Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 9, Issue 2, 2021

Physicochemical properties of thermally synthesized biogenic hydroxyapatite for tissue engineering application

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ABSTRACT

A lot of studies have presented synthesis of biogenic hydroxyapatite using different techniques, but this study presents a modified thermal treatment method by replacing boiling stage of the synthesis with carbonization. Raw bovine bones (RB) were collected and first heated with fired charcoal to remove the some organic matters present. The bones were heated until no appearance of smoke. The fired bones were further taken into an electric furnace for complete synthesis at 900, 1000, and 1100 ^{o}C with a ramp rate of 5 ^{o}C/min holding for 2 h. The heated samples were allowed to furnace cool afterwards. Fourier transform infrared (FTIR), bulk density, and porosity analysis were conducted on the synthesized hydroxyapatite (HAp). The FTIR result revealed variations in the degree of carbonate ion substitution of the RB and the synthesized HAp. Result obtained from FTIR shows that the modified thermal treatment method is a good synthesis technique for biogenic HAp. Physical properties result showed that increase in temperature increased the density of Hap and in turns reduced its porosity. Essentially, the porosity of HAp synthesized at 900 ^{o}C is within the required porosity for cell proliferation, which makes the material a promising candidate for tissue engineering application. Further study can be conducted on the structural, mechanical and biological properties of the synthesized HAp.

Keywords: Hydroxyapatite, chemical group analysis, bulk density, porosity, bovine bones, thermal treatment

INTRODUCTION

In tissue designing, it is all around reported that HAp is the steadiest type of calcium phosphate. It is considered as quite possibly the most utilized material in bone recovery on the grounds that

primarily of it is non-cytotoxic to bone tissues. HAp is more advantageous compared to other material due its close affinity with the native bone. Aside from that, HAp additionally has many wanted biocompatibility qualities like non-harmfulness, no fiery impacts and immunologic reactions associated with it [1-4].

In ongoing works, there are HAp powders synthesized from both biogenic and chemical sources through different processing techniques [1, 5-9]. Because of the special and efficient method of the crystal arrangement of apatite in bone, there are contrasts between the natural and synthetic HAp. Synthetic HAp will in general display smaller crystal size and thus, have higher surface region [10-11]. Be that as it may, Hap synthesized through chemical do exhibit some disadvantages. Among the disadvantages are; high cost of base materials, the presence of some undesired chemical compounds synthesis, and the absence of essential trace elements like sodium (Na), silicon (Si), iron (Fe) or minerals like carbonate in biogenic derived HAp. Notwithstanding, being viewed as contaminations in the HAp structure, these seemingly foreign elements are helpful in bone development and supporting natural capacities, as seen in organic HAp.

A lot of researchers have dealt with the advancement of HAp from biogenic sources. Among the biogenic sources that are right now being assessed incorporate cow-like bones [12-13], fish bones [14-16], porcine bones [17-18], eggshells [19-20] and seashells [21-22]. Despite the fact that there were numerous normal sources that were considered, cow-like bones stay the most grounded source because of the simplicity of this waste biogenic material.

In contrast to synthetic HAp, carbonate particles usually exist in biogenic HAp as an impurity. The carbonate particles discovered in biogenic HAp have all the earmarks of being amazing for bio-resorbable bone substitutes [23]. The attributes of Hap synthesized from biogenic sources depend essentially on the employed synthesis technique. On account of biogenic apatite, it was discovered that there are various replacements just as inadequacies at all ionic compounds. B-type carbonate HAp is an example of the compounds, in which the phosphate particles were subbed via carbonate [24].

This study presents the synthesis of biogenic Hap ceramic derived from bovine (cow) through thermal treatment. This study also investigated the chemical groups, bulk density and porosity the synthesized HAp. Bovine bone was selected because it is the most abundant biowastes in Abattoirs. This will reduce air pollution and the rather untransformed bovine bones

will become high valued clinical product. In addition, this study presents a modified, low-cost and facile calcination synthesis method. The modification introduced in this study is the use of charcoal firing to remove organic compounds from the raw bones in place of boiling of the bones which is hectic, time consuming and expensive.

MATERIALS AND METHODS

Hydroxyapatite thermal synthesis

Raw bovine bones were collected from an Abattoir in Zaria, Nigeria, and were used as a biogenic source to synthesize HAp. The collected bovine bones were first heated with fired charcoal to remove some organic matters present like protein, fat and oil, collagen, etc. The bones were heated until no appearance of smoke. The first firing was done to avoid smokes and probable build-up pressure within the closed furnace, which may damage the furnace. The fired bones were further taken into an electric furnace for complete synthesis. The bones were heated to different temperatures namely: 900, 1000, and 1100 ^{o}C (denoted as HAp-900, HAp-1000, and HAp-1100, respectively) at a ramp rate of 5 ^{o}C/min and were held for 2 h and allowed to furnace cool afterwards.

Chemical analysis

The functional or chemical groups present in the raw bovine bones and the heated samples at various temperatures were identified by FT-IR equipped with UATR sampling accessory in the range of 650–4000 cm^{-1}. Samples were ground and mixed with dried KBr using ceramic mortar and loaded into a sample holder mounted in the instrument.

Physical properties analysis

The heated samples were crushed with a metallic mortar and pestle and sieved through a 300 μm mesh sieve to obtain a fine powder. The powdery sample was cold-compacted in cylindrical shape (25 mm diameter and 10 mm thickness) under 5 KN with Universal testing machine (UTM). The fabricated scaffolds were left at atmospheric drying for 24 h. The bulk density and porosity measurements were conducted on the dried scaffolds. Bulk density and porosity were computed as follows:

The porosity of the sintered samples was calculated using Equation (1):

$$\text{Porosity } (\%) = \left(1 - \frac{Weight\ of\ HAp}{Volume\ of\ HAp \times Density\ of\ HAp}\right) \times 100 \quad (1)$$

3.16 g/cm^3 was used as the typical density of HAp [25-27].

The density of the sintered samples was calculated using the Equation (2):

$$\text{Density of the sintered powders } (g/cm^3) = \frac{mass\ of\ the\ sintered\ powders}{Volume\ of\ the\ sintered\ powders} \quad (2)$$

RESULTS AND DISCUSSION

Hydroxyapatite chemical groups' analysis

Figure 1 shows the Fourier transform infrared spectra of RB and the synthesized HAp made as a singular overlay plot. It is observed that the FT-IR data revealed phosphate and hydroxyl peaks in the as-received and thermally treated samples, and all the samples produced characteristic stretching modes of O–H bands at about 3400 cm^{-1}. These are noticed in all the FT-IR spectra of the RB and HAp. From the FT-IR spectrogram, differences between the spectrum of the raw and the synthesized HAp samples are noticeable. The bands at around 1400 and 1460 cm^{-1} for HAp-1000 and HAp-1100 samples show a clear variation compared to the bands ascribed to RB and HAp-900 samples. These differences are indicative of the degree of carbonate ion substitution. The bands at around 1640 and 3400 cm^{-1} for all samples with broader bands for RB and HAp-900 samples correspond to the disappearance of absorbed water after heat treatment. This observation is similar to the observation made by Abifarin *et al* [1, 34], which made the modified method a good technique for the synthesis of biogenic HAp.

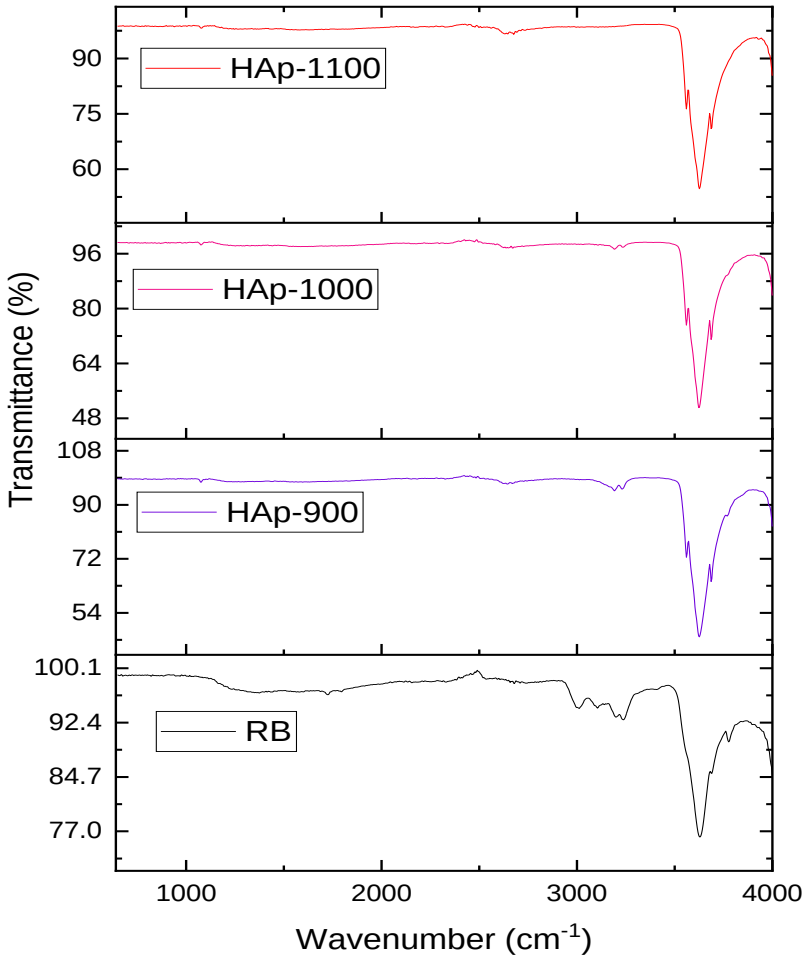

Figure 1: FTIR patterns of RB and Hap samples

Bulk density and porosity of the synthesized HAp

Figures 2 and 3 respectively reveal the deviations in bulk density and porosity of the synthesized biogenic HAp at 900, 1000, and 1100 ^{o}C. It is observed that the HAp density increased with increase in sintering temperature, while the porosity reduced. Bulk densities of the synthesized Hap at 900, 1000, and 1100 ^{o}C are respectively 1.67, 2.02, and 2.56 g/cm^3, while their corresponding porosities are 46, 38.04, and 30.01%. Clearly, the result revealed inverse relationship between density and porosity, which is in line with literature [28-30]. Previous reports have revealed that porosities in the range of 40–90% enhances osteointegration on the implant surface and enhanced adhesion of implants with bone [31]. Although, literature has it

that, increase in density improves the mechanical properties of a material [2, 5, 32-33], however, the porosity of HAp synthesized at 900 ^{o}C is within the required range and it is close to the minimum required range. It can be inferred from this study that the HAp-900 is a promising candidate for tissue engineering application.

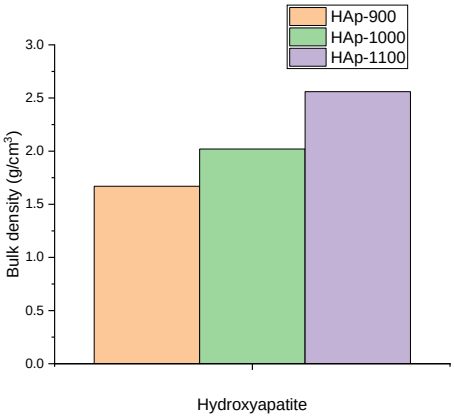

Figure 2: HAp bulk density

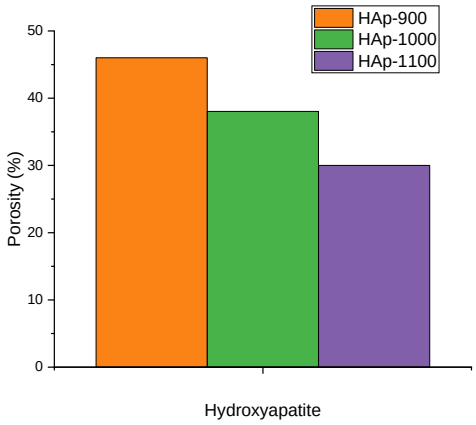

Figure 3: HAp porosity

CONCLUSION

Physicochemical properties of thermally synthesized biogenic HAp have been successfully conducted. The FTIR result revealed variations in the degree of carbonate ion substitution of the RB and the synthesized HAp. The broader bands observed at around 1640 and 3400 cm^{-1} for RB and HAp-900 samples indicated the disappearance of absorbed water after heat treatment. This result shows that the modified thermal treatment method in this study is a good technique to

synthesize biogenic HAp. Also, it is observed that increase in temperature increased HAp density but decreased its porosity. It is important to note that the porosity of HAp synthesized at 900 ^{o}C is within the range of porosity required for the enhancement of cell proliferation. Therefore, HAp synthesized at 900 ^{o}C is a promising candidate for tissue engineering application. Further study can be conducted on the structural, mechanical and biological properties of the synthesized HAp for tissue engineering application.

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