Utilizing *Anadara granosa* Shells and Poly Vinyl Alcohol (PVA) for Porous Hydroxyapatite Synthesis

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ABSTRACT

The majority of the inorganic material that makes up teeth and bones is called hydroxyapatite (HAp) with the chemical formula Ca10(PO4)6(OH)2), and it is produced by isolating calcium oxide (CaO) from the high calcium carbonate content of Anadara granosa shells, which is over 98%. The porous HAp is the type of hydroxyapatite that can be used for bone repair. The aim of this work is to use the polymer polyvinyl alcohol to synthesis porous hydroxyapatite from Anadara granosa shell. The Anadara granosa powder (AGP) was furnaced for 3 hours at 800oC. CaO powder was produced. Then, the CaO powder was treated with (NH4)2HPO4 at a mol Ca/P 1.67 to obtain the HAp. HAp is combined with a Polyvinyl alcohol (PVA) polymer at a 10:1 ratio. Compressive strength tests, FTIR, SEM, and XRD are carried out. The compressive strength resulted for HAp and porous HAp were14.58 and 50.43 Mpa., respectively. The results indicated that PVA achieved a good compressive strength with Hydroxyapatite – Anadara granosa shell.

1. Introduction

Osteoporosis, occupational accidents, and traffic accidents can all result in bone injury. Not only that, but an increase in the demand for bone implants can also be brought on by natural calamities, such as buildings that collapse after an earthquake. As a result, individuals with bone loss are increasingly in need of bone implants [1]. Most of the material that makes up human teeth and bones is called hydroxyapatite, or simply HAp ($Ca_{10}(PO_4)_6(OH)_2$) because of its biocompatibility, bioactivity, and osteoconductive. Synthetic HAp is regarded as one of the most significant implantable materials. It is employed as a substitute material for human hard tissues [2].

High calcium sources are needed to make HAp, and some examples of these sources are 31.48% of cow bones [3], 50.814% of mackerel bones [4] , 66.70% of ash Anadara granosa shells [5], and 76.6% of eggshells [6]. These are the main components of HAp. Porous hydroxyapatite (HAp) is particularly helpful in rebuilding broken bones because its pores would stimulate the formation of new bone cells. Using the sol gel method, research on porous HAp has been conducted utilizing chitosan porogens from the eggshell [7]. In earlier research, HAp was synthesized from a porous shell utilizing a chitosan polymer and a sediment reaction in a chemical reactor. HAp synthesis has also been performed on a virgin shell (*Anadara granosa*) using hydrothermal methods [8-9].

Porogen materials that will disappear during the calcination process can be used to form porous HAp. Paraffin, Naphthalene, Starch or several polymers such as PLA (Polylactic acid), PGA

(Polyglycolic acid), Gelatin, Alginate and Chitosan are materials that can be used as porogens [7] Polyvinyl alcohol is highly hydrophilic, forms films easily, and has good chemical stability. PVA is also non-toxic, water soluble, biocompatible, biodegradable, and exhibits low cell adherence. PVA interacts with bodily tissue at hydrophilic surfaces and can generate pores.

This study conducted the precipitation method. The most straightforward technique which may produce high composition at low temperatures and tends to produce particles with good size and uniformity [10]. This research was developing low cost and environmentally friendly technologies. *Anadara granosa* shell and polyvinyl alcohol are utilized to create porous hydroxyapatite, which is then utilized as a medical implant material.

2. Research Methodology

2.1 Reagents and Instruments

Anadara granosa shell, distilled water, $(NH_4)_2HPO_4$, NaOH 1M, HNO_3, Polyvinyl Alcohol, and Ammonia were used in this research. All reagents were analytical grade Sigma-Aldrich chemicals purchased from Chemical Regaent Co., Ltd. Filter paper, mortar and pestle, mixer bars, drop pipettes, spatels, pH meters, furnace, oven, hot plate stirrer, sieve with 80 mesh, and analytical balance are the instruments also involve in this study.

2.2 Preparation of *Anadara granosa* powder (AGP)

Anadara granosa shell was collected and washed due to remove the dust and other debris. Dried it under the sun, crusher the Anadara granosa shell to obtain the Anadara granosa shell powder. Then sieve it with 80 mesh. After that, the AGP was calcined in the furnace with the temperature 800ºC for 5 hours to obtain CaO. Analysed the element oxide of AGP by XRF (X-Ray Fluorescence).

2.3 Hydroxyapatite synthesis

20 g of CaO BSP shell were dissolved in distilled water. Stirred the solution for 30 min at 300 rpm to create a $Ca(OH)_2$ suspension. Diammonium hydroxy phosphate ($(NH_4)_2HPO_4$), with a concentration ratio of 1.67 Ca/P, is the phosphate precursor. After mixing the solution, then heated for an hour to 90°C. After that, use a NaOH I M solution to bring the pH down to 12. The solution is allowed to be aging (tempered) at room temperature for a full day. After that, the deposits are filtered and allowed to dry for five hours at 120°C in the oven. The weight of dry deposits is counted and weighed. Furnace is used to calcinate the dry precipitates at 900°C for five hours to obtain the Hydroxyapatite (HAp) from BSP. Analysed the HAp using FTIR, XRD, SEM, and compressive strength test.

2.4 Porous Hydroxyapatite (HAp) synthesis

Polyvinyl alcohol solution is prepared using 16 mg/ml in distilled water with the addition of hydroxyapatite to the polymer Polyvinyl Alcohol. Comparison of the addition of polyvinyl alcohol and hydroxyapatite results in a 10:1 adjusted pH of 11 using an ammonia solution. After that, mix for five hours at 300 rpm to create sediment, and soak for 21 hours. After that, rinse and filter several times to get a neutral solution, and then dry for 15 hours at 110°C in the oven. After four hours of sintering, stir at 1100°C. The resulting powder was analyzed using FTIR, XRD, SEM, and Compressive Strength Test.

3. Results and Discussion

a. Results of the synthesis of CaO, hydroxyapatite, and porous hydroxyapatite

Calcium Oxide (CaO) is obtained by calcined *Anadara granosa* powder (AGP) at a temperature of 800ºC, which forms the CaO powder. In a furnace, calcination is a high-temperature heating process used to lower the water content. At this point, calcium carbonate ($CaCO_3$) starts breaking down into calcium oxide (CaO).

$CaCO_3 \rightarrow CaO + CO_2$ is the reaction that takes place [4]

216.5 grams of CaO powder are produced after 300 grams of shells are calcined for three hours at 800ºC. To produce HAp at a mol Ca/P 1.67 variation, 20 grams are required, and the outcome is applied to 15 grams. 11 grams are required to produce porous hydroxyapatite with PVA polymers.

Tabel 1. Synthesis Results of CaO, HAp, and Porous HAp

	CaO	**Hap**	**Porous Hydroxyapatite**
Initial weight (g)	300	20	11
Outlet wieght (g)	216.5	15	9
Yield (*%)	72,1667	75	81,81
Color	grayish white	greenish white	greenish white

b. X-Ray Fluorescence (XRF) Analysis

According to the results of the XRF study, *Anadara granosa* powder (AGP) contained 84.548% CaO. This indicates that *Anadara granosa* powder (AGP) is a good source of calcium oxide, which is needed to make hydroxyapatite. Previous studies revealed that the CaO concentration of pearl oyster shells was 52.23% [11], egg shells were 76.6% [6], and mackerel fish bones had a CaO value of 50.814% [12]. The degree of shell hardness is what causes the variation in CaO composition that is obtained; the carbonate's calcium content increases with the hardness of the shell [13].

Table 2. XRF analysis

Oxide compound	**Composition (%)**
CaO	84.548
P_2O_5	4.023
MgO	3.911
K_2O	0.448

c. X-ray Diffraction Analysis of Polyvinyl alcohol, Hydroxyapatite and Porous Hydroxyapatite

A high intensity of polyvinyl alcohol (PVA) was obtained from the XRD examination at position 2Ɵ. This angle 2 Ɵ matches to the ICSD standard No. 00-056-1717, which indicates that PVA is the peak obtained. Regarding the powder obtained from the precipitation method is hydroxyapatite because the XRD analysis results show that the sample is hydroxyapatite. The peaks that appear are hydroxyapatite peaks with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$. The highest peaks at position 2θ: 31.71; 32.92 and 34.01 have high intensity in accordance with ICSD standard No. 01-072-1243. Similarly, the porous hydroxyapatite sample analysis shows a high intensity that matches position 2θ: 31.76 of the ICSD hydroxyapatite standard No. 01-072-1243. The sizes of hydroxyapatite crystals are 26.6146 nm and 26.6173 nm for porous hydroxyapatite.

Table 3. XRD analysis of PVA, HAp and Porous HAp

PVA		**HAp**		**Porous HAp**	
2 θ sample	ICSD Standard 00-056-1717	2 θ sample	ICSD Standard 01-072-1243	2 θ sample	ICSD Standard No. 01-072-1243
19.77	19.92	31.72	31.74	31.76	31.74
40.50	12.18	32.92	32.86	32.98	32.86

	34.78	34.01	34.04	25.90	25.87
	26.31	25.78	25.87	39.83	39.75
	22.09	46.67	46.66	46.84	46.66

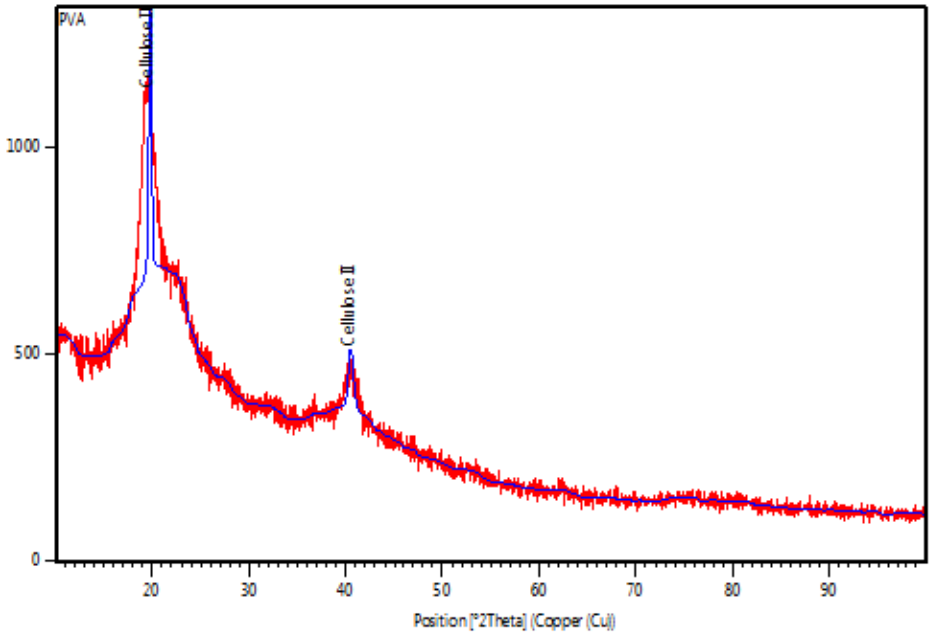

Fig. 1. XRD analysis of Polyvynil alcohol (PVA)

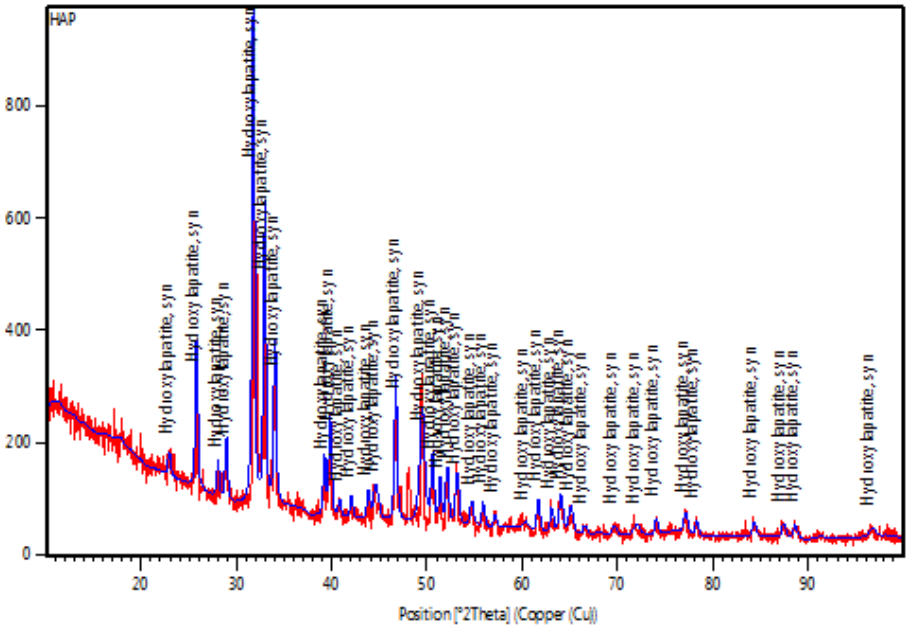

Fig. 2. XRD analysis of HAp from Anadara Granosa Powder (AGP)

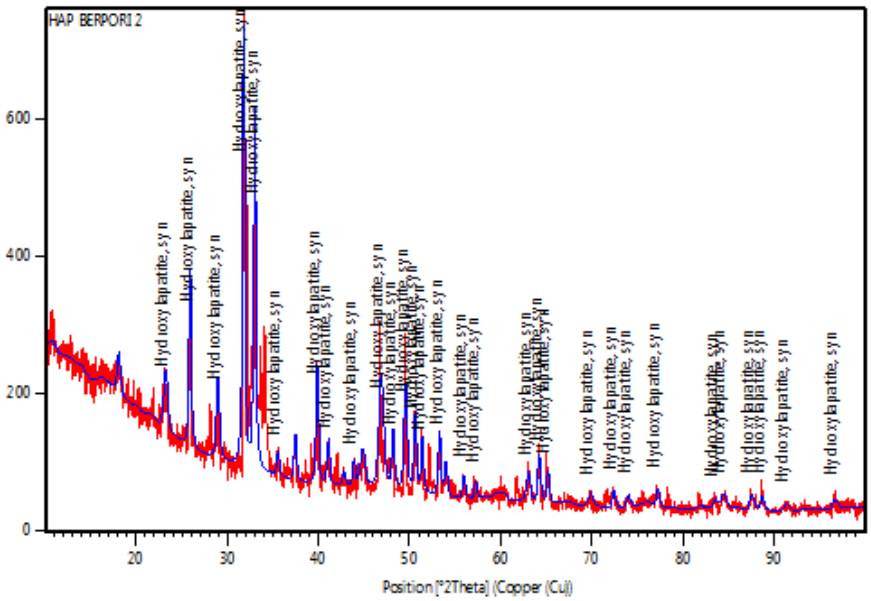

Fig. 3. XRD analysis of Porous HAp

The presence of the OH^- and PO_4^{3-} functional groups in the FTIR analysis of hydroxyapatite indicated that hydroxyapatite was present in the synthesized material. The Fourier transform infrared spectra (FTIR) revealed that the absorption of PO_4 is 1090 cm^{-1}, 960 cm^{-1}, 600 cm^{-1}, 560 cm^{-1}, and 470 cm^{-1}. These spectra have properties specific to hydroxyapatite. The FTIR band of CO_3^{-2} at 1400 cm^{-1} provides information about the characteristics of carbonate in HAp [14]. There are no PVA polymer functional groups in the functional group analysis of porous hydroxyapatite because the PVA polymer vanishes and leaves pores in the hydroxyapatite during calcination.

The purpose of hydroxyapatite's holes is to allow cells from bone tissue to pass through them and interact with the mineral to form a strong connection. Porous hydroxyapatite is the type of hydroxyapatite that works well for bone repair because the pores it forms serve as a medium for the development of new bone cell tissue. In order to create porous hydroxyapatite, the composition of hydroxyapatite is typically formed using polymers or organic materials, which are typically referred to as porogen. The organic material is then removed through calcination. Since polyvinyl alcohol is a naturally occurring polymer with limited cell adherence, biodegradability, non-toxicity, and biocompatibility, it was chosen as the porogen in this study. PVA interacts with bodily tissue at hydrophilic surfaces and has the ability to generate pores [15].

Table 4. FTIR results of PVA, HAp and Porous HAp

Sample	Wavenumber (cm^{-1}) theoretical	Functional Group	Wavenumber (cm^{-1}) Experiment	Characterization
PVA	3700 - 3100	O – H	3317.14	O – H stretching
	3100 – 2840	C – H	2922.64	C – H stretching
	1260 – 1000	C – O	1250.15	C – O stretching
	1000 – 650	C – H	834.34	C – H bending
HAp	3700 – 3100	O – H	3353.49	O – H stretching
	1303 – 794.67	P – O	1013.28	PO_4 stretching
	1400	C – O	1413	CO_3^{-2}
Porous HAp	3700 – 3100	O - H	3638.35	O – H stretching
	1303 – 794.67	P – O	1020.63	PO_4 stretching
	1303 – 794.67	P – O	836.20	PO_4 stretching
	1400	C – O	1454	CO_3^{-2}

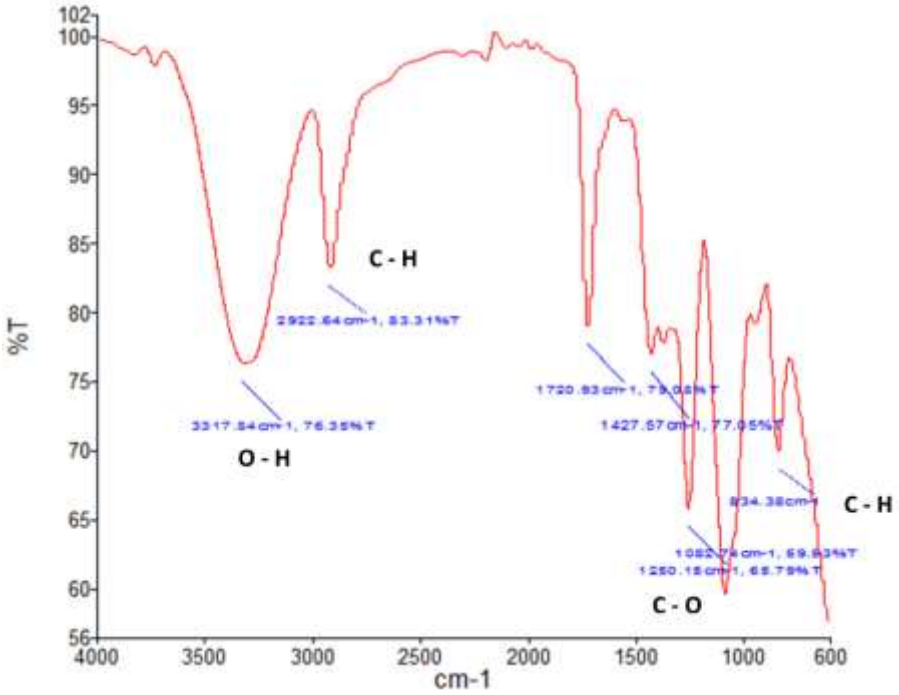

Fig. 4. FTIR analysis of PVA

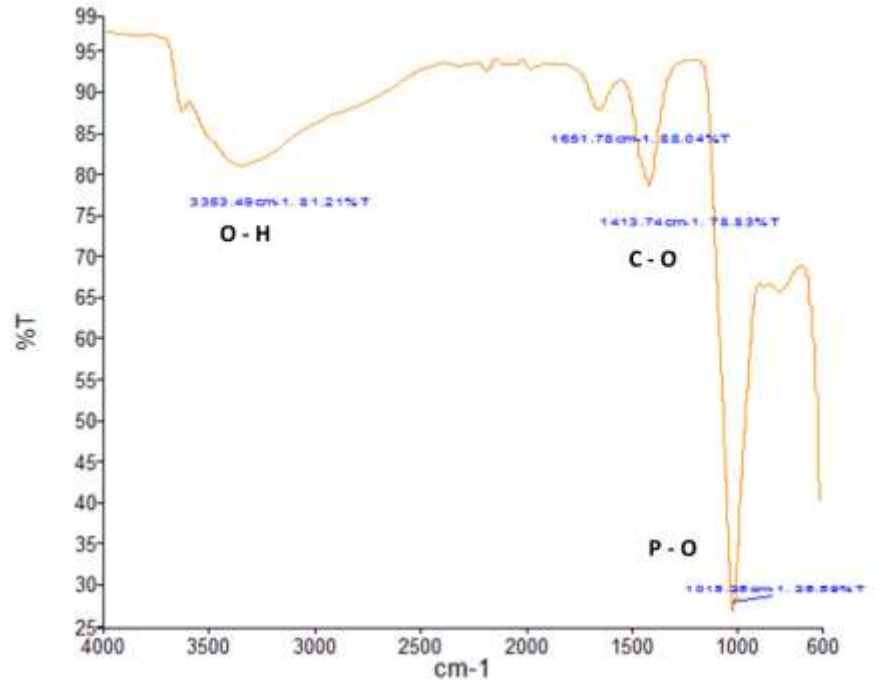

Fig. 5. FTIR analysis of Hydroxyapatite

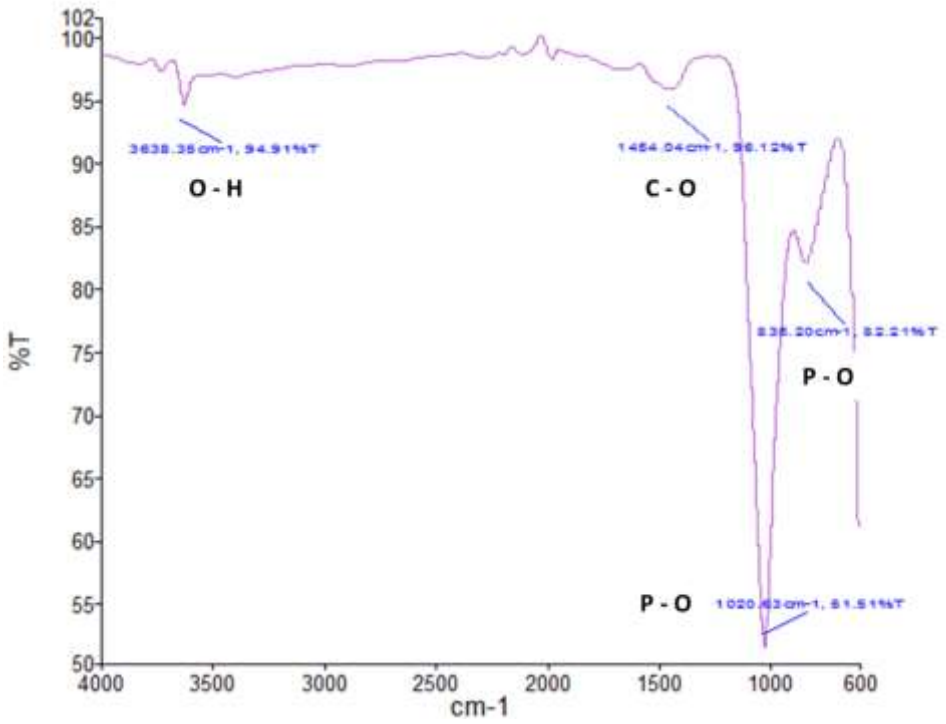

Fig. 6. FTIR analysis of Porous Hydroxyapatite

d. SEM Analysis

The hydroxyapatite compound's particle surface morphology was examined using SEM examination. In this study, lumps of irregular sizes were generated during the production of hydroxyapatite with the ratio of Ca/P is 1.67, larger chunks were formed upon the addition of PVA polymer. Hydroxyapatite generated at pH 8 is needle-shaped with a length of 0.25 µm, but ball-shaped particles with a size of 20 µm-30 µm would occur at pH 10 [16]. At pH 10, pure hydroxyapatite can be produced [17].

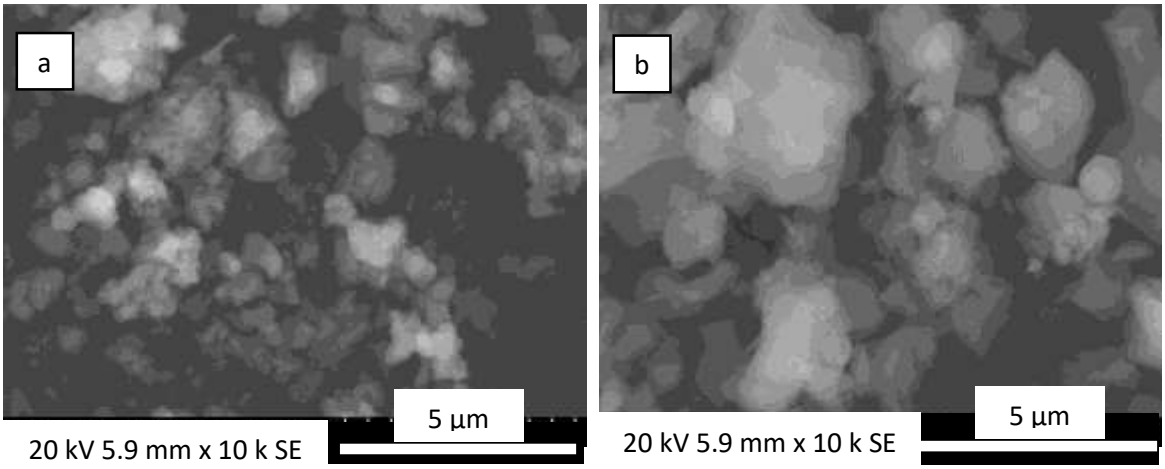

Fig. 7. SEM images of (a) Hydroxyapatite, (b) Porous HAp

e. Compressive Strength Test

According to the results of compressive strength hydroxyapatite increased when polymer was added. The material's compressive strength was 14.5833 Mpa for hydroxyapatite and 50.43 Mpa for porous hydroxyapatite. When used as a bone porosity, hydroxyapatite has a compressive strength value of 0.5–50 MPa [18]. The porous HAp samples used in this investigation had compressive strengths that are within the acceptable range for porous HAp. A higher compressive strength number indicates a higher capacity to bear loads.

Table 5. Compressive Strength Test result

Sample	θ/ mm	P/ mm	N/ mm	Compressive strength (MPa)
Hap	80	6.6	700 N	14.58
Porous HAp	8.2	6.5	150 N	50.43

4. Conclusion

Based on the study's findings, porous hydroxyapatite with a strong compressive strength of 50.43 MPa can be made from *Anadara granosa* shells by utilizing poly vinyl alcohol (PVA) polymer because PVA can create pores and interacts with biological tissue at hydrophilic surfaces.

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