Antibiotic effects of silver nanoparticles  
Contra Costa Community College  
Biology 110  
Dr. Pam Muick Spring 2013

**Introduction to Nanotechnology**

CCC has partnered with LHS (Lawrence Hall of Science), UC Berkeley on a nanotechnology grant. The purpose of the grant to share information about nanotechnology with STEM students in local community colleges.

In the past 10 years, a new technology has emerged – nanotechnology – technology at a tiny scale. Nanotechnology usually refers to objects or devices between 1 n to 100 nanometers in size. It was inspired by biology and requires a multidisciplinary team approach. Biologists, physicists, chemists and engineers work together to produce materials that utilize new, novel nanostructures.

Today, you have probably used products that incorporate nanotechnology. Nano sized particles are commonly found in makeup and sunscreen; used to make socks smell-resistant; and to create stain and water resistant fabrics.

**Silver and Nanotechnology**

Silver is a chemical element, \textbf{Ag} on the periodic table. Silver is easily manipulated (shaped) and has been used for millennia to make coins, jewelry, “silverware”, dishes and tools. Also, silver has a long and important history in medicine and healing going back to ancient Greece and China where acupuncture needles were made of precious metals including silver.

In modern medicine and before antibiotics were invented (pre-1890’s) silver was used commonly to prevent infection and treat burns and wounds (silver sutures). Today, many hospitals and clinics use silver containing and silver coated materials to thwart antibiotic-resistant superbugs like MRSA. For example, silver coated catheters (to drain bladders) have been demonstrated to result in fewer urinary tract infections.

More recently, silver nanoparticles have become a hot research area. Two of the applications with greatest promise are water purification and, in medicine, wound care.

**Review of metric scale and nano**

But first, let’s refresh our understanding of the nano-scale. Earlier in the semester we reviewed the metric scale. On the metric scale, nano is one billionth of a meter and represented by the symbol “n.” In scientific notation it is written as $10^{-9}$.

So, can we see nano-sized objects with the light microscope? No. Nano objects are smaller than cells and even most bacteria; this means they are smaller than the nuclei and chloroplasts we have observed so far in class. Therefore, in terms of biological molecules, nano describes the size range between viruses and antibodies.

**What are potential uses and benefits of nanosilver particles?**

In preparation for lab, let’s discuss the potential uses and benefits of nanosilver particles. Silver nanoparticles are being used and tested for water purification and in medicine, particularly wound care.
Water purification Both High & Low Technologies

As we discussed earlier in the semester, all life on earth exists in aqueous solutions. Water is naturally purified through the hydrologic cycle. Yet, due to the number and density of human populations we exceed the natural cleansing capacity of the hydrologic cycle. According to the World Health Organization (WHO) one of the seven billion people on earth (14%) do not have access to clean drinking water. Unfortunately, one of the most common causes of death of children under 5 is due to effects of drinking contaminated drinking water (diarrhea, parasites, etc.).

Water filtration using silver nanoparticles has been implemented widely at both high tech and low tech levels.

High Tech
IN SPACE – High Tech: Two space stations – the Russian Mir and the International Space station - use electrolytically-dissolved silver to disinfect water drinking water in space.

ON EARTH – High Tech: New technologies enable hospitals and other large institutions to filter hot water through copper-silver filters. The goal is to control and prevent outbreaks of E. coli, Listeria spp. and Legionella spp. that cause disease in humans.

Low Tech
ON EARTH – Low Tech: In order to address the concern that 1 in 7 humans do not have access to clean drinking water, a number of low cost water filtration systems have been developed. World Health Organization (WHO) recommends several of the silver nanoparticle technologies for use in developing countries. One American example that has spread worldwide is the product of teamwork between a Guatemalan chemist and an American potter.

Ron Rivera, a Puerto Rican American potter, and Fernando Mazriegos, a Guatemalan chemist, developed and popularized a ceramic pot filter that utilizes silver nanoparticles. The silver is used to keep the filter from clogging up; the silver is not used to disinfect the water. Local potters make ceramic pot filters which are placed into buckets or other containers.

Families pour contaminated stream or well water into the filter which drains into the bucket. Studies have shown that the filters are able to remove a large percent (50-98 %) of the diarrhea causing bacteria and micro-organisms.

Even though the inventors could have patented this technology, they decided to make it freely available. Details are available for viewing and downloading via the internet, books, etc) (see Potters for Peace http://pfp.he207.vps.webenabled.net/wp-content/pdfs/Best%20Practice%20Recommendations%20for%20Manufacturing%20Ceramic%20Pot%20Filters%20June2011.pdf) to any individual or organization interested in addressing local clean water problems. Thousands of filters have been distributed worldwide by organizations such as Doctors Without Borders, Unicef, Oxfam, Red Crescent and Red Cross and many others.
Medical Applications

The most common medical use of silver nanoparticles (AgNP) is for wound healing – a continuation of an ancient application. For most medical applications, silver nanoparticles smaller than 100 nm in diameter are preferred because they are considered be less toxic to human cells than silver ions. When tested in the lab, silver nanoparticles disrupt the cell membranes of bacteria growing in petri dishes resulting in their death. However, the specific mechanism by which nanoparticles kill bacteria is not fully understood.

A major area of concern is the ability of nano-particles to cross the blood-brain and blood-testes barriers. In studies on mice and rats, nanoparticles were able to cross these barriers. Currently is seems that the type of substance as well as its size are important criteria.

“The blood brain barrier (BBB) is an anatomic-physiologic feature of the brain thought to consist of walls of capillaries in the central nervous system and surrounding astrocytic glial membranes. The barrier separates the parenchyma of the central nervous system from blood. The blood-brain barrier prevents or slows the passage of some drugs and other chemical compounds, radioactive ions, and disease-causing organisms such as viruses from the blood into the central nervous system.” (Mosby's Medical Dictionary, 8th edition. © 2009, Elsevier)

Finally, some of you may have heard about people drinking colloidal silver solutions as an “alternative medical” treatment (Dr. Oz). To date there are no studies supporting this as helpful or healthful. However, at the extreme, excessive consumption of silver can cause argyria, a non-reversible blue gray color changes to skin, eyes, internal organs, nails and gums.

Link to lab

Our biology lab will utilize student work completed in Dr. Sidharta’s Chemistry class. Students imbedded filter paper with silver nanoparticles. In our lab, we will use these filters to purify water containing yeast, representing bacteria. The less growth of yeast indicates the better quality of the filter paper in killing yeast. Conversely, more growth of yeast indicates that the nanoparticles were not imbedded successfully. This lab is part of an interdisciplinary study with chemistry, biology and materials engineering at CCC in partnership with LHS.
Experiment

Materials for instructor
Weigh boat and weighing machine
Yeast
Molasses
DI water
Spatula (weighing spoon)
Large beaker

Shared materials
Yeast/molasses solution (prepared by the instructor)
Water Bath(s)

Materials for each group of 2:
DI water
Fermentation Tubes (2)
Glass beakers (2)
Glass stirring rods (2)
Nanoparticle solution
Markers,
ruler,
watch to record time

PREPARATION & SAFETY
Remember that gloves and goggles must be used in preparing the solutions and that any solutions containing silver nanoparticles must be disposed of as hazardous waste (pink bucket).

PROCEDURE
• Working in groups of 2, get two fermentation tubes and label one of them “variable” and the other one “control”; also label them with your name.
• Get two glass beakers and label these in the same way.
• Draw horizontal lines on the vertical sections of each fermentation tube, as shown in Fig. 1 below. Aim for a spacing of about 0.5cm between each pair of lines, and continue drawing the lines until you reach the bottom of the tube. These lines will allow you to track the progress of the bubbles.
• Mark every 5th line with its respective number, as shown in Fig. 1 below. Measure the distances between the lines and record these in your notebook.
• To the “control” beaker, add 20mL of distilled/deionized water and 20mL of the aqueous yeast/molasses solution. Stir solution until it is mixed.
• To the “variable” beaker, add 10mL of distilled/deionized water, 10mL of the silver nanoparticle solution, and 20 mL of the aqueous yeast/molasses solution. Using a new or cleaned stirring stick, stir solution until it is mixed.
• Now transfer 30 ml of the solutions from the beakers into the fermentation tubes.
  o Each beaker contains 40mL of solution – you should transfer approximately 30mL of each solution into the respective fermentation tube. If there are
bubbles on top of the solution in the beaker, try to avoid transferring the bubbles.

- Transfer the contents of the “control” beaker into the “control” fermentation tube
- Transfer the contents of the “variable” beaker into the “variable” fermentation tube

- Move your fermentation tubes to a water bath. Record the time (minutes as well as seconds).
- Every time the solution in one of your fermentation tubes reaches a line, record the line number and the corresponding time, as shown in Fig. 2 below. (Fig. 2 shows the data for one tube – you will make two tables – one for each tube.)
  - If you see very little movement in your “variable” fermentation tube, inform your instructor.
  - If the solutions in your two tubes are behaving very similarly, inform your instructor.
  - If you notice either of these behaviors your instructor may tell you to re-start your experiment, using a different ratio of silver particle solution to water in your “variable” sample.

- Make notes about what you see, such as the bubble size.
- Keep monitoring your fermentation tubes until the bubbles have reached the last line, or your instructor tells you to stop.

**Figure 1:** Draw horizontal lines on the fermentation tubes. Number every fifth line.

**Figure 2:** Record the time (minutes and seconds!) at which the solution reaches each line.