

Article

STUDY OF A BIOACTIVE HYDROXYAPATITE/GELATIN COMPOSITE. PART I - SYNTHESIS AND CHARACTERIZATION OF THE MATERIAL

Paul Albu^{1,2}, Gabriela Vlase^{1}, Titus Vlase¹*

¹ West University of Timisoara, Research Center for Thermal Analysis in Environmental Problems, 16 Pestalozzi St., Timisoara, RO-300115, Romania

² „Vasile Goldiș” West University of Arad, Faculty of Medicine, Pharmacy and Dentistry, Department of Pharmacy, 86 L. Rebreanu St., 310414 Arad, Romania

ABSTRACT

A biomimetic bioactive composite based on hydroxyapatite (HA) was prepared by using inorganic precursors for the mineral structure of the composite and a gelatin aqueous solution to provide the organic matrix as template for the HA crystal growth. The samples of the HA were characterized before and after the sinterization process by FTIR, XRD, SEM and EDAX analysis. The results indicate that the crystallization process ends only after sinterization at high temperature. This sinterization process also has an effect on the size and shape of the HA crystals.

Keywords: biomimetic hydroxyapatite, FTIR, SEM, EDAX, XRD.

1. INTRODUCTION

In the last two decades an important part of the research on bone substitute materials was focused on ceramic materials, like calcium phosphates [1] because of their biocompatible, bioactive, biodegradable and osteoconductive properties [2-6].

The mineral phase of the bone tissue consists in natural hydroxyapatite crystals with a general shape of needle or rod, that are distributed within the polymeric matrix of type I collagen. Research shows that these natural nanoparticles formed in physiological

* Correspondent author: Tel: +40256/592627, Fax:, E-mails: gabriela.vlase@e-uvt.ro

environment exhibits a more dynamic response in comparison with other synthetic materials, offer with a larger crystal size [7]. Despite their small size, bone nanoparticles are stable and resist to dissolution because of the interactions that occur between the acidic peptide sequence in the extracellular matrix and the Ca^{2+} ions present in the mineral nanocrystals [8].

Synthetic bone regeneration materials do not present problems regarding immunological incompatibility, sterility or limited availability. This type of materials that are used as bone substitutes include inorganic materials (i.e. calcium phosphates), polymers or polymer-ceramics composite materials [9-11]. The ideal material that should be used in order to induce complete bone regeneration should be: osteoconductive, osteoinductive, osteogenic, biocompatible, bioactive and biodegradable [9].

Biomimetic preparation of synthetic bone materials [12] such as hydroxyapatite has received an increased attention of the researchers and so they have tried to prepare hydroxyapatite based composites using collagen, denatured collagen or other polymers as template for the biomimetic synthesis.

In the current study, the biomimetic HA synthesis was carried out by wet-chemical precipitation of inorganic precursor into an organic gelatin matrix. Many factors can influence the properties of HA obtained by this method, for example the precursors, pH value, stirring speed, temperature of preparation or ageing time.

2. EXPERIMENTAL METHOD

HA was prepared by wet-chemical precipitation method. In a round three-neck flask a gelatine aqueous solutions is added, under magnetic stirring at 50°C. After 30 min the calcium solution was added to the biopolymer mix, under continuous stirring and then the phosphate solutions was added dropwise with an $1 \text{ cm}^3 \cdot \text{min}^{-1}$ flow. The pH value was set to 11-12 by adding NH_4OH 25%. The reflux process was employed at 50°C for 3 h under continuous stirring at 300 rpm. After 3 hours the white precipitate was washed and filtered under vacuum for 20 min. The precipitate was aged at room temperature for 24h and then dried for another 48 h at 35°C. The sample batch was divided in two, so we could perform a comparison between the characterization of HA and sintered HA at 900°C.

The Fourier Transform Infrared spectra of the samples were recorded on a Spectrum 100 Perkin Elmer device by U-ATR technique. They were used to determine if there are some structural differences of HA samples before and after sinterization process.

The X-ray Diffraction diffractograms were recorded on a X'Pert Pro MPD by Philips – FEI company PANalitical BV, The Netherlands, operating at 65kV and 55 mA. This diffractograms are useful to characterize the crystallization phase of the ceramic composite before and after the sinterization process.

Scanning Electron Microscopy and EDAX investigations were performed on a Fei Quanta 250 electron microscope, at 15 and 20 kV, in order to determine the shape and crystal size of the HA sample.

3. RESULTS AND DISCUSSIONS

FTIR Spectrometry

Figures 1 and 2 show the recorded spectra of the HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, samples before and after the sinterization process. Spectrum has a peak at 3647 cm^{-1} corresponding to $-\text{OH}$ stretching vibration and a peak at 668 cm^{-1} due to bending vibration of $-\text{OH}$. Between 1209 and 1138 cm^{-1} there are several peaks associated with $\text{P}=\text{O}$ vibration. There is a clear band splitting, showing a high cristallinity of HA, between 1100 - 936 cm^{-1} there are eight peaks corresponding to stretching and bending vibrations of $(\text{PO}_4)^{3-}$ groups.

The main features observed in the spectrum for HA before sinterization and differing from the spectrum recorded after sinterization are the broad band in the range of 3250 cm^{-1} , attributed to absorbed water during the synthesis process, and the presence of only 2 peaks corresponding to $(\text{PO}_4)^{3-}$ group vibrations at 1060 cm^{-1} and 1042 cm^{-1} , respectively, showing that HA is not fully crystallized at this stage.

Figure 1: FTIR spectrum for biomimetic HA: before sinterization

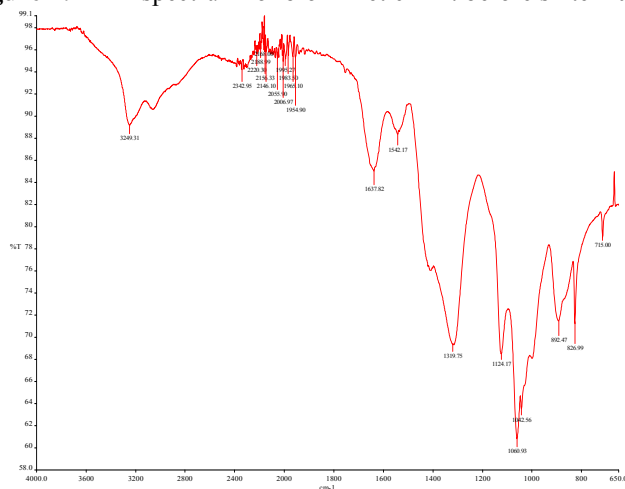
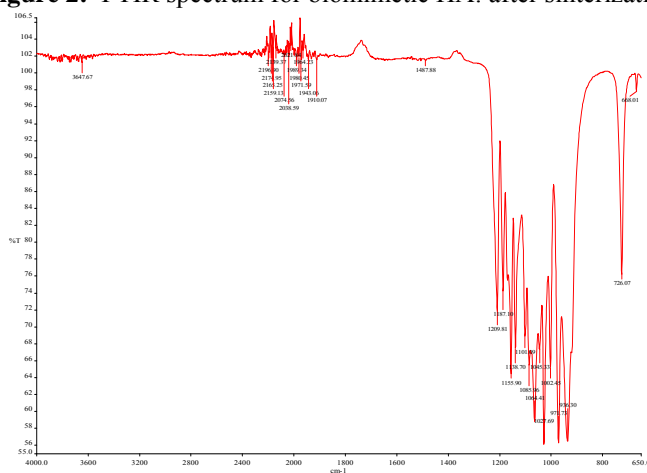


Figure 2: FTIR spectrum for biomimetic HA: after sinterization



XRD Analysis

The XRD patterns were recorded to determine if there is a higher degree of crystallinity after sinterization. The diffractograms for the sintered HA (Fig.4) show the expected increase of the crystallinity for the sintered HA. Slightly broad peaks are implying small size crystallites [4], in the range of some hundred nanometers. The pattern of HA also shows that there are several directions for the growth of HA crystallites.

Figure 3: XRD patterns for biomimetic HA: before sinterization

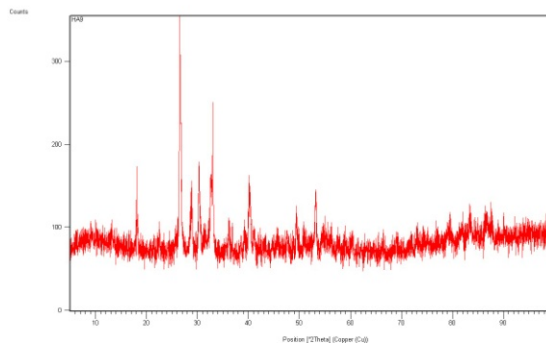
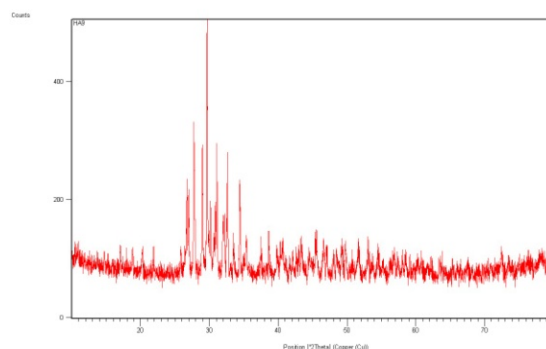


Figure 4: XRD patterns for biomimetic HA: after sinterization



SEM and EDAX analysis

SEM micrographs of the HA before and after sinterization are shown in Fig.5 and Fig.6, respectively. In the first case, due to the biopolymer matrix the crystallites have needlelike shape and with nanodimensional size. After sinterization the nanocrystallites have needlelike and spherical shape, most likely due to interaction between the organic matrix and the Ca^{2+} ions.

EDAX spectra (Fig.7) show that nitrogen atoms are present in the sample which confirms the growing of the HA particles in the biopolymer matrix. After calcination (Fig.8) the nitrogen is not present in the spectrum, which is expected because at 900°C organic matter

volatilises. Carbon cannot be measured quantitatively, because the support of the sample is also carbon based.

Figure 5: SEM image of biomimetic HA: before sinterization

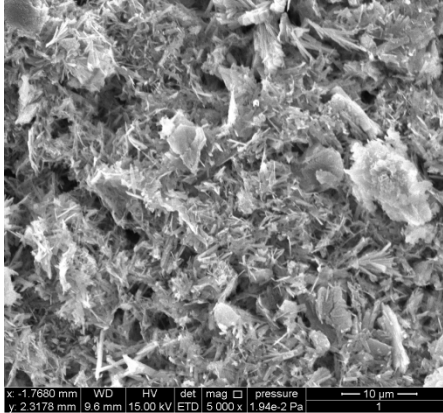


Figure 6: SEM image of biomimetic HA: after sinterization

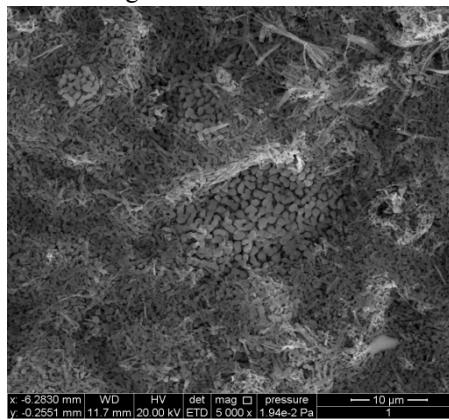


Figure 7: EDAX spectra of biomimetic HA: before sinterization

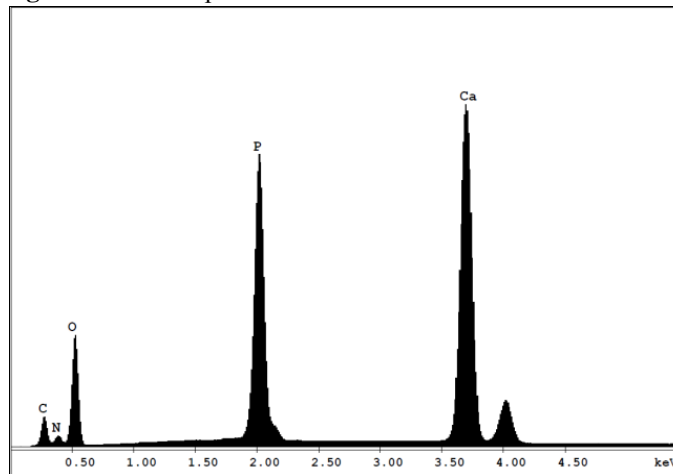
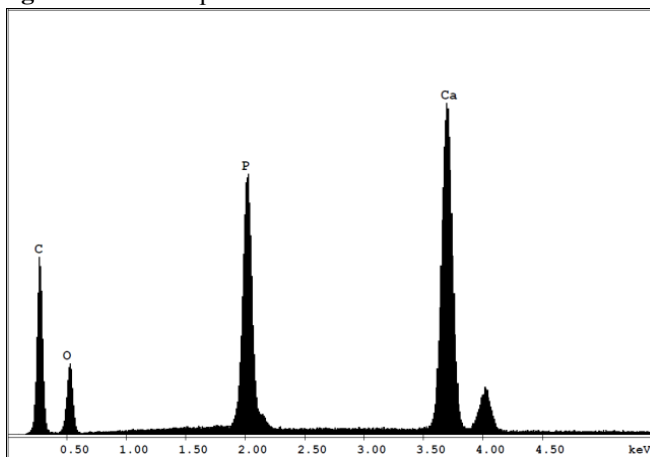


Figure 8: EDAX spectra of biomimetic HA: after sinterization

4. CONCLUSION

A biomimetic prepared hydroxyapatite was synthesized and characterized by several instrumental analytical methods: FTIR, XRD, SEM and EDAX. The results of the analyses confirmed that stoichiometric hydroxyapatite was prepared in respect with the conditions imposed by the molar ratio of calcium and phosphorous compounds ($\text{Ca:P} = 1.67$) used in the synthesis process, according to data collected from the literature. The crystallization process was not complete after the first step of the synthesis, only after the sinterization process at 900°C .

ACKNOWLEDGEMENT

This paper was supported by grant POSDRU/159/1.5/S/133391

REFERENCES

1. Mateus A.Y.P., Barrias C.C., Ribeiro C., Ferraz M.P., Monteiro F.J., Comparative study of nanohydroxyapatite microspheres for medical applications. *Journal of Biomedical Materials Research Part A*, **2008**, 86A(2), 483–493.
2. Bucholz R., Carlton A., Holmes R., Hydroxyapatite and tricalcium phosphate bone graft substitutes, *Orthop Clin N Am*, **1987**, 18, 323-334.
3. Daculsi G., Passuti N., Martins S., Deudon C., LeGeros R., Raher S., Macroporous calcium phosphate ceramic for ling bone surgery in humans and dogs: Clinical and histological study. *Journal of Biomedical Materials Research*, **1990**, 24, 379-396.
4. Gauthier O., Bouler J., Weiss P., Bosco J., Daculsi G., Aguado E., Kinetic study of bone in growth and ceramic resorption associated with the implantation of different

- injectable calcium-phosphate bone substitutes. *Journal of Biomedical Materials Research*, **1999**, *47*, 28-35.
5. Rey C., Calcium phosphate biomaterials and bone mineral: Differences in composition, structures and properties. *Biomaterials*, **1990**, *11*, 13-15.
 6. Yuan H., Kurashina K., de Bruijn J., Li Y., de Groot K., Zhang X., A preliminary study on osteoinduction of two kinds of calcium phosphate ceramics. *Biomaterials*, **1999**, *20*, 1799-1806.
 7. Liou S., Chen S., Liu D., Synthesis and characterization of needlelike apatitic nanocomposite with controlled aspect ratio. *Biomaterials*, **2003**, *24*, 3981-3988.
 8. Alves Cardoso D., Jansen J.A., Leeuwenburgh S.C.G., Synthesis and application of nanostructured calcium phosphate ceramics for bone regeneration. *Journal of Biomedical Materials Research B: Applied Biomaterials*, **2012**, *100B*, 2316-2326.
 9. Dorozhkin S., Calcium orthophosphate-based biocomposites and hybrid biomaterials. *Journal of Material Science*, **2009**, *44*, 2343-2387.
 10. Navarro M., Michiardi A., Castano O., Planell J.A., Biomaterials in orthopaedics. *Journal of The Royal Society Interface*, **2008**, *5*, 1137-1158.
 11. Seal B.L., Otero T.C., Panitch A., Polymeric biomaterials for tissue and organ regeneration. *Materials Science and Engineering R*, **2001**, *34*, 147-230.
 12. Chang M.C., Modification of hydroxyapatite/gelatin composite by polyvinylalcohol. *Journal of Materials Science*, **2005**, *40*, 505-509.