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Biomimetic crystallization of calcium oxalate monohydrate in the presence of group B vitamins

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Studied is the influence of group B vitamins (B1, B6, B12) on the morphology of calcium oxalate monohydrate (COM), the nucleation processes and the value of surface energy. It is revealed that the addition of B1 and B6 vitamins (c = 5–50 mM) into COM solution leads to inhibition of the growth of the crystals and diminution of their size proportionally to the increase of the addition concentration. The degree of COM crystal growth inhibition at the introduction of B1 and B6 exceeds 95 %. The value of COM surface energy is found to decrease in the presence of the vitamins in 40–50 mM concentrations.

Keywords: calcium oxalate monohydrate, crystal growth, vitamins, inhibitory effect, induction time.

Біоміметична кристалізація кальцію оксалату моногідрату у присутності вітамінів групи В. *Ю.В.Таранець, І.М.Притула, О.М.Безкровна*.

Досліджено вплив вітамінів групи В (В1, В6, В12) на морфологію кристалів кальцію оксалату моногідрату (COM), процеси нуклеації та величину поверхневої енергії. Виявлено, що додавання вітамінів В1 і В6 (c = 5–50 ммоль/л) в розчин COM призводить до пригнічення росту кристалів і зменшення їх розмірів пропорційно збільшенню концентрації домішки. Ступінь інгібування росту кристалів COM при введенні В1 і В6 становить понад 95 %. Встановлено зниження величини поверхневої енергії кристалів COM у присутності вітамінів у концентрації 40–50 ммоль/л.

В работе исследовано влияние витаминов группы В (В1, В6, В12) на морфологию кристаллов кальция оксалата моногидрата (COM), процессы нуклеации и величину поверхностной энергии. Выявлено, что добавление витаминов В1 и В6 (c = 5–50 ммоль/л) в раствор COM приводит к ингибированию роста кристаллов и уменьшению их размеров пропорционально увеличению концентрации добавки. Степень ингибирования роста кристаллов COM при введении В1 и В6 составляет более 95 %. Установлено снижение величины поверхностной энергии COM в присутствии витаминов в концентрации 40–50 ммоль/л.

1. Introduction

Human organism is a complicated biosystem with continuously running significant biochemical processes. These processes are closely interconnected, so a disruption in one of them leads to violation of integrity in functioning of the whole of the system followed by numerous consequences. A vivid example of such violations is onset of pathogenic crystallization which results in formation of urinary stones, kidney stones, gallstones, etc. Therefore, establishment of mechanisms of their formation and search for the molecules which influence such a

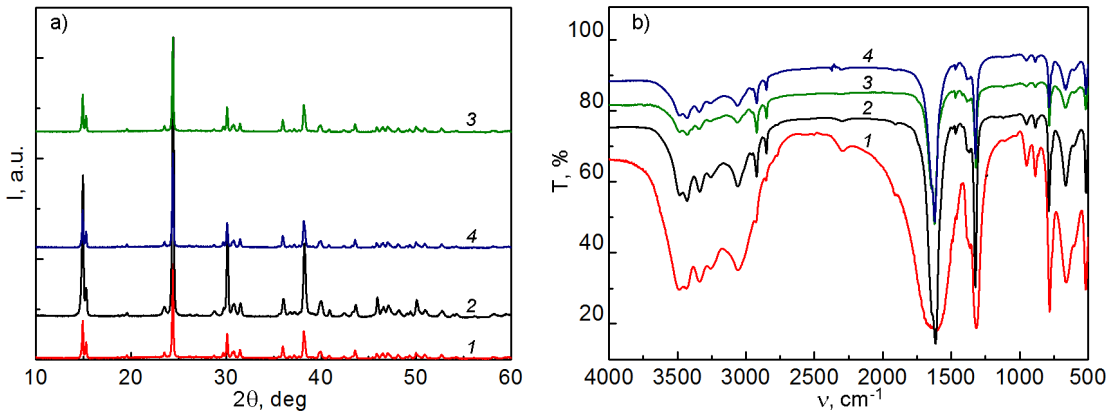

Fig. 1. XRD pattern (a) and IR-spectra (b) of COM crystals grown ($[Ca^{2+}]/[C_2O_4^{2-}]$ = 20:1, s = 4.6) without additives (*1*), with 10 mM B1 (*2*), with 10 mM B6 (*3*), with 20 μM B12 (*4*).

phenomenon, belong to topical present-day problems.

In the present work we studied the processes of crystallization of calcium oxalate monohydrate (COM), one of main components of kidney stones [1, 2], in the presence of water-soluble group-B vitamins (B1, B6, B12). Earlier in the literature there were reported investigations of the influence of proteins, polysaccharides and surface-active substances on the formation of COM crystals [3–7]. Up to now the role of vitamins in the processes of nucleation and growth of COM has been described insufficiently. There are only available clinical trials concerning the influence of C, E and A vitamins [8, 9]. As is known, vitamins essentially influence various physiological processes which take place in human organism. In particular, they maintain the functioning of nervous, immune, cardiovascular systems, participate in hematopoiesis, raise resistance to radionuclides. Many vitamins are not synthesized in human organism, so they are to come from food, or to be introduced in the form of nutritional additives. Shown in [10] is the relation between vitamin deficiency and development of stone formation in animals. In particular, for 43 % of animals that did not receive vitamin A with food there was revealed the appearance of stones in kidneys and bladder. According to clinical trials, A and E vitamins are able to raise excretion of oxalate in physiological fluid and risk of COM crystallization [8, 11]. At present the influence of the vitamin C is considered ambiguous. As reported in [11], intake of this vitamin is not associated with risk of the formation of pathogenic aggregates. The influence of group-B vitamins (B1, B6, B12) on the processes of COM formation has not been studied so far.

In this connection, the goal of the present work was to investigate the influence of water-soluble group B vitamins on the crystallization processes, morphology and nucleation kinetics of COM crystals.

2. Experimental

COM crystals were obtained by the reaction of interaction between potassium oxalate and calcium chloride under controlled conditions close to physiological ones [12–14]. The molar concentration ratio for calcium and oxalate ions $[Ca^{2+}]/[C_2O_4^{2-}]$ was 20:1. The solution supersaturation (s) was calculated according to the formula earlier applied in [2, 15]. B1, B6, B12 vitamins were used in liquid form as the solutions of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamine, respectively. The concentration of B1 and B6 vitamins in COM model system varied within the range from 5 to 50 mM, the concentration range of B12 being 10–100 μM, that did not exceed the biological concentrations of each of these vitamins in the organism [16, 17]. Formation of the phase of calcium oxalate was confirmed by the methods of X-ray structure analysis (using a Siemens D500 diffractometer) and IR-spectroscopy (on a Spectrum One PerkinElmer spectrophotometer). The morphology of COM crystals was studied by means of scanning electron microscopy (using a scanning microscope JSM-6390LV). The nucleation kinetic for the model COM solutions was established by the turbodimetric method (on a spectrophotometer Optizen 3220UV type) by measuring the optical density of the solutions at 620 nm wavelength [18–22]. The degree of COM crystal growth inhibition by the molecules of the vitamins was determined from the time dependence of the optical absorption of

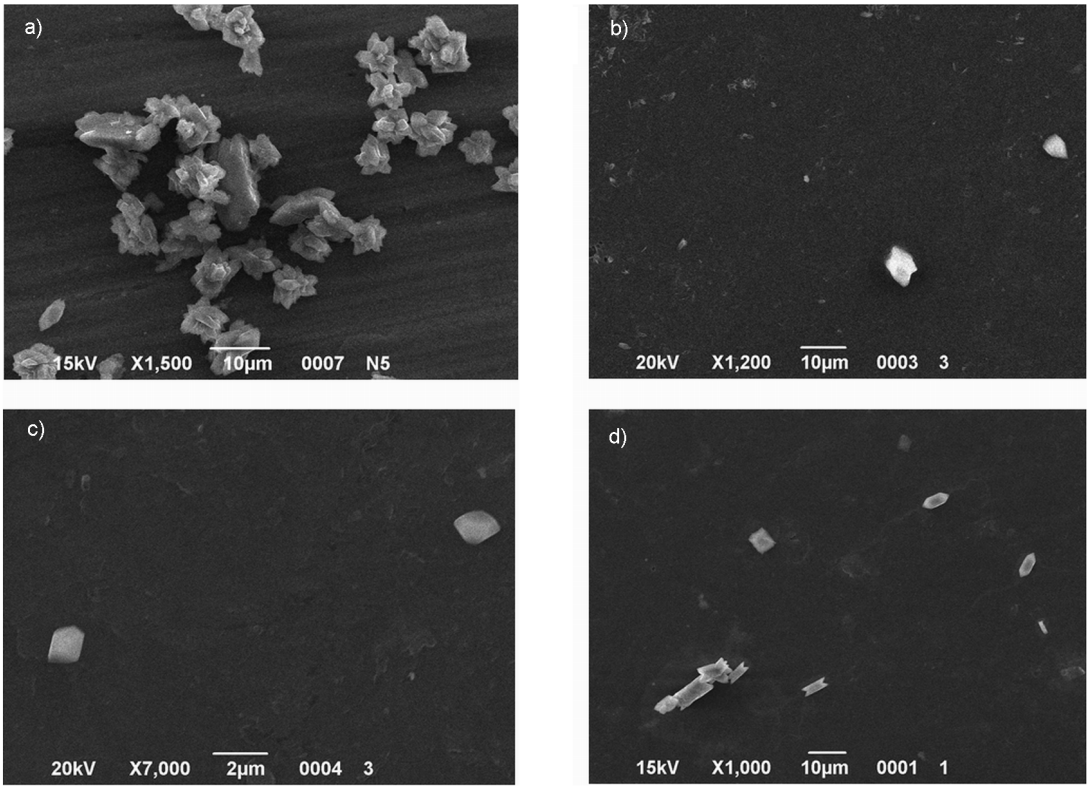

Fig. 2. The morphology of COM crystals grown without additives (a) and in the presence of 50 mM vitamin B1(b), 50 mM of vitamin B6 (c), 100 μM of vitamin B12 (d) (I = 0.15, pH = 5.8, s = 4.6).

the solutions at $\lambda = 620$ nm [18]. The value of the surface energy (σ) of the crystals was calculated from the Gibbs-Thomson equation [23].

3. Results and discussion

The samples of calcium oxalate synthesized during the present research were presented in the form of the phase of monohydrate, other phases were not revealed (Fig. 1a). The main diffraction peaks of COM appeared at 2θ values equal to 14.93° for the reflection (101), 15.29 for the reflection (110) and 24.37° for the reflection (202) [4, 24].

The IR-spectra of calcium oxalate without additions (Fig. 1b) contain all the absorption bands characteristic of COM [24]. The group of bands in the region of 3477–3047 cm^{-1} is bound up with asymmetric and symmetric stretching vibrations of OH-groups in the crystallization water, whereas the absorption bands at 1618 and 1316 cm^{-1} are caused by the corresponding vibrations of the group C=O. The peak at 780 cm^{-1} is connected with vibrations of the group O–C=O; the absorption bands at 947 cm^{-1} are due to symmetric vibrations of C–O, those at 883 cm^{-1} are caused by vibrations of the bond C–C, the bands at 660 cm^{-1} are connected with vibrations of OH-groups. The peak at 516 cm^{-1} is due to the presence of the bond metal-oxygen [24]. The IR-spectra of COM crystals grown in the presence of B1, B6, B12 vitamins (Fig. 1b) contain the absorption bands in the range from 3100 to 2800 cm^{-1} that correspond to the aromatic and aliphatic vibrations of the bond C–H. In the IR-spectra of the crystals grown in the presence of B1 vitamin there are also observed the bands characteristic of thiamine. The absorption band at 1472 cm^{-1} is caused by vibrations of pyrimidine ring. According to our data, the peak at 1658 cm^{-1} that corresponds to vibrations of the bond C=N [25] of B1 vitamin is revealed at 1613 cm^{-1}, and there is observed a shift of the absorption peak with a change in its intensity. This is caused by the interaction between B1 vitamin and the surface of the growing faces of COM crystal. The IR-spectra of the crystals grown with the addition of B6 vitamin contain the peaks at 1464 cm^{-1} and 1395 cm^{-1} that correspond

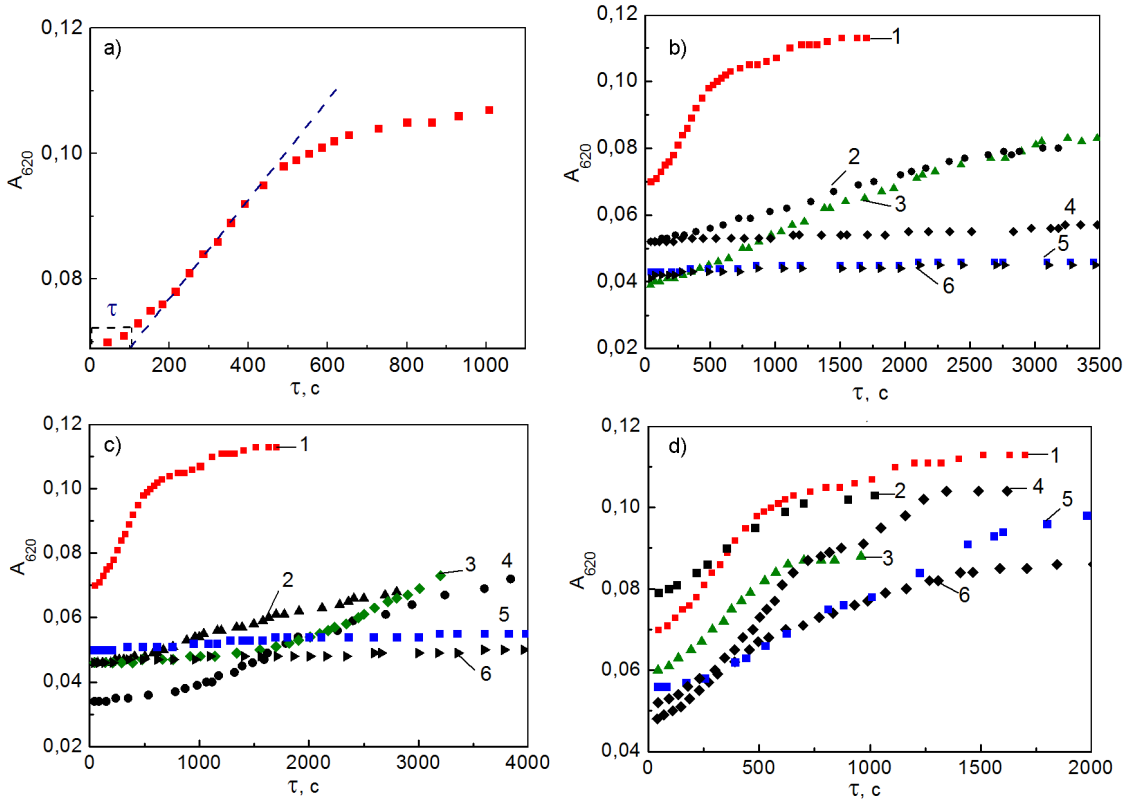

Fig. 3. Typical kinetic curve of pure COM crystallization (s = 6.0) (a); kinetic curves of COM crystallization (s = 6.0) in the presence of B1 (b) and B6 (c) with the concentrations of: 0 (*1*), 5 (*2*), 10 (*3*), 20 (*4*), 40 (*5*), 50 (*6*) mM; and in the presence of B12 (d) with the concentrations of: 0 (*1*), 10 (*2*), 20 (*3*), 40 (*4*), 70 (*5*), 100 (*6*) μM.

to the phenolic vibration of O–H and the aliphatic vibrations of C–OH. Pyridoxine has a high-intensity absorption band at ~ 1545 cm^{-1} due to C=C/C=N plane vibrations of pyridine ring [26]. In our case the given band is shifted, and we observe changes in the width and intensity of the peak at 1625 cm^{-1}, that confirms the interaction of B6 vitamin with COM. B12 vitamin is characterized by the absorption band at 1664 cm^{-1} bound up with stretching of the propionamide side of the corrine ring, as well as with the peaks at 1063 and 998 cm^{-1} caused by stretching of the phosphate fragment [27]. The spectra of the crystals obtained in the presence of B12 vitamin (Fig.1b) contain the peak at 1615 cm^{-1}, but the ones clearly seen at 1063 and 998 cm^{-1} are absent. That testifies to participation of B12 vitamin in intermolecular interactions. However, due to a complex chemical structure of B12 the mechanism of its interaction with COM crystals has not been completely established so far, and requires further investigations.

The SEM microphotographs of COM crystals with the addition of the vitamins (Fig. 2) make it possible to establish the tendency in the influence of the introduced vitamin additions on the morphology of the crystals. It is found that the introduction of 5–50 mM of B1 into the model system of COM leads to the formation of aggregates, twins and individual crystals with a size of 7–8 μm which have the morphology typical of COM [28]. Individual COM crystals start to form predominantly at 50 mM amount of the introduced vitamin B1 (Fig. 2b). The introduction of B6 vitamin in 5–50 mM concentrations leads to inhibition of the growth of COM crystals and diminution of their size proportionally with the vitamin concentration. At 50 mM (Fig. 2c) there is observed the formation of individual crystals with a size of about 2 μm, that is five times less than the size of pure COM. Such an effect is caused by the adsorption due to the formation of hydrogen bonds between the B6 molecules and the faces of COM. B12 vitamin in 10–70 μM concentrations does not

Table 1. Effect of B1, B6 and B12 on COM induction time

s	τ_{COM}, s	$\tau_{(COM+vit.)}$, s		
		B1 C_{max} = 50 mM	B6 C_{max} = 50 mM	B12, C_{max} = 100 μM
4.6	300	>7200	>7200	250
6.0	100			
8.0	75	>6600	>6600	90
10.0	50	300	400	50
12.0	40	140	300	40
14.0	25	100	100	

influence the size and morphology of COM crystals, however at 100 μM aggregation in COM decreases (Fig. 2d).

Investigation of the kinetic crystallization parameters permits to reveal the influence of the vitamins on the processes of COM nucleation. A typical curve of COM crystallization includes the stages of nucleation, subsequent growth and aggregation of the crystals (Fig. 3a). The kinetic curves of COM crystallization in the presence of the vitamins B1, B6 (5–50 mM) and B12 (10–100 μM) are shown in Fig. 3. The induction time (τ) for COM without additions is 100 s and rises proportionally with the growth of the concentration of B1 vitamin (Table 1). At B1 concentrations of 5–10 mM τ = 280–300 s, with the rise of the concentration up to 20 mM the time of COM induction becomes tenfold larger with respect to the one for COM without additions, whereas in the case of B6 it grows 15 times. The introduction of B1 and B6 in 40–50 mM concentrations raises τ by seventy times — up to 7200 s, that testifies to an essential inhibiting influence of the introduced molecules on COM nucleation. B12 vitamin weakly influences the kinetics of COM crystallization: at 70–100 μM of B12 the induction time is 250 s. Thus, among the studied vitamins B1, B6, B12 two of them — B6 and B1 — were found to exert an essential inhibiting effect on the processes of COM nucleation. Seventyfold rise of the induction time is caused by the formation of hydrogen bonds between the nitrogen atoms of the vitamin molecules and the hydrogen atoms that reach the surface of COM crystal faces.

To estimate the quantitative effect of the introduced concentrations of the vitamins on the processes of COM nucleation there was determined the degree of growth inhibition (I). According to the obtained results, at the introduction of 5–20 mM of B1 and

Table 2. Effect of the concentrations of the vitamins on the induction time (τ) and the degree of inhibition (I) of COM crystal growth

	$C_{vit.}$, mM	τ, s	I, %
Pure COM		100	–
COM + B1	5	280	57
	10	300	73
	20	1000	75
	40–50	>7200	95
COM + B6	5	400	69
	10	700	72
	20	1500	72
	40-50	>7200	95
COM + B12	0.01–0.06	100	0
	0.07–0.1	250	35

B6 vitamins the inhibition degree rises with the growth of their concentration (Table 1). The introduction of 50 mM of B1 and B6 leads to an essential inhibition of the growth of the crystals and raises the inhibition degree up to 95 %. The peculiarities of COM nucleation in the presence of B6 and B1 vitamins are bound up with the absorption caused by the formation of hydrogen bonds between the hydrogen and oxygen atoms of the vitamins and the oxalate groups that reach the surface of the COM faces {100} and {010}. The influence of B12 vitamin on COM crystallization is insignificant (Table 1). This is caused by the chemical structure of the vitamin: B12 molecule is larger and more branched in comparison with the ones of B1 and B6. This prevents its absorption on COM faces with subsequent crystal growth blocking.

To confirm the interaction of the introduced vitamins with COM crystals there was estimated the value of surface energy (σ) of

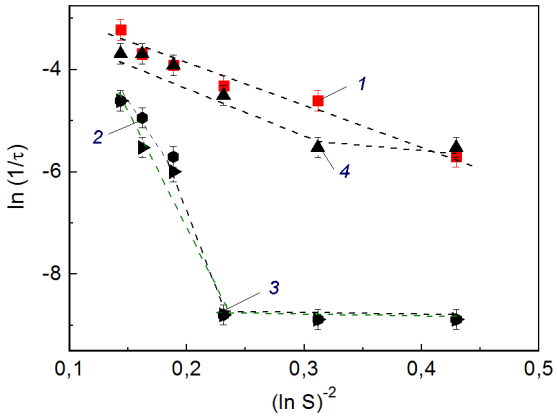

Fig. 4. Kinetic curves of nucleation of COM crystals without additives (*1*) and in the presence of: 50 mM of vitamin B1 (*2*), 50 mM of vitamin B6 (*3*), 100 μM of vitamin B12 (*4*) with S = 4.6–14.0.

the crystals. It was determined from the dependence of the nucleation rate on the supersaturation ranging from 4.6 to 14.0 by means of the Gibbs — Thomson equation (Fig. 4). The value of surface energy of COM crystals without additions determined for s = 4.6–14.0 was found to be 22.0 mJ/m^2 (Table 3).

The introduction of B1, B6 and B12 vitamins diminishes the surface energy of COM (Table 3). When B1 and B6 are introduced in a concentration of 10–20 mM the surface energy of COM diminishes to 20.8 and 20.7 mJ/m^2, respectively (Table 3). The rise of B1 and B6 concentration to 50 mM in the model system at s = 4.6–8 decreases the value of surface energy to 8.2 mJ/m^2, at s = 8.0–14.0 the surface energy of COM rises to 33.1 mJ/m^2. The kink (s = 8) of the kinetic curve points to the presence of the change-over from heterogeneous to homogeneous nucleation (Fig. 4). The value of the surface energy at 20–100 μM content of B12 vitamin ranges between 21.7 and 21.9 mJ/m^2 and practically correlates with the one of COM without additions. The distinction of COM nucleation in the presence of B6 and B1 vitamins from that in the presence of B12 is caused by the adsorption due to the formation of hydrogen bonds between the hydrogen and oxygen atoms of B1 and B6 molecules and the oxalate groups which reach the surface of the faces {100} and {010} of COM crystal.

4. Conclusions

Studied is the influence of group B vitamins (B1, B6, B12) on the crystallization processes, morphology and nucleation of COM crystals. The introduction of B6 and B1 vitamins in a concentration of 40–50 mM into the model system of COM leads to inhibition of the crystal growth by 95 % and favors more than seventyfold increase of the time of COM induction. It is established that B6 vitamin in 40–50 mM concentration promotes the formation of individual 2 μm COM crystals (the size of COM crystals without additions is ~ 10 μm). It is established that the value of COM surface energy diminishes at the introduction of B1 and B6 vitamins. The kinetic curve of COM crystallization in the presence of 50 mM of B1 and B6 has a kink (at s = 8) that points to the change-over from heterogenic to homogeneous nucleation (at s > 8).

Table 3. Effect of vitamins on the surface energy (σ) of COM crystals

	$C_{vit.}$, mM	σ, mJ/m^2
Pure COM	–	22.0
COM + B1	10–20	20.8
	50	8.2 (S = 4.6–8.0)
		32.4 (S = 8.0–14.0)
COM + B6	10–20	20.7
	50	8.2 (S = 4.6–8.0)
		33.1 (S = 8.0–14.0)
COM + B12	0.02–0.04	21.9
	0.1	21.7

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