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SYNTHESIS OF HYDROXYAPATITE IN A CONTINUOUS REACTOR: A REVIEW

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Because of excellent properties, similar to natural bone minerals, and variety of possible biomedical applications, hydroxyapatite (HAp) is a valuable compound among the calcium phosphate salts. A number of synthesis routes for producing HAp powders have been reported. Despite this fact, it is important to develop new methods providing precise control over the reaction and having potential to scale-up. The main motivation for the current paper is a view of continuous synthesis methods toward medical application of produced hydroxyapatite, especially in the form of nanoparticles.

Keywords: hydroxyapatite, synthesis in continuous reactor, continuous precipitation, microparticles, nanoparticles

1. INTRODUCTION

Hydroxyapatite plays an important role as a regenerative material in bioengineering. Synthetic HAp has been explored in tissue engineering applications as a material for hard tissue repair, regeneration or reconstruction (Suchanek and Yoshimura, 1998). Chemical and structural resemblance of synthetic HAp to human bone made hydroxyapatite commonly used as an additive in bone cements, scaffolds, orthopedic and dental implant coatings, and drug delivery applications (Chaudhury et al., 2014). Hydroxyapatite is the most thermodynamically stable compound in near-physiological conditions of temperature (37 °C) and pH (7) from a group of calcium phosphates. Stoichiometric HAp: $Ca_{10}(PO_4)_6(OH)_2$ has Ca/P ratio equal to 1.67 (10/6) (Uskoković and Uskoković, 2011).

70% of human bones is made up of hydroxyapatite, which is the most of the inorganic part of the bone. Natural origin HAp is non-stoichiometric with a Ca/P ratio less than 1.67. It exhibits nanostructured crystals, low crystallinity, and needle-like morphology and is responsible for mechanical strength of bone. Low amounts of ions such as Mg^{2+}, K^+, F^-, Cl^-, CO_3^{2-} built into its structure play an important role in bone metabolism (Dorozhkin, 2009). Natural nanohydroxyapatite (nHAp) belongs to B-type hydroxyapatite where some of PO_4^{3-} groups are replaced by carbonate groups. Among the types of hydroxyapatite, one can distinguish A-type substituted HAp, where hydroxide groups OH^- are replaced by CO_3^{2-} groups, and a third type known as AB when both substitutions are allowed in the HAp structure. Hydroxyapatite in natural bone and teeth has a nanoscale structure while the most types of HAp synthesis produce microscale aggregates. In synthetic HAp, the difference in scale between natural and synthetic material causes worse osteogenic and mechanical properties of bone implants. Nanosized particles have greater specific surface

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area and more uniform distribution than microparticles of HAp, thus it is crucial to design methods for synthesis of HAp in nanoscale form (Dorozhkin, 2010).

Hydroxyapatite as a biomaterial is biocompatible with hard tissue. It means that HAp interacts with the human tissue without any response of the immune system and has no toxic effect on biological system. What is more, HAp is well-known for its outstanding properties like osteconductivity and osteoinductivity. It positively influences processes of bone regeneration. HAp interacts with the surrounding bone tissue and creates a direct bond with host living bone as a result of bone remodeling (formation of new bone tissue in damaged spot). Moreover, it possesses high bioactivity and promotes the adhesion and the proliferation of bone cells (Catros et al., 2010).

Synthesis of hydroxyapatite in a controlled way represents a challenge. Nucleation, growth accompanying Ostwald ripening, and aggregation are three stages during particle formation (Rodríguez-Clemente et al., 1998). There have been comprehensive studies carried out on the effects of changing process parameters such as temperature, pH, Ca/P ratio, initial solution concentration on particle properties even though the reaction kinetics is still unknown. The various preparation methods of HAp also have significant influence on its properties and resulted in different size distribution, morphology, purity, crystallinity, crystallite size and are directly related to HAp biological characteristics (Pham et al., 2013).

The type of hydroxyapatite application demands a particular size range. Therefore, many batch or semi-batch techniques have been developed to industrial scale production, for example: mechanochemical (Fahamiet al., 2011), sol-gel (Kunjalukka et al., 2009), chemical precipitation (Wojasiński et al., 2015), micro-emulsion (Ponomareva et al., 2010), sonochemical (Cóta et al., 2016), hydrothermal (Sadat-Shojai et al., 2013). Precipitation is the most promising method and the most widely studied route due to low cost, simplicity and easy application in industrial production (Afshar et al., 2003). Despite this, batch methods provide only limited amounts of powder. Wide residence time and heterogeneous supersaturation in the reaction medium are problems with these techniques. Even small changes in stoichiometry can affect the material properties leading to problems with further application. These difficulties are significant especially as the scale rises from laboratory to industrial production.

The synthesis of hydroxyapatite in a continuous process improved control over the reaction conditions. Nevertheless, synthesis in continuous flow reactor is not a common way to produce hydroxyapatite. The literature reports describe the need to use special equipment, N_2 atmosphere inside the reactor or high process temperature. Thus, methods described in reports might be demanding and some of them could be restricted to laboratory scale. On the other hand, the approach based on continuous synthesis in a microreactor is characterized by high surface to volume ratio, short residence time, efficient mass transfer which promote more homogenous reaction conditions and thereby better monodispersity of the resulting product. What is more, continuous production allows higher productivity to be achieved. This article presents continuous synthesis methods of hydroxyapatite micro- and nanoparticles focused on biomedical applications. We also pay attention to the economic aspects. In this context, HAp needs to be produced as nanoscale particles with high purity and narrow size distribution using low-cost equipment.

2. PREPARATION OF HAp IN CONTINUOUS REACTOR

Among the various HAp preparation methods, most of them consider batch or semi-batch synthesis. The aim of this review is to present and compare HAp synthesis routes in continuous systems. We also put an emphasis on the possibility of scaling up of the presented procedures. The main advantages of these methods, over batch processes, are better control over the reaction and higher yield. On the other hand, the methods are associated with a number of difficulties in reactor geometry, size distribution, crystallinity, stoichiometry and degree of particle agglomeration.

2.1. Type of reactor and synthesis

Gomez-Morales et al. (2001) synthesized HAp by precipitation method in continuous system called Mixed Suspension Mixed Product Removal Reactor (MSMPR), maintaining temperature at 85 °C and pH equal to 9. The aqueous solutions were prepared by dissolving $CaCl_2$ (or $Ca(NO_3)_2$) and K_2HPO_4 in distilled water with a Ca/P ratio of 1.67. Thereafter, the starting solutions were pumped into the reactor maintaining continuous flow of N_2 inside the reactor to avoid the presence of atmospheric CO_2. The residence time (τ) was 30 or 60 minutes. Samples were collected at the outlet of the reactor. Post-processing treatment involved filtration, washing with distilled water and drying in an oven. They pointed out, that the method accomplishes high production rate, up to 1.17 g/min, which increases with the rise in the concentration of reagents and decreases when the residence time is longer. In short, this research was focused on the synthesis of pure HAp with stoichiometric Ca/P ratio under careful control of the pH and temperature.

Another production system is given in Fig. 1. This type of reactor was described by Fujii et al. (2015). The researchers used a tube reactor (2 mm in inner diameter, 10 m in length) equipped with a mixing unit containing a T-shaped connector. To control reaction temperature the test system was placed in an incubator. Aqueous solutions of $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ with flow rate of 2 ml/min each were pumped into the reactor. The residence time was about 8 min; pH was maintained at 6.4 or 9 and temperature ranged from 0 to 100 °C. The collected products were filtered, rinsed with distilled water and subjected to vacuum freeze-drying. They intended to control phase purity and morphology of the resulting HAp particles.

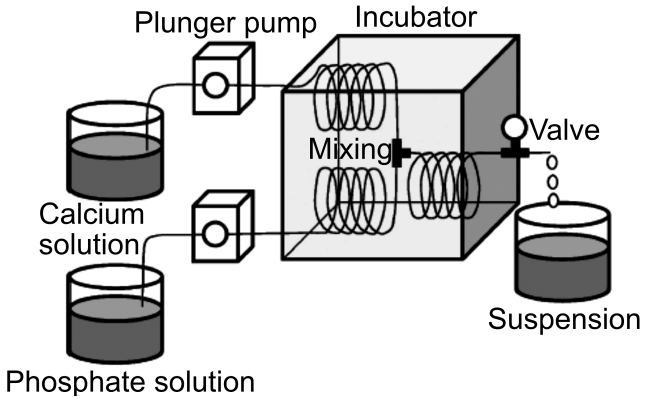

Fig. 1. Production of hydroxyapatite in tube reactor. Reprinted from (Fujii et al., 2015) with permission from The Ceramic Society of Japan (Copyright, 2015)

A successful approach to produce hydroxyapatite was presented by Lester et al. (2013). They created continuous hydrothermal rig to produce high quality powders. Fig. 2 shows a scheme of this reaction system. The aqueous solution of $(NH_4)_2HPO_4$ was superheated and pumped (20 ml/min) into the nozzle reactor as a downflow. At the same time, the cold aqueous $Ca(NO_3)_2 \cdot 4H_2O$ was used as the upflow and pumped (10 ml/min) to form particles at the interface of two solutions. The temperature of the preheater varied between 200 and 400 °C. The pressure was maintained at 240 bar and pH at 8 and 5 for $(NH_4)_2HPO_4$ and $Ca(NO_3)_2 \cdot 4H_2O$, respectively. They described the influence of altering parameters such as pH and temperature on the morphology of produced particles.

Anwar et al. (2016) used a continuous plastic flow synthesis (CPFS) to obtain Zn substituted HAp. They conducted the reaction at pH 11 and at the temperature of 70 °C. Different weight percent (wt%) of Zn ions from 0 to 4 wt% were used. The procedure involved preparing an aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$. Then, prepared reagents were contacted into T-piece and passed through 8 m long tube. Flow rates were tailored to give a total residence time of 5 minutes. Then, obtained powders were filtered

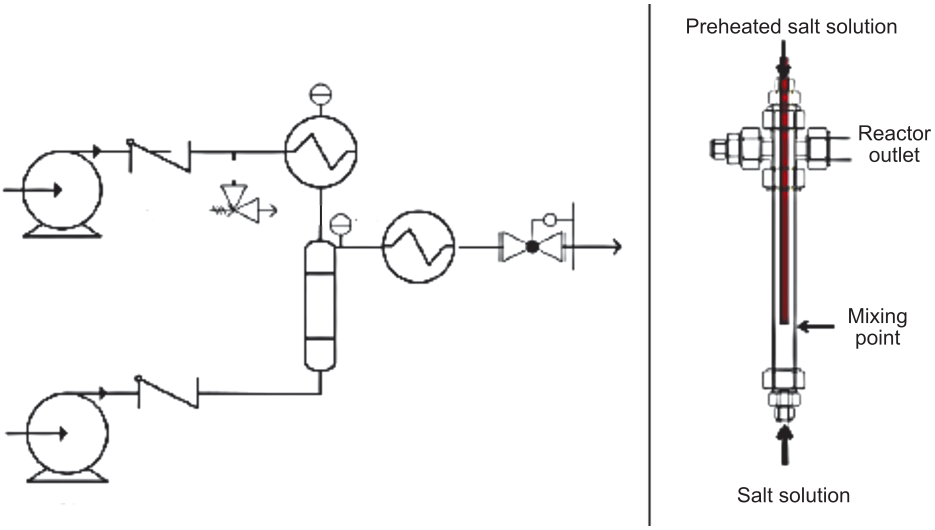

Fig. 2. Scheme of continuous hydrothermal synthesis (left) and hydrothermal rig (right). Reprinted from (Lester et al., 2012) with permission from Elsevier (Copyright, 2011)

and washed. In the next step, powder was dried in an oven at 90 °C for 12 h. The aim of this process was a synthesis of modified hydroxyapatite with antibacterial activities.

An interesting way to produce hydroxyapatite particles is a microporous tube-in-tube microchannel reactor (MTMCR) used by Yang et al. (2010). Scheme of this system is presented in Fig. 3. The main parts of the system are two coaxial tubes which form an annular cross-sectional microchannel. The inner tube had micropores that ensure dispersion of reagents. The width between the inner and outer tube is 500 μm. Aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$ as a continuous phase (outer tube) and $(NH_4)_2HPO_4$ as a dispersed phase (inner tube) were adjusted to pH 9.5 and pumped at room temperature. The produced HAp was carried forward to a Teflon autoclave and hydrothermally treated at 220 °C for 4 h. Then product was filtered, washed and dried at 80 °C for 12 h.

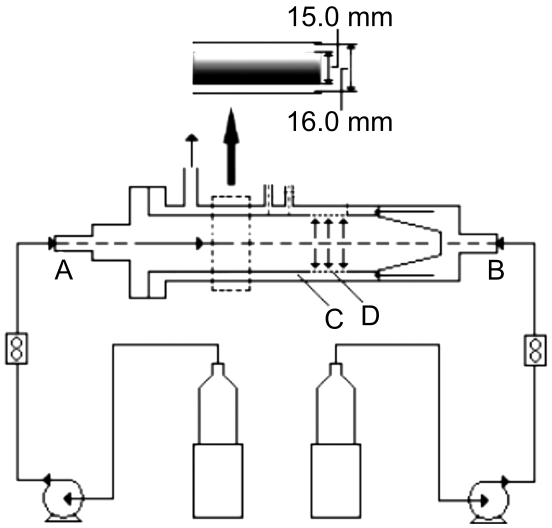

Fig. 3. Scheme of tube-in-tube microchannel reactor. A-inlet of inner tube; B-inlet of outer tube; C-microchannel; D-micropores. Adapted from (Yang et al., 2010) with permission from American Chemical Society (Copyright, 2010)

Kandori et al. (2011) described a microreactor system presented in some detail in Fig. 4. Reactants like aqueous solutions of $Ca(OH)_2$ and H_3PO_4 were prepared. The temperature of the bath was modified in

the range of 20–70 °C and flow rate varied from 5–75 ml/min. The ratio of Ca/P was determined to 1.67. Microreactor with 48 channels was used as a mixing part. Streams from all channels were merged at a center channel with a diameter of 1 mm. Solutions of reactants flowed along channels and mixed into the center channel. The authors conducted experiments with the increasing flow rate and temperature and investigated their influence on product properties.

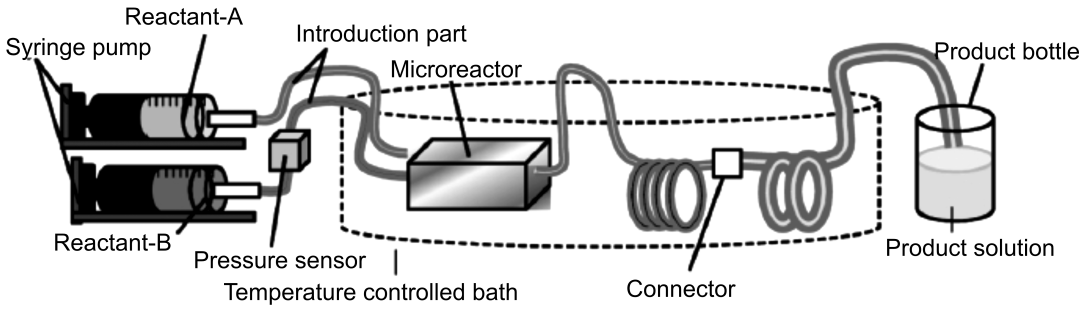

Fig. 4. Scheme of microsystem reactor. Adapted from (Kandori et al., 2011) with permission from American Chemical Society (Copyright, 2011)

Castro et al. (2013a) reported the preparation of HAp in near-physiological conditions. They used an ultrasonic tubular reactor inserted in an ultrasonic bath (Fig. 5). The authors clearly defined the meaning of the ultrasound application as a way to avoid clogging and to decrease level of particle aggregation. Single-phase flow (SPF, laminar) and gas-liquid flow (GLF, segmented) were used to compare monodispersity of synthesized HAp. Reactants contacted in T-mixer (SPF) or cross-mixer (GLF) chamber, then flowed through tubular reactor with inner diameter of 1.02 mm. $Ca(OH)_2$ and H_3PO_4 aqueous solutions were used in Ca/P molar ratio 1.33. Different liquid flow rates (0.152–4 ml/min) and gas flow rates (1.2–4 ml/min) were experimented.

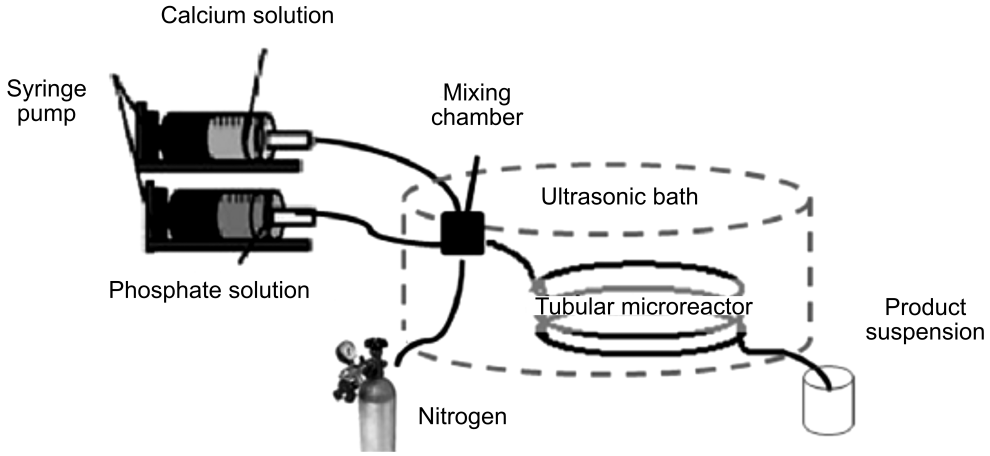

Fig. 5. Scheme of production HAp in ultrasonic tubular reactor. Reprinted from (Castro et al., 2013a) with permission from Elsevier (Copyright 2013)

Using the same reactants in the same conditions, Castro et al. (2013b) carried out synthesis in a meso oscillatory flow reactor (meso-OFR) and in a scaled-up meso-OFR (Fig. 6). To obtain a better production rate, the scale-up reactor was employed. The scaled-up reactor is a series of eight connected vertical meso-OFRs. One meso-OFR contains a glass jacked tube with 4.4 mm in inner diameter and 35 cm in length. It is equipped with smooth periodic cavities and baffles. Fluid moves from the walls to the center of the tube with intensity controlled by the oscillation frequency and amplitude. HAp precipitation was carried

out, for both systems, with the same liquid flow rate of 4.5 and 9 ml/min (but different residence time, due to differences in reactor construction).

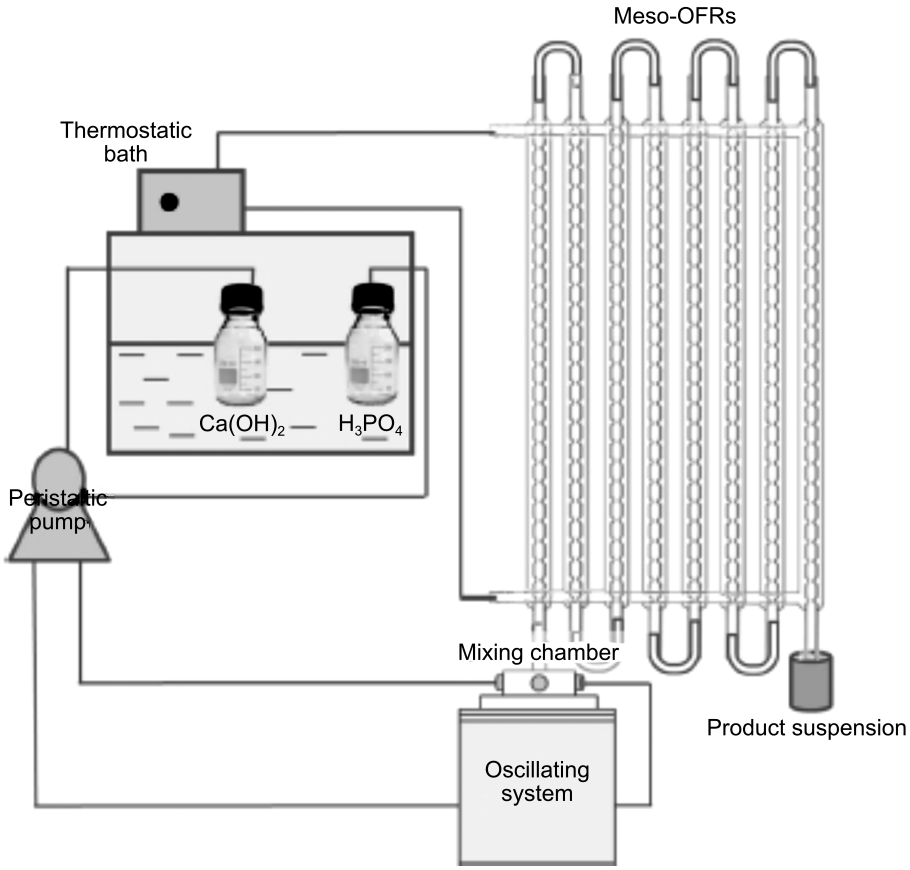

Fig. 6. Scheme of HAp production in scaled-up meso-OFR. Adapted from (Castro et al., 2013b) with permission from American Chemical Society (Copyright, 2013)

2.2. Phase purity and chemical analysis

All research groups characterized obtained powders by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) in order to determine the crystalline structure and chemical composition of the product. This kind of analysis is crucial to determine the type of calcium phosphate obtained in the synthesis based on crystalline structure (XRD) and to determine characteristic chemical groups or possible substitutions in the chemical composition of the product (FTIR). Gomez-Morales et al. (2001) indicated that suggested synthesis conditions allow to obtain pure hydroxyapatite without other calcium phosphates. After the time equal to residence time times four, steady state with Ca/P ratio was equal or close to the stoichiometric value. Chemical analysis also confirmed presence of functional group characteristic for HAp structure. Despite N_2 atmosphere inside the reactor, some signals from carbonated group were observed. Different results presented Fujii et al. (2015). They obtained powders prepared at pH 6.4 and temperature range from 0 to 60 °C which contained two phases: dicalcium hydrogen phosphate dehydrate (DCPD) and octacalcium phosphate (OCP). What is more, OCP phase increased with higher temperature synthesis. Using the temperature in the range of 80–100 °C caused formation only of one phase of calcium phosphate – HAp. In contrast, under pH 9, no matter what synthesis temperature was used, only HAp with improved crystallinity was obtained. At pH 6.4 the Ca/P ratio was correlated with temperature. When the temperature was raised, the Ca/P ratio was higher achieving 1.66 in 80 °C. Results of Lester et al. (2013) showed that powder produced in various conditions of temperature and pH

was identified as hydroxyapatite. It should be pointed out here that using the continuous hydrothermal rig allows to produce doped HAp too. Hydroxyapatite can easily exchange ions, so Zn^{2+} can replace Ca^{2+} in HAp lattice. Analysis of crystalline structure indicated a mixture of phases but hydroxyapatite was the main phase. Anwar et al. (2016) reported that synthesized powders had good correlation with stoichiometric hydroxyapatite. A slight change in unit cell lattice in comparison with HAp without Zn indicated that Zn substitution (replacement of Ca with Zn) was possible. Consequently, Ca/P ratio decreased by increasing Zn substitution. Samples collected in experiments in MTMCR by Yang et al. (2010) contained hydroxyapatite without any other phase present. Kandori et al. (2011) obtained hydroxyapatite with low crystallinity which confirmed structural and chemical analyses. Parameters of crystal lattice came close to the literature data. The Ca/P ratio was determined to be between 1.57 and 1.64. In addition, HAp prepared with a microreactor system contained CO_3^{2-} in crystal lattice. That indicates the presence of B-type HAp. In an ultrasound tubular reactor Castro et al. (2013a) received hydroxyapatite in SPF conditions and mixed hydroxyapatite, dicalcium phosphate dihydrate (DCPD), and β-tricalcium phosphate (β-TCP) using GLF. Breadth of peaks of all powders differed from commercial HAp. Chemical analysis indicated that all tested samples had a typical HAp structure. Due to the presence of carbonated ions, some of them can be classified as B-type hydroxyapatite. In an experiment with meso-OFR and scaled-up meso-OFR all prepared powders were identified as single-phased. All peaks in XRD diffractograms matched with reference and commercial HAp, which suggested that pure HAp was obtained for all the samples prepared at varied conditions. What is more, all the conditions of the synthesis used in that experiment make it possible to obtain carbonated HAp (Castro et al., 2013b).

2.3. Size and morphology

It is known that hydroxyapatite can occur in different morphology. The most common shapes are rod (or needle), sphere and sheet. Gomez-Morales et al. (2001) synthesized HAp and obtained aggregates of needle-like particles with na average size of 0.48-1.5 μm. It was observed that the size of particles increased with increasing residence time. The average crystallite size was between 81 and 53 nm. This difference in size could confirm the mechanism of particle formation: nucleation-aggregation-agglomeration growth. Fujii et al. (2015) obtained sheet-like particles with size larger than 200 nm at pH 6.4 and the temperature range of 20–60 °C. In 80 and 100 °C rod-like morphology appeared with particle size between 100 nm and 200 nm. In contrast, under pH 9 and temperature below 80 °C the particles were heavily agglomerated with undefined shape. In the temperature range of 80–100 °C particles were smaller than 100 nm and seemed oval and spindle-shaped. Results of Lester et al. (2013) showed that particles produced in 200 °C had sheet morphology with widths and lengths up to 2 μm and thicknesses < 80 nm. By changing only pH from 8 to 10 of the downflow particles had rod morphology with 30–40 nm in diameter and variable lengths. What is more, increasing reaction temperature to 350 °C produced particles with tube morphology and inner diameter between 30–70 nm. In case of changing other conditions, they observed mixed morphology, whereas HAp with Zn addition was sheet-shaped. Researchers from Anwar's group obtained rod-like nHAp particles with an average crystal length of 80 ± 15 nm and width of 12 ± 5 nm, while nHAp with 2 wt% Zn had about 70 ± 10 nm in length and 12 ± 5 nm in width (Anwar et al., 2016). Increasing the level of Zn substitution in particles caused decrease in size and appearance of semispherical morphology. Moreover, particles were very prone to aggregate. Size measurement showed that particles had 100–300 nm in diameter. Yang et al. (2010) indicated that at a high reactant flow rate of 1667 or 2500 ml/min, HAp had more homogenous rod-like morphology with smaller size and narrower size distribution in comparison to particles obtained at a lower flow rate (500 or 833 ml/min). On the other hand, the mean particle size increased with increasing the concentrations of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ solutions. Kandori et al. (2011) synthesized rod-shaped nanoparticles in their microreactor system. Increasing both flow rate and temperature caused decrease in size while maintaining the same morphology of particles. This can be explained by particle growth only along the longest axis (c-axis). The smallest

particles had 2 nm in width and 15 nm in length. Castro et al. (2013a) also obtained particles with rod-like morphology but samples obtained under GLF seem to have a more defined shape. The size of their HAp particles was found to decrease with the increasing flow rate. What is more, when residence time of reaction mixture in the reactor decreased, tendency to aggregation of particles was more visible. In GLF configuration, applying a gas flow to the reactor generates segmented flow and creates small reacting entities. As a result, formation of aggregates in those small entities was reduced. Using meso-oscillatory reactor with longer residence time resulted with not obvious morphology of particles. They seemed as rod-shaped and plate-shaped for the meso-OFR and the scaled-up meso-OFR, respectively. Regarding shorter residence time, obtained particles had a more uniform morphology, mostly rod-shaped. Received powders possessed micro-size aggregates composed of nano-size particles. The mean size and the level of aggregation increase with increasing residence time. At the residence times $\tau = 0.4$ and 3.3 min, for the meso-OFR and the scaled-up meso-OFR, respectively, the mean size of particles was 77 nm for both reactors (Castro et al., 2013b).

3. DISCUSSION

Precipitation is the most frequently reported method of synthesis of HAp among all reports described above. It is not a wonder that this method is so popular, because it exhibits advantages like simplicity, and low energy consumption, just to name the most important (Sadat-Shojai et al., 2013). In addition, using a microreactor as a tool to contact two streams gives a possibility to better control the reaction conditions. Despite small volume of a reactor, it is possible to obtain a high throughput of a product. By contrast, hydrothermal method, another one described above and presented as a possible continuous production method for HAp, requires high temperature and is energy-intensive. Comparison of these two methods, including cost-benefit analysis, indicates that precipitation, as a method of continuous synthesis of hydroxyapatite, would have the better potential to perform as large-scale industrial production of nanoparticles.

Transfer of the same reaction conditions from a batch to a continuous reactor does not ensure you can receive comparable particles. However, some research groups carried out HAp synthesis in batch reactor in order to compare the product with one synthesized in continuous reactors. In a microreactor and a batch reactor, Kandori et al. (2011) obtained the same rod-shaped morphology but particles from the batch system had a bigger size (2×15 nm – microreactor; 12×75 nm – batch). Castro et al. (2013b) manufactured HAp in a meso oscillatory reactor. This process was described both on a batch and continuous level (Castro et al., 2013c). They strongly suggest that particles had better crystallinity, smaller size, narrow size distribution in continuous synthesis, rather than in the batch type. A similar conclusion can be drawn considering the ultrasound tubular reactor.

The above described methods of continuous synthesis mainly produce rod-like, carbonated HAp. Despite particles having similar features, it is not possible to compare them. The authors reported either the size of crystallites or the size of particles (particle size distribution), or both. Moreover, they used different measurement methods and post processing treatment. A solution of the problem with comparing products from different processes and produced by different groups may be conducting the measurement in accordance with, for instance, an ISO Standard.

Based on abovementioned results, it can be concluded that the ‘standard’ synthesis condition enabling receiving a selected kind of particle (rod, tube, sphere) does not exist. However, one can distinguish that the main variables in continuous systems affecting particle properties are geometry of the reactor, pH, reactants concentration, flow rate, etc. One of the major factors that can change the structure of HAp is pH. It is possible to control phase purity of the product through precise adjusting pH. At acidic condition

DCPA and DCPD (Ca/P = 1) are likely to appear, while OCP (Ca/P = 1.33) is more probable in neutral environment of synthesis. TCP (Ca/P = 1.5) and HAp (Ca/P = 1.67) are stable in basic condition (Vallet-Regí and González-Calbet, 2004). Another factor bringing changes in synthesized HAp is reactant concentration. Higher concentrations of solutions of starting materials led to a high supersaturation level. This causes very fast nucleation and growth processes, thereby affecting the generation of small particles. What is more, the enhancement of flow rate increases Reynolds number and improves mixing effectiveness. That also leads to faster nucleation and creates a possibility to obtain small particles with uniform size distribution (Yang et al., 2010).

In order to improve properties of hydroxyapatite Lester et al. (2013) and Anwar et al. (2016) synthesized HAp with additions. The Lester's research group investigated influence of HAp substituted with Zn on cell viability. The results of cell proliferation assay show that independently of HAp concentration in the medium, no changes of proliferation were observed 3 days after exposure on mouse embryonic stem cells (mESC). Anwar et al. (2016) conducted antibacterial testing using disc diffusion method on pure HAp and HAp substituted with Zn. Results demonstrate that Zn doped hydroxyapatite has greater antibacterial activity in comparison to pure HAp. They suggested that the mechanism of antibacterial bioactivity is connected with the ability of Zn ions to create strong bonds with carboxylic, thiol, amine functional groups. These groups are present in the cell membrane of bacteria. When metal ions contact with cells it generates damage in cell membrane. Essential components of the cytoplasm leak out of the cell, thereby causing bacteria's death. Because of the complicated relationship between properties of HAp, biological experiments are not primary research in design of HAp synthesis processes. First of all, the appropriate approach is to produce and characterize general properties of HAp. Then, choose a well-defined powder to use in biological assays, and then, eventually, tailor process parameters to change HAp powder properties.

3.1. Potential application and economical assessment

The majority of research groups have seen a potential application of their HAp in bone regeneration, orthopedic implants or bioceramics. Besides the potential application, equally important are economical aspects and possibility to scale-up systems to produce HAp in a continuous way. Gomez-Morales et al. (2001) obtained stoichiometric HAp by precipitation method. This kind of apatite, due to its biodegradability, is more desirable in production of dense HAp ceramic rather than Ca-deficient (non-stoichiometric) HAp. Production in MSMPR in conditions proposed by Gomez-Morales et al. allows better productivity. It requires relatively high temperature (85 °C) and additional stream of N_2 which increases the overall cost of production of HAp. Fujii et al. (2015) in their type of the reactor can control crystalline phase and shape of particles depending on the range of initial temperature and pH. Simple system and possibility to change conditions in simple way, allowed to obtain wide range of products, thereby used them in different application. But in case of scaling-up of this process, it is important to control pH and temperature, which in terms of the latter may generate additional cost. Yang et al. (2010) achieved rod-like particles with small size (55–95 nm). Nano-sized HAp has better properties in bone regeneration and can repair repairing damaged enamel surface. The main advantages of this method are cost-effectiveness and high quality of powder. However, this method employs complicated, and a costly, tube-in-tube system, but with the ability to produce HAp in large scale, balancing the equipment cost. Castro et al. (2013a) obtained rod-like, carbonated nHAp in near-physiological conditions. This HAp mimics apatite present in human bone. Using their production systems (meso-OFR, ultrasonic tubular reactor) requires control of additional parameters like: gas flow and frequency of ultrasound in an ultrasonic tubular reactor or oscillation amplitude and frequency in meso-OFR. What is interesting, scaled-up meso-OFR has a similar amount of precipitate to single meso-OFR. Lester et al. (2013) synthesized nanotubes, which are less common in literature. These particles have potential application in drug delivery systems and as a material for porous bone scaffolds. However, the main disadvantages of the method proposed by this group are high synthesis temperature (200–400 °C) and pressure, which makes it not a commercially viable way of HAp production.

Hydroxyapatite can easily exchange ions so substituted HAp gathered a great interest in research (Šupová, 2015). Bioactivity of HAp can be boosted by applying a different level of Zn substitution, for instance. Undoubtedly an advantage of this approach is the strong inhibition effect on the growth of many kinds of bacteria and fungi. Anwar et al. (2016) synthesized Zn doped HAp. Using this kind of HAp in biomaterials can reduce infection around implants. The advantages of this production method are not only limited to their biological aspects. Production in a CPFS system offers facile, rapid, one step synthesis route which is undoubtedly important for large scale production. Another interesting application of HAp is protein adsorption. Kandori et al. (2011) have seen potential in the use of synthesized HAp in blood purification therapy to remove pathogenic proteins from blood. They obtained small particles with good adsorption properties but using the system is reported to be complicated. In our experiments we obtained particles with similar characteristics to natural HAp. It is useful because of wide applications of HAp in biomedical application.

The summary of the methods and with their potential application is presented in Table 1.

Table 1. The summary of production systems, particle properties, methods of synthesis and potential applications of products

Production system	Morphology	Synthesis method	Potential application	References
Mixed suspension mixed product removal (MSMPR)	needle	precipitation	bioceramics	Gomez-Morales et al., 2001
Tubular reactor	sheet, rod	precipitation	bone regeneration, implants, bioceramics	Fujii et al., 2015
Microporous tube-in-tube microchannel reactor (MTMCR)	rod	precipitation	enamel surface damage	Yang et al., 2010
Ultrasonic tubular microreactor	rod	precipitation	bone regeneration, implants, bioceramics	Castro et al., 2013a
Meso oscillatory flow reactor (meso-OFR)	rod	precipitation	bone regeneration, implants, bioceramics	Castro et al., 2013b
Continuous hydrothermal synthesis	nanotube	hydrothermal	drug carrier	Lester et al., 2013
Continuous plastic flow synthesis (CPFS)	rod, semispherical	precipitation	antibacterial activity	Anwar et al., 2016
Microreactor with 48 microchannels	rod	precipitation	protein adsorption, blood purification therapy	Kandori et al., 2011

4. CONCLUSIONS

Precipitation is the most common method of HAp synthesis owing to its scale-up possibilities. This method produces lead to receive rod-like, carbonated hydroxyapatite. The wet chemical precipitation method is cheaper and simpler than other methods. However, when a process becomes continuous, the level of complication increases and systems could become multi-faceted. Beyond the control of pH and temperature the process requires monitoring of extra parameters including, gas flow or frequency (depending on the reported method). However, the continuous process enables obtaining smaller particles and can reduce

aggregation problem of resulting particles. Preparation of HAp is connected with difficulties in controlling size, morphology, purity and degree of particle aggregation. In continuous flow systems all variable parameters are linked and it is difficult to change one without affecting the others. However, after proper optimization, either experimental or by modelling, the continuous flow processes can lead to well defined hydroxyapatite products, with properties suitable for application in biomedical filed. What is more, choosing the proper system and balancing the energy consumption with high productivity, the continuous production of hydroxyapatite can be a cost-effective process.

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SYMBOLS

β-TCP	β-tricalcium phosphate
CPFS	continuous plastic flow synthesis
DCPA	dicalcium phosphate anhydrate
DCPD	dicalcium phosphate dihydrate
FTIR	Fourier-transform infrared spectroscopy
GLF	gas-liquid flow
HAp	hydroxyapatite
meso-OFR	meso oscillatory flow reactor
MSMPR	mixed suspension mixed product removal
MTMCR	microporous tube-in-tube microchannel reactor
nHAp	nanohydroxyapatite
OCP	octacalcium phosphate
SPF	single-phase flow
XRD	X-ray diffraction

REFERENCES

Afshar A., Ghorbani M., Ehsani N., Saeri M.-R., Sorell C., 2003. Some important factors in the wet precipitation process of hydroxyapatite. *Mater. Des.*, 24, 3, 197–202. DOI: 10.1016/S0261-3069(03)00003-7.

Anwar A., Akbar S., Sadiqa A., Kazmi, M., 2016. Novel continuous flow synthesis, characterization and antibacterial studies of nanoscale zinc substituted hydroxyapatite bioceramics. *Inorg. Chim. Acta*, 453, 1, 16–22. DOI:10.1016/j.ica.2016.07.041.

Castro F., Kuhn S., Jensen K., Ferreira A., Rocha F. , Vicente A., Teixeira J.A., 2013a. Process intensification and optimization for hydroxyapatite nanoparticles production. *Chem. Eng. Sci.*, 100, 352–359. DOI: 10.1016/j.ces.2013.01.002.

Castro F., Ferreira A., Rocha F., Vicente A., Teixeira, J.A., 2013b. Continuous-flow precipitation of hydroxyapatite at 37 °C in a meso oscillatory flow reactor. *Ind. Eng. Chem. Res.*, 52, 29, 9816–9821. DOI: 10.1021/ie400710b.

Castro F., Ferreira A., Rocha F., Vicente A., Teixeira J.A., 2013c. Precipitation of hydroxyapatite at 37 °C in a meso oscillatory flow reactor operated in batch at constant power density. *AIChE J.*, 59, 12, 4483–4493. DOI: 10.1002/aic.14193.

Catros S., Guillemont F., Lebraud E., Chanseau C., Perez S., 2010. Physico-chemical and biological properties of a nano-hydroxyapatite powder synthesized at room temperature. *IRBM*, 31, 226–233. DOI: 10.1016/j.irbm.2010.04.002.

Chaudhury K., Kandasamy J., Kumar H.S., RoyChoudhury S., 2014. Regenerative nanomedicine: Current perspectives and future directions. *Int. J. Nanomed.*, 9, 4153–4167. DOI: 10.2147/IJN.S45332.

Cóta L.F., Licona K.P.M., Lunz J.N., Pereira L.C., 2016. Hydroxyapatite nanoparticles: Synthesis by sonochemical method and assessment of processing parameters via experimental design. *Mater. Sci. Forum*, 869, 896–901. DOI: 10.4028/www.scientific.net/MSF.869.896.

Dorozhkin S.V., 2009. Nanodimensional and nanocrystalline apatites and other calcium orthophosphates in biomedical engineering, biology and medicine. *Materials*, 2, 1975–2045. DOI: 10.3390/ma2041975.

Dorozhkin S.V., 2010. Nanosized and nanocrystalline calcium orthophosphates. *Acta Biomater.*, 2, 1975–2045. DOI: 10.1016/j.actbio.2009.10.031.

Fahami A., Ebrahimi-Kahrizsangi R., Nasiri-Tabrizi B., 2011. Mechanochemical synthesis of hydroxyapatite/titanium nanocomposite. *Solid State Sci.*, 13, 135–141. DOI: 10.1016/j.solidstatesciences.2010.10.026.

Fujii E., Kawabata K., Shirosaki Y., Hayakawa S., Osaka A., 2015. Fabrication of calcium phosphate nanoparticles in a continuous flow tube reactor. *J. Ceram. Soc. Jpn.*, 123, 101–105. DOI: 10.2109/jcersj2.123.101.

Gomez-Morales J., Torrent-Burgues J., Boix T., Sainz J.F., Clemente R.R., 2001. Precipitation of stoichiometric hydroxyapatite by a continuous method. *Cryst. Res. Technol.*, 36, 15–26. DOI: 10.1002/1521-4079(200101)36:1<15::AID-CRAT15>3.0.CO;2-E.

Kandori K., Kuroda T., Togashi S., Katayama E., 2011. Preparation of calcium hydroxyapatite nanoparticles using microreactor and their characteristics of protein adsorption. *J. Phys. Chem. B*, 115, 653–659. DOI: 10.1021/jp110441e.

Kunjalukkal S.P., Balakrishnan A., Chu M.C., Jai C., 2009. Sol-gel synthesisand characterization of hydroxyapatite nanorods. *Particuology*, 7, 466–470. DOI: 10.1016/j.partic.2009.06.008.

Lester E., Tang S.V.Y., Khlobystov A., Rose V.L., Buttery L.D., Roberts C.J., 2013. Producing nanotubes of biocompatible hydroxyapatite by continuous hydrothermal synthesis. *Cryst. Eng. Comm.*, 15, 3256–3260. DOI: 10.1039/c3ce26798a.

Lester E., Aksomaityte G., Li J., Gomez S., Gonzalez-Gonzalez J., Poliakoff M., 2012. Controlled continuous hydrothermal synthesis of cobalt oxide (Co_3O_4) nanoparticles. *Prog. Cryst. Growth Charact. Mater.*, 58, 3–13. DOI: 10.1016/j.pcrysgrow.2011.10.008.

Pham T.T.T., Phuong N.T., Pham T.N., Dinh T.M.T., 2013. Impact of physical and chemical parameters on the hydroxyapatite nanopowder synthesized by chemical precipitation method. *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, 4, 035014. DOI: 10.1088/2043-6262/4/3/035014.

Ponomareva N.I., Poprygina T.D., Karpov S.I., Lesovoi M.V., Agapov B.L., 2010. Microemulsion method for producing hydroxyapatite. *Russ. J. Gen. Chem.*, 80, 1070–3632. DOI: 10.1134/S1070363210050063.

Rodríguez-Clemente R., López-Macipe A., Gómez-Morales J., Torrent-Burgués J., Castaño V.M., 1998. Hydroxyapatite precipitation: A case of nucleation-aggregation-agglomeration-growth mechanism. *J. Eur. Ceram. Soc.*, 18, 1351–1356. DOI: 10.1016/S0955-2219(98)00064-8.

Sadat-Shojai M., Khorasani M.T., Dinpanah-Khoshdargi E., Jamshidi A., 2013. Synthesis methods for nanosized hydroxyapatite with diverse structures. *Acta Biomater.*, 9, 7591–7621. DOI: 10.1016/j.actbio.2013.04.012.

Suchanek W., Yoshimura M., 1998. Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. *J. Mater. Res.*, 13, 1, 94–117. DOI: 10.1557/JMR.1998.0015.

Šupová M., 2015. Substituted hydroxyapatites for biomedical applications: A review. *Ceram. Int.*, 41, 9203–9231. DOI: 10.1016/-j.ceramint.2015.03.316.

Uskoković V., Uskoković D.P., 2011. Nanosized hydroxyapatite and other calcium phosphates: Chemistry of formation and application as drug and gene delivery agents. *J. Biomed. Mater. Res. Part B*, 96 B, 152–191. DOI: 10.1002/jbm.b.31746.

Vallet-Regí M., González-Calbet J.M., 2004. Calcium phosphates as substitution of bone tissues. *Prog. Solid State Chem.*, 2, 1–31. DOI: 10.1016/j.progsolidstchem.2004.07.001.

Wojasiński M., Duszyńska E., Ciach T., 2015. Lecithin-based wet chemical precipitation of hydroxyapatite nanoparticles. *Colloid. Polym. Sci.*, 293, 1561–1568. DOI: 10.1007/s00396-015-3557-0.

Yang Q., Wang J.X., Shao L., Wang Q.A., Guo F., Chen J., Gu. L., An Y.T., 2010. High throughput methodology for continuous preparation of hydroxyapatite nanoparticles in a microporous tube-in-tube microchannel reactor. *Ind. Eng. Chem. Res.*, 49, 140–147. DOI: 10.1021/ie9005436.

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