

Research of Calcium Phosphates Using Fourier Transform Infrared Spectroscopy

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1. Introduction

In the biomaterial research field, nowadays a great attention is driven onto calcium phosphates synthesis and obtaining of ceramics that can be used in orthopedics and dentistry, in the form of coatings, granules, porous or solid blocks, as well as in the form of various composite materials. The most frequently studied, clinically tested and used synthetic materials based on calcium phosphate (CaP) are hydroxyapatite [HAp - $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$], β -tricalcium phosphate [β -TCP - $\text{Ca}_3(\text{PO}_4)_2$] and biphasic HAp/ β -TCP mixture. CaP ceramic demonstrates high biocompatibility and bioactivity while it contacts bone cells, builds a direct chemical connection between bone tissues and ceramic implant. As practice shows, purchased materials, most often commercial CaP materials, not always have properties and qualities defined by the manufacturer. Frequently, the manufacturer's information about the offered product is not complete or precise, by it troubling usage of raw CaP material for development of implants. The most common imperfections of CaP materials are – unpredictable properties after the high temperature treatment (composition and clarity of crystal phases, chemical composition, thermal stability, etc.) which become clear only after the high temperature treatment of the ready implant material has occurred. For several years, Riga Biomaterial Innovation and Development Centre (RBIDC) of Riga Technical University perform a wide range of property studies of various commercial CaP raw materials.

Properties of bioceramic implants obtained from various commercial and laboratory synthesized calcium phosphate precursors are different, since behavior of those precursors is different within the thermal treatment processes, which are a significant stage of obtaining ceramics.

CaP synthesis methods and their technological parameters can significantly impact stoichiometry of the synthesis product, its grade of crystallization, particle size, bioceramic phase composition, thermal stability, microstructure and mechanical properties. The important technologic parameters that impact properties of calcium phosphate synthesis product and then also of bioceramic, are temperature of synthesis, pH of synthesis environment, reagent type and concentration, as well as selection of raw materials, their purity and quality. All of the above mentioned also brings a significant impact on the tissue response of these bioceramic implants.

Fourier transform infrared spectroscopy (FTIR) is one of the methods which, systematically monitoring variations of structural characteristic groups and vibrations bonds, can provide an indirect evaluation of the synthesized Ca/P implant materials from TCP up to HAp and bioceramics, obtained from these materials.

FTIR spectroscopy has numerous advantages when used for chemical analysis of CaP products. First of all, an obtained spectrogram provides useful information about location of peaks, their intensity, width and shape in the required wave number range. Secondly, FTIR is also a very sensitive technique for determining phase composition. In the third place, FTIR is a comparatively quick and easy everyday approach.

During recent years, many authors' attention is turned onto synthesis of CaP and research of structure of the synthesized products depending on their technological parameters, with various methods, including an X-ray diffraction (XRD) and FTIR methods. However, the data of the literary sources is often incomplete or sometimes even contradictory. Studying various literary sources and analyzing the taken spectra of laboratory synthesized and commercial CaP products was aimed onto creating summary IR spectrum tables for the characteristic calcium phosphate chemical groups absorption bands. HAp stoichiometry is very important if the material has undergone a high temperature treatment.

A minor misbalance of synthesis product in a stoichiometric ratio (standard molar ratio of Ca/P is 1.67) during high temperature treatment can lead to composition of β -, α -TCP, or other phases. Thermally treating the stoichiometric calcium phosphates, it is possible to obtain stable phases at temperatures up to 1300 °C. One of the main non-stoichiometry reasons is inclusion of impurities, often substitutions of Ca^{2+} or interpenetration of other ions in the crystal lattice. In total, biological calcium phosphates are defined as calcium hydroxyapatites with deficient of calcium, $\text{Ca}_{10-x}(\text{PO}_4)_{6-x}(\text{HPO}_4)_x(\text{OH})_{2-x}$ ($0 < x < 2$), including the substituting atoms or groups, as, for example, Mg^{2+} , Na^+ , K^+ , Sr^{2+} , or Ba^{2+} substitute Ca^{2+} , CO_3^{2-} , H_2PO_4^- , HPO_4^{2-} ; SO_4^{2-} substitute PO_4^{3-} ; F^- , Cl^- , CO_3^{2-} , PO_4^{3-} substitute OH^- .

The main target of our work was to perform a FTIR spectroscopy analysis of the CaP products synthesized in RBIDC laboratory and make summarizing conclusions about chemical groups of calcium phosphates, their variations under impact of synthesis parameters and further thermal treatment, as well as creating summary tables for:

- CaP powders synthesized in the laboratory with a chemical solution precipitation method with different synthesis parameters (temperature, final suspension pH, maturation time) as-synthesized and then thermally treated at various temperatures from 200°C up to 1400°C;
- Commercial CaP products;
- CaO containing materials of various origins (marble, eggshells, land snail shells) and FTIR spectra of obtained products;

2. Calcium phosphates and FTIR absorption bands of their chemical groups

2.1 Calcium phosphates

Calcium phosphates as chemical compounds arise interest of the numerous fields of science, like geology, chemistry, biology and medicine. Many forms of calcium phosphates are

determined by their Ca/P molar ratio. From the point of view of chemistry, they are formed by three main elements: calcium, phosphorus and oxygen. Many calcium phosphates also contain hydrogen in an acidic phosphate anion (for example, HPO_4^{2-}), hydroxyl groups (for example, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) or in a form of bonded water (for example, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$). Majority of compounds of this class are poorly soluble in the water and non-soluble in alkaline solutions, but all of them easily dissolve in acids. Chemically pure calcium orthophosphates are white crystals with an average hardness, while natural materials are always of some other color which depends on the type and amount of impurities. Biologic calcium phosphates are main mineral components in the calcified tissues of the vertebrates (Dorozhkin, 2009a).

Main components of the natural bone tissues are calcium phosphates which, along with the other elements (Na, K, F, and Cl) form $\sim 70\%$ of the bone tissue mass. Also, bone tissues contain water (10% of mass) and collagen along with the other organic materials in small amounts. In living system CaP are found in the form of crystalline hydroxyapatite (HAp) and in the amorphous calcium phosphate (ACP) form.

As bone substitution materials, calcium orthophosphates are researched for more than 80 years. The most significant characteristics of calcium phosphates are their bioresorption and bioactivity. They are non-toxic and biocompatible. Bioactivity shows as an ability to create a physical chemical bond between an implant and a bone. This process is called osteointegration (Dorozhkin, 2009b).

Depending on the calcium/phosphorus (Ca/P) molar ratio and solubility of the compound, it is possible to obtain numerous calcium phosphates of different composition. Molar Ca/P ratio and solubility are connected with the pH of the solution. Majority of materials of this class are resorbable and dissolve when inserted in a physical environment. Calcium phosphates that are most frequently used in the biomaterial field are demonstrated in Table 1 (Dorozhkin, 2009c; El Kady, 2009; Shi, 2006).

For biomedical application, the following calcium phosphates are most frequently used: HAp (Ca/P = 1.67) and β -TCP (Ca/P=1.5), as well as biphasic calcium phosphate which mainly consists of HAp and β -TCP mixture in various ratios.

2.2 FTIR absorption bands of the synthesized HAp

Hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is dominating and the most significant mineral phase in the solid tissues of the vertebrates. It consists of the same ions that form mineral part of teeth and bones.

A biological HAp usually has a calcium deficient; it is always substituted with a carbonate. Two types of carbonate substitution are possible: (1) direct substitution of OH^- with CO_3^{2-} (A-type substitution ($\text{CO}_3^{2-} \leftrightarrow 2\text{OH}^-$)) and (2) necessity after charge compensation, PO_4^{3-} substituting a tetrahedral group with CO_3^{2-} (B-type substitution). Substitution groups may provoke characteristic changes in the lattice parameters, crystallinity, crystal symmetry, thermal stability, morphology, and solubility, physical, chemical and biological characteristics (Shi, 2006).

The most characteristic chemical groups in the FTIR spectrum of synthesized HAp are PO_4^{3-} , OH^- , CO_3^{2-} , as well as HPO_4^{2-} that characterize non-stoichiometric HAp.

Name	Abbreviation	Chemical formula	Ca/P
Amorphous calcium phosphate	ACP	$\text{Ca}_x\text{H}_y(\text{PO}_4)_z \cdot n\text{H}_2\text{O}$	1.2-2.2
Dicalcium phosphate anhydride	DCPA	CaHPO_4	1.00
Dicalcium phosphate dehydrate	DCPD	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	1.00
Octacalcium phosphate	OCP	$\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$	1.33
β -tricalcium phosphate	β -TCP	$\text{Ca}_3(\text{PO}_4)_2$	1.50
α -tricalcium phosphate	α -TCP	$\text{Ca}_3(\text{PO}_4)_2$	1.50
Hydroxyapatite with calcium deficient	CDHA	$\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$ $0 \leq x \leq 1$	1.5-1.67
Hydroxyapatite	HAp	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	1.67
Tetra calcium phosphate	TTCP (TetCP)	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	2.00
β -Ca pyrophosphate	CPP	$\text{Ca}_2\text{P}_2\text{O}_7$	<1.5
Oxyapatite	OAp	$\text{Ca}_{10}(\text{PO}_4)_6\text{O}$	1.67

Table 1. Calcium phosphates used in the biomaterial field.

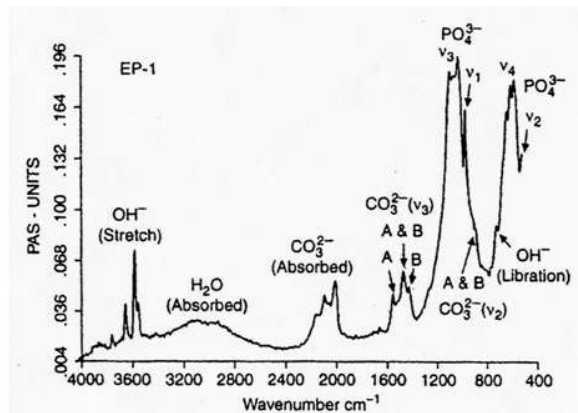


Fig. 1. A typical FTIR spectrum of hydroxyapatite (Ratner, 2004)

PO_4^{3-} group forms intensive IR absorption bands at 560 and 600 cm^{-1} and at 1000 – 1100 cm^{-1} . Adsorbed water band is relatively wide, from 3600 to 2600 cm^{-1} , with an explicit peak at 3570 cm^{-1} , a weaker peak is formed at 630 cm^{-1} . CO_3^{2-} group forms weak peaks between 870 and 880 cm^{-1} and more intensive peaks between 1460 and 1530 cm^{-1} . Absorption bands of chemical bonds of the synthesized HAp spectrum are summarized in Table 2.

Chemical groups	Absorption bands, (cm ⁻¹)	Description
CO ₃ ²⁻	873; 1450; 1640 (Meejoo, et al., 2006) 1650 (Raynaud, et al., 2002); 870 and 880; 1460 and 1530 (Ratner, 2004)	Substitutes phosphate ion, B-type HAp is formed (Meejoo, et al., 2006)
OH ⁻	3500 (Meejoo, et al., 2006) 630 and 3540 (Destainville, et al., 2003), (Raynaud, et al., 2002); 3570 and 3420 (Han J-K., et al., 2006); 1650 (Raynaud, et al., 2002)	OH ⁻ ions prove presence of HAp
Adsorbed water	2600 - 3600 (Meejoo, et al., 2006)	Under influence of thermal treatment, absorption band becomes narrower
HPO ₄ ²⁻	875 (Destainville, et al., 2003), (Raynaud, et al., 2002); 880 (Kwon, et al., 2003)	Characterizes HAp with deficient of calcium. (Raynaud, et al., 2002); Refers to non-stoichiometric HAp (Kwon, et al., 2003);
PO ₄ ³⁻	460 (Destainville, et al., 2003); (Raynaud, et al., 2002);	v2 (Destainville, et al., 2003);
	560 - 600 (Destainville, et al., 2003), (Raynaud, et al., 2002), (Mobasherpour & Heshajin, 2007); 602 un 555 (Han J-K., et al., 2006)	v4 (Destainville, et al., 2003); bending mode (Han J-K., et al., 2006)
	960 (Destainville, et al., 2003), (Raynaud, et al., 2002)	v1 (Destainville, et al., 2003);
	1020 -1120 (Destainville, et al., 2003), (Raynaud, et al., 2002); 1040 (Han J-K., et al., 2006); 1000 - 1100 (Mobasherpour & Heshajin, 2007);	v3 (Destainville, et al., 2003); bending mode (Han J-K., et al., 2006);
NO ₃ ⁻	820 and 1380 (Destainville, et al., 2003); (Raynaud, et al., 2002)	Synthesis residue that disappears during the calcifying process (Destainville, et al., 2003)

Table 2. FTIR absorption bands of synthesized HAp chemical groups.

2.3 FTIR absorption bands of thermally treated calcium phosphates

As a biomaterial, HAp is mainly used in its ceramic form that was obtained by sintering the powder at 1000 - 1350 °C or as a coating on the implant surface. During the process of thermal decomposition of HAp, sintering of ceramic or obtaining the coating, physical, chemical, mechanical and, most important, biomedical properties may be negatively affected. Thus, HAp and other CaP materials should be thoroughly studied while thermally treated.

Temperature, °C	Chemical groups and phases	Absorption bands, (cm ⁻¹)	Description
250	H ₂ O		Molecules of adsorbed water disappear (Mobasherpour & Heshajin, 2007);
600	NO ₃ ⁻	820 and 1380 (Raynaud, et al., 2002)	Synthesis impurities disappear (Raynaud, et al., 2002);
700	CO ₃ ²⁻	1450 (Meejoo, et al., 2006)	Intensity decreases;
	H ₂ O, OH ⁻	3500 (Meejoo, et al., 2006) 630 and 3540 (Destainville, et al., 2003);	Adsorbed water band becomes narrower (Meejoo, et al., 2006); Refers to variations of OH ⁻ (Destainville, et al., 2003);
800	CO ₃ ²⁻	1450 (Meejoo, et al., 2006)	Disappears (Meejoo, et al., 2006)
900	OH ⁻		Disappears (Meejoo, et al., 2006)
	β- TCP	947, 974 and 1120 (Meejoo, et al., 2006)	β-TCP shoulders begin to show up (Meejoo, et al., 2006);
	PO ₄ ³⁻	603 and 565; 1094 and 1032 (Meejoo, et al., 2006)	Shifts position at 1200 °C (Meejoo, et al., 2006);
1200	β- TCP		Can see the characteristic peaks better (Ratner, 2004);
	PO ₄ ³⁻	601 and 571; 1090 and 1046 (Meejoo, et al., 2006)	Indicates that under influence of temperature, phosphates decompose and β-TCP shoulders become wider (Meejoo, et al., 2006).
1200 - 1400			β- TCP transforms onto α-TCP (Mobasherpour & Heshajin, 2007);
1400	α-TCP	551; 585; 597; 613; 984; 1025; 1055 (Han J-K., et al., 2006)	

Table 3. FTIR absorption bands of thermally treated CaP chemical bonds.

During the thermal treatment, behavior of CaP is affected by various factors, like, atmosphere of sintering, ratio of Ca/P, method and conditions of powder synthesis, type and amount of impurities, sample size, particle size, etc.

During thermal treating HAp undergoes the following processes:

- dehydration (separation of adsorbed water);
- dehydroxylation (separation of structured water), forming oxy-hydroxyapatite (OHAp) and oxyapatite (OAp);
- HAp decomposition with formation of other phases.

In Tables 3-5, the data obtained from literary sources about the FTIR absorption bands of thermally treated CaP chemical groups, is summarized.

Temperature, °C	Chemical groups and phases	Absorption bands, (cm ⁻¹)	Description
650	TCP;		Synthesis residue is taken away, but the initial TCP remains unchanged (Destainville, et al., 2003);
750	TCP → β-TCP (Destainville, et al., 2003);		Agglutination begins (Destainville, et al., 2003);
950 - 1000			Maximum speed of compaction, comparing with HAp, β-TCP sintering occurs at a lower temperature. (Destainville, et al., 2003);
	OH ⁻	630 (Destainville, et al., 2003);	Disappeared; spectrum is similar β-TCP (Kwon, et al., 2003)
	P ₂ O ₇ ⁴⁻	727 and 1200 (Destainville, et al., 2003)	Lack of P ₂ O ₇ ⁴⁻ proves that there is no CPP phase and spectrum is similar to pure β-TCP (Destainville, et al., 2003);
1200	β-TCP → α-TCP		(Destainville, et al., 2003);

Table 4. FTIR absorption bands of thermally treated TCP chemical groups.

Losing the adsorbed water do not impact lattice parameters. The water adsorbed on the surface discharges under temperature of less than 250°C, when the moisture is discharged from pores up to 500°C. With temperature rising, wide water bands at 3540 cm⁻¹ become narrower and gradually disappear, but the sharp narrow peaks at 630 and 3570 cm⁻¹ refer to variations of structural OH⁻ groups, which is characteristic to structure of HAp. Depending on the synthesis condition, a carbonate containing apatite is often obtained. Then, it should be considered that CO₂ is discharged from the sample between 450-950°C.

Thermal stability is characterized by the decomposition temperature of HAp sample. The decomposition occurs when a critical dehydration point is achieved. In the temperatures less than the critical point, crystal structure of HAp remains unchanged in spite of the stage of dehydration. Achieving the critical point, a complete and irreversible dehydroxillation occurs, which results damage of HAp structure, decomposing onto tricalcium phosphate (β-TCP under 1200 °C and α-TCP in higher temperatures) and tetracalcium phosphate (TTCP).

At 900°C, β-TCP shoulders begin to show up at 947, 974 and 1120 cm⁻¹, but during heating at higher temperature as, for example, 1200°C, β-TCP phase becomes more visible and PO₄³⁻ peaks shift from 603 and 565 cm⁻¹ to 601 and 571 cm⁻¹, also from 1094 and 1032 to 1090 and 1046 cm⁻¹.

Temperature, °C	Chemical groups	Absorption bands, (cm ⁻¹)	Description
350	HPO ₄ ²⁻		Begins showing up (Raynaud, et al., 2002);
350-720	HPO ₄ ²⁻	875 (Raynaud, et al., 2002);	As a result of condensation, P ₂ O ₇ is formed (Raynaud, et al., 2002);
400	P ₂ O ₇ ⁴⁻	720 (Raynaud, et al., 2002);	Begins showing up if 1.5 < Ca/P < 1.677 (Raynaud, et al., 2002);
600	NO ₃ ⁻	820 and 1380 (Raynaud, et al., 2002);	Disappears (Raynaud, et al., 2002)
750	OH ⁻	630 and 3540 (Destainville, et al., 2003);	Proves presence of HAp (Destainville, et al., 2003);
800	P ₂ O ₇ ⁴⁻	715 (Meejoo, et al., 2006);	Forming of pyrophosphate groups (Meejoo, et al., 2006);
700-900			HAp with deficient of calcium, by decomposing, forms HAp and β-TCP (Raynaud, et al., 2002);
1000	P ₂ O ₇ ⁴⁻	720 (Raynaud, et al., 2002);	Disappears above 1000 °C, if 1.5 < Ca/P < 1.677 (Raynaud, et al., 2002);

Table 5. Analysis of FTIR absorption bands of thermally treated biphasic calcium phosphate (HAp/β-TCP) chemical groups

For stoichiometric HAp, HPO₄²⁻ group is not detected, even though it can appear from the synthesis impurities (NO₃⁻, NH₄⁺).

Various studies show that in the result of HAp (OAp) decomposition, apart from TCP and TTCP, also other calcium compounds may form, like calcium pyrophosphate (CPP, β-Ca₂P₂O₇) and calcium oxide (CaO).

Apatitic TCP (ap-TCP) Ca₉(HPO₄)(PO₄)₅(OH) is a calcium orthophosphate which, during thermal treatment at temperature higher than 750°C, transforms onto β-tricalcium phosphate Ca₃(PO₄)₂.

During the synthesis of TCP, the most important controllable parameters are temperature and pH. According to the literary sources, pH is almost neutral or slightly acidic, and is synthesized at lower temperatures.

A pure stoichiometric β-TCP with a molar ratio Ca/P=1.500, is formed in the result of temperature treatment. If Ca/P > 1.500, HAp is formed as the second phase. When Ca/P ratio is changed for 1%, HAp is formed for 10 wt%. If Ca/P < 1.500, then DCPA is formed, this is proven by presence of calcium pyrophosphate Ca₂P₂O₇.

In order to control speed of biodegradation, a biphasic calcium phosphate (BCP) bioceramic is developed, containing both HAp and TCP. By variation HAp/TCP ratio, it is possible to control bioactivity and biodegradation of implant. Since β -TCP is more soluble and HAp allows a biological precipitation of apatites, solubility of BCP depends on the ratio of HAp/TCP. Osteoconductivity among BCP, HAp and TCP does not significantly differ.

BCP is formed, if $1.500 < \text{Ca/P} < 1.667$, which refers to a hydroxyapatite with calcium deficient, its chemical formula is $\text{Ca}_{10-x}(\text{PO}_4)_{6-x}(\text{HPO}_4)_x(\text{OH})_{2-x}$ ($0 < x < 2$). Ca/P ratio of synthesis sedimentary is not directly connected with the initial Ca/P ratio. At the constant pH, molar ratio of calcium phosphates may be varied by changing the temperature during the synthesis process.

3. Materials and methods

3.1 Synthesis of calcium phosphates

In order to achieve higher assay of the obtained material, its thermal stability and predictability of other properties, the calcium phosphates were synthesized in a laboratory using a wet chemical precipitation method from CaO (or calcium hydroxide $\text{Ca}(\text{OH})_2$) of various origins (commercial, marble, eggshells, land snails shells) as precursors and orthophosphoric acid H_3PO_4 .

Main advantages of this method are a simple synthesis process, a relatively quick obtaining of end product, possibility to obtain large quantities of end product, relatively cheap raw materials and the only by-product it gives is water. It is also important that calcium phosphates with nanometric crystal size can be obtained at a low process temperature (from room to water boiling temperature).

During synthesis, technological parameters like final pH of calcium phosphate suspension and synthesis temperature ($T, ^\circ\text{C}$) were changed. Final suspension pH was stabilized in the range of 5-11, using solution of acid. Synthesis temperature was changed in the range from room temperature (21°C) up to 70°C , the following parameters were controlled: acid solution adding speed (ml/min), stirring speed (rpm), synthesis temperature ($T, ^\circ\text{C}$), final suspension pH, stabilization time (h), maturity time (τ , h), drying temperature and time ($T, ^\circ\text{C}$; h), calcifying temperature and time ($T, ^\circ\text{C}$; h).

For further obtaining HAp or biphasic bioceramic synthesized under impact of various parameters (final pH and synthesis temperature), powder is thermally treated. Samples are heated in different environments (air, vacuum and water vapor), varying thermal treatment temperature in range $200\text{-}1400^\circ\text{C}$ and processing time.

3.2 Analysis methods and sample preparation

In order to determine raw materials, structure of synthesized and heated powder, phase composition and functional groups, two important methods are used, complementing each other: Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometry (XRD). XRD method is widely used for apatite characterization, for it provides data about the crystal structure of material and its phase composition, however, it is not convenient to determine

amount of [OH] or [CO₃] groups in hydroxyapatite. FTIR method, in many cases is more sensitive than XRD when determining presence of new phases. Using FTIR, CaP can be characterized, considering three spectrum parameters:

- Location of absorption maximum indicates material composition, even slight variations of the composition influence energy of material bondings and, as follows, frequency of variations;
- Peak width shows degree of the atoms' order in the apatite elementary cell.
- Considering the absorption maximum of [OH] vibrations, presence of HAp and its thermal stability can be determined, as well as hydroxyl group concentration in the sample.

During the research process, *X'Pert PRO* X-ray diffractometer has been used (*PANalitical*, the Netherlands). Samples were measured in a spinning mode, in the 2θ angle range from 20-90°, with a scanning step 0,0334°, with a CuK α radiation. Ratio of HAp/TCP is determined using a XRD semi-quantitative method after calibration line.

Calcium phosphate spectra are measured and analyzed with a FTIR spectrometer „Varian 800” of Scimitar Series, with a wave length range from 400 - 4000 cm⁻¹, with precision of 4 cm⁻¹ and RESOLUTION software.

Samples are prepared by mixing powder with KBr and pressing the pellet. This method of sample preparation has some complications and requires a certain experience, in order to obtain a good quality spectrum in everyday work routine. Special factors should be considered in order to perform invariable sample analysis by using a KBr method, and these include pellet thickness, particle dispersion, ensuring vacuum state during the pressing, pressure influence, ion exchange, etc.

In a prepared KBr pellet, there should be material concentration of 2-10% from the total weight. For preparing a 300g KBr pellet, from 1 to 5mg of the sample is required, and the pellet size will be 13 mm.

Powder grain size should be ~150 μ m. The analyzed sample is crashed in a powder and thoroughly mixed with the KBr powder. A powder mixer „Pulverisette 23” was used for crashing and mixing of the powder. Prepared powder was located in a specific SPECAC (d = 13 mm) mould, and the required pressure was achieved by applying a uniaxial press (required pressure is ~5·10³ kg/cm², pressing time 1 min).

KBr attracts water molecules from the environment and they create wide water bands in the spectrum, so they are hard to or even impossible to analyze. KBr powder should be of the highest assay, Riedel-de-Haen KBr (Lot 51520) brand was used with an assay in the range of 99.5-100.5%. Usually, absorption bands of the main impurities in the KBr are: OH⁻ groups and H₂O molecules (3500 cm⁻¹ and 1630 cm⁻¹), NO₂ (1390 cm⁻¹), SO₄²⁻ (1160-1140 cm⁻¹). Spectrum of the KBr used in our research is demonstrated on the Fig. 2. Considering that the powder is hygroscopic, KBr powder is dried at 105°C and kept in special hermetic containers. Prepared pellets with the analyzed material are dried once again for 24 h. The obtained pellets should be transparent and equally colored. Weak bands connected with water can also be compensated by using a KBr pellet of the same thickness, but not containing the analyzed material, for background spectrum measuring.

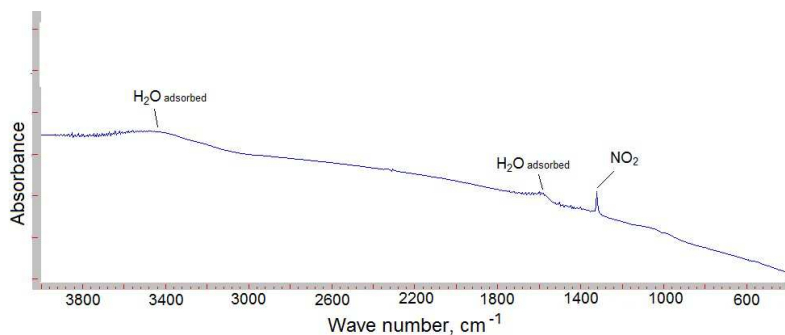


Fig. 2. FTIR spectrum of the KBr pellet

4. Results and discussion

4.1 Commercial HAp products description

While preparing bioceramic samples from various commercial materials available on the market, we have come across hardly predictable properties of the end product, like crystallinity degree, phase composition and, following, bioactivity and mechanical characteristics. One of the disadvantages while purchasing commercial calcium phosphate powders or commercial calcium phosphate ceramic materials is insufficient information about synthesis conditions of these calcium phosphates, raw materials and in which proportions these materials are taken.

It is significant to know if the purchased powder is thermally treated, and in which temperature range this thermal treatment was performed. Exactly temperature, at which the sample was obtained and processed, is one of the conditions that influences outcome of ceramic and phase composition.

For example, in spectra of several overviewed commercial hydroxyapatites, OH⁻ and PO₄³⁻ groups are observed, but band shape, width and intensity are different. Differences in spectra are also observed at CO₃²⁻ and HPO₄²⁻ groups location and intensity. On Fig. 3, there are three spectra from different commercial HAp compared: „Fluka” (F), „Riedel-de Haën”

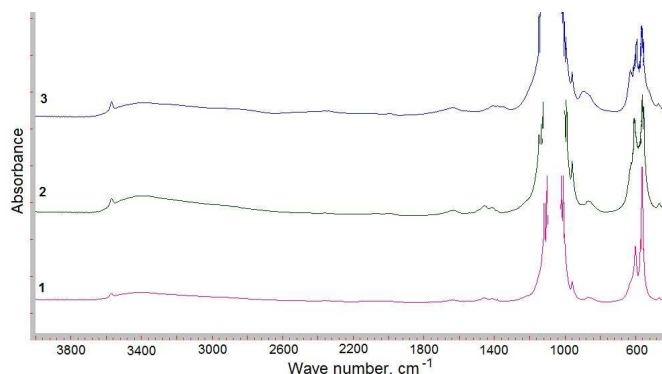


Fig. 3. FTIR spectra of commercial HAp products (1 - S-A; 2 - R-dH; 3 - Fluka)

(R-dH) and „Sigma Aldrich” (S-A). IR spectrum of commercial S-A product has a lower CO_3^{2-} and HPO_4^{2-} intensity that could mean a higher assay degree than the other materials have. These spectra also demonstrate that the products have a low crystallinity degree and they were not thermally treated.

Characteristic chemical groups of the commercial synthesized products are summarized in table 6.

Commercial HAp	Fluka	R-dH	S-A
Chemical groups	Absorption bands, cm^{-1}		
CO_3^{2-}	1386; 1411; 1635; 1997; (2359 $\text{C}\equiv\text{C}$)	1382; 1413; 1457; 1634; 1997	1382; 1417; 2457; 1639; 1990; 2359
H_2O adsorbed	3100 - 3600	3000 -3600	3200 - 3600
OH^-	635; 3568	3568	630; 3569;
HPO_4^{2-} ; CO_3^{2-}	891; 875	870	874
PO_4^{3-}	470; 553 - 600; 964; 1000 - 1156	470; 553 - 610; 964; 1000 - 1150	471; 561; 601; 605; 964; 1013 - 1120

Table 6. Characteristic chemical groups of commercial HAp FTIR absorption bands

In the FTIR spectra of commercial β -TCP products, PO_4^{3-} groups are observed, which is characteristic to β -TCP (Fig. 4). In HAp IR spectrum, OH^- group peaks are observed (at 630 and 3570 cm^{-1}), but there are no such in the IR spectrum of commercial β -TCP, which means that there is no HAp phase in this β -TCP product. In addition, at 725 cm^{-1} , presence of $\text{P}_2\text{O}_7^{4-}$ group can be observed, which is characteristic to calcium pyrophosphate phase.

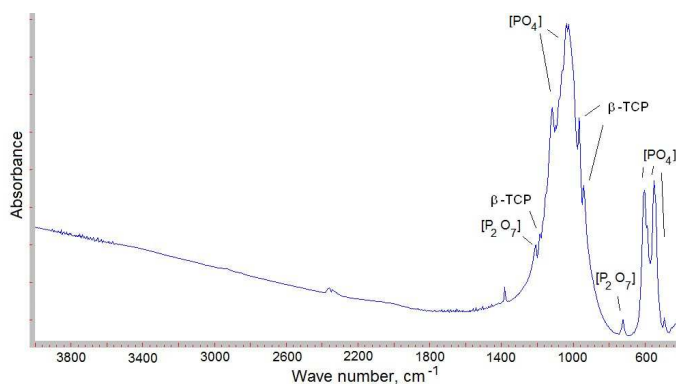


Fig. 4. FTIR spectra of commercial product (Fluka β -TCP)

4.2 Phase composition control during synthesis reaction

As above mentioned, CaP material synthesis was performed in our laboratory, varying synthesis and further sample heating parameters, searching for their optimal combination depending on the required properties of the obtained material.

In order to control reaction process and possible appearance of by-products, control samples were taken every 5 minutes and analyzed by measuring their spectra. After taking a sample from reactor with a plastic dropper, it was inserted in a glass bottle, hermetically sealed and frozen by putting it in the mixture of dry ice and acetone. After full freezing, the temperature is supported by storing the bottle in the dry ice. Before inserting in the cryogenic drying device, bottles are covered with a perforated plastic film. From the spectra of samples prepared this way (Fig.5), synthesis with a final pH=9,3 and synthesis temperature 45°C, it can be seen that the synthesized material is formed with an apatitic HAp structure with a slight Ca deficient, which is proven by presence of the CO_3^{2-} group, amount of which is constantly reducing along with reaction approaching its end. No by-products were detected, during the reaction, CaO has reacted fully which is proven by an OH- peak disappearing at 3642 cm^{-1} and forming OH- peaks at 3571 cm^{-1} and 631 cm^{-1} which is characteristic for HAp phase.

Such control is very important for scaling the synthesis, relatively increasing amount of synthesis and amount of obtained CaP.

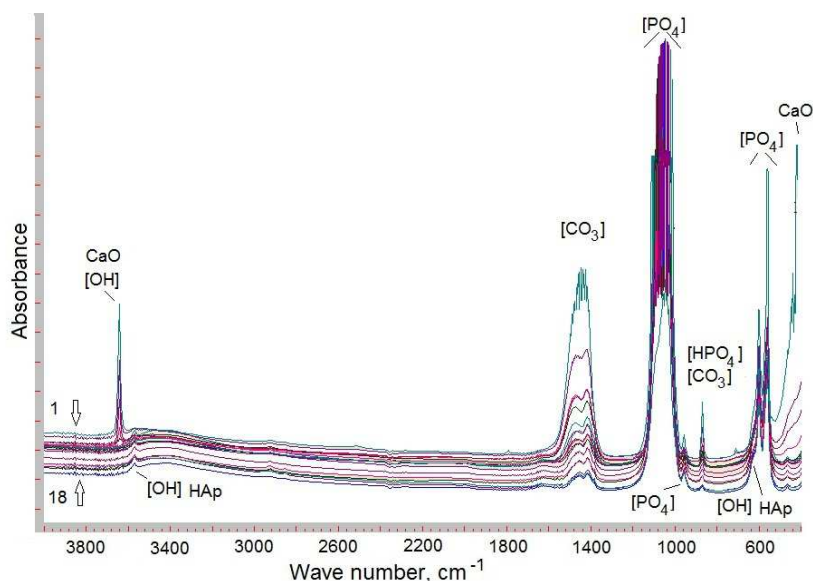


Fig. 5. FTIR spectra of the samples from the synthesis series with final pH=9,3 at temperature 45°C, depending on the reaction occurrence time (from 1 to 18).

4.3 Selection of CaO containing materials of various origins (marble, eggshells, snail shells) and its influence on the CaP product properties

Scientific literature contains very few information about research of how raw materials' (for example, CaO, $\text{Ca}(\text{OH})_2$) quality (chemical and physical properties) impacts properties of the obtained bioceramics.

Before starting synthesis of CaP products, selection of CaO containing raw material and complex research were performed. For „Ca” precursors, two commercial available synthetic

CaO powders from „Riedel-de Haën®“ (CaO_R) and „Fluka“ (CaO_F) and two materials of biogenic origin, widespread in the nature – egg shells and land snail (*Arianta arbustorum*) shells, were chosen. These materials were selected as raw materials for obtaining CaO and further usage in the synthesis process of CaP.

In composition of commercial CaO, presence of $\text{Ca}(\text{OH})_2$ phase, small amount of MgO phase, as well as small amount of polymorphous CaCO_3 modification – calcite phase (Fig. 6), is detected. Presence of CaCO_3 is undesirable in CaO, for during the process of suspension obtaining, $\text{Ca}(\text{OH})_2$ creates an error in preparation of precise amount of the reagent and, as follows, product reproduction, also preventing obtaining a homogenous $\text{Ca}(\text{OH})_2$ suspension which is required for further synthesis of CaP. After heating biogenic and commercial CaO at 1000°C , X-ray diagrams of all the synthesis materials demonstrated a CaO crystal phase with a small amount of MgO phase, along with $\text{Ca}(\text{OH})_2$ that formed in the result of CaO contact with air moisture; considering that CaO is hygroscopic (Siva Rama Krishna, et al., 2007).

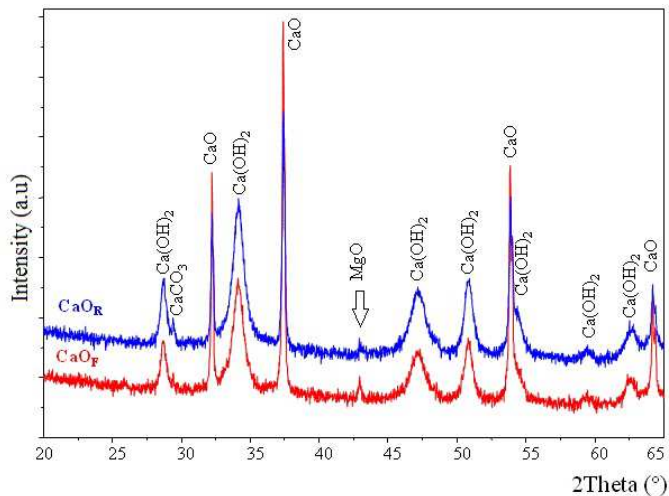


Fig. 6. Commercial CaO X-ray diffractograms before calcifying

FTIR spectra were also taken for the thermally treated CaO samples, and their compositions were similar (Fig. 7.). In all the IR spectra, an explicit absorption peak is visible at 3642 cm^{-1} , that indicates stretching variations of $\text{Ca}(\text{OH})_2$ (Ji, et al., 2009) and $[\text{OH}]$ groups (Siva Rama Krishna, et al., 2007). Presence of CaO is also proven by a wide intensive absorption band of $[\text{Ca-O}]$ group, which is centered at $\sim 400\text{ cm}^{-1}$ (Ji, et al., 2009). Absorption peaks at 874 cm^{-1} , 1080 cm^{-1} [113], as well as at 1420 cm^{-1} prove presence of the $[\text{CO}_3]$ groups in the samples which, therefore, shows a slight carboxilation of $\text{Ca}(\text{OH})_2$ from CO_2 of the atmosphere. Absorption band from $3430\text{--}3550\text{ cm}^{-1}$ proves presence of adsorbed water molecules in the samples.

FTIR spectra of the synthesized powders demonstrate absorption bands of the chemical functional groups, characteristic to HAp phase (Fig. 8.). Number of $[\text{CO}_3]$ groups in those is different, but after thermal treatment at 1100°C for 1 h, the spectra become very similar (Fig. 9).

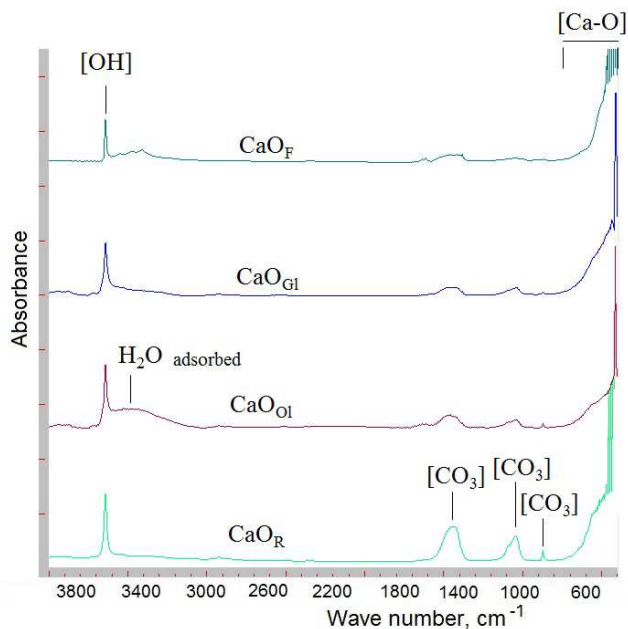


Fig. 7. FTIR spectra of biogenic and commercial CaO after heating at 1000°C for 1 h (F - „Fluka”, GI - land snail shells, OI - egg shells and R - „Riedel-de Haën®”)

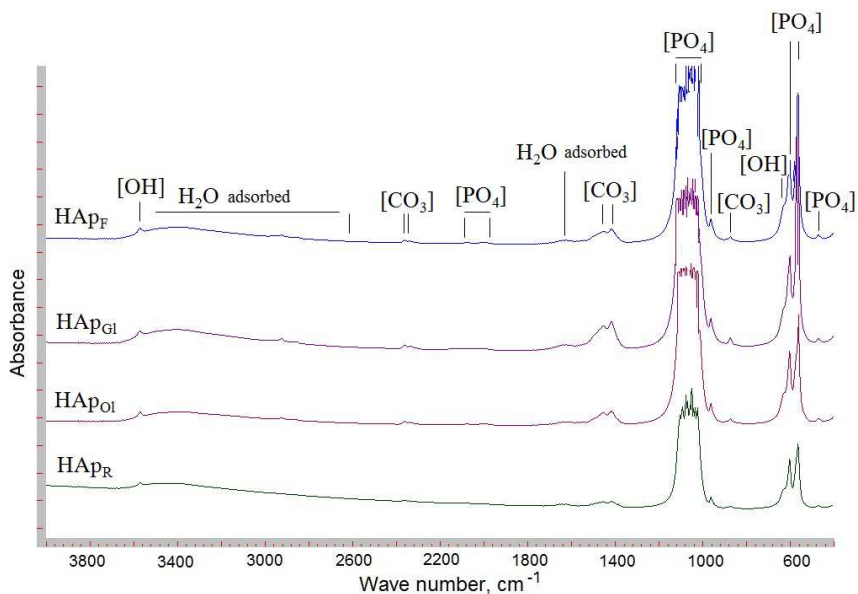


Fig. 8. FTIR spectra of HAp products as-synthesized after drying at 105°C for ~ 20 h from various CaO (F - „Fluka”, GI - land snail shells, OI - egg shells and R - „Riedel-de Haën®”).

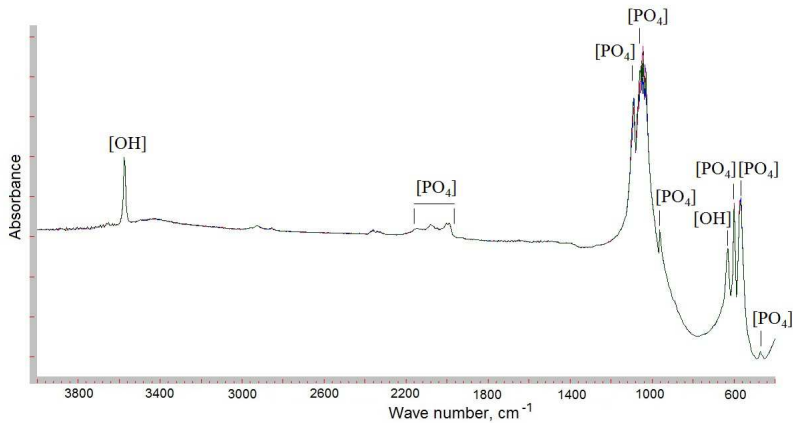


Fig. 9. FTIR spectra of HAp bioceramics obtained from various CaO after thermal treatment at 1100°C for 1 h.

Using CaO containing materials of various origins, including the commercial CaO available on the market that seemingly correspond with the assay specification, it is still impossible to obtain a completely reproducible HAp bioceramic materials. XRD and FTIR of the HAp products synthesized and heated at 1100°C, produce similar pictures, and post-synthesis morphology, phase composition and molecular structure of these bioceramics are identical. However, analyzing it with FE-SEM micrographs, it can be observed that obtained HAp products demonstrate different microstructures.

A homogenous, fine-grained microstructure with a small grain size – about 150-200 nm, is observed at the HAp bioceramic from commercial reagents, and non-homogenic grainy structure with irregular grain size in the range from 200 nm up to 1 μm is observed at the ceramic which was synthesized using Ca of natural origin.

Therefore, the samples from commercial reagents demonstrated color changing, from white in the synthesized and just dried powder to average aquamarine, at ceramic samples heated at 1000 °C, up to light blue color at 1300 °C. It can be explained by oxidizing of the manganese impurities from Mn^{2+} up to Mn^{5+} and substitution of hydroxyapatite (PO_4^{3-}) group with (MnO_4^{3-}) (Ślósarczyk, et al., 2010). Color change can be also explained by other microelements or defects in the crystal lattice, but, in this case, FTIR and XRD analysis cannot give a precise answer to this.

4.4 Thermal behavior of calcium phosphates depending on synthesis parameters

Sample structure, phase composition and thermal stability after heating depend on synthesis parameters, especially from final pH and synthesis temperature. Also, connection between pH value and temperature (with temperature increasing, pH decreases); so, by combining and analyzing those parameters, both pure HAp and TCP materials with a good thermal stability are obtained and biphasic materials with various Ca/P ratios and various percentages of phase composition.

Thermal behavior data of laboratory synthesized calcium phosphates with various synthesis parameters were summarized. Final reaction pH was varied in the range from 5.0 up to 10.7 and synthesis temperature was chosen as room (22°C), 45°C and 70°C (Table 7).

Nr. of synthesis	Final pH of synthesis	Synthesis temperature, °C	Phase composition by XRD after thermal treatment at 1100°C (1h)
1	5,0	room	TCP+CaHPO ₄
2	5,1	70	HAp/TCP (60/40)
3	5,3	45	HAp/TCP (35/65)
4	5,9	room	TCP
5	7	45	HAp/TCP (80/20)
6	9,3	45	HAp
7	10,7	45	HAp/CaO

Table 7. Variable parameters of some calcium phosphate synthesis and calcium phosphate products phase composition after thermal treatment at 1100°C for 1 h

It can be ascertained that, in spite of variable parameters of synthesis, all the spectra of samples and XRD diffraction diagrams, are similar, all the functional groups correspond with non-stoichiometric apatitic HAp structure with a low crystallinity degree (Fig. 10). The only slight differences that can be observed between spectra, are intensities of CO₃²⁻ group bands, however, no significant differences in the number of OH- groups, depending on the synthesis parameters at the non-calcified samples, are detected (Table 8).

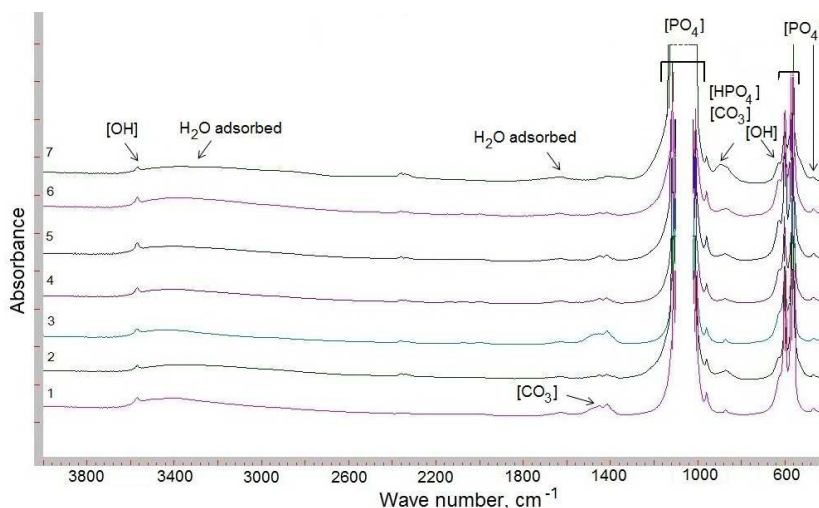


Fig. 10. FTIR spectrum of as-synthesized and drying at 105°C (20 h) CaP products of 1-7 synthesis

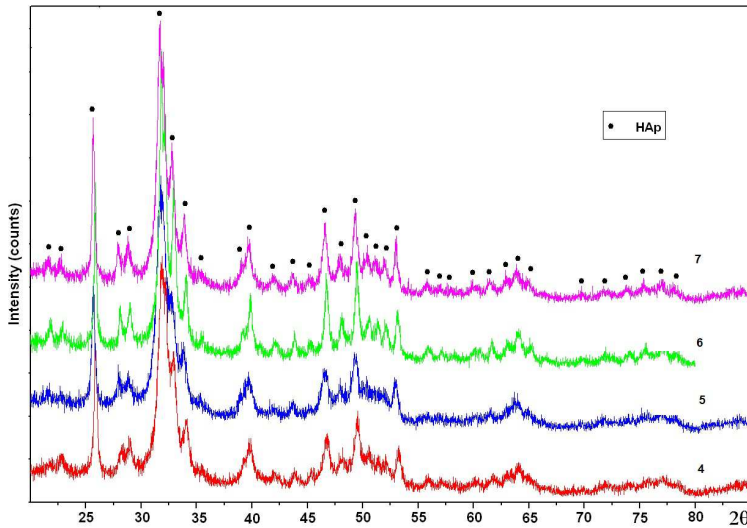


Fig. 11. XRD patterns of as-synthesized CaP products from 4, 5, 6, 7 synthesis.

Chemical groups	Absorption bands, cm^{-1}
PO_4^{3-}	472; 570; 602; 963; 1000 - 1140;
H_2O adsorbed	3100 - 3600;
OH^-	631; 3570;
HPO_4^{2-}	875 (identifies HAp with deficient of calcium and non-stoichiometric structure)
CO_3^{2-}	875; 1418; 1458; 1632 and 1650; 1994

Table 8. Absorption bands of as-synthesized and drying at 105°C (20 h) CaP products

Absorption bands of chemical functional groups characteristic to HAp phase can be defined as follows:

- Absorption bands at 3570 cm^{-1} and 631 cm^{-1} are referable to structural $[\text{OH}]$ groups (O-H) stretching and libration modes at the HAp crystallite surface or at the crystallites.
- Presence of $[\text{PO}_4]$ groups, characteristic to tetrahedral apatite structure, is proven by absorption bands at 472 cm^{-1} , which is characteristic to $[\text{PO}_4]_{\text{v}_2}$ group (v_2 O-P-O) bending variations; double band at 570 cm^{-1} and 602 cm^{-1} with a high resolution is referable to asymmetric and symmetric deformation modes of $[\text{PO}_4]_{\text{v}_4}$ group (v_4 O-P-O); absorption band at 963 cm^{-1} corresponds to a symmetric stretching mode; intensive absorption band in the range of $1040\text{-}1090\text{ cm}^{-1}$ corresponds to a band characteristic to $[\text{PO}_4]_{\text{v}_3}$ groups (v_3 P-O) at 1040 cm^{-1} and 1090 cm^{-1} asymmetrical stretching mode, which, as explicit maximums, can be observed after thermal treatment of the samples;
- An absorption band of weak intensity within the range between $1950\text{-}2100\text{ cm}^{-1}$ is connected with combinations of $[\text{PO}_4]_{\text{v}_3, \text{v}_1}$ modes.

These locations of absorption bands of the functional groups explicitly indicate forming of a typical HAp structure in the synthesized samples (Nilen & Richter, 2008; Siva Rama Krishna, et al., 2007; Kothapalli, et al., 2004; Landi, et al., 2000; Lioua, et al., 2004).

Weak absorption bands at 2365 cm^{-1} and 2344 cm^{-1} appeared due to attraction of CO_2 from the atmosphere.

Presence of $[\text{CO}_3]$ bands can be identified with explicitly visible absorption bands within the range between $1600\text{--}1400\text{ cm}^{-1}$ and at 875 cm^{-1} , which are observed in the spectra of synthesized samples. Absorption bands of weak intensity, centered at 1418 cm^{-1} and 1458 cm^{-1} correspond to symmetrical and asymmetrical stretching modes of the $[\text{CO}_3]_{\text{v}_3}$ groups (C-O). Absorption band at $\sim 875\text{ cm}^{-1}$ can prove presence of $[\text{CO}_3]_{\text{v}_2}$ stretching mode (at $\sim 872\text{ cm}^{-1}$), intensity of which is approximately 1/5 share of $[\text{CO}_3]_{\text{v}_3}$, or presence of $[\text{HPO}_4]$ group absorption maximum (Siva Rama Krishna, et al., 2007; Landi, et al., 2000). Considering that the $[\text{HPO}_4]$ group band partially covers $[\text{CO}_3]_{\text{v}_2}$, it is complicated to detect, of which group is this band. Presence of this absorption band ascertains solution of atmosphere CO_2 in the suspension, if synthesis occurs in the alkaline environment.

Combination of absorption bands – absorption bands of $[\text{CO}_3]_{\text{v}_3}$ groups at 1418 cm^{-1} and 1458 cm^{-1} , as well as at 875 cm^{-1} , proves substitution of „B-type“ $[\text{PO}_4]$ groups with $[\text{CO}_3]$ groups in the HAp crystal lattice (Barinov, et al., 2006; Siva Rama Krishna, et al., 2007).

Absorption maximum of $[\text{CO}_3]$ group at 875 cm^{-1} can also prove that „AB-type“ ($[\text{PO}_4]$ and $[\text{OH}]$ groups) substitution in the structure of HAp, as well as a weak absorption band at 3571 cm^{-1} in the synthesized HAp samples can mean the „AB-type“ substitution (Barinov, et al., 2006).

A wide absorption band within the range from $\sim 3600\text{ cm}^{-1}$ up to 3100 cm^{-1} points on v_3 and v_1 with H_2O molecules bonded with hydrogen for stretching modes and an absorption band at 1629 cm^{-1} is referable onto deformation mode v_2 of H_2O molecules (Siva Rama Krishna, et al., 2007), that proves presence of physically adsorbed water in the synthesized samples.

Processing the samples in the temperature range between $200\text{ }^\circ\text{C}$ to $1400\text{ }^\circ\text{C}$ in the air atmosphere, a similar sample behavior, even up to characteristic bioceramic sintering temperatures, is observed in all the syntheses. Dehydration of the samples occurs up to 500°C , and at 600°C , the spectra demonstrate that adsorbed water band disappears from the spectrum, adsorbed and capillary water is eliminated from CaP. Within the temperature range between 500°C and 800°C , amount of CO_3^{2-} groups in the samples also reduces, and bands of CO_3^{2-} group fully disappear at $900\text{ }^\circ\text{C}$. FTIR spectra (Fig. 12) and XRD diffractograms (Fig. 13) of the thermally treated at 1100°C samples considerably differ, comparing with the samples that just have been synthesized. A restructurization of functional $[\text{PO}_4]$ groups have occurred, and sample phase composition is considerably different from the combination of the initial synthesis parameters. It can be concluded that phases with a high crystallinity degree were formed.

A pure, stoichiometric and stable HAp in a wide temperature range is obtained using the following synthesis parameters: final $\text{pH}=9,3$ and synthesis temperature $T_s=45^\circ\text{C}$ (synthesis 6). Sample thermal treatment is performed in the air atmosphere, in the temperature range $200^\circ\text{C} - 1400^\circ\text{C}$ for 1 h. HAp spectra at various heating temperatures are demonstrated on the Fig. 14 and 15.

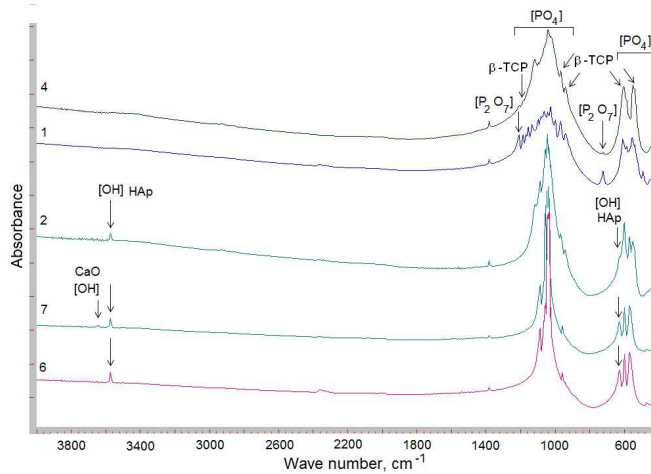


Fig. 12. FTIR spectra of calcium phosphates thermally treated at 1100°C for 1 h, depending on synthesis parameters (1, 2, 4, 6 and 7 synthesis)

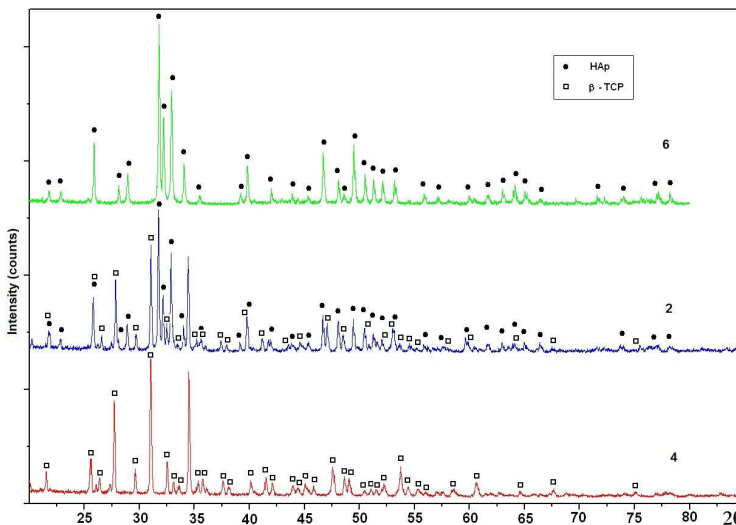


Fig. 13. X-ray diffraction patterns of different calcium phosphates thermally treated at 1100°C for 1 h with different phase compositions for synthesis 6 – pure HAp, 4 – pure β -TCP and 2 – biphasic mixture of HAp/ β -TCP

As can be seen after FTIR analysis which is often more sensitive than XRD, HAp begins to decompose when a sample is heated for 1 h at more than 1200°C, forming TCP shoulders at 948 cm^{-1} , 975 cm^{-1} and poorly intensive band at 432 cm^{-1} , which proves dehydroxylation of HAp and forming of α -TCP or OHAp phases. Therefore, XRD analysis shows that the decomposition occurs only at 1400°C, and is resulted with mixture of α -tricalcium phosphate (α -TCP, $\text{Ca}_3(\text{PO}_4)_2$), tetracalcium phosphate (TTCP, $\text{Ca}_4(\text{PO}_4)_2\text{O}$) and HAp. By

heating and then cooling a HAp sample in the air atmosphere, a complete HAp decomposition cannot be achieved, for during cooling [OH] groups get back in the structure from the air and therefore a partial reversible α -TCP and TTCP transform into HAp.

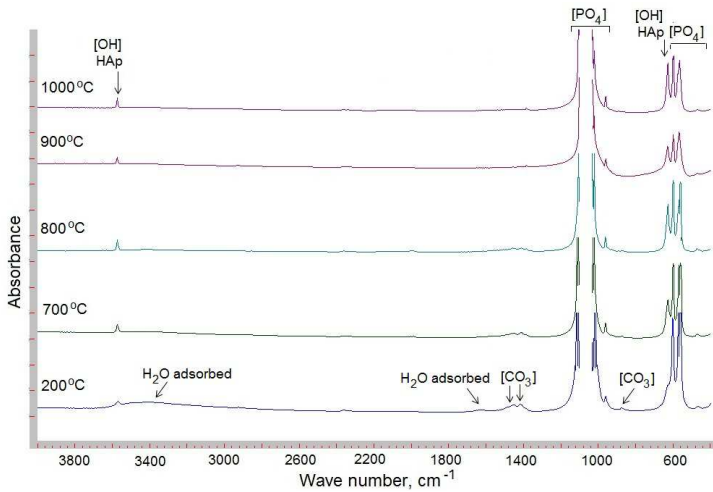


Fig. 14. FTIR spectra of HAp (synthesis 6) depending of thermal treatment temperature in range 200 - 1000°C for 1 h

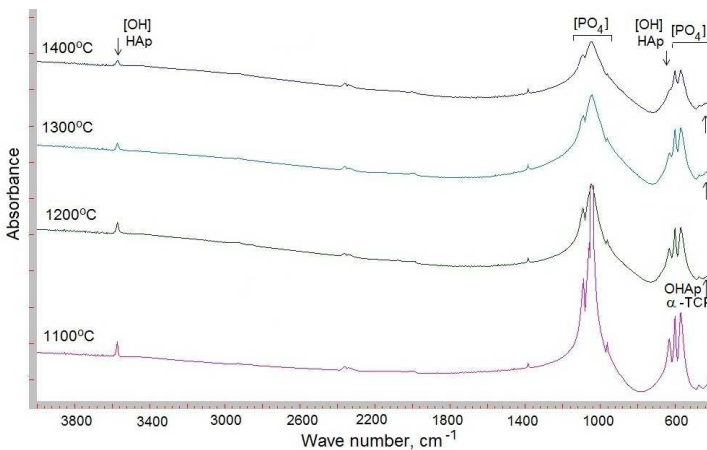


Fig. 15. FTIR spectra of HAp (synthesis 6) depending of thermal treatment temperature in range 1100 - 1400°C

A pure β -tricalcium phosphate phase $\text{Ca}_3(\text{PO}_4)_2$, obtained using the following synthesis parameters: final pH=5,8 and $T = 22^\circ\text{C}$ (synthesis 4), it is synthesized at low temperatures and pH is acidic.

OH- group bands completely disappear at 700°C with a wave length 3569 cm^{-1} and 631 cm^{-1} , which is characteristic for HAp. It can also be observed that at 900°C, bands that

correspond to a CO_3^{2-} functional group, disappear. At 700°C , a β -TCP phase begins to form, which is shown by characteristic shoulders which become more sharply explicit with increasing of temperature. Until 1100°C , also poorly intensive pyrophosphate (CPP) group bands at 727 and 1212 cm^{-1} , that disappear at higher temperatures.

Since the sample does not contain CPP and HAp any longer, a pure TCP is obtained in the result, and it is stable up to 1400°C (Fig. 16 and 17). A β -TCP phase can be identified in the spectrum by appearance of characteristic bands at 947 cm^{-1} and 975 cm^{-1} .

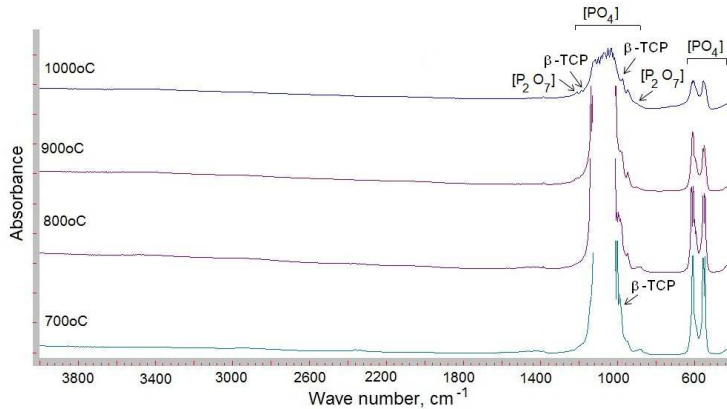


Fig. 16. FTIR spectra of β -TCP (synthesis 4) depending of thermal treatment temperature in range $700 - 1000^\circ\text{C}$ for 1 h

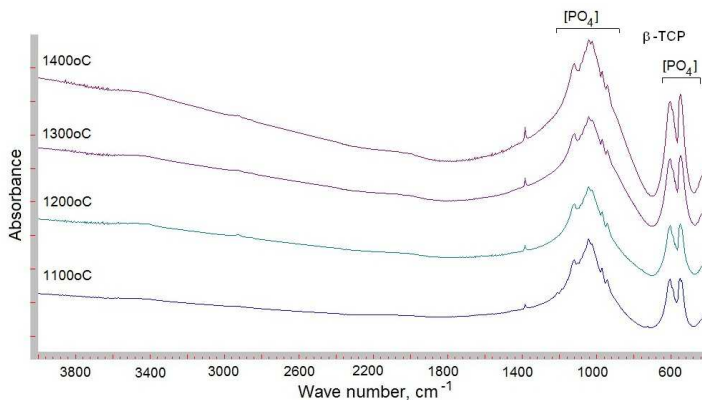


Fig. 17. FTIR spectra of β -TCP (synthesis 4) depending of thermal treatment temperature in range $1100 - 1400^\circ\text{C}$ for 1 h

In the CaP syntheses with final $\text{pH}=7$, $T=45^\circ\text{C}$ (synthesis 5), final $\text{pH}=5,1$ and $T=70^\circ\text{C}$ (synthesis 2), final $\text{pH}=5,3$ and $T=45^\circ\text{C}$ (synthesis 3), spectra demonstrate that a biphasic mixture with various HAp/TCP proportions, respectively 80/20, 60/40 and 35/65, measured at 1100°C with an XRD semi-quantitative method, is formed in the synthesized samples.

In the synthesis with final pH=5,00 and T=22 °C (synthesis 1), a complicated phase composition is formed from β -TCP, CaHPO₄ and TTCP phases, and it remains stable up to 1200 °C. CaHPO₄ phase can be recognized after a HPO₄²⁻ group band at 897 cm⁻¹, which is visible in the spectra already at 200 °C.

In the synthesis with a final pH=10,74 and T=45°C (synthesis 7), a HAp structure is formed but at 1000°C, and additional OH⁻ peak appears at 3644 cm⁻¹, which means that there is a unreacted CaO left. The reaction did not occur completely and this synthesis, as well as the above mentioned synthesis 1, cannot be used in practice.

Chemical groups and phases	T, °C							
	800	900	1000	1100	1200	1300	1400	
CO ₃ ²⁻	875; 1418; 1456; 1466; 1636	875; 1384; 1418; 1457; 1636	875; 1385; 1419; 1457	-	-	-	-	
HPO ₄ ²⁻	875	875	875	-	-	-	-	
H ₂ O adsorbed	3100 - 3600	3250-3600	3330 - 3595	-	-	-	-	
OH ⁻	3572; 634	3572; 634	3572; 633	3572; 632	3570	3570	3570	
PO ₄ ³⁻ β -TCP (OAp)	-	-	-	435;	436;	434	434	
PO ₄ ³⁻	1000 -1120	1006 - 1120	1046; 1091	1046; 1091	1046; 1091	1045; 1094;	1037; 1045; 1090	
PO ₄ ³⁻ HAp	473; 550 - 640; 963	471; 556-604; 963	473; 554-601; 963	472; 570; 602; 963	472; 569; 602; 962	472; 568; 602; 961	472; 568 601; 961	
PO ₄ ³⁻ β -TCP	946; 975	946; 975	946; 975; 1127	944; 971 1127	944; 970; 1121	944; 970; 1121	943; 970; 1120	
P ₂ O ₇ ⁴⁻	725; 1211	725; 1212	725;1210	-	-	-	-	

Table 9. FTIR absorption bands (cm⁻¹) of thermally treated laboratory synthesized HAp and β -TCP (synthesis 6 and 4) chemical groups.

4.5 Thermal stability of HAp in various environments

Dehydroxilation of HAp under temperature influence is proven by reduction of [OH] absorption maximums intensity. Absorption band of [OH] bonding at 632 cm⁻¹ is very

sensitive to temperature changes and at higher heating temperatures (over 1300°C) is only shown as shoulder of $[\text{PO}_4]$ absorption band at 601 cm^{-1} , when $[\text{OH}]$ bonding at 3572 cm^{-1} is more stable in the higher temperatures (Fig. 18).

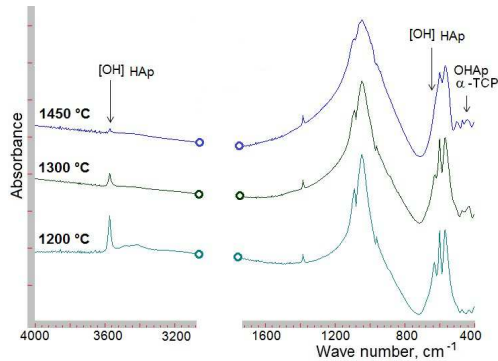


Fig. 18. FTIR spectra of HAp heated in the air at 1200, 1300, 1450°C (1h)

Temperature, °C	Heating time, h	Phase composition	
		After XRD	after FTIR
1000	8	HAp	HAp
1100	1	HAp	HAp
1200	1	HAp	HAp + TCP
1300	1	HAp	HAp + TCP
	12	HAp	HAp + TCP
1400	1	HAp + α -TCP + TTCP	HAp + TCP
	3	HAp + α -TCP + TTCP	HAp + TCP + TTCP
1450	1	HAp + α -TCP + TTCP	HAp + TCP + TTCP
	3	HAp + α -TCP + TTCP	HAp + TCP + TTCP
	6	HAp + α -TCP + TTCP	HAp + TCP + TTCP
1500	1	HAp + α -TCP + TTCP	HAp + TCP + TTCP

Table 10. Phase composition after HAp thermal treatment in the air

Stability of hydroxyapatite phase depends on the partial pressure of the water in the atmosphere, so, in an environment with no presence of water, by addition of sufficient amount of energy, HAp will turn into more stable calcium phosphates in the waterless environment. After thermally treating the sample in vacuum, there is an absorption band detected at 948 cm^{-1} in the FTIR spectrum already at $600\text{ }^\circ\text{C}$. It appears in the result of $[\text{PO}_4]$ group fluctuations and usually points onto TCP phase, however the literary sources mention that the absorption band at this wave length could also be characteristic to oxyapatite phase. In the result of thermal treatment, in vacuum at $1300\text{ }^\circ\text{C}$, HAp phase has completely decomposed and absorption bands, characteristic to TCP and TTCP phases, are visible in the FTIR spectrum (overlapping

of numerous $[\text{PO}_4]$ groups vibrations occurs, so it is not possible to distinguish, which absorption bands belong to TCP, and which to TTCP phase).

Temperature, °C	Processing time, h	Phase composition	
		after XRD	after FTIR
600	1	HAp	HAp + OAp/TCP
800	1	HAp	HAp + OAp/TCP
900	1	HAp	HAp + OAp/TCP
1000	1	HAp + α -TCP	HAp + OAp/TCP/TTCP
1100	1	HAp + α -TCP + TTCP	HAp + TCP + TTCP
1200	1	HAp + α -TCP + TTCP	TCP + TTCP
1300	1	α -TCP + TTCP	TCP + TTCP

Table 11. Phase composition after thermal treatment of HAp in the vacuum oven.

According to XRD data, supplying water vapor during heating of hydroxyapatite does not change phase composition, however slight changes in the sample structure are detected with FTIR analysis. After thermal treatment in the water vapor, absorption intensity of $[\text{OH}]$ group increases in ratio to $[\text{PO}_4]$, comparing with a sample heated in the air, which could improve HAp properties and make this phase more stable.

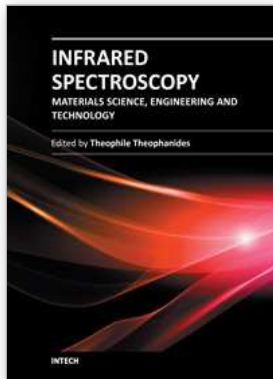
5. Conclusions

This summarizing work can help to evaluate both synthesized CaP and structure, phase composition and properties of the CaP bioceramic products. FTIR spectrometry along with XRD is one of the most important, quickest and most available methods for studying CaP materials and in many cases more sensitive than XRD in order to detect forming of new phases. Using these methods, it was possible to detect synthesis parameters in order to obtain a pure and thermally stable HAp and β -TCP, as well as biphasic mixtures with controllable ratio. From the results of above mentioned studies it can be concluded that a thorough selection of environment is required for processing HAp powder and it is also required to monitor behavior of the material during the heating, in order to obtain the desirable product. By varying conditions of thermal treatment, it is possible to improve structure of the synthesized HAp, for example, eliminate carbonate groups included in the structure during synthesis, increase number of $[\text{OH}]$ groups, as well as slow (by thermal treatment with water vapor presence) or quicken (by thermal treatment in vacuum) HAp decomposition.

6. References

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