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M. Akbarieh
University of Montreal

B. Dubuc
University of Montreal

R. Tawashi
University of Montreal

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SURFACE STUDIES OF CALCIUM OXALATE DIHYDRATE SINGLE CRYSTALS DURING DISSOLUTION IN THE PRESENCE OF URINE[1]

M. Akbarieh, B. Dubuc and R. Tawashi*

Faculty of Pharmacy, University of Montreal, P.O. Box 6128, Station A, Montreal (Quebec) H3C 3J7, Canada

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Abstract

Single crystals of Calcium Oxalate Dihydrate(COD) were grown from solution under controlled release of the reacting ions. Dissolution of COD was studied at different pH levels and in different dilutions of urine. The descriptors of the contour were determined during dissolution of COD using a quantitative morphological technique. The shape parameters and surface ruggedness were determined from Fourier and fractal analysis. The results obtained give quantitative information on the dissolution kinetics and the surface geometry of COD crystals in normal and diluted urine. Dissolution inhibition and morphological changes of COD crystals during dissolution were attributed to selective adsorption of urine non-ionic macromolecules on the crystal stepped surface. Surface etching of COD was found to depend on urine dilution and time of incubation. The data obtained suggest that the geometric structure of the surface is likely to be a potential factor in understanding crystal aggregation in stone formation.

KEY WORDS: Hydrated calcium oxalate crystals, Crystal growth, Calcium oxalate dihydrate dissolution, Surface ruggedness, Stone formation.

* Address for correspondence:
R. Tawashi
Faculty of Pharmacy,
University of Montreal,
Montreal, Quebec,
H3C 3J7, Canada.
Phone No: (514) 343-6455

Introduction

In recent years several studies have been reported on the role of different hydrated calcium oxalate crystals (COM, COD and COT) in the formation of calcium oxalate stones (Berg et al., 1979; Nancollas 1983; Nancollas and Gaur 1984; Tawashi 1983; Martin et al., 1984; and Deganello, 1986). Recently Heijnen and in about 30% of renal calculi of which COM is the principal constituent. Their findings demonstrated that COT is not rare but a common constituent of stones (Heijnen et al., 1985). The growth of these crystalline phases and the possible transformation between them is important for the understanding of mechanism of stone genesis. Detailed knowledge of the processes acting on the crystals after precipitation in the urinary tract such as erosion, dissolution, recrystallization and their surface reactions is a prerequisite for solving the problem of stone formation (Ismail and Tawashi 1982; Robertson et al., 1981; Khan et al., 1986).

In a recent paper from our laboratory, we described a method to grow single crystals of COD and COT by controlled interfacial crystallization using slow hydrolysis of diethyloxalate. The phase transformation of COT to COD was found to proceed through dissolution and subsequent surface nucleation of COD (Lachance and Tawashi 1987). The purpose of this work is to study the dissolution behavior of COD single crystal in different dilutions of normal urine. The specific aims are as follows:

1) to examine the effect of natural inhibitors in urine on the dissolution kinetics of COD single crystal.

2) to determine quantitatively the morphological changes of COD crystals during the dissolution process.

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3) to study the effect of the natural inhibitors of normal urine on COD crystal surface morphology.

Material and Methods

COD crystals used in this study were grown under slow diffusion of ions. The growth is based primarily on the slow hydrolysis of diethyloxalate in the presence of bidistilled water containing calcium chloride at pH:6 and at 4°C. The slow liberation of oxalate at the interface, which separates the two insolubles phases, controls the reaction and the growth of COD crystals at the interface. The crystals were harvested after 3-4 weeks and washed with absolute ethanol. The detailed technique of growth and identification has been previously reported (Lachance and Tawashi 1987).

COD single crystals (100-250μm) in dry form were transferred to a specially designed microscopical dissolution cell (Forget et al., 1981) which has been thermostatically controlled at 37°C. The dissolution medium (0.5ml) was added to the dissolution cell and the size and shape parameters of the crystals were determined during dissolution for a period of 2h. Urine used in this study is pooled from 10 normal subjects and filtered by 0.22μm millipore before being used. The pH of the dissolution media has been adjusted by the proper addition of NaOH or HCl to the required pH level.

Size and shape analyses of COD were performed using an image analysis system as previously described (Laurin et al., 1986, Akbarieh & Tawashi 1987). The system uses Fourier descriptors and is based on the digitization of the particle image by obtaining (x,y) coordinates of the particle boundary. Shape features characterizing the particle such as shape spectra and roundness were also determined (Dubuc et al., 1987). The boundary to be analysed was digitized on 512·512 pixel grid using a PIP-512/1024A Video-Digitizer by Matrox Electronic Systems Ltd (Canada), linked to an IBM-AT computer.

The dissolution rate was determined from the change in the boundary movement in the first 2h of dissolution and expressed in $cm \cdot s^{-1}$. This was conducted using a light microscope connected directly to the image analysis system. At least 10 crystals were used to determine each dissolution rate.

The surface geometry or surface ruggedness (roughness) of the dissolving face was studied using the concept of fractal dimension. The fractal dimension of a particle silhouette has been used (Clark 1986; Whalley and Orford 1982) to characterize surface roughness. Fractal analysis of self-similar profile relies on the fact that different step lengths will yield different estimates of the length of an edge. This relation is given by the classical expression (Mandelbrot 1982):

$$L_\lambda = k\,\lambda^{1-D} \qquad (1)$$

in which D is the fractal dimension, λ is the step length and L_λ is the length of the studied profile. The fractal dimension can be then calculated from the slope of the plot of the points $(\log\lambda, \log L_\lambda)$ which normally lie on a straight line. The algorithm used to compute L_λ for a given step length is the HYBRID algorithm (Clark 1986). This algorithm has shown to be computationally efficient and to provide a good approximation of the fractal dimension D. For practical reasons, the fractal dimensional increment H=D-1 [$0.0 \leq H < 1.0$] was used instead of D as a measure of surface ruggedness (Mandelbrot et al., 1984). The closer H is to 1, the more the COD crystal boundary is tortuous, convoluted and rugged.

In these experiments, COD crystal surface exposed to water and diluted urine at 37°C for 2h was subjected to fractal analysis. The SEM images of the crystals (SEM - JEOL: JSM 840) having the same degree of resolution were processed using the previously described image analysis system.

Results and Discussion

Figures 1-3 show an example of COD crystal used in the dissolution study, its morphology, as well as the {100} plane exposed for dissolution. The rate of dissolution in $cm \cdot s^{-1}$ as a function of pH, was determined from the boundary movements of {100} face and is given in Figure 4. The decrease in the dissolution rate is consistent with the decrease in solubility of COD at higher pH levels. At pH:6.3, the addition of normal urine to the dissolution media, reduced the dissolution rate. Dissolution inhibition becomes more prominent at higher concentration of urine (see Figure 5).

In this study the decrease in boundary movement of the {100} plane in diluted urine can be explained by the selective adsorption of the natural inhibitors present in urine - particularly the non-ionic macromolecules (Scurr and Robertson 1986) - on the stepped {100} plane. The dissolution

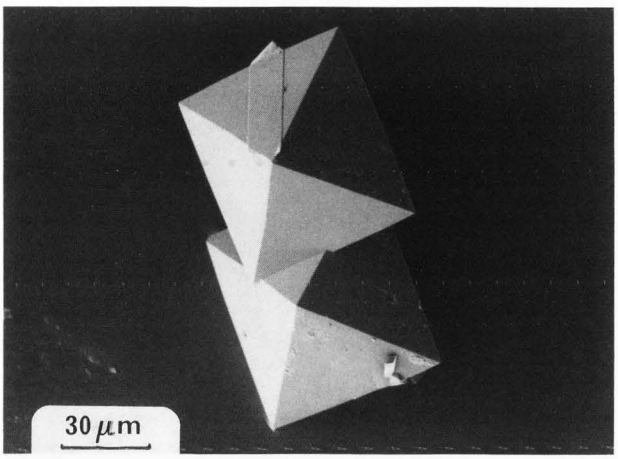


Figure 1. COD single crystals before the dissolution studies.

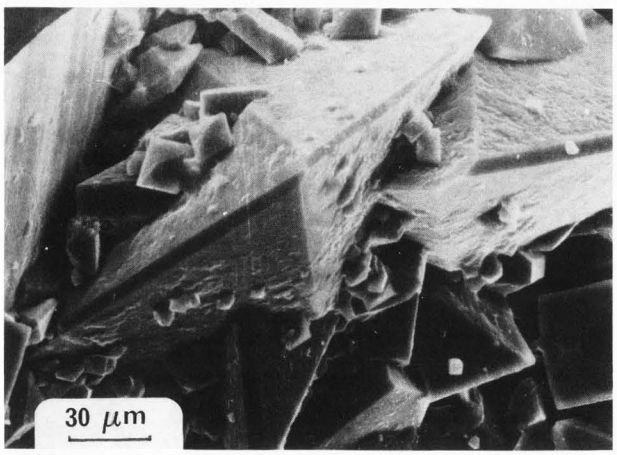
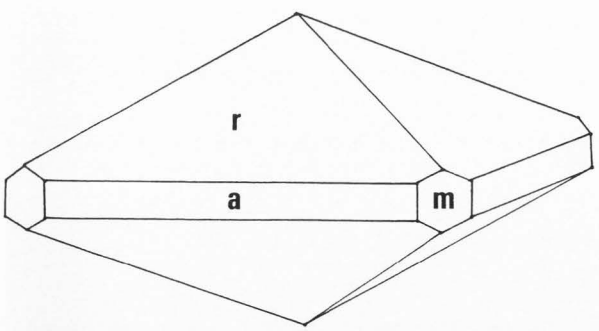


Figure 2. The morphology of COD crystal showing the {100} plane.



a{100}S ; m{110}F ; r{011}F

Figure 3. Diagram of COD crystal showing different faces (Franchini-Angela and Aquilano 1979).

from this face becomes controlled by the equilibrium concentration of the unfilled sites. As the concentration of these natural - inhibitors increases the number of open sites decreases and the rate of step motion decreases. This mechanism operates apparently at lower urine dilutions. At higher urine concentration, the dissolution inhibition becomes largely controlled by the concentration of lithogenic substances in urine namely the Ca^{++} and oxalate ions.

The effect of urine on the morphological characteristics of the dissolving faces is given in Figures 6-8. It is evident from these figures that while dissolution in water did not affect the corners of COD crystal, dissolution in the presence of urine resulted in more rounded crystals. The Fourier morphological analysis of the particle contour gives, quantitatively, the change in particle shape. Figure 9 shows difference in the shape spectra of COD in water and COD in 20% urine after 2h. From the Fourier descriptors at the Harmonic 8-16, we can see the difference in shape. The determination of the degree of roundness from Fourier shape descriptors of the contour shows that while COD crystal has maintained intact edges and sharp corners during dissolution in water, the crystal becomes more rounded in 20% urine. The erosion of the corners in the presence of diluted urine could be attributed to the lower affinity of inhibitor molecules to the corners. Although step motion has been hindered by selective adsorption on the dissolving plane,yet, the dissolution rate remained active on the corner, because of the higher surface energy of the corners and the dissolution process resulted in more rounded corner.

The effect of urine on COD surface structure and crystal micromorphology is another interesting observation. The main characteristic feature is the formation of hill and valley structure on the dissolving surface. Although urine significantly inhibited the rate of dissolution of COD, the study of the surface exposed to different dilution of urine, revealed remarkable etching on {100} plane. Etching appeared when COD crystal was incubated in diluted urine. This etching was practically absent in distilled water. The etching effect deepens by increasing the incubation time and increasing the concentration of urine (see Figures 10-13).

The determination of surface ruggedness using the concept of fractal analysis is given in Figure 14, where the fractal dimensional increment (H)

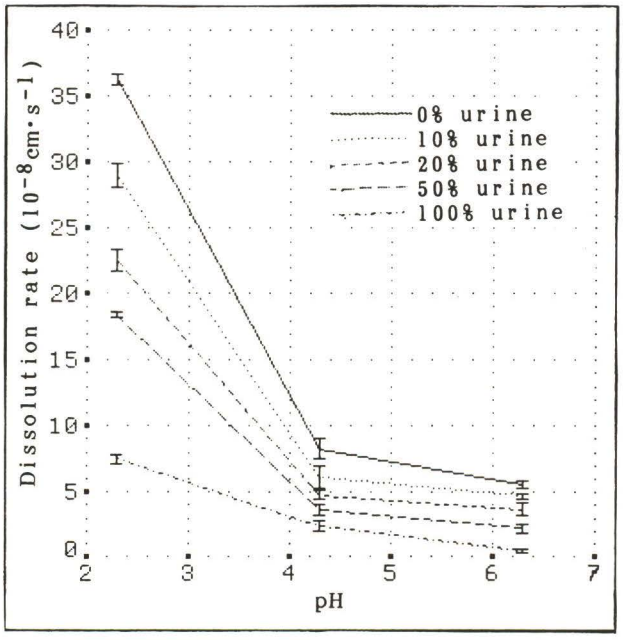


Figure 4. Dissolution rate of COD single crystal as a function of pH during 2h in different urine dilutions. Each point is the average of at least 10 determinations ± standard errors.

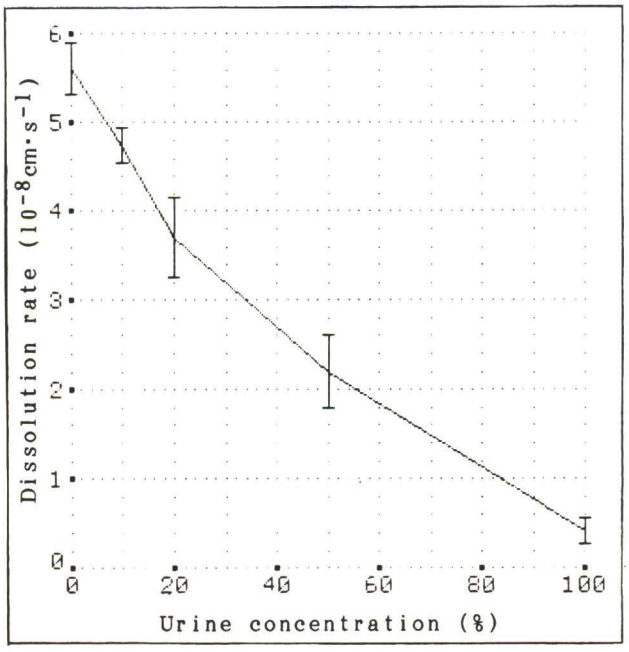


Figure 5. Dissolution rate of COD single crystal at pH:6.3 as a function of urine dilution. Each point is the average of at least 10 determinations ± standard errors.

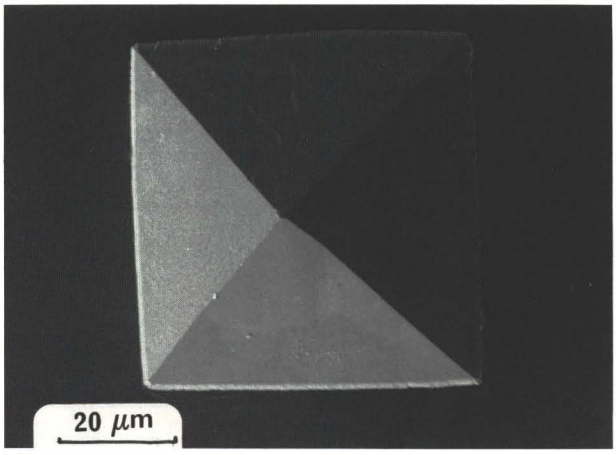


Figure 6. COD single crystal in distilled water after 2h.

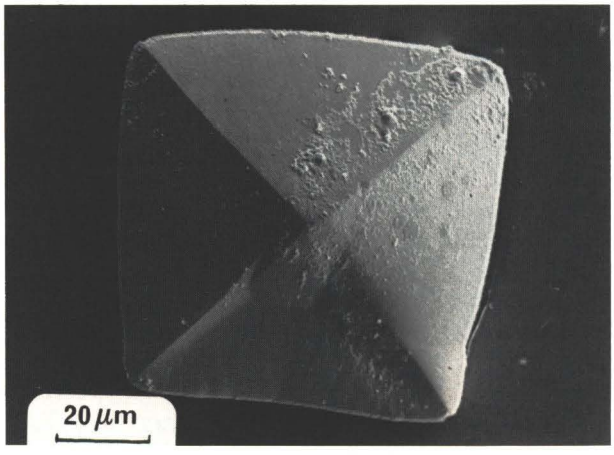


Figure 7. COD single crystal in 100% urine after 2h.

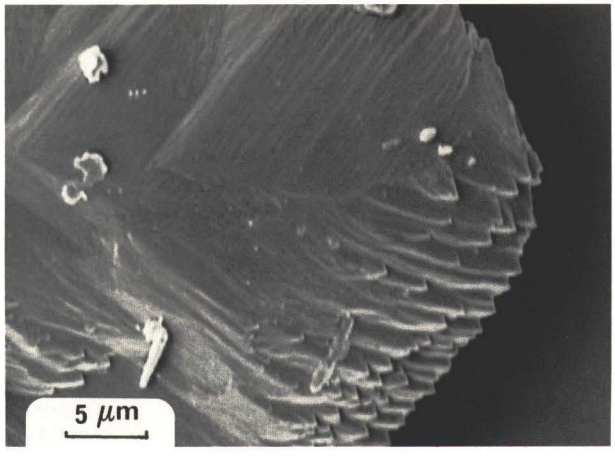


Figure 8. The rounded corners of COD crystal in 20% urine after 2h.

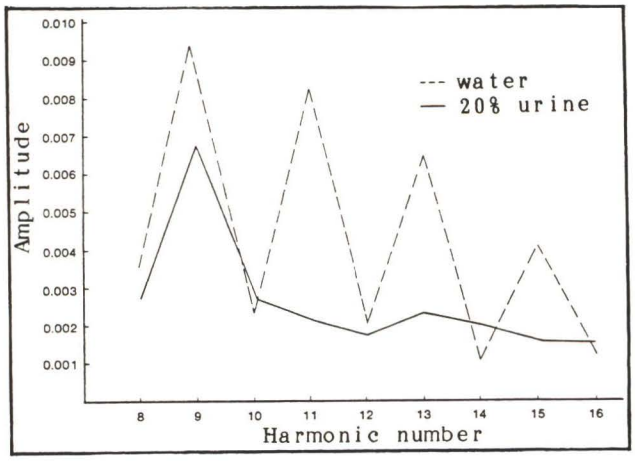


Figure 9. Shape spectra of COD crystal in distilled water and in diluted urine showing Fourier coefficients at harmonic 8-16.

of the etched surface is represented as a function of urine dilution. The results obtained suggest that etching of the surface is dependent on a higher rate of erosion from the unpoisoned steps and on the concentration of natural urine inhibitors.

In view of these findings, it appears that the biological circadian rhythmicity of kidney function suggested by Berg and coworkers (Berg et al., 1982) and the continuous change in concentration of urine in day and night, could be a potentially important factor in modifying the surface ruggedness of precipitated calcium oxalate crystals in the renal tubules. Our point of view is that the crystals like COD or other stone minerals interact with the environment through the

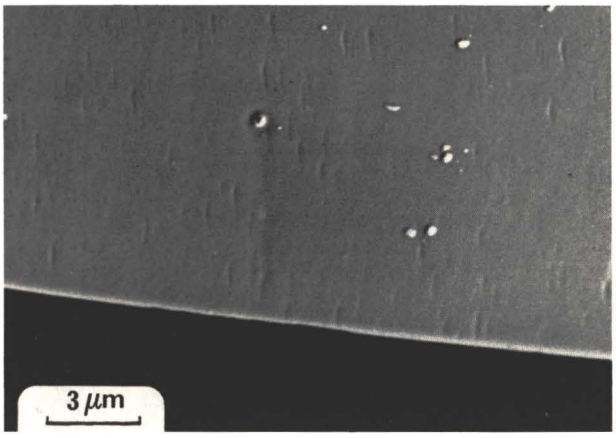


Figure 10. The surface of COD single crystal before dissolution.

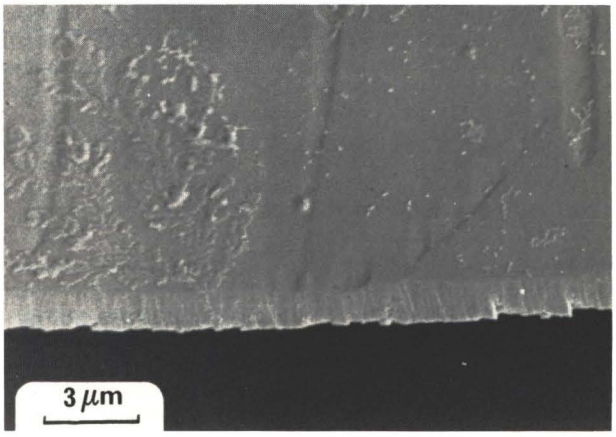


Figure 11. The surface of COD single crystal exposed to distilled water for 2h at 37°C.

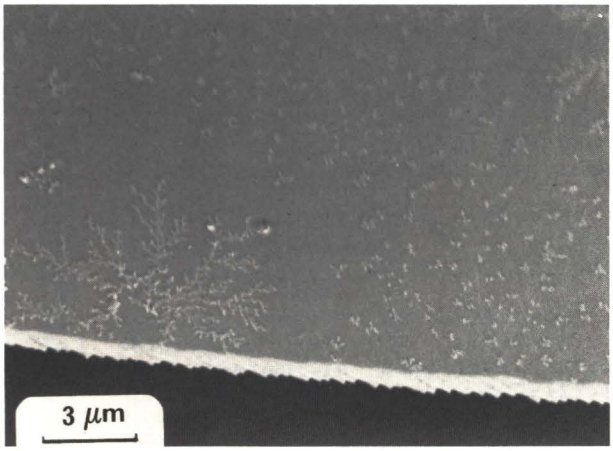


Figure 12. The surface of COD single crystal exposed to 20% urine for 2h at 37°C.

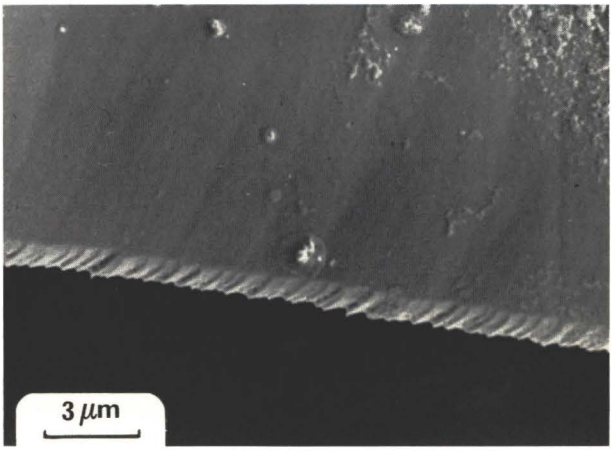


Figure 13. The surface of COD single crystal exposed to 100% urine for 2h at 37°C.

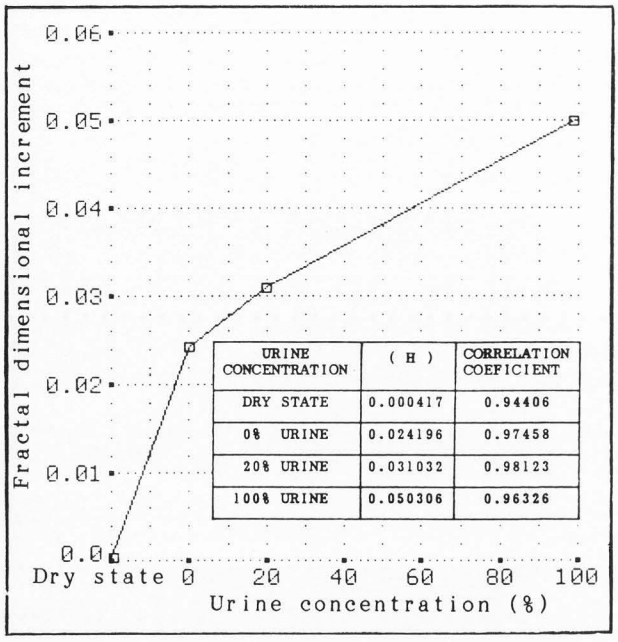


Figure 14.The fractal dimensional increment (surface ruggedness) as a function of urine dilution.

interface. Therefore, surface accessibility of these crystals seems to be of greater relevance to the understanding of stone formation. The change in the geometric structure of the surface influences processes such as adsorption of ions and macromolecules, distribution of the electrostatic forces, adhesion and agglomeration of individual crystallites. Studies are now in progress to measure the effect of the urine of stone formers on the surface geometry of COD and COT single crystals during dissolution.

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Discussion with Reviewers

W.G. Robertson: What do you estimate to be the relative roles of supersaturation and inhibitors concentrations on the rate of dissolution of calcium oxalate crystals in urine?
Authors: Assuming that crystal dissolution is diffusion controlled, the effect of the concentration of inhibitors becomes more important in diluted urine. However, when the concentration of lithogenic ions (Ca^{++} and $Oxalate^{--}$) increases to the saturation level, the degree of saturation becomes more important in controlling the dissolution rate.

K.M. Kim: The applicability of the image analysis to a "kinetic" study needs to be justified.
Authors: Using chemical analysis, the limit of detection in solution is about 1μg (10^{-6}g). On the other hand, the limit of detection in the image analyser, using a light microscope is 1μm. Taking a single crystal of 100μm in diameter, with a density of $2g \cdot cm^{-3}$, dissolution of 1μm from the surface means $6 \cdot 10^{-8}$g. Therefore, the limit of detection will be at least 10 times lower than the chemical limit of detection. Apart from accuracy, the method described in this study reveals detailed information on the nature of surface reactions that cannot be obtained by a chemical analysis.

Y.M. Fazil Marickar: "Addition of normal urine to the dissolution media reduced the dissolution rate" This statement is followed later in the text by the statement "Etching appeared when the COD crystal was incubated in diluted urine". How do the authors explain these contradictory statements?
Authors: We find no contradiction between dissolution inhibition in presence of urine and the etching of the crystal surface. Dissolution inhibition in presence of diluted urine was the result of selective adsorption of the natural inhibitors on the crystal surface. The selected adsorption on the primary dissolution sources resulted in the retardation of the step movement and the reduction of dissolution rate. In the same time, the presence of urine inhibitors resulted in the formation of etch pits on the crystal surface which is independent of boundary movement during dissolution.