The pH Level Influence on Hydroxyapatite Phase Composition Synthesized with Hydrothermal Method

K Chuprunov\textsuperscript{1}, A Yudin\textsuperscript{1}, D Lysov\textsuperscript{1}, E Kolesnikov\textsuperscript{1}, D Kuznetsov\textsuperscript{1}, D Leybo\textsuperscript{1}, I Ilinykh\textsuperscript{1} and A Godymchuk\textsuperscript{2,3}

\textsuperscript{1}Department of Functional Nanosystems and High-Temperature Materials, National University of Science & Technology "MISIS", 4 Leninsky avenue, Moscow 119049, Russia
\textsuperscript{2}Materials Science Department, Tomsk Polytechnic University, 30 Lenina street, Tomsk 634050, Russia
\textsuperscript{3}Chemistry Environmental Laboratory, Tobolsk Complex Scientific Station, Ural Branch of the Russian Academy of Science, 15 Akademian Osipov Street, Tobolsk 626152, Russia

E-mail: yudin@misis.ru

Abstract. This paper reports the pH level influence on hydroxyapatite phase composition synthesized with hydrothermal method in Ca(OH)\textsubscript{2}-H\textsubscript{3}PO\textsubscript{4}, Ca(NO\textsubscript{3})\textsubscript{2}-(NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}-NH\textsubscript{4}OH, Ca(OH)\textsubscript{2}-NH\textsubscript{4}H\textsubscript{2}PO\textsubscript{4}. The obtained samples were studied with X-Ray diffraction, Fourier Transform Infrared (FTIR) and Raman spectroscopy. The one phase Ca\textsubscript{5}H\textsubscript{2}O\textsubscript{13}P\textsubscript{3} high crystallinity hydroxyapatite was synthesized with hydrothermal method at pH equal to 11. The crystallinity degree was calculated from the X-Ray diffraction pattern and became 0.96. The increasing pH level from 7 to 11 provides obtaining one phase hydroxyapatite at pH level 11 instead the two phase Ca\textsubscript{9.04}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{1.68}, CaHPO\textsubscript{4} at pH level 9 and CaPO\textsubscript{3}(OH), Ca(OH)\textsubscript{2} at pH level 7.

1. Introduction

Biomaterials attract increasing interest owing to their applicability in a field of medicine [1]. The hydroxyapatite materials due to specific properties [2] find application as a biomedical material [3]. The hydroxyapatite properties, like crystallinity degree, crystal dispersity, porosity and stoichiometry make hydroxyapatite [4] widely used as scaffold for tissue engineering [4], fillers for healing defect in bones [5] and drug delivery systems [6].

Ceramic materials are a class of biomaterials used in biomedical devices. Ceramics based materials are widely used as implant materials due to their ability to be fabricated into a variety of shapes, along with their variable porosity, bioactive properties in the body and high compressive strength [7]. The calcium phosphates and hydroxyapatite have close chemical composition to human bone minerals [8]. Ceramic materials based on calcium phosphate and hydroxyapatite demonstrate high biocompatibility, perfect osteoconduction and bioactivity [9]. Also HAp finds application as biocompatible cover for implants made of metal materials [10]. The hydroxyapatite Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} is the most thermodynamically stable in its crystalline state. HAp could be integrated with bones without any local toxicity and inflammation of body response [11].
The synthesis methods and synthesis parameters provide the phase composition, morphological and dispersity control of the obtained materials. The two ways of hydroxyapatite fabrication exist extracting from nature sources and synthetic by different methods dry, wet and high temperature methods [9]. The HAp could be synthesized by the various methods including solid-state method [12], mechanochemical [13], chemical precipitation [14], hydrolysis [15], sol-gel [16], hydrothermal [17], emulsion [18], sonochemical [19], combustion [20], pyrolysis [21], synthesis from biogenic sources [22] and combined methods [23] methods. Any method provides its own special phase composition, dispersion and morphology of the hydroxyapatite. The hydroxyapatite properties depend on the synthesis parameters.

The aim of the presented paper is to estimate the initial precursor influence in precursor systems Ca(OH)$_2$H$_3$PO$_4$, Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_2$OH and Ca(OH)$_2$-NH$_4$H$_2$PO$_4$ on the hydroxyapatite phase composition, dispersion and crystallinity degree. Also, the pH level influence during synthesis process described at [24, 25] on the hydroxyapatite samples properties.

This paper discuss the first time using of chemical precipitation and hydrothermal synthesis method to obtain the calcium phosphate and hydroxyapatite powders. The synthesized hydroxyapatite powder will be used as an initial material in granulation process to produce hydroxyapatite spherical granules for 3D printing of the ceramic implants.

2. Materials and Methods

2.1. Hydrothermal synthesis of hydroxyapatite samples

Calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$•4H$_2$O, AR Grade), diammonium hydrogen phosphate (NH$_4$)$_2$HPO$_4$, AR Grade) ammonium hydroxide (NH$_2$OH, AR Grade), calcium hydroxide (Ca(OH)$_2$, AR Grade), phosphoric acid (H$_3$PO$_4$ AR Grade) produced by Reachem Co. (Russia) were used as initial precursors for obtaining HAp samples. The HAp samples were synthesized in three systems: Ca(OH)$_2$H$_3$PO$_4$, Ca(OH)$_2$-NH$_4$H$_2$PO$_4$ and Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_2$OH. In Ca(OH)$_2$-H$_3$PO$_4$ system calcium hydroxide was added to the phosphoric acid. The Ca/P element ratio was calculated equal to 1.67. Calcium hydroxide was added to diammonium hydrogen phosphate in system Ca(OH)$_2$-NH$_4$H$_2$PO$_4$. In Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_2$OH system the diammonium hydrogen phosphate solution was added to the calcium nitrate solution and ammonium hydroxide was used to increase pH level. The obtained suspensions were treated in stainless steel autoclave at 250 °C with 150 bar during 24 hours. The obtained suspensions were washed with distilled water till pH level became equal to 7 by decantation method.

The next step was samples washing suspensions with decantation method until pH level became equal to 7 using the distilled water. The vacuum filtration system separated the HAp samples from the dispersant liquid. The HAp samples dried at 90 °C during 12 hours in the drying chamber SNOL HS-80-01. The obtained samples are presented in table 1.

| Sample | Synthesis condition |
|--------|---------------------|
| HAp 1.1 | Ca(OH)$_2$H$_3$PO$_4$ | 7 |
| HAp 1.2 | Ca(OH)$_2$-NH$_4$H$_2$PO$_4$ | 7 |
| HAp 1.3 | Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_2$OH | 7 |
| HAp 1.4 | Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_2$OH | 9 |
| HAp 1.5 | Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_2$OH | 11 |

2.2. Analytical methods to study hydroxyapatite samples

Phase composition and crystallinity degree were characterized with X-Ray diffractometer Scientific Instruments Difray 401 (Russia) with Cr Kα emission (wave length of 2.2909 Å) using PDF-2004 and
COD (Crystallography Open Database). The diffraction patterns were detected in the range from 14 to 140° 2θ with the interval 58 degrees and equilibrium time 300 second. The crystallinity degree (Xc) was calculated with the equation (1). The V_{112/300} meant the minimum intensity between two intensity peaks reflected from the crystallography planes (112) and (300) consequently. The I_{300} meant the maximum intensity for the peak reflected from crystallography plane (300).

\[ X_c = 1 - \frac{V_{112/300}}{I_{300}} \]

Also, the Fourier Transform Infrared spectroscopy (FTIR spectroscopy) Thermo Scientific Nicolet 380 (USA) and Raman spectroscopy Thermo Scientific DXR Microscope (USA) with OMNIC operation system were used to determine hydroxyapatite samples phase composition. The specters determination was carried out via book [26].

3. Results and discussion

The Figure 1 demonstrates XRD patterns of the hydroxyapatite samples obtained via hydrothermal method. The HAp 1.1 sample is one phase Ca₅(PO₄)₂ [00-009-0169]. In the sample HAp 1.2 CaHPO₄ [00-001-0653] and hydroxyapatite Ca₁₀(PO₄)₆(OH)₂ [01-72-1243] phases were detected. Also, the HAp 1.3 sample consists two phases Ca(OH)₂ [00-050-0008] and CaPO₃(OH) [00-009-0080]. The HAp 1.4 sample has two phase composition with CaHPO₄ [00-003-0423] and hydroxyapatite phase Ca₉.0₄(PO₄)₆(OH)₁.6₈ [01-086-1203]. The sample HAp 1.5 is one phase sample hydroxyapatite Ca₃H₂O₁₃P₃ [96-900-2215].

![Figure 1. XRD Patterns of the hydroxyapatite samples.](image)

The phase composition of the HAp samples was presented in table 2.
Table 2. HAₚ samples phase composition.

| Sample | Phase composition |
|--------|-------------------|
| HAₚ 1.1 | Ca₃(PO₄)₂ [00-009-0169] |
| HAₚ 1.2 | CaHPO₄ [00-001-0653], Ca₁₀(PO₄)₆(OH)₂ [01-72-1243] |
| HAₚ 1.3 | Ca(OH)₂ [00-050-0008], Ca₃PO₄(OH) [00-009-0080] |
| HAₚ 1.4 | CaHPO₄ [00-003-0423], Ca₀.₉₄(PO₄)₆(OH)₁.₆₈ [01-086-1203] |
| HAₚ 1.5 | Ca₅H₂O₁₃P₃ [96-900-2215] |

The hydroxyapatite crystallinity degree can be analyzed only for the HAₚ 1.5 sample due to HAₚ 1.1 and HAₚ 1.3 haven’t hydroxyapatite phase. The HAₚ 1.2 and HAₚ 1.4 consists more than one phase. The presence of the second phase makes crystallinity degree analysis impossible due to intensity peaks of the second phase are overlaying on diffraction patterns. The hydroxyapatite samples crystallinity degree was calculated for the sample HAₚ 1.5 using figure 2. The figure 2 presents the HAₚ 1.5 remaster XRD pattern without background.

Figure 2. Hydroxyapatite HAₚ 1.5 XRD pattern without background.

The crystallinity degree calculated via equation (1) was equal to 0.96. This fact provides the ability to use high crystallinity hydroxyapatite as a material for the biomedical application. The results of FTIR and Raman spectroscopy are presented in figure 3.
The FTIR specters of the HAp obtained samples demonstrate peaks on 890, 960, 1020 and 1095 cm\(^{-1}\) reciprocal wavelength. Peak at 890 cm\(^{-1}\) refer to vibrations of OH\(^-\). Peak at reciprocal wavelength 960 cm\(^{-1}\) leads to valence oscillations of phosphate groups (PO\(_4^{3-}\)). Peaks at 1020 and 1095 cm\(^{-1}\) lead to phosphate group deformation oscillations.

Raman specters show the oscillation mode at 960 cm\(^{-1}\) determined as phosphate group.

The HAp 1.1 FTIR specter has additional three peaks which could be described as out-of-plane oscillations of carbonate groups (CO\(_3^{2-}\)) and peaks at 1340 and 1400 cm\(^{-1}\) described as carbonate group oscillations. The same results are on the FTIR specter of the HAp 1.3 sample.

The FTIR and Raman data support the results of X-Ray diffraction analysis.

The presented X-RAY diffraction FTIR and Raman spectroscopy data show the good perspectives of the Ca(NO\(_3\))\(_2\)-(NH\(_4\))\(_2\)HPO\(_4\)-NH\(_4\)OH precursor system using to obtain the one phase high crystallinity hydroxyapatite powder. Also, the combined chemical precipitation and hydrothermal synthesis method makes possible the hydroxyapatite synthesis. The chemical precipitation at pH level
11 with following hydrothermal synthesis method in stainless steel at 250 °C and 150 bar provide the one phase hydroxyapatite powder with high (0.96) crystallinity degree.

4. Conclusion
The high crystallinity one phase hydroxyapatite Ca$_5$H$_2$O$_{13}$P$_3$ was synthesized used hydrothermal method. The hydroxyapatite samples synthesized using three systems Ca(OH)$_2$-H$_3$PO$_4$, Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_4$OH, Ca(OH)$_2$-NH$_2$HPO$_4$-, shows the perspective of using Ca(NO$_3$)$_2$-(NH$_4$)$_2$HPO$_4$-NH$_4$OH to obtain one phase high crystallinity hydroxyapatite. The crystallinity was determined at for sample HAp 1.5 was equal to 0.96.

Acknowledgements
The authors acknowledge the financial support of the Ministry of Education and Science of the Russian Federation (Project no. RFMEFI57517X0168).

References
[1] Pu’ad N M, Koshy P, Abdullah H Z, Idris M I and Lee T C 2019 Syntheses of hydroxyapatite from natural sources Helioton 5 In press
[2] Okada M and Matsumoto T 2015 Synthesis and modification of apatite nanoparticles for use in dental and medical applications Jpn. Dent. Sci. Rev. 51 85
[3] Okada M 2012 Hydroxylapatite nanoparticles: fabrication methods and medical applicationsSci. Technol. Adv. Mater. 13 6
[4] Szczes A, Ho L, Chibowski E 2017 Synthesis of hydroxyapatite for biomedical applications Adv. Colloid Interface Sci. 1
[5] G M Raghavendra, K Varaprasad and T Jayaramudu 2015 Chapter 2 - biomaterials: design, development and biomedical applications, in: S. Thomas, Y. Grohens, N. Ninan (Eds.), Nanotech. Appl. for Tissue Engineering, William Andrew Publishing, Oxford, 21
[6] N Lertcuma, P Jaita, S Manotham, P Jarupoom, S Eitssayeam, K Pengpat and G Rujijanagul 2016 Properties of calcium phosphates ceramic composites derived from natural materials Ceram. Int. 10638
[7] M Sadat-Shojai, M T Khorasani, E Dinpanah-Khoshdargi and A Jamshidi 2013 Synthesis methods for nanosized hydroxyapatite with diverse structures Acta Biomater. 9 7591
[8] A A Hendi 2017 Hydroxyapatite based nanocomposite ceramics J. Alloy. Comp. 712 147
[9] P O’Hare, B J Meenan, G A Burke, G Byrne, D Dowling and J A Hunt 2010 Biological responses to hydroxyapatite surfaces deposited via a co-incident microblasting technique Biomaterials 31 515
[10] Teshima K, Lee S H, Sakurai M, Kameno Y, Yubuta K, Suzuki T, et al. 2009 Wellformed one-dimensional hydroxyapatite crystals grown by an environmentally friendly flux method Cryst Growth Des 9 2937
[11] Mochales C, Wilson RM, Dowker SEP, Ginebra MP. 2011 Dry mechano-synthesis of nanocrystalline calcium deficient hydroxyapatite: structural characterisation. J Alloys Compd, 509, 7389
[12] Sadat-Shojai M, Khorasani MT, Jamshidi A, Irani S 2013 Nano-hydroxyapatite reinforced polyhydroxybutyrate composites: a comprehensive study on the structural and in vitro biological properties Mater Sci Eng C 33 2776
[13] Sturgeon J L, Brown P W 2009 Effects of carbonate on hydroxyapatite formed from CaHPO$_4$ and Ca$_3$PO$_4$2O J Mater Sci: Mater Med 20 1787
[14] Montazeri N, Jahandideh R and Biazar E 2011 Synthesis of fluorapatite–hydroxyapatite nanoparticles and toxicity investigations Int J Nanomedicine 6 197
[15] Sadat-Shojai M, Khorasani M T and Jamshidi A 2012 Hydrothermal processing of hydroxyapatite nanoparticles – a Taguchi experimental design approach J Cryst Growth 361 3
[16] Ponomareva N, Poprygina T, Karpov S, Lesovoi M and Agapov B 2010 Microemulsion method for producing hydroxyapatite Russ J Gen Chem. 80 905
[17] Rouhani P, Taghavinia N and Rouhani S 2010 Rapid growth of hydroxyapatite nanoparticles using ultrasonic irradiation. Ultrason Sonochem 17 853
[18] Giardina M A and Fanovich M A 2010 Synthesis of nanocrystalline hydroxyapatite from Ca(OH)2 and H3PO4 assisted by ultrasonic irradiation Ceram Int. 36 1961
[19] Zhang J, Zhan X, Wen X, Song B, Ma L and Peng W 2009 Effects of ultrasonic and dispersants on shape and composition of hydroxyapatite by reflux method Inorg Mater 45 1362
[20] Aghayan M and Rodriguez M 2012 Influence of fuels and combustion aids on solution combustion synthesis of Bi-phasic Calcium Phosphates (BCP) Mater Sci Eng C 32 2464
[21] Itatani K, Tsugawa T, Umeda T, Musha Y, Davies I J and Koda S 2010 Preparation of submicrometer-sized porous spherical hydroxyapatite agglomerates by ultrasonic spray pyrolysis technique J Ceram Soc Jpn 118 462
[22] Huang Y C, Hsiao P C and Chai H J 2011 Hydroxyapatite extracted from fish scale: effects on MG63 osteoblast-like cells Ceram Int 37 1825
[23] Zyman Z, Goncharenko A, Rokhmistrov D and Epple M 2011 Nanocrystalline calciumdeficient hydroxyapatite prepared by a microwave-assisted solvent-free reaction. Mat-wiss u Werkstofftech 42 154
[24] Yudin A et al. 2019 Microwave treatment and pH influence on hydroxyapatite morphology and structure IOP Publishing Journal of Physics: Conference Series 1145
[25] Chuprunov K et al. 2018 The ultrasound effect on the morphological properties of hydroxyapatite MATEC Web of Conferences EDP Sciences 243
[26] Berzina-Cimdina L and Borodajenko N 2012 Research of calcium phosphates using Fourier transform infrared spectroscopy Infrared Spectroscopy-Materials Science, Engineering and Technology 12(7) 251-263