UDC 548.5.546.05

DOI: 10.15372/CSD2021274

Crystallization of Calcium Carbonates from Solutions Containing Bile

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(Received March 06, 2020; revised July 07, 2020)

Abstract

The process of calcium carbonate (calcite and vaterite modifications) crystallization from the solutions containing bile is investigated. It was found that calcite is formed in an uncontaminated environment and in the presence of 1 mass % bile, while an increase in bile concentration in the initial solution from 5 to 100 mass % promotes the crystallization of vaterite. It is shown that the mass of the solid phase increases in proportion to an increase in the bile concentration in the initial solution. The dissolution of the synthesized samples in 0.9 mass % NaCl and 0.05 M EDTA was studied. It was revealed that the presence of bile components in the composition of solid samples reduces their dissolution rate.

Keywords: crystallization, bile, calcium carbonate, calcite, vaterite, dissolution kinetics

INTRODUCTION

Cholelithiasis (CL) is currently a widespread disease [1–10]. About 10 % of the Earth's population suffers from this disease, which is usually diagnosed already at the stage of the formation of gallstones. In Russia, the rate of this disease is 3–12 %, and it occurs more frequently in women [9]. Analysis of the effect of various factors on the formation of CL allowed revealing a significant role of genetic factors which participate in the regulation of the metabolism of cholesterol, the major component of gallstones [11–13].

Gallstones (or chololiths) are organomineral formations; cholesterol, pigment, and mixed kinds of stones are distinguished [3, 5]. A complex kind of gallstones is also distinguished, which occurs in 10 % of cases and is a combination of all three kinds [1, 14]. Gallstones form in the bile cyst and in the biliary tract. Three stages of chololith formation may be revealed: saturation, crystallization, and growth [1, 15], however, the most important stage is the saturation of bile with cholesterol. There are many reasons of gallstone formation, but this process has not been studied in detail yet, since the initial stage of CL has not been revealed. In view of the high rate of occurrence, it is still urgent to study this disease for the purpose of developing new routes of diagnostics and treatment of CL.

Analysis of literature data [1, 2, 14–16] showed that the major component of gallstones is cholesterol in the majority of cases, only a small part of gallstones contain pigment (without cholesterol), and 30 % of gallstones contain calcium carbonate in three modifications: calcite, aragonite, and vaterite. In particular, previous analysis of gallstones (a collection of 75 species) removed operatively from the patients at surgical departments of the hospitals in the Omsk Region – the Regional Clinical Hospital and the Emergency Care Hospital No. 2, Omsk, carried out with the help of a set of physicochemical methods showed that cholesterol is present in 92 % of the studied stones;

cholesterol with the admixture of bilirubin component accounts for 6 %; various modifications of calcium carbonate (aragonite, vaterite, calcite) in cholesterol stones account for 16 % (with the domination of vaterite: 9 %) [1].

Carbonates are a group of minerals participating in many physical, chemical and biological processes. They are widespread in living nature and are components of biogenic and pathogenic minerals in human organisms [1–3, 17, 18]. The processes of calcium carbonate precipitation in biological fluids (in particular in the bile system) are complicated, so a solution of the problem of stone genesis in bile-containing media is not only of medical significance but also holds scientific interest from the viewpoint of determination of the physicochemical properties of these compounds [19].

The reasons for selective crystallization of a polymorphous modification of calcium carbonate in the process of pathogenic mineralization are of complicated nature and are to a high extent determined by the specificity of physicochemical and kinetic factors governing the formation of stones. In turn, the formation of calcium carbonate crystals as nucleation centres in bile saturated with cholesterol may promote cholesterol crystallization and the formation of gallstones. Therefore, experimental modeling of these conditions is an urgent and practically significant task.

The goal of the work was to study the crystallization of calcium carbonate from bile solution (with variations of its concentration), and to determine physicochemical properties of the synthesized samples with the help of a set of physicochemical methods.

EXPERIMENTAL

The synthesis of calcium carbonate was carried out through precipitation from the aqueous solution (experiment No. 1) and from bile solution with variations of its concentration (experiment No. 2) by means of spontaneous crystallization:

$$CaCl_2 + 2NaHCO_3 \rightarrow CaCO_3\downarrow + 2NaCl + CO_2 + H_2O \quad (1)$$

The precipitate was obtained by mixing the solution of calcium chloride ($CaCl_2 \cdot 2H_2O$, pure for analysis reagent grade) and sodium hydrocarbonate ($NaHCO_3$, chemically pure reagent grade) in equimolar concentrations at room temperature (22–25 °C). Initial reagents were the salts, twice distilled water, and bile (preserved medical bile, PC Samson-Med, Russia). Bile concentration was varied from 1 to 100 mass % with a step of 5. In each experiment, acidity (pH) was corrected to 7.0±0.1 by adding a solution of hydrochloric acid (1 mol/L).

All samples were kept during the synthesis in a Biotron-4 box (SibFTI, Russia) at a temperature of 36.6 °C, which corresponds to the average temperature of human organs.

After settling the heterogeneous system for 24 h to 12 days, the solution was filtered with a step of 24 h, the precipitate on the filter was washed with water (volume V = 50 mL), and dried in the drying box at a temperature of ~80 °C to the constant mass. Then the precipitate was weighted with the analytical balance and transferred into the marked container.

X-ray diffraction (XRD) of the precipitates was carried out by means of powder diffractometry with the help of a D8 Advance analyzer (Bruker, Germany) in monochromatic radiation of copper (wavelength 1.54 Å) and TOPAS 3.0 software (Bruker). The detection limit was 0.5–5 mass %. Crystallite size was estimated using the Debye–Scherrer equation [20].

After preliminary tableting of initial solid phases with KBr, IR spectra were recorded with the help of an IR Prestige-21 Fourier Transform spectrometer (Shimadzu, Japan). The spectra of samples under investigation were recorded within the range 4000–500 cm^{-1}.

The morphology of solid phases and particle shapes were determined with a monocular microscope XSP-140 (LC Armed, Russia). Crystal size was calculated with the help of ToupView software for image treating.

The specific surface area of two solid samples (one sample from each experiment 1 and 2) was studied using single-point nitrogen adsorption at 77.4 K with the help of a Sorbtometr adsorption analyzer (LC Katakon, Russia). The values of specific surface (m^2/g) were calculated according to Brunauer–Emmett–Teller (BET) procedure. The relative error of measurements was 5 %.

The residual calcium content in the liquid separated from the precipitate was determined according to RD 52.24.403–2018 “Mass concentration of calcium ions in water. The procedure of measurement using titration with Trilon B”, carbonates were determined according to GOST 31957–2012. “Water. Methods to determine alkalinity and mass concentration of carbonates and hydrocarbonates”). The indicated methods allow the determination of the mass concentrations of carbonates and hydrocarbonates within the ranges of 6.0–6000 and 6.1–6100 mg/dm^3, respective-

ly. The molar ratio of ions (Ca/CO_3) in the samples was estimated using "the introduced – found" procedure.

The synthesized samples were dissolved in two solvents to simulate the extracellular fluid of a human organism: sodium chloride (NaCl, 0.9 mass %) – modeling, and ethylenediamine tetraacetic acid (EDTA, 0.05 mol/L) – an efficient solvent for calcium compounds. The dissolution kinetics for the samples of the obtained solid phases, 0.1000±0.0001 g in mass (the samples were weighted with analytical balance with the accuracy of 0.1 mg) was studied in a thermostated cell at 37 °C for 2 h with the constant volume of the liquid phase under permanent mixing. The parameters that were controlled during experiment included acidity (pH), the concentration of calcium ions that passed into the liquid phase (pCa), and dissolution time (t) for calcium carbonate. The concentration of calcium ions was determined by means of direct potentiometry with the help of an I-160MI ionometer (LC Izmeritelnaya Tekhnika, Russia) using a Ca-selective electrode.

Kinetic curves were plotted relying on thus obtained experimental data, and mathematical processing of the data was carried out according to the algorithm described in [21].

RESULTS AND DISCUSSION

Synthesis of calcium carbonate from aqueous solutions

According to the XRD data, independently of the duration of synthesis, the samples synthesized from the aqueous solution (experiment No. 1) are represented by calcite phase. A typical X-ray diffraction pattern of the precipitate contains reflections in angle regions of 23.0, 29.4, 39.5, 43.0° over 2θ (Fig. 1), which corresponds to the line diffraction patterns of calcite [22, 23]. It is necessary to stress that calcite precipitated from aqueous solutions contains vaterite as an admixture. This is confirmed by the peak at 26.0° over 2θ. Vaterite content is not more than 5 %.

The IR spectrum of the synthesized sample (experiment No. 1, synthesis time 24 h) (Fig. 2) contains absorption bands (a. b.) characteristic of carbonate groups which are assigned to bending vibrations in CO_3^{2-} (702 cm^{-1}) and O–C–O bonds in CO_3^{2-} (880 cm^{-1}), stretching antisymmetrical vibrations of C–O bonds in CO_3^{2-} (1407 cm^{-1}). The absorption band at 1795 cm^{-1} corresponds to the high-intensity stretching vibrations of C=O in COOH groups, while the band at 2499 cm^{-1} is due to the low-intensity stretching vibrations of C=O in COOH groups (see Fig. 2).

Investigation of the synthesized samples by means of optical microscopy showed that the precipitate is composed of micrometer-sized cubic particles, which is characteristic of calcite crystals (Fig. 3, *a*) [24]. According to the literature data, calcite has more than a hundred natural forms, however, the synthesized calcium carbonate is characterized by a small number of forms. A typical cleavable rhombohedron and pinacoid are dominating units [23].

Synthesis of calcium carbonate in the presence of bile

The synthesis of calcium carbonates in the presence of bile was carried out in experiment No. 2. Bile concentration was varied from 1 to 100 mass %.

It was revealed in experiment No. 1 that synthesis time does not affect the phase composition of the synthesized samples. Similar results were obtained also in experiment No. 2 for vile concentration 10 mass %. For this reason, the optimal synthesis time chosen for subsequent experiments was 24 h.

It was determined by means of XRD that calcium carbonate precipitates synthesized in the presence of 1 mass % of vile are represented by calcite phase (see Fig. 1, *b*), while the phase dominating in the samples obtained from more concentrated solutions (5–95 mass % bile) or from pure bile is vaterite (reflections at 21.0, 24.9, 27.0, 32.7, 44.5, 49.0, 50.0, 56.0° over 2θ) [22, 23]. It should be stressed that the main reflections of cholesterol are to be observed within the region 2–24° over 2θ and are absent from the diffraction patterns of the synthesized samples. It may be concluded that if cholesterol actually gets crystallized, its concentration in the precipitate is not more than 5 mass %.

The size of calcium carbonate crystallites was estimated using the recorded diffraction patterns (Table 1). It was established that the size varies insignificantly with variations in synthesis conditions. In the case of low bile content in solution (1 mass %), crystallite size is larger than for the samples synthesized from the solutions containing no bile. The smallest crystallites are observed for the samples synthesized from the solution with bile concentrations of 70 and 100 mass %. This phenomenon requires further investigation.

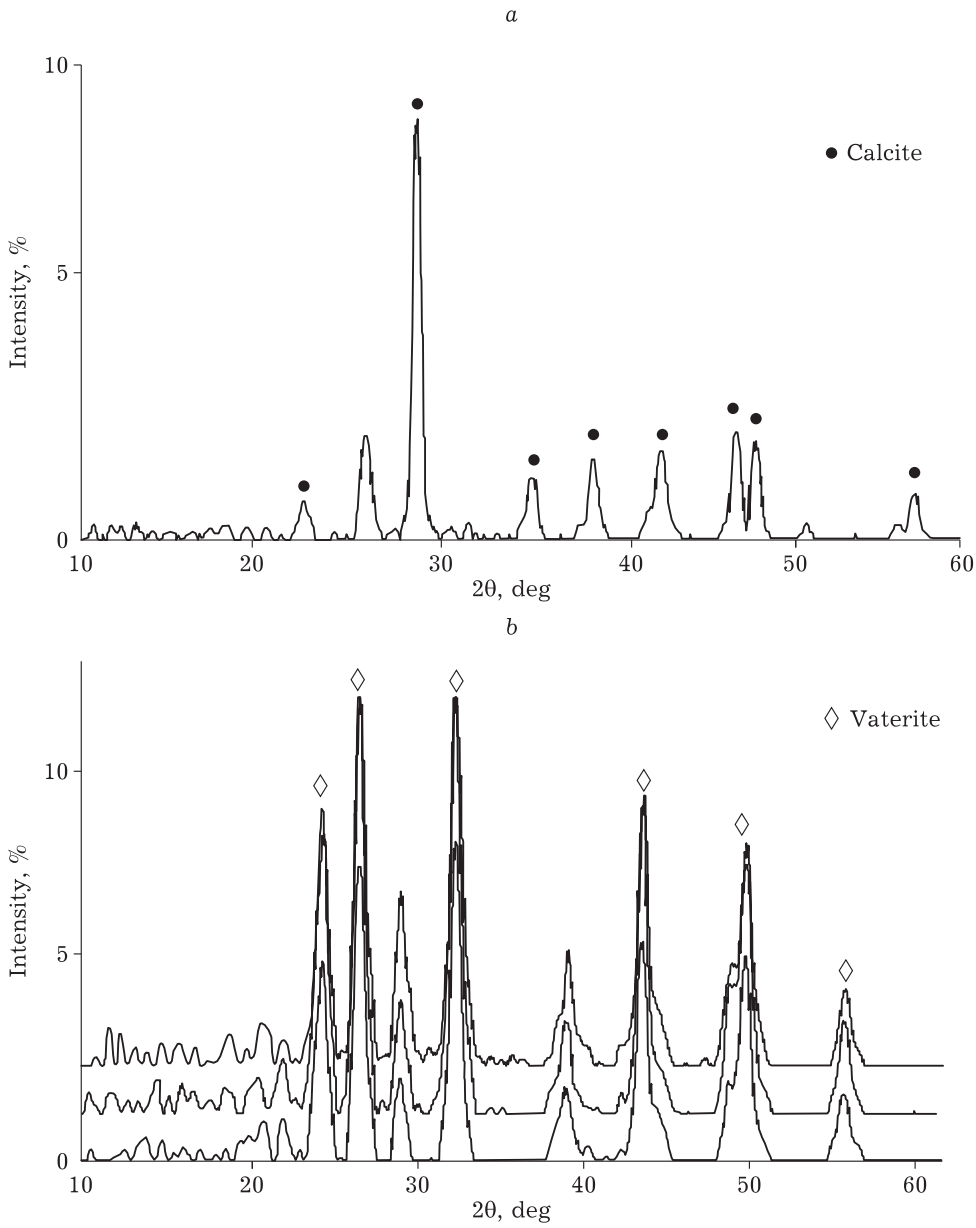

Fig. 1. Diffraction patterns of calcium carbonate synthesized from the aqueous medium (*a*) and from the solutions containing bile (*b*), mass %: *1* –10; *2* – 20; *3* – 30. Synthesis time 24 h.

It was determined by means of IR Fourier spectroscopy that the synthesized samples contain the entire set of bands characteristic of calcium carbonate, and some bands characteristic of bile components: a broad a. b. of the stretching vibrations of N–H bonds in the region of 3400 cm^{-1} corresponds to the vibrations of the amino group in bilirubin, while the band at 1050–1100 cm^{-1} relates to the stretching vibrations of O–H and C–N bonds, respectively, in bile pigments and in cholesterol incorporated in bile. This is confirmed by the formation of calcium carbonates and the presence of bile components in them (see Fig. 2).

Investigation of the morphology of particles formed from bile-containing solutions (5–100 mass %), by means of optical microscopy, showed (see Fig. 3, *b*) that vaterite aggregates have a globular shape [2].

It was established by means of low-temperature nitrogen adsorption that the precipitate obtained in the presence of bile (30 mass %) possesses the most developed specific surface (5 m^2/g) in comparison with calcium carbonate formed from the solution containing no bile, – 1 m^2/g. An increase in the specific surface area by a factor of 5 occurs due to the inclusion of bile components into the composition of the synthesized samples.

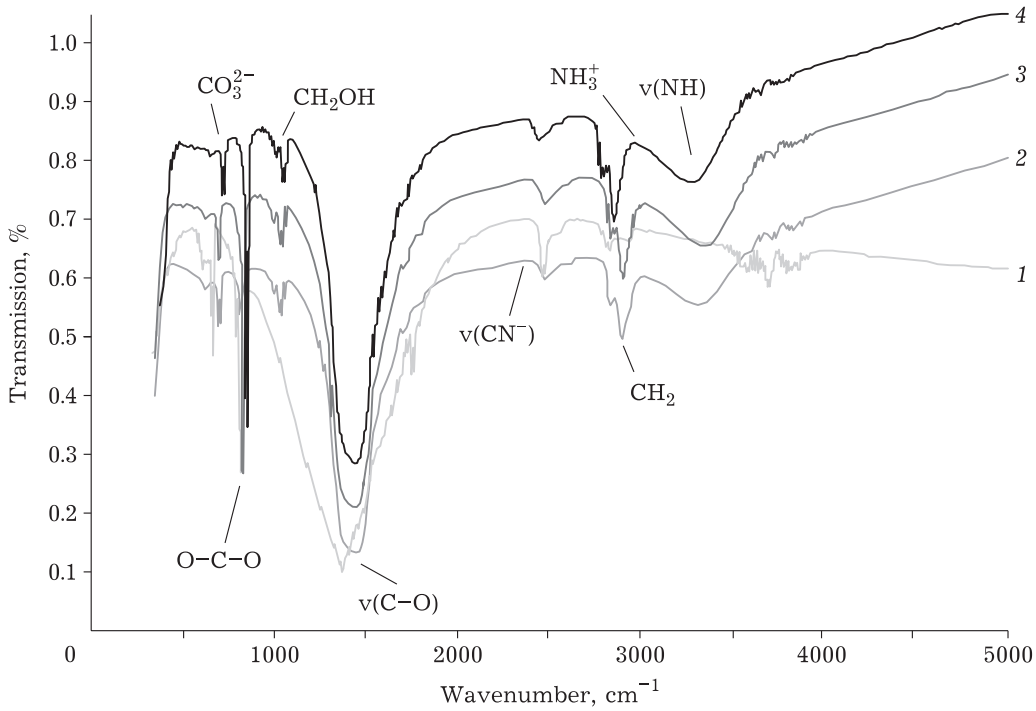

Fig. 2. IR spectra of precipitates obtained from solutions with different bile content: *1* – 0, *2* – 10, *3* – 20, *4* – 30 mass %.

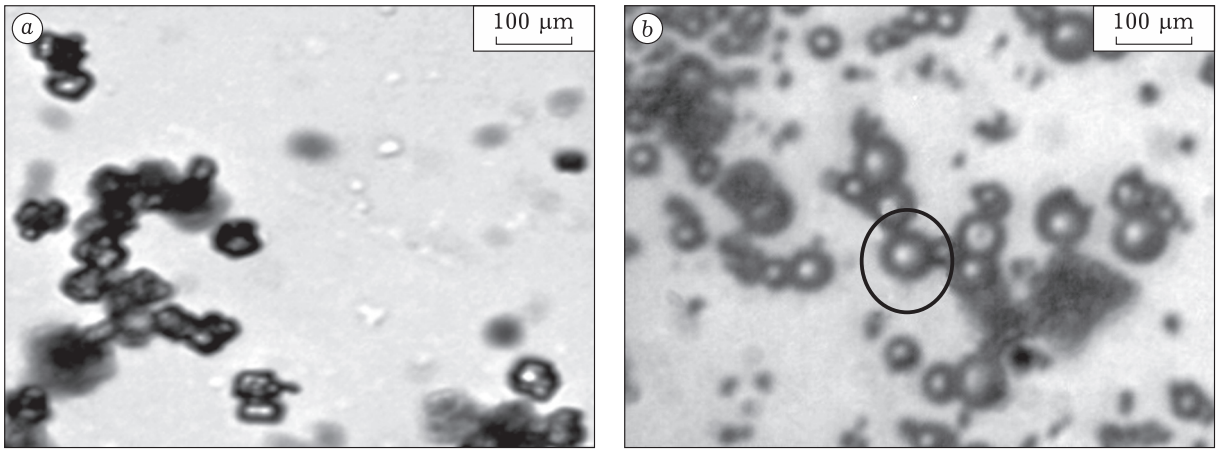

Fig. 3. Photographs of calcium carbonate obtained from solutions with bile content 1 (*a*) and 30 mass % (*b*). Magnification ×40.

This fact is confirmed by the recorded dependence of the mass of precipitate on bile concentration in solution (Fig. 4, *a*). An increase in the mass of precipitate with an increase in bile concentration in the initial model solution was detected.

It is known that the major bile components are biliary acids and bilirubin, which are able to interact with calcium ions forming the corresponding salts [1]. A confirmation of this interac-

TABLE 1

Size of the crystallites of calcium carbonate synthesized with different bile concentrations in solution

Synthesis conditions	Bile concentration, mass %					
	0	1	5–60	70	80–90	100
Crystallite size, μm	0.18	0.21	0.18	0.16	0.18	0.16

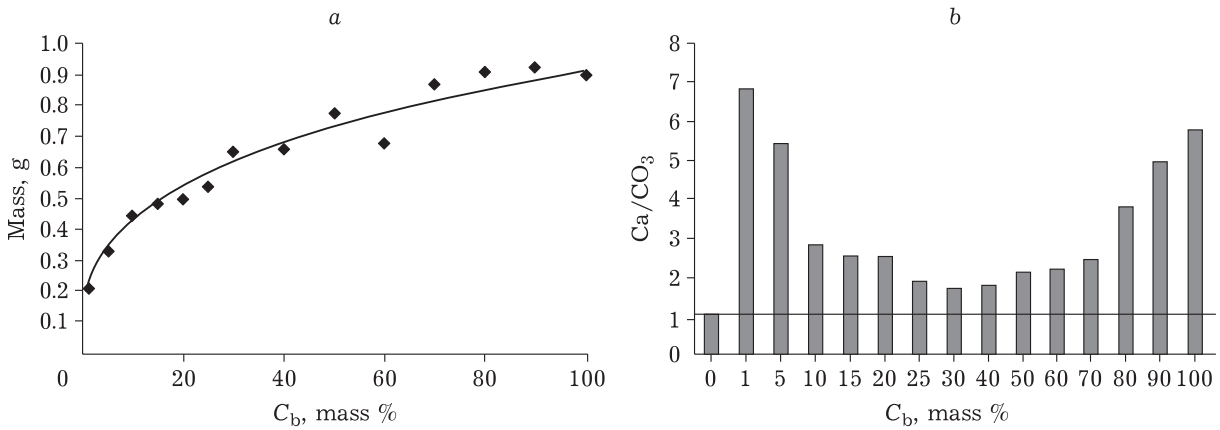

Fig. 4. Dependences of the mass of the formed precipitate (*a*) and the molar ratio Ca/CO_3 (*b*) on bile concentration (C_b, mass %) in solution.

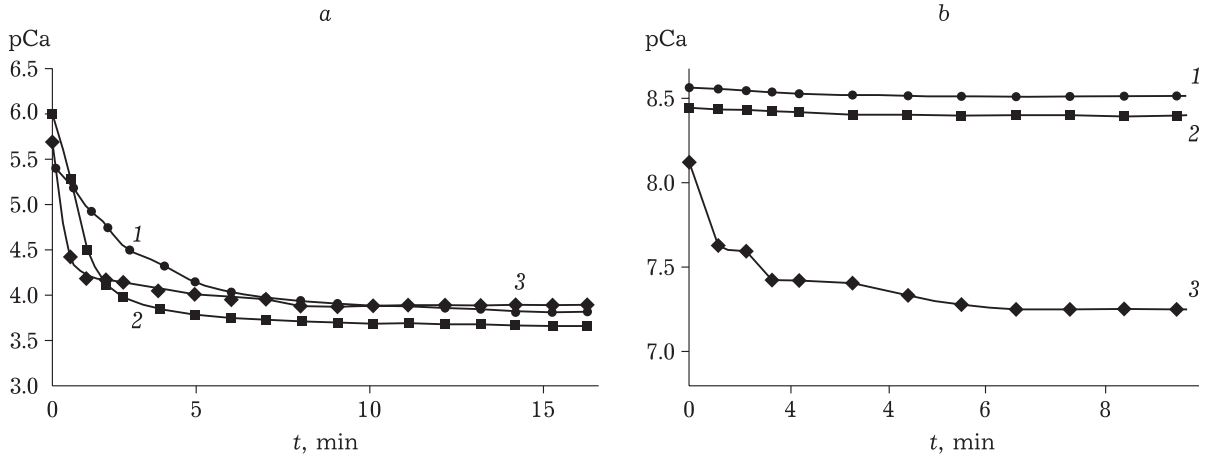

Fig. 5. Dependences of calcium concentration (pCa) on the time of dissolution (*t*) of solid phases (*a* – 0.9 mass % NaCl, *b* – 0.05 mol/L EDTA) for different concentrations of bile in solution: 0 (*1*), 50 (*2*), 100 (*3*) mass %.

tion may be the Ca/CO_3 ratio for the solid samples of calcium carbonate synthesized in the presence of bile (see Fig. 4, *b*). A parabolic dependence is traced with the minimum of calcium ion content in the solid sample for bile concentration in solution of 10–70 mass %. The obtained dependence of changes in the Ca/CO_3 ratio on bile concentration in the initial solution requires further refinement.

It is established that the interaction of the solvent (medium) with the samples involves the release of Ca^{2+} ions and their accumulation in the liquid phase. The concentration of Ca^{2+} increases rapidly in sodium chloride solution (Fig. 5, *a*) at the initial stage of dissolution. Later on, the intensity of Ca^{2+} ion release into the liquid phase from all samples decreases gradually. In the case of EDTA solution (see Fig. 5, *b*) the concentration of free calcium ions in solution is lower because the formation of EDTA complexes with calcium ions takes place ($\lg K_{st}$ = 10.7, where K_{st} is the stability constant of the complex).

The initial values of the rates of calcium release in the solution were calculated on the basis of the obtained experimental dependencies pCa = $f(t)$. The experimental kinetic curve (a change in the concentration of calcium ions) looks like an exponential dependence, that is, dissolution obeys the first-order kinetic equation. Because of this, the rate may be determined as a tangent of the slope of a linear region of the line. One can see (Table 2) that the initial rate of sample dissolution is higher for the samples synthesized from the solutions containing no bile than for the samples synthesized in the presence of bile.

It may be assumed that a decrease in the rate of dissolution of calcium carbonate formed in the presence of bile is promoted by the inclusion of the organic components of bile into the solid phase.

TABLE 2

Initial rates (V) of the dissolution of solid phases formed with different bile concentrations in solution

Bile concentration, mass %	Dissolution stages	t, min	Kinetic equation	$V \cdot 10^5$, min^{-1}
NaCl solution (0.9 mass %)				
0	1	0–1	$C(Ca^{2+}) = 4E\text{-}05x + 7E\text{-}06$	4.0
	2	2–7	$C(Ca^{2+}) = 4.79E\text{-}05 + 4E\text{-}05e^{0.0758x}$	4.79
50	1	0–2	$C(Ca^{2+}) = 3E\text{-}05x - 2E\text{-}06$	3.0
	2	3–15	$C(Ca^{2+}) = 7.94E\text{-}05 + 8E\text{-}05e^{0.0201x}$	7.94
100	1	0–4	$C(Ca^{2+}) = 1E\text{-}05x + 4E\text{-}06$	1.0
	2	5–15	$C(Ca^{2+}) = 5.94E\text{-}05 + 5E\text{-}05e^{0.0337x}$	5.94
EDTA solution (0.05 mol/L)				
0	1	0–2	$C(Ca^{2+}) = 5E\text{-}08x + 2E\text{-}08$	0.005
	2	3–6	$C(Ca^{2+}) = 1.24E\text{-}07 + 8E\text{-}08e^{0.1727x}$	0.012
50	1	0–2	$C(Ca^{2+}) = 2E\text{-}10x + 4E\text{-}09$	0.00002
	2	3–6	$C(Ca^{2+}) = 3.98E\text{-}09 + 4E\text{-}09e^{0.0113x}$	0.00039
100	1	0–2	$C(Ca^{2+}) = 1E\text{-}10x + 2E\text{-}09$	0.00001
	2	3–7	$C(Ca^{2+}) = 2.72E\text{-}09 + 3E\text{-}09e^{0.0051x}$	0.00027

Note. t – time of solid phase dissolution; $C(Ca^{2+})$ – concentration of calcium ions.

CONCLUSION

Calcium carbonate was synthesized from bile-containing solutions. It was determined that the samples synthesized in the absence of bile and with its concentration 1 mass % contain calcite. An increase in bile content in the initial solution from 5 to 100 mass % promotes vaterite crystallization.

Studies of the effect of bile on the mass of the solid phase showed that the mass of the precipitate increases in proportion with an increase in bile concentration in the initial solution.

Investigation of the dissolution of synthesized samples in NaCl (0.9 mass %) and EDTA (0.05 mol/L) solutions revealed that the presence of bile components in the solid samples causes a decrease in the rate of their dissolution.

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