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Leaf extract from the endemic plant *Peumus boldus* as an effective bioproduct for the green synthesis of silver nanoparticles

Gonzalo Recio Sánchez^{a,b}, Claudia Lagos Castilla^b, Noelia Benito Gómez^c, Alba García^d, Ricard Marcos^d, Erico R. Carmona^{b,e,*}

^a Departamento de Ciencias Matemáticas y Físicas, Facultad de Ingeniería, Universidad Católica de Temuco, Temuco, Chile

^b Núcleo de Investigación en Bioproductos y Materiales Avanzados (BioMA), Facultad de Ingeniería, Universidad Católica de Temuco, Temuco, Chile

^c Departamento de Física, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile

^d Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain

^e Escuela de Medicina Veterinaria, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile

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ABSTRACT

Green synthesis of nanoparticles (NPs) from plant extracts have emerged as a promising methodology for the fabrication of metallic NPs, as it involves an easy, fast, low-cost, and environmentally friendly bioprocess. In the present study, we propose aqueous extracts from the endemic-medicinal plant *Peumus boldus* ("Boldo") as an easy, fast, low-cost, and efficient bioproduct for the green synthesis of silver NPs (AgNPs). Our results indicate that room temperature conditions, short reaction time (only 3 h), low concentrations (1%) of leaf extracts of *P. boldus*, and silver nitrate salts (2 mM) were sufficient to biosynthesize AgNPs in a uniform size (18 nm) and shape distribution (spherical). In addition, stable and pure AgNPs were obtained in this process at the same time.

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1. Introduction

Green synthesis of silver nanoparticles (AgNPs) involves a chemical reduction of the silver salt solution, which makes use of plant extract. In this process, two phases are recognized: (1) the nucleation phase, where the silver atoms form small nuclei using high activation energy, (2) and the second phase, known as growth phase, in which these small nuclei are grouped, giving rise to the creation of nanoparticles (NPs) [1,2].

The features of these NPs obtained by using plant extracts are influenced by parameters such as temperature, reaction time, and pH; however, the nature of biomolecules present in the plant extracts could be the most relevant factor in the bioprocess [3,4]. Thus, the quality of the selected extract is of great importance [5,6].

In the present work, the South American endemic *Peumus boldus* Mol. (also known as "Boldo") was proposed to be a suitable and convenient plant to improve the efficiency of green synthesis

of AgNPs, since it shows high contents of polyphenols, and has a potent antioxidant capacity [7,8]. In addition, the major biomolecule constituents in infusions of *P. boldus* have been well described, being mainly proanthocyanidins, quercetin, kaempferol and isorhamnetin [9].

The aim of the present work was to describe the biosynthesis of AgNPs by using aqueous *P. boldus* extract, and to analyze the effectiveness of this bioprocess according to the chemical and physical properties of AgNPs synthesized from different AgNO₃ concentrations, and reaction time.

2. Materials and methods

2.1. Extracts

Samples of *P. boldus* were taken from the botanical garden of Universidad Católica de Temuco (38° 42' S, 72° 42' W) in October 2012 (austral spring season). Aqueous extracts were prepared by putting 10 g of the homogenized dried leaves and 200 mL of distilled water in a Soxhlet extractor for 4–5 h.

* Corresponding author at: Núcleo de Investigación en Bioproductos y Materiales Avanzados (BioMA), Facultad de Ingeniería, Universidad Católica de Temuco, Temuco, Chile.

E-mail address: ecarmona@uct.cl (E.R. Carmona).

2.2. Biosynthesis

Biosynthesis was carried out by mixing *P. boldus* extracts with aqueous solution of AgNO_3 (Merck Company, Darmstadt, Germany), and the solution was homogenized by stirring at room temperature. The final concentration of AgNO_3 used in the synthesis ranged from 0.1 to 10 mM with 1% volume of *P. boldus* extract.

2.3. UV–Vis-spectroscopy

UV–Vis absorption spectra were measured using a Shimadzu UV-mini 1240 model spectrophotometer. All measurements were performed within the range of 200 nm to 800 nm with a 1 nm interval and with 1 s of integration time.

2.4. X-ray photoelectron spectroscopy (XPS)

XPS spectra were measured using a hemispherical analyzer (Physical Electronics 1257 system). For the XPS, a twin anode (Mg and Al) with X-ray source was operated at 200 W of constant power and using Al $K\alpha$ radiation (1486.6 eV). The samples were placed in a sample stage with emission angle of 45° .

2.5. Transmission Electron Microscopy (TEM)

TEM was carried out with a JEOLJEM – 2011 instrument to determine the size of the AgNPs. Prior to carrying out the TEM measurements, AgNPs obtained from the biosynthesis were purified (by centrifugation) and sonicated.

2.6. Hydrodynamic size and Z-potential

Dynamic Light Scattering (DLS) and Laser Doppler Velocimetry (LDV) were performed with a Malvern Zetasizer Nano-ZS zen3600 instrument to measure the hydrodynamic diameter and Z-potential of AgNPs, respectively.

3. Results and discussion

Fig. 1a shows UV–Vis absorption spectra recorded as a function of time for an aqueous solution of 1 mM AgNO_3 and 1% volume of *P. boldus* extract in relation to the absorption of the extract. It can be observed that *P. boldus* extract showed absorption peak localized at 350 nm. After 2 min of reaction, the reduction of Ag^+ ions to Ag^0 showed a minor absorption peak localized at 454 nm, which is related to the localized surface plasmon resonance of AgNPs [10]. The properties of the absorption peaks related to surface plasmon resonance of metallic NPs are largely governed and influenced by the particle size, shape, distribution and also depend on the dielectric constant of the surrounding media. The absorption peak observed suggests that AgNPs have a spherical shape with an average diameter of 20 nm [11,12].

As the reaction time increased, the absorption peak steadily rose. Above indicates that the amount of AgNPs increased with the reaction time. However, any shift in the center of the absorption peak could be observed, suggesting that the average mean diameter of AgNPs was not affected by the reaction time. After 3 h, the absorption peak related to the plasmon resonance of AgNPs reached its maximum intensity, which confirmed that the biosynthesis of AgNPs was completed. After this time, the intensity of absorption peak slightly reduced, as was observed after 20 h. It should be noted that the biosynthesis of AgNPs with *P. boldus* extracts required less time to complete the process than other kind of leaf extracts previously studied [11,13–15]. The shorter process

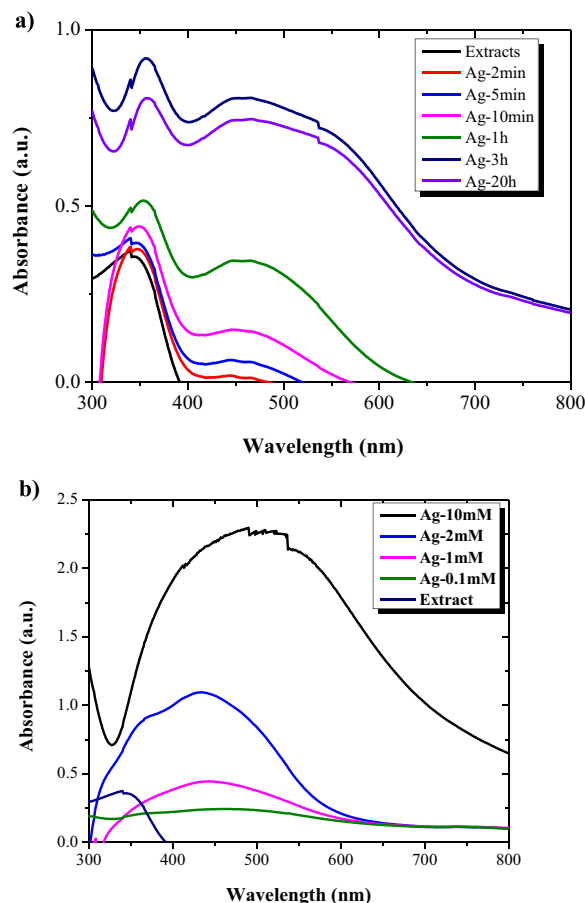


Fig. 1. UV–Vis absorption spectra of AgNPs as a function of the reaction time at room temperature. (a) The concentration of AgNO_3 was fixed to 1 mM, and the volume of *P. boldus* extract was 1% of the total solution volume. (b) UV–Vis absorption spectra of AgNPs fabricated with different concentrations of AgNO_3 keeping fixed the volume of *P. boldus* extract.

time of *P. boldus* could be explained by the potent antioxidant capacity of the leaf extract polyphenols, which facilitated the bioreduction process [9,16,17]. Although the mechanism associated with green fabrication of AgNPs remains unclear, there is a general consensus that synthesis of AgNP by biological entities is due to the presence of large number of organic chemicals like carbohydrates, lipids, proteins, enzymes, phenols, flavanoids, terpenoids, alkaloids, among others, which are capable of donating electrons for the reduction of Ag^+ ions to Ag^0 [18].

Fig. 1b shows UV–Vis absorption spectra after 1 h of reaction of AgNPs biosynthesized with the different concentrations of AgNO_3 and 1% of *P. boldus* extract fixed. A broad absorption band in the range of 430–475 nm could be observed for all concentrations of AgNO_3 , which are characteristic of surface plasmon resonance of AgNPs. In addition, as the AgNO_3 concentration was increased, the intensity of the absorption peak of AgNPs also increased, suggesting that the amount of AgNPs rises. Furthermore, a blue shift from 455 nm to 438 nm could be observed in the absorption peaks when the AgNO_3 concentration increased from 0.1 mM to 2 mM. This blue shift caused by quantum size effects indicates a smaller size distribution meaning that AgNPs do not agglomerate, which was in agreement with the TEM images. However, at 10 mM AgNO_3 , a wider absorption band was observed, suggesting a smaller diameter size of AgNPs, but a red shift occurred in the absorption peak at 472 nm, which actually indicates a large size distribution given by groups of agglomerated AgNPs, also in agreement with the TEM images.

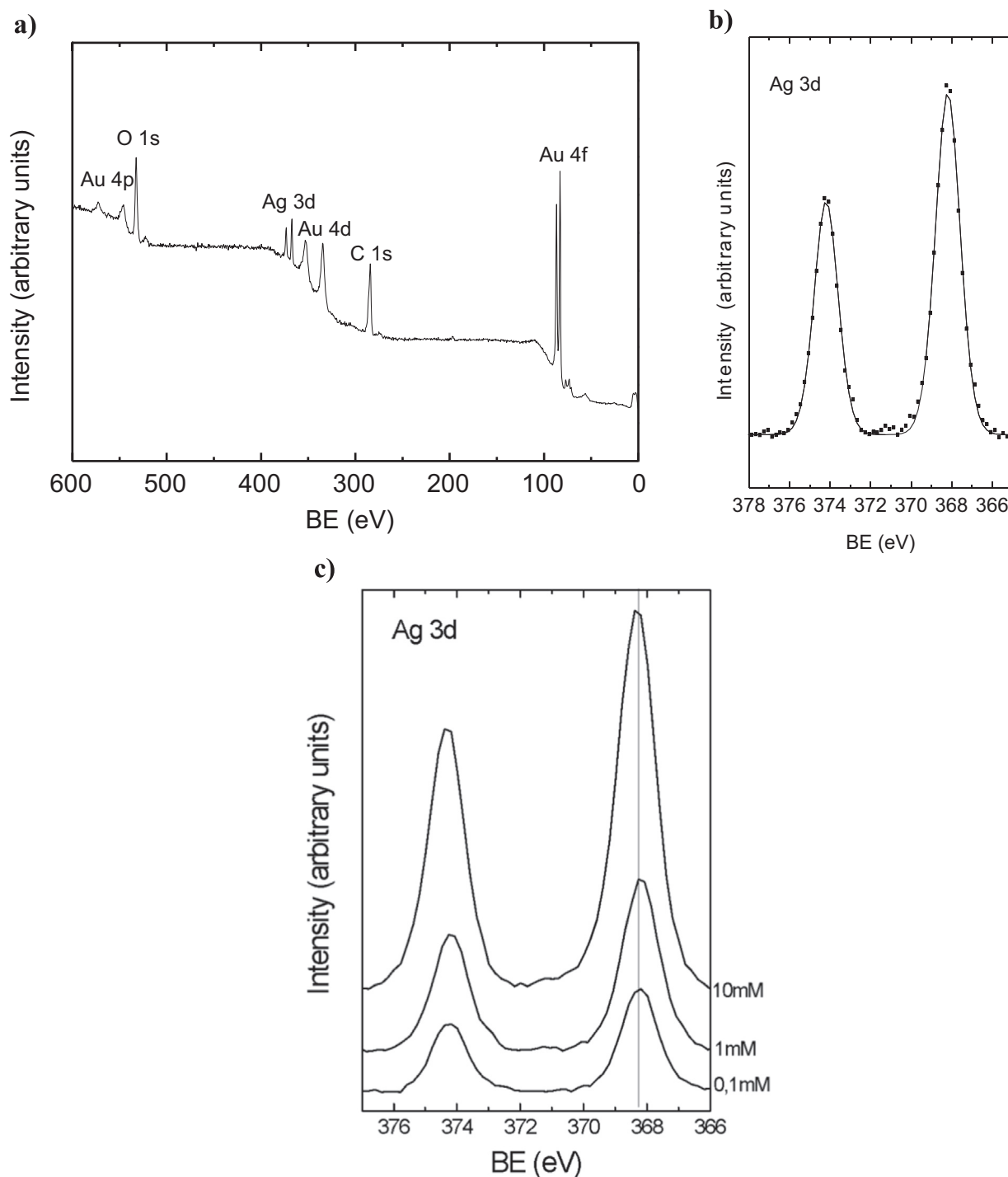


Fig. 2. XPS measurements of AgNPs formed by biosynthesis of 1 mM of AgNO₃ in aqueous solution and 1% volume of *P. boldus* extract. (a) XPS survey; (b) High resolution spectrum from Ag 3d band. (c) High resolution XPS measurement spectra from Ag 3d band of AgNPs biosynthesized at different concentration of AgNO₃ in aqueous solution and 1% volume of *P. boldus* extract.

Fig. 2a shows the XPS survey of the AgNPs obtained. XPS measurements of AgNPs colloids were carried out by suspending NPs on a gold film while the gold served as a metallic reference. Au 4f binding energy was 84 eV for samples without any charging effect. It can be seen that the only elements present on it were C, O, Ag, and the Au from the substrate. It should be noted that nitrogen was not detected from the AgNO₃, which is a clear indication of AgNPs formation. Fig. 2b shows a high-resolution spectrum from the Ag 3d band. This band was fitted with a doublet, with a spin-orbit separation of 6 eV, and an area ratio of 2/3. This doublet

was placed at 368.3 eV, which corresponds with the binding energy of metallic Ag [19].

Fig. 2c shows the Ag 3d band of the samples after Shirley background subtraction of biosynthesized AgNPs with different molar concentrations of AgNO₃ and 1% of Boldo extract. In all cases, the Ag 3d_{5/2} band appeared at 368.3 ± 0.1 eV (SD), which corresponds with the binding energy of metallic Ag [19], indicating that AgNPs were synthesized at different concentrations of AgNO₃.

TEM was used to characterize morphology and size of AgNPs in

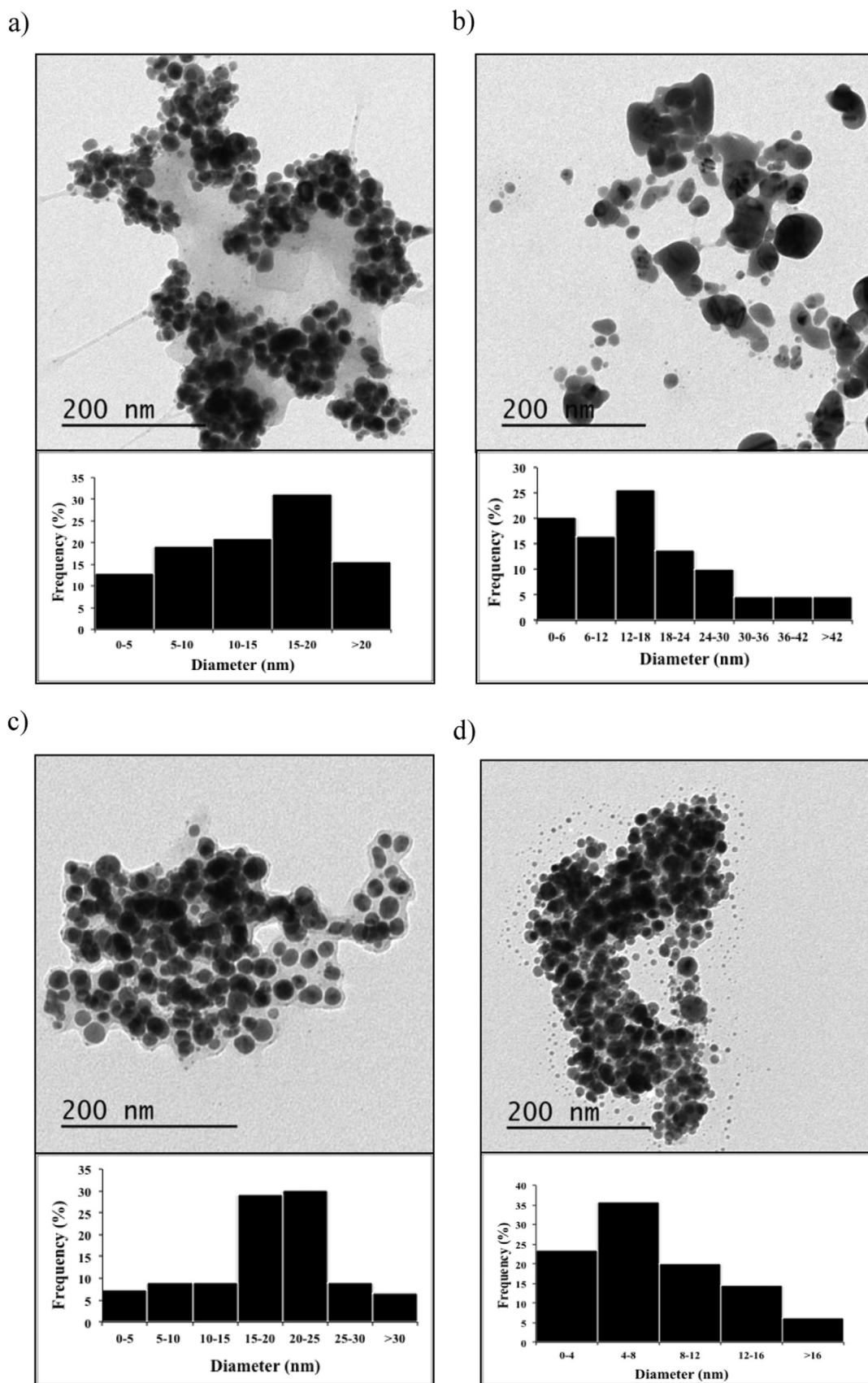


Fig. 3. TEM images and histogram diameter distributions of AgNPs obtained from 0.1 mM (a), 1 mM (b), 2 mM (c), and 10 mM (d) of AgNO_3 salt solution and 1% of *P. boldus* extracts. Scale bars represent 200 nm.

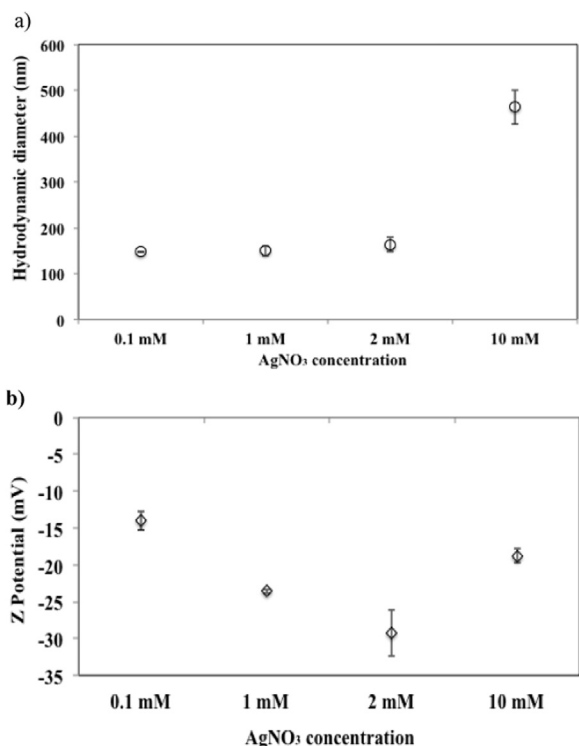


Fig. 4. (a) Hydrodynamic diameter average of biosynthesized AgNPs at different concentrations of AgNO₃ measured by DLS method. (b) Z potential measurements of AgNPs biosynthesized at different concentrations of AgNO₃ measured by LDV method.

aerodynamic form. Most of the NPs analyzed were spherical and showed some levels of agglomeration. The size of the AgNPs ranged from 2.2 nm to 67.7 nm ($n=100$), and the average diameter size was 17 ± 10 nm (SD). It should be noted that the diameter size and shapes of AgNPs were in agreement with the plasmon resonance absorption peaks observed by UV–Vis spectra.

Fig. 3 shows TEM images and the particle size distribution of biosynthesized AgNPs with different concentrations of AgNO₃ and 1% volume of Boldo extract. The data indicates that for all concentrations of AgNO₃ studied, the morphology of synthesized AgNPs was mostly spherical, and when the molar concentration of silver salt was increased from 0.1 mM to 2 mM, the average diameter size of AgNPs slightly increased from 15 nm to 20 nm. However, in the higher concentrations, the average diameter of AgNPs decreased, as can be observed for 10 mM of AgNO₃—where the fabricated AgNPs reached an average diameter of 7 nm, and a large grade of agglomeration was also observed. These results are in agreement with recent studies of green synthesis of AgNPs, as the higher concentrations of AgNO₃ resulted in bigger sized NPs, which sedimented down due to the agglomeration of smaller sized particles [20–22]. These results could be explained partially because polyphenols present in the extracts have the bioreduction capacity to form a higher number of silver nuclei in the system, and consequently, form smaller particle sizes [23–25]. It is important to note that high-pressure liquid chromatography with diode array detection analysis has demonstrated that rutin, and kaempferol-3-O- β were the most abundant flavonoid compounds, while cyanidin and peonidin were the most abundant anthocyanins from *P. boldus* aqueous extracts used in the synthesis of AgNPs (unpublished results).

DLS and LDV were used to measure hydrodynamic diameter and stability of AgNPs. The diameter average in water suspension was higher than TEM analyses. However, these differences are commonly explained by the tendency of NPs to agglomerate in an

aqueous medium, making them larger than in a dry state [26,27]. Synthesis with different concentrations of AgNO₃ showed an increase of average diameter size of AgNPs (range from 147 nm to 503 nm), especially in the high concentration of 10 mM (463 nm) of AgNO₃ (Fig. 4a). Likewise, the zeta potential of AgNPs measured with the LDV technique showed low mean values of zeta potential for AgNPs synthesized from 0.1 mM, 1 mM, and 10 mM of AgNO₃ (–14 mV, –23.6 mV, and –18.8 mV, respectively), indicating an incipient instability of AgNPs (Fig. 4b) [28]. However, a moderate colloidal stability was recorded for NPs synthesized at 2 mM [28]. Thus, the high hydrodynamic size found for NPs synthesized with 10 mM of AgNO₃ could be explained by the unstable NPs formation which agglomerates and forms clusters greater than 100 nm. In this sense, these results were in accordance with TEM images, where significant formations of agglomerates of AgNPs were observed at 10 mM of AgNO₃.

It should be noted that biosynthesized AgNPs showed negative charge, as was expected for the nucleation growth process. However, the OH[–] groups from polyphenol molecules capping around the surface of AgNPs may also be responsible of the negative charge of AgNPs, as was observed in previous studies [5,29].

According to the present study, *P. boldus* extract could be considered a promising candidate for the green synthesis of AgNPs since factors such as room temperature, short reaction time, low concentrations of leaf extracts of *P. boldus*, and silver nitrate salts are sufficient to biosynthesize stable and pure AgNPs in a uniform size and shape distribution.

4. Conclusion

We have developed a fast, convenient, and environmental friendly method for the synthesis of AgNPs from the endemic *P. boldus* aqueous leaf extract. These particles showed an average diameter of 18 nm, were spherical, and showed a good stability and dispersion. Surface plasmon resonance during the reaction with the biomolecules present in the plant leaf extract and AgNO₃ resulted in the formation of AgNPs, which were confirmed by UV–Vis, XPS, TEM, DLS, and LDV. Finally, these results could be related with the high antioxidant capacity given by polyphenol contents of *P. boldus* leaf extract, which were capable of inducing a chemical reduction of Ag⁺ ions to Ag⁰, allowing the nucleation, growth and stabilization process of AgNPs.

Acknowledgments

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