Synthesis of Silver-Alginate Nanocomposites Colloidal Assisted by Microwave Irradiation as Antibacterial Material

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Abstract. Synthesis of silver-alginate nanocomposite colloidal as an antibacterial material has been done. The colloidal is prepared through a chemical reduction method assisted by microwave irradiation with alginate as a reducing agent and stabilizer. Silver nitrate is used as a precursor and NaOH as an accelerator. The formation of silver-nanoparticles was indicated using a UV-Vis spectrophotometer based on localized surface plasmon resonance (LSPR) phenomenon. The shape and size of silver nanoparticles were characterized using TEM. Effect of storage in ambient temperature of silver-alginate nanocomposites colloidal was carried out during 14 weeks. Investigation of antibacterial activity of silver-alginate nanocomposite was carried out using diffusion method. The results showed that, the concentration of NaOH, irradiation time, AgNO₃ concentration affect to the absorption band of the LSPR which related with the number of nanoparticles produced. Based on the TEM images, the size of silver nanoparticles increased with increasing concentration of AgNO₃. Silver-alginate nanocomposites was stable for 12 weeks based on the absorption band of the LSPR. The greater the concentration of silver nanoparticles in silver-alginate nanocomposites, tends to the higher their antibacterial properties.

1. Introduction
Nanocomposites with filler silver nanoparticles as antibacterial material have been widely studied. The nanocomposite is a combination consisting of two or more material components, where the material components as a matrix (binder), and the other as a fillers, which are nano-sized with a diameter of less than 100 nm [1]. In this case, biopolymers as a matrix or binding component and nanoparticle as a filler. One of the most commonly used nanoparticles is metal nanoparticles [2]. Silver nanoparticles are a type of metal nanoparticles that are often used as objects of research and are used in the synthesis of nanocomposites [3] because of silver nanoparticles have good antibacterial activity, and also have low toxicity to cells of eukaryotic living things, such as humans. The dispersion of silver nanoparticles into the chitosan biopolymer will provide better antibacterial properties to the formed nanocomposite material. Based on research conducted by Du [4] the combination of chitosan-tripolyphosphate nanoparticles with Ag metal ions+ can provide higher antibacterial activity when compared to using other metal ions, such as Cu²⁺, Zn²⁺, Mn²⁺, and Fe²⁺. Meanwhile, according to research by kim [5] and Kumar-Krisnan [6] silver in the form of nanoparticles has a higher antibacterial activity when compared to silver in the form of ions or compounds. Because of its antibacterial effectiveness, silver nanoparticles are one of the antimicrobial materials that are often used and applied to food packaging products [7].
Silver nanoparticles can be synthesized using chemical reduction methods [8]. This method is effective and is often used for reasons of convenience, relatively affordable costs, and the possibility to be used for large-scale production. In the synthesis process using chemical reduction methods, the role of reducing agents and stabilizers is indispensable. Both are the main factors that will determine the characteristics of the nanoparticles produced. The utilization of chitosan as a reducing agent in the production of silver nanoparticles was effectively done by Raghavendra [9] using microwave irradiation for 11 minutes, where stopped each 45 second. In the investigation carried out by Darroudi [8] the utilization of accelerators agents can influence the number and size of the silver nanoparticles produced. NaOH as an accelerator agent can lessen the size of silver nanoparticles in line with concentration of NaOH utilized. The utilization of NaOH as a accelerator agent in the production of silver nanoparticles has additionally been done by Susilowati [10], by analyzing the synthesis results which are more or less the same. Thus, NaOH is proven to accelerate the process of forming silver nanoparticles.

The synthesis process by involving natural and environmentally friendly materials is commonly referred to as biosynthesis [8]. The use of reducing agents that tend to be toxic such as sodium borohydride (NaBH4) and hydrazine needs to be reduced because they can harm the environment. The use of natural reducing agents needs to be done as an alternative in the synthesis process of silver nanoparticles. In addition to being safe and environmentally friendly, these materials are also relatively cheaper. Several natural reducing agents (bioreactors) have been widely researched and proven to be able to provide good results in the synthesis process of silver nanoparticles, including glucose [11], apple waste [12], starch [9], cellulose [13], pith extract of sago stems[14], and pectin [15]. The other way, several natural stabilizers have also been extensively researched and developed.

Materials with biopolymer types such as cellulose, gelatin, chitosan, starch, and alginate are known to be stabilizers in the synthesis of silver nanoparticle materials, which can provide stability to metal nanoparticles from oxidation, agglomeration, and precipitation processes. Beside be able to act as a matrix and stabilizer, alginate can also act as a reducing agent [16]. They have succeeded in synthesizing gold nanoparticles using alginate as a reducing agent and stabilizer. Alginate is a biopolymer that is nontoxic, environmentally friendly, and more degradable. Alginate prevents the aggregation of metal nanoparticles and also acts as a reducing agent. Alginate can reduce the original gold nanoparticles charged 3−. In this study, we synthesis of silver nanoparticles use alginate assisted by microwave irradiation. To increase the reaction rate we use an accelerator of NaOH [16]. This is in line with research conducted previous research [8] which proved the effect of adding NaOH as an accelerator in green-synthesis of silver nanoparticles with glucose reducing agents which can increase the rate of formation of silver nanoparticles. The formation of silver nanoparticles can be identified using a UV-Vis spectrophotometer due to phenomenon Localized Surface Plasmon Resonance (LSPR). The LSPR phenomenon that occurs in silver nanoparticles can provide a characteristic absorption spectrum in the region UV-Visible. The silver nanoparticle LSPR band characteristics of the UV Vis absorption peak in the wavelength range of 400-450 nm [17,9]. The silver nanoparticles formed can be seen from the appearance yellow until brown color. Furthermore, colloidal silver nanoparticles were characterized using UV-Vis and TEM spectrophotometry. The colloidal stability of silver alginate nanoparticles was determined by UV-Vis spectra based on the LSPR absorption which is measured every two weeks for 14 weeks at room temperature storage. The Colloidal silver nanoparticles with alginate as stabilizer in this research was called silver-alginate nanocomposite then were tested for antibacterial activity against Escherichia coli and Staphylococcus aureus.

2. Research Methods
2.1 Material
This research used experimental methods in the laboratory. The materials were used in this research are sodium alginate, silver nitrate (AgNO3) and sodium hydroxide (NaOH) purchased from Merck, and distilled water was made in Chemistry Education Department Laboratory of Sebelas Maret University.
2.2 Synthesis of silver-alginate nanocomposites
Preparing 1% (w/v) sodium alginate solution in distilled water using a stirrer with medium speed till homogeneous. Put 12.5 mL each of 1% sodium alginate into six beakers and stir with a stirrer. Add AgNO3 to each beaker with various concentrations of AgNO_3 of 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0% (w/w, AgNO_3/Alginate) drop by drop while stirring with a medium speed stirrer for about five minutes. Adding 2M NaOH with a volume of 0.75 mL (0.11 M in solution) into each beaker glass drop by drop while stirring with a medium speed stirrer for about five minutes. Put the solution in the microwave and set the power to 100 watts in 6 minutes. Add 47.5 mL each of 1% alginate to six beaker cups that have been in the microwave and stir with a stirrer until completely dissolved. The resulting silver alginate nanoparticles were respectively coded of A1, A2, A3, A4, A5, A6. With the same working steps, nanoparticle synthesis can be carried out with different NaOH concentration variations to determine the effect of added NaOH concentration. The concentration variations of NaOH in the solution were 0 M (N1), 0.04 M (N2), 0.08 M (N3), 0.11 M (N4), 0.15 (N5). To determine the effect of irradiation time in the synthesis of nanoparticles, various irradiation times were carried out in the synthesis of nanoparticles using the same method with different irradiation times. The results of silver alginate nanoparticles with variations in irradiation time were respectively coded T1 (0 minutes), T2 (1 minute), T3 (2 minutes), T4 (3 minutes), T5 (4 minutes), T6 (5 minutes), T7 (6 minutes), T8 (7 minutes), T9 (8 minutes).

2.3 Analysis of UV-Vis spectroscopy
Visible Colloid was characterized by a UV-Vis spectrophotometer with a range of 300 nm-600 nm. The colloid was diluted 10 times with distilled water and then put in a cuvette so that the absorbance that occurs due to LSPR silver nanoparticles was observed. UV-VIS characterization was carried out using a UV-VIS Shimadzu UV3150 spectrophotometer.

2.4 Silver-alginate nanocomposite stability
The silver-alginate nanocomposite was stored at room temperature, then its stability was measured for 14 weeks with 10 times colloidal dilution and then characterized using a UV-Vis Shimadzu UV3150 spectrophotometer with a range of 300-600 nm.

2.5 TEM analysis
TEM is a technique used to analyze morphology, defects, crystallographic structure, particle size, and even the composition of a sample. The samples used were colloid silver-alginate nanocomposite of A3 and A6 codes. Samples were analyzed using the Transmission Electron Microscope (TEM) JIOL JEM-1400 series.

2.6 Anti-bacterial analysis
The test was carried out by the diffusion method. The sterilized MHA media was poured into each petri dish as much as 15-20 ml and left for a while until it solidified. On MHA solid media, 0.1 ml of bacterial suspension was spread which had been adjusted to the standard of 0.5 Mc Farland (McF) using a sterile spreading rod until the bacterial suspension was evenly distributed across the surface of the media. Colloidal samples were tested using the hole diffusion method. A hole was made in the MHA media that had been treated with bacteria and then poured 50 μL of colloidal nanocomposite samples in various of AgNO_3 concentration. Furthermore, the media was incubated at 37 °C for 24 hours and observed bacterial growth with inhibition zones in each area. The zone of inhibition marked with a clear color is measured using a caliper electric.
3. Result and discussion

3.1 Synthesis of silver-alginate nanocomposites

Silver-alginate nanocomposites were prepared by pre-preparing silver nanoparticles using chemical reduction methods. Furthermore, the silver nanoparticles formed are dispersed into the alginate biopolymer to form silver-alginate nanocomposites. From this process, a colloidal silver-alginate nanocomposite is produced in the form of a brownish yellow colloid as shown in Figure 1. In this study, the chemical reduction was carried out on the silver-alginate nanocomposite synthesis using microwave irradiation so that the distribution of the reaction is faster and spreads so that the reaction time is more efficient. This method uses AgNO₃ as a precursor, alginate as a stabilizer and matrix, and NaOH as an accelerator.

![Figure 1. Colloidal silver-alginate nanocomposite](image)

From the LSPR spectrum in Figure 2, the maximum wavelength absorption occurs between 403-407 nm, indicating that the absorption of the LSPR band cannot be read or observed without the addition of NaOH (0%) in the Nsample1. The more NaOH is added, the intensity of the LSPR band increases significantly. This indicates that the silver nanoparticle concentration is increasing. Whereas in the sample N5 with a concentration NaOH of 0.11 M showed a decrease in wavelength. This is due to the formation of a solid gel which causes inhibition of the reduction of silver nanoparticle ions [10].

![Figure 2. UV-Vis spectra of colloidal silver-alginate nanocomposite with varying concentration of NaOH: N1 (0%), N2 (0.04 M), N3 (0.08 M), N4 (0.11 M), N5 (0.15 M)](image)

In this study, microwave irradiation was used to accelerate the reaction time by heating and distributing it evenly. Based on the UV-Vis spectra of silver nanoparticles in Figure 3, it shows that the LSPR band significantly increases in samples T1 to T7. However, the samples T8 and T9 experienced a decrease. When the irradiation time is increased, the absorbance will increase up to the
maximum absorbance, then after that, the absorbance decreases. This indicates that the longer the microwave irradiation time will increase the absorbance. The increase in absorbance indicates that the concentration of silver nanoparticles formed is increasing, while the decrease in absorbance indicates that the nanoparticles are experiencing agglomeration or the accumulation of particles/substances into one where the colloidal nucleation process of silver nanoparticles has been completed and has reached a stable particle size\[18\]

![Figure 3](image3.png)

Figure 3. UV-Vis spectra of colloidal silver-alginate nanocomposite with time varying of microwave irradiation. T1 (0 minute), T2 (1 minute), T3 (2 minute), T4 (3 minute), T5 (4 minute), T6 (5 minute), T7 (6 minute), T8 (7 minute), T9 (8 minute)

3.2 Effect of AgNO₃ Concentration

The presence of silver nanoparticles in silver-alginate nanocomposites can be characterized with UV-Vis spectrophotometer due to LSPR phenomenon. LSPR band which represented UV-Vis spectra of silver nanoparticles in silver-alginate nanocomposites shown on Figure 4.

![Figure 4](image4.png)

Figure 4. UV-Vis spectra of colloidal silver-alginate nanocomposite with varying concentration of AgNO₃ (% w/w, AgNO₃/Alg) : A1 (0.5%), A2 (1.0%), A3 (1.5%), A4 (2.0%), A5 (2.5%), A6 (3.0%).

Figure 4 shows that absorption band of silver-alginate nanocomposites is in the range of 401-409 nm. This indicates that silver particles in nanosize have produced. The silver nanoparticles are
characterized by UV-Vis related with the LSPR phenomenon. LSPR is the excitation of electrons in the conduction band near the surface of the nanoparticles. Electrons are limited to specific modes of vibration by particle size and shape. Metal nanoparticles have optical absorption spectrum characteristics in the UV-Vis region. Therefore the LSPR phenomenon can be observed by UV-Vis spectroscopy[10].

3.3 Silver-alginate nanocomposite stability

The stability of silver nanoparticles in silver alginate nanocomposites was carried out by measuring the absorbance at various variations of AgNO₃ every two weeks for 14 weeks in room temperature storage. Based on the change in the intensity of the LSPR band from the UV-Vis spectra in Figure 5, it shows that the longer the storage time the absorbance tends to decrease, but there is a significant increase in week 14. At week 0 to week 12 there is no significant change in the number of silver nanoparticles. at 12 weeks of storage. This shows that the storage of silver alginate nanoparticles up to week 12 remains stable and are not aggregated significantly based on LRPR band [10]. For 14 weeks of storage, there was an increase in the intensity of the LSPR absorption band which was probably due to silver nanoparticle aggregation in the alginate matrix. This is because the alginate polymer as a stabilizer has degraded so that the silver particles were aggregated. To ensure the aggregation, it is necessary to confirm with TEM analysis.

3.4 Silver Nanoparticle Morphology

To analyze the shape and size distribution of nanoparticles in nanocomposites material, characterization was carried out using TEM. Figure 6 shows the distribution of particles of silver based on the TEM image for sample (a) namely A3 with 1.5% AgNO₃ and sample (b) namely A6 with 3% AgNO₃.

The concentration of AgNO₃ utilized as a percursor salt for synthesis of silver nanosize affects the particle size distribution as shown in Figure 7. A sample A3 shows the average particle diameter size
is (6.02 ± 1.47) nm, while in A6 the particle size is 10.59 ± 4.39 nm. This shows that at high concentrations (A6) the particle size is greater due to aggregation of silver particles. The higher deviation in A6 indicates that the engraving of particles is less homogeneous than at low concentrations (A3). When connected to the UV-Vis spectra, there is a congruence that the greater the addition of Ag concentration, the more nanoparticles produced are indicated by the increase in absorbance due to the LSPR phenomenon. The size of the silver nanoparticles is related with the maximum wavelength shift. The nanoparticles undergo a red-shift where the maximum wavelength shift is longer and the silver nanoparticles that are formed are bigger [10].

3.5 Antibacterial Activities of Silver-Alginate Nanocomposites

Nanocomposites Antibacterial testing of the biofilm and colloidal silver alginate nanoparticles produced was carried out by calculating the inhibition zone generated from each sample. The samples were tested against bacteria *E. Coli* and *S. aureus*. The results of the antibacterial activity test of silver-alginate nanocomposites can be seen in Figure 8 and Table 1. The greater the concentration of silver nanoparticles in the nanocomposite, tends to the higher the antibacterial activity, which is indicated by the larger the diameter of the inhibition zone.

E. Coli bacteria has a lower inhibition zone, which indicates that it is more resistant to silver nanoparticles than *S. aureus* bacteria. In this case the resistance of gram-negative bacteria, namely E. Coli, can be related to the structure of their cell walls. Gram-negative bacteria have an effective permeability barrier, namely a thin layer of lipopolysaccharide on the outer membrane that can limit the penetration of the silver nanoparticle solution. Meanwhile, gram-positive bacteria only have a peptidoglycan layer which is more accessible for the penetration of silver nanoparticles.
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The antibacterial abilities of silver nanoparticles, among others, damage bacterial cell walls, disrupt cell metabolism and inhibit bacterial cell synthesis. Silver nanoparticles have antibacterial activity due to their large surface area which allows for excellent contact with microorganisms. Silver nanoparticles approach the bacterial cell membrane and penetrate the bacteria. Furthermore, the silver nanoparticles diffuse and attack the bacterial respiratory chain, until the cells eventually die. Besides, the molecular mechanism of antibacterial silver nanoparticles is the interaction between silver ions and sulfide thiol groups on proteins. The cation of H\(^+\) in the sulfide thiol group will be replaced by an Ag\(^+\) ion so that a more stable S-Ag group is produced on the surface of the bacterial cell. This can deactivate proteins, decrease membrane permeability, and ultimately lead to cellular death [19, 20].

4. Conclusion
The silver-alginate nanocomposite was successfully prepared by utilizing chemical reduction techniques supported by microwave irradiation method. Alginate act as a reducing agent, stabilizer, and matrix-forming silver-alginate nanocomposites. NaOH act as an accelerator in the synthesis of silver-alginate nanocomposites. Based on the LSPR absorption band, silver nanoparticles identified form appearance peak between 365.40 – 422.00 nm. The silver nanoparticles were stable along 12 weeks storage based on LSPR absorption band. The greater the silver nanoparticle concentration, the larger the size of the particles and more heterogenous size. Based on TEM images, the particles are spherical, predominantly in the size range of 6-10 nm. The concentration of silver nanoparticles affect antibacterial properties, the greater the concentration of nanoparticles, tends to the higher their antibacterial properties.

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Table 1. Antibacterial activity of colloidal silver-alginate nanocomposite in various concentration of AgNO\(_3\) as precursor

| Sample | Inhibition Zone Diameter (nm) |
|--------|-------------------------------|
|        | E.Coli | S.Aureus |
| A\(_1\) | 15,10  | 17,05    |
| A\(_2\) | 14,32  | 17,24    |
| A\(_3\) | 16,52  | 18,35    |
| A\(_4\) | 15,61  | 17,86    |
| A\(_5\) | 16,94  | 19,80    |
| A\(_6\) | 15,65  | 18,55    |

Sample Inhibition Zone Diameter (nm)

E.Coli   | S.Aureus
---|---
A\(_1\) | 15,10    | 17,05 |
A\(_2\) | 14,32    | 17,24 |
A\(_3\) | 16,52    | 18,35 |
A\(_4\) | 15,61    | 17,86 |
A\(_5\) | 16,94    | 19,80 |
A\(_6\) | 15,65    | 18,55 |
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