Kalanchoe daigremontiana leaf extract: A green stabilizing agent in synthesis of Silver Nanoparticles.

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Abstract. The synthesis of silver nanoparticles (AgNPs) has been increasingly extended due to its potential applications in fields such as optics, environmental, catalysis, electronics and as an antibacterial agent. In this way it is necessary to develop methods framed in green chemistry to achieve greater stability over time of the AgNPs. The present work aims to show the synthesis of AgNPs using Kalanchoe daigremontiana leaf extract, as a reducing and stabilizing agent. UV–vis and transmission electron microscopy (TEM) were used to characterize AgNPs obtained. The absorbance of solutions was measured, evidencing of the formation of AgNPs due to the existence of plasmon resonance at that \( \lambda_{\text{max}} \approx 417 \) nm. The size distribution and morphology of the AgNPs by TEM shows stable, spherical and monodisperse nanoparticles with a size between 4 and 12 nm. The measurements were carried out immediately after the synthesis procedure, then the AgNPs solutions were stored at room temperature and darkness by 27 months and it could be corroborated the stabilizing capacity of Kalanchoe daigremontiana leaf extract, since the \( \lambda_{\text{max}} \) and the size of particle did not vary significantly in this period of time.

1. Introduction

Due to its potential applications, Silver nanoparticles (AgNPs) study has been increasingly extended in fields such as cosmetics[1, 2], catalysis [3], optics [4], and as an antibacterial agent [5-7] among others. Therefore, it is more common to use the green synthesis method to obtain AgNPs thanks to this method is eco-friendly, cost effective and rapid and avoid use toxic chemicals which are not friendly to environment.

Since the first study of Green synthesis of AgNPs was reported by Gardea-Torresdey et al [8], there have been numerous papers that describe different types of plant extracts as reductive and stabilizing agents of the silver nanoparticles; Ahmad et al., describe studies evaluated the use of plants and their tissues for AgNPs synthesis, they “explores the huge plant diversity to be utilized towards rapid and single step protocol preparatory method with green principles over the conventional ones and describes the antimicrobial activities of silver nanoparticles” [9].

The applications of AgNPs are dependent on their size, shape and stability [10]. Stability is the least studied parameter; we only found a few updated studies that address this issue, one of them achieved a stability of the AgNPs colloidal solution more than 300 days using microwave technology and Starch acting as both a stabilizing agent [11]. Other work found selenium nanoparticles syntetized by Aloe Vera leaf extract which are stable more than two months [12]. A study of 2010 reports silver
nanoparticles stability by one year, by borohydride reduction, in presence of citrate [13], these authors also mention others works about silver nanoparticles stability but too through not green processes, see references included in [13]. In the present study, green synthesis of AgNPs at room conditions has been reported, using the ethanolic extract of Kalanchoe daigremontiana leaf as stabilizing agent and reducing agent of the silver ions present in the silver nitrate solution. For the purpose of determining the capacity of Kalanchoe daigremontiana leaf extract to long term stability of AgNPs, UV-vis and transmission electron microscopy (TEM) were recorded immediately after synthesis and then 27 months later. The AgNPs solutions were stored in dark at room temperature (~25.0°C).

2. Experimental

2.1. Materials.
Analytical grade silver nitrate (AgNO₃) and ethanol absolute were purchased from Merck KGaA. The reagents were used as received.

2.2. Kalanchoe daigremontiana leaf extracts preparation.
Kalanchoe daigremontiana leaves are split into small pieces and was heated at 80°C for 1 hour and then dried. It was used for ethanolic extract, using a ratio of 0.1:3, dry material to solvent. The resulting extract was used in all synthesis after being filtered by gravity.

2.3. Synthesis of Silver nanoparticles (AgNPs) using Kalanchoe daigremontiana leaf extract.
In the present study, 30 mL of ethanolic Kalanchoe daigremontiana leaf extract was added with 50mL of 12 mM AgNO₃ solution in presence of air and neutral pH, it was remained under constant stirring on a magnetic stirrer at 75°C during 3 hours and then heated 2°C/min to reach 80°C holding for 2 hours until obtaining a translucent solution with small suspended particles that could be removed by simple filtration (0.45 μm).

2.4. Characterization
The synthetized AgNps were characterized by UV–vis absorption spectroscopy and Transmission Electron Microscopy (TEM). UV-visible absorption spectra were recorded whit UV-Visible Cary-100 VARIAN in the wavelength range from 350 to 700 nm, which included the maximum of absorbance of AgNPs to identify the surface plasmon resonance effect [14]. TEM images were obtained using a Tecnai F20 Super Twin TMP with field emission source, with resolution of 0.1 nm at 200 kV and 1.0 maximum magnification TEM MX camera GATAN US 1000XP-P. Samples for TEM measurements were suspended in ethanol and ultrasonically dispersed. Both measurements were carried out immediately after the synthesis procedure and 27 months later. Only for the measurement 27 months later Zeta potential measurements were made using a Malvern Zetasizer Nanoseries Nano ZS and Particle size and its distribution were assessed with a laser dynamic light scattering (DLS) instrument (Zetasizer Nanoseries, Malvern Instruments Ltd., Malvern, Worcestershire, UK).

3. Results and discussion
The absorption spectrum recorded for AgNPs solution after the synthesis procedure and 27 months later (see Figure 1) shows the characteristic surface plasmon resonance (SPR) band of silver nanoparticles with a maximum of ~ 417 nm, indicating the presence of lone spherical Ag nanoparticles. Both spectrums show a minimum at ~ 378 nm to fresh solution and ~ 384 nm to 27 months later, according to [15] this could correspond to the wavelength at which the real and imaginary parts of the dielectric function of silver almost vanish. A peak could be identified at a longer wavelength (shifted to 635 nm for the fresh solution and 634 nm to 27 months later), suggesting the possible presence of a population of anisotropic Ag particles [16] or the possible formation of aggregates [17]. The similarity of the spectra confirming that Kalanchoe daigremontiana leaf extract is a good stabilizing agent for AgNPs.
Figure 1. UV-vis spectrum of AgNPs recorded immediately after synthesis (•) and after 27 months (□).

TEM micrographs are shown in Figures 2(a) and 2(b) and corresponding size distribution histogram of AgNPs obtained for the fresh synthesis and 27 months later, respectively. The TEM image confirms the presence of nanoparticles mostly spherical, with a mean particle size ~5.9±2.2 nm for fresh solution and ~3.3±1.04 nm and 27 months later, although the formation of other forms of nanoparticles or larger particles or aggregates is not evident.

Figure 2. TEM micrographs along with the histogram of silver nanoparticles obtained for the fresh synthesis (a) and 27 months later (b)
In this study, approximately 168 particles for the fresh samples, and 81 particles 27 months later, were measured from several images using image analysis software (ImageJ) [18].

There is evidence of shape and size distribution stability when observed the AgNPs solutions stored in dark at room temperature 27 months later. However, the slight changes that were observed in size of AgNPs there are not statistically significant, and it was no evidence of other shape of the nanoparticles in TEM images, as suggested by second peak in the UV-Vis measurements. For sample 27 months later, the measurement of particle sizes was also performed using a DLS and Zeta Potential (ZP) to verify the stability to aggregation (see Figure 3(a) and (b)).

![Size Distribution by Intensity](image)

![Zeta Potential Distribution](image)

**Figure 3**: particle size determination by DLS (a) and ZP (b)

In the Figure 3(a) the size average of particles by DLS are approximately 32 nm, difference between DLS and TEM measures can be due to the fact that DLS gives its hydrodynamic size of the particle, i.e., DLS gives the particle size along with the adsorbed extract and solvation layer, whereas TEM gives the particle size without this solvation layer. ZP value was found to be -11.9 mV, indicating that the solution is relatively stable (for ranges of ±10-20 mV) [19]. The magnitude of the zeta potential will depend on the acidic or basic strength of the surface groups and as well as the pH of the solution [20], the composition of the *Kalanchoe daigremontiana* leaf extract is not yet known and may influence the measurements. However, it should be emphasized that no precipitation was detected in the solutions analyzed in this work.
4. Conclusions

Silver nanoparticles (AgNPs) were successfully prepared by the chemistry reduction method, using the *Kalanchoe daigremontiana* leaf extract as stabilizing agent and reducing agent. It was possible to obtain a diameter of the nanoparticles less than 10 nm, the *Kalanchoe daigremontiana* leaf extract stabilized AgNP solutions proved to be fairly stable for a period up to 27 months, indicating that these extract really is a green stabilizer for the AgNPs.

Solutions obtained were kept in dark and at room temperature and showing a large number of spherical AgNPs, and based on TEM measures there no significatively changes were observed in morphology, shape and size even after 27 months of storage.

Guaranteeing a stable AgNPs solution for so many months, without significant changes in its physical properties, opens up more possibilities of AgNPs application.

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