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# Reuse of organic waste from *Eucalyptus globulus* extract with high reducing potential in the green synthesis of silver nanoparticles

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**Abstract.** The research provides a new and sustainable methodology for the synthesis of silver nanoparticles, using *Eucalyptus globulus* extract, this due to the fact that it presents metabolites capable of acting as a reducing potential of our silver nitrate precursor, and thus obtaining nanostructured material. This is also associated with the reuse of this type of organic material, which currently abounds as waste in the Peruvian highlands. In the specific case of this research, the effect on the stability over time of the biosynthesized silver nanoparticles was evaluated by varying the pH, with values of 4.82, 8.05 and 10.15. It was observed that as the pH increases the production of nanoparticles is higher, having a saturation threshold close to pH 8. It was also found that for alkaline pH close to 10 a more complete reaction of the reducing agent occurs, but with a high dispersion.

## 1. Introduction

Nanobiotechnology is a growing field whose objective is to create and improve nanoscale structures for use in various applications, these nanoparticles have a high area/volume ratio which gives them unique physical and chemical properties compared to larger structures [1].

In recent years the biosynthesis of metallic nanoparticles has generated a lot of interest due to the growing need to develop more environmentally friendly synthesis methods. Metallic nanoparticles are of great importance due to the wide spectrum of applications that goes from bactericidal products to pharmaceutical products [2]. Some of the most studied metallic nanoparticles include gold, platinum, copper, and silver.

Silver nanoparticles have been used in sensor technology, disease diagnostic systems, and many other biomedical applications. Silver nanoparticles can be synthesized by various chemical and physical methods, the same ones that use toxic products such as sodium citrate or sodium borohydride (reducing agents) in the synthesis procedure [3], the use of these products brings with it a negative impact on the environment, as well as limitations in its use in the biomedical field. At present, it is necessary to develop biocompatible and ecological processes incorporating green chemistry methods, where various types of natural extracts are used, whose biological agents have reducing, stabilizing or both properties [4–6].



To carry out the biosynthesis of silver nanoparticles, several plants have been used by means of extracts obtained with various solvents, these contain chemical compounds such as essential oils, phenols, flavonoids, carbohydrates, proteins, alkaloids, glycosides, oligosaccharides, quinine, saponins, steroids, tannins, among others [7]. This work reports the biosynthesis of silver nanoparticles using eucalyptus to take advantage of the reducing properties of its components. Esmaili et al. they biosynthesized silver nanoparticles using ethanolic extract from dried *Eucalyptus comadulensis* leaves [8]. Thakur et al. reported the antibacterial activity of silver nanoparticles biosynthesized with *Eucalyptus globules* leaf extracts, using chloroform, acetone, and methanol as solvents, among others [9]. On the other hand, other researchers prefer to use aqueous extracts of eucalyptus leaves since, due to their applications, they turn out to be much safer for health and the environment than chemical solvents [10–16].

In the synthesis process, different parameters must be controlled for the formation of nanoparticles. The pH is one of the important parameters that allow controlling characteristics such as shape, size and stability in the biosynthesis of silver nanoparticles [2]. That is why the objective of this research was to study the stability in time according to the pH of the nanoparticles in suspension biosynthesized with aqueous extract of eucalyptus leaves.

## 2. Materials and methods

### 2.1. Process of obtaining the extract

The aqueous extract of eucalyptus leaves was prepared following the following procedure: Eucalyptus globulus leaves were harvested, washed with distilled water and then naturally dried under the sun for three days. The dried leaves were cleaned until they were free of impurities and then cut into small pieces and then shredded. 60 grams was weighed and placed in a beaker with 1 liter of distilled water. the mixture was heated for 30 minutes at 90 °C while stirring and then allowed to cool to room temperature. Finally, the mixture was filtered using whatman filter paper number 42. the extract was stored in a refrigerator at 4 °C for subsequent syntheses.

### 2.2. Silver nanoparticle synthesis process

The synthesis was carried out using the green chemistry method (biosynthesis), having as a precursor 50 mL of silver nitrate ( $\text{AgNO}_3$ ), from Merck, Germany (CAS: 7761-88-8) at a concentration of 1 mM. The solution was brought to a hotplate with stirring keeping it at 60 °C for 10 min. at 300 rpm, subsequently 2.5 mL of aqueous extract of eucalyptus leaves was added drop by drop, finally in the solution the pH was controlled by adding sodium hydroxide (CAS number 1310-73-2) drop by drop under magnetic stirring at 300 rpm. Aliquots with a pH of 4.82, 8.05 and 10.15 were collected to later be analyzed by UV-vis spectrophotometry.

### 2.3. Experimental tests

The colloidal samples were characterized by UV-vis spectrophotometry (Shimadzu, UV 1900, Tokyo, Japan) to evaluate the presence of the plasmon peak typical of silver nanostructures and in turn evaluate the stability of the colloid over time. The data from the spectrophotometry results was processed with Origin software, the same one that precisely defined the values of the optical absorbance peaks and their respective wavelengths.

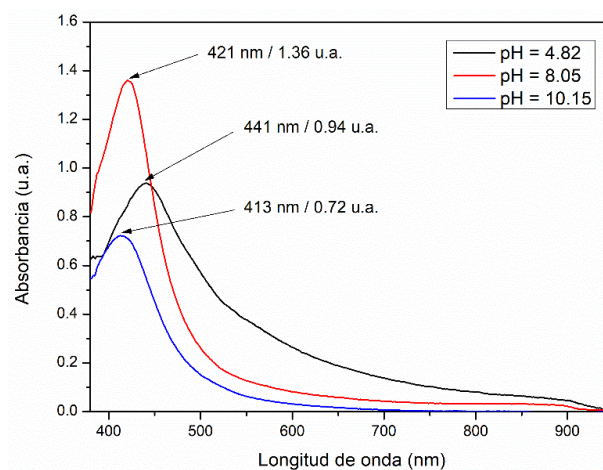
For the analysis in TEM, they were carried out in a JEOL equipment (JEM 2011 model) operated with a voltage acceleration of 120 Kv.

NP Ag samples for pH 10 were analyzed using a Nicolet iS50 FT-IR infrared spectrometer (Thermo Fisher Scientific). A scan was performed in the range of 500 - 4000  $\text{cm}^{-1}$ . Spectra were analyzed with OMNIC 8.1 software (OMNIC Series 8.1.10, Thermo Fisher Scientific).

### 3. Results and discussion

The results presented below show graphs of UV-vis spectrophotometry to find the location of the plasmon resonance peak and thus confirm the formation of silver nanoparticles manufactured under a biosynthesis process using aqueous extract of eucalyptus leaves. With the same characterization technique, the stability behavior of silver nanoparticles has been evaluated over time, observing their behavior on days 1 and 17 and thus confirming the influence of pH in the synthesis processes, understand day 1 like the day of manufacture of the nanoparticles.

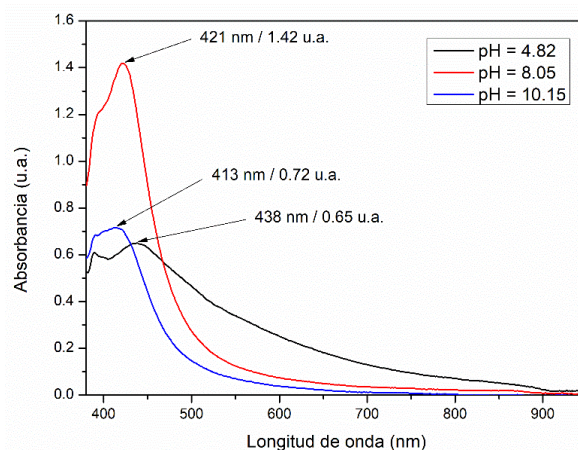
Figure 1 shows the results of UV-vis spectrophotometry for silver nanoparticle colloids at different pH (4.82, 8.05 and 10.15) on day 1. Nanoparticle formation is confirmed in all cases by the presence of the resonance peak. characteristic plasmon. The colloid with a pH 4.82 has a peak around 441 nm and an optical absorbance of 0.94 u.a., which still implies a low production of nanoparticles. By increasing the pH to 8.05, a greater production of nanoparticles is observed with a plasmon resonance peak located at 421 nm and 1.36 u.a. absorbance, also the shift towards the blueshift (shorter wavelength) would indicate the formation of smaller nanoparticles. Finally, a decrease in the absorbance peak is observed when pH 10.15 is reached, the absorbance fell to 0.72 u.a. with the peak at 413 nm. These results confirm the influence of pH on the production of nanoparticles, and also reveal the presence of a reducing agent saturation threshold that would be reached around pH 8. These results are confirmed by Veerasamy et al., In their study with extract of mangosteen leaf, reported that synthesis at acid pH favors the aggregation of silver nanoparticles on nucleation. However, a higher pH facilitates the nucleation and subsequent formation of a large number of smaller diameter nanoparticles [17].



**Figure 1.** UV-vis spectrophotometry of silver nanoparticles biosynthesized with aqueous extract of eucalyptus at different pH for day 1.

Figure 2 shows the UV-vis spectra of the silver nanoparticle colloids after 17 days of synthesis. The changes are significant for the sample with pH 4.82, a significant decrease in the absorbance peak is observed from 0.94 to 0.65 u.a. and a shift towards 438 nm, which implies that at acidic pH the reaction is incomplete and unstable. As the pH increases, the peaks remain at the same wavelength, highlighting a small increase in absorbance from 1.36 to 1.42 u.a. for the samples with pH 8.05, which means that the reaction was incomplete despite the high number of nanoparticles produced on day 1, however, stability with respect to the size of the nanoparticles is achieved. Dubey et al. reported that silver nanoparticles demonstrate a lower potential zeta value at strongly acidic pH compared to alkaline pH solutions, which indicates for the latter, greater stability and smaller size of the nanoparticles [18]. For colloids with pH 10.15 no changes in absorbance or peak shifts were observed, but a broadening which could be attributed to high dispersity. This same result is confirmed by Andreescu et al, who concluded that an increase in pH leads to the formation of highly dispersed

nanoparticles. This phenomenon could be related to electrostatic repulsion at high pH or attributed to the high magnitude of the potential zeta [19].



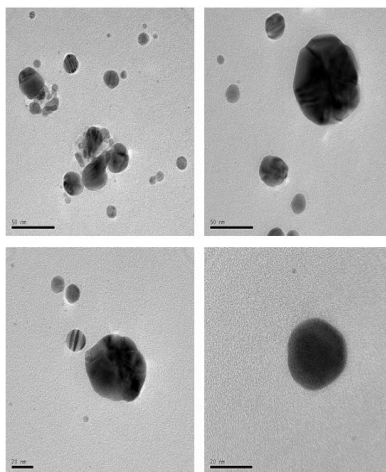
**Figure 2.** UV-vis spectrophotometry of silver nanoparticles biosynthesized with aqueous extract of eucalyptus at different pH for day 17.

According to the results obtained on stability in size of the silver nanoparticles, the colloids with pH 10 present an excellent resistance against agglomeration, however, in the case of pH 8.05, as the days pass there are still processes of reduction of the nitrate precursor of silver due to increased absorbance. Furthermore, the plasmon peaks are well defined and due to their bandwidth, they show high monodispersity and smaller diameter nanoparticles [20], the plasmon peak is relatively unchanged, a unique characteristic in relation to all other samples. With the increase in pH to 4.82, a tendency for the plasmon peak to widen is observed, which possibly implies processes of aggregation and cluster formation, generating even larger nanoparticles.

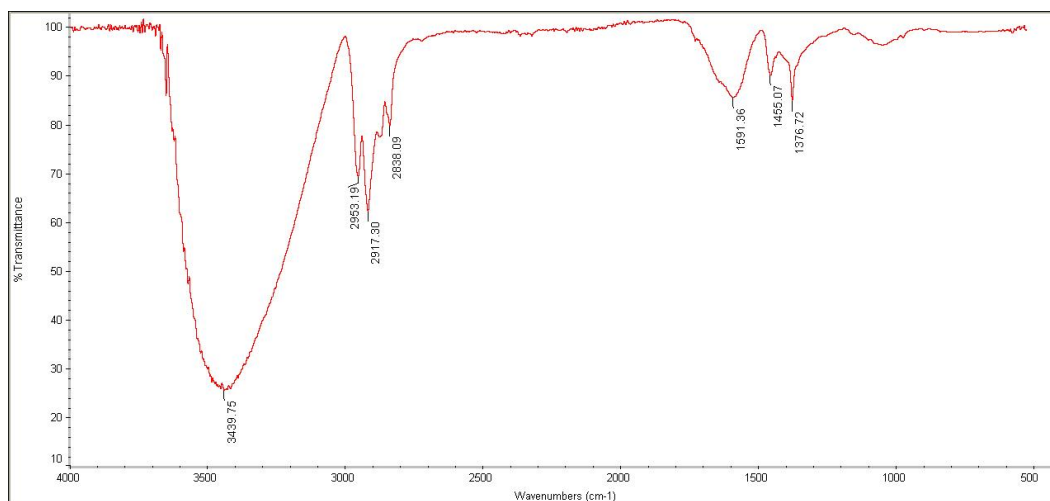
The variation of the pH in the synthesis process implies important changes. In this sense, it is significant to highlight that sample that is stable over time, the sample synthesized with pH 10.15, is the one characterized by being stable over time, the other two samples have a tendency to form agglomerations and have a variation in its absorbance.

Figure 3 shows the transmission electron microscopy (TEM) image of Ag nanoparticles at pH 10.15 17 days after the synthesis has been carried out, where its spherical morphology can be highlighted with an average size at 20 nm., without agglomerations, which to a certain extent justifies what was observed in the results by UV Vis spectrophotometry. The sample in question has a high tendency to be monodisperse, this linked to the fact that the majority of nanoparticulate material has values of average size to the aforementioned, this can be partially contrasted based on the absorbance spectra indicated in figure 2, in where the graph of the sample pH 10.15 has a leptokurtic-like bandwidth configuration.

Figure 4 shows results by FT-IR of the NP Ag sample at a pH of 10.15 after 17 days of having carried out the synthesis, the spectrum confirms that the carbonyl group of the amino acid residues and protein peptides has the greatest capacity to bind to the metal. In this sense, it is most likely that the proteins could form a layer that covers the metallic silver nanoparticles, to avoid agglomeration and therefore stabilize them in the middle. This evidence suggests that biological molecules could possibly perform the function of formation (reduction) and stabilization of silver nanoparticles in aqueous medium through carbonyl groups through free amine or cysteine groups in proteins. The proteins present on the surface of the silver nanoparticle act as protective agents for amino acid residues and the peptides have a great capacity to bind to the silver ion [21].



**Figure 3.** Transmission electron microscopy of silver nanoparticles (NP Ag) synthesized by the green route method at pH 10.15, after 17 days.



**Figure 4.** FT-IR of colloidal silver nanoparticles (NP Ag), synthesized by the green route method at pH 10.15, after 17 days.

#### 4. Conclusions

The reducing capacity of the aqueous extract of eucalyptus leaves for the biosynthesis of silver nanoparticles has been demonstrated and its stability in time for different pH's was studied. It was observed that as the pH increases the production of nanoparticles is higher, having a saturation threshold close to pH 8. At this point smaller and monodisperse nanoparticles were manufactured, the increase in the absorbance peak also indicates that the reaction was incomplete. It was found that for alkaline pH close to 10 a more complete reaction of the reducing agent occurs, but with a high dispersity. It is suggested that future work may be focused on having a better approach to the precursor reduction mechanisms, through techniques such as nuclear magnetic resonance.

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