

Special Issue of the 6th International Congress & Exhibition (APMAS2016), Maslak, Istanbul, Turkey, June 1–3, 2016

Characterization of Cu/Ag/Eu/Hydroxyapatite Composites Produced by Wet Chemical Precipitation

B. KOMUR^a, E.R. OZTURK^b, N. EKREN^{c,d}, A.T. INAN^e, O. GUNDUZ^{d,f,*}, E. ANDRONESCU^g,
A. FICAI^g AND F.N. OKTAR^{b,d}

^aKanuni Sultan Suleyman Training and Research Hospital, Department of Orthopaedics and Traumatology, Kucukcekmece, Halkali 34303, Istanbul, Turkey

^bMarmara University, Department of Bioengineering, Faculty of Engineering, Ziverbey, Kadikoy 34722, Istanbul, Turkey

^cMarmara University, Department of Electric Education, Technical Education School, Ziverbey, Kadikoy 34722, Istanbul, Turkey

^dMarmara University, Advanced Nanomaterials Research Laboratory, Faculty of Technology, Ziverbey, Kadikoy 34722, Istanbul, Turkey

^eMarmara University, Department of Mechanical Engineering, Faculty of Technology, Ziverbey, Kadikoy 34722, Istanbul, Turkey

^fMarmara University, Department of Metallurgy and Materials Engineering, Faculty of Technology, Ziverbey, Kadikoy 34722, Istanbul, Turkey

^gPolitehnica University of Bucharest, Faculty of Applied Chemistry and Material Science, 1-7 Polizu Str., 011061 Bucharest, Romania

In the first part of this study hydroxyapatite was prepared synthetically through classical wet precipitation, using $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (calcium nitrate tetrahydrate) as calcium source and $\text{HgN}_2\text{O}_4\text{P}$ as phosphate source. In the second part of the study HA compounds were prepared with different ratios (0.5, 1, 2, 5, 10 and 25 wt.%) of antibacterial materials like copper (Cu), silver (Ag) and europium (Eu), using the same wet precipitation method. The prepared pure HA material was utilized as the control group for comparison with Cu/Ag/Eu/HA composites. Studies of X-ray diffraction, FTIR spectroscopy, scanning electron microscopy, equipped with energy dispersive X-ray analysis were performed. The aim of this study was to investigate Cu/Ag/Eu/HA composites and the effect of metals on HA biomaterials, used as scaffold materials in bone disease treatment.

DOI: [10.12693/APhysPolA.131.392](https://doi.org/10.12693/APhysPolA.131.392)

PACS/topics: 81.05.-t, 81.05.Mh, 87.85.J

1. Introduction

The skeleton system of the human body provides not only support and movement, but also protection for the soft tissues. The skeleton system plays the role of a scaffold, which is comprised of bones. However, bone fracture is a very common challenge for human body. There are several ways to overcome bone fracture. The bone grafts is a common technique to heal bone fracture by bone repair or regeneration [1]. The autografts and allografts, which are types of bone material, do not fulfill the needs of bone graft to heal bone fracture. Autografts need more surgical time, and hence patients are exposed to anesthesia for longer time periods and multiple incision may increase the risk for nerve injury. Allografts, on the other hand, are not only expensive, but also introduce the risk of possibility that rejection will occur [2]. However, with the development of technology and with the yearly increase of the number of fractures, scientists come together

to produce new effective bone grafting materials, in order to get rid of any complications, rejections and failures. New bone graft materials supplant autografts, allografts and xenografts, which are also a type of bone grafting materials.

Natural bone comprises mainly hydroxyapatite (HA) (~ 70%). HA with the formula of $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ is a good example of new bone grafting biomaterial [3], since it is believed that it is important to understand composition of natural bone and its formation in order to get good bone substitutes. With understanding of bone biosynthesis, better bone substitutes could be made, which are very close to natural bone, structurally and chemically, by changing such parameters the size, shape and composition. This is why HA is a crucial biomaterial for hard tissue repairing. HA is an excellent biocompatible biomaterial, which is also bioactive, osteoconductive, nontoxic, non-inflammatory and a non-immunogenic agent. Osteoconduction makes HA a suitable material for hard tissue replacements. It is also easy to synthesize HA [4].

There are two commonly used methods for synthesis of HA, as stated by Boanini et al. [5]. One of them is based on the neutralization reaction between calcium hydroxide

*corresponding author; e-mail: oguzhan@marmara.edu.tr

slurry with phosphoric acid. The other is based on the precipitation method, in which a solution playing the role of phosphate source is added drop by drop into a solution that is as calcium source. E. Boanini, et al. have reported that ammonium or sodium monohydrate phosphate can be used as phosphate source and generally calcium nitrate or acetate [5] can be the source of calcium.

However, because of its poor mechanical properties, the applications of HA are limited. Despite the fact that HA has poor mechanical qualities and does not have antimicrobial properties, it can be improved and can obtain antimicrobial properties by adding to it diverse oxides, ions and metal or non-metal particles in order to develop some new properties of HA, such as the mechanical strength, solubility and other. In this study, copper, silver and europium were considered as an additional metal to improve mechanical properties of HA biomaterial.

The crucial properties of copper and silver are related with antimicrobial activity. Materials, which have antibacterial properties, especially silver, ward off bacterial colonization and biofilm growth on surfaces of medical devices and biomaterials. Antimicrobial properties of copper and silver were used in many medical fields. Despite the fact that copper in high amounts is toxic, copper in small amount is necessary for metabolic processes. During surgery there is a possibility to insert into bone an infected material. It requires extra procedures to prevent any kind of infection. Therefore it is preferable to have HA, which has an antimicrobial activity [6].

In the first part of this study, synthesis of pure HA was performed by using wet chemical method, using $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ as a calcium source and $\text{HgN}_2\text{O}_4\text{P}$ as a phosphate source. Then HA was synthesized with different amounts (0.5, 1, 2, 5, 10, 25 wt.%) of Cu/Ag/Eu, to find best amount of Cu/Ag/Eu for maximum bone grafting efficiency. To compare pure HA and HA with metal substitution, produced materials were characterized by X-ray diffraction (XRD), FTIR spectroscopy and scanning electron microscopy, equipped with energy dispersive X-ray analysis (SEM-EDX).

2. Materials and methods

2.1. Production of pure HA

Amounts of 4.83 g (in 500 ml) of $\text{HgN}_2\text{O}_4\text{P}$ and of 14.24 g (in 100 ml) of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were calculated in order to gain 6 g of $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ (hydroxyapatite), according to the following formula, $5\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O} + 3\text{HgN}_2\text{O}_4\text{P} = \text{Ca}_5(\text{PO}_4)_3\text{OH}$. 4.83 g of $\text{HgN}_2\text{O}_4\text{P}$ and 14.43 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were weighted out and dissolved in 500 and 100 ml of water, respectively. pH of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solution was calibrated to 7. $\text{HgN}_2\text{O}_4\text{P}$ solution then was transferred into $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solution, drop by drop. The total solution was divided into 6 beakers (labeled as pH7, pH8, pH9, pH10, pH11 and pH12), 100 ml each, and their pH were assayed according to their label. Solutions were left for one day and again, pH of six solutions was rearranged to same related pH value. The washing procedure was applied to six solutions

at the end of 3 days. After the washing procedure, solid parts of solutions in filter paper were put into oven, at 120°C , in order to dry. When the solid parts of solutions were dry, the solids were crushed into powder. These six samples that have had different pH value were analyzed using XRD, in order to determine crystal structure and obtain information about structure and phase of materials. Moreover, additional analyses were performed using SEM and FTIR.

2.2. Production of Hydroxyapatite with substitutions

Different amounts of Cu, Ag and Eu substitution (0.5, 1, 2, 5, 10 and 25 wt.%) were used besides $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{HgN}_2\text{O}_4\text{P}$ to gain 1 g of HA. Corresponding amounts of $\text{HgN}_2\text{O}_4\text{P}$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ / AgNO_3 / $\text{C}_6\text{H}_9\text{EuO}_6 \cdot \text{H}_2\text{O}$ were calculated in order to gain 1 g of $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ (hydroxyapatite) with different percentage of Cu/Ag/Eu substitution. $\text{HgN}_2\text{O}_4\text{P}$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and substituted metals were dissolved in 50, 30 and 20 ml of water, respectively, and their pH were assayed to 10. HA solutions with different percentage of Cu/Ag/Eu substitution were left for one day and again, the pH was rearranged to 10. The washing procedure proceeded after 3 days, solid parts of solutions in filter paper were dried and then crushed until they become powders. XRD, SEM and FTIR were performed for all samples of each substituted metal.

3. Results and discussion

Figure 1 shows the XRD diagram of the Cu, Ag, and Eu substituted HA, prepared by wet chemical method with different concentration of Cu, Ag, and Eu. All of the samples have revealed a typical pattern of HA (Fig. 1A–I). This indicates that the phase characteristic of HA remains stable in this work, at different Cu, Ag, and Eu concentrations in the reaction solution.

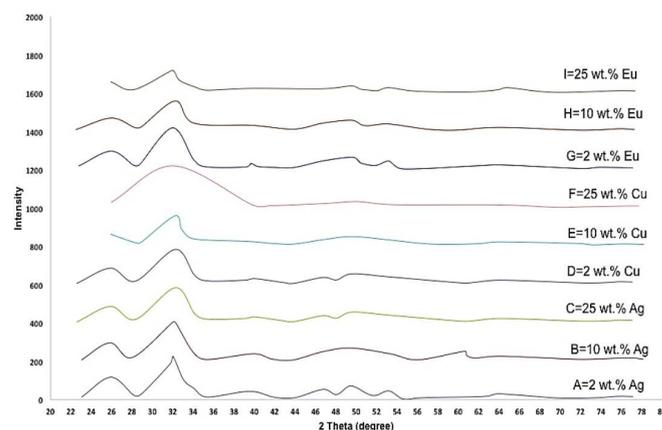


Fig. 1. The powder XRD patterns of different HA with various percentage of metal ions (A), (B) and (C) – 2, 10 and 25 wt.% of Ag; (D), (E) and (F) – 2, 10, and 25 wt.% of Cu; and (G), (H) and (I) – 2, 10, and 25 wt.% of Eu, respectively.

HA composite powders are obtained in weight percentage of 2, 10 and 25 in order to verify the synthesis of substituted HA and also for measurement of purity of substituted HA. When each diffractogram of substituted HA is analyzed, there are no differences, except in diffractogram of 25 wt.% Cu-substituted HA composite powder. The by-product detected in 25 wt.% Cu-substituted HA composite powder was phosphate.

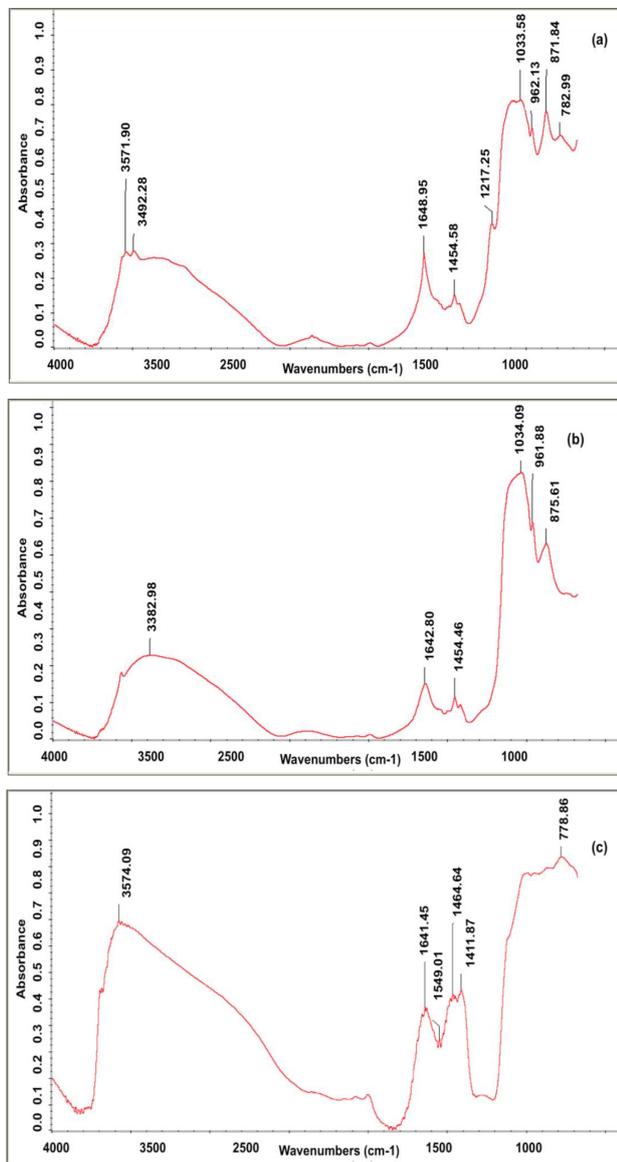


Fig. 2. FTIR spectra of Ag-substituted HA with different concentration of Ag, (a) 2, (b) 10 and (c) 25 wt.%.

The shape of XRD peaks shows that HA particles substituted with Ag, Cu, and Eu were nanosized. All samples have almost similar diffractograms. The infrared absorption spectra of HA with different concentrations of Cu, Ag and Eu are shown in Figs. 2–4, respectively. All samples show peaks in the vicinity of 2000.39 cm^{-1} and 1649.25 cm^{-1} , attributed to PO_4 symmetric and asymmetric vibrations. The bending vibrational modes of

PO_4 are observed in the region of 1455.87 cm^{-1} and 1163.15 cm^{-1} in Figs. 2–4. The FTIR spectroscopy shows further details of the Ag substitution in HA and the impurity phase formation.

Figure 2 contains the FTIR spectra of the HA powders with varying concentration of Ag. The characteristic bands for HA are present in all spectra for phosphate bending. Band at 3556 cm^{-1} corresponds to vibrational and stretching modes of hydroxyl vibration [7]. In addition, two broad bands are revealed at 1649 and 3445 cm^{-1} in Fig. 2a, b and c. The vibration band corresponding to the silver phosphate (bulk Ag_3PO_4 frequency of 975 cm^{-1} and nano Ag_3PO_4 frequency of 1017 cm^{-1}) was obtained in Fig. 2.

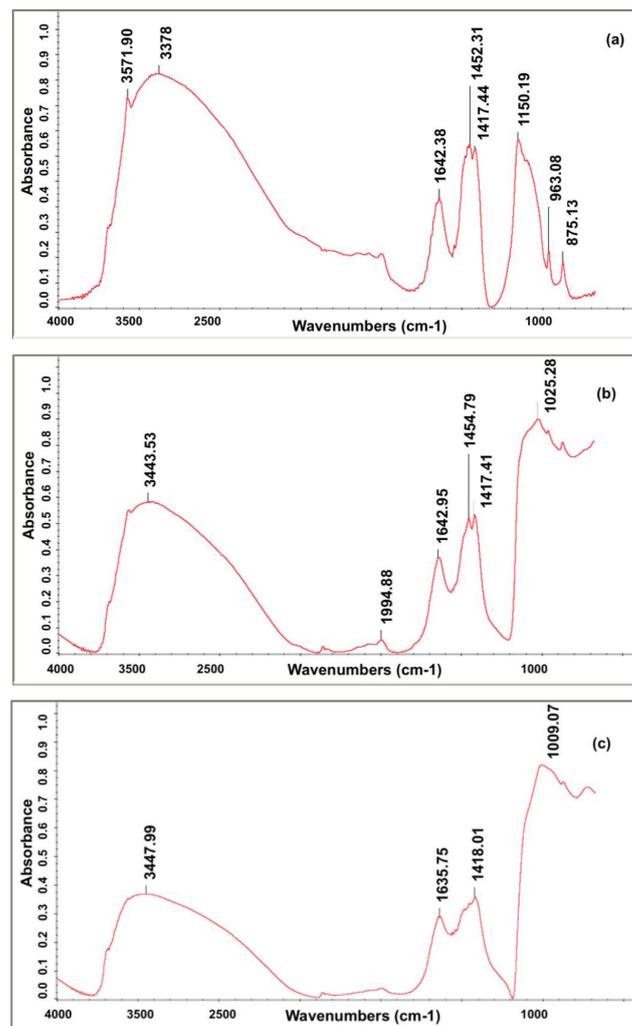


Fig. 3. FTIR spectra of HA with different concentrations of Cu, (a) 2, (b) 10, and (c) 25 wt.%.

The infrared spectra of HA with different percentages of Cu are given in Fig. 3. The peaks at 3570 cm^{-1} are attributed to stretching and bending modes of $-\text{OH}$ group. Moreover, the characteristic peaks of carbonate absorption bands are observed around 872 cm^{-1} and 14100 cm^{-1} in copper-substituted HA in Fig. 3a, b and c. However, the intensities of these bands are very weak [4].

Figure 4 shows the typical infrared spectra of HA prepared with varying concentration of Eu. In the FTIR spectrum of HA with high concentration of substituted Eu (Fig. 4c), the bands corresponding to the vibration of C–O were found at about 1411 cm^{-1} , which are a characteristic feature of the carbonate group [8]. It was also observed that bands at 2350 and 3492 cm^{-1} gradually disappear with the increase of Eu concentration.

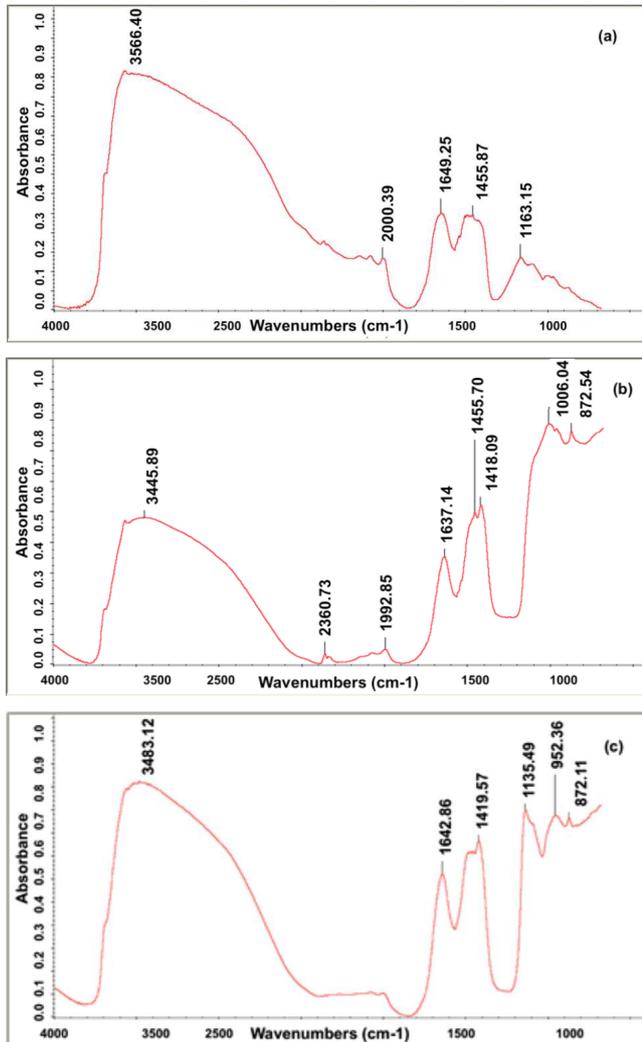


Fig. 4. FTIR spectra of different concentration of Eu-substituted HA. (a) 2, (b) 10 and (c) 25 wt.%.

The SEM images of various substituted HA samples are shown in Fig. 5. It is observed that the particles are agglomerated. The presence of Ca, P and Cu/Ag/Eu in the Cu/Ag/Eu-substituted HA were stated by SEM-EDAX. When comparing HA with different percentages of substituted Cu, it is clearly seen that 2 wt.% (Fig. 5a) is less multiparticulate and has bigger grains than 10 wt.% Cu (Fig. 5b) at $\times 1000$ and 25 wt.% Cu (Fig. 5c) at $\times 2000$ has more intense particles than others.

When comparing different percentage of Ag-substituted HA, it is clearly seen that 10 wt.% Cu

is more coarse-grained than sample with 2 wt.% at $\times 1000$ and sample with 10 wt.% Cu at $\times 2000$ has more intense particles than other samples in Fig. 5.

Comparison of Ag and Cu-substituted HA shows that Ag-HA samples have bigger particles and a more intense structure than those of Cu-HA (Fig. 5d–f). The examination Eu-HA SEM images tells that 10 and 25 wt.% Eu-HA (Fig. 5h–i) samples are denser and have remarkably larger particles.

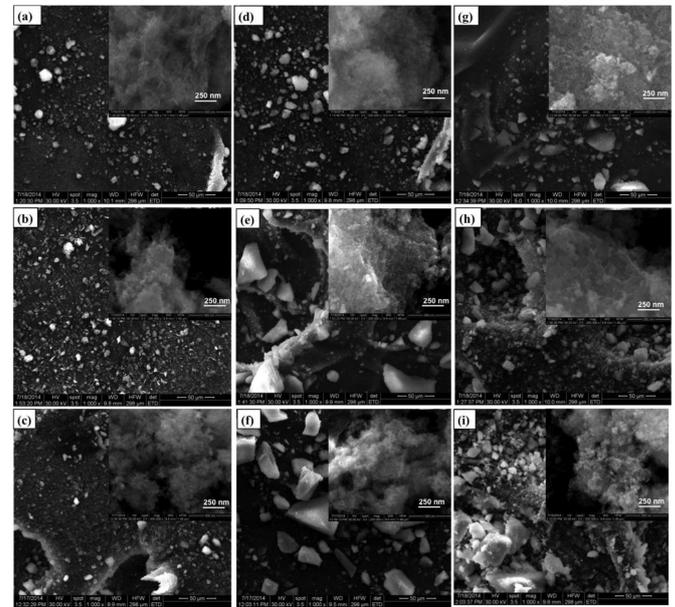


Fig. 5. SEM images of HA with different amounts of substituting metals, (a) 2 wt.% Cu ($\times 1000$ and $\times 200\,000$ magnification), (b) 10 wt.% Cu, (c) 25 wt.% Cu, (d) 2 wt.% Ag, (e) 10 wt.% Ag, (f) 25 wt.% Ag, (g) 2 wt.% Eu, (h) 10 wt.% Eu and (i) 25 wt.% Eu.

4. Conclusions

In the present work we contributed to study of the influence of the varying concentration of copper-, silver- and europium-substituted hydroxyapatite, synthesized by wet chemical method. All samples were analyzed by XRD, SEM and FTIR. These results and methods could be interesting for academic and industrial researchers of orthopedic biomaterials.

Acknowledgments

This study has been supported by Marmara University (BAPKO Project Number: FEN-B-080415-0117).

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