Antimicrobial Properties of Nanoparticles

Learning Objectives

- Understand that chemical, biological, and physical properties of materials change at the nanoscale
- Perform a controlled experiment to study the properties of silver nanoparticles including color and antibacterial properties
- Understand the reaction taking place to synthesize silver nanoparticles
- Become aware of the ethical debate surrounding nanotechnology and nano-enhanced products

Washington EALR Alignment

9-12 INQC — Explain *Conclusions* must be logical, based on *evidence*, and consistent with prior *established* knowledge.

9-12 APPA — *Science* affects society and *cultures* by influencing the way many people think about themselves, others, and the *environment*. Society also affects *science* by its prevailing views about what is important to study and by deciding what research will be funded.

9-12 APPE — Perfect *solutions* do not exist. All technological *solutions* involve trade-offs in which decisions to include more of one quality means less of another. All *solutions* involve consequences, some intended, others not.

9-11 PS2D — *Ions* are produced when *atoms* or *molecules* lose or gain *electrons*, thereby gaining a positive or negative electrical charge. *Ions* of opposite charge are attracted to each other, forming *ionic bonds*. Chemical formulas for *ionic compounds* represent the proportion of *ion* of each *element* in the *ionic crystal*.

9-11 PS2G — *Chemical reactions* change the arrangement of *atoms* in the *molecules* of substances. *Chemical reactions* release or acquire *energy* from their surroundings and result in the formation of new substances.

9-11 PS2H — *Solutions* are *mixtures* in which particles of one substance are evenly distributed through another substance. *Liquids* are limited in the amount of dissolved *solid* or *gas* that they can contain. *Aqueous solutions* can be *described* by relative quantities of the dissolved substances and acidity or alkalinity (pH).

9-11 PS2I — The rate of a physical or *chemical change* may be affected by *factors* such as *temperature*, surface area, and pressure.

9-11 LS1D — The cell is surrounded by a membrane that separates the interior of the cell from the outside world and determines which substances may enter and which may leave the cell.

Oregon Content Standards Alignment

H.2P.1	Explain how chemical reactions result from the making and breaking of bonds in a process that absorbs or releases energy. Explain how different factors can affect the rate of a chemical reaction.
H.2P.2	Explain how physical and chemical changes demonstrate the law of conservation of mass.
H.2P.3	Describe the interactions of energy and matter including the law of conservation of energy.
H.2L.1	Explain how energy and chemical elements pass through systems. Describe how chemical elements are combined and recombined in different ways as they cycle through the various levels of organization in biological systems.
H.2L.2	Explain how ecosystems change in response to disturbances and interactions. Analyze the relationships among biotic and abiotic factors in ecosystems.
H.2E.4	Evaluate the impact of human activities on environmental quality and the sustainability of Earth systems. Describe how environmental factors influence resource management.
H.3S.4	Identify examples from the history of science that illustrate modification of scientific knowledge in light of challenges to prevailing explanations.
H.3S.5	Explain how technological problems and advances create a demand for new scientific knowledge and how new knowledge enables the creation of new technologies.
H.4D.4	Recommend a proposed solution, identify its strengths and weaknesses, and describe how it is better than alternative designs. Identify further engineering that might be done to refine the recommendations.
H.4D.5	Describe how new technologies enable new lines of scientific inquiry and are largely responsible for changes in how people live and work.
H.4D.6	Evaluate ways that ethics, public opinion, and government policy influence the work of engineers and scientists, and how the results of their work impact human society and the environment.

Idaho Content Standards Alignment

8 – 9 Physical Science, 8 – 9 Earth Science, 9 – 10 Biology

1.2.1	Use observations and data as evidence on which to base scientific explanations.
5.2.1	Explain how science advances technology.
5.2.2	Explain how technology advances science.
5.2.3	Explain how science and technology are pursued for different purposes.

8 – 9 Physical Science, 8 – 9 Earth Science, 9 – 10 Biology

9-10.B.5.1.1	Analyze environmental issues such as water and air quality, hazardous waste, forest health, and agricultural production.

9 – 10 Biology

9-10.B.3.3.1	Identify the particular structures that underlie the cellular functions

11 – 12 Chemistry

11-12.C.1.3.1	Identify, compare and contrast physical and chemical properties and changes and appropriate computations.
11-12.C.1.3.7	Interpret how the presence of solute particles affects the properties of a solution and be able to do calculations involving colligative properties.
11-12.C.2.4.3	Describe the relationship between the structure of atoms and light absorption and emission.
11-12.C.2.5.3	Describe the factors that influence the rates of chemical reactions.
11-12.C.5.1.1	Demonstrate the ability to work safely and effectively in a chemistry laboratory.
11-12.C.5.2.1	Assess the role of chemistry in enabling technological advances.
11-12.C.5.3.1	Evaluate the role of chemistry in energy and environmental issues.

Antimicrobial Properties of Nanoparticles

<u>Background</u>

Nanotechnology is the study and manipulation of material at the nanoscale – particles or materials containing structures that are between 1 and 100 nm in length in at least one dimension. A nanometer is one billionth of a meter. Because of their extremely small size, nanoparticles have a number of unique properties that differ from the same material on the macroscale.

One of the main reasons nanoparticles act differently from bulk is due to their increased surface area. The same volume of silver has a much greater surface area when the particles are decreased in size. (See Figure 1.) Color, conductivity, and antimicrobial properties of silver vary depending on the size of the particle. As the size of the particle decreases more light is absorbed instead of reflected. This gives silver nanoparticles a yellow color that ranges based on particle size. Other property changes, such as increased electrical conductivity, occur as the size of silver particles decreases.

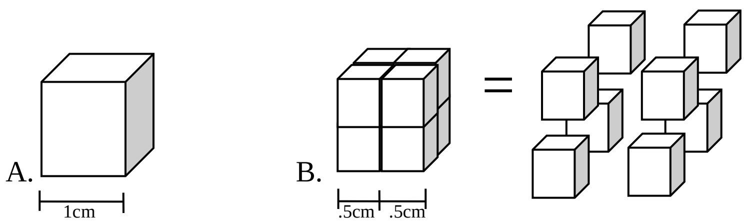

Figure 1. Sample A. and Sample B contain the same amount of silver. The surface area of Sample A is 6 cm^2. Although the volume is the same, the total surface area of the 8 particles that make up Sample B is 12 cm^2.

In this experiment, the synthesized silver nanoparticles form a colloid solution, in which the particles do not aggregate, but are evenly dispersed throughout the mixture. The silver nanoparticles are synthesized by mixing sodium citrate and silver nitrate at a high temperature. The silver nitrate provides Ag^+ ions, which are then reduced by the sodium citrate to form neutral silver particles (Ag°). As more Ag particles are synthesized, the mixture will begin to turn yellow. The remaining citrate molecules in the solution help to keep the Ag particles from growing into larger particles.

Silver has long been known to be antimicrobial. As far back as the middle ages, silver was used to keep food fresh, by using silver storage containers and even by putting silver coins in milk to slow spoilage. Today, silver is used in many bandages and products as an antimicrobial with increased results because of the ability to synthesize nanoparticles of silver that are more reactive than larger particles. Silver particles attach to proteins that are entering the cell or to the cell wall causing cells to rupture.

As particles of silver used for antimicrobial processes get smaller and smaller, ethical questions begin to be raised. For example, severe burns often result in serious infections in burn patients. Bandages for burn victims are being made with silver nanoparticles imbedded in them and greatly decreasing the incidence of infection. Severe burns, however, result in open wounds. Covering those open wounds with silver nanoparticle coated bandages can directly introduce those particles into the blood stream. Even if wounds are not open, the particles are small enough to make their way through the skin and into the blood stream.

Silver nanoparticles are also being researched for uses in fighting cancer. Currently, radiation and chemotherapy are some of the main ways cancer is fought. These therapies not only kill the cancer cells, but also attack the patient's healthy cells. Silver nanoparticles could be used to target and deliver drugs only to cancer cells.

Research is being done to see if these particles are safe and if there is a level of exposure that is harmful to the human body. One of the most important questions relating to nanoparticles is whether or not they are able to pass through the blood brain barrier. Like other antimicrobial agents, another question being raised with the increased use of silver nanoparticles is the effects of possibly killing bacteria that is beneficial as well as harmful bacteria. Like all new technologies the risks must be weighed against the benefits.

Applications

- Bandages and medicinal uses
- Sweat wicking clothing and socks
- Water treatment

Vocabulary

- Aggregate
- Antimicrobial
- Bulk
- Colloid Suspension
- Material properties
- Oxidize / Reduce (Redox Reactions)
- Surface area
- Synthesize (chemistry)

Materials

- 1 mM silver nitrate
- 10 ml Graduated cylinder
- 1% sodium citrate solution
- Small test tube
- 250 ml beaker
- Disposable transfer pipettes
- Agar plate
- Scissors
- Tweezers
- *E.coli* bacterial culture and/ or cheek sample
- Filter paper
- Cotton swab (sterile if using cheek cells)
- Plastic tray
- Incubator
- Hot plate
- Tongs
- Test tube rack
- Permanent marker
- Gloves
- Goggles
- Biohazard waste container
- Waste container

MSDS/Safety

MSDS are provided at the end of this unit for all chemicals used. Waste material for the silver nanoparticle solution should be collected in a waste container and then properly disposed of as hazardous waste. Special care should be taken in working with *E.coli* so as not to touch any skin. Instead of *E.coli*, a swab of the inside of a student's cheek may also be used to grow a bacterial culture. Students should wear gloves and goggles at all times. All surfaces and hands should be washed as soon as the procedure is complete. Both *E.coli* and the bacteria growth from the cheek samples and all disposable material that has come in contact with the bacteria (e.g. cotton swabs, gloves, pipettes, etc.) must be disposed of as biohazard waste. For information on medical waste programs in you state, see http://www.epa.gov/osw/nonhaz/industrial/medical/programs.htm

Pre-Lab Procedure

Determine if you will be using *E.coli* and/or cheek swabs in your procedure. Change the lab procedure to reflect the method of bacteria collection.

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Pre-Lab Questions

Why do you have to wait 24 hours to view the results of the lab?

What do you think will happen to the bacteria exposed to silver nanoparticles? The control?

The silver nanoparticles in this experiment are yellow in color. Why do you think they differ in color from bulk silver?

If the silver nanoparticle solution cools too long, it turns a greyish color. Why do you think this might happen? Do you think that the solution in this state would be as effective in killing bacteria?

Safety warning

Wear gloves at all times. For the chemicals used in synthesizing the silver nanoparticles, liquid spills on the skin can be washed off with water. Use care while heating the solution on the hot plate. Never leave the hot plate unattended. Do not remove the hot test tube from the boiling water with your hands. Special care should be taken when working with *E.coli* or other bacteria so as not to touch any skin. All surfaces and hands should be washed as soon as the procedure is complete. *E.coli* and the bacteria from the cheek samples and all disposable material that has come in contact with the bacteria (e.g. cotton swabs, gloves, pipettes, etc.) must be disposed of in a biohazard waste container.

Procedure

1. In a 250 mL beaker, heat about 50 ml of water to a boil.
2. In a 10 mL graduated cylinder, measure 2 mL of 1 mM silver nitrate solution then add the solution to a small test tube.
3. Place this test tube in the boiling water; Let heat for 10 minutes.
4. Add 7 drops of 1% sodium citrate to the hot silver nitrate solution.
5. Continue to heat until the silver nitrate solution changes to a yellowish color (~15 minutes).
6. Remove the test tube and set in a test tube rack to cool.
7. While waiting for the silver nanoparticles to form, cut filter paper into four small squares (about 2 cm across). Set aside two pieces of filter paper as controls.
8. Place two of the filter paper squares flat in a small plastic tray and pour the cooled silver nanoparticles over the squares. Let the filter paper squares soak for 10 minutes.
9. Using a permanent marker, divide the bottom of the agar plate into quarters and label two sections as controls and two as nanoparticles. Mark your initials on the bottom of the plate.
10. A. If you are using *E.coli*, put 1 to 2 drops of bacterial culture on the agar plate using a pipette. With a cotton swab, spread the bacterial culture on the agar plate coating it completely. **Place any disposable material (pipette, gloves, cotton swab, etc.) that has come in contact with the *E.coli* in a biohazard collection container. Do not throw these materials away in a trash can.**

    B. If you are using a cheek sample, use a sterile cotton swab to take a sample from the inside of your cheek. Spread the sample on the agar plate coating it completely. **Place any disposable material (pipette, gloves, cotton swab, etc.) that has come in contact with the cheek sample in a biohazard collection container. Do not throw these materials away in a trash can.**
11. Using tweezers, place your nanoparticle-soaked filter paper squares and your control(s) in the designated areas.
12. On your Data and Results page, describe the sample and predict what you think will happen after incubating the plate overnight.
13. Thoroughly wipe down your lab station and your wash hands.
14. Incubate your agar plate overnight at 37 °C.
15. Examine the agar plate. Record results. **Dispose of the agar plate in a biohazard collection container.**

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Data and Results

Describe what each section of the sample looks like before incubation in the table below.

	Description of sample section before incubation
Control 1	
Control 2	
Nanoparticle 1	
Nanoparticle 2	

Draw a picture of what you think the sample will look like after 24 hours. Make sure to label the sections of the sample.

Describe each sample after incubation.

	Description of sample section after incubation
Control 1	
Control 2	
Nanoparticle 1	
Nanoparticle 2	

Draw a picture of your plate after incubation. Make sure to label the section of the sample.

<u>Discussion questions</u>

From your observations, do silver nanoparticles exhibit antimicrobial properties?

Why is the formation of a colloid solution important to the increased ability of silver nanoparticles to be antimicrobial?

How might you design an experiment to test the effective antimicrobial efficiency of different sized silver particles?

Find a current product on the market that uses silver nanoparticles.

What do you think might be a problem with using silver nanoparticles in so many new products?

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Further Silver Nanoparticle Labs

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