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Physicochemical characterization of zinc-substituted calcium phosphates

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Abstract. Biocompatible and bioactive calcium phosphates can make chemical bonds with living bones. Improvement of their biological and physicochemical properties can be achieved by doping with various ions that are presented in natural apatites of bones. These substitutions influence lattice parameters, structure and morphology of apatites. In recent times great attention has been devoted to zinc ions that are the second most abundant trace element present in bones. Zinc embedded into calcium phosphate may enhance the bone formation and in addition exhibits antifungal and antibacterial properties. Therefore, it is rational to form structures incorporated with this ion. In this paper the incorporation of the Zn ions into natural and synthetic calcium phosphates has been reported. Natural hydroxyapatites (HAs) applied in this study were derived mainly from pork bones whereas both brushite and synthetic were formed using wet chemical methods. Ambient temperature synthesis leads to the formation of brushite, whereas the process performed at elevated temperature gives HA. Subsequently, attained structures were modified with Zn ions by using *in situ* or sorption procedures. Phase composition and morphology of obtained materials were determined by means of X-ray diffractometry, Fourier transform infrared spectroscopy and scanning electron microscopy equipped with energy-dispersive spectroscopy. Introduced XRD patterns depict changes of the crystallinity of HA with the increase in the amount of embedded zinc ions. On the contrary, no changes of the crystallinity were observed for the brushite doped with Zn ions. Morphology of attained powders, visualized using scanning electron microscopy exemplified structural changes between calcium phosphates conjugated with zinc ions. Many authors report that the addition of small amounts of Zn ions leads to loss of crystallinity and decrease of lattice parameters. Interestingly, upon addition of Zn ions to the natural and synthetic HAp by sorption procedures no crystallographic and structural changes were observed. Notably, upon increase of zinc ions also structure of brushite formed by the *in situ* method remains constant, indicating no influence of added ions. Our outstanding finding promotes sorption procedure as suitable route to form structures incorporated with various ions that can be further employed as potential implants.

Keywords. Hydroxyapatite; brushite; wet precipitation; Zn substitution; incorporation *in situ*.

1. Introduction

In the last few decades, calcium phosphate biomaterials have been used in dental and orthopaedic applications, such as, repair of bone defects, tooth root replacements, ear implants, spine fusion, and coatings on orthopaedic and dental implants [1,2]. Nowadays, there are two types of calcium phosphate cements (CPCs) apatite and dicalcium phosphate dihydrate (DCPD or brushite) [3,4]. Apatite is formed above pH 4.2, whereas brushite is mostly produced when the pH value is below 4.2 [5]. Among the various biocompatible calcium phosphates which are present in human bone, brushite (dicalcium phosphate dihydrate, DCPD) has a higher solubility at physiological pH in comparison with hydroxyapatite (HA), and an ideal *in vivo* resorption rate [6–8]. Brushite-based bone cements are well tolerated by the bone and soft tissue environment, so that the cement resorption may lead to the new bone formation. Moreover, brushite

structures possess high biocompatibility, osteoconductivity and bioresorbability, suitable for bone regeneration processes. Brushite cements have attracted great attention because they are resorbed *in vivo* much faster than apatite [6,7]. In addition, brushite is metastable under physiological conditions and brushite-based cements possess shorter binding times. The hard tissue of human bone is comprised of 69% inorganic calcium phosphate (HA), 20% collagen type I (organic matrix) and 9% water. Biological apatites, which comprise the mineral phases of calcified tissues (enamel, dentin and bone) and of some pathological calcifications (e.g., human dental calculi, salivary and urinary stones), are usually referred to as calcium hydroxyapatite, HA [9,10] defined as $Ca_{10}(PO_4)_6(OH)_2$. Interestingly, the biological apatites differ from pure HA in stoichiometry, composition, crystallinity (crystal size), dissolution, and in physical and mechanical properties, respectively. Biological apatites usually possess lower amount of calcium in comparison with stoichiometric structure (i.e., Ca/P ratio less than the stoichiometric ratio of 1.67 for pure HA) and are always

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substituted with carbonate [11,12]. In many studies calcium phosphates, in particular HA, can be used as a synthetic material for biomedical applications due to its bioactivity and osteoconductivity [13,14]. Furthermore, HA has a crystallographic and chemical composition similar to the natural bone mineral [15,16]. Moreover, HA is very often employed as a coating of metallic implants for bones for improving and accelerating the process of osseointegration [17]. Additionally, HA powder is often employed in dentistry (e.g., in the treatment of dental pulp and dentine hypersensitivity) and moreover is connected with the exposure of dentinal tubules [18]. Notably, HA present in toothpastes and dental gels, reduces the deposition of accretions on teeth. It can also be used as a component of dental cements and fillings [19].

HA has the disadvantage in which the rate of bone bonding ability is extremely slow [20]. Interestingly, HA does not prevent bacteria from adsorbing onto the surface, and this has implications for the bone healing required for recovery of patients [21]. Bone minerals are considered to be Ca-deficient apatite crystals with a variety of substituted elements. Thus, it is rational to synthesize nonstoichiometric HA with substituted elements, forming structures which may resemble bone apatite, to achieve improved bioactivity and osteoinductivity in comparison with pure HA ceramics. Indeed, considerable efforts have been devoted to modify synthetic HA to enhance its solubility, bioactivity and osteoinductivity by changing its composition through ionic substitutions [22]. The structure of apatites, determined by the formula as $Ca_{10}(PO_4)_6(OH)_2$, allows substitution of many ions without changing the hexagonal symmetry. Incorporations of various ions may lead to changes in their mechanical and biological properties. Among various ions K^+, Na^+, Mn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Cd^{2+}, Y^{3+}, La^{3+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ce^{3+}, Al^{3+} can substitute with Ca^{2+} ion. As, P, Si, V, Cr, (CO_3^{2-}) can replace (PO_4^{3-}) ion and F^-, Cl^-, O_2^-, OH^-, Br^- can be incorporated instead of (OH^-) ion [23]. Such substitution leads to changes in various structural properties, e.g., lattice parameters, crystallinity and morphology [24]. Nowadays, a great effort is devoted to application of the antibacterial properties of certain ions, including silver (Ag^+), copper (Cu^{2+}) and zinc (Zn^{2+}) [25]. Each of these ions play significant role in bone healing processes, hence it is imperative to form brushite or HA structure incorporated with the Zn, Ag or Cu ions, because such structures possess enhanced bioactivity and antibacterial properties in comparison with pure calcium phosphates. Zinc (Zn) is the most abundant trace metallic element in bone, and constitute an essential metal for biochemistry of bone tissues [26,27], furthermore zinc promotes bone formation and suppress bone resorption. Therefore, the Zn-substituted apatite might be useful in orthopaedics [28]. In addition, zinc is a crucial trace element for promoting osteoblastic cell proliferation and differentiation, possess a selective inhibitory effect on osteoclastic bone resorption *in vivo* and can be also involved in many metallo-enzymes and proteins. It was also shown that Zn incorporation into implants support bone formation around the implant and reduces the inflammatory response. Doping of high amount of zinc ions may result in cytotoxic effect, thus it is recommended to incorporate low amounts of zinc [28]. Moreover, doping high amounts (up to 15–20 mol%) of Zn^{2+} inhibits phase formation of HA [22]. LeGeros with co-workers reported that the incorporation of a small amount of Zn^{2+} into the HA structure leads to significant reduction in the degree of crystallinity of the apatitic phase, which allow to state that structure of apatite is unable to host greater amounts of Zn [9].

Miyaji *et al* [24] synthesized Zn-conjugated HA structure using precipitation method with specific experimental parameters (pH 8, 90°C) and determined the lattice parameters by X-ray diffraction (XRD) using an internal standard. They stated that the substitution limit of Zn was estimated to be about 15 mol%.

In this study, synthesis and detailed characterization of zinc-substituted HA (ZnHA) and Zn-brushite as an alternative biomaterial to natural calcium phosphates has been proposed. Synthesis performed at ambient temperature resulted in attainment of brushite whereas process carried out at elevated temperature leads to the formation of the HA. Subsequently, received structures were modified with Zn ions by *in situ* or sorption procedures. Several experimental techniques were employed to investigate the effects of the incorporation of Zn to brushite or HA structures, including X-ray diffractometry for determining the phase composition, scanning electron microscopy (SEM) observations and the Fourier transform infrared (FTIR) spectroscopy.

2. Experimental

Pork bones used for extraction of pure natural origin HAs were derived from a slaughterhouse. Reagents required to form synthetic calcium phosphates incorporated with Zn, such as zinc acetate and calcium acetate, 65% nitric acid, sodium hydrogen phosphate, trietanoloamine, 0.025 M EDTA, calcein indicator and 35–38% hydrochloric acid were purchased from POCh SA. Other reagents necessary for determining amount of calcium, phosphorus and zinc such as thymolphtalein indicator and eriochrome black T were delivered from ARGOS company. Accordingly, bismuth nitrate was provided by Chempur and potassium hydroxide was purchased from Stanlab Sp.j. company. All reagents were used as received.

The phase composition of the products was identified by powder X-ray diffractometry using XRD-Philips X'Pert diffractometer with Cu Kα radiation (λ = 0.15418 nm) and Ni filter, equipped with graphite monochromator PW 1752/00 operating at 30 kV and 30 mA. The diffraction spectra were recorded in the 2θ range from 10° to 60°.

FTIR spectroscopy measurements were performed with Scimitar Series FTS 2000 Digital spectrophotometer in the range of middle infrared 400–4000 cm^{-1} with the spectral resolution equal 4 cm^{-1}. Specimens for measurement were prepared by mixing 0.0007 g of sample with 0.2000 g of KBr and pressing to attain pellets suitable for FTIR.

The morphology of the samples was examined with SEM S-4700 Hitachi combined with chemical analysis carried out using X-ray energy-dispersive spectrometer (EDS) produced by Thermo Noran. The synthesized powders prior to SEM analysis were adhered on carbon tape slicked to a copper holder coated with chromium film.

Spectrophotometric determination of the amount of phosphorus in the field of UV–vis radiation were carried out on Thermo Scientific Evolution 220 Spectrophotometer over the wavelength range of 300–700 nm with a 2 nm resolution.

The amount of zinc ions was determined by titration with EDTA using eriochrome T as indicator. The number of calcium ions was determined using EDTA titration with calcein as indicator and the amount of phosphorus was determined spectrophotochemically.

Natural origin calcium phosphate structures applied for this study were derived mainly form pig bones whereas synthetic forms were fabricated using wet chemical methods. Synthesis at elevated temperature resulted in the production of HA while the formation at ambient temperature gave brushite structure.

Subsequently, attained powders were modified by the incorporation of the zinc ions into the main structures involving *in situ* or sorption procedures. Detailed description of the experimental stages required to perform synthesis and modification of the calcium phosphates was provided.

2.1 *Preparation of natural HA*

Preparation and extraction of the pure HA from pig bones is comprised of several stages. Primary step involves heating of the mixture which contains crushed bones with the addition of water and lactic acid at the temperature in the range of 125–130°C and under 0.26–0.3 MPa. Subsequently, bone sludge is separated from the solution. Next, the sludge is calcined in chamber furnace, electrically heated in the air atmosphere at 600°C for 3 h. Finally, the obtained homogeneous material is calcined again at 750°C for another 3 h [12].

2.2 *Preparation of synthetic HA*

In this study, synthetic HA was prepared by the simple wet chemical method.

Preparation of the synthetic HA or brushite is based on precipitation of the sludge from the aqueous solution. The formation of HA involves interaction of the calcium acetate and the phosphorus salts—here mainly the disodium hydrogen phosphate. The wet precipitation was carried out at ambient or elevated temperature according to the following reaction:

$$10Ca(CH_3COO)_2 + 6Na_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6OH_2 +$$
$$12NaCH_3COO + 8CH_3COOH. \quad (1)$$
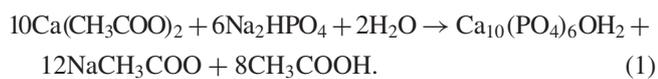
Detailed procedure of preparation of synthetic brushite at ambient temperature involves following steps: firstly, the amount of 650 cm^3 of the doubly distilled water and 100 cm^3 of the 0.2 M Na$_2$HPO$_4$ was poured into beaker and stirred magnetically. Secondly, 250 cm^3 of the 0.08 M Ca(CH$_3$COO)$_2$ was added drop-wise constantly mixing the solution. Subsequently, the attained suspension was magnetically stirred for another hour. Next, the mixture was aged for 24 h. Ultimately, the precipitate was separated from the solution by filtration, rinsed with distilled water to neutrality and dried at 105°C.

Preparation of synthetic HA at elevated temperature can be performed as follows: firstly, the amount of 650 cm^3 of the doubly distilled water and 100 cm^3 of the 0.2 M Na$_2$HPO$_4$ was poured into round bottomed flask and heated to boiling. Secondly, 250 cm^3 of the 0.08 M Ca(CH$_3$COO)$_2$ was added drop-wise to the solution with the speed of 1 drop min^{-1} keeping the temperature at boiling point. Subsequently, the attained suspension was magnetically stirred for 1 h at ambient temperature. Next, the mixture was aged for 24 h. Ultimately, the precipitate was separated from the solution by filtration, rinsed with distilled water to neutrality and dried at 105°C.

Successively, the natural and synthetic calcium phosphates were modified by conjugation of the zinc ions employing *in situ* or sorption procedures.

Modification of HA structure applying *in situ* method implies incorporation of the zinc ions during the synthesis of HA.

Synthesis of the HA structure modified with zinc ions by *in situ* method is performed as described below.

2.2a *Preparation of ZnHAp at elevated temperature by in situ procedure*:

Firstly, the amount of 650 cm^3 of the doubly distilled water and 100 cm^3 of the 0.2 M Na$_2$HPO$_4$ was poured into round bottomed flask and heated to boiling. Secondly, the appropriate amount of the Zn(CH$_3$COO)$_2$ was dissolved in 250 cm^3 of the 0.08 M Ca(CH$_3$COO)$_2$. Subsequently, the attained reactant was added drop-wise to the primary solution keeping the temperature constant at boiling point. After addition of the reactant the mixture was magnetically stirred for 1 h at ambient temperature. Next, the mixture was aged for 24 h. Ultimately, the precipitate was separated from the solution by filtration, rinsed with distilled water to neutrality and dried at 105°C.

2.2b *Preparation of Zn-brushite at ambient temperature by in situ procedure*:

Firstly, the amount of 650 cm^3 of the doubly distilled water and 100 cm^3 of the 0.2 M Na$_2$HPO$_4$ was poured into beaker with constant magnetic stirring. Secondly, the appropriate amount of the Zn(CH$_3$COO)$_2$ was dissolved in 250 cm^3 of the 0.08 M Ca(CH$_3$COO)$_2$. Subsequently, the attained reactant was added drop-wise to the primary solution and magnetically stirred for 1 h. Next, the mixture was aged for 24 h. Ultimately, the precipitate was separated from the solution by filtration, rinsed with distilled water to neutrality and dried at 105°C. The amount of zinc acetate used to modify powder brushite and HA structures are listed in table 1.

Alternatively, modification of the HA structure with Zn ions can be performed by the sorption method which is based on the incorporation of the zinc ions to the structure of the postsynthetic HA.

2.2c *Formation of the synthetic HA structure incorporated with zinc ions by sorption method*: The synthetic route can be described as follows:

Three grams of the synthetic HA was added to the 100 cm^3 of the zinc acetate solution prepared keeping appropriate Ca/P molar ratio. The mixture was poured into beaker and magnetically stirred for 2 h at 500 rpm. Subsequently, the mixture was transferred into Petri plate and dried at 105°C. The amount of zinc acetate used at this stage is listed in table 2.

Formation of the natural HA conjugated with zinc ions using the sorption method involves following steps:

Three grams of the natural HA formed during calcination of the animal bones was added to the 100 cm^3 of the zinc acetate solution prepared keeping appropriate constant Ca/P molar ratio. The mixture was poured into beaker and magnetically stirred for 2 h at 500 rpm. Subsequently the mixture was transferred into Petri plate and dried at 105°C. The amount of zinc acetate used in this synthetic route is listed in table 3.

It is known that the use of the chloride or nitrate of Ca as a starting reagent may cause the incorporation of Cl$^-$ or NO$_3^-$ into the apatite and then affect its structure. Consequently, the acetate salts of Ca and Zn were used in this work, because acetate ions are not incorporated into the apatite, i.e., they would not affect the apatite structure [9,24].

2.3 *Determination of the calcium, phosphorous and zinc ions*

Determination of the calcium ions was based on polish standard denoted as PN-97/R-64803 [29]. Such standard includes detailed information how to prepare the sample prior to analysis. Primarily, the 0.1 g of mineralized sample was mixed

Table 1. Amounts of zinc acetate added during formation of the Zn-HAp and Zn-brushite by *in situ* method.

Zn/Ca molar ratio	0.01	0.02	0.05	0.1
Mass of added Zn(CH$_3$COO)$_2$ (g)	0.0439	0.0878	0.2195	0.439

Table 2. Amounts of applied zinc acetate used to modify synthetic hydroxyapatite using sorption method.

Zn/Ca molar ratio	0.01	0.1
Mass of added Zn(CH$_3$COO)$_2$ (g)	0.0641	0.6409

Table 3. Amount of zinc acetate applied to modify natural hydroxyapatite using sorption method.

Zn/Ca molar ratio	0.01	0.1
Mass of added Zn(CH$_3$COO)$_2$ (g)	0.0705	0.7046

with 10 ml of 0.1 M nitric acid, and subsequently the mixture was boiled for 10 min. Successively, 20 ml of doubly distilled water was added to the primary solution and boiled for another 5 min. The attained solution was transferred quantitatively to the 100 ml volumetric flask and mixed with 6.25 ml of 0.4 M bismuth nitrate. The remaining volume of flask was filled with doubly distilled water to attain 100 ml. Prior to titration, 25 ml of the solution was retrieved from the flask and mixed with 25 ml of distilled water, 3 ml of 25% triethanoloamine and 20% of potassium hydroxide to reach finally pH from the range of 5–7. The solution was titrated with 0.025 M EDTA with the presence of calcein and thymolphtalein as indicators.

Determination of the phosphorus in the main samples was achieved by means of differential-photometric procedure. The samples prior to analysis were prepared according to polish standard termed as PN-80/C-87015 [30]. At the primary stage, 0.02 g of mineralized HA powder was dissolved in 2.5 ml of concentrated hydrochloric acid and 7.5 ml of concentrated nitric acid at the boiling point. Subsequently, the attained solution was transferred to 100 ml volumetric flask and the remaining volume was filled in with distilled water. Accordingly, 10 ml of the mixture was transferred to another 50 ml volumetric flask and mixed with 20 ml of solution termed as D and the remaining volume of flask was filled in with distilled water. The whole mixture was aged for 15 min. Consequently, the spectrophotometric measurement was performed employing 430 nm wavelength.

The amount of the zinc ions in the main specimens was evaluated using complexometric titration procedure. Primarily 0.05 g of the calcium phosphate was mineralized by boiling the sample with 5 ml of 2 M of nitric acid for 10 min. Subsequently, 10 ml of water was added and the whole mixture was boiled for another 5 min. At the next stage the attained solution was quantitatively transferred to the 100 ml volumetric flask and the remaining volume was filled with distilled water. The 20 ml of the main solution was transferred to Erlenmeyer flask. Successively, the solution of ammonium buffer was added drop-wise to the flask to reach formation of the white zinc hydroxide precipitate and finally reaching dissolution of precipitate. At the next stage, 80 ml of distilled water was added to the main solution and the whole mixture was titrated with 0.05 M EDTA with the presence of eriochrome black T.

3. Results and discussion

Synthetic calcium phosphates such as brushite and HA were formed using the wet chemical method at ambient or elevated temperature. Both structures were modified with zinc ions by the *in situ* method whereas substitution of the zinc ions into natural and synthetic HA was carried out using sorption procedure.

The main concept of the synthetic routes and amounts of Zn ions incorporated into HA and brushite structures are listed in table 4.
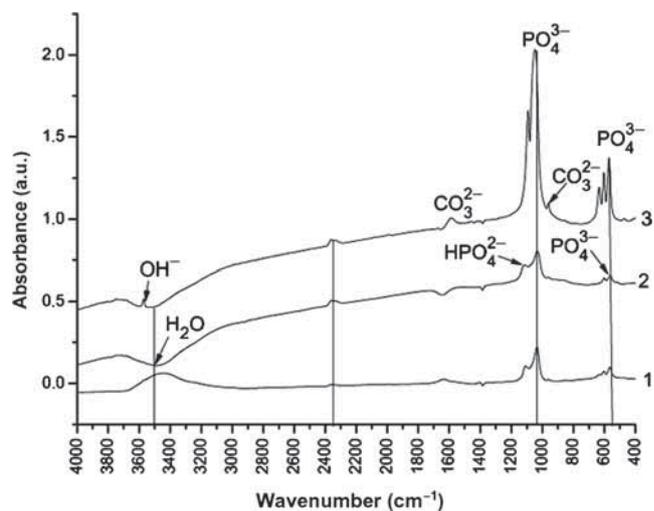
Table 4. Determination of the structures doped with Zn ions.

Name of the sample	Denotation	
	Types of calcium phosphate	Zn/Ca molar ratio
ZnB 0 N	Brushite synthesized at ambient temperature modified with Zn ions by the *in situ* method	0
ZnB 0.01 N		0.01
ZnB 0.02 N		0.02
ZnB 0.05 N		0.05
ZnB 0.1 N		0.1
ZnHAp 0 W	Hydroxyapatite synthesized at elevated temperature modified with Zn ions by the *in situ* method	0
ZnHAp 0.01 W		0.01
ZnHAp 0.02 W		0.02
ZnHAp 0.05 W		0.05
ZnHAp 0.1 W		0.1
ZnHAp 0syn	Synthetic hydroxyapatite, modified with Zn ions by the sorption method	0
ZnHAp 0.01syn		0.01
ZnHAp 0.1syn		0.1
ZnHAp 0nat	Natural hydroxyapatite, modified with Zn ions by the sorption method	0
ZnHAp 0.01nat		0.01
ZnHAp 0.1nat		0.1
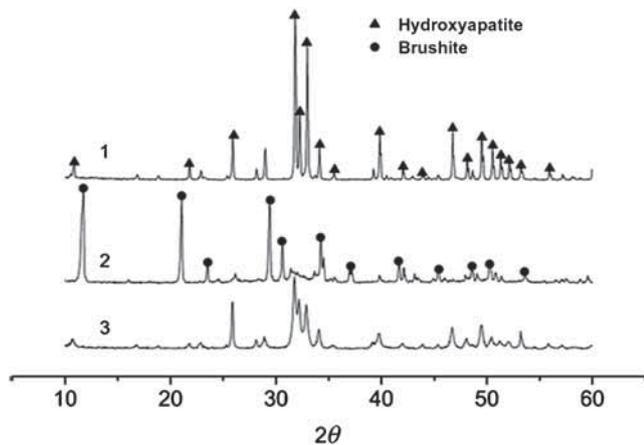
Figure 1. XRD patterns of calcium phosphate structures formed at various conditions. Patterns refer to: line 1—natural origin HAp, line 2—brushite and line 3—synthetic HAp.

Figure 2. FTIR absorbance spectra of calcium phosphate structures (formed at various conditions) in 4000–400 cm^{-1} region where the numbers denote, respectively: 1—natural HAp, 2—brushite and 3—synthetic HAp.

ZnB denotes brushite doped with zinc ions and ZnHAp is assigned to the HA conjugated with Zn. Zn/Ca molar ratio indicates applied amount of zinc ions introduced into the structures of various calcium phosphates. The postsynthetic structures conjugated with zinc ions were physicochemically characterized in order to determine differences in the main XRD patterns and FT-IR spectra (see figures 1 and 2) of natural and synthetic HA and the brushite structure.

Figure 1 depicts comparison of the XRD patterns of the natural and synthetic HA powders and the brushite structure

with no addition of zinc ions. The top pattern refers to the natural HA, middle refers to the brushite formed at ambient temperature and finally the bottom one indicates the synthetic HA formed at elevated temperature. In addition, figure 1 exemplify how the temperatures applied during the formation of the expected structures affect the final products. Synthesis carried out at ambient temperature leads to formation

Table 5. Calcium phosphate structures modified with Zn ions using *in situ* procedure.

Type of calcium phosphate	Theoretical molar ratio Zn/Ca	Molar ratio Ca/P	Content of Zn in the sample (%)	Adsorbed amount of Zn (%)
ZnHAp formed at elevated temperature W	0	1.55	0	0
	0.01	1.6	0.586	89.665
	0.02	1.54	1.148	87.819
	0.05	1.55	2.592	79.31
	0.1	1.37	4.951	75.738
ZnB formed at ambient temperature N	0	1.33	0	0
	0.01	1.21	0.5	76.488
	0.02	1.39	1.054	80.646
	0.05	1.16	2.176	66.589
	0.1	1.1	4.909	75.097

of the brushite instead of HA. Moreover, differences in the crystallographic structures of materials are confirmed by the differences in the Ca/P ratio which for synthetic HA equals to 1.55 whereas for brushite is equal to 1.33.

Notably, XRD pattern of the natural HA possess the highest relative intensity of most reflexes typical for high crystallinity. On the contrary, XRD patterns of the synthetic HA or brushite depict lower relative intensity which may be due to lack of calcination steps during preparation of the two synthetic structures.

Additionally FTIR spectroscopy analysis was used for determining characteristic vibrational modes of either brushite or HA structures.

Figure 2 exemplify FTIR spectra of the natural and synthetic HA and brushite structures with no addition of zinc ions, formed by the wet precipitation methods at ambient or elevated temperatures.

FTIR spectra shown in figure 2 indicate the presence of characteristics bands corresponding mainly to PO_4^{3-} and OH^- groups.

The natural origin HA depicted in spectrum 1 possess bands within wavenumber range 605–560 cm^{-1} which can be assigned to asymmetric deformation of O–P–O vibrational stretching mode of $(PO_4)^{3-}$ and additionally the most intensive absorption bands in the region of 1200–1000 cm^{-1} which correspond to asymmetric stretching band P–O mode of $(PO_4)^{3-}$.

The bands at 1091, 1044 and 959 cm^{-1} can be assigned to the $\nu3$, $\nu3$ and $\nu1$ vibrational modes of $(PO_4)^{3-}$ bond and confirm the formation of HA. The broad band with an average intensity within wavenumber range (3550–3350 cm^{-1}) correspond to the stretching vibration of OH^- ion group in HAp and additionally indicates the presence of water in HAp structure.

Natural origin HA possess weak peaks between 870 and 880 cm^{-1} and more intensive peak between 1460 and 1530 cm^{-1} which correspond to the CO_3^{2-} group. Absorbed water band is relatively wide from 3600 to 2600 cm^{-1}.

The spectrum in the middle depicts brushite structure. The band with wavenumber range 3540–3166 cm^{-1} is referred to stretching vibrations of water, whereas the 1135 cm^{-1} mode

is assigned to the asymmetric stretching band P–O mode of $(PO_4)^{3-}$. The band within wave number 576–408 cm^{-1} refer to bending O–P–O(H) mode of $(PO_4)^{3-}$ group. Notably, brushite structure exhibit split of the vibrational modes assigned to the $(PO_4)^{3-}$ bands between 870 and 1200 cm^{-1} while such split is not observed considering spectrum of synthetic HA. The bands at 873, 1125, 1134 refer to HPO_4^{2-} [31,32].

The bottom spectrum refers to the HA synthesized using *in situ* procedure.

According to the literature reports the most characteristic vibrational modes in the FTIR spectrum of synthesized HA are PO_4^{3-}, OH^-, CO_3^{2-} and HPO_4^{2-}, respectively, that characterize non-stoichiometric HAp. Notably, PO_4^{3-} groups form two intensive bands at 560–600 and 1000–1100 cm^{-1}. Absorbed water is relatively wide and covers the 3600–2600 cm^{-1} region. CO_3^{2-} group form weak peaks between 870 and 880 cm^{-1} and more intensive between 1460 and 1530 cm^{-1}. Finally hydroxyl group OH^- appears at 3500 cm^{-1}.

In this study, HA and brushite structures were modified with zinc ions by in situ or sorption methods. Table 5 summarizes the modification of the calcium phosphate structures by Zn ions using *in situ* process.

Titration procedure described in detail in section termed as determination of calcium, phosphorus and zinc allowed to quantify amounts of the calcium and phosphorus and the outcome of such analysis depicted as Ca/P molar ratio is introduced in table 5. Accordingly the amount of Zn was also determined using titration and the attained results are summarized also in table 5 above and denoted as Zn/Ca molar ratio.

Additionally, determination of the yield of substitution of zinc ions into various calcium phosphates is provided.

Figure 3 and table 5 indicate that the increase of the added the zinc acetate leads to changes of the adsorbed amount of zinc ions. With low Zn/Ca molar ratio, more zinc ions become embedded into HA than into brushite. With greater addition of zinc the differences of incorporated zinc ions become negligible. When the process is carried out at elevated temperature, the total yield of substitution of the zinc ions is decreasing with the increase of the Zn/Ca molar ratio. On the contrary, reaction performed at ambient temperature

shows no correlation between the yield of reaction and Zn/Ca molar ratio.

Subsequently, the experiments of incorporation of various amounts of zinc ions into structure of HA and brushite were carried out, and the received structures were determined using XRD and FTIR.

Samples of HA substituted with various amounts of zinc ions were analysed by means of X-ray diffractometry and the phase composition of the HA structures synthesized at elevated temperature doped with various amounts of the zinc ions applying *in situ* method is depicted in figure 4.

As exemplified in figure 4 synthesis at elevated temperature leads to the formation of HA (JCPDS file no. 9-432). The Zn/Ca molar ratios are denoted as 0, 0.01, 0.02, 0.05 and 0.1W, respectively, and the W indicates synthesis of the HA at elevated temperature. The higher content of the Zn ions results in decrease of the crystallinity of the HA. LeGeros and LeGeros [9] reported that the incorporation of a small amount of Zn^{2+} into the HA structure leads to reduction in the degree of crystallinity of the apatitic phase, which prevents from hosting a greater amounts of Zn. Such suggestion is also observed in figure 4. For all samples major peaks were found at 31.7°. Following structural analyses reported by Sobczak-Kupiec and Wzorek [12] on the basis of JCPDS 9-432 the diffractograms obtained contain only peaks of HA. There was no line related to tricalcium phosphate (JCPDS 9-348 and JCPDS 09-0169, respectively).

Additionally, the FTIR analysis was performed in order to determine characteristic vibrational modes of the HA structure formed at elevated temperature.

Figure 5 shows FTIR spectra of the synthetic HA attained using the wet chemical method and exemplify characteristic vibrations of the PO_4^{3-}, OH^- and water present in HA structure. The Zn/Ca molar ratios are assigned as 0, 0.01, 0.02, 0.05 and 0.1 W, respectively, and the W indicates synthesis of the HA at elevated temperature. The characteristic vibrational modes of the PO_4^{3-} can be observed at several regions starting from 1090–1032, 960 and 600–500 cm^{-1}, respectively. Bands corresponding to absorbed water are widely expanded between 3600 and 2600 cm^{-1}. Accordingly stretching vibration of hydroxyl group are visible OH^- at 3500 cm^{-1}, and carbonate bands at 1550, 1460, 1445 and 1415 cm^{-1}, respectively [33].

Synthesis of calcium phosphates was performed using various experimental conditions in order to exemplify how the parameters mainly temperature affect the formation of different structures. Synthesis of the calcium phosphates at ambient temperature leads to production of brushite. Incorporation of the zinc ions into brushite structure was physicochemically analysed by X-ray diffractometry, and the main outcome of this study is shown in figure 6.

The powder XRD patterns were recorded for all types of products with and without the addition of Zn.

As exemplified in figure 6 synthesis at ambient temperature leads to formation of the brushite which phase composition is confirmed by JCPDS file no. 9-77 [34].

Figure 3. Yield of the substitution of zinc ions as a function of Zn/Ca molar ratio.
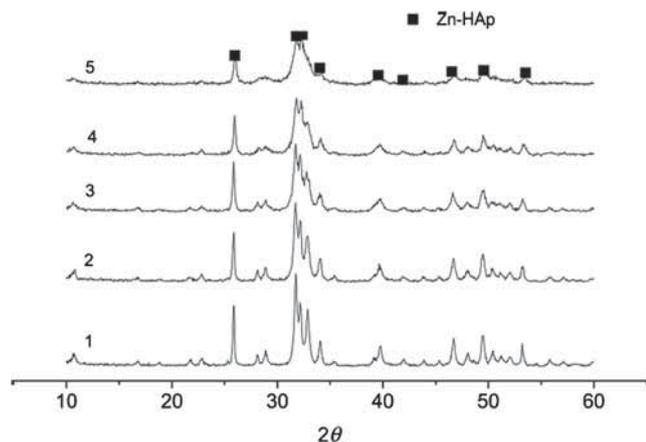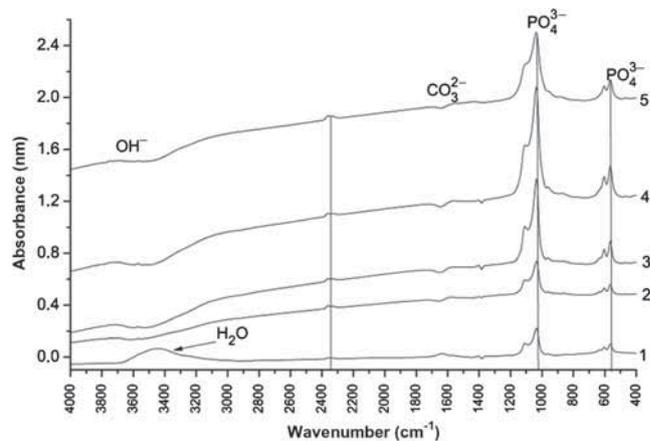

Figure 4. XRD patterns of the zinc-doped synthetic hydroxyapatite modified by using *in situ* method at elevated temperature where particular diffractograms are numbered as follows: 1—ZnHAp 0 W, 2—ZnHAp 0.01 W, 3—ZnHAp 0.02 W, 4—ZnHAp 0.05 W and 5—ZnHAp 0.1 W (denotation as in table 4).

Figure 5. FTIR spectra of the zinc-doped hydroxyapatite formed at elevated temperature.
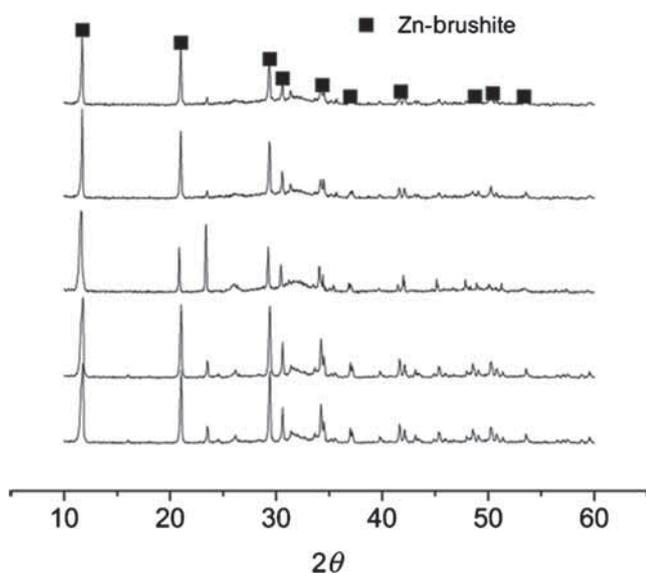
Figure 6. XRD patterns of the brushite doped with zinc ions applying *in situ* method. Patterns represent: 1—ZnB 0 N, 2—ZnB 0.01 N, 3—ZnB 0.02 N, 4—ZnB 0.05 N and 5—ZnB 0.1 N (denotation as in table 4).
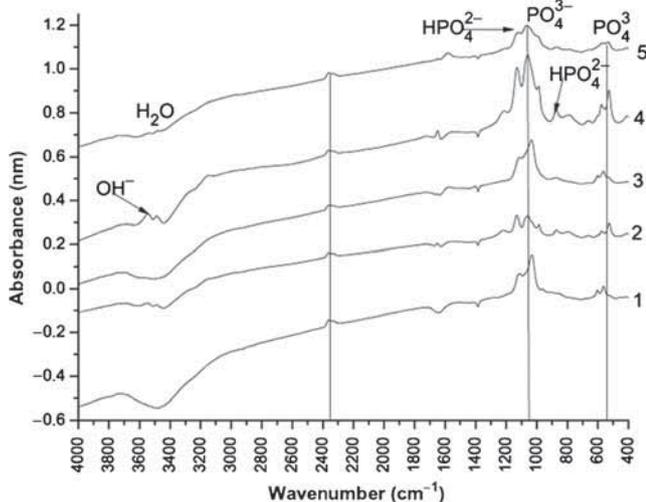

Figure 7. FTIR spectra of the zinc-doped brushite structure formed at ambient temperature where 1—ZnB 0 N, 2—ZnB 0.01 N, 3—ZnB 0.02 N, 4—ZnB 0.05 N and 5—ZnB 0.1 N (denotation as in table 4).

Figure 6 shows the powder XRD pattern of the CHPD (brushite) crystals grown without zinc 0N and with Zn/Ca ratio equal to 0.01, 0.02, 0.05 and 0.1, respectively. The diffraction peaks of the crystals grown with Zn have shown a slight change in the peak position towards the higher angle side. This suggests the partial substitution of zinc in calcium sites. Moreover, intensity of the peaks in the range of 27–40° is decreasing due to addition of the zinc ions. In the zinc doped brushite the crystallite was pure and the zinc doping did not alter the crystalline phase. For all samples the major peaks were found at 11.59°.

In addition, the structures of brushite powders incorporated with various amounts of the zinc ions were studied by means of FTIR analysis. Figure 7 shows characteristic vibrations of the groups typical of brushite phase.

Figure 7 depicts the FTIR spectra of products obtained at different amounts of doped zinc ions. The spectra exhibit easily distinguishable bands attributed to PO_4^{3-}.

Bands at around 525 and 575 cm^{-1} can be assigned to the $\nu4$ bending vibrational modes of the P–O–P mode. Bands at 873 and 1125 cm^{-1} which correspond to the HPO_2^{4-} group of brushite are observed in all samples. These analyses are in agreement with the previous studies reported by Vallet-Regi and Gonzalez-Calbet [31] and Lu *et al* [32]. A band at 985 cm^{-1} comes from the P–O(H) $\nu1$ symmetric stretching vibration of PO_4^{3-}. Notably, the peak at around 1060 cm^{-1} can be assigned to the $\nu3$ vibration of the PO_4^{3-} group. A peak close to 1134 cm^{-1} is correlated with the $\nu'6$ and $\nu''6$ of HPO_2^{4-} ions in brushite. Finally, a broad band between 3400 and 3500 cm^{-1} is depicted, due to the stretching (νs) mode of OH$^-$ or water [35].

Interestingly, vibrational modes which correspond to the PO_4^{3-} groups are shifted towards higher values of the wavelength with the addition of the zinc ions.
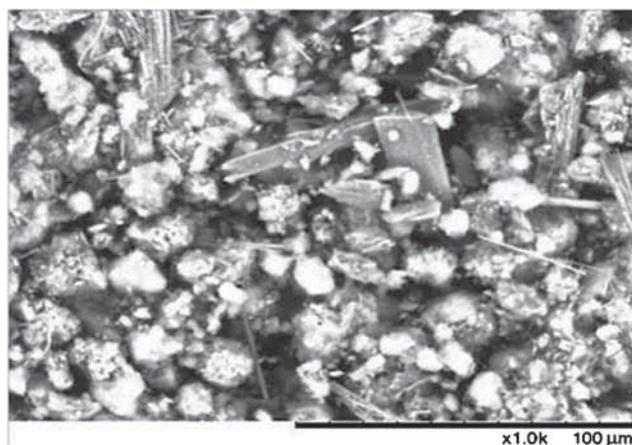

Figure 8. SEM micrography of the brushite doped with zinc ions where the Zn/Ca molar ratio equals 0.1 (*in situ* method).
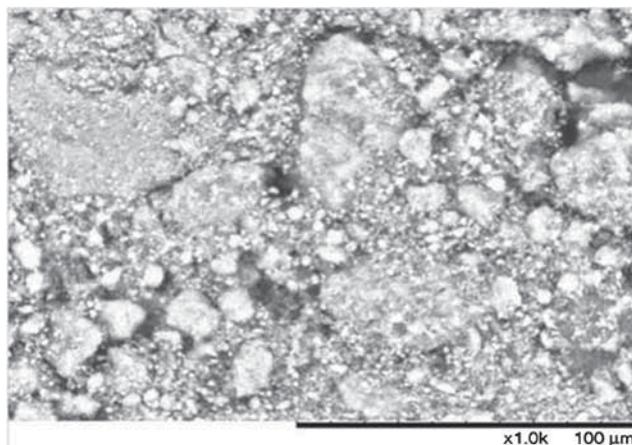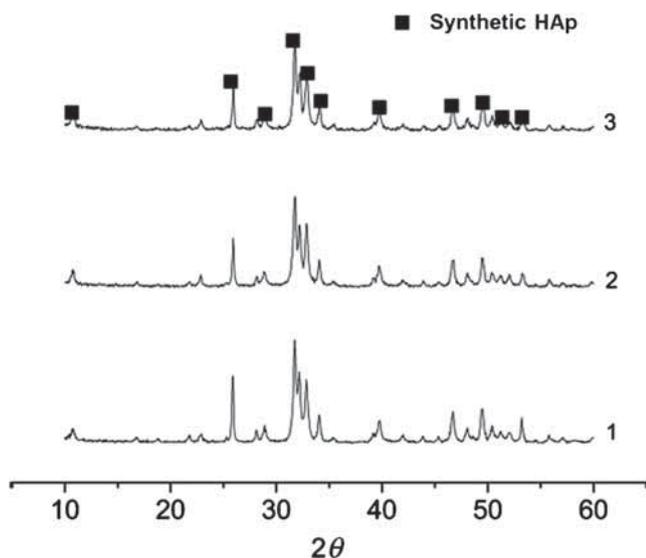

Figure 9. SEM micrography of the hydroxyapatite doped with zinc ions where the Zn/Ca molar ratio equals 0.1 (*in situ* method).

Table 6. Zinc-doped hydroxyapatites modified by the sorption method.

Hydroxyapatite type	Theoretical molar ratio Zn/Ca	Molar ratio Ca/P	Content of Zn in main sample (%)	Adsorbed amount of Zn (%)
ZnHAp syn.	0	1.55	0	0
	0.01	1.68	0.756	100
	0.1	1.7	4.83	75.867
ZnHAp nat.	0	1.84	0	0
	0.01	1.85	0.611	86.931
	0.1	1.9	4.676	66.499

Figure 10. XRD patterns of the synthetic HAp modified with Zn using sorption procedure where lines denote, respectively: 1—ZnHAp 0, 2—ZnHAp 0.01 and 3—ZnHAp 0.1.
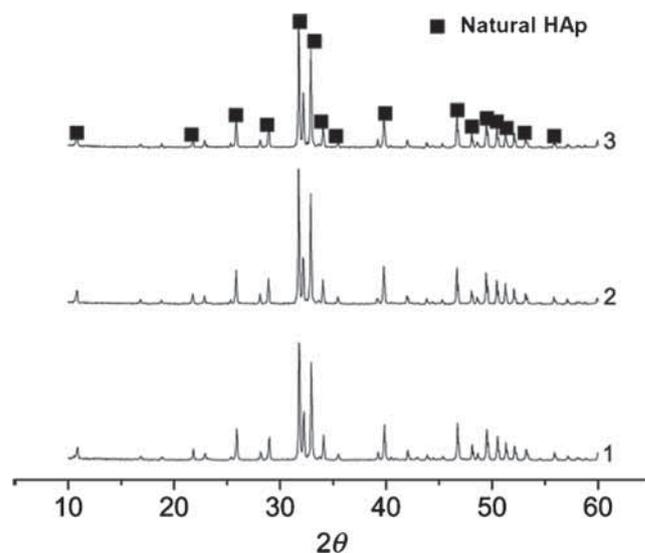
Figure 11. XRD patterns of the natural HAp modified with Zn using sorption procedure where numbers denote: line 1—Nat. HAp 0, line 2—Nat. HAp 0.01 and line 3—Nat. HAp 0.1.

Morphology of the attained powders incorporated with zinc ions was determined with SEM equipped with EDS system.

Figure 8 shows micrography of the brushite structure doped with zinc ions where the Zn/Ca molar ratio equals 0.1.

Additionally, the structure of HA doped with zinc ions is also visualized.

Figure 9 presents the SEM image of the zinc-doped HA structure where the Zn/Ca molar ratio equals 0.1.

Figures 8 and 9 exemplify morphology of the brushite and synthetic HA. Brushite possess freely bound crystals which form lumps, hence distinction of particular crystal fractions is facilitated. However, synthetic HA possesses more compacted structure, where the determination of particular crystals with lower size is hindered. It is stated that both analysed specimens contain at their surfaces calcium, phosphorus and zinc ions.

Modification of the HA structures by zinc ions using sorption procedure.

Alternatively, the synthetic and natural HAs were modified with zinc ions using sorption procedure. Amounts of zinc ions and the Ca/P molar ratios of particular samples were determined by titration procedures and the results are summarized in table 6.

As shown in table 6 with an increasing amount of added zinc ions the increase of the total Ca/P ratio is observed, nevertheless the content of adsorbed zinc ions is reduced and varies between synthetic and natural HA. This may suggest that origin of calcium phosphate and the amount of introduced zinc ions may affect the amount of embedded zinc into HA structures. When the synthetic HA is employed and with the addition of such amount of the zinc ions which leads to Zn/Ca molar ratio equal 0.01 the 100% adsorption of the zinc ions is observed. The data summarized in table 6 imply that the synthetic HA is regarded as the best sorption agent.

The phase composition of the specimens listed in table 6 was determined by X-ray diffractometry, and the received diffractograms are presented below.

Figure 10 depicts structure of the HAp conjugated with zinc ions using sorption procedure. Interestingly, the increasing amount of zinc ions added using sorption procedure does not influence the crystallinity of attained structures. The received diffractograms are comparable for all Zn/Ca ratio.

Similar observations are reported for synthetic HA incorporated with increasing amounts of zinc ions introduced by sorption procedure.

Figure 11 depicts structures of the HAp modified with zinc ions by the sorption method.

Notably, XRD pattern of the natural HA possess the highest relative intensity of most reflexes typical of high crystallinity. On the contrary, XRD patterns of the synthetic HA depict lower relative intensity.

From results exemplified in figures 10 and 11 one may generally conclude that the incorporation of ions by sorption procedure does not influence crystallinity and lattice parameters of received structures.

4. Conclusions

Various concentrations of zinc ions were applied in order to incorporate such ion with calcium phosphate structures. Both, brushite and HA were synthesized by the wet chemical method and subsequently attained structures were modified with Zn ions using *in situ* procedure. Alternatively, natural and synthetic HAs were modified with zinc ions applying sorption procedure. Physicochemical characterization of the attained powders confirms the formation of the relevant calcium phosphates.

XRD patterns depict the formation of the brushite structure at ambient temperature whereas formation at elevated temperature leads to the synthesis of HA. Incorporation of the zinc ions into the structure of HA results in reduction of the relative intensity and some loss of the crystallinity. On the contrary, the addition of zinc ions into the brushite does not affect significantly the crystallinity. FTIR analyses confirm the formation of HA and brushite structures, indicating characteristic bands typical of both structures.

Notably, experiments and the data analysis exemplified in this work showed that it is possible to attain with high reproducibility various structures of calcium phosphates when the chemical precipitation route was employed. Moreover the physicochemical characterization confirms the formation of appropriate phases. Incorporation of zinc ions into brushite structure result in no changes in the phase formation whereas embedding zinc ions into HA leads to crystallographic reorganization. However, further detailed studies are needed to elucidate the exact mechanisms of zinc doping into calcium phosphate structures. Many authors report that the addition of small amounts of Zn ions leads to loss of crystallinity and decrease of lattice parameters. Interestingly, upon addition of Zn ions to the natural and synthetic HAp by sorption procedures no crystallographic and structural changes are observed. Hence one can conclude that such procedure is suitable to form structures modified with various ions. Notably, upon increase of zinc ions also structure of brushite formed by *in situ* method remains constant indicating no influence of added ions. Our outstanding finding promotes sorption procedure

as suitable route to form structures incorporated with various ions, that can be further employed as potential implants.

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