

Ají Amarillo Chilli-Assisted Phytosynthesis of Silver Nanoparticles and Their H₂O₂ Sensing Ability

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Research Article

Keywords: Phytosynthesis, Ecuadorian chilli, *Capsicum baccatum* L, UV-Vis, TEM, H₂O₂ sensing

Posted Date: May 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-479226/v1>

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Abstract

Scope of green chemistry in nanobiotechnology is generating research interest in the development of an ecofriendly synthesis of nanoparticles and their wide range of applications in engineering and biomedical field. In the present study, we report a simple and ecofriendly phytosynthesis of silver nanoparticles (AgNPs) using Ají amarillo chilli (*Capsicum baccatum* L.) fruit, where phytoconstituents of *C. baccatum* behaves as a reducing as well as a stabilizing agent. The phyotsynthesized AgNPs were characterized through various spectroscopic and microscopic techniques. The produced AgNPs showed surface plasmon resonance (SPR) at $\lambda_{\max} = 458$ nm and stable for 10 days, confirmed by UV-vis spectroscopy. Transmission electron microscopy (TEM) explained that produced AgNPs were almost spherical in shape with average size of 10-30 nm along with small aggregation. Aggregation of AgNPs was indicated in the dynamic light scattering (DLS) analysis. The results also demonstrate that as-synthesized AgNPs are low-cost and rapid alternative as optical chemical sensors and showed >50% H_2O_2 quenching/ sensing activity for 15 mins at room temperature.

1. Introduction

Chilli peppers are one of the most popular vegetable and present in almost every diet throughout the world. There is archaeological evidence at sites located from the Bahamas to southern Peru that chilli peppers were domesticated more than 6000 years ago, [1] and were one of the first self-pollinating crops cultivated in Central and South America [2]. *Capsicum baccatum* L. (Aji amarillo chilli) is a traditional vegetable crop in Ecuador, Argentina, Bolivia, Brazil, Chile, Peru, Costa Rica, and Hawaii. *C. baccatum* fruit is distinguished from other species by the yellow, red, orange, brown, or dark green colour and valued for their sensory attributes of colour, flavor and high pungency [3]. *C. baccatum* fruit are economically important and good sources of many bioactive compounds, such as capsaicinoids, polyphenolic compounds, carotenoids, protein/enzymes, polysaccharides, amino acids and vitamins help the human body to function and are responsible for the growth of plants [4–6] (Fig. 1). Hence, these phytoactive contents of the *C. baccatum* fruit may be used as reducing and stabilizing agent for the synthesis of nanoparticles and other applicability in nanoscience field.

During last two decades, synthesis of noble metal nanoparticles (MNPs) via green methods has attracted much attention due to their ecofriendly nature and wide range of properties including a high surface-area-to-volume ratio, biocompatibility [7], and low toxicity [8]. Nanoparticles (NPs) are particles that range 1–100 nm in size. Green synthesized silver nanoparticles (AgNPs) are widely studied metal among other MNPs and efficiently utilized in a wide range of scientific and industrial applications including catalysis [9], sensing, molecular imaging [10], electronics [11], antimicrobial [12], therapeutics [13], tissue engineering [14], solar cells [15], drug and gene delivery [16], wastewater remediation [17], etc. Notably, absorption and scattering plasmonic spectra (colors) of colloidal gold nanoparticles (AuNPs) and AgNPs have been used as colorimetric assays for sensing of DNA, proteins and metal ions using the naked eye, showing the importance of design of colors (absorption and scattering optical properties) of colloidal NPs [18].

To address the environmental concern in nanoscience, scientists have been working throughout the year for improving the nano synthesis method by using biomolecules/ phytochemicals, and enhance the surface properties of AgNPs by functionalization of its surface with bioactive compounds. Surface functionalization of AgNPs having different morphology alters the physico-chemical properties [19, 20], which may play a crucial role in various bioengineering applications [9, 21]. Recently, different MNPs were synthesized by using *Capsicum* species of different geographical origin including *C. baccatum* [3] and *C. annuum* fruit [22] for AuNPs [3], *C. annuum* [23–24] and *C. frutescence* fruit for AgNPs [25], *C. annuum* leaves for AuNPs [26], *C. annuum var annuum* (Jalapeño Chili) for AgNPs [27], *Capsicum annuum* for ZnO nanoparticles [28, 29], etc has been already reported.

To the best of our knowledge, there are no reports on the synthesis of AgNPs using aqueous extract of *C. baccatum* L. (Aji Amarillo) cultivated in Ecuador and investigation of its H₂O₂ sensing activity. The current study illustrates an ecofriendly attempt made to phytosynthesize and characterize the synthesized AgNPs with UV–vis spectroscopy, transmission electron microscopy (TEM), dynamic light scattering (DLS), and fourier transform infrared spectroscopy (FTIR) to generate evidence of morphology, surface and optical property. The results could provide a new scope about the potential utility of *C. baccatum* fruit as a raw material source for the direct reduction of Ag⁺ ion, formation of AgNPs and their utilization as low-cost optical chemical sensors for detection H₂O₂.

2. Experimental

2.1. Materials

All chemicals were of analytical grade and used without any purification. Silver nitrate (AgNO₃ 99.8 %) and H₂O₂ (35 %) were purchased from Spectrum, USA. Milli-Q water was used in all experiments. Fresh Aji amarillo chilli /*C. baccatum* fruit was purchased from the popular market (January 2014) near Universidad de las Fuerzas Armadas ESPE, Sangolqui, Ecuador.

2.2. Aji amarillo chilli/ *C. baccatum* fruit extract and AgNPs synthesis

After being washed thoroughly, *C. baccatum* /Aji fruit (5g) was cut finely and stirred (23–25°C) in 50 mL of deionized water for 120 mins. After cooling, the light yellow color extract was filtered using Whatman No.1 paper and stored at 4°C for further use. For the phytosynthesis of AgNPs, 3 mL of *C. baccatum* fruit extract was mixed with 10 mL of AgNO₃ (1 mM) solution at room temperature (22–25°C). Phytoreduction of Ag⁺ to Ag⁰ indicated by the appearance of orange red colour after 3 hrs and studied the formation of the AgNPs at different time interval.

2.3. H₂O₂ sensing of AgNPs

The colorimetric H₂O₂ sensing activity of AgNPs was determined by the method explained by Kumar et al., 2020 [6] using H₂O₂ which introduced in the solution containing AgNPs. In this assay, 0.5 mL of 100 mM H₂O₂ was mixed with 2.5 mL of diluted AgNPs (25%, 0.25 mM) solution. For control/ blank assay, 0.5 mL H₂O was added with 2.5 mL of diluted AgNPs (25%, 0.25 mM) solution. The spectral absorbance was measured at 423.5 nm regular intervals after the addition of H₂O₂. Quenching/sensing activity percentage of H₂O₂ was calculated using Eq. (1):

$$\text{H}_2\text{O}_2 \text{ quenching/sensing activity (\%)} = \frac{A_o - A_t}{A_o} \times 100\% \quad (1)$$

A_o is the initial absorbance of the AgNPs solution and A_t is the absorbance of the AgNPs at time t.

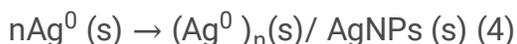
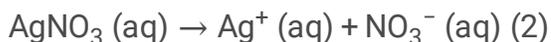
2.4. Spectroscopic and microscopic analysis of AgNPs

To confirm the synthesis AgNPs, UV–vis Absorbance spectrum was recorded using spectrophotometer (Thermo Spectronic, GENESYS™ 8, England and (SPECORD® S600 from Analytik Jena, Germany). Size and morphology of AgNPs were studied on TEM (FEI, TECNAI, G2 spirit twin, Holland). The particle size distributions and polydispersity index (PDI) of nanoparticles were monitored through DLS instrumentation (LB-550; Horiba, Japan). Fourier transform infrared spectroscopy- Attenuated total reflectance (FTIR-ATR) spectrum (650 – 4000 cm⁻¹) were recorded on Spectrum 100 IR spectrometer (Perkin Elmer, USA).

3. Results And Discussion

3.1. Visual and UV–visible analysis

The optical properties and progression of the reaction was monitored visually by the appearance of orange or wine red color in a reaction mixture from colorless solution, after addition of *C. baccatum* fruit extract to AgNO₃. This change of colour is the primary visual indicator of phytosynthesis of AgNPs (Fig. 3, Inset) and their formation further confirmed by UV – vis spectroscopy (Fig. 3a). This colour change of solution mixture may be attributed to the surface plasmon resonance (SPR) of the AgNPs appeared at 458 nm on completion of the reaction [20]. A broad single absorption band was observed between 300–600 nm corresponds to presence of larger size, spherical and polydisperse AgNPs [19]. Figure 3b showed the absorption spectrum of *C. baccatum* fruit extract and peak observed at 300–600 nm can be assigned to its bioactive compounds such as capsaicinoids, phenolic compounds, carotenoids, protein/enzymes, polysaccharides, amino acids and vitamins [19, 22, 27]. In Fig. 3b, the increase in absorption peak in the range of 400–600 nm confirming the involvement of bioactive compounds of *C. baccatum* fruit works as reducing and stabilizing agent for Ag⁺ to Ag⁰ conversion. No change in absorption peak of AgNPs at 458 nm was recorded for 10 days, indicating their stability. The reaction and phytosynthesis of AgNPs is depicted as follows in using Eq. (2–4):



3.2. TEM analysis

TEM is a primary means to determine the sizes and shapes of nanoparticles. Figure 4 show almost spherical shaped and polydispersed AgNPs with average size of 10–30 nm along with small aggregation. Figure 4b represent large clusters consisting of several small spherical particles of range 11–22 nm. This may be due to the association of bioactive molecules of *C. baccatum* on the surface of the AgNPs, and makes the surface attractive and polar. Some previous results on morphology of biogenic synthesis of AgNPs was noticed by Kumar et al., 2016 [30], Smekalova et al., 2018 [31] and Avitabile et al., 2020 [32] and also confirm the presence of aggregated spherical AgNPs. These results are in good agreement with UV–vis spectroscopic observations.

3.3. DLS analysis

DLS analysis showed a hydrodynamic diameter of dispersed nanoparticles. In Fig. 5, the average particle size of as-synthesized AgNPs was found to be 132.3 ± 75.5 nm. The PDI of the AgNPs was 0.326, i.e. PDI > 0.1, clearly indicating a broad size distribution and polydispersed nature of AgNPs. DLS displayed a size greater than TEM results because of the inter-particle interactions and causes high aggregation or screening of smaller molecules by a bigger one [3, 20, 30]. The obtained TEM images of AgNPs justifying the observed DLS results.

3.4. FTIR analysis

FTIR analysis was performed to recognize the possible phytochemicals involved in the reduction of the metal nanoparticles as well as the capping of the formed nanoparticles. In Fig. 6, the strong broad spectrum at 3350 cm^{-1} correspond to O-H stretching vibration of flavonoids/ polyphenolic/ capsaicin [26]. The peaks at 2131 and 1638 cm^{-1} represent the presence of CN/ –CO-Ag linkages and C = C/C = O groups, whereas 719 cm^{-1} attributed to the bending vibration of alkane (C-H) of corresponding saturated hydrocarbons [33]. A variety of IR vibration are present in as-synthesized AgNPs, indicated that -OH and –CHO groups may bind with the Ag^+ and responsible for the reduction and also confirm the presence of organic coatings/ *C. baccatum* fruit phytochemical on the surface of AgNPs [22].

3.5. H₂O₂ Sensing of AgNPs

The SPR based optical sensor for the determination of H₂O₂ represents an important topic in textile, paper, cosmetics, pharmaceutical and food industries [34]. H₂O₂ is an oxygen metabolite and exposure of

small amount in process streams result oxidative stress and associated with aging and cancer and also environmental hazards due to its toxicity [35]. The qualitative and quantitative sensing of H_2O_2 using noble MNPs has received enormous interest in the development of optical chemical sensor and biosensor. Figure 7 displays the optical sensing spectra of AgNPs with time in the visible region (300–700) due to the introduction of 100 mM hydrogen peroxide solution. It was evident the drastic change in its optical characteristics as the time increased, and eventually the characteristic SPR peak of AgNPs at 423.5 nm about disappeared. The quenching of H_2O_2 was found to be 13.33, 26.22, 34.28, 39.11, 42.64, 44.58, 45.44, 46.78, 49.28, 49.68 and 50.79 % for 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 15 mins. It is due to the spontaneous catalytic decomposition of H_2O_2 induces the degradation of AgNPs [34] and the higher redox potential of the H_2O_2/H_2O couple (1.763 V in acidic medium) or H_2O_2/OH^- couple (0.867 V in basic medium) than that of the Ag^+/Ag^0 couple (0.8 V) [9, 36]. Thus, as-synthesized AgNPs is a good candidate as renewable H_2O_2 optical sensors and could be potentially applied in the determination of reactive oxygen species (ROS●) and toxic chemicals.

4. Conclusion

The present study exhibited a simple, cost-effective and green method for the synthesis of AgNPs using *C. baccatum* fruit extract in aqueous media through the in-situ reduction pathway. As-synthesized AgNPs showed a SPR band at 458 nm in UV-visible spectroscopy and almost spherical, aggregated with size distribution from 10 to 30 nm is confirmed by TEM and DLS analyses. From the FTIR spectrum, the flavonoids/ polyphenolic/ capsaicin in the plant extract are more responsible in the reduction as well as capping/stabilization of AgNPs. The phytofunctionalized AgNPs showed a moderate colorimetric sensing activity for the decomposition of H_2O_2 (> 50%, 15 mins) at concentration of 100 mM. In future, *C. baccatum* fruit based AgNPs may be used as optical sensor for the determination of ROS● in different environmental, biomedical and food processing applications.

Declarations

Conflict of Interests

The authors confirm they have no conflict of interests.

Acknowledgements

This scientific work has been funded by the (a) Universidad de las Fuerzas Armadas ESPE, Ecuador, (b) Prometeo Project of the National Secretariat of Higher Education, Science, Technology and Innovation (SENESCYT), Ecuador and (c) TATA College, Chaibasa, India.

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Figures

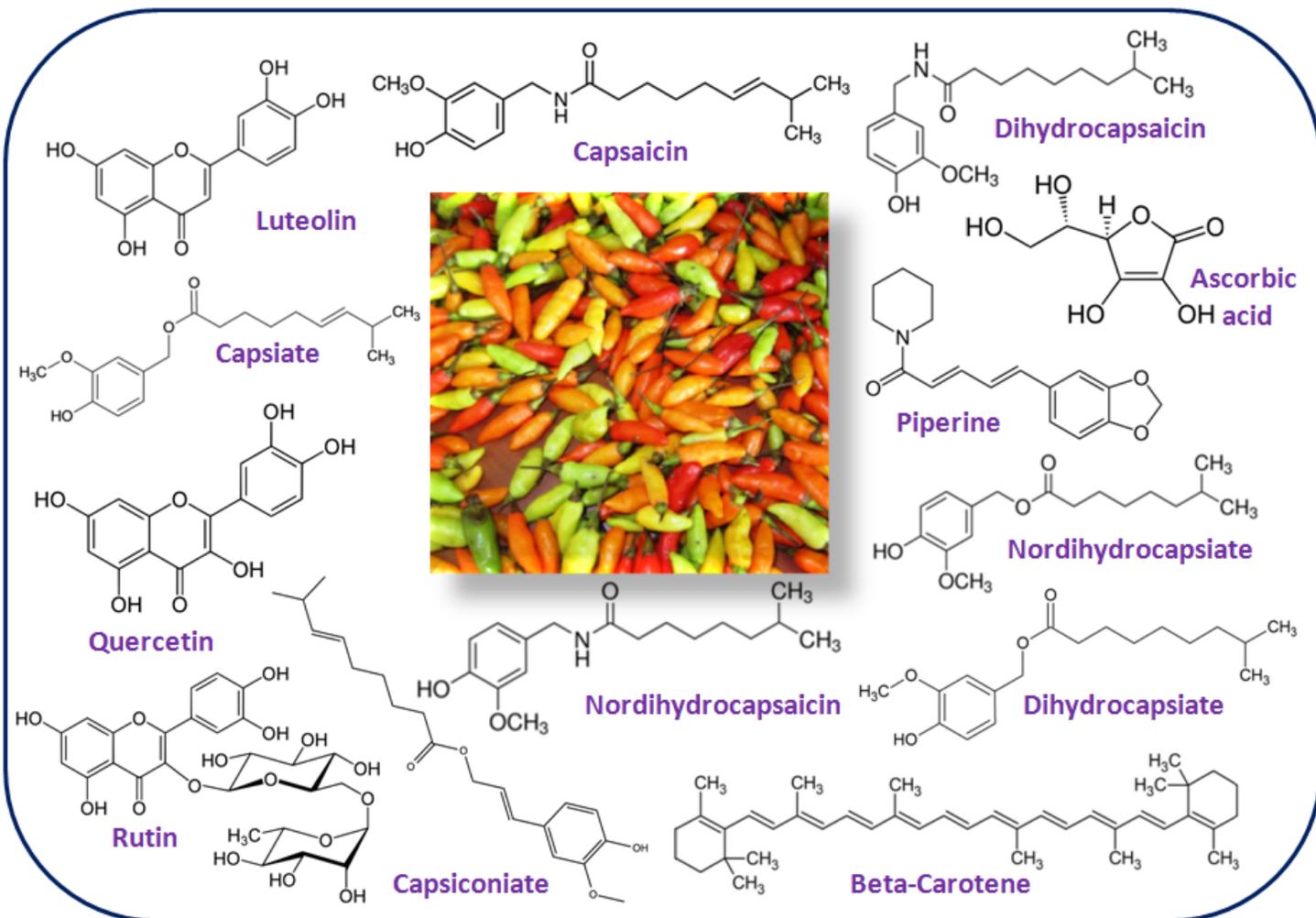


Figure 1

Major bioactive molecules present in Capsicum fruit.

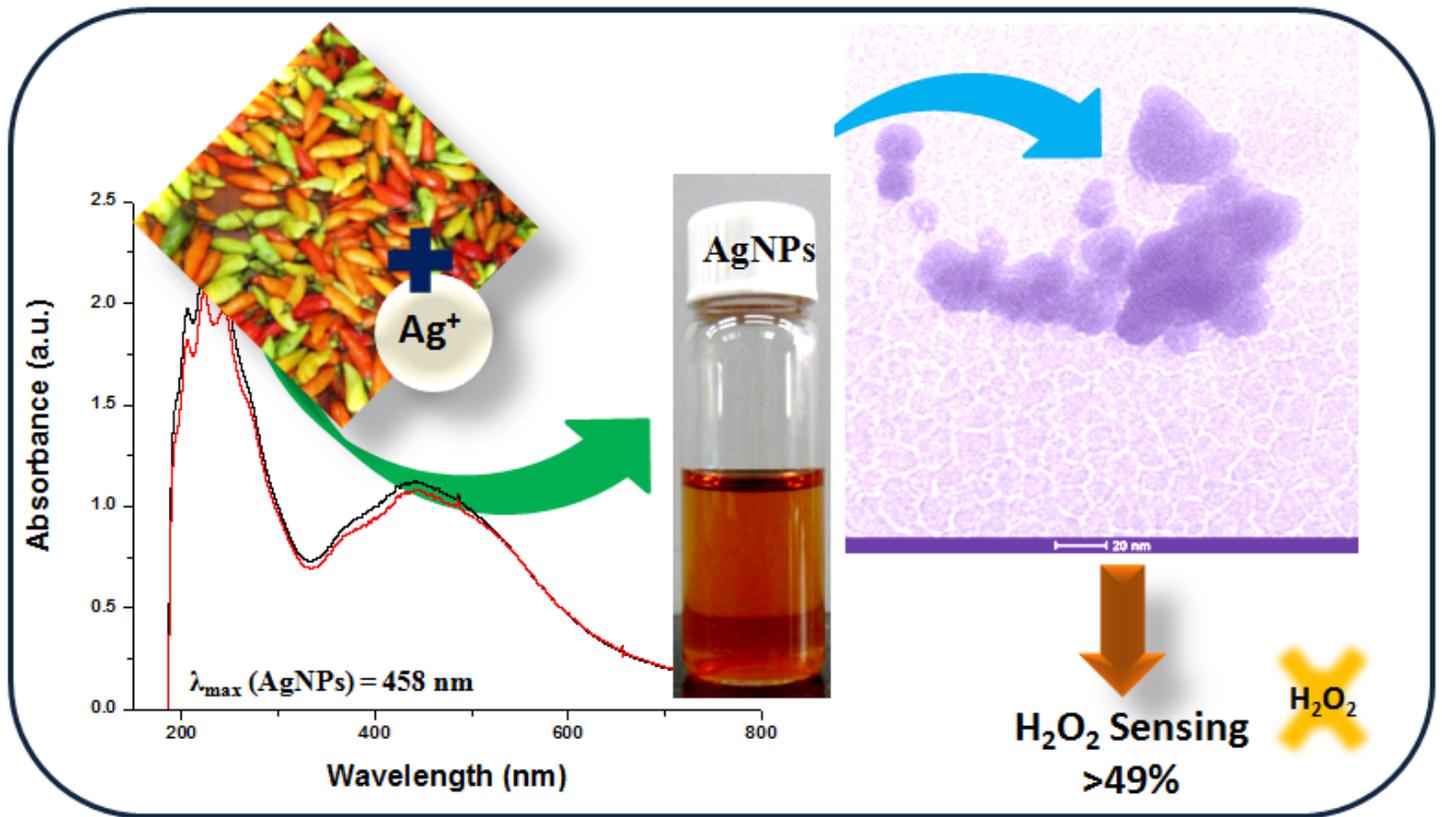


Figure 2

Scheme for the phytosynthesis of AgNPs using *C. baccatum* fruit extract and their H₂O₂ sensing ability.

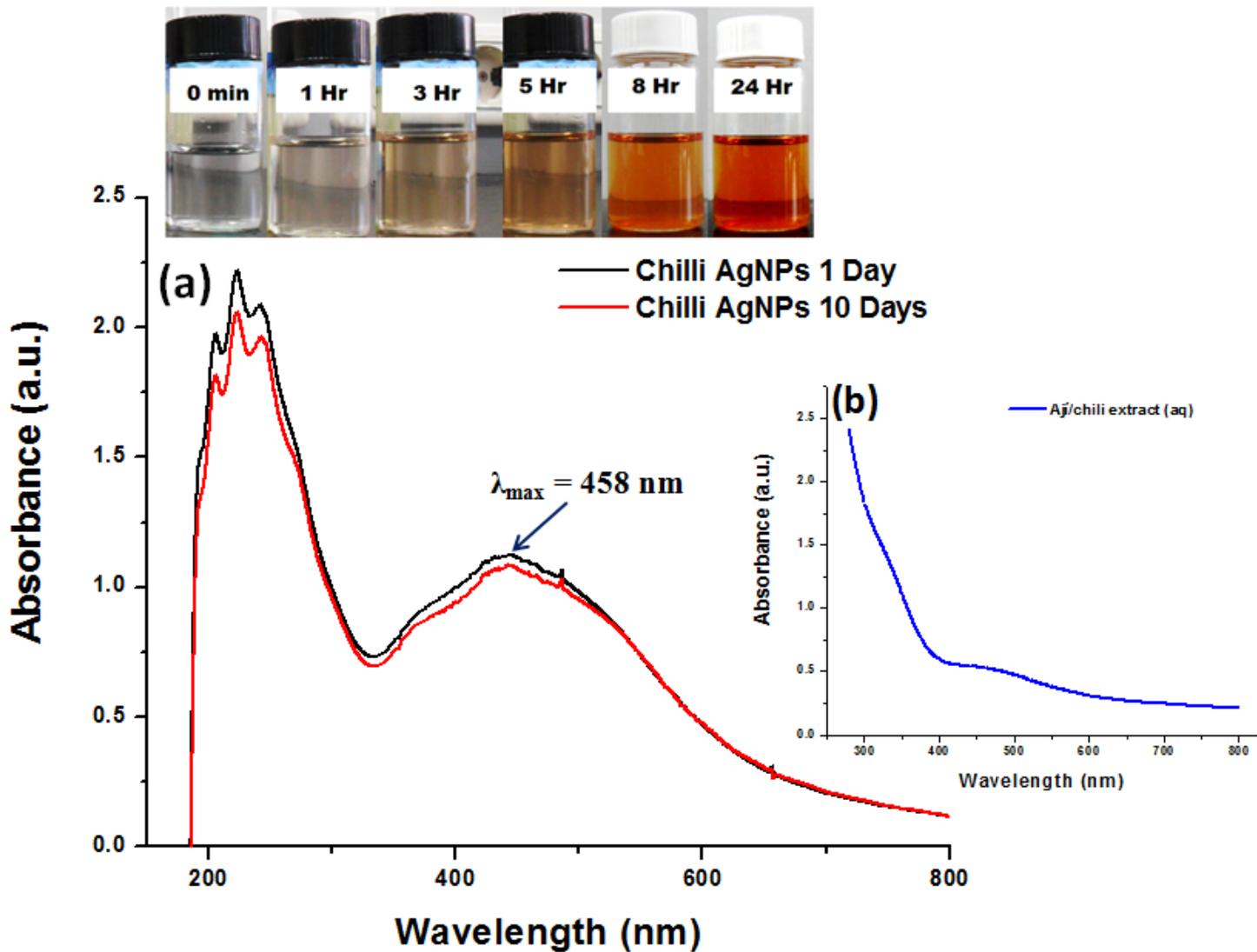


Figure 3

UV-visible spectrum of (a) as-synthesized AgNPs and (b) aqueous extract of *C. baccatum* fruit. [Inset: Photograph of as-synthesized AgNPs at different incubation time]

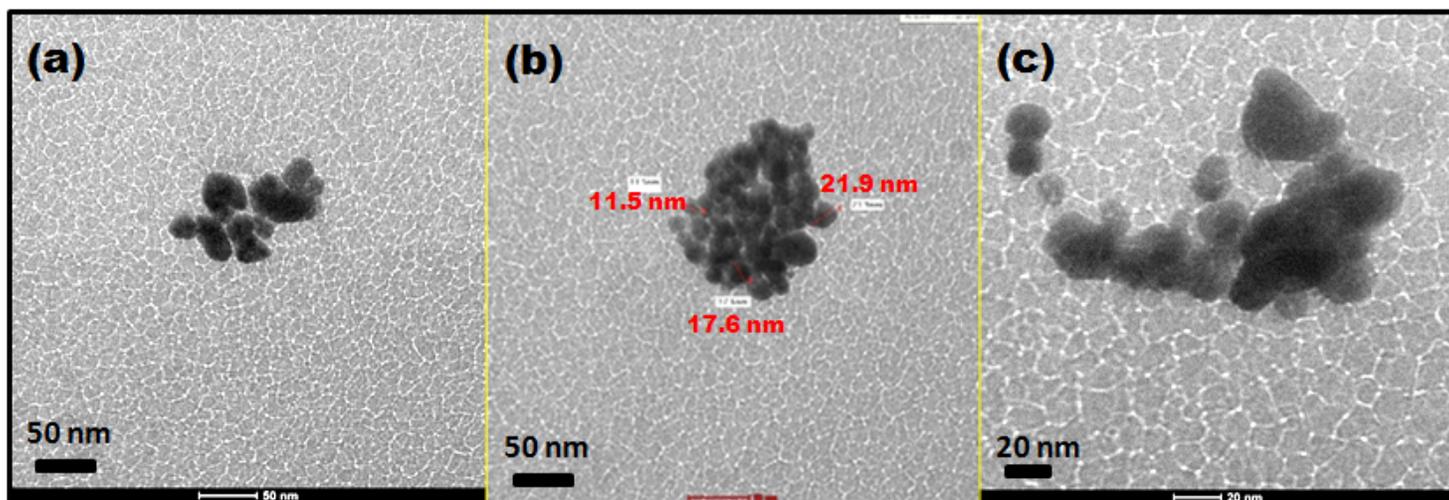


Figure 4

TEM images of as-synthesized AgNPs.

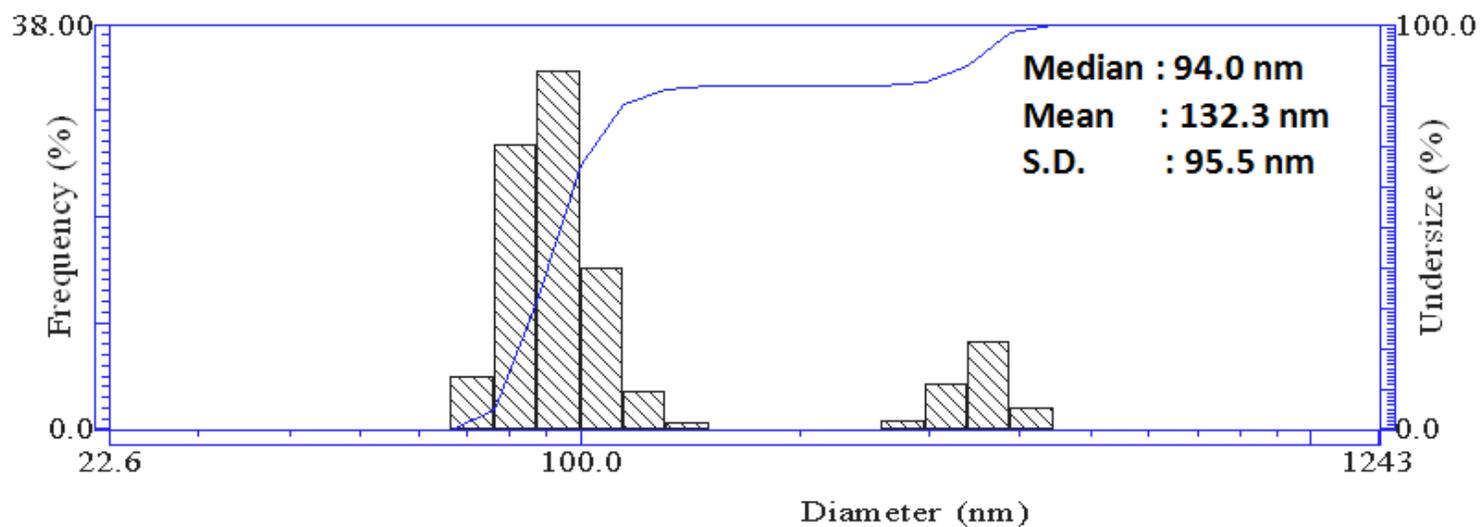


Figure 5

DLS pattern of as-synthesized AgNPs.

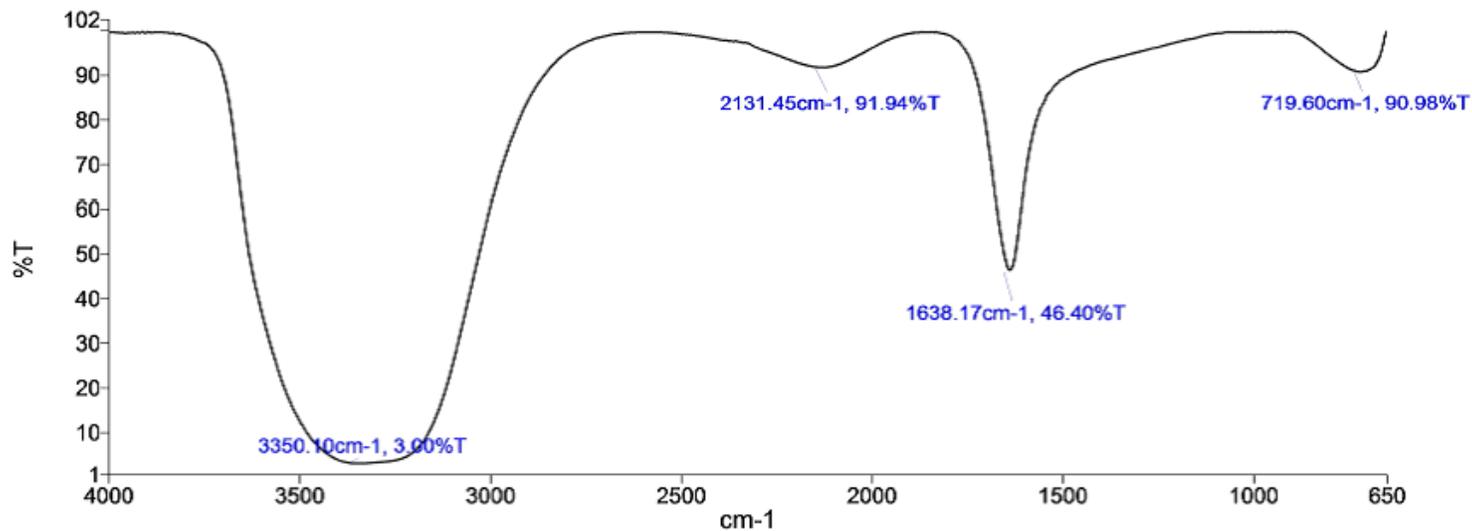


Figure 6

FTIR spectrum of as-synthesized AgNPs.

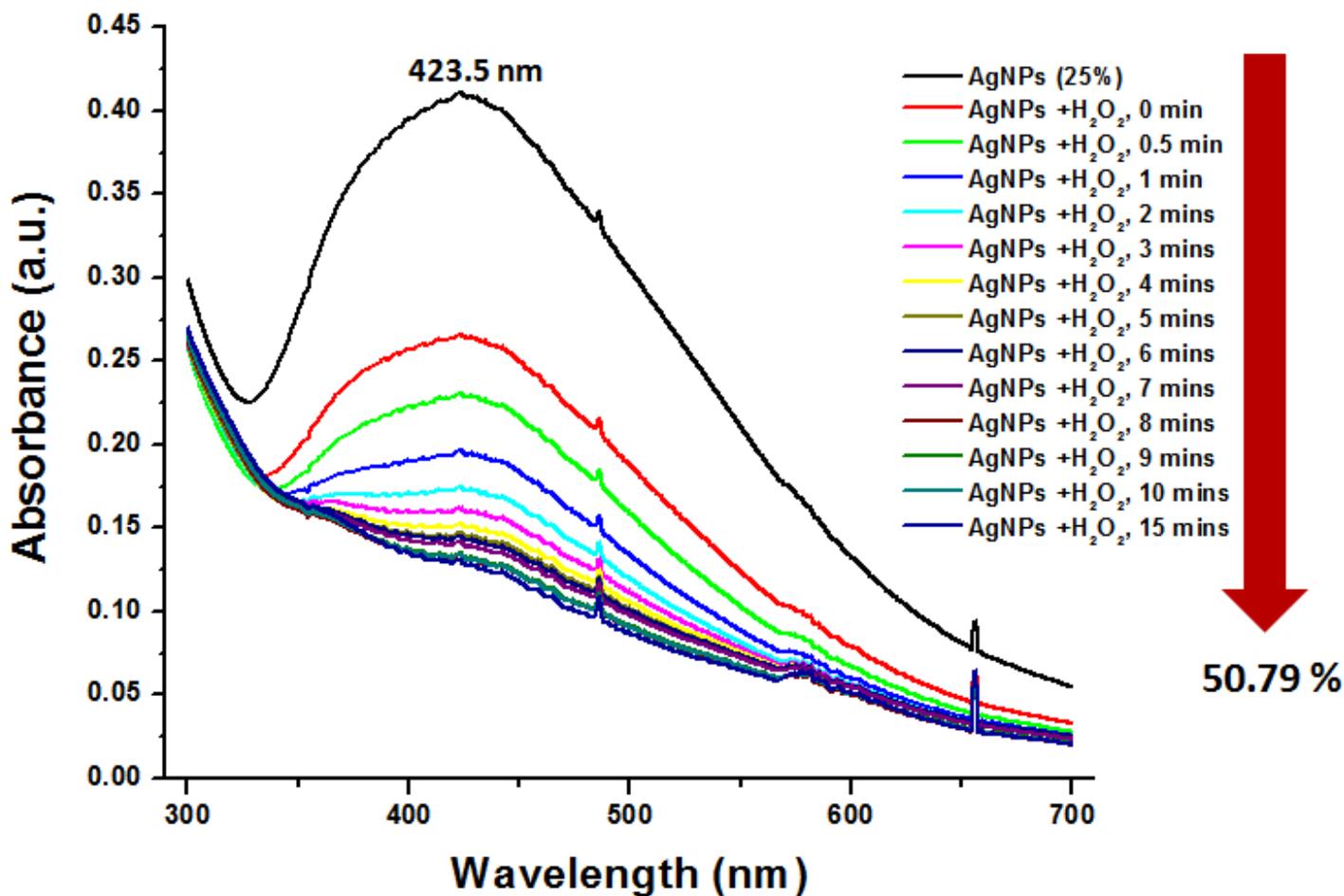


Figure 7

Optical sensing spectra of AgNPs with time due to the introduction of 100 mM H₂O₂.

Supplementary Files

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- [GA.png](#)