RIGA TECHNICAL UNIVERSITY
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INFLUENCE OF CALCIUM PHOSPHATE SYNTHESIS PARAMETERS ON PROPERTIES OF BIOCERAMICS

Summary of Doctoral Thesis

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THE DOCTORAL THESIS IS SUBMITTED FOR AWARD OF DOCTORAL DEGREE IN ENGINEERING SCIENCES AT RIGA TECHNICAL UNIVERSITY

The Thesis for the doctoral degree in engineering sciences is to be publicly defended on November 22, 2011 at Riga Technical University …….....

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CONFIRMATION

I confirm that I have developed the present Doctoral Thesis, which is submitted for consideration at Riga Technical University for scientific degree doctor of engineering sciences. The Doctoral Thesis has not been submitted at any other university for the acquisition of a scientific degree.

Kristīne Šalma-Ancāne.....(signature)

Date:.....

The Doctoral Thesis is written in Latvian language; it contains introduction, literature review (5 chapters), experimental part, results and discussion (5 chapters), conclusions, 149 references, 69 graphs and 23 tables, altogether 169 pages.

ACKNOWLEDGMENTS

I would like to express the deepest gratitude to the scientific supervisor of this Doctoral Thesis, professor, Dr.sc.ing Līga Bērziņa-Cimdiņa for the motivation and support provided during writing. Thanks for the unbiased critics and worthy advice during in all of those study years.

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The most whole-hearted thanks goes to my closest people – my parents, my sister and her family, my husband and son, to my husband's family, for the inspiration, trust and immeasurable support.

OVERVIEW OF DOCTORAL THESIS

Research Topicality and Motivation. The World Health Organization and 37 countries of the world have proclaimed the period of year 2000-2010 as Decade of Bones and Joints. The aim of this global initiative is to improve life quality for people with problems with musculoskeletal system.

After evaluation of information found in scientific literature, analyzed in the literature review, it can be concluded that, in spite of the long-term research of calcium phosphates and successful application of these in the field of biomaterials research, there is still a great potential for further development and usage of these materials in the various scientific disciplines: materials science, biology, medicine – those related to the restoration of functionality of human body.

In Latvia and Baltic region, there is still no manufacturing site established for production of calcium phosphate biomaterials – products with great added value (approximate price of one gram is 100 EUR), that could compete with European and world level manufacturers of biomaterials.

As practice has shown, not always purchased calcium phosphate materials, mainly commercial calcium phosphate starting materials, have got properties and quality promised by the manufacturer. Frequently, the manufacturer's information about offered product is insufficient or imprecise, making use of these materials complicated for fabrication implants. The usual imperfections of calcium phosphate starting materials are non-predictable properties after thermal treatment (phase composition and purity, chemical composition, thermal stability, etc). These can be found only after thermal treatment of ready to use implant materials. Therefore one of the goals of this Thesis was to develop a technology to obtain basic calcium phosphate materials with predictable and reproducible properties.

Aim of Doctoral Thesis: To develop a predictable, reproducible single or multiphase calcium phosphate

(hydroxyapatite, β-tricalcium phosphate, biphasic calcium phosphates) synthesis technology in a laboratory scale reactor for medical application, and to evaluate the influence of the synthesis technological parameters on properties of obtained calcium phosphate bioceramics.

Tasks of the Doctoral Thesis:

- Using the data found by analysis of scientific literature, to develop a calcium phosphate synthesis method scheme for obtaining hydroxyapatite, β-tricalcium phosphate and biphasic calcium phosphates after thermal treatment.
- Select variable technologic parameters of the synthesis which could influence phase composition and structure of synthesized products after thermal treatment.
- Synthesize calcium phosphate in series, systematically varying synthesis parameters.
- Determine limit values of the technological parameters that influence the composition and structure of the chosen synthesis product.
- Investigate the synthetic and biogenic calcium oxide containing starting materials, their influence on physical and chemical properties of hydroxyapatite bioceramics.
- Investigate influence regime of synthesis temperature and reagent concentration on composition and structure of the synthesized product.
- Characterize structure and composition of the synthesized and thermally treated (at temperatures ranging from 700 to 1300°C) calcium phosphates.

Scientific importance and novelty of the Doctoral Thesis.

For the first time, morphology of calcium hydroxide suspensions obtained from biogenic and synthetic calcium oxides after the “lime slaking” process and homogenization in a planetary ball mill were systematically investigated and compared.

For the first time, calcium phosphates are synthesized using modified wet chemical precipitation method with non-stoichiometric ratio of reagent concentrations $[Ca^{2+}]/[PO_4^{3-}]$ = 0.15 M/4.76 M.

For the first time, influence of the biogenic and synthetic calcium oxide starting materials on microstructure and color change of the hydroxyapatite bioceramics was investigated.

Influence of synthesis temperature regime, reagent concentration and final pH value on the phase composition, thermal stability, molecular structure, morphology and microstructure of the synthesized and thermally treated calcium phosphate product was evaluated.

Practical importance of Doctoral Thesis. An effective calcium phosphate multiphase synthesis technology was developed to obtain bioceramics (with controllable phase composition), that demonstrated a good perspective for manufacture of bioceramic implant materials with predictable composition and structure.

A technology to obtain homogenous, highly dispersed calcium hydroxide suspension for synthesis of calcium phosphates using wet chemical precipitation method was developed.

Publication and Approval of Doctoral Thesis. Scientific achievements and main results are presented on the 20 international scientific conferences, 8 full text scientific publications and 12 reviewed conference theses, mentioned in the summary.

LITERATURE REVIEW

Even though a very wide range of basic calcium phosphate materials are available on the market, many have relatively unpredictable properties after the thermal treatment – phase and chemical composition, microstructure, degree of crystallinity, thermal stability, etc., that are required for development of implant materials.

The main factor that influences characterization of these materials is the chosen manufacture technology and controlling technological multiparameters, which irreversibly influence properties of the end product.

The wet chemical precipitation method was chosen for synthesis of calcium phosphates in the experimental part. It is one of the methods to obtain hydroxyapatite; it demonstrates both complexity of the method from point of view of physical chemistry and variability of product properties by controlling synthesis parameters. Therefore developing materials based on calcium phosphates with a range of variable properties and a predictable, reproducible quality is still necessary.

After literature review, it can be concluded that use of various starting materials for synthesis of calcium phosphates, and their direct or indirect impact on properties of product, that can influence bioactivity of materials is important, but poorly investigated.

RESEARCH METHODOLOGY

To characterize starting materials and synthesis products multiple analytical techniques were used. Analysis of crystalline phase composition of starting materials and calcium phosphate (CaP) products was performed using *X-ray powder diffraction method (XRD)*; HAp/β-TCP phase ratio of thermally treated CaP products was determined using *semi-quantitative phase analysis by X-ray powder diffraction method using a calibration curve*; the mean (volume average) crystallite size of synthesized HAp products (D) in two Miller crystallographic planes (L_{200} un L_{300}) was calculated using *Sheerer equation* (crystallinity degree, that corresponds to the fraction of crystalline phase (volume) in thermally treated HAp samples was calculated using intensity of the characteristic diffraction peaks; molecular structure analysis of synthesized and thermally treated CaP products – infrared spectra (IR) of adsorption bands of characteristic chemical functional groups–was obtained using *Fourier transform infrared*

spectroscopy (FT-IR); investigation of the morphology and microstructure of synthesized and thermally treated CaP products and chemical analysis were performed using *field emission scanning electron microscopy (FE-SEM)* and *energy dispersive X-ray spectroscopy* (EDS); to determine microelement composition of the synthesized and thermally treated CaP products, *inductively coupled plasma mass spectrometry (ICP-MS)* was used; research of thermal stability of the synthesized CaP product phases was performed using *differential thermal analysis (DTA);* to investigate sintering process of synthesized CaP products, *high temperature microscopy* (ATM) was used; to investigate $Ca(OH)_2$ suspension morphology, *optical microscopy* was used; to calculate open and total porosity of HAp, β-TCP and BCP bioceramic samples *Archimedes (impregnation) method* was used.

EXPERIMENTAL PART

Choice and modification of synthesis method.

To synthesize calcium phosphates (HAp – hydroxyapatite, β-TCP – β-tricalcium phosphate and BCP – biphasic calcium phosphates) wet chemical precipitation method was chosen. This method is conventionally used for HAp ($Ca_{10}(PO_4)_6(OH)_2$) synthesis. The method is based on neutralization of $Ca(OH)_2$ suspension with solution of phosphoric acid:

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

Taking into account that $Ca(OH)_2$ is poorly soluble in the water and state of phosphate ions depends on pH of reaction synthesis media, calcium phosphate (CaP) precipitation includes dissolution of $Ca(OH)_2$, diffusion of calcium and hydroxy ions and dissociation of phosphate ions. Control of pH of the synthesis media is essential in order to avoid formation of undesirable CaP phases.

In comparison to other known methods, advantages of this method are as follows: it is applicable to obtain CaP in large quantities, it has a large outcome (~ 87%), it is low cost, it has a relatively simple technology, and it is environmentally friendly method with water as only by-product, properties of synthesis product are variable.

Choice of this method is based on possibility to perform scale up of synthesis process and to adapt the technology to manufacture large quantities. In such way this work could benefit further research and experiments.

The main disadvantage of this method is necessity for precise regulation of technologic multiparameters. Variations of these can significantly influence end product of synthesis and properties of bioceramic implant after thermal treatment.
Regulation of these multiple parameters can significantly impact reproducibility of synthesis, in order to obtain a product with constant quality. Possibility to reproduce synthesis process is the main precondition to manufacture large quantities.

In order to eliminate disadvantages of chosen method and to obtain pure HAp, pure β-TCP and BCP after thermal treatment, synthesis method was modified within experimental part. The following improvements were made to the chosen method:

1) Processing of $Ca(OH)_2$ suspension in a planetary ball mill, in order to obtain a homogenous and highly dispersed suspension, as well as to ensure the homogeneity of the suspension in the precipitation process of CaP phases.
2) Number of the variable technical parameters is minimized (variable parameters - final synthesis pH and synthesis temperature), in order to obtain a predictable and reproducible synthesis product and possibility to obtain a CaP product with controllable rate of HAp and β-TCP phases.
3) Chosen molar concentrations of $Ca(OH)_2$ suspension and H_3PO_4 solution differ from often applied principle of the classical „stoichiometric solution" method - using stoichiometric ratio of reagents. Syntheses were made with c = $[Ca^{2+}]/[[PO_4^{3-}]$ = [0.15 M]/[4.76 M]. A precise control of stoichiometry of synthesis solution does not guarantee the stoichiometry of obtained product. A higher concentration of H_3PO_4 solution increases dissolution of $Ca(OH)_2$ and influences kinetics of the CaP sedimentation. The main disadvantage of the „stoichiometric solution method" is eliminated – a requirement for large volumes of very dilute solutions.

Choice and investigation of starting materials.

Before beginning synthesis, a complex investigation of starting materials was made. Commercially available synthetic calcium oxides from Riedel-de Haën® (CaO_R) and Fluka (CaO_F) and biogenic materials that are common in nature – eggshells and land snail shells (*Arianta arbustorum*) after thermal treatment, respectively, CaO_{Ol} and CaO_{Gl} were used as „Ca" precursors for the CaP synthesis. Considering that the amounts of chemical impurities in CaO starting materials may directly influence quality of the

synthesized product, requirements of ASTM (American Society for Testing and Materials) standards F1185-1188, F1088-87 concerning amounts of heavy metals in HAp and β-TCP materials for medical application were checked. Concentration of Cd and Pb in all starting materials does not exceed the allowed amounts.

Development of synthesis scheme.

A detailed technological scheme of CaP synthesis and obtaining bioceramics is shown in Figure 1.

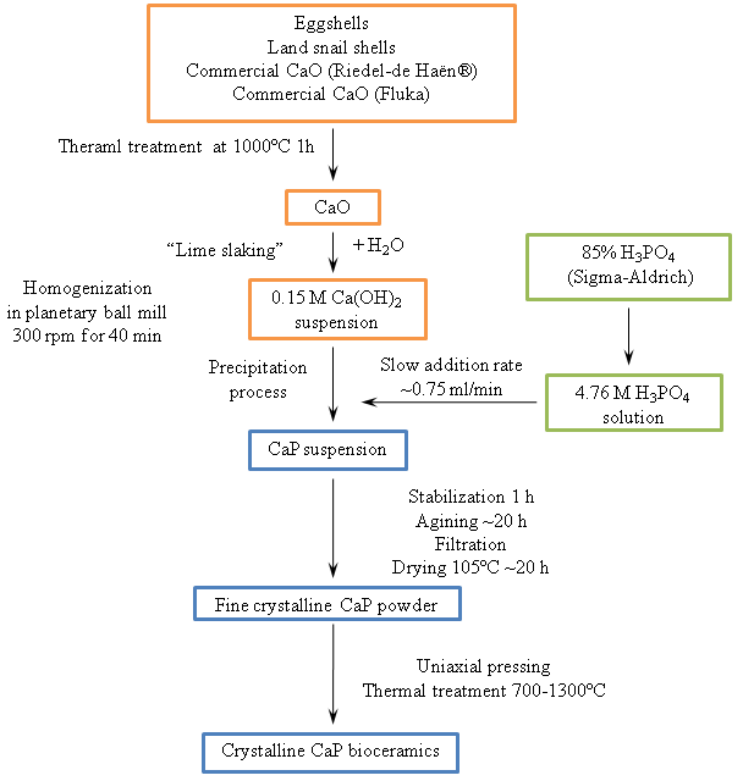

Fig. 1 A principal scheme to obtain CaP bioceramics

Preparation and processing of starting materials.

In experimental part $Ca(OH)_2$ suspension is homogenized in a planetary ball mill in addition to „lime slaking" process to obtain $Ca(OH)_2$ suspension (Fig. 2).

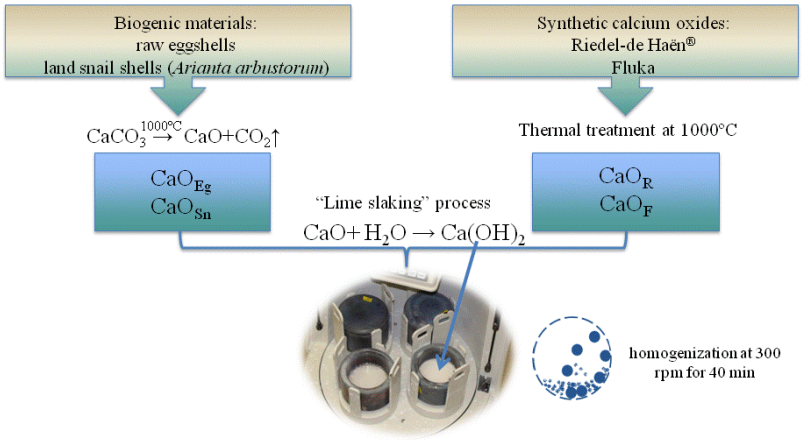

Fig. 2 Principal technological scheme for preparation and processing of CaO containing starting materials

After evaluation of morphologies of $Ca(OH)_2$ suspensions obtained from biogenic and synthetic CaO, it was found that it is problematic to avoid heterogeneous suspensions with multimodal size agglomerate formation, presence of unreacted CaO crystals and enlarged $Ca(OH)_2$ crystals (Fig. 3), that prevent formation of homogenous, highly dispersed $Ca(OH)_2$ suspensions. Furthermore, each $Ca(OH)_2$ suspension has quite different morphology. These phenomena can be explained by the fact that after thermal treatment each of CaO starting materials has different morphology (Fig. 4). This significantly influences further CaO reaction with water during the „lime slaking" process, formation kinetics and morphology of $Ca(OH)_2$.

Considering that CaO is only partially soluble in water, the dissolution process in water takes place according following reaction mechanisms:

$$CaO + H_2O \rightarrow Ca(OH)_2 \text{ (I)}$$

$$Ca(OH)_2 \rightarrow Ca^{2+} + 2OH^- \text{ (surface) (II)}$$

$$Ca^{2+} + 2OH^- \text{ (surface)} \rightarrow Ca^{2+} + 2OH^- \text{ (solution) (III)}$$

The dissolution process is very important to obtain homogenous, highly dispersed $Ca(OH)_2$ suspension.

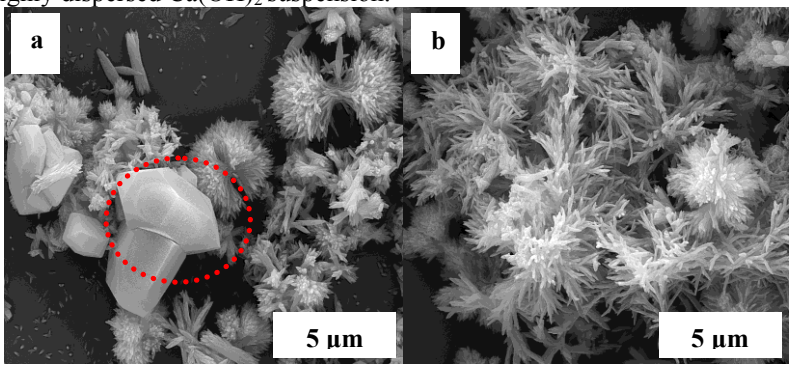

Fig. 3 FE-SEM microphotographs of $Ca(OH)_{2F}$ suspension morphology a) after „lime slaking" process; b) after homogenization in a planetary ball mill at 300 rpm for 40 min

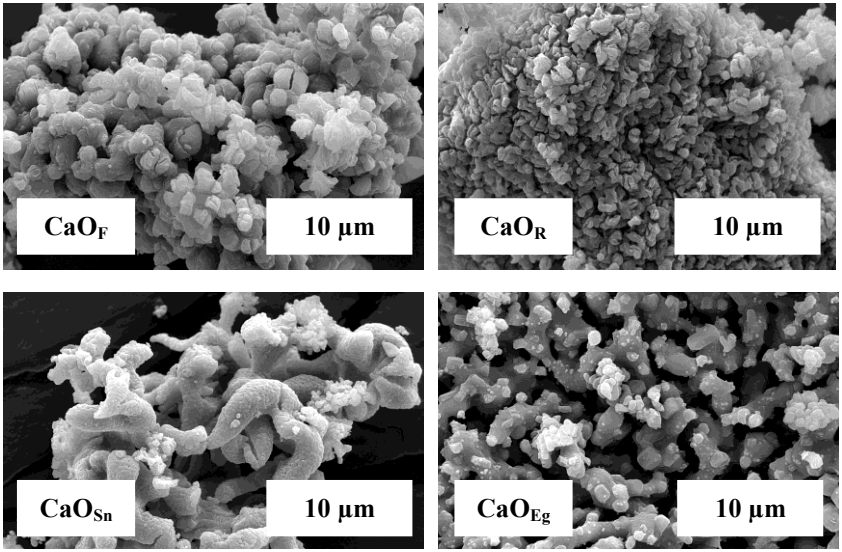

Fig. 4 FE-SEM microphotographs of overall morphologies of biogenic and commercial CaO after calcination at 1000°C for 1 h

It was experimentally proven that to obtain a homogenous, highly dispersed $Ca(OH)_2$ suspension after „lime slaking" process, extra technologic processing in planetary ball mill is required. Moreover, $Ca(OH)_2$ dispersion and dissolution time influences CaP precipitation process and depends on $Ca(OH)_2$ morphology.

The efficiency of processing $Ca(OH)_2$ suspension is proven by FWHM (full width at half maximum) measured value for the most intensive CaO (thermal treated at 1000ºC) and $Ca(OH)_2$ (milled in a planetary ball mill for 40 min) X-ray diffraction peaks (Fig. 5).

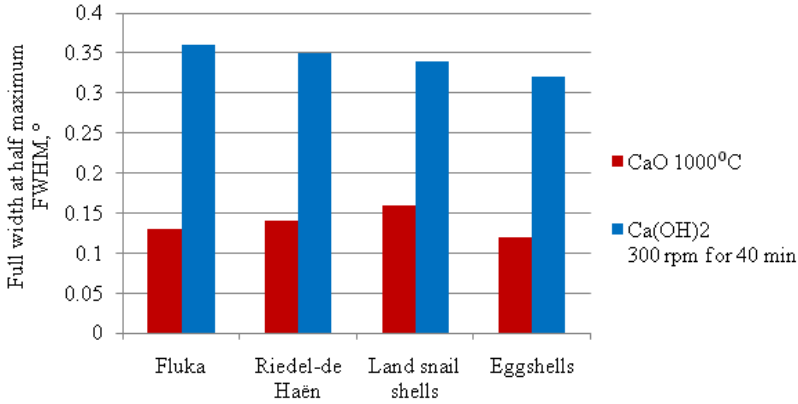

Fig. 5 Comparison of CaO and $Ca(OH)_2$ FWHM

RESULTS AND DISSCUSION

During experimental work, three syntheses series A, B, C were conducted using a modified wet chemical precipitation method. The pH_{final} (final pH of synthesis) and T_S (regime of synthesis temperature) were varied: 70ºC↓ - synthesis temperature that decreases in active synthesis phase (time of adding acid solution); 45ºC* - synthesis temperature that is constant in active synthesis time (time of adding acid solution); 22ºC – synthesis temperature is ambient air temperature. 15 CaP products were obtained (Tab. 1).

All syntheses were performed using following parameters:

- $Ca(OH)_F$ (obtained from CaO_F) suspension concentration 0.15 M;
- H_3PO_4 solution conc.: 4.76 M.

Average product yield per 2 liters synthesis solution was ~ 29 ± 2 g (losses during various stages of synthesis).

From the XRD patterns of as-synthesized CaP products it can be seen that all samples have a finely crystalline structure and low crystallinity degree, which is demonstrated by XRD patterns with relatively wide X-ray diffraction peaks of low intensity.

After performing XRD phase identification analysis of as-synthesized products, it was concluded that, in spite of the different technological parameters of the synthesis, there is an apatite structure formed in all samples at after synthesis.

XRD patterns of CaP products after the thermal treatment at 1100ºC were obtained. There was one to several crystalline phases identified in each sample (Tab. 1).

Tab. 1

Phases identified in CaP products after thermal treatment

Product No.	Variable parameters	Identified CaP phases after synthesis	Identified CaP phases at 1100ºC for 1h
A1	pH 5, 70ºC↓	CDHAp	HAp + β-TCP
A2	pH 6, 70ºC↓	CDHAp	HAp + β-TCP
A3	pH 7, 70ºC↓	CDHAp	HAp + β-TCP
A4	pH 9, 70ºC↓	apatite	HAp
A5	pH 10, 70ºC↓	CDHAp + $Ca(OH)_2$	HAp + CaO
B1	pH 5, 45ºC*	CDHAp	β-TCP + HAp
B2	pH 6, 45ºC*	CDHAp	HAp + β-TCP
B3	pH 7, 45ºC*	CDHAp	HAp + β-TCP
B4	pH 9, 45ºC*	apatite	HAp
B5	pH 10, 45ºC*	CDHAp + $Ca(OH)_2$	HAp + CaO
C1	pH 5, 22ºC	ap-TCP + DCPD	β-TCP + β-CPP
C2	pH 6, 22ºC	ap-TCP	β-TCP
C3	pH 7, 22ºC	CDHAp	β-TCP + HAp
C4	pH 9, 22ºC	CDHAp	HAp + β-TCP
C5	pH 10, 22ºC	CDHAp + $Ca(OH)_2$	HAp + CaO

Based on the goal set for experimental work – to obtain HAp, β-TCP and BCP products for medical application, CaP products with no presence of CaO and β-CPP phases after the thermal treatment were chosen

for the further description. Red colored CaP products in the Table 1 were not further evaluated because of unwanted phases.

CaO is not biocompatible with human body because $Ca(OH)_2$ can provoke an inflammation of neighboring tissues in a neutral or averagely acidic environment of body. It was experimentally proven, that the β-calcium pyrophosphate phase has a negative impact on sintering and microstructure of the β-TCP bioceramics (CaP product C1), creating a heterogeneous microstructure with enlarged grains, microcracks and enormous (~ 50 μm) β-CPP crystals (Fig. 6).

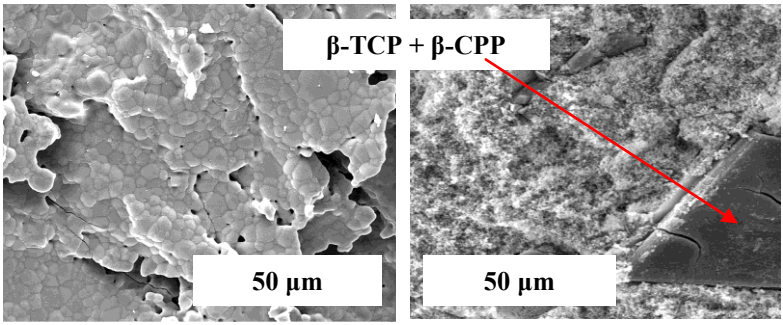

Fig. 6 FE-SEM microphotographs of obtained β-TCP + CPP bioceramic surfaces after thermal treatment at 1200ºC (left) and 900ºC (right) for 1h

Using XRD based semi-quantitative phase analysis (utilizing a calibration curve) of CaP products after thermal treatment at 1100ºC for 1 h, HAp and β-TCP phase content (%) was calculated (Series A, B, C in Fig. 7). For a HAp phase content (%) of each product relative error was calculated, which is 1.3-4.6%.

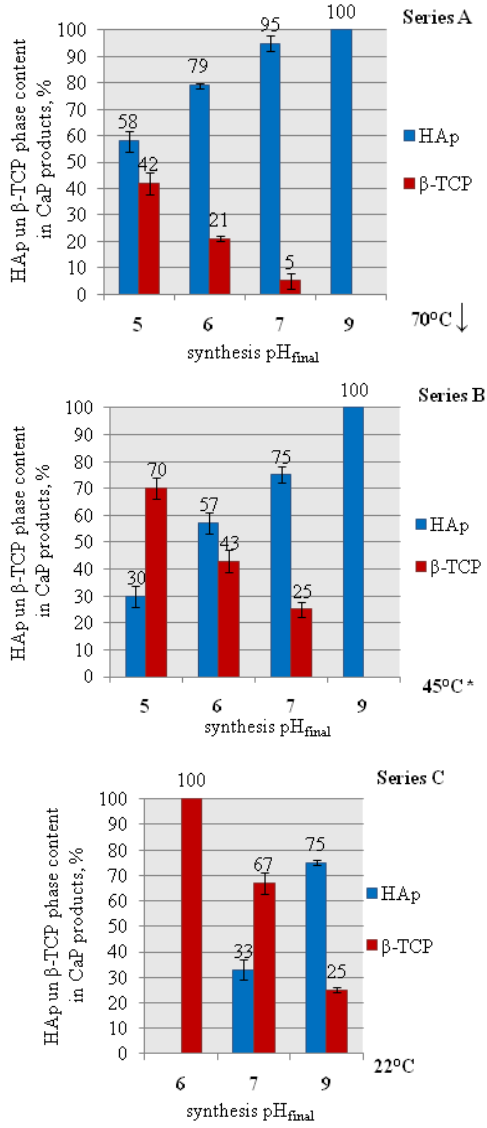

Fig. 7 Phase content (%) – HAp and β-TCP – for Series C products after thermal treatment at 1100ºC for 1h

After evaluation of content of HAp and β-TCP in each CaP product, it was concluded that the lowest pH_{final} favours formation of BCP with HAp as the minor phase or pure β-TCP phase after the high temperature treatment, but the highest pH_{final} – formation of BCP with HAp as the dominant phase or pure HAp phase in CaP product. The elevated T_S despite of pH_{final} favours formation of HAp as the dominant phase in the synthesis product. The ambient temperature of synthesis favours formation of β-TCP as the dominant phase in the synthesis product after thermal treatment. Dynamic of HAp phase formation depending on pH_{final} in different temperatures is shown in Figure 8.

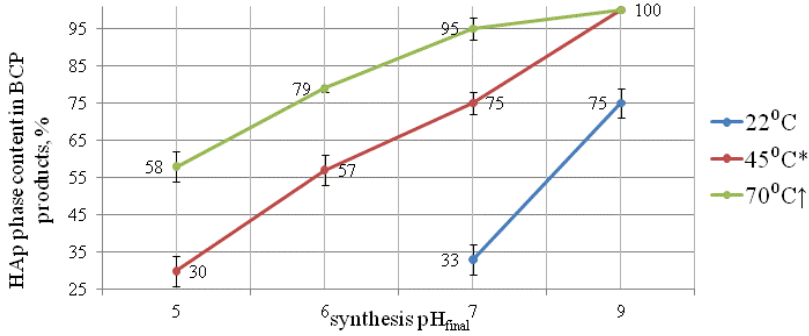

Fig. 8 HAp phase content (%) in BCP product depending on pH_{final} after thermal treatment at 1100ºC for 1 h

Development and characterization of biphasic calcium phosphate bioceramics.

BCP products from Series B are more precisely reproducible, than Series A products (evaluated with XRD and FTIR). FE-SEM microphotographs of microstructures of BCP products B1, B2, B3 (CaP products in Tab. 1) with HAp/β-TCP phase ratio, commonly used HAp/β-TCP phase ratio in commercial BCP products are shown in Figure 9. A high microporosity with partially sintered grain structure is characteristic to microstructure for BCP products. Comparing shrinkage of bioceramic, open and total porosity, it was shown that BCP bioceramic with dominant HAp phase has a higher total and open porosity, but not significantly different shrinkage of obtained bioceramic products.

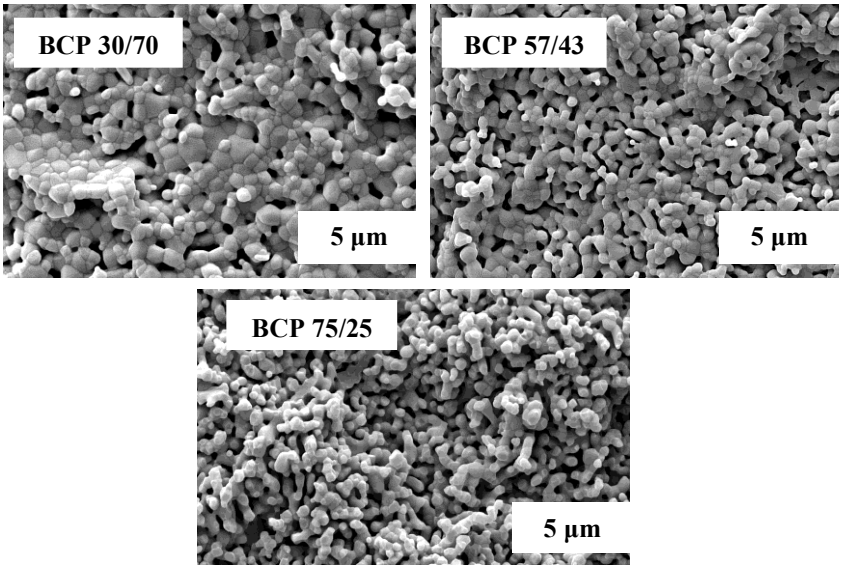

Fig. 9 FE-SEM microphotographs of BCP (HAp/β-TCP) bioceramics fracture B2 (57/43), B3 (75/25), B1 (30/70) after thermal treatment at 1100ºC for 1 h

Development and characterization of β-tricalcium phosphate bioceramics.

Experimentally obtained β-TCP bioceramic (CaP product C2 in Tab. 1) has dense and homogenous microstructure (Fig. 10) and is thermally stabile up to 1350ºC.

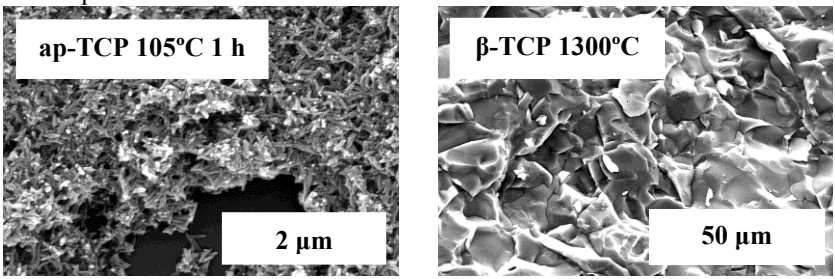

Fig. 10 Morphology of synthesized ap-TCP after drying at 105ºC for ~20 h and β-TCP bioceramic fracture after thermal treatment at 1300ºC for 1 h

This is a high value comparing with data from given by other authors. A FE-SEM microphotograph (Fig. 10) of the synthesized ap-TCP (apatitic tricalcium phosphate) suspension shows typical apatite powder with needle-like crystallite morphology.

A nanosize needle-like crystals, homogenous, thin, long (~ 150-200 nm) crystallites, which tend to form primary or secondary agglomerates, are characteristic for synthesized HAp suspension and CDHAp (calcium deficient hydroxyapatite) suspensions. It is known that the biological apatite exists in human bones in shape of needle-like crystals, dispersed in a collagen matrix.

Development of hydroxyapatite bioceramics from starting materials of different origin calcium oxides.

During the experimental work, hydroxyapatite bioceramics were developed from biogenic and commercial starting materials containing calcium oxides. HAp products (CaP product B4 in Tab. 1) were obtained: HAp_R, HAp_F, HAp_{Eg}, HAp_{Sn}.

Using ICP-MS data, it was determined that HAp products complies with ASTM F 1185-1188, ASTM F 1088-87 requirements about total amount of heavy metals (Cd and Pb) $\leq$ 50 mg/kg in the HAp and β-TCP materials for medical application.

XRD data demonstrates obtaining pure, thermally stable ~ 1450ºC HAp phase in each product. However, in HAp bioceramic obtained from CaO of different origins, significant differences in microstructure (Fig. 11) and color changes after thermal treatment were observed. Most explicit grain size and microporosity differences were observed in HAp_{Sn} and HAp_R bioceramics. The different microstructures of HAp bioceramics can also significantly influence mechanical properties and bioactivity of materials.

ICP-MS of HAp products showed presence of the transition elements Mn, Zn, Cu, Cd as well as microelements Sr, Ba. Additional chemical composition analysis using FE-SEM/EDS showed presence of Mg in tested HAp bioceramics in concentrations of 2700-5400 ppm. This can be compared with Mn concentrations – 2.83-100.54 ppm.

It should be considered that the microstructure parameters of obtained HAp bioceramics were influenced by presence of Mn, Zn, Cu, Pb, Cd, Sr, Ba and Mg microelements in HAp structure, which significantly influenced crystal lattice parameters.

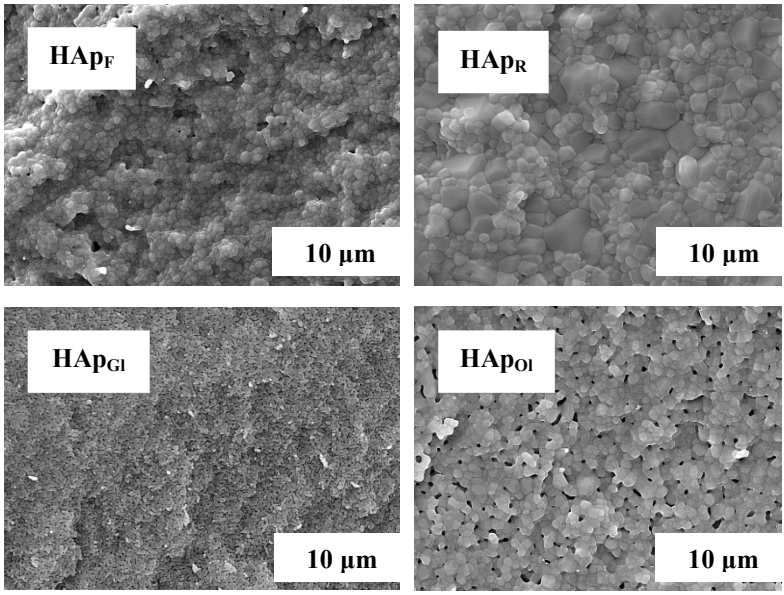

Fig. 11 FE-SEM microphotographs of HAp bioceramics surface obtained from CaO of different origins after thermal treatment at 1100ºC for 1h

After evaluation of crystallite size (L_{002} and L_{300}) of HAp products at 700ºC, it was concluded that average crystallite dimensions of synthesized HAp products are the same ~ 20 nm (Fig. 12).

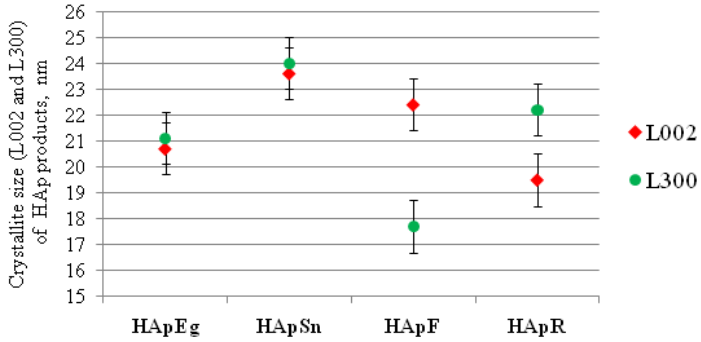

Fig. 12 Crystallite size (L_{002} and L_{300}) of HAp products after thermal treatment at 700ºC

HAp_F crystallite aspect ratio L_{002}/L_{300} shows the longest crystallites comparing with other HAp products. Calculated degree of crytstallinity of HAp bioceramic products at 1100ºC for 1 h is about 90%; this indicates the resultant dense ceramic.

Color changes of HAp bioceramics after thermal treatment are most likely caused by oxidation of transition elements located in HAp crystal lattice. It is possible that these elements do not have a negative impact on biocompatibility of HAp bioceramics in living organism. However, the criteria of FDA, USA (FDA – *Food and Drug Administration*) require HAp powder to have a clean white color. Considering that Mn concentration in all HAp samples was higher than that of other transition elements, it should be noted that white → light blue → dark blue color transition appeared as a result of [Mn^{2+}] ions oxidizing to [Mn^{5+}] ions at 700ºC and 1200ºC in ambient atmosphere.

Influence of acid solution concentration on properties of hydroxyapatite bioceramics.

Evaluating influence of H_3PO_4 solution concentration on properties of obtained HAp products (CaP product B4 in Tab. 1) 1.00 M, 2.00 M, 3.00 M, it was found that a dramatically different concentration of H_3PO_4 solution has not influenced phase composition, molecular structure, thermal stability and microstructure of the obtained products.

It was found that a higher concentration of H_3PO_4 solution promotes formation of products with a higher HAp crystallite aspect ratio L_{002}/L_{300} (Fig. 13). The higher aspect ratio L_{002}/L_{300} indicates formation of thinner and longer HAp crystallites.

The crystallinity degree of HAp bioceramic products is the same for all samples about 90%. After evaluation of HAp product microstructure sintering degree (1.00 M, 4.76 M), it was found that microstructure of product 1.00 M is microporous with explicit grain boundaries. Dense microstructure indicates fine homogenous grains and small crystallite size of HAp products after synthesis (HAp product 4.76 M).

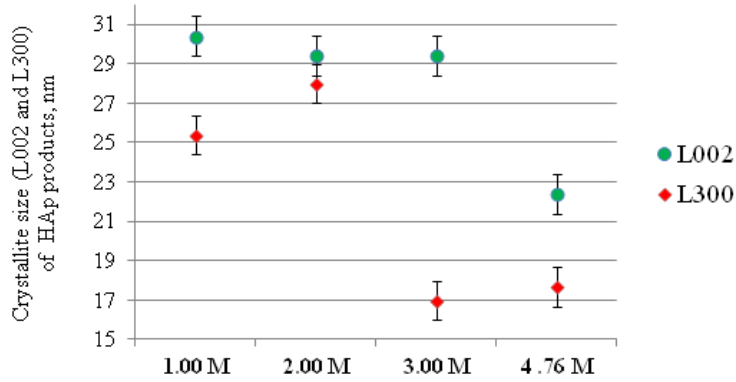

Fig. 13 Crystallite size (L_{002} and L_{300}) of HAp products after thermal treatment at 700ºC

Influence of synthesis temperature regime on properties of hydroxyapatite bioceramics.

Evaluating influence of synthesis temperature regimes 45ºC* and 70ºC↓ on morphology and microstructure of synthesized and thermally treated HAp products (CaP products A4, B4 in Tab. 1) HAp70, HAp45, it was found that temperature regime has influenced crystallite size of as-synthesized HAp products and bioceramics (Fig. 14).

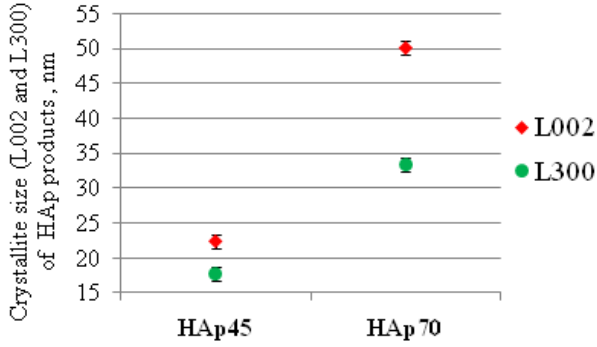

Fig. 14 Crystallite size (L_{002} and L_{300}) of HAp products after thermal treatment at 700ºC

Evaluating the crystallite size of obtained HAp products, it was found that elevated synthesis temperature has promoted crystallite growth along c axis. The crystallite size L_{002} of HAp product HAp70 is twice as large as crystallite size L_{002} of HAp product HAp45 in direction of c axis. Crystallite size L_{300} of HAp product HAp70 is larger than the crystallite size of the HAp product HAp45 in direction of c axis.

It was found that HAp crystallite aspect ratio L_{002}/L_{300} increases by elevating synthesis temperature. Higher aspect ratio L_{002}/L_{300} indicates on formation of longer and thinner crystallites. FE-SEM microphotographs of HAp70 bioceramic demonstrate a fine-grained (~ 200-300 nm), homogenous, partially sintered microstructure. The bioceramics microstructure of the HAp45 is also fine-grained (~ 250-500 nm) and homogenous, however the grain size and microporosity are higher and densification degree is less intense as bioceramics microstructure of HAp70. Evaluating the microstructures and crystallite size of obtained HAp products, it was found that crystallite size is not linearly related to grain size of HAp bioceramics. The HAp product HAp70 with longer and thicker crystallites shows more fine-grained structure.

CONCLUSIONS

1. A predictable, reproducible multi-phase technology with suitable technological parameters to obtain calcium phosphates – pure hydroxyapatite, pure β-tricalcium phosphate and biphasic calcium phosphate (with controllable ratio of hydroxyapatite/β-tricalcium phosphate) bioceramics has been developed.
2. Technological parameters necessary to control synthesis outcome were defined: final pH and temperature of synthesis, their boundary values to obtain pure hydroxyapatite ($8.48 \leq pH_{final} \leq 8.97$, $T_S = 70\pm1$ºC↓ or $8.83 \leq pH_{final} \leq 9.05$, $T_S = 45\pm1$ºC*), pure β-tricalcium phosphate ($5.90 \leq pH_{final} \leq 6.06$, $T_S = 22\pm1$ºC) and biphasic calcium phosphates ($5.00 \leq pH_{final} \leq 6.87$, $T_S = 70\pm1$ºC↓ or $4.97 \leq pH_{final} \leq 7.08$, $T_S = 45\pm1$ºC* or $6.95 \leq pH_{final} \leq 9.01$, $T_S = 22\pm1$ºC) after thermal treatment.
3. For the first time calcium phosphates have been synthesized using modified wet chemical precipitation method with non-stoichiometric ratio of reagent concentrations $[Ca^{2+}]/[PO_4^{3-}] = 0.15$ M/4.76 M.
4. Using commercially available synthetic and biogenic calcium oxides to obtain calcium hydroxide suspensions, during the „lime slaking" process, various heterogeneous suspension morphologies were

obtained. It was found that during a conventional „lime slaking" process, it is almost impossible to eliminate un-reacted calcium oxide crystals, enlarged calcium hydroxide crystals or agglomerates. By experiments, effectiveness of homogenization of calcium hydroxide suspension in a planetary ball mill was proven necessary to obtain a fine-dispersed and homogenous suspension.

5. Using commercially available synthetic and biogenic calcium oxides, hydroxyapatite bioceramics with a different microstructure and color after thermal treatment were obtained.
   At the same time, morphology of as-synthesized powders, phase composition and molecular structure of bioceramics are identical. For hydroxyapatite ceramics made from shells (blue colored), there is an explicitly homogenous and fine-grained microstructure is (grain size ~ 100-150 nm), while hydroxyapatite ceramics from Riedel-de Haën® (blue colored) have heterogeneous microstructure with enlarged grains (~ 500 nm - 5 μm). Formation of different microstructures of HAp bioceramics demonstrate the impact of microelements on HAp crystal lattice – creation of defects in crystal lattice – and brings a significant impact on the HAp product microstructure after thermal treatment. Blue color of HAp bioceramic samples is caused by oxidation of transition elements, possibly Mn, during thermal treatment of HAp.
6. Systematic research has proven that calcium phosphates with reproducible quality and a determined composition and structure bring a significant impact on structure and properties of bioceramic implants and can be obtained by precise control of composition and structure of calcium containing raw materials and properties of suspension for start of synthesis.

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