

DNA and Red Signals Lead: Part 1

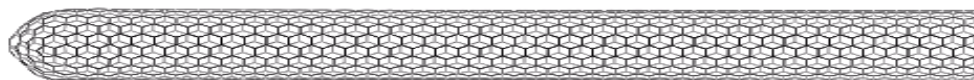
According to the Environmental Protection Agency, over 900,000 children in the United States currently have elevated levels of lead in their blood. Nancy Pavur became well aware of this fact after two weeks into a home renovation when her dog, Hero, died from lead poisoning. Three years later her daughter Elizabeth began to experience memory loss, one of the effects of lead poisoning. Her other two children are now showing other detrimental effects caused by lead poisoning. The lead present in her home in the form of paint and lead pipes was released into the air and water and subsequently taken in by the family.

In most cases, it is the younger children living in the inner city in homes built before 1978 who are affected by lead poisoning. Toddlers constantly test their environment through their senses, including taste, and tend to ingest the lead in the form of lead paint chips, dust on objects in the home, or in the soil around the home. Current studies have discovered that trace amounts of lead, one microgram per deciliter of blood, can result in a three quarters point drop in IQ. Damage to the brain and nervous system may result in the form of behavior and learning problems, slowed growth, hearing problems, and headaches. While small amounts of lead can cause brain damage, discovering even high levels of lead in the blood is difficult because traditional lead tests are expensive and inexact, oftentimes giving false-negative or false-positive results. To make matters worse, symptoms associated with lead poisoning are not immediately visible. Apparently healthy children may have very high levels of lead that acts as a neurotoxin in their blood streams. Thus, there is a clear need for easier, less expensive and more exact methods of testing for lead.

Task: Using this brief article and other resources develop answers to the following questions/ statements.

- What are some effects of lead poisoning?
- How much lead can a person or pet have in their system before falling ill or dying?
- Brainstorm some possible ways to quickly determine the presence of lead.

Reference: The WaterCAMPWS Center for Advanced Materials for Purification of Water with Systems at the University of Illinois at Urbana-Champaign. Red means lead. Retrieved March 12, 2007 from: http://www.watercampws.uiuc.edu/waterclear/labs/lessons/lead_teacher_guide.pdf



Laboratory Experiment

Introduction:

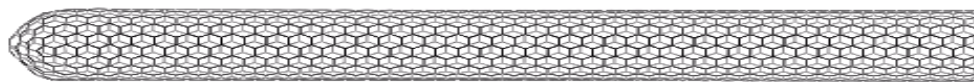
In this experiment, you will determine how to change gold nanoparticles from red to blue. You should keep complete records of your experimentation and the chemicals you are using. By the end of this experiment you should be able to answer the following questions in your laboratory report:

- If the solution is red to our eyes, what does this mean (what color is it absorbing)?
- How does a spectrophotometer relate to the color of the solution?
- Why does the solution change color (what are the particles doing)?
- What do the chemicals that change the nanoparticles have in common (what types of chemicals are changing the color)?
- How is this experiment a model of a biosensor (a device containing a biological sensing element that alerts you to the presence of particular molecules)?

Materials:

| Item | Number/Amount |
|---|---------------|
| Spectronic 20 | 1 |
| 1.0 mM H _{AuCl} ₄ | 30 mL |
| 38.8 mM Na ₃ C ₆ H ₅ O ₇ (sodium citrate) | 3 mL |
| Hot/ Stir plate | 1 |
| Magnetic stir bar (0.5 - 1 cm) | 1 |
| Distilled water | 33 mL |
| 50-mL beaker | 1 |
| 50-mL Graduated cylinder | 1 |
| 10-mL Graduated cylinder | 1 |
| Small clear cups, beakers, or vials | 6 |
| NaCl (table salt) solution | 3 mL |
| Sucrose (table sugar) solution | 3 mL |
| Vinegar (HC ₂ H ₃ O ₂) | 3 mL |
| Household ammonia | 3 mL |
| MgSO ₄ solution | 3 mL |
| KI solution | 3 mL |
| Isopropyl alcohol (C ₃ H ₇ OH) solution | 3 mL |
| Disposable pipet (or dropper) | 6 |
| Balance | 1 |
| Spectrophotometer tubes/ cuvettes | 4 |

WEAR GOGGLES and USE GLOVES in the lab!!!



Procedure:

Turn on the Spectronic 20 and let it warm up for at least 30 minutes while you complete this part of the experiment.

Part A: Preparing 13 nm-diameter gold nanoparticles

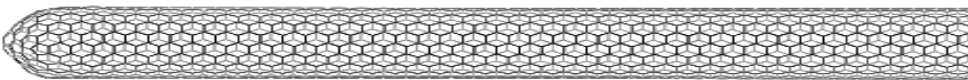
1. Into a 50-mL beaker, pour 33 mL of water. Add the stir bar. Carefully mark the water line on the beaker (you will need this calibration later). Empty the water and dry the beaker and stir bar.
2. Pour 30 mL of 1.0 mM HAuCl_4 into the marked 50-mL beaker. Add a magnetic stir bar. Heat the solution to boiling on a stir/ hot plate while stirring with the magnetic stir bar.
3. After the solution begins to boil, add 3 mL of 38.8 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$. Continue to boil and stir the solution until it is a deep red color (about 5 minutes). As the solution boils, add distilled water as needed to keep the total solution volume near 33 mL.
 - a. How does the solution visibly change?
4. When the solution is a deep red color, turn off the hot plate and stirrer. Cool the solution to room temperature before using in Part B.

Part B: Using gold nanoparticles as chemical selective sensors

1. For each chemical you choose to test (NaCl , sucrose, MgSO_4 , KI , vinegar, household ammonia, and isopropyl alcohol) place 3 mL of gold nanoparticles into a clear vial.
2. Use a dropper to add your chemical test reagents. As you only have a limited amount of chemicals, be sure to think about your procedure before adding chemicals. As you test, record all data and observations. Think about what variables you are changing, what should you change, what should you keep constant.
3. Using a spectrophotometer, determine the absorption vs. wavelength spectrum of three samples (original sample with no added chemicals, a sample that remained the original color, and a sample that changed color).
 - a. What does the absorption spectrum tell you?
 - b. Your initial nanoparticles are red, what color does it absorb? Why?

Data Analysis:

Using Excel, you should plot the absorbance vs. wavelength data for each of the three suspensions containing gold nanoparticles (original sample, sample that remained original color, sample that changed color). All three sets of data can go on the same graph. Determine the wavelength of light absorbed by each of the three samples. Relate these wavelengths to the colors that you see with your eyes.



Discussion:

- If the solution is red to our eyes, what does this mean (what color is it absorbing)?
- How does a spectrophotometer relate to the color of the solution?
- Why does the solution change color (what are the particles doing)?
- What do the chemicals that change the nanoparticles have in common (what types of chemicals are changing the color)?
- How is this experiment a model of a biosensor (a device containing a biological sensing element that alerts you to the presence of particular molecules)?

Results:

Using the data collected during the experiment and information gathered during the discussion, develop your results. What chemicals caused a color change? Why? What chemicals did not cause a color change? Why?

Conclusions:

The questions in the introduction and discussion as well as the questions below should assist in creating a good, solid conclusion.

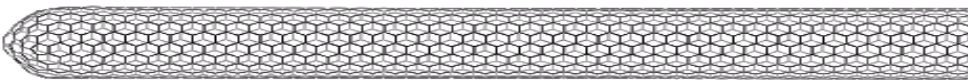
1. Based on the fact that the citrate anions cover the surface of each nanoparticle, explain what keeps the nanoparticles from sticking together (aggregating) in the original solution.
2. Why do some solutions produce different results than other solutions?
3. How could we use what we have found out to determine if lead (or another type of poison) is in a person's or animal's body?

Visiting the following site will also assist in developing your conclusions. Be sure to include what you have learned from them in your report.

1. Perform the NanoHub simulation: Nanosphere optics lab-biosensors from www.nanohub.org

McFarland, A.D., Haynes, C.L., Mirkin, C.A., Van Duyne, R.P., and Godwin, H.A. (2004). Color my nanoworld. *Journal of Chemical Education*, 81, pgs. 544A-544B.

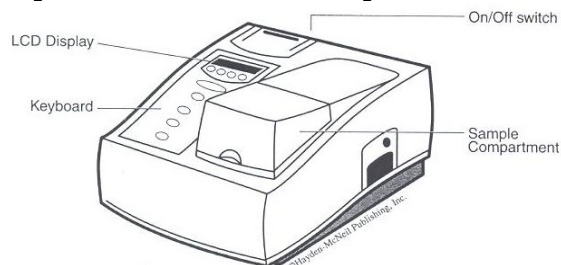
Purdue University Chemistry 115 Laboratory Manual. (Spring 2007). Chapter 13: Gold Nanoparticles



Calibrating the Spectronic 20 Genesys™ Spectrophotometer¹

Initial Preparation

- Make sure the cell holder in the sample compartment is empty before turning on the spectrophotometer.
- Turn the power switch (located at the rear lower left side) to ON (“I”).
- Allow the spectrophotometer to warm up for 30 minutes.



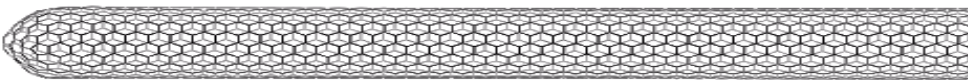
Making Measurements (general)

- Press the “A/T/C” pad to select the desired mode (A).
- Press the “nm (up)” or “nm (down)” pad to set the proper wavelength. (Holding down on the desired key changes the wavelength more quickly.)
- Insert the black reference solution into the cell holder in the sample compartment and close the door.
- Press the “0 ABS/ 100%T” pad to set the blank or reference. The spectrophotometer is now calibrated for the chosen wavelength setting. This procedure must be repeated if the wavelength is changed.
- Remove the blank and insert the sample into the cell holder. Close the sample compartment door. The measurement for the sample will appear on the LCD display.

Making Measurements (specific to lab)

- After the suspensions in cups have stood for at least 6 minutes collect absorbance data.
- Obtain two cuvetts. Rinse one cuvette with 2-3 mL of deionized water and discard the rinse into a waste beaker. Then fill the cuvette halfway with deionized water (this will be your blank). Rinse the other cuvette with 2-3 mL of the original gold nanoparticle suspension and discard this rinse into the waste beaker. Fill this cuvette halfway with the original gold nanoparticle suspension.
- Measure the absorbance of the suspension at 20 nm intervals from 370 to 810 nm, using the blank to recalibrate the Spectronic 20 after each change in wavelength (each data point). Discard the gold suspension in the waste beaker.
- Repeat this procedure for a suspension that remained the same color and one that changed color. Record absorbance data in your lab notebook.

¹Purdue University Chemistry 115 Laboratory Manual. (Spring 2007). Chapter 13: Gold Nanoparticles.

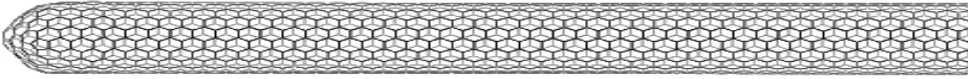


DNA and Red Signal Lead: Part 2

The public at large probably sees little relationship between DNA and testing of lead. Yet, the two have been merged through a relatively simple color change test, red indicating lead and blue indicating no lead. Dr. Yi Lu and his graduate student Juewen Liu, at the University of Illinois, Urbana/Champaign, have developed a seemingly simple test reliably indicating the presence or absence of lead. Basing their test upon earlier research using an increase of fluorescence as a positive indicator for lead, Lu and Liu wanted a simpler test: color.

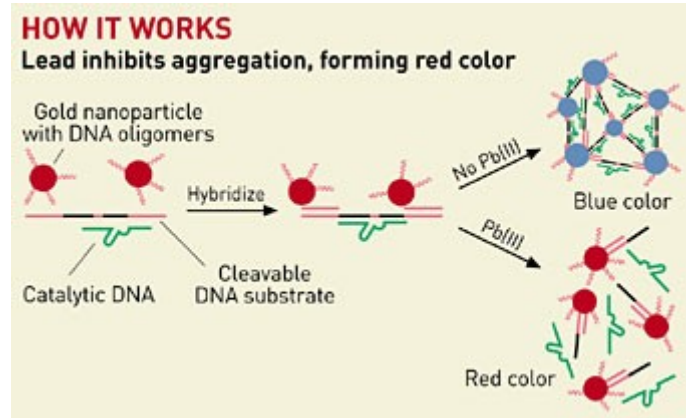
The ability to create a field test for lead coincided with the new technologies of the 1990's. The technological revolution and the resultant capability to create gold nanoparticles between 1 and 100 nanometers (nm) in diameter changed researchers' idea of "lead sensors". Gold nanoparticles can be adhered to a variety of substrates including silicon and bacteria cytoplasm. In addition when gold particles are linked with other substances, color changes occur. For example, the linking of gold nanoparticles to latex microspheres results in a color change from white to red; thus creating a reliable color change for that chemical reaction.

In addition to the work with gold nanoparticles, initial work in the early 1990's used DNA and RNA to recognize and bind to analytes or targets of interest. Oligonucleotides, a short DNA fragment typically of 15 or more nucleotides, are generally a single stranded, synthetic DNA molecule used as a probe or primer. Dr. Lu and Juewen Liu used these oligonucleotides and the color change associated with the gold nanospheres to create a new reliable simple test for lead detection. However, the research team of Lu and Liu took the known methods utilizing analytes to bind with DNA or RNA and changed the reaction process to an entirely different design to indicate the presence of lead. Their method was the first to use catalytic (cleavage or breaking of a substrate) events of DNA/RNA to design a lead sensor. The test itself involves a solution of catalytic DNA, a cleavable DNA substrate and gold nanoparticles with DNA oligomers. One might think of the reaction as the board game Clue in which there is a set number of characters with multiple outcomes. In the case of the DNA sensors, there are several players but only two outcomes, A and B. Under ideal conditions in which no lead is present the color blue is outcome A. If lead is present outcome B is the color red. While the test itself is seemingly simple, it is deceptively so, as anyone who has played Clue can tell you.



The set players in our game or “test” are:

- Gold nanoparticles with DNA oligomers
- DNA enzyme or DNAzyme (the catalytic DNA)
- Cleavable DNA substrate.
- Lead

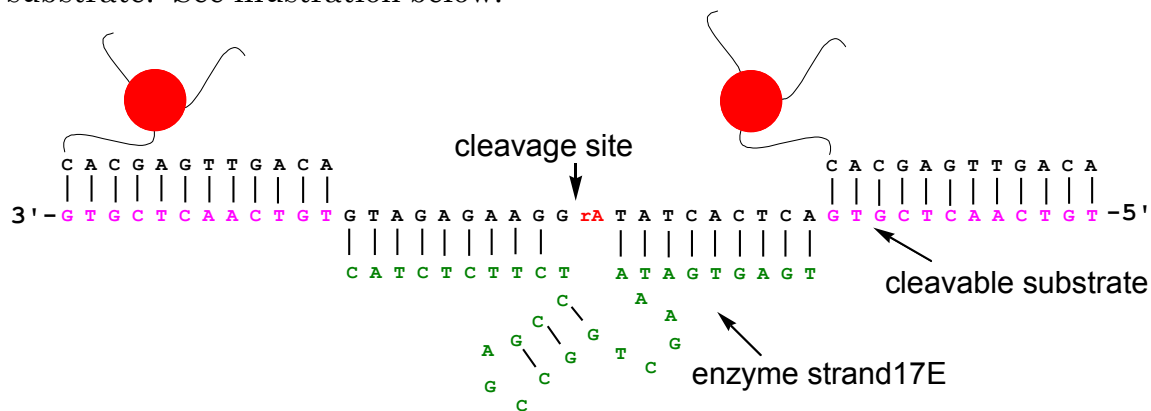


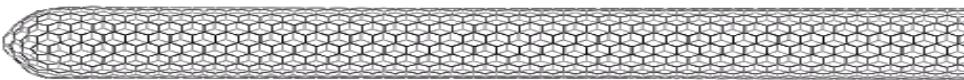
Dr. Lu and Juewen Liu chose gold nanoparticles because of previous work that demonstrated that different aggregate (crowded or massed into a dense cluster) states of the gold resulted in distinctive color changes.



Gold nanoparticles with oligonucleotides

The gold nanoparticles can be “functionalized” with DNA oligonucleotides, which are synthetically created to pair with the ends of the cleavable (breakable) DNA substrate. See illustration below.





The gold nanoparticles and associated DNA oligomers bond with the DNA substrate. The catalytic DNA acts just as a catalyst in chemistry, causing an increase reaction speed, but does not change themselves. If lead is not present in the solution, the gold nanoparticles, DNA oligomers and the cleavable DNA substrate aggregate causing the blue coloration. If lead is present, the reaction takes another turn. The lead causes the cleavable DNA substrate to break in two at the cleavage site shown above. Now, the gold nanoparticles, DNA oligomers and the cleavable DNA substrate cannot aggregate because the structures have been modified and the resultant solution color is red. Thus, the color change to either red or blue clearly indicates whether or not lead is present. This test can be easily conducted in the field by placing a solution containing the gold nanoparticles and DNA oligomers, the DNA substrate and the catalytic DNA in a test tube and adding the material to be tested. The test tube is held in the field testers hand at body temperature for about five minutes. The color change is then viewed for the presence of lead.

Further, the intensity of the red color in solution indicates the amount of lead present. The color range is likened to litmus paper in that it gives a definite color identifying the quantity of lead in solution. See figure below.

0% 0.02% 0.05% 0.08% 0.1% 0.5 % Pb(II) in leaded paint



Currently the EPA standard for lead paint is 0.05%. Paint containing less than 0.05% lead is not considered to be leaded paint. According to the figure above the color change is discernable at the 0.05% level.

The test developed by Dr. Lu and Juewen Liu is a dependable, reliable and simple field test for lead. Hopefully it can be used by people such as Nancy Pavur and those living in pre 1978 buildings to eliminate the potential harmful effects of lead.

To Watch the Video of this, GO TO:

<http://www.watercampws.uiuc.edu/waterclear/webquests/red-lead-webquest/RedSignalsLeadAnimation.html>

Reference: The WaterCAMPWS Center for Advanced Materials for Purification of Water with Systems at the University of Illinois at Urbana-Champaign. Red means lead. Retrieved March 12, 2007 from: http://www.watercampws.uiuc.edu/waterclear/labs/lessons/lead_teacher_guide.pdf