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Gold nanoparticle synthesis

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2 Works for me dx.doi.org/10.17504/protocols.io.bshhnb36

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ABSTRACT

This is a protocol for citrate reduction methods to synthesize gold nanoparticles. Gold nanoparticles (GNP or AuNP) are sub-micrometre particles with various sizes ranging from 1nm to 8 μm created from gold. Besides, they also have different shapes, including spherical, sub-octahedral, octahedral, decahedral, nano triangles, etc. Due to their properties, GNPs have been used in many areas such as electronics, nanotechnology and biomedicine. In biomedical application, spherical and rod shapes are mainly used. Spherical usually is synthesized using a chemical reduction method, while Rod-shape uses a seed-mediated synthesis method. Different shapes and sizes of GNP could give various optical properties and cellular uptakes; by this, GNPs have broader use in modern biomedical applications both in vivo and in vitro. The citrate reduction method was initially introduced by Turkevich, where we use trisodium citrate as a reducing agent. The method widely studied in 1951, a substantial amount of important information regarding this system has been deposited in literature. It was demonstrated that GNP's size variation could readily be realized by controlling the concentration of sodium citrate. Absorbance, zeta potential and size of gold nanoparticles (GNPs) will be determined by using UV/Vis, Zetasizer and Scanning Transmission Electron Microscopy (STEM), respectively.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

He, Z., Liu, K., Manaloto, E., Casey, A., Cribaro, G. P., Byrne, H. J., Curtin, J. F. (2018). Cold Atmospheric Plasma Induces ATP-Dependent Endocytosis of Nanoparticles and Synergistic U373MG Cancer Cell Death. Scientific Reports, 8(1).doi:10.1038/s41598-018-23262-0

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GUIDELINES

- The synthesis is considered to have failed if there is no colour change after 30 minutes. This could be due to ions present in the water or uncleaned glasswares.
- Gold nanoparticle batches must be stored in the refrigerator (2-8°C) to reduce the photo-induced oxidation and diminish heat influence. Do not freeze as they will irreversibly aggregate, the poor gold nanoparticles will have a blue-black colour. If the aggregate appeared, recover non-aggregated particles via filtration (0.2µm filter).
- Colloidal gold is stable for at least 1 year. However, GNPs stored for a long time might sediment at the bottom of the flask, so before use, swirling until having a homogenous solution.
- Use Tabular material presented in the Supporting information to determine the size and concentration of GNPs
- In Transmission Electron Microscopy, Regions containing gold will have a darker colour compared to regions containing carbon. This is because gold has a larger nucleus which scatters the electron beam much more when it passes through. Overlapping particles also have a darker image as they are thicker.

MATERIALS TEXT

A	B	C
Chemicals	**Equipment**	**Instrument**
0.086g HAuCl4	Duran bottle	Centrifugate
5g Na3C6H5O	Magnetic stirring	Ultraviolet Visible Spectroscop (UV/Vis)
Distilled water	Cuvettes	Zetasizer
	Pipette	Scanning Transmission Electron Microscopy (STEM)
	Folded capillary cell (DTS 10701)	Sonicator
	Micropipette	
	PCR tubes	
	TM grid	
	Tweezer	

BEFORE STARTING

All glassware and magnetic stir bars need to be cleaned through aqua regia (HCl/HNO3 3:1, v/v). Then oven-dried after rinsing with distilled water to avoid unwanted nucleation and aggregation of gold colloid solutions

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Synthesis of AuNP by Citrate Reduction

1 Dissolve **0.085 g of Chloroauric acid ($HAuCl_4$)** in **1 L Distilled water** into a clean Duran bottle

$HAuCl_4$ is corrosive so using glass to avoid contacting with metal

2 Heat with magnetic stirring and bring it to boiling. Keep the lid on loosely to remain evaporated water

3 Quickly add **5% (w/v)sodium citrate solution** to final **3:5:1 molar ratio** of citrate to **Au3+**

Sodium citrate solution is made by mixing **5 g trisodium citrate ($Na_3C_6H_5O$)** into **100 mL distilled water**

4 ⚠

Keep heating and stirring until the colour changes

The colour should change from purple to **wine red** in **00:20:00** to **00:30:00** . When it is stable, turn off the heat and keep stirring for **00:15:00** .

5 20m

Centrifugate gold colloid at **10.000 g** for **00:20:00** to concentrate the **AuNPs stock solution to 2500µg/ml**. Remove supernatant gently

Characterization of AuNP 1h 15m

6 AuNPs Absorbance Characterization by UV/Vis

The peak at around 550nm indicates the formation of Au particles

- Use the absorbance ratio of Table S-2 to determine the particle size of AuNPs. If the initial concentration of gold

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(CAu in mol per litre) used to synthesis the particles are known, then using the equation below can provide a precise way to calculate particle size in the range of 5-50nm

$$d = (A_{spr}(5.89\times10^{-6}) / C_{Au}\exp(C1))^{1/C2}$$

- Use table S-3 to determine the concentration (c) in mol per litre from the absorbance (A) at 450nm. The equation would be used is:

$$c = A_{450}/\varepsilon_{450}$$

Tables are in the Supporting Information link: ac0702084_si_001.pdf

6.1 Sample preparation

- Adding **a few crystals** into the bottom of the cuvette
- Fill with **Distilled water** until the mark
- Use a pipette to mix the solution into a homogenous solution

Only touch the frosted window to avoid dirty the clear window. This is because the light will pass through the clear window when processing

6.2 Set up UV/Vis

Turn on the instrument and the computer

- Open the software called Carry UV
- Choose SCAN
- Click SET UP
- Go to the BASELINE tab. Click BASELINE CORRECTION then press OK

6.3 Blank preparation

- Fill up two cuvettes with **Distilled water**
- Placing the cuvette into the Spectrometer

Before putting the cuvette into the machine, clean the clear side with tissue to make sure no fingerprint or any other residue left

- Close the lid

6.4 Sampling

- Using the blank sample as the control
- set up the wavelength in a range from 350-800nm, a resolution of 1nm

7 AuNPs zeta potential Characterization by Zetasizer

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7.1 Sample preparation

- Dilute GNPs with distilled water to the concentration of 0.1

If the mean counts rate < 100,000 counts/second, it means there is a low signal

7.2 Measurement

- Remove two tips of the folded capillary cell (DTS 10701) and rise with **Distilled water** to eliminate contamination
- Light shake and tap to clear the water out of the cell
- Slowly uptake **700 µl solution** into the micropipette
- Insert the micropipette tip into one of the two ports of the zeta cell and dispense the sample slowly and evenly

make sure to have no bubbles along the way. If there are bubbles in the cell, gently tap the size of the cell

- Plug each end of two ports back with the plastic tips
- Wipe the cell before insert into the instrument
- Insert the cell into the Zetasizer slowly

Two ports of the cell are facing left and right

- Close the lid

7.3 Sampling

- Open the Zetasizer software
- Go to SETTING and select **zeta potential** in the measurement type
- Put a sample name as “Gold nanoparticles”
- Choose **water** as in the DISPERSION
- The CELL TYPE is folded capillary cell
- For GENERAL OPTION, select **standard model**
- For MEASUREMENT, select **automatic**
- Insert the number of measurement then choose OK
- Click START

Always check the phase and the frequency plot during each run to avoid sample degradation. If there is a change in the phase or the frequency plot, it indicates that sample degradation has occurred. In case there is a sample degradation, reducing the number of runs or the voltage can be used.

8 AuNPs size Characterization by Scanning Transmission Electron Microscopy (STEM)

1h 15m

8.1 Sample preparation

- Use micropipette to add **50 µl distilled water** distilled water into

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50 µl gold nanoparticles solution

- Place the PCR tube into a beaker and put it in the Sonicator for **00:15:00** to get a homogenous solution
- TM grid is held by a tweezer
- Drop **20 µl solution** using the micropipette onto TM grid
- Leave the droplet evaporates for around **01:00:00**

Can leave under the light to speed up the process of evaporation

- The sample can go to the TM as soon as when it dry

8.2 Set up and measuring

- Release the clamp ease in the screws of the sample holder
- Place the sample into the sample holder
- Tighten the screws down
- Put the holder into the Microscope and wait for the Microscope to pump down the sample chamber
- Aligning the Microscope and Inserting camera
- Magnify the square of the grid that is interested
- Increase magnification to **40,000 time** of the image would be good for measuring size distribution of the nanoparticles

Do not choose the regions that are dense as they are not good for size determination

9 Dilute AuNPs stock solution in water or culture medium in desired concentration for testing

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