STUDENT LABORATORY WORKSHEET
EXPERIMENT C: COLORIMETRIC GOLD NANOSENSOR

Student name:……………………

Date:………………………………..

In this experiment you will study the properties of *nanoparticles of gold* and their application as colorimetric sensors. In this worksheet we provide you with some background information as well as instructions for running the experiment.

AIMS:

- Knowing how gold nanoparticles are produced in a school laboratory.
- Understanding the effect of size on the properties of a familiar material such as gold
- Understanding the use of gold colloids as sensors for medical diagnostics

This lab activity has three parts:
Part A: Synthesize a colloid of gold nanoparticles
Part B: Test for the presence of gold nanoparticles
Part C: Test the gold colloid as a colorimetric sensor

Before you start… answer the questions:

Q1. What colour is gold?

………..

Q2. What do you know about the properties of gold?

………..
………..

Q3. List three application or uses of gold

………..
………..

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 233433

Read paragraphs 1A & 1B

1A. Properties of gold

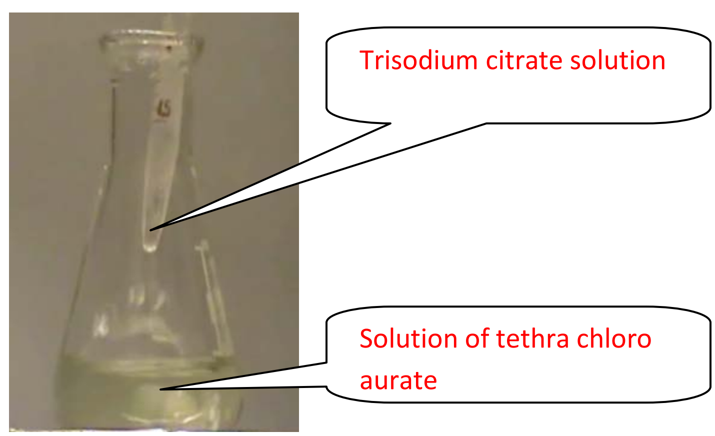
Gold (Au, atomic number 79) is the most malleable and ductile metal of all, it can be beaten to very thin sheets of material and rolled or bent as desired. This has been known and done for centuries. The **colour of pure** gold is metallic yellow ("golden"). You have probably seen or heard of "rose gold" or "white gold", but these are not made of pure gold, these are *alloys of gold*, they contain other metals such as copper and silver.

Gold is **very stable and not-toxic**, and for this reason is widely used in jewellery and dentistry because is air-inert and is not affected by most chemicals. Gold is also a **good conductor of heat and of electricity** (which is due to the fact that conduction electrons are free to move around the nucleus); it is **corrosion resistant** so it is used for electronic contacts and other electronic applications. Gold has also numerous other applications: for instance, thin layers of gold (so thin as to become transparent) are applied to the windows of large buildings to increase the amount of sunlight reflected back by the window. This way less air conditioning is required in summer to keep the building cool.

1B. Colloid and Solution

When gold nanoparticles are inside a material, such as water, they create a **colloid**. A colloid is different from a solution. A *solution* is a chemical mixture where the molecules of one substance are evenly dispersed in another (such as a salt solution); a *colloid* is another type of chemical mixture: the particles of the dispersed substance are only suspended in the mixture, they are not dissolved in it. A colloid is composed of particles in the range of 5-1000nm.

Colloidal gold can be made by mixing a *solution* of gold ions Au^{3+} and citrate *solution*. The size of the nanoparticles can be regulated by the concentration of the citrate solution, which acts also as a stabiliser.



Making Colloidal gold

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Q4. Does sugar mixed in water create a colloid or solution? Explain.

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……..

Q5. Do sand and water (muddy water) create a colloid or solution? Explain.

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……..

Colloids exist in nature and can be in the form of emulsion (such as milk), gel (gelatine), aerosol (fog), and many other forms. Even custard is a colloid! There are even nanoparticles in the natural colloids. For instance milk is a colloid, made of an emulsion of casein micelles (tiny tangles of casein protein) and liposomes (liquid fat droplets) in water. Casein micelles are a type of natural nanomaterial.

A simple way to **test whether a mixture is a solution or a colloid** is to shine a laser beam though the mixture:
The light will be scattered only by the colloid, as seen in the two colloids below. When shining a laser through a solution, the laser will be invisible.

WARNING: never shine a laser beam near the eyes nor look straight into the beam!!

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Experiment 1 A: Synthesize a colloid of gold nanoparticles

*** In this experiment you investigate the colour of nanoparticles of gold (or "nano-gold").**

Watch the teacher demonstration (or the video) and write your observations in the table. (Observe carefully the colour change during the time of the reaction.)

Q6. Record your observations in the table provided.

Colour of $HAuCl_4$ solution (*before* reaction)	**Colour *immediately* after the addition of the citrate**	**Colours *during* the reaction**	**Colour of the *final* gold colloid**

One of the interesting properties of nanoparticles is that their colour does not depend only on their composition (the material they are made of), but also on the area that their electrons are free to move in, that is – their size! So the colour changes observed in this experiment are not due to changes in the chemistry of the particles, but rather to the changes in their sizes.

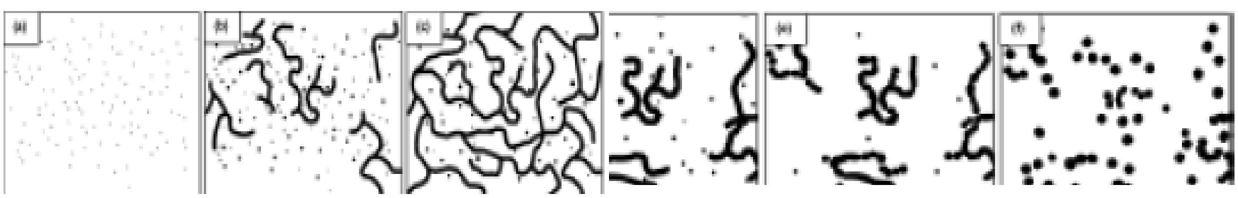

Figure 7. Illustration of the self assembly of nanoparticles : (Left) Growth mechanism of gold nanowires which were aggregated and then broke down to nanoparticles (Right) (Image credit: reprinted with permission from Pong et al., J. Phys. Chem. C 2007, 111, 6281-7. Copyright 2003 American Chemical Society.)

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Q7. Why do you think during the reaction some intermediate colours are seen?

……….

……….

Experiment 1B: Testing for the presence of gold nanoparticles

***In this experiment you will test whether a mixture is a solution or a colloid**

Q8. In the video clip (or the teacher demonstration), the gold began as a **solution** and became a **colloid**. What is meant by each of these terms [according to the information you read and the demonstration you have seen?

……….

……….

Shine a laser beam through each of the following samples. The presence of a colloid can be detected by the scattering of the laser beam from the particles.

(**WARNING**: never look straight into a laser beam!!!)

Q9. Record your observations in the table provided.

Sample	**Observed effect: laser beam scattered/not scattered**
Water	
Water with a few drops of milk	
Milk	
Salt water	
$HAuCl_4$ + water	
Trisodium citrate	
Gold nanoparticles (red)	

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Q 0. Based on your observations, which of the mixtures you have tested is a colloid? Why?

………

………

Note that the reaction of two solutions with no nanoparticles ($HAuCl_4$ + water+ Trisodium citrate), created by self assembly a colloid with nanoparticles (Gold nanoparticles).

Q11. Write in your own words what you learned from experiments 1A and 1B.

………

………

………

………

Experiment 1 C: Test the gold colloid as **colorimetric sensor**

***In this experiment you will test a colloid gold nanosensor to see if it can distinguish between an electrolyte (in our case salt) and sugar.**

Before you start the experiment… read paragraph 1.C

1C. Application of colloid gold in medicine (a biosensor)

Gold is now studied in many nanomedicine applications. Here we focus on one: its use as a colorimetric biosensor. Generally speaking, a *sensor* is a device capable of recognizing one or more specific chemical species within a mixture and “signalling” its presence through some chemical changes. A *biosensor* is a device that is capable of detecting a specific biomolecule, such as a type of antibody, a fragment of DNA, etc., which is chosen for its ability to confirm the presence of a certain type of virus or bacteria or a genetic problem which is responsible for a specific disease.

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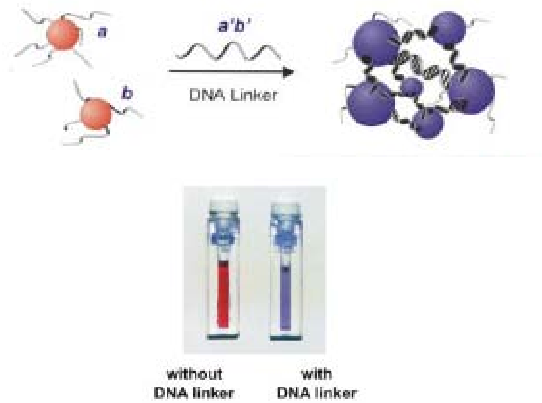

Figure 3. A colloidal gold sensor. (Image credit: reprinted with permission from Jin et al., Journal of American Chemical Society (2003), 125 (6), 1643- . Copyright 2003 American Chemical Society.)

In a *gold colloidal biosensor* the sensing event results in a change of aggregation among the nanoparticles that form the colloid. **Since the colour depends on size, this change of aggregation causes a colour change of the colloid.** For this reason the sensor is called *colorimetric* (from the word "colour").

Test your gold colloid and use it as a sensor!

MATERIAL NEEDED:

Chemicals:

- the gold colloid prepared in the synthesis (Part 3.), should be about 15 mL
- 0.5 g of NaCl, fine kitchen salt can be used as an alternative
- 2 g of sugar
- 1 fresh egg
- 1 litre of distilled water

Glassware/labware:

- Eye protection
- Latex or Nitrile gloves
- paper towels
- cylinders: 10mL cylinder, 50mL cylinder and 500 mL cylinder
- glass pipettes: 5 mL pipette and 25 mL pipette, 10 Pasteur pipettes

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- 50mL Erlenmeyer flask or beaker
- 2 disposable plastic capsules for weighing
- spatula
- glass bottles: bottle 500mL, 2 small bottles 25 mL or 2 beakers of 25 mL
- 6 glass vials
- 1 transparent plastic container

PRECAUTIONS

Use these materials with normal chemical precautions according to MSDS. Wear eye protection and gloves. Solids should not be inhaled and contact with skin, eyes, or clothing should be avoided. Wash thoroughly after handling.

METHOD:

1) Divide the ruby-red gold colloid into **six vials** each containing about 3 ml
2) Use one vial as control, and the other five to perform different colorimetric tests.

Now test your gold colloid and use it as a sensor for salt (NaCl).

Q12. Record your observations in the table provided

SAMPLE	**TEST**	**EFFECT OBSERVED (colour change)**
Control vial	-	
Vial 1	Add 6 droplets of NaCl solution	
Vial 2	Add 15-20 droplets of NaCl	
Vial 3	Add 10 droplets of sugar solution	

Note: Open a fresh egg and take with a Pasteur some egg white (about 1 mL or 2-3 full Pasteur), place it in an empty glass vial and add one Pasteur of distilled water. Mix gently together: it will form foam, so let it stand a minute to reduce the foam, then take the solution from the very bottom to avoid the foam/bubbles. Add this water/egg white mixture to the gold colloid.

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Test it!

SAMPLE	TEST	EFFECT OBSERVED (colour change)
Vial 4	Add some egg white	
Vial 5	Add 6 droplets of NaCl to Vial 4	

If an electrolyte is added, such as NaCl (salt), the nanoparticles stick together (aggregate), causing the solution to **turn deep blue**. If a high concentration of salt is added, the nanoparticles aggregate to the point that they precipitate, and the solution eventually **becomes clear and a black precipitate is seen**.

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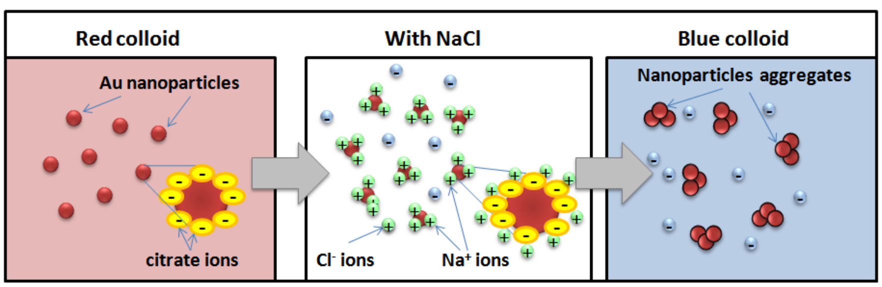
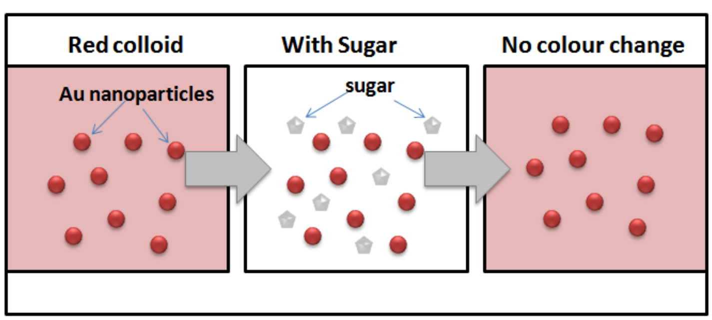


If a weak or non-electrolyte is added (e.g. sugar), the electrostatic repulsion between the gold and the citrate ions is not disrupted and the solution remains **red**.



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If a stabilizer of high molecular weight is added, such as a protein or polyethylene glycol, it adsorbs to the surface of the nanoparticles with the effect of inhibiting aggregation, even at high salt concentration.
*In this exercise egg white is used as a very economic source of protein (mainly ovoalbumin).

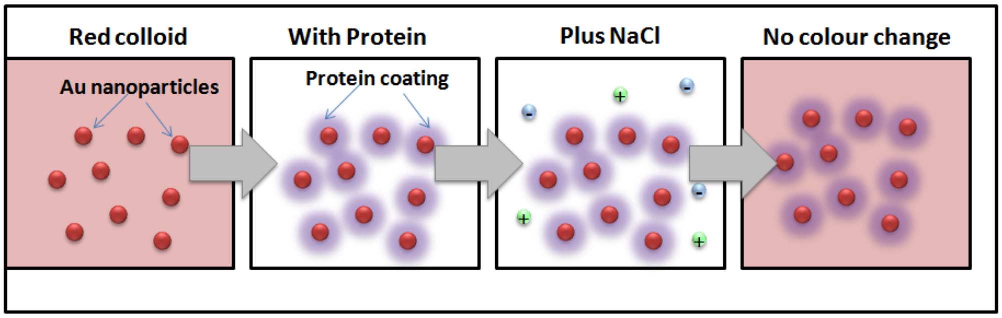

*Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0

Q13. How does colloid gold detect electrolytes, such as salt? (What indication do you have?)

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Q14. Why is there a difference in colour change in the experiment with salt and the experiment with sugar?

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The fact that the colour change depends only on aggregation makes the gold nanoparticles a useful tool in medical diagnostics. All that is needed is to attach the gold nanoparticles to a certain molecule that you want to find. These molecules are abundant and are relatively easy to attach to the gold nanoparticles. Once the target molecules are encountered, the different nanoparticles are drawn together, aggregate, and a colour change is visible.

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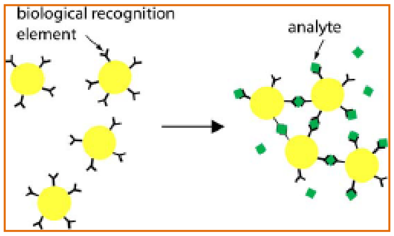

Schematic representation of a colloidal biosensor

One area where research is very active is the development of future miniaturized biosensors that doctors can use in their office to test if their patient has a specific disease.

For example: Rapid meningococcal test to save lives. The test uses nanoparticles of gold which are covered with antibodies that attract a protein present in meningococcal bacteria. If present, the particles bind and are readily detected by the colour change

http://mams.rmit.edu.au/otr11n41ry9q.jpg

DISPOSAL OF THE GOLD COLLOID

After the experiment, dispose of any gold colloid remaining as follows: add enough NaCl solution to the colloid to induce precipitation. Let the solution stand for at least 30 minutes (a black residue will form). Filter the residue on filter paper, and then dispose of it with solid normal waste. Dispose of the clear liquid in the wash basin with plenty of water.

CREDIT

This exercise was partly adapted from the experiment reported in: "Colour my nanoworld", Journal of Chemical Education, Vol. 81(4), 2004; a more detailed description of the synthesis of colloid gold is given in: Keating et al., Journal of Chemical Education 1999, Vol. 76, No. 7 pp. 949-955.

IMAGES TERM OF USE

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