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Comparisons of Silver Nanoparticle Synthesis Methods by Microwave and Non-Microwave using Hydrogen Gas as a Reducing Agent

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Abstract

Synthesis and characterization of silver nanoparticles are of current interest. This research project describes a simple and rapid synthesis of silver nanoparticles upon reduction of Silver (I) oxide (Ag_2O) by molecular hydrogen (H_2) at elevated temperature (70 – 100 °C) and a pressure of > 1 atmosphere by heating the aqueous mixture using a common kitchen microwave. This reaction generates naked silver colloids that contain no foreign stabilizers other than metal particles in aqueous media. Its application of an antibacterial effect was monitored by using biological cultures using the Kirby-Bauer Test.

Introduction

Metal nanoparticles are a well sought research topic due to their diverse applications in biotechnology, electronics, and textiles. Silver nanoparticles (AgNPs), specifically, are explored because of their many uses, a few of which are their anti-bacterial, bio-sensing, and water treatment purposes. Silver nanoparticles are also part of windshield coatings, cosmetics, energy capture in solar panels, and cancer therapy (Seku *et al.*). Though there are many purposes for these nanoparticles, synthesis requires time. Additionally, this does not account for other organic compounds that may have been added to the recipe, which indicates that the solution is not completely pure; the particles are not naked. Thus, purging the silver solution with hydrogen gas will act as a reducing agent where hydrogen bonds with the oxygen to create water, suspending the silver in an entirely aqueous solution (Bhatte *et al.*). Radiating through a microwave will also decrease the time of synthesis significantly and can produce pure silver colloid *(*Merga *et al.*)(Reaction 1).

$$Ag_2O\ (s) + H_2\ (g) \rightarrow 2Ag\ (s) + H_2O\ (l) \quad (1)$$

The synthesis of AgNPs could be seen in the color change of the solution. As particles are produced through heating, the solution turns from a clear liquid to a green-brown hue (Figure 1). The more time spent in heat, the population of the silver colloids multiply and the liquid becomes more viscous.

Figure 1: AgNPs produced using Ag_2O and citrate through microwave heating. This describes the color transition of AgNPs as heat time increases.

This color change allows the use of a UV-Visible spectrophotometer as the light in the instrument reflects the color of the solution and determine the wavelength of the color absorbed in nanometers. This wavelength is correlated to the particle size, and the differences in the plots such as, shifts to the left and right and narrowing and broadening of peaks, compared to the reference plot, determines the variations of AgNPs produced.

A frequently used method of AgNP synthesis involving aqueous citrate and boiling the solution using a hotplate was done to create a control or reference point for the different methods used (Yin et al.). Other studies have used different silver precursors; however, silver nitrate ($AgNO_3$) and Silver(I) oxide (Ag_2O) are the most common. This experiment will be focused on Ag_2O, as it will provide a better reference for methods involving naked particles.

Once the common method was established, synthesis with the common solutions through microwave heating was performed to confirm the production of particles. Particle formation indicates that a kitchen microwave is a reliable source of heat. The data for this can then be compared with the reduction reaction of H_2 using a microwave.

Methodology:

This project uses two different methods of synthesis: Method 1 which involves the preheating of the solution and Method 2 which does not preheat the solution. The purpose of using two methods was to indicate which order of heating was more favorable for size uniformity and show the significance of heat in the reaction.

To test for antibacterial properties, the Kirby-Bauer disk diffusion susceptibility test was performed on the synthesized AgNPs for Methods 1 & 2. The test utilizes bacterial colonies which are swabbed and grown on an agar plate (Hudzicki). Sterilized samples from both methods were placed on the cultures and incubated for at least 24 hours before measuring each zone of inhibition. These zones show areas that were minimally affected or not affected by the bacteria. Thus, the larger the zone of inhibition, the more potent the sample was.

In this project, the bacteria used are Gram-positive *Staphylococcus aureus,* Gram-negative *Escherichia coli, Pseudomonas aeruginosa,* and Gram-positive *Bacillus cereus*. This is similar to the bacteria used by Guzmán et al. and their experiment on the antibacterial activity of AgNPs by chemical reduction (Guzmán et al.). Infections due to *S. aureus* are commonly skin related, but can also be found in the nose, groin, and armpit areas. *E. coli* are seen in contaminated food or water, especially raw meats and produce. *P. aeruginosa* affects humans, animals, and plants, and can cause infections in the blood, lungs, and bones. Similar to *E. coli*, *B. cereus* is found in foods, more commonly, in soil vegetation, which causes food-borne illnesses if ingested. These four were selected to demonstrate the versatility of AgNPs for one of its many applications.

Procedures:

1. Production of AgNPs without microwave

In a 250 mL Erlenmeyer flask, 43 mg of Ag_2O was added with 100 mL of deionized water. In a separate container 59.3 mg of trisodium citrate was dissolved in 10 mL of deionized water. The Ag_2O solution was heated with constant stirring from a magnetic stirrer. Once the solution reached boiling temperature, about 5 mL of solution was transferred to a small glass vial. Then, the citrate mixture was pipetted in a slow stream to the boiling Ag_2O solution. 5 mL sample increments were obtained at 20 seconds, 30 seconds, 60 seconds, 120 seconds, 5 minutes, 7

minutes, 15 minutes and 20 minutes. Dilutions were carried for the 5, 10, and 20-minute sample where 1 mL was obtained and diluted to 10 mL. The Cary 5000 UV-Vis-NIR spectrophotometer was utilized to take a water background for the data baselining and all the samples were recorded from 200 nm to 800 nm with a quartz cuvette. The instrumental parameters were the following: single beam mode, full slit height, source changeover at 350.00 nm, detector changeover at 800.00 nm, and scan rate at 600.00 nm s^{-1}.

2. Production of AgNPs with microwave

a. $AgNO_3$ + Citrate

Using a Pyrex test tube (150 mm), 2.9 mg of $AgNO_3$ was added in 6.25 mL of deionized water. To this, a solution of 3.7 g of sodium citrate dissolved in 1.25 mL of deionized water was added and thoroughly mixed. A UV-Vis spectrum was taken for water in order to apply data baselining. Then, about 2 mL of the $AgNO_3$ solution was transferred to a quartz cuvette for the initial data set. After recording data, the contents in the cuvette were poured back in the original test tube. A kitchen microwave with 1800 watts was then used to radiate the unsealed tube for 30 seconds. Working quickly, 2 mL was transferred to the cuvette to obtain the UV-Vis spectrum then poured back into the test tube to be radiated for another 30 seconds. This radiation and collection of data was repeated for 90 seconds.

b. Ag_2O + Citrate

The preparation process was repeated from above, with the exception of using Ag_2O instead of $AgNO_3$, with the same measurements of sodium citrate. Radiation was done for 20, 40, 60, 80, and 140 seconds. In each radiation step, UV-Vis spectra were recorded. Since data starting from 60 seconds is too high of an intensity, the solution needed to be diluted with deionized water. Thus, a cooling step was added after each radiation to slow the reaction. For the data at 60 and 80 seconds, 3 mL was taken from the mother solution and was diluted to 10 mL. For the data at 140 seconds, 1 mL was taken from the mother solution and was diluted to 10 mL. A solution of sodium citrate was added to a plastic test tube with an aqueous solution of silver nitrate ($AgNO_3$) and was heated in a standard kitchen microwave with cooling and shaking intervals every 10 seconds. Sample data was taken at the 20, 30, 60, 80, and 140 second mark. The same procedure was performed with citrate and Ag_2O.

3. Method 1 & Method 2 of AgNPs production with H_2 and microwave

For Method 1, an aqueous solution of Ag_2O (100 mL) was preheated in the microwave until it boiled, which took 1:30 minutes and was purged with H_2 for 3 minutes, then heated again for 20 seconds. The purging and heating process was repeated for a total of 4 minutes (240 seconds) with shaking intervals every 10 seconds. During heating, the gas was contained inside the glass flask through parafilm and a cork. Sample data was taken after each time the solution was saturated with H_2.

Rather than covering with a cork and parafilm, Method 2 used a glass test tube which was fitted in the flask to create additional pressure and contain the gas within the container and does not include a preheating step. An aqueous solution of Ag_2O (100mL) was purged with H_2 for 3 minutes then microwaved for 2 minutes. The purging process was repeated and was heated for only 1 minute. Sample data was taken after every radiation stage.

4. Kirby-Bauer Test

Using the products from Methods 1 & 2, a Kirby-Bauer Test was performed to investigate their antibacterial effects. The bacteria used were Gram-positive *S. aureus,* Gram-negative *E. coli, P. aeruginosa,* and Gram-positive *B. cereus.* For each bacteria culture, a petri dish was prepared with agar and sectioned for swabbing. The bacteria were swabbed in their respective dishes and a sterilized paper was soaked into each of the AgNPs to be placed into a swabbed section of the bacteria. Both AgNPs solutions were filtered through a 0.2 μm filter for sterilization prior. The dishes were then stored in an incubator at 35°C. for 24 hours.

Another test was performed with a more concentration solutions. For each AgNP product, 2 ml of the sample was dried in a rotary evaporator until the solution left in the vial was 1 mL. These solutions were used for a second Kirby-Bauer Test.

Results and Discussion

1. Production of AgNPs without microwave

An absorbance plot was made for the time intervals, seen in Figure 2. Due to the strong absorptive capacity of the 5, 10, and 20-minute samples, there was not enough light for the spectrophotometer to record. Thus, dilutions were made. Considering the peak position at 430 nm, the wavelength of dilutions shifted right, which demonstrates the growth in size of particles. Additionally, the intensity of the peak increased up through the second minute and then began to decrease. This is due to the trend that as the particles grew in size, the distribution is size widened, as seen particularly in the 20-minute diluted sample.

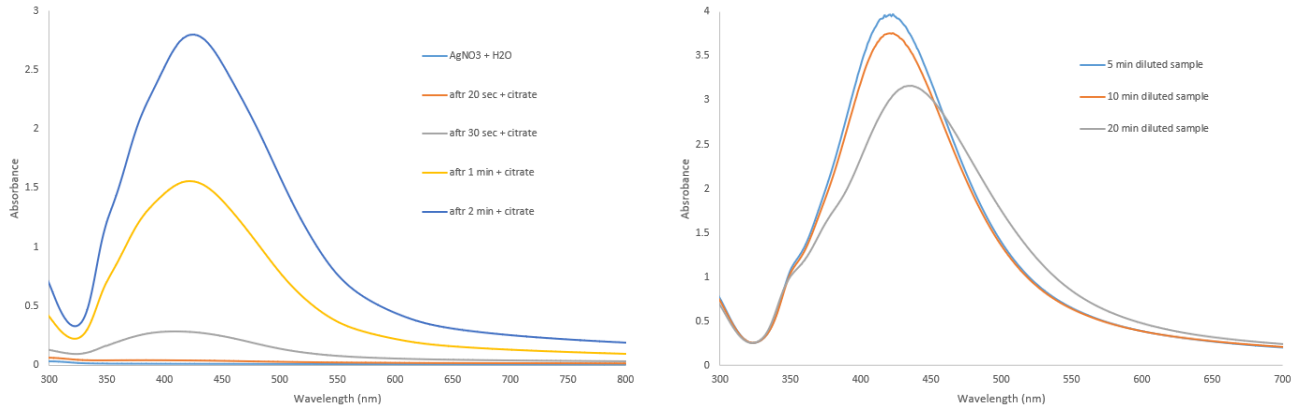

Figure 2: Reference plot for the project and absorbance plots for the synthesis of silver nanoparticles using Ag_2O and trisodium citrate. The plot on the left shows the undiluted samples while the plot on the right shows the diluted samples.

2. Production of AgNPs with microwave

Absorbance plots were again created for the AgNPs produced from using $AgNO_3$ and Ag_2O, both with citrate, found in Figure 3. Since the absorbance value for the 1.5-minute sample in the $AgNO_3$ data was high, it required a dilution.

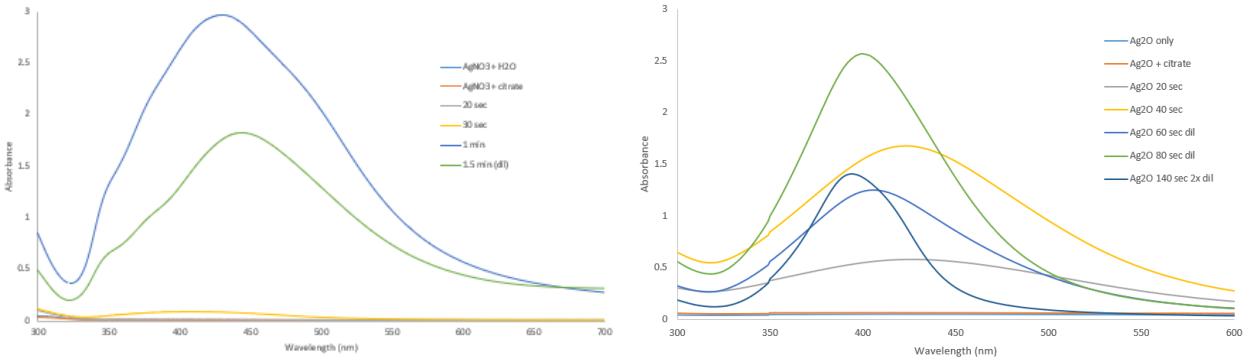

Figure 3: Absorbance plots for the microwave-assisted synthesis of silver nanoparticles using $AgNO_3$ and trisodium citrate (left) and Ag_2O and trisodium citrate (right).

In the $AgNO_3 + H_2O$ plot, a spike is seen at the 30 second mark, which indicates that some nanoparticles have been produced at this time. From this, it can be assumed that as time of radiation increases, intensity increases. The peak wavelength also shifted to the right and the peak appears to broaden slightly, indicating an expected growth in size. In comparison to the reference plot, the significant difference in broadened peaks does not make this a favorable product for specific applications such as biological tags.

For the data using Ag_2O and citrate, the population shifted left, early in the reaction, where large particles were made. Then, as the reaction proceeded, smaller particles were created at a faster rate leading the population to shift towards smaller particles. One thing to note is that the peak became more refined with the duration, which demonstrate the opposite of broadening. Compared to the previous absorbance plot, this product is more favorable for specific applications as it has more size uniformity.

3. Method 1 & Method 2 of AgNPs production with H_2 and microwave

Upon omitting organic support and using H_2 instead, the absorbance plots in Figure 4 demonstrate that the reaction is able to synthesize AgNPs.

In Method 1, a significant broadening is seen which indicates that there are larger nanoparticles in the solution. A reason for this could be attributed to the preheating step prior to saturating the solution with H_2. Heat is a major catalyst for the reaction and by initiating it without H_2 for support, the silver aggregates produced are larger. Another factor is the concept of agitation. As silver aggregates are formed, they could populate in larger groups if undisturbed. Thus, agitating the solution by stirring or shaking vigorously aids in the production of more uniformly sized particles.

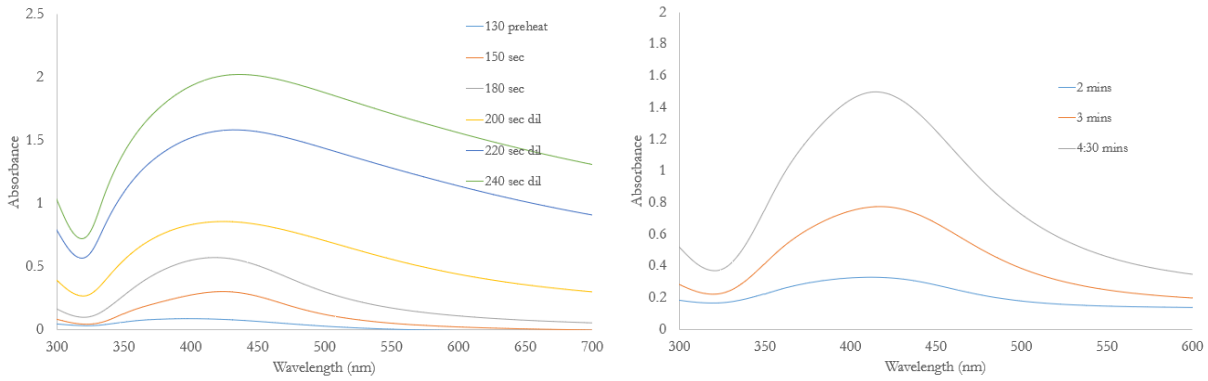

Figure 4: Absorbance plots for Method 1 (left) and Method 2 (right) of AgNPs production with H_2 and microwave.

In Method 2, the graph shows a more favorable product due to its narrower peaks. This could be explained through the saturation of H_2 prior to heating, and a duration which allows the solution to heat more evenly.

4. Kirby-Bauer Test

Figure 5: Kirby-Bauer disk diffusion susceptibility test results from Test 1, which comes from the mother solutions (left) and Test 2, which has the more concentrated samples (right). From top to bottom, the bacteria used were Gram-positive S. aureus, Gram-negative E. coli, P. aeruginosa, and Gram-positive B. cereus.

The antibacterial properties of the products were explored using AgNPs from Methods 1 and 2. Figure 5 shows the photographic results of the test and Table 1 documents the diameter measurements of the zone of inhibition for each bacterium and method.

BACTERIA	**TEST 1**		**TEST 2**	
	Method 1	Method 2	Method 1	Method 2
G(+) *S. AUREUS*	0	10	0	11
G(-) *E. COLI*	0	9	0	13
P. AERUGINOSA	0	9	0	12
G(+) *B. CEREUS*	0	10	0	10

Table 1: Diameter measurements of the zone of inhibition for each bacterium in millimeters.

Compared to the deionized water and AgNPs from Method 1, which exhibits no zone of inhibition, AgNPs from Method 2 demonstrates some antibacterial effects. Additionally, as the concentration increased, the zone of inhibition also increased.

As mentioned earlier, specific studies require specific sizes of AgNPs. Biological cultures such as bacteria tend to be more affected by smaller sized particles. Thus, the AgNPs in Method 1 were not favorable due to their larger size. Despite the lack of results, however, this confirms that particle size is important in the production of AgNPs and it is viable to synthesize them with a microwave.

Conclusion

The synthesis of AgNPs can be done using aqueous $AgNO_3$ and Ag_2O, both with the addition of equimolar citrate solution. However, due to the organic compounds assisted suspension of the nanoparticles and the nitrate that come with $AgNO_3$, the NPs produced with Ag_2O are more ideal to produce non-organic molecules suspensions. To synthesize these ideal NPs, hydrogen gas was used to bond with oxygen molecules from the water to suspend the silver colloid with negative zeta potential in a pure aqueous solution in the form of $Ag(OH^-)_x$. Nanoparticle synthesis was both produced by using $AgNO_3$, and Ag_2O by the common conventional method using citrate as well as hydrogen gas reduction by irradiating the solution using a kitchen microwave that resulted comparable outcomes yet tremendously decreased the duration of the waiting time of synthesis to 1-4 minutes. This method produced AgNPs, however, it also required a balance of heating times and agitation intervals in order to foster size uniformity.

Then, the antibacterial properties were explored through the Kirby-Bauer disk diffusion susceptibility test and provided expected results. The solution with larger particles, which are not ideal for biological testing, did not exhibit any zone of inhibition while the solution with smaller particles was seen to have zone inhibitions for each bacteria culture used. The second test performed demonstrates that the antibacterial properties of the nanoparticles is concentration dependent.

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