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Synthesis of Gold Nanoparticles Using Amino Acids by Light Irradiation

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Abstract

The synthesis of nanoparticles is generally carried out by chemical reduction, which is effective but uses a number of toxic substances, making the process potentially harmful to the environment. Thus, as part of the search for environmentally friendly or green synthetic methods, this chapter aimed to present the synthesis of gold nanoparticles (AuNPs) using only HAuCl_4 , Milli-Q water, white light from a xenon lamp, and amino acids. A total of 21 amino acids were studied, and the shapes and sizes of the resultant nanoparticles were evaluated. The products were characterized by ultraviolet-visible (UV-Vis) and fluorescence spectroscopy, zeta potential measurements, and transmission electron microscopy. The synthesis of the AuNPs was successful with 18 amino acids, and the best results were obtained with aspartic acid, arginine, threonine, tryptophan, and valine. The nanoparticles were spherical and their sizes ranged from 5 to 100 nm. Changes in pH were required to improve the stability of the colloidal suspensions.

Keywords: amino acid, gold, light, nanoparticle, photo reduction

1. Introduction

Over the past three decades, a significant growth in research involving nanotechnology has occurred worldwide. With the increased attention in this field, new perspectives have emerged for solving the major challenges faced by modern society, such as the treatment of cancer [1] and acquired immune deficiency syndrome (AIDS). These developments can also help

overcome obstacles in conventional microtechnology through the design of protein molecules for the fabrication of devices according to complex atomic specifications [2, 3].

Recently, gold nanoparticles (AuNPs) have attracted significant attention due to their advantageous surface characteristics that allow easy functionalization with biologically active molecules. The composition of the nanoparticles may vary. Materials for nanoparticles surface modification may be of biological origin, such as plants [4], bacteria [5], or peptides [6]. Amino acids have been shown to be useful in the synthesis of AuNPs, as first reported in the early 2000s. Mandal et al. [7] described the synthesis of AuNPs by the reduction of chloroaurate ions using aspartic acid. In 2003, Selvakannan et al. [8] showed that capping AuNPs with lysine enabled the storage of the lysine-stabilized AuNPs as a stable powder that could be readily redispersed in water. In 2004, Selvakannan et al. [9] demonstrated the spontaneous reduction of aqueous chloroaurate ions using tryptophan. The Bhargava and Wangoo groups also reported the synthesis of AuNPs using amino acids [10, 11]. Recently, Maruyama et al. demonstrated that 20 amino acids can act as reducing and capping agents for AuNPs [12]. The AuNPs were produced from the incubation of AuCl_4^- solution with the amino acids at 80°C for 20 min. The authors showed that the reaction conditions strongly affected the sizes of the AuNPs and their aggregates. Using that method only arginine, cysteine, and threonine did not form gold colloidal solutions. Further, although methionine, phenylalanine and tryptophan produced colloids, the products were easily precipitated.

AuNPs synthesized in water and subsequently capped with amino acids can contribute immensely in various applications such as drug delivery and gene transfer. Recently, Dubey et al. strategically synthesized stable gold and silver nanoparticles that were surface-functionalized with either tyrosine or tryptophan residues and examined their potential to inhibit the amyloid aggregation of insulin [13]. This result offers significant opportunities for developing nanoparticle-based therapeutics against diseases related to protein aggregation.

Ramezani et al. [6] investigated the adsorption of amino acids on AuNPs via molecular dynamics simulations and offered the following observations: all amino acids containing hydroxyl groups in their side chains (tyrosine, threonine, and serine) were adsorbed on the surface through Au–OH interactions. Alanine, valine, isoleucine, and leucine, having linear side chains, were adsorbed on the AuNPs surface by their methyl groups, and glycine was adsorbed through its carboxyl group. Histidine, arginine, asparagine, glutamine, and lysine adsorption on the AuNPs surface were enabled by the amino groups in their side chains. The interaction of AuNPs with the negatively charged amino acids, aspartic acid, and glutamine occurred through the side-chain carboxyl groups. The aromatic ring of phenylalanine participated in the adsorption on the AuNPs surface. Cystine, cysteine, and methionine were adsorbed on the AuNPs through their sulfur atoms. Proline interacted through its amine (Au–N) and carboxylic groups (Au–O and Au•••H–O).

For the synthesis of nanoparticles, electromagnetic radiation has the advantage of being free of environmentally negative effects (such as the need for toxic solvents or aggressive reducing agents) and is thus a green alternative. The use of light in the synthesis of nanoparticles first emerged in 1999 when Zhou et al. [14] reported the synthesis of shape-controlled AuNPs using ultraviolet irradiation at room temperature. Dong et al. [15] used sunlight to synthesize AuNPs

in suspension. Tomita et al. obtained AgNPs using tryptophan and light and observed their lethal effects against bacteria [16].

This study was aimed at reporting a simple, fast, cheap, and environmentally benign method for the synthesis of spherical AuNPs using aqueous solutions of amino acids, Au^{3+} , and white light (xenon lamp). No additives such as organic solvents, surfactants, or specific reducing agents were used.

2. Amino acids

Amino acids are natural molecules characterized by a chiral carbon that makes bond with a carboxylic acid group, an amine group, a hydrogen atom, and a side chain that is specific to each amino acid. As a quaternary compound, amino acids are a combination (primarily) of carbon, oxygen, hydrogen, and nitrogen. The general amino acid structure is shown in **Figure 1** [17].

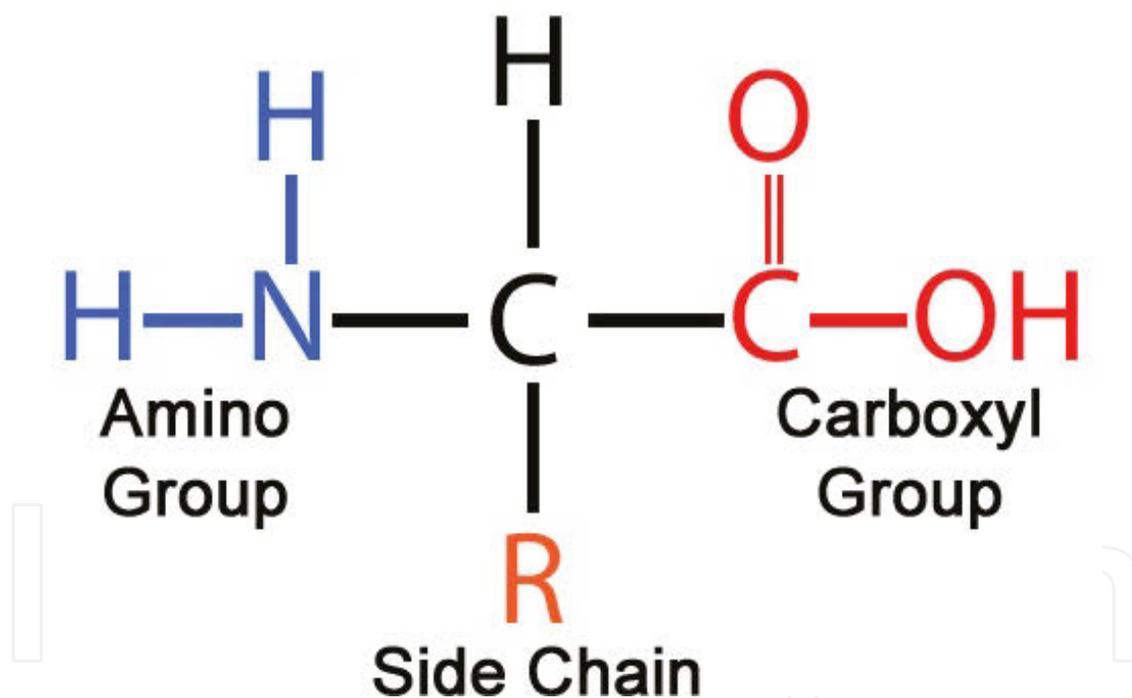


Figure 1. General amino acid structure.

There are 22 standard amino acids but only 21 are found in eukaryotes (**Table 1**). Amino acids play important roles both as building blocks of proteins and as intermediates in metabolism [18].

Amino acids are usually classified by the properties of their side chain into four groups: (1) weak acid, (2) weak base, (3) hydrophile if the side chain is polar, and (4) hydrophobe if the side chain is nonpolar [17].

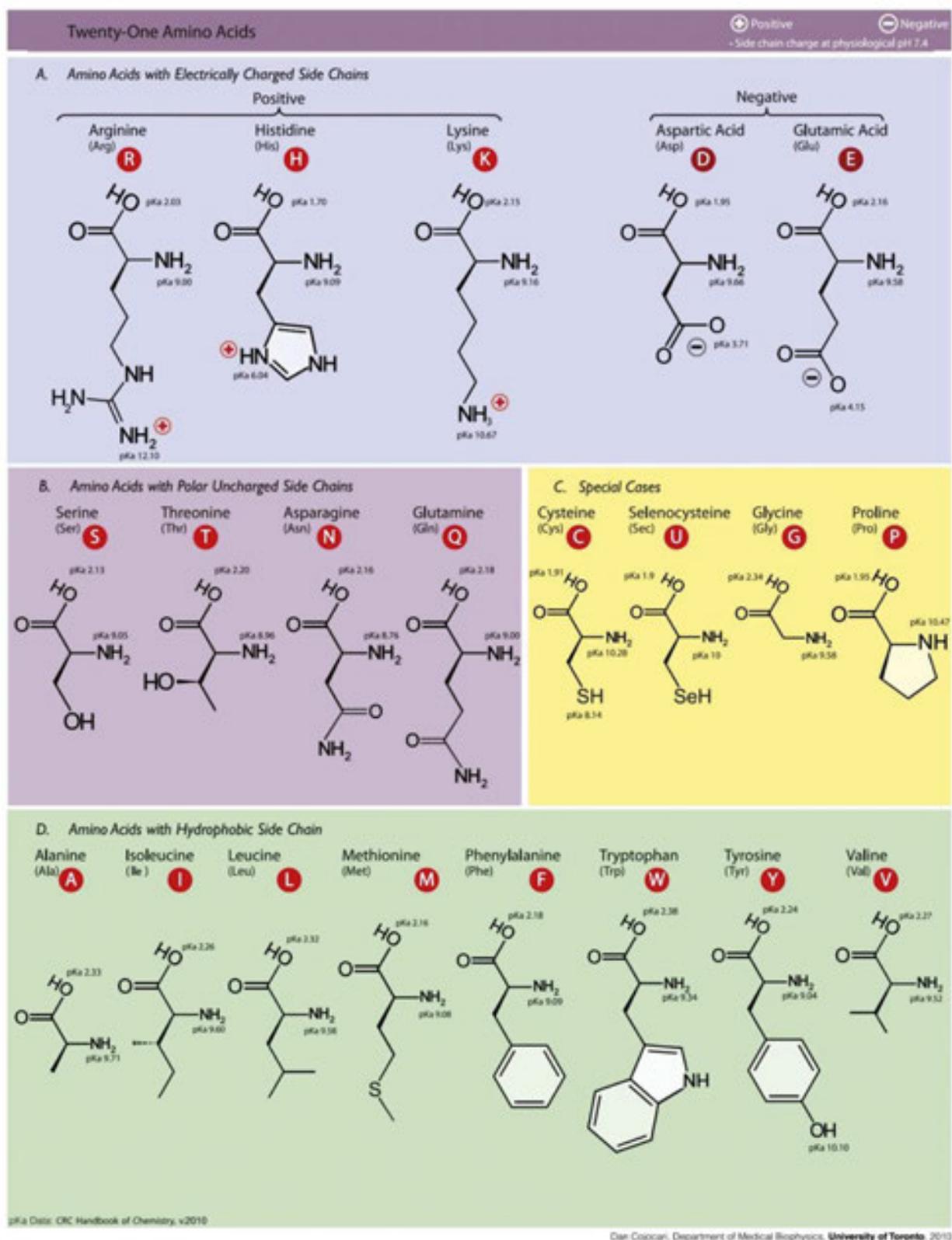


Table 1. Amino acids. The 21 amino acids found in eukaryotes [19].

The reagents used in the amino acids gold nanoparticles synthesis are described in **Tables 1** and **2**.

Gold nanoparticles									
Aspartic acid		Arginine		Threonine		Tryptophan		Valine	
T = 21°C/1' light		T = 21°C/1' light		T = 75°C/5 light		T = 21°C/0' light		T = 75°C/5' light	
pH final = 2.5		pH final = 6.8		pH final = 2.7		pH final = 3.2		pH final = 2.9	
[Au] mmol/L	[Asp] mmol/L	[Au] mmol/L	[Arg] mmol/L	[Au] mmol/L	[Tre] mmol/L	[Au] mmol/L	[Tri] mmol/L	[Au] mmol/L	[Val] mmol/L
1.78	10.15	1.78	10.05	1.77	8.59	1.78	9.75	1.69	8.74
Ratio: 5.70		Ratio: 5.65		Ratio: 4.85		Ratio: 5.48		Ratio: 5.17	

Table 2. Synthesis of AuNPs. For the synthesis of the nanoparticles, each amino acid was mixed with HAuCl_4 , followed by the addition of Milli-Q water. The solution was then stirred in a Fisatom vortex mixer (São Paulo, Brazil) for 5 min and exposed to a 400-W Cermax xenon lamp (Excelitas Technologies, Waltham, MA, USA).

The effects of the amino acid/metal concentration ratio, irradiation time, temperature, and pH were evaluated by ultraviolet-visible (UV-Vis) spectroscopy and transmission electron microscopy (TEM) for each of the 21 amino acids (and gold) as shown in **Table 3**.

Factors evaluated	Values (approximate)
Molar ratio amino acid/X (X = Au^{3+})	0.5–1–2–5
Time of illumination with white light	30''–1'–2'–3'–5'–10'
Temperature	25–75°C
Medium	Acid (pH ~ 4.0) and basic (pH ~ 9.0)

Table 3. Factors evaluated for the synthesis of gold nanoparticles.

A preliminary sorting study was carried out to select the most appropriate amino acids for the nanoparticle synthesis. The five best amino acids for gold were selected based on an evaluation of their stability after synthesis (as evaluated by zeta potential measurement) and the intensity of their UV-Vis spectra.

3. Nanoparticles synthesis with amino acids

3.1. Spectral behavior of HAuCl_4 in water

The spectral behavior of low and high concentrations of HAuCl_4 in Milli-Q water was analyzed by UV-Vis spectroscopy and the results are presented in **Figure 2**. In aqueous media, hydrogen tetrachloroaurate(III) is hydrolyzed, resulting in various species with differing chloride, aqua, and hydroxyl ligand complements (e.g., $[\text{AuCl}_4]^-$, $[\text{AuCl}_3(\text{H}_2\text{O})]$, $[\text{AuCl}_3\text{OH}]^-$, $[\text{AuCl}_2(\text{OH})_2]^-$, $[\text{AuCl}(\text{OH})_3]^-$, and $[\text{Au}(\text{OH})_4]^-$). The composition of the species depends on the pH and chloride concentration [20].

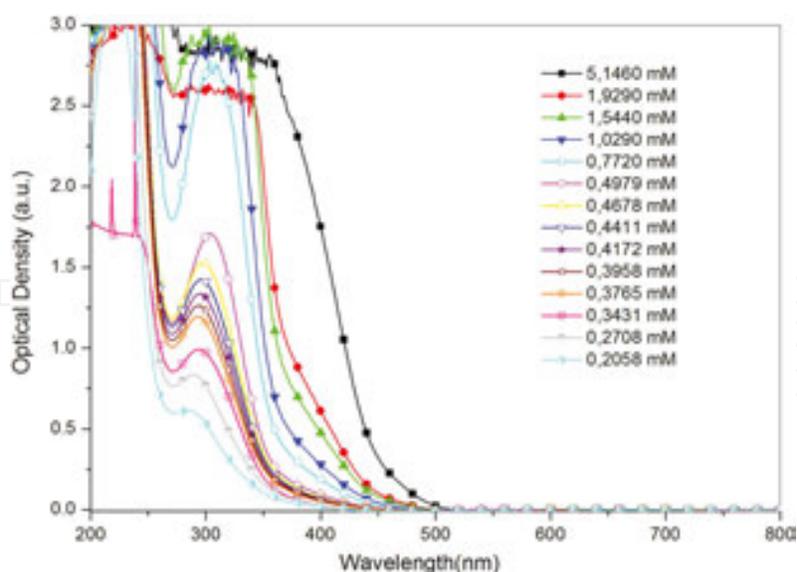


Figure 2. UV-Vis spectra of HAuCl_4 at different concentrations in water. The absorption measurements were carried out on a UV-Vis spectrophotometer (Shimadzu Multispec-1501). The samples were analyzed immediately after shaking at room temperature using quartz cuvettes with 10 mm optical paths.

The absorption spectra obtained after mixing HAuCl_4 with water at low concentrations (~ 0.1 – 0.5 mM) show a peak around 300 nm, which does not interfere with the absorbance of the AuNPs (~ 520 nm). As expected, there is a linear relationship between the absorbance and concentration. The 300 nm band is formed because of the replacement of the chloride ligands of the complex with hydroxyl groups; on increasing the concentration of the solution (>1.5 mM), the band is redshifted to ~ 311 nm because less hydrolysis of the chlorides in the initial complexes takes place [20].

3.2. Amino acids and HAuCl_4 in water and light

During the AuNPs syntheses, illumination with white light induces a color change from a slightly yellow to wine or purple, depending on the amino acid, as shown in **Figure 3** for five of the amino acid substrates. These color changes indicate the successful formation for the AuNPs.

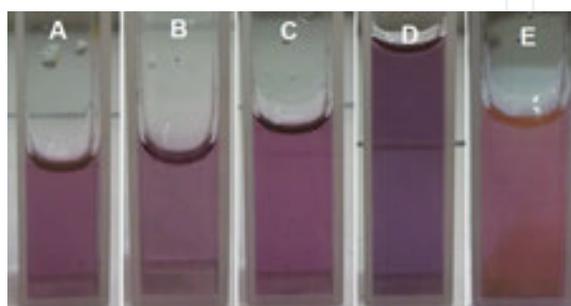


Figure 3. Color of the synthesized AuNPs prepared with (A) Asp, (B) Arg, (C) Thr, (D) Trp, and (E) Val.

The absorption spectra obtained after mixing the amino acids and HAuCl_4 in water followed by illumination with a xenon lamp are shown in **Figures 4** and **5**.

In the absorption spectra, the presence of a band at ~ 520 nm (surface plasmon resonance (SPR) band) indicates the formation of spherical AuNPs. From Mie theory, it is known that the maximum absorption wavelength is related to the size of the nanoparticle, wherein the smaller the wavelength, the smaller is the diameter of the nanoparticle [21]. The main parameters for the UV-Vis spectral analysis were the intensity of the band (which indicates the formation of large quantities of nanoparticles) and the full-width at half maximum (FWHM). The FWHM of the UV-Vis spectral bands indicates the size distribution of the colloidal dispersion [21]. The smaller the FWHM, the lower the polydispersity and more homogeneous the nanoparticle size.

From **Figure 4** we ascertained that AuNPs were obtained for 18 amino acids, each one under particular conditions such as the concentration ratio between the HAuCl_4 and the amino acids and the illumination time. AuNPs were not produced with L-cysteine hydrochloride, L-isoleucine hydrochloride, or L-lysine hydrochloride, which contained HCl as a stabilizer (because of excess of chloride ions) [22]. The best results considering stability, absorption intensity, and the FWHM were obtained with aspartic acid (Asp), arginine (Arg), threonine (Thr), tryptophan (Trp), and valine (Val).

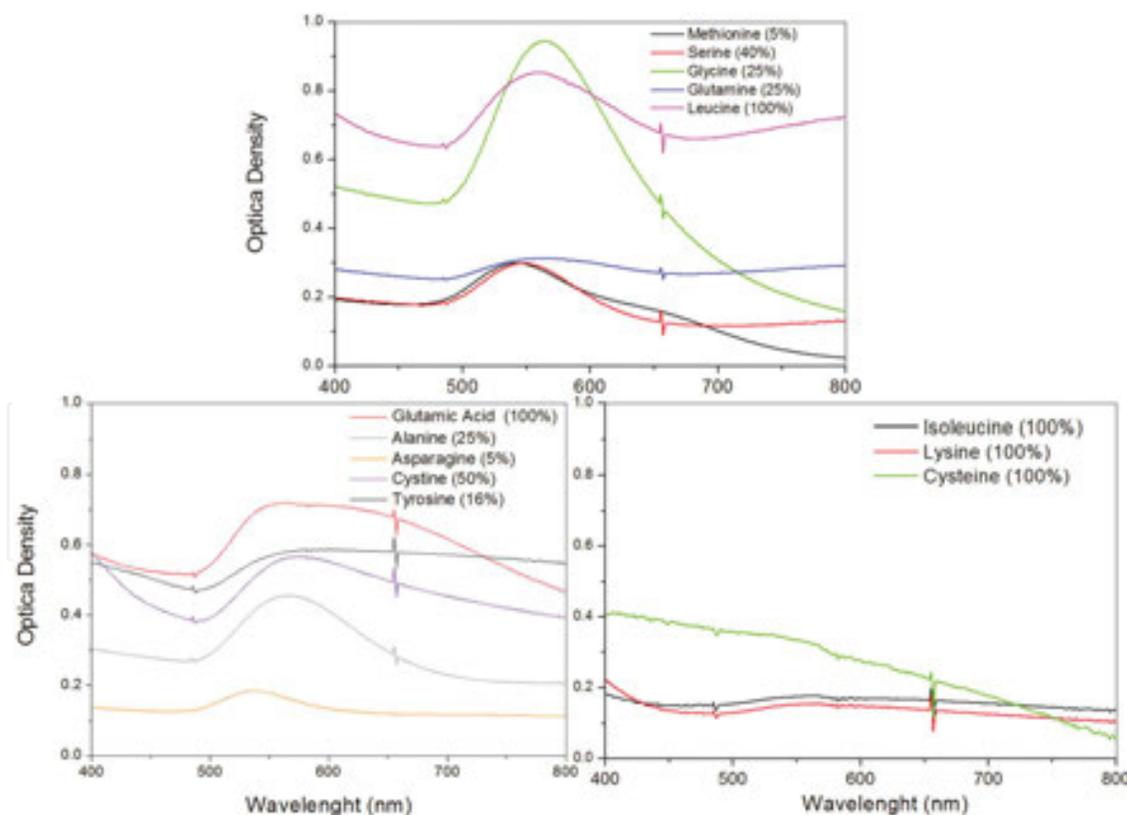


Figure 4. UV-Vis spectra (amino acid + HAuCl_4 + xenon light) of the reaction mixtures for the 18 amino acids that formed AuNPs and the three that did not. Each sample solution was prepared under different conditions depending on the amino acid, such as dilution, concentration ratios, and illumination times. Only the best results are shown.

The UV-Vis spectra obtained with Asp, Arg, Thr, Trp, and Val are shown in **Figure 5**. The position of SPR band was 554 nm for Val, 547 nm for Trp, 546 nm for Arg, 544 nm for Asp, and 540 nm for Thr, indicating that the sizes of the nanoparticles synthesized for these amino acids fall in the following order: Val > Trp > Arg > Asp > Thr.

TEM analyses were carried out to evaluate this size order and the results are presented in **Figure 5**. Microscopic analyses showed the formation of spherical AuNPs with the five selected amino acids, but the order of size was slightly changed: Asp > Trp > Val > Arg > Thr. Such

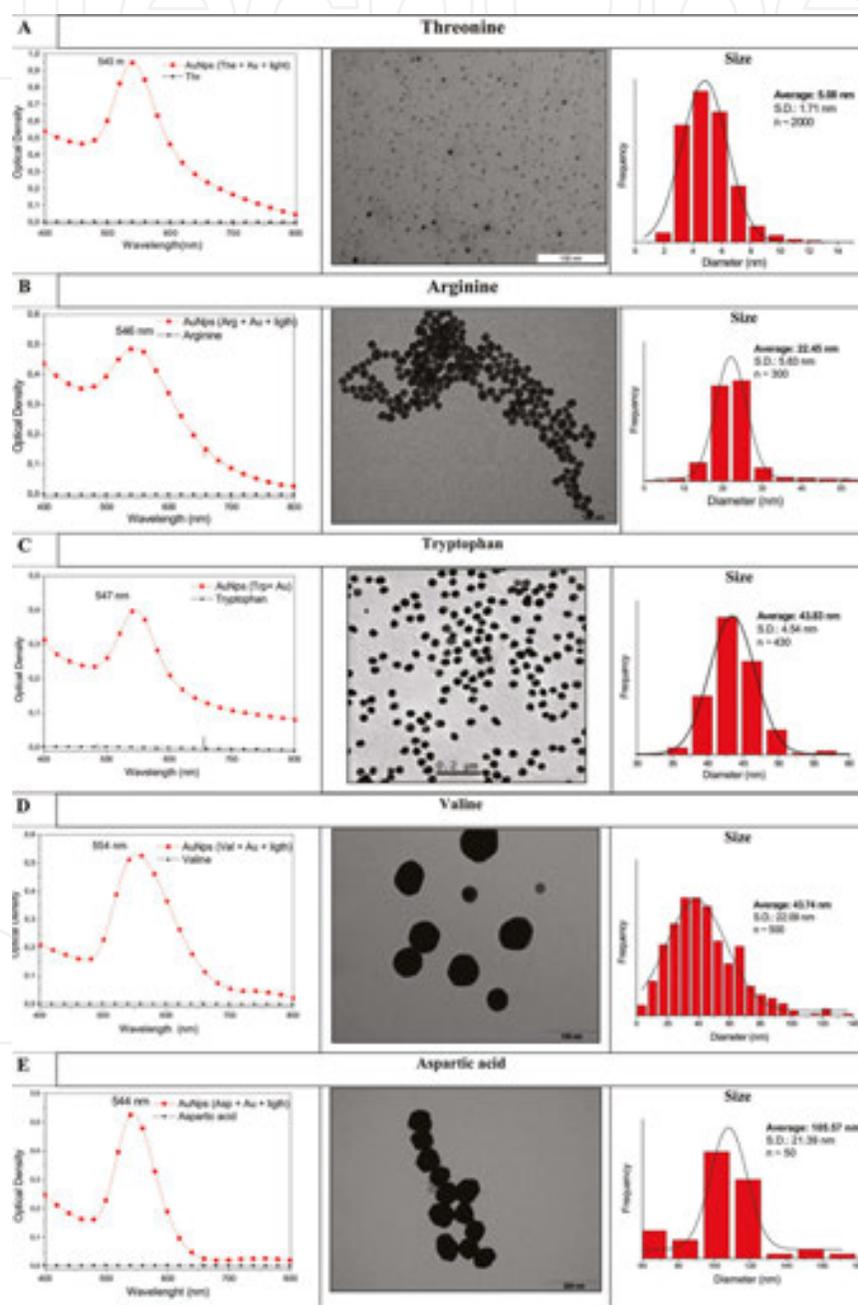
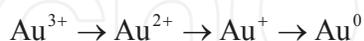


Figure 5. UV-Vis spectra, TEM images, and the average size of the AuNPs prepared with (A) Thr, (B) Arg, (C) Trp, (D) Val, and (E) Asp. Microscopic analyses were performed on a LEO 906E transmission electron microscope (Zeiss, Germany).

discrepancies may be due to variations in the refractive index of the medium [23], which directly influences the SPR band (cf. Mie theory) and can shift the wavelengths in the UV-Vis spectra. Alternatively, larger particles (or other shapes) may be present, as evidenced by the band near 750 nm observed for Val and Asp.

Three steps are required to completely reduce trivalent gold (Au^{3+}); each step involves the gain of an electron [24]:



After Au^{3+} is reduced to Au^0 gold clusters are formed with the subsequent nucleation and growth of the nanoparticles. It has been reported that ultraviolet radiation when interacting with a complex can make it more reactive and accelerate the reduction of ionic gold [24]. Therefore, for reducing Au^{3+} ions and forming nanoparticles, the presence of an auric complex with hydroxyls and the absence of chloride ions are essential. On the other hand, the oxidation of the amino acid is still required, and this reaction is catalyzed by light.

Illumination with white light is essential for the formation of nanoparticles. A xenon lamp radiates over a wide spectral range, from the ultraviolet to the infrared. The solution temperature also increases with the irradiation time. These interactions cause changes in the energy levels of the amino acids, leading to greater polarizability, even if temporary, and facilitating oxidation. There is a direct relationship between the oxidation potential and polarizability of an amino acid: the higher its polarizability, the greater its ease of oxidation and nanoparticle formation upon irradiation. In the cases of Trp, Arg, Thr, Val, and Asp, the polarizabilities are 54.1, 42.2, 40.3, 27.9, and 24.9 cm^{-3} , respectively. Thus, irradiation with light acts as a catalyst for the oxidation of the amino acids, which results in metal reduction (photo-oxidation/reduction).

Tryptophan does not require lighting; its color changes immediately after the mixing of reagents, confirming the results obtained by Selvakannan et al. [25]. In their paper, Maruyama et al. [12] observed that of the 20 amino acids Arg, Cys, and Thr did not result in a gold colloidal solution. Although Met, Phe, and Trp produced colloids, the products were easily precipitated. Our method realizes the possibility of producing stable nanoparticles with Arg, Cys, Thr, Met, Trp, and Phe. For the synthesis with Thr and Val, longer illumination times were required to produce AuNPs. Consequently, additional heating was required to facilitate the release of electrons (oxidation).

3.3. Importance of pH in nanoparticle stability

Once the colloidal nanoparticle suspensions are synthesized, their stability must be ensured. In this regard, the interaction of each amino acid with the metallic surface is very important. The excess amino acids are adsorbed on the metal surface; since amino acids are amphoteric, they can become electrically charged by gaining or losing electrons. The solutions become more acidic after synthesis because of the release of H^+ by the oxidation of amino acids. The evolution of pH before and after exposure to light is shown in **Table 4**.

	pH (before light)	pH (after light)
Aspartic acid	3.0	2.5
Arginine	8.9	6.8
Threonine	4.6	2.7
Tryptophan	6.9	3.2
Valine	5.0	2.9
H ₂ AuCl ₄	2.0	

Table 4. Evolution of pH with the formation of AuNPs.

The stability of the colloidal suspensions was satisfactory in acidic medium, with no precipitate formation or agglomeration over a period of 30 days. It was observed, in some cases, that the AuNPs reacted with the material of the Eppendorf tube (polypropylene) used for the samples producing a gold film and probably consuming the AuNPs. To resolve this issue, sodium hydroxide was added after the synthesis. This improved the stability of the colloidal suspension and avoided reaction with the Eppendorf tube, which increased the storage time (more than 30 days). To determine the stability of the colloidal suspensions, the surface charge of the nanoparticles was estimated by zeta potential measurements; the results are presented in **Figure 6**.

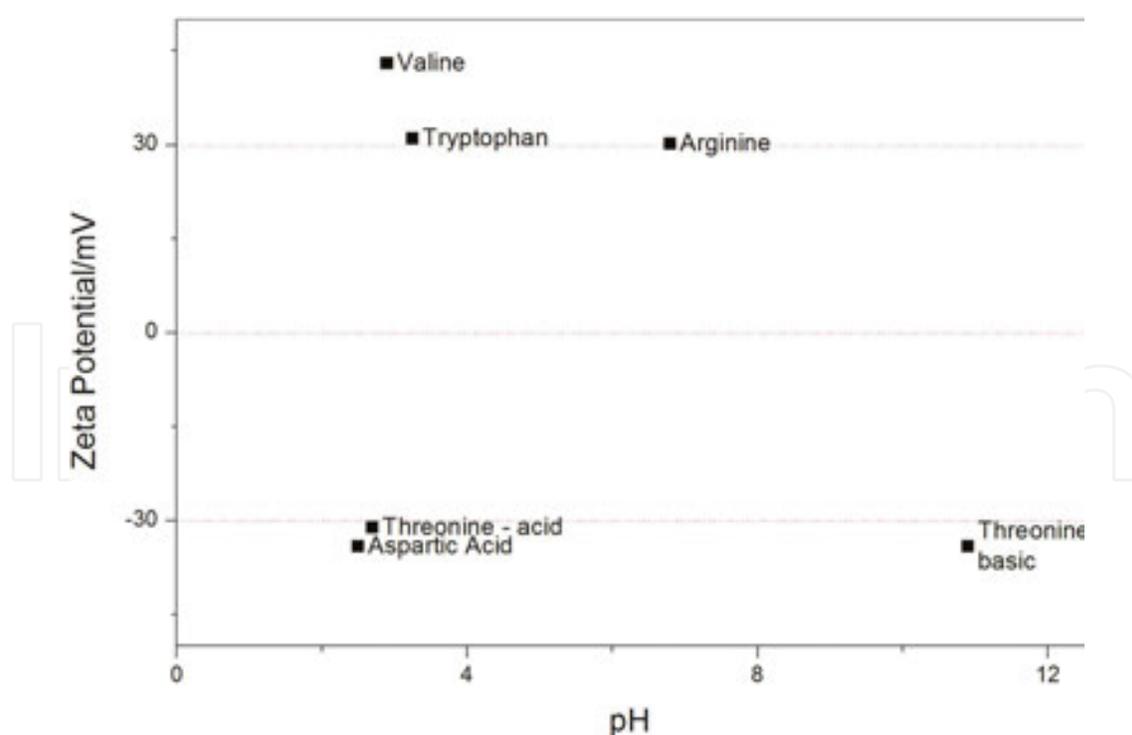


Figure 6. Zeta potentials of the AuNPs. Zeta potential measurements were performed on a Malvern Zetasizer NanoZS by focusing a 633 nm laser on the colloidal suspension. During analysis, the changes in the pH and zeta potential (mV) of the nanoparticles were measured.

From the figure, the zeta potentials for the five selected amino-acid-based AuNPs are always higher than $|30 \text{ mV}|$, for pH values ranging from ~ 3 to 11, showing that the colloidal suspensions are highly stable with a low tendency to aggregate.

The stabilization of a colloidal suspension depends on the electrostatic repulsion due to surface loads (load factor) and the electrostatic interactions with the amino acids (capping factor). Apart from the electrostatic interactions, ionic/hydrogen bonding between $-\text{NH}_3^+$ and COO^- -functionalized surfaces is also possible. The typical binding energies for such bonds are in the 10–30 kcal/mol range [11].

With an excess of chloride ions in the medium, hydroxide ligands are displaced by chlorides around the metal center, giving $[\text{AuCl}_4]^-$, which does not undergo reduction to form the AuNPs [22]. This effect was observed tentatively in the synthesis of AuNPs with Cys, Iso, and Lys (as their hydrochloride salts). The addition of chloride in the medium renders the formation of metal nanoparticles impossible. To prove this hypothesis, NaCl was added during the synthesis of AuNPs with aspartic acid. The addition of NaCl not only inhibited the formation of the AuNPs, but also modified the position of the auric complex band (redshift), as shown in **Figure 7**.

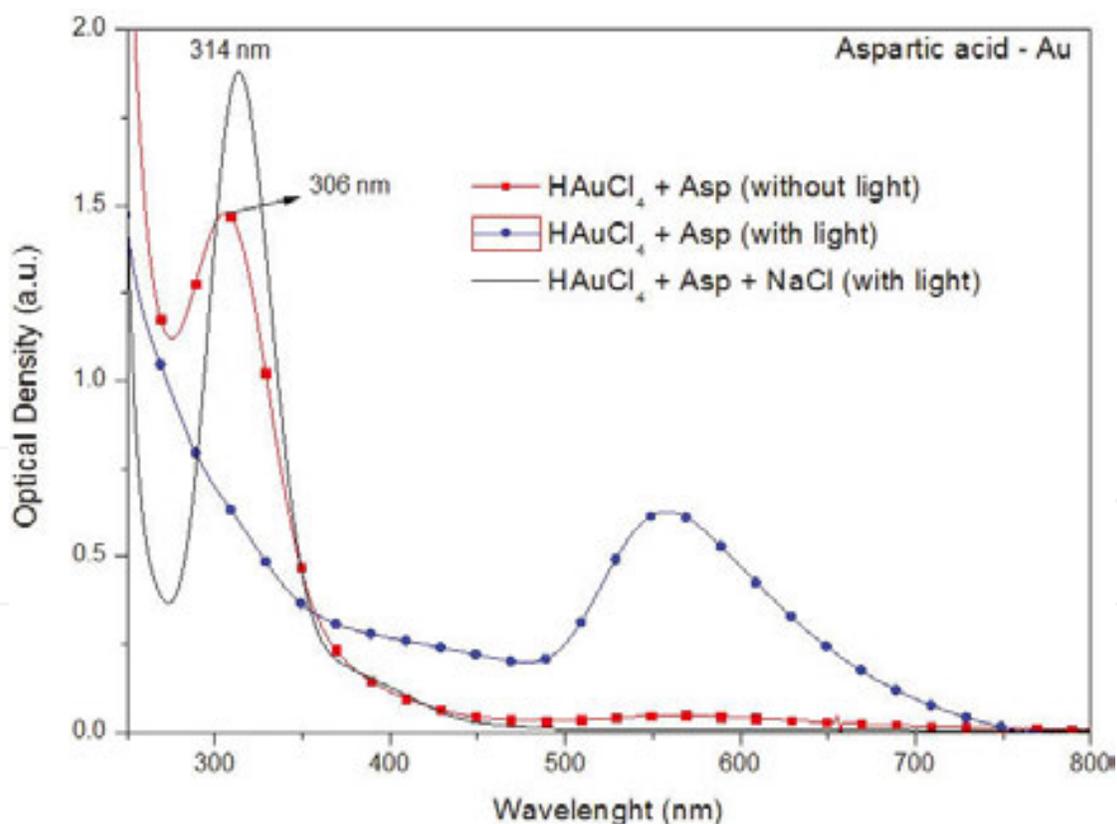


Figure 7. UV-Vis spectra of: (i) a hydroxylated gold complex with a small band at 550 nm (incipient formation of AuNPs with ambient light) (red/squares); (ii) AuNPs formed from the hydroxylated gold complex, Asp, and light (catalyst) (blue/balls); and (iii) addition of NaCl (chlorinated gold complex, in which the band position is changed and the formation of AuNPs is prevented) (black).

3.4. Tryptophan fluorescence

Of the 21 amino acids studied three are fluorescent (Tyr, Trp, and Phe). In the presence of the nanoparticles, only the fluorescence features of Trp are observed with a good signal-to-noise ratio for excitation at 280 nm. We varied the concentration of Au^{3+} while the concentration of tryptophan remained fixed (along with the irradiation time), and evaluated the changes by emission spectroscopy. The results are presented in **Figure 8**.

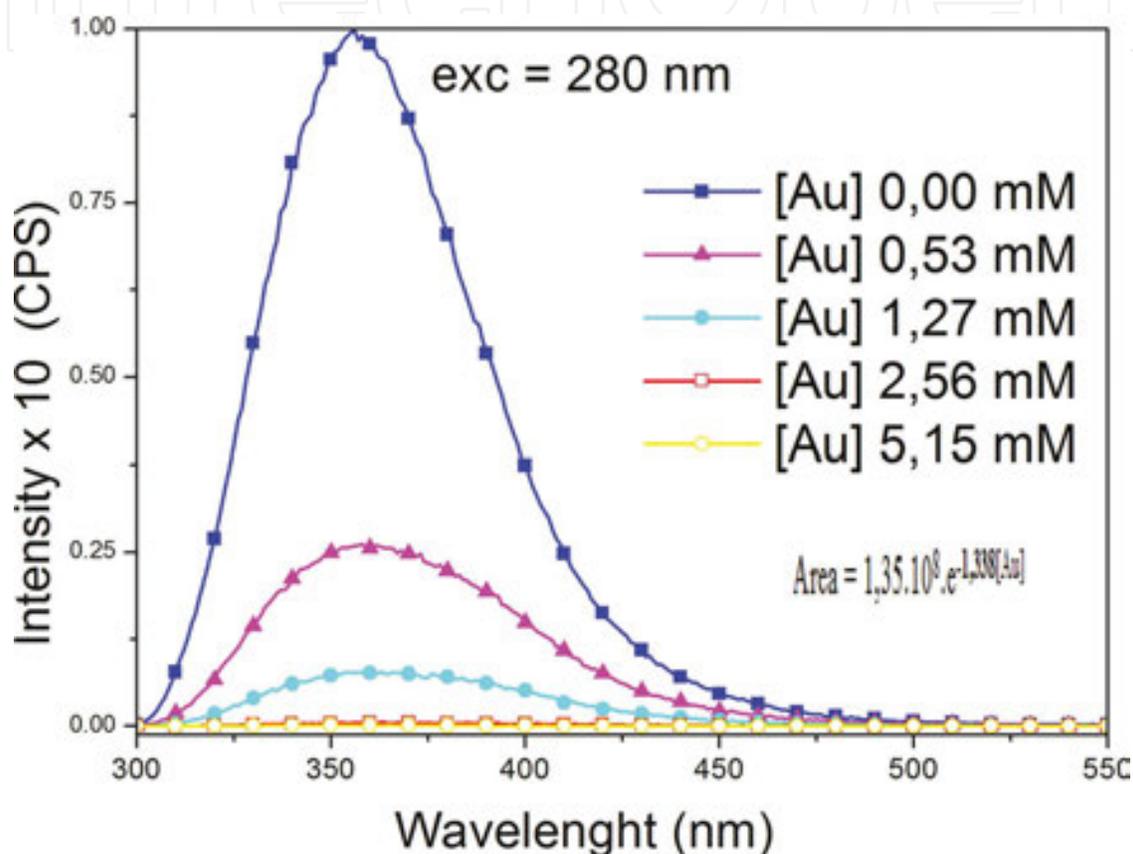


Figure 8. Fluorescence spectroscopy studies of AuNPs with Trp (12 mM) obtained by exciting Trp-AuNPs solution at 280 nm. Fluorescence measurements were performed on a fluorimeter (Horiba Jobin Yvon 3 Fluorolog) using quartz cuvettes with 10 mm optical paths. The analyses were carried out at room temperature after agitation of the samples.

Clearly, the Trp emission decreases with an increase in Au^{3+} concentration, indicating that in the synthesis of the nanoparticles part of the Trp is consumed or modified. Trp oxidation may occur at the nitrogen ring, forming kynurenine (reported as the largest product due to oxidation) [26]. Dimerization to form ditryptophan may also occur [26].

The spectra shown in **Figure 9** indicate the formation of AuNPs by varying the concentration of the species. The increase in HAuCl_4 concentration is observed to be linearly proportional to the area of the SPR band, with a high correlation, i.e., more nanoparticles are formed with more metal ions.

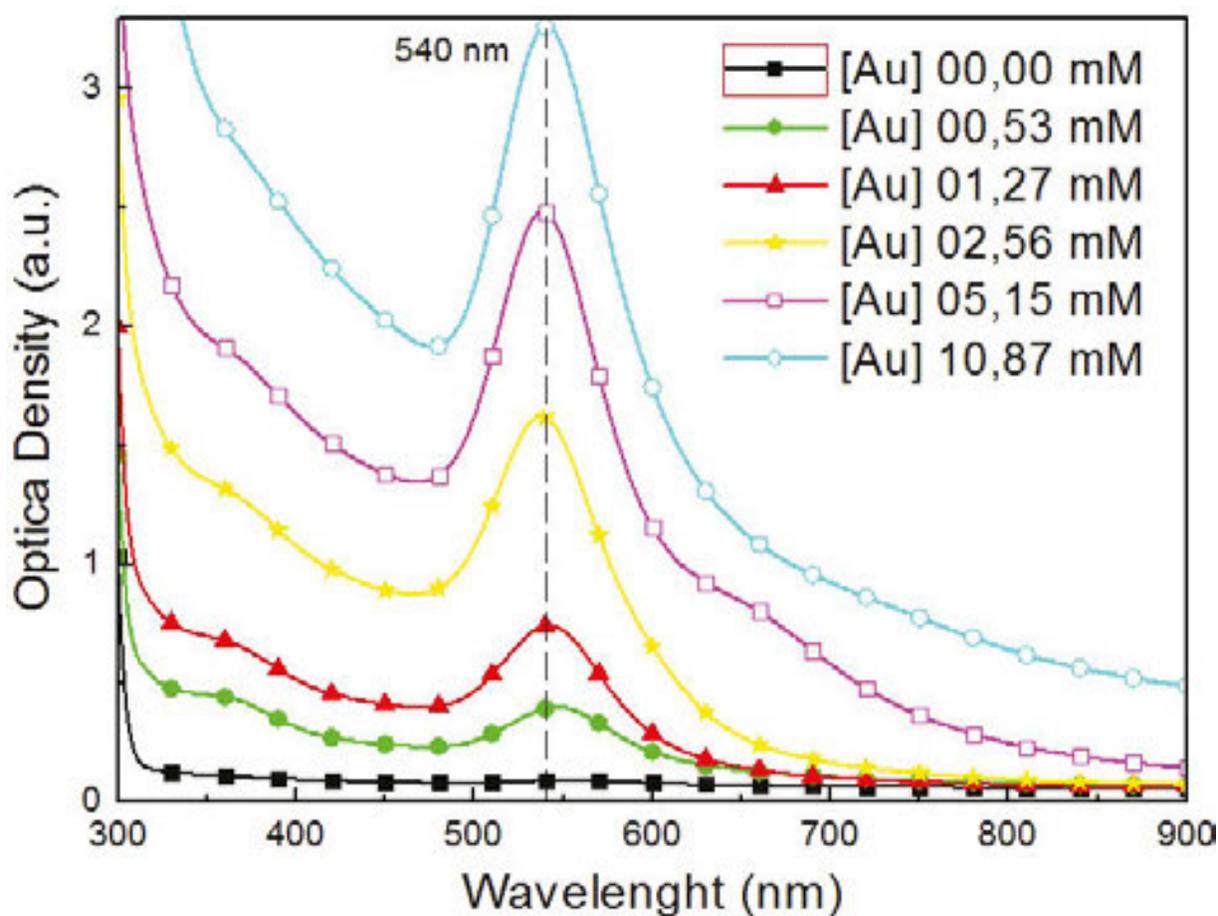


Figure 9. UV-Vis spectrum of AuNPs prepared with [Trp] = 12 mol/L.

4. Conclusions

AuNPs were obtained with 18 of the 21 studied amino acids under ideal synthetic conditions. The best results were obtained with aspartic acid, arginine, threonine, tryptophan, and valine. These amino acids reduced the metal ions (Au^{3+}) and prevented the agglomeration of nanoparticles adhering to the surface (steric effects and load). For the formation of the nanoparticles, the reduction of the metallic species in solution (Au^{3+}), with subsequent nucleation and growth of the metal crystals, was required. However, for reduction occur with the amino acids, their oxidation is required. Each amino acid has a different oxidation potential which depends on its size and the spatial arrangement of its atoms. In order to facilitate and/or accelerate the oxidation process, the use of electromagnetic radiation (xenon lamp) was necessary (tryptophan did not require lighting and the color changed immediately after mixing the reagents). The combination of the photons and temperature increase supplied by the xenon lamp facilitated the loss of electrons (oxidation), enabling the reduction of the metal species and nanoparticle formation. Thus, light was a catalyst for the formation of the AuNPs. From these experiments, a direct relationship was found between the oxidation potential and

polarizability of an amino acid: the higher its polarizability, the greater its ease of oxidation and nanoparticle formation upon irradiation. Zeta potential measurements indicated that the stability of the colloidal suspensions was higher in basic medium, wherein there is greater surface charge formation, particularly negative charge. The zeta potential, which exceeded ± 30 mV, is due to the deprotonation of the amino acids adsorbed on the nanoparticle surfaces in basic medium, leading to electrostatic repulsion between the particles. The presence of chloride ions was detrimental to the formation of nanoparticles. In the presence of chlorides, it was not possible to form nanoparticles.

As previously reported, the reactions with Arg, Cys, and Thr did not result in a gold colloidal solution. Although Met, Phe, and Trp produced colloids, the products were easily precipitated. Our method realizes the possibility of producing stable nanoparticles with Arg, Cys, Thr, Met, Trp, and Phe.

In short, the synthesis of AuNPs by the photoreduction of amino acids in water was simple, cheap, and fast. This study opens up the possibility of applying such nanoparticles in biological systems due to the biocompatibility of the amino acids, e.g., the in situ synthesis of nanoparticles or the functionalization of metal nanoparticles with amino acids/proteins. This could portend advances in areas such as biological markers and the detection/treatment of disease. With this study we can better understand the synthesis of nanoparticles with organic materials such as natural proteins and saliva secreted aspartyl proteinases (SAPs), which have been increasingly employed in the synthesis of metal nanoparticles.

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Abbreviations

AuNPs	gold nanoparticles
FWHM	full-width at half maximum
SPR	surface plasmon resonance
TEM	transmission electron microscopy
UV-Vis	ultraviolet-visible

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