Patterns of calcium oxalate monohydrate crystallization in complex biological systems

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Abstract. The paper presents the features of calcium oxalate crystallization in the presence of additives revealed through experimental modeling. The patterns of phase formation are shown for the $Ca^{2+} – C_2O_4^{2-} – H_2O$ and $Ca^{2+} – C_2O_4^{2-} – PO_4^{3-} – H_2O$ systems with the components and pH of the saline varying over a wide concentrations range. The effect of additives on crystallization of calcium oxalate monohydrate was investigated. It was found that the ionic strength and magnesium ions are inhibitors, and calcium oxalate and hydroxyapatite crystals are catalysts of calcium oxalate monohydrate crystallization. The basic calcium phosphate (apatite) was found to be most thermodynamically stable, which indicates its special role in kidney stone formation since it is found in virtually all stones.

1. Introduction

The data gathered through the analysis of human body fluids is the basis for creation and construction of physical-chemical models to reveal the regularities of physiological processes. The experimental modeling techniques are considered to be promising to predict the behavior of biological systems upon changing its parameters, which is especially important when studying the processes caused by functional impairment, such as pathogenic biomineral formation in human tissues and organs. These pathogenic formations are kidney stones (uroliths). Urinary stone disease (USD) is defined as a disease caused by endogenous (including hereditary) and/or exogenous factors due to formation of stones in the urinary tract. USD is one of the most common diseases that are prone to relapse, and in many cases it is a severe acute disease [1–4]. The reported prevalence and incidence of USD in adults in the world is 1–5% [5–7]. The annual incidence rate of urinary stone formation is 1200–1400 per 100 000 population [8], and the average risk for stone formation throughout one's life ranges from 5 to 10% [9]. USD mostly affects the working-age population [3, 10]. According to many researchers, an increased incidence of USD in all population groups is currently observed in most of the industrialized countries [11–13].

The most common mineral phases [14–16] of human kidney stones are spherulite aggregates of calcium oxalate monohydrate ($CaC_2O_4·H_2O$). These are regular products of crystallization caused by high urine supersaturation. The $CaC_2O_4·H_2O$ crystals nucleate mainly heterogeneously and their growth occurs in a kinetic mode. Oxalate is crystallized from aqueous saline in the form of various hydrates, thermodynamically stable monohydrate ($CaC_2O_4·H_2O$, whewellite; COM) and metastable tetragonal hydrate ($CaC_2O_4·(2+x)H_2O \leq 0.5$, weddellite), occurring in the tissues of urinary stones and deposits of biological origin. Triclinic hydrate ($CaC_2O_4·xH_2O$, $3 \geq x > 2.5$;

COT) was prepared as well, and its crystal structure was studied. It is known that the formation of poorly soluble compounds is affected by the ions present in the saline [17] including analogous ions with deposits. Therefore, an increased concentration of oxalate ions in urine promotes the formation of calcium oxalate. When the concentration of oxalate > 1 mmol/l, calcium oxalate monohydrate is formed in urine.

The aim of this paper is to study the crystallization of calcium oxalate and to establish the effect of a series of additives on the size, rate and the number of the crystals formed.

2. Materials and methods

The studied samples were prepared by the technique developed in [16] in accordance with the chemical equation $Ca^{2+}+C_2O_4^{2-} = CaC_2O_4\downarrow$. The value of the final concentration of calcium and oxalate ions was 20 times above the concentration of the saturated saline. All the experimental series were carried out under continuous stirring, and the samples were taken and placed on a preparation glass at appropriate intervals. The crystallization time in each series varied from 2 to 580 hours. The concentration of additives was prepared with respect to the biological fluid composition [17].

Calcium oxalate crystallization was studied by practical metallography techniques using a Neophot-2 optical microscope. We used a linear method to measure the size of the microparticle cross sections (to measure the particle diameter) and an intercept method to estimate the number and sizes of the microparticle cross sections [18]. The length of the chords was determined with allowance for a linear magnification of the image of the microparticle structure.

The phase composition of the deposits was investigated by XRD (DRON-3) and optical microscopy. To perform a qualitative analysis of the sample phase composition, the experimental values of the interplanar spaces and relative intensities of the diffraction peaks were compared to a set of corresponding table values for each of the assumed phases. The sensitivity of the XRD method to carry out the measurements was 3%.

3. Results and Discussion

In the first stage, the study was performed under conditions close to the biological medium, namely, the samples were prepared at supersaturation γ=7, and the ionic strength was varied from 0.003 to 0.4 (corresponds to a biological fluid (human urine)). It was found that under these conditions, only calcium oxalate monohydrate is crystallized (figure 1 and 2); at ionic strength greater than 0.15, calcium oxalate crystallization does not occur. At ionic strength equal to 0.003–0.1, the microparticle size varies slightly, the average size being 10.83 μm. However, at ionic strength I=0.15, the microparticles grow up to 13.5 μm. It is known that the greater the rate of nucleation and growth, the faster the crystallization, and this depends on the degree of the saline supersaturation. Therefore, further investigation of the calcium oxalate crystallization was conducted for three series. For the first series, supersaturation γ=15, for the second series γ=20 and for the third series γ= 25. The crystal size growth was not found within one series irrespective of the crystallization time, however, during transition from the second to the third series, the crystals were observed to increase in size (figure. 3).

The obtained data was used to evaluate the average rate of the $CaC_2O_4 \cdot H_2O$ particle growth. It was found that when γ=25, the predominant process was the growth (ϖ =0.46 μm/h), but not the nucleation. The initial supersaturation γ=20 was chosen to reveal its effect on the additive growth rate.

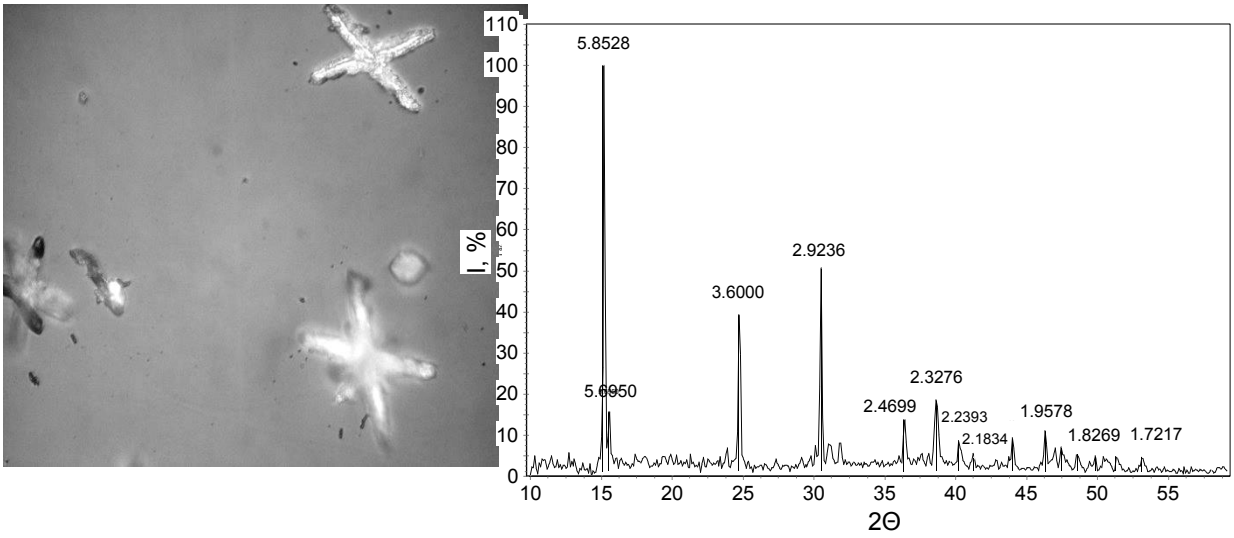
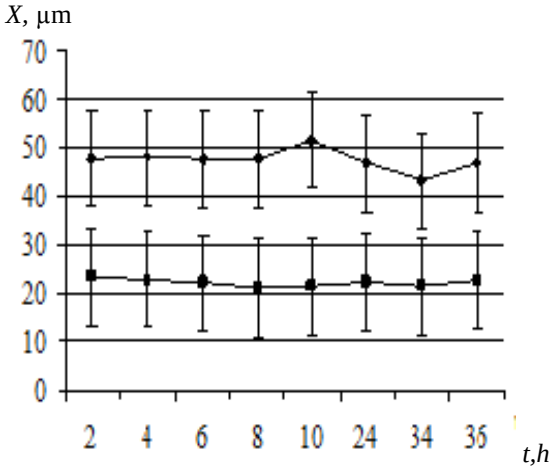


Figure 1. Optical image of calcium oxalate monohydrate crystals at γ=20 (x 450).

Figure 2. Diffraction pattern for CaC_2O_4 H_2O.



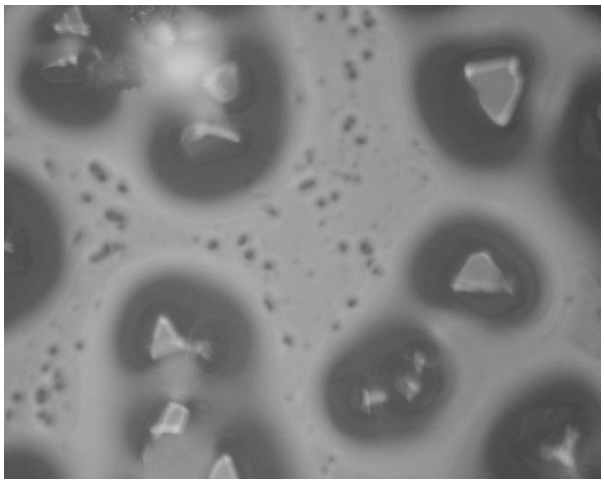


Figure 3. Dependence of the calcium oxalate monohydrate particle size x on t: γ=20 (1), γ=25 (2).

Figure 4. Microstructure of the particles (x450) crystallized from the saline solution after addition of magnesium chloride (=8.15 mmol/l, the crystallization time equal to 250 hours).

Biological fluids, in which kidney stones are formed, is a complex multi-component system [17] and a high ionic strength saline. To evaluate the effect of the ionic strength on the crystallization of calcium oxalate monohydrate, a series of experiments were conducted to study the patterns of the compound formation at constant ionic strength characteristic of the natural crystal-forming medium (urine) I=0.3. The formation of calcium oxalate monohydrate crystals at this ionic strength value was not observed. The obtained results can be attributed to a strong effect of repulsive electrostatic forces between similar ions in a high ionic strength saline, which inhibits chemical interaction between calcium and oxalate ions and hence reduces the nucleation probability [19].

Natural crystal-forming medium contains organic and inorganic compounds such as magnesium ions. Magnesium is one of the most important activators of many enzymatic processes. Although the substances that regulate the magnesium absorption are similar to those regulating the calcium

absorption, in particular, proteins and vitamin D, there is definite antagonism between calcium and magnesium ions in the human body [17]. Addition of magnesium chloride to the feed saline (figure 4) inhibits the formation of calcium oxalate monohydrate crystals irrespective of the concentration (4.07 mmol/l ÷ 0.041 mmol/l) of the additive and crystallization time. The magnesium ions were found to inhibit crystallization, and as a result, calcium oxalate monohydrate crystals are not formed.

It is known that during kidney stone formation calcium oxalate monohydrate microcrystallites (crystalluria products) and phosphate micro-individuals are found in the physiological solution that can affect pathogenic mineral formation. The crystallization of calcium oxalate in the saline with the addition of calcium oxalate and hydroxyapatite seeds was studied. The crystallization time was varied from 10 minutes to 580 hours. It was found that seeding causes a slight growth of the formed calcium oxalate monohydrate crystals at the initial time moment. When the seed was calcium oxalate monohydrate, the crystals grew by 1.5 times within 50 hours, and in case of hydroxyapatite, the crystals grew by 2.5 times within 150 hours as compared to the initially formed crystals (figure 5). Thus, it was found that the presence of calcium oxalate hydroxyapatite crystals in the saline catalyzes crystallization of calcium oxalate monohydrate. The experimental data were used to estimate the average rate (Table. 1) of calcium oxalate monohydrate crystal formation, which was 4 times higher in the presence of hydroxyapatite crystallites than that obtained through the addition of $CaC_2O_4 \cdot H_2O$. Thus, the presence of hydroxyapatite crystals in the urine initiates the crystallization of calcium oxalate monohydrate at a higher speed.

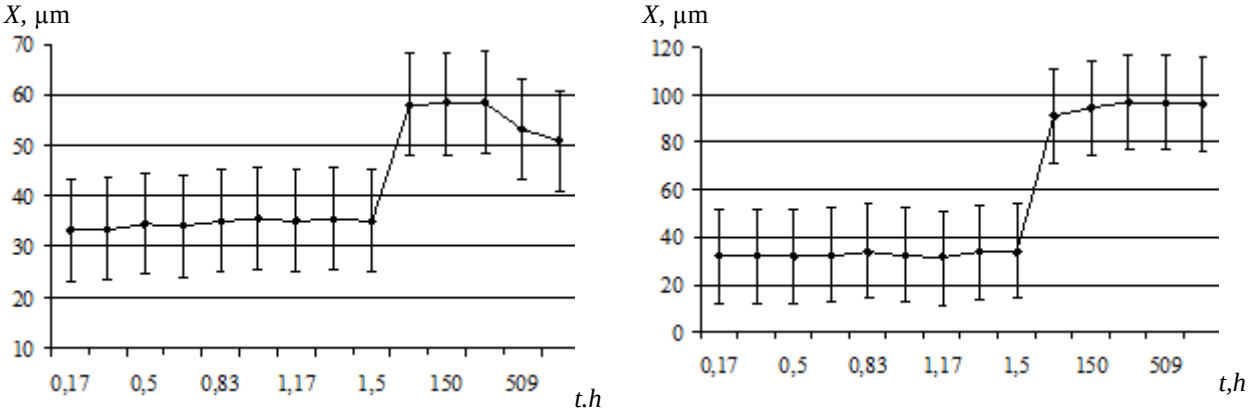


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Figure 5. Dependence of calcium oxalate monohydrate particle sizes on the crystallization time in the presence of seeds: a) calcium oxalate; b) hydroxyapatite.

Table 1. The average rate of calcium oxalate monohydrate crystallization in the presence of seeds.

Average rate ϖ, μm/h	
Calcium oxalate monohydrate	Hydroxyapatite
0.03	0.12

The stability fields constructed for CaC_2O_4 calcium oxalate using the thermodynamic data from [20] showed that the surface of the thermodynamic equilibrium in the concentration range characteristic of the physiological solution is substantially invariant with respect to pH variations (figure 6a). The range of the saline concentrations and pH values lies in the range of a stable compound. The stability range of the basic calcium phosphate $Ca_5(PO_4)_3OH$ (Figure 6b) is significantly extended if the pH increases up to 12. The concentration range characteristic of saline also corresponds to that of the compound stability. During oxalate phase formation, phosphates can be formed even if the concentrations of oxalate ion are significantly high. This is mainly hydroxyapatite,

especially if the pH of the saline s shifts to the alkaline range. The concentration range of oxalate and phosphate of the physiological saline lies almost entirely in the range of the $Ca_5(PO_4)_3OH$ stability.

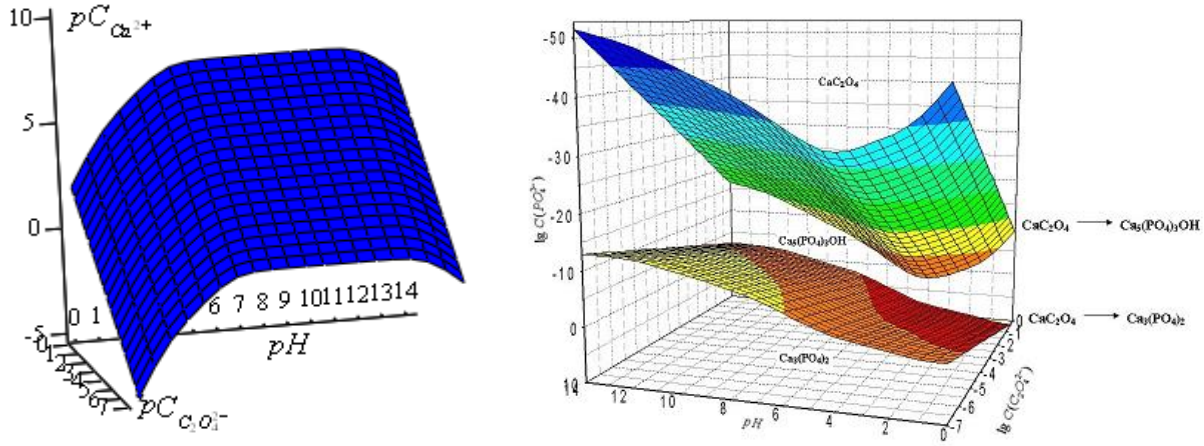

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Figure 6. Stability fields observed during formation of poorly soluble compounds from the saline solution at 37°C and ionic strength of 0.3 M for the systems: a) Ca^{2+}–$C_2O_4^{2-}$–H_2O; b) Ca^{2+}–$C_2O_4^{2-}$–PO_4^{3-}–H_2O.

The electron microscopy and microanalysis revealed separate apatite inclusions (figure 7), grains of hydroxyapatite, in oxalate type kidney stones (monomineral, according to XRD). The data yielded by the study showed that the Ca/P ratio in urolith apatites was less than that of stoichiometric apatite (1.67). Thus, the basic calcium phosphate apatite is the most stable compound, and it can be found in almost all kidney stones that indicates its special role in their formation. In real kidney stone samples, CaC_2O_4•H_2O plates form druse depositions on the kidney stone surfaces that traumatize the surrounding tissue upon the stone motion (figure 8).

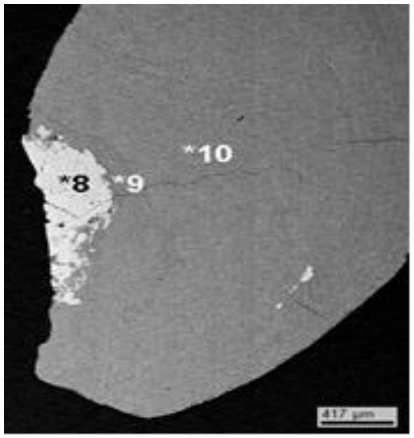

Figure 7. Apatite inclusions in oxalate type urolithes.

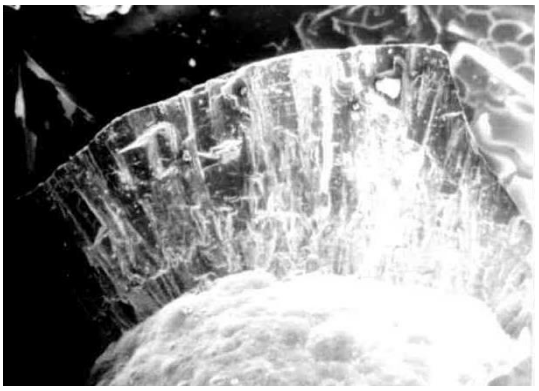

Figure 8. Whewellite crystals: on the apatite globule

4. Conclusion

The results of the investigation show that at supersaturation γ=20, 25 calcium oxalate monohydrate crystallizes only. The average size of its microparticles does not depend on the crystallization time. Ionic strength and magnesium ions are the inhibitors, and calcium oxalate and hydroxyapatite crystals are the catalysts of the calcium oxalate monohydrate crystallization. Basic calcium phosphate (apatite) is most thermodynamically stable and it can be found in almost all kidney stones that indicates its special role in their formation.

Acknowledgements

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