

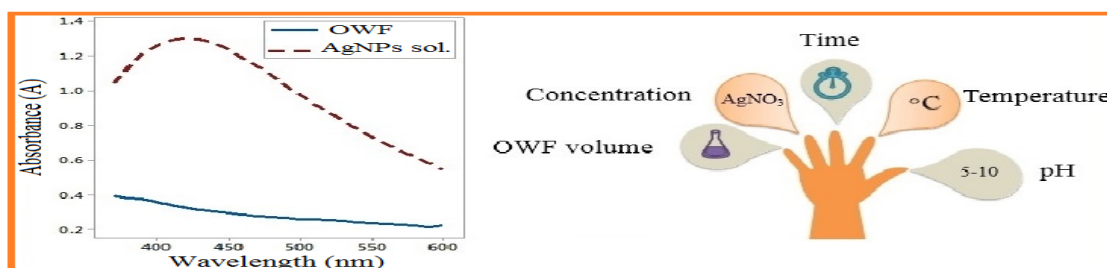
## Biosynthesis of Silver nanoparticles using Olive Wastewater

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Submitted on: 04-Feb-2021 Accepted and Published on: 29-Mar-2021

### ABSTRACT



Biosynthesis of the metallic nanoparticle is gaining importance because it is a single-step process, nontoxic, environmentally acceptable, and easily scaled up. The present study investigated the biosynthesis of silver nanoparticles (AgNPs) via reacting olive wastewater filtrate (OWF) with silver nitrate, and the formation of AgNPs was confirmed by a color change of the reaction mixture and visible spectrophotometry. Additionally, the influence of pH, reaction time, AgNO<sub>3</sub> concentration, temperature, and OWF volume on the proposed method was investigated. It was found that with increasing the mentioned parameters, the formation of the AgNPs was increased under the experimental conditions. The results showed that OWF represented a promising material for the biosynthesis of AgNPs.

*Keywords: silver nitrate, silver nanoparticles, olive wastewater, biosynthesis*

### INTRODUCTION

In recent years, nanotechnology has become a significant area of modern applications that deals with particle structures ranging from approximately 1-100 nm in one dimension at least.<sup>1</sup> Because of their size, shape, structure, and distribution, with no similarity to the corresponding individual bulk material, nanomaterials, including metallic nanoparticles (MNPs), have unique properties: optical, physical, chemical, electrical, thermal, and mechanical

properties.<sup>2</sup> As a result of these properties, MNPs have been used in a wide range of technological applications in several fields, for instance, healthcare, environment, energy, agriculture, consumer goods,<sup>3</sup> information, communication, and heavy industry.<sup>4,5</sup> In general, there are various approaches described in the literature to synthesize MNPs, including chemical, physical, and biological methods.<sup>6</sup> The route of biosynthesis has provided many benefits over chemical and physical synthesis as it is a simple, inexpensive, environmentally acceptable method, and can be scaled up for large-scale synthesis easily.<sup>7</sup> Moreover, in this route, it is not necessary to employ high pressure, temperature, energy, and hazardous chemicals as in the other methods.<sup>8</sup> Basically in biosynthesis, metallic NPs such as silver, gold, copper, zinc, platinum and titanium<sup>9,10</sup> could be formed by using bacteria, fungi, yeast,<sup>11</sup> and plants.<sup>12</sup> Among the biomaterials sources, plants that act as reducing and capping agents for synthesizing MNPs, are more advantageous due to their elimination of the elaborated processes required for the MNPs recovering.<sup>9,13</sup> Indeed, plants are safe to handle, usually non-toxic, cost-effective, and readily

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Cite as: *J. Mat. NanoSci.*, 2021, 8(1), 11-15.

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distributed so, they are better candidates for MNPs synthesis.<sup>14</sup> Furthermore, several parts of plant materials, including bark, callus, stem, seeds, leaves, flowers, fruit, peels, and roots, have been extensively studied for the MNPs production with different morphology.<sup>15-17</sup> Additionally, the bioreduction process of metal ions to MNPs has been ascribed to the active biomolecules found in plants, such as phenolic acids, alkaloids, proteins, enzymes, amino acids, alcoholic compounds, polysaccharides, polyphenols, and sugars exist in plant extracts.<sup>18-20</sup> Numerous studies have been performed to investigate the transformation of metal ions to MNPs using plant extracts from different parts during the green synthesis processes.<sup>9-16</sup>

Silver nanoparticles (AgNPs) are the most used nanomaterials among MNPs, with enormous applications including antibacterial agents,<sup>21</sup> antifungal agents, antioxidant agents, anticancer agents, and water treatment.<sup>20,22</sup> Presently, studies have reported the biosynthesis of AgNPs successfully by plant extracts such as banana,<sup>9</sup> *Dioscorea batatas*,<sup>23</sup> Carob,<sup>24</sup> Mangosteen,<sup>25</sup> Marigold flower,<sup>26</sup> *Terminalia arjuna*,<sup>27</sup> Olive tree.<sup>28-30</sup>

Although there is a large number of studies that have been reported on the synthesis of MNPs utilizing plant extracts, no previous report, so far, has studied, particularly, the biosynthesis of any MNPs by olive wastewater (OW), an agricultural by-product of the olive oil industry known as a resource of polyphenols,<sup>31</sup> which are active biomolecules acting as effective reductants in MNPs biological synthesizing.<sup>9,13</sup> In Libya, an African country bordered by the Mediterranean Sea to the north, OW (known locally as Morjeene) represents a serious environmental problem as it is discharged into the environment without any treatment. Thus, the present study aimed to utilize OW in the biosynthesis of AgNPs, and the formation of AgNPs was monitored by change of solution color and visible spectroscopy. Besides, the influence of pH, reaction time, silver nitrate ( $\text{AgNO}_3$ ) concentration, temperature, and the volume of OW, was investigated.

## MATERIALS AND METHODS

### Chemicals

$\text{AgNO}_3$  solution of 0.1 M (Winlab, UK) was used to prepare all  $\text{AgNO}_3$  solutions. Sodium hydroxide (NaOH) (BDH, UK) and Hydrochloric acid (HCl) (BDH, UK) were used to prepare solutions with 0.1 M concentration for both chemicals, which were used to adjust the pH of any reaction mixture used to prepare AgNPs, or to investigate the role of any factor (e.g., temperature) on the proposed method to biosynthesize AgNPs. For preparation of all solutions, double distilled water was used.

### Preparation of OW Aqueous Filtrate

A fresh sample of OW was collected from an olive mill located in Gharyan, Libya. The collected sample was transferred to the lab in a clean, sealed plastic bottle. At the lab, 1 mL of OW was added to 49 mL of water in a beaker, and then swirled to homogenize the mixture. After that, the mixture was filtered using Whatman filter paper no. 1, and the filtrate was received into a dry clean conical flask, which was then sealed and stored at 4°C. This filtrate of OW (OWF) was used later for AgNPs biosynthesis.

### Biosynthesis of AgNPs Using OWF

One mL of OWF was added to 9 mL of 1 mM aqueous solution of  $\text{AgNO}_3$ , after the pH of the solution was adjusted to 9, the resulting solution was left standing for 30 minutes at room temperature, and then it was examined for any color change and analyzed with visible spectrophotometry to confirm AgNPs formation.

### Monitoring of Green Synthesis of AgNPs

The reaction mixture was inspected for any color change, and Vis spectra, in 370-600 nm region, were recorded by Vis Spectrophotometer (Jenway 6300 spectrophotometer, Staffordshire, UK) using deionized water a blank. Whenever the absorbance (A) of AgNPs solution exceeded 2, the solution was diluted with deionized water, and the absorbance was multiplied by the dilution factor.

### Factors Affecting Green Synthesis of AgNPs

#### Effect of pH

To investigate the role of pH on AgNPs biosynthesis by OWF, five solutions were prepared as follows: 1 mL of OWF was added to 9 ml of 1 mM aqueous solution of  $\text{AgNO}_3$  and the pH of these solutions was adjusted to the desired pH (5, 7, 8, 9 or 10) by drops of either NaOH (0.1 M) or HCl solutions (0.1 M). The resulting solutions were mixed, and then left at room temperature for 30 minutes, and after that the color change of each solution was observed and its visible spectrum was recorded.

#### Effect of Reaction Time

Ten mL of OWF was added to 90 mL of an aqueous solution of  $\text{AgNO}_3$  (1 mM), and the pH was adjusted to 9 by NaOH solution, and then the mixture was allowed to react at room temperature. From this solution, aliquots of 5 mL was pipetted after 30, 60, 120, 180 minutes and 24, 48 hours and the visible spectrum was recorded to monitor the reaction with respect to time intervals. After one week and two weeks, the formed AgNPs were analyzed by a Vis spectrophotometer to investigate their stability.

#### Effect of $\text{AgNO}_3$ Concentration

One mL of OWF was added to 5 sample vials, each one containing 9 mL of  $\text{AgNO}_3$  with different concentrations, which were: 0.25, 0.5, 1, 2.5 and 5 mM. Each solution was mixed then left at room temperature for 20 minutes after its pH was adjusted to 9. Later, the visible spectrum of each solution was recorded.

#### Effect of OWF Volume

To study the effect of OWF, five solutions were prepared by adding different volumes of OWF to different volumes of  $\text{AgNO}_3$  (1 mM) provided that the volume percentage of OWF was different in all solutions (5, 10, 15, 20, and 30%). Then the pH of each solution was adjusted to 9, and after 30 minutes the visible spectrum of each solution was recorded.

#### Effect of Temperature

A volume of 9 mL of  $\text{AgNO}_3$  solution (1 mM) was added to five sample vials each containing 1 mL of OWF, and then the pH of each solution was adjusted to 9. One of the resulting solutions was kept at 20 °C, while the others were heated either to 30, 40, 50 or 60 °C. After 20 minutes, the visible spectrum of each solution was recorded.

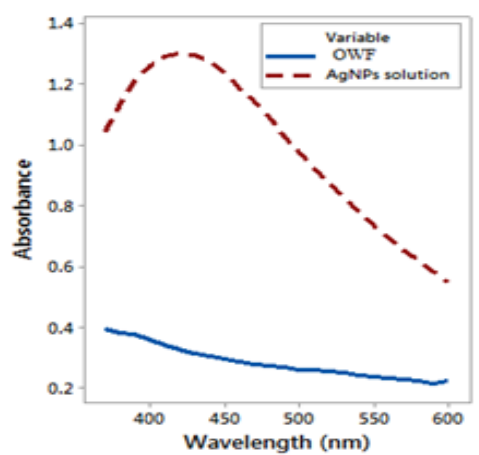
## RESULTS AND DISCUSSION

### Synthesis of AgNPs by OWF

After 1 mL of OWF was mixed with 9 mL of 1 mM  $\text{AgNO}_3$  at pH=9, the resulting solution devolved a pale yellow color at less than 1 minute. And as the reaction time increased, the color changed gradually from pale yellow to red to brown (figure (1)). This color change confirmed AgNPs formation. Most importantly, visible spectrum of AgNPs exhibited a peak at 425 nm (figure 2), which was not observed in OWF visible spectrum. The appearance of this peak was due to the Surface Plasmon Resonance (SPR) of free electrons in AgNPs<sup>9,23,25,32</sup>, and its appearance confirmed AgNPs synthesis. Similar results have been reported in many studies.<sup>9,25,33,34</sup>



**Figure 1.** Confirmation of AgNPs formation by colour change

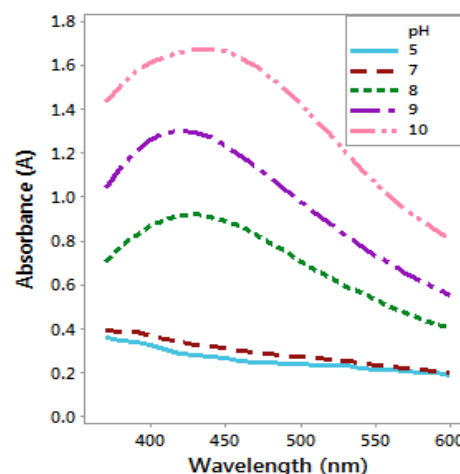


**Figure 2.** Visible spectrum of OWF (blue line) and AgNPs solution (brown line)

### Effect of pH

As shown in figure (3), increasing of pH values increased the absorbance of the SPR peak, with the highest absorbance at pH=10. Moreover, the color of reaction mixture became darker as the pH medium turned more basic. This indicated that AgNPs biosynthesis, in this study, preferred basic medium. Numerous studies have revealed that AgNPs biosynthesis is enhanced by

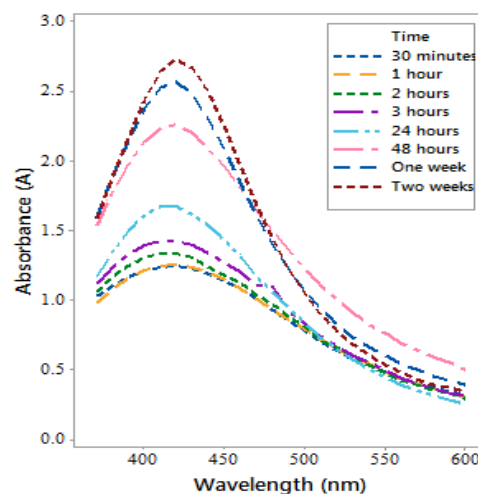
basic medium and suppressed in acidic medium.<sup>5,9,25,35</sup> It is crucial to note that at pH=5, there was no color change and, as shown in figure (3), the SPR peak was not observed at this pH, indicating that AgNPs were not formed in the acidic medium. This could be explained as follows: some functional groups (for instance, OH in polyphenols) did not ionize in that acidic medium; thus, these groups were unable to accomplish their role, which was reducing  $\text{Ag}^+$  ions and converting them into AgNPs.<sup>9,25</sup> The shape of SPR peak at pH=9 was narrower than the peaks at other pH values, suggesting that AgNPs at this pH were formed with less particle size distribution<sup>9,25</sup> and as this peak (at pH = 9) was at the lowest wavelength, compared to other SPR peaks at different pH values, therefore the shape of AgNPs formed at this pH was more identical to spherical shape.<sup>36</sup>



**Figure 3.** Increasing of AgNPs solution absorbance with increasing pH

### Effect of Reaction Time

The absorbance of SPR peak increased with increasing reaction time (Figure (4)), reflecting that more AgNPs formed as

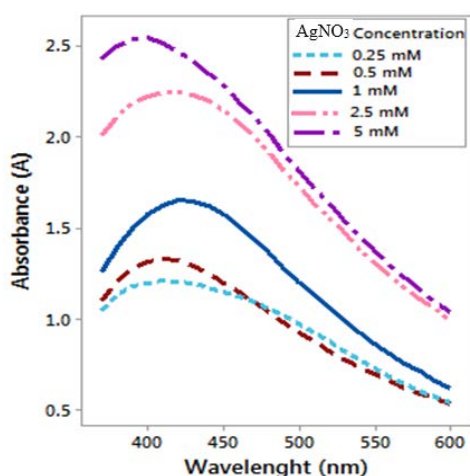


**Figure 4.** Increasing of AgNPs solution absorbance with increasing reaction time.

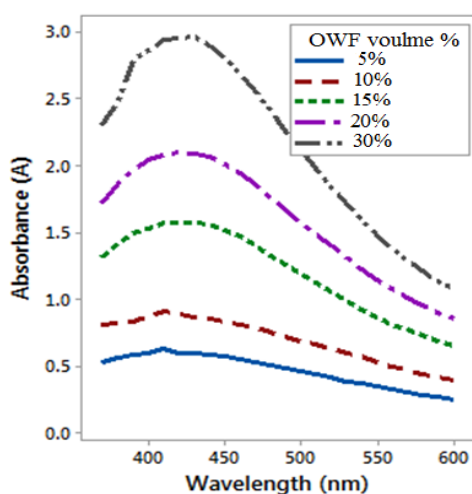
time passed.<sup>25,37</sup> Furthermore, the SPR peak became more beaked with increasing reaction time, and this pointed out the formation of AgNPs with less particle size distribution.<sup>9,38</sup> Completion of AgNPs formation was observed after two weeks, at which the maximum absorption of the SPR peak was recorded. After 2 weeks, there was only a slight change in SPR peak absorbance without changing its position. This pointed out the stability of the formed AgNPs.<sup>9,25,39</sup>

### Effect of AgNO<sub>3</sub> Concentration

When AgNO<sub>3</sub> solution was used with different concentrations (0.25-5 mM) to synthesize AgNPs, the SPR peak appeared in all experiment designs (Figure (5)), and with increasing AgNO<sub>3</sub> concentration the produced amount of AgNPs increased. Depending on SPR peak height and its shape, the suitable concentration was considered to be 1 and 2.5 as there was an appreciable amount of AgNPs with less particle distribution (smaller peak width).<sup>9,27,38-41</sup>



**Figure 5.** Increasing of AgNPs solution absorbance with increasing AgNO<sub>3</sub> Concentration.



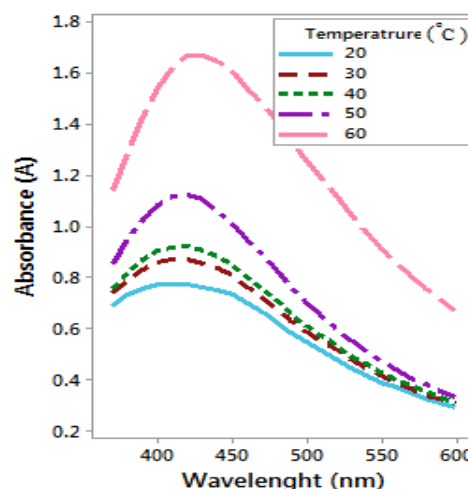
**Figure 6.** Increasing of AgNPs solution absorbance with increasing of OWF volume.

### Effect of OWF Volume

As shown in figure (6), SPR peak absorbance increased with increasing OWF, which was an indication of formation of more AgNPs amounts. Increasing OWF resulted in the presence of more bioactive compounds that responsible for the reduction of Ag<sup>+</sup> ions and forming AgNPs, consequently, the reaction occurred more rapidly, as the volume percentage of OWF increased.<sup>9,38,41,42</sup>

### Effect of temperature

As shown in figure (7) the absorbance of SPR peak increased remarkably as the temperature increased, especially when the reaction was carried out at 60 °C, suggesting that the reaction was endothermic. Furthermore, as the reaction temperature increased, the SPR peak became sharper, indicating that more AgNPs with smaller particle sizes were formed.<sup>9,38,40-43</sup>



**Figure 7.** Increasing of AgNPs solution absorbance with increasing of temperature

## CONCLUSION

Considering the results of this study, OWF represents a promising material for biosynthesis of AgNPs. In addition to that, the proposed method, which was applied in this study, proved to be a simple and eco-friendly method, with a lower cost compared to the conventional methods. Furthermore, the proposed method turns OW from a material associated with environmental concerns into a valuable one. Another study is being conducted to characterize the produced AgNPs by IR spectroscopy and scanning electron microscope.

## LIMITATIONS OF THE STUDY

Surface analysis was not conducted in this study which, if it was conducted, could have provided helpful information about the shape and size of the produced AgNPs.

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