Induction of Crystallization of Calcium Oxalate Dihydrate in Micellar Solutions of Anionic Surfactants

H. Füredi-Milhofer
The Hebrew University of Jerusalem, helga@vms.huji.ac.il

L. Tunik
The Hebrew University of Jerusalem

N. Filipovic-Vincekovic
The "Ruder Boskovic" Institute

D. Skrtic
The "Ruder Boskovic" Institute

V. Babic-Ivancic
The "Ruder Boskovic" Institute

See next page for additional authors

Induction of Crystallization of Calcium Oxalate Dihydrate in Micellar Solutions of Anionic Surfactants

Authors

H. Füredi-Milhofer, L. Tunik, N. Filipovic-Vincekovic, D. Skrtic, V. Babic-Ivancic, and N. Garti

INDUCTION OF CRYSTALLIZATION OF CALCIUM OXALATE DIHYDRATE IN MICELLAR SOLUTIONS OF ANIONIC SURFACTANTS

H. Füredi-Milhofer[1,2,*], L. Tunik[1], N. Filipovic-Vincekovic[2], D. Skrtic[2], V. Babic-Ivancic[2] and N. Garti[1]

[1]Casali Inst. Appl. Chem., Grad. Sch. Appl. Sci. and Tech., The Hebrew Univ. of Jerusalem, Jerusalem, Israel;
[2]Department of Chemistry, The "Ruder Boskovic" Institute, Zagreb, Croatia

Abstract

Calcium oxalate dihydrate $(CaC_2O_4 \cdot (2+x)H_2O;$ COD; $x \leq 0.5)$ does not readily crystallize from electrolytic solutions but appears as a component in crystalluria. In this paper, we review *in vitro* studies on the factors responsible for its nucleation and growth with special attention given to the role of surfactants. The following surfactants were tested: dodecyl ammonium chloride (cationic), octaethylene mono-hexadecylether (non-ionic), sodium dodecyl sulfate (SDS, anionic), dioctyl sulphosuccinate (AOT, anionic), and sodium cholate (NaC, anionic). The cationic and some of the anionic surfactants (SDS, AOT) induced different habit modifications of growing calcium oxalate crystals by preferential adsorption at different crystal faces. In addition, the anionic surfactants effectively induced crystallization of COD at the expense of COM, the proportion of COD in the precipitates abruptly increasing above a critical surfactant concentration, close to, but not necessarily identical with the respective CMC. A mechanism is proposed, whereby crystallization of COD in the presence of surfactants is a consequence of the inhibition of COM by preferential adsorption of surfactant hemimicelles (two-dimensional surface aggregates) at the surfaces of growing crystals.

Key Words: Urolithiasis, kidney stone, crystalluria, calcium oxalate, surfactants, micelles, crystallization, adsorption, morphology.

*Address for correspondence:
Helga Füredi-Milhofer
Casali Institute of Applied Chemistry,
The Hebrew University,
Givat Ram Campus,
91904 Jerusalem,
Israel

FAX number: 972-2-6528250
E-mail: Helga@vms.huji.ac.il

Introduction

Calcium oxalate hydrates, i.e., the monoclinic monohydrate $(CaC_2O_4 \cdot H_2O;$ COM) and the tetragonal dihydrate $(CaC_2O_4 \cdot (2+x) H_2O;$ $x \leq 0.5;$ COD) are among the main constituents of kidney stone. The triclinic calcium oxalate trihydrate $(CaC_2O_4 \cdot (3-x) H_2O,$ $x \leq 0.5,$ COT) is rarely found in kidney stone (Heijnen *et al.*, 1985) but has been extensively investigated in the laboratory (Gardner, 1975, 1976; Tomazic and Nancollas, 1979; Markovic *et al.*, 1984; Skrtic *et al.*, 1984, 1987; Babic-Ivancic *et al.*, 1985; Cody and Cody, 1994).

Crystallization of COD has been of particular interest because it does not readily crystallize from electrolytic solutions but appears as a component in crystalluria and the average sizes of crystals and/or crystal aggregates are greater than those of COM crystal habits (Elliot and Rabinowitz, 1980; Werness *et al.*, 1981). This paper will review *in vitro* studies which were carried out with the purpose to define the factors responsible for COD nucleation and growth from solutions supersaturated to all three crystal hydrates and to understand the underlying mechanisms. Special attention will be given the influence of surfactants which, because of their unique properties and ready availability in different designs, constitute ideal model additives for the study of interfacial phenomena which are the basis of interactions of stone-mineral with urinary organic molecules. The paper also includes new results on the influence of some anionic surfactants on the morphology and crystal hydrate distribution of calcium oxalates.

Factors Influencing Crystallization of Calcium Oxalate Hydrates

Many authors sought to define the factors responsible for crystallization of COD for preparation purposes because pure dihydrate crystals were needed for crystallographic, crystal growth kinetic, surface and/or other studies. Others were primarily interested in the mechanisms controlling nucleation, growth and aggregation of calcium oxalate hydrates. In the following sections, we

will describe some of the more comprehensive reports available on these subjects.

Crystallization from high ionic strength solutions

When calcium oxalate crystallizes from supersaturated solution, the distribution of crystal hydrates in the precipitate as well as the morphology, number and size distribution of the crystals depend on factors controlling the kinetics of one or several of the main crystallization processes, nucleation, crystal growth, aggregation and phase transformation. Such factors are the supersaturation, reactant concentration ratio, temperature, mode of mixing and stirring, additives, etc. (Füredi-Milhofer *et al.*, 1988, 1990). Babic-Ivancic *et al.* (1985) presented a comprehensive precipitation diagram showing the distribution and morphology of calcium oxalate hydrates as a function of the initial reactant concentrations (conditions: 298K, 0.3 M NaCl, pH = 6.5, unstirred systems). Under the given experimental conditions, typical octahedral bipyramids of COD formed at medium reactant concentrations and were stabilized by an excess of calcium ions. However, if COD crystallized, it always appeared as a minor component in mixtures with COM. Kinetic crystallization studies, carried out in high ionic strength solutions containing a large calcium to oxalate ratio, also demonstrated that regardless of other conditions in solutions without additives, COD always appears as a minor component {up to ~25 weight percent (w%)} in mixtures with COM and/or COT (Brecevic *et al.*, 1989). If calcium oxalate was precipitated in the presence of some amino acids (histidine, tryptophane; Brecevic and Kralj, 1986b), the yield of COD increased by approx. 20 w%.

Crystallization from urine and artificial urine

Many procedures for exclusive COD crystal growth involve the addition of calcium and oxalate solutions to urine and/or artificial urine (for a comprehensive list of relevant references, see, Brown *et al.*, 1989). Systematic studies of the influence of temperature, supersaturation and reactant concentration ratio on the crystallization of COD from artificial urine were carried out by Brown *et al.* (1989). The results were presented in the form of precipitation diagrams from which it appears that COD is favored by low temperature, high calcium to oxalate ratio, and low relative supersaturation.

In the search for likely promoters and stabilizers of COD crystallization from urine supersaturated to all three calcium oxalate hydrates, a number of different additives have been investigated. Brown *et al.* (1989) studied the effect of various concentrations of the components of artificial urine and some other macromolecules and concluded that magnesium ions, citrate, and RNA promote COD crystallization. Using these results, Brown *et al.* (1989) defined a simple solution (containing sodium citrate, magnesium ions, and potassium chloride) which was suitable for routine production of pure COD crystals. Hesse *et al.* (1976) reported that small amounts of divalent cations stabilize the dihydrate, but the formation of mixed crystal phases could not be excluded.

In a kinetic study Gardner and Doremus (1978) crystallized calcium oxalate from synthetic urine at conditions under which COT was the principal growth form. By adding a small amount of normal urine (10 vol%) the authors succeeded to change the composition of the solid phase from 100 w% COT to 100 w% COD. A similar effect was achieved by the addition of high molecular weight fractions isolated from the urine and/or high molecular weight mucopolysaccharides such as heparin. It was suggested that urine contains inhibitors which inhibit crystal growth of calcium oxalate monohydrate and/or trihydrate to the extent that the dihydrate is formed. Effective inhibitors of COT are negatively charged with high molecular weights, high charge density, and sulphonated functional groups as part of the molecule.

Control of crystallization of calcium oxalate phase(s) by synthetic organic molecules

In recent years, several groups presented additional evidence that the hydrate form of calcium oxalate, which precipitates from solution, can be controlled by negatively charged organic molecules (Skrtic and Filipovic-Vincekovic, 1988; Manne *et al.*, 1990; Cody and Cody, 1994). Manne *et al.* (1990) investigated a series of synthetic anionic polymers (carboxylates and sulphonates) and found that the type of the precipitating crystal hydrate depended on the concentration of the polymer. For all investigated polymers, inhibitor concentration at phase appearance followed the order COM < COT < COD. As an example, at low concentrations (up to 1 ppm) of low molecular weight (MW; 5000) polyacrylic acid, COM was the precipitating phase, at medium concentrations (between 1 and 5 ppm), COT crystallized, while at high concentrations (> 50 ppm), COD was exclusively formed. The efficiency of the respective polymer decreased with increasing molecular weight. In addition to the changes in the precipitating crystal hydrate, morphological changes were also observed.

Cody and Cody (1994) tested a large number of mono- di-, tri-, and polycarboxylic acids for their ability to change the crystallizing polymorph of calcium oxalate. While monocarboxylates showed no effect, the efficiency of di-, tri-, and polycarboxylates depended on the structure and concentration of the molecule. As in the work of Manne *et al.* (1990), increasing concentrations of the effective molecules induced phase changes in the order COM < COT < COD. In addition, mor-

phological changes of the precipitating crystals were also observed. Both groups explained their results by preferential adsorption of the additives at the crystal/ solution interface of COM (enabling the appearance of COT) and COT (enabling the appearance of COD), respectively. Cody and Cody (1994) supported their thesis by computer modeling, demonstrating a structural match between effective carboxylates and calcium spacings in the crystal lattices of COM, COT and COD, respectively.

In 1988, some of us (Skrtic and Filipovic-Vincekovic, 1988) reported the observation that micellar solutions of an anionic surfactant, sodium dodecyl sulfate, (SDS) cause almost complete reversal of the precipitating crystal hydrate, COM into COD. This result was significant for two reasons: (a) although it was known that surfactants interact with crystals controlling their size and morphology, this was the first time that control of the crystallizing phase by surfactants has been observed; and (b) as stated in the **Introduction**, surfactants lend themselves as ideal model additives for studies of the complex interactions that occur in pathological mineralization at mineral/matrix interfaces. For these reasons, we embarked on a program of systematic investigations of the possible role of surfactants in the crystallization of calcium oxalates.

In the following sections, we discuss some results of these studies. Since adsorption at the crystal/solution interface seems to be the underlying mechanism to most observed phenomena, a short discourse on the adsorption of surfactants at mineral/solution interfaces has been included.

Adsorption of surfactants at mineral/solution interfaces

Surfactants are amphiphilic molecules, comprising a hydrophilic head and hydrophobic tail(s), which cause them to exhibit unique properties in aqueous solutions and at solid/solution interfaces. At low concentrations, surfactants cumulate at the air/liquid interface causing lowering of the surface tension. An example is the biological surfactant molecules, such as bile salts, which are responsible for the lowering of the surface tension of urine (Mills *et al.*, 1988). When the chemical potential of monomers in solution has risen to an appropriate level, further addition of surfactant molecules causes their aggregation into micelles, while the surface tension at the air/solution interface stays nearly constant. The surfactant concentration at which aggregation commences is called the critical micellar concentration, CMC.

Our present understanding of the adsorption of surfactants at crystal/solution interfaces stems from investigations of their adsorption at silica and mineral oxide surfaces which were carried out with the purpose of better understanding flotation (Scamehorn *et al.*, 1982a, b;
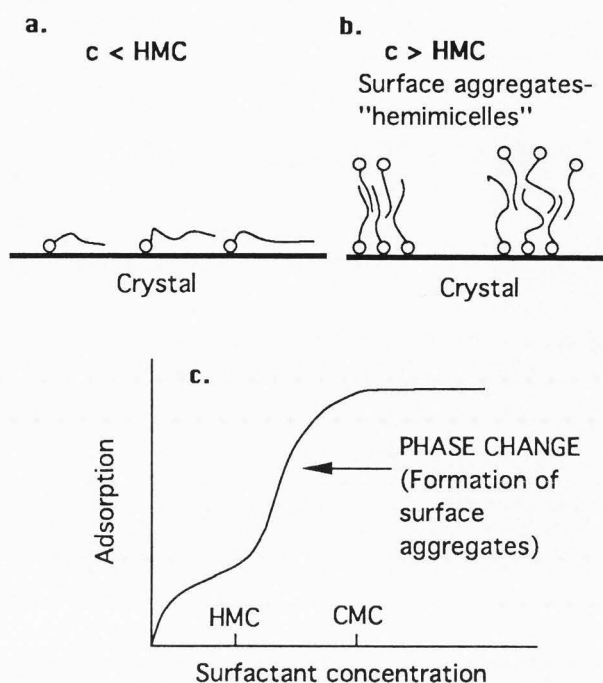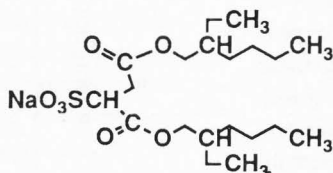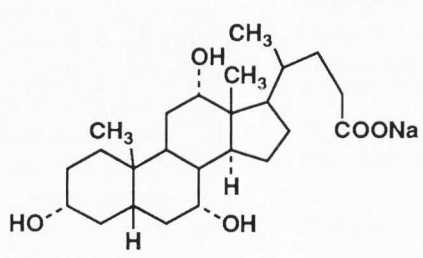

Figure 1. Schematic presentation of the mechanism of adsorption of ionic surfactants at polar crystal surfaces. (a) Adsorption of surfactant molecules at c < HMC; (b) formation of surface aggregates (hemimicelles) at c ≥ HMC; (c) adsorption isotherm showing the two-step adsorption process. CMC: critical micellar concentration; HMC: critical concentration for the formation of hemimicelles. After Scamehorn *et al.* (1982 a,b) and Rupprecht and Gu (1991).

Harwell *et al.*, 1985; Rupprecht and Gu, 1991; Zhu and Gu, 1991). In these systems, adsorption occurs in two steps. In the first step, surfactant molecules are adsorbed through direct interactions (electrostatic and/or van der Waals) with the solid surface (Fig. 1a). In the second step, the amount of surfactant adsorbed increases dramatically because of hydrophobic interactions between adsorbed and dissolved surfactant molecules which cause the formation of two-dimensional surface aggregates, so called, hemimicelles or admicelles (Fig. 1b). Micelles, that have been formed in solution, do not adsorb significantly at the mineral/solution interface (Scamehorn *et al.*, 1982a,b).

A typical adsorption isotherm (Fig. 1c, after Rupprecht and Gu, 1991) reflects these considerations by displaying a region of low and a region of high adsorption with a sharp transition between them. The low adsorption region corresponds to the gradual formation of surfactant monolayers while at higher concentrations, an abrupt transition indicates the formation of two-dimensional surface aggregates. The leveling off of the

Table 1. The effect of surfactants on the crystallization of COD at concentrations > CMC.

Name	Type	Formula	Effect	Reference
dodecyl ammonium chloride	cationic	$CH_3(CH_2)_{11}NH_3Cl$	none	Skrtic *et al.* (1991, 1993a)
octaethylene n mono hexadecyl ether	non-ionic	$C_{16}H_{33}-(CH_2-CH_2O)_8H$	none	Füredi-Milhofer *et al.* (1993, 1994)
Sodium dodecyl sulfate SDS	anionic	$CH_3-(CH_2)_{11}-SO_4Na$	yes	Skrtic *et al.* (1988, 1993a), Füredi-Milhofer *et al.* (1993)
Sodium dioctyl sulphosuccinate, AOT	anionic		yes	Tunik L, Füredi-Milhofer H, Garti N (in preparation)
sodium cholate	anionic		yes	Skrtic *et al.* (1991, 1993a, 1993b, 1994)

isotherm above the CMC has been attributed to the constancy of monomer concentration in this region (Scamehorn *et al.*, 1982a,b). Based on the two-step adsorption model and the mass action treatment, a general adsorption isotherm has been derived and successfully applied to various types of surfactant adsorption systems (Zhu and Gu, 1991).

Crystallization of calcium oxalates in the presence of surfactants

Strategy and experimental design. The investigations discussed in this and the following sections have been designed to understand the mechanism(s) that lead to preferential crystallization of COD in the presence of surfactants. With this question in mind, we investigated the influence of the head-group charge, aggregation state, and the structure of the hydrophobic group of the surfactant on the kinetics of precipitation and the morphology and composition of the precipitating solid phases. One cationic, one non-ionic, and three anionic surfactants with very different hydrophobic entities were chosen as representative (Table 1). Sodium cholate

(NaC) was included as a model for bile salts which are the main surfactants occurring in the urine. All surfactants were used at concentrations below, at, and above the CMC.

To keep the experiments relevant to urolithiasis research, physiological temperature (37 ± 0.1°C) was maintained throughout and precipitation was initiated at pH = 6.5 from high ionic strength solutions (in most cases, 0.3 molar in sodium chloride) which contained an excess of calcium ions and different concentrations of the respective surfactant. Typical initial reactant concentrations in mol dm^{-3} were $c(Ca) = 1 \times 10^{-2}$ and $c(C_2O_4) = 3 \times 10^{-4}$. In precipitates prepared without additives, COM was the dominant phase with less than 2 w% of COD coprecipitated. Electrophoretic mobility measurements of the COM particles indicated a heterogeneous distribution of charge densities (i.e., the presence of both negatively and positively charged patches on the surfaces) with the negative charges prevailing at the crystal/solution interface (Skrtic *et al.*, 1993a).

Data were collected by standard techniques: precipitation kinetics was followed by particle size analysis
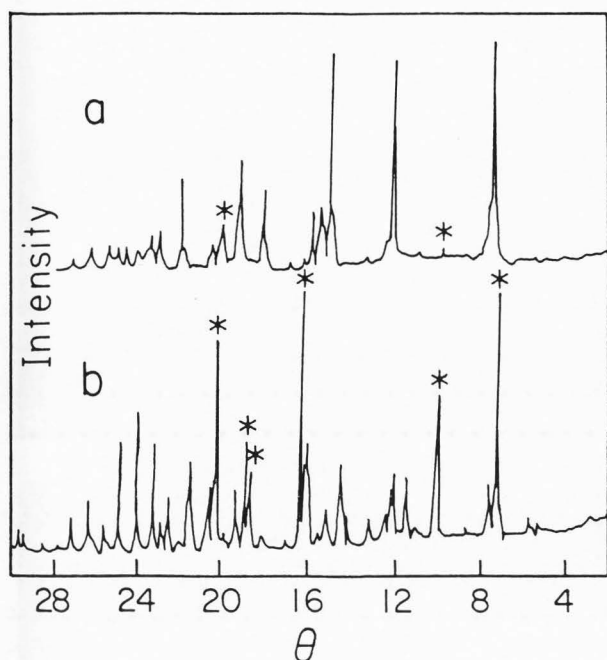
Figure 2. Typical X-ray diffraction powder patterns of calcium oxalate precipitated from supersaturated, high ionic strength solutions: **(a)** pattern characteristic of COM, obtained if calcium oxalate was precipitated without and/or in the presence of non-ionic, cationic and low concentrations (c < CMC) of anionic surfactants; and **(b)** pattern characteristic of intercrystalline mixtures of COM and COD (strong reflections corresponding to COD are marked with asterisks), as obtained at concentrations of anionic surfactants exceeding the CMC.

(Coulter counter), the composition of the precipitates was ascertained qualitatively by X-ray diffraction powder patterns and quantitatively by thermal analysis, and crystal morphology was observed by light and scanning electron microscopy. In a separate set of experiments, adsorption of SDS at the crystal/solution interfaces was determined by conditioning the precipitates with different concentrations of the surfactant and, after filtration, determining surfactant concentration in the supernatant (Skrtic *et al.*, 1993a).

Results

In general, all ionic surfactants inhibited precipitation of calcium oxalate, while in the presence of the non-ionic surfactant, precipitation was accelerated (Füredi-Milhofer *et al.*, 1993, 1994). However, only in the presence of the anionic surfactants at concentrations immediately preceding and/or above the CMC, did COD precipitate in any significant amount (Table 1 and Figs. 2 and 3). Figure 2 shows two typical X-ray diffraction
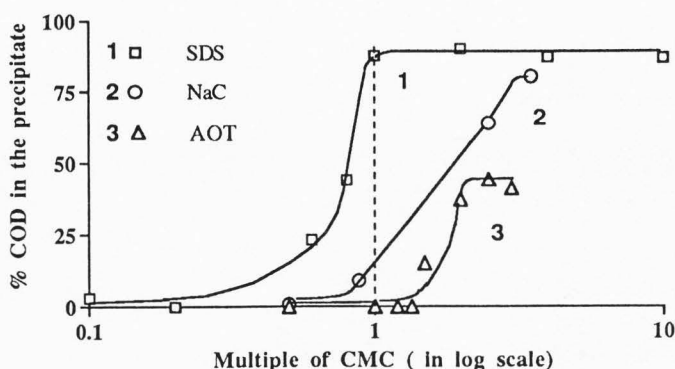

Figure 3. Comparison of the effect of different surfactants on the precipitation of calcium oxalate. The mass fraction of COD (in w%) in the precipitate is shown as a function of the surfactant concentration expressed in multiples of the respective critical micellar concentration. Curve 1: SDS (CMC(SDS) = 5×10^{-4} mol dm^{-3}); curve 2: NaC (CMC(NaC) = 2×10^{-3} mol dm^{-3}); and curve 3: AOT (CMC(AOT) = 1.1×10^{-4} mol dm^{-3}). Data after Füredi-Milhofer *et al.* (1993; curve 1), Skrtic *et al.* (1993b, 1994; curve 2); and L. Tunik, H. Füredi-Milhofer, N. Garti (in preparation; curve 3).

---

powder patterns obtained from calcium oxalate precipitates formed in the presence of surfactants. Pattern a, characteristic of COM, was obtained when calcium oxalate was crystallized in the presence of low concentrations (< CMC) of the anionic surfactants and/or in the presence of cationic and non-ionic surfactants at all concentrations. Pattern b, showing a significant amount of COD admixed with COM, is typical for precipitates formed in the presence of anionic surfactants at concentrations higher than the CMC (Skrtic and Filipovic-Vincekovic, 1988; Skrtic *et al.*, 1993a,b;, 1994; Tunik L, Füredi-Milhofer H and Garti N, in preparation).

In Figure 3, the proportion of COD (w%) precipitated in the presence of different concentrations of anionic surfactants is compared. In order to facilitate comparison, concentrations are expressed in multiples of the respective CMC (actual critical micellar concentrations are given in the figure caption). It is seen that an upsurge of the amount of COD in the precipitate occurred in the presence of all investigated anionic surfactants in the region around the CMC with the effect decreasing in the order of SDS > sodium cholate > AOT.

In Figure 4, the w% COD versus SDS concentration curve (same as curve 1 in Fig. 3) is correlated with the adsorption isotherm showing the amount of SDS adsorbed at the interfaces of crystals formed under the same experimental conditions. Clearly, the upsurge in w% COD corresponds to the inflection on the adsorption isotherm. At c(SDS) > CMC, the leveling off of the
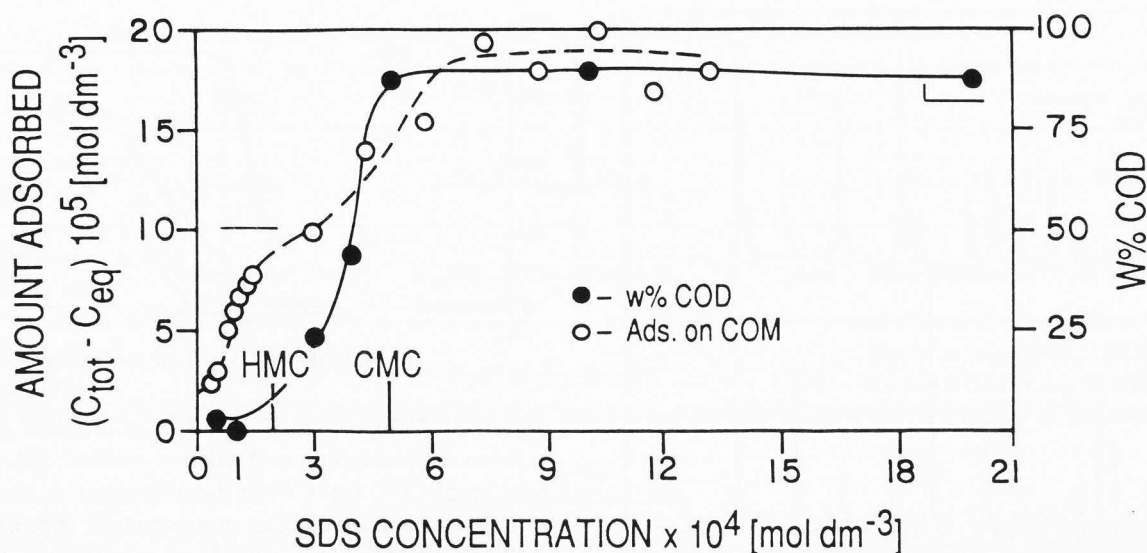
Figure 4. Precipitation of calcium oxalate in the presence of SDS. The mass fraction of COD (w%) in the precipitate as a function of the surfactant concentration (full dots corresponding to curve 1 in Fig. 3, after Füredi-Milhofer *et al.*, 1993). Superimposed is the corresponding adsorption isotherm (empty circles, data after Skrtic *et al.*, 1993a). HMC = 2.5 x 10^{-4} mol dm^{-3}; CMC = 5 x 10^{-4} mol dm^{-3}.

---

adsorption data coincides with that of the data obtained by thermal analysis which show that the COD/COM ratio stabilized at about 85 w% of COD.

The cationic and some of the anionic (SDS and AOT) surfactants induced habit modification of growing calcium oxalate crystals as represented by the scanning electron micrographs shown in Figures 5 and 6 (for molecular structure of the respective surfactants, see Table 1). At low concentrations of the surfactants, the crystal morphology of COM was unaffected (Fig. 5a) but at concentrations above the CMC, the cationic DDACl induced the formation of rombohedral platelets (Fig. 5b; see also, Skrtic *et al.*, 1991), while the anionic AOT induced the formation of elongated, needle-like COM crystals and, in addition, promoted their aggregation into peculiar structures (Fig. 5c). If crystal faces of COM are assigned after Deganello (1991), it follows that DDACl preferentially adsorbs at the {110} and {110} and AOT at the {010} crystal faces. (L. Tunik, H. Füredi-Milhofer and N. Garti, in preparation).

The habit of COD crystals grown in the presence of AOT was generally unaffected (Fig. 6a). When the same crystals were grown in the presence of SDS or high concentrations of AOT (c > 2.5 CMC) they appeared in the form of inverted bipyramids often with a hole in the middle (Fig. 6b), most probably, as a consequence of adsorption of the surfactant at the high energy tip of the bipyramid.

Discussion

From all the evidence discussed in this paper, it appears that organic molecules which effectively induce COD crystallization are negatively charged. They may be polymers with high charge density (Gardner and Doremus, 1978; Manne *et al.*, 1990), anionic surfactants (this paper), and high solution concentrations of di-, tri- or multicarboxylates which structurally match the ionic arrangement at crystal faces of the COM and COT, respectively (Cody and Cody, 1994). Most effective molecules induce, in addition, habit modification of calcium oxalate crystal hydrates. Consequently, it has been assumed (Manne *et al.*, 1990; Cody and Cody, 1994) that COD crystallization is promoted because of preferential adsorption of additive(s) at the crystal/solution interfaces of COM and COT respectively. The only direct evidence has so far been presented by Tomazic and Nancollas (1980) who showed, by example of polyphosphate ions, that the adsorption capacity of calcium oxalate seed crystals decreases in the order COM > COT > COD.

We have shown that anionic surfactants effectively induce COD crystallization from solutions from which, without surfactant, COM forms as the predominant crystal phase (Table 1). The formation of COT has not been observed under our experimental conditions which resemble the conditions in urine inasmuch as precipitates were formed at physiological temperature from solutions of high ionic strength and urinary pH.
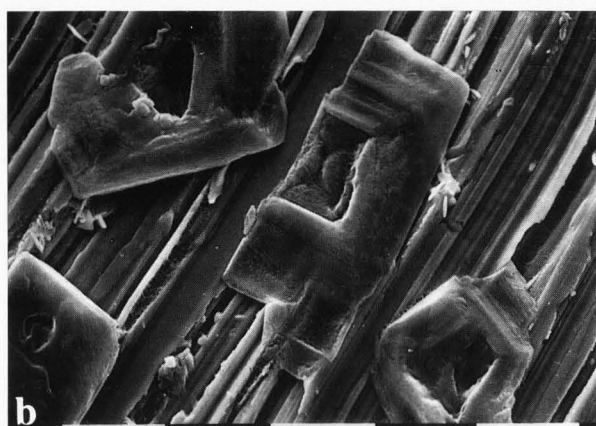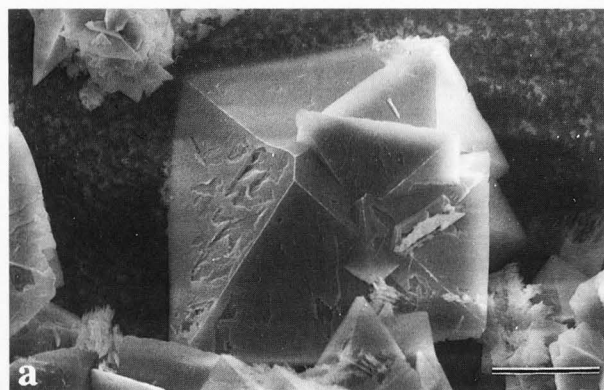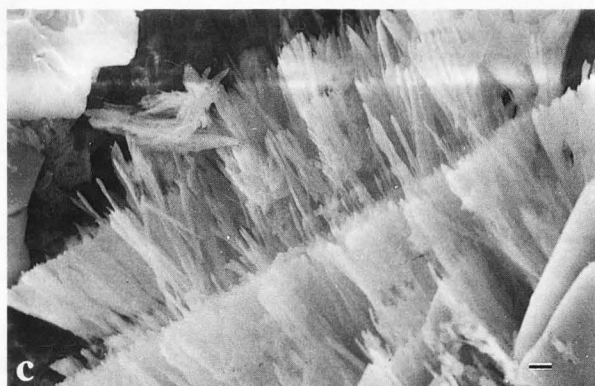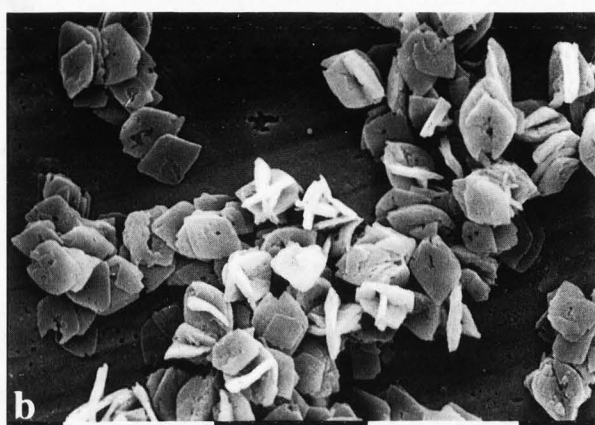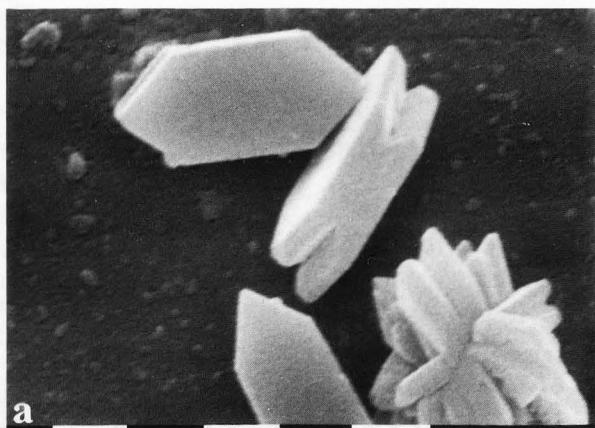
Figure 5 (above). Scanning electron micrographs showing COM crystals grown in the presence of DDACl (**a** and **b**) and AOT (**c**). Surfactant concentrations in mol dm^{-3}: (a) $c_{(DDACl)} = 3 \times 10^{-4}$ (unaffected crystals); (b) $c_{(DDACl)} = 5 \times 10^{-3}$; and (c) $c_{(AOT)} = 1.6 \times 10^{-4}$. CMC$_{(DDACl)} = 1 \times 10^{-3}$ mol dm^{-3} (Skrtic *et al.*, 1991); CMC$_{(AOT)} = 1.1 \times 10^{-4}$ mol dm^{-3}. Bars = 1 μm (in a and c) and 10 μm (in b).

Figure 6 (in column 2). Scanning electron micrographs showing COD crystals grown in the presence of 1.6 x 10^{-4} mol dm^{-3} AOT (**a**), and 1 x 10^{-3} mol dm^{-3} SDS (**b**). Bars = 10 μm.

Another significant result is the observation that the amount of COD precipitated at the expense of COM abruptly increases at a certain critical surfactant concentration which is close to, but not necessarily identical with, the CMC of the respective surfactant (Figs. 3 and 4). The isotherm characterizing the adsorption of SDS on the precipitates (empty dots in Fig. 4) shows that adsorption also proceeds in two consecutive steps, i.e., a region of relatively low and a region of higher adsorption divided by an inflection which coincides with the w% COD versus SDS concentration curve (black dots in Fig. 4). Similarly shaped adsorption isotherms, characterizing the adsorption of surfactants at silica and metal oxide/solution interfaces (Fig. 1c), have been interpreted by assuming the formation of hemimicelles (surface aggregates as shown schematically in Fig. 1b) commencing at a critical surfactant concentration, HMC (Scamehorn *et al.*, 1982a,b; Rupprecht and Gu, 1991; Zhu and Gu, 1991). It seems reasonable to propose a similar mechanism causing the preferred crystallization of COD above a certain critical surfactant concentration.

We thus assume that the upsurge in COD content is a consequence of preferred adsorption, in the form of two-dimensional surface aggregates, of the respective anionic surfactant at the COM/solution interface. Such

Table 2. The influence of micellar concentrations of SDS (2×10^{-3} mol dm^{-3}) and DDACl(1×10^{-3} mol dm^{-3}) on the kinetics of calcium oxalate precipitation (V_t = total precipitated volume).

Time (minutes)	V_t ($\times 10^{-6}$) control	V_t ($\times 10^{-6}$) SDS	V_t ($\times 10^{-6}$) DDACl
20	4.0	2.0	1.0
40	6.5	3.0	1.3
80	8.0	4.0	1.8
120	10.0	5.0	1.8

*Data after Skrtic and Filipovic-Vincekovic (1988) and Skrtic *et al.* (1991).

----

surfactant double layer could sufficiently inhibit growth of COM crystals to allow crystal growth of the less or uninhibited COD to proceed. The strong interaction of anionic surfactants with negatively charged COM particles may be explained by the involvement of calcium ions, which, as counter ions, move in close proximity to the surfactant head group and may serve as anchor at the crystal surfactant interface.

Although no direct comparison between the above *in vitro* results and the *in vivo* situation is possible, the information presented above nevertheless invokes certain important considerations concerning calcium oxalate lithiasis:

We assume that in the urine of healthy persons, the proper balance between the concentrations of various inhibitors exists, and therefore, crystalluria is either non-existent or, if present, consists of small non-aggregated calcium oxalate crystals. If this balance is upset in any way, for instance, by hyper excretion of some anionic inhibitors, such inhibitors may, by selectively inhibiting COM crystallization, effectively promote crystallization of large, inter-grown, and/or aggregated COD crystals which may be trapped in the nephron and serve as nuclei for stone growth. In such cases, the total inhibitory activity of the urine may appear appreciably reduced. This is illustrated in Table 2 (data after Skrtic and Filipovic-Vincekovic, 1988, and Skrtic *et al.*, 1991) in which the total volumes of calcium oxalate precipitated without surfactant and in the presence of SDS and DDACl, respectively, are compared. It is seen that between 20 and 120 minutes, the COD producing anionic surfactant, SDS, reduces the total precipitate volume by a factor of 2. If, however, calcium oxalate was precipitated in the presence of the cationic DDACl (no COD production; Skrtic *et al.*, 1991) the resulting total precipitate volume was about 4 to 6 times lower than in the controls.

In view of the above considerations, it would seem that one must look, not only for a lack of inhibitors in stone forming patients, but for an imbalance of the inhibitors which are beneficial in healthy individuals.

References

Babic-Ivancic V, Füredi-Milhofer H, Purgaric B, Brnicevic N, Despotovic Z (1985) Precipitation of calcium oxalates from high ionic strength solutions. III. The Influence of reactant concentrations on the properties of precipitates. J. Cryst. Growth **71**: 655-663.

Brecevic Lj, Skrtic D, Garside J (1986a) Transformation of calcium oxalate hydrates. J. Cryst. Growth **74**: 399-408.

Brecevic Lj, Kralj D (1986b) The influence of some amino acids on calcium oxalate hydrate transformation. J. Cryst. Growth **79**: 178-184.

Brecevic Lj, Kralj D, Garside J (1989) Factors influencing the distribution of hydrates in calcium oxalate precipitation. J. Cryst. Growth **97**: 460-468.

Brown P, Ackermann D, Finlayson B (1989) Calcium oxalate dihydrate (weddelite) precipitation. J. Cryst. Growth **98**: 285-292.

Cody AM, Cody RD (1994) Calcium oxalate trihydrate phase control by structurally-specific carboxylic acids. J. Cryst. Growth **135**: 235-245.

Deganello S (1991) Interaction between nephrocalcin and calcium oxalate monohydrate: A structural study. Calcif. Tissue Int. **48**: 421-428.

Elliot JS, Rabinowitz IN (1980) Calcium oxalate crystalluria: Crystal size in urine. J. Urol. **123**: 324-327.

Füredi-Milhofer H, Skrtic D, Markovic M, Komunjer Lj (1988) Kinetic analysis of the influence of additives in spontaneous precipitation from electrolytic solutions. Progr. Colloid Polym. Sci. **77**:, 195-200.

Füredi-Milhofer H, Babic-Ivancic V, Brecevic Lj, Filipovic-Vincekovic N, Kralj D, Komunjer Lj, Markovic M, Skrtic D (1990) Factors influencing nucleation from solutions supersaturated to different crystal hydrates. Colloids Surf. **48**: 219-230.

Füredi-Milhofer H, Bloch R, Skrtic D, Filipovic-Vincekovic N, Garti N (1993) Induction of crystallization of specific calcium oxalate hydrates in micellar solutions of surfactants. J. Dispersion Sci. Technol. **14**: 355-371.

Füredi-Milhofer H, Tunik L, Bloch R, Garti N (1994) The influence of surfactants on the crystallization

of calcium oxalate hydrates. Mol. Cryst. Liq. Cryst. **248**: 199-206.

Gardner GL (1975) Nucleation and crystal growth of calcium oxalate trihydrate. J. Cryst. Growth **30**: 158-168.

Gardner GL (1976) Kinetics of the dehydration of calcium oxalate trihydrate crystals in aqueous solution. J. Colloid Interface Sci. **54**: 298-310.

Gardner GL, Doremus RH (1978) Crystal growth inhibitors in human urine. Effect on calcium oxalate kinetics. Invest. Urol. **15**: 478-485.

Harwell JH, Hoskins JC, Schechter RS, Wade WH (1985) Pseudo phase separation model for surfactant adsorption: Isomerically pure surfactants. Langmuir **1** 251-262.

Heijnen WMM, Jellinghaus W, Klee WE (1985) Calcium oxalate trihydrate in urinary calculi. Urol. Res. **13**: 281- 283.

Hesse A, Berg W, Schneider HJ, Hienzsch E, (1976) A contribution to the formation mechanism of calcium oxalate urinary calculi. I Stabilizing urinary constituents in the formation of weddellite. Urol. Res. **4**: 125-128.

Manne JS, Biala N, Smith AD, Gryte CC (1990) The effect of anionic polyelectrolytes on the crystallization of calcium oxalate hydrates. J. Cryst. Growth **100**: 627-634.

Markovic M, Skrtic D, Füredi-Milhofer H (1984) Precipitation of calcium oxalates from high ionic strength solutions II Aggregation of calcium oxalate trihydrate. J. Cryst. Growth **67**: 645-643.

Mills CO, Elias E, Martin GHB, Woo TC, Winder AF (1988) Surface tension properties of Human Urine. Relationship with Bile Salt Concentration. J. Clin. Chem. Clin. Biochem. **26**: 187-194.

Robertson WG, Scurr DS, Bridge CM (1981) Factors influencing the crystallization of calcium oxalate in urine - Critique. J. Cryst. Growth **53**: 182-194.

Rupprecht H, Gu T (1991) Structure of adsorption layers of surfactants at the solid/liquid interface. Colloid Polym. Sci. **269**: 506-522.

Scamehorn JF, Schechter RS, Wade WH (1982a) Adsorption of surfactants on mineral oxide surfaces from aqueous solutions I Isomerically pure anionic surfactants. J. Colloid Interface Sci. **85**: 463-477.

Scamehorn JF, Schechter RS, Wade WH (1982b) Adsorption of surfactants on mineral oxide surfaces from aqueous solutions II Binary mixtures of anionic surfactants. J. Colloid Interface Sci. **85**: 479-501.

Skrtic D, Filipovic-Vincekovic N (1988) Inhibition of calcium oxalate crystallization by sodium dodecyl sulfate. J. Cryst. Growth **88**: 313-320.

Skrtic D, Markovic M, Komunjer Lj, Füredi-Milhofer H (1984) Precipitation of calcium oxalates from high ionic strength solutions. I. Kinetics of spontaneous precipitation of calcium oxalate trihydrate. J. Cryst. Growth **67**: 431-440.

Skrtic D, Füredi-Milhofer H, Markovic M (1987) Precipitation of calcium oxalates from high ionic strength solutions. V. The influence of precipitation conditions and some additives on the nucleating phase. J. Cryst. Growth **80**: 113-120.

Skrtic D, Filipovic-Vincekovic N, Füredi-Milhofer H (1991) Crystallization of calcium oxalate in the presence of dodecylammonium chloride. J. Cryst. Growth **114**: 118-126.

Skrtic D, Filipovic-Vincekovic N, Biscan J (1993a) Comparison of the effects of anionic and cationic surfactants on the precipitation of calcium oxalates. Ber. Bunsenges. Phys. Chem. **97**: 114-120.

Skrtic D, Filipovic-Vincekovic N, Babic-Ivancic V, Tusek-Bozic Lj (1993b) Influence of sodium cholate on the crystallization of calcium oxalate. J. Cryst. Growth **133**: 189-195.

Skrtic D, Filipovic-Vincekovic N, Babic-Ivancic V, Tusek-Bozic Lj, Füredi-Milhofer H (1994) Crystallization of calcium oxalate in molecular and micellar solutions of sodium cholate. Mol. Cryst. Liq. Cryst. **248**: 149-158.

Tomazic BB, Nancollas GH (1979) The kinetics of dissolution of calcium oxalate hydrates. J. Cryst. Growth **46**: 355-361.

Tomazic BB, Nancollas GH (1980) The kinetics of dissolution of calcium oxalate hydrates. II. The dihydrate. Invest. Urol. **18**: 97-101.

Tomazic BB, Nancollas GH (1981) Calcium oxalate hydrates. Dissolution, transformation and crystallization studies. In: Urolithiasis. Clinical and Basic Research. Smith LH, Robertson WG, Finlayson B (eds.). Plenum New York. pp. 453-457.

Werness PG, Bergert JH, Smith LH (1981) Crystalluria. J. Cryst. Growth **53**: 166-181.

Zhu BY, Gu T (1991) Surfactant adsorption at solid/iquid interfaces. Adv. Colloid Interface Sci. **37**: 1-32.

Discussion with Reviewers

A. Hesse: What role has your research revealed for calcium oxalate trihydrate in stone formation? Is there such a thing as primary crystallization of trihydrate with *in vivo* transformation via dihydrate to monohydrate?

Authors: Since urine is supersaturated with respect to all three calcium oxalate hydrates, the phase that will form first is determined by kinetic factors and by the presence of non-constituent ions and molecules (see also, Skrtic *et al.*, 1987; Brecevic *et al.*, 1989). Calcium oxalate trihydrate could thus play a role as precursor to monohydrate in calcium oxalate lithiasis (see also,

Gardner, 1975; Tomazic and Nancollas, 1981) and has indeed been found in several cases as a component in kidney stone (Heijnen *et al.*, 1985). However, the transformation of COT into COM **via COD** is unlikely. It has been shown by several authors (Gardner, 1976; Tomazic and Nancollas, 1981; Brecevic *et al.*, 1986a) that both COT and COD transform directly into COM, transformation of the trihydrate via the dihydrate has not been observed.

A. Hesse: Which surfactants are important in urine and how can they be influenced?

Authors: According to Mills *et al.* (1988), bile salts are probably responsible for most of the surfactant activity of urine. In our studies, sodium cholate was used as a model for these surfactants (Skrtic *et al.*, 1993b, 1994).

A. Hesse: Is crystalluria with COD a more critical signal than COM?

Authors: Unequivocal proof of a direct link between crystalluria and stone formation is not yet available. It has, however, been reported (Elliot and Rabinowitz, 1980), that the mean size of COD crystals and crystal aggregates found in crystalluria is significantly larger than the mean size of COM crystals. This appears also from the scanning electron micrographs of samples of crystalluria published by Werness *et al.* (1981). In addition, COD shows a tendency to form large aggregates or intergrown crystals with COM, uric acid and/or calcium phosphates (Werness *et al.*, 1981). Thus, it would seem that crystalluria consisting of large, aggregated COD and/or mixed crystal aggregates containing the dihydrate could signal an enhanced risk of stone formation.

J.P. Kavanagh: With SDS, the maximum COD was 85%, what are the corresponding values for sodium cholate and AOT?

Authors: 81% for sodium cholate and 38% for AOT (see also, Fig. 3).

J.P. Kavanagh: Have the authors any data on adsorption of the surfactants studied to differently hydrated seed crystals, and do these support their thesis that COD production is favored as a result of preferential inhibition of COM/COT nucleation?

Reviewer III: Did the data shown in Figure 4 come from the same experiment?

Authors: So far, data on the adsorption of surfactants on seed crystals of COM, COD or COT are not available but experiments to that effect are in progress. Our thesis is supported by data on the adsorption of SDS on precipitates formed under the same conditions as in the precipitation experiments (empty dots in Fig. 4) and by data in literature (Tomazic and Nancollas, 1980; Manne *et al.*, 1990, Cody and Cody, 1994).

Reviewer III: Are these COD crystals in Figure 6b?

Authors Indeed, these are COD crystals as follows from X-ray diffraction powder patterns (similar to pattern b in Fig. 2) and thermal analysis data obtained from the corresponding samples (Figs. 3 and 4).

S. Deganello: It is no longer necessary to achieve low temperatures to crystallize COD. Over the last four years, COD has been crystallized reproducibly and routinely from aqueous solutions in the temperature range 18-45°C (i.e., Deganello, Science and Technology for Cultural Heritage, **1**, 1-8, 1992).

Authors: Our aim was not to give an exhaustive list of methods for COD production but to define the factors which favor the crystallization of COD from electrolyte solutions and from urine. That low temperature is one of these factors is apparent from the precipitation diagrams published by Brown *et al.* (1989) described under the heading: **Crystallization from urine and artificial urine**.