, they would undergo a phase transition to COD in less than 24 h. This phase transition occurs via the surrounding humidity by dissolution-recrystallization. As COT transforms to COD, it releases water which forms a supersaturated solution, accelerating the growth of COD on the surface. This surface acts as a nucleating substrate for the growing COD (Figures 13 and 14). The transformation of COT to COD and COM depends on the presence of water, temperature, crystal size and degree of imperfection. The analysis of the transformed crystals on the surface by X-ray diffraction is rather difficult because of the limitation of size and quantity.

On the basis of this study, it is apparent that the rate of mass transfer of calcium and oxalate ions is an important factor in the growth of a specific calcium oxalate crystal. In the osmotic pump system and in interfacial crystallization, COT grew when the chemical potential of ionic species in the supersaturated solution was constant. This agrees with the results of Sheehan and Nancollas (1984) who grew COT by controlling potentiostatically the addition of titrant solutions containing the reacting ions.

Variation in the release kinetics and small changes in the level of other molecules in urine can bring about

Fig. 3: Surface of polyethylene matrix containing 60% W/W of $CaCl_2$. Bar=100 μm.

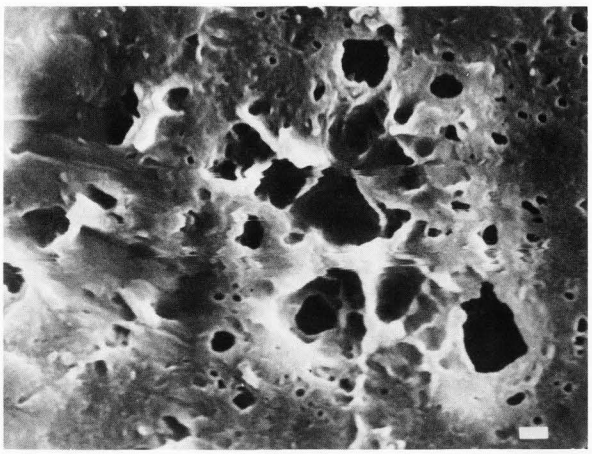

Fig. 4: Surface of polyethylene matrix showing pore structure after leaching $CaCl_2$. Bar=100 μm.

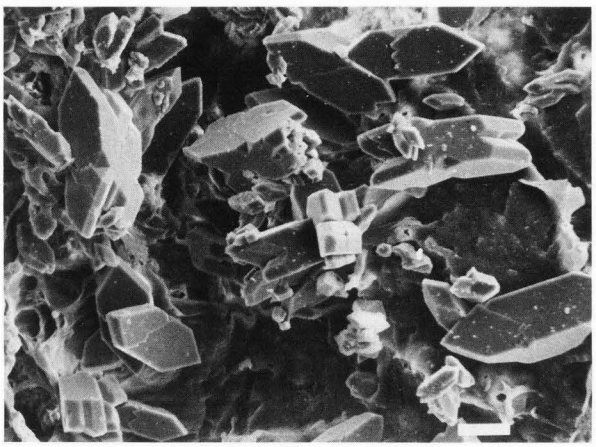

Fig. 5: Growth of COM on pores of polyethylene matrix (concentration of oxalate in external medium: 0.145mM). Bar=10 μm.

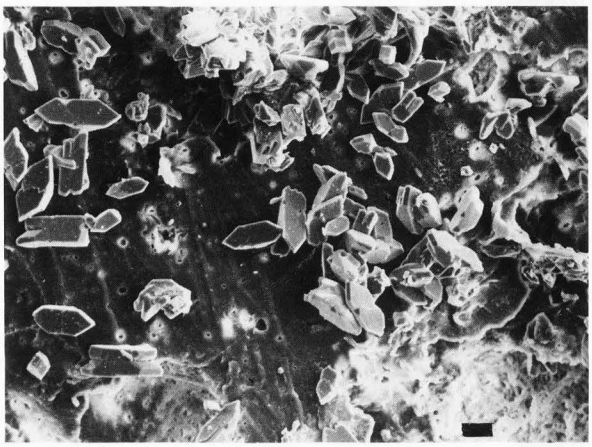

Fig. 6: COM growing in and on pores of polyethylene matrix (concentration of oxalate in external medium: 0.290mM). Bar=10 μm.

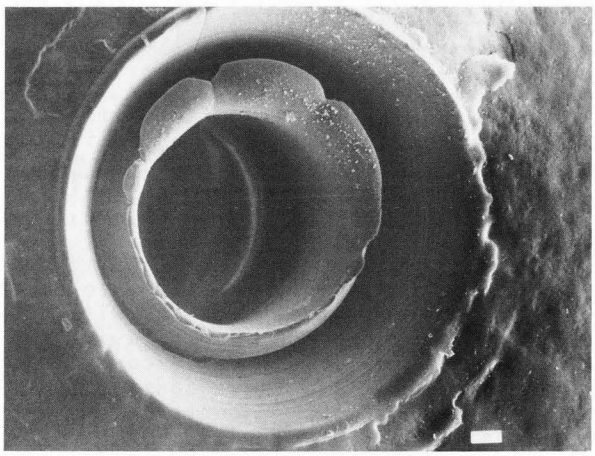

Fig. 7: Orifice of osmotic pump. Bar=100 μm.

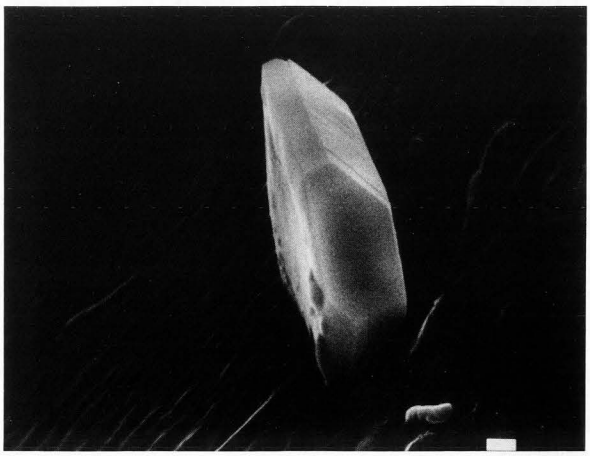

Fig. 8: COT crystal growing at orifice of osmotic pump. Bar=1 μm.

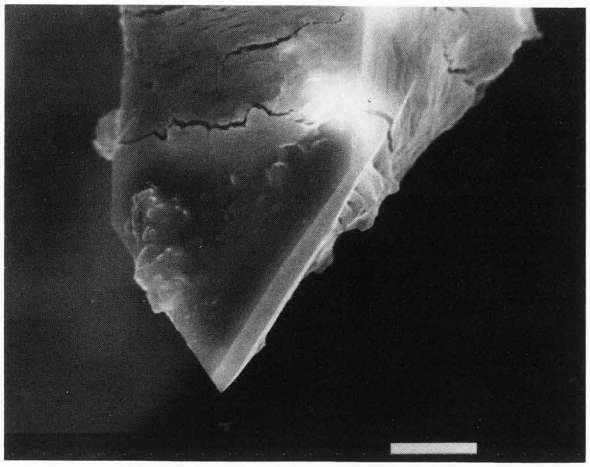

Fig. 9: COM crystal growing at orifice of osmotic pump. Bar=10 μm.

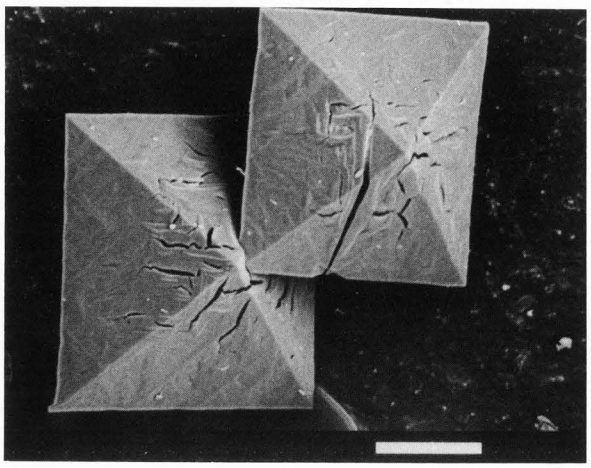

Fig. 10: COD obtained by interfacial crystallization at 4°C (see table 1). Bar=10 μm.

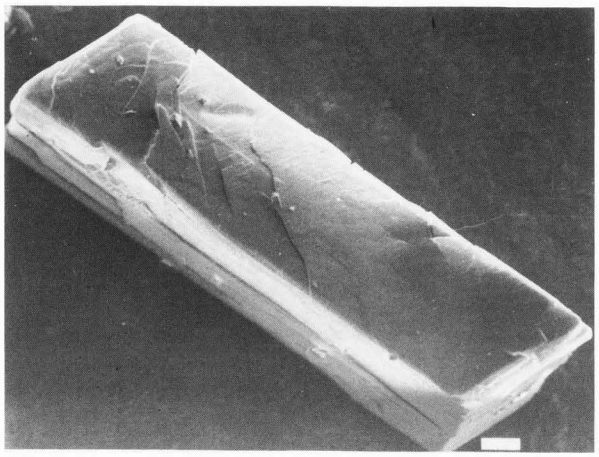

Fig. 11: COT grown by interfacial crystallization at 4°C. Bar=10 μm.

Fig. 12: COT obtained by interfacial crystallization. Bar=10 μm.

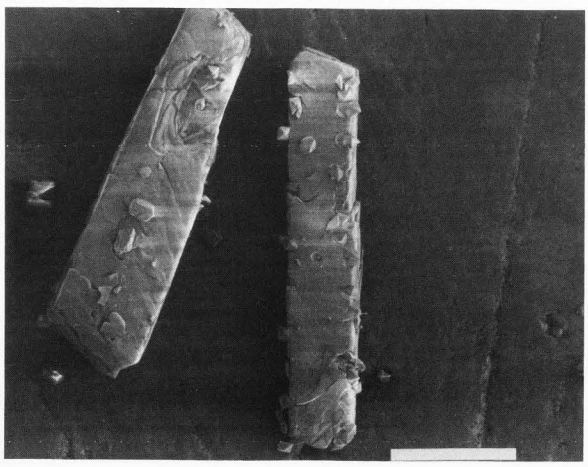

Fig. 13: Transformation from COT to COD at room temperature. Bar=100 μm.

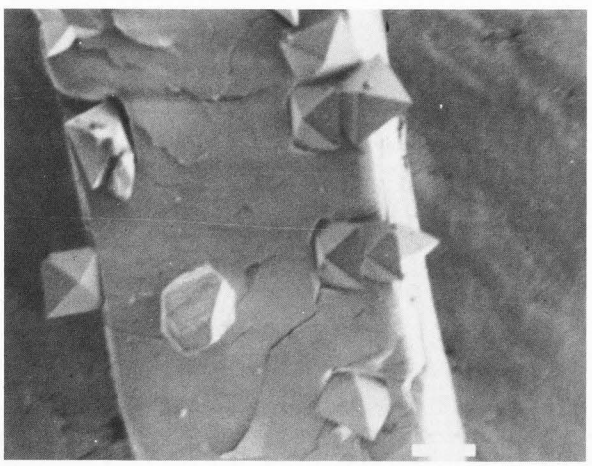

Fig. 14: COT acting as nucleation substrate for COD at room temperature. Bar=10 μm.

Table 1: Summary of results and conditions used to grow hydrated calcium oxalate crystals under controlled release of reacting ions.

MODEL	RELEASE PROFILE	RELEASED SOLUTE	EXTERNAL MEDIUM	CRYSTALS
POLYMERIC MATRIX	$CONC \propto t^{1/2}$	CALCIUM ($CaCl_2$)	$K_2C_2O_4$/NaCl 0.9% 0.145mM	COM
OSMOTIC PUMP	$CONC \propto t$	CALCIUM ($CaCl_2$)	$K_2C_2O_4$/NaCl 0.9% 0.145mM	COT
INTERFACIAL CRYSTALLIZATION	$CONC \propto t$	OXALATE	1.0M $CaCl_2/H_2O$ + 5.0 ml$(C_2H_5)_2C_2O_4$ (20.0 ml)	COD
		OXALATE	0.1M $CaCl_2/H_2O$ + 20.0 ml$(C_2H_5)_2C_2O_4$ (20.0 ml)	COT

remarkable variation in the degree of transition of crystals from the thermodynamically unstable COT and COD to the most stable COM. The ratio and distribution of these crystals in urinary stones would be under the control of these factors. Recently, the circadian course of lithogenic substances in urine has been repeatedly mentioned suggesting that the growth of natural concretions and the laminar structure of stones might be biorhythmically controlled (Berg et al. 1982; Bach et al. 1978). We believe that by the application of experimental or artificial systems that can deliver and control the release rate of both Ca^{++} and $oxalate^{--}$ simultaneously, it might be possible to clarify the role of the circadian course of lithogenic substances in the formation and the structure of calcium oxalate stones.

Conclusion

Using controlled diffusion of calcium and oxalate ions, it was possible to grow COM, COD and COT, suitable for single crystal experiments. Data obtained show that the degree of hydration of crystals formed is controlled by interfacial kinetic factors.

Acknowledgments

The authors are indebted to the laboratory of material testing and characterization. Ecole Polytechnique, Montreal, for the scanning electron microscope and X-ray diffraction and to the MRC of Canada for supporting this study.

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Discussion with Reviewers

G. S. Mandel: For experiment 3 what was the surface oxalate concentration?
Authors: The release kinetics of the oxalate ion at the interface has been determined indirectly by measuring the drop in Ca^{++} as shown in Figure 1 . The reaction between the oxalate and Ca^{++} to build the calcium oxalate crystals appears to be instantaneous and controlled by the rate of hydrolysis of diethyloxalate at the interface. However, the determination of the rate of oxalic acid formation at the interface in the absence of Ca^{++} under different experimental conditions of temperature and pH will be an important factor for future studies of COT formation.

H. T. Horner: Under what additional condition(s) do you think pure crystals of larger sizes could be produced?
Authors: We believe that the purity of the materials used in the growth experiments is extremely important to avoid crystal poisoning by impurities. In addition, maintaining a constant supply of the reacting ions either by increasing the capacity of osmotic pump and matrix, or by better control of the interfacial hydrolysis of diethyloxalate, will eventually form larger crystals.