Bachelor's degree project

Bachelor's degree in Chemical engineering

Green synthesis and characterization of silver and copper nanoparticles

Report

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Abstract

This Bachelor's project studied the eco-friendly synthesis of two different metallic nanoparticles (NP): silver (Ag) and copper (Cu) by using a green production route; mainly based on the grape stalk's extract in order to reproduce the production costs and make the final process more eco-friendly.

Within this context, the influence of temperature during the reaction change of NPs' solution over time for both synthesized NPs were characterized by means of advanced characterization techniques, like UV/Vis spectroscopy, scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM), energy-dispersive X-ray spectroscopy (EDX) techniques.

The changes of reduction potential of reagents during the storage have been observed. It's possible to synthesize NPs in room temperature; however, the concentration of metallic ion dissolution affects the NPs' size and it's the main variable to decide of the synthesis will take place or not.

Summary

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1. Glossary

NPs: nanoparticles

AgNPs: silver nanoparticles

CuNPs: copper nanoparticles

M: molar concentration (mol / L)

UV-Vis: Visible ultraviolet spectroscopy

SEM: Scanning electron microscopy

EDX: Energy dispersive X-Ray spectroscopy

STEM: Scanning transmission electron microscopy

ADF: Annular dark-field

BF: Bright-field

2. Preface

2.1. Project origin

This Bachelor's degree project is one part of the research project "Green synthesis of metal nanoparticles from acid mine water and agricultural and alimentary wastes' extracts", financed by the Ministry of Economy and Competitiveness, Madrid, and FEDER funds, EU, 2016-2018 (project CTM2015-68859-C2-2-R; MINECO / FEDER). The project is developed in the grup of techniques for the Separation and Treatment of Industrial Waste (Grup de Tècniques de Separació i Tractament de Residus Industrials, SETRI) of the Polytechnic University of Catalonia (Universitat Politècnica de Catalunya, UPC).

2.2. Motivation

Recently, the mining has become one of the most serious environment problems due to the formation of acid mine drainage (AMD). This is an acid fluent which contain high concentrations of heavy metals, such as iron (Fe), arsenic (As), manganese (Mn), aluminum (Al), nickel (Ni), zinc (Zn), cobalt (Co) silver (Ag), gold (Au) and copper (Cu), moreover, some anions, often sulphate and it cause a large part of nearby rivers' degradation.

Furthermore, Spain is one of the mainly wine producer country, together with Italy and France, produce more than half of all the wine in the world, according to data published by the International Organization of Vine and Wine (OIV), 2017 [1]. As consequence, a large amount of organic waste has been generated during this process. According to OIV, the wine production process generates 20% solid waste. In particular, 100 kilos of grapes generate approximately 25 kilos waste, 50% are grape skins, 25% stalks and the remaining 25% seeds 54].

Therefore, finding an eco-friendly technology to re-use these wastes generated for the treatment of contaminated water means to give another value to them. So, in one hand, using natural products as a source of reducing compounds, and in the other, the synthesis of metallic nanoparticles, since they are being widely used in different fields of industry, for example: development of sensors for determination of heavy metals or potentiometric sensors.

3. Introduction

3.1. Previous researches

The green synthesis of silver nanoparticles (AgNPs) experimental process used in present Bachelors' degree project took reference to the work done and presented during the last academic course by Miss Cristina Arena [3], where the organic substance extraction process is the same as employed in the current Bachelor's project, but slightly changes have been done related to the redox reaction employed to obtain the metallic nanoparticles.

3.2. Long term goal

The main objectives of this project are:

- To prove the reproducibility of the green synthesis of AgNPs and copper nanoparticles (CuNPs) using grape stalks' organic extract as reducer, taking previous research conducted in the SETRI research group as reference.
- To characterize the size and shape of synthesized metallic NP (AgNPs and CuNPs) by means of advanced characterization techniques.
- To decrease the energy consume during the experimental process by determinate the influence of the temperature during that.
- To discover how the content of metallic ions and temperature in the reaction affect the shape and size of final synthesized NPs.

3.3. Project scope plan

In this Bachelor's project, only the synthesis and characterization of metallic NPs have been studied. The separation, applications and final management of NPs are out of the Bachelor's scope, but they could be the initial part of further Bachelor's projects or even Master's thesis.

Many heavy metals are in AMD, only Ag and Cu have been chosen to be studied because of their higher reduction potential than Co, Ni and Mn.

Quantities of organic compounds in the extract of grape stalks have not been studied due to they have already been determined in previous studies [3][20][21], however, the evolution of reduction potential of extract has been observed.

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4. Introduction

4.1. Nano-science

4.1.1.   Different methods of nanoparticles (NPs) synthesis

The methods for making NPs consist of two major parts in general: *(i)* top-down and *(ii)* bottom-up. In top-down synthesis, NPs are produced by size reduction from a bigger material, which is achieved by various physical and chemical ways as it is depicted in **Figure 1**.

In bottom-up synthesis, NPs are built from smaller elements, such as atoms and molecules. The green synthesis used in this Bachelor's project is one example which followed the bottom-up method. To be concrete, it follows a redox reaction.
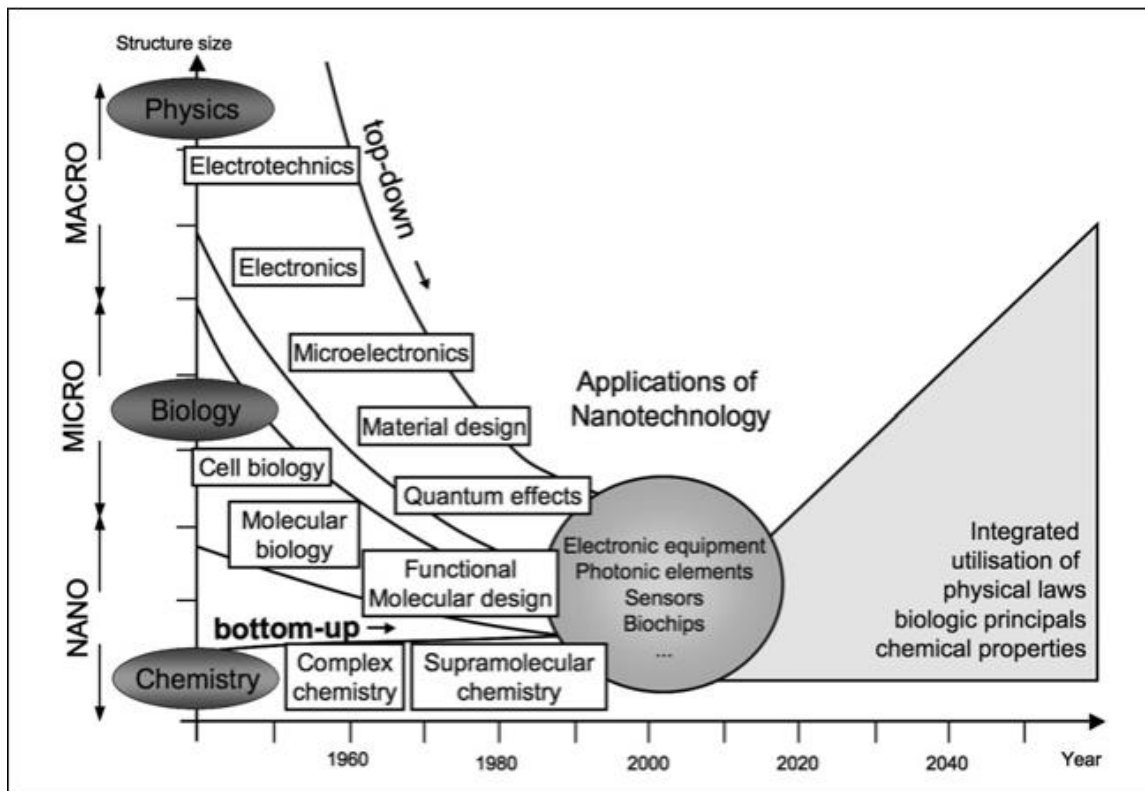

Figure 1: Top-down and bottom-up synthesis for different research fields [10].

4.1.1.1. Synthesis of NPs using plant extract

Using plant extracts to produce metallic NPs has been widely used in recent years because it is an economic, convenient and environmental-friendly way. Thanks to the high contain of organic compounds in plants, which can work as reducer and stabilizer in the same time. For example, glucose has been used as reducing agent to prepare AgNPs and gold nanoparticles (AuNPs), tea and coffee extract have been used to synthesize palladium NPs [12].

There are three key factors in the reducing process [12]:
1- *The solvent medium, to provide space for molecules movement.*
2- *The reducing agent, to provide electrons with metallic cations.*
3- *The stabilization agent, to avoid NPs' agglomeration and an uncontrolled growth.*

Luckily, they can be easily achieved by the plant extract. First of all, the extract is an aqueous solution; and the second, it contains natural reagents such as vitamins, sugars, polyphenols, terpenes, alkaloids, phenolic acids, proteins, biodegradable polymers and microorganisms as reductants and stabilization agents [13].
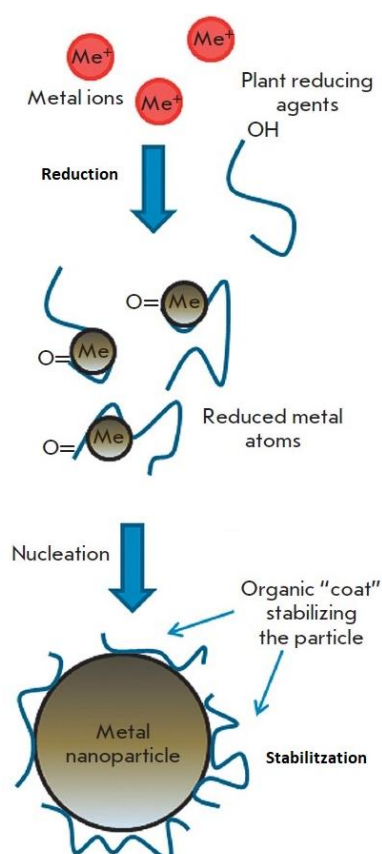
The process of NPs production can be divided into three phases [14] as it is shown in *Figure 2*:

1) The *activation phase* where the reducing agents reduce metal ions to metal atoms.

2) The *growth phase* where small NPs group together into larger particles, and this phenomenon increase the thermodynamic stability of NPs.

3) The *termination phase* determinates the final form of NPs. Studies show that the ability of stabilization of a plant extract determinates the final shape of NPs, possible shapes are hexagonal, triangular, spherical, etc.

Figure 2: Mechanisms of NPs synthesis [14].

4.1.2.   Applications of NPs synthesized by plants extract

Metal NPs have attracted much attention in nanoscale science and engineering technology over the past decade due to their unusual chemical and physical properties, such as catalytic activity, and novel electronic, optical and magnetic properties [12]. However, what could NPs synthesized by plants extract be used? Since they could have different shapes, size and particle size distribution, which are often strongly influenced by the variables of the synthetic methods, temperature, reducing agents and stabilizers [15]. Even so, because of the activation centers in NPs' surface, which provide the necessary conditions for NPs as catalyst. At present, the catalytic reaction with NPs, such as platinum, silver, aluminum and iron oxide, can greatly improve the reaction efficiency [11] as it is shown in **Figure 3**.
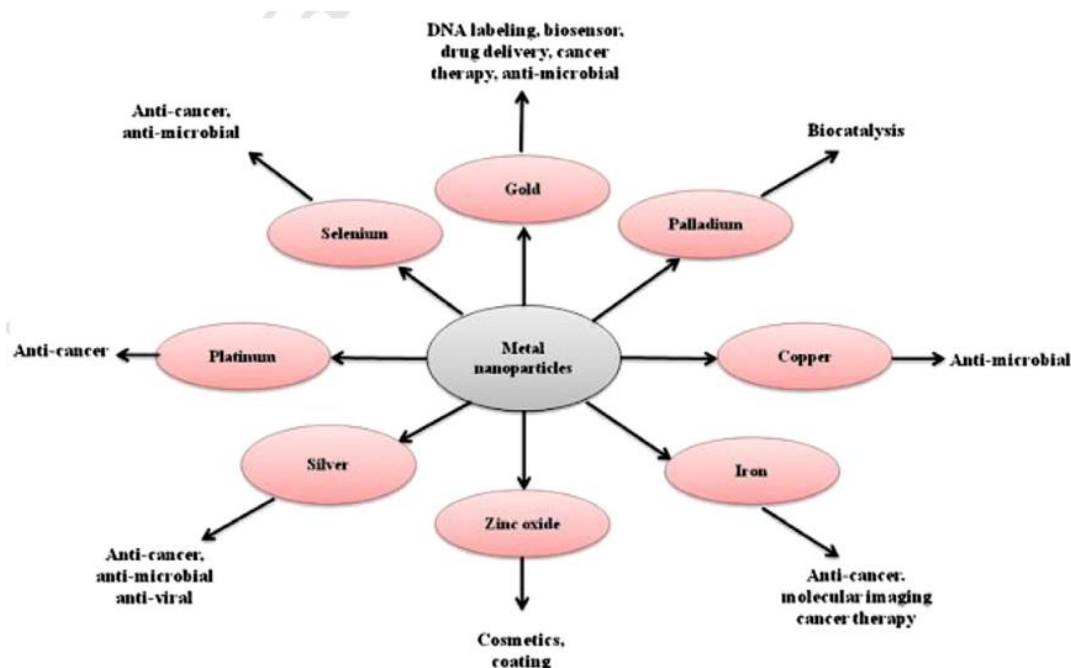

Figure 3: Principal applications of NPs [11].

4.2. Redox reaction

Oxidation-reduction reaction also known as redox reaction is a type of chemical reaction that involves a transfer of electrons between two reagents. In a redox reaction, there are two reagents, oxidant and reducer; one of them gains an electron while the other loses one as it is depicted in **Figure 4**.
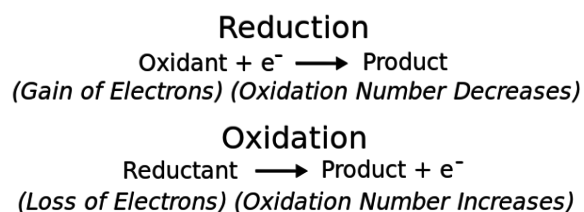
Reduction
Oxidant + e⁻ ⟶ Product
(Gain of Electrons) (Oxidation Number Decreases)

Oxidation
Reductant ⟶ Product + e⁻
(Loss of Electrons) (Oxidation Number Increases)

Figure 4: Electron change in a redox reaction [8].

To assure the redox reaction will take place, there has to be a significant difference between both reagents' standard known as reduction potential, which shows the tendency of the solution to either gain or lose electrons. A dissolution with higher standard reduction potential, more probably to gain electron, it means, to be reduced.

Therefore, as it is presented in **section 4.1.1.1**, the green synthesis are based on a redox reaction and the grape stalks extract actuates as a reducer, which means extract dissolution should has lower standard reduction potential than the metallic ion dissolution. In **table 1**, some metallic (acid mine drainage contains most of them) dissolution's standard reduction potential have been show.

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Table 1: Standard reduction potential of some metallic dissolution [9].

Reaction			E^o *(V)*
$Au^+(aq) + 1e^-$	$\rightarrow$	$Au(s)$	+1.83
$Ag^+(aq) + e^-$	$\rightarrow$	$Ag(s)$	+0.80
$Cu^+(aq) + e^-$	$\rightarrow$	$Cu(s)$	+0.52
$Cu^{2+}(aq) + 2e^-$	$\rightarrow$	$Cu(s)$	+0.34
$Ni^{2+}(aq) + 2e^-$	$\rightarrow$	$Ni(s)$	−0.25
$Co^{2+}(aq) + 2e$	$\rightarrow$	$Co(s)$	−0.28
$Fe^{2+}(aq) + 2e^-$	$\rightarrow$	$Fe(s)$	−0.44
$Zn^{2+}(aq) + 2e^-$	$\rightarrow$	$Zn(s)$	−0.76
$Mn^{2+}(aq) + 2e^-$	$\rightarrow$	$Mn(s)$	−1.18

4.3. Fundamental background: Techniques

In order to characterize the AgNPs and CuNPs produced through the eco-friendly synthesized route are presented along this section, where the theoretical background, limitations among other factors will be clearly presented.

4.3.1. Visible ultraviolet spectroscopy

The visible ultraviolet spectroscopy [4], also called UV-Vis spectrophotometry, consists in photon spectroscopy in the region of UV radiation. In this region of the electromagnetic spectrum, molecules are subjected to electronic transitions.

The principles of this technique are based on molecules that contain π-electrons or unlinked electrons (*n*-electrons) which can absorb energy in the form of UV or Vis light to excite these electrons to anti-linking orbitals higher molecules. The more easily electrons are excited, the longer the wavelength (λ) can be absorbed [4]. **Figure 5** exhibits the different transitions between the bonding and anti-bonding electronic states.
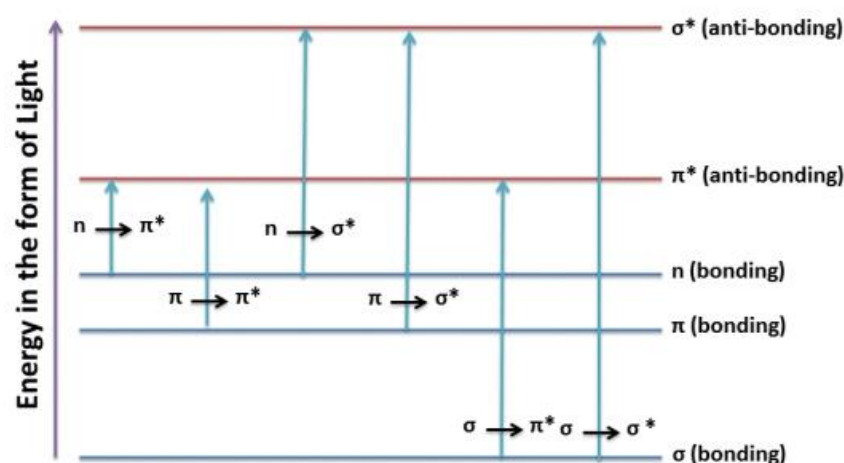

Figure 5: Different transitions between the bonding and anti-bonding electronic states when light energy is absorbed in UV-Visible Spectroscopy [6].

In this Bachelor's project, UV-Vis has been used to detect the metallic NPs' presence because of the surface plasmon band (SPB) phenomenon. This phenomenon is caused by the interacting of NPs with incident light and results a global scattering of it in a determinate frequency. SPB is a strong and broad band observed in absorption in the UV-visible spectrum for metallic NPs bigger than 2 nm [7].

Moreover, UV-Vis has been used to determinate NPs' quantity as well thanks to Lamber-Beer law, which relates the absorption of light with the properties of the material crossed by it by using the following expression:

$$A = \varepsilon \cdot \ell \cdot c$$ Equation (1)

where A is the absorbance, ε is the molar absortiveness (typically for each substance in L/(mol·cm)), l is the optical path (in cm), which is related to the distance crossed by the radiation and c is the substance concentration (in mol/L).

Synthesized samples have been analyzed with UV-Vis spectrophotometer Flame S, shown in **Figure 6**. The equipment is composed by *(i)* emisor of light *(ii)* support of cuvette and a plastic cuvette 1x1x2 cm *(iii)* receptor of light which is connected to a computer where software OceanView had been installed. Modules *(i)*, *(ii)* and *(iii)* are connected by light fibers, which are very delicate, so they shouldn't be bended.
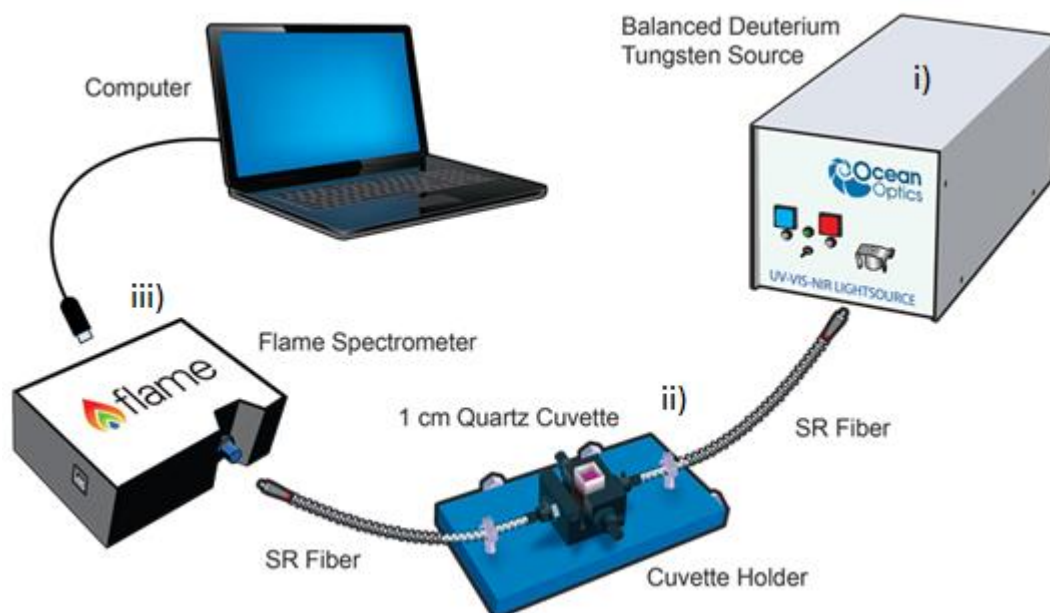

Figure 6: Representation of parts of the spectrophotometer [22]

4.3.2.   Scanning electron microscopy

The scanning electron microscopy (SEM) uses a focused high-energy electron beam to generate a variety of signals at the surface of solid specimens. These signals include secondary electrons (that produce SEM images because secondary electrons come from the region of 5-10 nm on the surface and have energy of 0-50 eV. It is very sensitive to the surface condition of the sample, which can effectively display the microstructure of the sample surface), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and continuum X-rays), visible light (cathodoluminescence–CL), and heat [16]. A schematic representation of the SEM equipment is presented in **Figure 7**, where in it appears the six main parts of this device: *A: generation of electrons by a metallic filament, B: beam of electrons, C-E: electromagnetic lenses to focus and orient the beam, F: sample, G: detector that amplifies the signals and sends it to a monitor, and H: monitor.*

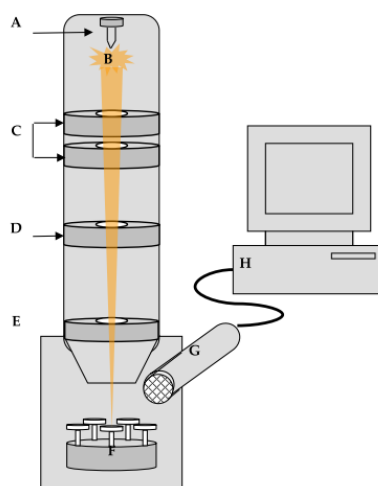**Figure 7***: Schematic presentation of different parts of a SEM [17].*

4.3.2.1. Sample preparation for SEM analysis

First at all, the sample to be analyzed by SEM should be dry. So in order to accelerate the drying process, ethanol has been used.

Sample preparation followed these steps:
1- Mix 1 mL of sample and 3 mL of ethanol in an eppendorf.
2- Stick a silicon wafer (see **Figure 8**) onto a SEM support by double side paste.
3- Deposit a drop of sample on the silicon wafer using pipet Pasteur.
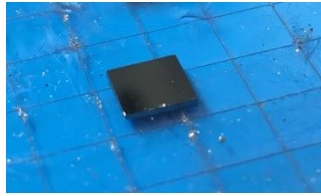4- Dry the sample in an oven at 50 $^{\circ}$C for 30 minutes.

Figure 8: Silicon wafer to support sample for SEM

4.3.3. Energy dispersive X-ray spectroscopy

Energy dispersive X-Ray spectroscopy (EDS, EDX, EDXS or XEDS) is an analytical technique that can be used together with SEM, Transmission Electron Microscopy (TEM) and Scanning Transmission Electron Microscopy (STEM) by changing detector of electrons.

EDX can provide elemental chemical analysis in very small areas, as small as nm in diameter, or to plot the lateral distribution of elements in the imaging area. However, it cannot distinguish between ions or solid metals.

4.3.4. Scanning transmission electron microscopy

Scanning transmission electron microscopy (STEM) combines the TEM and SEM [18]. The difference compare with SEM is that the detectors are placed below the sample and the detector receives the transmitted electron beam or the electron beam with the elastic scattering.

As shown in **Figure 9**, there are two types of detectors: Annular dark-field (ADF) and Bright-field (BF). With ADF, can get pictures of samples in black color with a bright background, and the inverse with BF, which obtain bright samples with dark background, the same be used in this Bachelor's project.

After electron beam focused on the sample surface, scan a region of the sample point by point through the coil control. At the same time as each scan, the detector under the sample simultaneously receives the scattered electrons. The signal received by the detector corresponding to each scanning position is converted into current intensity and displayed on a screen or a computer display. So each point on the sample will match the same point in the image [19].
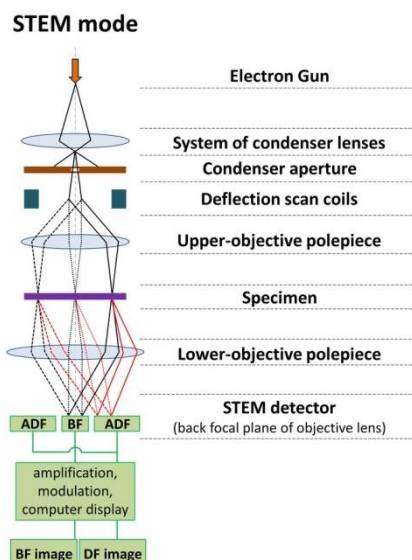
Figure 9: Schematic representation of STEM parts [18].

In order to quantify the particle size as well as the shape of the metallic NPs, a focused ion beam/field emission electron microscopy (FIB/FESEM) Neon 40 (Carl Zeiss) has been used working at 30 kV and with a constant working distance of 4.9 mm between the gold foil and the SEM detector.

4.3.4.1. Samples preparation for STEM analysis

First at all, the samples' thickness has to be taken into account, because it should be sufficient thin to allow the electrons to cross it.

Sample preparation consists in follow steps:

1- Shake the sample to homogenize the dissolution.
2- Take off one drop of sample with pipette Pasteur and deposit it on a gold grid from the Ted Pella brand, see **Figure 10**.
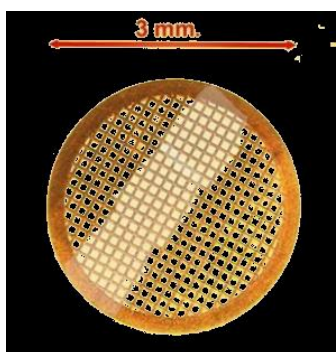

Figure 10: Sample's support for STEM [23].

3- Let the sample dry in the air for 15 minutes before collocate the gold grid on the sample holder of STEM.

Should ovoid hand contact with the gold grid in whole process and using special tweezer to manipulate grid, see **Figure 11**.

Figure 11: Dumont biology grade tweezer [23]

5. Experimental process

5.1. Preparation of extract

The main intention of this process is to extract maximum amount of polyphenol and possible reducing sugar from the grape stalks, since they are major reducing agents in the redox reaction. As mentioned in the experimental design section, this process has used the same variables as the previous researches, such as grains dimension, extraction time and etc. The detail laboratory equipment and material list has been shown in the **Table 2**.

Reagents:

- Distillated water.

- Dry grape stalks, from Subirats (Alt Penedès), 2014's harvest.

Table 2: Laboratory equipment and material for organic compounds extraction.

Two-neck round-bottom boiling flask	Ultrasound machine, Selecta 3000837, capacity 6.5 L	Electric sieving machine, Filtra
Büchner funnel	Oven	West Condenser of 40 cm
Digital scale	Heating mantle Fibroman-C	Spatula
Kitasato flask	Sieves of 0.8 mm and 1 mm	Pipette of 50 mL
5-Beakers of 300 mL	Thermometer	Pipette bulb
Plastic syringes of 5 mL, Luer-Lok Tip	Syringe filters of 0.2 µm, Rc Agilent	Coffee grinding machine, Waring commercial
2- Utility clamps	Whatman filter paper, pore size 11 µm	Centrifuge, Centronic BL-II
1- Ring stand	4- Centrifuge tubs, Oak Ridge	Micropipette of 5mL
2- Rubber tubs	Vaseline	Micropipette tips

5.1.1. Cleaning

First of all, all the impurities contained in the grape stalks that come from a vineyard need to remove manually, for being residue from wine making, they may contain leaves and grape skins.

5.1.2. Washing

The stalks washing process consists of 3 manual washes and 1 bath with ultrasound, with distilled water.

Manual washing consists of 3 different steps:

- *In the first wash, in the big recipient, leave water covers grape stalks, stir stalks manually for 3 min. and leave them rest for 6 min.*
- *In the second wash, change the water, stir stalks manually for 2 min. and leave them rest for 4 min.*
- *In the last one, repeat the same process, decrease the agitation and bath time to 1 and 2 min. separately.*
- *Once finished the washing process, dry the water and leave stalks under room temperature for 24 h.*

24 h later, repeat the third step of manual washing, use the same water for the ultrasound bath. Distribute equally stalks and water into 5 beakers of 300 mL; put them in the ultrasound machine for 5 min.

After washing, maintain cleaned stalks in an oven for 24 h with stable temperature at 110 ºC.

5.1.3. Grinding and sieving

Grind dry stalks with a home-use coffee grinding machine and separate ground particles by sieving. Using electric sieving machine and sieves of 0.8 and 1 mm. Set the machine with following conditions: *power level 6, 1 cycle and 5 min.*

The selection of sieves is according to previous researches, this particles dimension range is optimal because, on the one hand, it's small enough to ensure there will be an intimate contact between stalks and water to facilitate extraction of polyphenol, on the other, if particles are too small, they will obstruct pores of filter paper, which means un-efficiency on posterior experimental process.

5.1.4.   Organic substances extraction

The experimental mounting is showed in **Figure 12**. Weigh 3.000 g of grape stalks and measure 100 mL distillated water, add them into a round-bottom boiling flask and connect the West condenser and the thermometer, lubricate the connection with vaseline. Turn on the cooling system and the heating mantle. Use 10 min of reflux time, start counting from the moment of boiling. 10 min later, turn off the heater, and wait for 10 min more to turn of the cooling system to avoid the steam losses.
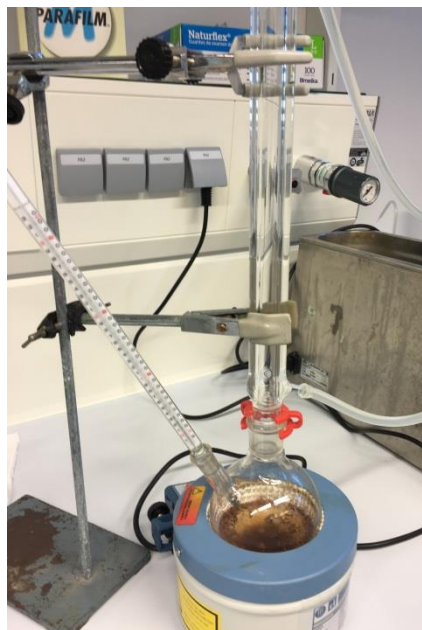

Figure 12: Experimental mounting for organic substance extraction

This contacting time between grape stalks and water has been chosen because in the previous research [3] it has been determinated that 10 minutes was enough to extract maximum polyphenol quantity, based on studied with 3 different reflux times: 10, 30 and 60 minutes.

5.1.5.   Filtering in vacuum

After 10 min of waiting, use Kitasato flask, Büchner funnel and Whatman filter paper to separate the stalks from the dissolution. See the experimental mounting in **Figure 13**. Try to take off all the dissolutions from the boiling flask.

Figure 13: Filtering in vacuum mounting

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5.1.6.   Centrifugal separation and filtering with syringe filter

Distribute equally the extracted solution into 4 (depend on quantity of the solution, but must be even number) centrifuge tubs, run the centrifuge machine during 10 min at 5000 rpm. After that, the Extract separated into two different parts as it is shown in **Figure 14**. Then, the supernate is separated by using a micropipette of 5 mL without touching the precipitate, and use the syringe filter to eliminate the precipitated part. Finally, the extract is storage in the fridge at 4 ºC until next day's use.

Figure 14: Extract after centrifuge

5.2. Synthesis of silver and copper nanoparticles

As was mentioned in the **section 3.2** two main objectives of this Bachelor's project are: *(i)* to prove the reproducibility of the green synthesis experimental process and *(ii)* to decrease the energy consume during the process. So, take reference to previous Bachelor's research project [3], the whole experimental part can separate into two big sections, one is synthesis of AgNPs and the other is to develop a new production route for CuNPs. There are two reagents, grape stalks extract and silver nitrate ($AgNO_3$) and copper (II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3\,H_2O$) dissolutions as source of metallic ions.

Laboratory equipment and material for NP synthesis can be found in the **Table 4** and the experimental details (variables and quantities) can be summarized in **Table 3**. The same extract volume (8 mL) has been used in all experiments, two different volumes of metallic dissolution (1 mL and 6 mL) have been employed to study how the quantity of metal ions will affect the form of NPs, and different volumes of distilled water have been added to assure all samples have the same concentration of organic compounds.

Table 3: Summary of the experimental conditions.

Temperatures (ºC)	# Sample	Extract volume (mL)	AgNO$_3$	Cu(NO$_3$)$_2$·3 H$_2$O	Distilled water volume (mL)
			0.01 M volume (mL)		
80 50 25 (room temperature)	0	8	0		6
	1	8	1		5
	2	8	1		5
	3	8	6		0
	4	8	6		0

Due to one of the reagents containing organic compounds, they also absorb light in the UV or Vis regions of the electromagnetic spectrum [5], so the samples 0 (which contains the same quantity of extract) has been used as a reference sample to calibrate the spectrophotometer.

Nomenclature of samples

Due to the huge amount of variables have been used in this Bachelor's project (for example: two metallic NPs have been studied in three reaction temperatures and five samples in each reaction condition), so a general nomenclature to all samples has decided as it is clearly presented in **Figure 15**.
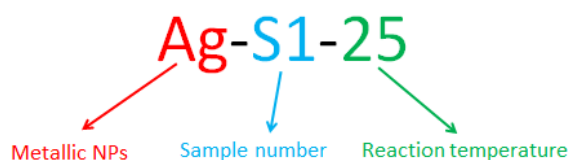
Figure 15: Nomenclature of samples.

As **Figure 15** shows, the first term indicates the NP, so may be Ag or Cu. The second term indicates the sample number, so would be from S0 to S4, S0 means no metallic ions has been added, S1 and S2 are duplicates, less volumes of metallic solution have been added, and S3 and S4 are duplicates too, but with more volumes of metallic solution as reagent. And the last term indicates the reaction temperature, could be 25, 50 or 80 ^{o}C.

Reagents:
-   Extract of grape stalks.
-   Silver nitrate, $AgNO_3$, Merck.
-   Copper (II) nitrate trihydrate, $Cu(NO_3)_2 \cdot 3\,H_2O$, Merck.
-   Distillated water.

Table 4: Laboratory equipment and material for NP synthesis.

Laboratory equipment and material:		
Digital scale	1- Glass rod	1- Spatula
1-Beaker of 100 mL	1-Volumetric flask of 100 mL	2- Pipette of 5 mL
Pipette Pasteur	2- Pipette of 1 mL	1- Pipette bulb
Plastic syringes of 5 mL, Luer-Lok Tip	Syringe filters of 0.2 µm, Rc Agilent	Centrifuge, Centronic BL-II
2- Graduated pipette of 10 mL	4- Centrifuge tubs, Oak Ridge	1- Test tub rack
5-Test tubs of 25 mL with caps	Thermostat, Selecta	Micropipette of 5mL
Micropipette tips		

5.2.1.   Ag and Cu cation's preparation

In this Bachelor's project, use $AgNO_3$ and $Cu(NO_3)_2$ dissolution of 0.01 M to simulate the content of Ag and Cu heavy metals in the water. According to the **Table 3**, 14 mL dissolution is needed in each synthesis of different temperature, so, it's been decided to prepare 100 mL $AgNO_3$ and $Cu(NO_3)_2$ dissolution. The detail of reagent preparation can be found in the **section 11.3**

5.2.2.   Redox reaction

In following text, a detail experimental process of synthesis of NPs has been summarized, the whole process is quite simple, the only thing should be considered is the reaction time has been started counting when two reagents have been mixed. So it's recommended to add extract in the test tubs first, then the water, and finally the metallic dissolution.

- Add volumes of reagents according to **Table 3** into 5 test tubs of 25 mL.
- Cover test tubs with caps and shake up to homogeneous dissolution.
- Place test tubs in a thermostat with water pre-heated at temperature 80ºC.
- Wait 2 hours, take off samples and cool them with tap water.
- Centrifuge samples in speed 6000 rpm during 20 min to eliminate nanoparticles bigger than 100 nm.
- Separate the supernate by using a micropipette of 5 mL without touching the precipitate, and use the syringe filter to eliminate the precipitated part. Finally, keep samples in the fridge at 4 ºC for 24 h (until next day's analysis).
- Repeat the same process with 25 (room temperature) and 50 ºC.

6.   Results and discussion

6.1.   AgNPs synthesis

6.1.1.   First synthesis

6.1.1.1. Color change

The first thing can be observed during the synthesis process is how samples change the color before and after 2 h being in a thermostatic bath with constant temperature.

As can see in **Figure 16** and **Figure 17**, S0 has not changed the color because doesn't contain metallic cations and actuates as the blank. S1 and S2 seem to be same, and S3 and S4. Not surprisingly S3 and S4 are darker than S1 and S2 caused by the higher contain of metallic cations.
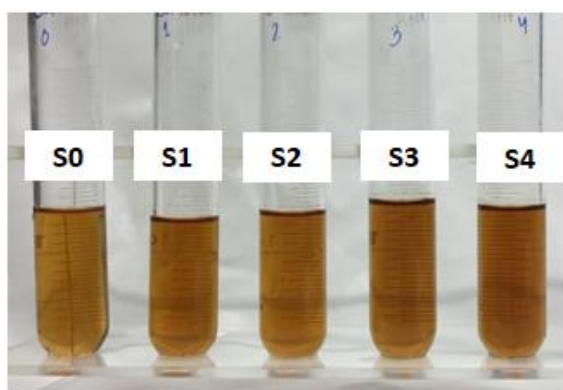
Figure 16: Ag samples when just have mixed two reagents.
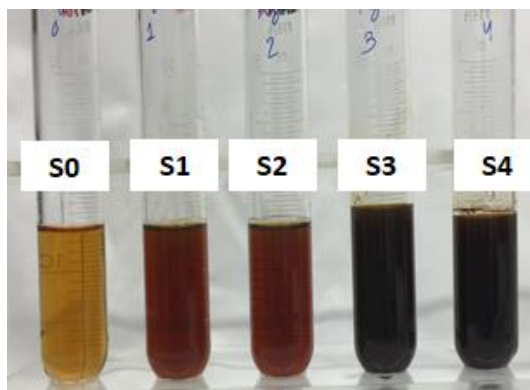
Figure 17: Ag samples after being in 80 ^{o}C during 2 h, without centrifuged either filtrated.

Furthermore, samples colors after centrifuge are lighter than before since NPs bigger than 100 nm have been separated and precipitated in the bottom of the tubs as it is evident in **Figure 18** and **Figure 19**.
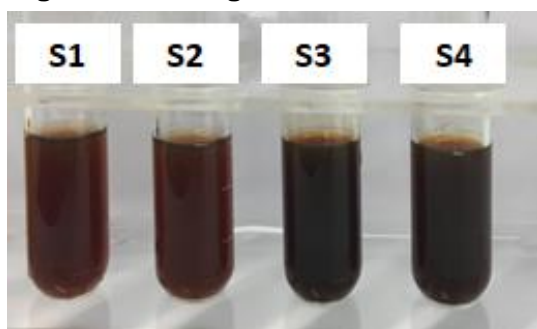
Figure 18: Ag-S1-S4-50 before centrifugation.
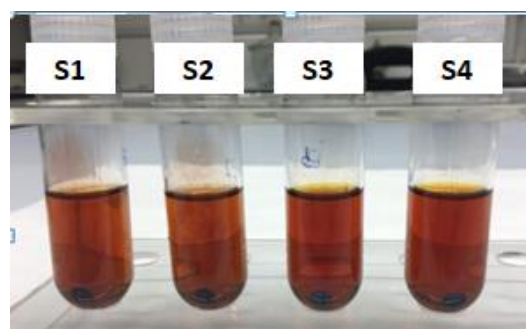
Figure 19: Ag-S1-S4-50 after centrifugation.

6.1.1.2. UV-Vis determination

Figure 20 shows spectra of all samples after 10 days (considering the synthesis day as day 0). A few conclusions can be reached by observing these spectras:

1- Reproducibility of this green synthesis method has been observed due to all duplicates (S1 with S2, S3 with S4) have similar spectra, see **Figure 20**.
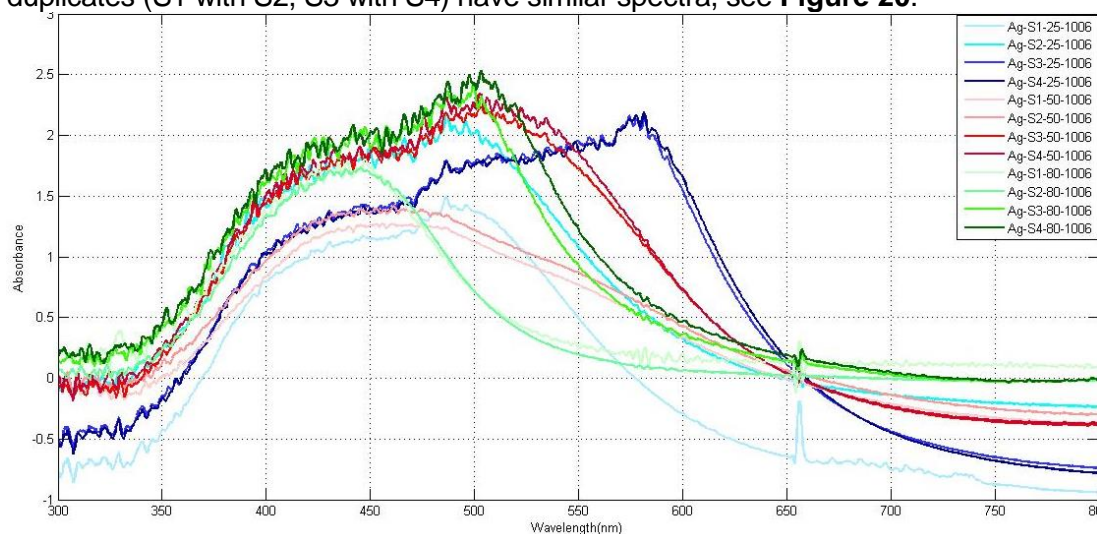
Figure 20: Spectras' comparison of all Ag samples 10 days after.

2- Samples synthesized with high contains of Ag ions have bigger absorbance than what have been synthesized with lower Ag contains. In the **Figure 20** and the **Table 5** it can be seen that samples 3 and 4 have higher maximum (max.) absorbance than samples 1 and 2. Moreover, a small displacement of the max. absorbance's λ has been seen when the concentration of metallic cations is higher. These conclusions have been summed up in previous works too [3][21].

3- Samples have been synthesized in higher temperature have higher absorbance, which can be observed more clearly in the **Table 5**.

Table 5: Summary of max. absorbance of samples after 10 days.

Samples	T=25ºC		T=50ºC		T=80ºC	
	λ (nm)	Absorbance	λ (nm)	Absorbance	λ (nm)	Absorbance
S1	485.9	1.4840	451.2	1.2620	440.5	1.7060
S2	488.7	2.0350	451.2	1.3760	446.3	1.7160
S3	576.0	2.0810	504.8	2.1800	500.3	2.3780
S4	581.4	2.1880	505.8	2.3140	503.1	2.5310

4- The maximum absorbance's λ is between 440 and 582 nm, corresponding to values found in published articles and previous thesis [3][7][11]. To be more specific, the λ of S1 and S2 is around 450 nm, and the λ of S3 and S4 is around 500 nm; however, spectra of samples of 25 ºC don't follow this rule, so they cannot be compared with other spectra.

Due to the limitation of the spectrometer, spectras with max. absorbance bigger than 2 demonstrate a lot of noise (small peaks). Some of them have been smoothed using software *Matlab*, but still no evident improvement.

A few days later, it's been accidentally found that samples were darker than before, so it's decided to verify with UV-Vis determination. In the following **Figure 21** shows the comparison of S2-25, S4-25, S2-50 and S4-50's spectras after 10 and 20 days. Samples of temperature 80 ºC's spectras haven't been represented because they present a lot of noise and impossible to determinate λ and max. absorbance.
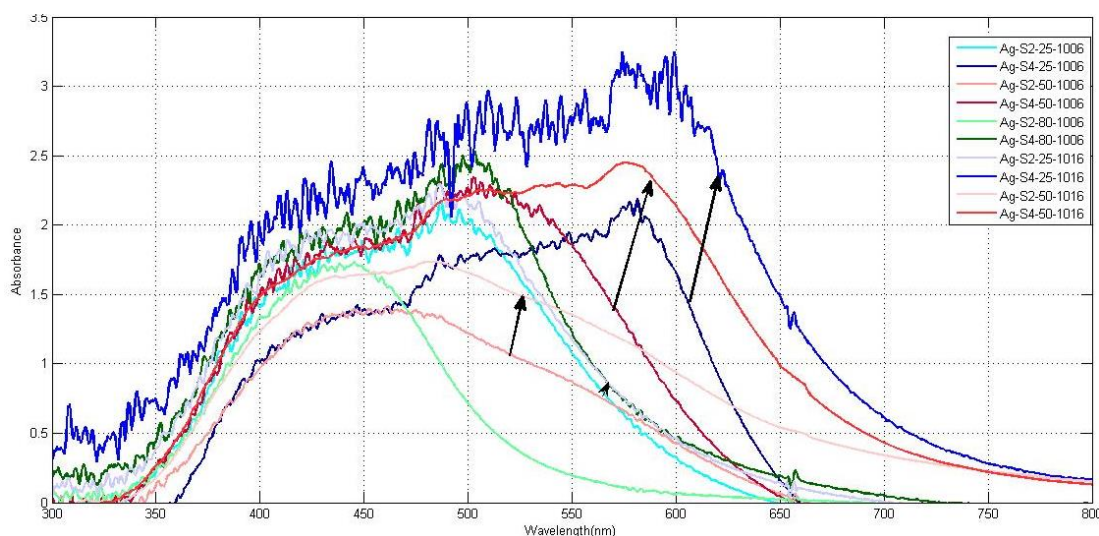

Figure 21: Spectras' comparison of samples after 10 and 20 days been stored in fridge.

Once noticed that time influence UV-Vis spectra of samples, or said in another way, that redox reaction could have continued once samples have been stored in the fridge, so it's been decided to study the evolution of samples by running UV/Vis tests from the first day.

6.1.2. Second synthesis of AgNPs

6.1.2.1. Color change

Same color change as the first synthesis' has been observed in the samples of the second synthesis; however, samples of 25 ºC were darker than the others, see **Figure 22**.

Figure 22: Color comparison of samples of the second synthesis.

6.1.2.2. UV-Vis determination

Due to the limitation of the spectrophotometer, samples have been diluted in 1:4 (mixed 0.25 mL of sample with 1 mL of water using micropipette) to make sure that the max. absorbance is lower than 2. The representations of all samples of second synthesis on the first day from **Figure 23**, together with **Table 6**, have verified again conclusions 1, 2 and 4 of the section **6.1.1.2**, but not the 3. On the contrary, samples of 25 ^{o}C have higher absorbance than the others, which corresponds to the colors of samples.

The possible reason is that the redox reaction is faster in higher temperature, so main part of Ag cations have been already reduced, precipitated or transformed to agglomerations, and have been eliminated by centrifuge. However, the samples synthesized in 25 ^{o}C, only a small part of Ag cations has been reduced, and a large part was still in the dissolution, redox reaction has been continued. This assumption should be verified by analyzing sizes of NPs in STEM sections.

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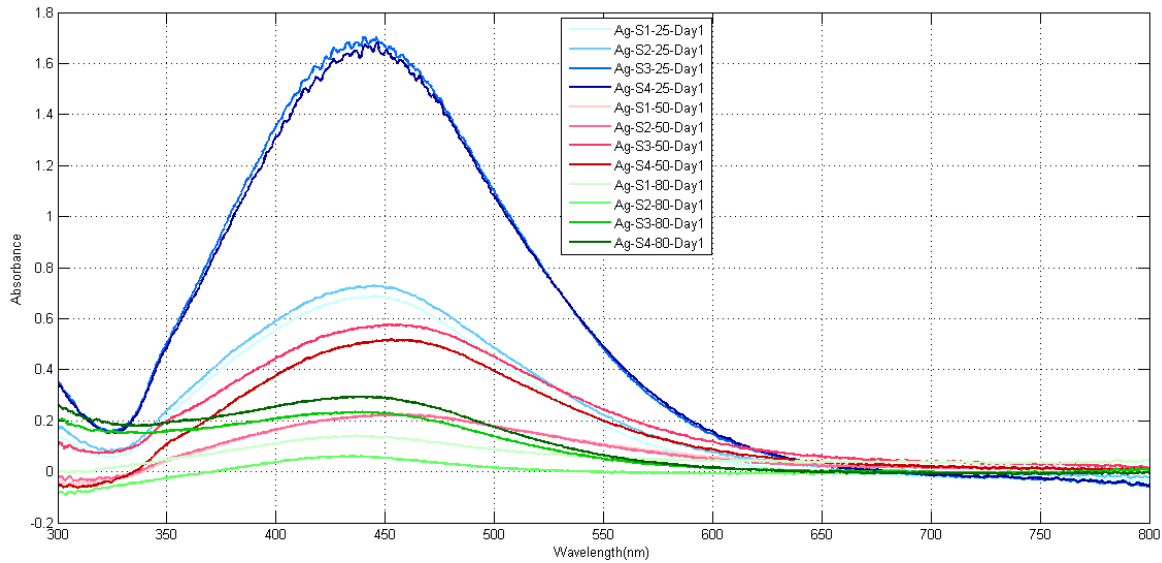
Figure 23: Spectra's comparison of all samples Ag on day 1.

Since this time, the samples have been diluted; an extra experimental error has been added. This phenomenon has been detected in S2-80. The max. absorbance of samples of 80 ºC were so small, which means samples were lighter, so big difference on spectra can be seen only because a small difference in sample's quantity (sometimes, a very small part of samples could stand at the pipet's wall). The spectrum of S1-50 has been overlapped with S2-50's.

Table 6: Summary of max. absorbance of samples on day 1.

Samples	T=25ºC		T=50ºC		T=80ºC	
	λ (nm)	Absorbance	λ (nm)	Absorbance	λ (nm)	Absorbance
S1	445.2	0.6868	459.4	0.2207	435.5	0.1395
S2	444.8	0.7182	452.0	0.2218	435.5	0.0607
S3	440.2	1.7040	457.3	0.5755	452.0	0.2297
S4	446.6	1.6760	453.7	0.5170	443.0	0.2926

Since the reproducibility has been proved again, only samples 2 and 4's spectra have been represented. See **Figure 24** and **Figure 25** the evolution of max. absorbance of samples S2-25 and S4-50, and the rest of figures can be found in the section **11.4** of the annex.
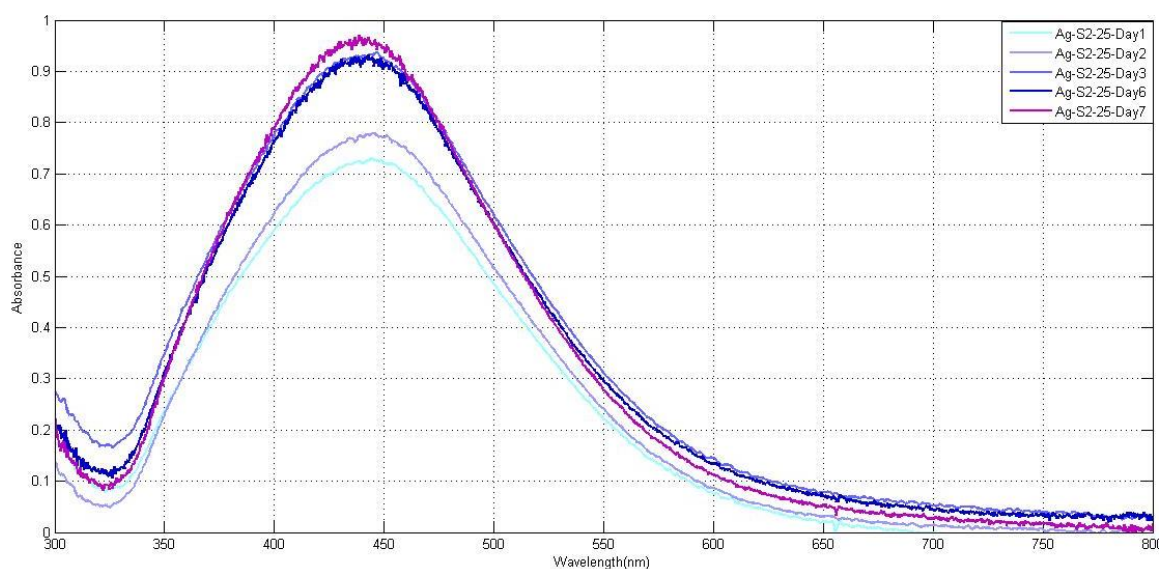
Figure 24: Comparison of S2-25's spectra in a week.

As the **Figure 24** shows, the sample S2-25 had a big change from day 2 to day 3, and then has been stabilized. However, the sample S4-50 seems to have the same change every day. See more samples' change in the **Table 7**. In this time, not significant λ displacement has been detected.

Table 7: Summary of max. absorbance change and its corresponding λ in a week.

#	Day 1		Day 2		Day 3		Day 6		Day 7	
	λ (nm)	Abs.	λ (nm)	Abs.	λ (nm)	Abs.	λ (nm)	Abs.	λ (nm)	Abs.
S2-25	444.8	0.7182	445.2	0.7788	441.3	0.9191	443.4	0.9326	442.7	0.9663
S4-25	446.6	1.6760	444.5	2.0300	449.1	2.4100	442.0	2.7120	451.6	2.9450
S2-50	452.0	0.2218	454.1	0.2091	456.9	0.2503	455.5	0.3030	454.4	0.2779
S4-50	453.7	0.5170	462.6	0.6582	464.7	0.7639	454.8	0.9123	453.4	1.0050
S2-80	435.5	0.0607	432.7	0.0838	435.5	0.1298	433.0	0.1234	434.8	0.1083
S4-80	443.0	0.2926	443.4	0.2757	445.9	0.3346	448.0	0.3770	449.5	0.4536
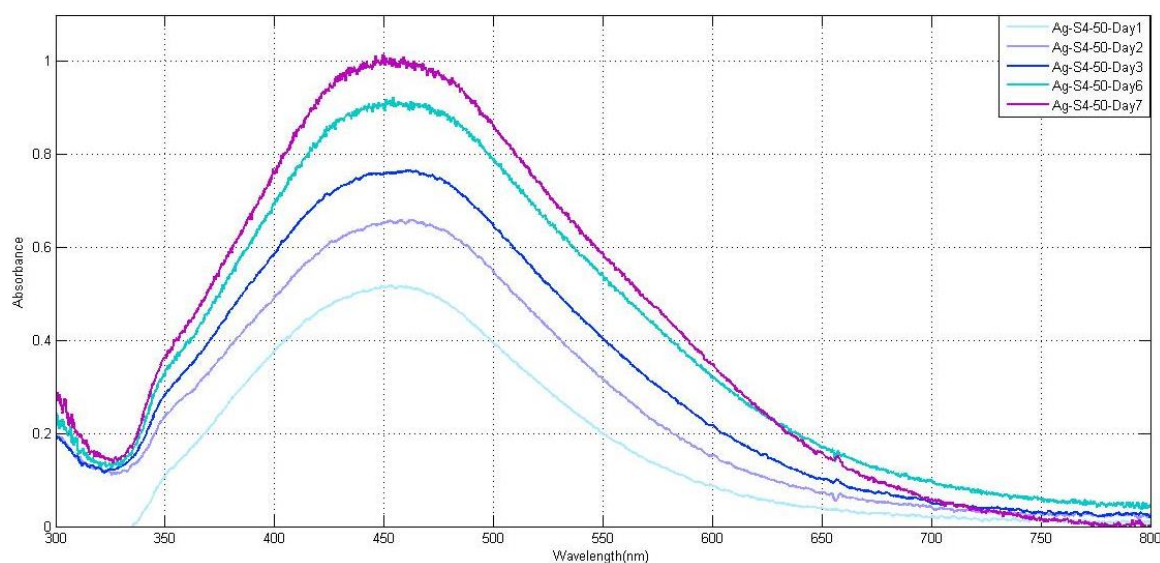
Figure 25: Comparison of S4-50´s spectra in a week.

6.1.2.3. pH control

Moreover, the pH of the samples has been measured during these 6 days, which values shown in the **Table 8** and represented in **Figure 26** to help understanding of these values.

Table 8: pH evolution in Ag samples

Samples	Day 0			Day 2			Day 5			Day 6		
	25°C	50°C	80°C	25°C	50°C	80°C	25°C	50°C	80°C	25°C	50°C	80°C
0	4.550	4.537	4.449	4.485	4.515	4.392	4.353	4.340	4.264	4.327	4.324	4.274
1	4.396	4.435	4.266	4.300	4.378	4.214	4.145	4.202	4.068	4.11	4.197	4.030
2	4.386	4.415	4.270	4.318	4.352	4.219	4.146	4.180	4.098	4.118	4.179	4.029
3	4.044	3.941	3.582	3.881	3.878	3.524	3.617	3.666	3.358	3.558	3.639	3.319
4	4.036	3.931	3.573	3.864	3.864	3.528	3.615	3.658	3.342	3.556	3.619	3.302
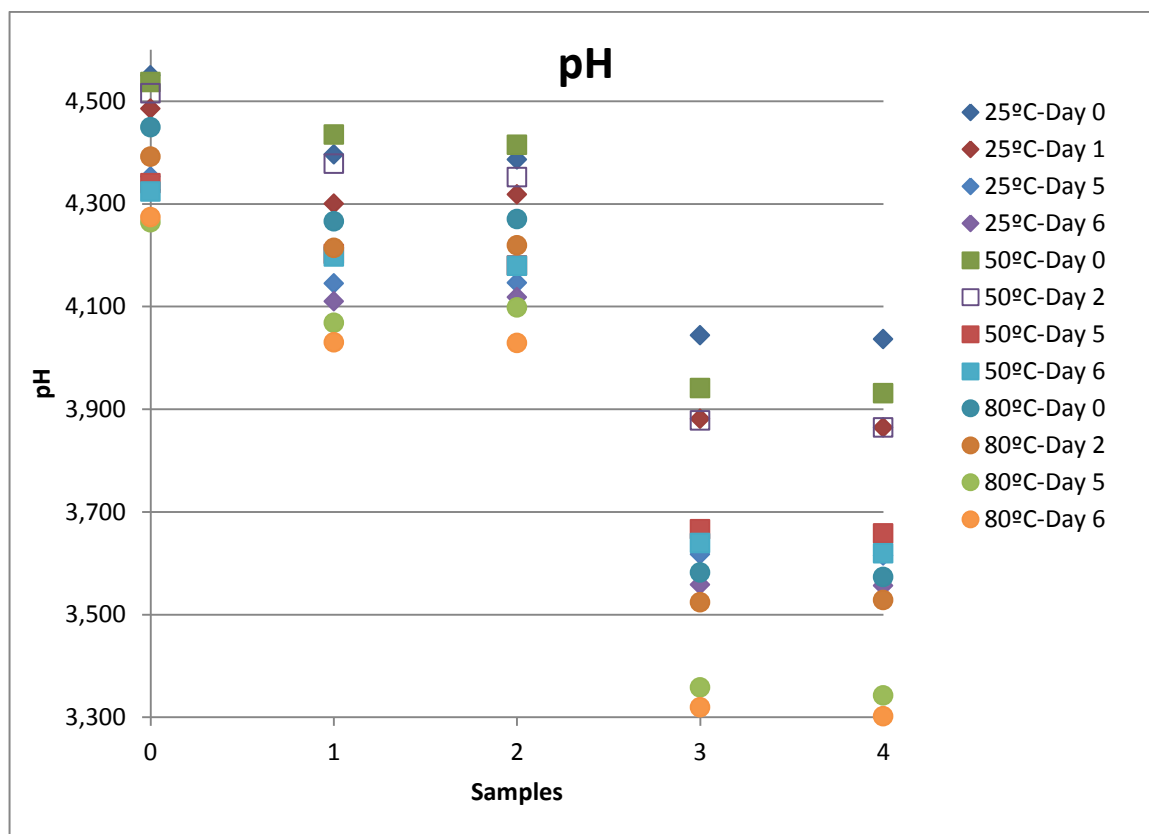
Figure 26: Presentation of Ag samples' pH evolution in a week.

Several conclusions can be reached by observing the **Figure 26**:

1- pH values show a decreasing trend.

2- pH value of S0 has been mostly maintained.

3- Samples of 80 ºC have lower pH than other.

4- S3 and S4 (which with more Ag ions) presented more decrease in pH value than S1 and S2.

6.1.2.4. Reduction potential of reagents

As mentioned in the **section 4.2**; to assure the redox reaction will take place, there must be a significant difference between both reagents' reduction potential. Therefore, it's decided to measure reagents' reduction potential. The electrodes used were Thermo Scientific Orion 900200 Epoxy double junction reference half cell (the one in the left of the **Figure 27**) and the one in the right is a redox electrode with platinum ring indicator. The reagents have been measured under agitation in 3 min on mode continue.

Extract's results have been shown in the **Figure 28.** Extract's reduction potential presents a growing trend, biggest difference of potential increase was between day 0 and 1.

Figure 27: Experiment mounting to measure reagents' reduction potential
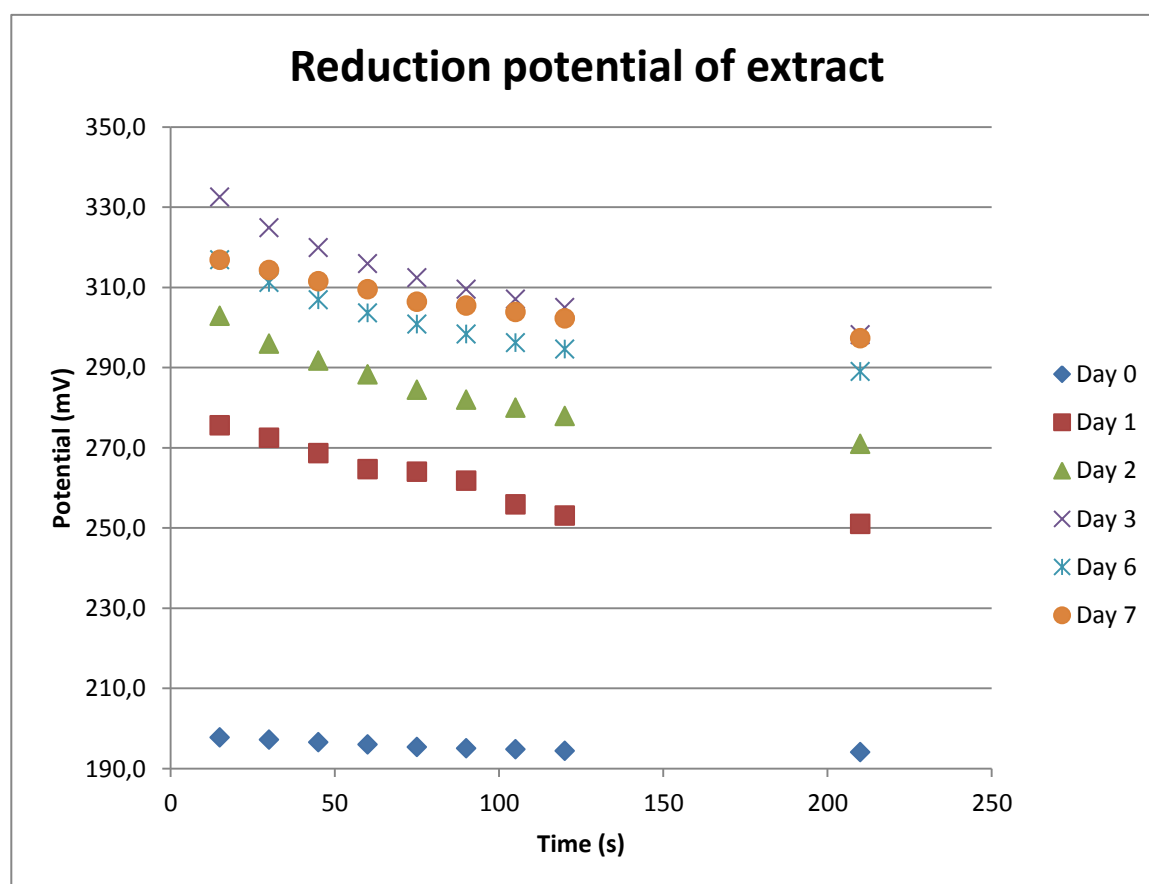
Figure 28: Evolution of extract's reduction potential.

Since AgNO$_3$ solution has already been stored several days before this measurement, so the evolution has been studied from day 7 on. Even so, a clear decreasing evolution of the

potential has been detected in the **Figure 29**.
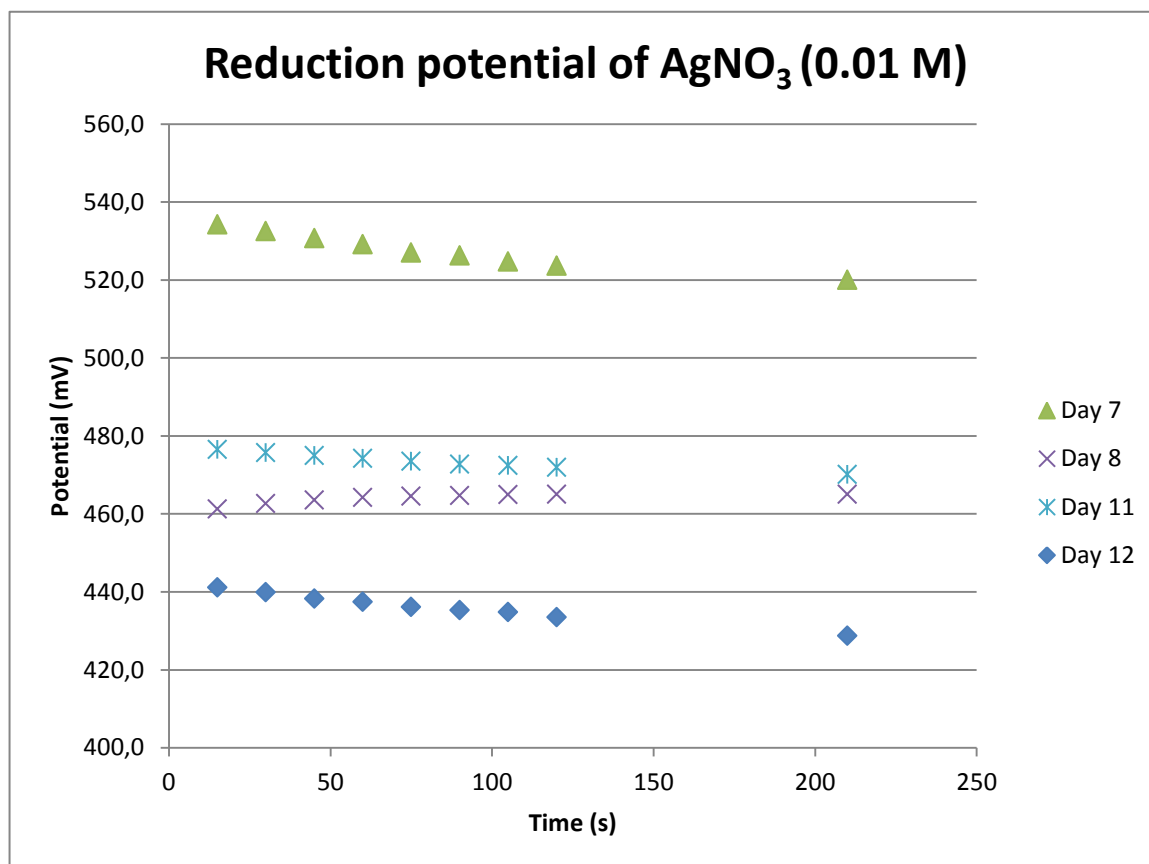
Figure 29: Evolution of Ag dissolution's reduction potential.

In conclusion, the recent prepared reagents are in the optimum condition for NPs synthesis. Because at that moment, metallic ion dissolution had highest reduction potential, which means metallic cations had higher chance to be reduced.

Ag dissolution has higher reduction potential even in the 12th day (440 mV approximately) than extract in the 7th day (315 mV), which probably means the reaction will take place too, maybe not so efficiently as the first day.

6.2. CuNPs synthesis

6.2.1. First synthesis

The first synthesis of CuNPs has been taken place at 80 ^{o}C, it followed the same experiment process as AgNPs' in order to be able to compare their results later. However, no CuNPs have been formed.

6.2.1.1. Color change

There was no color change in Cu samples after two hours of reaction; either precipitate has been seen after centrifugation, as it's evidenced in **Figure 30** and **Figure 31**.
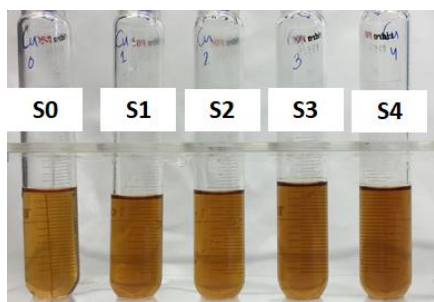
Figure 30: Cu samples after being in 80 ^{o}C during 2 h, without centrifuged either filtrated.
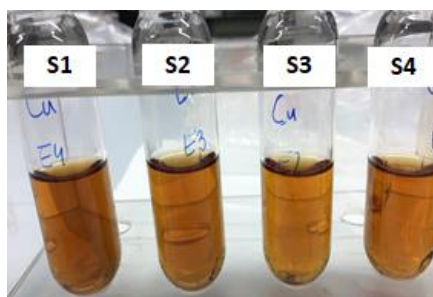
Figure 31: Cu-S1-S4-50 after centrifugation

6.2.1.2. UV-Vis determination

As consequence of the no color changing, spectra of S1 to S4 were same as S0's spectrum since they have same color. It verifies again that CuNPs synthesis has been failed using the experiment condition according to **Table 3**.

6.2.2. Second synthesis of CuNPs

6.2.2.1. Reduction potential of reagents

To discover the root cause of the fail experiment, it has been decided to measure Cu ion dissolution's reduction potential. In order to make the synthesis is feasible, higher concentration of Cu dissolution has been used to increase the reduction potential. In the

Table 9, the influence of dilution rate to the reduction potential has been seen. According to this table, new reaction´s conditions have been designed and summarized in the **Table 10**.

Table 9: Reduction potential of reagents.

	Only extract	1 mL extract +13 mL water	2 mL extract +12 mL water	8 mL extract + 6 mL water
Potential (mV)	328.70	220.63	221.30	242.18
	Only $Cu(NO_3)_2$ 0.5 M	1 mL $Cu(NO_3)_2$ +13 mL water	6 mL $Cu(NO_3)_2$ +8 mL water	8 mL $Cu(NO_3)_2$ +6 mL water
Potential (mV)	362.3	282.68	320.34	327.54

Table 10: New conditions experimental for CuNPs´ synthesis.

Temperatures (ºC)	# Sample	Extract volume (mL)	$Cu(NO_3)_2 \cdot 3\,H_2O$ 0.5 M volume (mL)	Distillated water volume (mL)
50	BS-12	8	0	6
25	S1	8	1	5
	S2	8	6	0
(Samples BSs actuate	BS-3	1	0	13
as reference in UV-Vis	S3	1	8	5
analysis.)	BS-4	2	0	12
	S4	2	8	4

S1 and S2´s variable are same as the first synthesis and only the content of Cu has been change. In S3 and S4 only a few quantity of extract has been added.

6.2.2.2. Color change

In **Figure 32** and **Figure 33** samples are showed, which have demonstrated the color change. Precipitates have been found in samples which Cu cations have been added, and with more extract in the dissolution, more materials have been precipitated.
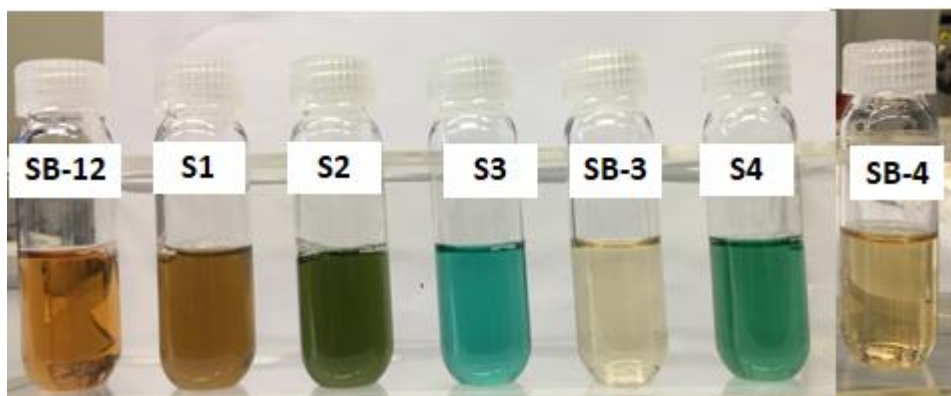
Figure 32: Cu samples when just have mixed two reagents.
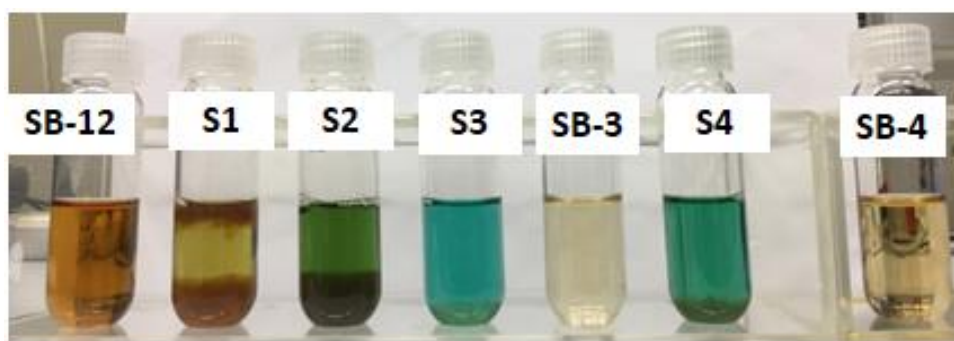
Figure 33: Cu samples after being in 25 ^{o}C during 2 h, without centrifuged either filtrated.

Synthesized samples after been centrifuged and filtrated have been test with UV-Vis. Spectra have been represented in the **Figure 34** and in the **Table 11**, absorbance of each sample can be observed.
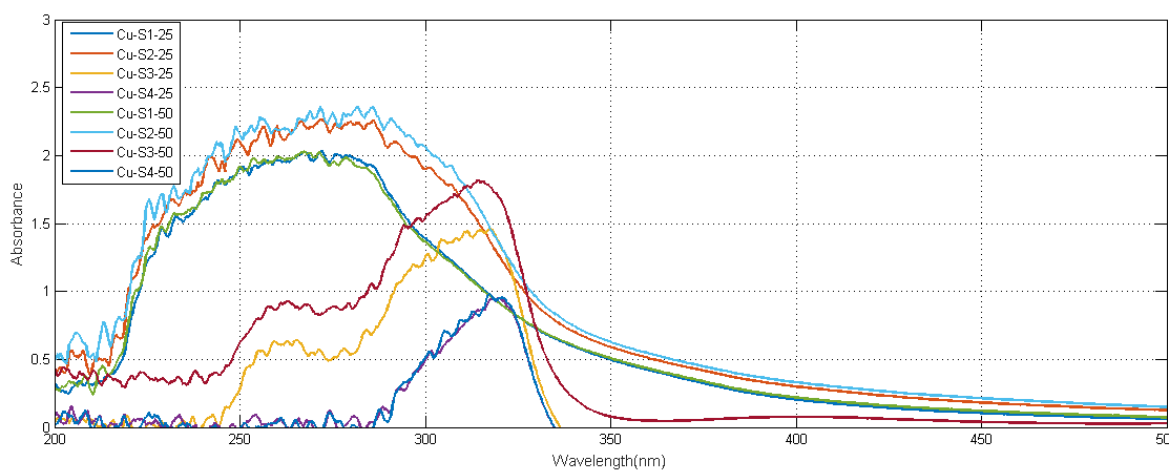
Figure 34: Cu spectra of second synthesis.

Table 11: Summary of max. absorbance and λ of spectra of CuNPs.

Samples	T=25ºC		T=50ºC	
	λ (nm)	Absorbance	λ (nm)	Absorbance
S1	272.0	2.0320	267.5	2.0280
S2	271.6	2.2650	271.6	2.3550
S3	317.3	1.4580	314.3	1.8150
S4	319.9	0.9488	317.3	0.9824

A few conclusions could be summed up from **Figure 34** and **Table 11**:

1. Samples with less quantity of extract (S3 and S4), their spectra show a max. absorbance in a higher λ (between 315 and 320 nm); moreover, the max. absorbance has been shown in the spectrum of S3, which contains less quantity of extract.

2. Samples of 25 ºC and 50 ºC have similar max. absorbance and its corresponding λ, it's approved again the reproducibility of the green synthesis of NPs.

6.3. Characterization of AgNPs by electron microscopy
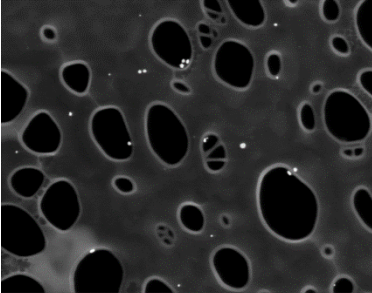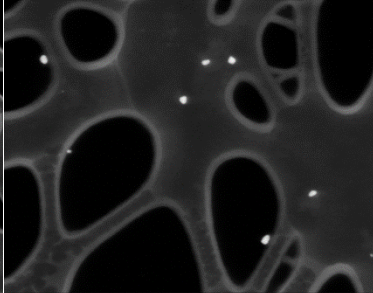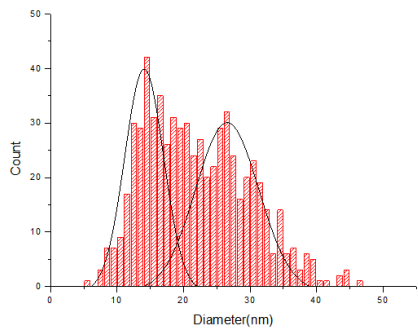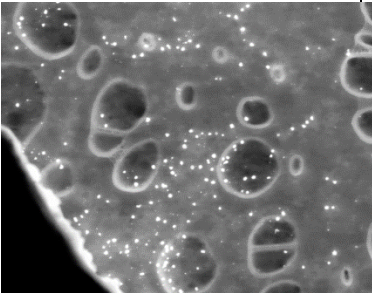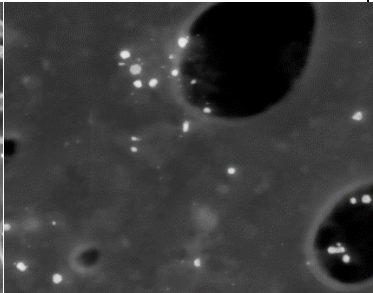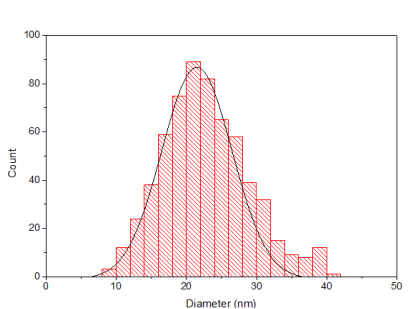
In the following

Table 12, Ag samples' photos taken by STEM have been shown; there are two magnifications, which mean two different scales. It's easier to measure NPs with bigger scale because the edged of NPs are clearer.

One samples of each temperature and metallic ion concentration has been chosen to study their influence to NPs' shape and size distribution, totally 6 samples should be analyzed. However, due to the breakage of the filament of the electron microscopy, S4-25 has not been able to be analyzed, either samples of Cu.

NPs' size has been measured using software ImageJ and detail measurement process can be seen in the section **11.1.** Size distribution has been analyzed by software Origin 9.0, see detail process in the section **11.2.** of the Annex.

Table 12：Study of samples with STEM after 15 days have been stored in the fridge.

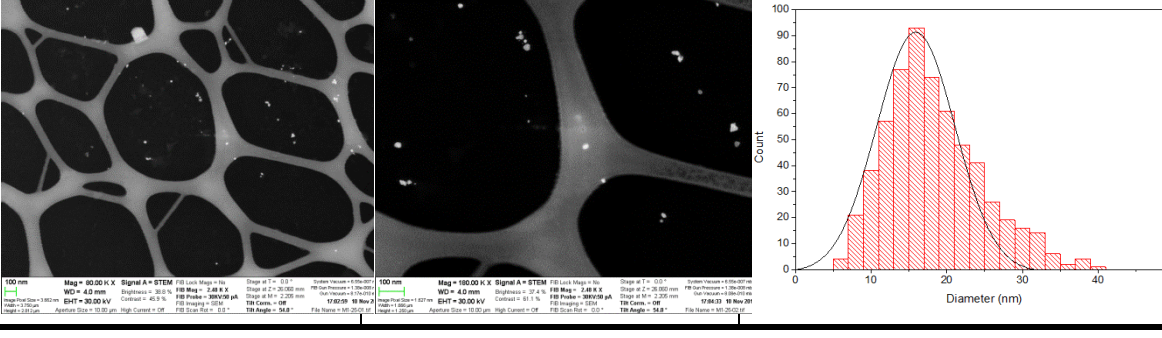| Magnification 80 000	Magnification 180 000	Size distribution
S2-80		
S4-80		

S2-50

S4-50

S2-25

S4-25

-

In the following **Table 13**, AgNPs' size distributions have been shown, which include the nominal and deviation of NPs' diameters. Samples with higher metallic contents present a single size distribution. However, samples with lower contents of Ag, have various size distribution.

Table 13: Summary of NPs' size distribution.

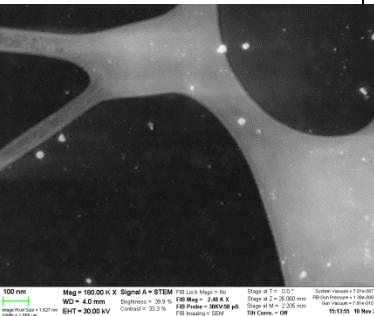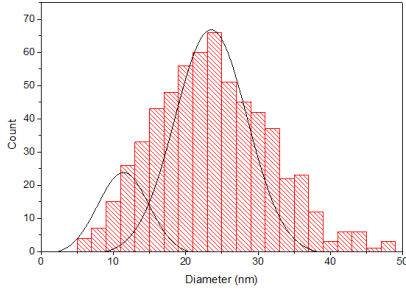| Samples #	Size distribution (nm)	Samples #	Size distribution (nm)
S2-80	14.07 ± 5.85 26.55 ± 9.50	S4-80	21.50 ± 10.00
S2-50	15.46 ± 9.93 24.76± 5.34 33.56 ± 8.57	S4-50	30.42 ± 10.36
S2-25	15.91 ± 10.37	S4-25	-
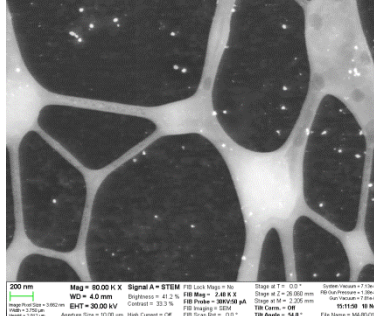
Size distribution of S2-80 and S4-80 (see **Table 14**) are similar to the results obtained in previous work. Only these two samples can be compared because they have the same experiment condition as previous work (have been synthesized in 80 ºC).

Table 14: Comparison of NPs' size distribution with previous work [3].

Samples	Present work (nm)	Previous work (nm)
S2-80	14.07 ± 5.85 26.55 ± 9.50	10 ± 5 25 ± 10
S4-80	21.50 ± 10.00	25 ± 10

See the **Table 15** the result of analysis of S4-80 after 30 days and the **Figure 16** the comparison with results before. Result shown that seems smaller NPs have been formed during the storage.

Table 15: Sample S4-80 after 30 days have been stored in fridge.

Magnification 80 000	Magnification 180 000	Size distribution

After 15 days (nm)	After 30 days (nm)
21.50 ± 10.00	11.40 ± 7.08 21.51 ± 10.00

7. Financial analysis

This is a 24 credits Bachelor's degree project, which corresponds to a 720 hours of work. In the *Table 16*, a simplified financial analysis has been shown.

Table 16: Distribution of badge

Concepts		Quantity	€/Quantity	Total (€)
Junior engineer		720	10	7200
Professors		45	50	2250
Synthesis of NPs				
	Reagents			200
	Syringes	40	2	80
	Filters for syringes	1 (box)		50
Characterization of NPs				
	STEM section	6	200	1200
	Gold grid	6	50	300
General laboratory material				50
Laboratory equipment amortization				300
Profit				500
	Before tax			12130
	V.A.T. (21%)			2547.3
	Total (€)			14677.3

8. Further investigation

Due to the breakage of the filament of the microscopy, STEM analysis of CuNPs hasn't been realized. So it's recommended to study CuNPs shape and size distribution by STEM.

The optimization of reagents' concentration to be used in future synthesis The samples storage should be taken in count because the optimum moment to use prepared reagents is the first day.

Synthesis of CuNPs using Cu^+ as metallic cation resource instead of Cu^{2+} may lead more efficiency process since Cu^+ dissolution has higher reduction potential than Cu^{2+} dissolution.

Finding the relation between sample's pH and its size or shape evolution could be interesting, because measurement of samples' pH is easier than test them by UV-Vis and microscopy.

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9. Conclusion

After studied several synthesis in the different conditions, following conclusions have been achieved.

-   The reproducibility of the green synthesis of AgNPs and CuNPs using grape stalks' organic extract as reducer has been proved based on all synthesis, same Ag as Cu.
-   Only the size and shape of synthesized metallic AgNPs have been determinated. There are different shapes of AgNPs, shown in the section **11.1.**; and the size distributions are depend on the reaction temperatures and the reagents concentration.
-   Experiments shown that it's possible to form NPs in room temperature, it's totally possible to decrease the energy consumes.
-   The optimum moment to use the reagents is the first day, should ovoid the reagents storage in the future.

10. Acknowledgements

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11. Annex

11.1. Measurement of NPs' size using ImageJ

ImageJ is a public domain, Java-based image processing program developed at the National Institutes of Health. This software allows to measure object's size (length or area) in a picture.

- First at all, open the program.
- Open a picture of samples which has been taken by STEM: File – Open - Select the picture.
- Set a scale according to the magnification which been used during the STEM section: Analyze – Set scale – Distance in pixels:1; Pixel aspect. ratio: it's should be indicated in the picture (1.627 if the magnification is 180 00 K); Unit of length: nm
- Click [icon] to draw lines inside NPs and click Ctrl + M to record values.
- Since the shape of synthesized NPs is not uniform, servel criterions have been used in order to measure NPs in different forms, see **Figure 35** to **Figure 39**.
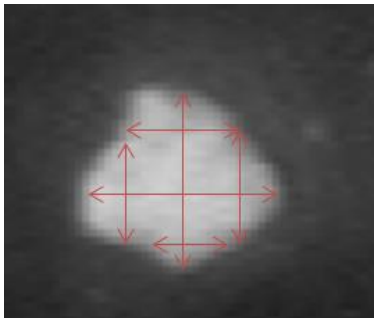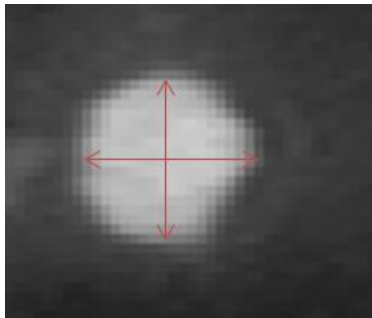

Figure 35: Irregular form NPs
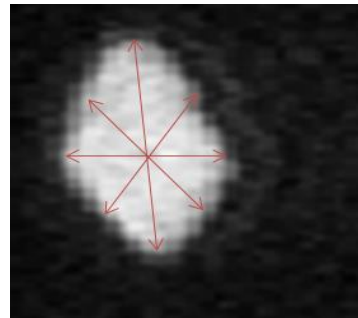

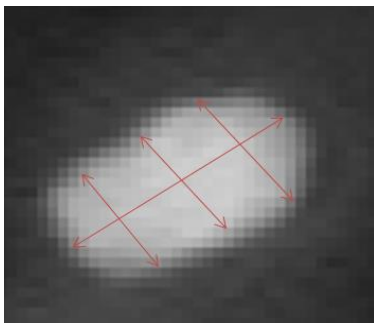**Figure 36:** Cycle form NPs

Figure 37: Rhombus form NPs

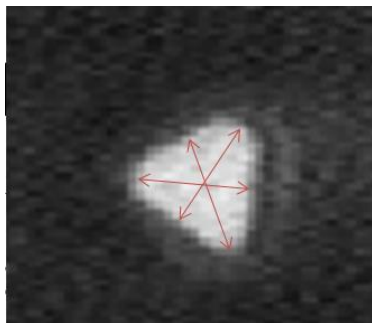**Figure 38:** Rectangle form NPs

Figure 39: Triangle form NPs

- Click Ctrl + D to mark measured particles to avoid double measurement of same particles.
- Save analyzed picture and size values.

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11.2. NPs' size distribution study using Origin 9.0

Origin is a proprietary computer program for interactive scientific graphing and data analysis. Data analyses in Origin include statistics, signal processing, curve fitting and peak analysis. Origin's curve fitting is performed by a nonlinear least squares fitter which is based on the Levenberg–Marquardt algorithm.

- First at all, open the program.
- Introduce values which obtained by ImageJ in the column A: Copy – Paste.
- Sort these numbers: Select all the column - Right click – Sort Column – Ascending.
- Represent these values in histogram: Plot – Statistics – Histogram – select variable A.
- Generate values of column B (CDF): Select the entire column - Right click – Set Column Values – From 1 to number of values in column A (it's should be more than 600) – Col(B) = i/ number of values in column A
- Represent column B into graphic1: Select the entire column - Right click - Plot – Symbol – Scatter.
- Fit the graphic1 with a curve: Analysis – Fitting – Nonlinear Curve Fit – New.
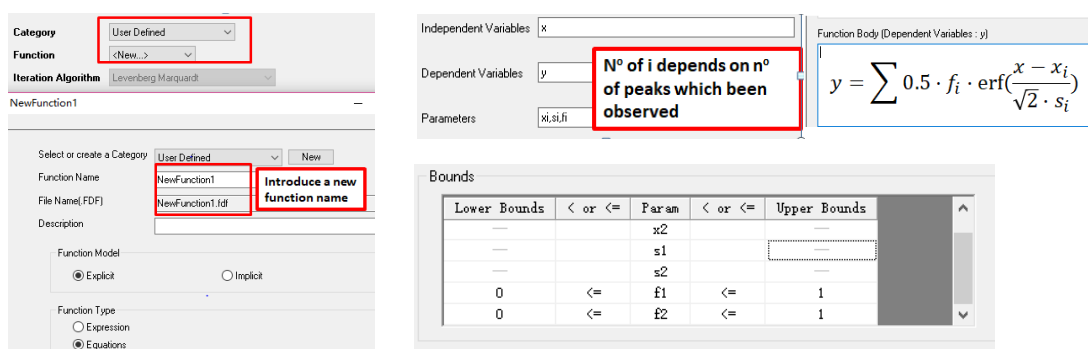
11.3. Reagent's preparation

Weigh 0.170 g solid $AgNO_3$ in a beaker of 100 mL with help of a spatula and digital scale, dissolve with distillated water and a glass rod. Drop the dissolution from beaker to a volumetric flask of 100 mL, dilute with distillated water to 100 mL using pipette Pasteur.

$$100 \text{ mL AgNO}_3 \cdot \frac{1 \, L}{1000 \, mL} \cdot \frac{0.01 \, mol}{1 \, L} \cdot \frac{169.87 \, g}{1 \, mol \text{ AgNO}_3} = 0.170 \, g \text{ AgNO}_3$$

Change the dissolution to an opaque plastic bottle to avoid silver degradation, mark the bottle with the dissolution information and conserve it in the fridge at 4 ºC.

The preparation of copper dissolution is similar, only the weight of solid $Cu(NO_3)_2 \cdot 3 \, H_2O$ is different, the calculation is following:

$$100 \text{ mL Cu(NO}_3)_2 \cdot 3H_2O \cdot \frac{1\ L}{1000\ mL} \cdot \frac{0.01\ mol}{1\ L} \cdot \frac{241.60\ g}{1\ mol\ \text{Cu(NO}_3)_2 \cdot 3H_2O}$$

$$= 0.2416\ g\ \text{Cu(NO}_3)_2 \cdot 3H_2O$$

11.4. Comparison of samples in a week

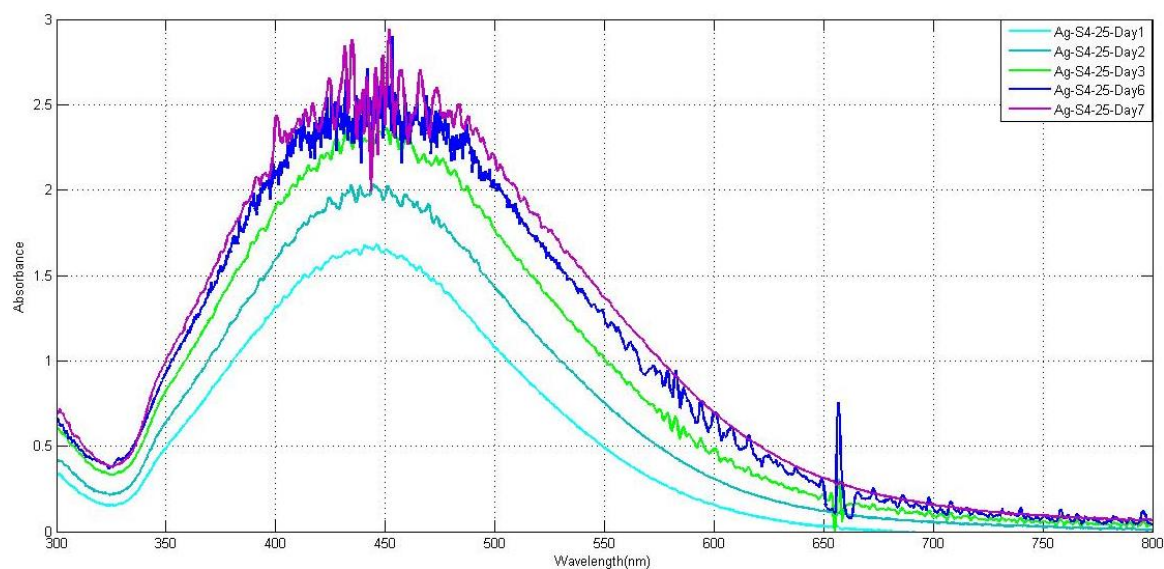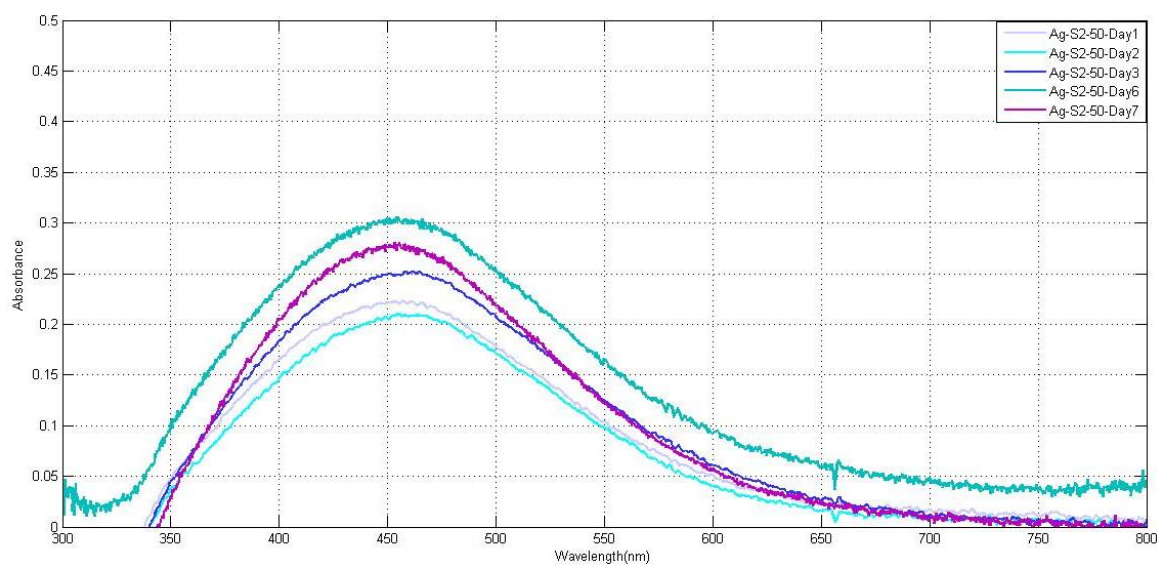**Figure 40:** Comparison of S4-25´s spectra in a week
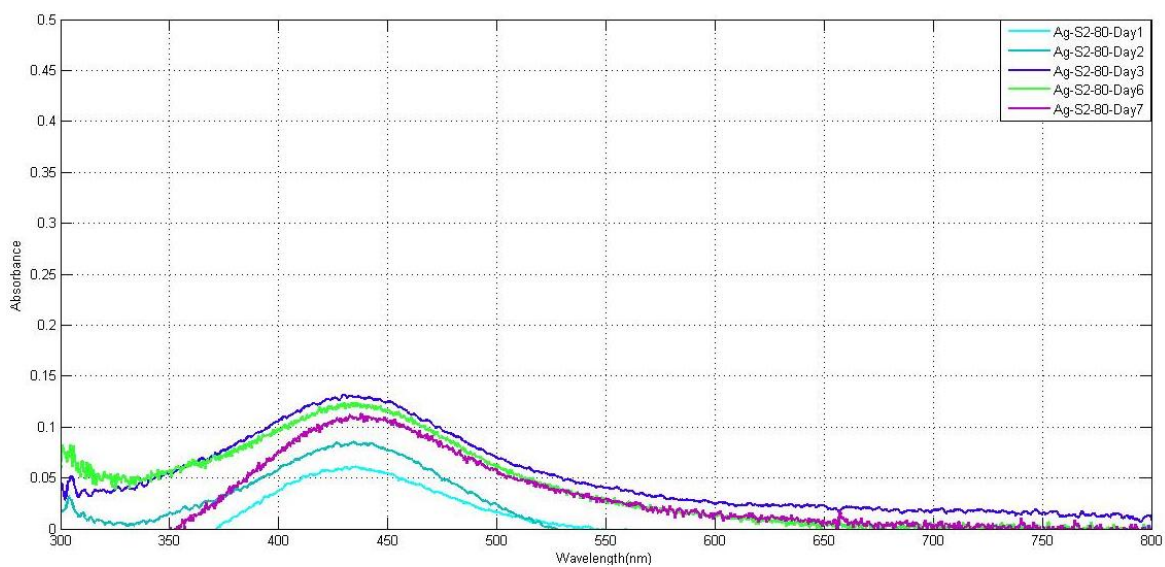

Figure 41: Comparison of S2-50´s spectra in a week

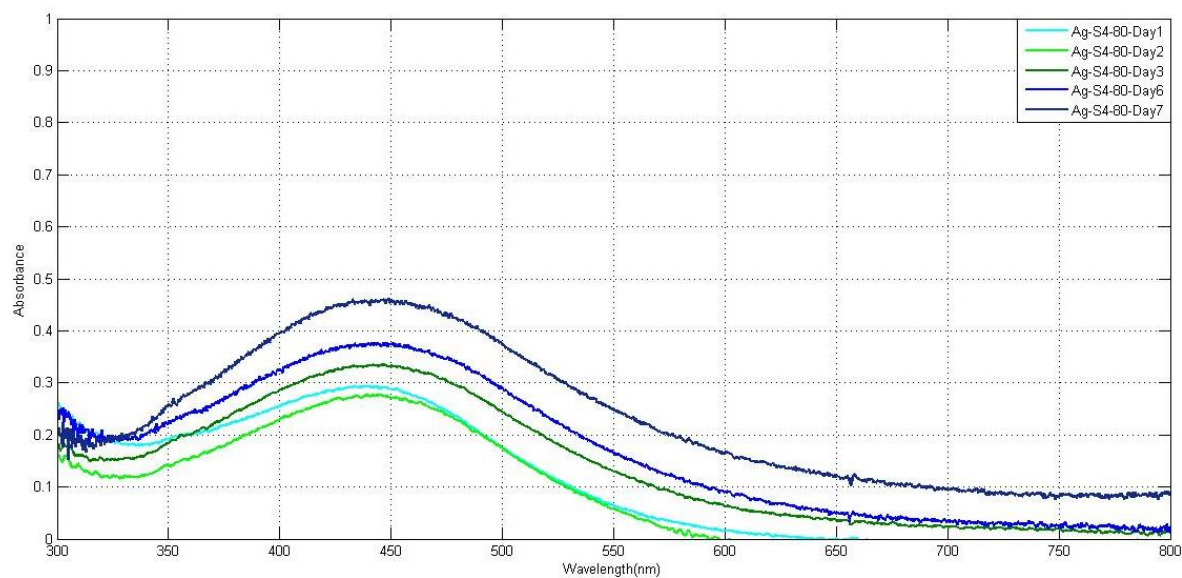**Figure 42:** Comparison of S2-80´s spectra in a week

Figure 43: Comparison of S4-80´s spectra in a week

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