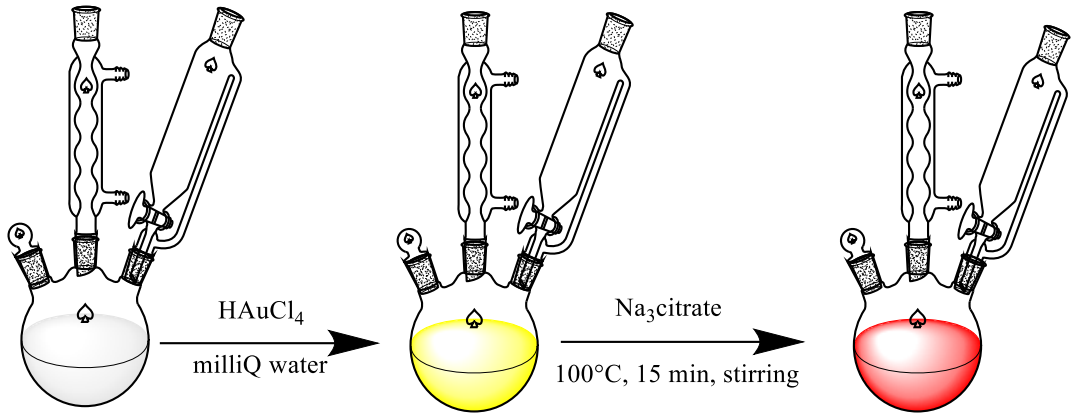
NANOBIOTECHNOLOGY LABORATORY

Academic year 2022-2023

Synthesis of 12-13 nm diameter colloidal Au nanoparticles.

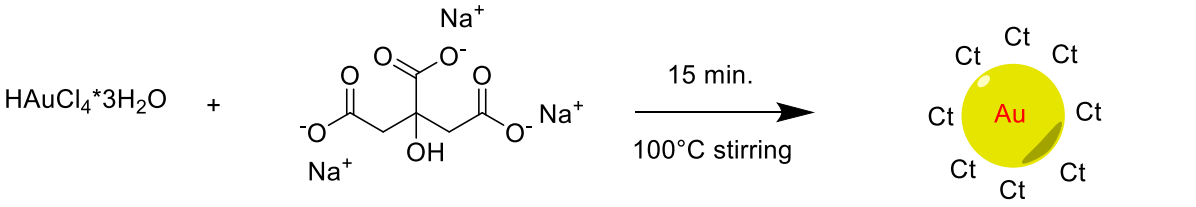
Rescaled procedure from *J. Chem. Educ.* **1999**, *76*, 949–955.



Reagents and solutions that will be used

Name	***Quantity***
Solution 1 mM $HAuCl_4$	100 mL (0,34 mg $HAuCL_4$ in 1 mL)
Solution 38.8 mM Na_3citrate	10 mL (0,1 g Na_3citrate in 10 mL H_2O milliQ)
PVP polyvinylpirrolidone 15K	0,1 mg/mL
α-chymotrypsin	1 mg/mL
NaCl 1 M	0,100 mL

Scheme of reaction



100 ml 1mM sol.

1% 38.8 mM sol.

12-13 nm gold colloid 17 nM

Procedure

1) In a three-necked round bottom flask put a magnetic stir bar and add 100 mL of 1 mM $HAuCl_4$ trihydrate. Bring the solution to a boil while vigorously stirring on a magnetic stirring hot-plate.

NOTE: the color of the solution is bright yellow.

2) Put 10 mL of 38.8 mM sodium citrate (Na_3citrate) in a dropping funnel. The funnel is then inserted in one of the necks of the flask. When the solution is boiling, open the sink so that all the solution is added all at once in the bottom flask, always under vigorous stirring.

3) In a few minutes the color of the solution changes from bright yellow to transparent, then dark blue, ultimately deep red-burgundy.

4) After 15 minutes turn off the heating and maintain the stirring for another 15 minutes, allowing the solution to cool down.

5) Once the solution has cooled down, transfer it in one Erlenmeyer flask with a funnel.

NOTE: close the flask with a stopper and mark the name of the group on it.

6) Take 9 UV-Vis cuvettes from the box paying attention not to touch the side where the optic beam of the instrument will incise (indicated with a transparent little triangle on the cuvette).

7) With a micropipette transfer 0.1 mL of the colloidal solution in 6 different cuvettes, then add 0.4 mL of milliQ H_2O to obtain a dilution factor of 1/5.

Group A) Steric stabilization of the colloids with α-chymotrypsin

1) In 8 cuvettes, add different aliquots with increasing volume of α-chymotrypsin (the concentration of the stock solution is 1 mg/mL) to obtain a different protein concentration in each cuvette. The range of the concentrations is 0.8-64 μg/mL (0.8 μg, 3.2 μg, 8 μg, 16 μg, 24 μg, 32 μg). In the last cuvette the protein is not added, but it will serve as control.

2) Close the cuvettes and wait ca. 10 minutes.

NOTE: mark the added protein concentration on the cuvette, the cuvette used as control will be signed as "control".

Group B) Stabilization of the colloids with a polymer, polivinylpirrolidone (PVP)

1) In 7 cuvettes, add different aliquots with increasing volume of PVP (the concentration of the stock solution is 0.1 mg/mL) to obtain a different polymer concentration in each cuvette. The range of the concentrations is 1-6 μg/mL (1 μg, 2 μg, 3 μg, 4 μg, 5 μg, 6 μg). In the last cuvette the polymer is not added, but it will serve as control.

2) Close the cuvettes and wait ca. 10 minutes.

NOTE: mark the added protein concentration on the cuvette, the cuvette used as control will be signed as "control".

Study of the stability of the protected colloids with UV-Vis spectroscopy

The purpose of this experience is to investigate the stability of the different colloidal solutions passivated by PVP or by **α**-chymotrypsin and determine which is the minimum concentration needed to stabilize the system.

1) Operations are carried out with the solutions inside the cuvettes previously prepared with the Au colloids and the different concentration of protein or polymer.

2) In the 9 cuvettes (excluding the control one) 0.1 mL of 1M NaCl solution are added.

NOTE: pay attention to the color changes compared with the control, they are a useful indication about the stability of the colloidal solutions.

3) Close the cuvettes.

9 different spectra will be acquired by the supervisor (one for each solution inside the cuvettes)

From the comparison of the different spectra obtained with the "control" it will be possible to determine the minimum concentration needed to maintain the colloidal solution stable.

The same procedure will be performed on the 7 cuvettes containing PVP.

Preparation of the solution for DLS and TEM

In order to prepare the solutions that will be used for preparing the sample for NTA, DLS and TEM the colloidal solution inside the Erlenmeyer flask is utilized.

1) Take 2x (0,5) mL of solution and transfer it into two different 1.5 mL Eppendorf.
2) One will be used as control in the DLS experience, whereas in the other one it is added the sufficient concentration of polymer or protein to ensure the stability (see UV-Vis experience). Wait 10 minutes and deliver them to the supervisor.
3) The solutions are centrifuged (14000 rpm, 15 min.) to precipitate the stabilized nanoparticles while the polymer or protein in excess will remain in solution.
4) Transfer the supernatants (0.2 mL) into two different UV-Vis cuvettes, paying attention not to re-suspend the precipitate.
5) Add 0.5 mL of milliQ H_2O to the precipitate. Close the Eppendorf and shake till a homogeneous solution is re-obtained.

DLS experience (Dynamic Light Scattering)

The purpose of this experience is to determine, via DLS, the average hydrodynamic radius of the synthesized Au colloids

The DLS instrument doesn't allow to directly visualize the nanoparticles but gives information about the dimension and the average distribution of the nanoparticles based on the intensity of the light scattering of the sample in an established range of time.

1) Take 200 µL from the previously prepared solution and transfer them inside a UV-Vis cuvette.

2) In a second cuvette transfer 200 µL of the solution prepared as control.

By the comparison of the two results, it will be possible to obtain important information about the dimension of the nanoparticles and observe mutual changes of dimension given by the steric hindrance of the protein or the polymer compared to the measures obtained by TEM.

Preparation of the sample for TEM analysis

1) With a tweezer (it is a special tweezer, ask the supervisor) take one TEM grid, lay down the tweezer on the laboratory bench paying attention not to fold or touch the TEM grid with bare hands.

NOTE: do not touch the center of the grid with the tweezer, stay on the edge.

2) With a micropipette take 5 µL of the solution from the Erlenmeyer flask and drop it at the center of the TEM grid. Then, cover up the TEM grid with a glass funnel or a watch glass to avoid the evaporation of the water during the night.

3) The day after, insert the TEM grid inside an Eppendorf flask with the tweezer, close it and write the name of the group on the flask to avoid confusion.