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CRYSTALLIZATION OF CALCIUM OXALATE IN MOLECULAR AND MICELLAR SOLUTIONS OF SODIUM CHOLATE

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Abstract The influence of sodium cholate on the crystallization of calcium oxalate from electrolyte solutions with a pH, ionic strength, temperature and concentrations of the constituent ions similar to those in urine has been studied. The additive has been chosen as a model for bile salts, which are considered to be the main factor responsible for lowering the urinary surface tension. Depending on the cholate concentration and consequently on its aggregation state in solution different effects on the overall crystallization process have been observed. Both in its molecular and partially associated state the additive inhibited growth and aggregation of the crystals by adsorption at the crystal/solution interface. In addition, micellar concentrations of the surfactant promoted crystallization of the metastable calcium oxalate dihydrate from solutions from which, without additive, calcium oxalate monohydrate was prevalently formed. The results of kinetic studies point to preferential inhibition of the thermodynamically stable monohydrate as the main reason for this effect.

INTRODUCTION

The research presented in this paper is part of a larger study on various physicochemical factors involved in urolithiasis, e.g. kidney stone formation. This crippling disease involves deposition of mineral within a macromolecular organic matrix consisting mainly of mucoproteins. Calcium oxalates (i.e. thermodynamically stable monohydrate, COM ($CaC_2O_4 \times H_2O$) and the metastable dihydrate, COD (CaC_2O_4 $(2+x\ H_2O)$ where $x \leq 0.5$) are among

the major and least containable mineral components of kidney stone.

It has been observed[1,2] that urolithiasis is related to crystalluria, a condition where crystals are formed from urines supersaturated with respect to the precipitating salts. This condition is not uncommon in healthy individuals, but while healthy people tend to void small, nonaggregated crystals of COM, the most common form voided by recurrent, idiopathic calcium oxalate stone-formers are large, often aggregated crystals of COD[1,2]. Mixed aggregates of COM and COD or COD and hydroxyapatite have also been frequently observed[1]. Such large crystals or crystal aggregates may be retained in the kidney to form a nidus for stone growth.

The physicochemical factors involved in urolithiasis are thus *thermodynamic* - pertaining to changes in urinary supersaturation - and *kinetic* - comprising rates of nucleation, growth, aggregation and phase transformation of crystals[3]. The latter are influenced by the conditions of precipitation, i.e. by changes in urinary flow rate, pH (which may vary between 5.0 and 7.8), ionic strength (approx. 0.3 mol dm^{-3}) and a wide variety of ions, molecules and macromolecules which may, by specific or nonspecific interactions at the crystal/solution interface, inhibit or promote one or more of the mentioned precipitation processes. In order to facilitate understanding of the complicated interfacial processes involved we have embarked on a program to investigate the influence of surface active agents on calcium oxalate crystallization[4-6]. It has been shown that in the presence of micellar concentrations of sodium dodecyl sulphate, an anionic surfactant, the composition of the precipitate changes in favor of the metastable COD, while neither cationic nor nonionic surfactant showed the same effect.

In this investigation sodium cholate (NaC) was used as a model for bile salts, which are the surfactants responsible for lowering the surface tension of urine[7]. It was of

interest to determine its influence on the rates of crystal growth, aggregation and phase transformation of calcium oxalate and from these parameters to estimate the possible role of bile salts in urolithiasis.

EXPERIMENTAL

Solutions were prepared from analytical grade chemicals and triply distilled water. Precipitation of calcium oxalate was initiated by mixing equal volumes of sodium oxalate solutions (6×10^{-4} mol dm^{-3}, adjusted to pH 6.5) with calcium chloride solutions (2×10^{-2} mol dm^{-3}) to which known concentrations of NaC solutions (1×10^{-3} - 7×10^{-3} mol dm^{-3}) were added. All solutions were 0.3 molar in sodium chloride. All experiments were conducted at 310 K in batch crystallizers. Precipitation kinetics were followed by particle number and size analysis (Coulter counter Mo TA fitted with a 140 μm orifice tube). Four hours after sample preparation precipitates were separated from the mother liquid and characterized by X-ray diffraction powder patterns (XRD, Philips X-ray diffractometer), thermogravimetric analysis (Cahn Rg electromicroanalytical balance) and IR spectroscopy (Perkin Elmer Mo 580 B).

RESULTS AND DISCUSSION

In aqueous or electrolyte solution sodium cholate exists in monomeric, or monomeric and micellar form. The molecule exhibits a three-step association pattern resulting in discontinuities in the pH vs NaC concentration curves[8,9]. Under our experimental conditions the first and second discontinuities (corresponding to the critical micellar concentrations CMC_1 and CMC_2) have been observed at c(NaC) = 2×10^{-3} and 1.5×10^{-2} mol dm^{-3}, respectively. At c(NaC) > 2×10^{-2} mol dm^{-3} significant association of calcium ions

with the micellar system also occured[9].

The results presented in this paper pertain to solutions in which NaC behaved as 1:1 electrolyte (below the CMC_1) or was partially associated in the form of dimers to tetramers (between the CMC_1 and the CMC_2, see ref. 8) and the association of the micelles with calcium ions was not significant[9].

Precipitates of calcium oxalate obtained under the same experimental conditions without additive consisted primarily of the thermodynamically stable monoclinic monohydrate, COM, with less than 1w% of the metastable polymorph, the tetragonal dihydrate, COD, admixed[4,10].

Keeping in mind the changes in association properties of NaC solutions the following concentrations of the additive were chosen for the experiments: below the CMC_1 (system 1: 1×10^{-3} mol dm^{-3}), at the CMC_1 (system 2: 2×10^{-3} mol dm^{-3}), and between CMC_1 and CMC_2 (system 3: 5×10^{-3} mol dm^{-3} and system 4: 7×10^{-3} mol dm^{-3}). The results of TGA and RTG of precipitates prepared in the presence of different concentrations of sodium cholate are given in Table 1 and Figure 1.

TABLE I Thermogravimetric analysis results

NaC concentration C /mol dm^{-3}	Precipitate composition COM (%)	COD (%)
0	98.2	1.8
1×10^{-3}	99.2	0.8
2×10^{-3}	90.9	9.1
5×10^{-3}	36.3	63.7
7×10^{-3}	19.8	80.2

It is shown that while in the controls and in the systems 1 and 2 COM was the prevailing solid phase, precipitates formed in systems 3 and 4 consisted of

significant amounts of COD which crystallized on account of COM. The relation of these phase changes to the CMC indicates that they were induced by molecular associates rather than single molecules of sodium cholate.

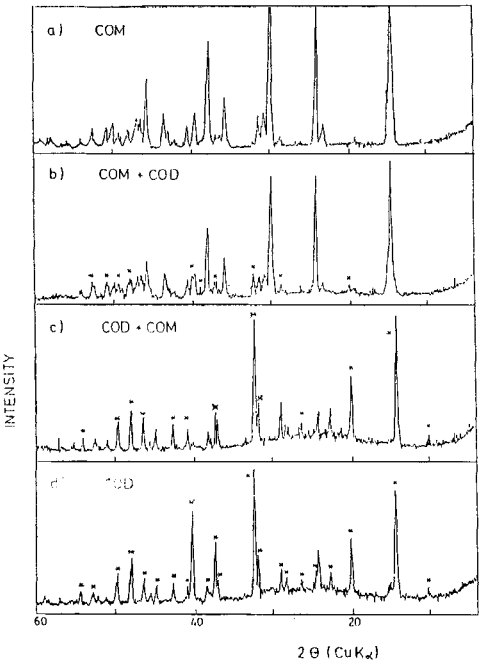

FIGURE 1 X-ray diffraction powder patterns of calcium oxalate formed in the presence of different NaC concentrations (in mol dm^{-3}): a) $1_3 \times 10^{-3}$, b) 2×10^{-3}, c) 5×10^{-3}, d) 7×10^{-3}. Peaks characteristic of COD are marked with asterisks. Precipitate were aged for four hours.

IR spectra of system 4 show primarily ionic interactions of cholate ions with the calcium oxalate crystals (Figure 2a, interpretation of absorption bands in accordance with ref. 11). Curve 2 in figure 2a shows significant changes in the spectrum of cholate associated with calcium oxalate crystals as compared to the spectrum of pure sodium cholate (curve 3). The changes are particularly evident in the 1800 - 1250 cm^{-1} region where stretching vibrations of the cholate and oxalate carboxylate groups occur. Sodium cholate

(curve 3) has a strong band at 1568 cm^{-1} associated with the antisymetric C-O vibration as well as a more complex band with four maxima (at 1467, 1449, 1408 and 1337 cm^{-1}) of medium to strong intensities for the symmetric vibrations of cholate ions could be detected. The multiple band is replaced by a medium band at 1324 cm^{-1} with two shoulders at 1372 and 1282 cm^{-1} and is overlapped with the corresponding symmetric C-O stretching vibration of the oxalate group. In

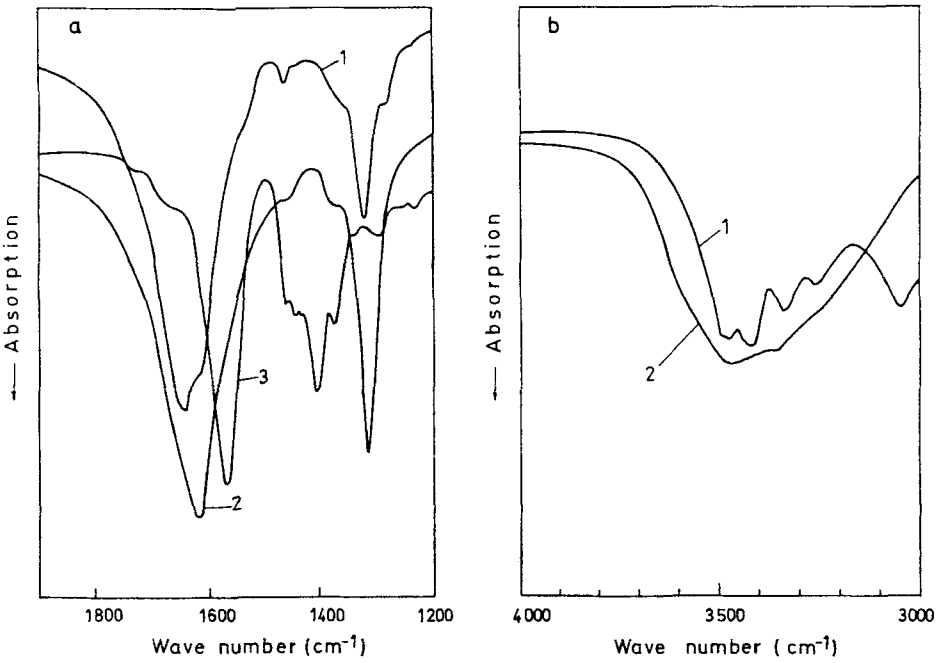

FIGURE 2 Comparison of IR spectra of the control system (COM, curves 1), crystals formed in system 4 ($c(NaC) = 7 \times 10^{-3}$ mol dm^{-3}, curves 2) and sodium cholate crystals (curve 3).

addition the antisymmetric stretching band is shifted to higher frequencies and by overlapping with the corresponding C-O stretching vibration of the oxalate groups a very strong and broad band appears at 1645 cm^{-1} with a shoulder at 1630 cm^{-1}. Absorption of cholate causes relatively small changes in the respective spectrum of COM (curve 1 in Figure 2) as evidenced by relatively small band shifts of both the

antisymmetric and symmetric C-O strecthing vibrations at 1621 and 1317 cm^{-1}, respectively[12]. In the υ (OH) region (Figure 2b) the spectrum of system 4 exhibits a broad band with shoulders of medium intensity between 3700 and 3050 cm^{-1} (curve 2) which replaces several sharper peaks characteristic of pure COM crystals (curve 1). This change is in accordance with preferential COD formation as well as with the assumption that the hydroxyl groups from cholate and water molecules are hydrogen bonded. The broadening of all the absorption bands of different vibrations of the O-C-O groups in the 800 -200 cm^{-1} region consequently could be ascribed to the increasing number of hydrogen bonds involving these groups.

In Figure 3 typical particle number, N_t, (a) and total precipitate volume, V_t, (b) vs time curves, showing the influence of molecular and micellar (between the CMC_1 and the CMC_2) concentrations of sodium cholate on the kinetics of calcium oxalate precipitation are represented. It is seen (curves 1) that molecular solutions of NaC inhibit both crystal growth and aggregation of calcium oxalate. Inhibition of aggregation is apparent from the respective N_t/t curve which is nearly parallel with the abscissa in contrast to the corresponding curve in the control system (curve 0) which exhibits a maximum and subsequent decrease in the number of particles (the decrease in N_t is due to aggregation). V_t/t curves representing calcium oxalate precipitation in the presence of micellar solutions of NaC (represented by curve 2 in Figure 3b) show discontinuities at about 60 min indicating a significant change in the precipitation kinetics (for a detailed analysis see ref. 9). Further kinetic analysis according to a method previously developed in our laboratory[13,14] gives linear rate vs supersaturation plots which show a significant change in the slope p corresponding to the time where discontinuities in the V_t/t curves appear (i.e. from $p = 15.4$ for $t < 60$ min to $p \approx 3.8$ (average from 3 systems) for $t > 60$ min as compared to $p_o = 3.5$ for the control system[9]). As previously

shown [14], such changes in slope indicate corresponding changes in the average crystal growth rate with higher p values corresponding to lower growth rates. It seems reasonable to attribute this change in precipitation kinetics to two successive, time resolved processes, i.e. we envisage strong inhibition of the growth of COM crystals in the first 50 - 60 min followed by almost uninhibited growth of the second phase, COD. Further experiments to prove this hypothesis are in preparation.

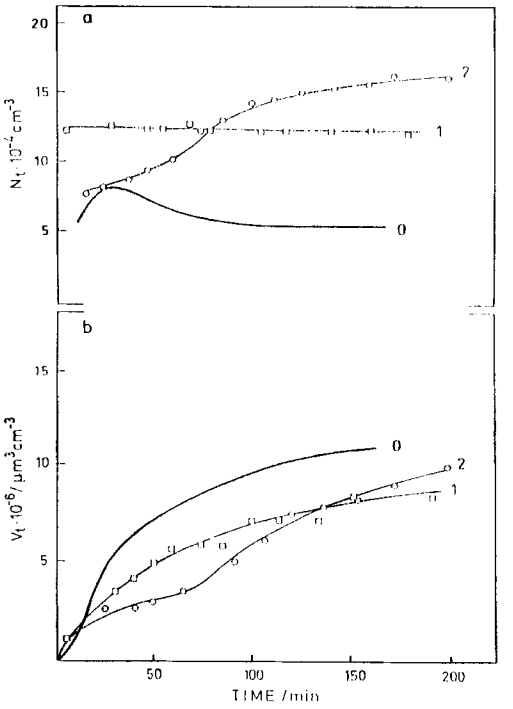

FIGURE 3 Kinetic curves showing changes in the number of particles (a) and total precipitate volume (b) with time for calcium oxalate formed in the presence of different concentrations (in mol dm^{-3}) of sodium cholate: 0 - control system (curves 0), 1 x 10^{-3} (curves 1), 5x 10^{-3} (curves 2).

The above experiments have no direct bearing on the situation in vivo. They facilitate, however our

understanding of the interactions at the crystal / surfactant / solution interface and thus contribute some ideas on the possible role of bile salts in the control or lack of control of stone diseases. In normal urines (bile salt concentration between 10^{-6} and 10^{-4} mol dm^{-3})[7] bile salts are most probably in the form of molecular dispersions and may thus have a beneficial effect as inhibitors of crystal aggregation (curve 1 in Figure 3a, see also ref. 9). On the other hand micellar solutions of bile salts may act as crystallization modifiers in gall-stone deposition. We are however lacking systematic information on the excretion of bile salts under pathological conditions (liver disease, urolithiasis) and also their association behavior in a complex environment such as human urine. It is therefore impossible to predict whether or not these compounds could contribute to the observed phase changes which seem to be characteristic of cystalluria in recurrent calcium oxalate stone-formers[1,2].

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