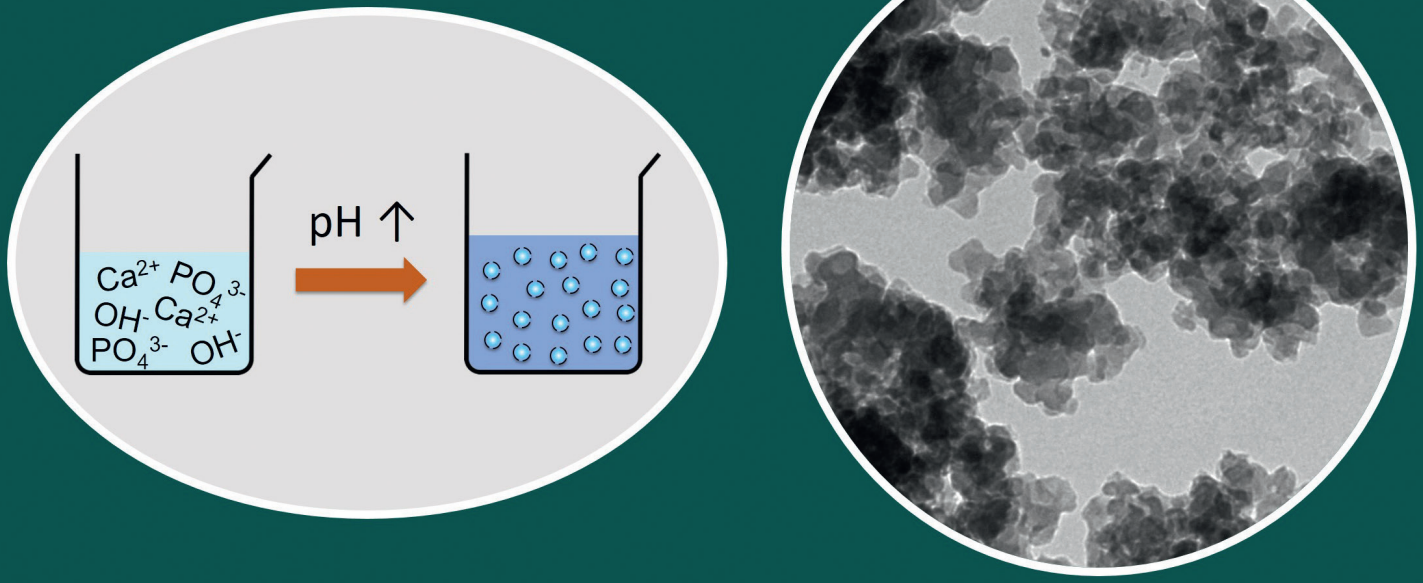
RIGA TECHNICAL UNIVERSITY

Jana Vecstaudža

AMORPHOUS CALCIUM PHOSPHATE BIOMATERIALS WITH HIGH SPECIFIC SURFACE AREA

Summary of the Doctoral Thesis



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RIGA TECHNICAL UNIVERSITY
Faculty of Materials Science and Applied Chemistry
Institute of General Chemical Engineering

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Doctoral Student of the Study Programme "Materials Science"

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Scientific supervisor
Professor Dr. sc. ing.
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EIROPAS SOCIĀLAIS FONDS

EIROPAS SAVIENĪBA

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DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR THE PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for the defence at the open meeting of RTU Promotion Council at 15:00 on June 21, 2021 at the Faculty of Materials Science and Applied Chemistry of Riga Technical University, 3/7 Paula Valdena Street, Room 272.

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DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for the review to Riga Technical University for the promotion to the scientific degree of Doctor of Science (Ph. D.) is my own. I confirm that this Doctoral Thesis had not been submitted to any other university for the promotion to a scientific degree.

Jana Vecstaudža ……. (signature)

Date: ……

The Doctoral Thesis has been written as collection of articles. It consists of summary in Latvian and English and four SCI publications. Publications are written in English with total volume of 34 pages including electronically available supplementary information.

CONTENTS

GENERAL OVERVIEW OF THE THESIS

Introduction

Due to increasing number of patients that encounter musculoskeletal system traumas, bone related diseases and pathologies worldwide [1], there is ongoing need for more effective biomaterials for bone treatment and replacement. These biomaterials should resemble the composition and structure of natural bone. Due to the unique chemical composition and structure of bone, it has not been artificially imitated so that the properties of produced biomaterial would be comparable to the bone [2]. The bone is composed of inorganic (75 wt. %, including 10 wt. % water) and organic (25 wt. %) components [3]. The inorganic part of the bone is composed of nanosized calcium phosphate particles that are embedded within the organic matrix. It is known that calcium phosphates have very high biocompatibility with human tissue and body environment [4]. Therefore, calcium phosphates are promising substituents of the inorganic part of the bone; and they can be synthesized in laboratory and used for the development of new and effective biomaterials.

However, the *gold* standard in bone regeneration is autograft, i.e., bone tissue from the same patient. Another popular option is to use xenograft, e.g., commercially available deproteinized bovine bone mineral (DBBM) materials, to avoid shortcomings of the autograft (several operation sites, pain, prolonged inability to work, etc.) [5]. DBBM materials are successful because of their micro and nano structures having high specific surface area (up to 88 m^2/g [6]), which is close to the value of specific surface area of the calcium phosphate particles found in bone (40–240 m^2/g [7]). Meanwhile, commercial calcium phosphate biomaterials are still far behind (up to 3 m^2/g [8]). High specific surface area is critical for bioactive molecule (e.g., protein and cytokine) adsorption on surface of biomaterials [9] that further promotes material integration and tissue regeneration. Synthetic calcium phosphates offer high purity, availability and repeatability and are not of animal origin. Therefore, scientific community carries out in-depth studies of calcium phosphates – synthesis, physicochemical properties and processing thereof, as well as performs *in vitro, in vivo* and clinical studies.

Amorphous calcium phosphate is the first inorganic phase that forms during a new bone formation [10]. Furthermore, amorphous calcium phosphate is designated as the first or an intermediate phase in formation of other calcium phosphates, e.g., hydroxyapatite. So far, the amorphous calcium phosphate is rarely used as stand-alone biomaterial due to its metastability; over time and under influence of other factors it crystallizes into other calcium phosphate phases [11]. In this PhD Thesis it is shown that it is possible to obtain stable amorphous calcium phosphate, thus significantly widening its potential applications in biomaterials research.

In the current PhD Thesis, novel synthesis method of stable amorphous calcium phosphate with high specific surface area (≥100 m^2/g) was developed and physicochemical properties were characterized. Crystallization of the amorphous calcium phosphate has been studied in conjunction with technological parameters of the synthesis (synthesis pH and drying method). Further, the amorphous calcium phosphate was successfully sintered into dense bioceramics using the principles of cold sintering, thus preserving the amorphous structure thereof.

Aim and Objectives

The aim of the Thesis was to develop a synthesis technology for obtaining stable amorphous calcium phosphate with high specific surface area and study formation, crystallization and sintering thereof. In order to fulfill the aim, the following objectives were set.

1. To study the impact of synthesis temperature and Ca/P molar ratio of reagents on the specific surface area of amorphous calcium phosphate obtained by the double salt decomposition method.
2. To develop a synthesis method for obtaining amorphous calcium phosphate with high specific surface area ($\geq$100 m^2/g).
3. To study the impact of drying method (freeze-drying or drying at 80 °C temperature) on the structure and crystallization of amorphous calcium phosphate.
4. To study long term stability of amorphous calcium phosphate at room temperature.
5. To study sintering of amorphous calcium phosphate using principles of cold sintering process.

Thesis to Defend

1. Rapid increase of pH up to pH = 10–11 of calcium and phosphate ions containing solution ensures precipitation of amorphous calcium phosphate with high specific surface area ($\geq$100 m^2/g).
2. Ca/P molar ratio larger than 1.5 of amorphous calcium phosphate precipitated at pH = 10–11 ensures its long-term stability at room temperature.

Scientific Novelty

New, simple, fast and cost-effective synthesis method of long-term stable amorphous calcium phosphate with high specific surface area has been demonstrated and physicochemical properties of the obtained products have been characterized.

Practical Significance

Long-term stable amorphous calcium phosphate was obtained and studied, and applicability thereof was found in the development of nanostructured granules[1]:

1) with strontium ions for improvement of performance of osteochondral implants[2],
2) with biomimetic chemical composition that will ensure biosynthesis of biological calcium phosphate after implantation *in vivo*[3].

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[1] Collaborative project between RTU and RSU "Development of nanostructured bone substituting materials and studies of immunologic aspects in bone regeneration", 2016–2019.

[2] EuroNanoMed III project "NANOstructured oSteoChOndral scaffold: novel biomimetic tRiggErS for enhanced bone regeneration", 2018–2021.

[3] Latvian Council of Science project No lzp-2018/1-0238 "Future of synthetic bone graft materials – *in vivo* guided biosynthesis of biomimetic hydroxyapatite", 2018–2021.

Structure of the Thesis

The Thesis is a collection of four SCI publications with a summary in Latvian and English. Publications are written in English; total volume of which is 34 pages, including electronically available supplementary information.

Publications and Approbation of the Thesis

Results of the Thesis are published in four SCI scientific publications

1. **Vecstaudza, J.**, Locs, J. Effect of synthesis temperature and Ca/P ratios on specific surface area of amorphous calcium phosphate, *Key Engineering Materials*, 721, 2016, pp. 172–176. doi: 10.4028/www.scientific.net/KEM.721.172 (Scopus).
2. **Vecstaudza, J.**, Locs, J. Novel preparation route of stable amorphous calcium phosphate nanoparticles with high specific surface area, *Journal of Alloys and Compounds*, 700, 2017, pp. 215–222. doi: 10.1016/j.jallcom.2017.01.038 (Scopus).
3. **Vecstaudza, J.**, Gasik, M., Locs, J. Amorphous calcium phosphate materials: formation, structure and thermal behaviour, *Journal of the European Ceramic Society*, 39, 2019, pp. 1642–1649. doi: 10.1016/j.jeurceramsoc.2018.11.003 (Scopus, Open Access).
4. Rubenis, K., Zemjane, S., **Vecstaudza, J.**, Bitenieks, J., Locs, J. Densification of amorphous calcium phosphate using principles of the cold sintering process, *Journal of the European Ceramic Society*, 41, 2021, pp. 912–919. doi: 10.1016/j.jeurceramsoc.2020.08.074 (Scopus, Open Access).

Results of the Thesis were presented at 18 scientific conferences

1. **Vecstaudza, J.**, Locs, J. Synthesis and characterization of amorphous calcium phosphate with biomimetic bone-like composition. *11th World Biomaterials Congress*, Virtual event, 11–15 December 2020 (poster).
2. **Vecstaudza, J.**, Locs, J. Amorphous calcium phosphate biomaterials for bone regeneration. *60th International Scientific Conference of RTU: Materials Science and Applied Chemistry*, Latvia, Riga, October 24, 2019 (oral presentation).
3. **Vecstaudza, J.**, Locs, J. Synthesis of amorphous calcium phosphate with biomimetic chemical composition. *30th Annual Conference of the European Society for Biomaterials*, Germany, Dresden, 9–13 September 2019 (poster).
4. **Vecstaudza, J.**, Locs, J. Thermally treated carbonated amorphous calcium phosphate. *30th Annual Conference of the European Society for Biomaterials*, Germany, Dresden, 9–13 September 2019 (poster).
5. **Vecstaudza, J.**, Locs, J. Biomimetic amorphous calcium phosphate. *XVI Conference of the European Ceramic Society*, Italy, Turin, 16–20 June 2019 (poster).
6. **Vecstaudza, J.**, Locs, J. Development of synthesis of amorphous calcium phosphate with biomimetic chemical composition. *Scandinavian Society for Biomaterials 2019 conference*, Finland, Kirkkonummi, 12–14 June 2019 (poster).

7. Makarova, E., **Vecstaudza, J.**, Vilskersts, R., Kupats, R., Kuka, J., Loca, D., Locs, J., Dambrova, M. Assessment of Biocompatibility and Osteoinductive Potential of Amorphous Calcium Phosphate in Mice: Experimental Model. *Rīga Stradiņš University International Conference on Medical and Health Care Sciences: Knowledge for Use in Practice*, Latvia, Riga, 1–3 April 2019 (poster).
8. **Vecstaudza, J.**, Locs, J. Reduction of specific surface area of amorphous calcium phosphate during gradual heat treatments. *29th European Conference on Biomaterials ESB 2018*, the Netherlands, Maastricht, 9–13 September 2018 (poster).
9. **Vecstaudza, J.**, Locs, J. Decrease of specific surface area of amorphous calcium phosphate during gradual heat treatments. *29th Symposium and Annual Meeting of the International Society for Ceramics in Medicine Bioceramics 29*: Book of Abstracts, France, Toulouse, 25–27 October 2017, p. 138 (poster).
10. **Vecstaudza, J.**, Locs, J. Impact of heat treatment on specific surface area of amorphous calcium phosphate. *58th International Scientific Conference of Riga Technical University*, Riga, Latvia, October 20, 2017 (**best poster award**).
11. **Vecstaudza, J.**, Locs, J. Calcium phosphate scaffolds with high specific surface area towards improved cell response in vitro. eCM Meeting Abstracts 2017, Collection 2; *TERMIS EU*, Switzerland, Davos, 26–30 June 2017, P809 (poster).
12. **Vecstaudza, J.**, Locs, J. Calcium phosphates with high specific surface area towards improved cell response *in vitro*, *10th annual meeting for Scandinavian Society for Biomaterials*, Norway, Hafjell, 15–17 March 2017 (poster).
13. **Vecstaudza, J.**, Locs, J. Novel preparation route of stable amorphous calcium phosphate nanoparticles with high specific surface area, *33rd Scientific Conference of the Institute of Solid State Physics*, Latvia, Riga, 22–24 February 2017 (poster).
14. **Vecstaudza, J.**, Locs, J. Effect of synthesis temperature and Ca/P ratios on specific surface area of amorphous calcium phosphate. *25th International Baltic Conference Baltmattrib 2016*, Latvia, Riga, 3–4 November 2016 (poster).
15. **Vecstaudza, J.**, Locs, J. Amorphous and low crystalline calcium phosphates for bone tissue regeneration, *57th International Scientific Conference of Riga Technical University*, Latvia, Riga, October 21, 2016 (oral presentation).
16. **Vecstaudza, J.**, Gasik, M., Locs, J. New biomimetic amorphous calcium phosphate biomaterials: structure and thermal properties. *The 2016 E-MRS Fall Meeting and Exhibition*, Poland, Warsaw, 19–22 September 2016 (oral presentation).
17. **Vecstaudza, J.**, Locs, J., Gasik, M. Biomimetic calcium phosphate nanoparticles with variable degree of crystallinity. *6th International congress on ceramics*, Germany, Dresden, 21–25 August 2016 (poster).
18. **Vecstaudza, J.**, Locs, J. Rapid reprecipitation of nanosized calcium phosphates. *24th International Conference Baltmattrib 2015*. Estonia, Tallinn, 5–6 November 2015 (poster).

Participation at other scientific conferences during development of the Ph. D. Thesis

1. Putnins, A., **Vecstaudza, J.**, Locs. J. Obtaining and characterization of C-shaped calcium phosphate granules for biomedical application. *9th International Granulation Workshop*, Switzerland, Lausanne, 26–28 June 2019, p. 194 (poster).
2. Putnins, A., **Vecstaudza, J.**, Locs. J. Development of designed shape calcium phosphate granules. *9th International Granulation Workshop*, Switzerland, Lausanne, 26–28 June 2019, p. 193 (poster).
3. Stīpniece, L., **Vecstaudža, J.**, Zālīte, V., Šalma-Ancāne, K., Loča, D., Bērziņa-Cimdiņa, L. Optimization of the Synthesis and Design of Calcium Phosphates for Biomedical Applications. *The 32nd Scientific Conference of the Institute of Solid State Physics*, Latvia, Riga, 17–19 February 2016, p. 136 (poster).

Other scientific publications published during development of the Ph. D. Thesis

1. Choudhary, R., Venkatraman, S. K., Chatterjee, A., **Vecstaudza, J.**, Yáñez-Gascón, M. J., Pérez-Sánchez, H., Locs, J., Abraham, J., Swamiappan, S. Biomineralization, antibacterial activity and mechanical properties of biowaste derived diopside nanopowders (2019) *Advanced Powder Technology*, 30 (9), pp. 1950–1964. doi: 10.1016/j.apt.2019.06.014 (Scopus).
2. Loca, D., Smirnova, A., Locs, J., Dubnika, A., **Vecstaudza, J.**, Stipniece, L., Makarova, E., Dambrova, M. Development of local strontium ranelate delivery systems and long term *in vitro* drug release studies in osteogenic medium (2018) *Scientific Reports*, 8 (1), art. no. 16754. doi: 10.1038/s41598-018-35197-7 (Scopus, Open Access).
3. Choudhary, R., Manohar, P., **Vecstaudza, J.**, Yáñez-Gascón, M. J., Sánchez, H. P., Nachimuthu, R., Locs, J., Swamiappan, S. Preparation of nanocrystalline forsterite by combustion of different fuels and their comparative in-vitro bioactivity, dissolution behaviour and antibacterial studies (2017) *Materials Science and Engineering C*, 77, pp. 811–822. doi: 10.1016/j.msec.2017.03.308 (Scopus).
4. Stunda-Zujeva, A., **Vecstaudža, J.**, Krieķe, G., Bērziņa-Cimdiņa, L. Glass Formation and Crystallization in P_2O_5-Nb_2O_5-CaO-Na_2O System. *Materials Sciences and Applied Chemistry*, 2017, 34, pp. 21–28. doi: 10.1515/msac-2017-0003.
5. Choudhary, R., **Vecstaudza, J.**, Krishnamurithy, G., Raghavendran, H. R. B., Murali, M. R., Kamarul, T., Sasikumar, S., Locs, J. *In-vitro* bioactivity, biocompatibility and dissolution studies of diopside prepared from biowaste by using sol-gel combustion method (2016) *Materials Science and Engineering C*, 68, pp. 89–100. doi: 10.1016/j.msec.2016.04.110 (Scopus).

MAIN RESULTS OF THE THESIS

Effect of Ca/P Molar Ratio of Reagents and Synthesis Temperature on the Properties of ACP

Literature analysis showed that synthesis technologies of amorphous calcium phosphate (ACP) could be modified in order to obtain product with high specific surface area (SSA) (≥100 m^2/g). Further analysis on calcium phosphate (CaP) synthesis protocols highlighted two technological parameters of synthesis – Ca/P molar ratio of reagents and synthesis temperature, that could have impact on the SSA of the product of ACP synthesis. Usually, ACP is obtained by precipitation method, e.g., double salt decomposition method. For this method calcium and phosphate ion containing water soluble salt solutions are used; both solutions are combined into one at a basic pH, thus ensuring precipitation of ACP. In such synthesis variety of calcium and phosphate ion containing salts may be used, e. g., $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$, $Ca(NO_3)_2 \cdot 4H_2O$ and $NH_4H_2PO_4$, $CaCl_2 \cdot 2H_2O$ and K_2HPO_4, $CaCl_2 \cdot 2H_2O$ and Na_2HPO_4, etc.

In the PhD Thesis ACP samples were synthesized by the double salt decomposition method where solutions of $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ (both salts purchased from Sigma Aldrich) were rapidly poured together under rapid mixing with magnetic stirrer (Fig. 1). Molarity of the $Ca(NO_3)_2$ was kept 0.45 M in all experiments. In order to evaluate whether it is possible to obtain ACP with high SSA using double salt decomposition method, effect of the following technological parameters was studied: 1) **Ca/P molar ratio of reagents** (1.5, 1.67 and 2.2) and 2) **synthesis temperature** (0 °C and 20 °C).

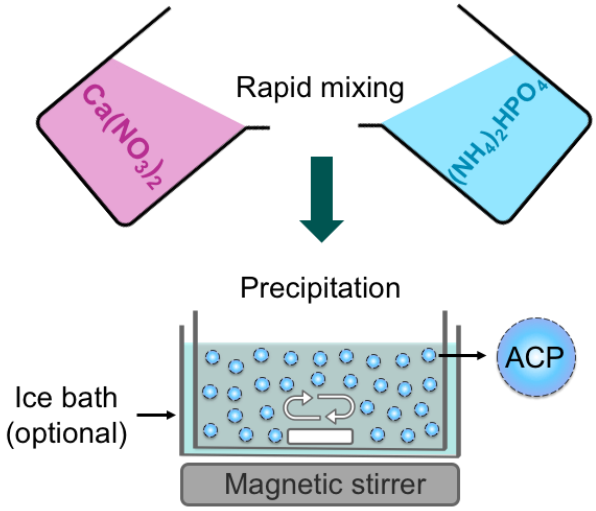

Fig. 1. General scheme of ACP synthesis by double salt decomposition method.

As-synthesized and heat treated (1100 °C, 1 h) samples were studied for their phase composition using x-ray diffractometer X'Pert Pro (PAnalytical, the Netherlands). Crystalline phase identification was done using PDF-2 database from International Centre for Diffraction Data (ICDD). SSA of the as-synthesized samples was determined using Brunauer–Emmett–Teller (BET) method and Quadrasorb SI (Quantachrome, USA) instrument.

Regardless of Ca/P molar ratio (1.5, 1.67 or 2.2) of reagents and synthesis temperature (0 °C or 20 °C), all synthesis products were x-ray amorphous (Fig. 2 (a)). In x-ray diffraction (XRD) patterns of the heat-treated ACP samples (Fig. 2 (a)) only β-tricalcium phosphate (β-TCP, β-$Ca_3(PO_4)_2$) or mixture of β-TCP and α-tricalcium phosphate (α-TCP, α -$Ca_3(PO_4)_2$) phases were identified. In samples where mixture of α-TCP and β-TCP was identified the dominant phase was β-TCP. Hence, the Ca/P molar ratio of all ACP samples was 1.5 regardless of the Ca/P molar ratio of the reagents and synthesis temperature.

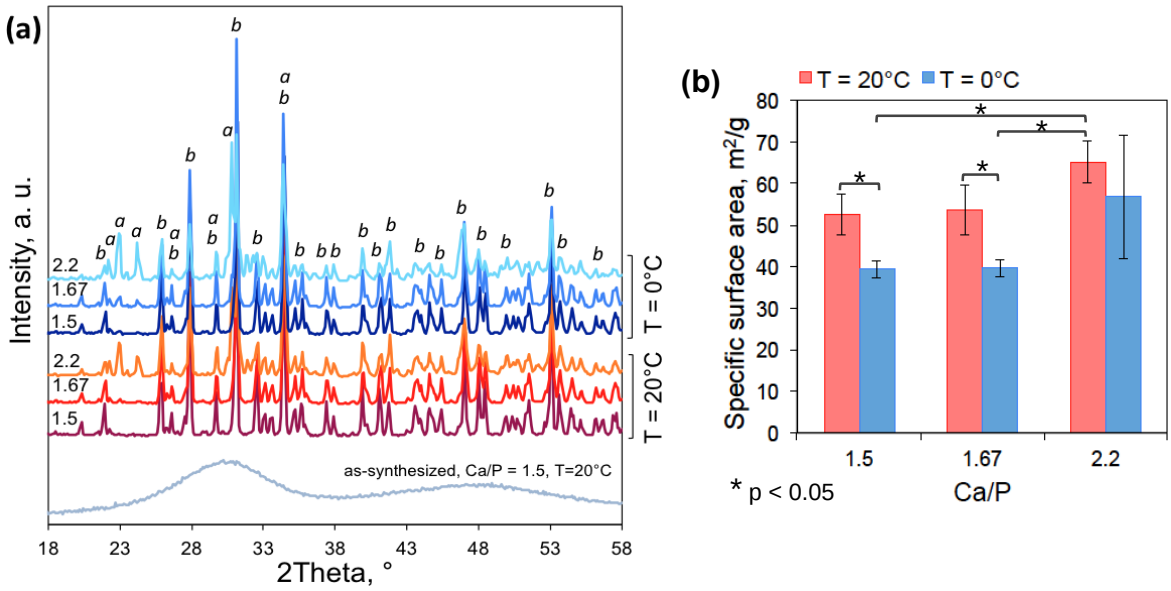

Fig. 2. XRD patterns (a) of as-synthesized and heat treated (1100 °C, 1 h) ACP, where *a* – α-TCP, *b* - β-TCP; (b) specific surface area of ACP samples as a function of synthesis temperature and Ca/P molar ratio of reagents [12].

Fig. 2 (b) presents the influence of Ca/P molar ratio of reagents and synthesis temperature on SSA of ACP. Both Ca/P molar ratio of reagents and synthesis temperature have influence on SSA of ACP obtained by double salt decomposition method. Higher initial Ca/P ratio produced ACP with higher average value of SSA both at 0 °C and 20 °C temperature. Overall, it was possible to increase SSA of ACP from (53 ± 5) m^2/g to (65 ± 5) m^2/g at 20 °C and from (39 ± 2) m^2/g to (57 ± 15) m^2/g at 0 °C by varying Ca/P molar ratio of reagents from 1.5 till 2.2. As all samples had Ca/P of 1.5, it suggested that excess of calcium ions in synthesis media has fostered formation of particles with higher SSA. The highest SSA value was for ACP synthesized from reagents with Ca/P molar ratio of 2.2 at 20 °C – 65 m^2/g which did not reach the expected value of 100 m^2/g. Therefore, other synthesis methods of ACP should be explored and modified in order to synthesize the product with intended value of SSA.

The novelty of the obtained results is twofold: 1) synthesis of ACP using double salt decomposition method without stabilizing agents at a lower temperature (0 °C) than reported in the literature before and 2) assessment of an impact of Ca/P molar ratio of the reagents on the SSA of ACP. Furthermore, as systematic studies on SSA of CaPs, especially ACP, were lacking in the literature, the current PhD Thesis partially filled the identified gap of knowledge.

Development of Synthesis Method for Obtaining ACP with High Specific Surface Area

In the double salt decomposition method of ACP synthesis **two solutions** are joined into *one*, thus it was hypothesized that use of a **single solution** containing both calcium and phosphate ions is efficient for obtaining CaPs with higher SSA. The initial homogeneity of calcium and phosphate ions would lead to uniform formation and growth of precipitated particles and thus to CaP with high SSA. Similar hypothesis of homogenized reactant solution was presented by E. D. Eanes and E. L. Meyer [13], where basic solution of KOH was rapidly added to an acidic mix of calcium and phosphate salts inducing precipitation of CaP.

In the current PhD Thesis, the developed synthesis method for obtaining ACP with high SSA consists of two steps: 1) obtaining of homogenous solution containing both calcium and phosphate ions by dissolving hydroxyapatite in hydrochloric acid and 2) rapid addition of strong base to the solution of calcium and phosphate ions and rise of pH (Fig. 3). The second step is immediately proceeded with precipitation of ACP.

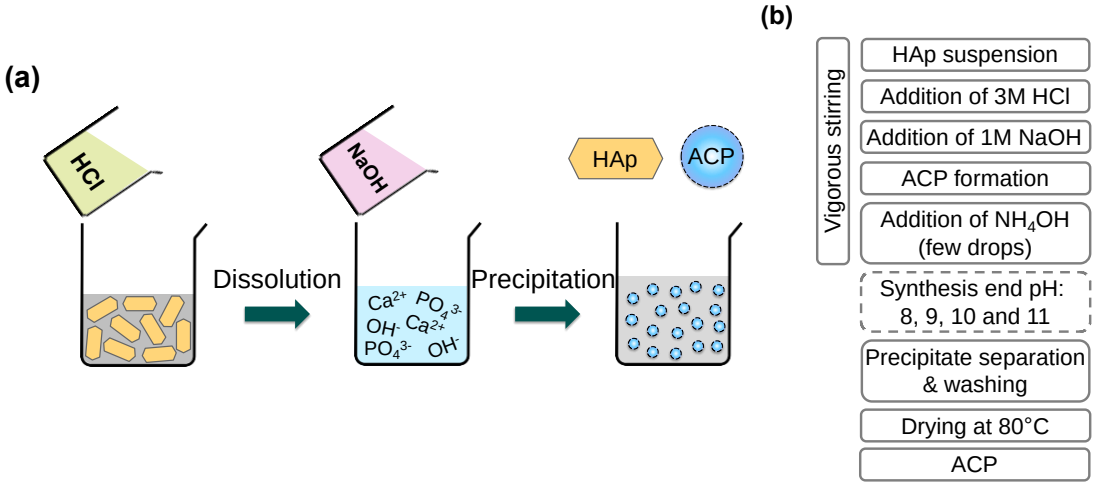

Fig. 3. Main steps (a) and detailed overview (b) of ACP synthesis (developed by author). Synthesis scheme is based on the image by J. Vecstaudza and J. Locs [14].

It is known that pH of the synthesis medium has a significant effect on CaP phase composition obtained by precipitation methods, therefore effects of synthesis end pH (8, 9, 10 and 11) on phase composition, chemical structure and SSA of the products obtained by the developed precipitation method were studied. The synthesized materials were characterized using XRD (X'Pert Pro, PAnalytical, the Netherlands), Fourier transform infrared spectrometry (FT-IR, Scimitar 800, Varian Inc., USA) and BET (Quadrasorb SI, Quantachrome, USA) methods. In addition, long-term phase stability of synthesized materials was studied using XRD. Crystalline phases were identified using ICDD PDF-2 database; phases were quantified using Rietveld refinement. Ca/P molar ratio of the samples was calculated based on quantitative phase analysis. Calculation of average particle diameter d_{BET} was done according to Equation (1) assuming particles to be spherical and nonporous:

$$d_{BET} = 6/(\rho \cdot SSA), \quad (1)$$

where ρ is the density of R-HAp (2.81 g/cm^3), SSA is a specific surface area (determined by BET).

Nanosized carbonated calcium phosphates (CaPs) with amorphous or low crystalline structure were obtained by a novel method based on fast pH increase of calcium and phosphate ions containing solution and drying of precipitates at 80 °C temperature. Phase of ACP was obtained at synthesis end pH values of 10 and 11; in turn, low crystalline CaPs were obtained at synthesis end pH values of 8 and 9 (Fig. 4 (a)). Chemical composition of respective materials was dependent on synthesis end pH as well (Fig. 4 (b)); differences observed in XRD patterns were complemented by information gained from FT-IR spectra. Furthermore, FT-IR spectra revealed presence of carbonate groups within all studied samples. Both the phase and chemical group composition analysis showed that ACP synthesized by the developed method is valid for drying at 80 °C temperature, as it remains amorphous when precipitated at certain pH. Later advancement holds promise for upscaling of ACP synthesis and later commercialization due to substantially shorter processing time, as usually, the metastable ACP is dried using lyophilization technique for 48–72 h.

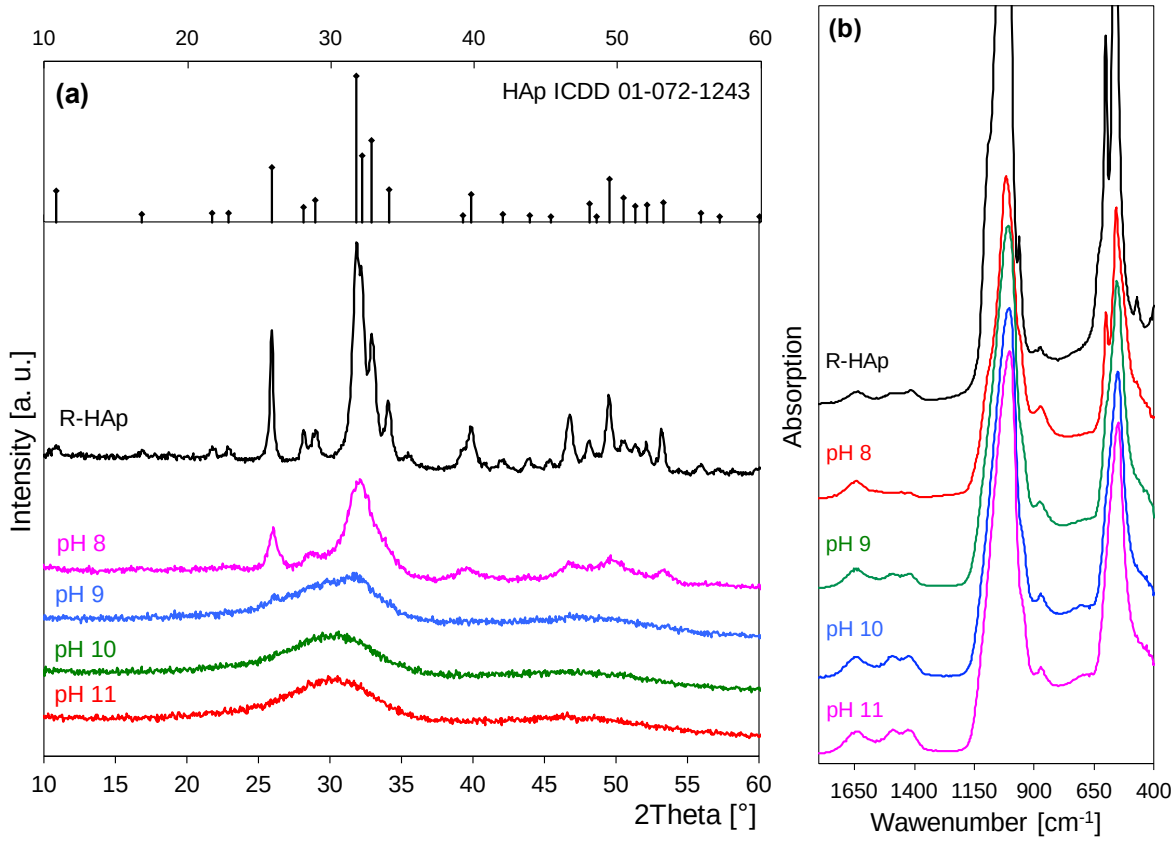

Fig. 4. X-ray diffraction patterns (a) and FT-IR spectra presented in 1700–400 cm^{-1} range (b) of calcium phosphates obtained at different pH values and R-HAp, samples dried at 80 °C temperature [14].

The obtained CaPs have SSA within a range from 133 m^2/g to 154 m^2/g (Table 1), thus fulfilling the set goal of obtaining ACP with SSA ≥ 100 m^2/g. Next, the calculated particle size d_{BET} was in the range from 14 nm to 16 nm. However, correlation between SSA and the synthesis end pH of ACP was not found (results were not statistically significant).

Table 1

Characteristics of the Obtained Calcium Phosphates, where d_{BET} – Particle Size, SSA – Specific Surface Area [14]

Sample	Synthesis end pH	d_{BET}, nm	SSA, m^2/g
ACP-8	8	14 ± 1	154 ± 9
ACP-9	9	15 ± 1	141 ± 8
ACP-10	10	16 ± 3	133 ± 25
ACP-11	11	15 ± 3	150 ± 28
R-HAp	8.8	22 ± 1	95 ± 3

Regardless the initial Ca/P molar ratio in synthesis solution (in all cases Ca/P = 1.67), different crystalline phases formed upon heat treatment (1100 °C, 1 h) of ACP: β-TCP (pH = 8 and pH = 9) or β-TCP/HAp (pH = 10 and pH = 11) was identified. Ca/P molar ratio of the heat-treated samples can be seen in Table 2. Ca/P molar ratio of the samples was affected by synthesis end pH value – it increased with increasing synthesis end pH. The increase of Ca/P molar ratio of the sample may be due to carbonate ions that left the structure of CaP during the heat treatment. It is known that in CaP synthesis pH is one of the main factors that contributes to obtaining of the product with specific composition; it is confirmed in the current study as well.

Table 2

Crystalline Phase Composition of Heat-Treated Samples and Ca/P Molar Ratios Thereof [14]

Sample	Synthesis end pH	β-TCP, wt. %	Hap, wt. %	Ca/P molar ratio
ACP-8	8	100	–	1.50
ACP-9	9	100	–	1.50
ACP-10	10	95.2 ± 1.5	4.8 ± 1.5	1.51
ACP-11	11	90.3 ± 1.6	9.7 ± 1.6	1.61

The obtained CaPs are stable in dried state at least up to 7 months (Fig. 5) when synthesis end pH values thereof are pH = 8, pH = 10 and pH = 11. After 5 months, the sample obtained at pH = 9 started to crystallize because of internal hydrolysis mechanism. The stability of samples obtained at pH = 10 and pH = 11 could be explained by incorporation of carbonate ions within the structure of the samples, thus increasing the Ca/P molar ratio. Therefore, ACP samples with increased Ca/P molar ratio (>1.50) demonstrated a long-term stability of amorphous phase.

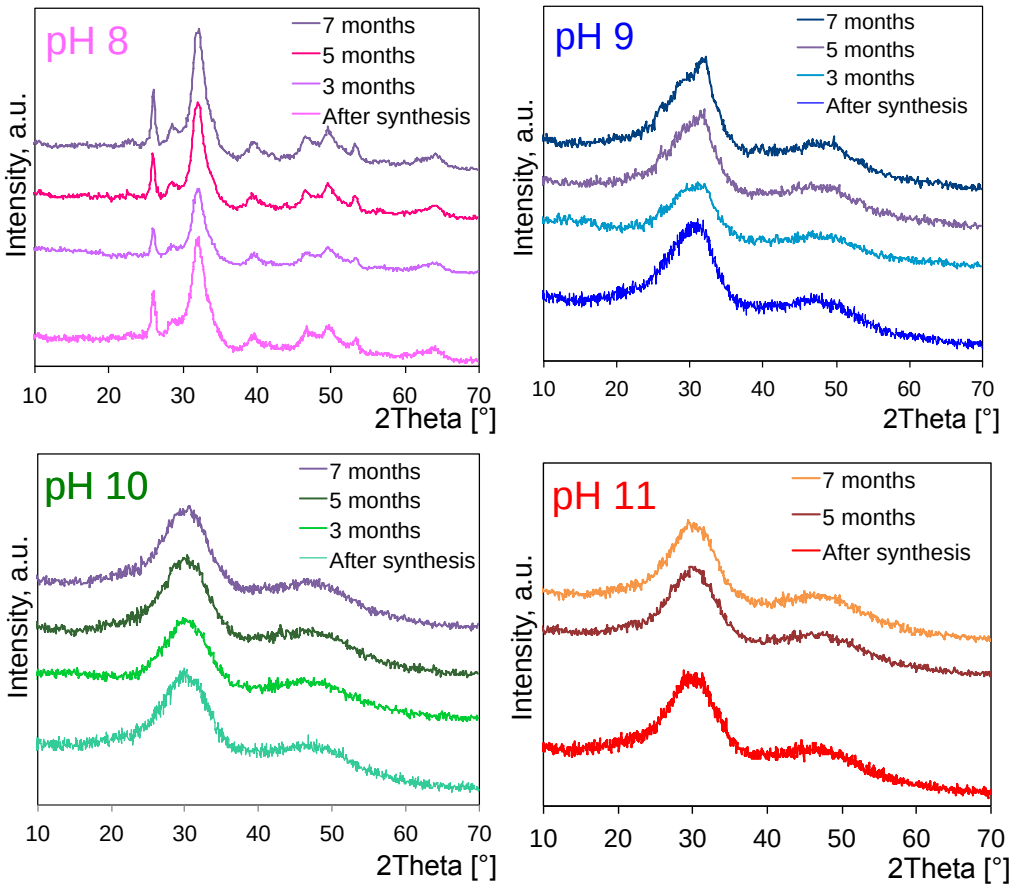

Fig. 5. XRD patterns of low crystalline and amorphous CaPs obtained at different pH and dried at 80 °C temperature: as-synthesized, 3, 5 and 7 months after synthesis [14].

Thermal Behaviour of Differently Dried ACP Materials

The developed synthesis method allowed drying of ACP even at 80 °C temperature (1 h) without the need for the time-consuming freeze-drying (72 h) to preserve the amorphous structure. However, CaPs synthesized using the developed method were freeze-dried as well, and their physicochemical characteristics were compared to CaPs dried at 80 °C temperature.

All freeze-dried samples were x-ray amorphous, regardless of synthesis end pH value. However, the freeze-dried powders visually differed from CaPs dried at 80 °C temperature (Fig. 6), where free flowing voluminous powders for freeze-dried and agglomerated powders for oven dried materials were obtained. The samples dried at 80 °C temperature (abbreviated as "Ov") had a larger particle size and occupied less volume than the freeze-dried (abbreviated as "FrD") samples. A hypothesis was formulated: differently dried ACP will crystallize into different phases because of structural differences that were not detectable by means of routine analysis tools (e.g., XRD and FT-IR). Possible differences of differently dried CaPs were analyzed within crystallization process thereof using thermogravimetry (TGA) and differential scanning calorimetry (DSC) methods.

DSC-TGA measurements were performed with STA449C Jupiter® (Netzsch, Germany) instrument. Two samples were used for comparison: n-HAp (nanocrystalline HAp, Sigma Aldrich) and R-HAp (produced at RTU RBIDC), both contain only HAp.

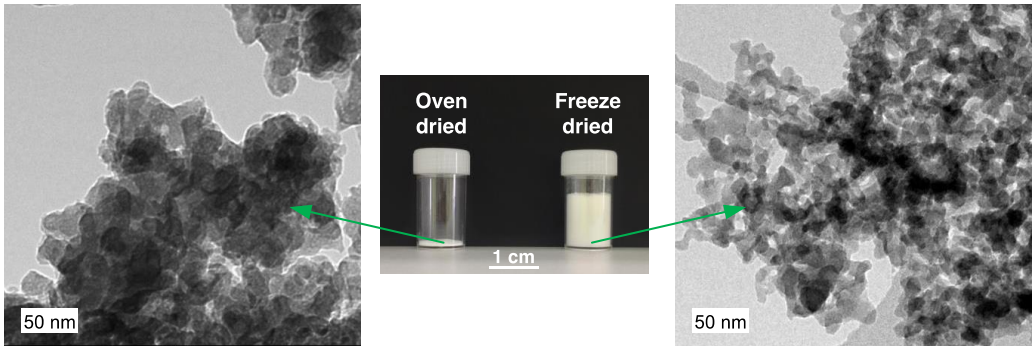

Fig. 6. Photographs and TEM images of oven dried at 80 °C (left) and freeze-dried (right) ACP samples of the same weight [15], synthesis end pH = 10.

TGA curves of Ov and FrD ACP samples are shown in Fig. 7. The observed mass losses are gradual and without abrupt changes for all Ov and FrD samples. However, the total mass loss value differs for samples that were obtained at synthesis with different synthesis end pH value, e.g., see mass loss at 800 °C temperature.

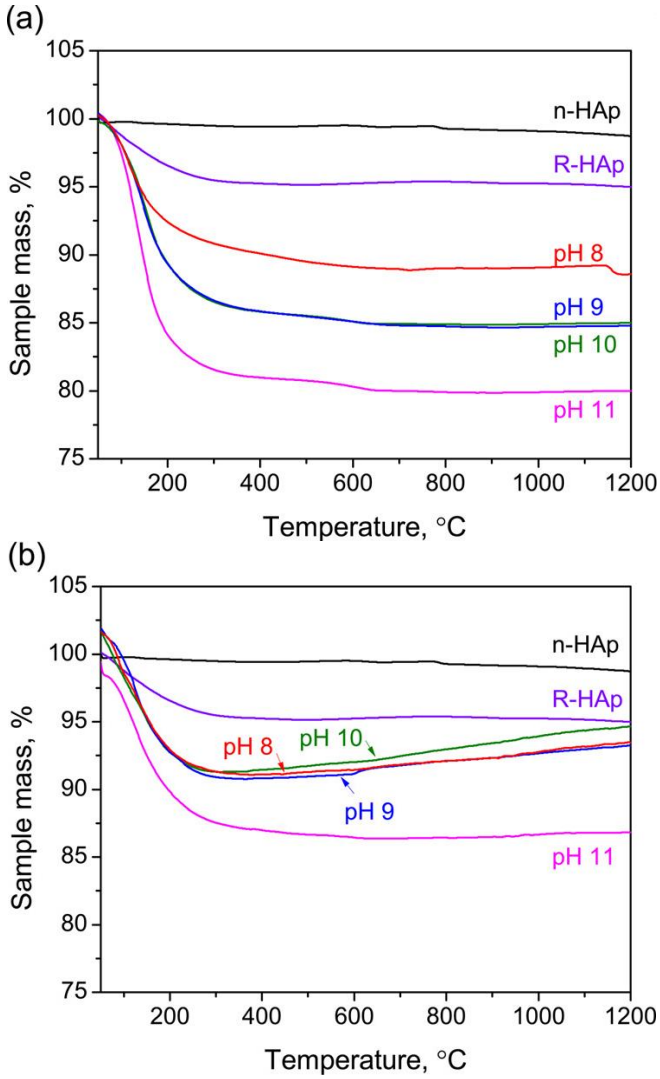

Fig. 7. TGA curves of (a) 80 °C temperature dried and (b) freeze-dried ACP shown in the temperature range of 50 °C to 800 °C; n-HAp and R-HAp are for reference purpose [15].

The comparison of both drying methods revealed that Ov samples had larger amount of physically adsorbed water that was lost up to 200 °C temperature (Fig. 7). The same observation applies for the total mass loss determined at 1200 °C. The total mass loss of Ov samples ranged from 11 wt. % to 20 wt. %, where higher values of mass loss were for Ov samples that were obtained at higher synthesis end pH. In turn, the total mass loss of FrD samples reached maximum of 14 wt. % for the sample that was obtained at pH = 11. The total mass loss values were approximately the same for FrD samples obtained at pH = 8–10; the effect of synthesis end pH here was not observed.

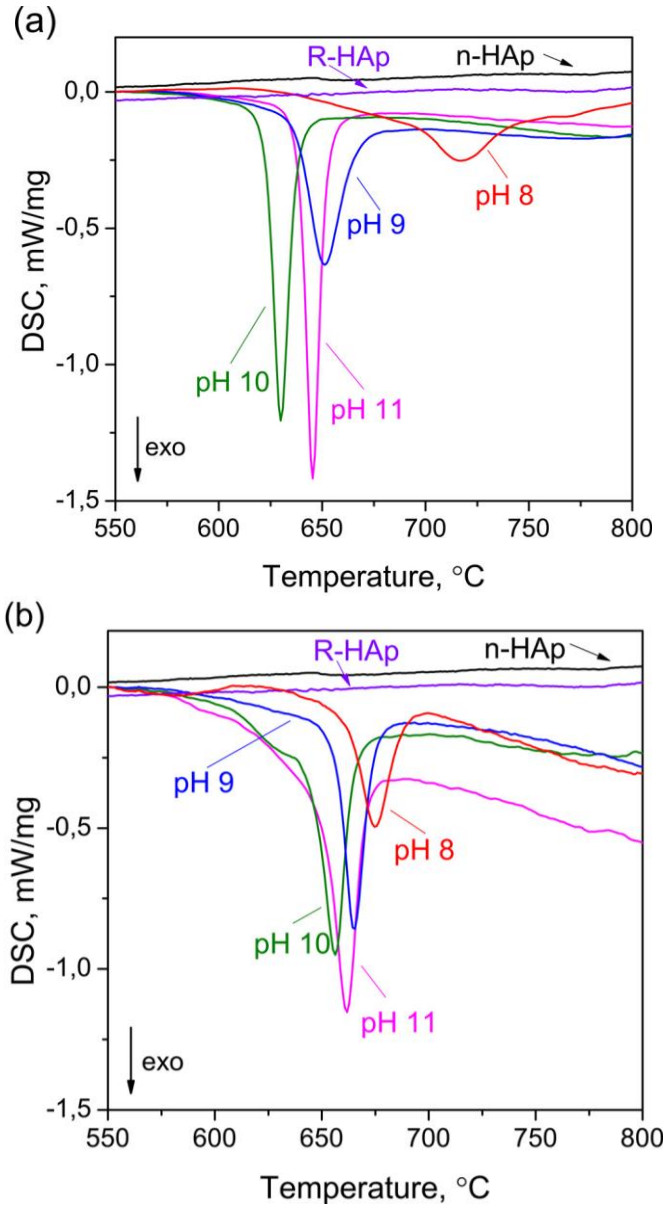

Fig. 8. DSC curves of (a) 80 °C temperature dried and (b) freeze-dried ACP in temperature range from 550 °C to 800 °C; n-HAp and R-HAp are for reference purpose [15].

In all DSC curves of Ov and FrD samples exothermic crystallization effects were observed (Fig. 8). Within the temperature range of these effects at 630–720 °C ACP transformed into crystalline CaPs with dominant crystalline phase being β-TCP. $T_{cryst.onset}$ of

the studied ACP was in the range from 600 °C to 650 °C; correlation between crystallization temperatures ($T_{cryst.onset}$ and $T_{cryst.end}$) and drying method or synthesis pH was not observed. The analysis of both TGA and DSC curves indicated that thermally induced crystallization of ACP was not associated with simultaneous mass loss. Crystallization of ACP started at 150–200 °C higher temperature from the point where in TGA curve the greatest mass loss (up to 400 °C) was observed.

Densification of ACP Using the Principles of Cold Sintering Process

Besides the unique phase and chemical composition, the bone has a unique physical structure as well, which is comprised of both dense and porous bone regions. So far it has remained a challenge to sinter ACP bioceramics to high relative density without affecting its amorphous, hydrated structure and properties. Therefore, alternative sintering techniques such as hydrothermal hot-pressing and low-temperature spark plasma sintering have been used for densification of ACP. However, densification of ACP is challenging even with these technologies. Recently, cold sintering process (CSP) has been used for low-temperature (≤300 °C) densification of various powders. In CSP transient liquid, applied uniaxial pressure and heat is used to densify a powder compact. Thus, use of the CSP technique for densification of ACP has been explored in the current Thesis.

In order to determine optimal technological parameters of CSP for obtaining dense ACP bioceramics, two technological parameters were tested: 1) presence or absence of a transient liquid (water, 20 wt. %) and 2) sintering temperature (room temperature, 100 °C, 120 °C and 150 °C). For each CSP experiment 0.5 g of previously prepared ACP powder (dried at 80 °C, 24 h) were transferred to an easy retrieve pressing die (*ID* = 13 mm, Across International, USA). Here ACP with high SSA was used, that was synthesized according to the developed synthesis protocol [14] and dried using freeze-drying. Moistening of the ACP powder was done manually using agate mortar and pestle. CSP process was done using a two-column lab press PW 40 (P/O/WEBER, Germany) by applying uniaxial pressure of 500 MPa. At least three samples were made for each sintering condition. See overview of CSP experimental conditions in Fig. 9.

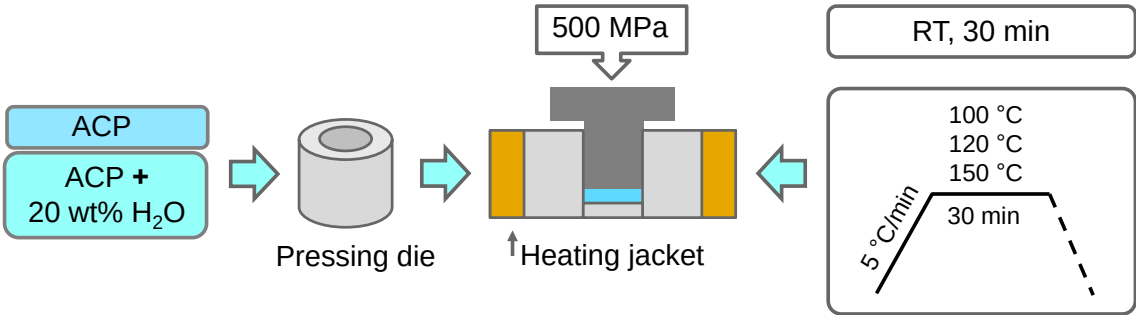

Fig. 9. Densification of ACP using principles of CSP, where RT – room temperature.

Starting ACP powder and CSP-sintered samples were analyzed using XRD (X'Pert Pro, PAnalytical, the Netherlands) and BET (Quadrasorb SI, Quantachrome, USA) methods to determine the phase composition and SSA. In order to evaluate the effect of CSP on densification of the samples, bulk and relative density values of CSP-sintered samples were determined using helium pycnometer Micro UltraPyc 1200e (Quantachrome Instruments, USA). Before each measurement the CSP-sintered samples were pre-dried (80 °C, 24 h) and milled to fine powder using Mini-Mill Pulverisette 23 (FRITCH, Germany) one ball mill for 3 min.

The impact of CSP on phase stability of ACP was evaluated using XRD (Fig. 10). Presence of transient liquid (water, 20 wt. %) led to crystallization of ACP into nanocrystalline HAp phase. The crystallization occurred at lower temperatures (crystallized at 100 °C) when compared to the dry ACP powder (crystallized at 150 °C). Both the temperature and pressure could have activated the surface diffusion processes leading to the phase transition from ACP to nanocrystalline HAp.

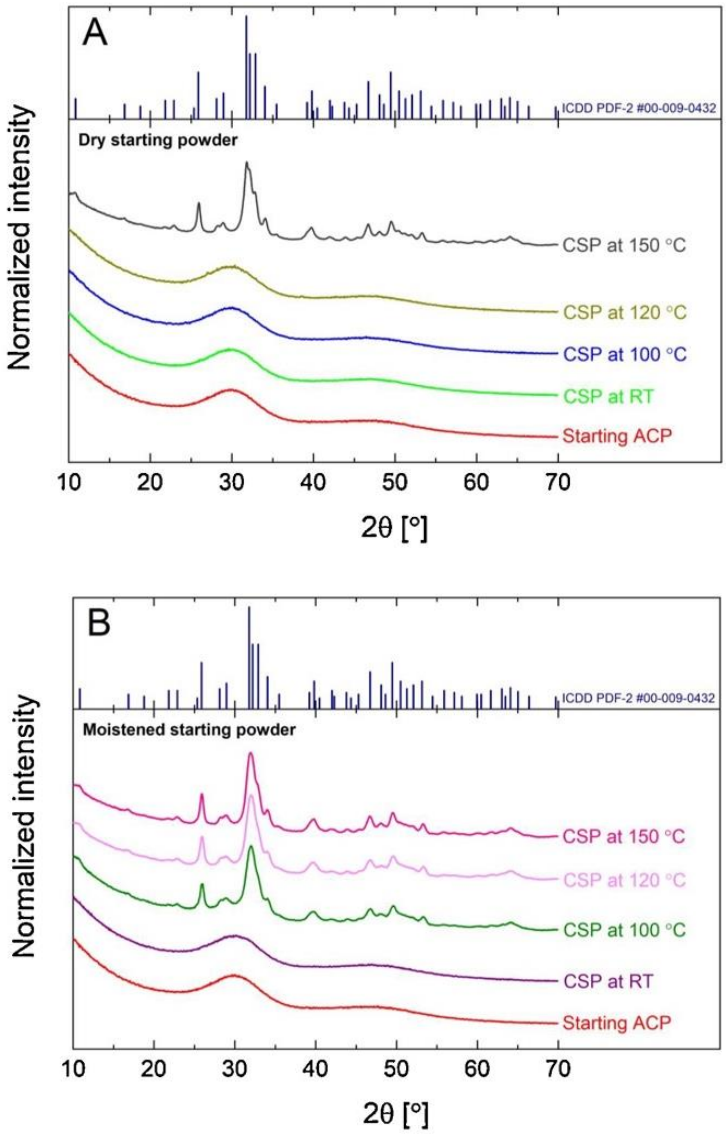

Fig. 10. XRD patterns of the starting ACP and the CSP-sintered samples produced from dry (A) and from moistened starting powder (B) [16].

SSA of the CSP-sintered samples was reduced approximately 5-fold (Fig. 11) comparing to one of the as-synthesized ACP powder (109 $m^2/g \pm 11$ m^2/g). The observed reduction of SSA indicates to particle coalescence during the CSP. Further, insufficient densification produces interparticle (closed) porosity with a surface that may be inaccessible to nitrogen gas that was used in BET measurements.

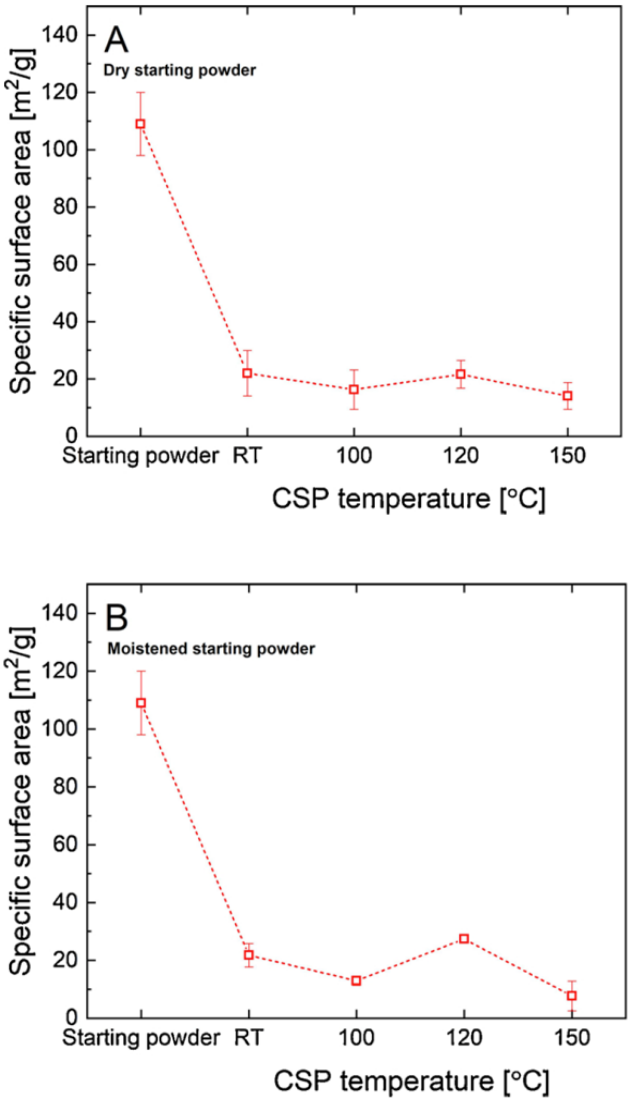

Fig. 11. Specific surface area of the starting ACP powder and the CSP-sintered samples produced from dry (A) and from moistened starting powder (B) [16].

Application of a moderate uniaxial pressure of 500 MPa at room temperature enabled sintering of ACP to relatively high density by using principles of the CSP (Fig. 12). Increase of sintering temperature had only a slight effect on the density values of the samples that remained amorphous after the CSP (refer to Fig. 10). Apparently, the low sintering temperatures were not sufficient in order to increase densification by diffusion processes. The highest relative density value reported for ACP ceramics so far was ~45 % [17]. In current study, relative density of ACP CSP-sintered ceramics was over 75 %.

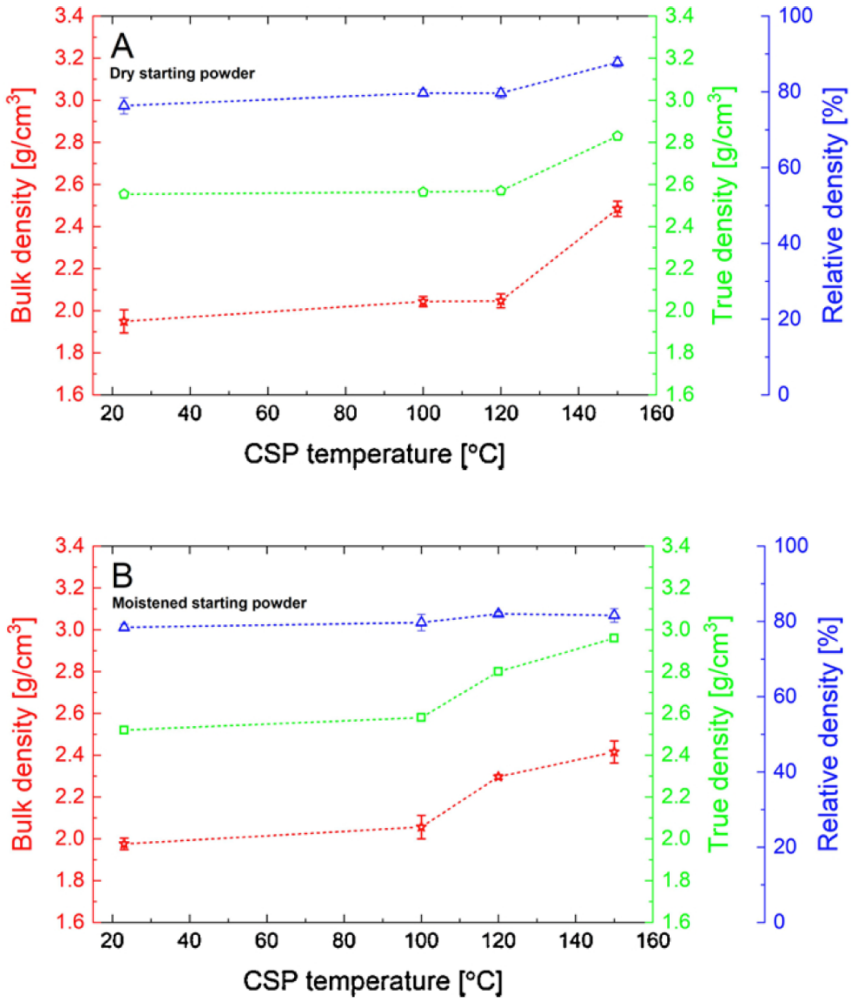

Fig. 12. Bulk, true and relative densities of the CSP-sintered samples produced from dry (A) and from moistened (B) ACP powder [16].

CONCLUSIONS

1. Synthesis of amorphous calcium phosphate by double salt decomposition method from $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ by varying Ca/P molar ratio of the reagents (1.5, 1.67 or 2.2) and synthesis temperature (0 °C or 20 °C) produces negligible increase of the specific surface area; furthermore, Ca/P molar ratio of the reagents does not affect Ca/P molar ratio of the produced ACP.
2. A simple, fast and cost-effective synthesis method for preparation of nanosized calcium phosphates, amorphous or low crystalline, with a high specific surface area ($\geq$100 m^2/g) has been developed based on a fast increase of pH value in synthesis media containing both calcium and phosphate ions.
3. Synthesis pH (8–11) and drying method (freeze-drying or drying at 80 °C temperature) of amorphous calcium phosphate does not affect crystallization temperature (600–650 °C) of amorphous calcium phosphate during heat treatment, however mass losses associated with the removal of water are affected.
4. Amorphous calcium phosphate materials with increased Ca/P molar ratio (>1.50) exhibit long term stability when stored in room temperature.
5. Use of cold sintering process principles enables obtaining dense amorphous calcium phosphate ceramics at very low temperatures (room, 100 °C and 120 °C) by uniaxial pressing at a pressure of 500 MPa.

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