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The Novel of Chitosan-Hydroxyapatite of Chicken Eggshells Composite as Methylene Blue Adsorbent

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Abstract

High egg consumption in Indonesia, particularly in South Sulawesi, presents a significant opportunity to utilize eggshells as precursors for hydroxyapatite. This study aims to characterize hydroxyapatite derived from chicken eggshells and to evaluate the absorption properties of chitosan-hydroxyapatite composites for methylene blue removal. The precipitation method was used for hydroxyapatite synthesis, as it produces hydroxyapatite with a high level of purity and low contamination. X-ray diffraction (XRD) analysis revealed that eggshells contain approximately 30% $CaCO_3$*. The XRD diffractogram of hydroxyapatite from eggshells showed a peak at* $2\theta = 31.94^{o}$*. Fourier-transform infrared (FTIR) spectroscopy identified key functional groups in hydroxyapatite, including hydroxyl (-OH) at 3434* cm^{-1}*, carbonate (*$-CO_3^{2-}$*) at 1421* cm^{-1}*, phosphate bending (*$-PO_4^{3-}$*) at 565* cm^{-1}*, and phosphate stretching (*$-PO_4^{3-}$*) at 1035* cm^{-1}*. The chitosan-hydroxyapatite (Cs-HAp) composite showed the incorporation of chitosan functional groups, including amine (-NH) at 1577* cm^{-1} *and methylene (-CH) at 2928* cm^{-1}*. The chitosan-hydroxyapatite composite demonstrated effective removal of methylene blue dye, with an absorption efficiency of 96% at a Cs-HAp ratio of 2:2 within 180 minutes. Based on the high adsorption efficiency, the chitosan-hydroxyapatite composite shows great potential for absorbing methylene blue.*

Keywords: *Eggshell; hydroxyapatite; chitosan; precipitation; adsorbent.*

Introduction

Purebred chicken eggs are highly consumed in Indonesia, making them a significant component of the country's diet [1]. The by-product of egg consumption is egg shells, which have not been optimally utilized. Chicken egg shells have been used as liquid waste metal absorbents in the electroplating industry [2], chromium absorbents and reactive dye absorbents, cationic dyes, azo and melathion dyes [3]. Each eggshell has around 10,000-20,000 pores, so it is believed to absorb solutes and act as an absorbent [4]. The eggshell is equivalent to 11% of the total egg weight and contains 91%-94% calcium carbonate ($CaCO_3$), 1% calcium phosphate, and organic matter. Therefore, eggshells have great potential as the main ingredient of hydroxyapatite [5].

Hydroxyapatite, with molecular formula ($Ca_{10}(PO_4)_6(OH)_2$ or hap, is a calcium phosphate ceramic or bio-ceramic that functions as an absorbent that can manage wastewater from dyes (Hamzah & Salleh, 2014). Hydroxyapatite has chemically stable properties compared to metal and polymer materials, and is non-toxic, bioactive, and biocompatible. In addition, hydroxyapatite can be used as an absorbent because it has pores, is wear-resistant, and is inert (Nascimento et al., 2007). One method often used to synthesize hydroxyapatite is precipitation. This method is effective because it can produce hydroxyapatite, which is mostly amorphous, and the by-product is only water so that the possibility of contamination in the treatment process is very low [8]. It has synthesized hydroxyapatite using the precipitation method from the calcination results of eggshells obtained CaO levels of 76.6%. However, hydroxyapatite hasweaknesses, namely, it is not osteoinductive, has low mechanical properties [9], and is fragile, so it is necessary to innovate composites to improve these mechanical properties [10]. Chitosan, in this case, serves to perfect the mechanical properties of hydroxyapatite, which is quite fragile [11], stable, and nontoxic [12]. Chitosan can be used as an adsorbent, too. The ability of chitosan is due to the nature of chitosan, which is associated with amine and hydroxyl groups that are bonded so that chitosan has strong chemical reactivity and cation polyelectrolyte properties [13]. Both chitosan and hap have the functionality required for strong interaction and compatibility, represented by hydrogen bonding. The main objective of this article is to prepare a novel composite of chitosan-hydroxyapatite as an adsorbent for dyes. This research was conducted on the utilization of calcium sources found in eggshells, which can be synthesized into hydroxyapatite compounds by the precipitation method composite with chitosan as a methylene blue absorbent.

Experimental

Materials

Chicken Eggshell, chitosan (Aldrich), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (Merck), methylene blue (Merck), aquades, Whatmann filter paper no 24.

Instruments

The tools used in this research are X-Ray Diffraction Shimadzu maxima XRD 7000, Fourier Transform Infra-Red (FT-IR) nicole iS10, Varian 50 Conc Uv-Vis spectrophotometer, furnace, oven, analytical balance, sieve shaker, centrifugation, magnetic stirrer, shaker, Erlenmeyer, volumetric flask, stative and clamps, burettes, measuring cups, beakers, pestles and mortars, stirring rods and funnels.

Methods

Calcining of chicken eggshells

The eggshell is cleaned of 1 kg of mucous membranes and dirt. Then the eggshells are washed using clean water. After that, the eggshells are dried at 105 °C for 2 hours in an oven. Next, the eggshell is mashed into powder and then sifted using a 100 mesh. Then the eggshell powder that escaped the sieve was weighed and then dried at 105 °C for 1 hour. After that, the eggshell was calcined at 900 °C for 5 hours using furnance to obtain calcium oxide (CaO) (Mawadara et al., 2016). Furthermore, CaO compounds are converted to $Ca(OH)_2$ by being allowed to come into contact with air for one week. Then analyzed using XRD [14].

Hydroxyapatite Synthesis

$Ca(OH)_2$ and $(NH_4)_2HPO_4$ are prepared with Ca/P ratio of 1.67. 14.7410 grams of $Ca(OH)_2$ was dissolved with 100 mL aquades in the Erlenmeyer as the first solution. After that, 15.7840 grams of diammonium hydrogen phosphate $(NH_4)_2HPO_4$ was dissolved in 100 mL of an Erlenmeyer flask for the second solution [15]. Furthermore, the second solution is dripped into the first solution with a flow rate of 5 mL/minute for 100 minutes using a burette and then stirred at a speed of 350 rpm using a magnetic stirrer until homogeneous. After that, the mixture is decanted for 24 hours. Next, the precipitate is filtered using Whatman 42 filter paper. Then the precipitate is washed three times using Aquadest. Then the precipitate is dried at 110 °C for 3 hours [16]. After drying, the precipitate is crushed until smooth with a mortar. The phase and size of the resulting powder crystals are stored in a desiccator [17]. Further characterized by FTIR and XRD.

Chitosan-hydroxyapatite synthesis

Chitosan hydroxyapatite was weighed in a variation of 2:4, 2:2, and 4:2 (w/w) ratios. Then, hydroxyapatite was dissolved in 100 mL of aquades, and then chitosan was dissolved in 100 mL of 2% acetic acid solution [18]. Furthermore, each ratio of chitosan hydroxyapatite solution was mixed for 1 hour using a magnetic stirrer. Then each mixture is centrifuged at a speed of 3500 rpm for 15 minutes. After that, dry the composite at 80 °C in the oven. Furthermore, it is characterized by FTIR [6].

Methylene Blue Absorption

A solution of methylene blue is prepared with a concentration of 40 ppm. HAp/chitosan composites with variations in ratios of 4, 2:2, and 4:2 (w/w) were weighed 0.05 grams, then 15 mL of methylene blue solution from each concentration in different Erlenmeyer flasks. The mixture is homogenized with a *shaker* with time variations of 120 minutes, 180 minutes, and 240 minutes at a speed of 150 rpm [19]. After that, the mixture is separated by centrifuging for 15 minutes at a speed of 3500 rpm to separate the precipitate. Then the filtrate will be measured for adsorption using a UV-Vis spectrophotometer at a wavelength of 664.5 nm [20].

Results and Discussion

Calcining of chicken eggshells

The calcium content in chicken egg shells is calcium carbonate ($CaCO_3$). The calcination process of chicken egg shells at a temperature of 900 ºC aims to release organic compounds and decompose $CaCO_3$ to form CaO and CO_2. Temperatures of 850 ºC to 1000 ºC break the chemical bonds in $CaCO_3$, so that CaO is formed [21]. In addition, calcination also aims to eliminate organic compounds and impurities that can inhibit the process of forming hydroxyapatite compounds (Suci and Yulius, 2020). The color of the eggshell produced after the calcination process turns pure white. This color change from grayish white to pure white is caused by a change in the arrangement of filler elements at the time of calcination [8]. The reaction equation is as follows:
$CaCO_{3(s)} \rightarrow CaO_{(s)} + CO_{2(g)}$.

Conversion of Calcium Oxide (CaO) to Calcium Hydroxide ($Ca(OH)_2$)

$CaCO_3$ after calcining is converted to CaO. Furthermore, the hydration process is achieved by allowing CaO powder to come into direct contact with water vapor in the open air for a week so that $Ca(OH)_2$ is formed. This process is known as a hydration reaction. The formation of $Ca(OH)_2$ is caused by the absorption of water vapor in the vicinity [16]. The reaction equation is as follows:

$$CaO_{(s)} + H_2O_{(g)} \rightarrow Ca(OH)_{2(s)}$$

CaO is in direct contact with air to form $Ca(OH)_2$. Because $Ca(OH)_2$ is not volatile, this reaction will continue until all the CaO is hydrated or until the water vapor in the air is used up. The results of XRD characterization of $Ca(OH)_2$ compounds obtained as follows:

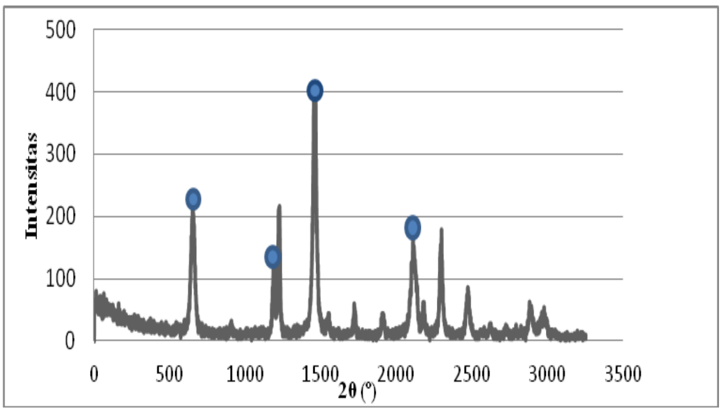

Figure 1. XRD Diffractogram of $Ca(OH)_2$

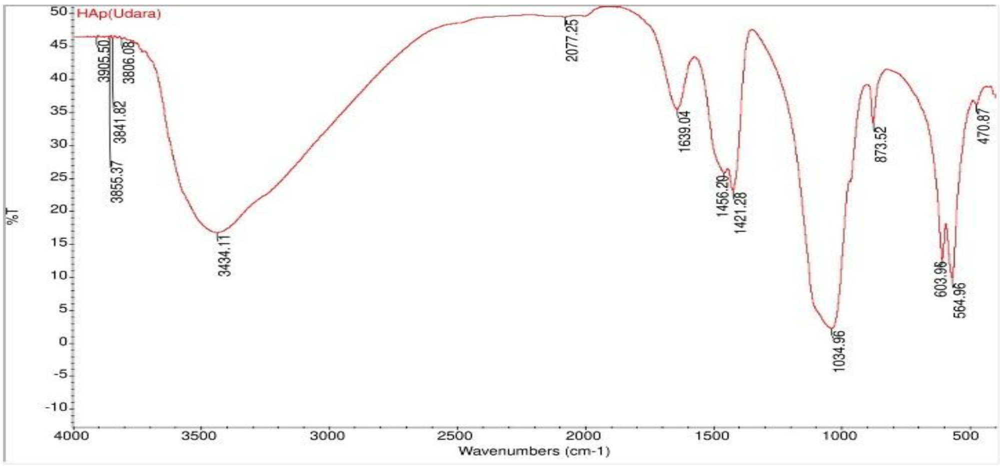

Figure 2. FTIR spectrum of hydroxyapatite

Based on the diffractogram, the results of XRD analysis in Figure 1 indicate the formation of the main fraction of $Ca(OH)_2$ according to JCPDS No. 84-1263, as evidenced by the presence of peaks at angles 2θ= 18.02º, 28.66º, 34.13º, and 47º [22].

Determination of Calcium (Ca) Levels

Analysis of calcium (Ca) levels in purebred chicken eggshells after calcination using *X-Ray Diffraction* (XRD). The results of the analysis of purebred chicken egg shell powder contain calcium of 49%. Measurement of calcium levels is carried out to determine the amount of calcium contained in chicken egg shells [17].

Hydroxyapatite synthesis

Hydroxyapatite particles are obtained by reacting $Ca(OH)_2$ with $(NH_4)_2HPO_4$ through the mole parameter Ca/P 1.67: 1 with the reaction equation as follows:

$$10Ca(OH)_2 + 6(NH_4)_2HPO_4 \longrightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O + 12NH_4OH$$

Synthesized hydroxyapatite particles produce a high HAp powder of 91.30 %. Hydroxyapatite particles are produced in relatively high quantities using the precipitation method [23].

Characterization of Hap using FTIR is carried out to provide information regarding the typical functional groups possessed by the hydroxyapatite particles obtained. The results of FTIR HAp analysis in Figure 2 have typical functional groups belonging to hydroxyapatite, namely groups (-OH) and ($-PO_4^{3-}$). The wavenumber 3434 cm^{-1} indicates the vibration of the cluster (-OH). The presence of a weak peak that is the vibration of the cluster ($-CO_3^{2-}$) is shown at wavenumber 1421 cm^{-1}. The vibrations of the cluster ($-PO_4^{3-}$) are at wavenumbers 565, 603, and 1035 cm^{-1}.

XRD analysis is performed to determine the characteristics of the crystal structure derived from the obtained hydroxyapatite. Based on the results of the XRD diffractogram of hydroxyapatite eggshell of purebred chickens by precipitation method, the peaks formed appear at angles of 2θ= 25.88º; 31.94º; 32.96º; 34.04º; 39.88º; 46.88º; 49.54º. The characterization results prove that the peaks formed are hydroxyapatite peaks in accordance with JCPDS standard No. 09-432. The results of the hydroxyapatite diffractogram of purebred chicken eggshells show that the highest peak is located at an angle of 2θ = 31.94º with a very strong peak intensity and produces 100% hydroxyapatite.

The results of the resulting hydroxyapatite XRD analysis are as follows:

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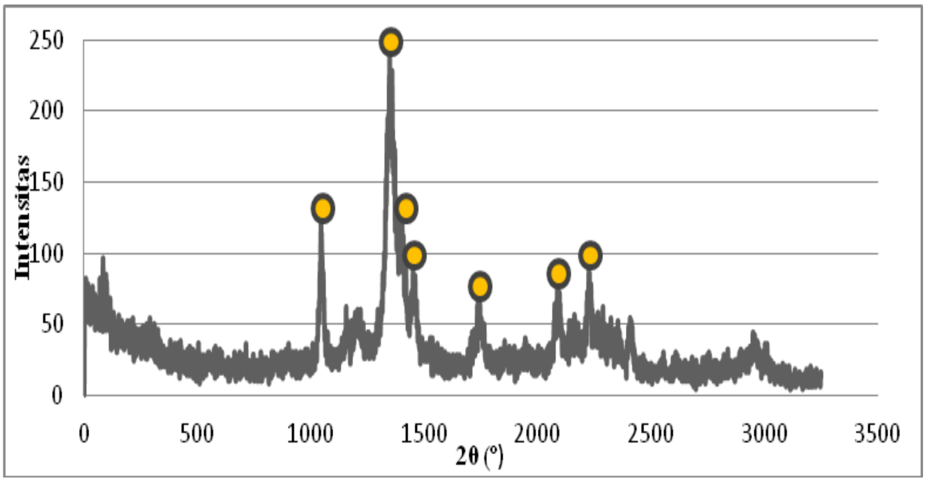

Figure 3. XRD Diffractogram of Hydroxyapatite.

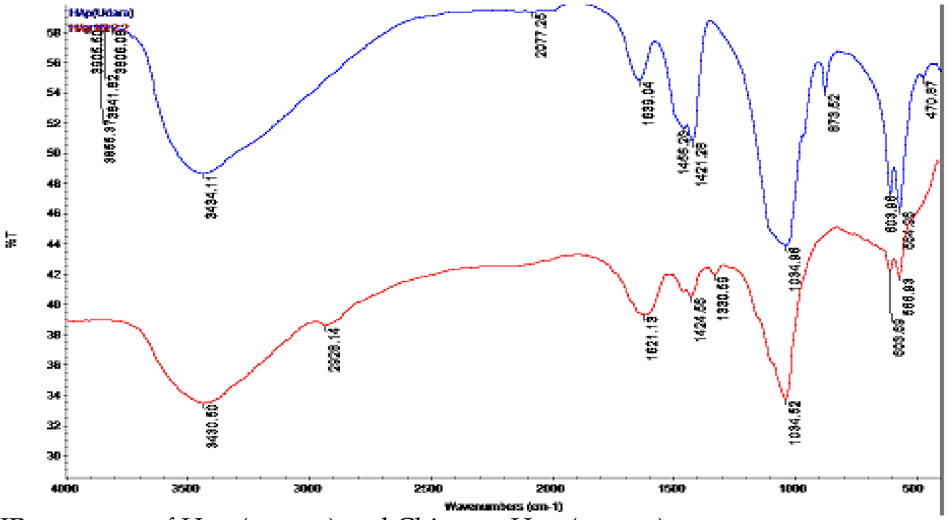

Figure 4. FTIR spectrum of Hap ( ) and Chitosan-Hap ( ).

Based on the results of the analysis conducted by [24]. It is stated that the crystal structure of hydroxyapatite in chicken egg shells is identical to hexagonal hydroxyapatite. This is because the parameters of the hydroxyapatite crystal lattice of chicken egg shells (a= 9.4232 Å, c= 6.8833 Å) are identical to the stoichiometry of hydroxyapatite (a= 9.423 Å, c = 6.875 Å) [25]. The term apatite is currently used to refer to a family of compounds that have similar structural characteristics (hexagonal system, space group, and P63/m) despite having a wide variety of chemical make-ups [26].

Chitosan-Hydroxyapatite Composite Synthesis

FTIR spectral results between hydroxyapatite and bentonite peaks show that hydroxyapatite particles are dispersed in the chitosan matrix. The peak of the spectrum in hydroxyapatite widens at wavelength 3433 cm^{-1}, forming the -NH group. In addition, the absorption band of hydroxyapatite at wavelength 2077 cm^{-1} shifts to wavelength 2928 cm^{-1} due to the presence of chitosan dissolved in Hap, which is marked by the formation of the -CH group. This proves that the -NH and -CH groups indicate chitosan [27].

Methylene Blue Absorption

The absorption ability of Cs-Hap against methylene blue was carried out using contact time variation parameters of 120 minutes, 180 minutes, 240 minutes, and absorbat concentrations of 40 ppm. The resulting filtrate was analyzed using a UV-Vis spectrophotometer to determine the absorbency of the composite mass contact time variation parameter.

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Table 1. Contact Time Relationship with Absorbance

Time (Minutes)	HAp: Cs Cs: Hap	Unabsorbed Residual Methylene Blue Concentration (ppm)	Absorbed Methylene Blue Concentration (ppm)	Uptake Efficiency (%)
120	4:2	19.63	20.36	50.92
	2:2	1.79	38.20	95.52
	2:4	6.55	33.45	83.62
180	4:2	21.41	18.58	46.45
	2:2	1.55	38.45	96.12
	2:4	6.13	33.86	84.67
240	4:2	19.81	20.18	50.46
	2:2	2.33	37.66	94.15
	2:4	6.45	33.54	83.87

Contact times of 120 minutes, 180 minutes, and 240 minutes experienced a fairly high increase. Especially at a contact time of 180 minutes with a ratio of Cs/Hap 2: 2 with absorption efficiency reaching 96.12%. The curve below shows that the longer the contact time, the higher the absorption efficiency. However, the Cs/Hap composite at a contact time of 240 minutes experienced a decrease in absorption efficiency of 94.15%. The occurrence of decreased absorption ability of methylene blue due to too long contact time can cause desorption, which is the release of methylene blue that has been bound by the absorbent. Therefore, the absorption capacity of the Cs/Hap composite will decrease if the contact time exceeds the optimal time. However, if the optimal contact time is achieved, then all dyes will be absorbed by the absorbent [28].

Conclusions

The results of FTIR characterization of hydroxyapatite compounds are characterized by the presence of functional groups -OH at wavelengths 3434 cm^{-1}, $-CO_3^{2-}$ cm^{-1} at wavelengths 1421 cm^{-1}, PO_4^{3-} bending at wavelengths 565 cm^{-1}, $-PO_4^{3-}$ cm^{-1} *stretching* at wavelengths 1035 cm^{-1}. XRD Diffractogram of hydroxyapatite eggshell of purebred chickens by precipitation method in this study showed the highest peak formed appeared at an angle of $2\theta=31.94^{o}$. The characterization results prove that the peak formed is a hydroxyapatite peak in accordance with JCPDS standard No. 09-432.

Test results show that the chitosan hydroxyapatite composite is able to remove methylene blue dye with absorption efficiency reaching 96% at a ratio of Cs/HAp 2: 2 within 180 minutes. Based on the absorption efficiency value, the chitosan hydroxyapatite composite has the potential to absorb methylene blue.

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