Synthesis and characterization of silver nanoparticles as a potential sensor for volatile organosulfides for visual detection of postharvest storage in garlic

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Abstract. Garlic, one of the most popular spices used in manufacturing many food products, is subjected to fungal and bacterial diseases resulting to substantial losses in quantity and quality. Specific detection tools for garlic spoilage will be an asset in relieving these losses during postharvest storage. In this study, stable yellow-colored silver nanoparticle colloidal solution was studied as a potential sensor for volatile organosulfides in garlic. The silver nanoparticles (AgNPs) were synthesized by chemical reduction method where silver nitrate was taken as metal precursor, sodium borohydride as reducing agent, and polyethylene glycol as stabilizing agent. The silver nanoparticles were characterized by UV-Vis Spectroscopy and Dynamic Light Scattering (DLS). The spectral analysis revealed the formation of silver nanoparticles by exhibiting the typical surface plasmon absorption peak at around 395 nm. The DLS results show two populations of AgNPs with particle sizes of 3 nm and 50 nm. The volume ratio of sodium sulfite to be added in the silver nanoparticle and the reaction time was also determined. Synthesized AgNPs could detect sodium sulfite at 0.52 to 50 µM concentration range. This simple method indicated the potential of silver nanoparticles as a powerful detection tool for volatile organosulfides in garlic.

Keywords: silver nanoparticles, chemical reduction, volatile organosulfides.

1. Introduction
Nanotechnology-enabled sensors (nanosensors) are developed to provide new solutions for a wide variety of health, safety, and environmental assessments [1]. Silver nanoparticles are one of the widely used nanoparticles in nanosensors as they have features such as high extinction coefficient, unique optical properties, good stability, low toxicity and good biocompatibility [2,3]. The concept of silver nanoparticles as an analytical and bioanalytical sensor is based on the optical excitation of the surface plasmon resonance (SPR) caused by a collective excitation of the conduction band electrons of the nanoparticle [4]. Mainly, silver nanoparticles are in colloidal suspensions that display brilliant colors due to intense light absorption and scattering [5].

Several physical and chemical methods have been reported when it comes to synthesizing and stabilizing silver nanoparticles. Among these methods, the chemical reduction method is considered a leading method because the processes that lead to obtaining nanostructures involve only the mixing of solutions...
and does not require the use of specialized laboratory equipment. The synthesis of AgNPs by chemical reduction involves the reduction of silver ion of an inorganic soluble silver salt with a reducing agent in the presence of a surfactant as stabilizer. The short time of synthesis and easiness of transfer from the laboratory scale to the industrial scale are advantages of this method [6]. Spectral properties of nanoparticles depend strongly on its size, shape, interparticle spacing and environment so it is important to characterize nanoparticles to examine the different parameters. Some of the characterization techniques used for AgNPs are UV-Vis spectroscopy and Dynamic Light Scattering (DLS). These techniques give information about the optical property and size of silver nanoparticles [7].

As an optical sensor, one of AgNPs applications is its ability to detect chemical vapors like volatile organic compounds (VOCs). Nanotechnology sees this development as an alternative pathway to monitor deterioration that occurs during post-harvest spoilage of fruits and vegetables [8].

Garlic, the second most widely consumed Allium crop after onion, is mostly composed of water (65%), and the bulk of the dry weight is composed of fructose-containing carbohydrates, sulfur compounds, protein, fiber, and free amino acids. It has an annual production of 24 million tons of dry bulbs [9]. Garlic's high-quality still depends mostly on the adequate storage conditions [8]. In the Philippines, postharvest losses of garlic range from 20 to 42%, which is higher compared to other countries [10]. Due to the increase of globalization trends and policies, Philippine postharvest technology faces a big challenge to make sure that fresh produces of garlic are handled well and can compete to the global market [11].

Specific detection tools for garlic spoilage will be assets in the development of postharvest technologies in the Philippines. Researchers from the Philippine Center for Postharvest Development and Mechanization (PhilMech) use Gas Chromatography Mass Spectroscopy (GCMS) to separate healthy garlic from infected ones based on their volatile metabolite profile. However, GCMS is very bulky and expensive. Also, it uses complex calculations and their preparation may have operational problems, which can result in erroneous output [12]. Other technique such as irradiation treatment has also been used to check microbial growth [11]. There is only one irradiation facility in the country, which is operated by the Philippine Nuclear Research Institute (PNRI). However, the facility cannot operate on commercial quantity [13].

This study tends to address the slow development of postharvest technology in the country, particularly for garlic. Silver nanoparticles was synthesized and characterized to be used as a potential colorimetric sensor for volatile organosulfides in garlic.

2. Materials and Methods

2.1 Chemical reagents and equipment

All the reagents used in this study are of analytical grade. The reagents are silver nitrate (AgNO₃, Sigma Aldrich), sodium borohydride (NaBH₄, Sigma Aldrich), polyethylene glycol 400 (PEG, Sigma Aldrich), sodium sulfite (Na₂SO₃, LESO) and ultrapure water from Millipore Elix Advantage 10 without preliminary preparations. The equipment used in this study were PerkinElmer UV-Vis Spectrophotometer, and Malvern Instruments Zetasizer Nano-ZS90. All the glass wares were washed with aqua regia and rinsed with ultrapure water right before every experiment.
2.2 Methodology

2.2.1 Synthesis of Silver nanoparticles (AgNPs)

The silver nanoparticles were synthesized by chemical reduction method using silver nitrate (AgNO$_3$) as the metal precursor, sodium borohydride (NaBH$_4$) as the reducing agent, and polyethylene glycol (PEG) 400 as stabilizing agent. Thirty (30) mL of 0.002 M NaBH$_4$ is poured in an Erlenmeyer flask and placed in an ice bath on a stir plate. A magnetic stir bar is added and the solution is vigorously stirred (600 rpm) and cooled for 20 minutes. Four (4) mL of 0.001 M AgNO$_3$ is then added at approximately 1 drop per second into the NaBH$_4$ solution that is kept on ice. The entire addition process took about 2 minutes after which the stirring was stopped and the stir bar was removed as soon as all the AgNO$_3$ is added. Four (4) drops of PEG 400 was added to 5 mL of synthesized silver nanoparticles. The sample is stored in an amber bottle and placed in an ice bath before characterization tests.

2.2.2 Characterization of synthesized AgNPs

The size and optical property of the synthesized silver nanoparticles were determined by UV-Vis Spectroscopy and Dynamic Light Scattering technique. UV-Vis Spectroscopy was used to verify the presence of silver nanoparticles in the sample and to check its Surface Plasmon Resonance property. Using a PerkinElmer Model UV-Vis Spectrophotometer Lambda 25 program, pure AgNPs and water-diluted sample were pipetted in a plastic cuvette and scanned from 700 nm to 300 nm. Dynamic Light Scattering measurement technique was also used to determine the particle size distribution of the synthesized silver nanoparticles with and without stabilizing agent. The suspensions were subjected to particle size analysis using Malvern Instruments Zetasizer Nano-ZS90. Particle size analyses were done in automatic mode wherein parameters are optimized by the instrument. Average particle size was determined through three (3) measurements at 90° measurement angle using water as dispersing medium.

2.2.3 Response of AgNPs in the presence of Sulfite solution

To corroborate our endeavor of employing AgNPs as a potential sensor for volatile organosulfides, analogous behavior of spectroscopic changes was studied wherein colloidal AgNPs solution was incubated with sodium sulfite solutions.

Reaction time was determined by performing time drive experiments and was done to determine how long the AgNPs must be incubated with sulfite solutions. The sample composed of water-diluted AgNPs with 50 µM sodium sulfite solution was scanned using the time drive program of the PerkinElmer UV-Vis Spectrophotometer. The scanning process was stopped when the absorbance became constant with time.

The synthesized silver nanoparticles were also mixed with different volumes of 50 µM sulfite solutions. In a vial, 500 µL of 50 µM sulfite solution was added to 1500 µL of water-diluted silver nanoparticle solution and stored in a dark cabinet. The sample was then tested using the UV-Vis Spectrophotometer. The procedure was repeated for 1000 µL and 1500 µL of 50 µM sulfite solutions.

Different concentrations of sodium sulfite solutions (50 µM, 20 µM, 10 µM, 5 µM, 1 µM, and 0.5 µM) were also mixed with the colloidal AgNPs solution in 1:1 ratio. Five samples were prepared for each concentration of sodium sulfite. The change in absorbance was then plotted against sodium sulfite concentration. The change in absorbance was computed by subtracting the average absorbance of each batch from the absorbance of the water-diluted AgNPs. Performance characteristics of the AgNPs as colorimetric sensor such as repeatability, reproducibility, and stability were also determined.
3. Results and Discussion

3.1 Characterization of AgNPs

Silver nanoparticles were synthesized by chemical reduction method using silver nitrate as metal precursor and sodium borohydride as reducing agent. PEG 400 was added as a stabilizing agent to inhibit aggregation of reduced silver nanoparticles.

Fig. 1(a) shows the absorption peak of pure AgNPs at 392 nm which slightly shifted to 393 nm on diluting with water. The absorption peak of AgNP solution with stabilizing agent was recorded at 397 nm and shifted to 399 nm after diluting with water as shown in Fig. 1(b). The slight shift of the absorption peak when the silver nanoparticles solution was diluted with water may be due to the presence of water ligands near the metal center, which slightly altered the refractive index of the surrounding medium [8]. Based from previous studies, silver nanoparticles exhibit characteristic Surface Plasmon Resonance (SPR) peak at 380 to 450 nm depending on the size and shape of the nanoparticles [14]. The absorption peaks of the solutions fall within the range which confirms the formation of silver nanoparticles.

The particle size distribution of the synthesized nanoparticles for the three trials is given in Fig. 2. The DLS results show two peaks at 3 nm and 50 nm with a polydispersity index of 0.803 ± 0.002. The results verify that the silver ions were reduced to Ag within the nanoscale dimension.

3.2 Repeatability and Stability test of the Silver nanoparticles

The repeatability of silver nanoparticle synthesis by chemical reduction method was evaluated by preparing silver nanoparticle solution at two different weeks.
The UV-Vis spectra of the synthesized nanosilver solutions are shown in Fig. 3(a) and Fig. 3(b). The percent relative standard deviation (%RSD) was computed to be 2.34%, which was calculated based from the absorption peaks of the spectra. At lower concentrations, the acceptable range is within 10%, although some reports are within 20% [15]. The %RSD of the data indicates that the synthesizing method is repeatable.

The stability of the synthesized silver nanoparticles was also tested by observing the change in absorbance of the samples after a specified period. The %RSD of trials 1, 2 and 3 are shown in Table 1. The %RSD of all the samples are lower than 10% indicating that the AgNPs are stable and can be employed as a sensor system [16].

| Table 1. Stability test of AgNPs synthesized on different weeks |
|---------------------|---------------------|---------------------|
|                      | Absorbance          | %RSD               |
| Trial 1              | 0.7192              | 0.17%              |
| Trial 2              | 0.7032              | 3.26%              |
| Trial 3              | 0.7191              | 6.43%              |

### 3.3 Performance Characteristics of AgNPs as a Colorimetric Sensor for Sulfite

In order to evaluate the distinct property of silver nanoparticles as a visual sensor for sulfite, AgNP solutions were incubated with sulfite solutions and spectroscopic changes were studied. Sodium sulfite was chosen for interaction studies with colloidal AgNPs since sulfite in the medium also acts as a scavenger for oxygen which further confirms that the change in absorbance is only due to the presence of the sulfur in sulfite form which transforms silver ions into stabilized Ag$_2$S nanoparticles and not due to the presence of atmospheric oxygen [8].
Fig. 4 shows the absorbance of the diluted AgNP solution with 50 μM sodium sulfite solution versus time. It can be observed that the absorbance of the sample stabilized after 2400 seconds (40 minutes) at an absorbance of 0.2656. Therefore, the optimized parameter for incubation time is 40 minutes.

Table 2 presents the absorption peak and change in absorbance of silver nanoparticles diluted with different volumes of sodium sulfite. It can be observed that the absorption peak (390 nm) of freshly prepared silver nanoparticles shifted to 395 nm when it was incubated with sodium sulfite. This red-shift is due to the stable silver-sulfur complexes which results from higher stabilization constant between silver and sulfur [17]. The largest change in absorbance was observed when 1500 μL of 50 μM sulfite solution was incubated with silver nanoparticles solution. Therefore, the synthesized AgNPs must be diluted with sulfite solution by 1:1 volume ratio to achieve the highest possible absorbance change.

| Peak (λ) | Absorbance | Δ Absorbance |
|----------|------------|--------------|
| AgNPs    | 390 nm     | 0.7074       | 0            |
| AgNPs with 500 μL sulfite | 395 nm | 0.4115 | 0.2959 |
|          | 395 nm     | 0.3273       | 0.3801       |
| AgNPs with 1500 μL sulfite | 395 nm | 0.2638 | 0.4436 |

To test the potential application of silver nanoparticles as a colorimetric sensor for sulfur for visual detection of postharvest spoilage in garlic, different concentrations (50, 20, 10, 5, 1, and 0.5 μM) of sodium sulfite solutions were incubated with AgNPs solution in 1:1 ratio by volume.
Figure 5. Absorption spectra of AgNPs at varying sulfite concentrations.

Fig. 5 shows the spectral changes of AgNPs in presence of different concentrations of sulfite solution after incubation for 40 minutes. It can be observed that the addition of sulfite solution to colloidal AgNPs solution decreased its absorbance value. This distinctive sensitivity of SPR towards sulfur addition occurred due to the change in nanostructural morphology.

Figure 6. Repeatability test of AgNPs with 50 μM of sodium sulfite

The repeatability of AgNPs as a colorimetric sensor was also validated by evaluating the response of the AgNPs to 50 μM of sodium sulfite. Five AgNP solutions with 50 μM of sodium sulfite were prepared and analyzed by UV-Vis spectroscopy. The change in absorbance of the solution for the five trials is shown in Fig. 6. A relative standard deviation of 2.31% was computed which is indicative of good repeatability. The overlapping error bars for the difference trials show that there may not be significant difference between the trials with regards to their change in absorbance.
Fig. 7 displays the variation in absorbance depression of the AgNP solution with respect to the concentration of sodium sulfite solution at $\lambda_{\text{max}}$ of 395 nm. It is shown that the change in absorbance increases as the sulfite concentration increases exhibiting a linear relationship. The curve obtained a slope of 0.0073 and a coefficient of determination ($r^2$) of 0.9807. The slope shows the sensitivity of the absorbance change to changes in the sulfite concentration. As a potential visual sensor, it is also important to determine the lowest concentration of the sulfite concentration that can be detected in the AgNP solution. The approach to be used in determining the limit of detection (LOD) is based on the standard deviation of the response and the slope of the calibration line given in Eq. 1.

$$LOD = \frac{3 \times \text{Std dev blank}}{\text{slope}} = 0.523 \text{ µM/ÅAbs}$$

A value of 0.52 µM was obtained through the calculations. This means that this is the lowest concentration that AgNP could detect.

4. Conclusion

The study presents the synthesis and characterization of silver nanoparticles and its potential application as sensor for volatile organosulfides in garlic. Silver nanoparticles were synthesized by the chemical reduction method where silver nitrate was taken as the metal precursor, sodium borohydride as the reducing agent, and polyethylene glycol as stabilizing agent. These AgNPs were characterized by UV-Vis Spectroscopy and Dynamic Light Scattering (DLS). The spectral analysis showed that the absorption peak of the silver nanoparticle solution was around 395 nm. The DLS results show two populations of AgNPs with particle sizes of 3 nm and 50 nm. The performance characteristics of stable AgNPs as colorimetric sensor for sulfite were also determined in this study and based from the results, it can be concluded that the synthesized nanoparticles can be employed as a sensor system for sulfite and has a potential application in detecting volatile sulfur compounds in garlic.

5. Acknowledgment

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