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CHEMISTRY

Synthesis of Nanocrystalline Calcium Hydroxyapatite from Calcium Saccharates and Ammonium Hydrogen Phosphate

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The synthetic hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HAP) is a mineral component of the bone tissue of mammals. HAP is biocompatible with the human body, capable of biointegration with the bone tissue, has no negative influence on the immune system, is nontoxic, and shows osteoconductive behavior. Therefore, HAP is used to design ceramic materials for bone implants [1].

The activity of powders toward sintering in the production of ceramic materials increases with a decrease in the particle size of the powder [2, 3]. For bioceramics, an important feature is the absence of acute cytotoxicity, which is determined by the chemical purity of the initial powder. To prepare HAP containing no toxic or associated products difficult to remove, the reaction of calcium hydroxide with ammonium phosphate is used. In this case, one component, $Ca(OH)_2$, is taken as a suspension [4]. An obvious drawback of the synthesis of calcium phosphates from a poorly soluble compound is that dissolution of $Ca(OH)_2$ is the rate-limiting step, and this increases the duration of synthesis. The formation of calcium phosphates may also occur on the surface of $Ca(OH)_2$ particles, which shifts the reaction to a region of slower diffusion through a solid calcium phosphate bed and gives rise to an inhomogeneous target product. The use of soluble calcium compounds for preparing highly dispersed powders is preferred, because this implements the known bottom-up approach to the creation of a dispersion system (from small to large) [5]. In the presence of saccharose, the solubility of calcium hydroxide increases due to formation of soluble calcium saccharates [6]. Organic compounds being adsorbed on the surface of the inorganic crystals formed prevent their growth and thus ensure the formation of a finely crystalline powder [7].

The purpose of the present work was to prepare nanocrystalline HAP containing a biocompatible associated product, saccharose, from ammonium hydrogen phosphate and calcium saccharates. The study was carried out with solutions of calcium saccharates $Ca_nC_{12}H_{22-2n}O_{11}$ at different n (n is the Ca : $C_{12}H_{22}O_{11}$ molar ratio in the saccharate synthesis) and HAP samples synthesized from different calcium saccharates and ammonium hydrogen phosphate.

Calcium saccharate was synthesized according to the reaction

$$n\mathrm{CaO} + \mathrm{C_{12}H_{22}O_{11}} = \mathrm{Ca}_n\mathrm{C_{12}H_{22-2\mathit{n}}O_{11}} + n\mathrm{H_2O}. \quad (1)$$

The reaction of a mixture of 0.5 mol calcium oxide with 1, 0.5, or 0.25 mol of saccharose with water (1 L) for seven days gave yellow-brown viscous solutions (hemicalcium or monocalcium saccharates) or an ivory-colored suspension (dicalcium saccharate). These products were used for the synthesis of HAP according to reaction

$$\frac{10}{n}\mathrm{Ca}_n\mathrm{C_{12}H_{22-2\mathit{n}}O_{11}} + 6(\mathrm{NH_4})_2\mathrm{HPO_4} + 2\mathrm{H_2O}$$
$$= \mathrm{Ca_{10}(PO_4)_6(OH)_2} + 12\mathrm{NH_3}\uparrow + \frac{10}{n}\mathrm{C_{12}H_{22}O_{11}}. \quad (2)$$

The required volume of a 0.3 M aqueous solution of ammonium hydrogen phosphate was added dropwise at room temperature at a rate of 16–22 mL/min over a period of 50–60 min. Within 10 min after the beginning of the synthesis, noticeable smell of ammonia appeared, which persisted up to the end of the synthesis. During the synthesis, pH in the reaction zone was higher than 11. After addition of the solution of $(NH_4)_2HPO_4$, the sol formed was gradually heated to 60°C and kept for 30 min. The filtered-off gel-like precipitate containing HAP and adsorbed saccharose was dried in air in thin film at room temperature for about five days.

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X-ray diffraction studies were carried out on a D/MAX-2500 Rigaku diffractometer (Japan) with a rotating anode using CuK_α radiation. The size of HAP particles was estimated using the Scherrer formula proceeding from analysis of (002) peak broadening (d = 3.44 Å). The peak shape was approximated by a Gaussian function, the diffractometer instrumental broadening was estimated from the X-ray diffraction pattern of ground fine crystalline silicon to be equal to 0.1° in the analyzed range of angles on the 2θ scale.

The electron microscopic study was carried out using a field-emission Supra 50 VP Carl Zeiss scanning electron microscope (Germany) at accelerating voltages of 3–10 kV with an InLens type secondary electron detector for surface topography analysis and in reflected electrons to elucidate chemical contrast (Compo mode) under low vacuum (not less than 100 Pa for effective charge sink as the samples were not metal plated to avoid thermal action). This study makes it possible to distinguish sample areas with different chemical composition.

Thermal analysis of the samples was carried out on a Perkin Elmer Pyris thermal analyzer (United States). The heating rate was 5 K/min.

The compositions of solutions and suspensions of calcium saccharates were determined by electrospray ionization mass spectrometry with ion trap mass analyzer. Electrospray ionization (ESI) is one of the mildest ionization methods used in mass spectrometry that allows effective generation of ions of polar compounds from their solutions in polar solvents supplied continuously under atmospheric pressure [8]. The electrospray ionization was carried out under the following conditions: flow rate 120 μL/h, capillary voltage 3.5 kV, cutoff voltage 3 kV. Ion trap (IT) mass analyzers ensure high sensitivity for ions with a mass to charge ratio m/z of up to 4000. The measurements were carried out on an Esquire 4000 Bruker Daltonics instrument (United States). Aqueous solutions with a constant concentration of calcium ions (0.0005 mol/L) prepared by the described procedure according to reaction (1) were used for analysis. The electrospray ionization method is inapplicable to insoluble compounds; however, for the purpose of this study, it is more important to elucidate the state of calcium saccharates in the solution. Since the composition of the initial compounds is known, the mass measurement to an accuracy of tenths of amu was enough for the purpose of this study.

It was found that most of the intense peaks in the mass spectra of calcium saccharates correspond to the formulas $[Ca_xC_{12y}H_{22y-2x-z}O_{11y}]^{z-}$ in the negative ion mode or $[Ca_xC_{12y}H_{22y-2x+z}O_{11y}]^{z+}$ in the positive ion mode ($x \geq 1$, $y \geq 1$, $z = 1, 2$); this supports the view on calcium saccharates as salt compounds of the alcoholate type formed upon proton abstraction from saccharose molecules. The positive ion spectra exhibit mainly ions with undissociated ($[CaC_{12}H_{22}O_{11}]^{2+}$) and singly deprotonated ($[CaC_{12}H_{21}O_{11}]^{+}$) saccharose, whereas the negative ion spectra exhibited ions with saccharose deprotonation degree of up to four ($[Ca_6C_{48}H_{84}O_{44}]^{2-}$) or five ($[Ca_2C_{12}H_{17}O_{11}]^{-}$).

The mass spectral pattern (Fig. 1) changes qualitatively as the initial calcium oxide to saccharose molar ratio increases to 2 : 1 irrespective of the ionization mode. In particular, the ion current proportional to the overall content of ions in solution sharply decreased. Simultaneously, the proportion of cluster ions $[CaOH(CaO)_x(H_2O)_y]^+$ formed by calcium hydroxide increased stepwise in the positive ion mode. This indicates that excess calcium hydroxide is present in the reaction mixture, apparently, as a suspension; according to mass spectrometry, the solution also contains calcium saccharates with $n = 1$ and $n = 2$. Incomplete reaction between a large amount of CaO and a saccharose solution is apparently due to kinetic reason and has been noted previously in the literature [6].

Note that under ESI conditions almost no formation of hydroxy saccharate ions $[(CaOH)_xC_{12n}H_{22n-x-z}O_{11n}]^{z-}$ was observed. This is understandable because saccharose is a weak acid (pK_1 = 13.1 ± 0.3 at 25°C [9]); i.e., anion hydrolysis takes place. A similar result was obtained for sodium saccharate solutions [9].

Saccharose-containing ions are highly prone to form associates stable under ESI conditions (Table 1). Noticeable amounts of cluster ions containing up to 11 formula units of saccharose $[Ca_4C_{132}H_{232}O_{121}]^{2-}$ were found. The content of calcium in these ions is highly variable; thus ions containing eight formula units of saccharose were found to contain 0 to 8 calcium atoms ($[C_{96}H_{174}O_{88}]^{2-}$ and $[Ca_8C_{96}H_{158}O_{88}]^{2-}$, respectively).

The obtained experimental data can be used to consider the ionic composition of the solution at the qualitative level. A typical component of saccharate solutions is the $C_{12}H_{21}O_{11}^-$ anion; i.e., abstraction of one proton from the saccharose molecule is not difficult; species indicative of saccharose dissociation to the second and third steps were also found (according to published data [9], pK_2 is in the range of 13–15 and pK_3 should not differ much from these values). No noticeable saccharose dissociation in higher steps was found in our experiments, although in the negative ion modes equivalent to the conditions pH $\gg$ 14, ions with quintuply deprotonated saccharose were obtained. The relative contents of different products of acidic dissociation of saccharose are nearly the same for solutions with calcium oxide : saccharose ratios of 0.5 to 1.5. The major complex species present in the positive ion spectra are also the same, namely, singly and doubly charged saccharates $[CaC_{24}H_{43}O_{22}]^+$ and $[Ca_2C_{36}H_{64}O_{33}]^{2+}$ (note that in the negative ion mode, the ion current from $[CaC_{24}H_{41}O_{22}]^-$ is rather low, the most intense peak in the mass spectra (Fig. 1a) corresponds to $[C_{12}H_{21}O_{11}]^-$,

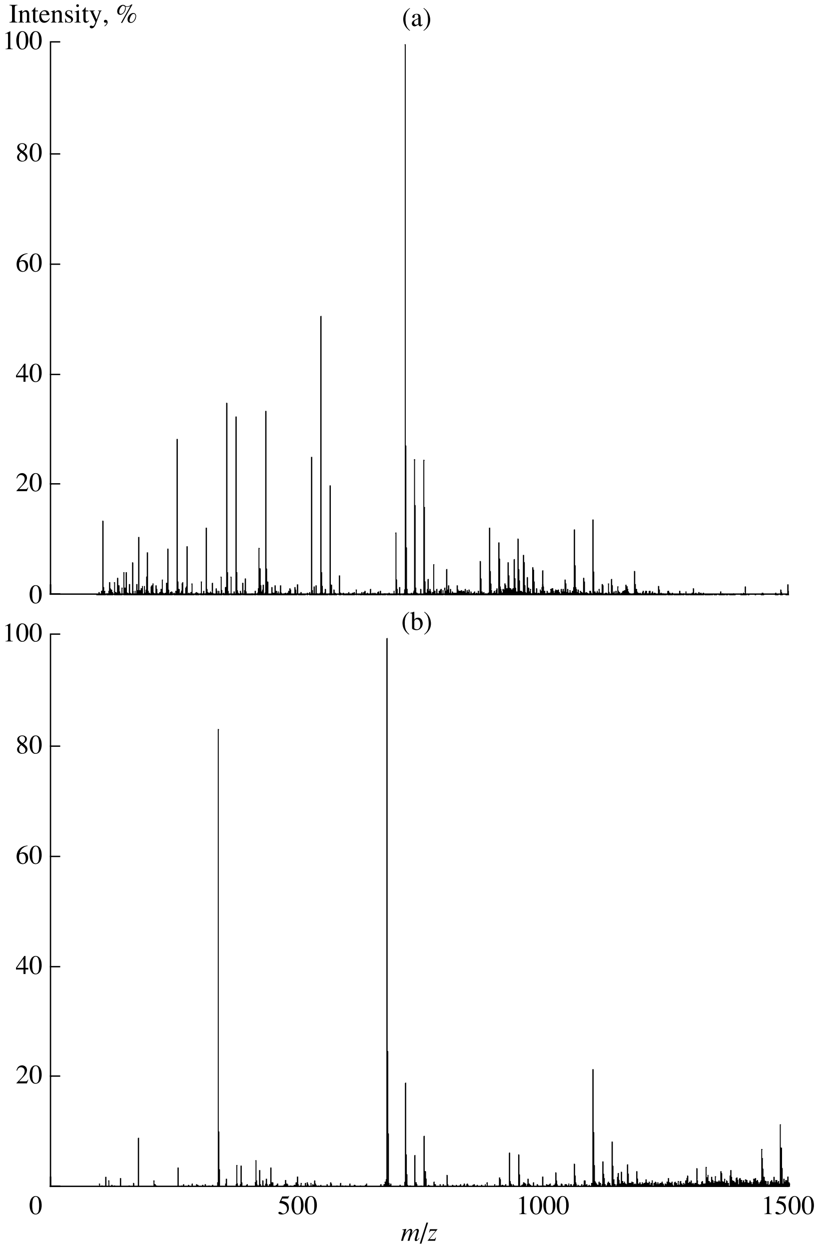

Fig. 1. (a) Positive and (b) negative ion mass spectra of monocalcium saccharate.

the second most intense peak is that of $[Ca_2C_{36}H_{61}O_{33}]^-$ anions). This fact indicates that the major component in solutions with such composition is semicalcium saccharate $CaC_{24}H_{42}O_{22}$, the protolytic equilibrium with its participation being shifted to the right

$$CaC_{24}H_{41}O_{22}^- \underset{OH^-}{\overset{H_2O}{\rightleftarrows}} CaC_{24}H_{42}O_{22} \underset{OH^-}{\overset{H_2O}{\rightleftarrows}} CaC_{24}H_{43}O_{22}^+. \quad (3)$$

Deprotonation of hemicalcium saccharate results apparently in the formation of the binuclear complex anion more stable under ESI conditions and the saccharate anion

$$2CaC_{24}H_{41}O_{22}^- \longleftrightarrow Ca_2C_{36}H_{61}O_{33}^- + C_{12}H_{21}O_{11}^-. \quad (4)$$

In the synthesis with calcium oxide : saccharose = 2 : 1, the presence of a poorly soluble compound is likely (this may be the dicalcium saccharate and/or unreacted calcium hydroxide). The data presented above demonstrate that the solutions under study represent mixtures of a large number of compounds; therefore, the formulas of calcium saccharates appearing in equations (1) and (2) are arbitrary and rather reflect the general stoichiometry and not the actual chemical composition of all of the products.

It can be concluded that in the reaction of sugar syrup with calcium oxide, the solubility of CaO increases due to the acidic dissociation of saccharose to give hemicalcium saccharates (reaction (1), $n = 1 : 2$). As this takes place, the concentration of hydroxide ions decreases and the concentration of Ca^{2+} increases ($Ca(OH)_2 = [Ca^{2+}] \cdot [OH^-]^2 = 6.5 \times 10^{-6}$ [10]; a decrease in the pH from 12 to 11 would induce an increase in the $[Ca^{2+}]$ concentration from 0.065 to 6.5 mol/l). The resulting saccharate is hydrolyzed by reaction (3); therefore, the pH of saccharate solutions does not differ much from that of calcium hydroxide solutions, which was also noted previously [6]. The outcome of reactions (1) and (3) is actually binding of calcium atoms by the saccharose molecule to give a complex cation, which increases the solubility of calcium hydroxide in water. Note that calcium saccharate–saccharose system has a buffering action in highly alkaline medium (pH ~ 12). The maintenance of high pH at early stages of HAP synthesis from solutions may prove useful for the formation of nano-sized apatite crystals, because their recrystallization and growth occurring by the dissolution–precipitation mechanism are substantially suppressed in the highly alkaline medium.

Table 1. Average number of formula units of saccharose in observed ions

n	Positive ionization	Negative ionization
0.5 : 1	3.11	2.19
1 : 1	3.21	2.50
2 : 1	2.75	3.88

Table 2. Peak intensity of some cluster ions observed in the positive ion mass spectra, % relative to the most intense peak

Ion	*m/z*	*n*		
		0.5 : 1	1 : 1	2 : 1
$CaOH^+ \cdot 3H_2O$	111	11	14	92
$CaOH^+ \cdot 7H_2O$	183	10	11	54
$CaOH^+ \cdot 3Ca(OH)_2 \cdot 9H_2O$	441	28	34	47

The powder X-ray diffraction data (Fig. 2) indicate that each of the obtained powders contains HAP and does not contain any other calcium phosphate in any significant amount. High background intensity in all

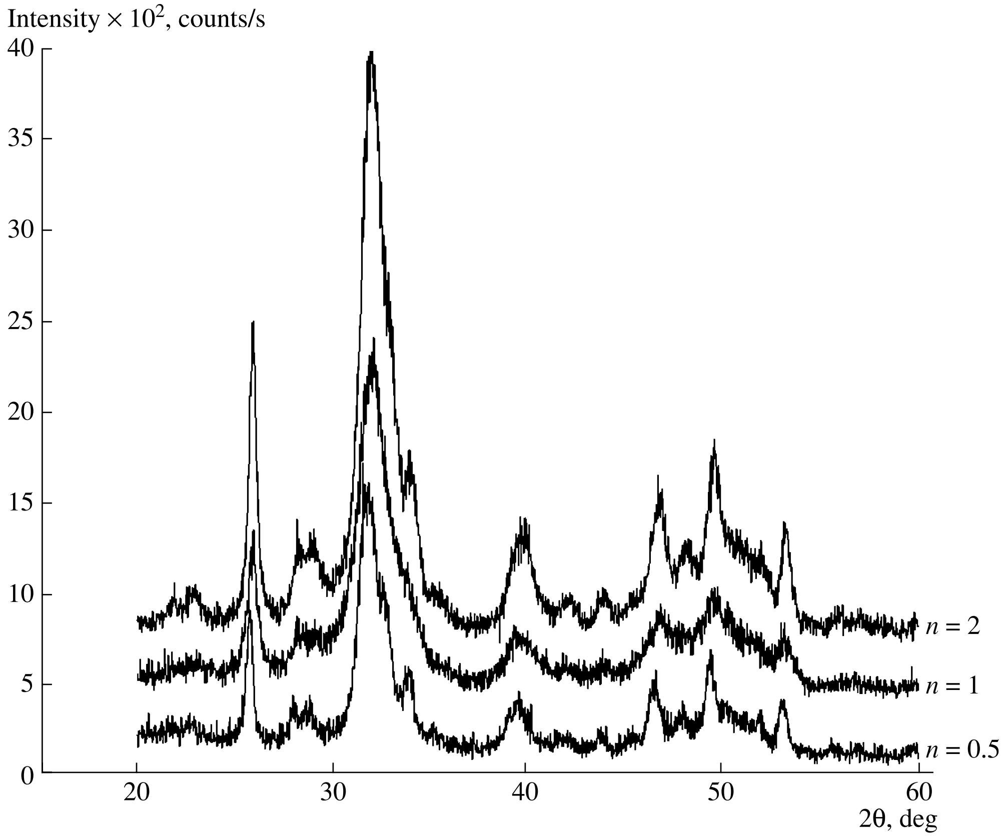

Fig. 2. Powder X-ray diffraction data for HAP synthesized from different calcium saccharates and ammonium hydrogen phosphate; *n* is the calcium oxide : saccharose ratio.

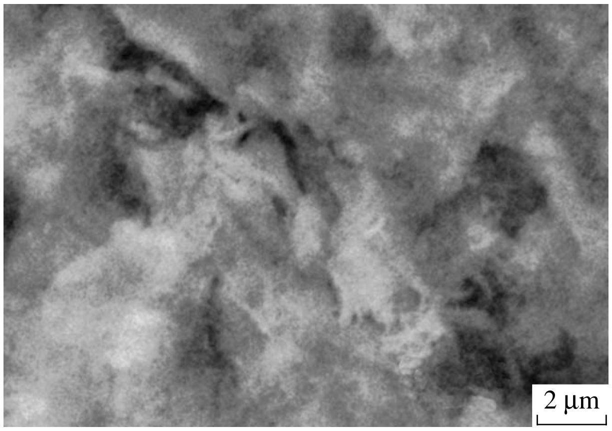

Fig. 3. Photomicrograph of a HAP sample synthesized from hemicalcium saccharate and ammonium hydrogen phosphate.

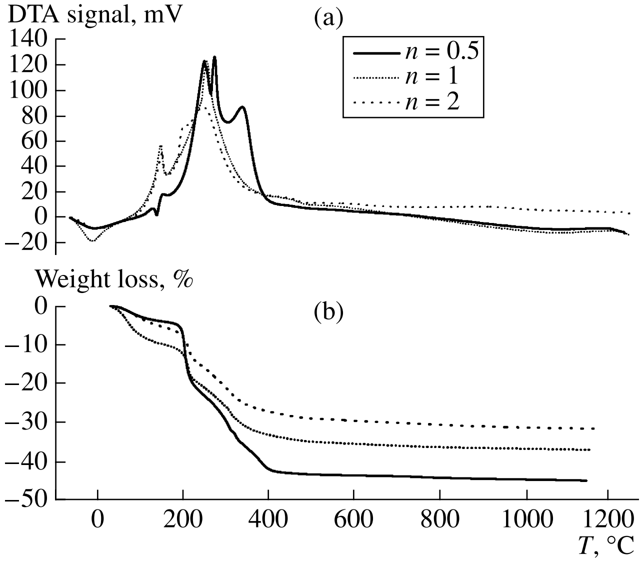

Fig. 4. (a) Differential thermal analysis data of synthesized HAP containing saccharose as the associated product; (b) weight loss vs. temperature.

X-ray diffraction patterns may be due to the amorphous nature of saccharose. The presence of crystalline saccharose and the possible products of its decomposition, glucose and fructose, is not confirmed. Thermal analysis points to a substantial (up to 45% for $n = 0.5$) content of saccharose in the powders after the synthesis. Peak broadening attests to the formation of nanosized HAP particles. Using the Scherrer equation, it is possible to determine the largest size of HAP crystals along the [001] direction: $D = 230 \pm 12$, 160 ± 10, and 210 ± 10 Å for compositions with n of 0.5, 1, and 2, respectively. The relatively small crystal size may be due to the increase in the solution viscosity upon an increase in the saccharose concentration, adsorption of saccharose on the surface of HAP particles, and high pH caused by the buffering properties of the saccharate solutions.

The electron photomicrograph (Fig. 3) shows a spall of the sample with $n = 0.5$ prepared after air drying of the precipitates. The HAP particles (light spots) are distributed in the saccharose template (dark background).

Decomposition of the organic component, saccharose, takes place in the range of 300–450°C (DTA exotherms related to pyrolysis and oxidation of the carbohydrate, Fig. 4a). In all cases, weight loss at 100°C caused by the removal of physically bound water was observed (Fig. 4b). The flat section in the weight loss curve (150–200°C) is obviously due to saccharose melting [11]: the melt coats the HAP particles, thus hampering water evaporation and decreasing the total evaporation surface. For all powders, the maximum rate of weight loss was observed at about 200°C. Decomposition of the organic component occurs apparently in two stages, 200–300°C and 300–450°C. The first stage corresponds to evolution of water and formation of anhydrides and the second stage involves decomposition of the organic residue. Note that further burning of pyrolyzed saccharose residues does not take place up to 800°C (constant weight in the TG curve and the absence of exotherms in the heat evolution curve).

Thus, this study represents the first attempt to synthesize HAP containing only biocompatible saccharose as the associated product and to determine the qualitative composition of solutions of calcium saccharates with a calcium-to-saccharose ratio of 0.5, 1, and 2. The use of saccharose allows one to synthesize nano-dispersed HAP powder free from cytotoxic inorganic impurities in high yield owing to the use of concentrated (up to 0.5 M) solutions of calcium saccharates.

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