Student Guide

Part 2: Do Silver Nanoparticles Inhibit Bacterial Growth

Introduction: In this lab you will explore how effective silver nanoparticles are in inhibiting bacterial growth. You will use the silver nanoparticles you synthesized in Part 1 or nanoparticles provided by your teacher.

Silver nanoparticles are used to control spoilage of foods, to fight bacterial infections, and to limit bacterial growth in clothing. In minute concentrations, silver is nontoxic to human cells. The high surface area to volume ratio of the nanoparticles allows for increased contact with the bacteria with minimum exposure to silver.

Materials:

Needed for all three procedures:

Synthesized silver nanoparticles (teacher made or student synthesized in part 1)

- 100 uL micropipetter with tips
- *E. coli* in nutrient broth, at an optical density of 0.03 at 600 nm
- Table disinfectant
- Marking pens
- Bunsen burner
- Incubating oven (optional)

Needed for zone of inhibition procedure:

- Sterilized filter paper dots
- Ethanol with bent glass elbow
- Forceps
- Dilute hand soap solution
- 8 sterile nutrient agar Petri dishes
- Sterile deionized water
- Metric ruler with mm calibration

Needed for colony counting procedure:

- 14 sterile nutrient agar Petri dishes
- Ethanol with bent glass elbow

Needed for growth in nutrient broth:

Spectrophotometer

- 13 Sterile cuvettes containing 5 mL sterile nutrient broth – set aside one cuvette of sterile broth as a blank to zero the spectrophotomer
- Sterile synthesized AgNPs

Safety:

Use aseptic technique when handling the *E. coli.* Wear nitrile/latex gloves when working with *E. coli*

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Procedure: There are three options for this lab and your teacher wil direct you to the one you will do.

1. Zone of Inhibition Procedure

1. Disinfect table.
2. Use marking pens to label the bottoms of Petri dishes – two control, two silver nanoparticle, two hand soap, and two disinfectant dishes.
3. Aseptically pipette 100 uL of the *E. coli* stock solution to a control Petri dish. Use a sterile bent glass elbow to aseptically spread the bacteria over the surface of the agar. Close the dish. Using the same aseptic techniques, pipette 100 uL of *E. coli* to each of the remaining seven dishes.
4. Dip tips of forceps in ethanol and flame to sterilize. Use sterile forceps to pick up a sterile paper dot. Soak the dot in sterile deionized water. Place the paper dot in the center of the control inoculated Petri dish. Repeat this procedure to place the appropriate paper dots in the Petri dishes.
5. Place lids on petri dishes and tape closed.
6. Wash hands with soap and water.
7. Incubate Petri dishes upside down at room temperature or place in an oven at 37° C for 24 hours.
8. Observe Petri dishes through the plastic. Do NOT open the Petri dishes. Using the ruler, measure the zone of inhibition around the paper dots and record in the data table.

2. Colony Counting Procedure

1. Disinfect table.
2. Use marking pens to label the bottoms of Petri dishes with the varying volumes of silver nanoparticles solutions to be used – 0 uL AgNPs, 10 uL AgNPs, 25 uL AgNPs, 50 uL AgNPs, 100 uL AgNPs, 200 uL AgNPs, and 300 uL AgNPs. Label two Petri dishes for each volume of silver nanoparticles, for a total of 14 Petri dishes.
3. Aseptically pipette 10 uL AgNPs to the appropriately labeled Petri dish. Use a sterile bent glass elbow to aseptically spread the nanoparticles over the surface of the agar. Be careful to spread the nanoparticles over the entire surface. Repeat this procedure(with aseptic pipette), pipetting the appropriate volumes of nanoparticles to each of the Petri dishes.
4. Aseptically pipette 100 uL of *E. coli* stock solution to the first Petri dish, 0 uL AgNPs. Use the sterile bent glass elbow to spread the *E. coli* over the surface of the agar. Be careful to spread the bacteria over the entire surface. Repeat this procedure to aseptically inoculate and spread 100 uL *E. coli* onto each of the remaining Petri dishes.
5. Cover Petri dishes with lids and tape closed. Allow Petri dishes to sit for 10 minutes undisturbed.
6. Wash hands with soap and water.
7. Incubate the Petri dishes upside down at room temperature or in an oven at 37° C for 24 hours.
8. Observe colonies through the plastic – do NOT open the Petri dishes. Count the colonies on each Petri dish and record in the data table.

3. Growth in Nutrient Broth Procedure

1. Disinfect table.
2. Zero the spectrophotometer at 600 nm using a sterile broth cuvette as the blank.

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3. Use small pieces of marking tape to label the cuvettes of sterile nutrient broth with the varying volumes of silver nanoparticles to be used – 0 uL AgNPs, 10 uL AgNPs, 25 uL AgNps, 50 uL AgNPs, 75 uL AgNPs, and 100 uL AgNPs. Label two cuvettes for each volume of nanoparticles. Put each piece of labeled ape near the top of the cuvette.
4. Aseptically pipette 10 uL of sterile AgNPs to the appropriate sterile cuvette of broth. Repeat this procedure for the varying volumes of the AgNPs solutions, aseptically pipetting the sterile AgNPs to the sterile broth cuvettes. (12 total)
5. Aseptically pipette 100 uL *E.coli* to a cuvette with 0 uL AgNPs and gently finger vortex to mix. Record the absorbance of this solution at 600 nm in the data table at time 0 hours. Repeat this procedure, aseptically inoculating *E. coli* into each of the cuvettes and recording the time zero absorbances.
6. Wash your hands with soap and water.
7. Incubate in an oven at 37° C for 24 hrs. Gently finger vortex & take absorbance readings at 24 hrs. (If possible, take readings at recorded time intervals during the 24 hrs. Before each reading, gently finger vortex.)

Analysis:

Table 1. Zone of Inhibition

Plate	Test Reagent	Diameter of Zone of Inhibition (mm)	Average Zone (mm)
1	Control		
2	Control		
3	AgNPs		
4	AgNPs		
5	Soap		
6	Soap		
7	Disinfectant		
8	Disinfectant		

Table 2. Colony Counting

Plate	Amount of AgNPs (μL)	Colony Count	Average Colony Count
1	0		
2	0		
3	10		
4	10		
5	25		
6	25		
7	50		
8	50		
9	100		
10	100		
11	200		
12	200		
13	300		
14	300		

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Table 3. Growth in Nutrient Broth

Tube	Amount of AgNPs (µL)	Absorbance at 600 nm							
		0 hrs	Avg. 0 hrs	2 hrs	Avg. 2 hrs	12 hrs	Avg. 12 hrs	24 hrs	Avg. 24 hrs
1	0								
2	0								
3	10								
4	10								
5	25								
6	25								
7	50								
8	50								
9	75								
10	75								
11	100								
12	100								

Questions:

1. Prepare a graph showing the relationship between the independent and dependent variables.:

2. Describe the relationship between independent and dependent variables.

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3. What is the control in the experiment? What is the purpose of this control?

4. Why is the aseptic technique critical for accurate reults? Give there examples of aseptic practices used in this experiment.

5. Suggest possible sources of error in your experiment. These sources of error may NOT be mistakes in reading the measurements or in calculations.

6. Why are silver nanoparticles more effective than bulk silver in inhibiting bacterial growth?

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