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SURFACE STUDIES OF CALCIUM OXALATE DIHYDRATE SINGLE CRYSTALS DURING DISSOLUTION IN THE PRESENCE OF STONE-FORMERS' URINE

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Abstract

Dissolution of calcium oxalate dihydrate (COD) single crystals was studied at different pH levels and in different dilutions of stone formers' (SF) urine. The Fourier descriptors of the contour were determined during dissolution of COD using a quantitative morphological technique. The surface ruggedness of COD single crystals was determined from fractal analysis. The results obtained were compared with COD single crystals behavior in different dilutions of normal urine previously reported. The shape parameters and surface geometry of the dissolving COD crystals were significantly different in normal and SF urine. The data suggest that the shape descriptors and fractal geometry is likely to be a potential factor in identifying the urine of stone formers.

Key words: Hydrated calcium oxalate crystals, crystal growth, calcium oxalate dihydrate dissolution, surface ruggedness, surface transition, stone formation.

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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 (Berenyi et al., 1972; Hesse et al., 1976; Hienzsch et al., 1979; Tomazic and Nancollas, 1979; Berg et al., 1979; Lepage and Tawashi, 1982; Ismail and Tawashi, 1982; Singh et al., 1988). The formation of these phases and the possible transformation between them is important from the urinary calcification standpoint (Hesse et al., 1976; Hienzsch et al., 1979; Tomazic and Nancollas, 1982; Nancollas, 1983; Nancollas and Gaur, 1984; Tawashi 1983; Deganello 1986; Khan et al., 1986). The relation between shape features and surface geometry of the calcium oxalate crystals and the process of their aggregation and cohesion to form stone is still unclear (Martin et al., 1984; Marickar and Koshy, 1987). In a recent work from our laboratory, we reported on the dissolution kinetics and the surface geometry of calcium oxalate dihydrate (COD) single crystal in normal urine. Dissolution inhibition and morphological changes of COD crystals during dissolution were attributed to selective absorption of normal urine non-ionic macromolecules on the crystal stepped surface. 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 (Akbarieh et al., 1987; Singh et al., 1987).

The purpose of this work is to study the dissolution rate of COD single crystal at different pH levels and different dilution of stone formers' (SF) urine, to quantify the morphological and surface changes during the dissolution process, and to establish a quantitative difference in surface reaction between normal and SF urine.

Materials and Methods

In this study we used COD crystals grown under slow liberation of reacting ions. The growth is based primarily on the slow hydrolysis of diethyloxalate (lot: 16F-3418, Sigma, St. Louis MO, USA) in the presence of bidistilled water containing calcium chloride (lot: 733229, Fisher Scientific, Canada) at pH 6 and at 4°C. The slow diffusion of oxalate at the interface, which separates the two insoluble phases, controls the reaction and the growth of COD crystals. The crystals were harvested after 3-4 weeks and washed with absolute ethanol. The detailed technique of growth and identification has been

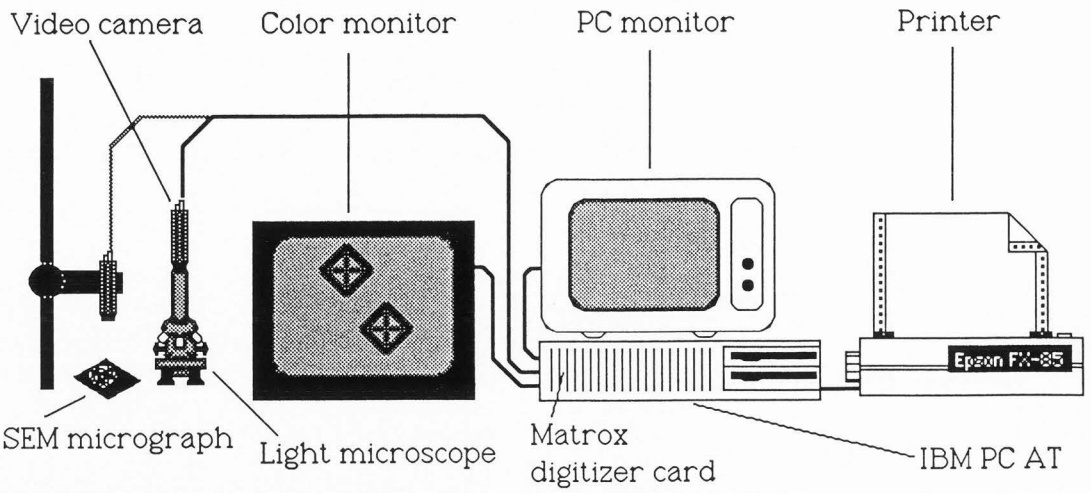


Figure 1. Diagram of the components of the image analysis system used for the digital processing of COD crystals.

previously reported (Lachance and Tawashi 1987).

COD single crystals (100-250 μm) in dry form were transferred individually to a specially designed microscopical dissolution cell (Forget et al,. 1981) which has been thermostatically controlled at 37°C. The dissolution medium (0.5 ml) was added to the dissolution cell and the size and shape parameters of the crystals were determined during dissolution for a period of 2 hours. Urine used in this study is pooled from 12 well characterized recurrent calcium oxalate stone formers. These subjects were under normal diet and no medications for at least two weeks. In all cases, stone-formers or non-stone-formers, first urine of the day was collected and immediately frozen. Before using, each sample was warmed-up at room temperature, and diluted with distilled water for required concentrations. The pH of the dissolution media was adjusted by the proper addition of NaOH or HCl. Urines were filtered by 0.22 millipore before incubation at 37°C.

Size and shape analysis of COD were performed using an image analysis system as previously described (Laurin et al., 1986; Akbarieh & Tawashi, 1987; Akbarieh et al., 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. Using Fourier coefficients, the shape parameters characterizing the dissolving COD crystals in different conditions such as diameter, shape spectra, boundary variation, and roundness were computed. For example, the degree of roundness (P_1) was determined from the normalized Fourier coefficients using the following expression:

$$P_1 = \frac{|\ a_1\ |}{\sum_{n=1}^{P} |\ a_n\ | + |\ a_{-n}\ |} \qquad (1)$$

where $|\ a_n\ | + |\ a_{-n}\ |$ is the normalized amplitude of each coefficient; and $|\ a_1\ |$ is the amplitude of the first Fourier coefficient (which represents the amplitude of a real circle and has the highest value). The shape descriptors obtained have the desired invariant properties (Chen and Shi, 1981). For instance, they are independent of the starting point, rotation, and orientation of the particles. The detailed technique has been previously reported (Dubuc et al., 1987).

Figure 1 is a schematic diagram of the image analysis system used in this study. The boundary to be analyzed was digitized on 512-512 pixel grid using a PIP-512/1024A Video-Digitizer by Matrox Electronic Systems Limited (Montreal, Canada), and linked to an IBM-AT computer.

To determine the dissolution rate we measured the boundary movement in the first 2 hours 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. Fractal analysis of boundary lines is more suitable to characterize the rugged surface of solids since conventional Fourier analysis has difficulty with very jagged or reentered silhouettes (Clark, 1986; Whalley and Orford, 1982). The fractal concept of Mandelbrot (1982) offers the theoretical basis for the understanding of the changes in boundary lengths due to differences in resolution. In fact, the fractal dimension is a measure of the space filling ability of a curve (Clark, 1986). Fractal analysis relies on the fact that the perimeter of the silhouette edge is dependent on the step length with which we measure it. Thus, the smaller the step-length, the larger the perimeter measured. An ideal fractal structure should yield a linear plot, at all resolutions, when $\log L_\lambda$ is plotted against $\log \lambda$, where L_λ is the perimeter and λ is the step-length. The length estimated L_λ tends to increase without limit as the step size λ decreases. The slope of this straight line is **S**, where **S** = 1-D and D is the fractal dimension (see Akbarieh et al., 1987). By this approach, the degree of irregularity is given by D so that $1 \leq D \leq 2$ for lines and $2 \leq D \leq 3$ for surfaces. The more irregular and wiggly an object is, the higher the value of D. Therefore, a particle contour with D = 1.2 will have less rugged and smoother boundary than a particle with D = 1.5.

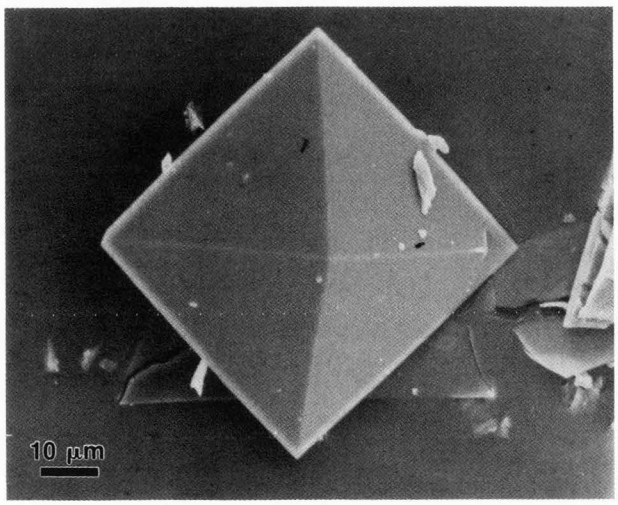


Figure 2. COD single crystal before dissolution.

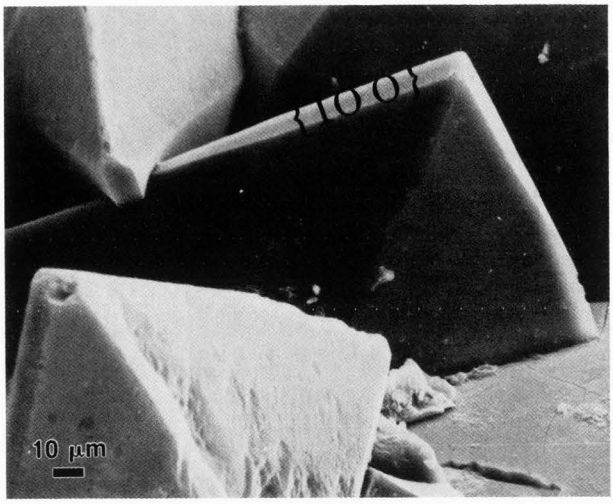


Figure 3. The morphology of COD crystal showing the {100} planes according to Franchini-Angela and Aquilano, 1979.

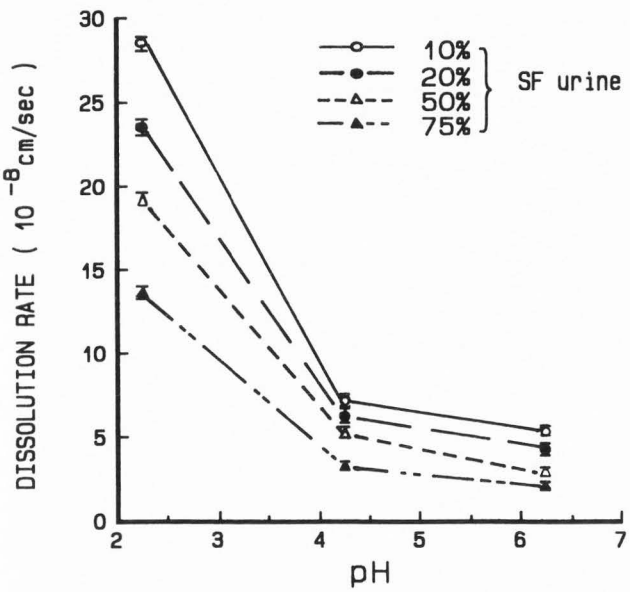


Figure 4. Dissolution rate of COD single crystal as a function of pH during 2 hours in different SF urine dilutions. Each point is the average of at least 10 determinations ± the standard errors.

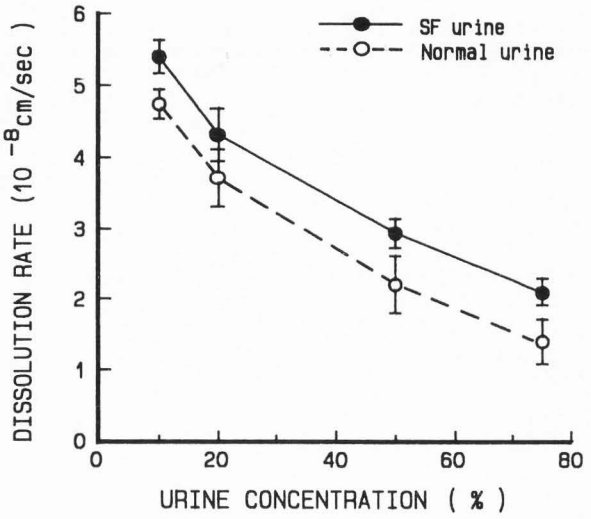


Figure 5. The rate of the boundary movement of COD single crystal at pH = 6.3 as a function of SF urine dilutions. Each point is the average of at least 10 determinations ± the standard errors.

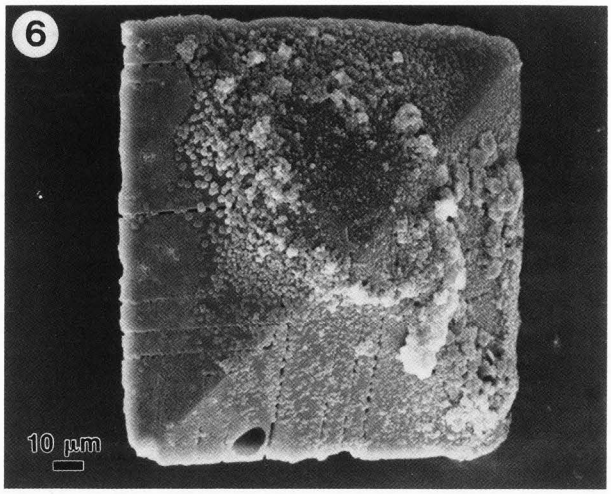
In this work, the algorithm used to compute L_λ for a given step length is the HYBRID algorithm (Clark, 1986). This algorithm has shown to be computationally efficient and to provide a good approximation of the fractal dimension D. For practical reasons, the fractal dimensional increment $H = D - 1$ [$0.0 \leq H \leq 1.0$] 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 different concentrations of SF urine at 37°C for 2 hours was subjected to fractal analysis. The scanning electron microscope (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 2 and 3 show an example of COD crystal used in the dissolution study, its morphology, as well as the {100} planes exposed for dissolution. From the boundary movements of {100} faces, we determined the dissolution rate in $cm \cdot sec^{-1}$ as a function of pH (Figure 4).

In diluted SF urine, the dissolution rate decreases with the increase in pH which is consistent with the decrease in solubility of COD at higher pH levels (Figure 4). At pH = 6.3 the dissolution rate of COD crystals is higher in SF urine than in normal urine (Figure 5). Phase transition of COD



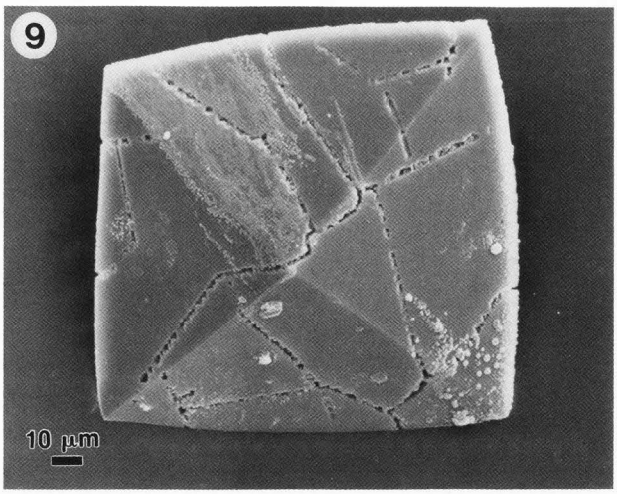


Figure 6. COD single crystal in 100% SF urine after 2 hours.

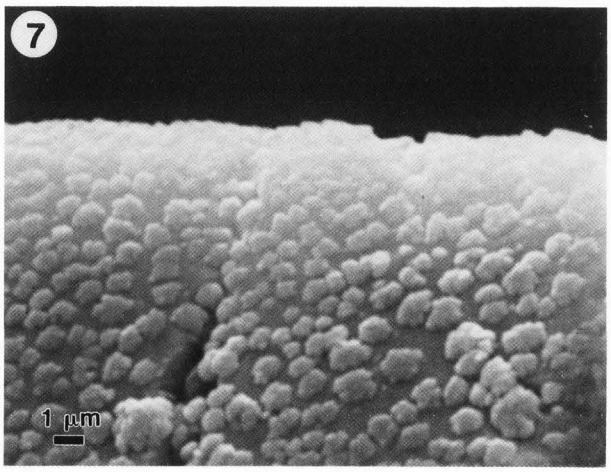
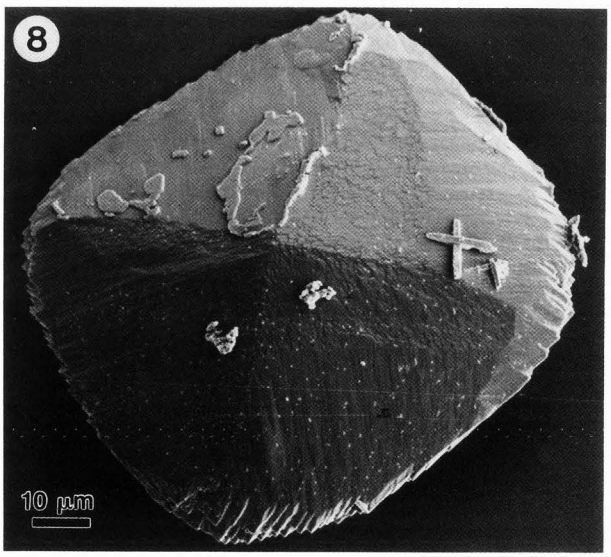


Figure 7. A portion of COD single crystal in 100% SF urine after 2 hours at higher magnification.

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

Figure 9. The intact corners of COD crystal in 20% SF urine after 2 hours



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to calcium oxalate monohydrate (COM) together with diffusion appears to control the mass transfer from the surface. The secondary nucleation of the new phase (COM) on the surface of COD becomes more significant (see Figures 6 and 7) and is accelerated in the presence of low concentration of stabilizing factors (ionic and non-ionic macromolecular inhibitors). The transformation of the thermodynamically unstable COD to the more stable COM was previously reported (Doremus et al., 1976; Tomazic and Nancollas, 1982; Nancollas, 1983; Tawashi, 1983). A recent experiment from our laboratory using SEM and X-ray microanalysis proved the presence of COM on the surface of COD crystal incubated in SF urine, which will be the subject of a future paper.

The effect of the urine of stone formers and normal subjects on the morphological characteristics of the dissolving face is given in Figures 8 and 9). These figures reveal some striking observations. While dissolution in the presence of normal urine produced rounded corners, the urine of stone formers did not affect the corners of COD crystal in the same way. The Fourier morphological analysis of the particle contour measure, quantitatively, the change in particle roundness. Figures 10 and 11 clearly show the differences in the shape spectra and the degree of roundness in 20% SF and in 20% normal urine after 2 hours. 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 analysis of the contour shows that while COD crystal has maintained intact edges and sharp corners during dissolution in SF urine, the crystal becomes more rounded in the urine of normal subject. The erosion of the corners in the presence

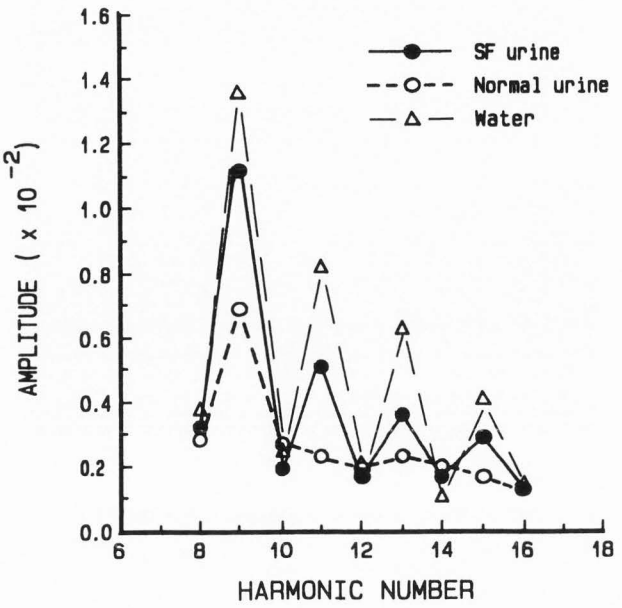


Figure 10. shape spectra of COD crystal in distilled water, 20% normal urine and 20% SF urine, showing Fourier coefficients at harmonic 8-16.

of diluted normal urine could be attributed to the lower affinity of inhibitor molecules to the corners while acting on the dissolving face. On the other hand, in the urine of the stone formers apparently there was less active inhibitors to act on the dissolving surface. Therefore, phase transition and spontaneous surface erosion were active peeling the crystal face layer by layer. Consequently the degree of roundness obtained in SF urine is much lower than in normal urine.

The effect of dilute SF urine on COD crystal surface is given in Figures 12-15. The main characteristic feature observed is the formation of hill and valley structure on the dissolving face. The examination of the face exposed to different dilution of urine, revealed remarkable etching on {100} planes. Such etching was absent on the surface of COD crystals incubated in distilled water but present in normal urine (Akbarieh et al., 1987). The etching effect in the presence of SF urine is more pronounced and it deepens by increasing the incubation time at lower concentration of urine (see Figures 12-15).

In SF urine, the surface rugosity decreased with the increase in the urine concentration (see Figure 16). This decrease could be attributed to the fact that in 10% SF urine, the natural inhibitors or stabilizers are not present in sufficient concentration to stop surface etching. The surface reaction which controlled boundary movement and surface erosion is retarded by the increase of SF urine concentration, i.e., the concentration of natural inhibitors.

We reported earlier that normal urine produced surface ruggedness at all levels of concentration (Akbarieh et al., 1987). The surface ruggedness obtained in this study was concentration dependent. However, when we compare normal urine with SF urine, the change of surface roughness in normal

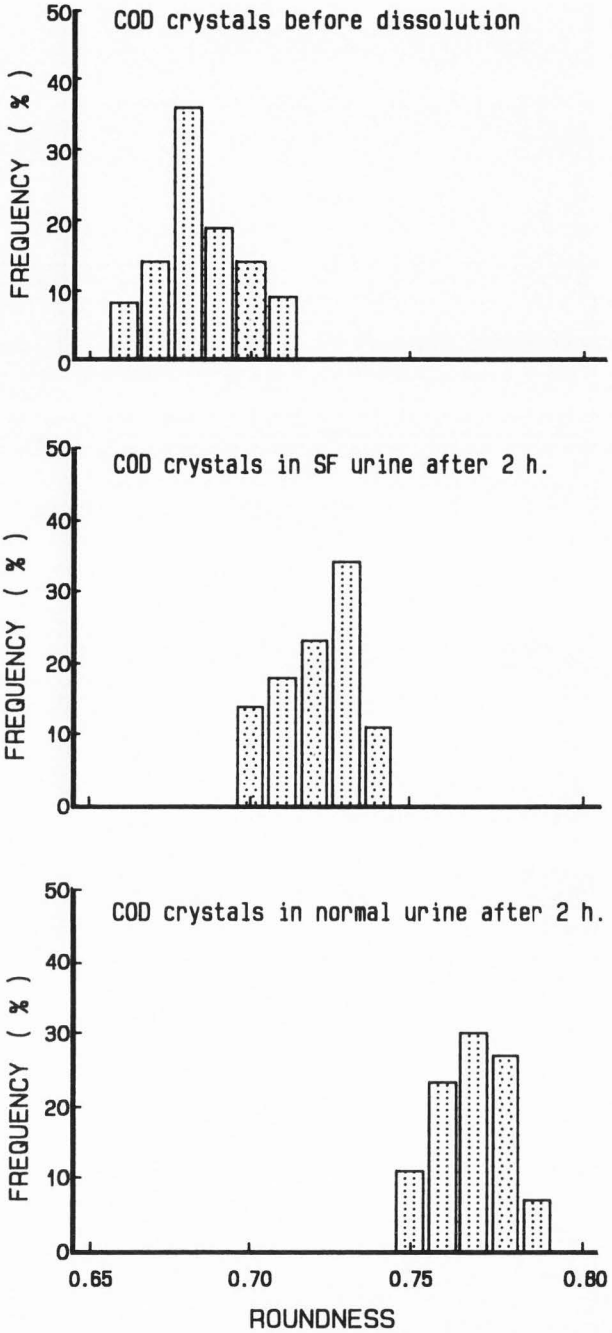
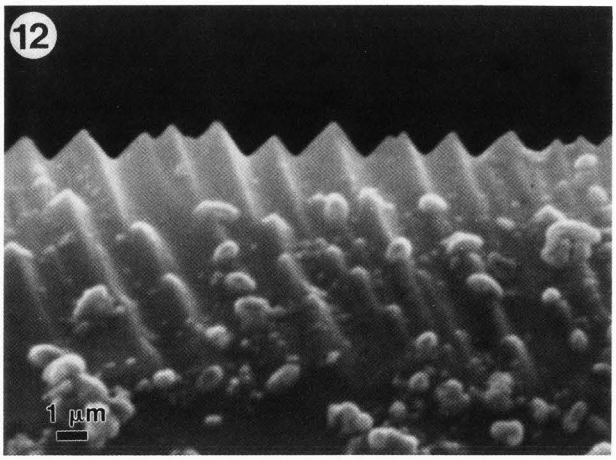
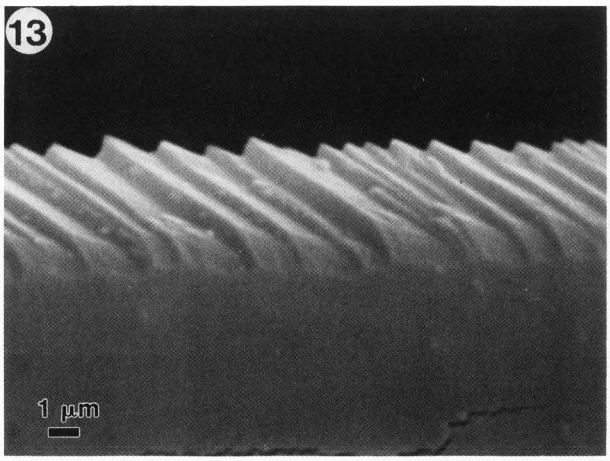


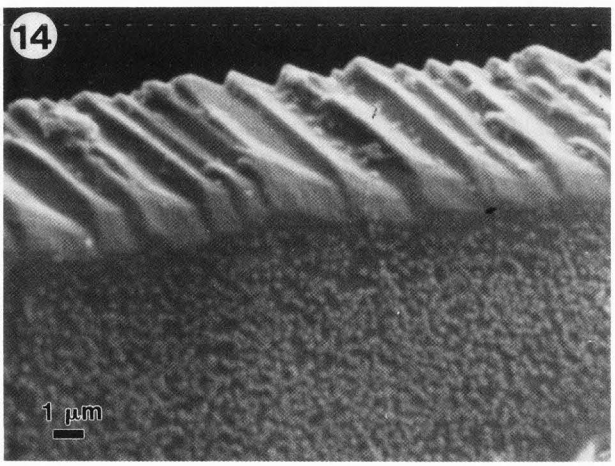
Figure 11. Roundness (P_1) frequency distribution of COD crystal before dissolution and after dissolution in normal and SF urine for 2 hours.

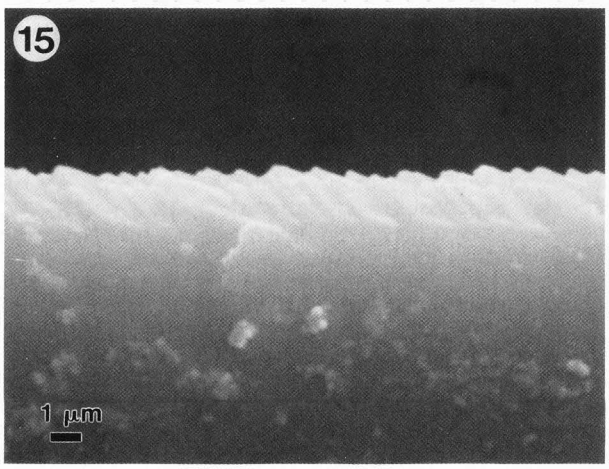
urine appears insignificant (see Figure 16).

In fact, boundary movement of the {100} faces of COD crystals in urine is a complex process. The exact role of inhibitors (stabilizers) on the crystal surface has not been intensively investigated (Marickar and Koshy, 1987). The overall rate is controlled by different operating mechanisms, namely, diffusion, chemisorption, and phase transition. The contribution of each step in the overall reaction is a matter of speculation. In SF urine, the









Figures 12 -15. The surfaces of COD single crystals exposed to:

10% (Figure 12),
20% (Figure 13),
50% (Figure 14), and
75% (Figure 15),

SF urine for 2 hours at 37°C.

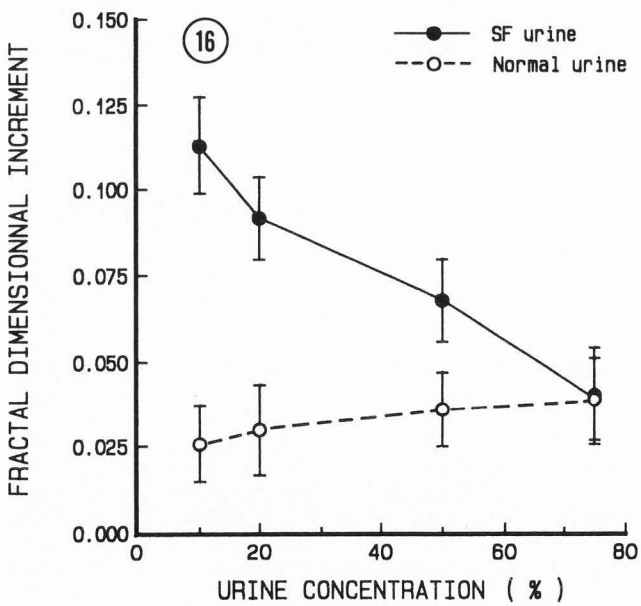


Figure 16. The fractal dimensional increment (surface ruggedness) of COD crystal in normal and SF urine as a function of urine dilution.

absence of natural inhibitors (Robertson et al., 1981; Berg et al., 1979, 1982) might be responsible for the increase in the rate of surface etching and phase transition of COD to COM, leading to higher degree of erosion.

Conclusions

In view of these findings, quantitative differences between normal and SF urine based on surface reaction of COD single crystal with diluted urine could be established. The differences are based on the change in the degree of roundness of COD single crystals determined from Fourier analysis, and the change in surface geometry determined from fractal dimension. In our opinion, single crystal experiments will offer new possibilities for identification of SF urine and for better understanding of stone formation.

Acknowledgements

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Discussion with Reviewers

W.G. Robertson: In view of the fact that most urines are well supersaturated with respect to COM and COD, what effect would the authors expect to see when crystal growth (rather than dissolution) is occurring in the presence of inhibitors?

Authors: The crystals of COM and COD will grow in the supersaturated urine. However, the rate of crystal growth will be dependent on the concentration of lithogenic ions (Ca^{2+} and O^{2-}) and the concentration of inhibitors. We expect that the selective adsorption of inhibitors on a specific crystal phase will lead to habit modification.

W.G. Robertson: Since Tamm-Horsfall mucoprotein appears to compete with the macromolecular inhibitors for binding to calcium oxalate crystals and since its effect is highly dependent on ionic strength and pH, what influence would the authors anticipate it having in their system at different dilutions?

Authors: We believe that the following will probably take place: a) retardation of dissolution of calcium oxalate crystals, b) less etching and erosion on the crystal surface, c) more tendency towards aggregation and adhesion. The experimental study of Tamm-Horsfall mucoprotein in diluted urine on calcium oxalate single crystal is certainly an interesting approach for the better understanding of stone formation.

S.R. Khan: Did you test the levels of Alcian blue positive material, RNA, magnesium, etc., in the urine?

Authors: We determined the concentration of Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+} and we found that only Mg^{2+} concentration was significantly lower in the tested urines. Working with frozen urines, we were not able to determine the levels of RNA or proteins.

S.R. Khan: Am I right in understanding that inhibitors that are responsible for retardation of crystal growth are also responsible for retardation of crystal dissolution?

Authors: Our experience with growth and dissolution of calcium oxalate crystals indicates that crystal growth inhibitors act also as crystal dissolution inhibitors. However, for the same inhibitory effect, dissolution rate retardation requires a higher concentration of inhibitor than in crystal growth.