Original Research Article

Using aqueous extract of *Eucalyptus grandis* to synthesize iron oxide nanoparticles

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ABSTRACT

This work presents the evaluation of iron oxide nanoparticles obtained from the aqueous extract of Eucalyptus grandis. Twenty-three experiments were carried out where the synthesis of nanoparticles was performed by using the aqueous extract together with salts of iron (II) chloride tetrahydrate and iron (III) chloride hexahydrate. A characterization was carried out by IR, TEM and BET, where bands were presented at 3,440.77, 1,559.26 and 445.31 cm^{-1}, indicating the presence of iron oxide nanoparticles. A relatively high monodispersity was evidenced with particles around 9 nm. By means of BET analysis it was found to present a surface area of 131.897 m^2/g. Obtaining nanoparticles by this green method presents yield values of 98%, with application in nanotechnology, biomedicine, environmental treatment, among others, making them highly versatile and their production cost is relatively low.

Keywords: Green Synthesis; Magnetite Nanoparticles; Plant Extract

1. Introduction

Nanotechnology refers to research and technological development at the atomic, molecular or macromolecular level, in the range of 1 to 100 nm, to provide a fundamental understanding of nanoscale phenomena and materials and on the other hand to create and use structures, devices and systems with novel properties and functions due to their sizes compared to bulk size materials[1].

In recent years the interest in the use of magnetic nanoparticles has been increasing due to the wide range of applications they have, ranging from applications in biomedicine, nanotechnological construction of DNA, development of electronic devices, even environmental remediation treatments[1–3]. Among the nanoparticles that have been studied are iron nanoparticles given their properties such as magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3)[4–7]. Their nanoferrite structure is characterized by having a spinel crystal structure (**Figure 1**), in which oxygen ions form the compact cubic packing and iron ions are located in the tetrahedral (Td) and octahedral (Oh) interstices. Equation (1) shows the formula of magnetite, in which Fe^{2+} cations occupy only Oh positions, while Fe^{3+} ions are distributed between Td and Oh positions[8].

$$Fe_3O_4 : \left[Fe^{3+} \right]_{Td} \left[Fe^{3+} Fe^{2+} \right]_{Oh} O_4$$
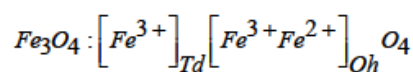
$$\tag{1}$$

The magnetization of Fe_3O_4 nanoparticles arises from the antiferromagnetic coupling between Fe^{3+} ions at Oh and Td interstices, leaving
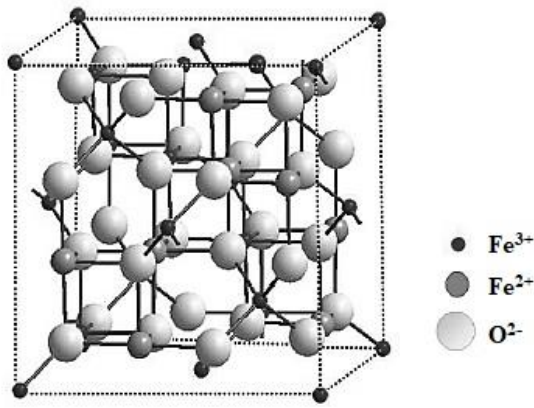
Figure 1. Spinel structure of Fe₃O₄.
Source: Noval *et al.*[9].

magnetic moments of Fe^{2+} ions, at Oh positions, as the ones responsible for the unit cell magnetization[8,9].

There are many methods that can be used for the synthesis of nanoparticles, some of these methods are classified as mechanical-chemical methods consisting of a top-down system, i.e. a method consisting of the division of a larger solid to reduce it to smaller portions of the same, although unfortunately this method is not recommended when a homogeneous synthesis is sought because it tends to form unequal and amorphous particles[10]. Other methods are classified as chemical-physical methods and are called bottom-up system, which consists of the fabrication of nanoparticles through the condensation of atoms, molecules or molecular entities in gas phase or in solution, this type of synthesis being the most popular in obtaining nanoparticles[1,3,10–12]. In the latter case, conditions such as working temperature, solvent used or stabilizing agents and reproducibility are two very important aspects to take into account when choosing the appropriate method, and the most common method used for the synthesis of iron oxide nanoparticles is chemical co-precipitation[1], which is perhaps the simplest and most chemically efficient method to obtain iron oxide nanoparticles (Fe_3O_4 or γ-Fe_2O_3), which are generally prepared by a stoichiometric mixture between ferrous and ferric salts in aqueous medium[1]. Equation 2 shows the chemical reaction that takes place in the formation of Fe_3O_4.

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O$$

$$(2)$$
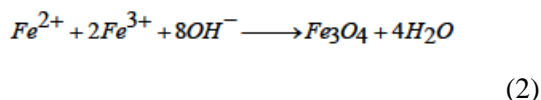
According to the thermodynamics of this reaction, complete precipitation of Fe_3O_4 is achieved at pH between 8 and 14, with a stoichiometric ratio of 2:1 (Fe^{3+}/Fe^{2+}) in an oxygen-free environment to avoid iron oxidation[11]. However, magnetite (Fe_3O_4) is not very stable in the environment and is sensitive to oxidation. Therefore, in the presence of oxygen, it transforms into maghemite (γ-Fe_2O_3) as shown in Equation 3[11].

$$Fe_3O_4 + 2H^+ \longrightarrow \gamma Fe_2O_3 + Fe^{2+} + H_2O$$

$$(3)$$
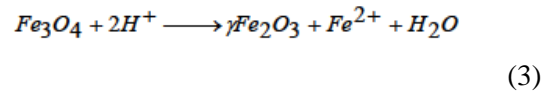
Oxidation with air not only transforms magnetite (Fe_3O_4) into maghemite (γ-Fe_2O_3), but also transfers various electrons or ions, depending on the pH of the solution, as shown in Equation 3. Under acidic and anaerobic conditions, the surface of Fe^{2+} ions form hexaaqua complexes in solution, whereas, under basic conditions, the oxidation of magnetite involves oxidation-reduction reactions on the magnetite surface. The main advantage of this method is the large amount of nanoparticles that can be synthesized, however, the control of the particle size distribution is limited because kinetic factors control the crystal growth[11]. Thus, the size and shape of the nanoparticles can be controlled with relative success by adjusting parameters such as pH, ionic strength, temperature, nature of the salts used (perchlorates, chlorides, sulfates and nitrates) or the concentration ratio of the Fe^{2+}/Fe^{3+} species. In addition, the addition of chelating organic anions (carboxylates or α-hydroxy carboxylate ions such as, citric, gluconic or oleic acids) or surface polymers as complexing agents (dextran, carboxydextran or polyvinyl alcohol) during magnetite formation can help to control particle size[11,13].

Similarly, the synthesis of nanoparticles is achieved by using aqueous plant extracts such as Stan *et al.* in 2017 using grape or lemon peel extracts, Robles *et al.* in 2019 using papaya peel extracts, Awwad and Salem in 2013 using plants such as carob, and Ahmed *et al.* in 2013 also using papaya peel; they used citrate in order to achieve the synthesis of these materials, reducing the cost of manufacturing, requiring the use of many reagents, as these extracts have multiple functions in the process, in addition to reducing hazardous waste when

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using this type of extract[4-6,14].

This work is carried out with the purpose of testing the effectiveness of a eucalyptus extract in the synthesis of iron oxide nanoparticles, which can be used in processes for the uptake of heavy metals such as mercury or selenium, or in processes for the degradation of organic molecules present in aqueous media[3,15].

2. Materials and methods

2.1 Sample collection and processing site

The foliage sample of Eucalyptus grandis was obtained from the forest nursery of the Cooperativa Agroforestal del Cauca (COOTRAFORC), located in Vereda Gonzales, municipality of Popayán, department of Cauca, coordinates 2°28'34.9" N 76°34'03.2" W. The study was carried out in the facilities of the Laboratory of the Environmental Analytical Chemistry Research Group (EACRG) and the Industrial Analysis Unit of the Chemistry Program of the Universidad del Cauca.

Since only the leaves would be used, the sample was subjected to a previous classification and manual cleaning in order to separate the foliage from dust, branches or seeds that could later interfere with the development of the research. Subsequently, the wet sample was left to dry for about 2 weeks to eliminate excess moisture, moving the leaves periodically to avoid moisture retention in some of the leaves that could generate losses of material, obtaining a dry biomass ready to be ground. Subsequently, the sample was completely ground by means of a conventional mill and then classified by size in order to achieve a small particle size of biomass by sieving through a No.18 mesh sieve of 2 mm thickness, thus obtaining a biomass with a very fine particle size of about 2 mm, finally the sieved sample was stored at room temperature.

2.2 Obtaining the aqueous extract of *Eucalyptus grandis*

For the preparation of the aqueous plant extract, a ratio between plant material and solvent of 1:5 weight/volume, respectively[4], for which the extract is prepared starting from the use of the plant material in this case of foliage of the species *Euca-*

lyptus grandis in the solvent in this case deionized water, heating at a temperature of 80 ℃ for 5 minutes, then filtered under vacuum and centrifuged at 1,250 rpm for 5 minutes, deionized water is used in order to complete the total volume of the extract and stored at room temperature for later use[5].

2.3 Preparation of magnetite nanoparticles

In a typical execution, the coprecipitation method uses two iron salts, in this case iron (II) chloride tetrahydrate ($FeCl_2·4H_2O$) and iron (Ill) chloride hexahydrate ($FeCl_3·6H_2O$) in a ratio of 1:2 weights, respectively, heated for 10 minutes with constant stirring, after which a volume of the previously prepared eucalyptus extract is added turning the solution slightly brown, and finally 20 mL of a known concentration of sodium hydroxide (NaOH) solution was added after 5 minutes. After this process, since the particle obtained is insoluble in water, it is precipitated, filtered and dried in an oven at 50 ℃ for 24 hours. Finally, the dried particle is macerated and stored in a dry, cool place at room temperature[4,5]. The conditions of both working temperature, volume of the extract used and hydroxide concentration were optimized to find the maximum yield point of the synthesis.

Table 1. Variables considered for the synthesis

Variable	Definition	Units
Performance	Particle synthesis performance	%
Extract volume	Amount of extract to be used	mL
Temperature	Temperature for synthesis	℃
NaOH Concentration	Concentration used to complete the reaction	mol/L

Source: Authors.

2.4 Statistical analysis of the synthesis

For the analysis of the design and optimization of the synthesis, the statistical program STATGRAPHICS CENTURION XVII.II was used, with which a central composite experimental design with star points 2^3 was carried out. **Table 1** presents the variables taken into consideration for the design and the procedure followed by Awwad and Salem for obtaining magnetite nanoparticles[5], so that through this experimental design the best synthesis parame-

ters for the temperature, the volume of extract used and the concentration of the base can be established and thus achieve the highest yield in the process of obtaining magnetite nanoparticles.

2.5 Characterization of nanoparticles

The characterization of the nanoparticles was performed by instrumental analysis using Transmission Electron Microscopy (TEM), FT-IR spectroscopy by KBr pellet and surface area analysis by BET technique.

2.5.1 Transmission electron microscopy (TEM)

To obtain the respective micrograph of the synthesized magnetite, a JEOL JEM 1200-EX transmission electron microscope was used in the microscopy laboratory of the Universidad del Cauca, dispersing the nanoparticles by ultrasound, measuring with a micrograph resolution of 4 nm and using the Image-Pro Plus 3.0 image processing program.
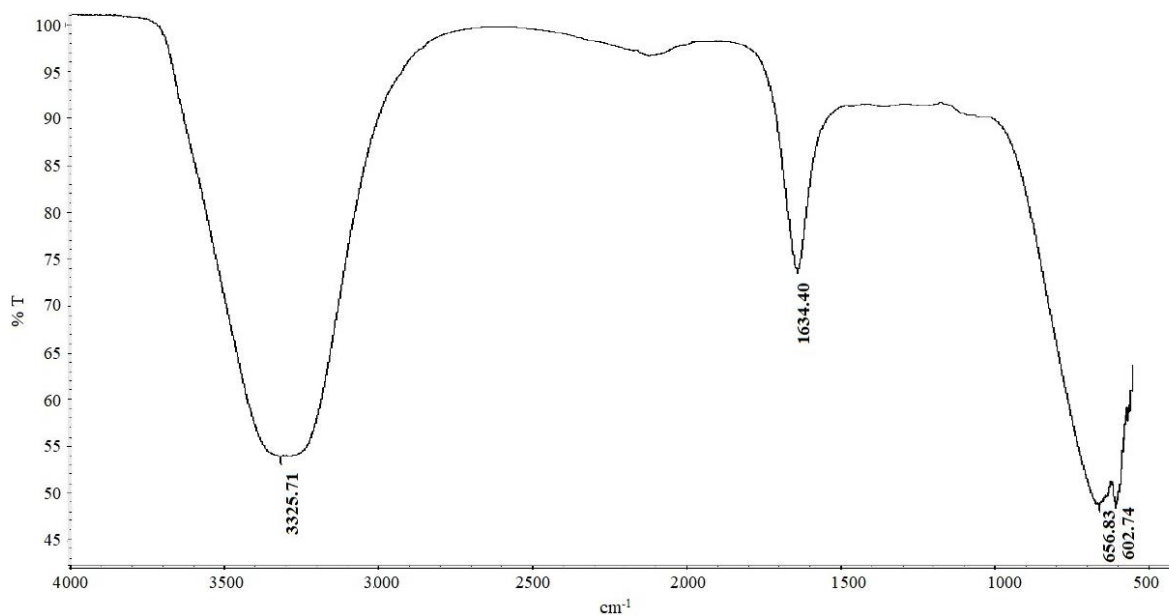
2.5.2 Infrared Spectroscopy

The spectra were measured in a Thermo$^{®}$ IR-NICOLET iS10 spectrophotometer with iTR (ATR) accessory, using the KBr pellet analysis method. In this case, both the infrared spectra for the aqueous extract and the synthesized magnetite nanoparticle were taken by this technique.

2.5.3 BET analysis

To determine the estimated surface area of the magnetite nanoparticle, a BET analysis was performed at the Universidad del Valle in a Nova 1000e, Quantachrome Instruments, using nitrogen (N_2) grade 5.0 as adsorbate, a run range between 0.05 to 0.3 P/P0 for an acquisition of 11 points, pressure tolerance of 0.100 mm Hg, equilibrium time of 60 seconds, tolerance time of 240 seconds and a liquid N_2 temperature of 77 K.

Figure 2. Infrared spectrum of *Eucalyptus grandis* extract.

Source: Authors.

3. Results and discussion

The aqueous extract of *Eucalyptus grandis* foliage obtained presented a slightly brown coloration which was analyzed by infrared spectrometry (**Figure 2**), finding a band at 3,319.61 cm^{-1} which is indicative of the hydroxyl groups (-OH) of the polyphenols found in the extract, a band at 1,634.74 cm^{-1} is also observed which represents the carbonyl group (-CO), which together with the hydroxyl group, expresses the presence of carboxylic groups (-COOH) within the extract of *Eucalyptus grandis*.

Table 2 shows the results obtained from the analysis of variance (ANOVA), performed with the help of the STATGRAPHICS CENTURION XVII.II software. The statistical significance of

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each effect was determined by comparing its mean square with the estimated values of the experimental error; in this way, the values of the significant parameters in the yield process of nanoparticles obtained with *Eucalyptus grandis* extract are obtained. For the synthesis yield, a confidence level of 95% was established (maximum permissible error 5%) and, therefore, those effects or parameters with an error (*P* value) of less than 0.05 are accepted as significant.

Table 2. Results of the analysis of variance for yield

Source	Medium Square	F-Ratio	*P*-Value
A: NaOH conc.	589.627	6.35	0.0256
B: Temperature	139.913	1.51	0.2412
C: Vln extract	10,895.2	117.41	0.0000
AB	91.7335	0.99	0.3382
AC	7.66361	0.08	0.7784
BC	338.65	3.65	0.0784
Total error	92.7934		

Source: Authors.

The ANOVA Table partitions the variability of the percentage of nanoparticle synthesis into separate pieces for each of the effects. Testing the statistical significance of each effect by comparing its mean square with an estimate of the experimental error. The *P* value of the respective experiments was compared with a significance level of $\alpha = 0.05$ and it is observed that factors A (NaOH concentration) and C (Extract volume) reject the null hypothesis because it presents a *P* value less than 0.05, which indicates that they are significantly different from zero with a confidence level of 95%, that is to say that effects A and C do have an influence on the synthesis process of nanoparticles. Factor B (Temperature) presents a *P* value greater than 0.05, therefore the null hypothesis is accepted and it is concluded that it has no effect on the percentage of synthesis, in the same way it is established that the correlation coefficient obtained for this experiment reached 91.67% adjusted for the synthesis yield.

Analyzing the Pareto diagram (**Figure 3**), which presents each of the studied effects in decreasing importance, the length of each bar is proportional to the standardized effect, which refers to the estimated effect divided by its standard error. The vertical line presented is used to judge which effects are statistically significant, so that any bar extending beyond this vertical line corresponds to effects that are statistically significant at a confidence level of 95%[16].
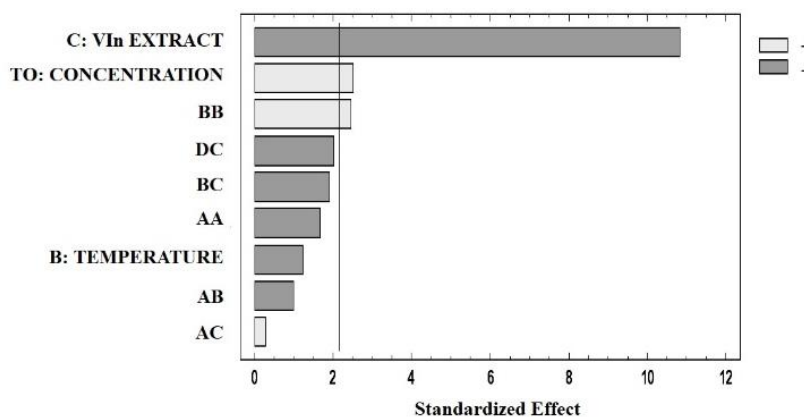

Figure 3. Pareto diagram for nanoparticle synthesis performance.

Source: Authors.

Therefore, in this case the factors that exceed the significance line are A and C, namely, the concentration of NaOH and the volume of extract, respectively, so they are the effects that significantly influence the synthesis process of the nanoparticle, and results that can be corroborated with the analysis of variance. According to the sign presented, the effects can be both positive and negative, therefore, in the case of factor A, it is indicated that there will be a higher percentage of synthesis at higher concentrations of NaOH, while on the other side in the case of factor C, there will be a lower percentage of synthesis with higher volumes of eucalyptus extract. The ANOVA made it possible to observe

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the effects of the factors studied, as shown in **Figure 4**, which presents the main effects for the yield; this analysis makes it possible to establish the optimum points for the synthesis of the nanoparticles.

$$Rendimiento = 183,295 + 109,183A - 6,1855B + 13,5384C - 17,4276A^2 - 0,67725AB + 0,783AC + 0,058457B^2 - 0,26025BC - 0,779835C^2$$
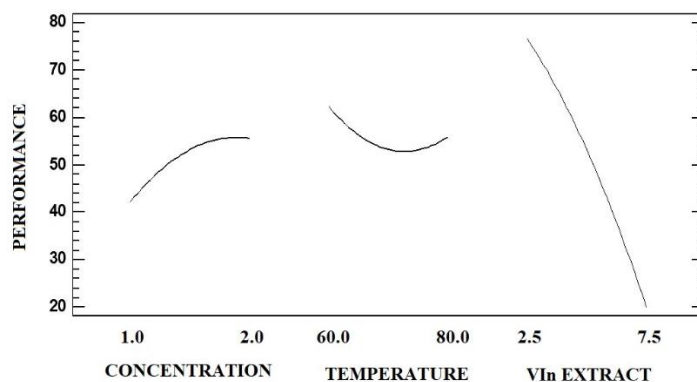
(4)

Figure 4. Main effects plot for yield.

Source: Authors.

It is observed then that as previously mentioned in the case of factor A (NaOH concentration) and C (Volume of the extract), the higher the NaOH concentration the higher the yield because the -OH group of the base interacts with the nucleus of the particle in formation generating iron hydroxides which by dehydration allow the formation of the iron oxide crystals of interest (Fe_3O_4), while as the volume of the extract used increases, the yield decreases to a great extent, because a greater number of organic molecules will come into contact with the iron ions, increasing the impediment of the base to interact with the nucleus of the particle in formation[5]. On the other hand, in the case of temperature the values start high, drop to a minimum point and at the end rise, but to a value that is lower. This trend presumably is due to the fact that after a certain temperature, the compounds in the eucalyptus extract degrade, reducing the proportion of them present during the synthesis, however, the yield of the particle synthesis remains above 50% in this case.

Based on these results, Equation 4 is generated which models the performance behavior of the synthesis based on the three parameters previously stipulated.

The function led to an estimated response surface plot[16], which is shown in **Figure 5**. It is observed that with a NaOH concentration of 1.7 M, with temperatures of 87 ℃, with an extract volume close to 1 mL, a % yield of approximately 96% is obtained. At the end of the experimental design and having the values for the optimization of particle synthesis, tests were performed in triplicate of those points, obtaining on average a yield of 98.99% ± 0.21, results that resemble those expressed by Alvear *et al.*, who reported yields in the synthesis of magnetite nanoparticles of 94% and exceed the results expressed by Robles *et al.*, whose yields in the synthesis reached a maximum of 35.03%[14,17].

For the determination of the nanoparticle size, transmission electron microscopy (TEM) was employed and using Image-Pro Plus 3.0 image analysis software with which it is obtained that the average size of the nanoparticles is approximately 8.97 nm, which is an average size close to that exhibited by Alvear *et al.* of 7 nm and that of Awwad and Salem of 8 nm[5,17]. **Figure 6** shows the micrograph obtained for the synthesized particle in the nanometer range.

To determine the functional groups present in the synthesized magnetite nanoparticle, an analysis was performed by infrared spectroscopy (IR) technique, this technique can be applied for the identification of surface functional groups, which is important to have a general idea of the chemical structure of the possible biomolecules that are responsible for the coating and stability of the nanoparticle[5]. In this case, several functional groups are found on the particle, in **Figure 6**, we see the IR
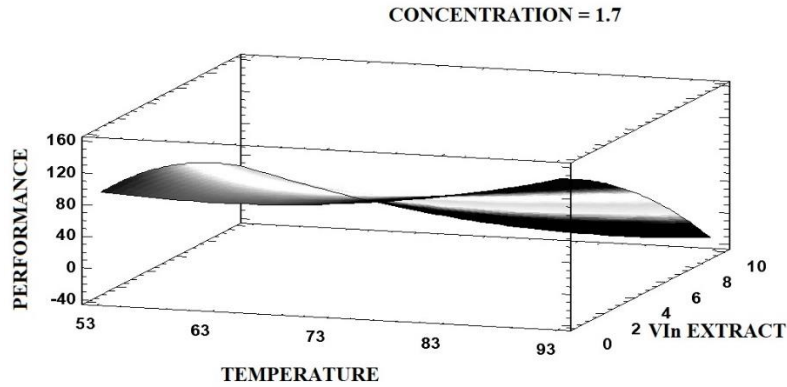
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Figure 5. Estimated response surface plot of nanoparticle synthesis.
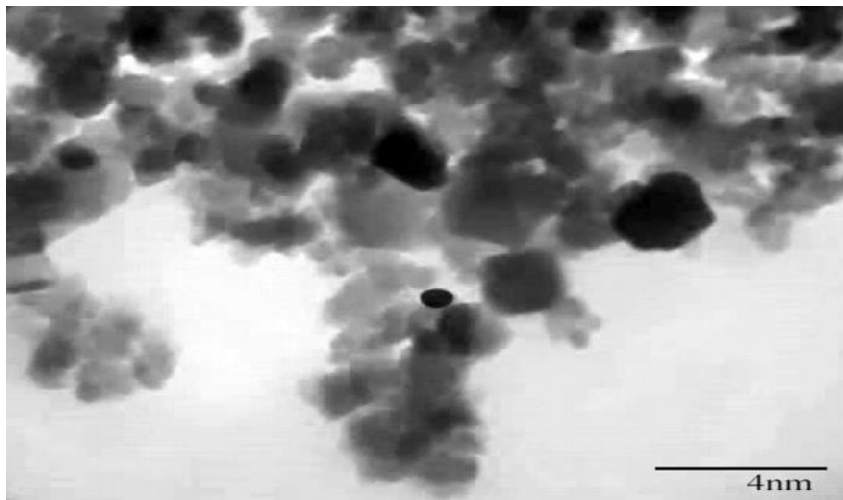
Source: Authors.

Figure 6. Transmission electron microscopy of nanoparticles.

Source: Authors.

spectrum of the synthesized nanoparticle which shows that around 3,440.77 cm^{-1} a band of stretching of the hydroxyl group (-OH) is found, although in this case the band is quite attenuated, likewise a band can be seen at 1,559.26 cm^{-1}, which refers to the stretching of the carbonyl group bond (-CO). The presence of magnetite nanoparticles can be seen by the appearance of a band at 445.31 cm^{-1}, which corresponds to the stretching band of the Fe-O bond of the magnetite core[5].

With these results it can be established that the carboxyl (-COO^{-}) has interacted with the core by binding on the surface of the magnetite nanoparticles. From this we can say that the polyphenols of the eucalyptus extract have interacted with the particle acting as a reducing and stabilizing agent for the magnetite nanoparticles.

A comparison is also made between the spectra of the eucalyptus (*Eucalyptus grandis*) leaf extract

(**Figure 2**) and the synthesized magnetite (**Figure 7**), in order to compare the bands between both spectra finding that the band of the hydroxyl groups remains around 3,440.77 cm^{-1}, although in the case of the magnetite spectrum this band, as already said, is greatly attenuated, a fact that is also presented in the studies carried out by Awwad and Salem for the synthesis of magnetic particles[5]. In addition it can be observed how the band belonging to the carboxyl is also presented in both spectra although slightly displaced, a fact that could be attributed to the same magnetic properties of the nanoparticle that generate this fact. The BET analysis provided a surface area of 131.9 m^2/g for the synthesized magnetite nanoparticles, values that are verified by the studies performed by Stan *et al.* where surface area values of 137.4 m^2/g are obtained in their experiments of nanoparticle synthesis with aqueous plant extracts[4].

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4. Conclusions

It was possible to synthesize the magnetite nanoparticle with the aqueous extract of *Euclayptus grandis* foliage with a synthesis percentage of 98.99% and with the experimental design the optimal conditions for the synthesis reaction were established (NaOH solution 1.7 M, extract volume 0.8 mL, temperature 87 ℃), improving the characteristics of the nanoparticles.
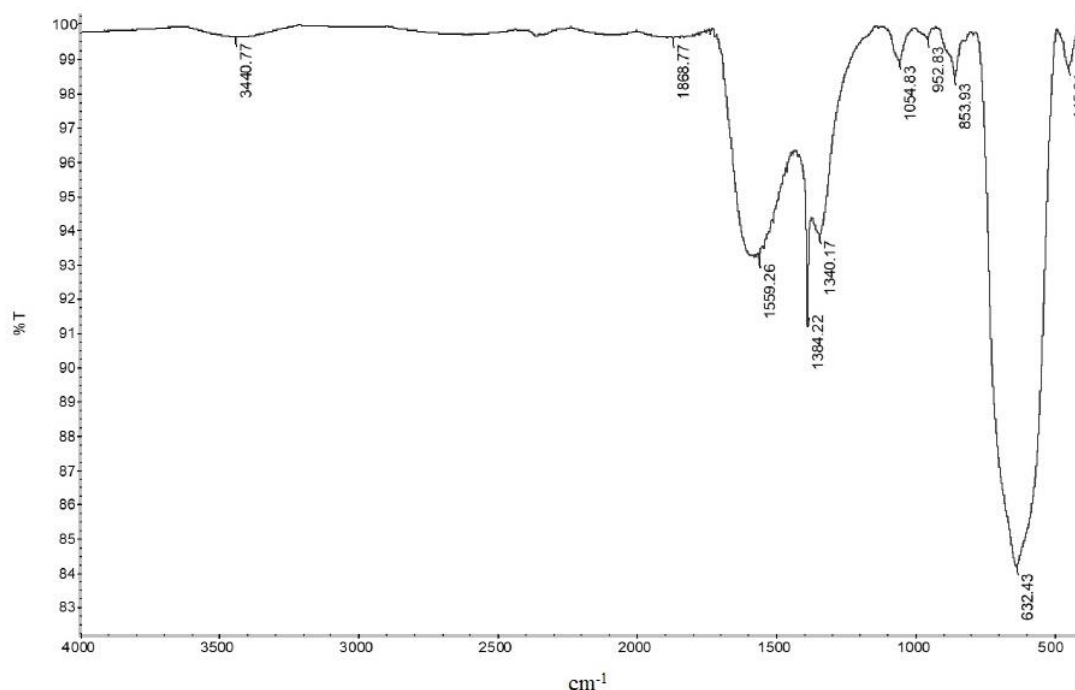

Figure 7. IR spectra of the synthesized magnetite nanoparticles.

Source: Authors.

From the results obtained, it is concluded that the iron oxide nanoparticle prepared from aqueous extract of *Eucalyptus grandis* foliage, due to its chemical stability, as well as the nature of the biomass and its low cost, in addition to its large estimated surface area, is presented as an alternative for obtaining this type of materials taking into account the guidelines of green chemistry.

Conflict of interest

The authors declare that they have no conflict of interest.

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