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Chemical Synthesis and Characterization of Hydroxyapatite Prepared from *Cypraea Annulus*

Serap AYAZ SEYHAN[1*], Dilek BİLGİÇ ALKAYA[1]

Highlights:

- Green and biocompatible waste-derived HA powders were prepared from sea shell Cypraea Annulus
- HA powders were produced by three different methods
- Microwave assisted method was determined as the ideal production method for HA powder synthesis

Keywords:

- Bioceramic,
- Calcium phosphate powders
- *Cyprae annulus*
- Hydroxyapatite

ABSTRACT:

In the last decade, the processes involved in biomineralization has greatly developed, leading to the production of a new generation of biomaterials. Calcium phosphate ceramic materials attract special interest due to their bioactive and biocompatible properties in biomaterials. Most of marine structures contains calcium carbonate (calcite or aragonite) and they can be easily converted to bioceramic material. The application of calcium phosphate ceramics as useful biocompatible materials largely depends on the purity and morphology of the powder. In this study calcium phosphate bioceramics (as raw materials for bone-scaffolds) were obtained via hot-plate, ultrasound-assisted, and microwave assisted method using the sea shell *Cyprae Annulus* as a calcium source. The characterization of the produced materials was carried out via FT-IR, SEM, XRD analysis. It was found that the calcium phosphate powders (hydroxyapatite) produced by three different methods were predominantly monetite and hydroxyapatite as the secondary phase. According to the SEM results, the overall morphology for CaP powder bioceramics shows the regular distribution of spherical and rice-shaped and CP powders produced by microwave assisted method have better morphology. The used methods are safe and inexpensive. Moreover, the raw materials (*Cypraea Annulus*) feature the advantages of the unlimited source as well as the biological origin. These methods were compared takes attention due to it is economical and easy method to obtain hydroxyapatite.

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INTRODUCTION

Biomaterials derived from marine derivated are generally abundant, renewable, inexpensive, and environment-friendly as compared to artificial biomaterials synthesized chemically (Bhattacharjee et al., 2019; Wan et al., 2021). Therefore, the research on seeking different possibilities and methods of recycling the waste material into useful product is quite necessary. By using this idea worldwide, a large number of industries are working for apatite products from biological resources (Bhattacharjee et al., 2019), Calcium phosphate (CaP) ceramic materials are used in orthopedics and dentistry (Eliaz et al., 2017). Due to its biocompatibility, non-toxicity, and non-inflammatory response, it has a very important place in implant coatings and bone graft studies (Eliaz et al., 2017). Hydroxyapatite (HA), is a mineral of the calcium phosphate family, is produced from various natural sources, as its pure powder is quite expensive (Antoniac et al., 2015).

Many researchers have been studied to use natural -based materials because of their comprehensive availability (Antoniac et al., 2015) Therefore, different bioactive compounds can be produced from marine derived resources such as Tiger Cowrie- *Cypraea Tigris* (Sahin et al.,2015), *Cypraea cervus* Linnaeus (Gunduz et al.,2013), *Cerithium vulgatum* (Gunduz et al.,2014), *Pseudo ceratinidae*, Verongiida (Shaala et al., 2019) and land snails (Kel et al., 2012). Also, our previos studies were conducted, calcium phosphate nanostructures which have have been synthesized from natural biowastes, like, sea urchin (Ağaoğullari et al., 2012) oyster (Seyhan et al.,2022; Cesur et al., 2020), and snail shells (Alkaya et al., 2022).

Cypraea annulus, the gold ring cowry, is common throughout its range in the West Indo-Pacific and can be found in shallow intertidal zones, hidden under coral or rock boulders and amongst seagrass (Figure1).

Figure 1. *Cypraea Annulus*

The synthesis of CaP and their production methods have been authored. Chemical precipitation method takes attention because it is simple, environmental, low-cost methods. The reaction of calcium and phosphate resources with different Ca/P ratios in aqua medium results calcium phosphate ceramics (Fathi et al., 2015; Castro et al., 2020). Additionally, the ultrasound method has proved to be rather easy procedure for the HA and TCP production from calcite/aragonite powders (Seyhan et al.,2022; Kel et al., 2012; Tamasan et al., 2013).

However, the synthesis CaP with microwave assisted method is performed by microwave energy conversion into heat. Smaller particle size, good purity and a closer size distribution is obtained by this technique (Hassan et al., 2016; Castro et al., 2020). In this study, hydroxyapatite was prepared by three synthesis method from marine derivative waste, cyprae annulus, was used as a calcium source effects of the proposed three methods (hot-plate, ultrasound-assisted, and microwave assisted) on structure and characterization was compared.

MATERIALS AND METHODS

Materials

Sea snail shells (*Cypraea Annulus*) used in this study were obtained from Marmara University Advanced Nanomaterials Research Laboratory. Orthophosphoric acid (85%) was purchased from Sigma-Aldrich. SEM (EVA MA 10, ZEISS, USA), FT-IR (Jasco FT / IR-4700) and XRD (Bruker, USA) apparatus were used for the characterization of bioceramics. Hot-plate (IKA, RCT, Germany), Microwave-Digestion System (MWS-2 by Berghof) and ultrasonic bath (EFLAB, KUDOS-SK 3310NP) apparatus were used for the production of bioceramics.

Experimental procedure

The samples were cleaned by removing the surface contaminants and then milled in mortar until a powder of 100 µm particles size was obtained. DSC TGA, was used to determine the exact CaO content of shells during dissociation.

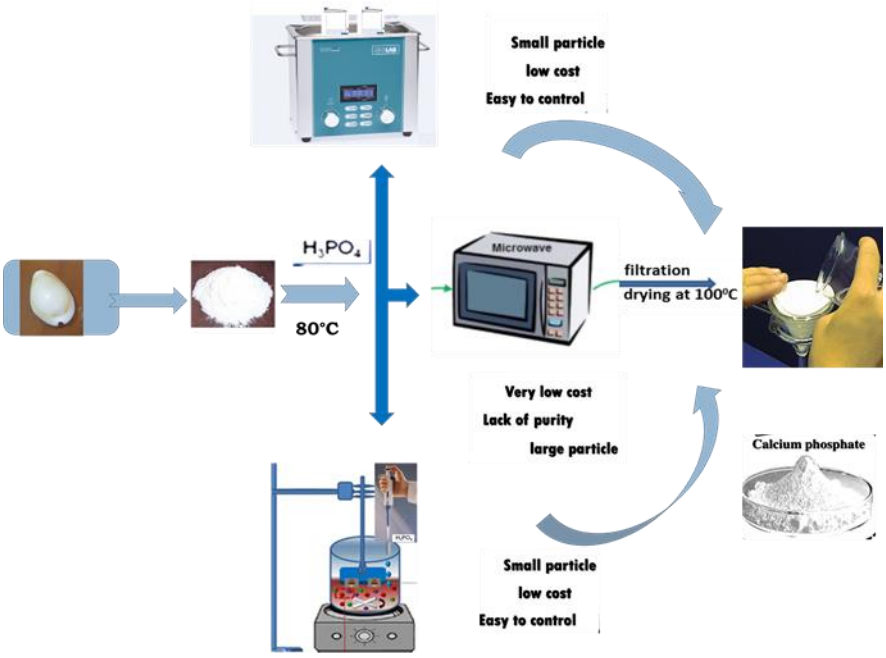

Figure 2. Schematic diagram of the method

The snail samples were converted to calcium phosphate by three different synthesis methods: hot-plate, ultrasonic and microwave-assisted co-precipitation method (Figure 2) and the natural powders containing solution were performed adding, orthophosphoric acid solution slowly. The obtained amount of Ca/P was 1.67. Firstly, wet chemical precipitation was modified for CaP synthesis. The reaction was conducted under a stirring rate of 800 rpm at 80 ^{o}C under hot-plate (IKA, RCT, Germany) for 2 h. Afterwards the same procedure was carried out for the ultrasound-assisted (with a nominal power of 100 W and a frequency of 35 kHz; EFLAB, KUDOS-SK 3310NP) method. The obtained solution was kept overnight at 25 °C. Then, the settled precipitates, were filtered and washed three times with distilled water. Finally, the solid was oven dried at 100 ^{o}C for a period of 24 h. This last process was adopted on the products obtained from the two synthesis methods (Castro et al., 2020). As a third section microwave synthesis was performed at the end of aging time, at 80 ^{o}C. The suspensions transferred to microwave- Digestion System (MWS-2 by Berghof) keeping the temperature at 80 ^{o}C for 30 min; the chemical reaction was occured inside the microwave oven. At the end of the reaction, the products were washed with distilled water and recovered by centrifugation (Pena et al.,2001; Hassan et al., 2016).

RESULTS AND DISCUSSION

Characterization of Bioceramics

In Figure 3 are shown DTA-TG results of the sea snail shells powder. As it can be seen DTA-TG result showed that snail shell is mainly composed of calcium carbonate ($CaCO_3$) (Alkaya et al., 2022; Seyhan et al., 2022). A weight loss of about 42 % following the decomposition reaction is observed (Figure 3). DTA-TG graphics gave the idea of the calcination temperature to which the snail shell powder must be heated for the formation of the apatite as biomaterial (Bhattacharjee et al., 2019; Kel et al., 2012). DTA curve shows an exothermic peak at ∼700 °C that may be due to the formation of biological HA.

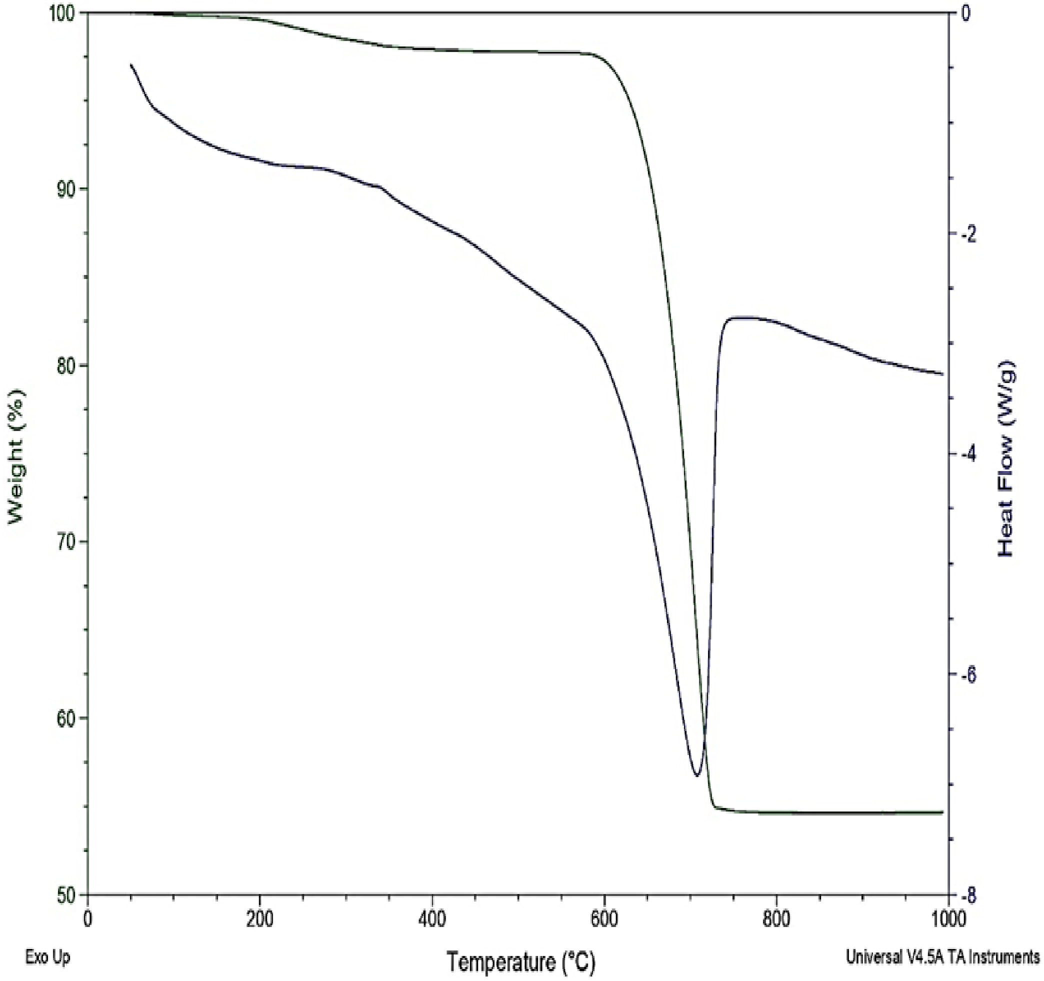

Figure 3. DTA-TG analysis for the sea snail shell sample

XRD patterns of the samples produced by hot plate, ultrasonic, and microwave-assisted method are included in Fig. 4. X-ray diffraction (XRD) test results show the presence of monetite and apatite structures in the synthesized product. In Figure 4a and 4b, HA showed its characteristic peaks at 28, 31, 32. A diffraction peak located at 30.31° was assigned to monetite ($CaHPO_4$) according to ICDD card No. 01-071-1759, indicating the presence of a predominant phase (Seyhan et al., 2022). Monetite is an important calcium phosphate precursor that promotes cell proliferation, being recognized for its osseoinductive and osteoconductive character (Zhou et al., 2021). In Figure 4c contains of the hydroxyapatite peaks and it is also detected that there are other phases such as monetite, aragonite (Alkaya et al., 2022).

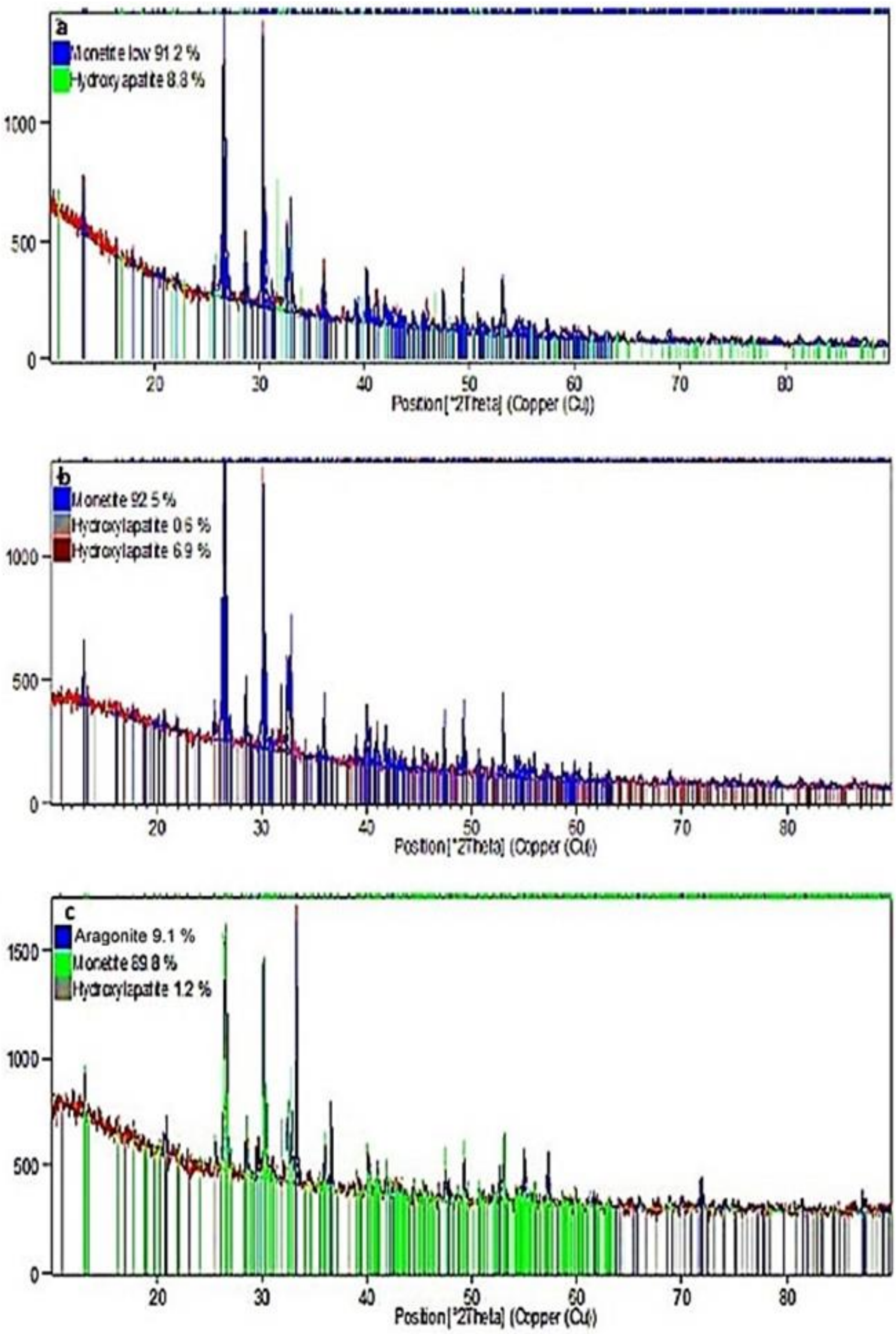

Figure 4. X-ray diffractograms of HA samples; a. microwave method, b. hot-plate method, c. ultrasonic method

The chemical constituents of the shells for all time measurements in all experiments were analyzed by FT-IR which is based on the principle of absorption of infrared energy at specific frequencies or wavelengths of chemical bonds. Absorbance value was recorded in the region of 650-4000 cm^{-1} and functional groups of the samples were relevant from the literature (Seyhan et al., 2022; Kel et al., 2012; Sahin et al.,2015).

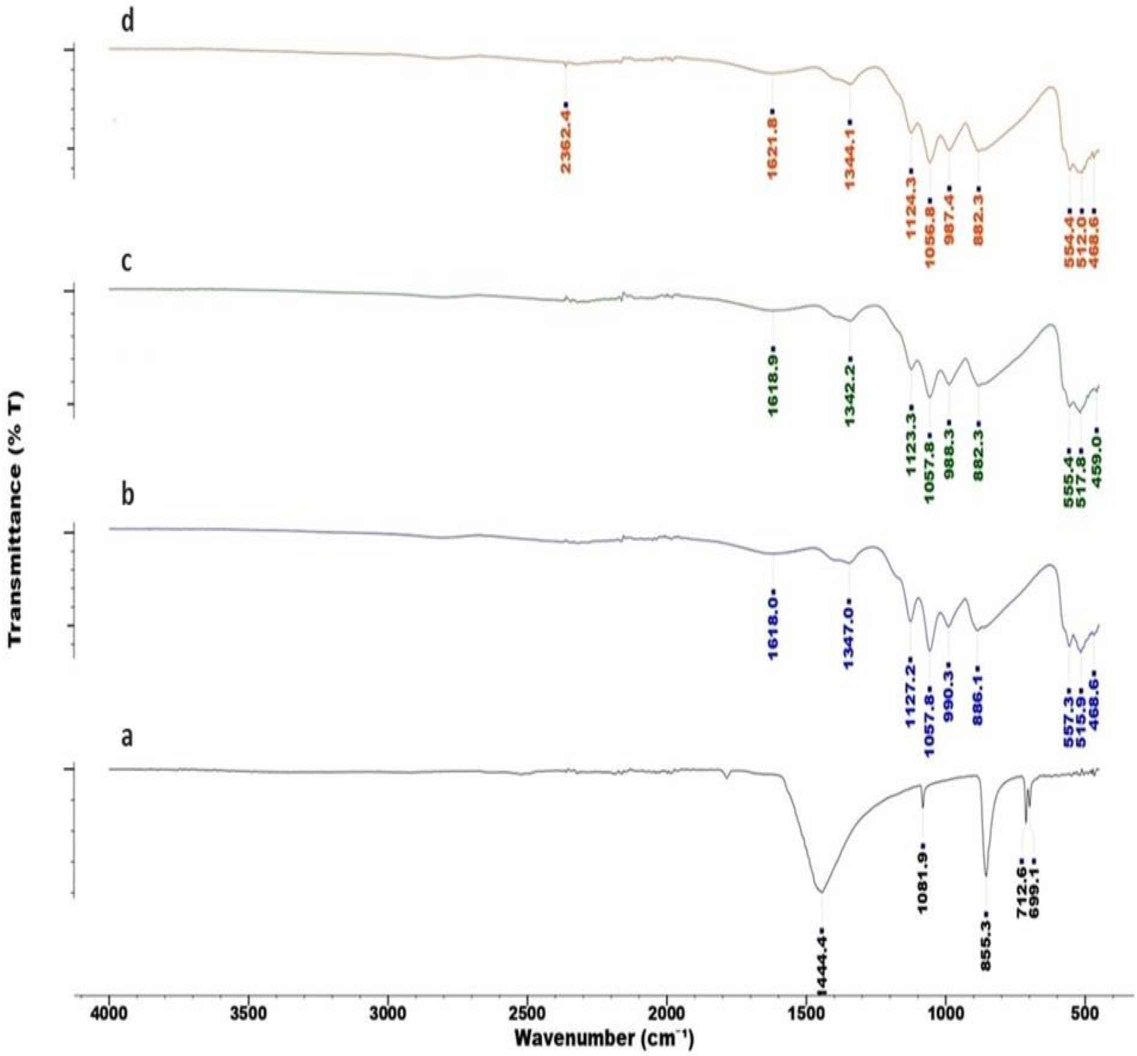

Figure 5. Comparison of FTIR spectra of HA samples; a. raw sample, b. ultrasonic method, c. hot-plate method, d. microwave method

Figure 5 displays the FT-IR spectra of the converted shell samples by proposed three synthesis method. The absorption band of the PO_4^{3-} group is recorded at wave number 990 cm^{-1}, the absorption band for the CO_3^{2-} group is at wave number 1344 cm^{-1} by ultrasound and hotplate method (Seyhan et al., 2022; Kel et al., 2012; Sahin et al.,2015). Otherwise, the HA sample originating from *cyprae annulus* synthesized by microwave assisted method sample PO_4^{3-} and CO_3^{2-} group absorption bands were recorded at wave number 988 cm^{-1}, 1342 cm^{-1} respectively. The CO_3^{2-} groups were recorded in each sample. The CO_3^{2-} group can replace PO_4^{3-} or OH^- and cause changes the CaP crystal structure.

SEM is one of the most preferred instruments that is available for the examination and analysis of the structure morphology and chemical composition. Figure 6 represents SEM micrographs of prepared HA bioceramics. According to the SEM scanning, the overall morphology for HA bioceramics shows the regular distribution of spherical and rice-shaped nanoparticles (Sadjadi et al., 2010). When SEM images are examined, microwave assisted method, one of the synthesis methods, can be applied as the ideal production method for CaP powder synthesis. As can be seen in Figure 6c, better morphology of the materials is presented.

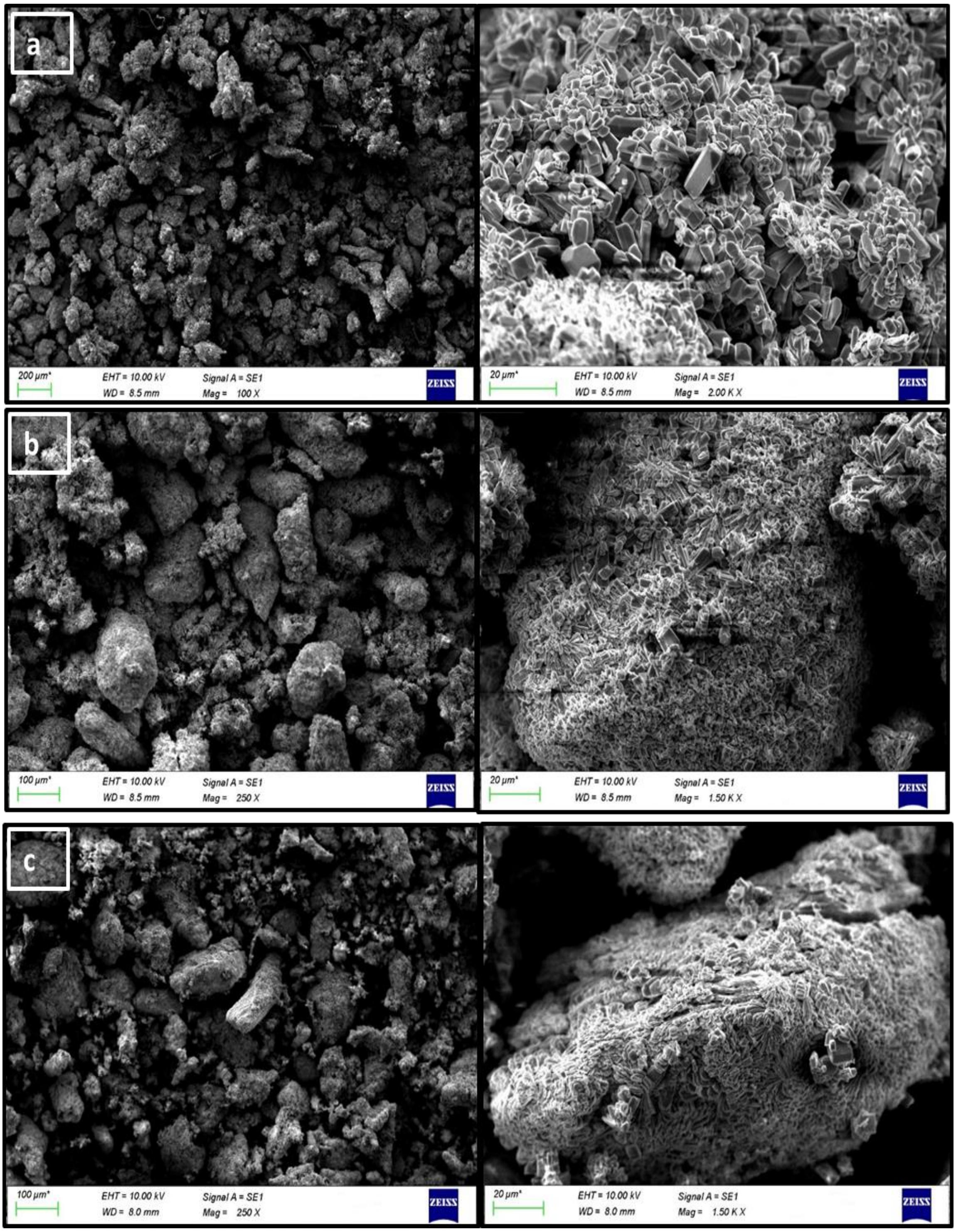

Figure 6. SEM micrographs of prepared HA bioceramics at high and low magnification; a. Hot-plate b. Ultrasound assisted and c. Microwave assisted chemical precipitation

CONCLUSION

In this study, calcium phosphate material was obtained from sea snail shell (*Cypaea Annulus*) waste by three synthesis methods (hot-plate, ultrasound and microwave assisted). Thus, the aragonite structure of the seashell was transformed into hydroxyapatite and monetite. Monetite is an important precursor of calcium phosphate, which promotes cell proliferation, known for its osseoinductive and osteoconductive character. When SEM and FT-IR images were examined, smaller particle size, good purity and closer size distribution were obtained with microwave assisted technique. Thus, green and biocompatible waste-derived CaP powders were prepared from snail shells. This shows that microwave assisted method can be applied as the ideal production method for CaP powder synthesis.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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