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# Effects of Silver Nanoparticles on Growth of *E. coli*: A Study Examining Various Antibiotic Factors

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## ABSTRACT

Nanomedicine, an emerging and revolutionary scientific field, warrants greater scientific investigation on the antibacterial applications of AgNPs; hence, this research serves to assess AgNPs' antibacterial effectiveness on the growth of *E. coli* while establishing the antibiotic's optimal conditions for drug delivery. The hypothesis expects that optimal drug delivery of AgNPs will occur at higher concentrations of AgNPs and neutral pHs. To demonstrate this, 1:10 serial AgNP dilutions were placed in *E. coli* cultures and were treated with the varying environmental factors of concentration and pHs. The results supported our hypotheses with the exception of our pH hypothesis, for the data showed that having a basic pH increased the efficiency of the nanoparticles for all concentrations, except for the 500 µg/L concentration. Nevertheless, higher concentrations of nanoparticles have the highest and fastest increases in their zones of inhibitions. The concentration and pH experiments yielded statistical significance. With nanomedicine's ability to diagnose, detect, and deliver drugs, its research applications are endless, proving it has a significant impact in the fields of cancer drug delivery, *E. coli* toxicity, and ectopic pregnancies, and ultimately, having the potential to save over two million lives every year.

## Introduction

Nanotechnology has recently been recognized as a medical field that may revolutionize the way scientists diagnose and treat human injuries and diseases. It functions on the basis of nanoparticles, a collection of atom-sized particles that can enhance the strength of a chemical reaction and the electrical conductivity of a substance and can "mimic naturally occurring biological processes or structures" (Galligan, 2018). Silver ions' antimicrobial properties have been applied in hand washes, bandages, household products, healthcare medicines, and drug delivery through "liposomes, dendrimers, polymeric, and carbon-based nanocarriers" (Bharathala & Sharma, 2019).

Due to their large surface area to volume ratio, nanoparticles can change the chemical, physical, and biological properties of other substances. "AgNPs have optical properties which make them strongly interact with specific wavelengths of light" (Zhang et al., 2016).

UV-Visible Spectroscopy, a method of measuring the properties of AgNPs, helps to provide information on the synthesis and stability of the silver nanoparticles. Additionally, X-Ray Diffraction can help determine the molecular and crystal structures of the particle while also measuring the amount of resolution, salinity, crystallinity, purity, and particle size (Zhang et al., 2016).

Although nanoparticles may be a reliable treatment source, most of its characteristics are unknown, such as effectiveness in varying pH or solute concentration. pH stands for the potential of hydrogen and measures how acidic or basic a solution is by identifying how many hydrogen atoms/hydroxyl ions are present in the solution. The pH scale runs from 0 to 14, and a neutral solution has a pH of 7. Any quantity below 7 counts as an acid, and any level above 7 counts as a base. Acidic solutions can conduct electricity because they have more hydrogen atoms compared to basic solutions and can react with active metals to produce hydrogen gas (LibreTexts Chemistry, 2021). Silver nanoparticles have a neutral pH of 7. "pH" is measured using pH strips which contain indicators, or chemicals that change color

depending on how acidic or basic a solution is. A scientist must match the color of the pH strip to its corresponding color on the key in order to identify the pH level of the solution (LibreTexts Chemistry, 2021).

"*E. coli* is a rod-shaped bacterium of the Enterobacteriaceae family" that helps animals digest food (Cleveland Clinic, 2020). Infections stem from the toxin, Shiga, affecting those with weak immune systems; these strains are called Shiga-toxin-producing *E. coli*, or STEC (Cleveland Clinic, 2020). Shiga travels down the intestine track and damages the lining of the small intestine, which is responsible for digesting food and absorbing nutrients. Although most strains, a genetic variant or subtype of a microorganism, are harmless to our bodies and actually help keep our intestinal pathway healthy, some cause detrimental symptoms, such as fatigue, nausea, diarrhea, stomach pain, low fever, and kidney failure. Most symptoms can arise after one day of exposure to ten days of exposure, and one can usually expect to develop symptoms of a STEC infection within three to five days after drinking or eating contaminated foods (Cleveland Clinic, 2020). "*E. coli* outbreaks have been linked to contaminated municipal water supplies." (Mayo Clinic, 2022). *E. coli* is extremely contagious, especially when proper hygiene isn't maintained (Mayo Clinic, 2022).

Children under the age of five who develop STEC have a condition called hemolytic uremic syndrome (HUS) where the "toxins in the intestines cause diarrhea and travel into the bloodstream, destroying red blood cells and the kidneys" (Cleveland Clinic, 2020). Stomach acid provides some protection against *E. coli*; however, if one takes medication to reduce stomach acid pH to aid in the digestion of various proteins such as lactose, and kill bacteria, then they put themselves at risk of developing HUS, depending on age, and other *E. coli* infections, including STEC ones (Mayo Clinic, 2022).

Antibiotic resistance can be defined as "when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them, [causing] the germs to continue to grow. Data has shown that *E. coli's* exposure to silver colloidal nanoparticles decreases antibiotic resistance. "The combined results suggested that AgNPs may damage the structure of bacterial cell membrane and depress the activity of some membranous enzymes, which cause *E. coli* bacteria to die eventually" (Li et al., 2010). Furthermore, *E. coli* were shown to be substantially lessened by AgNPs, and the antibacterial activity of AgNPs did not falter with pH (Kim et al., 2011).

"Meanwhile, AgNPs resulted in the leakage of reducing sugars and proteins and induced the respiratory chain dehydrogenases into an inactive state, suggesting that AgNPs were able to destroy the permeability of the bacterial membranes. When the cells of *E. coli* were exposed to 50 μg/mL AgNPs, many pits and gaps were observed in bacterial cells by transmission electron microscopy and scanning electron microscopy, and the cell membrane was fragmentary, indicating the bacterial cells were damaged severely. After being exposed to 10 μg/ml AgNPs, the membrane vesicles were dissolved and dispersed, and their membrane components became disorganized and scattered from their original ordered and close arrangement based on TEM observation." (Li et al., 2011). Silver nanoparticles have a direct effect on the cell wall and plasma membrane due to the damage structurally as well as the destruction of the permeability of the membrane, which affected what materials could and couldn't pass through.

This research will study the possibilities of treating *E. coli* strains with AgNPs in various conditions: differing AgNP concentration and pH, seeing as HUS affects the pH of the intestines (Cleveland Clinic, 2020). This research will help the community gain an understanding of nanotechnology's applications, the function of pH and concentration in bacterial growth, and the role of *E. coli* in our intestinal pathways. This project is worth studying because it recognizes new applications of treatment options in the medical field and tests the abilities of nanoparticles by exposing them to various conditions.

The question at hand is "How efficient is the treatment of silver colloidal nanoparticles on *E. coli*?". The null hypothesis is that there will be no difference in the performance of the silver nanoparticles regardless of the conditions applied. The alternative hypothesis is that there will be a difference in the performance of the silver nanoparticles in the conditions applied, with respect to AgNP concentration and pH.

## Methods and Materials

Three separate experiments were conducted, and each had its own procedures and materials. The experimental units in each study remained constant: *E. coli* growing on agar plates. However, by changing various properties of the nanoparticles, the response of the growth of *E. coli* was measured.

### Effects of Nanoparticles' Solute Concentration on *E. coli* Growth

Our solute concentration procedure was adopted and adapted from *Science Buddies*. All materials and the workspace were sterilized with 70% isopropyl alcohol to ensure no bacteria contaminated the results of the experiment; rubbing alcohol sterilizes microorganisms and will prevent anything that's not *E. coli* from growing on the Agar plates. Five baby food jars were labeled with a permanent marker: #1-5. Each jar represented a different solute concentration, where the solute is the silver nanoparticles and the solvent is distilled water.

22.5 mL of distilled water was poured into jars #2-5 using the 25 mL graduated cylinder. 25 mL of colloidal silver was poured into jar #1 using the 25 mL graduated cylinder, so Jar #1 had a concentration of 500,000 µg/L (undiluted) concentration of silver. 2.5 mL of the colloidal silver solution was transferred from jar #1 into jar #2 using the 10 mL graduated cylinder, so Jar #2 had a concentration of 50,000 µg/L concentration of colloidal silver. 2.5 mL of the colloidal silver solution was transferred from jar #2 into jar #3 using the 10 mL graduated cylinder, so Jar #3 has a concentration of 5,000 µg/L concentration of colloidal silver. 2.5 mL of the colloidal silver solution from jar #3 was transferred into jar #4 using the 10 mL graduated cylinder, so Jar #4 has a concentration of 500 µg/L concentration of colloidal silver. 2.5 mL of the colloidal silver solution from jar #4 was transferred into jar #5 using the 10 mL graduated cylinder, so Jar #5 has a concentration of 50 µg/L concentration of colloidal silver. Five different concentrations of nanoparticles have been made of which each has a total volume of 22.5 mL. The formula $C_1V_1=C_2V_2$ further exemplifies how, when performing dilutions, the starting concentration multiplied by the starting volume is equal to the final concentration multiplied by the final volume (Bradburn 2022).

Three sterile plates were laid in front of each jar, and 7.5 mL of each concentration was poured into its corresponding jar. Three plates had 7.5 mL of 500,000 µg/L; another three plates had 7.5 mL of 50,000 µg/L; and so on. Three circles were drawn on the base of a coffee filter and cut out. Each circle was placed into one plate of the solution. For example, the three plates with 7.5 mL of 500,000 µg/L will each have one circle in them; the three plates with 7.5 mL of 50,000 µg/L will each have one circle in them; the three plates with 7.5 mL of 5,000 µg/L will each have one circle in them; and so on. A total of fifteen circles were cut out in which each circle had its own plate to soak in. The filter circles were soaked in the solution for 1 minute until used in a later step. One agar plate was placed in front of every jar (a total of five agar plates). Using a permanent marker, the agar plates were labeled #1-5 on the back of the plate to correspond with a food jar number. For example, agar plate #1 will have a concentration of 50,000 µg/L, and agar plate #5 will have a concentration of 50 µg/L.

After reconstituting the *E. coli* culture with distilled water, the bacteria was spread on the agar plates by streaking a vertical line down the center of the plate and continuously rotating the plate while spreading. The surface of the agar was not pierced during this process. This step was completed for every agar plate. The lid of agar plate #1 was opened and using sterilized tweezers, a soaked filter paper circle was picked up from a plate that has 7.5 mL of 500,000 µg/L and was placed it in agar plate #1. This step was repeated for the other two circles in the remaining two plates of 500,000 µg/L. The configuration of the circles should be similar to that of a triangle, with each circle evenly spaced out from one another (about 3 cm). These steps were repeated for the four remaining concentrations, starting with the least concentrated solution to the most concentrated solution to help minimize error in spreading the colloidal silver solutions.

The agar plates were secured with a few pieces of tape and left in a sterile, undisturbed area where there was an absence of sunlight. The following information was recorded every day for one week to check on the progress of

the growth of *E. coli*: shape, elevation, margin, surface, opacity, zone of inhibitions, and pigmentation of the bacterial colony. A zone of inhibition around a paper circle suggests that the specific concentration of nanoparticles has anti-bacterial effects (Science Buddies, 2021).

## Effects of Nanoparticles' pH on E. coli Growth

Our procedure for this experiment is original, as we found that in our background research this has not been previously studied. The same concentrations as made in experiment #1 were replicated, and three plates containing 7.5 mL of every concentration were made. One plate of each concentration was treated with 10 mL of lemon juice to make the solution acidic. Another five plates were treated with 10 mL of dish soap to make the solution basic. The remaining five plates were not treated with any extra solutions (control). The same procedure was repeated in experiment #1 for the process of soaking the filtered circles and spreading the *E. coli* on the plates. After the plates had been thoroughly mixed with their substance, the filtered circles were placed in the plates in the same fashion as experiment #1.

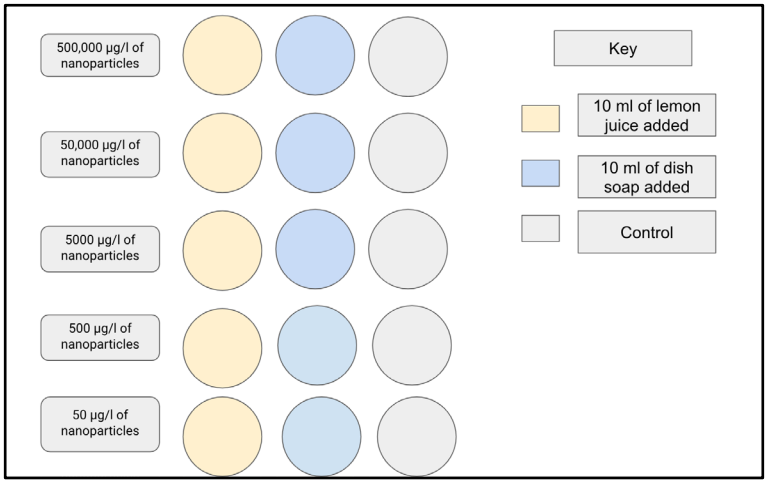


**Figure 1**. Layout of Experiment #3

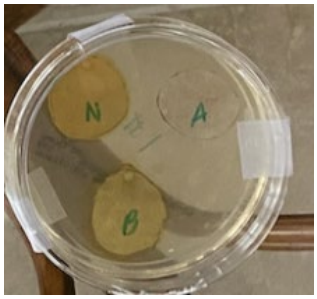


**Figure 2.** Labeling Diagram of Agar Plate #1 in the pH experiment.

After allowing the circles to soak for1 minute, they were moved to their respective agar plate; the following labels were placed above each soaked filtered circle to track the pH: A for acid, B for base, and N for neutral as shown in the diagram to the left. The same information recorded in the concentration experiment was recorded in the pH experiment but based on the pH of the filtered circles.

## Results

After conducting a series of tests, the following results were obtained.

### Phases of Data

1. The concentration experiment studied the effect of the concentration of silver colloidal solution on the growth of the average zone of inhibition over the course of the observed four days.
2. The pH experiment studied the effect of pH on different concentrations of silver colloidal solution and how this affects the growth of the zone of inhibition over the course of the observed four days.
3. Comparing and Contrasting: Because concentrations #1 (500,000 µg/L) and #2 (50,000 µg/L) delivered the highest efficacy in all conditions of pH, and concentration, phase 4 deals with the comparison of the two to identity which may yield optimal results in clinical settings.

Figure 3 summarizes the growth of the average zone of inhibition in centimeters over the observed four-day period in different concentrations of the silver colloidal solution.

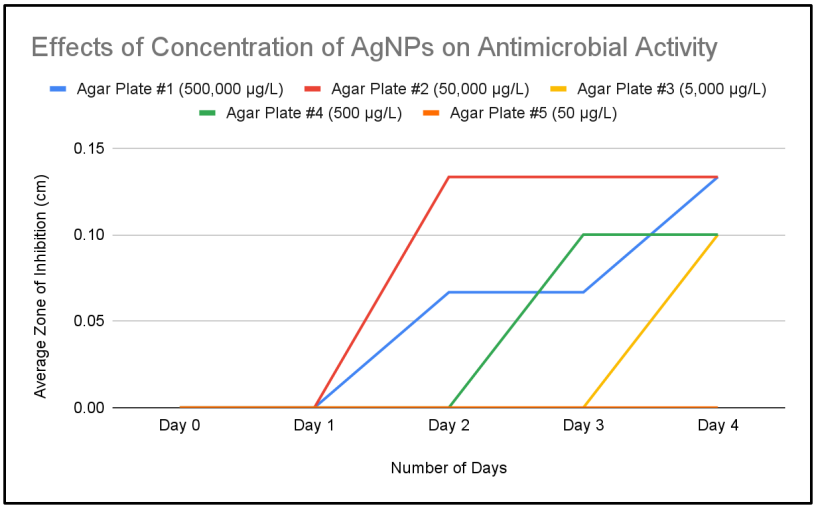


**Figure 3**. Effect of Concentration of Average Zone of Inhibition

While Figure 3 visually illustrates the effect of concentration of AgNPs on the growth of the zone of inhibitions, Table 1 gives the qualitative characteristics of the growth of *E.* coli, proving when and where on the agar plate, antibacterial activity was present. Table 2 demonstrates experiment 1's statistical significance using an Anova test, validating the results. An ANOVA Test for Mean Zone of Inhibition was run due to the presence of one categorical independent variable and one quantitative dependent variable.

**Table 1**. Growth of the *E. coli* colony

| # of Days | Agar Plate #1 (500,000 µg/L) | Agar Plate #2 (50,000 µg/L) | Agar Plate #3 (5,000 µg/L) | Agar Plate #4 (500 µg/L) | Agar Plate #5 (50 µg/L) |
|---|---|---|---|---|---|
| Day 0 | no growth | no growth | no growth | no growth | no growth |
| Day 1 | no growth | no growth | no growth | no growth | small patch of bacteria forming around filter circles |
| Day 2 | no growth | no growth | no growth | no growth | small patch of bacteria continues to form around filter circles |
| Day 3 | rhizoid shape, bumpy surface, translucent, whitish-yellow color | no growth | circular shape, raised, opaque, whitish-yellowish color, growing on edge of plate | small bacteria colonies forming around filter circle | circular & filamentous shape, flat surface, opaque, pale-yellow color |
| Day 4 | irregular shape, no elevation, smooth surface, translucent, white ovals forming on filter circles | circular shape, flat elevation, white color, smooth surface | circular shape, raised, opaque, whitish-yellowish color, growing on edge of plate | circular bacteria, no elevation, white color, translucent | bacteria has grown larger, same physical properties as before |

**Table 2**. Statistical Analysis Anova Single Factor

| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
|---|---|---|---|---|---|---|
| Column 1 | 3 | 6 | 2 | 1 | | |
| Column 2 | 3 | 0.44 | 0.1466666667 | 0.008533333333 | | |
| Column 3 | 3 | 0.4 | 0.1333333333 | 0.003333333333 | | |
| Column 4 | 3 | 0 | 0 | 0 | | |
| Column 5 | 3 | 0.34 | 0.1133333333 | 0.006533333333 | | |
| Column 6 | 3 | 0 | 0 | 0 | | |
| **ANOVA** | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 9.292377778 | 5 | 1.858475556 | 10.94938466 | **0.0003853148126** | 3.105875236 |
| Within Groups | 2.0368 | 12 | 0.1697333333 | | | |
| Total | 11.32917778 | 17 | | | | |

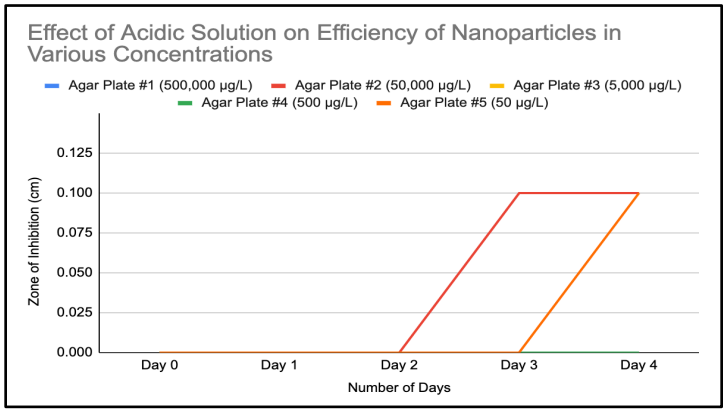


**Figure 4**. Effect of Acidic pH of AgNPs on Growth of the Zone of Inhibition

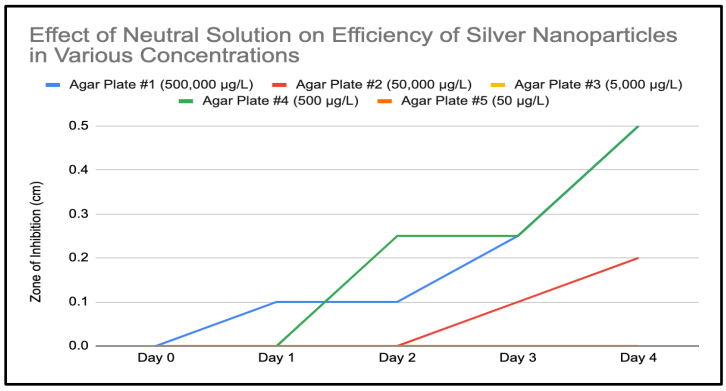


**Figure 5**. Effect of Neutral pH of AgNPs on Growth of the Zone of Inhibition

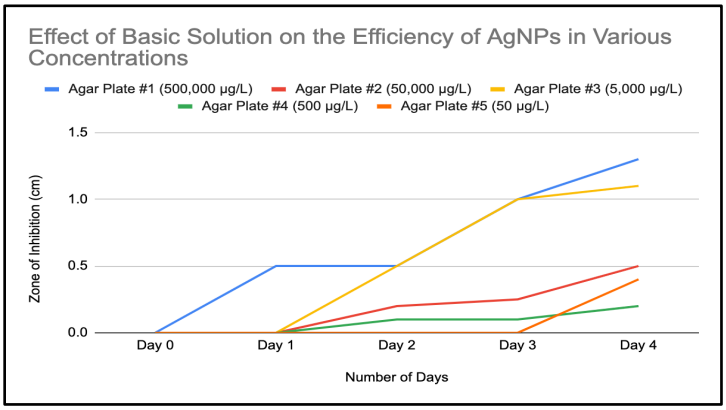


**Figure 6.** Effect of Basic pH of AgNPs on Growth of the Zone of Inhibition

Figures 4 through 6 provide contrasting visual representations of the effect pH has on the growth of zones of inhibitions in different concentrations as well as information as to which concentrations were able to maintain antibacterial activity under certain controlled conditions of pH of the AgNPs. While Figures 4-6 provide visual representations of the pH experiment's results, Table 3 gives the qualitative characteristics of the growth of *E.* coli, proving when and where on the agar plate antibacterial activity was present.

**Table 3**. Growth of *E. coli* Colonies in Presence of Varying pH Solutions

| Number of Days | Agar Plate #1 (500,000 µg/L) | Agar Plate #2 (50,000 µg/L) | Agar Plate #3 (5,000 µg/L) | Agar Plate #4 (500 µg/L) | Agar Plate #5 (50 µg/L) |
|---|---|---|---|---|---|
| Day 0 | no *E. coli* growth | no *E. coli* growth | no *E. coli* growth | no *E. coli* growth | no *E. coli* growth |
| Day 1 | no *E. coli* growth | no *E. coli* growth | no *E. coli* growth | no *E. coli* growth | no *E. coli* growth |
| Day 2 | showing some signs of *E. coli* growth under acidic and basic filter circles | no growth, cotton swab marks somewhat visible | no growth, cotton swab marks somewhat visible | no growth, cotton swab marks somewhat visible | no growth, cotton swab marks somewhat visible |
| Day 3 | no *E. coli* growth yet, showing signs, cotton swab marks opaque | no *E. coli* growth yet, showing signs, cotton swab marks opaque | no *E. coli* growth yet, showing signs, cotton swab marks opaque | no *E. coli* growth yet, showing signs, cotton swab marks opaque | no *E. coli* growth yet, showing signs, cotton swab marks opaque |
| Day 4 | cotton swabs are opaque, and some *E. coli* is growing around the basic zone of inhibition | cotton swabs are opaque, and some *E. coli* is growing underneath the acidic and neutral-soaked filtered circles | cotton swabs are somewhat transparent and *E. coli* is growing on side of plate | Cotton swab marks are opaque, showing signs of bacterial growth | cotton swab marks are somewhat transparent, small amounts of *E. coli* are visible |

**Table 4**. Statistical Analysis Anova Two Factor Without Replication

| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* | | |
|---|---|---|---|---|---|---|
| Acidic | 5 | 0.2 | 0.04 | 0.003 | | |
| Neutral | 5 | 1.2 | 0.24 | 0.063 | | |
| Basic | 5 | 3.5 | 0.7 | 0.225 | | |
| Concentration 1 | 3 | 1.8 | 0.6 | 0.43 | | |
| Concentration 2 | 3 | 0.8 | 0.2666666667 | 0.04333333333 | | |
| Concentration 3 | 3 | 1.1 | 0.3666666667 | 0.4033333333 | | |
| Concentration 4 | 3 | 0.7 | 0.2333333333 | 0.06333333333 | | |
| Concentration 5 | 3 | 0.5 | 0.1666666667 | 0.04333333333 | | |
| **ANOVA** | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 1.145333333 | 2 | 0.5726666667 | 5.577922078 | **0.03041968884** | 4.458970108 |
| Columns | 0.3426666667 | 4 | 0.08566666667 | 0.8344155844 | **0.5398010773** | 3.837853355 |
| Error | 0.8213333333 | 8 | 0.1026666667 | | | |
| Total | 2.309333333 | 14 | | | | |

Table 4 validates the pH experiment's results after completing an Anova two factor test to check for statistical significance. For this specific experiment there were two sets of hypotheses, since an ANOVA Double-Factor Without Replication Test for Mean Zone of Inhibition was run. The rationale for this test was due to the presence of two categorical independent variables yielding one dependent quantitative variable. "Without Replication" was utilized since there was only one row of data per categorical variable.

## Discussion

This experiment tested the antibacterial effects of nanoparticles under several conditions, including concentration, and pH, to create the strongest antibiotic against *E. coli* bacteria. The hypotheses predicted that these factors would be statistically significant to the results and that higher concentrations of nanoparticles would cause a faster immunity response than lower concentrations, and nanoparticles function at an optimal pH range. The data supports the concentration hypothesis, however we found that basic pHs provided more antibacterial activity. All of our *p-values* were statistically significant except for the second factor in the ANOVA test for pH, and that was between the factor of concentration of AgNPs and antibacterial activity. The factor of pH of AgNPs and antibacterial activity was significant.

### Effects of Nanoparticles' Solute Concentration on *E. coli* Growth

As shown in Figure 4, concentrations #1 (500,000 µg/L) and #2 (50,000 µg/L) display the highest and fastest increases in their zones of inhibitions: concentration #1 achieved an average zone of inhibition of about 0.13 cm by Day 4, and concentration #2 had an average zone of inhibition of about 0.13 by Day 2. Concentration #3 (5,000 µg/L) and concentration #4 (500 µg/L) demonstrated signs of zones of inhibitions forming during days 3 and 4 to a max of 0.1 cm; however, considering it took at least three days for the nanoparticles to achieve this optimal antibacterial activity as compared to achieving a wider zone of inhibition in similar time, concentration #1 and #2 are more effective. Concentration #5 shows no signs of a zone of inhibition across all days, therefore, displaying no signs of antibacterial activity with a nanoparticle concentration of 50 µg/L.

The null hypothesis was that there is no difference in the performance of the plates with higher concentrations of silver nanoparticles compared to that of lower concentrations. The alternative hypothesis proposed was that higher concentrations of nanoparticles will have more antibacterial effects compared to lower concentrations. At a significance level of 0.05, the resulting p-value was approximately 0. Therefore, the null hypothesis was rejected, and the data observed was statistically significant.

Table 1 displays the growth of *E. coli* in the presence of various concentrations. 50 µg/L has proven to be an ineffective concentration for optimal clinical treatment outcomes as signs of bacterial growth appear earlier than other concentrations. Agar plates #1, 3, and 4, however, did not show signs of *E. coli* growth until day 3, proving limited sustained antimicrobial properties. Concentration #2 (50,000 µg/L) was more effective compared to the other concentrations as it prevented the growth of bacteria from forming for longer periods of time over the observed four days than its neighboring concentrations, including concentration #1 (500,000 µg/L).

Research has shown that increasing concentration will increase the rate of the reaction, but bacteria can become resistant to some antibiotics when prescribed continuously at high concentrations (Nebraska Medicine, 2020). Additionally, "some antibiotics cannot distinguish between normal body bacteria and disease-causing bacteria," which causes a disturbance in the natural body levels as well as symptoms, such as severe diarrhea or gastrointestinal issues (Nebraska Medicine, 2020). Similar symptoms may also arise from an allergic reaction to the antibiotic, so concentration #1, the highest concentration, may not be optimal in an antibiotic. If concentration #2, then the chances of symptoms or the severity of allergic reactions are reduced, making the solution a more viable option for treatment. Its gradual increase in antibacterial activity may prevent the body's homeostasis from being disrupted.

Effects of Nanoparticles' pH on E. coli Growth

As shown in Figure 8, acidic pH decreased the efficiency of the nanoparticles for all concentrations, for figure 2 shows a much greater increase in the efficiency of the nanoparticles' antibacterial activity under a neutral pH. Only concentrations #2 and #5 showed any signs of zones of inhibitions under acidic conditions, proving that concentration #2 has antibacterial effects.

The first set of hypotheses for pH of the antibiotic is as outlined: The null hypothesis was that there is no difference in the performance of the plates (compared to concentrations) with higher pH of silver nano-particles compared to that of lower pH values. The alternative hypothesis proposed was that there is a difference in the performance of the plates with higher pH of silver nano-particles compared to that of lower pH values. The second set of hypotheses for the concentration of silver nanoparticles is as outlined: The null hypothesis was that there is no difference in the performance of the plates with higher concentrations of silver nano-particles compared to that of lower ones. The alternative hypothesis proposed was that there is a difference in the performance of the plates with higher concentrations of silver nano-particles compared to that of lower ones. At a significance level of 0.05, the resulting p-value for the pH was 0.03, and the p-value for the concentration was 0.5. Thus, due to the p-value for pH being less than or equal to the significance level, the null hypothesis is rejected for pH of an antibiotic. However, due to the p-value for concentration being greater than the significance level, the null hypothesis failed to be rejected. Therefore, the data observed is significantly significant for pH, and shows no significant difference in mean zone of inhibition across various concentrations for this experiment.

## Conclusion

Altering pH and concentration of AgNPs had a statistically significant effect on antibacterial activity against *E. coli.* We find that AgNPs function optimally at basic pHs and high concentrations (that aren't saturated), most specifically concentration #1 (500,000 µg/L) and concentration #2 (50,000 µg/L). With nanotechnology's unique biochemical properties, future applications will significantly contribute to the biomedical, manufacturing, pharmaceutical development of the nation's economy (Bharathala & Sharma, 2019). "Nanotechnology has impacted all aspects of biomedicine that includes both in vivo [imaging] and in vitro modes of diagnostic procedures, nanoformulations and drug delivery systems for therapeutics, tissue engineering, and regenerative medicine" (Bharathala & Sharma, 2019). Studies have shown that not only are silver colloidal nanoparticles an effective target-antibiotic for a variety of diseases but also it is more reliable and cost-efficient (Bharathala & Sharma, 2019). Understanding the antibacterial factors of nanomedicine, not only is applicable to microbiology but lends itself to learning about the chemical composition and biological structure of AgNPs as a whole. This information is vital, as the basics of science are rested upon these fundamentals. The more time that is invested in research of AgNPs the closer the medical field will get to saving more lives in an innovative and revolutionary way, including but not limited to cancer diagnoses (Herb 2020), safer ectopic pregnancies (Henderson 2022) and E. coli toxicity (Jones 2016).

## Limitations

Even though our experiments yielded statistical significance, we would have loved to run more trials for both the concentration and pH experiment if it wasn't for our access to limited funds. Running even more trials will only bolster our convictions. Similarly, we didn't have access to a centrifuge, so the largest source of error occurred when reconstituting the *E. coli* as the amount of water was not standardized for each experiment, since the state of the *E. coli* changed as well. Specific to the concentration experiment, the dilutions for concentrations #2 (50,000 µg/L) and #3 (5,000 µg/L) were 1 mL short from the total 22.5 mL. Moreso, in the pH experiment, the neutral circle with silver

nanoparticles had the greatest brown hue in concentration #1 (500,000 µg/L) however the reason why remains unknown. To add on, in the pH experiment we had to use dish soap as a basic pH indicator. If it wasn't for our limited access to funds, we would have gotten more sophisticated materials. However, we have strong warranting for our data, nonetheless, based on background research and how soap itself isn't a sanitizer and won't kill microorganisms such as salmonella and *E. coli* (Bogart 2019).

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