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Research Letter

Eco-Friendly Synthesis of Silver Nanoparticles Using Brown Sugar and Their Potential to Inhibit the Growth of *Escherichia Coli*

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Abstract: Silver Nanoparticles (AgNPs) were synthesized in a brown sugar solution at three different temperatures (23 °C, 40 °C and 60 °C) with a concentration of 2.5 mM of $AgNO_3$ and 0.02 $g \cdot mL^{-1}$ of brown sugar. The sample prepared at 60 °C exhibited the highest nanoparticle yield, with an average diameter of 22 nm, as determined by analyses of micrographs. The bactericidal effect of these nanoparticles was evaluated against *Escherichia coli* American Type Culture Collection (ATCC) 25922 using the microdilution method, monitoring bacterial growth via absorbance at 600 nm. After 24 hours, nanoparticles formed at 60 °C demonstrated a significant inhibitory effect on *E. coli* growth when used at volumes of 300 µL and 400 µL in the assay.

***Keywords*:** green synthesis, silver nanoparticles, brown sugar, *Escherichia coli*

1. Introduction

In recent years, green synthesis methods for Silver Nanoparticles (AgNPs) have gained considerable attention due to their simplicity, cost-effectiveness and environmental friendliness. AgNPs are getting special attention in biomedicine, medicine and agricultural science for their antibacterial activity and the fact that their preparation does not require hazardous reagents.[1-3]

Escherichia coli is more than just a laboratory workhorse or harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen.[4] Therefore, it is important to study new alternatives to kill this bacterium. For instance, the growth of *E. coli* was inhibited in the presence of AgNPs on the culture agar plates where the inhibition solely depended upon the AgNPs concentration.[5,6] Also, AgNPs with a size smaller than 10 nm enhanced the antibacterial efficacy against *E. coli*.[7]

Studies have reported the synthesis of Silver Nanoparticles (AgNPs) from $AgNO_3$ in aqueous solution using different types of sugars, such as maltose and sucrose. Among these, sucrose has been shown to promote an increase in nanoparticle size.[8] In contrast, rapid formation of AgNPs was observed when glucose or white sugar was dissolved in NaOH, highlighting the influence of the sugar type and reaction conditions on nanoparticle nucleation and growth.[9,10] Furthermore, AgNPs have been synthesized using artificial sweeteners, white sugar and brown sugar. However, little has been done on the research and application of this kind of nanoparticles obtained using brown sugar.[11] Basically, different organic molecules with functional groups transfer electrons, enhancing the number of reactions of $Ag^+ + e^- = Ag^0$. As a

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result, silver atoms are linked through metallic bonds, leading to the formation of nanoparticles.[12]

Silver Nanoparticles (AgNPs) can be synthesized using only $AgNO_3$ and sugar dissolved in water, representing an eco-friendly and straightforward method of preparation. In this study, AgNPs were synthesized using brown sugar as the reducing agent, with reaction temperature as the variable parameter. Nanoparticle formation was compared at 23 °C, 40 °C, and 60 °C, and the resulting AgNPs were assessed for their efficacy in inhibiting bacterial growth.

2. Materials and methods

Synthesis of AgNPs was carried out using brown sugar dissolved with the $AgNO_3$ using distilled water as a solvent. $AgNO_3$ was acquired from Sigma Aldrich company and brown sugar was purchased from a commercial store.

Stock solution with a molar concentration of 0.05 M of $AgNO_3$ prepared by dissolving 0.1698 g of $AgNO_3$ in 20 mL of distilled water. Meanwhile, 6 g of brown sugar was dissolved in 60 mL of distilled water. Both solutions were stirred at 300 rpm for 5 minutes ($AgNO_3$ solution) and 15 minutes (brown sugar solution). Three flasks were prepared, each containing 2.5 mL of the $AgNO_3$ stock solution and 10 mL of the brown sugar solution, along with 37.5 mL of distilled water, resulting in a final working volume of 50 mL. This corresponded a molar concentration of 2.5 mM of $AgNO_3$ and 0.02 $g \cdot mL^{-1}$ of brown sugar in each flask. Figure 1 presents an illustration of the 3 samples obtained at different temperatures: 23 °C, 40 °C, and 60 °C. The solution was heated on a hot plate until the desired temperature was reached.

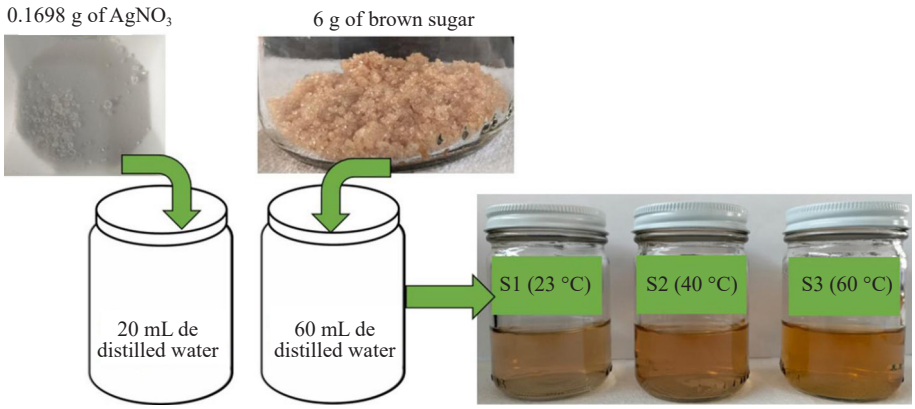

Figure 1. S1, S2 and S3 were prepared to 23 °C, 40 °C and 60 °C, with a molar concentration of 2.5 mM of $AgNO_3$ and 0.02 $g \cdot mL^{-1}$ of brown sugar

The absorbance spectra of the samples were recorded in the wavelength range of 300-800 nm with a Genesys 150 Ultraviolet-Visible (UV-Vis) spectrophotometer. Transmission Electron Microscopy (TEM) JEOLJEM2010 was employed to acquire micrographs of the nanoparticles synthesized at 40 ℃ and 60 °C. Then, the micrographs were prepared using Adobe Photoshop software and with Image J software, the areas in pixels were quantified, and their diameters were subsequently estimated by approximating each particle as a circle. Finally, the size distribution was obtained with the aid of Origin software.

3. Results and discussion

On the first day of the experiment, the value of the maximum absorbance around 400 nm was very low, suggesting little nanoparticle formation. In this sense, as previously mentioned in the introduction, adjusting the pH with NaOH helps accelerate the formation of AgNPs.[9,10]

Figure 2a shows the UV-Vis absorption spectra of the samples synthesized at different temperatures, recorded on the seventh day after preparation. The specific mixing proportions are detailed in the Materials and Methods section. All samples displayed an absorption peak at approximately 438 nm, indicating the formation of quasi-spherical AgNPs.[12]

The absorption peak intensity increased with temperature, which, according to the Lambert-Beer law, suggests a higher nanoparticle concentration in sample S3 synthesized at 60 °C. Despite this, in the first antibacterial assay, the amounts of samples S1, S2, and S3 were insufficient to fully inhibit bacterial growth. To address this limitation, the second antibacterial test employed two higher concentrations of sample S3, as described later.

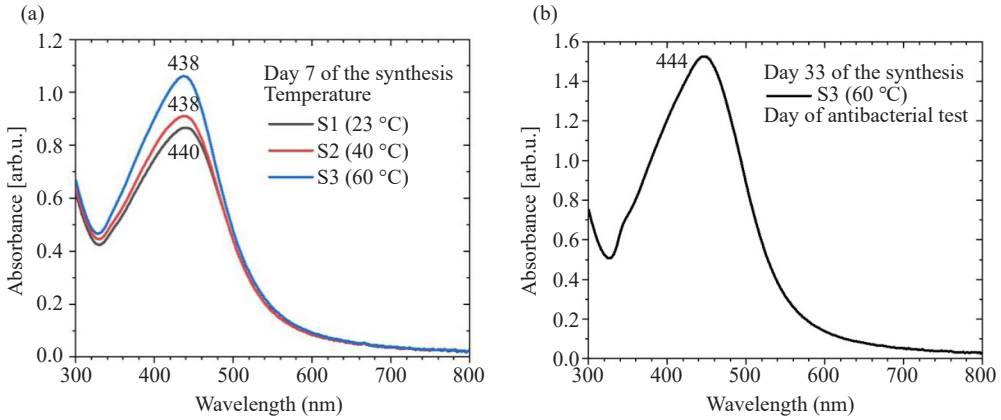

Figure 2. UV-Vis absorbance of AgNPs synthesized using a different temperature (a), UV-Vis absorbance of AgNPs synthesized at 60 °C (b)

Figure 2b shows the absorbance of the sample S3 over a period of 33 days after its preparation and corresponds to the solution used on the first day of the second antibacterial assay against *E. coli.* In this case, the absorption peak is further intensified, exhibiting a shift to approximately 444 nm and a broader profile. This behavior can be attributed to the combined effects of increased concentration, a wider particle size distribution and nanoparticle agglomeration.[12]

Figure 3a shows the size distribution of AgNPs corresponding to sample S3. In this analysis, a total of 344 nanoparticles were included in the size distribution, resulting in an average size of 22 nm. Furthermore, most nanoparticles are smaller than 30 nm, and this size can influence bacterial damage.[13,14] Alongside the size distribution are three of several micrographs at different magnifications, where it can be observed that the AgNPs have a quasi-spherical shape.

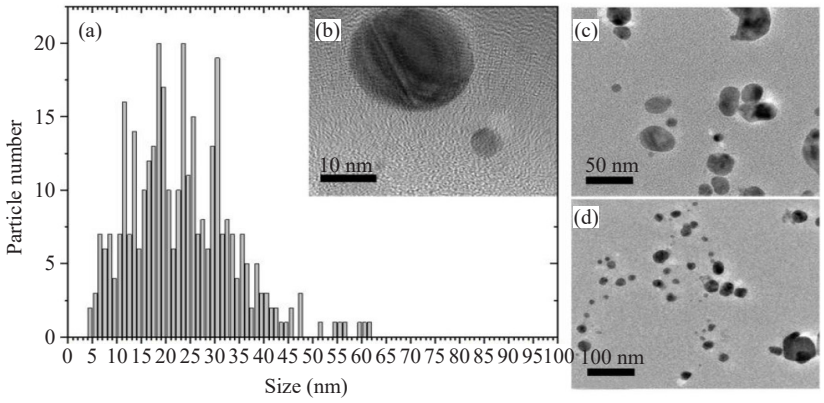

Figure 3. Size distribution (a), and micrographs of AgNPs corresponding to sample S3 obtained at 60 °C (b), (c), (d)

In addition, Figure 4 presents the size distribution of AgNPs corresponding to sample S2, based on the analysis of 255 nanoparticles, with an average diameter of 21.9 nm. The distribution is comparable to that of sample S3, indicating similar morphological characteristics. Consequently, the observed increase in the absorption peak can be attributed to a

higher nanoparticle concentration (see Figure 2a).

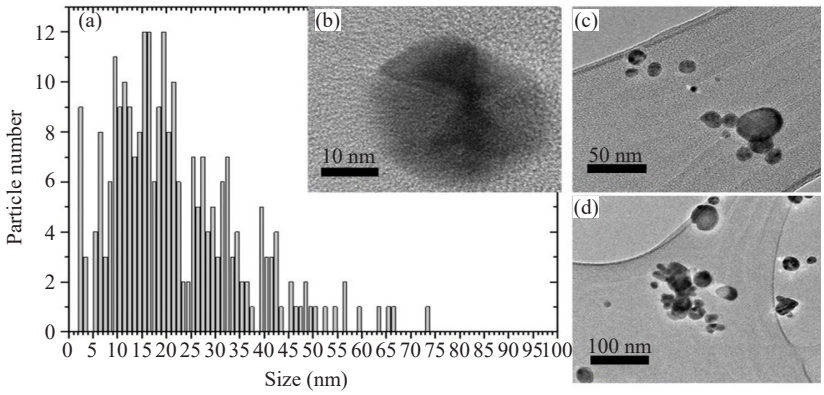

Figure 4. Size distribution (a), and micrographs of AgNPs corresponding to sample S2 obtained at 40 °C (b), (c), (d)

For the effect of AgNPs against *E. coli* ATCC, 25922 of the Kawik-Stik™ brand was analyzed using the micro dilution method, meaning that the antibacterial effect was detected with the unaided eye.[15] *E. coli* was grown overnight in Luria-Bertani medium (LB) at 37 °C and adjusted to 10^8 CFU mL^{-1}. In the first antibacterial test, six tubes were prepared, each containing 10 mL of LB medium. Tube 1 contained only LB, representing the positive control. Tube 2-6 contained 100 µL of the *E. coli* at a concentration of 10^8 CFU mL^{-1}. In tube 3, 100 µL of brown sugar solution was added. Instead of the brown sugar solution, tubes 4-6 received 100 µL of samples S1, S2, and S3, corresponding to AgNPs obtained at 23 °C, 40 °C, and 60 °C, respectively. These dilutions were prepared in triplicate to ensure reproducibility. All tubes were incubated at 37 °C with shaking (180 rpm) and measure Optical Density $(OD)_{600}$ of 2 mL aliquots from each tube was measured using a spectrophotometer (blanked with LB medium) to quantify bacterial growth over time. Table 1 shows the contents in each tube.

Table 1. Contents of the tubes to analysis bactericidal effect firstly

Tube	LB (mL)	*E. Coli* (µL)	Sugar (µL)	Sample (µL)
1	10	-	-	-
2	10	100	-	-
3	10	100	100	-
4	10	100	-	S1 (100)
5	10	100	-	S2 (100)
6	10	100	-	S3 (100)

Figure 5a shows a photograph of the test tubes taken 8 h after the dilutions. Tube 1, which solely contains Luria Bertani broth, presents a translucent yellow color to the unaided eye; it has a similar appearance to the tubes 4, 5 and 6, which additionally contain 100 µL of *E. coli* and 100 µL of AgNPs obtained at 23 °C (S1), 40 °C (S2) and 60 °C (S3), respectively. At this time, all tubes with AgNPs inhibited the growth of the *E. coli*. However, after 24 hrs, these dilutions exhibited an opaque color, showing bacterial growth, being more evident in tubes 4 and 5, as can be observed in Figure 5b. Hence, the amount of the sample with AgNPs obtained at 60 °C (S3) was used in a second assay to study its effect

against *E. coli.*

Additionally, to further assess this effect, absorbance measurements at 600 nm were performed, as this wavelength does not adversely affect the bacterial culture.[7] Figure 5c shows the corresponding absorbance as a function of time (in hours). These results show a certain correlation with the visual interpretation, since at 8 h in tubes 4, 5, and 6, the absorbance is very low, but this behavior changes at 24 h due to an increase in absorbance, being more notable in tubes 4 and 5 with AgNPs obtained at 23 °C (S1) and 40 °C (S2).

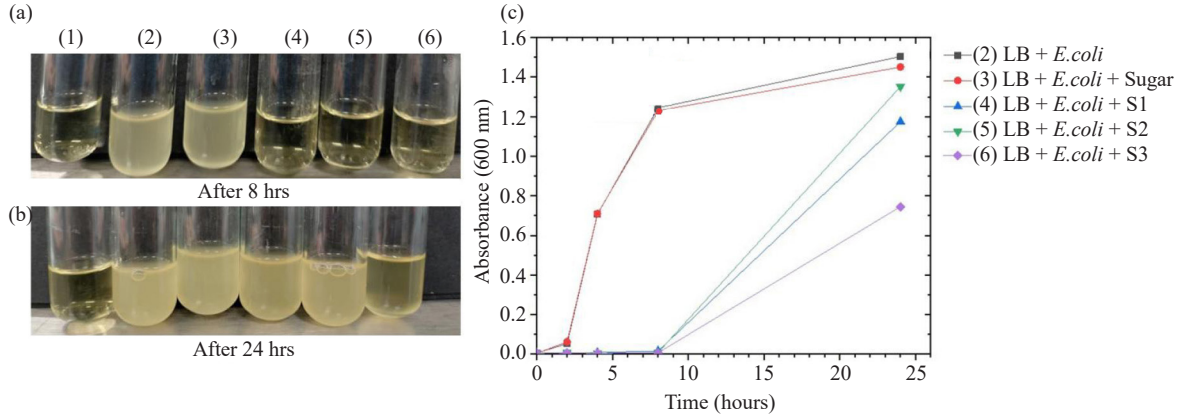

Figure 5. (a) and (b) tube (1), positive control: LB, tube (2) negative control: LB medium and 100 µL *E. coli* strain; tube (3) LB medium, 100 µL *E. coli* and 100 µL brown sugar solution; tube (4), (5) and (6) LB medium, 100 µL of *E. coli* strain, and 100 µL of the samples S1, S2 and S3, respectively. (c) Absorbance measured at 600 nm with time in hours

With respect to the second antibacterial test, Figure 6a presents a photograph of the test tubes taken 24 h after the dilutions. Table 2 shows the contents of each tube. Tube 1, which solely contains Luria Bertani broth, presents a translucent yellow color to the unaided eye; it has a similar appearance to the tubes 5 and 6, which additionally contain 100 µL of *E. coli*, 300 µL and 400 µL of AgNPs obtained at 60 °C (S3). Therefore, these amounts of AgNPs inhibited the growth of the *E. coli*. On the other hand, the tubes 2, 3 and 4 exhibit an opaque color evidencing bacterial growth. his implies that in tube 4, the 100 µL of added AgNPs was not enough to cause damage to *E. coli*, giving similar results to those of the first assay. This means that the doses of AgNPs used in tubes 5 and 6 in dilution had a good bactericidal effect.

Table 2. Contents of the tubes to analysis bactericidal effect secondly

Tube	LB (mL)	*E. Coli* (µL)	Sugar (µL)	S3 (µL)
1	10	-	-	-
2	10	100	-	-
3	10	100	100	-
4	10	100	-	100
5	10	100	-	300
6	10	100	-	400

Figure 6b shows the corresponding absorbance as a function of time (in hours). These results also corroborate visual observations, as the absorbances in tubes 2 and 3 increases over time, indicating an increase in bacterial concentration. However, in tube 4, which contains AgNPs, the absorbance recorded up to 8 h presents an effect in inhibit

the growth of *E. Coli*, but this behavior changes at 24 h. Meanwhile, in tubes 5 and 6 the absorbance values over time remain very low, which means that there is no visible bacterial growth, indicating that the amount of AgNPs used was effective.

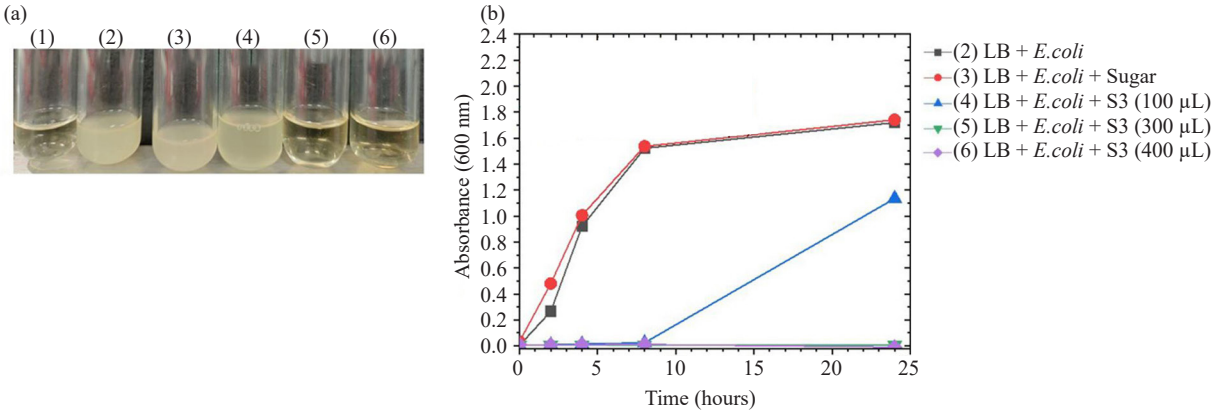

Figure 6. (a) Tube (1), positive control: LB, tube (2) negative control: LB medium and 100 µL *E. coli* strain; tube (3) LB medium, 100 µL *E. coli* and 100 µL brown sugar solution; tube (4), (5) and (6) LB medium, 100 µL of *E. coli* strain, and 100 µL, 300 µL and 400 µL of AgNPs. (b) Absorbance measured at 600 nm with time in hours

4. Conclusion

A simple, eco-friendly method was developed to synthesize silver nanoparticles using brown sugar as the reducing agent. By varying the reaction temperature (23 °C, 40 °C, and 60 °C), it was found that 60 °C yielded the highest nanoparticle concentration, with an average diameter of 22 nm. AgNPs prepared at 60 °C exhibited antibacterial activity against *E. coli* ATCC 25922, achieving complete inhibition at dosages of 300 µL and 400 µL in a 10 mL LB broth dilution. The method for preparing AgNPs in this study was simple and cost-effective, requiring only $AgNO_3$, brown sugar, and distilled water as reagents. The preparation of the brown sugar solution did not require a filtration step, and the commercial product is readily available. Moreover, the synthesized AgNPs remained stable at room temperature for several days, and even small quantities were sufficient to inhibit the growth of *Escherichia coli* ATCC 25922. Future work will explore the efficacy of these AgNPs against antibiotic resistant *E. coli* strains and other clinically relevant pathogens.

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Conflict of interest

The authors declare no competing financial interest.

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