INSTITUTO TECNOLÓGICO Y DE ESTUDIOS SUPERIORES DE MONTERREY

Characterization of Materials

Characterization Techniques for Silver/ Gold Nanoparticles for its applications against *Staphylococcus aureus*

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Abstract:

Although it is still in the experimental phase and considering the fact that the metals from which it is made have already been used for the treatment of breast cancer, it is possible that the silver, gold and bimetallic nanoparticles (NP's) could be used for the treatment of several diseases. Through the years, several methodologies of synthesis and characterization have been proposed depending on the desired application.

In this report, it is discussed how and why silver/ gold NP's could be studied by XRD, UV-vis, TEM, SEM, FT-IR, DLS and other techniques with the purpose of determine if said NP's may be useful as antibacterial agents. Each technique is analyzed in detail and the expected results are discussed in order to conclude which of the mentioned techniques provide the most important information. Said investigation will be used for thesis "Evaluation of the antibacterial properties of bimetallic silver/ gold NP's against *Staphylococcus aureus*"

Introduction:

Since ancient times, silver and gold have been given a wide variety of uses, including medicine. It is also possible to find natural alloys of these two metals, such as the electrum, which has a specific composition of 20-80% gold and silver or 20-80% silver (Ag) and gold (Au) plus a smaller percentage of other metals such as copper (Cu) and palladium (Pb), although it can also be artificially synthesized. [1] Since ancient times, it has been used for making coins, jewelry. For their value, the Egyptians also used it to cover monuments and important artifacts, although the knowledge and techniques of modern synthesis allow to obtain this same alloy.[1] Nowadays there is a great amount of synthesizing techniques that allow to obtain alloys of Ag and Au with the purpose of using the properties of both metals for the diagnosis, treatment of diseases and nanomedicine, including cancer.[2]

Modern techniques to synthetize Ag and Au nanoparticles include physical chemical methodologies and green synthesis. The latter one is friendly with the

environment and therefore it has become the most common technique to extract and synthetize both Ag and Au NP's as there is a large variety of plants and substances that contain these two metals.

After the synthesis, the next step is to study the resulting NP's through characterization to known their properties and structure in order to make sure that they are going to be able to produce an ideal performance. It is known that depending on the applications or desired uses, characterization is necessary, since the properties of the nanoparticles and in this specific case, a bimetallic alloy of Silver/ Gold nanoparticles (Ag/Au NP's) tend to vary, since said properties are affected by the surface area, size, shape / morphology (nanoparticle, nanoplate, nanowires , nanocubes or nanospheres) [3], agglomeration, surface charge, coating, Zeta potential [4], dissolution rate and purity of the particle. [2]

Objectives:

1. Describe in the depth some characterization techniques that could be used to produce an accurate characterization of Ag/Au NP's which antibacterial against *Staphylococcus aureus* will be tested.

2. Select the most ideal characterization techniques to use on the thesis project

Bimetallic NP's synthesis:

The synthesis process consists on a mixture of 500 µL of starch, 50 µL of NaOH and Ag and Au precursors heated at 70 ºC for 3 hours, which where then centrifuged (1 hour, 12,500 rpm). After removing the supernatant, the NP's are once again diluted on water and in order to ensure stability, another 500 µL of starch are added.

At the end of the reaction process, the solution should have a distinctive coloration depending on the proportion of the metals in said solution (20:80 Ag/Au, 40:60 Ag/Au, 60:40 Ag/Au and 80:20 Ag/Au); a reddish if gold is more abundant and yellowish if silver is more abundant. If the proportion is 50:50, then the color of the solution is brownish.

Characterization Techniques:

In order to identify all the desired characteristics, there are several analytical techniques which work with these types of nanoparticles in colloidal state. Some of them can be complemented in order to have a more accurate lecture.

Main Techniques:

1. **X-Ray Diffraction (XRD):** This technique is used to confirm the crystalline nature of the bimetallic NP's and to study the structural properties of the NP's.[5,9] 6-8 mL of colloidal sample are evaporated dropwise on a microscope slide, and X-ray diffraction (XRD) patterns are obtained using a Rigaku Miniflex 600 diffractometer operating with a voltage of 40 kV, current of 15 mA, and Cu-Kα radiation (λ = 1.542 A). The patterns for the three samples are to be obtained at room temperature, using a step width of 0.05◦(2θ) and scan speed of 1◦/min. [5,6,7] The expected results should be similar to the ones seen in Fig. 1 even of the synthesis method and the source of the metals is different form the one to be used on the thesis, as the crystalline nature of the metallic NP's does not changes and just the size of said crystals may differ. [8]

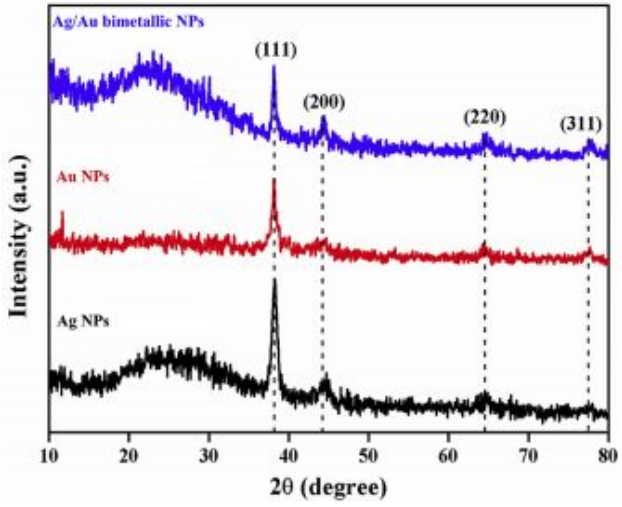

Fig 1: Expected patterns of the Ag/Au NP's according to the article "Green synthesis of silver, gold and silver/gold bimetallic nanoparticles using the *Gloriosa superba* leaf extract and their antibacterial and antibiofilm activities". Because of its composition, the bimetallic NP's peaks are similar (or should be very similar) to the ones of Ag and Au.[8]

2. **UV-vis Spectroscopy:** It is used to visualize the reproducibility of the synthesis, as well as the stability of the colloids over time by comparing the absorption spectra measured through time. It can also be used to give an estimation of concentration, degree of agglomeration and size of the particles. Said measurements shall be taken on 0, 5, 10, 20 and 30 days after the sample was synthesized, and to ensure the reproducibility of the synthetization process, which was done by comparing the absorption spectra of different samples for Ag/AuNPs.

   Absorption spectra of the colloidal Ag/AuNPs needs to be measured in the range between 300 and 700 nm, as the absorption spectrum of both Ag and Au is located between this two wavelengths. The samples should be diluted in a proportion 1:5 (0.4 mL of the NP's samples and 1.6 mL of milli Q water) to measure their absorption band. Depending on the width of the band we can know if the solution contains monodispersed or poly dispersed NP's.[5,6,7]

   The wavelength of the 20:80 AgAu samples are supposed to be titled to the right, while the 80:20 AgAu should be titled to the left and there should be little or not changes on said lectures with the pass of time, which means that the NP's are stable.

   This is the most basic technique to utilize, yet it is cheap, easy to use and it is ideal with colloids.

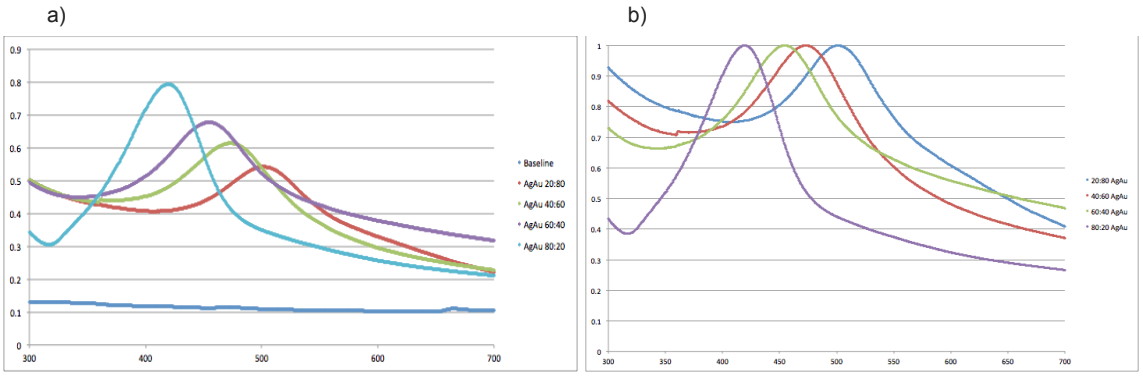

Fig 2: Expected bands of Ag/Au NP's for different concentrations. a) Corresponds to absolute absorbance, (Red: 20:80, green: 40:60, purple 60:40 and light blue 80:20) while b) represents the normalized spectra where the max. of each curve is 1 (Blue: 20:80, Red: 40:60, Green 60:40 and purple 80:20). Axis X corresponds to the wavelength, while Axis Y corresponds to the absorbance.

3. **Transmission Electron Microscopy (TEM):** It is used to reveal the exact size, shape, morphologies and dispersion of the nanoparticles, as well as crystallization, stress and magnetic domains [5,10] and it provides images in 2D with high resolution (*Rajan, R., 2013*). Due to the nature of the synthesis process, it is expected that the the NP's should be spherical or quasi-spherical with no agglomeration, with a size between 50 and 100 nanometers.

   The main advantage of TEM over the very similar SEM, it is that it has a better resolution and provides information of the internal composition, although in this case this information is not particularly useful as we already know the composition of the NPs. As it is extremely costly, it should only be used for its ability to produce high quality images.

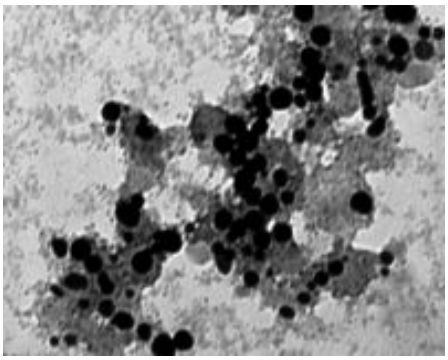

Fig 3: TEM image of bimetallic NP's extracted synthetized from *Swietenia mahogani.* Most of the NP's are spherical with uniform size distribution. [13]

4. **Scanning Electron Microscopy (SEM):** It is used to reveal the exact size, shape, morphologies and dispersion of the nanoparticles focusing on the sample's surface, as it provides images stereoscopic pairs in high definition.

   Although TEM should be enough to visualize the shape and size of the NP's, which are the only visual characteristics that actually matter in this test, SEM can also be used as it is it is cheaper and easier to use than the former. Just as with TEM, it is expected to visualize spherical or quasi-spherical NP's with no agglomeration, with a size between 50 and 100 nanometers.

5. **Fourier-Transform Infrared Spectroscopy (FT-IR):** FT-IR measurements are carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the bimetallic NPs synthesized. In this case, it is simply used to confirm the presence of starch as the stabilizing agent of the noble metal NP's. [5,9,14]

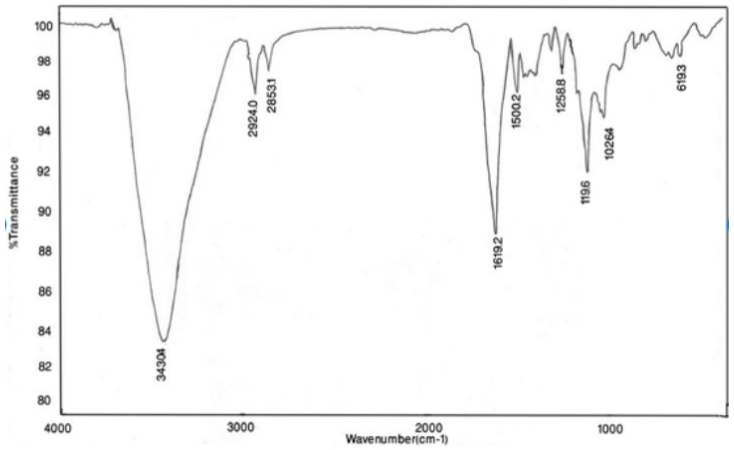

Fig 4: Expected bands of, Ag/Au NP's according to *Biological Synthesis Au-Ag Bimetallic Nanoparticles by A-Amylase,* which shows peaks that corresponds to OH/NH and C=O groups. [14]

Technically, UV-vis should provide enough information about the stability but if we were to document specifically the one of starch with the pass of time, the FTIR shall be used. The results can also be compared to the ones of UV-vis in order to have a more concise information. However, the sample must consists on dry powders, which is this technique main disadvantage against UV-vis. [14]

Although bands may vary to the ones seen on Fig. 4 depending on the synthesis process, the type of material used to stabilize the bimetallic system or primary source of the metals, it is expected to see similar molecular interactions and bands, even if starch is used as the stabilizer.

6. **Dynamic Light Scattering (DLS):** This technique helps to determine the distribution of particle sizes in suspension (hydrodynamic size) and even the diffusion coefficient of these. The measurement in question takes into consideration the size of the particle's core, the size of the surface

structures, the concentration and the type of ions in the medium.[5,15] It is expected that the size of the NP's will be between 50 and 100 nm.

The Ag/AuNPs samples are prepared by being diluted in a ratio 1:10 with deionized (DI) water For all the samples, the refractive index to be used is 0.135 with an absorptivity value of 3.39. For the DLS measurements, several runs must be carried out to obtain the mean particle size and standard deviation. The values obtained should be comparable to the ones obtained with the XRD and TEM for the crystallite and particle size respectively. [5,6]

Alternative and Optional Techniques.

Energy-dispersive X-ray spectroscopy (EDX): It is a technique that can provide the composition and the percentage of each element.[5,8,16,20] Although the composition of the bimetallic alloy is known, this study could help to verify if the composition matches exactly to the one that we are assuming.

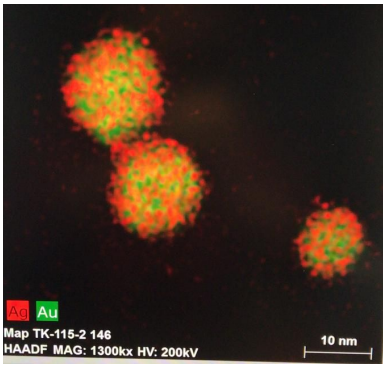

Fig 5: Expected EDX spectrum images from bimetallic nanoparticles with a composition of 10:90 %at Ag, where the red dots correspond to Au and green to Ag. Image provided by Jorge Luis Cholula Díaz (02/28/2019).

Zeta Potential: It is the static electric potential that exists in the double layer at the boundary between the grain (NP) and the fluid in the colloids. It increases or decreases according to the shape of the particle, but the higher its value, the greater the stability of the dispersion. In other words, it tells us how easy it is to penetrate the ionic layer around the NP's. [5, 6, 17]

Zeta potential measurements is obtained for each of the samples, carrying out 10 runs and 100 sub runs to calculate the mean value and standard deviation.

Raman Spectroscopy: A technique used for analyzing chemical structure, crystallinity, molecular interactions and possible contaminants.[7,18]

How this information will help to the thesis?

Bimetallic NP's can be characterized through several techniques. Because the NP's are to be used against bacteria, it is important to make sure that the NP's comply with some requirements, being the most important ones the size, shape and composition, since it is desired to know which of the 4 bimetallic types of NP's that are being designed is the most effective one; the ones with a higher concentration of silver or those with a higher concentration of gold. Therefore, of all the techniques mentioned the most effective ones will be UV-vis, XRD and SEM, as the most important information comes from knowing the shape and size of the NP's because from the morphology and diameter depends the bioaccumulation, cytotoxicity and efficiency of their interactions and inhibition properties against *S. aureus,* but it is also important to make sure that the NP's will not be harmful to normal cells because of the presence of Ag^+ ions, as it is expected that they will act inside the human body. UV-vis and XRD specifically can be used to make sure that the composition and structural properties of the NP's after the synthesis is exactly the one desired, and because this two techniques are ideal for colloids, it is not necessary to make special preparations for the lecture. On the other hand, SEM confirms the size and morphology of the NP's.

From these techniques it will be possible to know if the synthesis methodology proposed for the thesis will produce NP's with the necessary characteristics to be effective against *S. aureus.*

Conclusions

Characterization techniques allows us to know and understand better the samples and materials to be used on future tests and experimentations. By

having the larger amount of information about the NP's, it becomes easier to know what results to expects and make corrections on the synthesis techniques that may allow to achieve more efficient results. Efficiency lies is knowing which characterization techniques will provide the larger amount of information without the need of using all of them and spend unnecessary resources and for this particular case, UV-vis, XRD & SEM provide the most vital information for the purpose of this thesis.

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