

INCUBATION-BASED HYDROXYAPATITE SYNTHESIS METHOD USING SHELLS AS Ca²⁺ SOURCE

Cosmin Pascariu^{1,2}, Bogdan-Ovidiu Taranu², Alexandra Ioana Bucur², Radu Banica², Cristina Mosoarca², Raul Bucur²

¹"Vasile Goldiș" Western University of Arad, Faculty of Pharmacy, Liviu Rebreanu street, No. 86, RO-310414, Arad, Romania

²National Institute for Research and Development in Electrochemistry and Condensed Matter, Dr. A.P. Podeanu street, No. 144, 300569, Timisoara, Romania
e-mail: b.taranu84@gmail.com

Abstract

Hydroxyapatite, the well-known calcium phosphate, was obtained on the surface of shells fragments by partial conversion of the latter, which served both as template and also provided the calcium ions required for synthesis. Confirmation of hydroxyapatite formation was acquired by recording and analyzing X-ray diffraction patterns. The degree to which the phosphate ions were consumed during the incubation-based synthesis was determined using a phosphate minicolorimeter, and the results indicate that a nucleation process takes place in the first 4 hours.

Introduction

The unique osteoconductive, osteoinductive, osseointegrative, biodegradable and corrosion protective properties of hydroxyapatite [1-5] make it one of the most widely used calcium phosphates in the biomedical field. An important quality possessed by this ceramic material is its ease of synthesis, which has led to the development of several methods for obtaining it, including the hydrothermal, solvothermal, homogeneous precipitation and electrochemical deposition techniques [6,7]. On the other side, depleted shells are an abundant renewable resource, which in some cases may represent an ecological problem [8]. The use of shells as Ca source for the synthesis of useful materials is one way to use this resource in a highly desired manner. Herein, we present an incubation-based method for obtaining the specified material that employs a significantly lower temperature than the ones used in the standard hydrothermal synthesis, for example. Furthermore, the calcium precursor is provided by the exoskeleton fragments of sea shells that also serve the role of hydroxyapatite deposition substrate. The synthesized product was characterized by X-ray diffraction and the results show the presence of hydroxyapatite. The phosphate ions consumption degree during the incubation experiments was determined as a measure of hydroxyapatite formation.

Experimental

Shells were collected from the Black Sea, washed repeatedly with distilled water and dried in the oven at 80 °C for 12 h. The fragments of shells used for synthesizing hydroxyapatite were selected by grinding and sieving, and the retained fraction is the one between 1 and 1.6 mm. The phosphate precursor was Na₂HPO₄ x 12H₂O (Reactivul București) and the calcium precursor was the CaCO₃ from the shells. Incubation experiments were performed with a Hygiena dry bath incubator and the shells together with the phosphate precursor were introduced in glass vials that were subsequently closed (Figure 1). The phosphate precursor helped to ensure the initial solution pH of 9.18. The Na₂HPO₄ x 12H₂O concentration in the solution was set so as to obtain a calcium carbonate conversion degree of maximum 5%. The

effect of the time period on the reaction between the two precursors was monitored at 90 °C, and the experiments lasted between 4 and 112 hours.



Figure 1. Glass tube containing a sample of shells, fraction 1-1.6 mm, and the phosphorus precursor solution, before the experiment

Results and discussion

After the experiments ended, the shells samples were collected, washed and dried, and they were structurally characterized *via* X-ray diffraction. The obtained spectra were analyzed in order to determine their composition. The comparison of the diffraction maxima recorded on the samples and their theoretical positions (Figure 2) indicates that during the incubation period hydroxyapatite with low crystallinity was synthesized on the surface of the exoskeletons fragments.

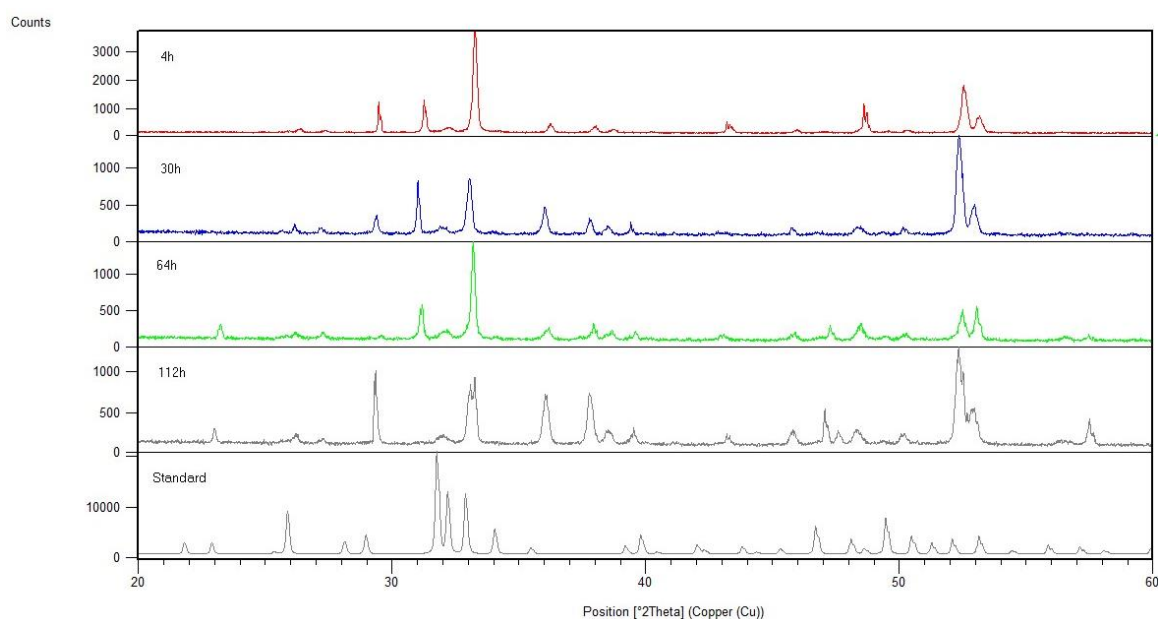


Figure 2. X-ray diffraction spectra of samples obtained at 4 h, 30 h, 48 h, and 112 h. The spectrum at the bottom is a standard from the ICDD database, file no. 9-432

Accurate identification of the presence of hydroxyapatite is difficult, due to a number of factors: the low quantity, the presence of large amounts of aragonite and calcite with high crystallinity, as well as the overlapping of hydroxyapatite maxima positions with those corresponding to the other phases present in the sample.

In order to raise the degree of confidence regarding the formation of the desired product, the sample obtained in the incubator at 112 h was further calcined at 200 °C and 350 °C, and the spectra recorded on these samples are shown in Figure 3. With the increase in temperature, the improvement of the reflections corresponding to the hydroxyapatite crystalline structure can be observed, together with a clearer delimitation of the signals and an increase in their resolution.

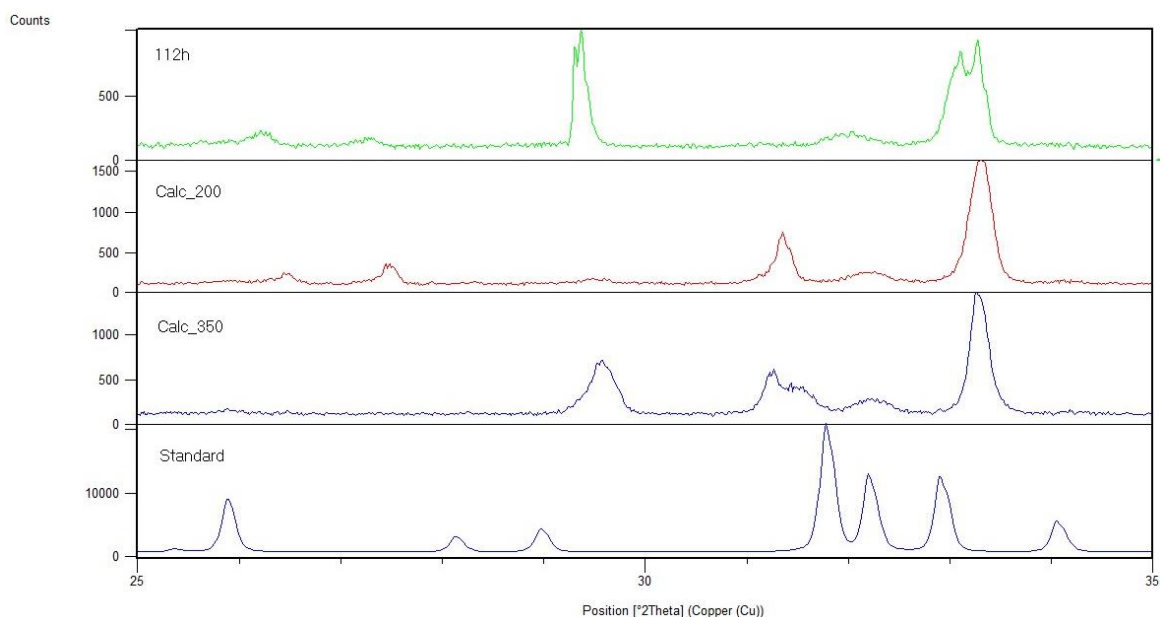


Figure 3. X-ray diffraction spectra of: the sample obtained at 112 h, the same sample calcined at 200 °C and 350 °C, and (at the bottom) the standard hydroxyapatite spectrum from the ICDD database

The degree of phosphate ions consumption was determined as a measure of hydroxyapatite formation, by first finding out the level of phosphate ions for the precursor solution and then for the solutions collected after the completion of the experiments. In order to do this, a phosphate minicolorimeter (Hanna Instruments) and the standard method of the apparatus were used. A total of three determinations were made for each sample and their arithmetic mean was calculated. After the incubator synthesis at 90 °C, for different time periods, each sample was washed repeatedly with small fractions of distilled water. These fractions were collected and the obtained solution was filtered through a 0.22 µm cellulose filter. Each resulted solution was used to fill a volumetric flask to its marking and subsequently, *via* successive dilutions, its concentration was brought in the measuring range of the apparatus. The degree of phosphate ions consumption obtained based on the performed determinations and calculations is presented in Table 1.

Table 1. Consumption degree of phosphate ions as a function of the time allocated for each experiment

Experiment duration (h)	4	30	64	112
Degree of consumption (%)	94.7	97	97.7	97.8

As can be seen, there was a high degree of consumption in all cases, but the results also show that in the first 4 incubation hours the phosphate ions concentration decreases to a much larger extent than it does after this time period. It is probable that a nucleation process takes place during these 4 hours in which most of the phosphate ions are consumed. Once this process comes to an end, the consumption rate diminishes substantially and keeps decreasing inversely proportional to the incubation time.

Conclusion

Hydroxyapatite was obtained on the surface of exoskeleton fragments of sea shells following an incubation-based synthesis method, in which the shells provided the Ca precursor. Shells also represent the substrate for the synthesis of composites with hydroxyapatite. X-ray diffraction structural analysis was used to confirm the presence of hydroxyapatite on the shells surface. The phosphate ions consumption degree during incubation was also determined and it indicates the existence of a nucleation process that takes place in the first 4 hours.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2019-2116, within PNCDI III

References

- [1] J.R. Woodard, A.J. Hildore, S.K. Lan, C.J. Park, A.W. Morgan, J.A.C. Eurell, S.G. Clark, M.B. Wheeler, R.D. Jamison, A.J.W. Johnson, *Biomaterials*. 28 (2007) 45.
- [2] L. Lin, K.L. Chow, Y. Leng, *J. Biomed. Mater. Res. A*. 89 (2009) 326.
- [3] J. Chamrad, P. Marcian, J. Cizek, *PLoS One*. 16 (2021) e0254837.
- [4] S.U. Zaman, M. Irfan, M. Irfan, N. Muhammad, M.K.U. Zaman, A. Rahim, S.-U. Rehman, *Biomed. Lett.* 6 (2020) 84.
- [5] A.A.E. Hadad, E. Peon, F.R. Garcia-Galvan, V. Barranco, J. Parra, A. Jimenez-Morales, J.C. Galvan, *Materials (Basel)* 10 (2017) 94.
- [6] G. Ma, *IOP Conf. Ser.: Mater. Sci. Eng.* 688 (2019) 033057.
- [7] A.I. Bucur, E. Linul, B.-O. Taranu, *Appl. Surf. Sci.* 527 (2020) 146820.
- [8] M. Jovic, M. Mandic, M. Sljivic-Ivanovic, I. Smiciklas, *Studia Marina* 32 (2019) 47.