Investigation of the Process of Hydroxyapatite Synthesis

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Abstract

Transformations taking place in the system $Ca(NO_3)_2-Na_2H_2Y-(NH_4)_2HPO_4-NH_4OH-H_2O$ during the homogeneous precipitation of hydroxyapatite were studied by means of conductometric, potentiometric and photometric analysis. It was established that the synthesis of hydroxyapatite from the aqueous solutions of calcium and phosphorus salts proceeds as a sequence of several stages, and the use of disodium ethylenediaminetetraacetate promotes the formation of microcrystalline powder with Ca/P ratio close to the biological value (1.67).

Key words: hydroxyapatite, sodium ethylenediaminetetraacetate, conductometric, potentiometric and photocolorimetric analysis methods, X-ray fluorescence analysis

INTRODUCTION

The material based on calcium phosphates, in particular hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA), is the subject of perennial investigation in the aspect of the possibilities to use it in medicine. Hydroxyapatite is a natural mineral and the predominant component of bones and tooth enamel [1]. At present, biological hydroxyapatite obtained from the bones of cattle [2] is widely used as dental implants, layers of endoprostheses, and also as a dietary additive of calcium to the ration of pregnant women and nursing mothers.

The synthesis of artificial HA is carried out mainly by precipitation from aqueous solutions of calcium salts with ammonium hydrophosphate [3–5]. However, with this approach, amorphous HA precipitations are formed within broad ranges of the concentrations of initial reagents, temperatures and methods of add-ing the precipitating agent. The use of the standard methods of ageing the precipitates in order to increase its crystallinity implies essential time consumption and does not provide reproducible results with respect to the Ca/P ratio.

To form microcrystalline HA and achieve the ratio Ca/P ~ 1.67 (close to the biological value), the synthesis of HA in the mode of homogeneous precipitation was carried out with the use of calcium complexing agent – the sodium salt of ethylenediaminetetraacetic acid (EDTA, Na_2H_2Y).

To optimize the proposed synthesis method, it is necessary to perform detailed investigation of the processes involved in the formation of chemical precipitates (HA), in particular the course of chemical interaction, the composition of the formed solid phases depending on the ratio between the interacting components etc. These tasks can be solved using the methods of physicochemical investigations to study the properties and structure of the precipitate.

EXPERIMENTAL

To synthesize HA, the solutions of 0.17 M $Ca(NO_3)_2$ (ch. d. a. reagent grade) and 0.02 M Na_2H_2Y (os. ch. reagent grade) were mixed in the stoichiometric ratio (the stoichiometry for the formation of Ca-EDTA complex). It is known that EDTA forms a strong complex with calcium and interacts with Ca^{2+} ions stoichiometrically at a ratio of 1 : 1. Then the 0.01 M solution of ammonium hydrophosphate (ch. d. a.) was added under stirring to the resulting mixture. During the synthesis, the reagents ratio (precipitator /precipitating ion) was varied from 0.1 to 1 (by volume) to achieve the stoichiometric ratio Ca/P = 1.67. The acidity of the medium was maintained at a level of pH 8–9 by adding the aqueous solution of ammonia. The precipitate was kept under the mother solution with excess Ca^{2+} at a temperature of 20 °C for 24 h, filtered, washed on the filter with hot distilled water, and dried on the filter at 100–150 °C. The resulting powder was placed in a crucible, heated at 250 °C for 1 h to remove the residual NH_4NO_3, and annealed at 700 °C to the constant mass for 1 h.

Synthesis processes were studied using conductometry, potentiometry and photometric analysis. Investigation of the system $Ca(NO_3)_2$–Na_2H_2Y–$(NH_4)_2HPO_4$–NH_4OH–H_2O was carried out at the constant temperature of 35 °C and pH 8. To decrease the experimental error, the experiment was repeated three times. On the basis of the data obtained, the dependencies were plotted for each analysis method; the curves from three parallel experiments were processed to obtain averaged curves characterizing the process of HA formation.

Investigation of the change of electrical conductivity was carried out with the help of a Multitest KSL-101 conductometer. Measurements were performed after each addition of a portion of precipitating agent (1 mL) to the mixture of solutions $Ca(NO_3)_2$ and Na_2H_2Y in the conductometric cell [7]. Three readings of the instrument were taken at the intervals of 2 min. The results of the experiment were plotted in the coordinates: electrical conductivity *versus* the volume of precipitating agent (V_{prec}); the plot was used to find the cross points of the sections of straight lines corresponding to the final points of precipitation (equivalence point). One straight line (to the equivalence point) reflects the change of the concentration of the precipitating ion and the ions of the precipitator, while another straight line (after the equivalence point) is connected with an increase in the concentration of precipitator ions [7].

Measurements of the optical density were carried out with KFK-3 spectrophotometer at the wavelength of $\lambda = 500$ nm. The choice of the wavelength was performed using the known procedure [8]. On the basis of the readings of the photometer, we plotted the dependence of optical density (D) on the volume of the precipitator agent V_{prec}.

We also studied the system $Ca(NO_3)_2$–Na_2H_2Y–$(NH_4)_2HPO_4$–NH_4OH–H_2O by means of potentiometry with the help of an I-500 pH-meter. The titration curve shows the changes of the potentials (pH) in the unit volume of the reagent between the solution under investigation and the electrode submerged into it [9]. The points of equivalence were determined from the jumps of the potential of indicator electrode on the curve of potentiometric titration.

RESULTS AND DISCUSSION

The results of experiments on the studies of HA synthesis are presented in Figs. 1–3. It was established during the experiments that even at the components ratio HPO_4^{2-} /Ca^{2+} = 0.3–0.4 (V_{prec} = 3–4 mL) the formation of the solid phase takes place, which is evidenced by jogs in the curves of electrical conductivity and optical density (see Figs. 1, 2). Nonlinear changes of the electrical conductivity of the solution are explained by the fact that the ion equilibria get shifted during the reaction, and the equilibrium concentrations of the ions of the compound to be precipitated but remaining unreacted yet are changed [7] (in the case under consideration, the concentration of calcium ion bound in the complex with EDTA).

We also observe a jump of potential at the potentiometric curve in the region V_{prec} = 3–4 mL (see Fig. 3), in the vicinity of which a decrease in the drift accompanying the potential change is observed [9] (Table 1). The equivalence point can be determined within the indicated range
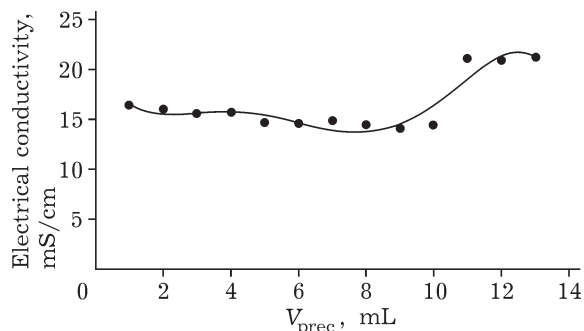
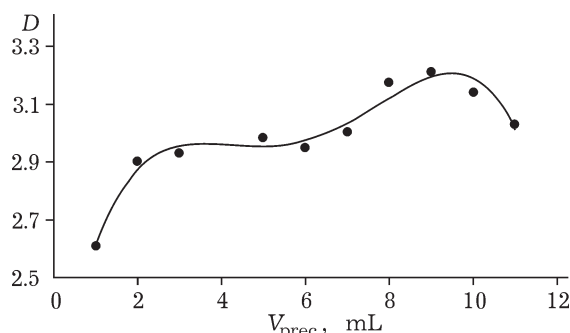Fig. 1. Conductometric curve of the synthesis of hydroxyapatite.

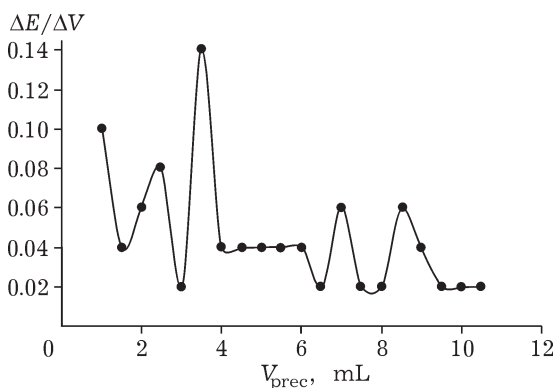Fig. 2. Dependence of the optical density (D) on the volume of added precipitating agent (V_{prec}).

Fig. 3. Curves of potentiometric titration.

TABLE 1

Data of potentiometric titration

V_{prec}, mL	E (pH)	$\Delta E/\Delta V$
1.0	9.59	0.05
1.5	9.54	0.013
2.0	9.52	0.015
2.5	9.49	0.016
3.0	9.45	0.0033
3.5	9.44	0.02
4.0	9.37	0.005
4.5	9.35	0.0044
5.0	9.33	0.004
5.5	9.31	0.0036
6.0	9.29	0.0033
6.5	9.27	0.0002
7.0	9.26	0.004
7.5	9.23	0.0013
8.0	9.22	0.00125
8.5	9.21	0.004
9.0	9.24	0.002
9.5	9.26	0.0011
10.0	9.27	0.001
10.5	9.36	0.00095

with the help of the plot built using the calculated parameter of potential change per unit change of the reagent volume $\Delta E/\Delta V$ (see Table 1). The value reaches its maximum at 3.5 mL (see Fig. 3). This equivalence point indicates the formation of the solid phase, presumably amorphous; of the acid calcium phosphate (pH of the system regularly decreases due to the formation of acidic reaction products).

The addition of the new portions of precipitating agent into the system leads to the appearance of the second break in the region corresponding to the ratio $HPO_4^{2-}/Ca^{2+} = 0.5–0.6$ at the curves of electrical conductivity and optical density (see Figs. 1, 2). This points to the secondary reactions taking place in the solution, and to the formation of calcium phosphate having variable composition. The nonlinear character of the changes of electrical conduction and rounded regions of the conductometric curves near the equivalence points can be due to the reversibility of these reactions [7].

According to the data obtained in [6], the use of the conductometric and photometric methods allows one to uncover the stages of the chemical interaction in a more complete manner during the investigation of many systems. However, it is a difficult problem to establish the detailed chemical mechanism of interaction with the help of the potentiometric method; as far as the system under consideration is concerned, this method fails to give information about all the stages of the formation of calcium phosphates. The position of the third bend on the curves (see Figs. 1, 2) corresponding to the ratio $HPO_4^{2-}/Ca^{2+} = 0.8–0.9$ (the consumption of the precipitating agent 80–90 % of the stoichiometric amount) points to

the formation of the basic salt having the composition $Ca_{10}(PO_4)_6(OH)_2$. With the approach of the system to the equivalence point, the complex Ca-EDTA gets decomposed completely, and non-stoichiometric apatite is formed; its presumable composition is $Ca_9HPO_4(PO_4)_5(OH)$. As a result of secondary inter-phase reactions in the excess of HPO_4^{2-} and Ca^{2+} ions increasing with an increase in the distance from the equivalence point, this compound is transformed into the basic salts of the stoichiometric composition. The excess reaches $50-75\%$ of the stoichiometry. Similar processes are observed during chemo-aging of non-stoichiometric precipitates.

It is known [6] that the synthesis of compounds by means of chemical precipitation causes complications of the chemical scheme of precipitation. The system Ca−P includes a number of non-stoichiometric compounds that are inevitably formed in the reaction medium during the precipitation of HA. In the system $Ca(NO_3)_2-(NH_4)_2HPO_4-NH_4OH-H_2O$, the process initially runs under the conditions of the large excess of Ca^{2+}. The chemism of the process includes the formation of the primary precipitate of calcium hydrophosphate as a result of the exchange interaction of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$. Further on, as the precipitated Ca^{2+} ions are consumed in solution with respect to the amount of precipitating agent HPO_4^{2-} entering the system, the reaction between the active freshly formed precipitate and the solution proceeds with the formation of non-stoichiometric apatite $Ca_9HPO_4(PO_4)_5(OH)$. The secondary process running in part through the topochemical mechanism leads to the formation of the precipitate of variable composition and to an increase in the fraction of calcium in its composition. In the system without the complexing agent, a small amount of basic salt (HA) is synthesized due to the local excess of Ca^{2+} arising at the site of input of one of the reagents.

Homogeneous precipitation with EDTA allows one to avoid local supersaturation of the solution. As a rule, this method belongs to diffusion-based slow processes. The sodium salt of EDTA forms a strong water-soluble complex with Ca^{2+} at a ratio of 1 : 1. Under the action of the precipitating agent calcium gradually gets liberated from this complex and forms water-insoluble precipitate HA. This method allows one to obtain HA with required composition and necessary properties.

The HA powder synthesized using EDTA was analyzed by means of X-ray fluorescence analysis. It was established that the powder corresponds to the composition of biological HA (the ratio Ca/P = 1.67). Microscopic examination showed that along with the amorphous structure HA contains inclusions having microcrystal composition.

CONCLUSION

Complex physicochemical studies showed that the proposed synthesis method results in an increase in HA crystallization time and complication of the process chemism. However, in the case of dosed input of calcium ions the process is controlled, so that the formation of HA with microcrystal structure and the ratio Ca/P close to the biological value is provided. The use of widespread analysis methods allows us to perform control during the introduction of this synthesis method into industrial production.

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