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CUTTLEFISH (*Sepia Sp.*) DERIVED HEAT RESISTANCE HYDROXYAPATITE/ALGINATE COMPOSITES THROUGH THE SOL-GEL METHOD

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ABSTRACT

This work aims to synthesize hydroxyapatite/alginate (HAp/Alg) composites with varied alginate concentrations using the sol-gel method and evaluate the effect of alginate addition on the features of generated HAp/Alg composites. Calcium precursor was obtained from cuttlefish shell calcined at 900°C for 5 hours. FTIR spectrum demonstrated the presence of hydroxyapatite and alginate functional groups. XRD data revealed the typical peaks of HAp and alg in all of the alginate concentration variation composites, indicating the formation of HAp/Alg composites. The SEM image of HAp/Alg-15 exhibited agglomerated rod-like and spherical-like particles, while EDX results identified the elements Ca, O, P, and C with a Ca/P ratio of 1.77. The TGA-DTA curve demonstrated increased heat resistance and no weight loss above 802°C. Finally, the HAp/Alg-15 composite has the potential to be employed in the biomedical field as a bone implant.

Keywords: Hydroxyapatite, Alginate, Hydroxyapatite, Composite, Cuttlefish Shell.

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INTRODUCTION

Hydroxyapatite(HAp), $Ca_{10}(PO_4)_6(OH)_2$ is a ceramic that is part of the calcium phosphate group and has a physical and chemical mineral composition similar to vertebrate bone and teeth.[1,2] Because of that, HAp could be used in medical applications such as biomedical implants, bone grafts, and bone tissue regeneration. Besides, HAp has other excellent properties like non-toxic, bioactive, biodegradable, biocompatible, osteoconductive, and non-immunogenic.[3] Hydroxyapatite synthesis includes natural sources and synthetic sources of precursor. The natural source of hydroxyapatite can be obtained from cuttlefish shells[4], egg shells[5,6], animal bones[7], and scallop shells.[8] In this research, cuttlefish was employed as a biosource of calcium ions. There has been no report about cuttlefish-derived hydroxyapatite before. Unfortunately, cuttlefish waste is only used as bird feed and animal feed mixtures, which have low economic value, even though cuttlefish bone contains 89.61% calcium carbonate($CaCO_3$) aragonite while the organic compound is in the form of β chitin.[9] Macro minerals, especially $CaCO_3$ and β chitin contained in cuttlefish, have great potential to be applied in biomaterial and advanced material. However, HAp is brittle and has low mechanical strength, so improving its mechanical properties can be done by adding polymer as fiber/filler like Alginate. The biodegradable and biocompatible properties of alginate, along with its ability to form a gel after reacting with divalent cations, make alginate very useful in biological applications such as developing tissue for tissue engineering, this is also the reason researchers use polymer alginate, which has the potential as a fiber/filler in making hydroxyapatite composites as a biomaterial.[10] The sol-gel method is used in the synthesis of hydroxyapatite because it can produce a homogeneous layer, is more efficient in increasing interfacial contact and stability, has high purity, and uses low temperatures.[11,12] Nanoparticle engineering has become a new material field in recent years. Several studies showed that HAp/Alg composites using the ex-situ synthesis method experienced an increase in compressive strength values but experienced a decrease in degradability as the amount of alginate added increased. Other research was conducted by Sukhodub *et al.* (2018) carried out the synthesis of HAp/Alg

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as drug release using the coprecipitation method, showing that the addition of alginate reduced the average crystal size of hydroxyapatite and increased the degree of lattice deformation and increased the adsorption volume. A recent study showed that HA/Alg composites using the coprecipitation synthesis method had good crystallinity and high thermal resistance. Another study by Hartatiek *et al.* (2020) showed an increase in the hardness value of hydroxyapatite in hydroxyapatite-polyethylene glycol composite.[13–16] This research carried out the synthesis of cuttlefish-derived heat resistance hydroxyapatite/alginate using the sol-gel method to investigate the effect of alginate on the characteristics of the hydroxyapatite composite and has excellent potential as a biomedical material. The hydroxyapatite/alginate composite was identified using characterization; FTIR and XRD were used to prove that it has formed synthesized HAp/Alg composite, then SEM-EDX characterization results to identify the surface morphology of the HAp/Alg composite and confirm the appearance of Ca, P, O, and C elements, TGA-DTA test to determine the thermal resistance properties of HAp/Alg composite compared pure HAp.

EXPERIMENTAL

Materials

The materials used in this research were cuttlefish shell powder, nitric acid (HNO_3) (Merck), ammonium hydroxide (NH_4OH) (Merck), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (Merck), alginate, filter paper, and distilled water.

Preparation of CaO from Cuttlefish Shells

The cuttlefish shells are cleaned from impurities with distilled water and dried, then ground using a grinder to become cuttlefish powder. The cuttlefish powder was heated to 900ºC for 5 hours to get CaO powder. XRF then characterized this CaO powder.

Synthesis of Hydroxyapatite using the Sol-Gel Method

The 4.2 g of CaO powder was mixed with 75 mL of 2 M HNO_3 while being stirred at 65ºC for 15 minutes at 250 rpm. After that, a $Ca(NO_3)_2$ solution is formed and filtered. The NH_4OH solution added the $Ca(NO_3)_2$ filtrate until the pH ten and $Ca(OH)_2$ sol was obtained. It was done drop by drop, adding $Ca(OH)_2$ sol to a 250 mL solution of 0.18 M diammonium phosphate ($(NH_4)_2HPO_4$). The solution was heated at 60ºC while stirring for 5 hours to form the HAp sol. After aging for 24 hours, the HAp sol was filtered and dried at 110ºC for 5 hours. Lastly, it was fire-blasted for three hours at 600ºC.

Hydroxyapatite/Alginate Synthesis

A 75 mL solution of 2 M HNO_3 was mixed with CaO powder and then stirred at 65˚C for 15 minutes at 500 rpm. This mixture made a $Ca(NO_3)_2$ solution. After filtering the solution, 250 mL of a solution of diammonium phosphate ($(NH_4)_2HPO_4$) was slowly added. The pH was adjusted using NH_4OH until pH ten. Next, the solution was heated at 60˚C, and alginate was added with varying concentrations of 5, 10, 15, and 20(wt%) while stirring for 5 hours until hydroxyapatite sol is formed. The HAp sol was agitated for 24 hours, then filtered and dried at 110ºC for 5 hours. Finally, it was calcined at 600ºC for 3 hours. Then, the samples were coded according to concentration variations as HAp/Alg-5, HAp/Alg-10, HAp/Alg-15, and HAp/Alg-20.

Characterization of HAp/Alg Composite

A study was conducted to analyze the oxide composition of the cuttlefish carapace utilizing X-ray fluorescence (XRF). Fourier-Transform InfraRed (FTIR) assessed the functional groups; meanwhile, the crystal structures were examined using X-ray diffraction (XRD). SEM-EDX, or energy dispersive X-ray, was analyzed to determine the elemental and morphological compositions. Finally, differential thermal analysis using thermogravimetric analysis (TGA-DTA) was employed to ascertain the thermal resistance.

RESULTS AND DISCUSSION

The Reaction in the Formation of Hydroxyapatite

Calcination of cuttlefish shell powder is carried out to break up the organic groups contained in cuttlefish shells to produce calcium oxide (CaO) and release carbon dioxide (CO_2). The reaction that occurs is:

$$CaCO_3 \rightarrow CaO + CO_2 \quad (1)$$

The reaction that occurs in dissolving CaO with HNO_3 is as follows:[17]

$$CaO_{(s)} + 2HNO_{3(l)} \rightarrow Ca(NO_3)_{2(aq)} + H_2O_{(l)} \quad (2)$$

$Ca(NO_3)_2$ was added with NH4OH p.a until pH 10 to form $Ca(OH)_2$ sol. The pH value is ten, corresponding to the ideal pH for hydroxyapatite formation. A colorless gel is produced by combining a $Ca(OH)_2$ solution with a diammonium hydrogen phosphate $(NH_4)_2HPO_4$ solution. The white gel signifies the formation of hydroxyapatite. The gel was left for 24 hours, filtered, and heated to obtain hydroxyapatite powder. The reactions that occur at this stage are:[17]

$$10Ca(NO_3)_{2(l)} + 6(NH_4)_2HPO_{4(l)} + 8NH_4OH_{(l)} \rightarrow Ca_{10}(PO_4)_6(OH)_{2(s)} + 10NH_4NO_3 + 6H_2O \quad (3)$$

The synthesized HAp/Alg composite was obtained as a white powder with mass variations, as displayed in Table-1.

Table-1: Mass of HAp and HAp/Alg Composite

Samples	Mass (gram)
Pure Hap	6.8519
HAp/Alg-5	7.5908
HAp/Alg-10	7.6744
HAp/Alg-15	7.7052
HAp/Alg-20	8.0034

The HAp/Alg composite synthesis showed an increase in mass with each increase in alginate concentration. Besides, the mass of the composite formed is greater than that of pure HAp because the added alginate will interact with hydroxyapatite in forming HAp/Alg composites.[15]

Composition Analysis of Cuttlefish Shells

Table-2 displays the XRF analysis of the oxide composition of cuttlefish shells that were calcined for five hours at 900°C.

Table-2: Oxide Composition of Cuttlefish Shell (*Sepia sp*.)

Compound	Compositions (%)
CaO	96.305
SrO	0.778
Al_2O_3	0.325
Ag_2O	0.190
SO_3	0.584
P_2O_5	0.765
Fe_2O_3	0.132

Table-2 displays that cuttlefish shells contain 96.303% calcium oxide (CaO), so they have great potential as a substitute for calcium precursors in synthesizing hydroxyapatite.

Functional Group Analysis

FTIR analysis was carried out at wavenumbers 4000-400 cm^{-1}. The FTIR spectra of the HAp/Alg composite and pure HAp are displayed in Fig.-1.

The absorption band at 560-600 cm^{-1} and 1022 cm^{-1} was assigned to the bending and stretching vibration of the phosphate group (PO_4^{3-}) of HAp in pure HAp and HAp/Alg composite. The band at 3277 cm^{-1} was related to the symmetric stretching vibrations of hydroxyl (OH). Moreover, the spectrum of Figure 1(c-e) shows a band at 788 cm^{-1}, which could be attributed to the carboxyl group of alginate.[16] The characteristic bands of alginate include carboxylic group bands at 1613 cm^{-1} (–COO–asymmetric) and 1421cm^{-1} (–COO–symmetric) bands.[18,19] Thus, this is evidence of HAp/Alg composite formation.[15]

Crystal Structure Analysis

XRD analysis was carried out to identify the presence of hydroxyapatite and alginate in the composite sample by comparing the HAp/Alg composite sample data with pure hydroxyapatite data. XRD data was

used to strengthen the FTIR analysis, which showed the presence of hydroxyapatite and alginate functional groups. Besides, XRD data was used to determine the crystal size. The results of the XRD analysis are shown in Fig.-2.

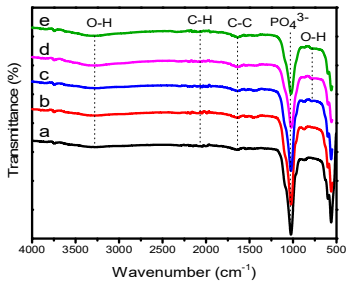

Fig.-1: FTIR Analysis of (a) HAp, (b) HAp/Alg-5%, (c) HAp/Alg-10%, (d) HAp/Alg-15%, and (e) HAp/Alg-20%

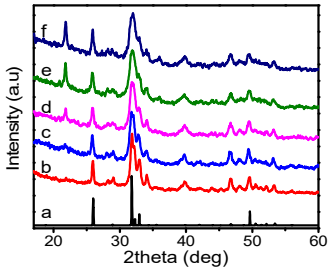

Fig.-2: XRD Patterns of (a) Hydroxyapatite Standard (b) HAp, (c) HAp/Alg-5, (d) HAp/Alg- 10, (e) HAp/Alg-15, and (f) HAp/Alg-20

Figure-2 confirms the formation of hydroxyapatite and HAp/Alg composite where the XRD pattern shows the diffraction peak of hydroxyapatite is at 2θ: 25.92º; 31.77º; 34.11º. It shows that the diffraction peak has similarities with the hydroxyapatite standard ICSD #97849. Therefore, it can be confirmed that hydroxyapatite still existed in the composite. Moreover, the appearance of a new peak at 2θ: 21.82º in all of the composite patterns has confirmed the attendance of alginate in the formation of HAp/Alginate composite.[16] The new peak's intensity gets robust with each alginate concentration increase. Other information obtained from the XRD pattern is that there is a level of aggregation that indicates the interaction of Ca^{2+} with alginate, which follows research by Tong (2017).[20]

Observation of Morphology and Elemental Composition

SEM characterization was performed on HAp/Alg composites because surface morphology is essential for cell adhesion and proliferation.[21] SEM analysis was carried out by coating gold through a coating process first because hydroxyapatite has low electrical and thermal conductivity properties. SEM-EDX magnification analysis was carried out on the HAp/Alg-15 composite because the XRD pattern had a change from the HAp standard and the appearance of alginate peaks starting from concentrations of 10, 15, and 20(wt%) with an intensity that was increasingly higher with the addition of alginate, so it was taken 15% HAp/Alg composite which is the midpoint for the SEM test. SEM tests carried out with magnifications of 100,000× are shown in Fig.-3.

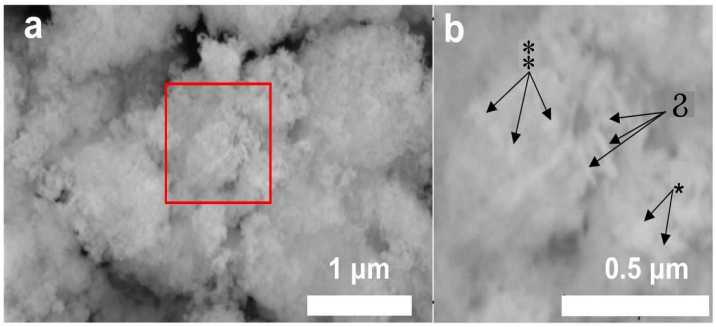

Fig.-3: Morphology of (a) HAp/Alg-15 at 100.00 kx, and (b) Magnification of the Red Spot

Figure-3 shows that the HAp/Alg-15 composite has some particle shapes, namely rod-like (ƨ) and spherical-like (*), where both of these forms are included in the form of hydroxyapatite which can be used as an implant material[22], aside from that, the alginate particles are evenly spread out over the HAp particles(⁑).[16] Differences in the shape of hydroxyapatite particles can influence the body's biological response cytotoxicity, the size of the hydroxyapatite, and the adaptation of bone cells to the hydroxyapatite itself. Rod and spherical-shaped hydroxyapatite is suitable and easy to use in biological tissue engineering, unlike needle-shaped hydroxyapatite, generally used in compact bone/dense bone. In addition, the HAp/Alg composite showed agglomeration, where agglomeration indicated that it had high osteoinductive properties for cell adhesion and proliferation.[21] It is different from the research of Mahmoud (2020), where the HAp/Alg composite synthesized using the coating method had a spherical-like shape and a lower level of agglomeration.[23] Composite particles tend to agglomerate because hydroxyapatite has hygroscopic properties, causing a physisorption process that forms O-H bridges in the hydroxyapatite group through Van Der Waals bonds, thus forming a physical bond between the particles. In addition, the hydroxyapatite produced does not have a dispersing agent, limiting the physiosorption process.

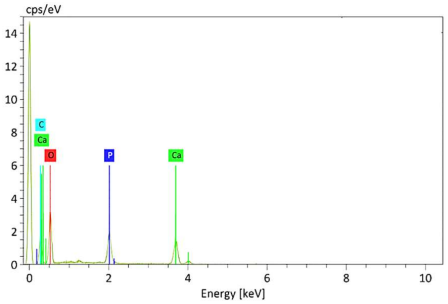

Fig.-4: EDX Spectrum of HAp/Alg-15 Composite

The elemental composition of the HAp/Alg-15 composite sample was determined using EDX analysis, as illustrated in Fig.-4. The conclusions drawn from the EDX analysis indicate that the elemental composition contained in the HAp/Alg composite is C (carbon) as much as 30.19%; O (oxygen) as much as 50.36%; Ca (Calcium) as much as 12.44%; and P (Phosphorus) as much as 7.01% with a Ca/P ratio of 1.77, where the hydroxyapatite ratio (1.67-2)—which can be utilized as a material for bone implants—still includes this ratio.[24] Apart from that, the results of EDX analysis also confirm the presence of carbon elements originating from organic compounds, namely alginate.[10]

Thermal properties analysis

TGA-DTA analysis was performed to ascertain the mass reduction in the HAp/Alg composite. In this research, tests were carried out on HAp/Alg-15 composites,where the TGA-DTA plot can be seen in Fig.-5.

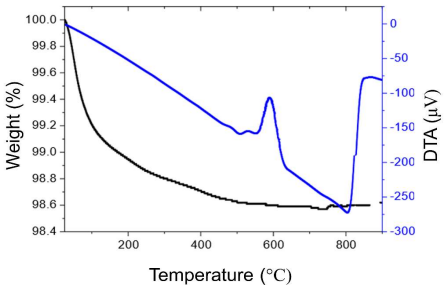

Fig.-5: TGA-DTA Plot of HAp/Alg-15 Composite

Figure-5 shows the TGA curve, which shows that weight loss is divided into three stages. The initial phase involves a substantial reduction in weight at temperatures below 165°C due to water molecule evaporation within the specimen. Weight loss transpires during the second stage due to alginate decomposition occurring between 230 and 350°C. In the third stage, there is a slight decrease in mass at a temperature of 450-600º, which occurs due to complex decomposition between alginate residues and their interaction with

Ca^{2+} from hydroxyapatite, which proves the existence of interactions between alginate and hydroxyapatite. Furthermore, at a temperature of 802°C, no further weight loss occurred; this demonstrates the high thermal resistance of the HAp/Alg composite that was synthesized.[22] DTA analysis shows an exothermic peak at a temperature of 590°C, possibly caused by the crystallization process due to the loss of alginate in the composite. The endothermic peak at 802°C indicates an endothermic event that may be correlated with alginate elimination in the composite.[22]

CONCLUSION

HAp/Alg composites have been synthesized through the sol-gel method. FTIR and XRD results revealed the formation of a HAp/Alg composite with the characteristic peak and absorption band of hydroxyapatite and alginate. Additionally, adding alginate affects the particle shape and thermal resistance. SEM-EDX analysis shows composites with varying particle shapes, namely rod-like and spherical-like, with agglomeration. The TGA-DTA analysis results show no further weight loss at 802°C. The HAp/Alg-15 composite has the potential to be utilized as a bone implant in the biomedical field, as this demonstrates that the HAp/Alg composite has a high thermal resistance.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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[RJC- 8745/2023]