Applied Chemical Engineering (2025) Volume 9 Issue 1 ISSN: 2578-2010 (O)
doi: 10.59429/ace.v9i1.5845

RESEARCH ARTICLE

Sustainable synthesis of nano-hydroxyapatite from chicken bones: Effect of microwave treatment on structural and electrochemical performance

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ABSTRACT

Hydroxyapatite (HAp) is a bioactive calcium phosphate ceramic, which is the major inorganic constituent of natural teeth and bone. The current research paper involves the production of hydroxyapatite using chicken bone waste through a sustainable process and its pre- and post-processing with the use of microwave post-treatment. The originality of the work is the synthesis of a biogenic source of calcium with the irradiation of microwaves in order to adjust the structural and electrochemical characteristics of HAp. HAP1 was prepared as a sample by grinding and then by calcification, and then HAP2 was prepared by treating HAP1 with microwave. X-ray diffraction (XRD) ensured that the two samples are crystalline, and the microwave treatment caused slight changes in the peak and the size of crystallites. FESEM and TEM observations showed that HAP2 had smaller and more homogenous particles with lower agglomeration than HAP1. EDS identified the Ca, P, and O as the significant elements with minor traces of Mg and Na being biogenic in nature. Electrochemical characterization showed better ionic mobility, charge-transfer behavior, and capacitance of HAP1, but the treatment of microwave treatment raised the internal resistance and lowered ionic conductivity. In general, the research illustrates that microwave processing can increase the level of morphological homogeneity of biogenic HAp, but it has a comparable negative effect on the electrochemical activity.

Keywords: Hydroxyapatite (HAP); microwave-assisted synthesis; crystallite size; XRD analysis; FESEM; TEM; EDS; BET; electrochemical properties; ionic mobility; charge-transfer resistance.

ARTICLE INFO

Received: 28 November 2025
Accepted: 29 December 2025
Available online: 08 January 2026

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1. Introduction

Hydroxyapatite (HAp) is a naturally occurring mineral of calcium phosphate and has a chemical formula of Ca10(PO4)6(OH)2, which is the main inorganic component of the human bone and dentin, and contributes to about 60-70 percent of the bone mass. HAp has found wide use in biomedical applications as bone grafts, dental implants, coating of metallic implants, drug delivery systems, and tissue engineering scaffolds due to its excellent biocompatibility, bioactivity and chemical similarity to native bone tissue[1].

HAp synthesis has been applied to many synthesis pathways; classic chemical precipitation, sol-gel technology, hydrothermal synthesis, microemulsion, and solid-state reaction[2]. Even though phase purity and morphology are easily regulated in such chemical routes, it may be grounded upon synthetic precursors and may contain no trace of the ionic composition that occurs in the biogenic apatite [2].

The use of natural resources such as eggshells and animal bones in the process of synthesis has been a popular approach towards sustainable and bio-derived synthesis methods and has been widely used in the last couple of years [3]. As demonstrated by the recent ultimate reviews, biogenic HAp is more similar to the human bone apatite chemical composition and structure, and thereby enhances osteogenic competency and biological efficacy. Chicken bone waste as a source of calcium is a rich, affordable, and environmentally friendly source of calcium used to produce HAp[4]. The bone-derived HAp that is equivalent to the chemically synthesized HAp contains trace elements such as Mg and Na that are known to influence the crystallinity, bioactivity, and ionic transport property[5]. The modification of HAp properties other than the selection of precursors is also very important through the post-synthesis treatments[6]. Although many studies have been conducted on biogenic HAp and a reduction of energy used in the synthesis under microwave irradiation[7], very little research has been done on the systematic attribution of the correlation between the post-treatment of microwave and morphological homogeneity, in addition to electrochemical characteristics of chicken-bone-derived Hap[8]. The current research fills this gap since it will include a comparative study of a traditionally calcined and microwave-treated biogenic hydroxyapatite[9-11].

This paper aims at synthesizing hydroxyapatite using chicken bone waste materials and to determine how the post-treatment with microwaves affects the structural, morphological, elemental, and electrochemical characteristics of hydroxyapatite[12,13].

1.1. Procedure / methods

Hydroxyapatite was produced using the residues of poultry bones using a two-step procedure based on other biogenic HAp production procedures mentioned in the literature. To start with, chicken bones were washed in detail in distilled water, dried, and the ground into fine powder. The powder (5 g) was placed in the 0.1 M sodium hydroxide solution to leave it under 24 h to deproteinize and purify the phases as in prior research on bone-derived HAp. To obtain the sample HAP1, the precipitate was filtered and washed 6 to 8 times with deionized water until a neutral pH was reached after which it was calcined at 600 degC over a period of 30 min.

Some modifications to the HAp microstructure of the synthesized sample were then performed by using the microwave post-treatment. It was mixed by adding 1 gram of HAP1 to 100 mL of deionized water and 10 mL of 1 M nitric acid was added to enhance the stability of the suspension. The solution duration was 30 min of ultrasonic treatment, which is considered an implementation of the typical nanoparticle processing procedures to break up agglomerates and improve the dispersion of particles. Intermittent microwave exposure (30 minutes on / 30 minutes off) to the suspension was then done to prevent the excessive rise in temperature and the formation of bubbles under controlled conditions to produce sample HAP2.

The suspension was centrifuged (10 min, bench-top centrifuge) after it was heated by a 2.7-kHz microwave. The sample of the collected sediment was washed with deionized water and ethanol to cleanse the sediment content and the sample dried at 70 degC over a period of 8 h. Both the synthesis and post-treatment methods adopted are in-line with the accepted guidelines on the preparation of biogenic HAp and processing of the material using the microwave method.

2. Results and discussion

2.1. XRD analysis

The X-ray diffraction (XRD) spectra of hydroxyapatite produced by grinding and calcination (HAP1) and following the exposure to microwaves (HAP2) have been indicated in Figure 1. Both spectra affirm crystallinity of the samples with a minor shift in 2 th angle (0.05deg -0.12deg) and slight reduction in the intensities of several peaks in HAP2. The identification of the reflection peaks was made in the 2 th regions and attributed to the respective Miller indices of compound HAP 1: 25.9391deg (002), 28.9909deg (210), 31.791deg (211), 32.9323deg (112), 34.1555deg (300), 35.472deg (202), 39.7148deg (310), 46. The associated intensities of HAP 2 were 25.9081deg (002), 28.8711deg (210), 31.7407deg (211), 32.8351deg (112), 34.0503deg (300), 35.5250deg (202), 39.734deg (310), 46.6590deg (222), 48.013 These values prove the crystal structure of the compound[14, 15].

Calculation of the crystallite sizes of HAP1 and HAP2 was done with the Debye-Scherrer equation. [15-18] as given in equation (1). When microwave was used, it was found that the average crystallite size became smaller as compared to before, and it has decreased to 21.6 nm.

$$D=\frac{0.9\lambda}{\beta cos\theta} \tag{1}$$

Here l is the wavelength of the monochromatic beam of X rays, B is the full width at half maximum (FWHM) and th is the angle of diffraction in which the peak is located.

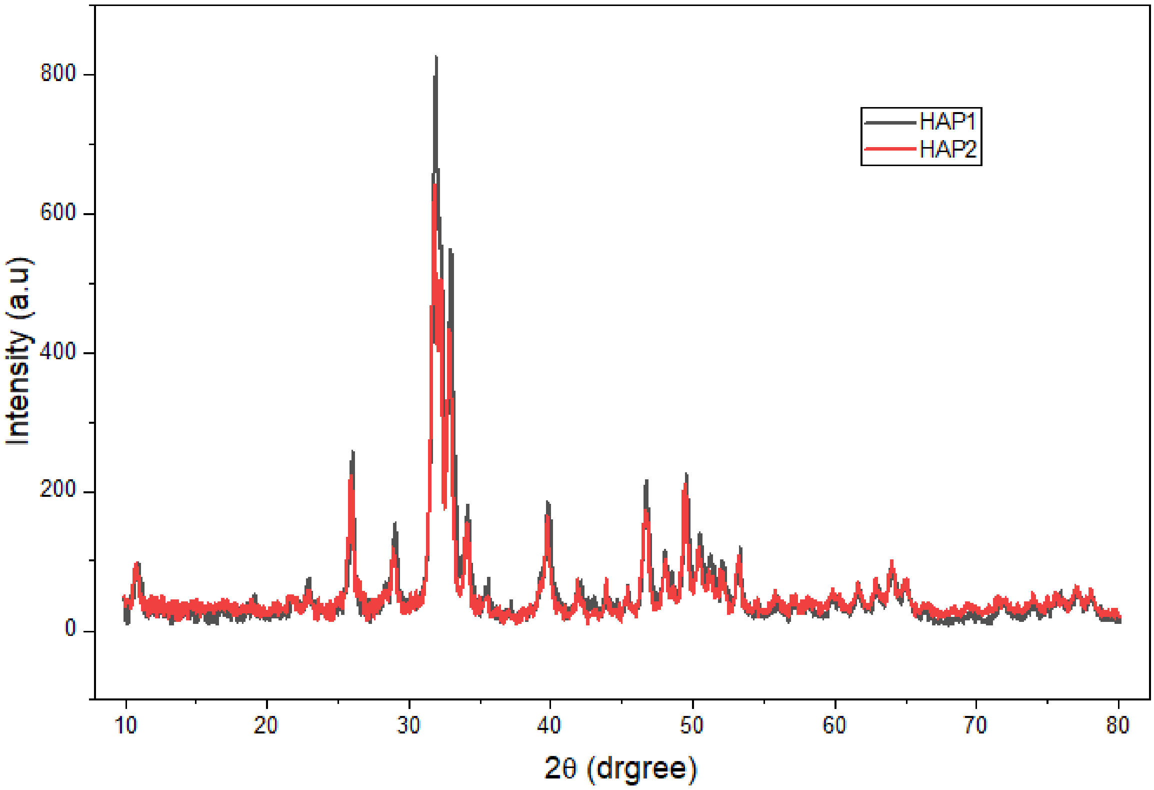

Figure 1. X-ray diffraction (XRD) spectra of hydroxyapatite

2.2. EDS analysis

An energy-dispersive analysis of the sample HAP1 and HAP2 showed strong peaks of calcium (Ca) and phosphorus (P), which supports the evidence of hydroxyapatite, mineralised bone phase. The relative Ca and P peaks, as compared with other elemental indications, represent the anticipated stoichiometric ratio of bone-mineral constituents, and indicate an efficient demineralisation, rearranged, and stabilisation of the calcium-phosphate structure. Minor components, such as magnesium (Mg) and sodium (Na) were also found; these components are noted to play a role in formation of the bone, and enhance biocompatibility of hydroxyapatite. The fact that oxygen (O) and carbon (C) has been found indicates the existence of remnants of the phosphate groups and remains of the sample holder or the left-over organic materials. Communally, these data indicate that the relentless procedure and calcination steps produced a high-purity multifarious in the similar quality as the natural hydroxyapatite.

The EDS spectrum of the microwave-treated sample (HAP2) indicated a significant reduction in the strength of most elemental peaks (in comparison to the traditionally calcined sample), but the most common constituents were calcium and phosphorus. This observation reveals that the basic chemical structure of hydroxyapatite was not affected by microwave treatment; however; surface rearrangements and alterations in the porosity took place due to the quick heating of the material as well as the evaporation of moisture and uncombustible remains [19]. Mineral components like magnesium and sodium also were still present, therefore, proving that the inherent biochemical attributes are maintained in the material. All in all, these findings indicate that microwave treatment maintains the major mineral arrangement of hydroxyapatite and improves its surface nature

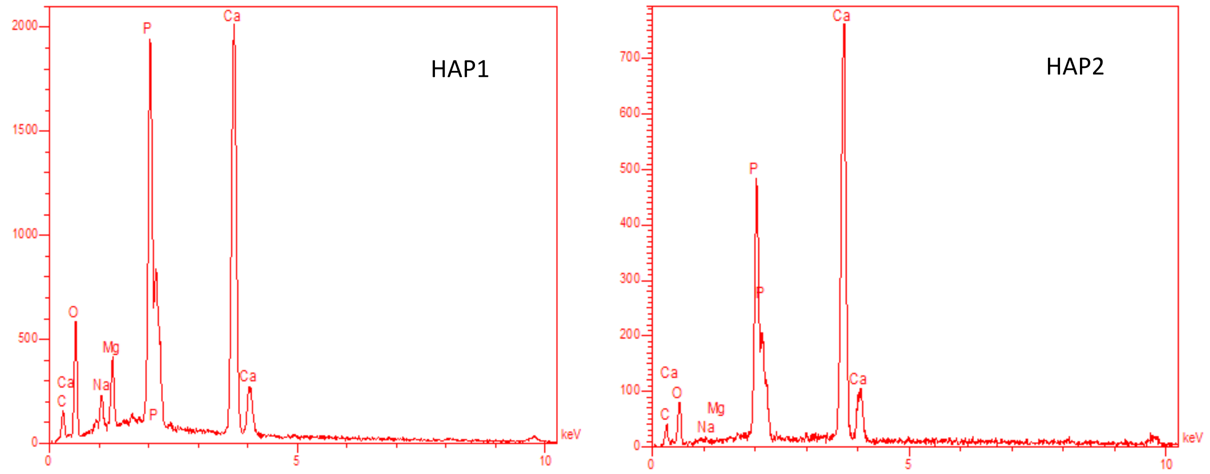

Figure 2. EDS spectrum of hydroxyapatite

2.3. FESEM analysis

In **Figure 3**, the images of field emission scanning electron microscope of hydroxyapatite nanoparticles are shown. Agglomeration of nanoparticles with heterogeneous spatial distribution is observed with the sample HAP1, which is explained by the thermal effects of the traditional calcification; the diameter of the particles is 69-95nm.

Sample HAP2, proved to be subjected to post calcination microwave treatment, on the contrary, shows nanoparticles of smaller size (51 56nm) and a more homogenous distribution of space with a high reduction in the flower-like agglomeration echoed in HAP1. These measurements testify to the effectiveness of microwave processing in morphology refinement and kaleidoscopic structure improvement of nanoparticles of the hydroxyapatite material [20]

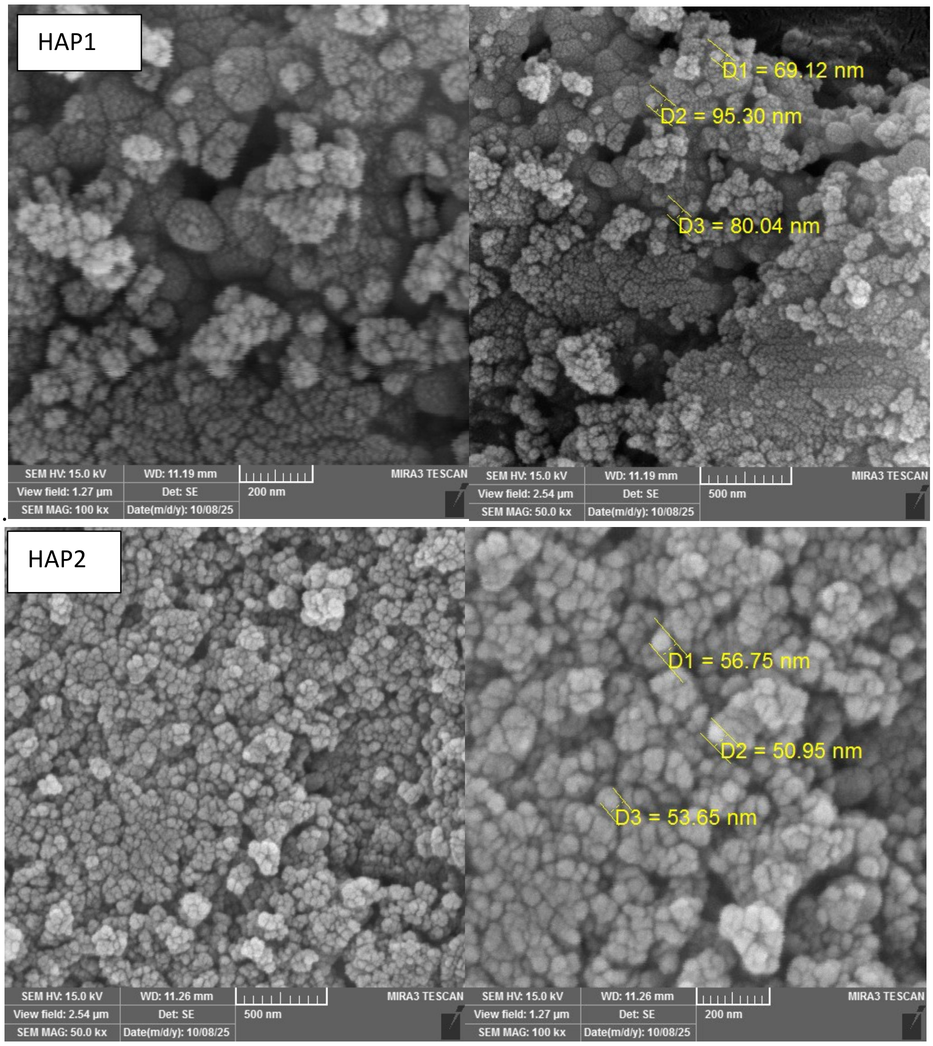

Figure 3. FESEM Images of hydroxyapatite

2.4. TEM analysis

Figure 4 of the transmission electron micrographs of sample HAP1 reveals clusters of nanoparticles that are densely packed, and that are distributed unevenly. The particles have wide size distribution and distorted morphology with a strong level of agglomeration. These findings suggest that there is some crystallization and that the unstable surface moieties remain. Also, the changes in electron contrast show regions of reduced crystallinity or unreacted impurity which most probably goes back to the biotic precursor.

On the other hand, the TEM images on the nanocomposite HAP2 hydroxyapatite clearly show improvement in morphology which can be credited to microwave mediated treatment. The effects of microwave irradiation were crystal restructuring with smaller dimensions of crystals and higher homogeneity in comparison to HAP1. The decrease in agglomeration is visible that indicates more stable surfaces and the weakened interparticle bonding. As a result, the comparative study of HAP1 and HAP 2 proves that microwave treatment is one of the effective methods of enriching the morphological and crystalline properties of biosynthesized nanohydroxy apatite according to reference [21].

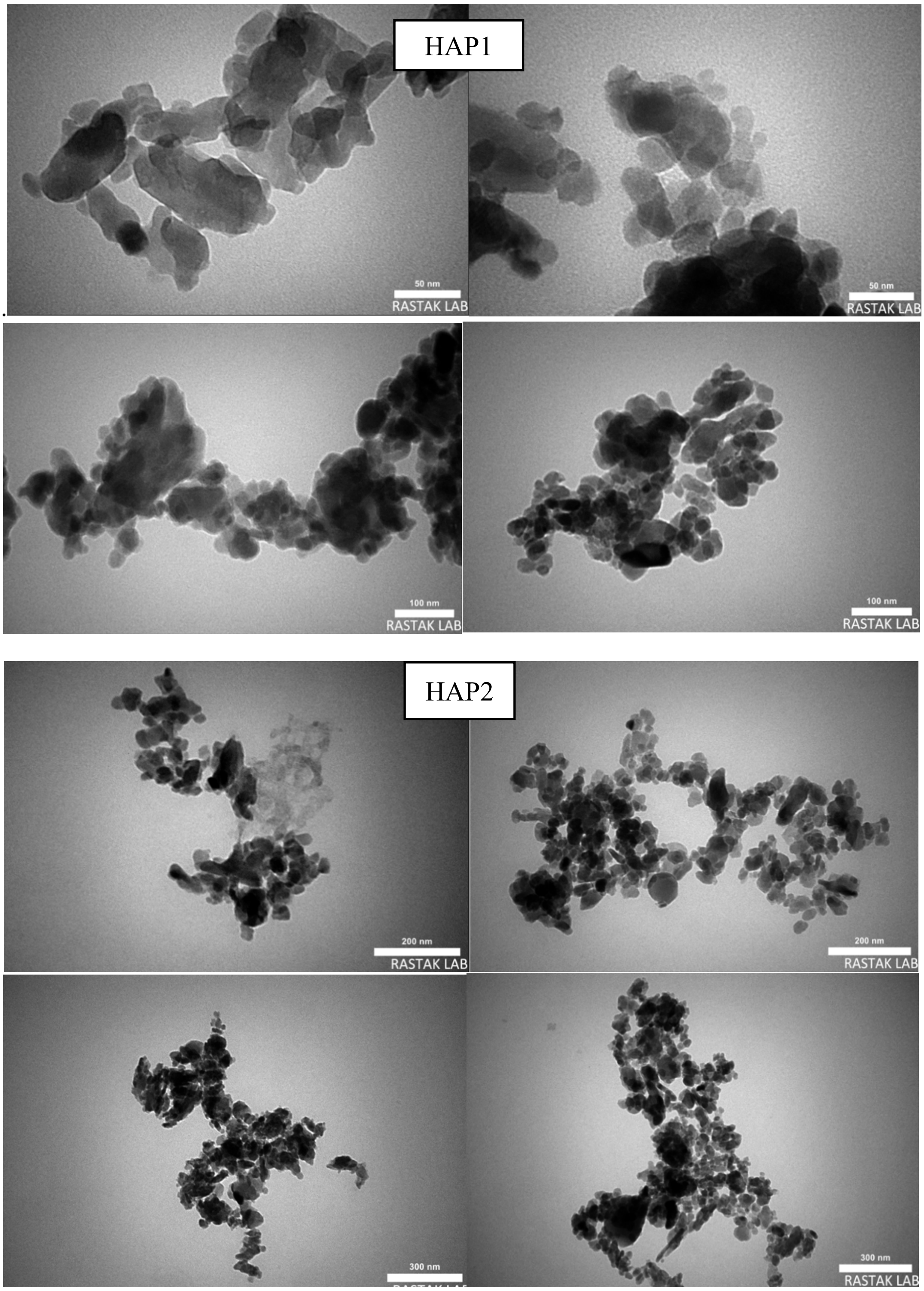

Figure 4. TEM Images of hydroxyapatite

Brunauer - Emmett-Tellers Specific Surface Area (BET) Analysis.

The comparative evaluation proves that microwave post-treatment has a binding effect on the shaping of physicochemical features of biogenic hydroxyapatite. Morphological and structural characterisation Hydraulic reduction in the size of the particles, increased uniformity of crystallites, and alleviation of agglomeration of particles have been achieved with microwave irradiation, resulting in a more homogenous nanostructure of HAP2 compared to HAP1. Elemental analysis indicates that microwave irradiation has no effect on the basic chemical structure or stoichiometry of hydroxyapatite, which means that the impact is limited to the changes in surface morphology and other structural properties, but does not affect the bulk inorganic structure.

On the other hand, at the point when the comparison is made to textural and electrochemical properties, an opposite tendency is observed. Despite the fact that HAP2 has been found to have an improved homogeneity of surfaces and a larger specific surface area, electrochemical analysis shows that HAP1 has a high ionic mobility, reduced charge-transfer resistance, and an increased electrochemical activity.

The comparison shows that structural rearrangement and reduced microporosity of HAP2 caused by the action of microwave negatively influence ion-transport pathways and thus suppress electrochemical activities.

On the whole, the aim of the study is directly achieved with this comparative study due to the clarification of the selective enhancement of structural and morphological uniformity with the reduction of the electrochemical functioning as comorbidities.

This has prompted the need to consider application-oriented synthesis and post-treatment approaches to hydroxyapatite using waste materials to compromise morphological refinement versus an electrochemical efficiency in harmony with the targeted end use.The results are listed in **Table 1** shown that the surface area in the table below for all the prepared.

Table 1. The characteristic surface for all the prepared in the BET method

Compound	Surface area m^2/g
HAP1	22.16
HAP2	41.48

2.5. Nyquist / EIS

Figure 5 shows the Nyquist diagrams of hydroxyapatite. The HAP1 curves have relatively low internal resistance and generally well-defined ionic diffusion traits. The EIS profiles also suggest that there were several layers on the surface thus leading to high porosity and a nanostructure that was rich in active sites [22]. Contrarily, HAP2 diagram shows significantly greater resistance and no clear semicircular area. Rather, the curves turn to a quasi-linear profile that is typical of Warburg impedance, which is a significant decrease in ionic mobility, an elevation of surface resistance, and a decline in porosity. These results suggest that microwave treatment has reduced the charge transfer capacity of the material and it has also raised its topical resistivity.

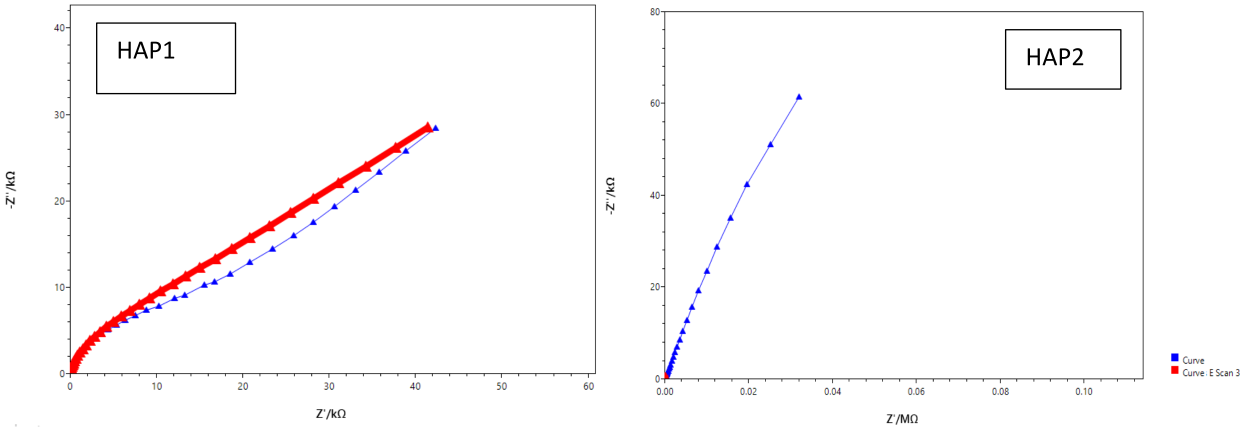

Figure 5. Nyquist diagrams of hydroxyapatite

Impedance vs Frequency

The association between frequency and impedance can be seen in **Figure 6**. The HAP1 curves show a clear peak in Z between at a certain frequency, the shape of the curve has the cross-shape formation, which shows that the system is electrically active and able to efficiently transport ions [23]. This finding indicates an evident dynamic reaction to frequency, indicating that HAP1 has quite strong conductivity and well-defined internal relaxation mechanisms [24].

As a contrast, curves of compound HAP2 are more slender and do not have any significant peak in Z. Both the Z' and Z" have a decreasing trend with frequency. It is indicative that HAP2 has a high impedance without significant relaxation frequency. There is no peak, which indicates that there is not a lot of ion transport and characteristic time-constant processes in the system [25]. Generally speaking, HAP2 is a highly resistive material or semi-insulating.

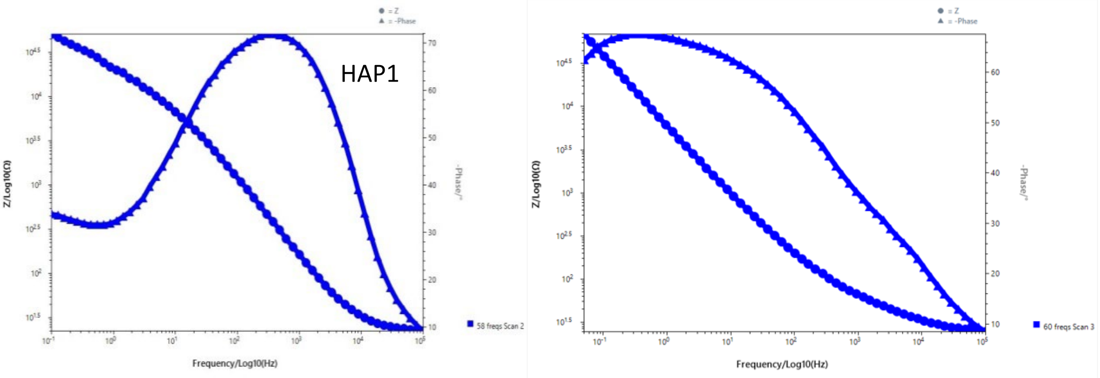

Figure 6. Relationship between impedance and frequency of hydroxyapatite

2.6. Cyclic voltammetry

The curves of cyclic voltammetry are provided in **Figure 7**. The current relative to the curve that is related to HAP1 is significantly higher than the one related to HAP2, which reflects an improved conductivity and electrochemical activity [26]. The pure oxidation and reduction peaks are signal of the effective electron transfer. The curve is neither smooth nor straight and, conversely, it has slight oscillations on it, which is indicative of the surface layer, which is highly electrochemically active and with a reversible or semi-reversible redox behaviour. The intense current at negative potentials is also a further indication of strong reduction process in the HAP1 sample [15].

On the contrary, the curve of HAP2 is more smooth and has significantly lower current densities, indicating worse conductivity and lower-level electron transport. Thepeaks of oxidation and reduction are blurred and not very well defined meaning low redox activity [14]. The general direction of the curve nearly follows semi-resistive behaviour which means that electroactive sites are not as abundant on the surface of HAP2. Such results prove that the compound HAP 2 is a relatively weak electrochemical system in comparison with HAP1.

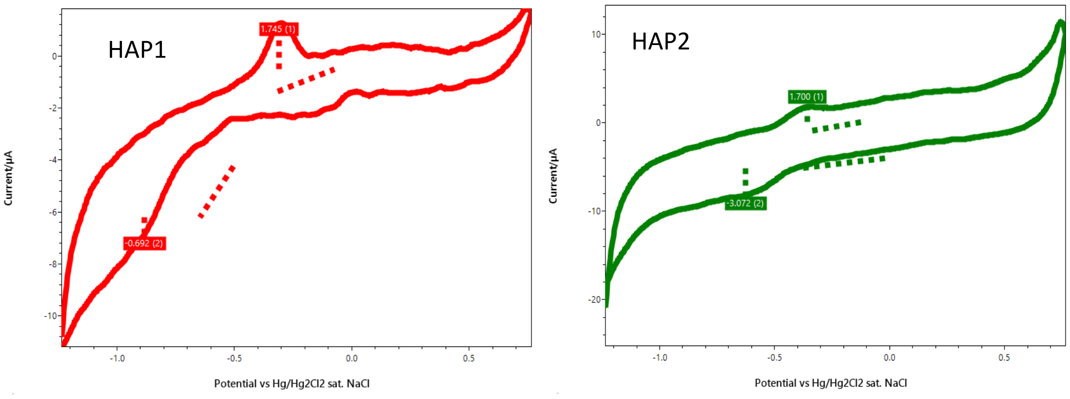

Figure 7. Cyclic voltammetry curves of hydroxyapatite

2.7. GCD analysis

The charge–Discharge (GCD) curves show that there is a clear difference in the electrochemical behavior between HAP1 and HAP2. HAP1 presents periodic triangular shapes with prolonged charge-discharge times and lowered slope which are indicative of an increment in electrochemical capacitance. This finding can be explained by the fact that there are more effective sites of charge-storage and more favorable nanostructured surface which helps in transporting ions.

In contrast, microwave-modified HAP1 to produce HAP2 shows significantly shorter charge release times, and a more steep slope, which depicts a decrease in electrochemical capacitance. The reduced value can be attributed to structural changes by the irradiation of microwave, including recrystallization to some degree, reduced microporosity and increased internal resistance, which together hinder ion movement through the nanostructured face.

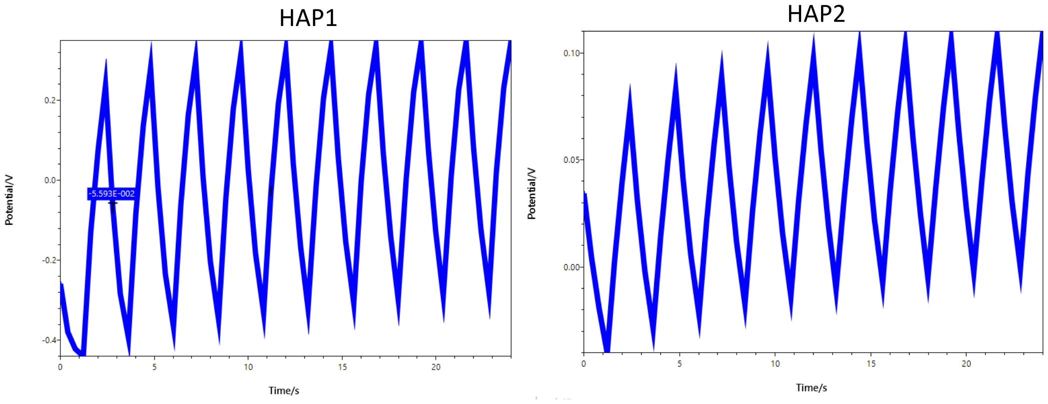

Figure 8. The charge–discharge (GCD) curves of hydroxyapatite

3. Conclusion

It entails comparative analysis of the syntheses of hydroxyapatite through the application of conventional grinding-calcination process (HAP1) and post-calcination (HAP2) by means of the application of microwaves. The structural investigations revealed a minor reduction of the crystallite size and homogeneity of the particles yielding small and less agglomerated nanoparticles due to the microwave modification. Nevertheless, despite these morphologic improvements, electrochemical investigations revealed that HAP1 was more appropriate than HAP2 with increased ionic mobility and enhanced redox activity and reduced charge-transfer resistance. It appears that this mechanism leads to rearrangement of the structure that causes high level of resistance and inhibits electrochemical responsiveness.

Overall, the findings indicate that, even though the refined morphology of particles can be obtained by following microwave treatment, this treatment is likely to damage electrochemical properties. Consequently, the selection of a synthesis route of hydroxyapatite must be based on the desired application: should one dwell on structural homogeneity, or the electrochemical functionality be much more significant.

Conflict of interest

The authors declare no conflict of interest

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