International Interdisciplinary Congress on Renewable Energies, Industrial Maintenance, Mechatronics and Informatics
Booklets

RENIECYT 1702902 CONACYT

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Title: Biosynthesis of Metallic Nanoparticles and their Applications

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Editorial label ECORFAN: 607-8695
BCIERMMI Control Number: 2021-01
BCIERMMI Classification (2021): 271021-0001

Pages: 16
RNA: 03-2010-032610115700-14

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Introduction

- ✓ Nanotechnology is a fascinating multi-disciplined field that involves the design and engineering of functional systems on the molecular scale.
- ✓ It can be defined as the art and the science of the manipulation of matter at the nanoscale to create new and unique materials.
- ✓ Its main characteristic is a dimensional structure smaller than 100 nm, which is important in the fields of materials science, medical and life sciences, and physical and chemical sciences.
- ✓ They mainly present themselves in characteristics such as catalytic reactivity, thermal conductivity, nonlinear optical behavior, and chemical stability, owing to their high surface-area-to-volumen.

(Agarwal et al., 2017; Nayantara & Kaur, 2018; Vijayaraghavan & Ashokkumar, 2017)

Introduction

Basic Approaches

The 'Top down' approach is based on mechanical size reduction methods, gradually breaking down the volume of the material into structures on the nanoscale, using lithographic techniques such as: grinding, milling, sputtering, and thermic or laser ablation.

The 'Bottom up' approach is based on assembly through smaller entities in the nanoscale range (10-100 nm) such as atoms or molecules. Based mainly on chemical and biological methods, this approach increases the possibility of producing more chemically homogenous metallic particles with less defects.

(Agarwal et al., 2017; Chinnasamy et al., 2018; Gan & Li, 2012; Narayanan & Sakthivel, 2010b; Vijayaraghavan & Ashokkumar, 2017).

Top down approach

- Laser ablation
- Evaporation-condensation
- High-energy ball milling
- Plasma arc technique

Bottom up approach

- Chemical reduction
- Microemulsion process
- Thermal decomposition
- Sol-gel technique
- Solvothermal method
- Electrochemical deposition

Introduction

The biological methods involving the reduction of metallic ions using extracts or live biological mass have been investigated, such as sources of reducers, equally so with those that are intracellular and extracellular.

The **intracellular** processes take place within the cell. The process is based on metabolic pathways that are probably responsible for synthesis, such as photosynthesis, respiration, and nitrogen fixation)
Extracellular processes are referred to as the processes that take place outside the cells, mainly supported by the exudates of cellular metabolism that comprise metabolites, ions, pigments, many proteins (enzymes) and non-protein entities such as DNA, RNA, microbial subproducts (hormones, antioxidants) and lipids.

Biosynthesis methods surpass other classic procedures thanks to their advantages **- the wide availability of biological entities, ecological procedures, cost-effectiveness and easy scaling.**

Bacteria, fungi, yeasts, algae, and plants are among the different organisms that are used.

(Agarwal et al., 2017; Carrillo-Inungaray et al., 2018; Jamkhande et al., 2019; Khanna et al., 2019; Kharissova et al., 2013; Kuppusamy et al., 2016; Nasrollahzadeh et al., 2020; Sharma et al., 2015)

Introduction

Compared with the synthesis of nanoparticles mediated by microorganisms, the use of plants presents diverse advantages owing to the rich biodiversity and easy availability of vegetative organisms that have been explored for the synthesis of nanomaterials.

They also represent less biological risks during production and eliminate the laborious process of cell cultivation, which has led to plants being considered the better option to reduce metallic ions, as well as being ideal candidates for the production on a great scale.

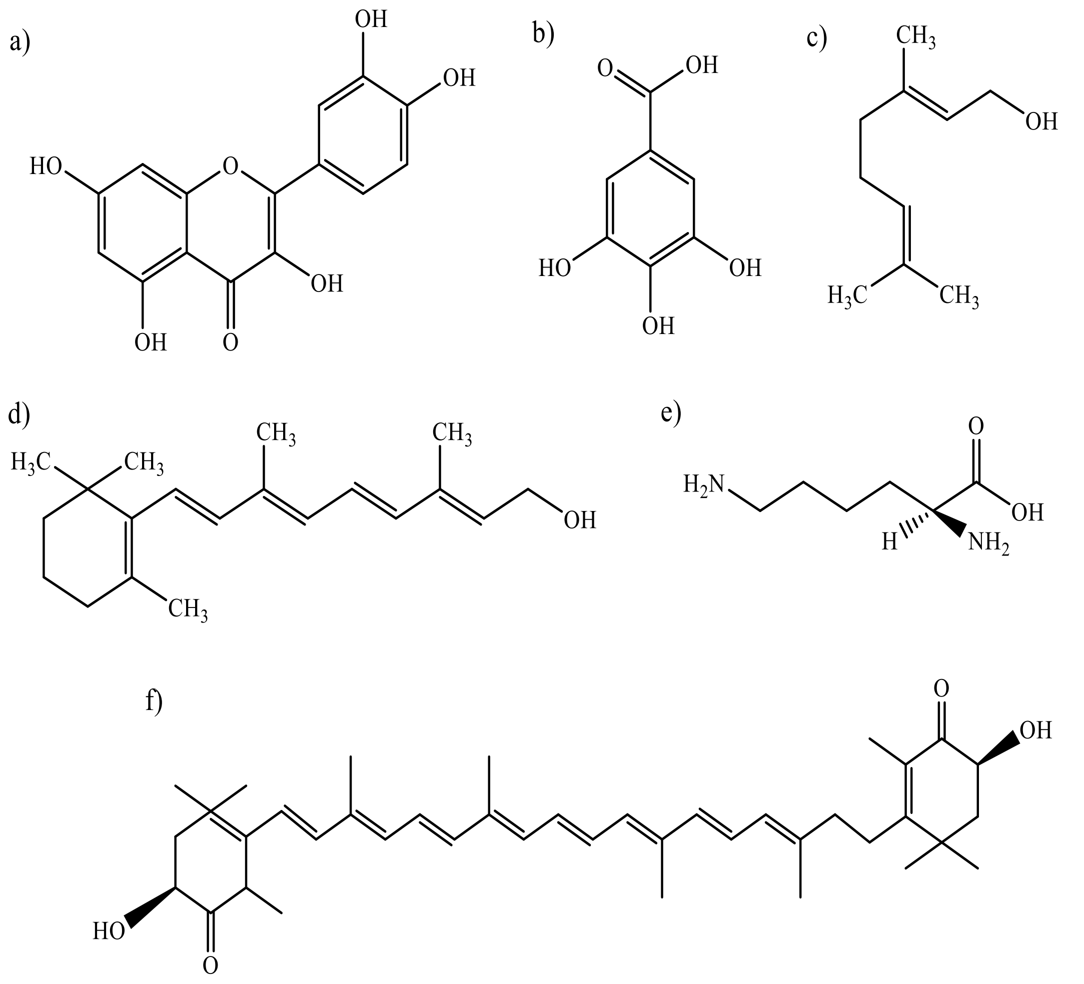


Figure 1. Examples of phytochemicals present in plants: a) Quercithin (flavonium); b) Gallic acid (phenolic compound); c) Geraniol (terpenoid); d) Retinol (vitamins); e) L-lysine (amino acid); f) Carotenoid (pigment).

(Agarwal et al., 2017; Gan & Li, 2012; Kuppusamy et al., 2016; Nasrollahzadeh et al., 2020).

Introduction

Different factors such as pH, temperature, concentration of metal salts, or the quantity of vegetal extract, play an important role in the control of nucleation, formation, and stabilization of NPs. Changes in these parameters can induce changes in the size and shape, as well as preventing the agglomeration of NPs.

- Regarding the concentration of metallic salts, it is suggested that the working concentrations be considered to be in the range of 1 to 10 mM mainly in the case of noble metals. In accordance with that revised for other metallic ions, it is possible to increase the range to 0.1 M.

- In the case of vegetal extract volume upon increasing this factor, the size of the NPs decreases. As such, the use of percentages higher than 5% is recommended.

- For the pH, it is recommendable to work between the values of 5.0 and 12.0, given that lower values will not have a reduction process.

- For the temperature, it is better to work with high temperatures, or those that are at least higher than 50°C and lower than 100°C.

(Khanna et al., 2019; Rai & Yadav, 2013).

Introduction

Characterization techniques

Once prepared, the metallic nanoparticles conform to diverse characterization techniques in order to determine their size, shape, distribution, morphology, and surface area.

UV-vis

Various investigators agree that one of the first signs of the reduction of metals is the change in color between the solutions before and after the reduction reaction. This change indicates the formation of nanoparticles, confirmed by the visible surface plasmon resonance (SPR) in the UV-vis absorption spectrum.

FTIR

Analyses through Fourier-transform infrared spectroscopy (FTIR) are relevant for determining the functional groups present in vegetal extracts, which allows the investigation of the underlying synthesis mechanism, and the surface chemical.

XRD

The purity, crystalline size, geometry, orientation, and phases can be determined through XRD data. Generally, the diffraction patterns are compared with a standard crystallographic database such as JCPDS in order to have the structural information.

(Asghar et al., 2018; Dada et al., 2019; Khanna et al., 2019; Kuppusamy et al., 2016)

Introduction

XPS

It can also shed light on the interaction between NPs and their adjacent biomolecules, as well as signaling the presence of secondary or undesired elements that could reduce its efficiency or lead to a secondary reaction and process contamination.

SEM/TEM

Scanning electron microscopy (SEM) offers information on particles at the nanoscale and helps to determine the surface morphology and the dispersion of free NPs or those in the matrix.

Transmission electron microscopy (TEM) is more commonly used for the size and shape and can also offer information on the number of layers of the material, given that it varies from a low or high increase.

(Ealia & Saravanakumar, 2017; Khanna et al., 2019)

Introduction

Applications

Metal nanoparticles are of great interest to several disciplines including biotechnology / biomedicine, bioremediation, agriculture, catalysis, biosensors, among others.

Monometallic Particles (Ag, Au, Fe, Cu, Zn)

Bimetallic Particles

- ✓ Has been evaluated the bactericidal activity, against both gram negative and gram-positive pathogenic bacteria like *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Salmonella infantis* and *Enterobacter aerogenes*.
- ✓ Also has been evaluated the anticancer activity of the NPs produced and the results of cytotoxicity against HeLa cancer cells getting promising results.
- ✓ They have also been evaluated for the treatment of domestic and industrial wastewater.

(Albeladi et al., 2020; Balasubramanian et al. 2020; Huang et al., 2014, Korkmaz et al., 2020; Rai & Yadav, 2013)

Introduction

Toxicity

- ✓ The toxicity of nanomaterials depends on several factors such as: size, shape, size and shape of their distribution, surface charge and surface chemistry.

- ✓ NPs can enter the body through various means, like inhalation, skin and digestion; depending on their physicochemical characteristics and their method of preparation, NPs can access vital organs through blood flow and induce tissue and cell damage.

- ✓ Several investigations indicate that NPs activate oxidative stress by increasing the generation of reactive oxygen species (ROS), intervening in the expression of genes involved in inflammation, consequently generating damage to proteins, cell membrane and DNA.

- ✓ The presence of NPs in the environment gives rise to transformations capable of modifying their degree of toxicity, which still requires studies and test methods to single out the processes underway in different environmental spheres that allow identifying risk levels.

(Ajdary et al.,2018; Amini, 2019; Fard et al., 2015; Sajid et al., 2015; Zhang et al., 2018).

Methodology

Preparation of the vegetal extract

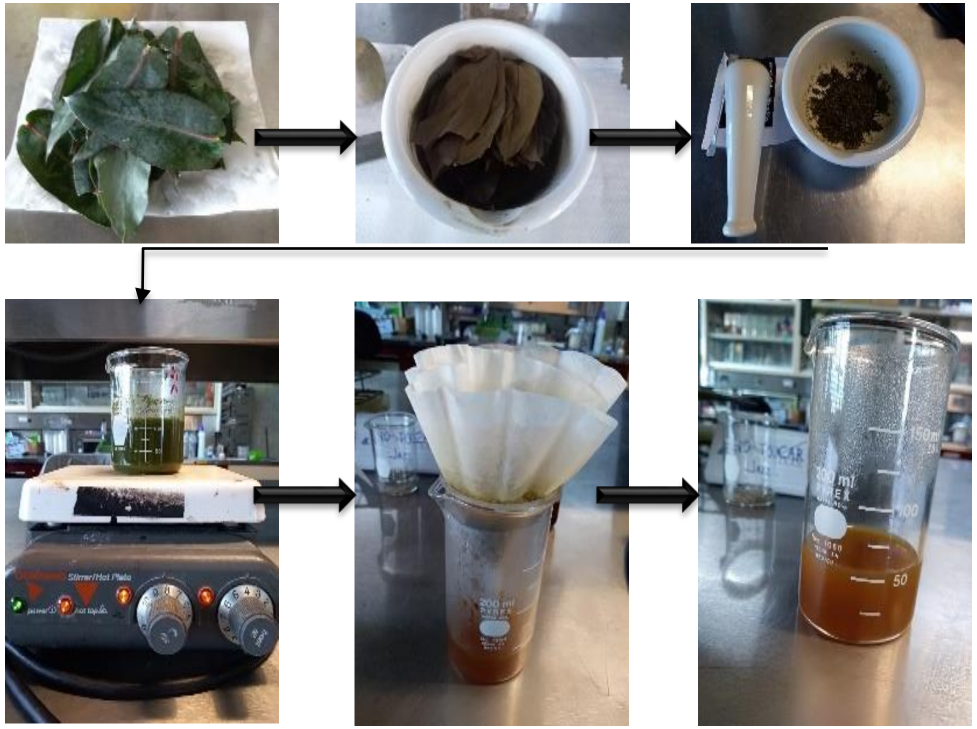


Figure 4. Procedure for the elaboration of the vegetal extract from eucalyptus leaves *(own elaboration)*.

Reduction reaction

For the reduction reaction, 150 mL of $CuSO_4 \cdot 5H_2O$ 0.05 M were prepared with distilled water in a volumetric flask.

The reduction reaction was carried out with the vegetal extract (moringa, hibiscus and eucalyptus) plus the copper solution in a 1:1 volume ratio, at room temperature under constant stirring for one hour.

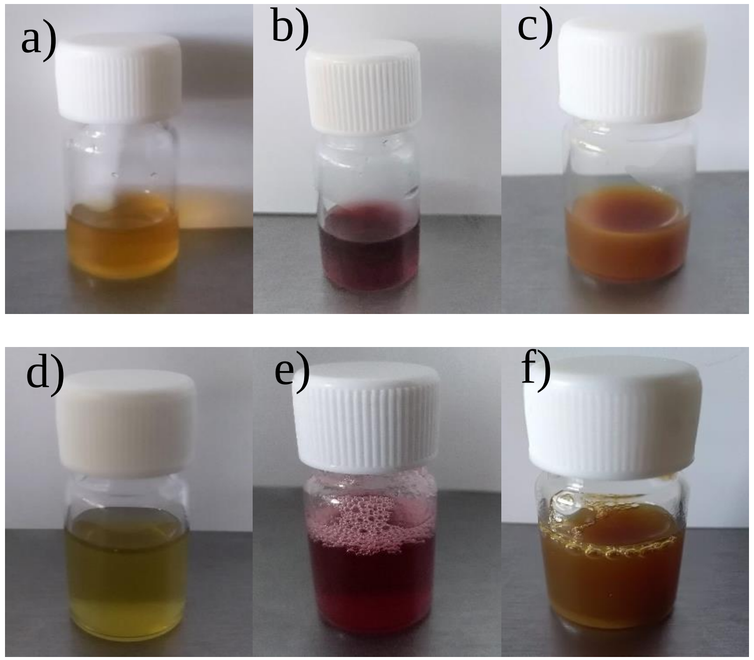


Figure 5. a), b) and c) infusions of moringa, hibiscus and eucalyptus before the reduction reaction; d), e) and f) solutions after one hour of reduction reaction with copper.

Methodology

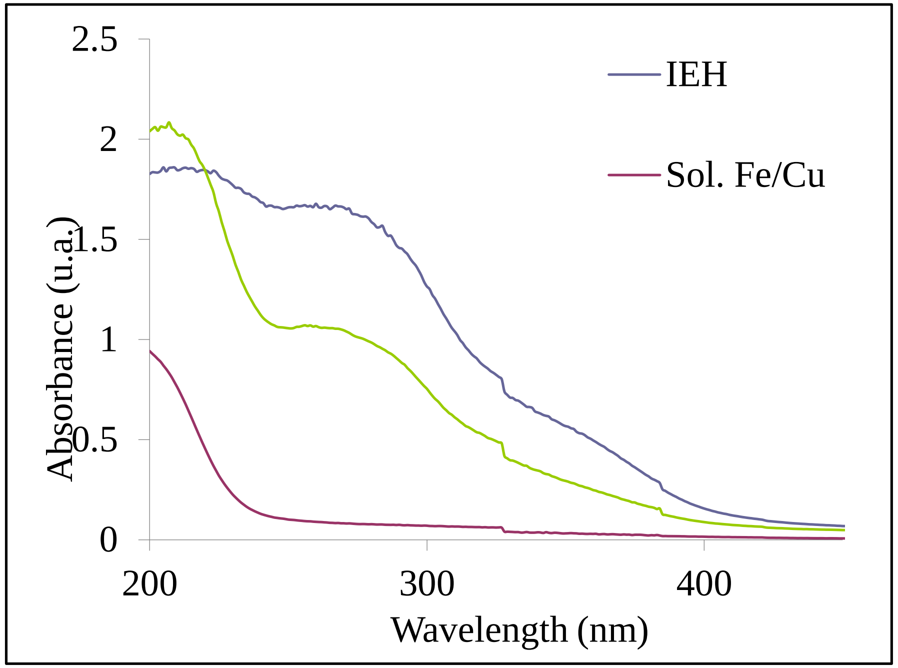
Characterization of the Fe/Cu NPs

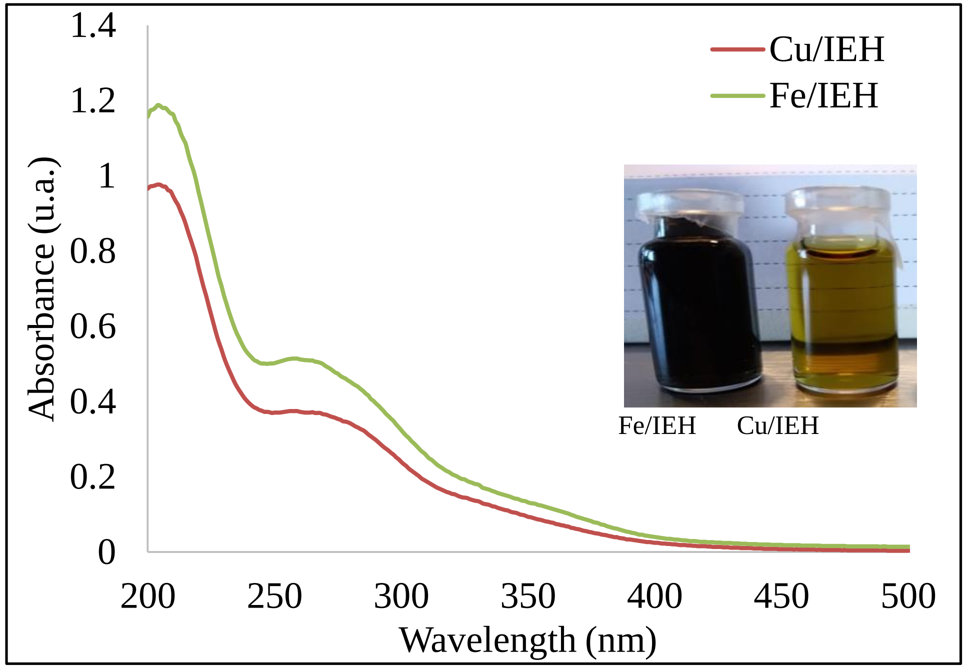
Determination of the SPR of the IEH-Sol containing Fe / Cu solution by means of UV-visible with a Perkin-Elmer Lambda 25 spectrophotometer.

Indigo carmine removal

Indigo carmine reagent grade was added with distilled water to set the pH with 4 M sulfuric acid and hydrogen peroxide at 30% analytical grade, the removal of the dye was monitored by UV-visible spectrophotometry with the Perkin-Elmer Lambda spectrophotometer 25, applying equation, the results obtained were fed into the statistical software Minitab Ver. 18.0 to design and carry out a factorial experimental 2^3; the factors evaluated were: dye concentration (200 - 400 mg / L), dose of 30% hydrogen peroxide (5 and 10 μL) and catalyst dose (1 and 5 mg). The volume used for each test was 10 mL at a pH of 3.0.

Results





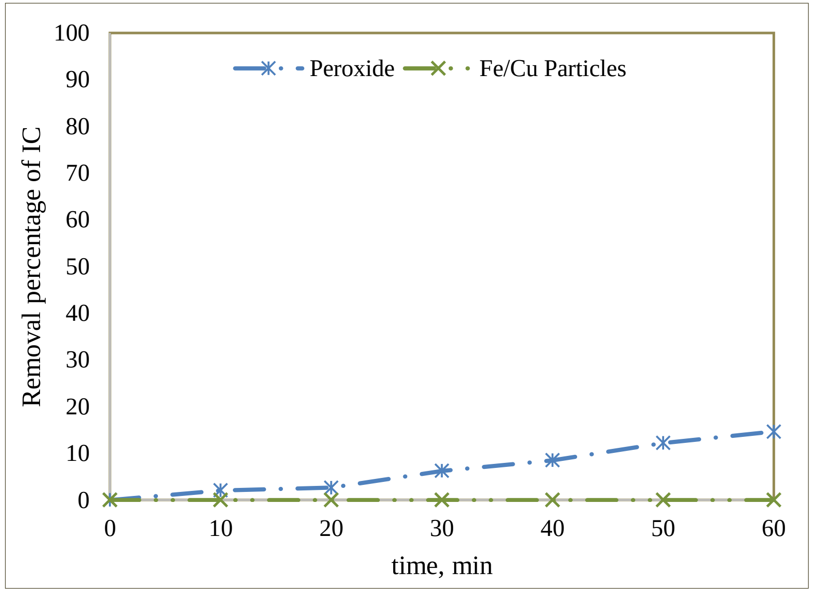
Graph 1. UV-Vis spectra: a) spectrum of the reduction reaction solutions in the green infusion of eucalyptus leaves plus iron and in the red infusion of eucalyptus leaves plus copper; b) spectra in the blue infusion of eucalyptus leaves (IEH), red bimetallic solution (Fe / Cu) and in green reduction solution (IEH-Sol. Fe / Cu).

Proposed reduction mechanism

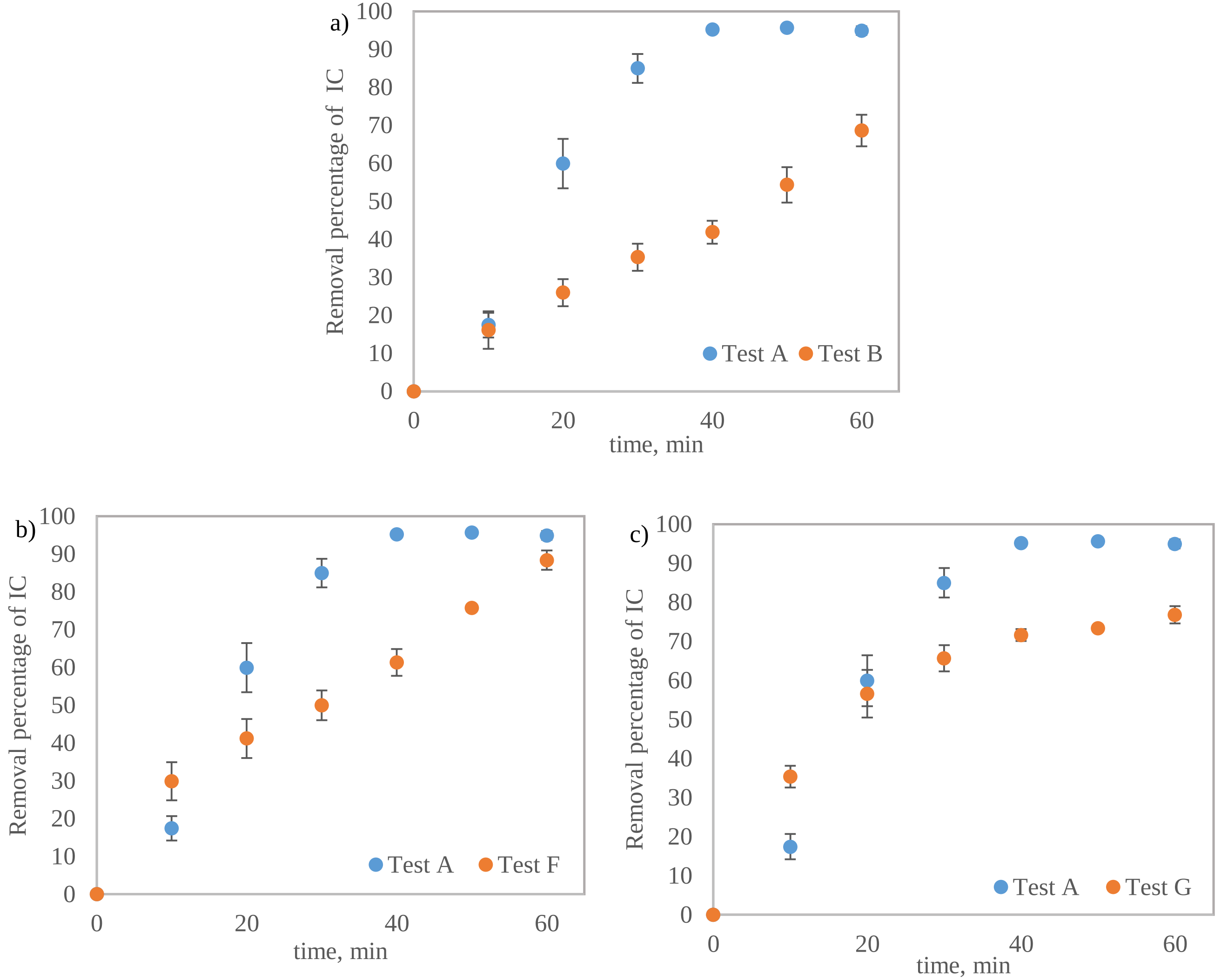
$$nCu^{2+} + nFe^{2+} + 4nAr\text{-}(OH)_n \rightarrow nFe(Cu)_{(s)} + 4n\ Ar{=}(O)_n + 4nH^+$$

Results

Indigo carmine removal

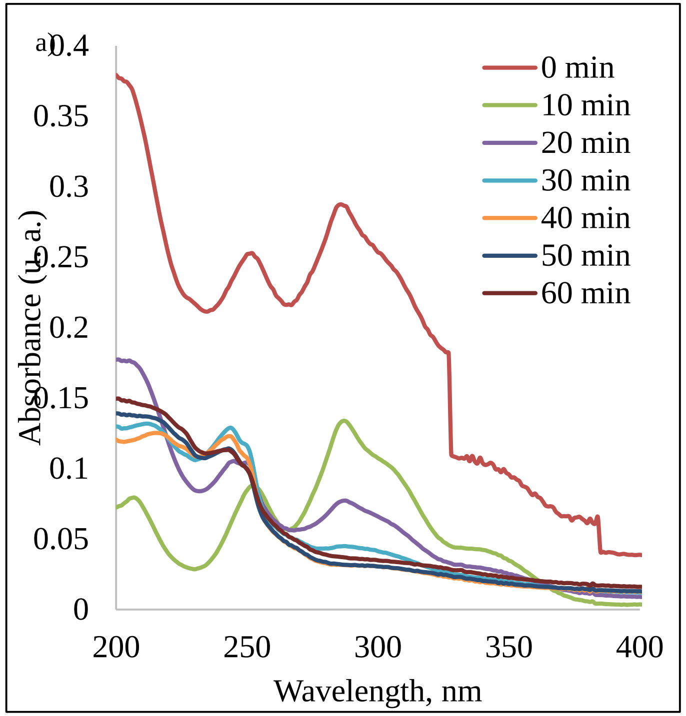


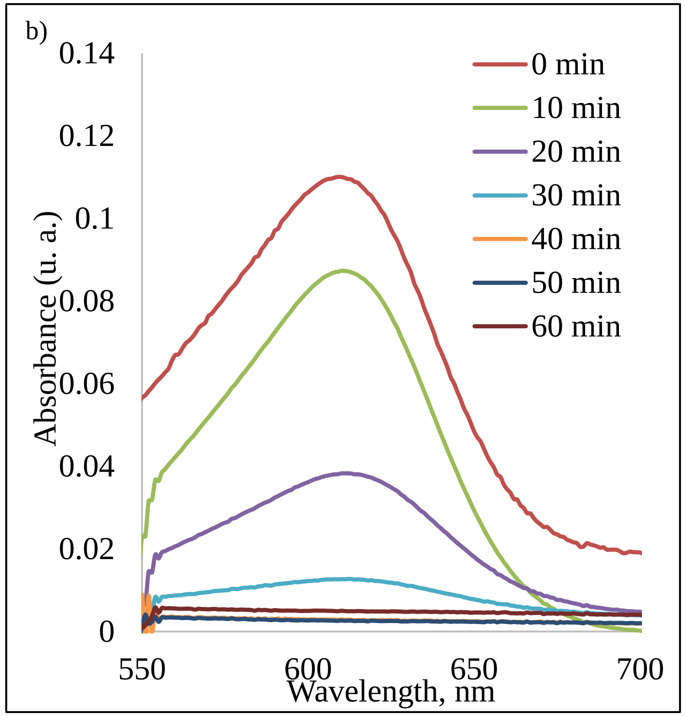
Graph 2. Evaluation of the individual reagents for removal of the IC dye. Working conditions, volume: 10mL; dye concentration: 200 mg / L; pH: 3.0; amount of particles: 1mg; amount of H_2O_2: 10 μL.

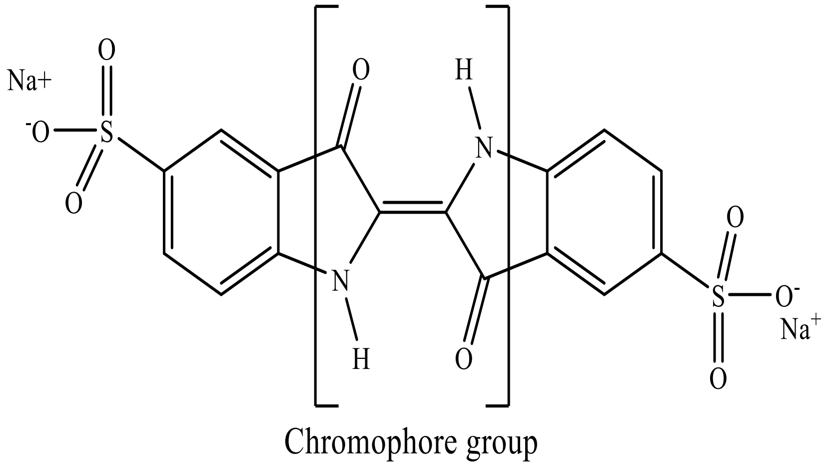


Graph 3. Indigo carmine removal with Fenton-like reactions with Fe / Cu bimetallic nanoparticles: a) evaluation of the influence of the IC dye concentration (200 - 400 mg / L); b) evaluation of the influence of the amount of NPs Fe / Cu (1 - 5 mg); and c) evaluation of the influence of the H_2O_2 dose (5 - 10 μL). Working conditions: volume of 10 mL, pH 3.0, constant stirring (ANOVA analysis $p < 0.05$).

Results







Graph 4. Indigo carmine UV-visible spectra: a) segment of the 200-400 nm range; b) segment of 550-700 nm characteristic absorption of the blue of the indigo carmine.

$$Fe^{2+} + Cu^{2+} + 3H_2O_2 \rightarrow 3HO^{\bullet} + HO^{\bullet}_2 + H_2O + Fe^{3+} + Cu^{2+}$$

Conclusions

Bioreduction processes using plants show broad advantages over other methods related to the availability of plant species with antioxidant capacity, the complex matrix of the extract that provides not only chemical species with reducing capacities, but also provides stabilizing species that when associated with the surface of the NPs, can influence positively the application to be evaluated, mainly generating high expectations in the medical and catalytic area.

The manufacturing processes must consider the aforementioned aspects like pH, temperature, metal ion concentration, as well as the amount of plant extract, so it is important to carry out an optimization process to synthesize nanoparticles displaying the best qualities, which can be corroborated with the fundamental characterization studies such as UV-vis Spectrophotometry, X-ray Diffraction (XRD) and microscopy, either scanning electron microscopy (SEM) or transmission electron microscopy (TEM) to ensure the success of the tests to be carried out.

The application of bimetallic NPs obtained by bioreduction, as catalysts for advanced oxidation processes such as Fenton-like, is a viable field in development where high efficiencies can be found in the removal of organic pollutants; subsequent and complementary studies are required to shed light on the degree of mineralization of the pollutants and the toxicity of the particles used in the process.

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