# Calcium Oxalates: Thermodynamic and Kinetic Conditions of Their Formation in the Presence of Organic Components

**Vyacheslav V. Korolkov, Olga A. Golovanova and Marina V. Kuimova**

**Abstract** The paper researches a range of problems related to the peculiarities of calcium oxalate crystallization in the presence of amino acids on the bases of thermodynamic calculations and experimental data. The regularities of phase formation in the system $Ca^{2+}$–$C_2O_4{}^{2-}$–$H_2O$–amino acid in a widely variable interval of component concentrations and pH are theoretically researched. The influence of pH on the thermodynamic stability of crystalline compounds is considered. The kinetic parameters of crystallization are determined: the induction period, the rate constant, the order of crystals' growth, and the influence of amino acids on the kinetics of crystallization of calcium oxalate is established.

**Keywords** Crystallization · Calcium oxalate · Thermodynamic simulation · Kinetics · Amino acids

## 1 Introduction

The problem of stone formation is becoming more prominent and is a real threat to modern residents of all countries, in particular in metropolitan cities. According to statistics, throughout the twentieth century, various diseases leading to the formation of stones (organo-mineral aggregate—OMA) in human organs has only increased (Golovanova 2007; Larina et al. 2006; Tiktinsky and Alexandrov 2000). Stones are formed at any age, even in very young children from birth. The urinary system stones are the most common that form minerals of pathogenic nature. However, modern medicine has not yet been able to fully understand the causes for the formation of various OMA in the human body.

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Urinary stone disease (urolithiasis), leading to the formation of stones in the organs of the urogenital system, ranks third in prevalence among all urological diseases. About 5 % of the world's population suffers from urolithiasis, and experts predict that in the future their number will only grow. In Russia, up to 3 % of people suffer from this disease. In 7 % of all cases of urolithiasis, disease occurs in children under the age of 16 years. However, according to statistics, the majority of cases are among adults of working age—from 20 to 49 years; the peak of disease incidence occurs in people aged 35–45 years. This illness is about three times more likely in men than in women (Al Zahrani et al. 2000; Golovanova 2008; Golovanova and Pyatanova 2002; Palchik et al. 2006; Pospekhova et al. 2001; Sevostyanova and Polienko 2004; Zuzuk 2005). Physicians attribute the increased prevalence of urolithiasis in cities with increased consumption of foods rich in fats and animal proteins, as well as with negative environmental conditions in the major metropolitan areas (Assimos and Holmes 2000; Al Zahrani et al. 2000; Arias Funez et al. 2000; Bailly et al. 2000; Bak et al. 2000; Golovanova 2008; Golovanova et al. 2004b; Zuzuk 2005).

Pathogenic organo-mineral aggregates are of complex and heterogeneous composition (Elnikov et al. 2007; Golovanova 2007; Golovanova et al. 2006a; Sevostyanova and Polienko 2004). However, overall, the most common of urinary stones are calcium oxalate stones, composed of minerals $CaC_2O_4 \cdot H_2O$ whewellite and $CaC_2O_4 \cdot 2H_2O$ weddellite (Golovanova 2007, 2008; Pospekhova et al. 2001; Sevostyanova and Polienko 2004; Tiktinsky and Alexandrov 2000). These minerals may also be a part of dental and salivary stones, gallstones, and others. Moreover, they are found in the composition of mineral deposits in lungs, blood vessels, spleen, prostate and pancreatic glands, muscle, and others (Golovanova 2008). These data highlight the importance of studying the crystallization of calcium oxalate in conditions close to physiological.

Organic materials of proteinic nature, which are components of physiological fluids, have a great influence on the character of the crystallization process of minerals, particularly calcium oxalate (Golovanova et al. 2006b, 2010). Current information about the nature of the crystallization of calcium oxalate complex by composition of physiological solutions is insufficient. New data are needed on the study of crystallization of these minerals from fluids in the presence of amino acids, as they are required from the viewpoint of fundamental understanding of bio-mineralization processes and also medically, to prevent calcium oxalate stone formation in the human body.

The aim of this work is to establish the thermodynamic and kinetic regularities of formation of calcium oxalate in the presence of a number of amino acids.

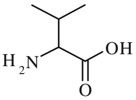
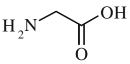
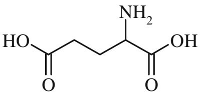
## 2 Materials and Methods

Model solution, mineral composition (inorganic macro components), temperature, ionic strength, and pH of urine corresponding to that of an average healthy adult person were taken as a thermodynamic model of processes of phase formation (in the system $Ca^{2+}$–$C_2O_4{}^{2-}$–$H_2O$–amino acid, as a prototype of body fluid) (Tiktinsky and Alexandrov 2000). Additives of amino acids were used to research the influence of organic components on the formation of the solid mineral phase. The choice of this set of amino acids is based on their similarities to the physiological fluids of concern, which are the high salinity and organic structures observed in urinary stones (Table 1).

The calculation of amino acids' influence was limited to the description of the process of complexation with calcium ions (Berthon 1995; Kiss et al. 1991; Lurie 1989; Vasiliev 2003; Yamauchi and Odani 1996); while for the calculation of the conditional solubility products, an amendment was introduced, characterized by the fraction of ions $Ca^{2+}$ that are not bound in a complex with amino acids. In calculation, the range of pH was varied over a wide range from 0 to 14. The coefficients of ion activity in these conditions were calculated using the Davis equation (Lurie 1989).

During the calculations to determine the possibilities and conditions of calcium oxalate deposition, the values of the thermodynamic solubility products (Lurie 1989) and the bases of constants of instability of *SC-database* complexes were used. Theoretical definition of the possibilities and conditions of deposition of calcium oxalate monohydrate was made based on the values of the indices of supersaturation of the (SI) system. Stability fields were used for the thermodynamic description of sediment–solution equilibrium in the system, wherein the formation of poorly soluble combination occurs. The principle of design of such diagrams is in determining the functional dependence of the parameter of the minimum concentration of the cation within the sediment; this dependence must be created for the start of the deposition phase at a given pH of the solution and given concentration of the anion: $pM^{q+} = f(pX^{q-}, \text{pH})$.

**Table 1** The amino acids used as impurities, and their characteristics (Dawson et al. 1986)

| Amino acid | Structure | Isoelectric point pI | $pK_a$ |
|---|---|---|---|
| DL-Valine (Val) |  | 5.96 | 2.27<br>9.52 |
| Glycine (Gly) |  | 5.97 | 2.34<br>9.58 |
| DL-glutamine acid (Glu) |  | 3.22 | 2.16<br>4.15<br>9.58 |

The field located above the obtained critical plane characterizes the conditions under which the formation of this phase is thermodynamically impossible. On the basis of the constructed three-dimensional diagrams in the coordinates $pM^{q+}-pX^{q-}-\mathrm{pH}$, conclusions can be drawn about the stability of the system and the prediction of the nature of the change of the balance under different conditions.

The process of crystallization of calcium oxalate monohydrate was studied at 37 °C and the basic values of a supersaturated solution $\gamma = C_0/C_s = 5,7$; this choice of the basic value of $\gamma$ is justified by the presence of supersaturation in biological media, namely that of the urine of an average healthy adult person (Golovanova 2007; Tiktinsky and Alexandrov 2000).

Supersaturation with calcium oxalate was created in the chemical reaction:

$$\mathrm{Ca^{2+} + C_2O_4^{2-} \rightarrow CaC_2O_4},$$

which is implemented by mixing stock solutions of stoichiometric ratio of soluble compounds—calcium chloride and ammonium oxalate.

The visual method, based on measuring the induction periods ($\tau_{\mathrm{ind}}$), was used to determine the parameters of the nucleation. As it is known, the induction time is inversely proportional to the nucleation rate: $\tau \sim 1/J$. In turn, the dependence of the nucleation rate on supersaturation of the solution is expressed by an exponential function, which includes the surface energy at the interface $\sigma$:

$$J \sim \exp\left[-\frac{16\pi\sigma^3 v^2}{3k_B^3 T^3 (m \ln\gamma)^2}\right] = \exp\left[-\frac{B}{(\ln\gamma)^2}\right], \tag{1}$$

where $v$ is the volume of the molecule; $k_B$ is the Boltzmann constant; $T$ is the temperature; $m = 2$ is the number of ions on which a molecule dissociates in the solutions; $B$ is a constant.

The timing of the induction was carried out in a visual way, as the solution becomes cloudy. The study was conducted in vitro, in a medium close to that of the physiological solution of human urine.

The conductometric method of measurement of solutions' concentration during their crystallization was used to research the crystal growth. Conductometer Anion-4154 was used (Izatulina et al. 2006). According to conductometric analysis, the degree of completeness of the process of crystallization, $\alpha$, as a function of time, was determined from:

$$\alpha = \frac{C_0 - C_\tau}{C_0 - C_s}, \tag{2}$$

where $C_0$ is the initial concentration of calcium oxalate in a supersaturated solution, $C_\tau$ is the concentration of calcium oxalate at time $\tau$, and $C_s$ is the solubility of calcium oxalate.

To determine the kinetic parameters of the growth of crystals of calcium oxalate according to $\alpha = f(\tau)$ dependence, deposition rate was calculated as a function of current absolute supersaturation by the formula:

$$\frac{\mathrm{d}\alpha}{\mathrm{d}\tau} = kA \cdot (C_\tau - C_s)^n, \tag{3}$$

where $A$ is the total surface area of the precipitate, $k$ is the rate constant, and $n$ is the order of the reaction. The estimation of the total surface area for a constant shape of particles is given as

$$A = \beta \cdot N_\tau^{1/3} \cdot V_\tau^{2/3}, \tag{4}$$

where $\beta$ is the form factor, $N_\tau$ is the total number of particles, and $V_\tau$ is the sludge volume at time $\tau$. Considering that $\alpha = V_\tau / V_{\max}$ and $V_{\max}$ is the maximum amount of sediment in full supersaturation, after transformations we obtain the formula for calculation of the kinetic characteristics of the process of crystallization of calcium oxalate (assuming a constant number of particles $N_\tau = N =$ constant):

$$\left(\frac{\mathrm{d}\alpha}{\mathrm{d}\tau}\right) \cdot \alpha^{-2/3} = k'(C_\tau - C_s)^n, \tag{5}$$

where $k'$ includes all constants ($V_{\max}$, $\beta$, $N$, and $k$) and is constant for the given initial conditions. Applying the logarithm we obtain

$$\lg\left(\frac{\mathrm{d}\alpha}{\mathrm{d}\tau}\right) - \frac{2}{3}\lg\alpha = \lg k' + n\lg(C_\tau - C_s). \tag{6}$$

The structure of dependence in the coordinates $\lg(d\alpha/d\tau) - 2/3\lg\alpha = f(\lg(C_\tau - C_s))$ should give a straight line. The jog, chipped off by the straight line on the $Y$-axis, gives the value lg $k'$, and the slope ratio corresponds to the order of the growth rate $n$.

The kinetics of crystallization was researched both in solutions of calcium oxalate without organic components and with additives of the amino acids in the concentration of 0.004 mol/l, corresponding to their location in physical solution.

The phase composition of precipitate deposits obtained during synthesis was studied by X-ray diffraction (XRD), the diffraction patterns were obtained by powder diffraction technique on X-ray DRON-3, and phase identification was carried out using a database ICDD PDF for powder diffraction. IR spectra were obtained on the spectrophotometer "FT-801", the samples were prepared by pressing tablets with KBr. The spectrum of test samples was recorded in the range of 4000–470 $\mathrm{cm}^{-1}$. In order to obtain information about the interaction of amino acids with calcium oxalate crystals, experiments were carried out on adsorption of amino acids on the synthesized samples (Golovanova and Vysotsky 2011).

# 3 Results and Discussion

## 3.1 Thermodynamic Simulation

Based on the available data about the thermodynamic values of solubility products at 310 K, functional dependences were determined $p\mathrm{Ca}^{2+} = f\left(p\mathrm{C_2O_4^{2-}}, \mathrm{pH}\right)$, and three-dimensional diagrams ("stability field") were constructed for calcium oxalate monohydrate $CaC_2O_4{\cdot}H_2O$ (Fig. 1).

To evaluate the influence of acidic medium and the concentration of amino acid additives on the possible formation of sparingly soluble compounds in solution, we constructed graphical dependences (Fig. 2) of the index of supersaturation of these factors: $\mathrm{SI} = f(\mathrm{pH})$ and SI = $f(pC$ amino acid) It is assumed that if SI > 0, precipitation of this phase from the solution is thermodynamically more likely.

Medium acidity has the greatest influence on the thermodynamic stability, the state of the system, and processes of crystallization of the substances. Thus, with increasing pH values, the driving force of crystallization of $CaC_2O_4{\cdot}H_2O$ is greatly increased. The described regularity is explained by the fact that when pH increases, relative concentration of $C_2O_4^2$—in the system increases, so a positive correlation between the supersaturation of the medium and pH is observed.

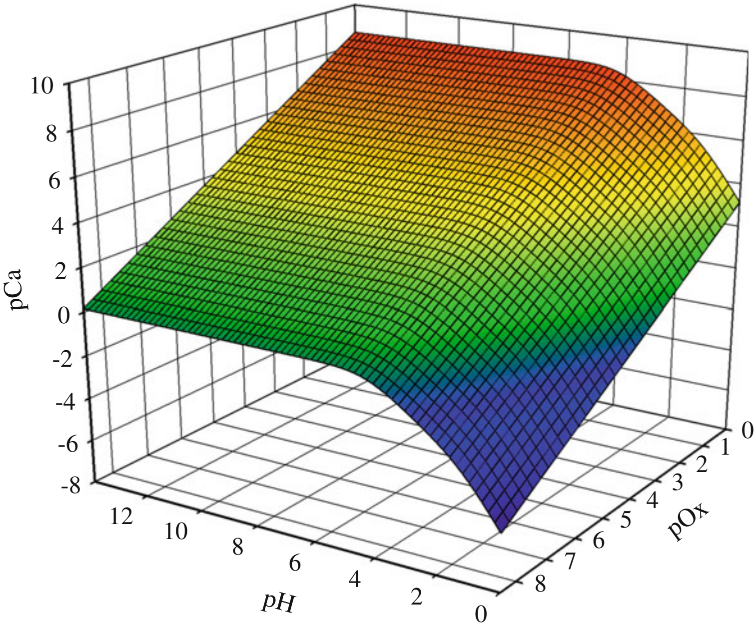


**Fig. 1** Field of stability in the formation of the poorly soluble compound system $Ca^{2+}–C_2O_4^{2-}–H_2O$

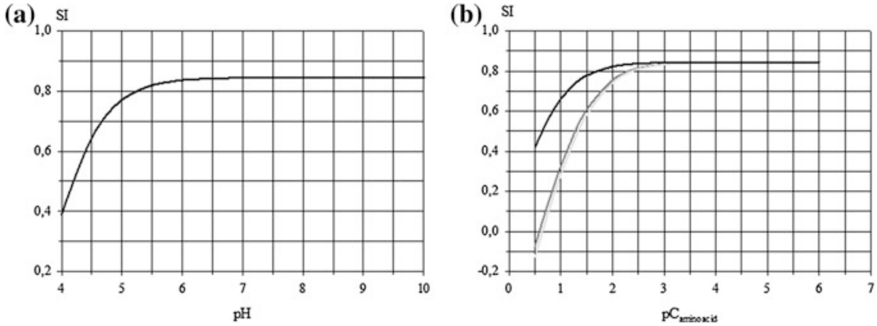


**Fig. 2** Plots of the SI index of supersaturation of calcium oxalate: **a** from pH solution; **b** from amino acids concentration: DL-glutamic acid; glycine; L-lysine

It was established that the introduction of amino acids in concentrations corresponding to physiological solution (0.004 mol/l) in the system, has little effect on the formation of mineral solids due to low values of stability constants of complexes with calcium ions for all considered amino acids.

It should be noted that the constructed thermodynamic model reflects the possibility of phase formation, only on the basis of data on their thermodynamic stability in the standard state, and does not take into account, in particular, kinetic factors influencing the formation of the solid phase in the real world. Therefore, conducting a model experiment in vitro is a necessary condition for determining the possibility of the formation of sparingly soluble compounds in solution, ionic composition (inorganic macro components), temperature, and pH close to the parameters typical to the biological fluid.

The study, with the help of X-ray analysis of the solid phases formed during crystallization from the model solution, showed that the obtained precipitate in all cases is calcium oxalate monohydrate (Rashkovich and Petrova 2006); whereas the presence of other phases, as impurities, was not detected (Fig. 3). Additionally, several studies have reported the formation of di- and tri-hydrate calcium oxalate in similar experiments.

The influence of pH (4.5; 6.0; 7.5) and temperature (20, 37, 58 °C) on the crystallization of calcium oxalate was studied. It is shown that the obtained precipitates are calcium oxalate monohydrate in all cases. The synthesis with additives of glycine, glutamic acid, and urea (their concentrations were chosen corresponding with their content in the biological fluid, human urine) indicate the formation of calcium oxalate monohydrate.

Thus, it can be concluded that the composition of deposition precipitate $CaC_2O_4 \cdot H_2O$ does not change under the chosen conditions and in the presence of these additives. The obtained results are consistent with the known data (Golovanova 2007; Palchik et al. 2006; Sevostyanova and Polienko 2004; Zuzuk 2005) that oxalate urinary stones, more than any other (phosphate stones and uric acid), are conservative with respect to the accumulation of micro-impurities.

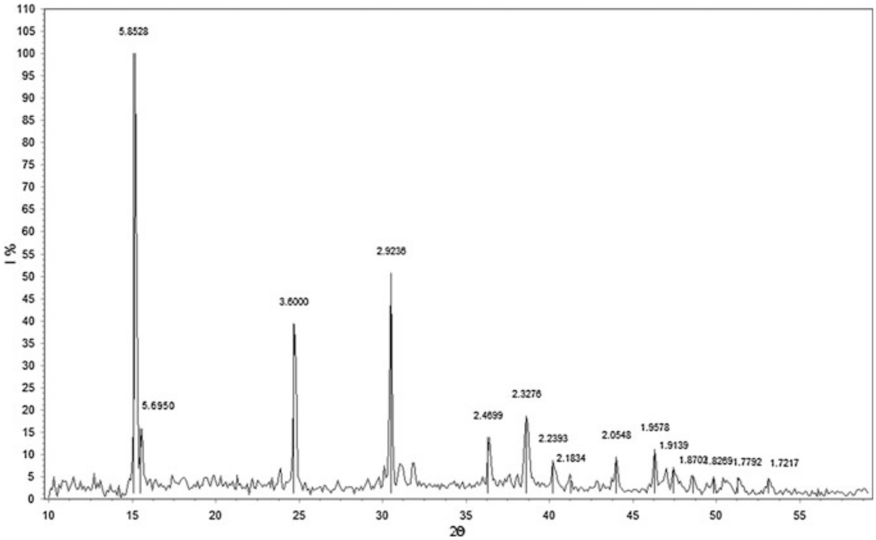


**Fig. 3** XRD pattern of calcium oxalate monohydrate

### 3.2 *Kinetics of Nucleation*

In the first stage the influence of supersaturation on the values of the induction periods of calcium oxalate monohydrate in the absence of extraneous additives was studied (Golovanova et al. 2004a). A linear relationship was obtained $\ln\,\tau = f\left((\ln\,\gamma)^{-2}\right)$. This nonstandard dependence, as it was shown, is a composition of two exponential dependences with differing values of the exponent. The determination of surface energy $\sigma$, a member of the constant $B$ for two plots of the kinetic curve, gives the values of 15.3 and 36.0 mJ/m$^2$ corresponding to heterogeneous and homogeneous nucleation, respectively.

These values are close to those obtained earlier (Golovanova et al. 2013), but significantly different from those found here, which can be explained by the absence of impurities in the system. The influence of organic additives of amino acids on the induction period of calcium oxalate monohydrate was very diverse and depended on the type of acid. The obtained data are shown in Fig. 4, illustrating the values of induction period of calcium oxalate monohydrate in the presence of amino acids.

Figure 4 shows that amino acids can both inhibit the crystallization of calcium oxalate monohydrate (DL-glutamic acid, glycine, L-lysine) and promote it (DL-valine). It is assumed that the inhibitory effect of amino acid is due to their adsorption on the active centers of the surface of the formative crystals. Adsorption is carried out due to the interaction between the positively charged surface of the crystals of calcium oxalate and the amino acid which is in the most probable conformation

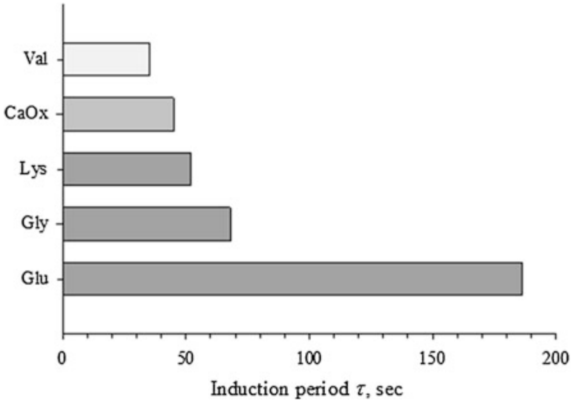


**Fig. 4** The influence of amino acids on the kinetics of nucleation of calcium oxalate: *Val* DL-valine; *Lys* L-lysine; *Gly* glycine; *Glu* DL-glutamic acid; *CaOx* calcium oxalate

under these coitiondns. In this case, we can expect that with the growth of inhibitory action, the content of amino acids in the kidney stones will increase. Indeed, at least for the basic amino acids, this is true (Golovanova et al. 2013b).

Comparative analysis of the effects of the studied amino acids on the induction period shows that this effect is probably connected with the main characteristics of the amino acids defining their adsorption on the surface of the crystals of calcium oxalate; i.e., with the structure (in particular, with the number of carboxyl groups) and with protolytic properties which determine the state and forms of locations of the amino acids in the solution at various values of pH. However, it is not yet possible to identify any general correlation between the characteristics of the amino acid and its effect on the nucleation of calcium oxalate monohydrate. Thus, the opposite effect of amino acids (similar in structure and properties) on the nucleation of calcium oxalate can be explained by the fact that both the inhibition and promotion of nucleation are realized by a single mechanism—tight binding of the amino acid with calcium ions on the surface of the nucleus (inhibition) or in the solution (promotion).

## *3.3 Kinetics of Crystallization*

According to the obtained kinetic curves (Fig. 5), the dependences were constructed according to the form $\lg(d\alpha/d\tau) - 2/3\lg\alpha = f(\lg(C_\tau - C_s))$ in which one can select several linear sections with different angles of inclination (Fig. 6). Plot A corresponds to the increase in the total number of particles due to the formation of nuclei; plot B corresponds to the growth of formed particles without increase in their total number; section C corresponds to secondary processes: reduction of the overall number of generating particles owing to the dissolution of small and growth of larger crystals, as well as aggregation of particles (Golovanova et al. 2013a).

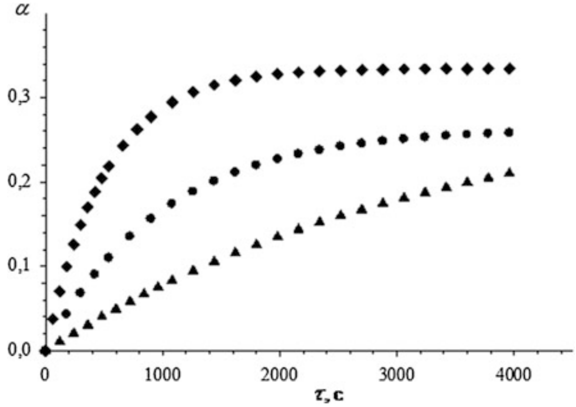


**Fig. 5** Kinetic curves of crystallization of calcium oxalate ($\gamma = 7$) in the presence of amino acids: *filled circle* without additives; *filled triangle* L-lysine; *filled diamond* DL-valine

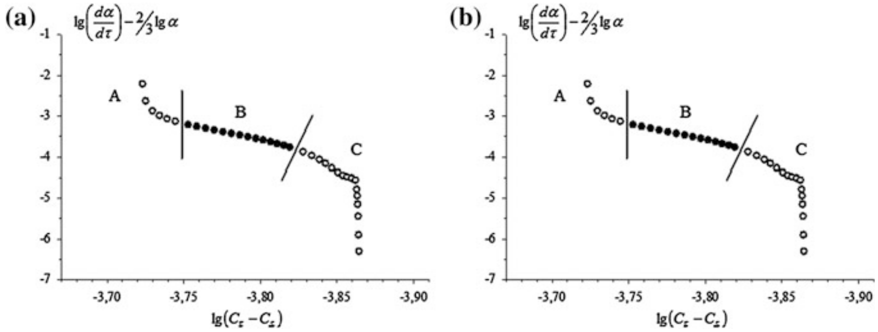


**Fig. 6** Kinetic parameters of crystallization calcium oxalate ($\gamma = 7$) in the presence of amino acids: **a** without additives; **b** in the presence of glycine

Plot B has the greatest interest for kinetics, which is why it is used for the calculation of basic kinetic parameters of calcium oxalate crystallization. The intersection of this segment with the ordinate axis gives the rate constant of deposition reaction, and the obliquity of the segment determines the order of the reaction. The constants lg $k'$ and $n$, defined during the processing of experimental data, are shown in Table 2.

First of all, one should note the great importance of the order of $n$-reaction in the crystallization of calcium oxalate. Apparently, this is a consequence of the approximation of power law dependence on the exponential law, describing kinetics of crystal growth by a mechanism of two-dimensional nucleation. For high supersaturation this mechanism of growth is quite real. It can be seen that with the increase of the initial supersaturation, the rate of crystallization increases. This can be explained by both the increase of the total number of nucleation centers and by the increase of the average rate of crystal growth.

**Table 2** Kinetic parameters of the process of calcium oxalate crystallization in the presence of amino acids

| Additive | Time of induction $\tau$, *c* | $\gamma = 5$ | | $\gamma = 7$ | | $\gamma = 10$ | |
|---|---|---|---|---|---|---|---|
| | | *n* | lg *k*′ | *n* | lg *k*′ | *n* | lg *k*′ |
| CaOx | 45 | 7.9 | 26.5 | 10.1 | 33.1 | 12.0 | 38.6 |
| Glu | 186 | 5.4 | 16.3 | 7.5 | 23.9 | 9.1 | 28.6 |
| Gly | 68 | 7.1 | 23.5 | 10.0 | 32.8 | 9.9 | 31.0 |
| Lys | 52 | 5.3 | 16.9 | 7.5 | 23.6 | 9.3 | 28.4 |
| Val | 35 | 8.0 | 27.0 | 10.2 | 34.1 | 11.6 | 36.9 |

The presence of amino acids in the solution has, as in the case of nucleation, a different effect on the process of crystallization of calcium oxalate. Glutamic acid, lysine, and glycine have an inhibitory effect; valine has an expressed promoting effect.

The inhibitory effect of amino acids is more obviously explained by their adsorption on the growing crystals of calcium oxalate monohydrate. Analyzing the structure of the amino acids and their state in the solution (Table 1), it can be concluded that the inhibition of the growth of the crystals of whewellite increases with the increase of the length of the hydrocarbon radical, growth of the number of carboxyl groups, and the amino acid presence in the solution at physiological values of pH in the form of charged ions.

On the other hand, the amino acids can serve as new centers of whewellite nucleation, which increase the number of crystals, as they are able to fix calcium ions. In addition, being adsorbed on the surface of crystals of calcium oxalate, the amino acids may stimulate two-dimensional nucleation, thereby increasing the growth rate of crystals. These effects explain the promotional effect of amino acids on the crystallization of whewellite.

These data indicate that the same amino acid can affect the crystallization both as an inhibitor and as a promoter. The predominance of a particular impact determines the subdivision of amino acids on promoters and inhibitors. This dual effect explains the contradictions observed in the literature in the views of different authors on the role of amino acids in the process of crystallization of calcium oxalate.

With the increase of initial supersaturation, inhibition of the growth of amino acids is enhanced. This behavior is unexpected, as usually an increase in supersaturation reduces the inhibition of crystallization with impurities. Thus, the dual effect of amino acids is due to the competition of effects of inhibition and promotion of crystallization. If the proposed mechanism for the acceleration of crystal growth is valid, then for a given amino acid, with increase of supersaturation, the promoting effect should be attenuated and the rate of two-dimensional nucleation increased rapidly). Consequently, the inhibition of crystallization of whewellite by amino acid is enhanced

It is assumed that the inhibitory effect of amino acids is due to their adsorption on active centers of the surface of the forming crystals, owing to the interaction between the positively charged surface of calcium oxalate and the amino acid, which is in the most probable conformation. Hereby, in the initial stage, the interaction of uncharged amino acid with positively charged surface of crystals of calcium oxalate is possible. This can lead to the binding of a carboxyl group with the calcium ion, which causes deprotonation of the second carboxyl group and its interactions with another calcium ion. Additional stabilization of forming surface compounds may be due to the ability of amino acids with two or more carboxyl groups to form a multidentate chelate complex with calcium ions. Thus, the internal complexation, protonation of amino acid, and stereochemical factors play a significant role in the process of adsorption of amino acids on the surface of crystals of calcium oxalate.

To confirm the possibility of the adsorption influence of amino acids, the adsorption of glutamic acid on synthesized calcium oxalate monohydrate has been studied. The obtained experimental data show that adsorption can be described within the Langmuir model ($R^2 = 0.994$), and the Freundlich model ($R^2 = 0.985$).

According to the Langmuir equation $1/G = 1/G_\infty + 1/G_\infty КC$, $G_\infty$ value was calculated corresponding to the complete monomolecular coating of surface (Table 3).

It can be considered that in experiments at the maximum concentration of glutamic acid in the solution equal to 0.020 M, almost saturated monolayer is formed on the surface of the crystals, as the value of $G_{max}$ is very close to the calculated $G_\infty$ ($G_{max} = 0.010$ mol/kg, $G_\infty = 0.012$ mol/kg). The constant of adsorption, obtained from the Langmuir equation is very high ($K = 227.7$ 1/M), which indicates a strong adsorption of glutamic acid on the crystals of calcium oxalate.

The Freundlich equation $\ln G = \ln k + (1/n) \ln Cp$ enables indirect characterization of the value of the specific surface area of the adsorbent through the constant $k$ (Table 4).

The low value of the coefficient $k$ in the Freundlich equation indicates good crystallinity of the adsorbent. The adsorbent is synthesized from calcium oxalate monohydrate.

The obtained results of adsorption explain the strong inhibitory effect of amino acids on the processes of crystallization of calcium oxalate. Based on this, the

**Table 3** The calculation of the coefficients in the Langmuir equation

| Equation | $1/G_\infty$, kg/mol | $G_\infty$, kg/mol | $1/К \cdot G_\infty$, kg/mol | К, 1/M |
|---|---|---|---|---|
| $Y = 83.5X + 0.366$ | 83.5 | 0.012 | 0.366 | 227.7 |

**Table 4** The calculation of coefficients in the Freundlich equation

| Equation | $1/n$ | $n$ | $\ln k$ | $k$ |
|---|---|---|---|---|
| $Y = 0.38X + 3.01$ | 0.38 | 2.63 | −3.01 | 0.5 |

concentrations of amino acids in saline are sufficient to inhibit the processes of nucleation and for the growth of crystals of whewellite. In the process of stone growth, the concentration of lithogenic components and pH of the physiological solution change. Temporary decrease in supersaturation of urine in calcium oxalate to level $\gamma = 7$ will result in the normal amino acid composition of urine, in a complete inhibition of the stone growth owing to the adsorption of the impurity and deposition of organic matter on the surfaces of the stone. The increase in supersaturation (according to the obtained data to $\gamma \geq 10$) will cause a zone of regrowth with the capture of organic matter. As a result, there is a thin oscillating zone of organic matter which is characteristic of calcium oxalate stones (Izarulina et al. 2014).

Strong affinity of amino acids with dicarboxylic group to oxalate monohydrate indicates that the proteins, rich in these amino acids, play a functional role in the pathogenic formation of kidney stones.

With IR spectroscopy it was shown that these amino acids are actually adsorbed on powders of calcium oxalate monohydrate: on the spectra of the adsorbent there are characteristic amino acid bands at 1200–1000 cm$^{-1}$, 3300–3200 cm$^{-1}$ and 1400–1300 cm$^{-1}$ (Fig. 7).

The study of the conditions of calcium oxalate formation at reduced concentration of glutamic acid, up to 11 times its physiological pH, enables establishing the fact of its formation, within these conditions, of the mixture of monohydrate and dihydrate calcium oxalate (Fig. 8). Such combination is often found in the composition of urinary stones (Golovanova 2007, 2008; Palchik et al. 2006; Sevostyanova and Polienko 2004; Zuzuk 2005).

In the samples at the concentration of glutamic acid less than the physiological pH of 6–8, crystals of calcium oxalate dihydrate of various sizes appear. And at the concentration of 10–11, the microparticles have approximately the same sizes, although their number increases.

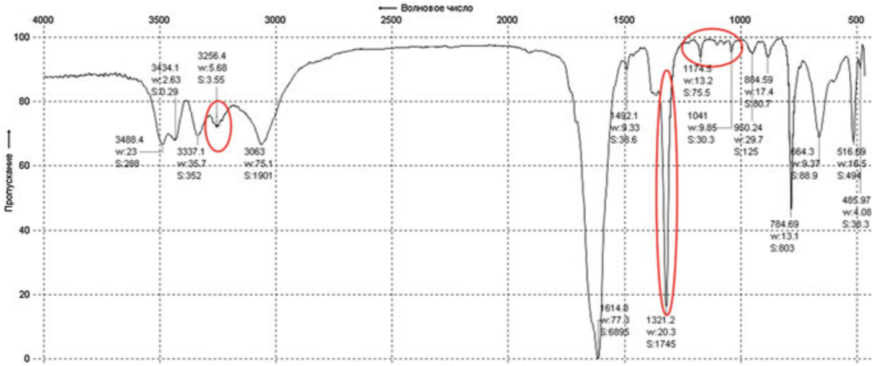


**Fig. 7** IR-spectrum of the synthesized powder of whewellite after the adsorption experiment with glutamic acid. The bands of absorption of amino acids are *marked*

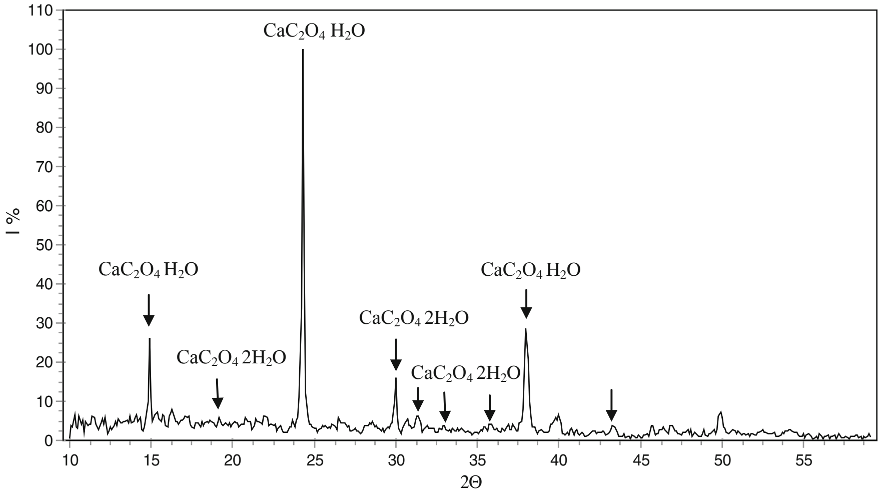


**Fig. 8** X-ray powder diagram of calcium oxalate in the presence of the glutamic acid in the solution

It is known that in urinary stones, rhythmic alternation of monohydrate and dihydrate zones of calcium oxalate is often observed. The structure of the stone may be of bi-zonal texture with whewellite nucleus and weddellite outer area that sometimes takes up to 3/4 diameter of the stone. Weddellite is a metastable phase in the presence of stone formation, and usually its crystals are replaced by fine-grained crystals whewellite (Izarulina et al. 2014).

# 4 Conclusion

1. The possibility of phase formation in the $Ca^{2+}$–$C_2O_4^{2-}$–$H_2O$–amino acid, the nature of crystallizing compounds, their thermodynamic stability, as well as the depth of the deposition reaction is determined mainly by the value of medium acidity. It was established that the role of amino acids as a ligand in the processes of complexation in the system has a slight impact on the formation of mineral rigid phase due to low values of the constants of stability of complexes with calcium ions.
2. In the study of the nucleation processes of calcium oxalate monohydrate in model solutions without impurities and with additives of amino acids in concentrations close to physiological, the following results were obtained:

- in solutions without impurities, the transition from heterogeneous to homogeneous nucleation at the increase of the supersaturation in excess of $\gamma = 12$ is observed; the effective surface energy increases more than twofold;
- various amino acids have both inhibiting and promoting action on the nucleation of calcium oxalate monohydrate;
- the increase of inhibitory properties of amino acids correlates with the increase of their content in calcium oxalate kidney stones.

3. Studies of the kinetics of crystallization of calcium oxalate monohydrate in the presence of amino acids additives in concentrations close to the physiological, gave the following results:
   - the growth of crystal of whewellite occurs by the mechanism of two-dimensional nucleation;
   - different amino acids have both inhibiting or promoting action on the crystallization of whewellite;
   - the influence of amino acids on the growth of crystals of whewellite coincides with their effect on the induction time of nucleation of whewellite;
   - the inhibiting properties of amino acids increase and promoting ones fall with the increase of supersaturation owing to the competition of these effects;
   - both effects can be explained in the adsorption of amino acids on the crystals of whewellite: inhibition by preventing the points of growth, promotion by the creation of centers of two-dimensional nucleation on the surface of crystals.

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