Synthesis and Characterization of Gold Nanoparticles

Version 01/2014

by Damian Brock

1. Introduction

Gold nanostructures have been a subject of intensive research, particularly for their surface plasmon resonance (SPR) properties. The SPR is an optical phenomenon arising from the interaction between an electromagnetic wave and the conduction electrons in a metal. Under the irradiation of light, the conduction electrons in a gold nanostructure are driven by the electric field to collectively oscillate at a resonant frequency relative to the lattice of positive ions. At this resonant frequency the incident light is absorbed by the nanostructure. Some of these photons will be released with the same frequency in all directions, and this process is known as scattering. Another part of the photons are converted into vibrations of the lattice (phonons), and this process is referred to as absorption.

In general, the SPR peak of a gold nanostructure should include components from scattering and from absorption. The cross-sections of these two components can be substantially different depending on the size and shape of the nanostructure. For gold nanospheres about 50 nm in diameter, the SPR peak is positioned at 520 nm, and this peak is responsible for the ruby red colour displayed by conventional gold colloids. Michael Faraday was the first person to observe this spectacular phenomenon. In 1857, he prepared the first stable suspension of gold colloids by reducing gold chloride with phosphorus in water. Some of his original samples are still preserved and on display at the Faraday Museum in London. The history of gold nanoparticles is nevertheless much older. Gold nanospheres have been used to make coloured glasses for almost two thousand years. This is probably the first practical application of nanomaterials in history, although clearly the people who made the glasses had no idea about the mechanism of colouration. Another example comes from the Lycurgus cup (Roman 400 A.D.), which reflects green light and transmits ruby red light. This is again built upon the unique scattering and absorption properties of gold nanospheres embedded in the glass.

Present applications of gold colloids are in the fields of colorimetric sensing, bioassay applications, as contrast enhancing agents for optical imaging, and for cancer phototherapy treatment. Bioassays utilize the strong absorption of biomolecules, especially via thiolate/thioether or imine functions to gold surfaces and nanoparticles.

In this manner particles can be functionalised with antigens or antibodies. The binding of nanoparticle loaded antibodies or antigens to their biological partners is conveniently monitored by electron spectroscopy in the optical or near infrared. The goal in photothermal therapy is to use near infrared light to image and kill cancer cells via selective heating. For the phototherapy treatment of cancer cells gold nanocages are bioconjugated with antibodies for selective targeting of cancer cells. Exposure of the nanocages to light of appropriate energy converts the absorbed photons into phonons, leading to a temperature increase of the lattice. If brought on surfaces with poor heat conduction, excitation by laser pulses increases the temperature of the nanocages beyond their melting point! In biological systems, the tissues and cells are essentially in a water environment. Irradiation with short laser pulses of appropriate energy creates high lattice temperatures of up to 1100 K without destroying the particles. Heat dissipation from the hot particles creates a temperature jump to far beyond where the cancer cells survive (ca. 330 K) and selectively damages the targeted cancer cells. Thus, the combination of high repetition rate of near infrared and ultrafast laser sources with gold nanocages is very promising for photothermal therapy.

2. Theory

For biomedical or other applications, it is essential to selectively produce gold nanoparticles that absorb in a spectral region where the surrounding tissue is more or less transparent, such as it is the case for $\lambda > 800$ nm in the near infrared. Absorption and scattering properties of gold nanoparticles depend on their size and shape. The surface plasmons can be quantitively calculated by solving Maxwell's equations as it was introduced by Gustav Mie. The total extinction cross section composed of absorption and scattering is given as a summation over all electric and magnetic multipole oscillations. The dependence of the surface plasmons on the shape of the metal nanoparticles is accounted for by introducing the appropriate

boundary conditions. For spherical particles that are small compared to the wavelength λ of the exciting light ($\lambda \gg 2R$, for gold $2R < 25$ nm), only the dipole absorption of the Mie equation contributes to the extinction cross section C_{ext} of the nanoparticles. The Mie theory then reduces to the following relationship (quasi-static or dipole approximation):

$$C_{ext} = \frac{24\pi^2 R^3 \varepsilon_m^{3/2}}{\lambda} \frac{\varepsilon_2}{(\varepsilon_1 + 2\varepsilon_m)^2 + \varepsilon_2^2}$$

(1)

In this equation, ε_m is the dielectric constant of the surrounding medium, and $\varepsilon = \varepsilon_1 + i\varepsilon_2$ is the complex dielectric constant of the particle. From equation (1) a resonance peak occurs whenever the condition of $\varepsilon_1 = -2\varepsilon_m$ is satisfied. This is the SPR peak, which accounts for the brilliant colours of various metal nanoparticles. Fig. 1 shows the extinction spectra calculated using Mie theory for gold nanospheres of different sizes. Their SPR peaks are located at ca. 520 nm. The peak slightly shifts to the red and becomes broader as the particle diameter increases to 100 nm. The broadening can be attributed to enhanced radiation damping for large particles. It is important to note that the position of SPR peak of gold nanospheres depends on the dielectric constant of environment, thus, different solvent or adsorption of a capping agent onto the nanospheres may result in slight variation for the SPR peak position. In addition, aggregation of gold nanospheres will lead to a pronounced colour transition from red to purple. This is due to plasmonic coupling between particles. These colour changes are instrumental in fabricating colorimetric sensors or biosensors.
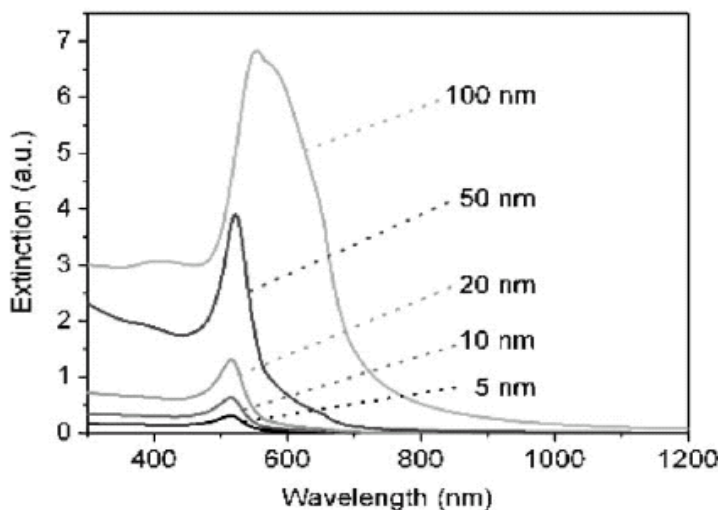

Figure 1: Extinction spectra calculated using Mie theory for gold nanospheres with diameters varying from 5 nm to 100 nm.

The shape of a particle can drastically change the SPR properties. For rod-like nanoparticles (nanorods), the boundary conditions in Mie theory have to be modified to describe their plasmonic properties. Therefore, it is essential to know which shapes are formed by different routes of synthesis (Fig. 2,3 and 4).
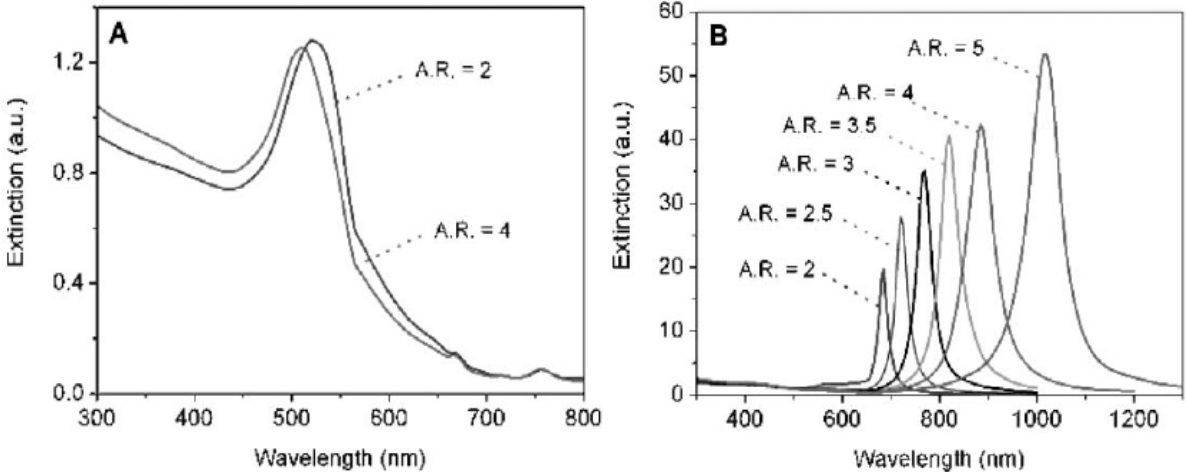

Figure 2: Extinction spectra calculated using the DDA method for gold nanorods of different aspect-ratios with a fixed width of 20 nm: (A) the transverse and (B) longitudinal mode, respectively.
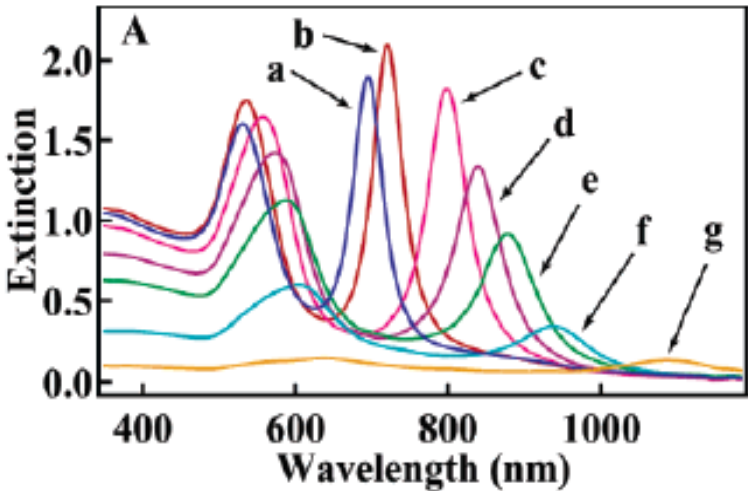

Figure 3: Extinction spectra of gold bipyramids grown with varying amounts of seed solution and different aspect ratios.
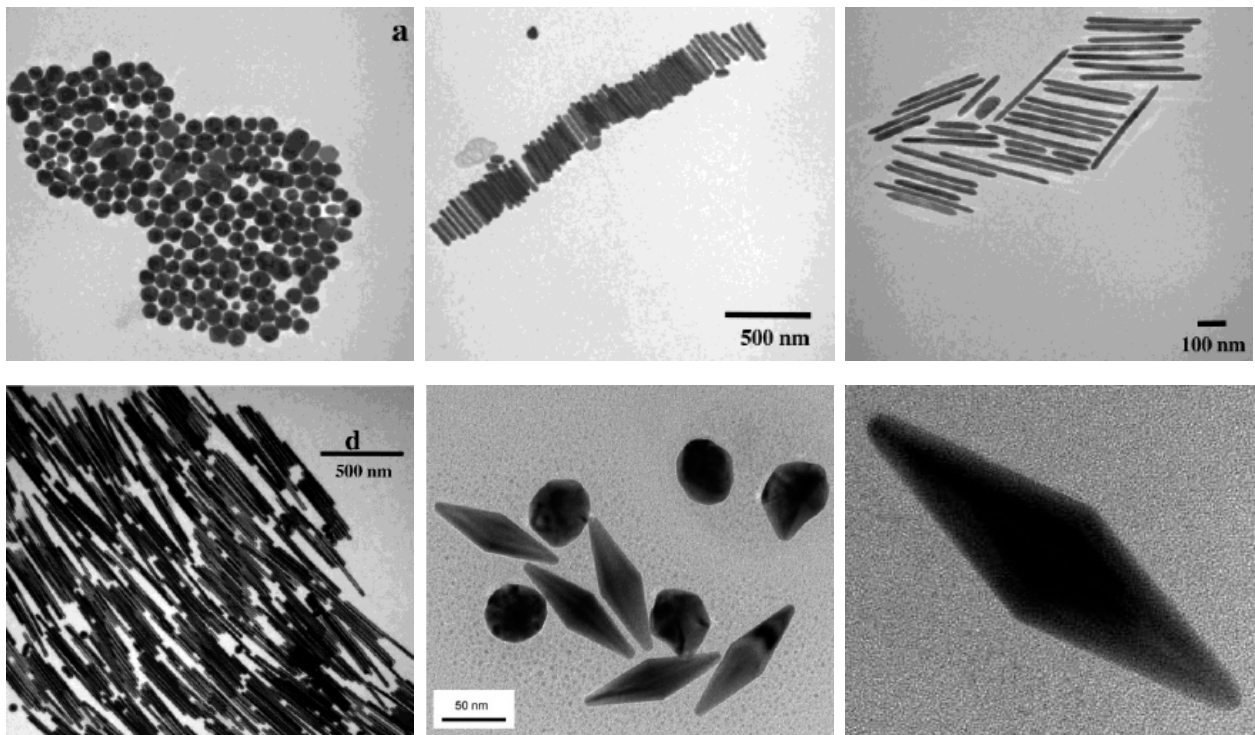
Figure 4: Differently shaped gold nanoparticles. Upper right: Spherical nanoparticles. Upper middle, right and lower left: Au nanorods with different aspect ratios. Lower middle: bipyramidal and spherical nanoparticles. Lower right: Pentagonal bipyramidal nanoparticle (magnified).

Another particle shape that can selectively be manufactured is that of a pentagonal bipyramid. In this case one also observes two different SPR bands. One important difference to the nanorods is that the longitudinal plasmon resonance is much sharper due to a more uniform aspect ratio (that is height to diameter ratio of the bipyramid). Typical spectra are shown in Figure 3, while figure 4 depicts TEM (transmission electron microscopy) snapshots of differently shaped gold nanoparticles. The broader transversal mode at ca. 520 nm also has contributions from irregular shaped or spherical Au nanoparticles that are also formed during the reduction process.

From all the above it is clear that the custom preparation of gold nanoparticles with desired spectroscopic or any other properties requires the experimentalist to exert control over their size and overall shape. It is only during the past years that a more detailed understanding of the factors governing their nucleation and growth from

molecular precursors has been reached, which finally enables more rational syntheses. Recent studies have shown that the nature of the primary nucleation particles (the so-called seeds), modification of the particle surface, for example by surfactants, control over the faces, along which the growth proceeds and the rate of the further growth are equally important in controlling the outcome of such experiments.

3. Preparation

3.1 The particle seeds

The most popular method that allows for shape and size control of metal nanoparticles is the seed mediated two-step growth process. The basic principle of shape-controlled synthesis involves two steps: First, the preparation of small size spherical gold nanoparticles, and second, growth of the prepared spherical particle in a micellar or surfactant controlled environment. Initial addition of preformed seeds has two advantages: first, it increases the overall reaction rate, and hence the growth rate; second, the particle size is controlled by varying the ratio of metal salt to seed, thus restricting the particle size to the nanometer regime.

The seeds are produced by treating a molecular gold source, mostly $HAuCl_4$, with a reducing agent in the presence of a surfactant or ionic substrate that adsorbs onto the nanoparticle surface. The role of the surfactant is twofold: On the one hand, it restricts the size of the primary particles by preventing agglomeration due to steric effects or ionic stabilization. In the latter case, the surfactants are ionic and thus create a surface charge that leads to Coulombic repulsion between individual particles. In this manner, primary particles of rather uniform sizes between 3 to 20 nm can be grown selectivley. On the other hand, every of these adsorbates may prefer a unique face of the growing nanoparticle leaving only a specific one open for later growth.

Typical surfactants/adsorbates are trisodium citrate, cetyltrimethylammonium bromide (CTAB) or other tetraalkylammonium salts featuring longer alkyl chains, or long chain alkyl thiols. As reducing agents, sodium borohydride (NaBH$_4$), hydroxylamine, ascorbic acid, citrate and others have been used. Depending on the exact reductant / surfactant / solvent combination, highly crystalline, spherical, monodisperse seeds that can be developed into spherical particles or nanorods (CTAB surfactant) or crystalline, pentagonally twinned particles that are prone to further develop into bipyramids (citrate as "capping agent") can be grown.

3.2 The growth process

In the seed mediated growth method, small amounts of the seeds grown as above are added to a growth solution which contains more gold(III) precursor (mostly HAuCl$_4$), surfactant, a mild reducing agent and, in some cases, Ag(I) salts as a growth mediator. The reducing agent is chosen such its reducing power is just not sufficient to directly reduce the Au(III) precursor to elemental gold. Once the gold complex precursor adsorbs onto the surface of a metallic gold particle, its redox potential is lowered to such an amount that it now gets reduced. This enables a controlled growth of the primary seeds into larger particles. With increasing ratio of Au(III) precursor to seed particles, the Au nanoparticles obtained tend to grow larger.

Although the exact mechanism of formation of rod-shaped nanoparticles in aqueous surfactant media remains still somewhat unclear, some principles have emerged that may serve as a guide to a customized growth. Based on the idea that surfactants like CTABs adsorb onto gold nanorods in a bilayer fashion, with the trimethylammonium headgroups of the first monolayer facing the gold surface, it was proposed that the C$_{16}$TAB headgroup preferentially binds to the crystallographic faces of gold existing along the sides of pentahedrally twinned rods, as compared to the faces at the tips. The growth of gold nanorods would thus be governed by preferential adsorption of CTAB to different crystal faces during the growth process, rather than acting as a soft micellar template. The influence of CnTAB analogues in which the length of the hydrocarbon tails was varied, keeping the headgroup and the counterion constant, was also studied. It was found that the length of the surfactant tail is critical for

controlling the length and the yield of the nanorods, with shorter chain lengths producing shorter nanorods and longer chain lengths leading to longer nanorods in higher yields. Thus, the spheres and the different rods in Figure 4 were produced with C_8TAB, C_{12}TAB, C_{14}TAB, and C_{16}TAB. Considering the preferential adsorption of C16TAB to the different crystal faces in a bilayer fashion, a "zipping" mechanism was proposed. This mechanism takes the van der Waals interactions into account between surfactant tails within the surfactant bilayer on the gold surface that may promote the formation of longer nanorods from more stable bilayers (see Figure 5). The results on the different surfactants suggest that the longer the tail, the more stable the bilayer. Tail-tail interactions between the long-chain alkyl groups even seem to be more important than the headgroup interactions with the gold surface. The overall growth rate also decreases with increasing size of the ammonium headgroup, because more voluminous ones hinder the approach of further gold atom precursors to the growing particle.
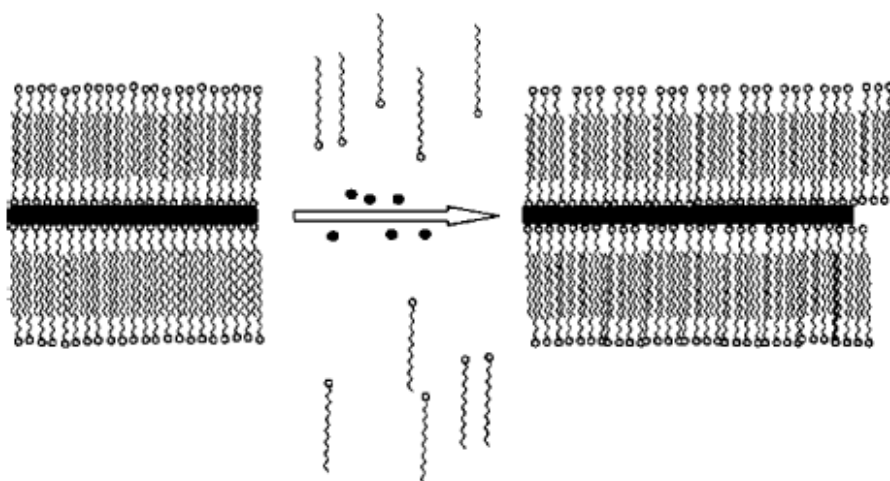

Figure 5: Schematic representation of "zipping": the formation of the bilayer of C*n*TAB (squiggles) on the nanorod (black rectangle) surface may assist nanorod formation as more gold ions (black dots) are introduced.

Some experiments in this lab course explore different methods for the preparation of primary particles and the dependence of their spectroscopic properties on particle size. Other experiments intend to illustrate some of the interdependencies of growth conditions, surfactant and reductant on the optical properties of the gold nanoparticles that are obtained in stepwise growth processes.

4. Experimental

In the first section, spherical nanoparticles with an average size of 10 ± 10 % and 20 ± 10 % nm are prepared. The 20 nm particles are used as seeds for growing larger particles. The size of the particles is controlled by the amount of gold ions in the solution. The resulting particle size can be calculated by the formula of Turkevich,

$$D_f = D_n \left(\frac{Au_n + Au_g}{Au_n} \right)^{\frac{1}{3}}$$

where D_f is the final diameter, D_n is the diameter of the nucleus, Au_n is the amount of gold in the nuclei and Au_g is the amount of gold in the growth medium. In order to obtain 40 nm particles from 20 nm particles, one thus would have to use seven times the amount of gold in the growth medium as in the 20 nm nucleating gold solution. The UV/Vis-spectra of all samples are measured and the influence of the particle size on the energy of the plasmon band is investigated.

In the second part, rod-shaped particles are produced in two ways. Rodlike particles show two plasmon bands in the UV/Vis/NIR-region, one corresponding to the longitudinal and the other to the transverse electron oscillation. The aspect ratio can be estimated by the energy of the longitudinal plasmon band. The particle size and shape ranges from spherical to rodlike with an aspect ratio from 1 to 6.

The following solutions have to be prepared at the start and are needed in all parts.

			c [mmol/L]	V_{H2O} [mL]	n [mmol]	m [mg]
"gold solution"	$FW_{HAuCl4\cdot3 H2O}$	: 393.8 [g/mol]	0.25	60	0.015	6
"Ascorbic acid solution"	FW	: 176.1 [g/mol]	10.0	4	0.040	7

4.1 Spherical nanoparticles

4.1.1 *"Seed solution"*: 20 nm – Gold nanospheres

A volume of 47.5 mL of a chloroauric acid solution containing 5.0 mg of $HAuCl_4 \cdot 3\,H_2O$ are refluxed in a 250 mL round bottom flask and 2.5 mL of 1% (w/w) sodium citrate solution (i.e. 50 mg in 4.95 g H_2O) are added. The reduction of the gold ions is supposed to be complete after 5 minutes, while the solution is further boiled for 30 minutes and is then left to cool to room temperature. This method yields spherical particles with an average diameter of 20 nm. This solution is used as the *"seed solution"* for the growth of the larger spheres in part 4.1.3.

4.1.2 10 nm – Gold nanospheres

In a 50 mL round bottom flask - equipped with a reflux condenser and a stirring bar - 14 mg of sodium citrate is dissolved in 22 mL water and heated to reflux. Then 0.21 mL of an aqueous solution containing 2 mg of $HAuCl_4 \cdot 3\,H_2O$ is added. Boiling is continued for 15 minutes. This method yields spherical particles with a diameter of 10 nm.

4.1.3 Growth of larger spherical nanoparticles

The particles are prepared in a sample vial. 3.5 mg of hydroxylamine hydrochloride are dissolved in 15 mL *"gold solution"* (Au_g = 3.75 µmol). After adding 2 mL of the *"seed solution"* (Au_n = 0.54 µmol) the sample vial is left to stay for at least 30 minutes.

4.2 Rodlike nanoparticles

The *"growth solution"* is prepared by dissolving 1.13 g of CTAB, 0.45 mL cyclohexane and 0.6 mL acetone in 30 ml *"gold solution"* in a warm water bath (The Krafft-temperature of CTAB is 27 °C, therefore temperature control is very important!).

The *"seed solution 2"* used in part 4.2 and 4.3 of the experiment is prepared by the supervisor according to the following procedure:

For the *"seed solution 2"* a 20 mL aqueous solution containing 18.4 mL of deionized water, 0.5 mL of an aqueous 0.01 mol/L $HAuCl_4 \cdot 3H_2O$ solution and 0.5 mL of a 0.01 mol/L aqueous trisodium citrate solution is prepared in a conical flask. Next, 0.6 mL of a freshly prepared 0.1 mol/L $NaBH_4$ solution is added all at once with stirring. The solution turns pink immediately after the addition of sodium boronhydride, indicating formation of 4 nm gold nanoparticles. The solution can be used between 3 and 10 hours after its preparation.

The *"growth solution"* and *"seed solution 2"* are mixed consecutively in 5 sample vials. The reaction is started by the addition of the aqueous "*ascorbic acid solution*" and mixed thoroughly by turning the sample vial upside down. After that the sample vials are left to stay for at least 30 minutes.

Sample	Growth solution	Seed solution 2	Ascorbic acid solution
1	3 mL	300 µL	150 µL
2	3 mL	150 µL	150 µL
3	3 mL	75 µL	150 µL
4	3 mL	22.5 µL	150 µL
5	3 mL	15 µL	150 µL

4.3 Aspect ratio control by directing surfactant

In the first step, the growth solution containing 7 mL of *"gold solution"* and 0.7 mmol (251 mg in 7 mL water) of the surfactant Hexadecylpyridiniumchlorid-Monohydrate (**T+**, check the safety data sheet!) is prepared.

This surfactant is most suitable for this application as its Krafft-temperature is below 20 °C, which allows us to work at room temperature. Furthermore it nearly does not foam, which is useful due to the mixing processes.

2 mL of the growth solution are placed in three sample vials labelled **X**, **Y** and **Z**, respectively. Then 100 µL of the *"ascorbic acid solution"* (the same as in part 4.2) is added to each vial. After mixing the solutions carefully 200 µL of *"seed solution 2"* is added to vial **X**, which must be mixed again and then left to stay for 30 seconds. Afterwards 200 µL from vial **X** are added to vial **Y**, which is inverted to mix. After 60s 200 µL of the vial **Y** solution is withdrawn and added to vial **Z**. Vial **Z** is inverted in order to mix again. After approximately 0.5 hours an UV/Vis-spectrum is recorded from vial **Z**.
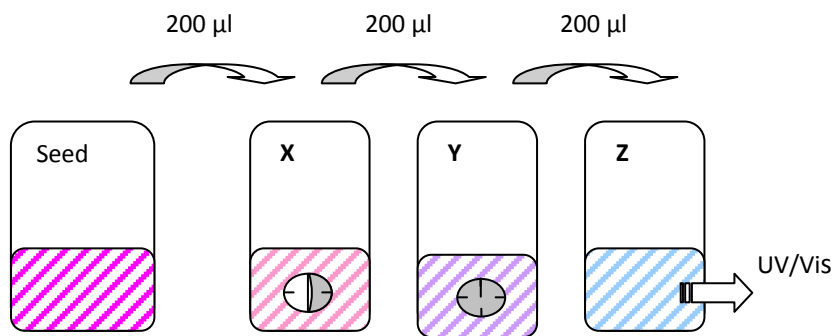
5. Analysis

5.1 Spherical nanoparticles

- Record the UV/Vis-spectra of all samples and summarize the peak maxima in nm and eV in one table.
- Calculate the expected particle size by the formula of Turkevich and describe qualitatively the effects of particle size on the energy of the SPR-band.
- Which process is responsible for the plasmon band?

5.2 Rodlike nanoparticles

- Summarize the extinction maxima of each solution in a table and compare the UV/Vis-spectra of all samples with each other.
- Discuss the influence of the amount of seeds on the aspect ratio.

5.3 Aspect ratio control by directing surfactant

- Record the UV/Vis-spectrum and compare it with those of spherical particles.
- Describe the growth mechanism and the role of the surfactant.

6. Sources

- Topp, K. *Kolloidale Goldnanopartikel – Synthese, Charakterisierung und Wirkung in Polymer/Fulleren-Solarzellen*; Dissertation **2011**, 19-29.
- Mahl, D. *Synthese, Löslichkeit und Stabilität von Gold-Nanopartikeln in biologischen Medien*; Dissertation **2011**, 20-26.
- N. Li, P. Zhao, D. Astruc, *Angew. Chem.* **2014**, DOI: 10.1002/ange.201300441