

# Factors Influencing the Formation of Calcium Oxalate Hydrates *in vitro*\*

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Precipitation from supersaturated solutions is in general affected by the physical chemical properties of the solution, by the hydrodynamics of the system, i.e., by the processes of mixing reactants and stirring the system, as well as by soluble impurities present in the system. Therefore, such a knowledge should be considered in a number of areas where precipitation plays an important role. Since calcium oxalates have been found to be a major crystal constituent of urinary stones, their investigations, as well as investigations of physical chemical conditions relevant for their formation are of particular importance for fundamental research in the field of pathological mineralization. Calcium oxalate forms three hydrates. These three hydrates are the thermodynamically stable monohydrate (COM) and the metastable modifications, dihydrate (COD) and trihydrate (COT). The results on investigations of the influence of a number of thermodynamic and kinetic factors, as well as the potential inhibitors, on the hydrate formation and transformation under different experimental conditions had been obtained and published by the present authors during 1980<sup>th</sup>.

**Key words:** Calcium oxalate monohydrate; Calcium oxalate dihydrate; Calcium oxalate trihydrate; Amino acids; Chemical precipitation; Transformation

## INTRODUCTION

It has been generally accepted that mild hyperoxaluria is a major factor in the formation of idiopathic kidney stones and that calcium oxalate ( $\text{CaC}_2\text{O}_4$ ,  $\text{CaOx}$ ) is the most frequent constituent of urinary calculi; about 70 % of all kidney stones contain  $\text{CaOx}$ . It has also been known that urine is supersaturated with respect to  $\text{CaOx}$ , especially the urine from the recurrent stone formers. Because of its importance in urinary stone formation, the interest in calcium oxalate metabolism and precipitation conditions has increased in the course of time.

Calcium oxalate forms three hydrates: two metastable modifications, trihydrate (COT) and dihydrate (COD), and a thermodynamically stable one, monohydrate (COM). Although COT can exist as a component in urine, it has never been found in the urinary stone composition. Only COD and COM have been reported to be crystal constituents of urinary stones.

By the time we started to investigate  $\text{CaOx}$  precipitation, a number of batch precipitation (1,2) and the growth kinetic

studies on seed crystals (3,4), as well as dehydration of the higher hydrates (5,6), had been undertaken. As urine naturally contains components the role of which is to prevent calculi formation, the so-called inhibitors, the inhibitory effect of several types of inhibitory compounds had also attracted considerable attention (1,7).

In order to get better understanding of the mechanisms of stone formation and also to study problems related to fundamental precipitation processes of hydrated solid phases,  $\text{CaOx}$  provided a convenient model system. Herein we report our own contribution to the study of calcium oxalate hydrates. Results reported below had been obtained and published during 1980<sup>th</sup> and demonstrate the influence of a number of thermodynamic and kinetic factors, as well as the potential inhibitors, on the hydrate formation and transformation under different experimental conditions.

## FORMATION OF CALCIUM OXALATE HYDRATES

In the systems in which the formation of different metastable phases is possible, as it is the formation of different calcium oxalate hydrates, the composition of the precipitate could be changed by altering the mode of nucleation and affecting the

\* Dedicated to Prof. Dr. Sc. Helga Füredi-Milhofer on the occasion of her 80<sup>th</sup> birthday.

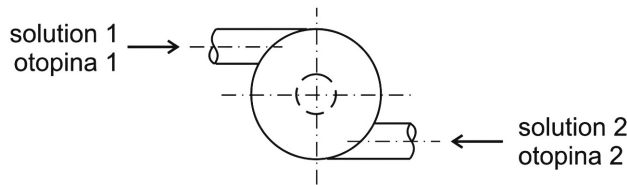


FIGURE 1  
 Schematic demonstration of solution mixing chamber.  
 SLIKA 1  
 Shema uređaja za miješanje otopina.

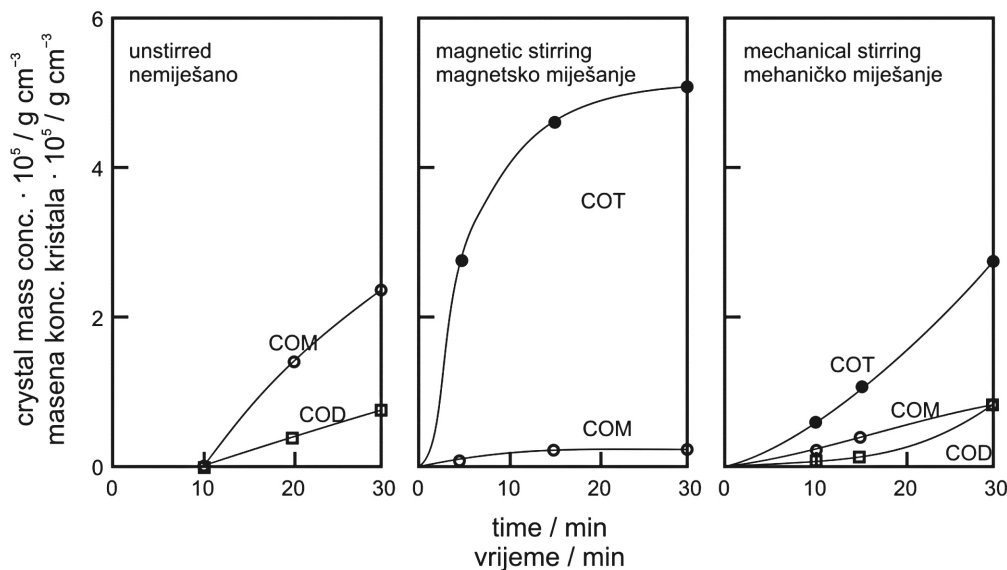


FIGURE 2  
 Effect of type of stirring on the calcium oxalate hydrate distributions.  
 SLIKA 2  
 Učinak miješanja sustava na raspodjelu hidratnih oblika kalcijeva oksalata.

process of transformation from one solid phase into another. This is of great importance in biomedical research and particularly in pathological mineralization of kidney stones.

In some papers (8,9) it has been demonstrated that the initial distribution of CaOx hydrates in a precipitation system was affected by the method of stirring the system. Therefore, the investigation of factors that are important in determining the formation of CaOx hydrates seemed to be a worth-while effort. Batch experiments have been undertaken ( $t = 25\text{ }^{\circ}\text{C}$ ,  $\text{pH} = 6$ ) in which we studied the effect of initial mixing of reactants, initial composition of the system (*i.e.* initial supersaturation), mode of stirring the system and the presence of an external magnetic field during the initial 30 minutes of the precipitation process (10). Besides, the influence of some amino acids on the precipitation of COD was studied, as well as the effect of temperature on the formation and stability of CaOx hydrates in a continuous crystallization process. Crystal size distribution was measured by an electronic counting device and the composition of the precipitate was analysed by means of X-ray diffraction (XRD), thermogravimetry (TG), optical and scanning electron microscopy (SEM).

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clear, it is obvious that the initial supersaturation profile played an important role.

### Effect of Stirring and Magnetic Field

The effect of changing the mode of agitation the system was investigated by using the identical reactant concentrations and the way of their initial mixing by using the mixing chamber (see Figure 1) as mentioned above. The initial solutions were supersaturated with respect to all three CaOx hydrates. The experiments were conducted under following conditions: (i) no stirring was applied after mixing the reactants; (ii) magnetic stirring (rotating at  $n = 13 \text{ s}^{-1}$ ) succeeded the mixing process and (iii) mechanical agitation with a flat-bladed stirrer was applied subsequent to mixing.

Figure 2 shows representative results of these experiments. By introducing stirring, not only induction times but also the composition of the precipitate was affected. Since the applied stirring compared with the unstirred system was shortening of the induction times, it is to be assumed that the better probability of contact between the system constituents contributed to such an effect. Stirring also favoured the formation of the least stable hydrate, COT, which was more pronounced in the magnetically stirred system. Obviously, "grinding" of COT crystals by the magnetic stirrer, the most easily fragmented CaOx hydrate, increased the surface area of COT and thereby the mass deposition on its surface. This was also confirmed by adding glass beads to the crystallizer in which the system was agitated with the mechanical stirrer. The mass of COT doubled with the addition of 3 beads, compared to the experiment in which only mechanical stirring was applied, and came closer the value for magnetic stirring. The thermodynamically stable COM appeared in all experiments, most probably as a result of direct nucleation. It is not likely that a transformation process was responsible for its formation, because such a process appears to be relatively slow and takes place when the system is supersaturated with respect to COM and undersaturated with respect to the higher hydrates, COD and COT (8). Because of the very low nucleation rate, COD precipitated only in the unstirred and mechanically stirred systems after relatively long induction times (5-10 min). In the magnetically stirred system the fall of supersaturation in the system was fast as a consequence of the formation of the other two hydrates, so that the resulting supersaturation was not high enough to make nucleation of COD possible.

In order to examine the role of the magnetic field present when the magnetic stirrer was used, the experiments were performed in the same way as described above, with the crystallizer placed on the magnetic stirring device switched on, but without using the stirring bar. In the first set of these experiments, the system was kept unstirred. The total mass of the precipitate and the distribution of hydrates obtained were very similar to those of unstirred system with no magnetic field applied. A notable number of small crystals were produced but no COT was detected. The second set of experiments was conducted with mechanical stirring in the pres-

ence of 3 glass beads. An increased number of small crystals also appeared but the distribution of hydrates were found to be similar to that obtained with using magnetic stirring bar; no COD was formed, the total mass, as well as the proportions of the other two hydrates, COT and COM, were resembling the magnetically stirred system.

Although the role of magnetic field in crystallization processes is rather controversial (11,12), our results give some evidence that magnetic field influences in a certain degree the nature of the calcium oxalate precipitation.

### Effect of Initial Supersaturation

Compared with the experiments defined above, these experiments were performed at twice as high reactant concentrations. All other conditions (temperature, mixing chamber, and unstirred system) remained the same.

In the case of doubling only the calcium concentration, the distribution of hydrates was not affected, i.e. the major component was COM (mass fraction,  $w = 75 \%$ ) with some COD ( $w = 25 \%$ ). When only oxalate concentration was doubled, the content of COM increased to about  $w = 90 \%$  and the crystals appeared mostly in the form of dendrites. By doubling concentrations of both reactants, the proportion of COM in the mixture remained high ( $w = 93 \%$ ) and the shape of dendrites was retained. The results indicate that the additional increase of oxalate concentration influences the amount and even the shape of COM crystals, since there was practically no difference regarding COM crystals between the two experiments at high concentration of oxalate.

### Effect of Amino Acids

Based on the reports on urine analyses of healthy persons and urinary stone-formers (13), we have chosen five amino acids (ornithine, Orn, histidine, His, tryptophan, Trp, serine, Ser, and glutamic acid, Glu) for investigations, in order to assess their influence on calcium oxalate precipitation (14). It is to be emphasized that among the investigated amino acids, Orn and Trp are not found in the urines of healthy individuals (in stone-formers their concentrations are respectively about  $c_{sf} = 3 \times 10^{-5}$  and  $1 \times 10^{-4} \text{ mol dm}^{-3}$ ), the concentrations of His and Ser are higher ( $c_{sf} = 5 \times 10^{-4}$  and  $c_{sf} = 2.5 \times 10^{-4} \text{ mol dm}^{-3}$ , respectively) and of glutamic acid lower ( $c_{sf} = 4.5 \times 10^{-5} \text{ mol dm}^{-3}$ ) than that in urine of stone-formers. In our experiments, we have used the amino acid concentrations similar to those excreted in stone-former urines ( $c_{sf}$ ), which were always added to the oxalate solutions prior to pH adjustment. The other experimental conditions were identical to those mentioned above (batch experiments conducted at  $t = 25 \text{ }^\circ\text{C}$ , pH = 6.0, reactant mixing by mixing chamber, no further agitation applied).

The composition of calcium oxalate precipitate in the control system (containing the same reactant concentrations, but without additives addition) at the maximum content of COD (approximately 15 h after mixing reactants) and before the

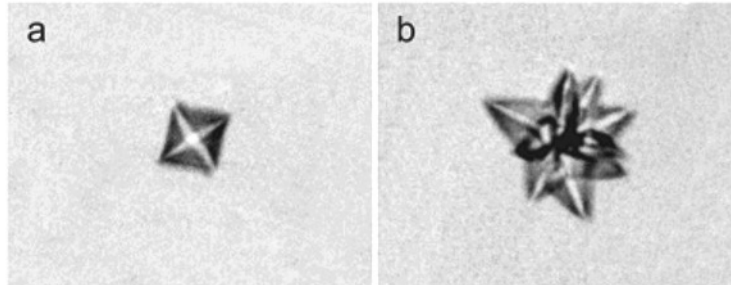


Figure 3

Micrographs of COD crystals precipitated in the pure system (a) and in the presence of Trp or Ser. Magnification 490 $\times$ .

Slika 3

Mikroskopske snimke COD kristala dobivenih taloženjem u čistom sustavu (a) i uz Trp ili Ser. Povećanje 490 $\times$ .

transformation process COD  $\rightarrow$  COM started, was found to be a mixture of COM ( $w = 67\%$ ) and COD ( $w = 33\%$ ). The results showed that most of the investigated amino acids promoted the formation of COM ( $w = 84\%$ ,  $86\%$  and  $89\%$  in the case of Orn, Ser and Glu, respectively), the effect of which cannot be correlated either to their isoelectric points ( $pI_{\text{Orn}} = 9.72$ ,  $pI_{\text{Ser}} = 5.68$  and  $pI_{\text{Glu}} = 3.22$ ) or nature of side chains. Only two amino acids, Trp and His ( $pI_{\text{Trp}} = 5.88$ ,  $pI_{\text{His}} = 7.58$ ), promoted the formation of COD ( $w = 44\%$  and  $54\%$ , respectively), while the presence of His also caused the formation of small amount of COT ( $w = 5\%$ ). The observed promotion of higher hydrate formation in relation to COM, could be explained as being specific because of the similarity in side chains of the two amino acids (His - imidazole and Trp - indole).

Apart from the influence on the composition of precipitate, the amino acids caused an increase in crystal size distribution. Thus for instance, while in the control system more than  $40\%$  of all crystals were less than  $8\ \mu\text{m}$  in size and approximately  $45\%$  were in the range  $10\text{-}30\ \mu\text{m}$ , in the presence of amino acids the content of crystals of  $< 8\ \mu\text{m}$  was about  $6\%$  in the case of Ser, Glu or His and  $10\%$  of Orn addition, while the portion of crystals of the volume within the range  $10\text{-}30\ \mu\text{m}$  was between  $75$  and  $90\%$ . Obviously, the addition of selected amino acids favours the formation of larger crystals. This is in agreement with some observations (15) that the differences between the stone-formers and healthy persons are also in the size of crystals found in urine. The urine of calcium oxalate stone-formers contains much more aggregates of COD octahedral crystals  $10\text{-}12\ \mu\text{m}$  in diameter, while the crystals found in urine of healthy persons do not exceed  $3\text{-}4\ \mu\text{m}$ .

The morphology of crystals was also affected by the addition of some of the amino acids. A considerable number of intergrown COD crystals were formed in the presence of Trp and, somewhat less, in the presence of Ser (Figure 3); the common shape of COD being octahedral bipyramid. The same coalescence of COD crystals was reported when CaOx precipitated from urine (16). Such a similar influence on the shape of COD crystals of the two amino acids with quite different side

chain structures is somewhat unexpected. A possible explanation of the effect is related to the fact that both amino acids were present in the system as zwitter ions at the pH at which experiments were performed so they can increasingly adsorb on the crystal or the primary particles.

A significant effect on the average particle size, morphology and precipitate composition of calcium oxalate hydrates (COM and COD), depending on the additive type and concentration (amino and carboxylic acids, urinary proteins and model compounds), was also reported recently (17-21). The effects are mostly explained as the result of adsorption, selective binding, non-specific electrostatic interactions between  $\text{Ca}^{2+}$  of the crystal and the additive or even incorporation (occlusion) of the additives on the particular crystallographic face(s).

### Effect of Temperature

One of the variables that can influence a number of properties of a precipitation system is the temperature. In order to investigate the effect of temperature on the nucleation and growth kinetics, as well as on the CaOx hydrate(s) formed, we used continuous laboratory experiments (22). Under certain experimental conditions this type of experiments enables to obtain information on the apparent nucleation kinetics and the crystal growth kinetics from single experiment. Moreover, concerning the specific problem of urinary calculi formation, a continuous crystallizer has been considered as a useful model of the urinary tract (23,24) and a number of studies has been conducted in the so-called mixed-suspension mixed-product removal (MSMPR) crystallizers (25-27). In our study, calcium oxalate was precipitated by mixing calcium chloride and sodium oxalate solutions in a continuous MSMPR crystallizer in which the flow rate of each feed solution was kept constant and equal during the experiment. The experiments were performed at mean residence times of  $\tau = 1.8$  to  $16.2\ \text{min}$  ( $\tau = V/Q$ ,  $V$  being the crystallizer volume and  $Q$  the volumetric flow rate) over the temperature range  $9$  to  $45\ ^\circ\text{C}$ . The main kinetic parameters, crystal growth rate,  $G$ , and apparent nucleation rate,  $B_0$ , were determined from the relationship between the crystal population density,  $n$ , crystal

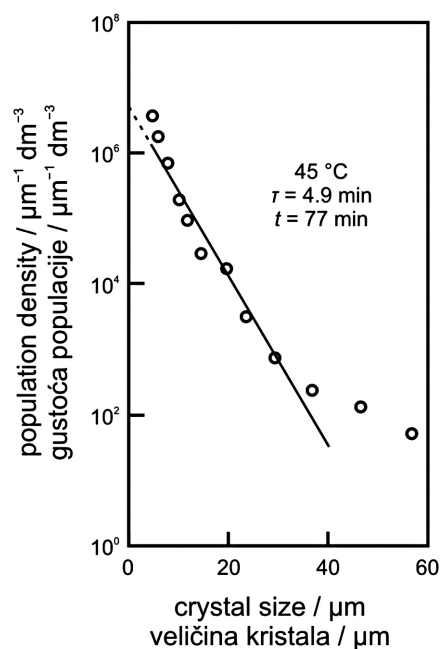


FIGURE 4  
 Typical steady state population density plot.  
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Tipičan grafički prikaz gustoće populacije kao funkcije veličine kristala pri uspostavljenom stabilnom stanju u sustavu.

size,  $L$ , crystal growth rate and residence time when plotted on semilogarithmic coordinates (28).

$$n = n^0 \exp(-L/G\tau) \quad (1)$$

From the slope of thus obtained straight line the crystal growth rate can be calculated, while the intercept of the line (at  $L = 0$ ) gives the value of  $n^0$  which is the measure of the apparent nucleation rate according to the equation

$$B_0 = n^0 G \quad (2)$$

In such a way, for a given residence time the crystal size distribution is described by the two kinetic parameters,  $G$  and  $B_0$ . Figure 4 shows a typical population density plot obtained at steady state conditions in the system, i.e. at the constant total number of crystals with identical crystal size distribution. These were determined for each experiment by using an electronic counting device (29). The steady state total dissolved calcium concentrations in the solutions were determined by means of atomic absorption spectrophotometry and the precipitates were characterized by X-ray diffraction.

The solution in the crystallizer under steady state conditions at all temperatures examined was found to be supersaturated with respect to both COT and COM. The precipitate obtained at  $t = 37$  and  $45$  °C consisted entirely of COM and at  $t = 9$  °C only COT was formed. At  $t = 25$  °C COT was the predominant hydrate modification with about 5 % COM and at  $30$  °C the major component in the precipitate was found to be COM comprising also about 20 % COT. Those results showed that during the continuous process of CaOx precipitation under

steady state conditions, the thermodynamically unstable hydrates could be formed. That was in accordance with the reports on batch experiments (5) suggesting that COT has been the initially formed hydrate in the precipitation systems in which the initial equimolar reactant concentrations were in the range  $c = 10^{-2}$  to  $10^{-4}$  mol  $\text{dm}^{-3}$ , as was used in our experiments (5,6).

The calculated growth rates,  $G$ , when plotted against the supersaturation,  $\sigma$ , gave straight lines, the slopes of which were: 2.04 (9 °C), 1.76 (25 °C), 2.20 (30 °C), 2.14 (37 °C) and 2.12 (45 °C), confirming the second order of the growth process. By expressing the nucleation and growth processes as a power function of the same supersaturation the apparent nucleation rates,  $B_0$  (in  $\text{dm}^{-3} \text{s}^{-1}$ ) and the growth rates,  $G$  (in  $\mu\text{m} \text{s}^{-1}$ ), were correlated empirically giving the following equations referring respectively to the temperatures 9, 25, 30, 37 and 45 °C:

$$B_0 = 1.35 \times 10^8 G^{1.62} \quad (3)$$

$$B_0 = 1.62 \times 10^6 G^{0.99} \quad (4)$$

$$B_0 = 4.68 \times 10^4 G^{0.29} \quad (5)$$

$$B_0 = 2.51 \times 10^5 G^{0.43} \quad (6)$$

$$B_0 = 4.47 \times 10^5 G^{0.54} \quad (7)$$

It is evident that there is a significant difference between the nucleation rates of COM (37 °C and 45 °C) and COT (9 °C), the latter being much higher. The nucleation rates obtained at 25 and 30 °C are not consistent with either of the two trends which is most probably a consequence of the formation of two hydrate mixtures. In such a case it is difficult to define a

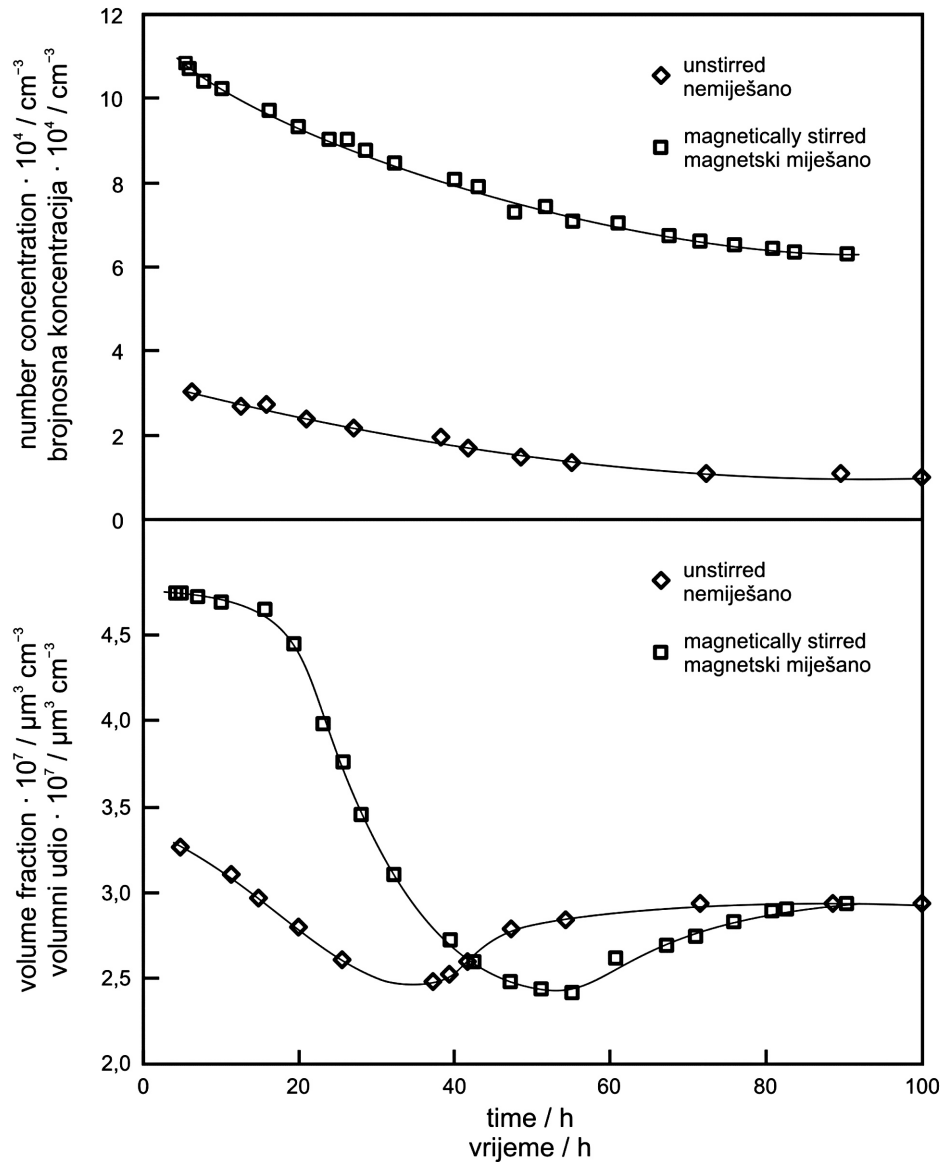


FIGURE 5  
 Variations of total crystal number concentrations and volume fractions with time in the unstirred and magnetically stirred systems.

SLIKA 5  
 Promjene ukupne brojnosne koncentracije i volumnog udjela kristala s vremenom u nemiješanom i magnetski miješanom sustavu.

proper supersaturation, so that the kinetic data may not be completely reliable.

The presented results clearly show that the formation of a particular hydrate(s) is a result of kinetic rather than thermodynamic factors and that it depends on the temperature of precipitation.

#### TRANSFORMATION OF CALCIUM OXALATE HYDRATES

The calcium oxalates are a convenient model system for the study of hydrate transformation, among others, because of

being the major crystal constituent of urinary stones. Since by the time of our investigations there were no reports on the mechanisms of transformation from the metastable CaOx hydrates, COT and COD, to the thermodynamically stable one, COM, we had undertaken investigations of the kinetics of the processes involved and of the effect of amino acids on the formation of COD and the kinetics of its transformation.

#### Transformation of COT and COD

Experiments were performed in a batch crystallizer under conditions as mentioned above. In order to obtain COD and COT in separate systems, two series of experiments were car-

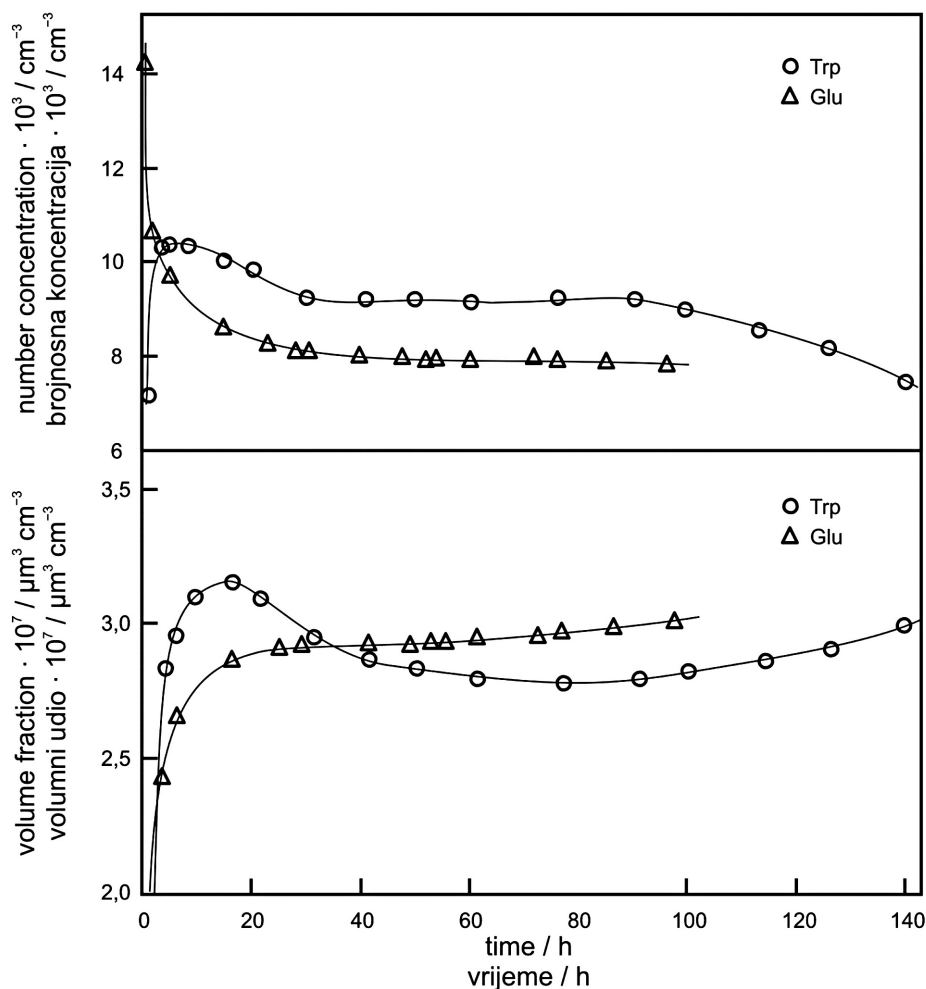


FIGURE 6

Changes of total crystal number concentrations and volume fractions with time for systems containing Trp (o) and Glu (Δ).

SLIKA 6

Promjene ukupne brojnosne koncentracije i volumnog udjela kristala s vremenom za sustave s dodatkom Trp (o) i Glu (Δ).

ried out, the method of agitation differing for each series: no stirring and magnetic stirring with a Teflon coated stirring bar (8). To characterize the course of the overall process, the samples were removed from the system at intervals and analysed by a range of techniques (X-ray diffraction, crystal size distribution, atomic absorption spectroscopy).

As known also from the results mentioned above, in the unstirred system a mixture of COD and COM precipitated and the magnetically stirred system gave COT and COM. During the transformation process, first an increase in a number of small crystals was observed, which was a result of the unstable hydrates (COD and COT) dissolution and/or the formation of new crystals of the stable hydrate (COM). Later on, as the COM crystals grew, the number of small crystals decreased and the number of larger sizes increased. This was also evidenced by the photographs, which illustrated the COD and COT crystals becoming smaller with rounded corners. The redistributions of COD and COT to the COM crystals during the transformation processes are given in Figure 5.

Such changes in the system, evidenced by the size distributions, time variations of total crystal number and volume, as well as the shape of crystals during the process, point at a solution mediated transformation of both COT and COD to the thermodynamically stable COM. This means that the metastable phase (COT or COD) dissolves in the solution with which it is in contact, and simultaneously an independent nucleation and growth of the stable phase (COM) from the same solution occur (30).

### The Influence of Amino Acids

Transformation of a solid phase or its precipitation in general, can be affected by the impurities present in the system. In order to change one or more properties of the precipitate, or the course of the processes involved in precipitation, some impurities can be used intentionally. Such additives can act either as promoters or inhibitors at specific cases. It is well known that in urine some substances are present which promote or inhibit the formation of CaOx hydrates. Thus for instance, it is much easier to crystallize COD from urine or

artificial urine (31) than from pure calcium oxalate solutions. Obviously, that some components, other than calcium and oxalate ions, play their role in its formation. Some amino acids have been found to be urinary constituents (13), among which some of them showed inhibitory effects (32).

We have studied the effect of amino acids (Orn, His, Trp, Ser and Glu) on the transformation of COD to the thermodynamically stable COM in the unstirred system in which the two hydrates precipitated simultaneously (33), as mentioned above.

Figure 6 shows typical changes in the precipitate volume,  $V_t$ , and the total number of crystals,  $N_t$ , during the overall process in the control-system and the systems containing Trp and Glu. System with Trp shows the plots of  $V_t$  and  $N_t$  versus time similar to the control-system. Systems containing Ser and His (not shown here) behave similarly. These plots of the Glu-containing system, as well as the one with Orn (not shown here), which produce small amounts of COD, look somewhat differently. The total volume of the precipitate in the control-system and the systems containing amino acids do not differ greatly. A significant difference appears in the total number of crystals. The much higher number of crystals in the control-system with approximately the same total volume of precipitates in the control- and amino acids-containing systems indicates that the crystals in the presence of amino acids must be larger. This is also in accordance with some earlier reports (15) from which in the urines of patients with calcium oxalate stone disease large individual COD crystals and their aggregates are formed while in the urines of healthy persons only tiny particles (crystals) form.

The influence of amino acids on the kinetics of COD transformation was examined as described previously for the control-system (8). As the overall transformation process comprises the dissolution of COD and the growth of COM, the given amino acids are expected to influence the rates and/or mechanisms of these two main processes. The separate examination of COD dissolution and COM growth showed the following:

- His kept the diffusion controlled dissolution process of COD, the rate constant being a bit lower than in the control-system, thus suggesting the possible incorporation of this amino acid into COD crystals;
- The presence of Ser and Trp changed the rate-determining mechanism of COD dissolution from diffusion to the surface controlled rate, most probably because of the amino acid's adsorption at the solid/solution interface;
- In the presence of Orn and Glu the amount of COD formed was too small for quantitative analysis;
- His and Trp showed practically no effect on the growth process of COM;
- Ser, Glu and Orn changed the order of the kinetics of COM growth from 2 to 4;
- The rate constant of COM growth kinetics in the presence of Glu was found to be noticeably lower.

The shown results indicated that the given amino acids interact with calcium oxalate hydrates. The type and proportions of these interactions are most probably due to the specific structure of the particular amino acid, its dipole behaviour and interactions with specific crystal structure of the CaOx hydrate.

Some recent investigations of the influence of water-soluble polymers, with different molecular weight and structure, on calcium oxalate crystallization (34) report on the inhibition effect of these additives on crystal growth and their influence on both the morphology and transformation of CaOx crystals. The effect is, like that of amino acids, explained in terms of the adsorption of inhibitors on the active growth sites on the crystal surface.

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## ČIMBENICI KOJI UTJEČU NA NASTAJANJE HIDRATÂ KALCIJEVA OKSALATA *IN VITRO*\*

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### SAŽETAK

Općenito uzevši, na proces taloženja iz prezasićenih otopina utječu fizikalno-kemijska svojstva tih otopina, hidrodinamički čimbenici - procesi miješanja reaktanata i taložnoga sustava, kao i prisutnost topljivih nečistoća u sustavu. Zbog važnosti procesa taloženja u brojnim područjima znanosti i tehnike, od izuzetnoga su značaja temeljna istraživanja tih procesa kao i međudjelovanja koja dovode do stvaranja taloga. Obzirom na činjenicu da su kalcijevi oksalati glavni sastojci mokraćnih kamenaca, njihovo izučavanje, kao i izučavanje fizikalno-kemijskih čimbenika koji dovode do njihovoga nastajanja jesu od posebnog značaja u području patološke mineralizacije. Kalcijevi oksalati tvore tri hidratna oblika od kojih je monohidrat (COM) termodinamički stabilan, dok su dihidrat (COD) i trihidrat (COT) metastabilni. Rezultate istraživanja utjecaja brojnih termodinamičkih i kinetičkih čimbenika, kao i potencijalnih inhibitora na nastajanje i transformaciju kalcijevih oksalata pri različitim eksperimentalnim uvjetima, prikazane u ovom pregledu, sadašnji su autori ostvarili i objavili tijekom 1980-ih.

**Ključne riječi:** Kalcijev oksalat monohidrat; Kalcijev oksalat dihidrat; Kalcijev oksalat trihidrat; Aminokiseline; Kemijsko taloženje; Transformacija

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