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Preparation and Characterization of Hydroxyapatite and Optimizing Its Properties Using Regression Model

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Abstract. This study clarified the importance of hydroxyapatite in the biological and medical field as alternatives for damaged parts of the bones and teeth. The aim of this study is to preparing the hydroxyapatite from a biological source, bovine femur bones, for its efficiency and low cost. The bovine bone has been converted to hydroxyapatite by a heat treatment method at 1000 °C for 3 hrs. The resulting powder was characterized by X-ray diffraction, Fourier transform infrared spectroscopy and particle size. The regression models used for the optimization mechanical and physical properties, by taking density as an independent variable, porosity, hardness and compressive strength as dependent variables. Through regression analysis, it was found that the value of the correlation coefficient (R) is (0.990), while the value of (F) in the ANOVA test was (15.97). In addition to the T-test, which indicates the presence of statistical differences between the variables.

Keywords: Hydroxyapatite, FTIR, XRD, hardness, density, porosity, regression model

1. Introduction

As a bone replacement, hydroxyapatite (HAP) with the chemical composition of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ has been extensively studied. If inserted in any soft tissue, it shows excellent biocompatibility [1, 2, 3], or hard tissue [4]. HA is a bioactive material majorly because its ratio of calcium to phosphorus is close to that of natural bone and teeth, making this material an ideal candidate for clinical applications either as a fully dense calcified material [5]. HA is not only a biocompatible, osteoconductive, non-toxic, non-inflammatory and non-immunogenic agent, but also a bioactive agent that is capable of forming a direct chemical bond with living tissues [6]. HA crystallizes in a hexagonal system, although with some exception in a monoclinic system. The system belongs to the hexagonal space group P63/m, with hexagonal rotational symmetry and a reflection plane and with cell parameters of $a=b=9.418 \text{ \AA}$ y $c=6.884 \text{ \AA}$ [7].

HAP can be derived from bones for instance; bovine and fish bones, fish eggs, oyster shells, chicken egg shells and corals, both naturally and economically. Waste can be recycled, reused and channeled into the creation of value-added goods for sustainable growth. Bovine bone and a biowaste is a cost-effective source of HAP for hard tissue replacement in medical applications. [8]. Intensive HA study including a wide variety of techniques for powder processing, composition and experimental conditions has been studied in order to determine the most efficient method of synthesis and conditions for the development of well-defined particle morphology. The wet precipitation process, mechanochemical technique, sol-gel technique and hydrothermal



method are among the more popular methods used to synthesize HA [9]. Applications of hydroxyapatite are Drug delivery [10] Surface coating on metal parts for implantation, Tissue engineering[11] and Other applications such as fillers, spacers and bone graft substitutes in orthopedic, maxillofacial applications and bone replacement[12].

The regression analysis is a set of statistical procedures that can be used to draw conclusions about the relationships between variables. Regression analysis is today possibly the most widely used data analysis tool, with applications in practically every field of study, including physical and biological sciences and engineering [13].

The assumption that the data is sampled from a Gaussian distribution underpins many statistical tests. Parametric tests are the name given to these types of testing. The first column of the table lists the most often used parametric tests, which include the t test and analysis of variance. Non-parametric tests are those that do not make any assumptions about the probability distribution. [14].

ANOVA is currently the most commonly used statistical approach for hypothesis testing. It covers a wide range of topics and can accommodate a bigger number of experimental designs. [15.16]. There are several types of ANOVA are (1) one-way ANOVA, 'random effects' model (2) two-way ANOVA in randomised blocks (3) three-way ANOVA (4) factorial ANOVA (5) factorial ANOVA, split-plot design, and (6) factorial ANOVA [17].

The aim of this study is to prepare and characterize hydroxyapatite from bovine bone and optimize (mechanical and physical) properties by regression analysis.

However, in the Previous Study, Lü, X. & et al, (2007) the hydroxyapatite was prepared and characterized from natural sources. The study was used pig bones, using the calcination method, with temperatures ranging from 650°C to 1050°C. According to the results of XRD and FTIR, the best temperature gives a high degree of crystallinity and the required quality is 850 °C [18]. While, Manalu, J. & et al, (2015) In this study, the hydroxyapatite was prepared from a biological source, which is bovine bone, by a heat treatment method at different temperatures, and it was characterized using XRD, EDX and FTIR, where 850°C was the best temperature gives desired quality. In addition, SEM indicated the granular size of HA and that it tends to crystal agglomeration with increasing temperature [19]. As well, Khoo, W., & Kurniawan, D. (2015) In this study, hydroxyapatite was extracted from bovine femur bone, where fresh bovine bone was used after washing, cleaning and calcining it at different temperatures of 700°C, 900°C and 1100°C for 3 hrs and sizes of granules. HA was characterized by XRD, FESEM, and FTIR, as it was found that by increasing the temperature, the crystallinity and granular size increases and free of organic matter. Hydroxyapatite above 700°C is suitable for use in structural applications [20].

2. Materials and Methods

The preparation process steps show in Figure 1, include boiling the bovine femur bone with water for 6 hours to remove the fat and other contaminants. After that, it was washed with distilled water and dried. A gas torch under direct ignition was used to clean the bones at a temperature of 700 °C for 5 hours. Bone ash was heated in an electric furnace under ambient conditions, at 1000 °C with a heating rate of 10 °C /min and 3 hours holding time. After heat treatment, the hydroxyapatite was milled in a planetary ball mill system (consisting of an alumina jar and balls) to prepare fine particles. The milling duration was set at three hours and the speed was set at 360 rpm.

To prepare compact hydroxyapatite sample was mixed with 2% wt. polyvinyl alcohol (PVA) as a binding material. Green ceramic specimens were then formed in stainless steel cylinder molds with a diameter of 20 mm using uniaxial semi-dry pressing procedures. The pressure used was 150 MPa, and the solid samples

was sintered at temperatures of 1250 °C with a heating rate of 5 °C/min for 3 hrs, and cooling down has been done inside the furnace. The specimens were ground on a (YMP – 2 Machine in the ceramics laboratory / College of Material Engineering/ Babylon University) at a speed of 300 revolutions per minute using SiC grinding papers of different grit sizes (180, 320, 600, 800, 1200, 1000, 1500, 2000). The grinding duration was 10 min for each grit size, with water as a coolant.

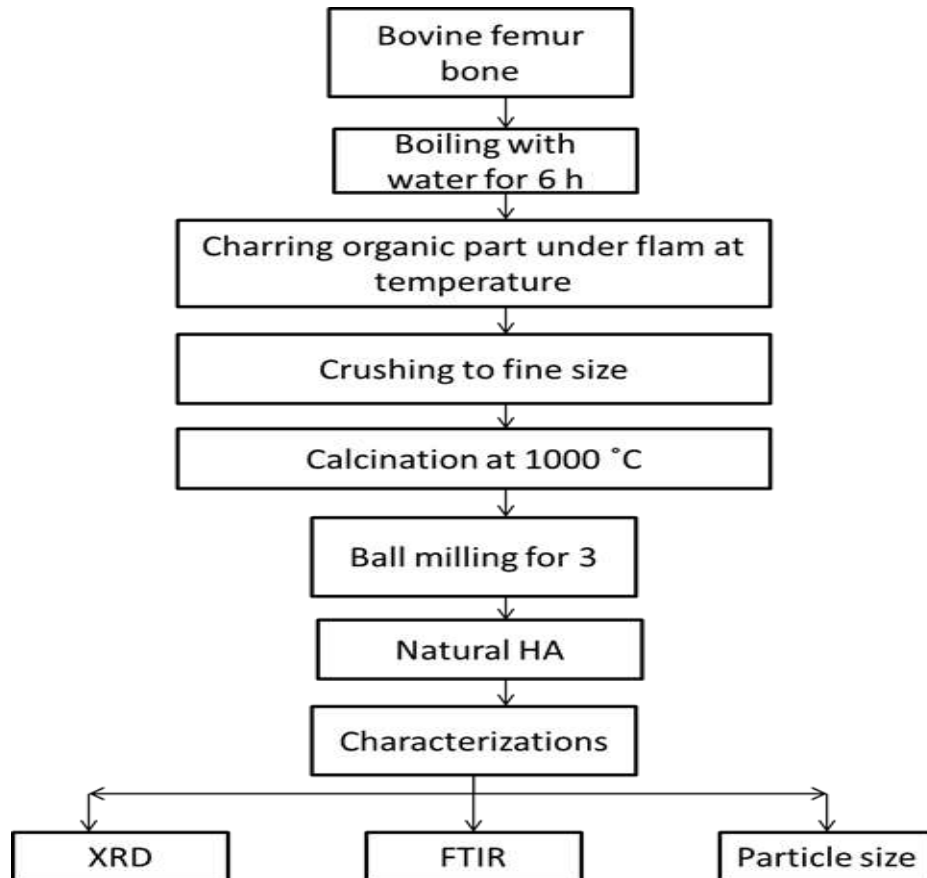


Figure 1. Flowchart of extraction HA from bovine bone.

3. Results and Discussion

3.1 XRD diffraction results.

The phase and purity of derived HAp crystals were confirmed with XRD analysis. Figure 2 shows the bovine bone calcined at 1000°C . The results show that all peaks are corresponded with HA phase and matching with (JCPDS, card NO. 01-074). Figure 3 shows the hydroxyapatite powder after sintering at 1250°C and there is no decomposition of HA to β -tricalcium phosphate phase .The results matching with (JCPDS, card NO. 01-074).

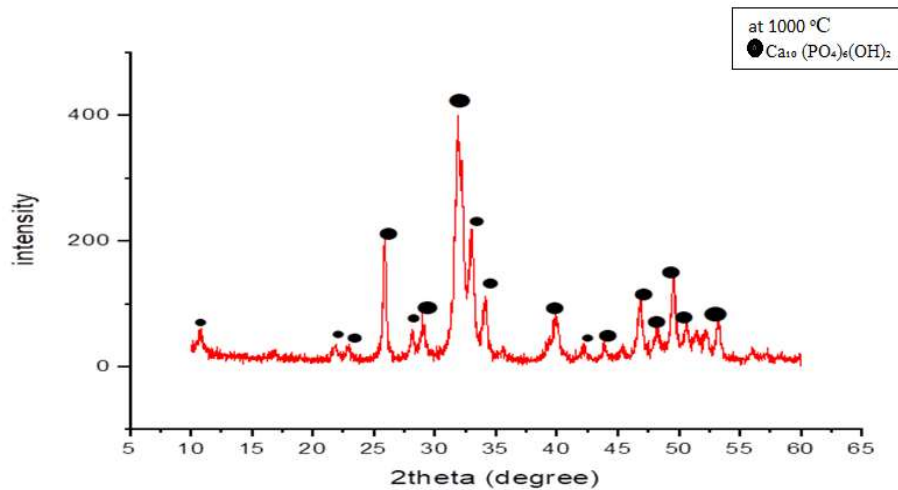


Figure 2. shows the XRD of raw bovine bone after calcination.

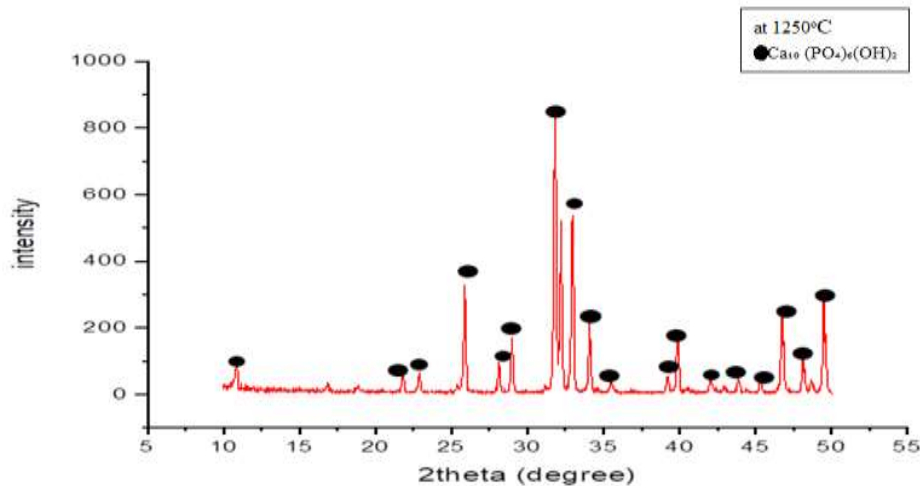


Figure 3. shows hydroxyapatite powder after sintering at 1250 °C.

3. 2 The Fourier transforms infrared Spectrometer (FTIR)

The Fourier transform infrared FTIR (Shimaduz 1800, Japan, Department of Polymers Engineering and petrochemical industries, College of Materials, University of Babylon) was used to evaluate molecular structure or functional groups in inorganic materials. The wavenumber in the range (500-4000 cm^{-1}). Figure 4 displays the FTIR test of HA and the broad band at 3417 and 1620 cm^{-1} were attributable to adsorb water, while sharp beak at 3572 cm^{-1} was attributable to stretching vibration of lattice $-\text{OH}$ ions and medium sharp peak at 632 cm^{-1} assign to the O-H deformation mode. The characteristic bands for PO_4^{3-} appear at 570, 601, 964, 1049 and 1089 cm^{-1} . The results show that the powders of hydroxyapatite free from carbonate groups. The result of FTIR of HA powder were calculated with reference [21].

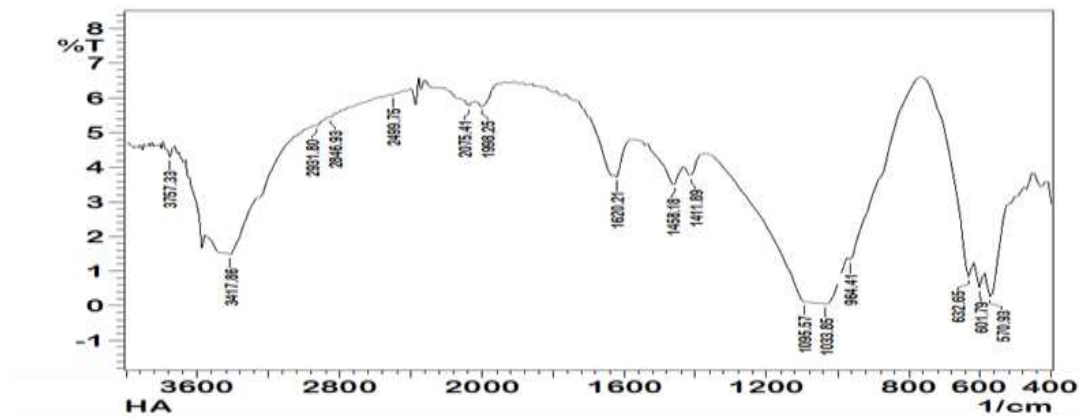


Figure 4. shows FTIR of hydroxyapatite

3.3 Particle size analysis

The distribution of particle size of hydroxyapatite powder was measured using the laser particle size analyzer Bettersize2000 (Better size Instruments Ltd., China). The system was located in Department of Ceramics Engineering and building materials in Faculty of Materials Engineering - University of Babylon

Figure 5 displays the particle size distribution analysis results for hydroxyapatite powder. The distribution of the hydroxyapatite particles was in range 0.3 to 3 μ m, and the D10, D50, and D90 were 0.302, 0.848, and 3.779, respectively.

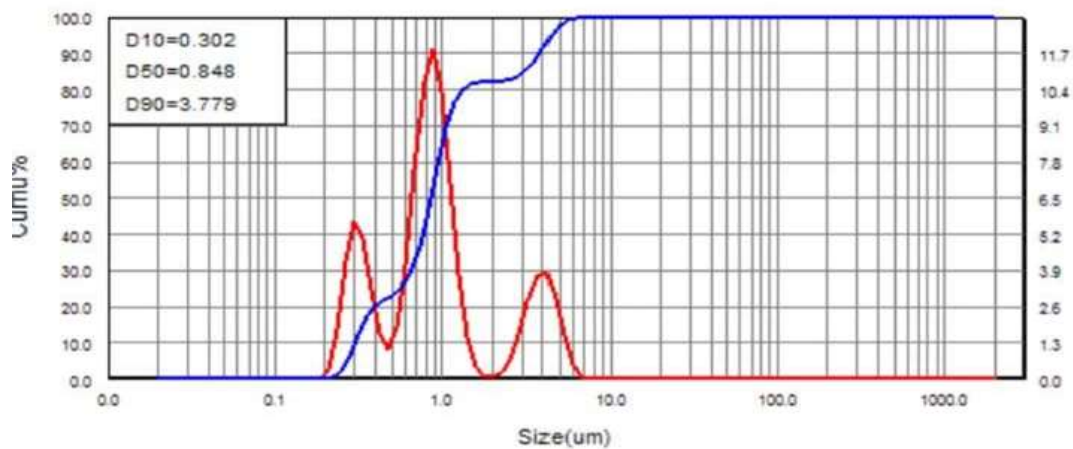


Figure 5. Illustration particle size of hydroxyapatite.

3.4 Physical and mechanical properties

The Archimedes method was used to calculate the apparent porosity and bulk density in accordance with ASTM C373-88. Compression strength was determined using an electronic universal testing machine (Department of Ceramics and Building Materials, Faculty of Materials Engineering, University of Babylon). The cylindrical specimens were prepared with dimensions D=20 mm, H=40 mm, and testing was done according to ASTM standard C-1424.

Vickers hardness was measured using disc specimens with a diameter of 20 mm and a height of 5 mm. The test was performed using a digital micro Vickers hardness tester (TH-717) at 1 kg with a dwell time of 15 seconds, in accordance with ASTM standard C1327-90.

3.5 Regression Models

A) Regression analysis

Table 1 display result of regression analysis for the study variables.

Table 1. Result of regression analysis

dependent Variable	Predictor variables	R	R ²	F	Sig.F	Beta	t	Sig.t
Density	Porosity	0.990	0.918	15.970	0.181	0.796	4.593	0.136
	Hardness					0.176	0.790	0.574
	Compression					-1.113	-4.663	0.134

The results were statistically analyzed by SPSS (one-way ANOVA) to determine the mean value and showed a significant difference for each particle size. According to Table (1), which considered density as a dependent variable and (porosity, hardness and compression) as predicted variables. The results represented that the density has an effect on the rest of the properties (porosity, hardness and compression) as explained in R value (0.990) and R² value (0.918).

B) Analysis of Variance

Table 2 exhibits the results of the presence of statistically significant differences because that the value of *F* (15.970) and its significance (0.181) is a statistically significant sign at the level of significance (0.05). Based on that, we reject the zero hypothesis and accept the alternative hypothesis that states the existence of statistically significant differences. Therefore, the study used one-way analysis of variance (ANOVA) because there are three groups within current study.

Table 2 . Result of ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	0.168	3	0.056	15.970	0.181 ^a
	Residual	0.004	1	0.004		
	Total	0.172	4			

a. Predictors: (Constant), Compression, porosity, hardness

b. Dependent Variable: Density

C) Factor analysis

Factor analysis was performed with 1 as the Eigen value to improve the strength of the factors. Then, two factors were extracted when the rotation converged in. The analysis extracted a two-factor solution, each with Eigen values above one. (Table 3)

Table 3 .Component Matrix^a

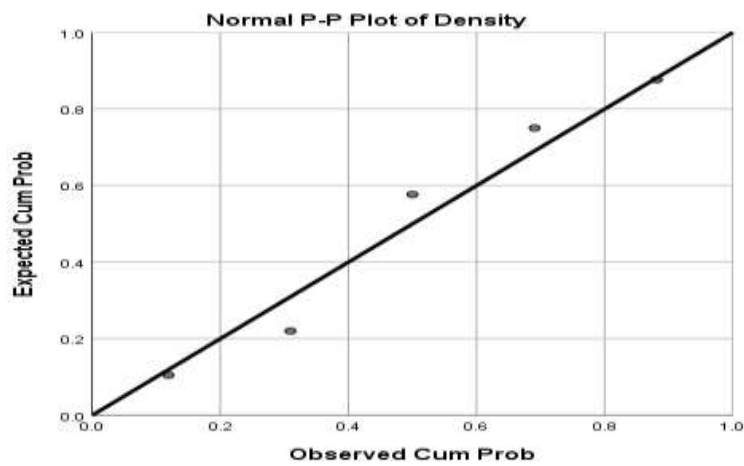
	Component	
	1	2
Density	-0.895	0.336
Porosity	-0.065	0.997
Hardness	0.877	-0.005
Compression	0.889	.0416

Extraction Method: Principal Component Analysis. 2 components extracted.

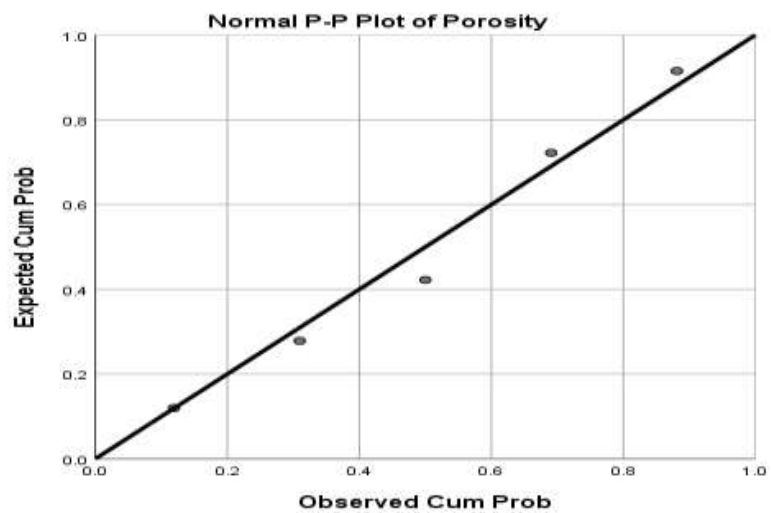
D) P-P plot

Figure 6 show a result of p-p plot Which represents an abnormal distribution of the variables .

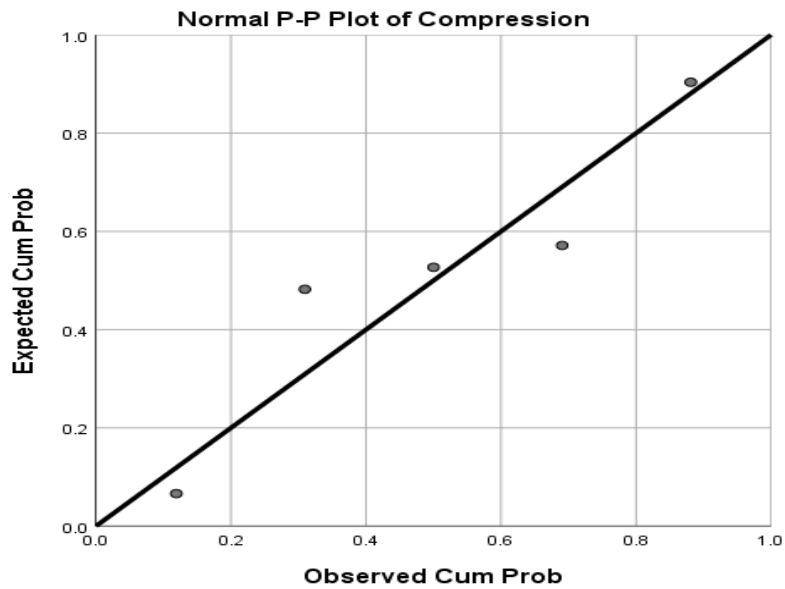
(a)



(b)



(c)



(d)

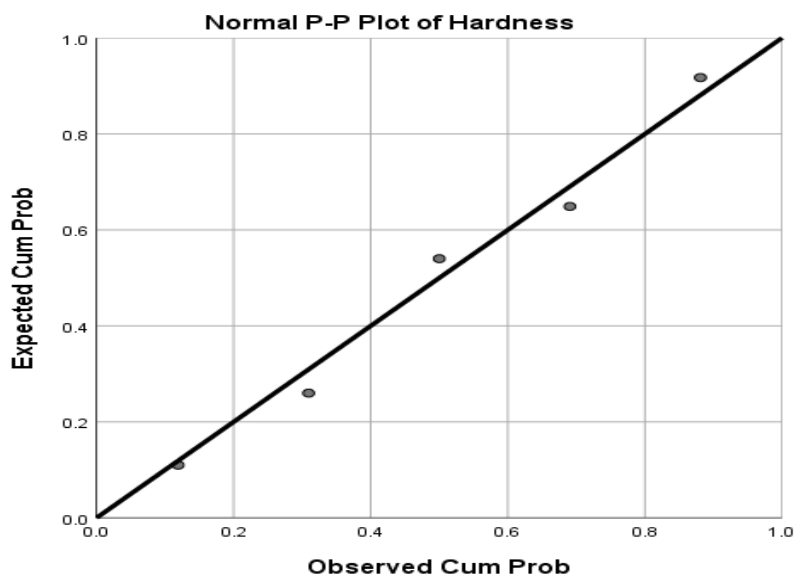
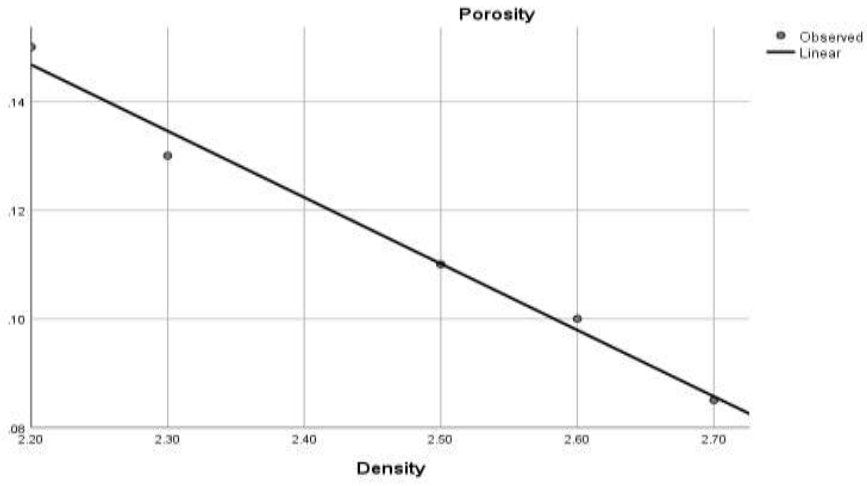


Figure 6. display P-Plot of (a) density, (b) porosity, (c) compression, (d) hardness variables.

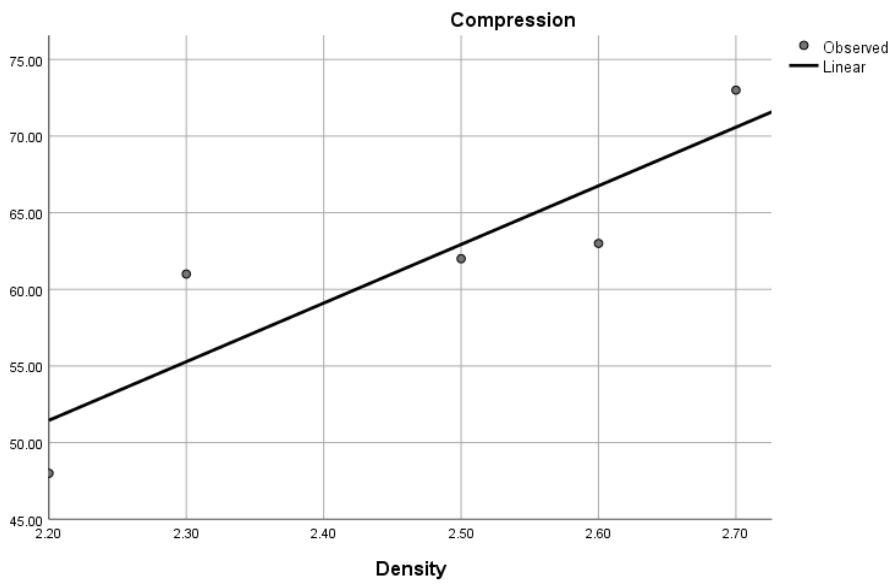
E) Curve fitting

Figure 7 shows the relationship among the independent variable (density) and the dependent variables (porosity, compression and hardness) it was found that the inverse relationship between density and porosity and a direct relationship between density, compression, density and hardness in addition to the correlation coefficient (R^2) among the independent variable and the dependent variables is 98 %

(a)



(b)



(c)

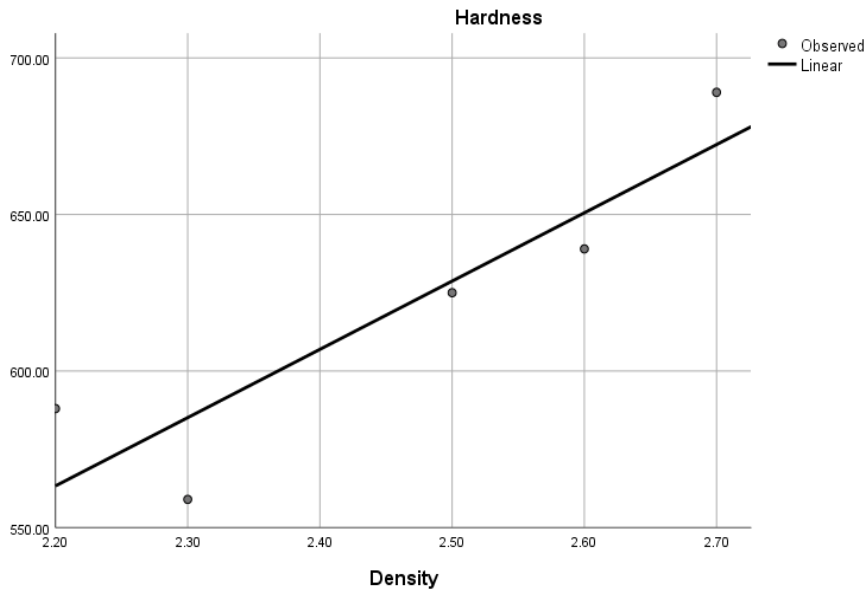


Figure 7. Regression curve fitting (a) porosity (b) compression (c) hardness.

F) T-Test

The performance of indentation Density and Porosity, Hardness and Compression strength tests were undertaken on the four samples that were prepared of hydroxyapatite the laboratory. The aim of this test to know if there is a strong linear correlation among density index value and Porosity, Hardness and Compression strength indexes values. Accordingly, from Table 4 conclude that there are statistical differences between the variables because the significance value (sig. (2-tailed) < 0.05).

Table 4. One-Sample Test

Test Value = 0						
	T	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Density	26.527	4	0.000	2.46000	2.2025	2.7175
Porosity	10.304	4	0.001	0.11560	0.0845	0.1467
Hardness	27.878	4	0.000	620.00000	558.2530	681.7470
Compression	15.418	4	0.000	61.40000	50.3429	72.4571

4. Conclusions

This study shows the solid state method is effective and feasible for preparation of natural HA from bovine femur bones at 1000°C. The results of FTIR and XRD shows that the chemical components of the prepared materials are hydroxyapatite. After hydroxyapatite samples sintering at 1250°C for 3 hrs. by regression model density was taken as an independent variable, porosity, compressive strength and hardness as the

dependent variables. Through the analysis values, density has an effect on other properties, as the relationship between density and porosity is an inverse relationship and among density, compressive strength and hardness is a direct relationship.

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