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Evaluation of the influence of microwaves radiation on a biomaterial composed of three phases of calcium phosphates

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ABSTRACT
The chemical similarity with the bone components that calcium phosphates present have been the fundamental cause of their use as biomaterials in the regeneration processes, presenting good biocompatibility, bioactivity and osteoconductivity. Among the biomaterials studied from calcium phosphates, there are multiphase biomaterials, which are those that contain two or more phases of calcium phosphates in their composition. These mixtures improve the chemical-physical and biological properties of these materials. The use of biomaterials in the regeneration process requires previously eliminating the microorganisms present in the material, through a sterilization process. Different sterilization methodologies have been applied to decontaminate biomaterials, including the use of microwave radiation. The objective of this study was to evaluate the influence of the MW radiation application of a multi-phase biomaterial made of a three-phase mixture of calcium phosphates synthesized by wet chemical method. The results of this study showed that the application of MW radiation to ceramic samples containing the OCP phase causes the thermal decomposition of this phase, modifying its crystallinity index. This aspect should be taken into account when applying sterilization by applying MW radiation to ceramic samples with characteristics similar to those studied in this work.
Keywords: *Biomaterials; Calcium phosphates; microwave radiation; mixture of calcium phosphates; synthesis.*

1. INTRODUCTION
Due to the chemical similarity with the bone constituents, calcium phosphates have been widely used as a biomaterial in regeneration processes, presenting good biocompatibility, bioactivity and osteoconductivity [1- 4].

Calcium phosphates alone do not mimic the composition and properties of the bone, so to overcome this difficulty have studied the preparation of multiphase biomaterials, containing a mixture of two or more phases of calcium phosphates. These mixtures improve their solubility and bioabsorbibity [1, 4].

In the preparation of these biomaterials, mixtures are usually made with calcium phosphate phases that have similar Ca/P ratio values, one of the ways being the homogeneous mixture of powders of different phases of calcium phosphates [1].

The use of biomaterials in the regeneration process requires previously eliminating the microorganisms present in the material, through a sterilization process. An efficient sterilization guarantees the effective elimination of microorganisms without affecting the chemical-physical and biological properties of the biomaterial [5]. Several works have been published on the study of sterilization of biomaterials [3-11]. Among the methodologies studied is the use of microwave radiation (MW radiation) [12].

Microwaves are electromagnetic radiation with a wavelength range between 1 m and 1 mm and a frequency range that ranges between 300 MHz and 300 GHz [13, 14]. Electromagnetic waves transfer their energy through open space, this transfer depending on the intensity of the magnetic field, the frequency of oscillations and the dielectric properties of the material [14]. The main advantage of dielectric heating is its volumetric interaction with the material, heating as electromagnetic energy is absorbed [13, 15, 16].

The objective of this study was to evaluate the influence of the MW radiation application of a multi-phase biomaterial made of a three-phase mixture of calcium phosphates synthesized by wet chemical method [17-20].

2. MATERIALS AND METHODS
Synthesis of calcium phosphate. Amorphous calcium phosphate (ACP), tricalcium phosphate (TCP) and octacalcium phosphate (OCP) were synthesized by applying the wet chemical method according to Rodriguez-Chanfrau et al. [17] with the following modifications. In cases of synthesis ACP and OCP at the end of the reaction, the suspension was filtered and dried by vacuum freeze-drying process. To obtain the TCP sample, ACP sample dried by vacuum freeze-drying were subjected to a sintering process at 800 °C for 3 hours.

Preparation of tablets. Tablets (300 mg by weight) were prepared with a mixture of the three phases of calcium phosphate previously synthesized (100 mg of each phase) and mixed for 5 minutes. Subsequently, the homogeneous mass was pressed in a manual hydraulic press (SPECAC, USA) at a pressure of 10 TN for 5 minutes.

Microwave irradiation application. The elaborated tablets were divided into four groups. Three groups were applied MW radiation and one group was used as a control group. Times of 20, 40 and 60 seconds of MW application were studied.

Sample characterization. The samples treated with MW radiation and the control sample were characterized by X-ray powder diffraction and FTIR spectroscopy.

X-ray powder diffraction studies. The XRD spectra were recorded at room temperature (25 ºC) with a SIEMENS D5000,

DIFFRAC PLUS XRD diffractometer (Germany) with BRAGG-Brentano geometry, Cu Kα radiation (λ=0.154 nm), Flicker detector and graphite monochromator. A scattering angle range from 4º to 80º with 2θ step interval of 0.02º was used. The samples were placed in the glass sample holder, analyzed under plateau conditions. An operating voltage of 40 kV and current of 30 mA was utilized, and the intensities were measured in the range of $5° < 2\theta < 30°$. Peak separations were carried out using Gaussian deconvolution. The d-spacings were calculated using the Bragg equation.

The crystallinity index for each of the samples was calculated according to the method defined by Person *et al*. [21] For this semi-quantitative analysis method the reflections (2 0 2), (3 0 0), (2 1 1) and (1 1 2), appearing between the 2Θ values of 30° and 35°, were used.

FTIR spectroscopy. FTIR spectra of the samples were measured on a FTIR-VERTEX 70/BRUKER spectrometer (Germany). Transmission mode was used with 64 cumulative scans and a resolution of 4 cm^{-1}, in the frequency range of 4000 to 400 cm^{-1}.

3. RESULTS

Tablets with a diameter of 13.0 ± 0.1 mm and height of 2.5 ± 0.2 mm were obtained.

Figure 1 shows the X-ray diffractogram of the sample without treatment with MW radiation (control tablets). A mixture of phosphate phases OCP, ACP and hydroxyapatite was observed.

The presence of carbonate ions in the sample is also observed.

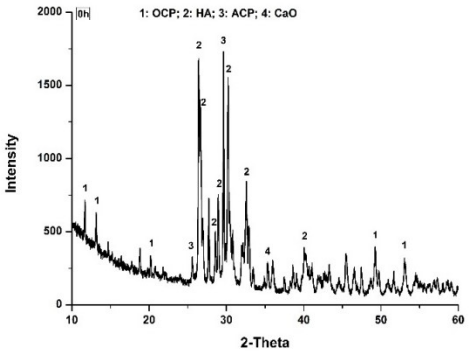

Figure 1. X-ray diffractogram of the untreated sample with MW radiation.

On the other hand, Figure 2 shows the FTIR spectrum of the control sample. Bands at 1207 cm^{-1} (dOH mode of the HPO_4^{2-} linked by H), 1186 cm^{-1}, 1136 cm^{-1}, 1064 cm^{-1}, 1028 cm^{-1} and 1001 cm^{-1}, typical of the presence of phosphate ions were observed. The bands at 1643 cm^{-1} and 725 cm^{-1} confirm the presence of carbonate ions in the sample.

Figure 3 shows the results of the analysis by X-ray diffraction of the samples treated at different times with MW radiation. Apparently no major changes in the spectra are observed, except a decrease in peak intensity to $\theta = 11.5$ which corresponds to the OCP phase.

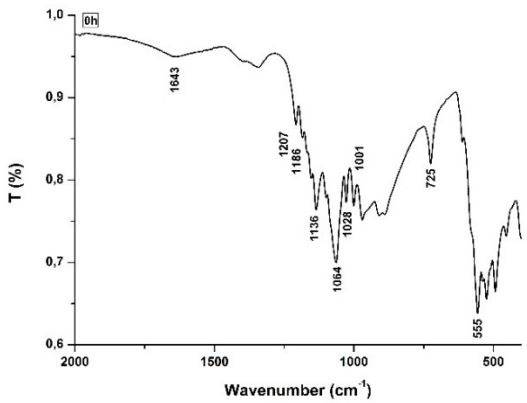

Figure 2. FTIR spectrum of the untreated sample with MW radiation.

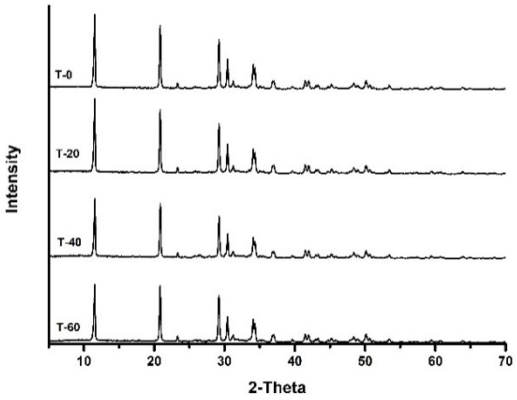

Figure 3. X-ray diffractogram of the treated sample with MW radiation at different times.

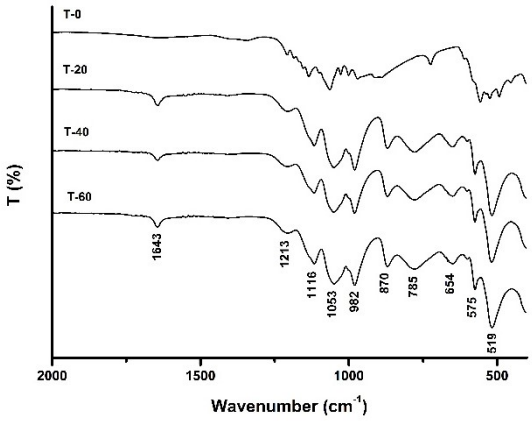

Figure 4. FTIR spectrum of the treated sample with MW radiation.

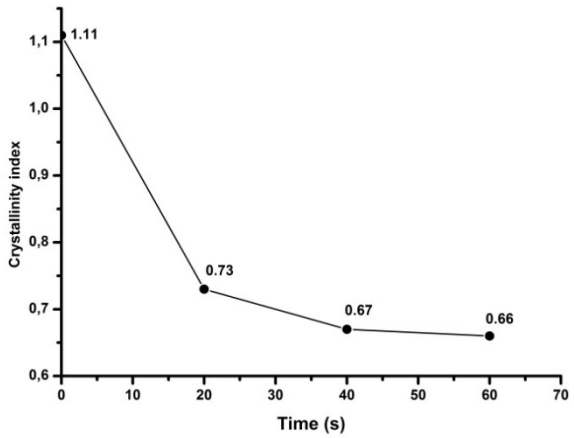

Figure 5. Behavior of the index of crystallinity in the exposure time to MW radiation.

Figure 4 shows the results of the FTIR analysis of the samples treated with MW radiation at different times. In general, no modifications were observed between the different spectra,

except that the band at 1643 cm^{-1}, corresponding to the presence of carbonate, is slightly intensified, while the band at 725 cm^{-1} is overlapped by one more band wide at 785 cm^{-1}. The latter may be due to the formation of water vapor during the process, which causes hydration of the CaO present in the sample [5].

Figure 5 shows the behavior of the crystallinity index in the exposure time to MW radiation. This parameter decreased in time, the decrease being less sharp after 40 seconds.

The biomaterial evaluated in this work was made up of a three-phase mixture of calcium phosphates (ACP, TCP and OCP). Analyzing the results achieved during the characterization of the samples treated with MW radiation at different times, it was corroborated in the X-ray diffractogram that the signal corresponding to the OCP phase decreased the intensity over time. Studies reported by Elliot [22] showed that the OCP phase in the presence of heat undergoes thermal decomposition, depending on the resulting products of the range of heating temperature and time of heat application. This author defined that during the thermal decomposition process the OCP crystalline phase collapses, with the consequent formation of poorly crystallized hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and anhydrous calcium phosphate hydrogen (CaHPO4).

On the other hand, Morejon *et al*, reported a study on the influence of different types of sterilization (ethylene oxide; autoclaving and dry oven) on a sample composed of a mixture of calcium deficient hydroxyapatite, octacalcium phosphate (OCP), and β-tricalcium phosphate, that the peak intensity corresponding to the OCP phase in the X-ray diffractogram, decreased with heat, due to a process of thermal decomposition of the material. This process of decrease was more significant when they applied sterilization by dry oven at a temperature of 190 ºC [5].

In this study, the samples were treated in a conventional microwave oven at 100% power during different times. It is known that these furnaces, at the power studied, reach temperatures above 200 ºC, with the advantage that the heating is dielectric, which depends on the dielectric properties of the material [13].

Based on this, it is possible to assume that during the MW radiation treatment process, the temperature reached during the process was high enough to cause thermal degradation of the OCP phase present in the sample, which would justify the decrease in peak intensity OCP in the analysis by X-ray diffraction.

When analyzing the behavior of the crystallinity index over time, a decrease is observed as the treatment with MW radiation increased. In our opinion, the thermal decomposition process of the OCP phase, with the consequent obtaining of the degradation products, can modify the crystalline structure of the material evaluated and therefore modify the crystallinity index, which would justify the results achieved.

4. CONCLUSIONS

The results of this study showed that the application of MW radiation to ceramic samples containing the OCP phase causes the thermal decomposition of this phase, modifying its crystallinity index. This aspect should be taken into account when applying sterilization by applying MW radiation to ceramic samples with characteristics similar to those studied in this work.

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6. ACKNOWLEDGEMENTS

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