

Effect of silica

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Effect of Silica Addition on Mechanical Properties of Eggshell-Derived Hydroxyapatite

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Abstract – Eggshell is a solid waste that is available in abundance but is being left unused. Eggshell containing calcium in a high amount. Calcium can be used as a precursor for hydroxyapatite (HAp). Modification of HAp with SiO₂ is expected to improve its low mechanical properties for biomedical applications. In this study, HAp is synthesized from the eggshell. Then, it was modified by adding SiO₂ utilizing the coprecipitation method with concentrations of 10%, 20%, 30%, and 40%, respectively. The HAp and HAp/SiO₂ were characterized using X-ray diffraction and Fourier transform infrared spectroscopy. The analysis HAp and HAp/SiO₂ were density, compressive strength, and hardness. The best mechanical properties of HAp/SiO₂ were characterized using SEM-EDS. The HAp were prepared successfully with a ratio of Ca/P was 1.673, close to the theoretical 1.67. The addition of SiO₂ caused a decrease in crystallite size and density but increased compressive strength and hardness. The best mechanical properties of HAp/SiO₂ were obtained with SiO₂ of 30% and 40% with similar values.

Keywords: Eggshell, hydroxyapatite, SiO₂, mechanical properties

Introduction

Hydroxyapatite (HAp) is one of the materials that has received considerable attention for its potential to apply in various applications, including biomedical products (Gomes *et al.*, 2019), drug delivery (Taha *et al.*, 2020), and skin regeneration (Pal *et al.*, 2020). Besides, it is also promising in waste treatment (Ibrahim *et al.*, 2020; Hariani *et al.*, 2020) and heterogeneous catalysts (Saha *et al.*, 2018). HAp has the molecular formula Ca₁₀(PO₄)₆(OH)₂, which belongs to the type of apatite material compound [M₁₀(XO₄)₆Z₂], which has a crystal phase of the most stable polycrystalline calcium phosphate compound (Wang *et al.*, 2017). The molar ratio of calcium to phosphorus (Ca/P) is about 1.67.

HAp is also beneficial for bone tissue engineering applications such as bio-scaffolds (Xiao *et al.*, 2019), bioactive glasses (Bazli *et al.*, 2017), bioceramics (Hubadillah *et al.*, 2020), and implants (Shakir *et al.*, 2018). It is similar to human bones and teeth in crystal structure and composition (Xu *et al.*, 2004; Amaechi *et al.*, 2019). HAp is applicable in biomedicine because it has high biocompatibility (Aktug *et al.*, 2017), osteoconductivity (Bovand *et al.*, 2019), and bioactivity (Szczer *et al.*, 2017), and is non-inflammatory, non-immunogenic, and non-

toxic (Dan *et al.*, 2019; Coelho *et al.*, 2019). Its ability to absorb hydrophilic and hydrophobic compounds is vital in drug delivery and environmental applications (Barbosa *et al.*, 2020). However, HAp has shortcomings, especially in the medical field. It has low mechanical properties, namely the low strength due to the influence of the applied compressive and tensile forces (Noviyanti *et al.*, 2021), its relatively slow reactivity, and integration with cells (Bohner, 2009; Latifi, 2012). Synthetic HAp has mechanical properties poorer than human cortical bone's (Hsu *et al.*, 2021). The application of HAp in the medical field must meet certain standard parameters, including good mechanical properties (Chakrawaty *et al.*, 2020). Structural modifications such as substituting or incorporating other materials can increase mechanical strength (Noviyanti *et al.*, 2021) and improve the quality of tissue repair. Several researchers have modified HAp with other materials such as magnesium (Noviyanti *et al.*, 2021), chitosan (Pighinelli and Kucharska, 2013), chloride (Hsu *et al.*, 2021), and zirconia (Es-saddik *et al.*, 2021).

Several researchers have modified HAp by adding silica. The addition of silica can improve HAp's mechanical properties and increase in vivo bioactivity (Latifi, 2012). Silica is biocompatible and non-toxic (Sadeghzade *et al.*, 2015; Thian *et al.*, 2006). Latifi (2012) reported synthesizing HAp/SiO₂ with silica with 20% and 40% concentrations and finding that increasing SiO₂ concentration can decrease crystallinity. Hydroxyapatite is synthesized from the reaction of phosphoric pentoxide with calcium nitrate tetrahydrate in an alcohol medium. The other research that synthesized HAp/SiO₂ by mechanical alloying followed by sintering showed that increasing SiO₂ concentration increased densification, mechanical properties, and in vitro bioactivity of HAp/SiO₂ nanocomposite sintered at 900 °C (Taha *et al.*, 2020).

Hydroxyapatite can be synthesized from raw materials or solid wastes that are readily available in quantity, such as fish bones (Pamungkas *et al.*, 2019), clamshells (Pu'ad *et al.*, 2019), eggshells (Noviyanti *et al.*, 2021), camel bones (Alqodami *et al.*, 2018), and animal bones (Obada *et al.*, 2020). One of the potential sources for HAp is chicken eggshells. Chicken eggs are usually used in large quantities in the food industry, restaurants, households, and even the pharmaceutical industry (Curkovic *et al.*, 2017). An eggshell, which accounts for about 11% of the egg's weight, is almost always disposed of as solid waste. The main element is calcium carbonate (95–97 wt%) with a minor amount of calcium phosphate, magnesium carbonate, organic, strontium, sodium, iron, potassium, and chlorine (Witoon, 2011; Curkovic *et al.*, 2017). The high calcium content in chicken eggshells is the potential to be used as HAp material.

In this study, HAp was synthesized with CaO that originated from chicken eggshells. The reaction of CaO with H₃PO₄ produces HAp. Later, HAp was modified with SiO₂ at various concentrations with the coprecipitation method. This method is a simple, inexpensive, and high success (Curkovic *et al.*, 2017). The resulting HAp and HAp/SiO₂ were characterized using XRD, FTIR, and SEM-EDS. The effect of SiO₂ concentration was determined by the density and mechanical tests, including compressive strength and hardness.

Materials and Methods

Materials

The materials used were chicken eggshells (Hyline brown chicken) from local chicken farmers in Palembang city, chemicals including H₃PO₄, TEOS (C₈H₂₀O₄Si), NH₄OH obtained from Sigma Aldrich (Germany), and distilled water.

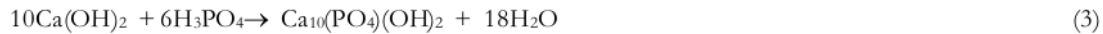
HAp Preparation

Eggshells were cleaned using distilled water until free of impurities. The clean eggshells were boiled for ±1 hour, separated from the membrane, and dried in the sun for a day. After being dried, they were mashed with a grinding machine to be a fine powder of ±100 mesh. A total of 100 g of the eggshell powder obtained was calcined at around 900 °C for 3 hours at a speed of 10°/min to change the CaCO₃ phase into CaO. The calcined CaO was added with 250 mL of deionized water, stirred using a magnetic stirrer for 30 minutes. Then, 250 mL of 1 M H₃PO₄ was added slowly while being stirred. The reaction produced HAp. The HAp produced was dried at a temperature of 105°C using an oven for 1 hour (Hariani *et al.*, 2020; Asadipour *et al.*, 2019).

HAp/SiO₂ Preparation

The preparation of HAp/SiO₂ in this study refers to the procedure reported by Asadipour *et al.* (2019). In preparing HAp/SiO₂, TEOS was used as a precursor with different weights to obtain SiO₂ with 10%, 20%,

30%, and 40% concentrations. Each mixture of HAp and TEOS solutions was added with 1 M ammonia solution gradually until the pH of the solution lies between 9-11 while stirred with a magnetic stirrer and given a stream of nitrogen gas. The mixture was allowed to stand for about 96 hours, then filtered and washed with deionized water. The powder obtained was dried using an oven at 40 °C for 72 hours. The reaction of calcium hydroxide with phosphoric acid produced HAp, as follows (Curkovic *et al.*, 2017):



The reaction of SiO₂ formation from the precursor TEOS was as follows:



Characterization of HAp and HAp/SiO₂

The HAp and HAp/SiO₂ were characterized using X-ray diffraction (XRD Malvern Panalytical) using Cu-Kα = 0.154 nm, in the range 2θ of 5-80°. The HAp and HAp/SiO₂ functional groups were identified using the Fourier transform infrared (FTIR Prestige 21) instrument at a wavenumber of 400-4000 cm⁻¹. A scanning electron microscope-energy dispersive spectrometer (SEM-EDS JOEL JSM 6510 LA) was used to observe the morphology and composition of the elements.

Analysis of Density, Compressive Strength, and Hardness

Density (ρ) was analyzed using the ratio of mass divided by total volume m/v (g/cm³), while the compressive strength was tested used the Autograph SHIMADZU AG-10 TE, Vickers Hardness Test (Leco M-400-H1).

Results

Characterization of HAp/SiO₂ using XRD and FTIR

Figure 1 shows the XRD spectra of HAp and HAp/SiO₂, with varying concentrations of SiO₂. The XRD analysis was performed at 2θ = 5-80°. The crystallite size HAp and HAp/SiO₂ were determined using Scherrer's equation, according to the following formula:

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (5)$$

Where *D* is the crystallite size (nm), *K*s the Scherrer constant (0.91), λ is the wavelength of X-rays (1.54056Å), β is the value of Full Width at Half Maximum (rad), and θ is the diffraction angle (FWHM) (rad). Table 1 shows Crystallite sizes of HAp and HAp/SiO₂. From the table, it can be seen that the greater the concentration of SiO₂, the smaller the crystallite size. Figure 1 shows the diffractograms of HAp and HAp/SiO₂.

Table 1. Crystallite sizes of HAp and HAp/SiO₂

SiO ₂ content (%)	The average crystallite size (nm)
0	51.8
10	47.8
20	39.4
30	35.7
40	34.8

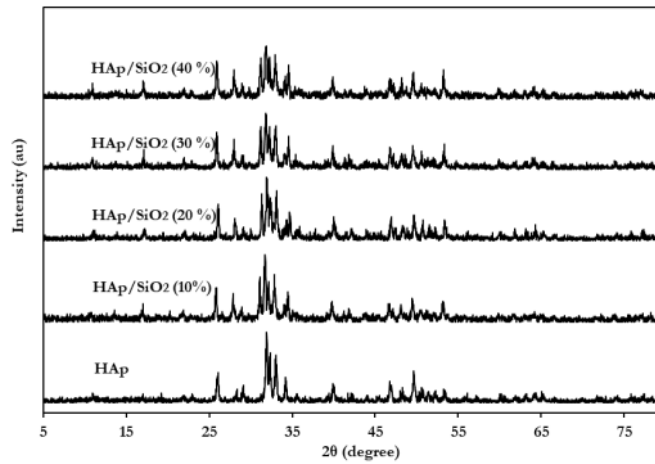


Figure 1. XRD patterns of HAp and HAp/SiO₂

Figure 2 shows the FTIR spectra of HAp and HAp/SiO₂ at wavenumbers 400-4000 cm⁻¹. All peaks indicate the similarity of phosphate functional groups observed at wavenumbers 900-1200 cm⁻¹ (Hariani *et al.*, 2020). There are OH groups on a wide peak at wavenumbers 3280-3600 cm⁻¹ (Balamurugan *et al.*, 2013). Symmetric stretching vibrating bands of Si-O-Si were observed at a wavenumber of about 800 cm⁻¹. The addition of SiO₂ causes changes in peak intensity, especially those identified at wavenumbers below 800 cm⁻¹.

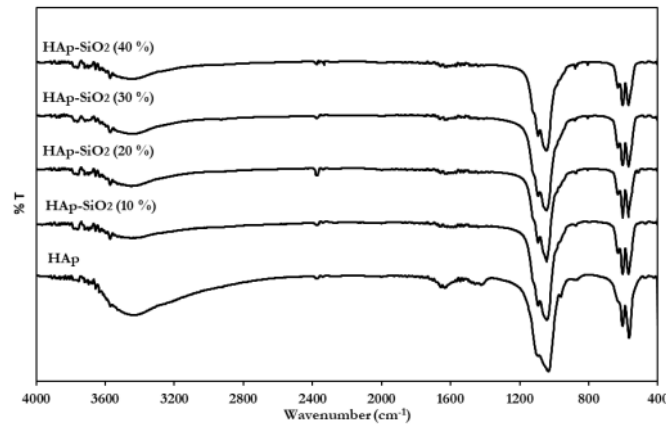


Figure 2. FTIR spectra of HAp dan HAp/SiO₂

Density, Compressive Strength, Hardness of HAp and HAp/SiO₂

Figure 3 shows the density of HAp and HAp/SiO₂ that decreased along with the addition of SiO₂. Compressive strength and hardness are critical properties of a material to be compatible with biomedical biomaterials. Figures 4 and 5 indicate the compressive strength and hardness values of HAp and HAp/SiO₂.

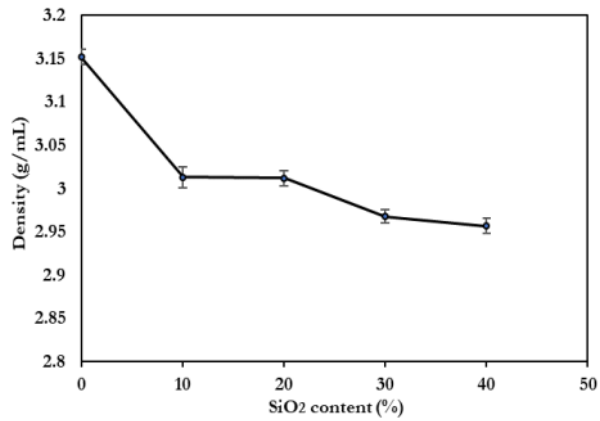


Figure 3. The density of HAp and HAp/SiO₂ at different SiO₂ content

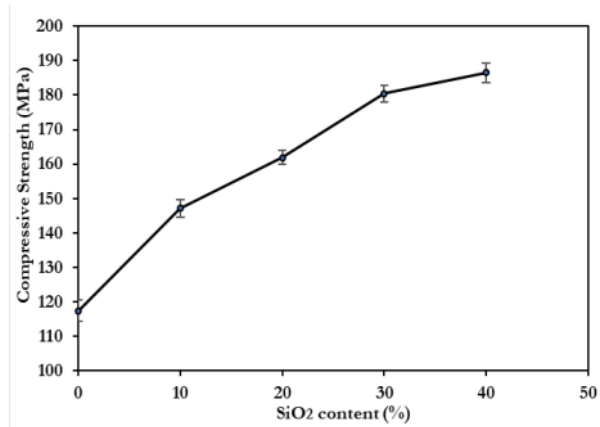


Figure 4. Compressive strength of HAp and HAp/SiO₂ at different SiO₂ content

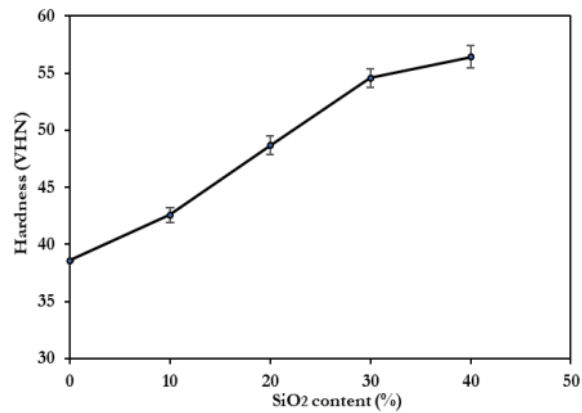


Figure 5. The hardness of HAp and HAp/SiO₂ at different SiO₂ content

SEM-EDS Characterization of HAp and HAp/SiO₂

In this study, SEM-EDS analysis was performed on HAp and HAp/SiO₂ (30%). HAp/SiO₂ (30%) was selected because both HAp/SiO₂ (30%) and HAp/SiO₂ (40%) show the best result with similar mechanical properties. Figure 6 shows the morphologies of HAp and HAp/SiO₂ with the addition of 30% SiO₂ as measured by a magnification of 5000 x. The compositions of HAp and HAp/SiO₂ based on EDS results are presented in Table 2.

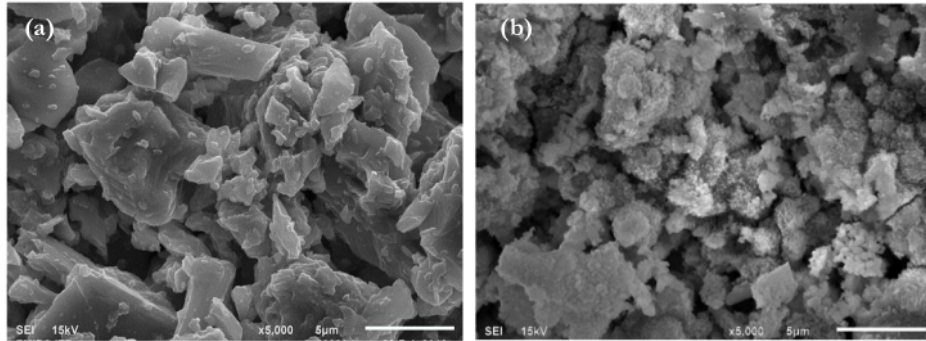


Figure 6. Morphologies of (a) HAp and (b) HAp/SiO₂ (30%)

Table 2. Compositions of elements of HAp dan HAp/SiO₂ (30%)

Elements	Percentage (%)	
	HAp	HAp-SiO ₂ (30 %)
O	45.16	46.08
P	17.36	12.70
Ca	37.48	27.45
Si	-	13.77

Discussion

Characterization of HAp and HAp/SiO₂ using XRD and FTIR

According to JCPDS No. 09-432, HAp has a major peak at 2θ 25.879°, 31.773°, 32.196°, 32.902°, and 49.468°. The HAp manufactured from chicken eggshells appears to agree with these data, namely at 2θ angles at 25.52° (201), 31.94° (211), 32.22° (112), 32.98° (300), and 49.66° (213). The addition of SiO₂ caused the peaks to widen and the intensity to decrease, as seen at the 2θ around 31-32° (Figure 1). The amorphous nature of silica causes silica peaks not to be observed. Silica peaks should be observed at 2θ around 23° (Hariyani et al., 2020). The increasing silica content in HAp brought the crystallite size to decrease, indicating the amorphous silica phase in the composite (Latifi, 2012), which caused a decrease in intensity. Table 1 shows the average crystallite sizes of HAp and HAp/SiO₂ calculated based on Scheerer's equation. Based on the table, the higher the SiO₂ content, the smaller the crystallite size. The research by Hanora *et al.* (2021) showed that the greater the concentration of SiO₂ (25%, 30%, 40%, and 50%), the smaller the crystal size. In this study, the crystallite sizes of HAp and HAp/SiO₂ are small (< 100 nm).

The characterization of HAp and HAp/SiO₂ using FTIR shows the peaks of HAp and HAp added with SiO₂ of 10%, 20%, 30%, and 40% contained OH groups, as identified at wavenumbers 3421.7 cm⁻¹, 3435.2 cm⁻¹, 3431.6 cm⁻¹, 3435.2 cm⁻¹, and 3431.4 cm⁻¹. These hydroxyapatite structures containing OH groups. Besides that, OH groups could also be from water molecules absorbed. The peak also strengthened the presence of H₂O in HAp at 1622.4 cm⁻¹. The presence of phosphate groups (PO₄³⁻) in HAp could be identified at wavenumbers 1032.8 cm⁻¹, 960.5 cm⁻¹, and 565.1 cm⁻¹. The peak also appeared in HAp/SiO₂, but the intensity

decreased. Peaks that indicate the presence of Si-O-Si asymmetric stretching groups in HAp/SiO₂ can be identified in wavenumbers 1070.5 cm⁻¹, 1072.4 cm⁻¹, 1072.6 cm⁻¹, and 1070.5 cm⁻¹.

Density, compressive strength, and hardness of HAp and HAp/SiO₂

The statistical tests using ANOVA and LSD showed that the density of HAp was significantly different from that of HAp/SiO₂, while HAp/SiO₂ with the addition of 10-40% of SiO₂ was not significantly different. Theoretically, the density of HAp is 3.156 g/cm³ (Ramesh *et al.*, 2013). Taha *et al.* (2020) reported that the density of SiO₂ was 2.65 g/cm³, and that of HAp prepared by the mechanochemical synthesis method was 3.15 g/cm³. Jouda *et al.* (2021) obtained a density of HAp from the cow bone of 2.982 g/cm³ and was considered compatible with biomedical applications. The density values of HAp and HAp/SiO₂ are similar to previous researchers, namely between 2.957-3.152 g/cm³.

The mechanical properties determined included compressive strength and hardness, as shown in Figures 4 and 5. In Figures 4 and 5, it can be seen that the addition of SiO₂ to HAp increased both compressive strength and hardness. The statistical tests using ANOVA and LSD test showed that the compressive strength of HAp/SiO₂ with variations in SiO₂ concentration also showed significant differences. The nature of silica can improve the brittleness of hydroxyapatite (Kailasanathan and Gangadharan, 2016). The compressive strength values for human compact bone are in the range of 170-193 MPa. HAp/SiO₂ with concentrations of 30% and 40% met these criteria. The compressive strength values were included in the compressive strength value of cortical bone, ranging from 100 to 230 MPa (Ficai *et al.*, 2011). In this study, the compressive strength of HAp/SiO₂ with concentrations of 30% and 40% were 180.44 ± 2.55 MPa and 186.5 ± 3.12 MPa, respectively.

The hardness of materials that will experience frictional force and plastic deformation should be known. Plastic deformation is the condition of a material in which the microstructure of the material cannot return to its original shape when given a force. The HAp and HAp/SiO₂ hardness tests showed that the addition of SiO₂ increased the hardness (Figure 5). The SiO₂ added filled the HAp pores. If the porosity is too high, the biomaterial will be easily porous because many pore cavities are formed.

In contrast, low porosity will inhibit the intake of nutrients from the blood vessels to the bones. According to Jouda (2021), the value of hardness is highly dependent on density and porosity. Hardness values of cortical bones range from 53.7 to 77.9 VHN (Ahn *et al.*, 2001; Rho *et al.*, 1997). In this study, the hardness values of HAp/SiO₂ with SiO₂ compositions of 30% and 40% met these criteria. The statistical tests using ANOVA and LSD test showed that HAp with the addition of SiO₂ of 30% and 40% were not significantly different obtained 54.56 ± 0.83 VHN and 56.43 ± 0.98 VHN, respectively. The best properties of the compressive strength and hardness values of HAp/SiO₂ were achieved at 30% and 40%.

Characterization of HAp and HAp/SiO₂ using SEM-EDS

HAp and HAp/SiO₂ with the addition of 30% SiO₂ were analyzed using SEM-EDS, as shown in Figure 6. The results of EDS are presented in Table 2. The surface morphologies of HAp and HAp/SiO₂ are different from each other. HAp has an oval shape; its surface becomes tighter with the addition of SiO₂ because SiO₂ fills the pores of HAp. The molar ratio of calcium to phosphorus in HAp was 1.673, close to the theoretical ratio of 1.67, indicating the synthesis of HAp has been successful. The addition of SiO₂ by 30% increase the Si element and decreased the P and Ca elements with a molar ratio of Ca/P = 1.670. The elements of HAp/SiO₂ was O=43.08, P = 12.70, Ca = 27.45 and Si = 13.77%.

Conclusion

The synthesis of hydroxyapatite (HAp) from eggshells and its modification by SiO₂ with concentrations of 10%, 20%, 30%, and 40% have been carried out. The HAp obtained had a Ca/P ratio of 1.673. The addition of SiO₂ reduced the average crystallite size and density. The modification also improved the mechanical properties of HAp by increasing its compressive strength and hardness. The concentration of SiO₂ for the best mechanical properties were achieved at 30% and 40% with a similar value. It can be reckoned that eggshell-derived HAp with SiO₂ modification has the potential as a biomaterial for biomedical applications.

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