Teacher Guide
EXPERIMENT C – COLORIMETRIC GOLD NANOSENSOR

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

BACKGROUND INFORMATION

In this experiment students use a *plasmonic colorimetric sensor made of nanoparticles*: the gold colloid (containing gold nanoparticles) is the sensing material, and the addition of an analyte (such as salt or sugar) induces a change in the aggregation of the nanoparticles in the colloid, which is reflected in a change in its colour. Here we give some background information of the properties of gold nanoparticles and their use in medical diagnostics, and we provide some details of the synthesis of the gold colloid.

1) About gold

In this experiment the sensor is made of **gold nanoparticles**. Most students will be very familiar with the colour of bulk gold and with some of its properties, but not with its properties at the nanoscale. 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. Gold creates alloys with many metals. The colour of pure gold is metallic yellow ("golden"), but other colours can be conferred by alloying gold with metals such as copper and silver. For instance "rose gold" is an alloy of gold and copper in high mass percentages (**Figure 1**).

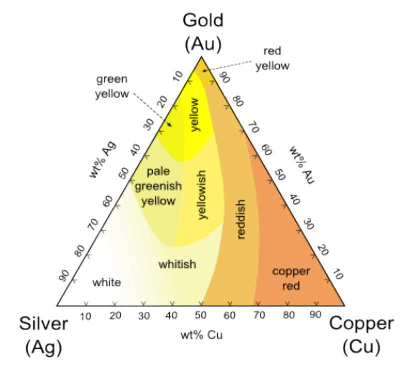

Figure 1. Approximate colours of Ag-Au-Cu alloys. (Image credit: Wiki commons, Creative commons Attribution ShareAlike 3.0)

NOTE: Some gold alloys have a colour different from the metallic yellow of pure gold but this is due to the presence of two metals in the alloy. Colloids of gold nanoparticles might have colours such as red, purple or blue, but are made only with gold.

Gold is very stable and not-toxic, and for this reason widely used in jewellery and dentistry because is air-inert and is not affected by most reagents. Gold is also a good conductor of heat and electricity (due to the fact that conduction electrons are free to move around the nucleus); it is corrosion resistant so it is used for electrical contacts and other electronic

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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 reflectivity of sunlight (and reduce transmittance), so that less air conditioning is required in summer to keep the building cool.

2) From bulk metals to nanosized metals ("nano-metals")

One of the distinguishing properties of metal nanoparticles in general is their *optical properties*, which are different from those of their bulk counterpart. This is due to an effect called localised surface plasmon resonance (LSPR), which is described in **Chapter 4 of Module 1 "Fundamental 'Nano-effects'"**. One of the consequences of the LSPR effect in metal nanoparticles is that they have very **strong visible absorption** due to the resonant coherent oscillation of the plasmons. As a result, colloids of metal nanoparticles such as gold or silver can display colours which are not found in their bulk form, like red, purple or orange, depending on the nanoparticles' shape, size and surrounding media. The energy of LSPRs is sensitive to the **dielectric function** of the material and the surroundings and to the shape and size of the nanoparticle. This means that if a ligand, such as a protein, attaches to the surface of the metal nanoparticle, its LSPR energy changes. Similarly the LSPR effect is sensitive to other variations such as the **distance between the nanoparticles**, which can be changed by the presence of surfactants or ions. The fact that the LSPR depends on the dielectric environment means that the refractive index can be used as the sensing parameter: changes in the local dielectric environment, induced by the sensing process, are used to detect the binding of molecules in the particle nano-environment.

3) Plasmonic colorimetric gold colloid sensor

In a plasmonic biosensor the nanoparticles can be dispersed in a medium (in which case the biosensor is a **colloidal plasmonic biosensor**) or supported on a surface (**surface plasmonic biosensor**). Both types of sensors exploit the fact that the sensing event changes the LSPR of the metal nanoparticles, but they use different readout report strategies:

In a **colloidal plasmonic biosensor** (for instance made of gold nanoparticles) the sensing event results in a change of aggregation among the nanoparticles that form the colloid (**Figure 2**), which can determine a colour change of the colloid.

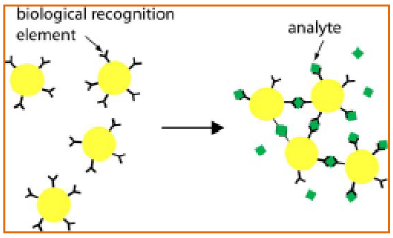

Figure 2. Schematic representation of a colloidal plasmonic biosensor.

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Absorption spectroscopy is used to quantify the biosensing event. In the case of gold colloid, which is normally red, the sensing event can result in the colloid becoming blue. Thus metal colloids can be used as **plasmonic colorimetric biosensors**. In nanomedicine this effect is used for instance for **genetic screening**, where scientists look for a specific gene sequence in a sample which can be indicative for a specific disease. **How is this done?** First, the sequence of bases in the target DNA is identified. Then two sets of gold particles are prepared – one has DNA attached that binds to one end of the target DNA, and the second set carries DNA that binds to the other end (**Figure 3**). The nanoparticles are dispersed in water. When the target DNA is added, it binds both types of nanoparticle together, linking them together to form an aggregate. The formation of this aggregate causes a shift in the light-scattering spectrum from the solution, that is, a colour change in the solution that can easily be detected. The example is illustrated in **Figure 3**. Since the colloid changes colour as a result of the sensing event, this is called **colorimetric sensing**. Gold colloids can be used for colorimetric sensing specifically because they have different colours depending on the environment that surrounds them.

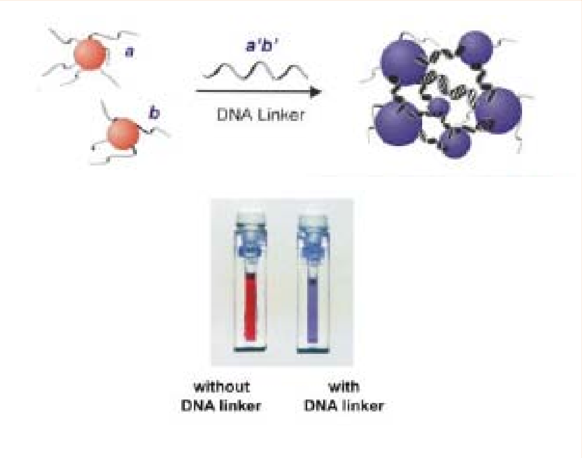

Figure 3. A plasmonic colloidal nanosensor. (Image credit: reprinted with permission from Jin et al., Journal of American Chemical Society (2003), 125 (6), 1643- . Copyright 2003 American Chemical Society.)

The method is not limited to sensing DNA strands but also other complementary biomolecules such as antigen-antibody systems; it is used to study the stability of proteins. In this experiment student will only use a *qualitative approach*: the outcome of the sensing process is a change in colour of the solution which is visible with the naked eye (visible absorption).

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4) Does nano-gold have other properties different from bulk gold?

Yes, it does. Nano-gold is extremely reactive and is now being studied as a **new catalyst**. Nano-gold has been shown to be an extremely efficient catalyst in numerous pollution control studies. For example, a company has announced an engineered nano-gold oxidation catalyst which can reduce diesel hydrocarbon emission 40% more than commercially available materials. Considering that there are over 14 million light-duty diesel vehicles worldwide, and 2 million heavy-duty ones, the impact of this nanotechnology could be enormous.

5) About the gold colloid synthesis

In the **present experiment** students prepare a colloid of gold nanoparticles in water starting from a solution of **gold chloride hydrate and a solution of sodium citrate**. This is the simplest reaction to synthesise gold nanoparticles, which was pioneered by J. Turkevich et al. in 1951 and refined by G. Frens in the 1970s. The method described in this reaction is adapted from Ref 1. This synthetic method generally produces nanoparticles 10-20nm in size, so the end-product is a **gold colloid**[1]. In the reaction, the citrate acts as a weak reducing agent (reducing $AuCl^{4-}$ to Au) and as a stabiliser. A layer of citrate anions adsorbs around each nanoparticle and prevents these from aggregating: the anions' electrostatic repulsion keeps the nanoparticles separated. In this state, the colloid appears ruby-red owing to the absorption of light by the free electron oscillations (the surface plasmon). The position of the surface plasmon λ_{max} for colloid gold is between 500 and 600nm depending on the particles' size and shape, as well as the solvent refractive index and inter-particle distance. In this experiment λ_{max} is around 520nm (green) and the solution appears red. A colour wheel can be used to show the correlation between the colour a substance absorbs and the colour it appears.

[1] In a *colloid* a substance is dispersed evenly throughout another one but the particles of the dispersed substance are only suspended in the mixture, they are not completely dissolved in it. The particles have dimensions in the 10-100 nm range. Natural colloids are in the form of emulsion (milk), gel (gelatine), aerosol (fog), etc.

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If the anion layer is removed, the nanoparticles start to approach and agglomerate. This effect can be used to *sense* a certain chemical. If a strong electrolyte is added, such as NaCl, the ions of the salt shield the negative charges on the particles, allowing them to approach and aggregate into larger and larger clumps. The formation of agglomerates is reflected in a change of the optical spectrum and the appearance of a second absorbance peak around 650-750nm, causing the solution to **turn deep blue**. If a high concentration of salt is added, the nanoparticles aggregate to a point at which they precipitate, the solution becomes clear, and a black precipitate is seen.

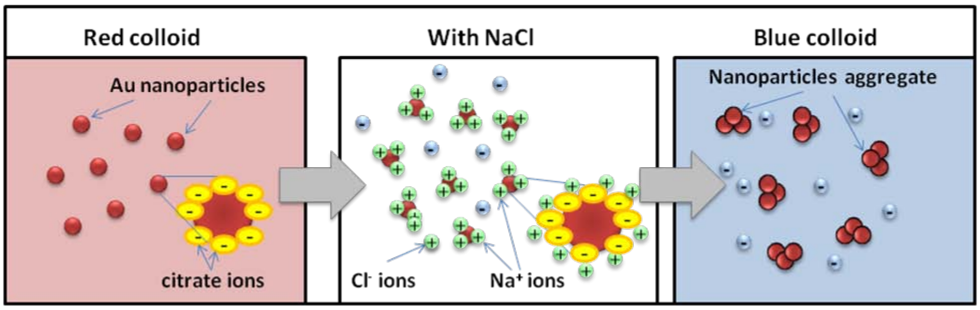

Figure 4. Schematic representation of the optical changes of a colloidal gold as a solution of salt is added. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0)

Other perturbations that can be easily observed are:

- **If a stabiliser 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 (**Figure 5**). In this exercise egg white is used as a very economic source of protein (mainly ovoalbumin).

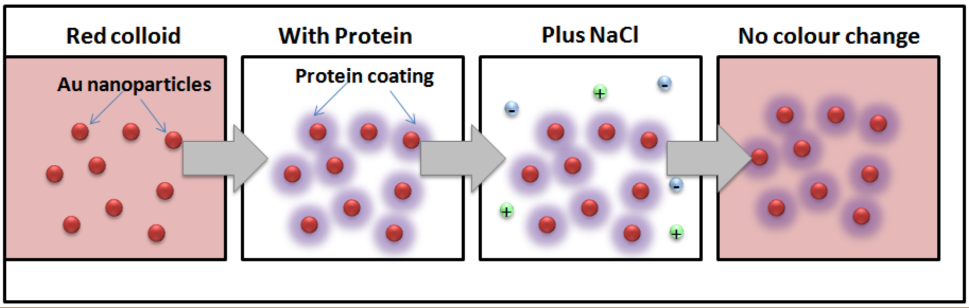

Figure 5. Schematic representation showing that a protein coats the gold nanoparticles and prevents them from aggregating as salt is added to the colloid. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0.)

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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. A small change in colour (e.g. from ruby-red to slightly pink) can be observed, which is the consequence of a very small agglomeration that occurs as the protein is added (**Figure 6**).

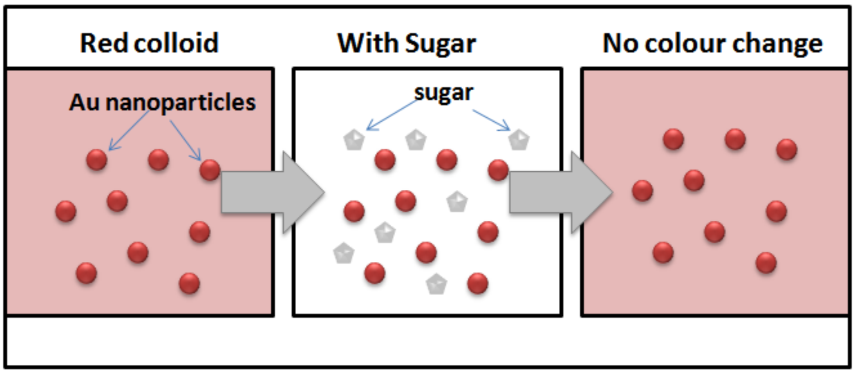

Figure 6. Schematic representation of the optical changes of a colloidal gold as a solution of sugar is added. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0)

One peculiarity of this synthesis is the colour change that is observed as the citrate is added to the gold chloride hydrate solution before it reaches the final ruby-red colour. The reaction starts with a solution which is yellowish (solution of $HAuCl_4$), and after the citrate is added, the colour changes first to clear, then light grey, dark blue-grey, then purple, dark purple, and finally ruby-red. Only recently have these intermediate colours been understood and correlated to the **formation of intermediate nanostructures before the formation of the final gold nanoparticles**. Specifically, TEM images have shown that after few seconds of citrate addition, **gold nanowires 5 to 8 nm are formed**, which are responsible for the dark purple colour. Beyond a certain threshold, the nanowires disintegrate into nanoparticles, and the solution turns ruby-blue.

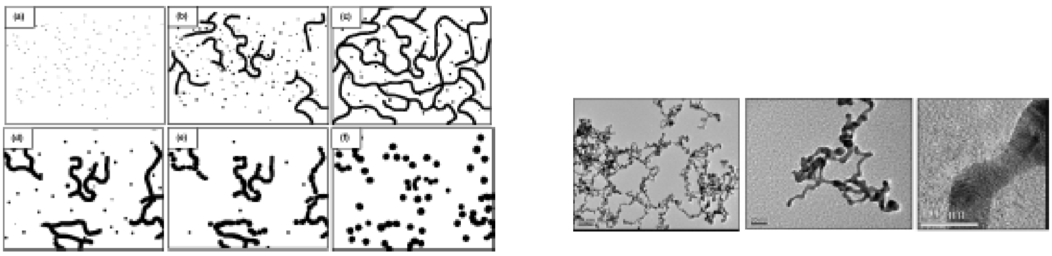

Figure 7. (Left) Growth mechanism of nanospherical gold particles synthesised by reduction of aqueous $AuCl_4^-$ by sodium citrate. (Right) TEM images of the dark intermediate showing an extensive network of gold nanowires which were isolated from the dark purple solution. (Image credit: reprinted with permission from Pong et al., J. Phys. Chem. C 2007, 111, 6281-7. Copyright 2003 American Chemical Society.)

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Although the chemistry and kinetics of this reaction would be too advanced for class teaching, this rapid and dramatic colour change during the reaction should be closely observed and this observation used to further support the notion that **size and shape at the nanoscale are very important factors** (**Figure 7**).

EXTRA TEACHERS' READING: Chapter 4 of Module 1 "Fundamental 'Nano-effects'" and Chapter 1 of Module 2 "Application of Nanotechnologies: Medicine and Healthcare" in the NANOYOU Teachers Training Kit in Nanotechnologies.

ABOUT THIS EXPERIMENT:

To visualize the concept of gold colloids as sensors for medical diagnostics students will:

1.A: watch a video clip on making gold nanoparticles made by Dr Luisa Filipponi, Aarhus University. Students will see during the chemical reaction process the formation of gold nanoparticles. In the process some intermediates are formed that have different colours. Or watch the experiment you will demonstrate.

1.B: Test the presence of a colloid by shining a laser beam through different materials.

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

1.C: Test the gold Nanoparticles as colorimetric sensor

DISCLAIMER: The experiments described in the following training kit use chemicals which need to be used accordingly to MSDS specifications. Follow school lab safety guidelines. Personal protection must be taken as indicated. As with all chemicals, use precautions. Solids should not be inhaled and contact with skin, eyes, or clothing should be avoided. Wash hands thoroughly after handling. Dispose as indicated. All experiments must be conducted in the presence of an educator trained for science teaching. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOYOU consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

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SUGGESTED STRATEGIES FOR TEACHING

Warm up activity: Questions & discussion:

1. Ask the students to answer questions 1-3

Q1. What colour is gold ?

Lustrous Yellow

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

- *lustrous–it has a shiny surface when polished*
- *Malleable–it can be hammered, bent or rolled into any desired shape*
- *Ductile–it can be drawn out into wires*
- *good conductor of heat and electricity*
- *gold alloys generally have high density*
- *gold alloys have a range of melting points but most are quite high*
- *gold alloys are often hard and tough with high tensile strength, meaning that they offer high resistance to the stresses of being stretched or drawn out and therefore*

Q3. List three applications or uses of gold

- *Gold is a soft metal usually alloyed in jewellery to give it more strength, and the term carat describes the amount of gold present (24 carats is pure gold)*
- *Metallic gold is applied as a thin film on the windows of large buildings to reflect the heat of the sun's rays*
- *Gold electroplating is used in the electronics industry to protect copper components and improve their solderability.*

2. Discuss and pose the question:

'**Are the properties of gold the same at the nanoscale?'**

Gold has been around for Ages

Show pictures of medieval stained glass.

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- Medieval artisans were the first nanotechnologists, without knowing it
- They made stained glass by mixing gold chloride into molten glass
- They created tiny gold spheres, which absorbed and reflected sunlight in a way that produces a variety of colours

After students have read paragraphs 1A & 1B in the students' worksheet document:

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

Sugar creates a solution, there are no sugar particles suspended in the water, the sugar grains have either dissolved completely (only separated sugar molecules left), or sunk to the bottom.

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

Although sand in water is technically not a colloid, since the grains are large enough to see, it is very similar to a colloid and is useful as mental visualisation of a colloid, as particles are not dissolved at all, but are suspended within the water.

1.A: Synthesize a colloid of gold nanoparticles

Show the video: synthesis for gold colloid

or

Demonstrate: synthesis for gold colloid (see appendix A).

* The video can be downloaded from the NANOYOU portal http://www.nanoyou.eu/ and is called VIDEO 1_gold colloid

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Q6. Record your observations in the table

Colour of $HAuCl_4$ solution (*before* reaction)	**Colour immediately after the addition of the citrate**	**Colours change during the reaction**	**Colour of the *final* gold colloid**
Pale yellow (very faint)	*Clear*	*Light grey* *Dark grey* *Purple* *Dark purple*	*Ruby red*

Q7. Why do you think during the reaction some intermediate colours are seen? (hint: explain in terms of particle size)

During the reaction the size of the nanoparticles is changing constantly and a whole range of colours can be observed, depending on the size and shape of the intermediate nanostructure, which includes gold nanoparticles and gold nanowires. At the end of the reaction gold nanoparticles about 15 nm are formed.

1.B: Testing for the presence of gold nanoparticles

Test samples of different mixtures with a laser beam.

Q . 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?

A colloid is different from a solution. A solution is a chemical mixture where the molecules of a substance are evenly dispersed in another one (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 completely dissolved in it. A colloid is composed of particles in the range of 5-1000nm.

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

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Q9. Record your observations in the table provided:

Sample	**Observed effect-laser beam : Scattered/Not scattered**
Water	*Not scattered*
Water with a few drops of milk	*Scattered (the path of the laser can be seen)*
Milk	*Light is scattered back (back scattered)*
Salt water	*Not scattered*
* $HAuCl_4$ + water	*Not scattered*
Trisodium citrate	*Not scattered*
Gold nanoparticles (red)	*Scattered (the path of the laser can be seen)*

* Note1: **HAuCl4** is the solution that was used in experiment 1A (before the reaction with Trisodium citrate).

* Note2: All solutions should be made with distilled water and once prepared they should be left still for a few minutes before being tested, because if they contain bubbles of air the test might give an erroneous result.

Q10. Based on your observations, which of the mixtures you have tested is a colloid? Why?

Nanoparticles are large enough to scatter visible light, while they are much too small to be seen by the naked eye (as well as too small to be seen with an optical microscope). With our eyes, however, we can see the colour change they produce.

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

- ***Colloidal gold is a suspension of nanoparticles of pure gold in water or a solution.*** *The colloid particles may have a size in the range of 5 to 1000nm*

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(and in the final product made in this experiment particle sizes are in the range of 15 nm).

- ***The colour** of gold changes at the nano scale.*
- *Gold nanoparticles appear ruby red in solution. **In fact a whole range of colours can be observed depending on the size of the gold nanoparticles.***
- *If you shine a laser beam through the colloidal solution the particles will scatter light.*
- ***Colloids exist in nature** and can be in the form of emulsion (such as milk), gel (gelatine), aerosol (fog), and many other forms.*

1.C: Test the gold colloid as a **colorimetric sensor** (biosensor)

1) Divide the ruby-red gold colloid into 5 vials each containing about 3 ml
2) Use one vial as control, and the other four to perform different colorimetric tests.

Q12. Record your observations in the table provided

SAMPLE	**TEST**	**EFFECT OBSERVED (colour change)**
Control vial	-	*Ruby-red gold colloid*
Vial 1	Add 6 droplets of NaCl solution	*Blue*
Vial 2	Add 15-20 droplets of NaCl	*Dark grey, and after a while, clear with black (aggregate) on the bottom*
Vial 3	Add 10 droplets of sugar solution	*No colour change (ruby-red)*

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SAMPLE	**TEST**	**EFFECT OBSERVED [colour change]**
Vial 4	Add some egg white	*Brighter red*
Vial 5	Add 6 droplets of NaCl to Vial 4	*No colour change (ruby-red)*

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

The nanoparticles aggregate in the presence of electrolytes, and this induces a change in their colour. The change in colour is a clear indication of aggregation.

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

The gold nanoparticles are covered by negative charges. Same charges repel each other, and this keeps the particles separated in the colloid. The salt (NaCl) is an electrolyte, meaning that it brings positive and negative charges inside the colloid (in the form of Na^+ and Cl^-). The positive charges that come from the NaCl shield the negative charges on the particles; in a sense they neutralize them. The result is that the particles can now approach each other, and this is reflected in a colour change (from red to blue). If one adds a lot of salt, the particles approach so much that they aggregate and precipitate. However, sugar is not an electrolyte, it is not charged and thus it does not change the distance between the particles and does not cause aggregation, so no colour change should be observed for sugar.

Summary (optional – can be used for final assessment)

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1. Is nano-gold different from the gold we are familiar with?

Compare bulk gold and nanoparticle gold

	Gold	**Nanoparticles gold**
colour		
Size		
Optical properties		
Applications		

2. Name a few natural colloids

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3. Why are gold nanoparticles so important in medical research?

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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.

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Appendix A - SYNTHESIS PROCEDURE for making a gold colloid

Below is the synthesis procedure for EXPERIMENT C

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MATERIAL NEEDED:

Chemicals:

- 0.1 g of $HAuCl_4$ * $3H_2O$ (Sigma Aldrich #G4022, 1g costs 144.50 Euro);
- 0.5 g of $Na_3C_6H_5O_7$*$2H_2O$ (Sigma Aldrich #S4641, 25g costs 24 Euro),
- 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
- 50mL Erlenmeyer flask or beaker
- 4 disposable plastic capsules for weighting
- Spatula
- Glass bottles: bottle 500mL, 2 small bottles 25 mL or 2 beakers of 25 mL
- Aluminium foil,
- 1 laser pen
- 1 hot and stirring plate
- 1 magnetic stir bar
- 1oven gloves

Also needed for laser test:

- 1 glass of milk

PRECAUTIONS

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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. **Be aware that $HAuCl_4 * 3H_2O$ is corrosive and must be handled with caution**.

DISPOSAL OF THE GOLD COLLOID

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

PROCEDURE:

A. Preparation of stock solutions

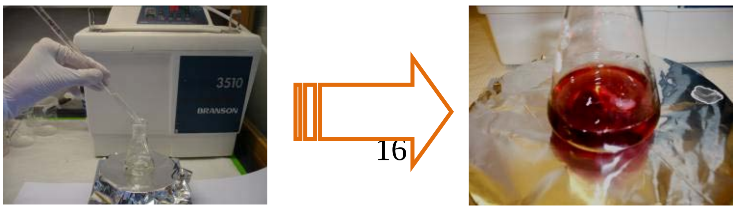
- 1.0 mM hydrogen tetrachloroaurate: Dissolve 0.1 g $HAuCl_4 * 3H_2O$ (orange solid) in 500 mL distilled water. Solution appears light yellow.

- **1% trisodium citrate**: Dissolve 0.5 g $Na_3C_6H_5O_7*2H_2O$ in 50 mL distilled water
- **~1 M NaCl**: Dissolve 0.5 g of NaCl (or table salt) in 10 mL distilled water.
- **~ 1 M sugar**: Dissolve 2 g of sugar in 10 mL distilled water.

B. Synthetic procedure of the gold colloid

1) Add 20 mL of 1.0 mM $HAuCl_4$ stock solution to a 50 mL Erlenmeyer flask on a stirring hot plate (turned off). The solution appears light-yellow. Add a magnetic stir bar inside the flask; turn the stirring on and the temperature on (about 120 C). Bring the solution to boil. To minimize the volume loss due to evaporation, once the solution is boiling, reduce the temperature to about 100 °C.

2) To the **boiling solution**, add all in one 2 mL of a 1% solution of trisodium citrate dihydrate, ($Na_3C_6H_5O_7*2H_2O$). The gold colloid gradually forms as the citrate reduces the gold(III). Observe the colour change as the citrate is added. Once the solution is ruby-red, turn the hot plate off and stop the stirring. Remove the flask from the hot plate. **WARNING**: the flask will be hot so use an oven glove to avoid burning your fingers!! Place the flask in a safe surface (e.g., table bench with a piece of aluminium foil)



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Before	End of

CREDIT

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