

Synthesis and characterization of silver nanoparticles using as a reducing agent plant extract of Dandelion (*Taraxacum officianale*)

Síntesis y caracterización de nanopartículas de plata utilizando como agente reductor extracto vegetal de Diente de León (*Taraxacum officianale*)

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Abstract

The objective of this work was to synthesize the synthesis of silver nanoparticles using as a reducing agent of ionic silver the plant extract of Dandelion (*Taraxacum officianale*), vegetable of high availability and low cost, as an alternative to the processes conventional, based on the antioxidant capacity of plant extracts that reduce metals in solution. The nanoparticles prepared by this method were characterized by the golden yellow color characteristic of silver nanoparticle solutions. Measurements with UV-Vis spectroscopy of aqueous solutions of Ag⁺ ions after coming into contact with plant extracts of Dandelion at different pHs showed an intense absorption band around 400-450 nm, characteristic of the resonance of the Plasmon of silver nanoparticles. Through the scanning of the samples by means of AFM (*atomic force microscopy*), morphological information of the nanoparticles is obtained, from 3D topographic images of them, such as distribution, size and shape of the silver nanoparticles. Finally, its antibacterial activity was tested against the *Escherichia coli* strain.

Resumen

El objetivo de este trabajo fue realizar la síntesis de nanopartículas de plata empleando como agentes reductores de la plata iónica el extracto vegetal de Diente de León (*Taraxacum officianale*), vegetal de alta disponibilidad y bajo costo, como una alternativa frente a los procesos convencionales, basada en la capacidad antioxidante de los extractos vegetales que reducen metales en disolución. Las nanopartículas preparadas por este método fueron caracterizadas por el color amarillo dorado característico de las disoluciones de nanopartículas de plata. Las mediciones con espectroscopia UV-Vis de las disoluciones acuosas de iones Ag⁺ después de entrar en contacto con los extractos vegetales de Diente de León a diferentes pH, mostraron una banda de absorción intensa alrededor de los 400-450 nm, característica de la resonancia del plasmón de las nanopartículas de plata. A través del escaneo de las muestras por medio de AFM (*atomic force microscopy*) se obtiene información morfológica de las nanopartículas, a partir de imágenes topográficas 3D de las mismas, como distribución, tamaño y forma de las nanopartículas de plata. Finalmente, se comprobó su actividad antibacteriana frente a la cepa de *Escherichia coli*.

Silver, Plant extracts, Nanoparticles

Plata, Extractos Vegetales, Nanopartículas

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Introduction

In recent years the synthesis of nanoparticles of noble metals has gained great importance because, on a nanometric scale, the physical and chemical properties of metals change with respect to bulk materials, properties that give them multiple applications, in the areas of medicine, technology and nanotechnology. Due to their large surface area on a nanometric scale, several metals have biocidal properties which have promoted the study of metal nanoparticles in order to use them as new antimicrobial agents (Sondi, 2004).

Among the noble metals, silver has excelled because in addition to presenting interesting optical, magnetic, electrical and catalytic properties, in the form of nanoparticles it also has biocidal or antimicrobial properties (Petica *et al.*, 2008). At present, silver nanoparticles have attracted the attention of many researchers because, depending on their size or shape, they have differentiated properties, which have been used in different industrial and commercial areas such as bactericides, in food packaging, in the immobilization of proteins, and in the development of optoelectronic materials and sensors, or even in the textile industry due to the different colorations that silver can present depending on its nanometric shape and size (Khodashenas, 2015).

In this context, silver is a very attractive material to create nanoparticles focused on the treatment of various diseases caused by viruses or bacteria. The fact that the size of silver on a nanometric scale causes a significant increase in its antimicrobial potential is attributed to the fact that nanoparticles have a greater surface area in relation to volume compared to silver on a larger scale (Han, 2012), since Silver nanoparticles can bind to biological tissue proteins causing structural changes in the cell membrane and in the cell wall of bacteria, thus generating cell distortion and death (Ravindran *et al.*, 2013). Therefore, it is evident that the antimicrobial activity or potential of silver nanoparticles strongly depends on the size and shape of the nanoparticles. (Pal *et al.*, 2007).

Most of the methods described to date for the synthesis of silver nanoparticles use reducing agents such as hydrazine, sodium borohydride (NaBH₄) and dimethylformamide (DMF).

All these are highly reactive chemicals and present potential environmental and biological risks (Monge, 2009). Due to this and due to its wide field of application there is a growing interest in developing environmentally friendly synthesis processes that avoid the use of toxic chemicals, such as biological synthesis that has emerged as an option to obtain nanoscale materials, this process It implies the use of microorganisms (bacteria, yeasts, fungi) or plant extracts (Sastry *et al.*, 2003).

Plant extracts are a promising option because they contain reducing agents such as polyphenolic and flavonoid compounds with low redox potentials, suitable for the synthesis of silver nanoparticles (García, 2001). They belong to the group of polyphenolic compounds those whose structure has at least one aromatic ring substituted with one or more hydroxyl groups, and are found in nature mainly in fruits, vegetables, seeds and derived products (Naczka, 2006).

This paper presents an alternative synthesis of economical silver nanoparticles, simple and environmentally friendly, using water as a solvent and does not require strong and toxic reducing agents. The synthesis of silver nanoparticles was carried out using silver nitrate (*Sigma-Aldrich*) and as a reducing agent aqueous extract of Dandelion (*Taraxacum officinale*), vegetable chosen for its low cost and wide availability, within its chemical composition present polyphenols with high antioxidant capacity and low redox potentials such as Luteolin belonging to the flavonoid group. These compounds correspond to a group of polyphenolic secondary metabolites that are formed by an aromatic ring attached to at least one oxhydryl group (Bedascarrasbure *et al.*, 2004). In Figure 1, the structure of the Luteolin (flavonoid) compound present in the Dandelion plant (*Taraxacum officinale*) (Cataya, 2001) is seen.

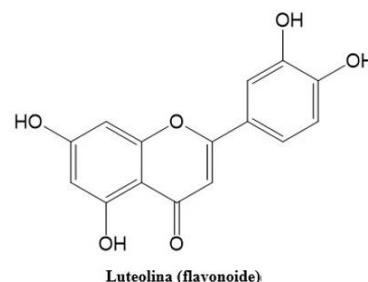


Figure 1 Chemical structure of Luteolin (flavonoid)

In order to verify the antibacterial activity by the silver nanoparticles prepared with the dandelion plant extract and determine whether they have the property to inhibit the growth or development of bacteria, they were tested against the *Escherichia Coli strain*. (Rai *et al.*, 2012).

Methodology

The synthesis of metallic nanoparticles requires following an extremely rigorous synthesis protocol with the reagents used, such as their purity, the form and the order in which they are mixed and all kinds of physical and chemical factors, especially the concentration of the reagents, the temperature, the form and intensity of the stirring and the pH. To obtain the AgNPs silver nanoparticles, an aqueous solution of AgNO_3 10^{-3} Molar (Sigma-Aldrich) was used, as the reducing agent of the ionic silver Ag^{1+} to Ag^0 , the aqueous vegetable extract of Dandelion (*Taraxacum officinale*) was used). In order to determine the effect of the pH variation on the formation of silver nanoparticles, pH values of 5, 7 and 8 were worked, under the same concentration and temperature conditions.

Preparation of plant extracts

The dried leaves of Dandelion were crushed and the water-soluble components were extracted using the traditional methodology called infusion consisting of a solid-liquid extraction. 0.5 g of the ground plant material were taken and contacted with 100 mL of distilled water at 80°C until a volume of 70 mL was obtained, the Whatman No. 3 filter paper was filtered. Finally, each was preserved of the extracts in bottles in the refrigerator at 4°C until use (Arunachalan *et al.*, 2012).

Synthesis of silver nanoparticles

The synthesis of silver NPs was carried out at room temperature, through the reduction of silver ions with the dandelion plant extract. The chemical reagents used and their concentrations were: 1×10^{-3} M AgNO_3 silver nitrate (Sigma-Aldrich), the dandelion plant extract and deionized water ($18\text{ M}\Omega\text{ cm}^{-1}$). For the pH adjustment, drops of a solution of sodium hydroxide (NaOH) (J. T. Baker) 0.5 M in water were added to the Dandelion extract until the desired pH value was adjusted.

The method consists in preparing the indicated solutions and combining them in glass vials in the following order: At 30 mL of the Dandelion extract at different pH values (5, 7, and 8) 10 mL of the AgNO_3 solution is added (1×10^{-3} M) and kept under gentle stirring at room temperature for 10 minutes, thus promoting the formation of silver nanoparticles by reduction of Ag^+ ions.

Characterization

The silver NPs were characterized by UV-Vis spectrophotometry, a technique that has proven to be very useful for the rapid analysis of colloidal solutions of silver nanoparticles allowing to know if the synthesis process has concluded with the formation of nanoparticles. UV-Vis spectra were recorded with a double beam Lambda 35 (Perkin Elmer) spectrophotometer in a wavelength range between 350 and 800 nm using 1 cm optical path quartz cuvettes.

It is based on the fact that the reduction of metal ions produces solutions that in the case of silver have a yellow color with an intense band (plasmon resonance) between 400-450 nm indicating the presence of silver nanoparticles, behavior attributed to excitation collective of electrons on the surface of the particles (surface plasmonic absorption) (Kapoor, 1998). Said absorption spectrum has high sensitivity to changes in the morphology, size and dispersion medium of the nanoparticles (Cruz *et al.*, 2012). This optical property of the appearance of plasmon resonance allows to characterize the metal nanoparticles including those of silver, in addition to being able to determine their formation and growth mechanisms.

On the other hand, through the scanning of the samples by means of atomic force microscopy AFM (*atomic force microscopy*) from 3D topographic images of them, information about the distribution, size and shape of the silver nanoparticles is obtained prepared (Oncins, 2014). These studies were carried out using a JEOL microscope, model JSPM-5200, equipped with a silicon nitride tip for scanning, of the μ -mash brand. The measurements were made at 20°C and at atmospheric pressure. Each sample in aqueous suspension was placed on a silicon wafer and allowed to evaporate at room temperature for one hour.

The scanning conditions for the cantilever were: peak frequency of 275.393 KHz and a Q factor of 530.49, for the image acquisition conditions a speed of 666.67 μ s was used up to 333.33 μ s per line, the feedback filter was varied from 0.75 up to 1.6 Hz and the closed loop gain varied from 2 to 8.

Antibacterial activity

The method used for the bactericide test was disc sensitivity (Bernal, 1984). In Müller-Hinton agar plates, a mass of *Escherichia coli* strains was made, and the filter paper discs were placed at three points on the plate. Whatman No. 5 filter paper discs were previously impregnated for 8 hours in the dissolution of the silver nanoparticles prepared with the Dandelion extract, incubated at 24°C for 24 Hours.

Results

The interaction of aqueous dandelion plant extracts at pH = 5, pH = 7 and pH = 8 with the silver nitrate solution resulted in golden yellow solutions in all cases, characteristic color of the silver nanoparticles, this as a consequence of the reduction of the Ag^{1+} ionic silver to Ag^0 metallic silver. Figure 2 shows the golden yellow color of the nanoparticles prepared with the Dandelion extract at pH = 8.



Figure 2 Dissolution of silver nanoparticles prepared with Dandelion extract at pH = 8

Measurements of UV-Vis spectroscopy of the aqueous solutions of AgNO_3 after coming into contact with the plant extracts of Dandelion at pH = 5, pH = 7 and pH = 8, showed intense absorption bands around 400-450 nm, characteristics of the plasmon resonance of silver nanoparticles, a result attributed to its formation.

In Figure 3, the maximum absorbance band corresponding to the surface plasmon that appears with the samples in which the Dandelion extract was used at different pH values is observed, the band appearing at 424 nm for the case of the sample at pH = 5, at 412 nm at pH = 7 and at 403 nm at pH = 8.

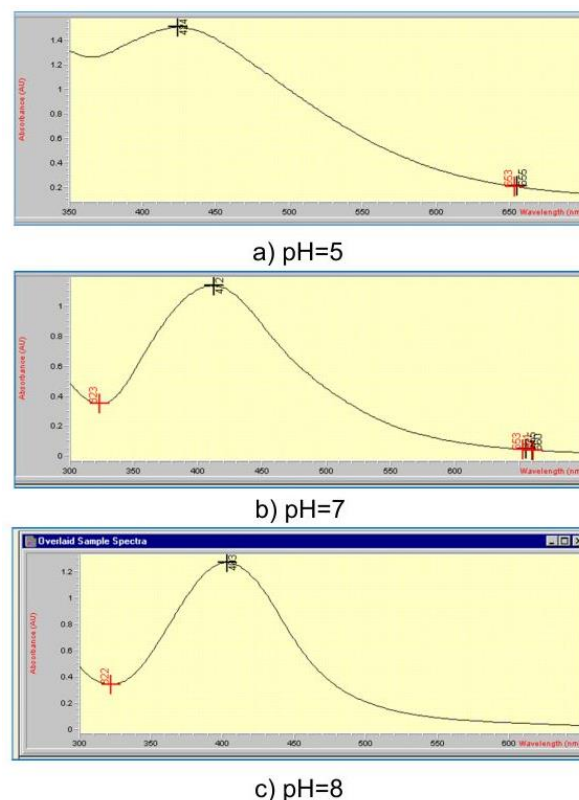


Figure 3 Absorbance spectra showing the surface plasmon band of silver nanoparticles formed with Dandelion extract a: a) pH = 5, b) pH = 7 and c) pH = 8

The shapes and positions of the absorbance bands obtained from the solutions of the prepared nanoparticles, in all cases reveal that they are polydispersed, the width of the bands indicates a wide distribution of the size of the nanoparticles, this effect is less in the sample with the pH = 8, and it can be inferred that the pH of the samples strongly influences their size and polydispersity.

From the comparison of the UV-Vis spectra of each of the samples of silver NPs obtained using Dandelion extract (*Taraxacum officinale*) at pH = 5, pH = 7 and pH = 8, the displacement of the band at shorter lengths with the increase in pH, accompanied by a decrease in the size of the nanoparticle, that is, resulting in smaller AgNPs size for the sample with pH = 8. This demonstrates the dependence of the particle size on the pH.

On the other hand, the appearance of a single shoulderless band (Sosa *et al.*, 2003) is evidence that the nanoparticles obtained are spherical and, due to the position of the bands around 400 nm, it can be inferred that the size of the nanoparticles is approximately between 30 and 20 nm.

Table 1 presents a summary of the results corresponding to the color of the prepared solutions and wavelength in which the maximum absorbances corresponding to the surface plasmon resonance appear.

AgNPs prepared at different pH	Observed color	Wavelength corresponding to the peak absorbance of plasmon
pH= 5	Golden yellow	424 nm
pH = 7	Golden yellow	412 nm
pH = 8	Golden yellow	403 nm

Table 1 Color and wavelength of the absorbance band corresponding to the plasmon resonance of AgNPs obtained with Dandelion extract at different pH values

To confirm the size and distribution of the silver nanoparticles obtained, they were characterized by AFM Atomic Force Microscopy (JSPM-5200 Equipment). The topographic study carried out on the samples makes it possible to determine the presence of nanoparticles in the form of aggregates, as well as their morphology and their size distribution intervals.

Figures 3, 4 and 5 show the photographs of the different scans performed on the sample of silver nanoparticles obtained with the dandelion aqueous extract at pH = 8.

Figure 4 shows the topographic AFM image (5.50 μm x 5.50 μm x 170.4 nm) in shaded mode of the silver nanoparticles obtained with the Dandelion extract, where submicrometric and semi-parchromatic morphology particles are seen, disseminated homogeneously on the surface of the silicon wafer.

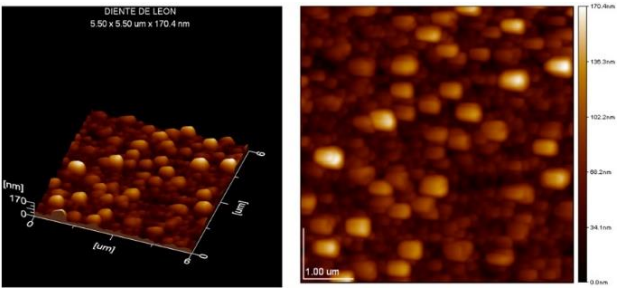


Figure 4 Scanning image of 5.5 μm x 5.5 μm x 170.4 nm, in shaded mode of nanoparticles prepared with Dandelion extract

Figure 5 shows the topographic AFM image with a scanning scale of 2.01 μm x 2.01 μm x 140 nm, in which an amplification of Figure 4 is observed, where the particle cluster distributed in more detail can be seen Silicon wafer. In the images, hemispherical and semiprismatic particles, apparently nanometric, can be seen.

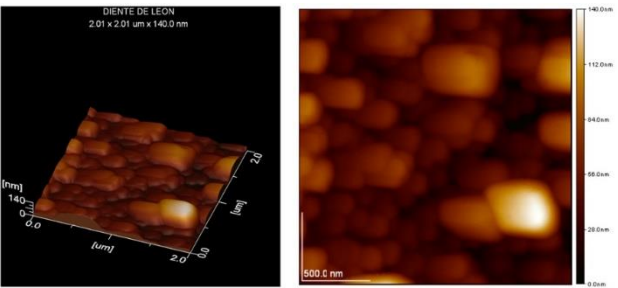


Figure 5 Scanning image at 2.01 μm x 2.01 μm x 140.0 nm "shaded" mode of nanoparticles prepared with Dandelion extract

In Figure 6, the AFM image is presented by topography (1.00 μm x 1.00 μm x 73.0 nm), in an amplification of approximately 1 μm^2 , where two morphologies are observed, which were previously seen as semi-prismatic can be seen with greater detail in this amplification so it can be determined that they have the shape of flakes of the order of 180 to 200 nm long and 100 to 150 nm wide approximately, and the second morphology consists of hemispherical nanometric particles of the order of 20 to 30 nm.

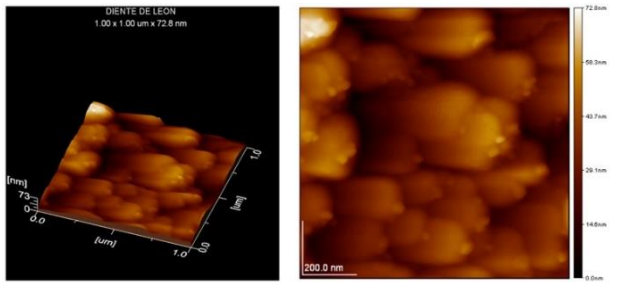


Figure 6 Scanning images at 1.0 μm x 1.0 μm x 72.8 nm "shaded" mode of silver nanoparticles prepared with Dandelion extract

An amplification of the same area of the film (256 nm x 256 nm x 28.9 nm) is shown in Figure 7, in which the hemispherical morphology of the particles deposited on a film formed by flakes, probably due, is observed in greater detail to some other component of the solution, on the other hand, it is confirmed that the diameter of one of these particles is 25.9 nanometers. The image allows to detect the shape of small hemispherical particles as expected according to the results of the electronic absorption spectra in the visible region.

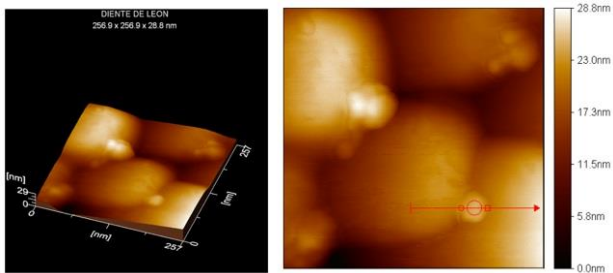


Figure 7 Scanning images at 256.9 nm x 256 nm x 28.8 nm, shaded mode of silver nanoparticles prepared with Dandelion extract, showing the size of a 25.9 nanometer nanoparticles

Test of silver nanoparticles obtained with Dandelion extract as a bactericide

After 24 hours of sowing, the plate was verified with the *Escherichia.coli* strain, clearly showing halos of inhibition in the three points where the filter paper discs impregnated with the colloid of silver nanoparticles prepared with the Tooth extract were placed. Lion.

The antibacterial action was quantified using Bauer's methodology (Bauer *et al.*, 1966), which consisted of measuring the diameter of the bacterial growth inhibition halo around Whatman No. 5 filter paper discs impregnated with the prepared silver nanoparticles. Figure 8 shows the plaque where inhibition halos are clearly observed, and Table 2 shows the results of their measurements.

A moderate activity is observed on the *E. coli* strain, in the three points of the application of the nanoparticle colloid, considered as evidence of its effectiveness as a bactericide against the *Escherichia coli* strain.



Figure 8 Plaque showing the halos of inhibition of AgNPs against *E. coli*.

Number	Diameter of inhibition halos.
1	10 mm
2	12 mm
3	8 mm

Table 2 Measurements of the halos of inhibition of AgNPs against *E. coli*.

Conclusions

The synthesis of silver nanoparticles was developed by applying an environmentally friendly method, compared to the chemicals that originate unwanted by-products. For this reason, the use of the Dandelion aqueous extract is economical and less harmful to the environment and with successful results for the synthesis of nanoparticles. The colloids obtained in all cases presented the golden yellow color characteristic of silver nanoparticles.

The UV-Vis analysis allowed to corroborate the formation of silver nanoparticles in a state of zero oxidation through the absorption response in the blue-violet region (400 nm - 490 nm) that characterizes this metal. The prepared nanoparticles were also characterized by AFM Atomic Force Microscopy, a study that confirmed the formation of silver nanoparticles, revealing the areas where the nanoparticles are added, as well as their morphology and their varied sizes. A moderate activity of the AgNPs prepared on the *Escherichia coli* strain, considered as evidence of its effectiveness as a bactericide against this strain is observed.

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