Teacher's Preparatory Guide

The Effects of Gold and Silver Nanoparticles on Brine Shrimp: A Toxicology Study

Overview: Students will synthesize and prepare serial dilutions of gold and silver nanoparticles in order to determine their individual toxicity on brine shrimp. The brine shrimp are used as a model organism to examine the potential toxicity effects of nanoparticles.

Purpose: This lab is designed to help students understand the following: the interdisciplinary fields of science, a practical application of chemistry in the real world, and the need to carefully conduct experiments and evaluate data in a collaborative environment. The lab explores the effects of exposing living organisms to different nanoparticles at various concentrations. In this experiment, students will compare the toxicity of different concentrations of nanoparticle suspensions on brine shrimp, along with controls, to perform toxicity assays and data analysis. Students will understand the importance of conducting toxicity assays before releasing nanoparticles into the environment.

Time Required: Three 55 minute classes

Level: High school and community college/ beginning undergraduate chemistry, environmental science, and biology

Big Ideas: Size Dependent Properties, Models and Simulations, and Science and Technology

Teacher Background: Nanoparticles are particles that are less than 100 nm in size. Because of their unique physical and chemical properties, which differ from the bulk form of the same material, they are increasingly incorporated into a variety of products and medicines. Their 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 antimicrobial effects. Gold nanoparticles are used in chemical and biological sensing as well as in medical diagnostics and therapeutics. This lab will attempt to study the toxicity that gold and silver nanoparticles have on brine shrimp. The procedure for this lesson and its use with a high school chemistry class has been published in the Journal of Chemical Education (online) – see Maurer-Jones reference in resource section.

Equipment: (per group of two students)

- goggles
- gloves (optional)
- 10 and 20 mL graduated cylinders

*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1363
Rev: 10/2013

- 24-well plates
- glass vials w/ covers or test tubes with stoppers
- combination hot plate/stirrers
- magnetic stir bars
- amber storage bottles or aluminum foil to cover bottles
- thermometers
- glass stir rods
- tongs, test tube holder, or "hot hands"
- timers
- permanent markers
- volumetric pipets marked at 0.5 and 1.0 mL
- small beakers
- Pasteur pipets
- incandescent 40 watt lamp
- ring stand
- aquarium air pump
- fish tank or large bowl to hatch brine shrimp
- disposable pipets
- noble metal waste container

Materials:

1. *Materials requiring advance preparation*
   - 6g brine shrimp eggs
   - 10 gallons Instant Ocean
   - 250 mg gold (III) chloride
   - 10 g sodium citrate dehydrate
   - 10 g silver nitrate

2. Gold Nanoparticle Synthesis
   - 15 mL gold (Au) stock solution
   - 1.5 mL 50 mM sodium citrate solution for gold

3. Silver Nanoparticle Synthesis
   - 13 mL deionized water
   - 1 mL 7.5 mM silver (Ag) stock solution WARNING: Ag stock solution will stain hands and clothing
   - 1 mL 7.5 mM sodium citrate stock solution for silver
   - 1 mL 10 mM sodium borohydride

4. Dilution of Gold Nanoparticles
   - 20 mL deionized water

*Development and distribution partially funded by the **National Science Foundation***

- 5 mL gold stock solution
- 10 mL gold nanoparticles

5. Dilution of Silver Nanoparticles
- 20 mL deionized water
- 5 mL silver stock solution WARNING: Ag stock solution will stain hands and clothing
- 10 mL silver nanoparticles

6. Viability Assay

Part A: mark Pasteur pipette with .5 mL and 1 mL markings
- water
- Protoslo quieting solution (if desired)

Part B: Count and record living brine shrimp in 12 spot wells
- Pasteur pipet with .5 mL and 1.0 mL markings
- pipet pump dial
- 6 mL brine shrimp

Part C: Nanoparticle Exposure
- 15 mL serum solution (bovine calf serum or fetal bovine serum)
- synthesized nanoparticle solutions

7. Brine Shrimp Viability
- chlorine bleach

Advance Preparation: Obtain materials from the sources below.

Material	Cas #	Source	Product No.	Price
6g Brine Shrimp Eggs (best hatching results obtained w/San Francisco Bay Brand)		San Francisco Bay Brand, Inc. 8239 Enterprise Drive Newark, CA 94560 www.sfbb.com/Brine-Shrimp-Eggs_51.php	65031	5.99
10 gallons Instant Ocean		Instant Ocean 800-822-1100	Part Number: SS15-10 UPC: 051378012003	5.99
250 mg Gold (III) chloride trihydrate	13453-07-1	Sigma-Aldrich Co. www.sigmaaldrich.com	379948-250MG	63.00

Page 3

10 g Sodium citrate dihydrate	0006132043	Sigma-Aldrich	W302600-SAMPLE-K	40.00
10 g silver nitrate	0007761888	Sigma-Aldrich	204390-10G	89.20
25 g sodium borohydride	16940-66-2	Sigma-Aldrich	480886-25G	99.20
500ml bovine calf serum solution		Sigma-Aldrich	12133C-500ML	36.40
15 ml Protoslo (R) quieting solution order 2 bottles		Carolina Biological Supply Co 2700 York Road Burlington, NC 27215 www.carolina.com	885141	6.75

To be Prepared in Advance:

1. **Hatching brine shrimp.** Mix 0.4g brine shrimp eggs and 35 g Instant Ocean into 1 liter of deionized water. Place under an incandescent lamp and insert an aquarium air pump. Eggs will be hatched in 2-3 days.

2. **Gold nanoparticle stock solutions.** To make stock solutions for 25 batches:
   a. Gold chloride solution (0.010 M $HAuCl_4$): dissolve 1.0 g of $HAuCl_4$-$3H_2O$ in 250mL DI water to make a 10mM stock solution of gold (III) ions that can be kept for years if stored in a dark colored, light-tight container. Dilute 27.5 mL of stock to 250 mL to make the 1.1 mM solution needed for this activity.
   b. trisodium citrate solution #1 (0.050M $Na_3C_6H_5O_7$): dissolve 0.5g $Na_3C_6H_5O_7$-$2H_2O$ in 50mL DI water

3. **Silver nanoparticle stock solutions.** Stock solutions for 8 batches:
   a. Silver stock solution (0.0075 M $AgNO_3$): dissolve 0.128g of $AgNO_3$ in 100mL DI water.
   b. Sodium borohydride solution (0.010 M $NaBH_4$): dissolve 0.095g of $NaBH_4$ in 250mL DI water.
   c. trisodium citrate solution #2 (0.0075 M $Na_3C_6H_5O_7$): Dilute 2 mL of trisodium citrate solution #1 (above) in 13.3 mL DI water.

   Sodium borohydride should be used the day of synthesis. The sodium citrate and the gold and silver stock solutions can be stored in the dark for 4-6 months.

4. **Making serum solution.** The serum solution consists of 25% serum (e.g. bovine calf serum, fetal bovine serum, etc.) and 75% artificial sea water by volume. Prepare a small quantity (10mL) for use later as an additive to the nanoparticle suspensions.

Nanoparticle Synthesis Procedures:

1. Gold nanoparticles

Students will synthesize citrate-reduced Au nanoparticles from sodium citrate and $HAuCl_4$ stock solutions as modified from a previously published method.{Marquis, 2008}

In glass vials, students add 15 mL of 1.1 mM $HAuCl_4$ stock solution and begin heating on medium-high heat, stirring the solution with either a magnetic stir bar or every 30 seconds with a stir rod. When the Au solution is boiling, or after 10 min, add 1.5 mL of 50 mM sodium citrate stock solution. Au nanoparticles should form within a couple minutes, if not immediately, and the solution should change from clear to a deep purple. Keep heating the Au nanoparticle solution for 10 min after the color change, making sure to stir every 30 seconds or continuously stir with stir bar. Nanoparticles can be stored so long as they are kept from light by amber colored storage bottles or aluminum foil.

2. Silver nanoparticles

Citrate-capped Ag nanoparticles will be synthesized by students using a $NaBH_4$ reduction of $AgNO_3$ to Ag(0) modified from a previously published method.{ Chinnapongse, et.al., 2011}. In a glass vial or beaker, 13 mL of deionized or distilled water is added and heated, ideally under constant stirring, until the water is beginning to boil, after which 1 mL of 7.5 mM $AgNO_3$ and 1 mL of 7.5 mM sodium citrate should be added to the vial and stirred. Next, 20 drops of 10 mM $NaBH_4$ is added and the solution should immediately turn from clear to yellow as the nanoparticles form. Continue to heat and stir nanoparticle suspension for 10 minutes after color change. Unlike the other stock solutions, $NaBH_4$ solutions should be freshly prepared for each experiment. Once formed, silver nanoparticles can be stored for many weeks as long as they are kept from light.

Safety Information and Procedures:

All nanoparticle mixtures, reagents, and exposed brine shrimp should be collected as noble metal waste and disposed in accordance with hazardous waste procedures. $NaBH_4$ solution can be disposed down the drain. Obtain relevant Materials Safety Data Sheets and follow school procedures for all chemical handling and disposal.

Day 1

equipment: wear goggles (optional: gloves and lab apron)
procedures: wash hands completely after lab; rinse skin with water if contact
disposal: place unused nanoparticles in "Noble Metal" waste container

Day 2

equipment: wear goggles (optional: gloves)
procedures: wash hands completely after lab; rinse with water if contact
disposal: place unused brine shrimp in 10% bleach for 30 minutes, then dispose down drain
place unused nanoparticles in noble metal waste container
unused serum solution may be flushed down the drain

Day 3

equipment: wear goggles (optional: gloves)
procedures: wash hands completely after lab; rinse with water if come into contact
disposal: Add 10% bleach to brine shrimp solution, then dispose in the noble metal waste container

*Development and distribution partially funded by the **National Science Foundation***

Suggested Instructional Procedure:

Time	Activity	Goal
Day 1	Nanoparticle synthesis and dilution	Students perform assigned roles within groups to create test conditions
Day 2	Brine shrimp counting and exposure	Students conduct live shrimp assays and then expose shrimp to appropriate assigned conditions
Day 3	Counting surviving brine shrimp and data analysis	Students conduct live shrimp assays. Students perform statistical measures on their own data and then combine data into group data and finally classroom data. Students complete graphs and questions as homework.

Teaching Strategies: This lab explores the effects of exposing living organisms to different materials at various concentrations. The number of possible combinations of exposure materials and concentrations leads to a substantial number of individual tests to run. Thus it is a good idea to break the class up into subgroups so that all the combinations of variables can be tested as a class. After members of the class have carried out their tests, they will share data and produce the results of the larger study, a situation similar to how professional scientists work.

As with many real experiments involving living organisms, a single measurement in this experiment cannot be expected to give a reliable result. Instead, it is necessary to make repeated measurements on a large number of similar systems, and then calculate values for mean and standard deviations. Students will make multiple measurements on the same system as part of this lab. When the students analyze their data, they will need to produce the mean values of measured quantities. This is an opportunity to address the concepts of statistical variation, measures of variance (standard variation, standard error, etc.), and experimental uncertainty.

Guided Dialog: Before beginning the lab, review the meaning of these terms:

- nanoparticle- *a particle less than 100 nanometers in size*
- toxicity- *a measurement of a substance's ability to kill*
- synthesis- *making new materials*
- solution – *a material dissolved in a liquid solvent that exists in the form of ions (charged atoms or small molecules)*
- suspensions – *small solid particles in a liquid. The suspension is stable if the particles do not settle under gravity*
- dilutions- *lowering the concentration of a substance*

Activity Procedure:

1. Prepare Exposure Solutions and Nanoparticle Suspensions

In this experiment, students will be comparing the toxicity of different concentrations of nanoparticle suspensions on brine shrimp, along with controls consisting of pure water and a

*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1363

Rev: 10/2013

Page 6

metal ion (i.e. Ag+ or Au+) solution. To achieve the varied concentrations, a serial dilution is performed from the stock suspension of particles as synthesized, yielding the following concentrations: as-synthesized nanoparticle concentration (100% original value), 50% original concentration, 25% original concentration, and 12.5% original concentration. While the exact concentration of nanoparticles may differ in the syntheses, the concentration of the as-synthesized and diluted nanoparticles will be comparable for the toxicity assay.

Table 1 indicates the solutions/suspensions that will be prepared and subsequently exposed to the brine shrimp. Students should prepare solutions in vials/test tubes labeled a-f (see table 1) and these solution can either be stored for future use or used immediately.

Table 1:

Sample Label	Sample Name	Water Volume (mL)	Nanoparticles Volume (mL) - from vial X	Other specified solution Volumes
a	Control	5	-	-
b	ion control	-	-	5 mL Ag or Au stock solution
c	100% nanoparticles	-	10 - synthesis vial	-
d	50% nanoparticles	5	5 - vial c	-
e	25% nanoparticles	5	5 - vial d	-
f	12.5% nanoparticles	5	5 - vial e	-

2. Perform Live/Dead Brine Shrimp Assay

The brine shrimp assay entails a 24 hour exposure to two control solutions and four nanoparticle suspensions described above, followed by determining if the brine shrimp are viable after exposure. Viability of the brine shrimp is assessed based on movement: live brine shrimp are those that move whereas dead shrimp do not. Two to three days prior to the viability experiment, brine shrimp should be hatched from eggs by placing ~0.4g eggs in 1 L of artificial sea water (35 g Instant Ocean(R) in 1 L DI water) with constant aeration, either from an air pump or in a fish tank, and illuminated by a lamp to keep them warm.

The assay is best performed in a multi-well plate where the wells can hold at least 2 ml each,

*Development and distribution partially funded by the **National Science Foundation***

although other containment is possible. A Pasteur pipet marked with lines for measuring 0.5 and 1 mL can be prepared by pre-measuring these volumes of water with a volumetric pipet, sucking the water up into the Pasteur pipet, and recording the height of the water with a permanent marker. While this task can be completed by the instructor prior to the students' experiment, it is also possible for the students to perform this task; instructions are included in the student worksheet. Students will measure 0.5 mL brine shrimp suspension and count the moving brine shrimp while they are still in the pipet, then transfer the brine shrimp to an empty well and record the value and the well in which it is placed. This part of the experiment has the greatest potential for error and therefore, students should take their time counting. It may be easier to count the moving brine shrimp by holding the pipet against a dark surface. The addition of Protoslo to the brine shrimp to slow them down can make it easier for students to count (add 10 drops Protoslo to 20 mL brine shrimp aliquot). Students should get 15-20 brine shrimp per 0.5 mL so if the brine shrimp are too dense from the hatching solution, add artificial sea water solution (35 g Instant Ocean in 1L water) to the suspension of brine shrimp to dilute. Students should perform the viability in quadruplicate so that each control or nanoparticle concentration has multiple data points. This means that if a student/lab pair within a larger group is testing the viability of 3 sample solutions, they should fill a total of 12 wells.

After counting and recording the number of shrimp per well (the most time-consuming portion of this experiment), students should add 1 mL serum solution to each well of brine shrimp to prevent nanoparticle aggregation and 1 mL of the control or nanoparticle suspension to each well. The serum solution consists of 25% serum (e.g. bovine calf serum, fetal bovine serum, etc) and 75% artificial sea water by volume. Upon exposure, brine shrimp should be covered with a clear cover (i.e. that provided with the well plates or plastic wrap), and placed under a lamp, such as a desk or utility lamp, approximately 9 inches from the bulb. Incubate the wells for 24 h, after which the brine shrimp should be counted again, only counting the shrimp that are moving (i.e. alive).

3. Data Analysis

An important component of chemistry, and science in general, is data analysis and statistics. In this experiment, students use the viability data to perform simple statistics.

First, viability is calculated per well by equation 1:

$$\text{viability (in \%)} = \frac{\text{\# brine shrimp after exposure}}{\text{\#brine shrimp before exposure}} \times 100 \qquad (1)$$

Once the viability is calculated per well, data can be combined for each exposure condition and students can calculate the average, standard deviation, and other statistical transformations (e.g. t-testing). Using the combined data, students can then plot the data to see a graphical representation of the class results, making conclusions about the relative toxicity of Au and Ag nanoparticles based on the results. Variations to the viability experiment can be performed, such as varying the time of exposure (e.g. 24 vs 48 or 72 h) and number of replicates, depending on the aim of this experiment.

Page 8

Enhancing Understanding: Cover this section *after* the activity.
Discuss the observed results and then pose the question, "Should the use and disposal of silver nanoparticles be regulated?"

Expected Results:
Students should find a significantly lower viability rate for the brine shrimp in the 100% silver nanoparticles compared to the gold nanoparticles or the controls. The toxicity effects decrease as the concentration of the silver nanoparticles decrease.

Review the findings with students: Post the class combined data table and graphs. Ask students to discuss trends they notice. Ask for reasons for discrepancies between actual and expected results.

Assessment:
In addition to lab questions, students will be graded individually based on answers to the lab questions, quality of the data tables, data analysis, and presentation of results to class.

Resources:

1. Posgai,R; Cipolla-McCulloch,C.B;Murphy,K.R; Hussain,S.R; Rowea, J.J; Nielsen, M.G., (2011) "Differential toxicity of silver and titanium dioxide nanoparticles on *Drosophila melanogaster* development, reproductive effort, and viability: Size, coatings and antioxidants matter." *Chemosphere* Volume 85, Issue 1: 34-42
2. Tiwari, DK; Jin,T, and Behari,J., (2011) "Dose-dependent in-vivo toxicity assessment of silver nanoparticle in Wistar rats." *Toxicology Mechanisms and Methods*, 21:13-24.
3. Luoma, S.N, "Silver Nanotechnologies and the Environment : Old Problems or New Challenges?." *Project on Emerging Technologies*, Volume 15 (2008): 1-72. Available online at www.nanotechproject.org/publications/archive/silver/.
4. Marquis, B. J.; McFarland, A. D.; Braun, K. L.; Haynes, C. L. (2008) Dynamic measurement of altered chemical messenger secretion after cellular uptake of nanoparticles using carbon-fiber microelectrode amperometry. Anal. Chem. 80, 3431−3437.
5. Maurer-Jones, M.A., Love, S.A., Meierhofer, S., Marquis, B.J., Liu, Z., and Haynes, C.L. (2013). Toxicity of Nanoparticles to Brine Shrimp: An Introduction to Nanotoxicity and Interdisciplinary Science, *Journal of Chemical Education*, 90,475-478. Accessed at: http://pubs.acs.org/doi/pdfplus/10.1021/ed3005424.
6. Chinnapongse, S.L., MacCuspie, R.I., and Hackley,V.A., (2011). Persistence of singly dispersed silver nanoparticles in natural freshwaters, synthetic seawater, and simulated estuarine waters. *Science of the Total Environment*, 409(12):2443-2450.

Additional Resources:

Nanoparticles and Nanoparticle Toxicity

- Project on Emerging Nanotechnologies - http://www.nanotechproject.org/
- Center for Biological and Environmental Nanotechnology – http://cben.rice.edu
- International Counsel on Nanotechnology – http://icon.rice.edu/

*Development and distribution partially funded by the **National Science Foundation***

Page 9

• The Science Museum (UK): Nanotechnology: Small Science, Big Deal – http://www.sciencemuseum.org/uk/antenna/nano/

Nanoparticles in the Environment

• Center for the Environmental Implications of NanoTechnology (CEINT) – http://www.ices.cmu.edu/ceint/home.asp
• University of California Center for Environmental Implications of Nanotechnology (UC CEIN) http://www.cein.ucla.edu

Risk Assessment and Regulation

• Society of Toxicology Risk Assessment Primer - http://www.toxicology.org/ai/fa/riskassess.pdf
• Federal Drug Administration - http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/default.htm
• Environmental Protection Agency - http://epa.gov/oppt/nano/
• National Institute for Occupational Safety and Health – http://www.cdc.gov/niosh/topics/nanotech

Relevant Additional Experiments

• "Leaching of Silver from Silver-Impregnated Food Storage Containers." J.F. Hauri and B.K Niece. *Journal of Chemical Education* **2011,** 88 (10), 1407-1409.

National Science Education Standards (NSES) **Grades 9-12**

Content Standard A. Science as Inquiry
- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard B. Physical Science
- Structure and properties of matter
- Chemical reactions

Content Standard E. Science and Technology
- Understandings about science and technology

Content Standard F. Science in Personal and Social Perspectives
- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

Next Generation Science Standards

HS. Structure and Properties of Matter
- HS-PS2-6. Communicate scientific and technical information about why the molecular-level structure is important in the functioning of designed materials.

HS. Interdependent Relationships in Ecosystems
- HS-LS2-7. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity

Page 10

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
(with answers in red)

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

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

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)

*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1363
Rev: 10/2013

Page 11

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?____clear_____
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?_should change to purple ___
   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)

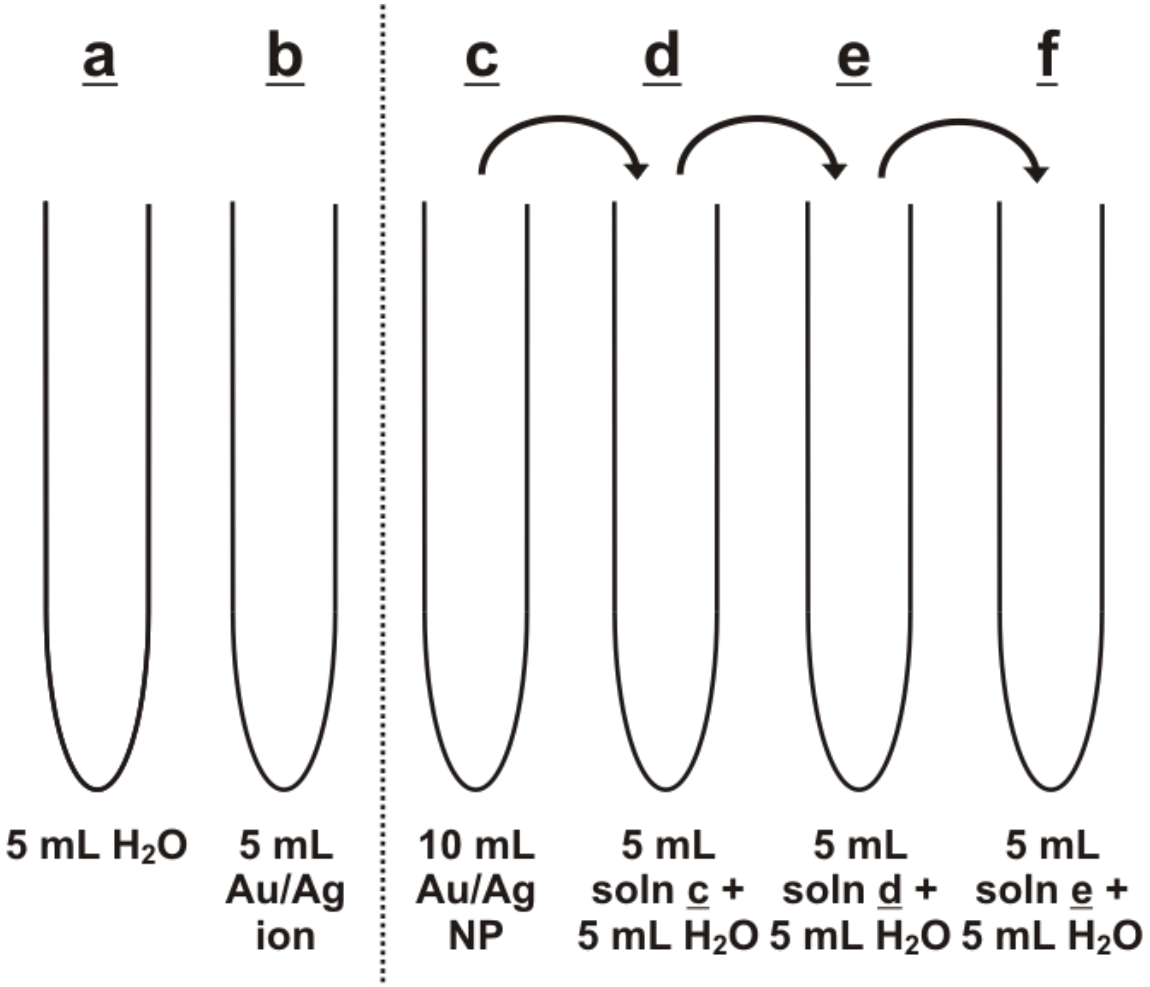
1) Add 13 mL deionized (d.i.) water to glass vial using a graduated cylinder
   Actual volume of water added: _____mL
2) Heat water on hot plate until small bubbles begin forming on side of vial (beginning to boil)
3) Add 1 mL of the 0.0075 M Ag solution and 1 mL trisodium citrate solution #2 (0.0075 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?_____clear_____
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 $NaBH_4$?__should change to yellow__
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 vial c + 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?_____50%_____
   b) vial e as compared to vial d?_____50%_____
   c) vial e as compared to vial c?_____25%_____

Page 12

d) vial f as compared to vial c?_____12.5%_____



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? *Nanoparticles are parictles that have at least one dimension between 1 and 100nm. They often have properties that differ from the bulk material. Au and Ag*

*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1363

Rev: 10/2013

Page 13

nanoparticles have different optical properties and large surface area in comparison to volume.

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. *Serial dilution.*

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.)

Page 14

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 vial a (control): _____
   Class average for vial b (ion): _____
   Class average for vial c :_____
   Class average for vial d:_____
   Class average for vial e : _____
   Class average for vial f:_____

Concluding Questions

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

*Development and distribution partially funded by the **National Science Foundation***

Page 15

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?

*Development and distribution partially funded by the **National Science Foundation***

Page 16

Guiding Lab Questions

Name_____ **Class period**_____

_____

_____

1. What is the research question for your experiment?

How do gold and silver nanoparticles affect brine shrimp viability?

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

If the concentration of nanoparticles increases, then the number of brine shrimp that survive will decrease (or remain the same or increase).

3. What is the independent variable in your experiment?

The concentration of nanoparticles

What is the dependent variable in your experiment?

The percent of brine shrimp that survive

4. What are the experimental groups for your experiment?

The brine shrimp samples that are exposed to different concentrations of gold and silver nanoparticles

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

The brine shrimp that are not exposed to nanoparticles; one group just to water and one group just to gold and silver ions in solution, i.e., the Au and Ag stock solutions.

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

The control serves as a basis of comparison OR the control shows that other factors such as the water, light, or other factors are not responsible for the effects observed.

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

The species of brine shrimp, the amount of liquid, the exposure time, the amount of light, the temperature, the serum solution.

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

Page 17

So that you know that only the independent variable (nanoparticle concentration) caused any observed differences in brine shrimp viability.

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

So that you have "repeated trials" or so that you can be sure that there was not something wrong with the brine shrimp .

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			

*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1363

Rev: 10/2013

Page 18

Table 2: Data for Gold 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 % 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 (<u>not</u> 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?

Student answers will vary. Look at their data table and graphs. Expected results would show least viability in the 100% Silver nanoparticles and then increasing viability to the

Page 19

50%, 25% and 12.5 % silver nanoparticles. The gold nanoparticles not expected to impact viability.

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

Student answers will vary. Look at their initial hypothesis, data table and graphs.

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.

Student answers will vary. Look at their data table and graphs.

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

ex: Have all group members count the brine shrimp and average the results

ex. Complete more rounds of the experiment

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.

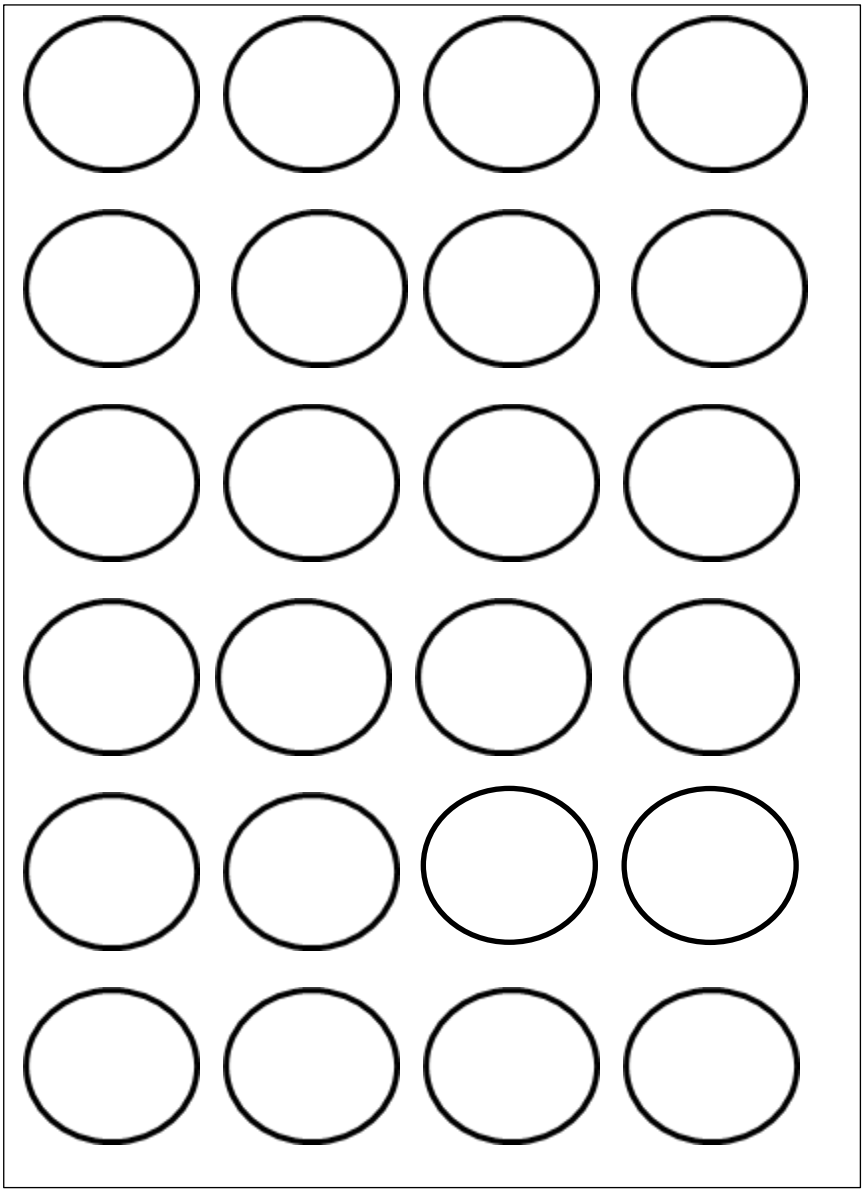
*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1363

Rev: 10/2013

Page 20

Day 3 Well Plate



*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1363

Rev: 10/2013

Page 21