UDC 544.774.4.548.5

Effect of Amino Acids on the Crystallization Kinetics of Calcium Oxalate Monohydrate

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(Received December 10, 2012; revised July 2, 2013)

Abstract

An integrated set of problems connected with the features of calcium oxalate crystallization in the presence of amino acids is considered. It is established with the help of X-ray phase analysis that the formed precipitates are represented by calcium oxalate monohydrate (wavellite). The kinetic parameters of crystallization were determined. It was shown that the growth of crystals follows the mechanism of two-dimensional nucleation. It was established that amino acids have different effects on crystallization: glutamic acid, arginine, glycine, and lysine inhibit the process, while proline, serine, asparagine serve as the catalysts of the process; some amino acids (alanine, phenylalanine *etc.*) have insignificant effect on the kinetics of crystal growth. With an increase in supersaturation, the promoting action of amino acid weakens, while the inhibiting action enhances, which is connected with the competition of these effects.

Key words: calcium monohydrate oxalate, growth, adsorption, amino acids, inhibition, crystallization

INTRODUCTION

The problem of stone formation is becoming increasingly tangible and real threat to modern inhabitants of all countries, especially in megapolitan areas. According to the statistical data, the rate of propagation of various diseases leading to the formation of stones in human organs [1?5]. The risk group included American and industrially developed countries of Europe including Russia. In the opinion of a number of official experts, at present every seventh person can potentially suffer from these diseases. So, stone formation is an urgent problem of the global scale.

At the same time, modern medicine has not established in full the reasons of the formation of various stones in human organism. A wide spread occurrence of urolithiasis in cities is considered to be connected with an increase in the consumption of food rich in fat and animal proteins, as well as with negative ecology in large cities.

Pathogenic organomineral aggregates (OMA) have complicated and inhomogeneous composition [3–6] and are composed mainly of calcium oxalates – wavellite $CaC_2O_4 \cdot H_2O$ and weddellite $CaC_2O_4 \cdot 2H_2O$ [4–8] the most frequent components of the stones of urogenital system [2–7]. In addition, calcium oxalates can be components of dental and bile stones, salivary gland calculi. In addition, calcium oxalates were detected in mineral deposits in lungs, vessels, spleen, prostate gland, pancreas, in muscles and joints [8].

It was established that the formation of OMA of any composition, in particular oxalate

calculi, is to a substantial extent determined by the organic components of physiological fluids [9]. Especially important role is played by amino acids; their concentration in the organic matter of nephroliths reaches 70 % [10–15].

By present, many works have been published on crystallization of wavellite and weddellite in physiological solutions of complicated composition, in particular in the presence of amino acids [16–22]. At the same time, available data on the effect of amino acids on the nucleation and growth of calcium oxalate crystals are very contradictory, and their amount is surely insufficient to understand the regularities of stone formation in human organisms. The necessity of further investigation of this problem both from the medical viewpoint, to prevent the formation of oxalate biominerals in human organism, and for the development of the fundamental problem of biomineralization.

Previously we studied phase formation and crystallization in oxalate solutions containing amino acids [18, 21–23]. For example, in [23] we studied the effect of a broad range of amino acids found in nephroliths (14 compounds) on wavellite nucleation process.

The goal of the present work was to study the growth kinetics of wavellite crystals in the presence of amino acids of the same set in order to obtain complete information on the action of these physiological admixtures on the regularities of oxalate mineralization.

EXPERIMENTAL

Calcium oxalate crystallization was studied at a temperature of 37 °C and three values of solution supersaturation $\gamma = C_0/C_s$ equal to 5, 7, 10 (C_0 is oxalate concentration in supersaturated solution, C_s is solubility of calcium oxalate, which is equal to $0.5 \cdot 10^{-4}$ mol/L). The chosen values are characteristic of biological media, namely for urine of a healthy adult average statistical human [2].

Supersaturation with respect to calcium oxalate was created due to the chemical reaction $Ca^{2+} + C_2O_4^{2-} \rightarrow CaC_2O_4$, which was carried out by mixing initial solutions with the stoichiometric composition of readily soluble compounds – calcium chloride and aluminium oxalate. Twice distilled water was used as a solvent. Salts of the ch. d. a. grade were taken to prepare the solutions. In view of the fact that calcium chloride is able to get hydrated during storage, standardization of its solution by complexometric titration with Trilon B was carried out before experiments [24].

Initial solutions were mixed in equivalent volumes. The ready solution was poured into a conical flask and mixed for 5–10 s. Crystallization was carried out *in vitro* both in the solutions of pure calcium oxalate and in its solutions with amino acids added in the concentration of 0.004 mol/L, which corresponded to their concentration in physiological solution. Amino acid admixtures were introduced into calcium chloride solution before mixing the initial solutions.

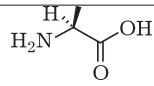
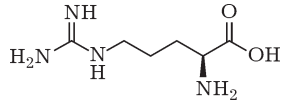
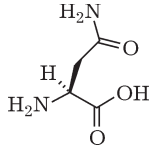
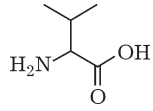
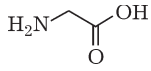
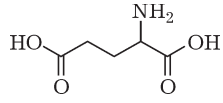
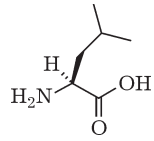
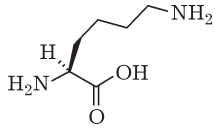
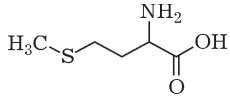
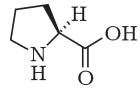
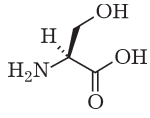
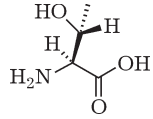
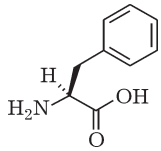
The mineral (phase) composition of the precipitates obtained during the synthesis was studied by means of X-ray phase analysis. Diffraction patterns were obtained using the powder method with the help of DRON-3 X-ray apparatus. Qualitative analysis of the phase composition of samples was carried out by comparing the experimental values of interplanar spacings and relative intensities of diffraction maxima with the set of the corresponding reference values for each of the assumed phases. Phase identification was carried out using the international database ASTM.

In order to obtain the information on the interaction of amino acids with calcium oxalate crystals, experiments on amino acid adsorption on the synthesized samples were carried out. The procedure and experimental results were considered in [26]. To confirm adsorption of amino acids, we used infrared spectroscopy. The IR spectra were recorded with a FT-801 spectrophotometer. The samples were prepared by means of pressing in tablets with KBr. The spectra of samples under study were recorded within the range from 4000 to 470 cm^{-1}. Interpretation was performed with the help of ZaIR 3.5 software with the database containing more than 130 000 spectra.

The kinetics of crystallization was studied by monitoring the concentration of solution during the precipitation of calcium oxalate. The concentration was determined using the conductometric method by measuring specific conductance (SC) of solution in the Kohlrausch cell with automatic multiplication of the results by

TABLE 1

Amino acids used as admixtures and their characteristics [25]

Amino acids	Structure	Isoelectric point pI	pK_a
L-Alanine		6.00	2.33 9.71
L-Arginine		10.76	2.03 9.00 12.10
L-Asparagine		5.41	2.16 5.41 8.72
DL-Valine		5.96	2.27 9.52
Glycine		5.97	2.34 9.58
DL-Glutamic acid		3.22	2.16 4.15 9.58
L-Leucine		5.98	2.32 9.58
L-Lysine		9.74	2.15 9.16 10.67
DL-Methionine		5.74	2.16 9.08
L-Proline		6.30	1.95 10.47
L-Serine		5.68	2.13 5.68 9.05
L-Threonine		5.60	2.20 5.53 8.96
L-Phenylalanine		5.48	2.18 9.09

the cell constant. Anion-4154 conductometer was used. The range of measured EC of solution was 0.001–100 mS/cm. The limits of admissible values of basic error during SC measurement was ±2.0 % (but not less than 0 μS/cm), the error of automatic temperature compensation during SC measurement did not exceed ±2.5 %. The sensor constant $K = (1\pm0.2)\ \mathrm{cm}^{-1}$. Experiments were carried out within the supersaturation range with the linear dependence of SC on concentration.

The completeness of crystallization (α) depending on time was calculated from the conductometric data:

$$\alpha = C_0 - C_\tau/(C_0 - C_s) \quad (1)$$

Here C_0 is the initial concentration of calcium oxalate in supersaturated solution; C_τ is calcium oxalate concentration at a moment of time (τ); C_s is solubility of calcium oxalate.

To determine the kinetic parameters of the growth of calcium oxalate crystals, precipitation rate was calculated from the dependence $\alpha = f(\tau)$ as a function of the current absolute supersaturation $(C_\tau - C_s)$ according to equation

$$d\alpha/d\tau = kA(C_\tau - C_s)^n \quad (2)$$

where k is the rate constant of the reaction; A is total area of the precipitate; n is reaction order. Total area of surface A for the constant shape of particles is defined as

$$A = \beta N_\tau^{1/3} V_\tau^{2/3} \quad (3)$$

where β is shape factor; N_τ is total number of particles; V_τ is the volume of precipitate at time (τ). Taking into account that $\alpha = V_\tau/V_{max}$ and V_{max} is the maximal volume of precipitate after complete drop of supersaturation, finally after transformations we obtain the equation to calculate the kinetic characteristics of calcium oxalate crystallization (accepting a constant number of particles $N_\tau = N = \mathrm{const}$):

$$(d\alpha/d\tau)\alpha^{-2/3} = k'(C_\tau - C_s)^n \quad (4)$$

where parameter k' includes all constants (V_{max}, β, N and k) and is constant for the given initial conditions. Taking the logarithm we obtain

$$\log(d\alpha/d\tau) - 2/3\log\alpha = \log k' + n\log(C_\tau - C_s) \quad (5)$$

Plotting the dependence in the coordinates $\log(d\alpha/d\tau) - 2/3\log\alpha = f(\log(C_\tau - C_s))$ is to give a straight line. The intercept at the ordinate axis is $\log k'$, while the tangent of the slope angle corresponds to the order of growth rate (n).

RESULTS AND DISCUSSION

The XPA investigation of solid phases formed during crystallization showed that the precipitate is calcium oxalate monohydrate – wavellite – in all the cases; other phases were not detected as admixtures. At the same time, some works [27, 28] report the formation of calcium oxalate di- and trihydrates in similar experiments.

The examples of recorded kinetic curves are shown in Figs. 1, 2. One can see that the transformation degree increases monotonously with time; the process slows down gradually. This is caused, first of all, by saturation drop and corresponding decrease in the driving force of crystallization, second, by the ability of calcium oxalate to form stable supersaturated solutions. It is typical that even for small a values crystallization is almost ceased.

The kinetic data thus obtained were treated with the help of the algorithm described in the

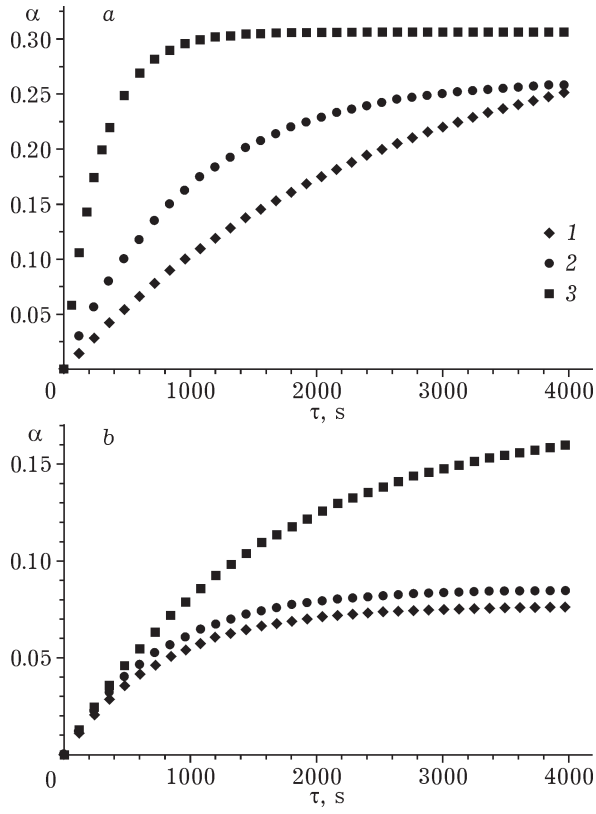


Fig. 1. Kinetic curves of calcium oxalate crystallization (α) at different supersaturation values γ without amino acids (*a*) and in the presence of DL-glutamic acid (*b*); γ values: 5 (*1*), 7 (*2*), 10 (*3*).

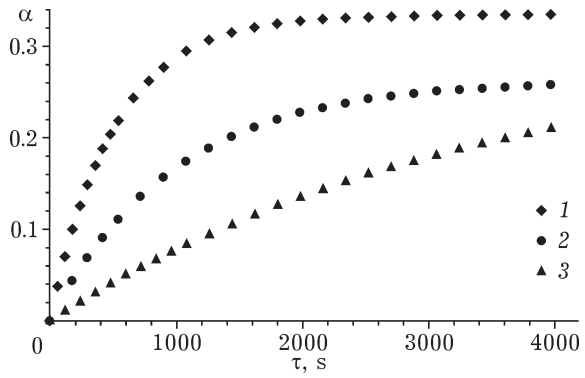


Fig. 2. Kinetic curves of calcium oxalate crystallization (γ = 7) in the presence of amino acids: *1* – without additives; *2* – L-lysine; *3* – DL-valine.

previous section describing the procedures. Dependences plotted in coordinates $\log(d\alpha/d\tau) - 2/3\log\alpha = f(\log(C_\tau - C_s))$ are represented by curves on which several linear regions with different slopes may be distinguished (Fig. 3). Region A corresponds to an increase in the total number of particles due to the formation of crystallization nuclei; region B corresponds to the growth of the formed particles without an increase in their total number; region C corresponds to secondary processes – a decrease in the total number of formed particles due to the dissolution of small crystals and the growth of larger crystals, and particle aggregation [21]. To study the regularities of crystal growth, region B is of interest, so it is used further on to calculate the basic kinetic characteristics of calcium oxalate crystallization.

The constants $\log k'$ and n, determined as a result of the treatment of experimental data, are listed in Table 2. One can see that with an increase in initial supersaturation crystallization rate increases. This may be explained by an increase in the total number of crystallization centres. Assuming the number of crystallization centres (N) to be proportional to the nucleation rate (J_s): $N \sim J_s \sim \exp[-B/(\ln\gamma)^2]$ [29], a linear dependence of $\log k'$ on $1/(\ln\gamma)^2$ should be obtained. Indeed, for pure calcium oxalate and a half of amino acids studied in the present work (L-arginine, DL-valine, DL-glutamic acid, L-lysine, L-proline, L-threonine) this dependence is fulfilled (Fig. 4, *a*). For other amino acids, the

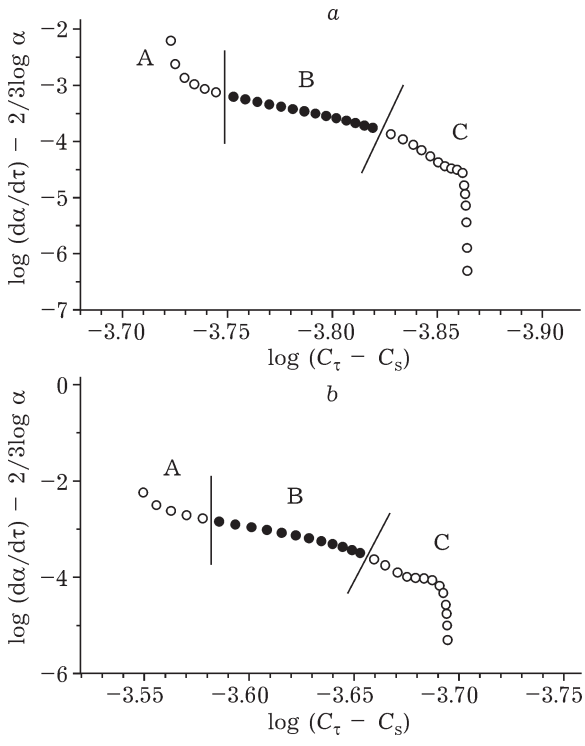


Fig. 3. Determination of the kinetic parameters of calcium oxalate crystallization (γ = 7) without amino acids (*a*) and in the presence of L-proline (*b*).

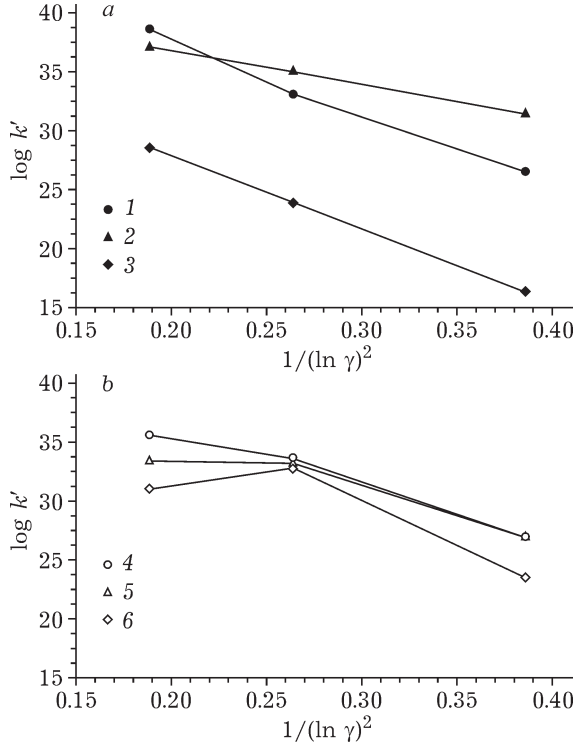


Fig. 4. Example of the $\log k' = f(1/(\ln\gamma)^2)$ dependence behaviour: *a* – straight line dependence (*1* – without additives; *2* – L-proline; *3* – DL-glutamic acid); *b* – dependence differing from the straight line (*4* – DL-methionine; *5* – L-alanine; *6* – glycine).

TABLE 2

Kinetic characteristics of calcium oxalate crystallization in the presence of amino acids

Additives	$\gamma = 5$		$\gamma = 7$		$\gamma = 10$	
	n	$\log k'$	n	$\log k'$	n	$\log k'$
Without additives	7.9	26.5	10.1	33.1	12.0	38.6
L-Alanine	8.0	26.9	10.1	33.2	10.7	33.4
L-Arginine	5.7	17.2	7.6	23.9	9.0	28.1
L-Asparagine	8.0	27.3	10.0	32.9	10.9	34.3
DL-Valine	8.0	27.0	10.2	34.1	11.6	36.9
Glycine	7.1	23.5	10.0	32.8	9.9	31.0
DL-Glutamic acid	5.4	16.3	7.5	23.9	9.1	28.6
L-Leucine	7.9	26.7	10.1	33.6	10.5	33.4
L-Lysine	5.3	16.9	7.5	23.6	9.3	28.4
DL-Methionine	7.9	26.9	10.1	33.6	11.2	35.6
L-Proline	9.1	31.4	10.6	35.0	11.8	37.1
L-Serine	8.1	27.0	10.1	33.5	10.9	34.5
L-Threonine	8.0	26.5	10.0	33.2	12.1	38.5
L-Phenylalanine	8.0	26.8	10.2	33.5	10.6	33.3

dependence comes to the plateau for $\gamma = 10$, which is connected with the increase in the inhibiting effect of amino acids with an increase in supersaturation (see Fig. 4, *b*).

For the dependence of $\log k'$ on supersaturation (γ) $n >> 2$, which is incompatible with the dislocation mechanism of crystal growth. In addition, fort his system, parameter n increases with an increase in initial supersaturation. This may be due to the power approximation of the exponential law describing the mechanism of two-dimensional nucleation [30]. Favouring this assumption is the fact that for large residual a values crystallization is ceased due to the existence of critical supersaturation for the case of two-dimensional nucleation.

The presence of amino acids has different effects (inhibiting or promoting) on the growth of calcium oxalate crystals. For instance, glutamic acid, lysine, arginine and glycine possess the strongest inhibiting effect. Clearly pronounced promoting action is exhibited by proline, valine, serine and asparagine. Other amino acids have only insignificant effect on the kinetics of calcium oxalate crystallization. With an increase in initial supersaturation, braking of the growth by amino acids increases; promoters and neutral amino acids become inhibitors (see Table 2). This is a contradiction to the known regularities [31] and is likely to be connected with the competition between the effects of promotion and inhibition of the growth by one and the same amino acid. In reality, an increase in supersaturation weakens the promoting effect; as a result, inhibition increases.

A comparison of the action of amino acids on the kinetics of wavellite crystal growth and on induction time determined by us previously [23] shows that for the majority of the studied amino acids these effects coincide. Only three of them (leucine, alanine and methionine) are neutral with respect to growth kinetics, and are substantial inhibitors with respect to induction

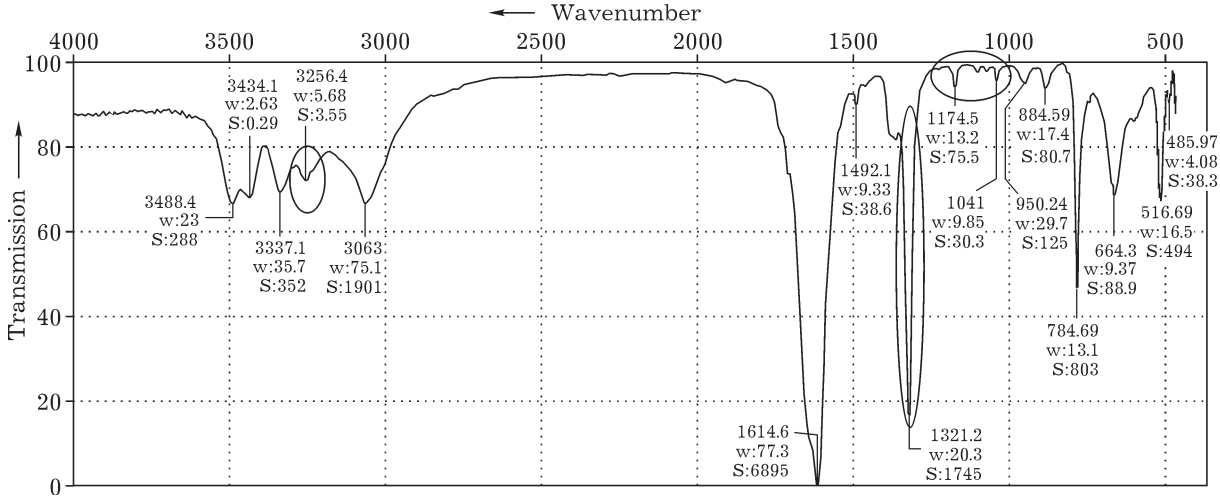


Fig. 5. IR spectrum of wavellite with glutamic acid as additive.

times. However, these admixtures, too, poison the growth at maximal supersaturation values ($\gamma = 10$). Only threonine falls out of the general regularity: it does not affect growth kinetics but decelerates crystal nucleation strongly. This may be connected with the structure of this compound and requires further investigation.

The inhibiting action of amino acids may be explained by their adsorption on the growing crystals of calcium oxalate monohydrate. Adsorption of so strong inhibitors as glutamic acid and lysine on wavellite crystals is confirmed by means of IR spectroscopy (Fig. 5).

As a result of identification of the spectra of calcium oxalate monohydrate preparations on which the sorption of amino acids was carried out, the bands at 1200–1000, 3300–3200 and 1400–1300 cm^{-1} were detected. These bands are characteristic of amino acids [32]. It was also established that adsorption is satisfactorily described within Langmuir's model [26].

At the same time, amino acids adsorbed on crystals may serve as the centres of formation of two-dimensional nuclei because they are able to bind calcium ions. This may explain the promoting action of amino acids and a competition between inhibition and promotion.

Analyzing the structure of amino acids and their state in solution at pH 5.0–7.0 [25], it may be concluded that the inhibition of wavellite crystal growth increases with an increase in the length of hydrocarbon radical, an increase in the number of carboxyl groups in amino acid and the presence in the form of charged ions in the solution at physiological pH values.

Analysis of the plotted ion diagrams showed that amino acids inhibiting the crystallization (glutamic acid, lysine, arginine) at pH values thermodynamically optimal for the formation of calcium oxalate monohydrate [4] are present in solution in the form of charged ions (Fig. 6, *a* and *b*). Other amino acids are present at these pH values in the form of neutral molecules (zwitter ions) (see Fig. 6, *c* and *d*).

CONCLUSION

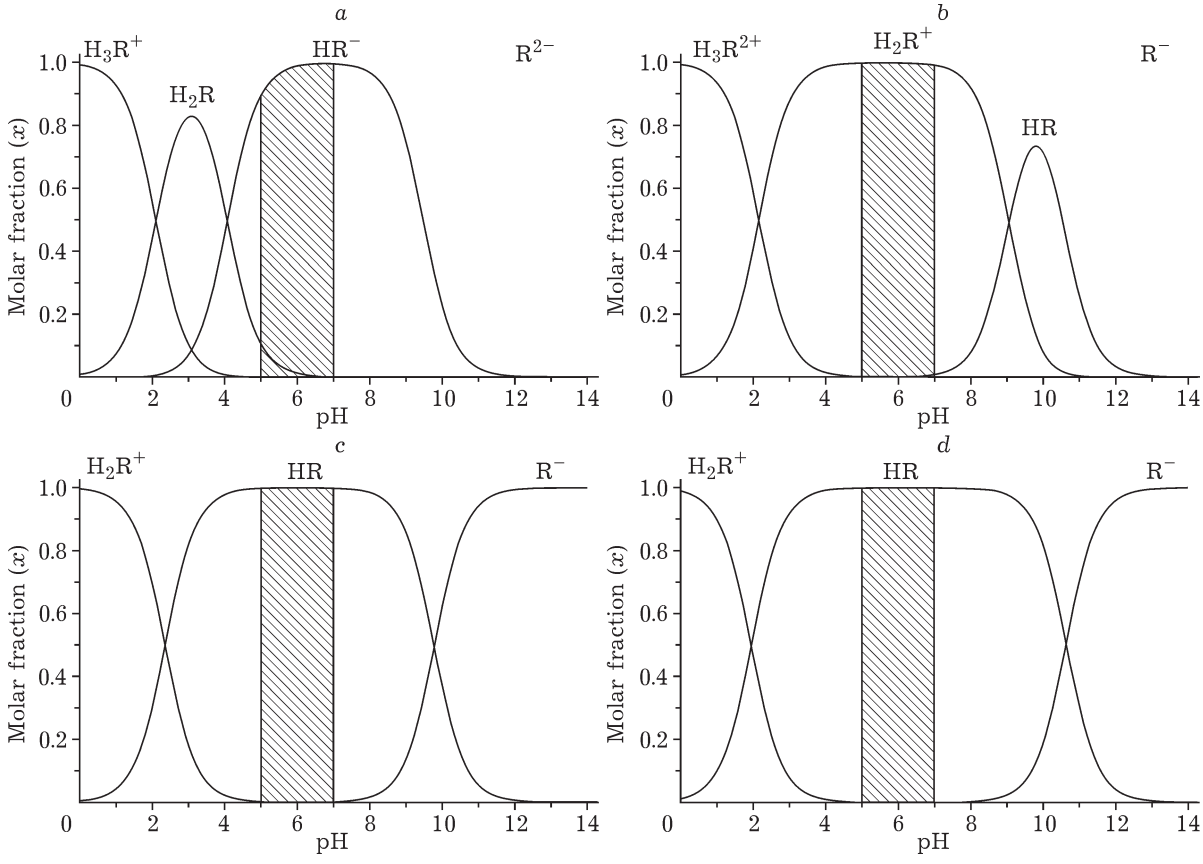


Fig. 6. Ion diagrams of amino acids: *a* – glutamic acid; *b* – lysine; *c* – glycine; *d* – proline.

The following conclusions may be drawn from the results of the investigation into the kinetics of calcium oxalate monohydrate crystallization kinetics in the presence of 13 amino acids as additives in the concentrations close to the physiological ones.

1. The growth of wavellite crystals proceeds according to the mechanism of two-dimensional nucleation.

2. Different amino acids have either inhibiting or promoting action on wavellite crystallization.

3. The effect of amino acids on the growth of wavellite crystals mainly coincides with the previously established effect of amino acids on the induction time of wavellite nucleation.

4. Inhibiting action of amino acids increase and promoting properties decrease with an increase in supersaturation, which is connected with the competition between these effects.

5. Both effects are explained by the adsorption of amino acids on wavellite crystals: inhibition is due to blockage of growth sites, while promotion is due to the formation of the centres of two-dimensional nucleation on the surface of crystals.

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