Microwave-assisted synthesis and characterization of nanocomposite hydroxyapatite-chitosan

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Abstract. In this study, the synthesis of hydroxyapatite-chitosan composites has carried out using the microwave-assisted method. The hydroxyapatite-chitosan composite is interesting to develop based on the application in many fields. Porous hydroxyapatite is very useful for reconstructing damaged bones because it will stimulate the growth of new bone cells. The synthesis of hydroxyapatite utilized waste of Geloina coaxans shells as the calcium source both phosphate sources are ammonium dihydrogen phosphate NH4H2PO4 and KH2PO4 while for synthesis hydroxyapatite-chitosan composites will be carried out using the waste of Scylla olivacea shell as a source of chitosan. The rapid pathway synthesis is very crucial for producing composites. Synthesis has done using the microwave 450 W with a retention time of 20 minutes. Based on the results, the hydroxyapatite powder can be produced with specific peaks of hydroxyapatite at 31.7°. Mass of chitosan influenced pore of the hydroxyapatite-chitosan composite increased the pore diameter of hydroxyapatite-chitosan composite 0.28 to 5.0308 nm and 4.3182 and 7.0268 using KH2PO4 and NH4H2PO4. The functional group also shows the specific bands of hydroxyapatite.

1. Introduction
Hydroxyapatite or Ca10 (PO4)6(OH)2 mainly consists of calcium and phosphate with a ratio of Ca/P 1.67. The bioactive, biocompatible, and osteoconductivity are some of the properties that support its application. It’s has been widely applied as a biomaterial for repairing or replacing human hard tissues although it has some disadvantages. Composite is a combination of matrix and reinforcement that has different properties resulting in the physical and mechanical properties of each component. The hydroxyapatite is a matrix while some natural polymers such as starch, alginate, and chitosan are reinforcement to produce hydroxyapatite composites. This material was synthesis to enhanced several properties of pure hydroxyapatite.

Chitosan or (C6H11O4N)n is a product of the deacetylation of chitin compounds. Chitosan is a porogen and natural polymer that has biodegradable, non-toxic, and biocompatible properties [1]. In previous research, several invertebrate animal shells such as (Parapeneopsis stylifera) [2], (Nototodarus sloanii) [3] have been extracted to produce chitosan. The waste of animal shell which contains chitin and chitosan compounds can be optimized as a porogen on a synthesis of hydroxyapatite composite. The porous hydroxyapatite-chitosan composite is interesting to develop based on the application in many fields. Porous hydroxyapatite is very important for reconstructing damaged bones because it will stimulate the growth of new bone cells [3]. The hydroxyapatite-
chitosan composites have also successfully synthesized hydroxyapatite-chitosan composites with a pore diameter of 3.87 nm for the removal of heavy metals [4] while hydroxyapatite-polysaccharide composites prepared with a pore volume of 0.25 for the adsorption of Sr$^{2+}$ ions [5].

In this study, the synthesis of hydroxyapatite utilized Geloina coaxans shells as the calcium source and both phosphate sources are ammonium dihydrogen phosphate NH$_4$H$_2$PO$_4$ and KH$_2$PO$_4$ while for synthesis hydroxyapatite-chitosan composites will be carried out using the microwave-assisted method and Scylla olivacea shell as a source of chitosan. The rapid pathway synthesis using microwave-assisted is very crucial and helpful for producing composites. The powder obtained will be analyzed and characterized to learn mineral phases, surface area, and pore size. Besides, functional groups of samples are also investigated.

2. Material and Methods
The purpose of this study was to utilize the waste of Geloina coaxans shell as a source of calcium To produce hydroxyapatite. The hydroxyapatite obtained will be composited with chitosan to obtain a porous material. The material dan methods can be seen below

2.1. Chemical Materials
The materials used in this research were Geloina coaxans shell, Scylla olivacea shell, NH$_4$H$_2$PO$_4$ (Merck), KH$_2$PO$_4$, NH$_3$ (Merck), NaOH (Merck), HCl (Merck), CH$_3$COOH (Merck), Whatman 42, and DDW.

2.2. Synthesis Procedures

2.2.1. Preparation of Geloina coaxans shell (GCS) as calcium precursor
Geloina coaxans shell (sample) was collected and washed for removing its inner membrane. After that, the sample was dried out and mashed. The particle size of the sample was adjusted at 200 mesh.

The powder of geloina coaxans shell was analyzed by X-Ray Fluorescence technique to determine its chemical composition. In the next step, the sample was calcined using a furnace to remove organic composition and decomposed calcium carbonate into calcium oxide at 1000°C for 12 hours.

2.2.2. Preparation of Scylla olivacea shell (SOS), as chitosan source
Scylla olivacea shell is collected from seafood restaurants, washed, dried, and then mashed using a mortar. The sample is adjusted to 200 mesh. The powder that has been obtained is followed by a process of demineralization, deproteination, and deacetylation to obtain chitosan.

2.2.3. Synthesis of hydroxyapatite-chitosan composite
The synthesis of hydroxyapatite was carried out using potassium dihydrogen phosphate (KH$_2$PO$_4$) as a phosphate source while the source of Ca was derived from the Geloina coaxans shell. The Ca/P ratio used was 1.67. The pH of the solution is adjusted to pH 10 with NH$_4$OH. Then, the Ca/P was added chitosan with 0, 10, 20, and 30%. In the next step, the solution is put in the microwave 450 W with a retention time of 20 minutes. The resulting sample is filtered to separate the precipitate and the filtrate. The precipitate was dried using an oven at 105 °C. The precipitate was calcined at 900°C for 1 hour. The hydroxyapatite powder obtained was analyzed using several instruments. (the same procedure for NH$_4$H$_2$PO$_4$)

2.3. Characterization
Fourier Transform-Infra Red Spectroscopy (Shimadzu -0265), X-Ray Diffraction (Gbc Emm), X-Ray Fluorescence (S2 Ranger Burker), and SAA.
3. Results and Discussion
The sample synthesized were analyzed using several instruments. Firstly, the raw material (GCS) and (SOS) was prepared and examined with XRF and FTIR technique. Hydroxyapatite and hydroxyapatite-chitosan composite (HCC) were analyzed using FTIR, XRD and SAA.

3.1. Analysis chemical composition of GCS using XRF and functional groups of SOS using FTIR, XRD
The chemical composition analysis of the Geloina coaxans shell was carried out using X-Ray Fluorescence (XRF). The results of the analysis can be seen in Table 1.

| No | Chemical Composition | Percentage (%) |
|----|----------------------|----------------|
| 1  | CaO                  | 98.719         |
| 2  | Al₂O₃                | 0.321          |
| 3  | Fe₂O₃                | 0.007          |
| 4  | K₂O                  | 0.014          |

Geloina coaxans shell was taken from Panipahan Village, Pasir Lima District, Rokan Hilir Regency. The sample is crushed and adjusted to 200 mesh, then calcination is carried out at a temperature of 1000 ºC for 12 hours to convert the CaCO₃ to CaO. In the next step, the sample was analyzed using X-Ray Fluorescence (XRF). The results of the chemical composition analysis were CaO, which is 98.719; Al₂O₃ 0.321; Fe₂O₃ and K₂O 0.014 %. The large percentage of CaO is the potential to be used in the synthesis of hydroxyapatite as a source of calcium.

The Fourier Transform Infra-Red (FTIR) analysis was carried out to determine the functional groups of chitin and chitosan. Scylla olivacea shells have three important components including minerals, protein, and chitin. Minerals found in crab shells are 66.58%, 16.68% protein, and 16.73% chitin. Scylla olivacea shell can be extracted to produce chitosan which is processed through several steps including demineralization, deproteination, and deacetylation. The results of the analysis can be seen in Figure 1 and Table 2.
**Table 2.** The results of the functional groups analysis of chitin and chitosan

| Functional groups | Results | Previous Research |
|-------------------|---------|------------------|
|                   | a       | b    | a    | b    | a    | b    |
| -NH               | 3181    |     | 3107 |     |      |      |
| C=O (amide I)     | 1793    | 1792 | 1647 | 1654 | 1647 |      |
| -NH (amide II)    | 1543    | 1654 | 1560 | 1580 | 1560 |      |
| -OH               | 3696    |     | 3462 |     |      |      |
| -CH₂              | 2878    | 2957 | 2925 | 2921 |      |      |
| -CO               | 1029    | 1030 | 1029 |      |      |      |
| -NH₂ (amine)      | 3695    |     | 3420 |     |      | 3260 |

(a) chitin; (b) chitosan

The specific wavenumber of chitin is indicated by the appearance of the N-H group at 3181 cm\(^{-1}\). The vibration appears at 1793 and 1543 cm\(^{-1}\) which indicates the presence of amide I (C = O) and amide II (-NH). The absorption band of the CH₂ group was observed at 2878 cm\(^{-1}\) and the absorption band for the C-O group appeared at wavenumber 1029 cm\(^{-1}\). The hydroxyl or OH- group absorption observed at 3696 cm\(^{-1}\). The chitin from shrimp shells Parapeneopsis stylifera have synthesized specific wavenumber of chitin (-NH) was at 3107 cm\(^{-1}\) [2]. The bands at 1647 and 1560 cm\(^{-1}\) indicate the presence of amide I (C = O) and amide II (-NH) groups. The absorption band of the -CH₂ group was observed at 2925 cm\(^{-1}\) and 1020 cm\(^{-1}\) indicated C-O group. The hydroxyl group or OH- appears at 3462 cm\(^{-1}\).

Chitosan can be observed with the appearance of the -NH₂ group at the wave number 3695 cm\(^{-1}\). The wavenumbers at 1792 and 1654 cm\(^{-1}\) indicate the presence of amide I (C = O) and amide II (-NH) groups. The absorption band of the -CH₂ group was observed at wave number 2957 cm\(^{-1}\) and at wave number 1030 cm\(^{-1}\) the presence of a C-O group. The chitosan from squid Nototodarus sloani have also analyzed, the bands of chitosan appear -NH₂ at wave number 3260 cm\(^{-1}\). The wave numbers 1647 and 1560 cm\(^{-1}\) indicate the amide I (C = O) and amide II (-NH) groups. [3]

Identifying the mineral phase of chitosan from Scylla olivacea shell was carried out using X-Ray Diffraction (XRD). The results of XRD analysis can be seen in **Figure 2** and **Table 3**

![Figure 2. XRD diffractogram of chitosan synthesized](image-url)

**Figure 2.** XRD diffractogram of chitosan synthesized
Table 3. The mineral phase of chitosan from crab shells or Scylla olivacea shell

| JCPDS   | Results | Previous research (2θ°) |
|---------|---------|------------------------|
| 29.985  | 29.3398 | 29.75°                  |
| 34.224  | 34.0575 | 34.85°                  |
| 17.703  | 17.9885 | 40.93°                  |
| 37.964  | 37.8823 | 37.62°                  |
| 22.041  | 22.9512 | 46.03°                  |

*JCPDS No. 39-1894

The chitosan diffractogram in Figure 2 shows the specific peak of chitosan at 2θ = 29.3398°, 34.0575°, 17.9885°, 37.8823° and 22.9512° which is compared to JCPDS No. 39-1894. The specific chitosan peaks indicate that the conversion of Scylla olivacea shell to chitosan has been successful. The chitosan from shrimp shells or Penaeus monodon shell have successfully synthesized with specific peaks of chitosan at 2θ = 29.75°, 34.85°, 17.9885°, 40.93° and 46.03° [6]; 2θ = 10° and 20°, [7] while at 2θ = 9.3°, 11.4°, 18.1°, and 22.6° [8].

3.2. Analysis hydroxyapatite and composite-chitosan hydroxyapatite (HCC) using XRD, FTIR and SAA

Synthesis hydroxyapatite were done using KH₂PO₄ and NH₄H₂PO₄ as phosphate sources and GCS as calcium source. Analysis of sample were examined using X-Ray Diffraction (XRD), Fourier Transform Infra-Red (FTIR) while hydroxyapatite-chitosan composites (HCC) are analyzed with FTIR and. The results of the mineral phase analysis hydroxyapatite using KH₂PO₄ can be seen in Figure 3.

![Figure 3. XRD diffractogram of hydroxyapatite using KH₂PO₄ precursor](image)

The hydroxyapatite diffractogram in Figure 3 shows the specific peak of hydroxyapatite at 2θ = 25.8002° while the other hydroxyapatites also appear at 2θ = 32.0033°, 34.0393°, 46.6801°, 44.9680°, 49.4606° according to JCPDS No. 1206-06-5. The average of crystal size of 11.81 nm. Other researchers have also succeeded in synthesizing hydroxyapatite with the help of a microwave (1100 W) with the precursors Ca (NO₃)₂ and KH₂PO₄ which obtained a particle size of 35 nm [9]. The hydroxyapatite was successfully prepared with the help of a microwave (450 W for 20 minutes) through the precursors Ca (NO₃)₂ and (NH₄)₂HPO₄, specific peaks of hydroxyapatite were obtained at 2θ = 25.76°, 31.71°, 32.15°, 32.74°, 33.79 ° and 39.82° [10], and Ca (NO₃)₂ and Na₂HPO₄, as
precursors resulted hydroxyapatite at 2θ respectively 25.88º, 28.97º, 31.77º, 32.20º, 32.90º, and 34.05º [11].

Reaction of synthesis hydroxyapatite

\[ \text{CaO} + \text{H}_2\text{O} \rightarrow \text{Ca(OH)}_2 \]  
(1)

\[ \text{KH}_2\text{PO}_4 + \text{H}_2\text{O} \rightarrow \text{KH}_2\text{PO}_4 \]  
(2)

\[ 10\text{Ca(OH)}_2 + 6\text{KH}_2\text{PO}_4 \rightarrow \text{Ca}_10(\text{PO}_4)_6(\text{OH})_2 + 6\text{KOH} + 12\text{H}_2\text{O} \]  
(3)

The results of the functional group of hydroxyapatite and hydroxyapatite-chitosan composites (HCC) can be seen in Figure 4, Figure 5 and Table 4. The specific characteristics of the functional group of hydroxyapatite can be observed by the appearance of PO\text{4}^{3-} groups 632 and 1088 cm\textsuperscript{-1}, while the hydroxyl groups (OH\textsuperscript{-}) appear at wave number 3570 cm\textsuperscript{-1}, while at 567 and 1043 cm\textsuperscript{-1} indicate the presence of PO\text{4}^{3-} groups [13]. The specific bands of hydroxyapatite also observed at 565 and 1000-1100 cm\textsuperscript{-1} were the specific bands of phosphate groups [14]. The functional group of synthesized hydroxyapatite using fish bones (Lates calcarifer) observed specific peak at 3574 cm\textsuperscript{-1} which indicates the presence of a hydroxyl and the vibration of PO\text{4}^{3-} at 569 and 1044 cm\textsuperscript{-1}[15].

Figure 4. FTIR spectra of hydroxyapatite with phosphate prekursor KH\textsubscript{2}PO\textsubscript{4}
Figure 5. FTIR spectra of hydroxyapatite-chitosan composite 20% with phosphate prekursor KH$_2$PO$_4$

Table 4. Functional groups of hydroxyapatite-chitosan composite with phosphate prekursor KH$_2$PO$_4$

| Functional Groups | Results (cm$^{-1}$) | Previous research |
|-------------------|--------------------|-------------------|
| O-H               | 3571               | 3200-3500         |
|                  |                    | 3425              |
|                  |                    | 3400              |
|                  |                    | 3140              |
| PO$_4^{3-}$       | 1092               | 1000-1100         |
|                  |                    | 1027              |
|                  |                    | 1033              |
|                  |                    | 1000-1100         |
| PO$_4^{3-}$       | 633                | 560-610           |
|                  |                    | 532               |
|                  |                    | 563               |
|                  |                    | 560-610           |
| CO$_3^{2-}$       | 1411               | -                 |
|                  |                    | 1375              |
|                  |                    | -                 |
| -NH$_2$           | 3571, 1688         | 3452, 1627        |
|                  |                    | 1582              |
|                  |                    | 1650              |
|                  |                    | 3140, 1634        |

The spectra of hydroxyapatite-chitosan composite show the bands of phosphate groups at 633 and 1092 cm$^{-1}$ and hydroxyl group at 3571 cm$^{-1}$. Based on the spectra of Hydroxyapatite-chitosan composite there was a decrease in the peak intensity of the hydroxyl group (OH$^{-}$), this was due to an overlap with the –NH$_2$ group found in chitosan. The presence of –NH$_2$ groups detected in the FTIR spectrum means that hydroxyapatite-chitosan composite material has been formed. Hydroxyapatite-chitosan composite can be used as adsorbents because they have a hydroxyl group (OH$^{-}$) and a –NH$_2$ group derived from chitosan which can be used to remove heavy metals in water [4].

3.3. Analysis Hydroxyapatite and Hydroxyapatite-Chitosan Composite Using (SAA)

To learn the effect of chitosan on the physical properties of hydroxyapatite (HCC) such as the surface area, diameter, and pore volume. Sample of the synthesized hydroxyapatite-chitosan composite was analyzed using SAA with the Brunauer-Emmett-Teller BET and BJH methods. The results of the BET analysis method can be seen in Table 5.

Table 5. The results of hydroxyapatite-chitosan composite analysis using the BET method

| Sample                        | Chitosan (%) |
|-------------------------------|--------------|
| Surface area (m$^2$/g)        | 2.532        |
|                               | 5.20753      |
|                               | 3.27315      |
|                               | 0.49542      |
|                               | 23$^d$, 41.0$^e$, 5.62$^e$ |
| Diameter pore (nm)            | 0.280        |
|                               | 5.0308       |
|                               | 4.3182       |
|                               | 7.0268       |
|                               | 10-50$^a$, 45-100$^b$, 35$^c$, 0.6$^d$, 18.2$^e$ |
| Pore volume (cc/g)            | 0.018        |
|                               | 0.0065496    |
|                               | 0.003533     |
|                               | 0.00087      |
|                               | 0.028$^e$, 0.2264$^b$ |

$^a$ (11) $b$ (24), $c$ (9), $d$ (19), $e$ (16), $f$ (25), $g$ (26), $h$ (27)
The results obtained from the surface area analysis of hydroxyapatite were 2.5632 m²/g, while the surface area of the HCC with the addition of chitosan 10, 20, and 30% were 5.20753, 3.27315 and 0.495429 m²/g respectively. The pore diameter and pore volume produced of hydroxyapatite were 0.28 nm and 0.018888 cc/g, respectively. The pore volume obtained for hydroxyapatite-chitosan composite with variations of 10, 20, and 30% was 0.0065496; 0.0035336; and 0.00087 cc/g respectively. The pore diameter was 5.0308; 4.318; and 7.0268 nm, respectively. The HCC also synthesized and obtained a pore diameter of 4.81 nm which can be used to adsorption of Ni and Co metals, the adsorption capacity were 213.8 mg/g and 180.2 mg/g respectively and explained that the decrease in the surface area could be caused by the agglomeration of hydroxyapatite particles which resulted in a decrease in the adsorption of nitrogen molecules in the pores [4]. The pore diameter obtained in this study could potentially be used for metal adsorption. Based on the research results, it can be seen that there is a decrease in surface area and pore volume due to the increase in the organic phase content. These results indicate that the porosity of the biocomposite is mostly related to the hydroxyapatite in the sample [16].

3.4. The mineral phases analysis of hydroxyapatite using NH₄H₂PO₄ as source of phosphate

The synthesized hydroxyapatite using NH₄H₂PO₄ as source of phosphate are analyzed for identifying mineral phase using X-Ray Diffraction (XRD), the results of mineral phase analysis can be seen in Figure 6. The hydroxyapatite diffractogram shows the specific peak of hydroxyapatite appears at 2θ = 31.7646 and other hydroxyapatite peaks also appear at 2θ = 32.9445°, 25.8836°, 49.5321°, 31.0539° and 46.7807° according to JCPDS No. 1206-06-5. The percentage of synthesized hydroxyapatite was 94.054% with an average crystal size 12.75 nm. The diffractogram also shows other calcium phosphate compounds such as α-TCP (α-tricalcium phosphate) at 2θ = 37.2944° with a percentage 0.3601% and β-TCP (β-tricalcium phosphate) at 2θ = 27.7974°, 41.0662° and 41.8852° with percentage 4.5366%. Previous researchers had successfully synthesized hydroxyapatite from the precursors Ca(NO₃)₂ and (NH₄)₂HPO₄ using 300 W microwave-assisted for 20 minutes, obtained 20 hydroxyapatite respectively 25.9°, 31.7°, 32.1°, and 32.9° [13], while hydroxyapatite prepared through Ca(NO₃)₂ and (NH₄)₂HPO₄ using microwaves-assisted, the peaks of hydroxyapatite were 25.87°, 28.12°, 28.88°, 31.74°, 39.75° and 46.61°[17]. Others researchers synthesized hydroxyapatite using microwaves-assisted method, with calcium and phosphate source used was CaCl₂ and Na₂ATP respectively. The peaks of hydroxyapatite were 25.9°, 28.9°, 31.8°, 32.2°, 32.9° and 34.1°[18].

![Figure 6. XRD diffractogram of hydroxyapatite using NH₄H₂PO₄ precursor](image-url)

Figure 6. XRD diffractogram of hydroxyapatite using NH₄H₂PO₄ precursor
Reaction on synthesis hydroxyapatite

\[
\begin{align*}
\text{CaO(s)} + \text{H}_2\text{O(l)} & \rightarrow \text{Ca(OH)}_2 \tag{4} \\
(\text{NH}_4)\text{H}_2\text{PO}_4(s) + \text{H}_2\text{O(l)} & \rightarrow (\text{NH}_4)\text{H}_2\text{PO}_4(aq) \tag{5} \\
10\text{Ca(OH)}_2(aq) + 6(\text{NH}_4)\text{H}_2\text{PO}_4(aq) + \text{NH}_4\text{OH}(aq) & \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2(s) + 12\text{H}_2\text{O(l)} + 7\text{NH}_4\text{OH(l)} \tag{6}
\end{align*}
\]

To study the functional groups of hydroxyapatite and hydroxyapatite-chitosan composites (HCC) synthesized was carried out using Fourier Transform Infra-Red (FTIR). The results of the analysis can be seen in Figure 7.

![FTIR spectra of hydroxyapatite using phosphate precursor NH₄H₂PO₄](image)

**Figure 7.** FTIR spectra of hydroxyapatite using phosphate precursor NH₄H₂PO₄

The specific characteristics of hydroxyapatite can be observed with the appearance of the PO₄³⁻ group at wavenumbers 631 and 1085 cm⁻¹, it’s was supported by previous research at 599 and 1036 cm⁻¹ [19], and 565 and 1000-1100 cm⁻¹ [20] while the hydroxyl group (OH⁻) appears at wave number 3642 cm⁻¹.

To study the surface area, size, and pore volume of the hydroxyapatite-chitosan composite was examined using surface area analysis method. The results of the BET and BJH analysis can be seen in Table 6.

**Table 6.** The results of hydroxyapatite-chitosan composite analysis using the BET method

| Parameter             | 0     | 10   | 20   | 30   | Previous research |
|-----------------------|-------|------|------|------|-------------------|
| Surface area (m²/g)   | 12.1831 | 2.66774 | 1.1403 | 5.9041 | 20 and 16⁺, 24⁺, 67⁺ |
| Pore diameter (nm)    | 8.7114 | 5.7792 | 8.7782 | 4.4634 | 20-60 b, 4.90, 5.12, 4.52, 4.10, 3.51 d |
| Pore volume (cc/g)    | 0.026533 | 0.0038544 | 0.002502 | 0.006587 | 0.487, 0.512, 0.470, 0.451, 0.410 d, 0.489, 0.512, 0.549, 0.495, 0.453 c, 0.3450, 0.0174 f |

a (27), b (28), c (29), d (30), e (2), f (31)

The result of surface area analysis for hydroxyapatite is 12.1831 m²/g. Meanwhile, the pore diameter and pore volume were 8.7114 nm and 0.026533 cc/g, respectively. The analysis results
obtained for the surface area for hydroxyapatite-chitosan composite with the addition of 10, 20 and 30% chitosan were 2.66774; 1.1403; and 5.9041 m²/g, respectively. The pore diameter obtained for the hydroxyapatite-chitosan composite with the addition of 10, 20 and 30% chitosan was 5.7792; 8.7782 and 4.4634 nm, respectively. Previous researchers have succeeded in synthesizing hydroxyapatite-chitosan composites through the deposition method with HAp variations 2, 5, 10, 15 and 20%, the pore volumes were 0.489, 0.512, 0.549, 0.495 and 0.453, cm³/g respectively [4].

The hydroxyapatite-chitosan composites also obtained with pore diameter of 0.1-0.2 µm which is used for drugs delivery [19]. The bond that occurs between the hydroxyapatite-chitosan composites is a hydrogen bond. The bond occurs between the H atoms in HAp with the O atoms in chitosan and other hydrogen bonds are also formed between the H atoms of HAp and the N atoms in chitosan as shown in Figure 8.

![Figure 8. Interaction of hydroxyapatite and chitosan](image)

Composites with natural polymers such as chitosan combined with hydroxyapatite will increase fit and hard tissue growth to treat fractures and bone demineralization. Its superior tissue compatibility, functionality, and non-antigenic properties make chitosan an ideal material for medical applications in tissue regeneration.

4. Conclusion

Based on the results, the utilization of the waste of Geloina coaxans shells as the calcium source to produce hydroxyapatite has been done. The hydroxyapatite is matric and chitosan as reinforce combined to prepare a composite. The hydroxyapatite-chitosan composite is interesting to develop based on the application in many fields. These materials can be used as adsorbents to remove heavy metals and dye. Composites with natural polymers such as chitosan combined with hydroxyapatite also can be applied to increase fit and hard tissue growth to treat fractures and bone demineralization. The production of hydroxyapatite-chitosan composites is carried out using the waste of Scylla olivacea shell as a source of chitosan. The rapid pathway synthesis using microwave-assisted is very crucial and helpful for producing composites. Synthesis has done using the microwave 450 W with a retention time of 20 minutes. Mass of chitosan influenced pore of the hydroxyapatite-chitosan composite increased the pore diameter of hydroxyapatite-chitosan composite 0.28 to 5.0308 nm and 4.3182 and 7.0268 nm using KH₂PO₄ and NH₄H₂PO₄. The functional group also shows the specific bands of hydroxyapatite-chitosan composite The presence of –NH₂ groups detected in the FTIR spectrum means that hydroxyapatite-chitosan composite material has been formed. This composite also can be synthesis using other natural polymers with various reaction parameters.

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