

Evaluación de los métodos de centrifugación y de tratamiento hidrotérmico para la obtención de nanopartículas de fosfatos de calcio

BIOENGINEERING – BIOMATERIALS ENGINEERING

Evaluation of methods for centrifugation and hydrothermal treatment for obtaining of calcium phosphate nanoparticles

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(Recibido: Noviembre 25 de 2015 – Marzo 04 de 2016)

Resumen

Los fosfatos de calcio son materiales cerámicos empleados en la fabricación de sustitutos óseos, ya que tienen una composición similar a la del hueso, son bioactivos, osteoconductivos y favorecen la conformación de estructuras porosas permitiendo vascularización y adherencia celular; además se destacan por su biocompatibilidad. En la actualidad son empleados para sustitución de tejido óseo en diferentes aplicaciones clínicas como cementos óseos y agentes de relleno. En el presente trabajo se obtuvieron y caracterizaron nanopartículas de diferentes fosfatos de calcio por medio de dos métodos reportados en la literatura, centrifugación y tratamiento hidrotérmico a partir de reacción de precipitación. Los polvos fueron caracterizados por DRX y FE-SEM. Los resultados demuestran que ambos métodos son adecuados para la obtención de nanopartículas de fosfatos de calcio, lo que se verifica en las micrografías FE-SEM donde se muestran partículas en escala nanométrica con diversas morfologías para la mayoría de polvos obtenidos con diámetros promedio entre 44.98 y 82.21 nm y longitudes promedio entre 123.91 y 151.48 nm. Los difractogramas para ambos métodos evidencian la presencia de diversos fosfatos de calcio con potenciales aplicaciones en ingeniería de tejido óseo, para el método hidrotérmico se encontró que la temperatura y el tiempo son factores determinantes en la estabilización de las fases. Del presente estudio se concluyó que ambos métodos de síntesis son adecuados para la obtención de nanopartículas y la estabilización de diferentes fases de fosfatos de calcio, siendo los protocolos 1, 3 y 4 los más adecuados para aplicaciones biomédicas.

Palabras clave: *Centrifugación, fosfato de calcio, nanobarras, nanopartículas, tratamiento hidrotérmico.*

Abstract

Calcium phosphates are ceramics materials used in the manufacture of bone substitutes, due to their composition which is similar to the bones, they are bioactive, osteoconductivity and works in favor of forming porous structures, allowing vascularization and cell adhesion; furthermore they stand out for their biocompatibility. They are currently employed in the replacement of bone tissue in several clinical applications such as bone cements and fillers. Within this paper, nanoparticles of different calcium phosphates were obtained and characterized by two methods reported in the literature, centrifugation and hydrothermal treatment from precipitation reaction. The powders were characterized by XRD and FE-SEM. The results prove that both methods are suitable for the obtaining of nanoparticles of calcium phosphates, which is verified in the micrographs obtained where different morphologies are observed. Particles in nanoscale for most powders obtained have average diameter between 44.98 and 82.21 nm and average length between 123.91 and 151.48 nm. Diffractograms by both methods show the presence of calcium phosphates with different potential applications in bone tissue engineering, for the hydrothermal method was found that the temperature and time are major factors during stabilizing of phases. From this study it was concluded that both synthesis methods are suitable for obtaining nanoparticles and stabilization of different phases of calcium phosphate, being protocols 1, 3 and 4 the most suitable for biomedical applications.

Keywords: *Calcium phosphate, centrifugation, hydrothermal treatment, nanorods, nanoparticles.*

1. Introduction

Calcium phosphates belong to the bioceramics family, which are also classified as biomaterials. Biomaterials are used to make a function in the human body or any living tissue, without them causing any adverse reaction can injure the host tissue. The development of these materials has increased, the need for humans to improve the life quality mainly his health, wheraby it is necessary to search for biocompatible materials; the biocompatibility is described as the biological acceptance of organism tissues as for example: interaction material/tissue, mechanical factors and degradation reaction (Amor-Márquez, 2005).

Calcium phosphates have great importance in biomaterials, considering that they represent the mineral phase and inorganic in most of human body hard tissues, and resembles their composition and structure. In recent years, congenital bone defects, traumatic and oncological have been a public health problem; due to the fact that biomaterials, specifically calcium phosphates, are promising materials for the study and development of applications where is necessary the replacement or interaction with bone tissue, since calcium and phosphorus are in their composition, represent a bone precursor phase and they are excellent donor of calcium ions. (Amor-Márquez, 2005; Combes & Rey, 2010; Zárate & Reyes, 2006; Tejada Montes et al., 2014).

For tissue regeneration, it is necessary than the materials should be more similar to possible mechanical and morphologically to the tissue, where they will be applied. Bone tissue, specifically if the bone is constituted by collagen fibers type I and calcium phosphate elongated nanocrystals called hydroxyapatite, for this reason it is very important to work in nanoscale, the nanomaterials can improve adhesion and cellular response to be more similar to natural tissue, also it was demonstrated that nanoscale particles, with sizes less than 100 nm have better cell acceptance namely adhesion and proliferation (Sadat-Shojai et al., 2012; Jiang & Zhang, 2009).

Calcium phosphates such as hydroxyapatite and tricalcium phosphate can be readily fabricated synthetically in the laboratory from different reactions, there are different routes for synthesis instead of using minerals present in nature, the

most prominent are the solid-state synthesis, wet or dry method, hydrothermal method, sol-gel methods, thermal processing in the case of tricalcium phosphate pass from α to β in two ways. However, these methods were implemented to obtain size of particles, generally in the micrometer scale and different morphologies irregulars forms, sphere, semisphere, elongated particles, homogeneous and heterogeneous forms; and in many cases the particles formed clusters or large agglomerates. For this reason it is important to research about methods which can be reduced particle sizes (Park, 2008; Combes & Rey, 2010; Carrodegua & De Aza, 2011).

There are two techniques with excellent characteristics such as centrifugation method and hydrothermal treatment. The first one involves precipitation reactions mainly to obtain an aqueous solution, which can have a posterior maturation period; after that, the material is carried out in a series of centrifugation processes at a specific rpm number, in order to obtain elongated particles, by the centrifugal force generated with the machine rotation. (Chen et al., 2008; Tan et al., 2009). The hydrothermal technique is a heterogeneous reaction in the presence of aqueous solvents under high pressure and temperature, in this process the water is usually a catalyst reaction. The method is valid for metals and ceramics, nevertheless it has not exactly defined the pressure and temperature values which favor an appropriate process, however some reports employ temperatures above 100 °C and pressures above 1 atm, mainly to achieve new synthesis phases and complex stabilization, inorganic crystal compounds growth, preparation of nanomaterials with special morphology for specific applications, materials leaching in metals extraction and the materials changes such as decomposition, corrosion and etching (Byrappa & Masahiro, 2001).

In this research two methods for obtaining calcium phosphates from wet and precipitation reaction, reported in the literature for elongated nanoscale particles were evaluated. The methods were centrifugation and hydrothermal treatment applied after wet reaction. According to reports, these methods have shown good results because it is possible to obtain hydroxyapatite nanometric particles, nonetheless it is necessary to emphasize that the reports concerning centrifuge method are very limited and the main focus is on the phase separation after the

reaction was completed. While for the hydrothermal synthesis, the reports mainly focus on obtaining hydroxyapatite phase.

2. Methodology

For development of calcium phosphate nanoparticle two methodologies found in bibliographic reports were evaluated, these have demonstrated their effectiveness in the nanoparticles production with different morphologies: centrifugation and hydrothermal treatment. The influence of method in the calcium phosphate type and the particle morphology were analyzed.

2.1 Preparation and synthesis of calcium phosphate nanoparticles

The wet method and precipitation reaction were used, and the calcium carbonate CaCO_3 and diammonium phosphate $(\text{NH}_4)_2\text{HPO}_4$ were used as precursors, it tried to maintain a molar Ca/P ratio of 1.5. For the precipitation reaction, solutions in distilled water were prepared CaCO_3 0.6 M and $(\text{NH}_4)_2\text{HPO}_4$ 0.4 M; pH was adjusted to 10 with the ammonium hydroxide addition. Subsequently, the diammonium phosphate solution 0.4 M was added drop by drop in a calcium carbonate solution 0.6 M; the addition velocity was 0.5 ml/min under constant magnetic stirring at 600 rpm (Carrodeguas & De Aza, 2011; Sadat-Shojai et al., 2013).

The centrifugation method used in this research is a modification of different methodologies found in

scientific reports (Tan et al., 2009; Chen et al., 2008). After precipitation reaction described in the preceding paragraph, the solutions were centrifuged in a 3-16 Sygma centrifuge machine for 30 min at 4000 rpm, the procedure was done twenty times. Then, after each centrifuge cycle, the material was washed with distilled water to a final pH 7. The final solutions were dried at 60 °C for 24 h and the powder obtained was divided into two samples, the first sample was characterized after drying (protocol 1) and the other sample was subjected to thermal treatment (protocol 2), the goal of thermal treatment was the stabilization phases, and it consisted in 1 h at 300 °C, 1 h at 600 °C and finally 5h at 950 °C, each ramp had a heating rate of 10 °C/min and the cooling was made in the oven.

For the hydrothermal treatment, after precipitation reaction three protocols were established with variation of time and temperature, the obtained solutions were placed in a sealed teflon autoclave, the first solution during 3 h at 120 °C (protocol 3), the second solution had the same treatment but after being dried the following thermal treatment was applied with sustain temperatures to obtain the stabilization phase: 1 hour at 100 °C, 1 h at 450 °C, 1 h at 750 °C and finally 2h at 900 °C, each ramp with rate of 10 °C/min (protocol 4), and the third solution during 2 h at 100 °C (protocol 5). The vessel was cooled in oven until approximately 60 °C, then the vessel was removed from the oven and cooled to room temperature. The solutions were washed with destiled water until reaching a pH 7.0, finally each solution was dried for 24 h at 60 °C for their further characterization (Jokic et al., 2007; García et al., 2012; Byrappa & Masahiro, 2001).

Table 1. Description of all protocols evaluated by both methods.

Centrifugation					
Protocol	Cycles of centrifugation	Revolutions per minute	Thermal treatment		
			Maximun temperature	Time	
1	20	4000 rpm	----	----	
2	20	4000 rpm	950°C	5 h	
Treatment hydrothermal					
Protocol	Temperature in autoclave	Time	Thermal treatment		
			Temperature	Time	
3	120 °C	3 h	----	----	
4	120 °C	3 h	900 °C	5 h	
5	100 °C	2 h	----	----	

2.2 Characterization of calcium phosphates

The morphology of the obtained powders were evaluated by Field Emission Scanning Electron Microscopy (FE-SEM), on a microscope JEOL JSM 6701, the particles were analyzed and measured using the software Analysis Image-Processing. The crystallinity and phases were analyzed by X-ray diffraction (XRD) using a Rigaku X-ray diffractometer equipped with a source of Cu at an angle of 2θ between 0° and 60° . Additionally the diffractograms were compared and analyzed using databases of software HighScore Plus.

3. Results and discussion

Figure 1 shows the micrographs of the powers of calcium phosphates obtained by centrifugation. In protocol 1 without thermal treatment, it has been seen that the particles formed agglomerates of irregular shapes, which, predominated elongated particles or nanorods, that is to say, the length prevailed on the diameter, with average measures 136.34 nm and 82.21 nm respectively. For protocol 2 after thermal treatment shows agglomerates, which were not possible to perceive a specific morphology because the particles were observed sintered due to the temperature employed on the thermal treatment, for this reason the particles were not measured.

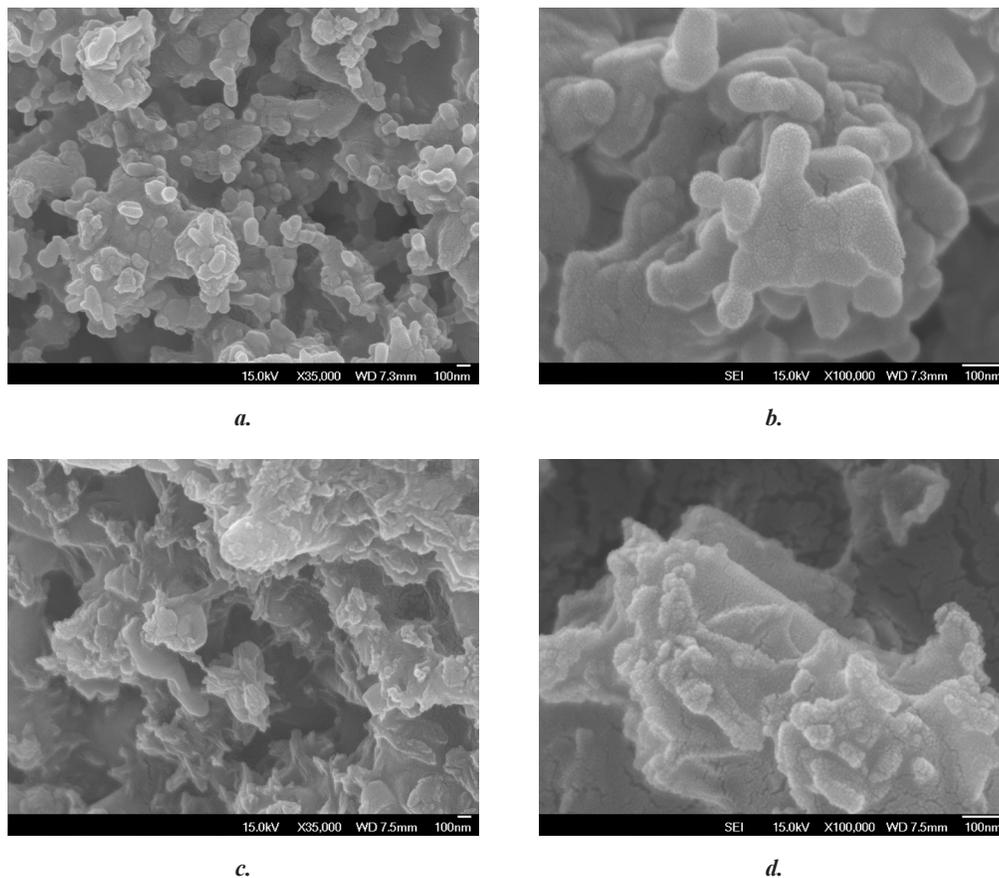


Figure 1. Micrographs FE-SEM for calcium phosphates obtained by centrifugation method in different magnifications. a) and b) protocol 1, c) and d) protocol 2.

Results obtained in this investigation showed minor sizes compared with other investigations like Chen et al. (2008), they synthesized hydroxyapatite nanorods with wet precipitation method, employing calcium nitrate tetrahydrate and ammonium phosphate dibasic like precursors of the reaction, also, they added gelatin at the aqueous solution of calcium nitrate to serve like covering of fibers, the obtained aqueous solutions were centrifuged to separate phases during 30 min at 10000 rpm and 8000 rpm. The TEM images show morphologies of elongated crystals with length between 150 and 250 nm for gelatin assay and between 350 and 500 nm for assay without gelatin (Chen et al., 2008). Tan et al. (2009) synthesized HA fibers using precipitation reaction with aqueous solutions of calcium nitrate, sodium citrate, hydroxide sodium and triammonium phosphate, the final solution was centrifuged for separating phases during 30 min at 10000 rpm and after, was dried by lyophilization, finding particles with elongated morphology with measures between 300 and 400 nm (Tan et al., 2009). For the previous results, it can be affirmed that the centrifugation method is convenient for nanoparticles formation, although majority of particles were close to nanorods, also hemispherical particles were found, highlighting the importance of the revolutions and repetition cycles, these parameters influence the elongation process of the particles until the obtaining rods in nanometric scale. Figure 2 shows diffractograms calcium phosphates obtained by centrifugation.

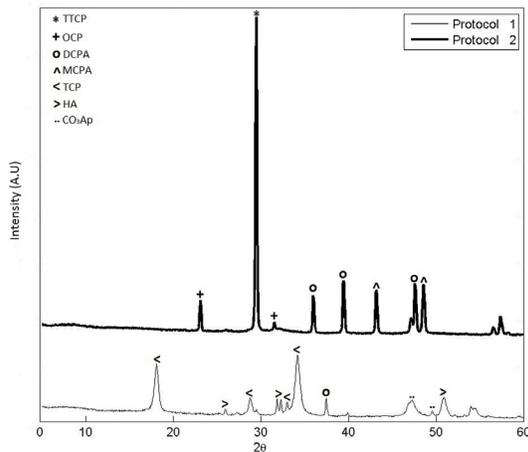


Figure 2. Diffractograms calcium phosphates obtained by centrifugation.

For protocol 1 tricalcium phosphate can be seen (TCP) JCPD 09-0169, finding a main peak approximately in $2\theta=34.3^\circ$ and secondary peaks and minor intensity, also is evident the presence of other phases corresponding to other calcium phosphates like carbonapatite (CO_3Ap) JCPDS 09-272, hydroxyapatite (HA) JCPDS 73-0294 and anhydrous dicalcium phosphate (DCPA) JCPDS 70-1425. For protocol 2 which had a thermal treatment, is evident the other stabilization phases, finding main peak approximately in $2\theta=29.7^\circ$ corresponding to tetracalcium phosphate (TTCP) JCPD 25-1137 and some secondary peaks of octacalcium phosphate (OCP) JCPDS 74-1301, anhydrous dicalcium phosphate (DCPA) and anhydrous monocalcic phosphate (MCPA) JCPDS 09-080.

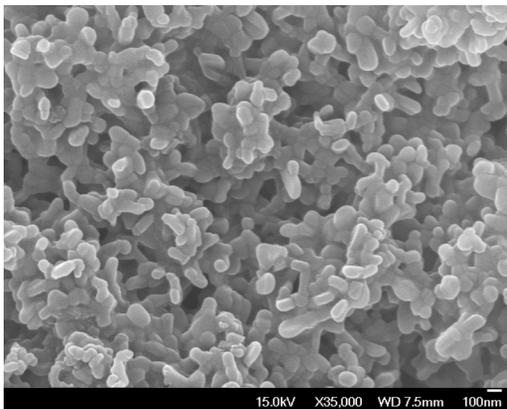
DCPA has been used amply in bone cements fabrication like composites with other calcium phosphates as α -TCP, TTCP and HA. The analysis shows biocompatibility as to cell proliferation (Ginebra et al., 2012). OCP is considered like HA precursor and help *in vivo* bone regeneration (Ito et al., 2014), also has been studied in nanometric particles shape for fabrication of scaffolds with gelatin, used in *in vivo* assays, resulting in a quick bone regeneration in defects (Miura et al., 2013). TCP has been extensively studied and clinically used like biomaterial for bone reparation, have the capability to convert in HA gradually once implanted, this property can stimulate bone remodeling (Yang et al., 2015; Duncan et al., 2014). HA is the calcium phosphate most similar to mineral component that bones have, reason why has been the most studied calcium phosphate, after several years, the implant allows complete degradation and has been used principally in bone fillers and small parts replacement of bones (Park, 2008; Kokubo, 2008). TCP is a important component of bone cements, dental composites and scaffolds for tissue engineering, is the most alkaline among all calcium phosphates and therefore has a potencial used like dental caries inhibitor (Jokanović & Čolović, 2014), also is used with HA and TCP in coating of metallic prosthesis, and its latest applications have been directed to use with composite materials like polymers reinforcement (Moseke & Gbureck, 2010). Likewise, carbonapatite for being HA with group OH^- or PO_4^{3-} , replaced for CO_3^{2-} , also has been used

in biomedical applications for bone restoration and tissue engineering (Resende et al., 2006). MCPA is frequently used in biomedical applications, but the main use is agricultural applications, for example fertilizers (Dorozhkin & Epple, 2002).

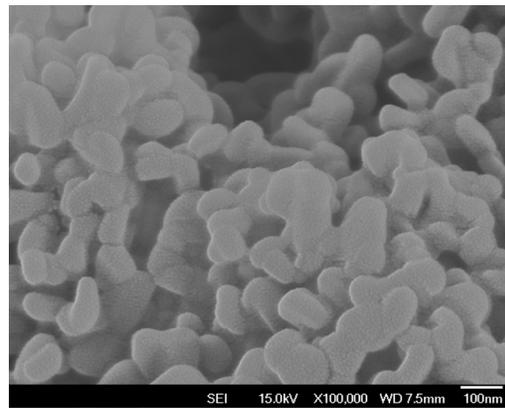
Each one of different obtained calcium phosphates have different Ca/P relation and at the same time the solubility constant K_{ps} varies, providing a diverse chemical behavior in contact with tissues, these characteristics combined with excellent properties like osteoinduction, reabsorption and tissue response, it makes them attractive for clinical applications (Fan et al., 2013; Dorozhkin, 2010).

The FE-SEM micrograph of the obtained powders from protocols 3 to 5 are shown in Figure 3. The par-

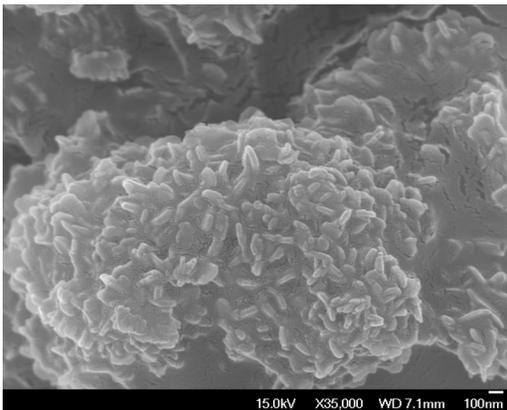
ticles are in big agglomerates, in these agglomerates was possible to distinguish nanoparticles of different morphology, for protocol 3 there are several morphologies with predominance of nanorods, in these structures, the length predominated over diameter, the measured values were the average diameter of 49.98 nm and average length of 123.91 nm. For protocol 4 there are agglomerates with prevalence of nanorods with strong bond due to thermal treatment, where a sintering process started that affected the size and morphology of the nanorods, with sizes in average diameter in 74.89 nm and average length 151.49 nm. For protocol 5 the obtained particles do not have a clear morphology, being as they are in agglomerates with irregular geometries like thin flakes with nanometric width.



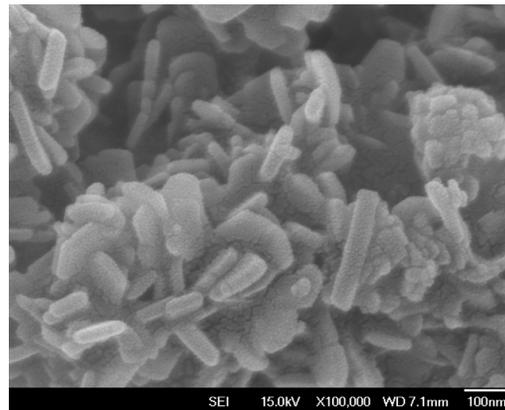
a.



b.



c.



d.

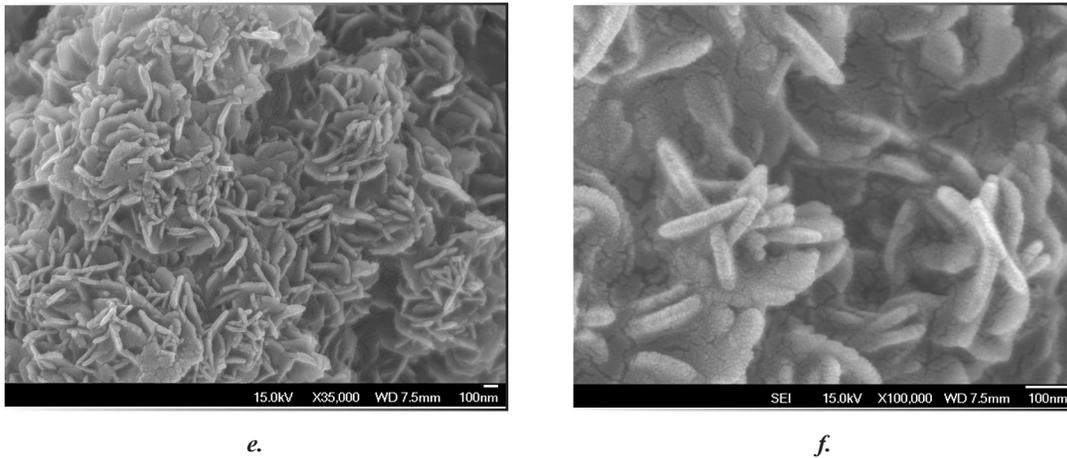


Figure 3. FE-SEM micrographs with several increases from calcium phosphates obtained through hydrothermal synthesis. a) and b) Protocol 3, c) and d) Protocol 4, e and f) Protocol 5.

The results in the hydrothermal synthesis process, show the temperature and time influence, these results demonstrate as morphology of particles are clearly affected for the variations of both parameters, which was evidenced in the protocols 3 and 5. This same result was obtained for Sun et al. (2007) who synthesized microemulsions of HA nanoparticles under hydrothermal conditions at 160 °C by 12 h. They also evaluated the pH between 7 and 11 noticed its effect above morphology, they found that in values for low pH, the particles were thinner and elongated, while for high pH the size particles were reduced. The particles in the protocol 3 had the same behavior, where at pH 10 the particles were thick and short (Sun et al., 2007). Nathanael et al. (2011) manufactured HA nanofibers with hydrothermal treatment at 180 °C by 24 h (Nathanael et al., 2011). Zhao et al. (2014) synthesized HA nanofibers, through wet reaction with hydrothermal treatment between 120-180 °C by 3-24 h (Zhao et al., 2014). These researchers corroborated that obtaining calcium phosphates nanorods is reached with temperatures above to 120 °C.

The superficial energy is a parameter which govern the particles shape, i.e. if are spherical, rods, flakes or other shapes. That energy should be minimum to give the possibility of having crystal in equilibrium with environment. In natural systems, the crystals growth is given by means of the union from atom to

atom, by adding organic templates or inorganic and by dissolution of unsteady phases or by precipitation of the more stable phase (Banfield et al., 2000). The energy generated during hydrothermal synthesis is necessary for nanoparticles formation to have enough energy to break the bonds, create divisions and produce new surfaces; during synthesis the superficial area minimizes the electromotive force in materials. Whereas is lower than the relationship between surface and volume, then material surface energy is reduced (Cao et al., 2010). In order to decrease the surface energy, the crystallization can happen in shape of rods or spheres like took place in this research.

In figure 4 shows X-ray diffraction patterns of calcium phosphates obtained by hydrothermal method. Protocols 3 and 4 had the same hydrothermal treatment (3 h at 120 °C), the results of HA were since main peaks are in $2\theta=31.7^\circ$, 32.2° y 32.9° , secondary peaks in $2\theta=25.9^\circ$, 34° , 39.7° , 46.7° y 49.4° and others peaks with lower intensity in $2\theta=28.88^\circ$ and 53.2° , protocol 4 has a higher crystallinity due to later thermic treatment, this crystallinity is shown in the diffraction peaks. In the protocol 5 carried out a hydrothermal treatment with the lowest time and temperature than protocols 3 and 4, demonstrating HA presence in formation process because in about $2\theta=31.7^\circ$, 32.2° y 32.9° there

is an amorphous phase coinciding with the HA main peaks, besides, are some secondary peaks are shown and others with lower intensity of HA as well. However, in figure 4 also shows that the main peaks and other peaks with lower intensity are of calcium carbonate, which suggest that used precursors had an incomplete reaction, maybe this is due to the pressure into reactor is low, because

there is not enough vapor owing to the low water temperature and is in the boiling limit. Moreover, protocol 5 had only two hours with hydrothermal treatment, therefore the reaction was inhibited, thus an incomplete nucleation of crystal was done and a poor stabilization of calcium phosphates (Byrappa & Masahiro, 2001).

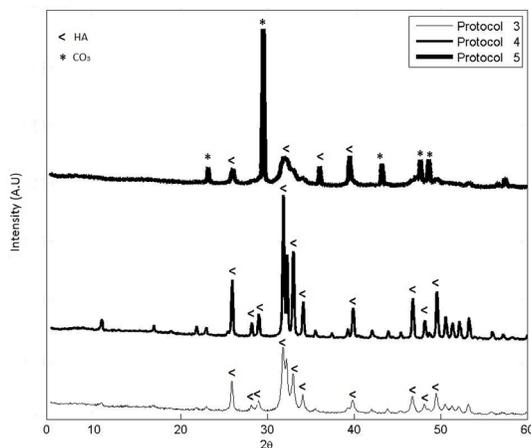


Figure 4. XRD patterns obtained to calcium phosphates through hydrothermal synthesis.

Table 2. Summary protocols and results.

Protocol	Centrifugation		Hydrothermal Treatment		
	1	2	3	4	5
Morphology	Nanorods and nanoparticles	Irregular	Nanorods and nanoparticles	Nanorods	Flakes
Phases	TCP, CO ₃ Ap, HA and DCPA	TTCP, OCP, DCPA and MCPA	HA	HA	HA and CO ₃
Average length	136.34 nm	---	123.91 nm	151.49 nm	---
Average diameter	82.21 nm	---	49.98 nm	74.89 nm	---

In table 2 shows a summary of protocols with obtained results about morphology and stabilized phases.

As is noted in table 2, both centrifugation techniques and hydrothermal treatment are adequate to obtain calcium phosphate, however is necessary to discard protocol 2 and 5: the protocol 2 because it has MCPA

but this calcium phosphate is not conventionally used in medical applications, and these applications are main focus of evaluated materials in this research. The protocol 5 because it did not have a complete reaction whereby had calcium carbonate in its final phases, and this carbonate is not suitable as a biomaterial. On the other hand, in protocols

1, 3 and 4 was possible to obtain particles with appropriate morphology and chemical composition, for later fabrication of scaffolds, bone substitutes, bone cement among others (Carrodeguas & De Aza, 2011; Dorozhkin & Epple, 2002).

4. Conclusions

According to the observed results in micrographs is possible concluding that the hydrothermal method as well as centrifugation allow to obtain nanoparticles with several calcium phosphates and when the parameters associated with each technique are modified, can achieve different morphologies.

The stabilized phases of calcium phosphates, except MCPA, they indicate a suitable formation of bioceramics which can be used as biomaterials, mainly for applications in bone tissue engineering, nevertheless to get monophasic or biphasic phosphates is necessary to know chemical reactions involved and the nature of precursors.

The nanorods and nanoparticles obtained in protocols 1, 3 and 4 are the most adequate to use in several medical applications, such as scaffolds, cements, substitutes and fillers of bone cavities; these applications are defined according to the kind of synthesized calcium phosphate.

5. Acknowledgments

The authors wish to thank the Biomaterials Research Group BIOMAT of the University of Antioquia for funding a part of this research, the University of Antioquia -CODI- Research Committee for funding the development of the project PR13-2-02 and to Lina Paola Higuera González for her collaboration.

6. References

Amor, A. (2005). Los materiales y su biocompatibilidad: Hidroxiapatita. *Materiales Avanzados* 3 (4), 44-48.

Banfield, J.F., Welch, S., Zhang, H., Ebert, T. & Penn, R.L. (2000). Aggregation-based crystal growth and microstructure development in natural

iron oxyhydroxide biomineralization products. *Science (New York, N.Y.)* 289 (5480), 751-754.

Byrappa, K. & Masahiro, Y. (2001). *Handbook of hydrothermal technology. A Technology for Crystal Growth and Materials Processing*. W. Andrew, ed., New York: Noyes.

Cao, H., Zhang, L., Zheng, H. & Wang, Z. (2010). Hydroxyapatite nanocrystals for biomedical applications. *Journal of Physical Chemistry C* 114 (43), 18352-18357.

Carrodeguas, R.G. & DeAza, S. (2011). α -Tricalcium phosphate: synthesis, properties and biomedical applications. *Acta Biomaterialia* 7 (10), 3536-3546.

Chen, M., Tan, J., Lian, Y. & Liu, D. (2008). Preparation of Gelatin coated hydroxyapatite nanorods and the stability of its aqueous colloidal. *Applied Surface Science* 254 (9), 2730-2735.

Combes, C. & Rey, C. (2010). Amorphous calcium phosphates: synthesis, properties and uses in biomaterials. *Acta Biomaterialia* 6 (9), 3362-3378.

Dorozhkin, S. (2010). Bioceramics of calcium orthophosphates. *Biomaterials* 31 (7), 1465-1485.

Dorozhkin, S. & Epple, M., (2002). Biological and Medical Significance of Calcium Phosphates. *Angewandte Chemie International Edition* 41 (17), 3130-3146.

Duncan, J., Macdonald, J., Hanna, J., Shirotsaki, Y., Hayakawa, S., Osaka, A., Skakle, J. & Gibson, I. (2014). The role of the chemical composition of monetite on the synthesis and properties of α -tricalcium phosphate. *Materials Science & Engineering C, Materials for Biological Applications* 34, 123-129.

Fan, R., Zhou, L., Song, W., Li, D., Zhang, D., Ye, R., Zheng, Y. & Guo, G. (2013). Preparation and properties of g-TTCP/PBS nanocomposites and its in vitro biocompatibility assay. *International Journal of Biological Macromolecules* 59, 227-234.

Ginebra, M.P., Canal, C., Espanol, M., Pastorino, D. & Montufar, E.B. (2012). Calcium phosphate

- cements as drug delivery materials. *Advanced Drug Delivery Reviews* 64 (12), 1090-1110.
- Ito, N., Kamitakahara, M., Yoshimura, M. & Ioku, K. (2014). Importance of nucleation in transformation of octacalcium phosphate to hydroxyapatite. *Materials Science and Engineering C* 40, 121-126.
- Jiang, D. & Zhang, J. (2009). Calcium phosphate with well controlled nanostructure for tissue engineering. *Current Applied Physics* 9 (3), 252-256.
- Jokanović, V. & Čolović, B. (2014). Synthesis of TTCP by using inverse micelle method. *Materials Chemistry and Physics* 143 (3), 1481-1488.
- Kokubo, T. ed. (2008). *Bioceramics and their clinical applications*. Boston, USA: JMM.
- Miura, K., Anada, T., Honda, Y., Shiwaku, Y., Kawai, T., Echigo, S., Takahashi, T. & Suzuki, O. (2013). Characterization and bioactivity of nano-submicro octacalcium phosphate/gelatin composite. *Applied Surface Science* 282, 138-145.
- Moseke, C. & Gbureck, U., (2010). Tetracalcium phosphate: Synthesis, properties and biomedical applications. *Acta Biomaterialia* 6 (10), 3815-3823.
- Nathanael, A., Mangalaraj, D., Hong, S.I. & Masuda, Y. (2011). Synthesis and in-depth analysis of highly ordered yttrium doped hydroxyapatite nanorods prepared by hydrothermal method and its mechanical analysis. *Materials Characterization* 62 (12), 1109-1115.
- Park, J. (2008). *Bioceramics Properties. Characterizations and Applications*. USA: Springer.
- Resende, N.S., Nele, M. & Salim, V.M. (2006). Effects of anion substitution on the acid properties of hydroxyapatite. *Thermochimica Acta* 451 (1-2), 16-21.
- Sadat-Shojai, M., Khorasani, M.T., Dinpanah-Khoshdargi, E. & Jamshidi, A. (2013). Synthesis methods for nanosized hydroxyapatite with diverse structures. *Acta Biomaterialia* 9 (8), 7591-621.
- Sadat-Shojai, M., Khorasani, M.T. & Jamshidi, A. (2012). Hydrothermal processing of hydroxyapatite nanoparticles - A Taguchi experimental design approach. *Journal of Crystal Growth* 361 (1), 73-84.
- Sun, Y., Guo, G., Tao, D. & Wang, Z. (2007). Reverse microemulsion-directed synthesis of hydroxyapatite nanoparticles under hydrothermal conditions. *Journal of Physics and Chemistry of Solids* 68 (3), 373-377.
- Tan, J., Chen, M. & Xia, J. (2009). Water-dispersible hydroxyapatite nanorods synthesized by a facile method. *Applied Surface Science* 255 (21), 8774-8779.
- Tejeda-Montes, E., Klymov, A., Nejadnik, M., Alonso, M., Rodriguez-Cabello, J.C., Walboomers, X. & Mata, A. (2014). Mineralization and bone regeneration using a bioactive elastin-like recombinamer membrane. *Biomaterials* 35 (29), 8339-8347.
- Yang, K., Zhang, J., Ma, X., Ma, Y., Kan, C., Ma, H., Li, Y., Yuan, Y. & Liu, C. (2015). β -Tricalcium Phosphate/Poly(glycerol sebacate) Scaffolds with Robust Mechanical Property for Bone Tissue Engineering. *Materials Science and Engineering: C* 56, 37-47.
- Zárate, B. & Reyes, A. (2006). Injertos óseos en cirugía ortopédica. *Cirugía y Cirujanos* 74 (3), 217-222.
- Zhao, X.Y., Zhu, Y.J., Lu, B.Q., Chen, F. Qi, C. Zhao, J. & Wu, J. (2014). Hydrothermal synthesis of hydroxyapatite nanorods using pyridoxal-5'-phosphate as a phosphorus source. *Materials Research Bulletin* 55, 67-70.



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