The pH role about synthesis, distribution and potential applications of gold nanoparticles

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Abstract: The synthesis of metallic nanoaprticles (NPs) is currently performed through the development of methodologies using microorganisms, plant extracts, and even organic waste, seeking to mitigate the impact on the environment. However, for both synthesis and biosynthesis it is necessary to consider physical and chemical factors such as temperature, stirring and pH, that allow controlling size and shape in an accurate way, as size and shape play an important role in the optical, electronic and thermal properties and determine many features of the structure and activity of molecules and biomolecules. In this work, we carry out chemical synthesis and biosynthesis of AuNPs at acidic (3-6), neutral (7) and basic (9) pH; the process is fast, simple and eco-friendly. We obtained AuNPs from 5-100 nm and determined that the pH of the solution is a key factor in the distribution and shape of the nanoparticles. Uniform AuNPs were obtained at neutral pH, or values of pH near neutrality. In addition, they have potential applications in various fields, due to their optical, magnetic, catalytic and electrical properties.

Keywords: synthesis; biosynthesis; pH; AuNPs applications.

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José Abraham Balderas-López studied Physics at Instituto Politécnico Nacional (IPN-México). He receive his Master and Doctorate in Physics from CINVESTAV-IPN. He worked as a Research Fellow in the Photothermal and optoelechtronic Diagnostic Laboratories (CADIPT) in Toronto, Canada from 1999 to 2002, He is now Full Professor Research and Leader of the Photothermal Laboratory in UPIBI-IPN, He is member of Mexican Academy of Sciences, since 2004. His current research areas are in the field of photothermal phenomena and nanotechnology developments and their applications in the biomedical field.

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1 Introduction

Research and development of science and technology at the nanoscale is booming worldwide as it has applications in diverse areas such as health, pharmaceuticals, new materials, environment, among others. One of the most important activities is the development of new materials with a concrete structure and surface suitable for a particular purpose, including nanoparticles (NPs), also the metal NPs have physicochemical properties and exceptional biological activities, compared to other materials; this is due to the electrons on their surface which interact in a very complex way with the electromagnetic radiation and is directly related to the shape, size and chemical nature of NPs as well as their surrounding environment (Duque et al., 2015).

However, is possible that nanomaterials obtained by biosynthesis will be the future alternative to biomedical, pharmaceutical, therapeutic and health care applications, due to their toxic chemical-free nature (Patil and Kim, 2016).

The synthesis of gold nanoparticles (AuNPs) is commonly performed using the methodology proposed by Turkevich et al. (1951). In this, the reduction of gold ions is performed by addition of sodium citrate, nevertheless there are processes that involve high temperatures and pressure, hazardous materials that are very harmful to health and the environment, therefore NPs of metals have been synthesised using microorganisms, plant extracts or organic waste (MubarakAli et al., 2013; Chavez-Sandoval et al., 2016; Arya et al., 2017; Ghosh et al., 2017) However, for both the synthesis and biosynthesis it is necessary to take into account several factors among which we emphasise temperature and pH. Li et al. (2011) synthesised AuNPs using sodium hydroxide (NaOH) as a reducing agent and obtain AuNPs concentrated and uniform, when used neutral pH

values. The pH determines many features of the structure and activity of molecules and biomolecules, also recent studies emphasise the fact that the pH of the solution is the crucial parameter which determines the distribution, size and shape of the nanoparticles, these parameters play important role for applications of AuNPs (Doyen et al., 2013; Chavez-Sandoval et al., 2015).

The rise in the use of NPs of noble metals in various fields, has resulted in the inorganic synthesis of metal NPs with different approaches: by ultraviolet radiation, technology spray, laser ablation, among others, but these methods are expensive and involve the use of high pressures or hazardous chemicals, which is why the development has recently increased of alternative methodologies sustainable and friendly to the environment, called biosynthesis or green synthesis. This is a simple, inexpensive way to obtain stable NPs of different size and shape (Kumar, 2008; Narayanan and Sakthivel, 2011; Philip, 2010; Chavez-Sandoval et al., 2016; Anand et al., 2017; Arya et al., 2017). Biomolecules present in plant extracts are involved in the reduction of metal ions to nanoparticles in a one step and eco-friendly synthesis process, in addition eliminate the need for a stabilising and capping agent; metabolites such as alkaloids, terpenoids, enzymes, carbohydrates and phenolic compounds, among others, are the possible reducing agents, nevertheless the specific compound from plant extract involved in the synthesis remains unclear (Patil and Kim, 2016). In this sense malic acid is one of the most abundant in nature, participates in the Krebs cycle to obtain ATP and provides the energy needed by organisms. Therefore, it is important to perform studies to analyse its participation as a reducing agent in biosynthesis.

In this paper, we carry out chemical AuNPs synthesis, using sodium citrate and biosynthesis using extracts of the following plants: nopal (*Opuntia sp.*); onion (*Allum sp.*); pear (*Pyrus sp.*); coffee (*Coffea sp.*); laurel (*Laurus sp.*), as a reducing agent at acidic, neutral and basic pH values. No toxic solvents or stabilising agents were added.

We obtained AuNPs 5 to 100 nm and determined that, in both syntheses, the pH of the solution is a key factor to the distribution and the shape of the nanoparticles, finally we obtained AuNPs that are concentrated and uniform when using neutral or close to neutrality pH values.

In relation to applications, AuNPs can be used in biomedicine such as gene therapy, anticancer activity, pharmaceuticals, sensors and biosensors, food packaging, among other applications (Bailey et al., 2004; Zhang and Noguez, 2008; Patil and Kim, 2016; Abbas et al., 2017; Anand et al., 2017).

2 Methods

2.1 Reagents and solutions

The trihydrate hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$, 99.99%) (520918), and dihydrate sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) were purchased from Sigma-Aldrich. The he formvar resin 15/95, copper grids 75 mesh (GilderGrids Cu) and mica muscovite V-1 quality were purchased from Electron Microscopy Sciences.

All reagents used were analytical grade and all solutions were prepared using double deionised water (Milli-Q, 18MW cm) from a Millipore purification system.

2.2 Materials and equipment

AFM images were obtained using a scanning probe microscope (SPM) Digital Instruments brand. The TEM images were taken using a HRTEM Jeol 2100F microscope, from Central Laboratory of Electronic Microscopy at UAM-Iztapalapa. Temperature measurements were performed with an infrared digital thermometer, Traceable brand.

UV-Vis, spectroscopic measurements, were conducted using a Perkin Elmer Lambda 35, spectrophotometer. The pH of the solutions and buffers was adjusted using a pH meter Mettler Toledo MPC 227 brand.

2.3 AuNPs chemical synthesis

This was performed using Turkevich et al.'s (1951) technique with some modifications. Glassware was washed used regia water (aqua regia) to remove any traces of metals that could interfere during the synthesis. In a 250 mL Erlenmeyer flask, 500 mL of hydrogen tetrachloroaurate were placed 1% (25 mM) in 50 mL of doubly deionised water and was placed on a hot plate with stirring to boiling at 83°C. Subsequently they were added 1.25 mL of sodium citrate 1%, which acted as reductant and stabiliser agent; once sodium citrate was added, the stirring and heating were maintained for no more than 15 minutes. Finally, the flask was allowed to cool to room temperature while stirring and stored in a sterile flask at 4°C for further characterisation, it should be noted the AuNPs were obtained with different pH and all tests were performed at the same temperature and the same reaction time (Table 1).

Table 1 pH of sodium citrate used as reducing agent in chemical synthesis of AuNPs

Chemical reagent		*pH*		*Temperature*	*Reaction time*
Sodium citrate	3	7	9	83°C	15 min.

2.4 AuNPs biological synthesis

This proceeded as described in Chavez-Sandoval et al. (2016) from plant extracts, nopal (*Opuntia sp.*); onion (*Allum sp.*); pear (*Pyrus sp.*); coffee (*Coffea sp.*); laurel (*Laurus sp.*). These plants were chosen because of their antioxidant content, and those are, in general, common foods. The AuNPs were obtained with plant extracts at different pH (acid, neutral and basic); all tests were performed at the same temperature and the same reaction time the same day, to avoid confounding variables (Table 2). The AuNPs were characterised by UV-Vis, transmission electron microscopy (TEM) and atomic force microscopy (AFM).

Table 2 pH of the extracts of different plants used as reducing agent for the biosynthesis of AuNPs, temperature and reaction time is also observed

Plant extracts		*pH*		*Temperature*	*Reaction time*
Opuntia sp. (nopal)	4.8	7	9	83°C	15 min.
Allum sp. (onion)	5.3	7	9	83°C	15 min.
Pyrus sp. (pear)	4.7	7	9	83°C	15 min.
Coffea sp. (coffee)	5.0	7	9	83°C	15 min.
Laurus sp. (laurel)	6.5	7	9	83°C	15 min.

2.5 Statistical analysis

The results were analysed using statistical methods with Statistica software (version 10), and a multivariate analysis of variance (ANOVA) was carried out to determine the relationship between and within samples.

3 Results and discussion

The use of different plant extracts allowed the reduction of gold ions in an aqueous matrix (Shankar et al., 2004). The UV-Vis spectra recorded for the obtained AuNPs, represent the absorbance levels in each treatment and the dispersion of the biosynthesised nanoparticles, which indicates the degree of resonance on the surface of the plasmons found in the matrix of the AuNPs, whose quantum nature is a direct consequence of their size (Dubey et al., 2010; Corzo, 2012).

3.1 Chemical synthesis

AuNPs were obtained synthesised by the method of Turkevich et al. (1951), with some modifications and at different pH (3, 7, and 9). This method is simple, cheap and relatively fast. Table 3 shows the comparison of the wavelength (λ nm), the size (Ø nm) and shape of different pH synthesised AuNPs.

Table 3 Comparison of the wavelength, size and shape of different pH synthesised AuNPs

Chemical reagent	*pH*	*λ (nm)*	*Ø (nm)*	*Morphology*
Sodium citrate	3	564	5 and 20	Heterogenous, aggregated
Sodium citrate	7	520	20	Homogenous, dispersed
Sodium citrate	9	525	20	Heterogenous, aggregated

Figure 1 shows the UV-Vis spectra of AuNPs obtained, lambda maximum was 564 nm at pH 3, 520 nm at pH 7, as previously reported; and 525 nm at pH 9. The lambda maximum is similar in the AuNPs obtained at pH 7 and 9 (520 and 525), which are homogeneous NPs and is displaced up to 564 nm at pH 3, corresponding to heterogeneous NPs. This also indicates that different shapes and sizes were obtained by changing the pH of the reducing agent (sodium citrate).

Figures 2(a)–2(f) show the characterisations obtained by TEM and AFM. It is noted that the AuNPs at pH 3 are aggregated and heterogeneous morphology (A and D), while AuNPs at pH 7, are homogeneous and dispersed [Figures 2(b) and 2(e)]. AuNPs at pH 3 and 9 observed aggregated.

Figure 1 UV-Vis spectra of AuNPs obtained by chemical synthesis at different pH (see online version for colours)

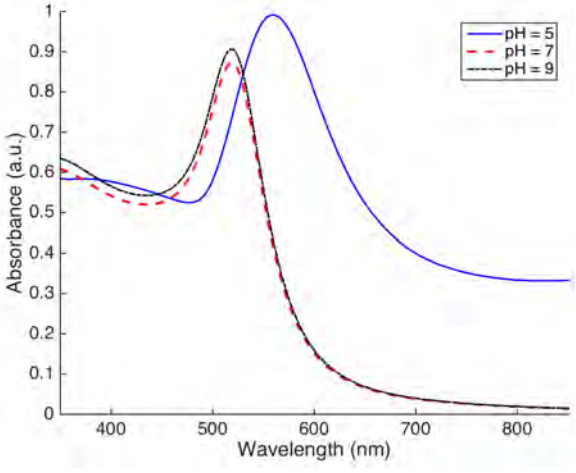
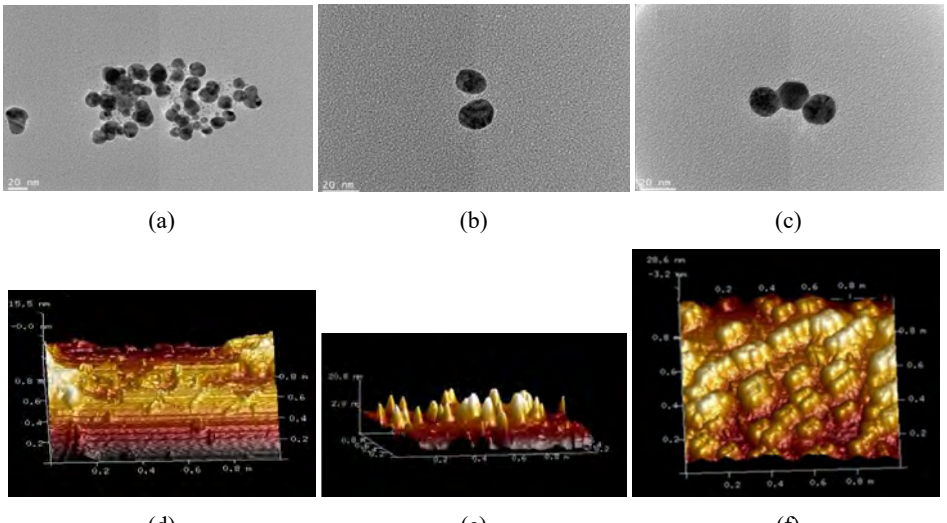


Figure 2 TEM images of AuNPs synthesised with sodium citrate: (a) pH 3 (b) pH 7 (c) pH 9 and AFM images of AuNPs synthesised with sodium citrate: (d) pH 3 (e) pH 7 (f) pH 9 (see online version for colours)



(a) (b) (c)

(d) (e) (f)

It is observed that the nanoparticles obtained at pH 3 are of varied sizes and of different shape, besides they are aggregated. The nanoparticles obtained at pH 7 are homogeneous and dispersed. Finally, the nanoparticles obtained at pH 9 are observed homogeneous but aggregated.

3.2 Biosynthesis at different pH

With biosynthesis at different pHs were obtained AuNPs in all cases, the results are shown in Figures 3–6. Table 4 shows the comparison of the wavelength, the size and shape of different pH biosynthesised AuNPs. UV-Vis characterisation showed the maximum lambda is displaced to 520 nm in all cases, oscillating between 533–560 nm for nanoparticles of nopal, pear, coffee, onion and laurel, confirming that different shapes and sizes were obtained by changing the pH of the reducing agent (plant extract).

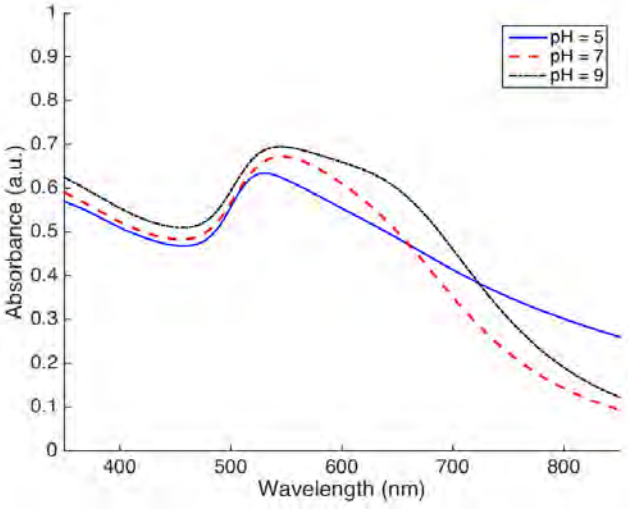
Table 4 Comparison of the wavelength, the size and shape of different pH

Plant	*pH*	*λ (nm)*	*Ø (nm)*	*Morphology and distribution*
Nopal	4.8	549	10 and 20	Heterogeneous and aggregated
Nopal	7	555	10 and 20	Heterogeneous and aggregated
Nopal	9	560	20	Heterogeneous
Onion	5.3	540	10 and 20	Aggregated
Onion	7	545	10 and 20	Heterogeneous
Onion	9	543	5 and 10	Aggregated
Coffee	5.0	539	5, 10, 20, 30	Heterogeneous
Coffee	7	532	100	Aggregated
Coffee	9	530	10 and 20	Heterogeneous
Laurel	6.5	535	20	Dispersed
Laurel	7	535	20	Heterogeneous
Laurel	9	537	20 and y 30	Aggregated
Pear	4.7	537	20	Heterogeneous
Pear	7	533	20 and 30	Aggregated
Pear	9	536	20 and 30	Heterogeneous and aggregated

3.3 Biosynthesis from Opuntia sp. (nopal) extract

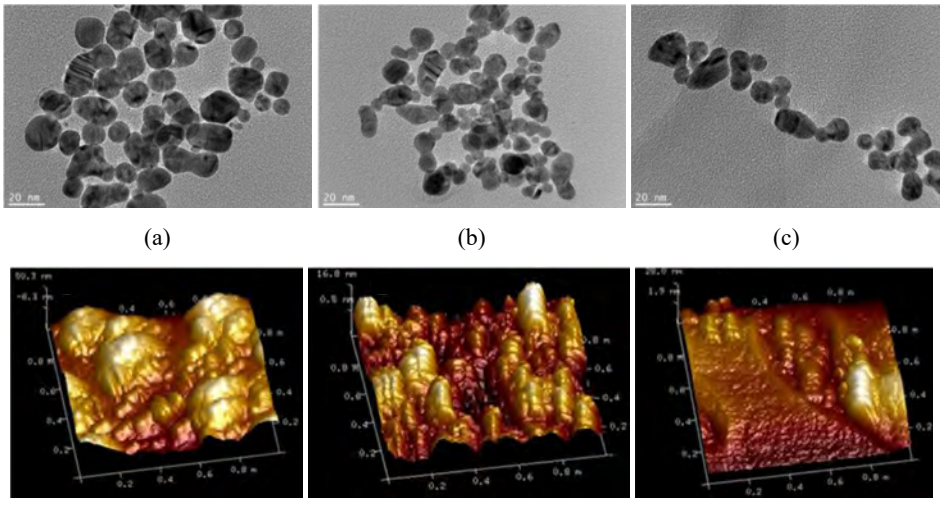
UV-Vis characterisation of the AuNPs obtained by biosynthesis from nopal extract, shows a lambda maximum of 549 nm at pH 4.8, 555 nm at pH 7 and 549 at pH 9 (Figure 3).

Figure 3 UV-Vis spectra of AuNPs obtained by biosynthesis nopal extract at different pH (see online version for colours)



The TEM and AFM images of the AuNPs biosynthesis from nopal extract [Figures 4(a)–4(f)] show different forms and sizes (10–20 nm), mostly at pH 4.8 [Figures 4(a) and 4(d)], in Figure 4(d) the scale reaches 50 nm; perhaps, this is because AuNPs are very aggregated; pH 7 [Figures 4(b) and 4(e)]; at pH 9 [Figures 4(c) and 4(f)], the sizes are similar. At all pHs AuNPs are aggregated.

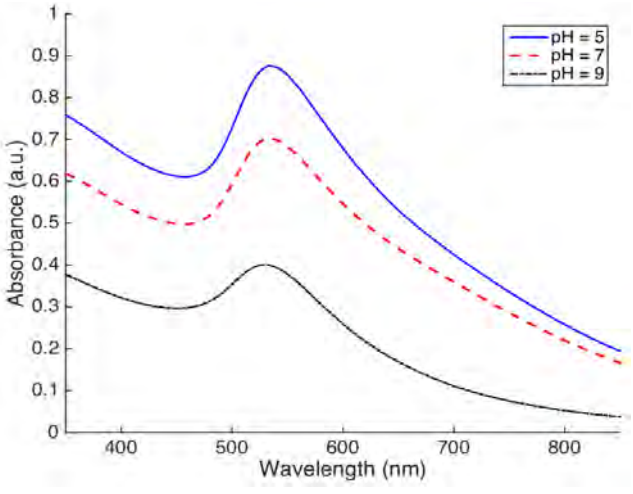
Figure 4 TEM images of synthesised AuNPs extract nopal: (a) pH 4.8 (b) pH 7 (c) pH 9 and AFM images of this AuNPs: (d) pH 4.8 (e) pH 7 (f) pH 9 (see online version for colours)



3.4 Biosynthesis from Allum sp. (onion) extract

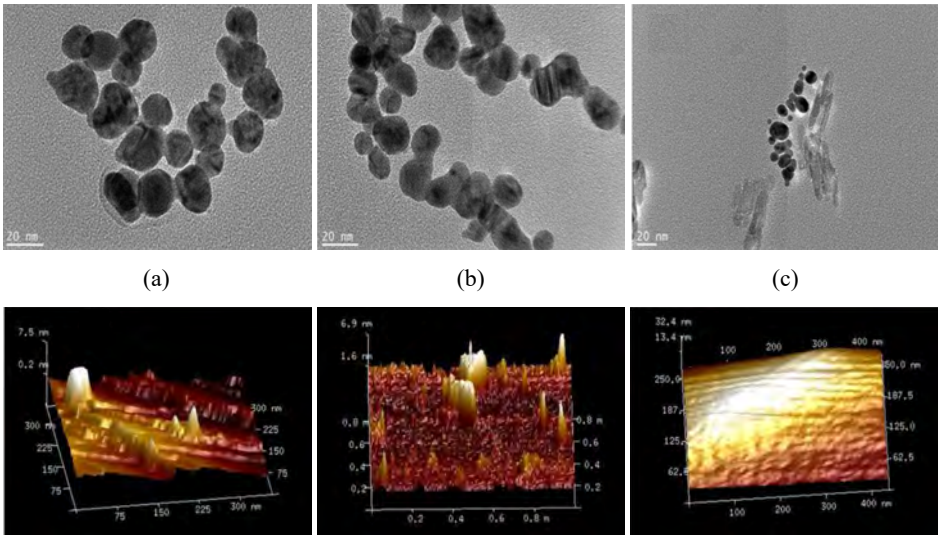
UV-Vis characterisation of the AuNPs obtained by biosynthesis from onion extract, shows differences in absorbance, indicating that AuNPs differ in concentration and size. The lambda maximum was 540 nm at pH 5, 545 nm at pH 7 and 543 at pH 9 (Figure 5).

Figure 5 UV-Vis spectra, biosynthesis onion extract at different pH (see online version for colours)



Figures 6(a)–6(f) show the TEM and AFM characterisation. In all cases the AuNPs are aggregated and have heterogeneous morphology.

Figure 6 TEM images of synthesised AuNPs onion extract: (a) pH 4.8 (b) pH 7 (c) pH 9 and AFM images of this AuNPs: (d) pH 4.8 (e) pH 7 (f) pH 9 (see online version for colours)



3.5 Biosynthesis from Coffea sp. (coffee) extract

Characterisation by UV-Vis the AuNPs obtained by biosynthesis from coffee extract (Figure 7) shows a lambda maximum of 539 nm at pH 5, 532 nm at pH 7 and 530 nm at pH 9.

Figure 7 UV-Vis spectra, biosynthesis coffee extract at different pH (see online version for colours)

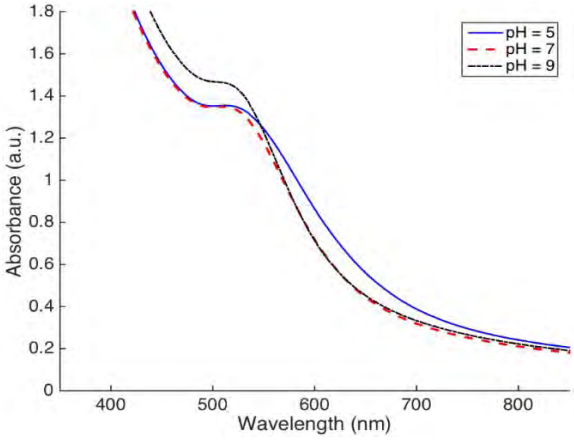
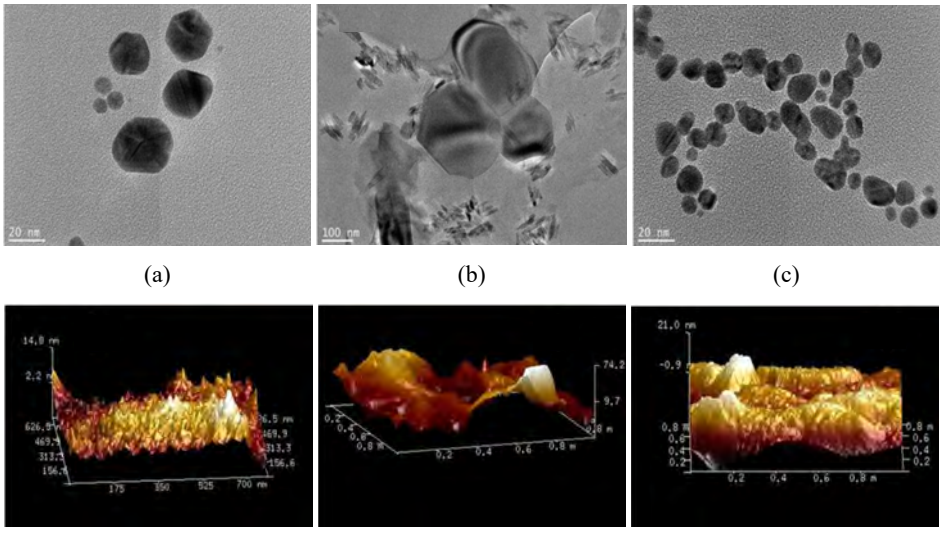


Figure 8 TEM images of synthesised AuNPs coffee extract: (a) pH 4.8 (b) pH 7 (c) pH 9 and AFM images of this AuNPs: (d) pH 4.8 (e) pH 7 (f) pH 9 (see online version for colours)



(a) (b) (c)

(d) (e) (f)

With the TEM and AFM characterisation [Figures 8(a)–8(f)] observed NPs of different sizes (5–30 nm) at pH 7; it is interesting that at pH 7 we obtained very large NPs of more than 100 nm; but at pH 9 there are NPs of 10–20 nm. In all cases the NPs are

heterogeneous; finally, at pH 5 AuNPs are dispersed, and at pH 7 and 9 they are aggregated.

The biosynthesis from coffee extract is peculiar because in the other syntheses made in this work at pH 7 NPs obtained do not exceed 30 nm.

3.6 Biosynthesis from Laurus sp. (laurel) extract

As for NPs synthesised with laurel extract the UV-Vis spectrograph shows a maximum wavelength of 535 at a pH of 6.5 and 7, and 537 to pH 9 (Figure 9).

Figure 9 UV-Vis spectra, biosynthesis laurel extract at different pH (see online version for colours)

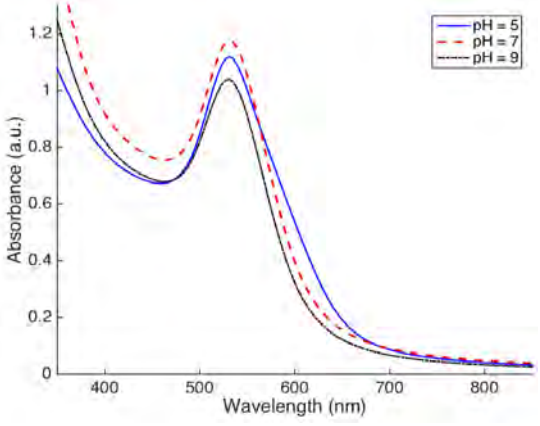
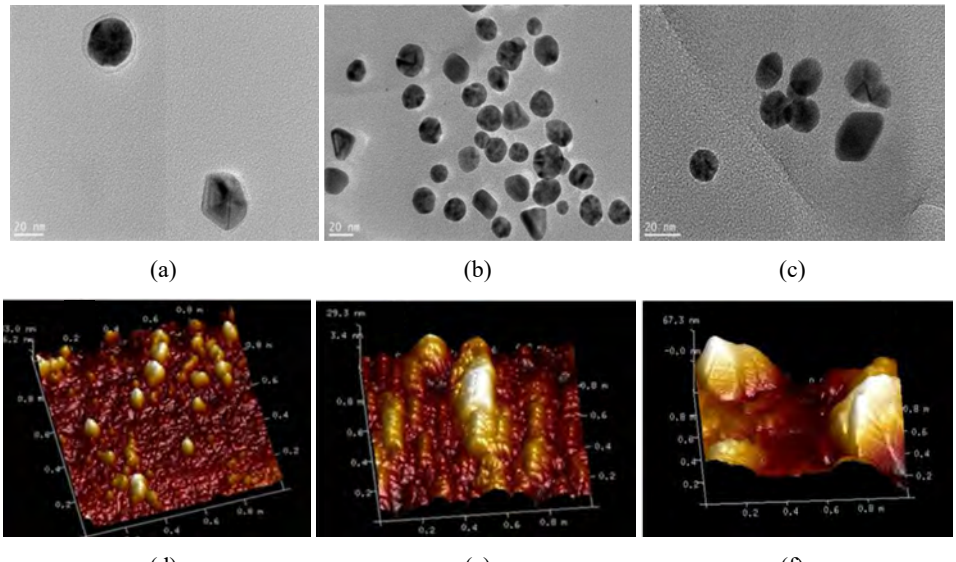


Figure 10 TEM images of synthesised AuNPs laurel extract: (a) pH 4.8 (b) pH 7 (c) pH 9 and AFM images of this AuNPs: (d) pH 4.8 (e) pH 7 (f) pH 9 (see online version for colours)



(a) (b) (c)

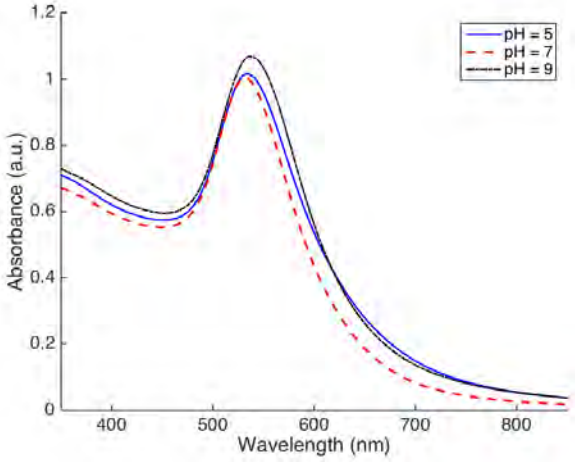
(d) (e) (f)

Characterisation of laurel extract AuNPs, by TEM and AFM [Figures 10(a)–10(f)] shows NPs of different shape, dispersed at pH 6.5 [Figures 10(a) and 10(d)], more concentrated at pH 7 [Figures 10(b) and 10(e)] and aggregated at pH 7 [Figures 10(b) and 10(e)] and 9 [Figures 10(c) and 10(f)]. They are of uniform size at pH 6.5 and pH 7 (20 nm), but larger at pH 9.

3.7 Biosynthesis from Pyrus sp. (pear) extract

The UV-Vis characterisation for AuNPs obtained from pear extract shows wavelengths of 537 for pH 5, 533 for pH 7 and 536 for pH 9, indicating that they are homogeneous in size and concentration (Figure 11).

Figure 11 UV-Vis spectra, biosynthesis from pear extract at different pH (see online version for colours)



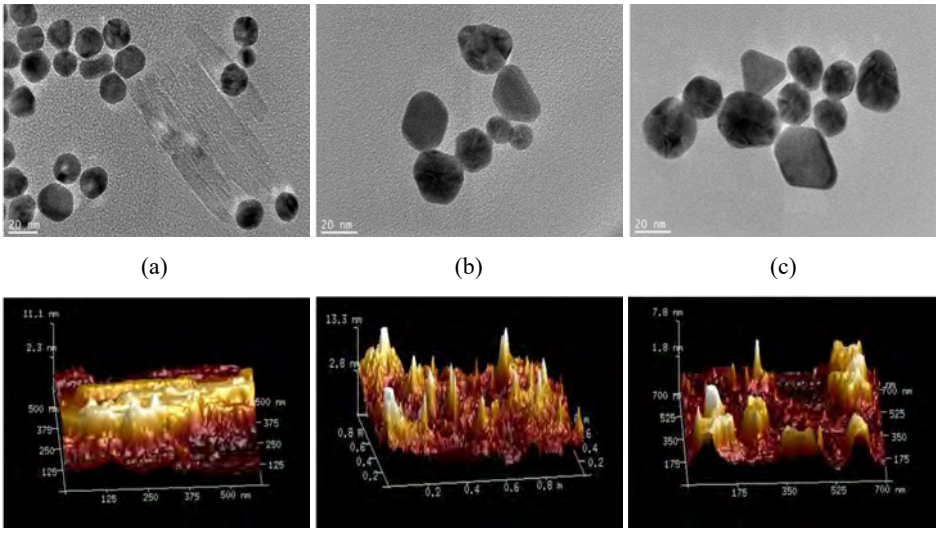
The characterisation by TEM and AFM for the AuNPs obtained from pear extract [Figures 12(a)–12(f)] shows they are aggregated and of different forms, but of uniform size. At pH 5 [Figures 12(a) and 12(d)] they are about 20 nm; at pH 7 [Figures 12(b) and 12(e)] and pH 9 [Figures 12(c) and 12(f)] of 10–40 nm approximately.

The colouring of the AuNPs is found in shades of blue, brown and reddish, in a colloidal solution the nanoparticles take a particular colour depending on the excitation of the surface of the plasmon, obtaining a determined wavelength within the region of the visible electromagnetic spectrum. This characteristic spectrum of the colloidal solution of the biosynthesised AuNPs for each extract provides information about its size and uniformity (Mulvaney, 1996; Shankar et al., 2004; Cruz et al., 2012; Duque et al., 2015).

The multivariate analysis (ANOVA) showed that there are significant differences in relation to the absorbances of each treatment ($p \leq 0.05$), which indicates that the sizes of nanoparticles are directly related to the type of extract and the pH (acid, neutral or basic) at which each synthesis was carry out (Figures 13–15).

For the chemical synthesis treatment (sodium citrate) existing significant differences between the treatments at pH 7 and pH 9 with respect to the treatment at pH 3 ($p = 0.00002174$), for the treatment with pH 7 the absorbance was 0.537 and for the treatment with pH 9 the absorbance was 0.574 (Figure 13).

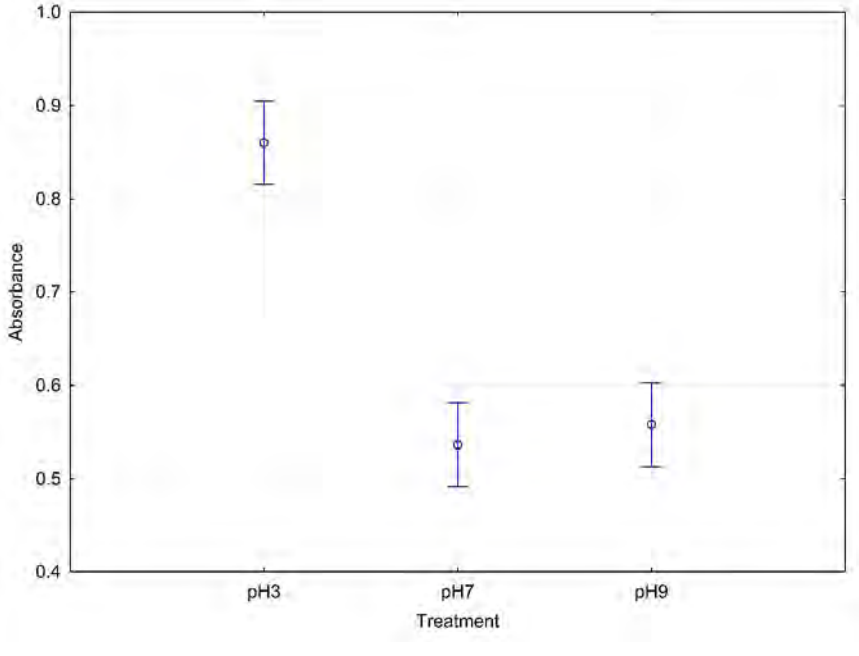
Figure 12 TEM images of synthesised AuNPs pear extract: (a) pH 4.8 (b) pH 7 (c) pH 9 and AFM images of this AuNPs: (d) pH 4.8 (e) pH 7 (f) pH 9 (see online version for colours)



(a) (b) (c)

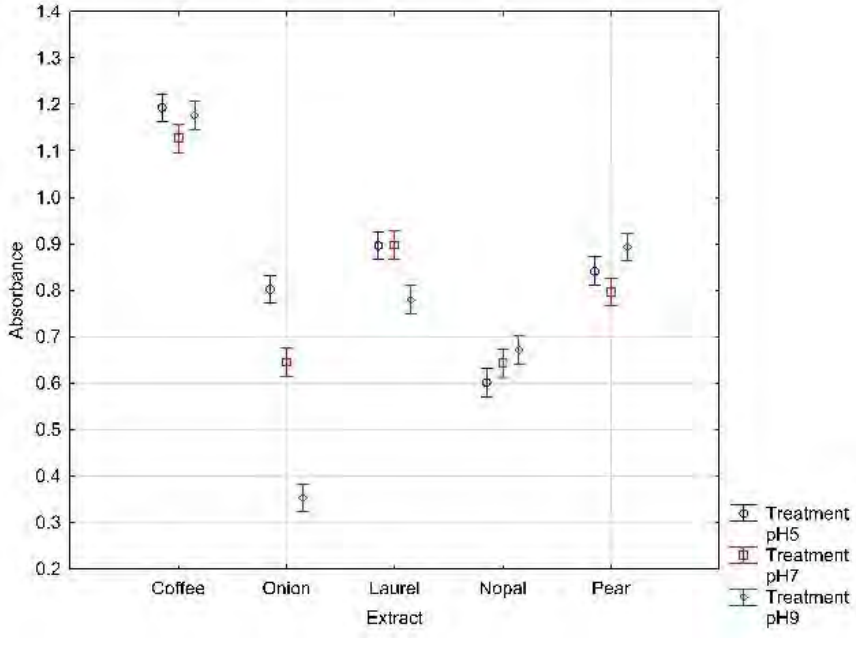
(d) (e) (f)

Figure 13 ANOVA for the treatments (pH) used to obtain AuNPs by chemical synthesis (see online version for colours)



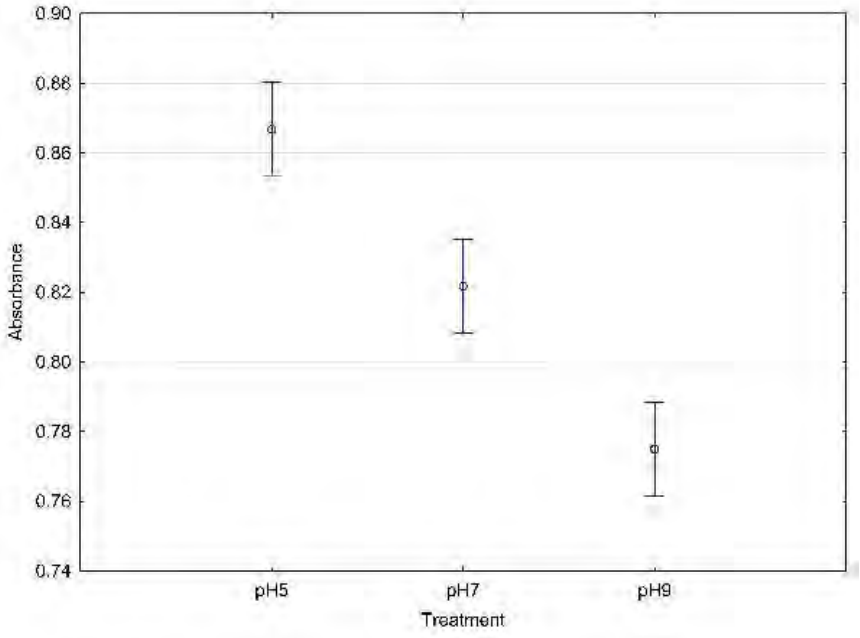
ANOVA of each extract shows that the AuNPs obtained from coffee extract did not show significant differences ($p > 0.05$) however, the highest absorbance ranges were recorded, reaching average values of 1.176. The AuNPs synthesised from onion extract showed the highest variability among the three pH treatments, registering significant differences ($p = 0.000035$), treatment with a pH of 5 presented the highest absorbance (Abs = 0.876), followed by treatment with pH of 7 (Abs = 0.703), and the treatment at pH 9 (Abs = 0.401). While the AuNPs synthesised with laurel extract, there was a lower absorption of pH 9 (Abs = 0.787). The AuNPs obtained from nopal extract did not show significant differences between the pH used ($p > 0.05$), with absorbances of 0.645 being observed on average. Finally, for the AuNPs obtained from the pear extract, significant differences were obtained between the treatment at pH 7 and pH 9 ($p = 0.0065$). The absorbances were recorded between 0.791 and 0.898 (Figure 14). These differences, according to Drude's model, indicate that changes in absorption are directly related to particle size and material properties (Mulvaney, 1996).

Figure 14 ANOVA between the experimental treatments, with different extracts from which AuNPs were obtained (see online version for colours)



Another general ANOVA was performed for the different treatments (acid, neutral and basic) and significant differences were observed ($p \leq 0.05$). The highest absorbances were recorded at pH 5, reaching, on average, absorbance values of 0.8638, for nanoparticles synthesised at pH 7, the absorbance was 0.8217, for treatment at pH 9 it was 0.775, being those with the lowest absorbance (Figure 15).

Figure 15 ANOVA between different treatments (pH) from which AuNPs biosynthesised were obtained



These differences may be because, during the synthesis with plant extracts, the reduction of metals is carried out by various biomolecules and biological components, such as primary or secondary metabolites, alkaloids, flavones, anthracenes, and various acids such as linoleic, oleic, palmic, gallic and ascorbic acids, among others, which also function as stabilisers due to the electrostatic interactions of the carboxylic, hydroxyl and carbonyl groups (Huang et al., 2007; Dubey et al., 2010; Corzo, 2012). In addition, Yoosaf et al. (2007) mention that during the biosynthesis of gold nanoparticles with organic compounds used as reducing agents, the organic molecules must have at least two carboxyl groups in the ortho position for the stabilisation of the AuNPs, which is through the interaction of carboxyl groups.

Several investigations have been carried out on gold biosynthesis using different plant extracts (fruit, stem, leaf, flowers, etc.), standing out as an alternative, simple, economical method that offers a valuable contribution towards the care of the environment, because organic compounds with low toxicity are used during biosynthesis (Shankar et al., 2004; Narayanan and Sakthivel, 2008; Smitha et al., 2009; Philip, 2010; Dubey et al., 2010; Thakkar et al., 2010).

4 Conclusions

The synthesis of nanomaterials requires diverse treatments both physical and chemical that allow to obtain specific forms and sizes besides suitable surfaces for different applications, as well as coating or anchoring procedures to present, introduce and even hide different qualities. In biosynthesis in addition, the large amount of metabolites

present makes it difficult to obtain homogeneous nanoparticles, thus it is important to conduct studies to determine that biomolecules act as reducing agents to obtain isotropic nanoparticles by biosynthesis.

In this work, we have successfully synthesised AuNPs at different treatments and we obtained different forms and sizes, depending on the pH used in the reducing agent (plant extracts); because of that, the pH is a key factor in the synthesis of AuNPs, however the nature of the extract also plays a significant role to control the structure and morphology.

We observed that when the nanoparticles are homogeneous in size and shape the wavelength in the UV-Vis spectrum is well defined, as in the NPs obtained with sodium citrate at pH 7, or the laurel extract NPs at pH 6.5; however, when the nanoparticles are of different shapes and varied sizes the wavelength is not well defined, as in the NPs obtained with nopal (*Opuntia sp.*) or coffee (*Coffea sp.*) extracts.

On another hand, due to their high stability, the AuNPs obtained can be functionalised with different reagents and might be suitable for various applications mainly in biomedicine as a gene therapy, phytonanotherapy, pharmaceuticals, biosensors, biological imaging, antimicrobial and antiproliferative activities, inclusive in environment analysis, among others.

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