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Direct Synthesis of Nanocrystalline Hydroxyapatite by Hydrothermal Hydrolysis of Alkylphosphates

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Summary. The influence of reaction conditions (temperature, type of catalyst, time) on the base-catalyzed reaction of mono-, di-, and trialkylphosphates (alkyl = methyl, ethyl, *i*-propyl, *n*-butyl) with Ca^{2+} ions and on the structure and composition of the reaction products was studied. The composition of the calcium phosphates depends mainly on the reaction temperature. At temperatures below 100°C, a nanocrystalline solid product transforming into dicalcium phosphate by heating (calcination) was found. Pure nanocrystalline hydroxyapatite was prepared by hydrothermal synthesis at 160°C from mono- and dialkylphosphates. The size of hydroxyapatite crystallites was about 1 nm, the particle size about 150 nm. Agglomerated particles of hydroxyapatite larger than 2 μm were prepared at 200°C. Hydrothermal reaction of trialkylphosphates with Ca^{2+} ions at 200°C produced $CaHPO_4$. The experimental results were used to propose a reaction mechanism for base-catalyzed hydrothermal reactions of alkylphosphates with Ca^{2+} ions.

Keywords. Ceramics; Total synthesis; Nanostructures; Reaction mechanism; Hydroxyapatite; Nanoparticles; Hydrothermal synthesis; Alkylphosphates.

Introduction

Natural calcium phosphates, in particular hydroxyapatites (*HA*), form the fundamental inorganic component of the skeletal systems of vertebrates. Synthetic hydroxyapatites have similar properties to those of natural hydroxyapatites and are therefore frequently used as biomaterial in orthopaedics and dentistry. Among the most frequent medical applications of hydroxyapatite are biologically active surface layers applied to biologically inert ceramic or metallic materials. The usual methods of applying such coatings, *i.e.* flame and plasma spraying, operate at temperatures that often decompose hydroxyapatite into calcium phosphates which are of low stability in the organism. Exploring new methods of applying hydroxyapatite coatings represents an important field in contemporary biomaterials research. Most of these methods, such as dip coating, spraying, and spin coating, employ liquid precursors of hydroxyapatite at normal temperature. Among the most interesting precursors of calcium phosphates or phosphate ceramics are

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alkylphosphates because of their easy preparation *via* the reaction of P_2O_5 with alcohols [1–3].

Alkylphosphates reacting with aqueous solutions of calcareous salts at normal temperature form gels or precipitates that transform into hydroxyapatite or a mixture of calcium phosphates ($Ca_2P_2O_7$, $Ca_3(PO_4)_2$) during heat treatment (sintering) [4–7]. Pure hydroxyapatites have been prepared by hydrothermal decomposition of inorganic phosphates (of ammonia or calcium) in the presence of Ca^{2+} ions at a temperature of 500°C. Usually, microcrystalline particles of 5–25 μm in size were obtained [8–10]. With the temperature reduced to just above 100°C, this hydrothermal decomposition yielded nanocrystalline hydroxyapatites whose particle size was between 15 and 300 nm [11–13]. The hydrothermal method was used successfully also in the synthesis of crystalline hydroxyapatite from trialkylphosphate and calcium acetate. Particles of hydroxyapatite prepared at 350°C were 10–30 nm in size [14].

From the above overview it can be seen that the hydrothermal reaction of alkylphosphates with Ca^{2+} ions at temperatures ranging from 100 to 200°C could yield pure nanocrystalline hydroxyapatite suitable for the preparation of bioactive pure hydroxyapatite coatings by the sol-gel method (dip coating, spraying). The present paper is concerned with the study of this problem.

Results and Discussion

Reaction of mono- and dialkylphosphates with calcium acetate at 60°C

Determination of the phase composition of the precipitates formed at 60°C by reaction of methyl-, ethyl-, *i*-propyl-, and *n*-butyl phosphate with calcium acetate under normal conditions by means of X-ray analysis was not possible. The spectra did not correspond to any of the expected products ($CaHPO_4$, $Ca_2P_2O_7$, $Ca_3(PO_4)_2$, *HA*). The calcination of precipitates yielded mostly $Ca_2P_2O_7$, a mixture of $Ca_2P_2O_7$ and $Ca_3(PO_4)_2$, or *HA*.

Figure 1 shows the IR spectra of precipitates formed by the reaction of mono- and diethylphosphate 1, 2, and 3 hours after the reaction. It is evident from Fig. 1 that after 1 hour of reaction the composition of the precipitate did not change. The band at 2970 cm^{-1} corresponding to the vibration of the CH_3 group in the P–OEt group shows that the precipitate contained part of the non-reacted P–OEt groups, which did not change with the reaction time.

Hydrothermal reaction of mono- and dialkylphosphates with calcium acetate at 160°C

Under conditions of hydrothermal synthesis at 160°C and 16 MPa the preparation of hydroxyapatite required 4 hours in all cases (Fig. 2). The size of crystallites calculated from RTG spectra was within a narrow interval from 1.0 to 1.1 nm. The size and shape of hydroxyapatite particles can be seen in Fig. 3. The particles are of almost globular shape. In the case of the product from methyl

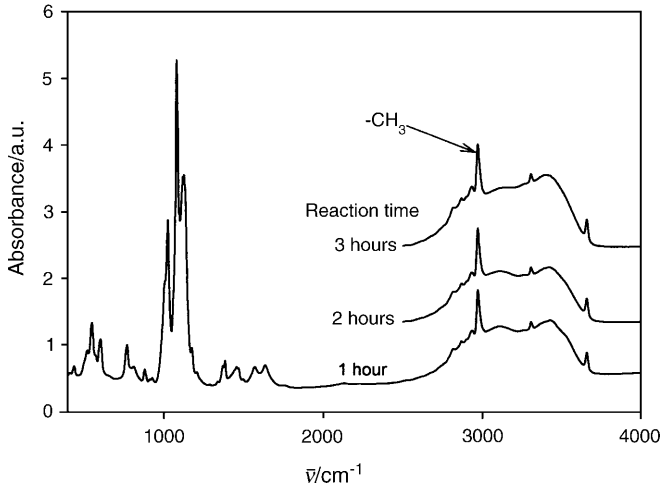


Fig. 1. FT-IR spectra of precipitates prepared by reaction of ethylphosphates with calcium acetate at 60°C for 1, 2 or 3 h

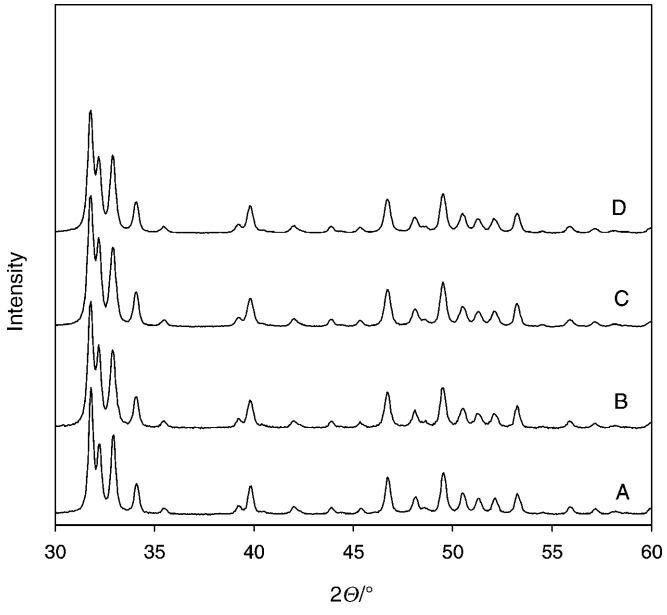


Fig. 2. XRD patterns of hydroxyapatite prepared by hydrothermal synthesis at 160°C from A) methylphosphate, B) ethylphosphate, C) *i*-propylphosphate, D) *n*-butylphosphate

phosphate the particle size was 180 nm, in the case of ethylphosphate 160 nm, in the case of *i*-propylphosphate 150 nm, and in the case of *n*-butylphosphate 160 nm (Table 1).

It follows from Fig. 3D that in particular in the case of hydroxyapatite prepared from *n*-butylphosphate the particles formed 'soft' agglomerates of more than 1 μm in size. The lowest mean size of agglomerates (measured by laser diffraction) was established in the case of hydroxyapatite prepared from methylphosphate

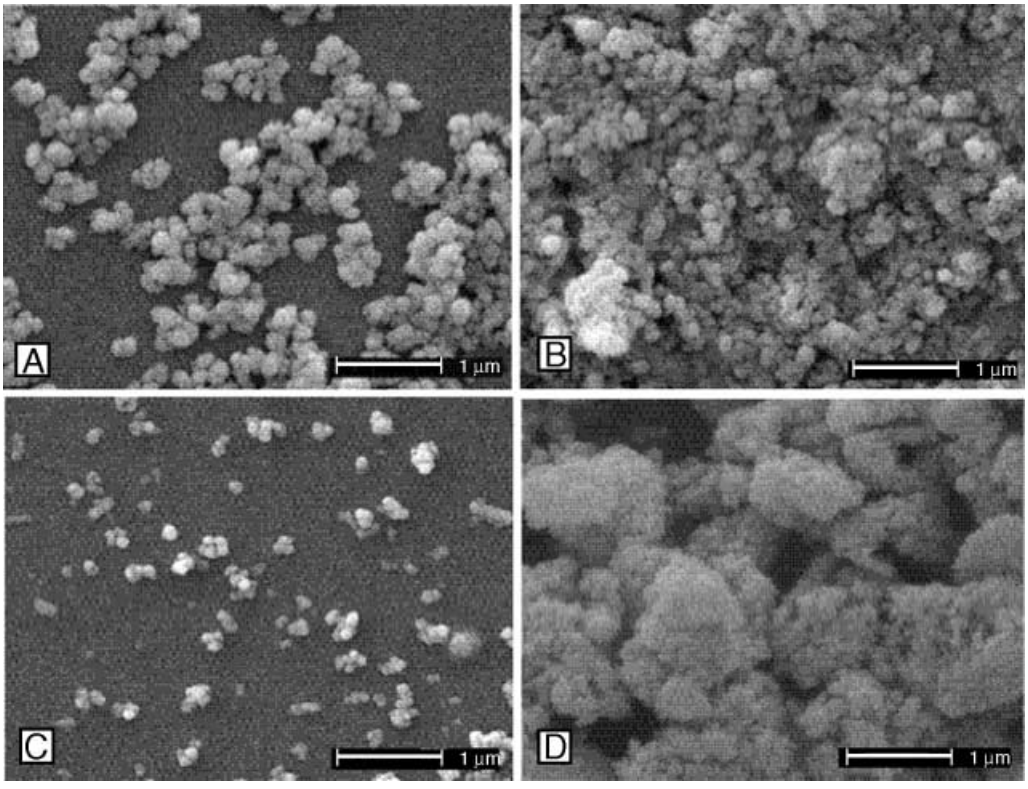


Fig. 3. SEM micrographs of hydroxyapatite particles prepared by hydrothermal synthesis at 160°C from A) methylphosphate, B) ethylphosphate, C) *i*-propylphosphate, D) *n*-butylphosphate

(0.59 μm), the highest mean size in the case of hydroxyapatite made from *i*-propylphosphate (2.2 μm).

Hydrothermal reaction of mono-, di-, and trialkylphosphates with calcium acetate at 200°C

Via hydrothermal reaction of mono- and dialkylphosphates at 200°C, hydroxyapatite was formed from all alkyl derivatives of phosphoric acid (Fig. 4). The size of hydroxyapatite crystallites from methylphosphate and ethylphosphate was the smallest (0.7 and 0.6 nm, respectively); the size of *HA* crystallites from *i*-propylphosphate and *n*-butylphosphate was 1.1 and 0.9 nm, respectively (Table 1, Fig. 4). The structure, size, and shape of *HA* particles are evident from Fig. 5. Hydroxyapatite particles formed from methyl- and ethylphosphate were of roughly globular shape, and their size was 230 and 210 nm, respectively. Hydroxyapatite particles formed from *i*-propyl- and *n*-butylphosphate had globular to elongated shape and 120 and 200 nm, respectively, in size. The particles of all hydroxyapatite products were agglomerated into 'hard' agglomerates whose sizes (established by laser diffraction) are given in Table 1. Agglomerates of methylphosphate were 2.74 μm in size, those of ethylphosphate 5.12 μm, those of *i*-propylphosphate 3.44 μm, and those of butylphosphate 4.95 μm. Breaking up the aggregates into separate particles could not be achieved by ultrasound but by colloidal milling for 6 hours.

Table 1. Results of analysis of Ca-phosphate products

Alkylphosphate temperature	Analysis			
	Phase composition	Size of prim. Crystallites (XRD)	Particle size (SEM)	Mean size of agglomerates (laser diffraction)
methylphosphate, 60°C	unknown	–	–	–
ethylphosphate, 60°C	unknown	–	–	–
i-propylphosphate, 60°C	unknown	–	–	–
n-butylphosphate, 60°C	unknown	–	–	–
methylphosphate, HT/160°C	*HA*	1.1 nm	180 nm	0.59 μm
ethylphosphate, HT/160°C	*HA*	1.0 nm	160 nm	1.31 μm
i-propylphosphate, HT/160°C	*HA*	1.0 nm	150 nm	2.22 μm
n-butylphosphate, HT/160°C	*HA*	1.0 nm	160 nm	1.70 μm
methylphosphate, HT/200°C	*HA*	0.7 nm	230 nm	2.74 μm
ethylphosphate, HT/200°C	*HA*	0.6 nm	210 nm	5.12 μm
i-propylphosphate, HT/200°C	*HA*	1.1 nm	120 nm	3.44 μm
n-butylphosphate, HT/200°C	*HA*	0.9 nm	200 nm	4.95 μm
trimethylphosphate HT/200°C	$CaHPO_4$	–	–	–
triethylphosphate HT/200°C	$CaHPO_4$	–	–	–

After hydrothermal reaction of trimethyl- and triethylphosphate with calcium acetate at 200°C the only crystalline phase found in the reaction product was $CaHPO_4$; no hydroxyapatite phase was detected.

Proposed reaction mechanism of hydrothermal synthesis of hydroxyapatite from alkylphosphates

The reaction of alkylphosphates with Ca^{2+} in aqueous medium proceeds in three basic steps. In the first step the hydrolysis of the P–O*R* bond takes place, in the second step the calcium salt of alkylphosphoric acid is formed, and in the third step the P–OH bond (formed by the hydrolysis of the P–O*R* bond) reacts with Ca^{2+} accompanied by the formation of Ca-phosphate [15]. Under normal temperatures the rate of the hydrolysis of P–O*R* bond is low [16, 17]. The reaction is reversible and, for example, in the product of the reaction of trialkylphosphate with Ca^{2+} one –O*R* group per one P was found [5]. These data from the literature are corroborated by our results obtained for the reaction of mono- and dialkylphosphates under

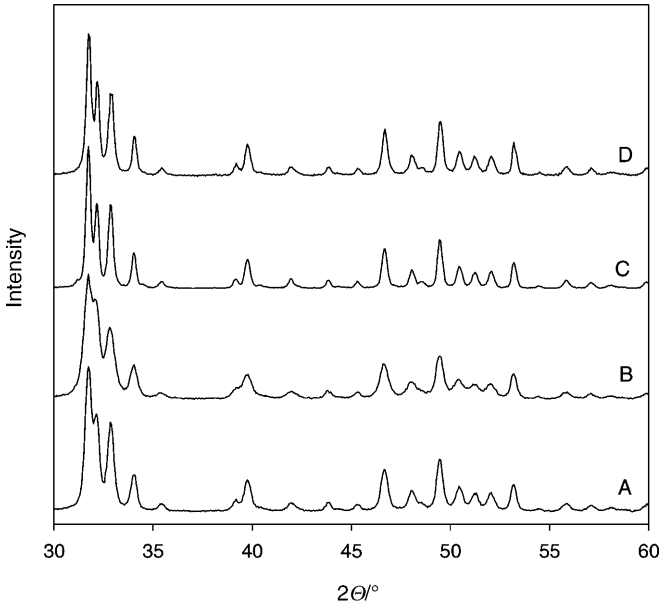
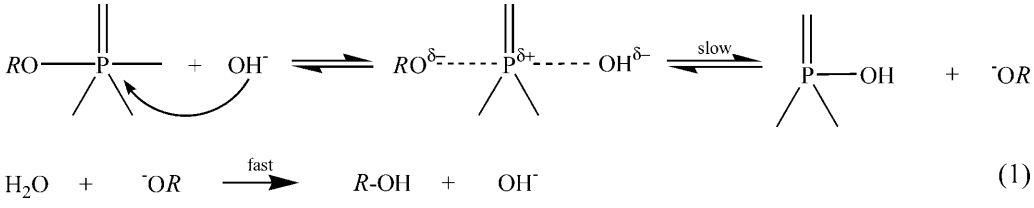


Fig. 4. XRD patterns of hydroxyapatite prepared by hydrothermal synthesis at 200°C from A) methylphosphate, B) ethylphosphate, C) *i*-propylphosphate, D) *n*-butylphosphate

normal conditions. The reaction product was not hydroxyapatite, but undefined crystalline compounds containing P–O*R* groups.

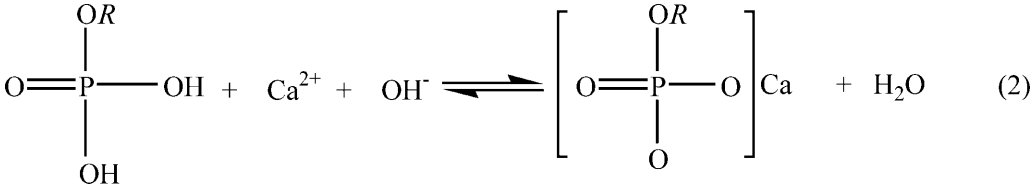
In the course of the hydrothermal reaction the rate of the hydrolysis of P–O*R* bonds increased, the stability of the P–O*R* bond was reduced, and the equilibrium was shifted towards the hydrolysis product. The base-catalyzed hydrolysis of the P–O*R* bond probably proceeds according to Eq. (1):



(1)

Under hydrothermal conditions, the hydrolysis of di- and trialkylphosphates proceeds gradually, and the final product is phosphoric acid.

In the presence of Ca^{2+} ions, however, the second step proceeds probably in parallel with the hydrolysis according to Eq. (2):



(2)

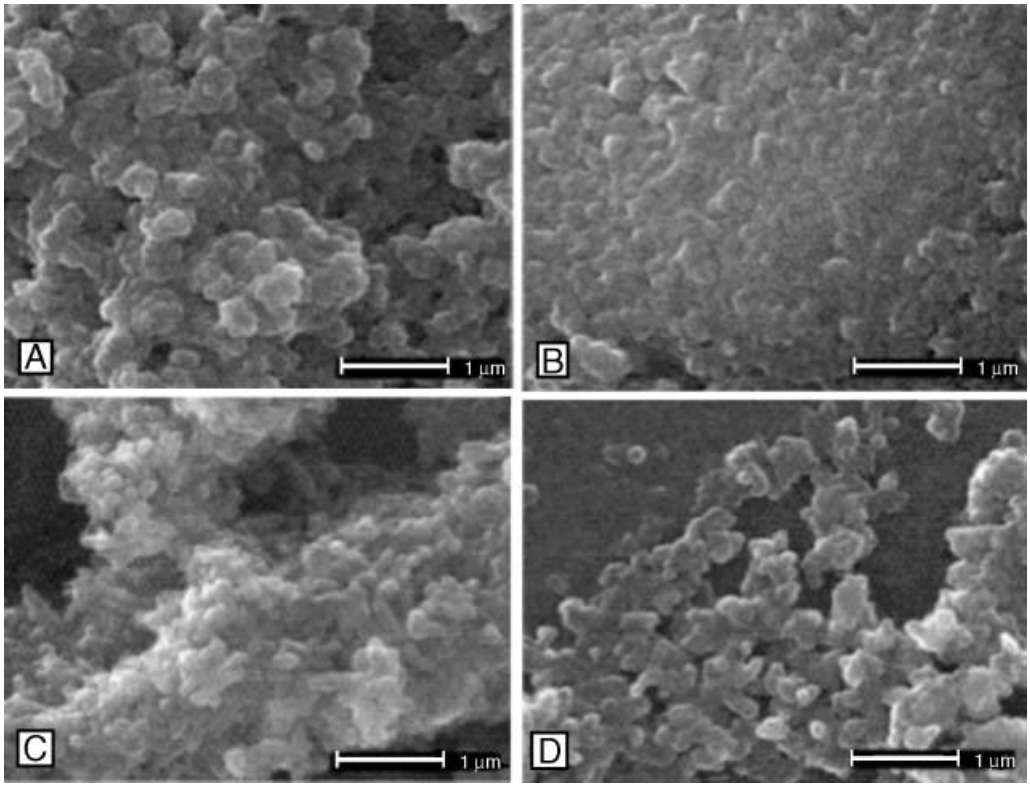
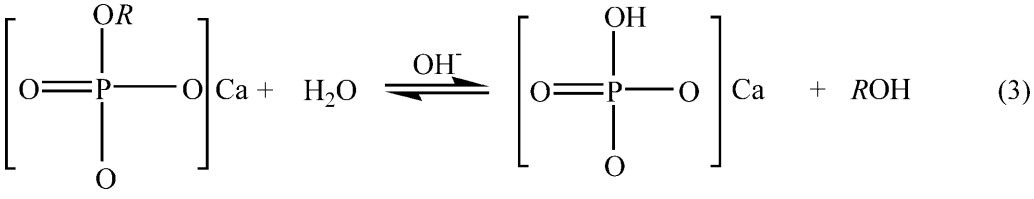


Fig. 5. SEM micrographs of hydroxyapatite particles prepared by hydrothermal synthesis at 200°C from A) methylphosphate, B) ethylphosphate, C) *i*-propylphosphate, D) *n*-butylphosphate

The existence of such or a similar intermediate product during hydrothermal synthesis can be inferred from the absorption of the CH_3 group in the IR spectrum (2970 cm^{-1}). Another fact that supports this hypothesis is the reaction product of the hydrothermal reaction of trialkylphosphates with Ca^{2+}, *i.e.* calcium hydrogenphosphate ($CaHPO_4$). It is assumed that the product is formed by hydrolysis of the last P-O*R* group of trialkylphosphate according to Eq. (3):



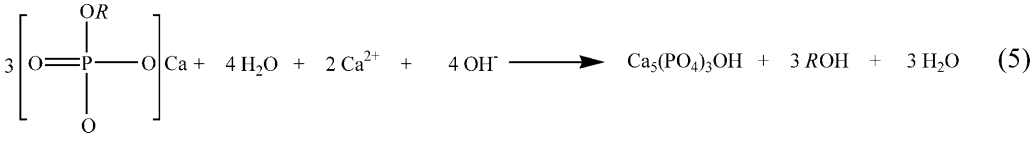
(3)

In the case of the hydrothermal reaction of mono- and dialkylphosphates, the transformation of $CaPO_3(OH)$ probably also proceeds according to the same equation.

The last step of the reaction mechanism is the hydrothermal transformation of $CaHPO_4$ into hydroxyapatite [18] according to Eq. (4):

$$3\,CaHPO_4 + 2Ca^{2+} + 4OH^- \longrightarrow Ca_5(PO_4)_3OH + 3\,H_2O \qquad (4)$$

A direct reaction of $CaPO_3(OH)$ according to the Eq. (5) cannot be excluded either:



Hydroxyapatite coatings applied by the dip coating method

In Fig. 6, the surface of hydroxyapatite layers prepared by applying hydroxyapatite sols to Al_2O_3 ceramics by the dip coating method and subsequent sintering at 800–1100°C is shown. The coatings sintered at 800°C and 900°C are formed by hydroxyapatite particles about of 150 nm in size. Figure 6C shows the surface of a *HA* layer sintered at 1000°C; the layer is more compact than in the preceding case, and the particle size increased to 220 nm. The surfaces sintered at 1100°C were formed by sintered particles of 660 nm in size. An XRD analysis of the coatings revealed that hydroxyapatite transformed into β-$Ca_3(PO_4)_2$ during sintering at 1100°C. Hence, it follows that the transformation of nanocrystalline hydroxyapatite into β-$Ca_3(PO_4)_2$ took place at a temperature some 300°C lower than that established for hydroxyapatite ceramics with particle size around 5 μm [19, 20].

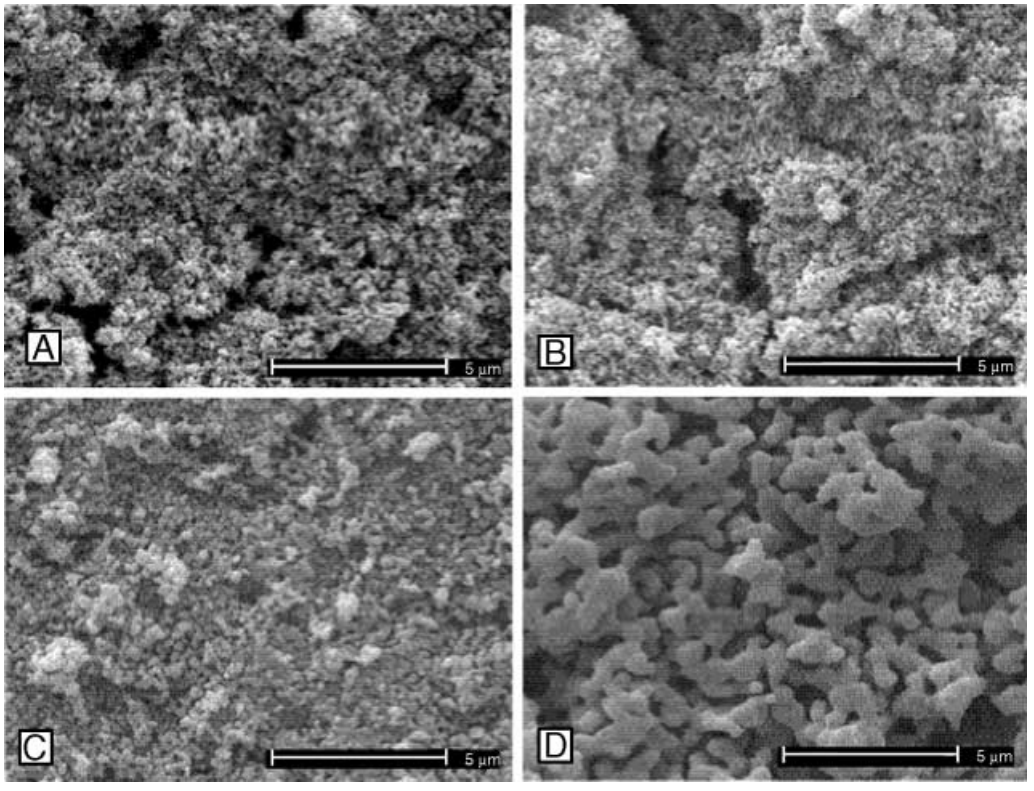


Fig. 6. SEM micrographs of hydroxyapatite coatings prepared by dip coating of hydroxyapatite sol on alumina substrate sintered at A) 800°C, B) 900°C, C) 1000°C, D) 1100°C

Conclusions

It was found that a hydrothermal reaction of mono- and dialkylphosphates with Ca^{2+} ions at a temperature of 160°C for 4 hours yielded nanocrystalline particles of hydroxyapatite of about 150 nm in size which are suitable for the preparation of colloidal suspensions and for applying bioactive layers by the dip coating method.

Experimental

Synthesis of hydroxyapatite

Hydroxyapatite was prepared by the reaction of calcium acetate with mono- and dialkylphosphate or trialkylphosphate (Fluka) at a molar Ca:P ratio of 1.67 in the presence of a base (*DEA*, NH_4OH). Mono- and dialkylphosphates (methyl, ethyl, *i*-propyl, *n*-butyl) were prepared by the reaction of P_2O_5 with the respective alcohol such that the resultant concentration of P in alkylphosphate was 2 mol/dm^3.

The synthesis of hydroxyapatite was conducted

i) under classical conditions: a solution of calcium acetate was added in drops into the reactor containing an alcoholic solution of the alkylphosphate. The *pH* value was maintained at 8–9 by addition of the base, and the reaction mixture was maintained at a temperature of 60–70°C for a period of 4 h.

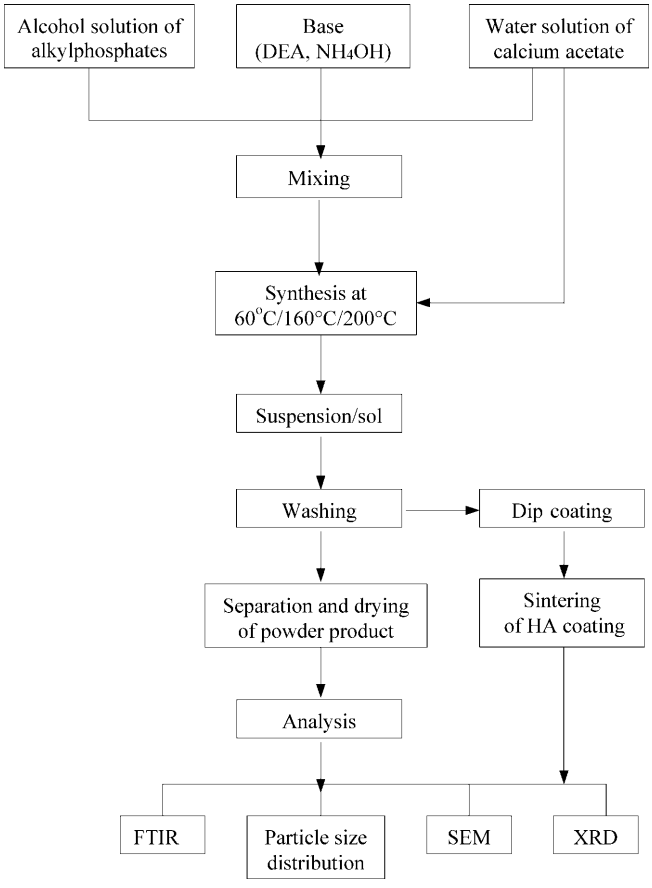


Fig. 7. Flow chart of nanocrystalline hydroxyapatite synthesis and analysis

Table 2. Conditions of hydroxyapatite synthesis

Synthesis type	Alkylphosphate	Base	Reaction time/h	Heat treatment
classical	mono- and dimethyl	NH_4OH	4	drying, calcination
classical	mono- and diethyl	NH_4OH	4	drying, calcination
classical	mono- and di-*i*-propyl	NH_4OH	4	drying, calcination
classical	mono- and di-*n*-butyl	NH_4OH	4	drying, calcination
HT/160°C	mono- and dimethyl	*DEA*	5	drying
HT/160°C	mono- and diethyl	*DEA*	5	drying
HT/160°C	mono- and di-*i*-propyl	*DEA*	5	drying
HT/160°C	mono- and di-*n*-butyl	*DEA*	5	drying
HT/200°C	mono- and dimethyl	*DEA*	5	drying
HT/200°C	mono- and diethyl	*DEA*	5	drying
HT/200°C	mono- and di-*i*-propyl	*DEA*	5	drying
HT/200°C	mono- and di-*n*-butyl	*DEA*	5	drying
HT/200°C	trimethyl	*DEA*	5	drying
HT/200°C	triethyl	*DEA*	5	drying

ii) under hydrothermal conditions (HT, see Fig. 7): the reactants were mixed together, poured into a hydrothermal reactor, and heated to 160°C (5 h, 16 MPa).

iii) under hydrothermal conditions (HT, see Fig. 7): a mixture of alkylphosphate and the base was poured into the reactor. After heating to 200°C, a solution of calcium acetate was added in doses by a pressure pump at 16 MPa. With the doses added, the mixture was heated to 200°C for 5 h.

The product was separated, repeatedly washed with distilled H_2O, and dried at 100°C. Synthesized *HA* in powder form was studied by RTG/X-ray phase analysis (Xpert, Philips), IR spectroscopy, scanning electron microscopy (XL 30, Philips); using laser diffraction, the particle sizes and the distribution of particle sizes were studied (LA-500, Horiba).

The types of reactants used, synthesis conditions, and types of heat treatment are summarized in Table 2.

Preparation of layers by the dip coating method

Hydroxyapatite layers were prepared by applying hydrothermally synthesized hydroxyapatite sol to ceramic substrates (Al_2O_3) by the dip coating method (Dip Master model 200, Chemat Technology Inc.). Three layers were applied to the substrate at a rate of 50 mm/min, and after drying they were sintered at temperatures of 800, 900, 1000, and 1100°C for 2 h. The coatings were studied by X-ray diffraction (Xpert, Philips) and structural analysis (SEM; XL 30, Philips).

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