Name:_____Date:_____Class:_____

Nanoparticle (circle one):   Au   Ag

Group role (circle one):   nanoparticle synthesis   dilution

Exposure conditions (circle two): a   b   c   d   e   f

Student Worksheet

Nanoparticle Toxicity to Brine Shrimp

It is critical to determine the toxicity of substances in order to determine their safety. This lab is designed to assess the toxicity of gold (Au) and silver (Ag) nanoparticles on brine shrimp viability. Viability is a term to describe whether an organism is alive or dead. That is, if an organism is living, it is said to be viable. In this lab, brine shrimp are a model organism to perform initial assessment of nanoparticle toxicity. If the nanoparticles are found to be non-toxic, scientists would likely move to toxicity test in an organism higher in the food chain.

Nanoparticles fast-growing use in medical, industrial, and commercial fields poses questions on their safety in products and in waste material. Silver nanoparticles are used specifically for their

> **Safety**
> Gloves and goggles should be worn when working with laboratory chemicals.

antimicrobial effects. Gold nanoparticles are used in chemical and biological sensing as well as in medical diagnostics and therapeutics.

Experimental Outcomes:
- Synthesize gold and silver nanoparticles and prepare nanoparticle suspensions
- Perform tests of nanoparticle toxicity on living organisms
- Analyze the toxicity results for statistical significance

Instructions: You will be working with one lab partner. One half of the class will be working with each type of nanoparticle that we are investigating (Au or Ag), and each lab pair will use that nanoparticle to perform brine shrimp toxicity tests at a number of concentrations. Regardless of the material you are using, you are to answer the questions below in the relevant sections. There are also questions at the end of each day's procedure that everyone must answer. **NOTE:** Please remember to circle the nanoparticle your group will be working with and the group role that you have at the top of this page.

Procedure – Day 1

A. Au Nanoparticle Synthesis (each group in the "gold" half of the class completes)
1) Add 15.0 mL of the 1.1 mM Au solution to a glass vial using a graduated cylinder
   Actual volume of Au stock solution measured: _____mL
2) Heat the Au solution to approximately 140°C (medium high heat)
   Actual solution temperature: _____
   What color is the solution before heating?_____ _____
3) Stir the solution with a stir rod every 30 seconds for the duration of the synthesis
4) When the Au solution is boiling (or after 10 minutes), add 1.5 mL of the sodium citrate
   solution #1 (0.050M $Na_3C_6H_5O_7$) using a graduated cylinder
   Actual volume of sodium citrate solution measured: _____mL
   What color is the solution after addition of sodium citrate?_____
   When did the solution change colors? (immediately? after some time?)_____
5) Keep the reagents heating for 10 minutes after color change, making sure to stir every 30
   seconds
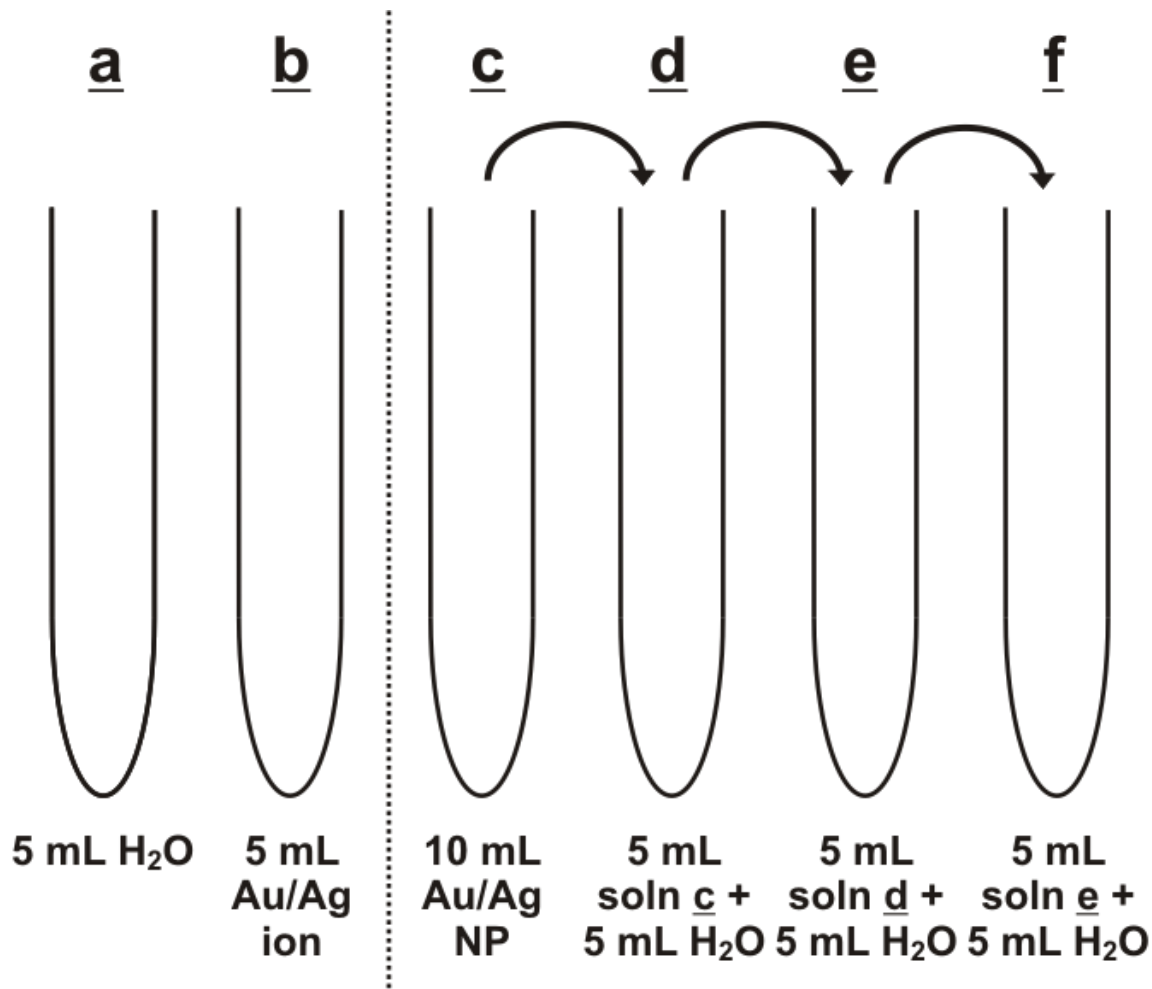 6) Remove the vial from the heat, and allow it to cool for a minute or two.

B. Silver (Ag) Nanoparticle Synthesis (each group in the "gold" half of the class completes)
1) Add 13 mL deionized (d.i.) water to glass vial using a graduated cylinder
   Actual volume of water added: _____mL
2) Heat water on hot plate until small bubbles begin forming on side of vial (beginning to boil)
3) Add 1 mL of the 0.0075 M Ag solution and 1 mL trisodium citrate solution #2 (0.0075 M
   $Na_3C_6H_5O_7$) to the hot water using a pipet (WARNING: Ag stock solution will stain hands
   and clothing)
   What color is the solution?_____ _____
4) Add magnetic stir bar to glass vial and begin stirring the solution
5) Add 20 drops sodium borohydride solution to vial
   What color is the solution after addition of $NaBH_4$?_____
6) Keep Ag nanoparticle mixture boiling for 10 minutes after color change, making sure to stir
   vigorously and continuously.
7) Remove the vial from the heat, and allow it to cool for a minute or two.

   Dilution of Au/Ag Nanoparticle Suspensions
1) Label six vials as a-f
2) Fill vials as follows
   a) Vial a: add 5 mL d.i. water with a graduated cylinder
   b) Vial b: add 5 mL Au or Ag stock solution with a graduated cylinder
   c) Vial c: add 10 mL of the Au or Ag nanoparticle suspensions with a graduated cylinder
   d) Vial d: add 5 mL of the suspension from <u>vial c</u> + 5 mL d.i. water with a graduated
      cylinder and stir
   e) Vial e: add 5 mL of the suspension from vial d + 5 mL d.i. water with a graduated
      cylinder and stir
   f) Vial f: add 5 mL of the suspension from vial e + 5 mL d.i. water with a graduated
      cylinder and stir
3) What is the concentration of :
   a) vial d as compared to vial c?_____
   b) vial e as compared to vial d?_____
   c) vial e as compared to vial c?_____

d) vial f as compared to vial c?_____

a   b   c   d   e   f

5 mL H_2O 5 mL Au/Ag ion   10 mL Au/Ag NP   5 mL soln c + 5 mL H_2O 5 mL soln d + 5 mL H_2O 5 mL soln e + 5 mL H_2O

Within the lab groups working on the gold nanoparticles, half the groups will investigate vials a, d, f and the other half will use vials b, c, and e to perform brine shrimp viability assay. Trade vials among the gold nanoparticle half to do this.

Likewise, within the lab groups working on the silver nanoparticles, half the groups will investigate vials a, d, f and the other half will use vials b, c, and e. Trade vials to set this up. **BE VERY CAREFUL NOT TO MIX UP THE GOLD AND SILVER PARTICLE SUSPENSIONS!**

Questions: (These questions have multiple components. Make sure to answer all parts of the question before day 2 of the procedure.)

1. What are nanoparticles? What are two chemical and/or physical properties that differ between the Au and Ag nanoparticles?

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2. What type of dilution is used to make the nanoparticle suspensions with varying concentrations? Describe a different dilution scheme/method to arrive at the same concentrations.

3. Assume the concentration of the nanoparticles you synthesized in the suspension is 100 nM (nanomolar, 10^{-9} M or 10^{-9} moles per liter of suspension). Based on the dilution factors, what is the concentration of nanoparticles in vial c, d, e, and f? (You may need to get dilution factors from members within the group)

Procedure – Day 2

Viability Assay (everyone completes)
1. Add 0.5 mL water to a small beaker using a volumetric or transfer pipet
2. Pipet all 0.5 mL of the water into the Pasteur pipet and mark the meniscus location on the pipet (see figure)
3. Dispose of the water but save pipet with the 0.5 mL marked upon it.
4. Add 1 mL water to the same small beaker.
5. Pipet all 1 mL of water into the *same* Pasteur pipet and mark the meniscus location on the pipet as done above
6. Dispose of the water. The pipet should now have a 0.5 and 1 mL marks on it and will be used to measure out solutions for the brine shrimp assay
7. Pipet up 0.5 mL brine shrimp into the glass Pasteur pipet
8. Count brine shrimp that are moving while in the pipet. Tip: it may be easier to see brine shrimp by holding pipet against a dark surface
9. After counting, put brine shrimp into one well of a 24-well plate
10. Record the number of shrimp below in the appropriate circle corresponding to the location of the brine shrimp in the 24 well plate (shown below).
11. Repeat steps 7-10 until 3 columns (a total of 12 wells) contain brine shrimp
12. Add 1 mL 25% serum solution to each well of brine shrimp
13. You will be given 2 exposure conditions per lab group, (as described in #8 of the dilution instructions), so 4 wells will be replicates of a single condition
14. Add 1 mL exposure solution (control, ion, or nanoparticles) to each well and label the well plate above with which solution (a-f) was placed in the well
15. Place well plate under lamp approximately 9 inches from the bulb
16. Rinse pipet out with water and save for tomorrow
17. Dispose of remaining nanoparticle and ion solutions (vials b-f) in the designated waste container
18. After exposure to nanoparticle solutions, the brine shrimp will be incubated for 24 hours before you do the viability test in tomorrow's class to see what fraction of brine shrimp survive exposure.

Questions: (These questions have multiple components. Make sure to answer all parts of the question before day 2 of the procedure.)

1. Hypothesize what will happen to the brine shrimp after exposure to all the conditions. That is, predict whether the shrimp with live or die upon exposure to solutions a-f.

Procedure – Day 3
Brine Shrimp Viability (everyone completes)
1. Pull solution from one well in the plate into glass Pasteur pipet.
2. Count the brine shrimp that are still moving in pipet as well as any moving shrimp that are left behind in the well. Do NOT include shrimp that are not moving (dead shrimp) in your count.
3. Record your brine shrimp count in the appropriate circle below for all 12 wells.
4. Using the values from yesterday (see the values you put into the well plate diagram yesterday), determine the fraction of brine shrimp that are alive today and convert it to a decimal fraction. For example, if yesterday in well 1A you had 20 brine shrimp alive and today you had 15 alive in well 1A, your fraction would be 15/20 and the decimal fraction would be 0.75.
5. Record the decimal fraction value in the correct well in the well-plate diagram below. Determine average decimal fraction for each exposure condition you performed
   Exposure Condition (e.x. a or e): _____ Average: _____
   Exposure Condition: _____ Average: _____
   Exposure Condition: _____ Average: _____
6. Upon completion of the viability assay, discard all well contents into the designated waste container.

Data Collection

1. Collect decimal fraction for all wells of each culture condition from your own data and the others working with the same concnetrations
2. Pool data with that of other groups that used the same nanoparticle (Au or Ag). Record the values on the class data graph
3. Determine the class average for each exposure condition (a-f) of the nanoparticle you worked with the following:
   Nanoparticle used: _____
   Class average for <u>vial a</u> (control): _____
   Class average for <u>vial b</u> (ion): _____
   Class average for <u>vial c</u> :_____
   Class average for <u>vial d</u>:_____
   Class average for <u>vial e</u> : _____
   Class average for <u>vial f</u>:_____

Concluding Questions

1. Why do we include the ion exposure as one of the conditions? Why do we include the water exposure?

2. What does a viability decimal fraction of 1 mean? What does a viability decimal fraction of 0 mean? Considering the data from your entire trio, did the exposure to nanoparticles affect brine shrimp viability?

3. How consistent was the data within your given group? Across groups? How might we represent this variability mathematically?

4. Do you think brine shrimp are a good model to predict human nanoparticle toxicity?

Guiding Lab Questions

Name_____ **Class period**_____

_____

_____

1. What is the research question for your experiment?

2. What is the hypothesis that you will be testing?
State your hypothesis as an If……., then……. statement.

3. What is the independent variable in your experiment?

   What is the dependent variable in your experiment?

4. What are the experimental groups for your experiment?

5. What are the control groups for in your experiment?

6. Why is it important to include a control in the experiment?

7. What are four controlled factors (things that are kept the same in all of the samples) for your experiment?

8. Why is it important to keep the controlled factors the same in each of the experiments?

9. Why was it important to use more than one brine shrimp in each sample?

10. Use the grid below to design a data table that you will use to collect data from your experiment.

- The data table should have a title that includes the independent variable, the dependent variable, and the organism studied.
- The independent variable (with units of measurement) is written in the left-hand column—arranged in increasing order from top to bottom.
- The dependent variable (with units of measurement) is written in the right-hand column. You will collect data later to complete the right-hand column.
- Be sure to include units of measurement for each variable.

Table 1. Data for Silver Nanoparticle Concentration and Brine Shrimp Viability

Nanoparticle Concentration	Initial number of brine shrimp (before exposure to nanoparticles)	Number of live brine shrimp (after exposure to nanoparticles)	% Viability of brine shrimp= # brine shrimp after exposure/ # brine shrimp before exposure * 100%
control			
Ion Control			
100 % Silver nanoparticles			
50 % Silver nanoparticles			
25 % Silver nanoparticles			
12.5 % Silver Nanoparticles			

Table 2: Data for Gold Nanoparticle Concentration and Brine Shrimp Viability

Nanoparticle	Initial number of brine shrimp (before	Number of live brine	% Viability of brine shrimp=

Concentration	exposure to nanoparticles)	shrimp (after exposure to nanoparticles)	# brine shrimp after exposure/ # brine shrimp before exposure * 100%
control			
Ion Control			
100 % Gold nanoparticles			
50 % Gold nanoparticles			
25 % Gold nanoparticles			
12.5 % Gold Nanoparticles			

Data Analysis
1. Record the number of surviving brine shrimp in the data table on the previous page.
2. Summarize the data you have collected in graph form.
    ● The graph should have a title that includes the independent variable, the dependent variable, and the organisms studied.
    ● Each axis should be clearly labeled with the variable and the units of measurement-- put the independent variable on the horizontal axis and the dependent variable on the vertical axis.
    ● Mark a scale (even intervals) on each axis.
    ● Use the data from your data table to create a **line (not bar)** graph.
    ● Use two different colors to represent the effects of the gold and silver nanoparticles

3. Look at the information represented in your graph. What conclusions can you draw from the data you collected? Describe any patterns or trends you see in the data. Are there any exceptions to these patterns or trends?

4. Does your data support or refute (disprove) your hypothesis? Explain.

5. Based on the results of your experiment, do you think the silver nanoparticles you were testing were harmful? At what concentrations? Explain your answer.

6. A good experiment is one that gives approximately the same results if it is replicated (repeated) by others. List at least <u>two</u> ways you could improve your experiment to be certain that it could be replicated (repeated) by others to give the same results?

7. During the next class period, you and your team members should be prepared to present your research findings to the class. You should prepare visuals (transparencies or PowerPoint slides) that show your:
- Data table
- Graph
- Conclusions

Be prepared to answer questions from your classmates and your teacher.

Day 3 Well Plate