Influence of the volume of ascorbic acid in the synthesis of copper nanoparticles mediated by chemical pathway and its stability over time

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Abstract. In the present investigation, the effect of ascorbic acid volume in the synthesis of copper nanoparticles (Cu NPs) mediated by chemical route and their stability over time was evaluated. For the synthesis, copper sulfate pentahydrate CuSO₄ (5H₂O) was used as a precursor agent and ascorbic acid (AA) as a reducing agent. Cu NPs was characterized by the following techniques: UV-Visible spectrophotometry to evaluate structural changes that are evidenced in the absorbance peak and atomic absorption spectrophotometry to define nanoparticulate concentrations material in the precipitated and supernatant phases generated.

On the methodology it was possible to observe a controlled formation based on the increase in the volume of ascorbic acid in the presence of sodium hydroxide, noticing a production of Cu nanostructures with a tendency to oxidation over time. The UV-visible results showed characteristic surface plasmon resonance peaks of metallic copper for the colloid containing 1.2 mL of A.A; as well as a specific copper concentration of 0.14 ppm in the supernatant and 1519.1 ppm in the precipitate. It is also evidenced that the solution exhibits a rapid reaction on exposure to air by shifting the absorbance peak to 386 nm. In addition, it does not present notable photosensitivity with respect to exposure to sunlight.

1. Introduction

There is currently a growing demand for nanomaterials due to the novel properties they exhibit and the wide field of application, among which optoelectronics, electronics, food industry, textile industry, biosensors, catalysts, and especially medical and biological applications [1–5]. Among these materials there is special interest in the metallic nanostructures of copper, silver and gold because they show a wide spectrum of antimicrobial activity against different species of microorganisms such as fungi, Gram positive and Gram negative bacteria. However, among these, copper turns out to be more attractive for some researchers since, in addition to its excellent physical and chemical properties, it has low cost and abundant availability [6–9].

Previous studies have confirmed that Cu NPs have antimicrobial activity against E. coli and Staphylococcus among other species, as well as antifungal properties. The ability of Cu NPs to interact with and neutralize microorganisms lies in their morphological characteristics of shape, size, and stability to form other compounds; however, its synthesis in an uncontrollable atmosphere continues to
be a challenge due to its electrical properties, which generate a rapid reaction on exposure to air [10–12]. It has been reported that copper oxidizes forming cuprous oxide (CuO) and cupric oxide (Cu2O) over time and its properties depend on a series of factors such as the type of reactants, concentrations, temperature, pH, among others. Nanostructures developed in aqueous medium or other fluids, for example, usually present a deposition phase which implies the existence of nanomaterial material both in the precipitate and in the suspension. Under these conditions, the atomic absorption spectrophotometry test performed on the sediment and the supernatant can quantify the concentration of the analyte in a sample [13].

Copper oxide (CuO) is also an accessible material compared to noble metals and polymeric compounds can be made due to its stability [14]. A series of methods have been tested, the best known being the wet chemistry method, electrochemical synthesis, sonochemical synthesis, laser ablation and heat treatment, among others [15–17]. There are reports of copper nanoparticles obtained from a copper salt as a precursor and AA as a reducing agent, revealing an XRD pattern of a mixture of metallic Cu and copper(I) oxide of cubic shape and average size of 28.73 and 25.19 nm respectively, results that were corroborated with EDX spectroscopy and SEM analysis, the process was carried out at a temperature of 80 °C and in aqueous solution, using a starch solution as dispersant without being able to avoid a later and slow oxidation process in which the role of AA plays an important role in slowing down oxidation and agglomeration, helping nanoparticles to obtain better stability. Different states of copper matrices have been identified by UV-vis spectroscopy: absorption bands at 250nm (Cu +), 320-370nm (cuprous peroxide), 400-440 (charge transfer bands of cuprous oxide), 510-580 (neutral Cu plasmon resonance) and 620-850 nm (dd transitions in Cu2 ions) [18–21].

One of the new areas that takes advantage of the antipathogenic properties of nanomaterials is agriculture. Copper and copper oxide ions have been reported as pesticides, fungicides, and fertilizers, being considered friendly control strategies because they require a low concentration of the metal [19]. This was proven in the effective inhibition of Xanthomonas axonopodis pv. Punicae (Xap), a bacterium that affects the cultivation of Punica granatum in India, inducing great economic losses for the producers of the sector. Nanomaterials with a particle size less than 100 nm not only influence important events in plant life but could also reduce the dangerous effects of pesticides by improving the quality and yield of crops by delivering the macronutrients and micronutrients required with a reduction in the use of conventional chemical fertilizers and pesticides [22,23]. Alternative pesticides were also explored in the control of S. littoralis based on oxides derived from essential nutritive elements of the soil, for their evaluation, nanostructures of CuO and CaO synthesized by means of wet chemistry methods were elaborated, both showed very efficient pesticidal activity against S. littoralis, being those of CuO those of fast effect, compared to those of CaO. The efficiency shown by metal oxides poses a new role for nanomaterials in the generation and formulation of pesticides [24]. Copper and copper oxide also have optical properties, in a report made on CuO, which was exposed to ultraviolet radiation, it was concluded that there was no change in the structure, this is important if it is to perform optical testing or use these nanomaterials in capturing light for solar cells [25–27].

The objective of the present investigation is to determine the influence of the volume of A.A. in obtaining copper nanoparticles by chemical route and their stability over time, determining if there is an influence of exposure to air and light on the stability of the nanostructure under uncontrolled atmospheric conditions, evaluated by the changes that can be generated in the absorbance peak by UV-vis spectrophotometry. For future application purposes in pest control on agricultural crops.

2. Materials and methods

2.1. Materials

The chemicals used in the experiment are described below: Copper Sulfate II pentahydrate (CuSO₄ * 5 H₂O) p.a. EMSURE® ACS, ISO, Reag. PH Eur (CAS number 7758-99-8), ultrapure water with 0.2 μm final filtration (reverse osmosis water purification system / UV light / activated carbon / Thermo
Scientific brand), Sodium hydroxide (NaOH) supplied by Merck Millipore (CAS number 1310-73-2), and Ascorbic Acid (CAS number 50-81-7).

2.2. Synthesis of Copper Nanoparticles
The experimental scheme for the synthesis of copper nanoparticles by chemical route consisted of adding, dropwise, to a solution of 10 mL of copper sulfate [C] 0.05 M, 0.5 mL of sodium hydroxide [C] 7.5 M, by means of vigorous stirring until convert it into a mixture of intense blue color and viscous consistency and then add a series of variant volumes of AA [C] 1.13 M as in Figure 1, which are 0.3 mL, 0.6 mL, 1.2 mL, 1.8 mL and 2.4 mL. This was done at room temperature and under conditions of uncontrolled atmosphere and lighting. The stability of the synthesis product was then analyzed after 4 days by recording the absorbance by UV-Vis spectrophotometry during days 0 and 4.

![Figure 1. Process that shows color changes in the sample as an effect of varying the volume of A. A with values of 0.3 mL, 0.6 mL, 1.2 mL, 1.8 mL, and 2.4 mL from top to bottom in the extreme left of the image](image)

2.3. Effect of light and air exposure on the absorbance peak
Due to the results reported on the influence of the atmosphere in the oxidation processes of Cu NPs, a follow-up was carried out to possible changes that the absorbance peak of a sample exposed to different light and air conditions may present, it was divided and stored in test tubes under the following conditions: one sample was left exposed to natural light and without a lid, a second sample remained with a lid and exposed to light, and the third sample was kept without exposure to light and with a lid; recording the ultraviolet absorption spectrum on days 0, 7 and 11 from the synthesis. Also due to the presence of two phases of the mixture formed over time, precipitate and supernatant, an atomic absorption test was carried out to determine the concentrations of copper in the respective phases of the colloid.

2.4. Characterization of copper nanoparticles
The synthesis and experimental tests were carried out in the Physics Laboratory of the Department of Sciences of the Universidad Privada del Norte (UPN), Trujillo. Of the characterizations carried out by UV-vis spectrophotometry, the absorbance spectrum was monitored by means of a UV-Vis spectrophotometer (Shimadzu, UV 1900), in the range of 200 to 900 nm for all reactions. Thus, an analysis of the copper concentration in the precipitate and supernatant was also carried out with an Atomic Absorption Spectrophotometry test.

3. Results and Discussion
The obtained colloid has two defined absorption bands, one in the visible spectrum region and the other close to the ultraviolet. In the UV-Vis absorption spectrum that characterizes each sample according to the variation in the volume of A.A added to the base solution (Figure 2). The results are an indication that the colloid contains copper nanoparticles formed for the volumes of 0.6 and 1.2 mL.
of A.A, since the peak close to the characteristic of metallic copper is observed in the visible range around 501nm. Thus, it can also be concluded that there is the presence of cuprous oxide nanoparticles, which are characterized by having an absorbance peak around 400 nm.

![Figure 2. UV-vis spectrum of samples containing 0.3 mL, 0.6 mL, 1.2 mL, 1.8 mL and 2.4 mL of A.A.](image)

However, volume additions above this value generate a shift towards the ultraviolet, evidence of a possible oxidation process in addition to a broadening of the peak indicating polydispersity in the size of the particles formed or agglomerations of them.

After four days, the spectrophotometry results obtained from the colloids stored in closed flasks, without exposure to air, show instability over time (Fig. 3), due to the shift of the 501 nm peak to the right for the samples with 0.3, 0.6 and 1.2 mL of AA, which register a shift to the left close to the ultraviolet range, which is an index of an oxidation process. A complete shift towards the 392 nm peak is also observed for the 1.8 and 2.4 mL volumes, indicating the possibility of the generation of cuprous oxide nanoparticles [15].

The spectrophotometric tests carried out to evaluate the stability to the exposure of air (Fig. 4) and light (Fig. 5) yielded the following results: After 7 days, the sample exposed to air (D-7-A) has a shift to the right at 385 nm unlike the unexposed sample (D-7-C) whose absorbance shows the existence of nanoparticles with a peak at 490 nm showing an incomplete oxidation process. In a similar way, the behavior is observed for the solution after 11 days (D-11-A and D-11-C) in which the sample open to air shows a greater tendency to shift the peak to the right side.

![Figure 3. UV-vis spectrum of colloids containing 0.3 mL, 0.6 mL, 1.2 mL, 1.8 mL and 2.4 mL of A.A after 4 days of synthesis.](image)
Regarding the effect of exposure to light, there are no notable differences in the wavelength that defines the absorbance peak, at seven days both samples (D7-CCL and D7-CSL) have a peak shifted to the right in the visible range of 490 nm, indicative of the oxidation process in progress; similar behavior is observed in the case of the samples with 11 days of aging (D11-CCL and D11-CSL), with a predominance in the order of 386 nm, close to the characteristic peak of cuprous peroxide.

Due to the presentation of two phases that are formed when the sample is left to rest for 14 hours, an atomic absorption spectrophotometry was performed on both the precipitate and the supernatant, in order to define in which phase we found the highest concentration of the desired metal.

Table 1. Precipitate and supernatant atomic absorption spectrophotometry of the NPS Cu Solution

| Samples   | [C]Cu (ppm) NPs | [C]Real ppm Standard |
|-----------|-----------------|----------------------|
| Supernatant | 0.14            | 295                  |
| Sediment  | 1519.1          | 30382                |

Figure 4. UV-vis spectrum of 1.2 mL AA samples at: 0 days (D-0), 7 days exposed to air (D-7-A), 7 days closed without air (D-7-C), 11 days exposed to air (D-11-A), 11 days closed without air (D-11-C).

Figure 5. UV-vis spectrum of 1.2 mL AA samples at: 0 days (D-0), 7 days with light exposure (D7-CCL), 7 days without light exposure (D7-CSL), 11 days with light exposure (D11-CCL), 11 days without light exposure (D11-CSL).
4. Conclusions
The results obtained in this article suggest that copper nanoparticles are formed by using a volume of 1.2 mL of AA as a reducing agent because it presents a well-defined absorbance peak, which resulted in the order of 501 nm. However, this solution is not stable over time as it shows a shift of peak to the right, evidencing a possible oxidation process that converts copper into cuprous oxide with the passing of days and can even form cuprous peroxide. If the sample is stored exposed to air, the compound formed is not stable and is evidenced by a spectrum whose absorbance peak undergoes a more accelerated shift to the left, stabilizing in the order of 380 nm characteristic of copper peroxide.

It was also determined that the nanoparticles obtained are not photosensitive, as they do not present differences in absorbance in samples exposed to and without light, but they do react to prolonged exposure to air.

Thus, it was also concluded that in the two phases that occur of the colloid, precipitate and supernatant, the highest concentration of the metal is found in the precipitate with 1519.1 ppm compared to 0.14 ppm of the supernatant (Figure 6).

5. References
[1] Padilla-Vaca F, Mendoza-Macías C L, Franco B, Anaya-Velázquez F, Ponce-Noyola P and Flores-Martínez A 2018 El mundo micro en el mundo nano: importancia y desarrollo de nanomateriales para el combate de las enfermedades causadas por bacterias, protozoarios y hongos Mundo Nano. Rev. Interdiscip. en Nanociencias y Nanotecnología 11 15–27
[2] Bordbar M M, Tashkhourian J, Tavassoli A, Bahramali E and Hemmateenejad B 2020 Ultrafast detection of infectious bacteria using optoelectronic nose based on metallic nanoparticles Sensors Actuators B Chem. 319 128262
[3] Elamathi R, Ramesh R, Aravindhraj M, Manivannan M, Liakath Ali Khan F, Mpahle K, Lethcholathebe D, Kaviyarasu K, Kennedy J and Maaza M 2020 Investigation of structural and electrical properties of lithium cobalt oxide nanoparticles for optoelectronic applications Surfaces and Interfaces 20 100582
[4] Kobayashi Y, Yasuda Y and Morita T 2016 Recent advances in the synthesis of copper-based nanoparticles for metal–metal bonding processes J. Sci. Adv. Mater. Devices 1 413–30
[5] Oliveira F R, Galvão F M F, Do Nascimento J H O, Silva K K O S, Medeiros J J and Zille A 2015 Photocatalytic Properties of Sisal Fiber Coated with Nano Titanium Dioxide Mater. Today Proc. 2 41–8
[6] Kiriyanthan R M, Sharmili S A, Balaji R, Jayashree S, Mahboob S, Al-Ghanim K A, Al-Misned F, Ahmed Z, Govindarajan M and Vaseeharan B 2020 Photocatalytic, antiproliferative and
antimicrobial properties of copper nanoparticles synthesized using Manilkara zapota leaf extract: A photodynamic approach Photodiagnosis Photodyn. Ther. 32 102058

[7] Bashir F, Irfan M, Ahmad T, Iqbal J, Butt M T, Sadeq Y, Umbreen M, Shaikh I A and Moniruzzaman M 2021 Efficient utilization of low cost agro materials for incorporation of copper nanoparticles to scrutinize their antibacterial properties in drinking water Environ. Technol. Innov. 21 101228

[8] Rojas-Michea C, Morel M, Gracia F, Morell G and Mosquera E 2020 Influence of copper doping on structural, morphological, optical, and vibrational properties of ZnO nanoparticles synthesized by sol gel method Surfaces and Interfaces 21 100700

[9] Yousefvand P, Mohammadi E, Zhuang Y, Bloukh S H, Edis Z, Gamasaee N A, Zanganeh H, Mansour F N, Heidarzadeh M, Attar F, Babadaei M M N, Keshtaii A B, Shahpasand K, Sharifi M, Falahat M and Cai Y 2021 Biothermodynamic, antiproliferative and antimicrobial properties of synthesized copper oxide nanoparticles J. Mol. Liq. 324 114693

[10] Cho K H, Park J E, Osaka T and Park S G 2005 The study of antimicrobial activity and preservative effects of nanosilver ingredient Electrochim. Acta 51 956–60

[11] Argueta-Figueroa L, Mesta-Pichardo D, Torres-Gómez N, Martínez-Alvarezo O, Paulino-González Á D and Arenas-Arrocuena M C 2018 Acrylic thermopolymerizable enriched with nanopartículas de cobre: evaluación antibacteriana y citotóxica Mundo Nano. Rev. Interdiscip. en Nanociencias y Nanotecnología 11 45–60

[12] Cohen D, Soroka Y, Ma’or Z, Oron M, Portugal-Cohen M, Brégégere F M, Berhanu D, Valsami-Jones E, Hai N and Milner Y 2013 Evaluation of topically applied copper(II) oxide nanoparticle cytotoxicity in human skin organ culture Toxicol. Vitr. 27 292–8

[13] Alsawafita M, Badilescu S, Packirisamy M and Truong V-V 2011 Kinetics at the nanoscale: formation and aqueous oxidation of copper nanoparticles React. Kinet. Mech. Catal. 2011 1042 104 437–50

[14] Ren G, Hu D, Cheng E W C, Vargas-Reus M A, Reip P and Allaker R P 2009 Characterisation of copper oxide nanoparticles for antimicrobial applications Int. J. Antimicrob. Agents 33 587–90

[15] Din M I and Rehan R 2016 Synthesis, Characterization, and Applications of Copper Nanoparticles http://dx.doi.org/10.1080/00032719.2016.1172081 50 50–62

[16] Lopez R, G A and Ataucuri S J C 2018 Estudio sobre metodologías aplicadas para la síntesis de nanopartículas de cobre (NPsCU) y plata (NPsAG) en diferentes formas y tamaños Rev. Cient. Tec. 27 45

[17] Granata G, Yamaoka T, Pagnanelli F and Fuwa A 2016 Study of the synthesis of copper nanoparticles: the role of capping and kinetic towards control of particle size and stability J. Nanoparticle Res. 2016 18 1–12

[18] Gawande M B, Goswami A, Felpin F X, Asefa T, Huang X, Silva R, Zou X, Zboril R and Varma R S 2016 Cu and Cu-Based Nanoparticles: Synthesis and Applications in Catalysis Chem. Rev. 116 3722–811

[19] Jain S, Jain A, Kachhawah P and Devra V 2015 Synthesis and size control of copper nanoparticles and their catalytic application Trans. Nonferrous Met. Soc. China 25 3995–4000

[20] Khan A, Rashid A, Younas R and Chong R 2015 A chemical reduction approach to the synthesis of copper nanoparticles Int. Nano Lett. 2015 61 6 21–6

[21] Pestryakov A N, Petranovskii V P, Kryazhov A, Ozhereliev O, Pfänder N and Knop-Gericke A 2004 Study of copper nanoparticles formation on supports of different nature by UV–Vis diffuse reflectance spectroscopy Chem. Phys. Lett. 385 173–6

[22] XG Z, CN X, Y T, E T, H Y and Y S 2000 Observation of charge stripes in cupric oxide Phys. Rev. Lett. 85 5170–3

[23] Mondal K K and Mani C 2011 Investigation of the antibacterial properties of nanocopper against Xanthomonas axonopodis pv. punicae, the incitant of pomegranate bacterial blight Ann. Microbiol. 2011 622 62 889–93
[24] Padhi S and Behera A 2021 Nano-enabled Approaches for the Suitable Delivery of Fertilizer and Pesticide for Plant Growth Plant Growth-Promoting Microbes Sustain. Biot. Abiotic Stress Manag. 355–94

[25] Ayoub H A, Khairy M, Elsaid S, Rashwan F A and Abdel-Hafez H F 2018 Pesticidal Activity of Nanostructured Metal Oxides for Generation of Alternative Pesticide Formulations J. Agric. Food Chem. 66 5491–8

[26] Parveen F, Sannakki B, Mandke M V. and Pathan H M 2016 Copper nanoparticles: Synthesis methods and its light harvesting performance Sol. Energy Mater. Sol. Cells 144 371–82

[27] Shi J, Abid A D, Kennedy I M, Hristova K R and Silk W K 2011 To duckweeds (Landoltia punctata), nanoparticulate copper oxide is more inhibitory than the soluble copper in the bulk solution Environ. Pollut. 159 1277–82