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RESEARCH ARTICLE

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Synthesis and Characterization of *Eugenia uniflora* L. Silver Nanoparticles and L-Cysteine Sensor Application

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L-Cysteine (Cys) is a non-essential sulfur-containing amino acid, crucial for protein synthesis, detoxification, and several metabolic functions. Cys is widely used in the agricultural, food, cosmetic, and pharmaceutical industries. So, a suitable sensitive and selective sensing approach is of great interest, and a low-cost sensor would be necessary. This article presents silver nanoparticles (*Eu*AgNPs) synthesized by a green synthesis method using *Eugenia uniflora L.* extracts and photoreduction. The nanoparticles were characterized by UV/VIS, transmission electron microscopy, high-performance liquid chromatography (HPLC), FTIR, and Zeta potential. With the addition of Cys in the *Eu*AgNPs solution, the terminal thiol part of L-cysteine binds on the surface of nanoparticles through Ag–S bond. The *Eu*AgNPs and CysAgNPs coexist until flavonoids bound the amino group of Cys, enhancing the red color of solutions. The *Eu*AgNPs provided selectivity to detect Cys among other amino acids, and its detection limit was found to be 3.8 nM. The sensor has the advantages of low-cost synthesis, fast response, high selectivity, and sensitivity.

Keywords: Pitangueira, silver nanoparticles, photoreduction, cysteine, HPLC.

1. Introduction

L-Cysteine (Cys) is a natural thiol (–SH) containing semi-essential amino acids and a critical player in oxidative stress conditions.[1] Cys is widely used in the medical, agricultural, food, cosmetic, and pharmaceutical industries.

Abnormal cysteine levels are responsible for many human diseases. Hyperhomocysteinemia is associated with atherosclerosis, congestive heart failure, age-related macular degeneration, Alzheimer's disease, and cancer.[2] Cysteine deficiency may lead to edema, liver damage, skin lesions, and weakness.[3] Consequently, precise assessment of Cys levels in human bodies may aid the early diagnosis of some diseases.

In industry, Cys is used in pharmaceuticals, cosmetics, baking, and as a flavoring additive.[4,5] The quantification of Cys in food matrices is laborious and involves sample preparation and specific analytical methods such as high-performance liquid chromatography (HPLC). These methods show good accuracy but are time-consuming and require expensive equipment operated by specialists. So, a low-cost, rapid, and simple platform to detect Cys is essential to overcome the limitations of traditional methods.

For cysteine quantification methods besides HPLC, electrochemistry, and fluorometric, nanosensors have been reported.[6–11] Recently, green synthetic routes were applied for the preparation AgNPs, used for Cys detection.[11–13] The interest in nanosensors for food

analysis is based on cost-effectiveness, portability, simplicity, and rapid detection.

Silver nanoparticles functionalized with quercetin were able to sense the cysteine up to 21.1 nM (in aqueous), 86 nM (in urine), and 230 nM (in FBS) concentration.[10] Quercetin is a member of the flavonoid family, a large group of natural metabolites with variable phenolic structures found in fruits, leaves, roots, stems, and flowers of plants.

Quercetin is one of the flavonoids present in *Eugenia uniflora Linnaeus* extracts.[14,15] *E. uniflora* belongs to the *Myrtaceae* family, with 142 genera and 5.500 species.[16,17] Native to South America, *E. uniflora L.*, the Brazilian cherry, is widely distributed in Brazil, Argentina, Uruguay, and Paraguay. The leaves and fruits (pitanga) of *E. uniflora L.* have many therapeutic properties, such as antimicrobial, antifungal, antiviral, antitumor, antidiarrheal, anti-helminth, insecticide, antihypertensive, and anti-rheumatic.[18–23] Besides, the flavonoids containing in the *E. uniflora L.* extracts includes other phenolic compounds, terpenes, tannins, anthraquinones, anthocyanins, and carotenoids.[16,17,23–26] Using plant extracts without purification makes the method very simple and inexpensive.

In this article, silver nanoparticles were synthesized by photoreduction, an inexpensive and eco-friendly green method using extracts of *Eugenia uniflora L.* (*Eu*AgNPs).[27–32] In this method, light can facilitate silver reduction using plant extracts, and phytochemical agents inhibit the ions agglomerations from producing nanoparticles.[33–39] The nanoparticles were characterized by UV/VIS spectroscopy, dynamic light scattering (DLS), FTIR, Zeta potential, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and HPLC. The influence of increased concentration of cysteine on the optical properties of *Eu*AgNPs was investigated to verify their potential as a cysteine sensor.

2. Materials and Methods

2.1 Materials and Synthesis

E. uniflora L. leaves, and fruits were collected from the spontaneous germination trees (named #1, #2, and #3) in São Paulo, SP, Brazil (Longitude: −46.6793941. Latitude: −23.623774). The leaves, fruits, and seeds were washed with distilled water and grossly chopped (leaves and fruits) into pieces, as shown in *Figure 1*. The leaves and fruits (1.1 ± 0.01 g) and a seed (0.7 ± 0.01 g) were infused for ~5 min in 100 mL of distilled water until they reached the temperature of 80 °C (± 2 °C). The colors of the extracts of leaves, fruits, and seed were light green, pink, and light yellow.

Figure 1. *E. uniflora L.* (pitangueira), preparation of leaves extract, Photoreduction, *Eu*AgNPs.

The silver nanoparticle suspensions (*Eu*AgNPs) were prepared by mixing 5.31 ± 0.02 mg of silver nitrate ($AgNO_3$) Sigma–Aldrich in 40 mL of the leaves, fruit, or seed extracts. The colors of the extracts changed in the presence of silver nitrate (light brown), indicating the rapid formation of silver nanoparticles. Immediately after mixing reagents, the solutions were illuminated with a 300 Watts Cermax xenon (Xe) lamp for 1 min. The Xe lamp was positioned at 10 cm of the transparent glass beaker containing the solution. The illuminated region covered exactly the beaker diameter and with an intensity of ~3.6 W/cm^2. The photoreduction reaction intensified the color of the solutions (deep brown). After the photoreduction process, the measured pH ranged from 3.9–5.3, depending on the extract, indicating the release of H^+ by the phytochemicals oxidation. The pH was then adjusted to ~7.0 using sodium hydroxide. The increase in the pH modified the phytochemical structures, enabling nanoparticle stabilization against agglomeration and precipitation. Some pictures of the synthesis process are shown in *Figure 1*.

The effect of cysteine in silver nanoparticles synthesis was evaluated, and cysteine silver nanoparticles were prepared by mixing $AgNO_3$ (2 mmol/L) with cystine (Cys-2) (from Merck) (0.23 mmol/L) in ultrapure water and 2 min of irradiation with Xe lamp.

2.2. Nanoparticles Characterization

High-performance liquid chromatography (HPLC) analysis was performed on a Shimadzu HPLC composed of a sequential auto-sampler and a high-sensitivity diode array detector (190–800 nm) that was managed by a LabSolutions System Manager software and Origin 8.0. The extracts and *Eu*AgNPs were analyzed by HPLC column Agilent Zorbax SB-C18 (4.6 mm × 150 mm × 5 μm). Mobile phases A and B were aqueous and 100% HPLC analytical grade methanol, respectively. UV detection was obtained at 254 nm.

Shimadzu MultiSpec 1501 spectrophotometer was used to measure the UV-vis region absorption spectra. For this, 500 μL of NPs were diluted in 3 mL of doubly distilled water, and the measurements were carried out in a 10 mm optical path quartz cuvette from 200 to 800 nm.

The Fourier transform infrared spectroscopy (FTIR) was performed with Shimadzu IRPrestige. In this case, 200 μL of the NPs and their extracts were deposited on microscope slides and dried. The process was repeated three times. The material deposited on the slides was scraped off to make KBr pellets.

The stability of the colloidal suspensions was analyzed by Zeta potential measurements using the Zetasizer Nano ZS Malvern apparatus. Three sizes were made for each sample.

Transmission Electron Microscopy (TEM) images of prepared nanoparticles were obtained. The samples were dripped onto a copper grid and analyzed on JEM 2100 – JEOL transmission electron microscope. Particle size distribution analysis was performed through images obtained by this technique with ImageJ software.

2.3. Cysteine Quantification

To determine a Cys calibration curve, a mother solution with 1 mM of L-Cysteine, purchased from Dinâmica (Meta Química, Santa Catarina, Brazil), was diluted serially with the dilution factor 2 (1 mM, 0.5 mM; 0.25 mM, 2 mM). Aliquots of 100 μL of Cys solutions were added to 3 mL of distilled water and 500 μL of *Eu*AgNPs. Optical measurements were performed after 10 min of solution reaction.

The selectivity of *Eu*AgNPs for Cys was determined based on its performance against other amino acids (L-arginine, L-glutamine, glycine, L-histidine, L-methionine, L-phenylalanine, L-serine, L-tryptophan purchased from Merck). In this case, 100 μL of prepared solutions (100 μM) were added to 3 mL of distilled water and 500 μL of *Eu*AgNPs. The UV/VIS spectra were obtained.

2.4. Preparation of Milk Samples

Tree commercial samples of milk (cardboard, 250 mL; pasteurized and full-fat dairy) were purchased from a local supermarket (São Paulo, SP). A dilution with deionized water 60 times was performed. A 100 μL milk sample solution was used the verify cysteine content by mixing 3 mL of water and 500 μL of *Eu*AgNPs. UV/VIS spectra for each sample were recorded in triplicate.

3. Results

3.1. Synthesis and Characterizations

The colorless solution of $AgNO_3$ instantly transformed into light brown after a reaction with aqueous *E. uniflora* extracts. The deep brown color could be observed after illumination of the solutions for 1 min with a Xe lamp (*Figure 1*). The brown color indicates the presence of silver nanoparticle surface plasmon resonance (SPR), which arises due to the conduction of free electrons around the nanoparticle surface.

The analysis by HPLC of *E. uniflora* (#2) leaf, fruit, and seed extracts shown in *Figure 2a* reveals distinct regions between 2 to 28 min, where significant peaks were observed in the chromatograms. The flavonoids were identified as myricitrin (MYR), gallic acid (GA), and ellagic acid, comparing the results obtained by Bezerra et al.[40] The presence of quercetin has also been reported in leaves of *E. uniflora*[26,40] and corresponds to the peak at the retention time of 20.2 min (*). It can be noticed that differences in the flavonoid composition for each extract. This was mainly observed in myricitrin (MYR – 19.3 min) content in the seed extract, gallic acid (GA – 17 min), and ellagic acid (EA – 20.7 min) in the leaf extract. The diminution and disappearance of peaks were observed in *Eu*AgNPs prepared with leaf, fruit, and seed extracts, as observed in *Figure 2b*.

*Eu*AgNPs prepared with leaves extract (*Figure 2b* green line) presented intense peaks with retention times between 20–21 min intervals. The height at ~16 min almost disappeared in *Eu*AgNPs. The polyphenols are prone to oxidation during the photoreduction process, forming hydrogen peroxide, quinones, and semiquinones, resulting in new peaks in the spectra. Certain oxidation products of quercetin have an antioxidant potency that vastly surpasses that of quercetin.[41]

Figure 3a shows the UV/VIS spectra of the extracts prepared with *E. uniflora* leaf, fruit, and seeds. All spectra show peaks from flavonoids around 240–280 nm and around 300–370 nm, attributed to conjugations in the B- and A-rings, respectively.[42] *Figure 3b* shows the UV/VIS spectra of the *Eu*AgNPs prepared with *E. uniflora* leaf, fruit, and seed extracts (#3). The spectra show the SPR band around 410 nm. The range with higher intensity was obtained with leaves extract.

Figure 3c shows the UV/VIS spectra of extracts prepared with leaves of three trees (#1, #2, #3). All spectra showed the same peaks from flavonoids with little variations in the signal intensities.

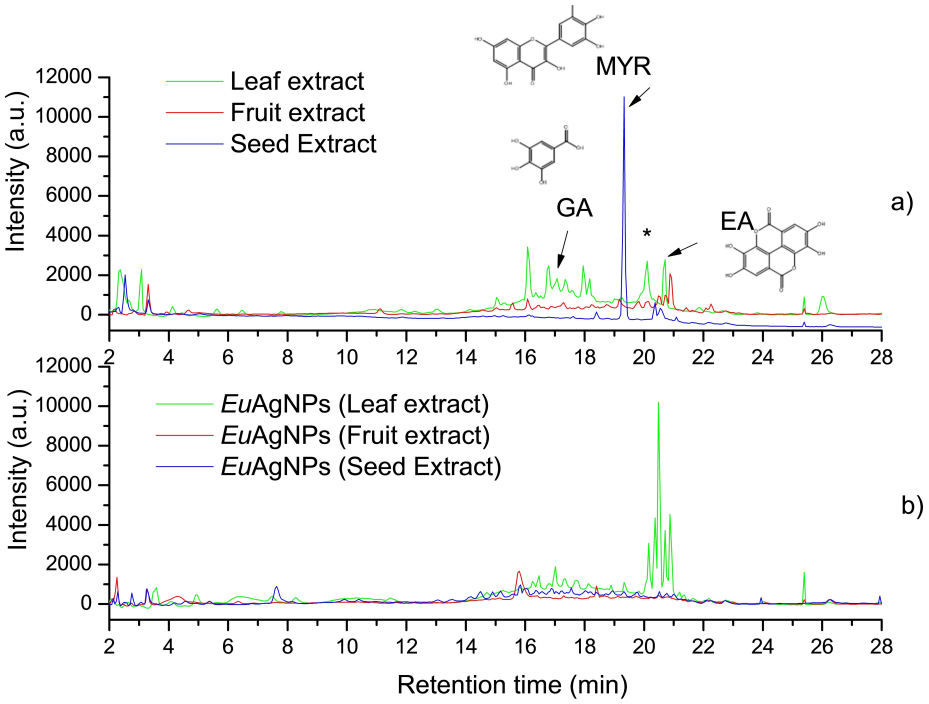

Figure 2. a) Chromatograms obtained for leaf, fruit, and seed extracts. b) *Eu*AgNPs prepared with leaf, fruit, and seed extracts. Detection at 254 nm.

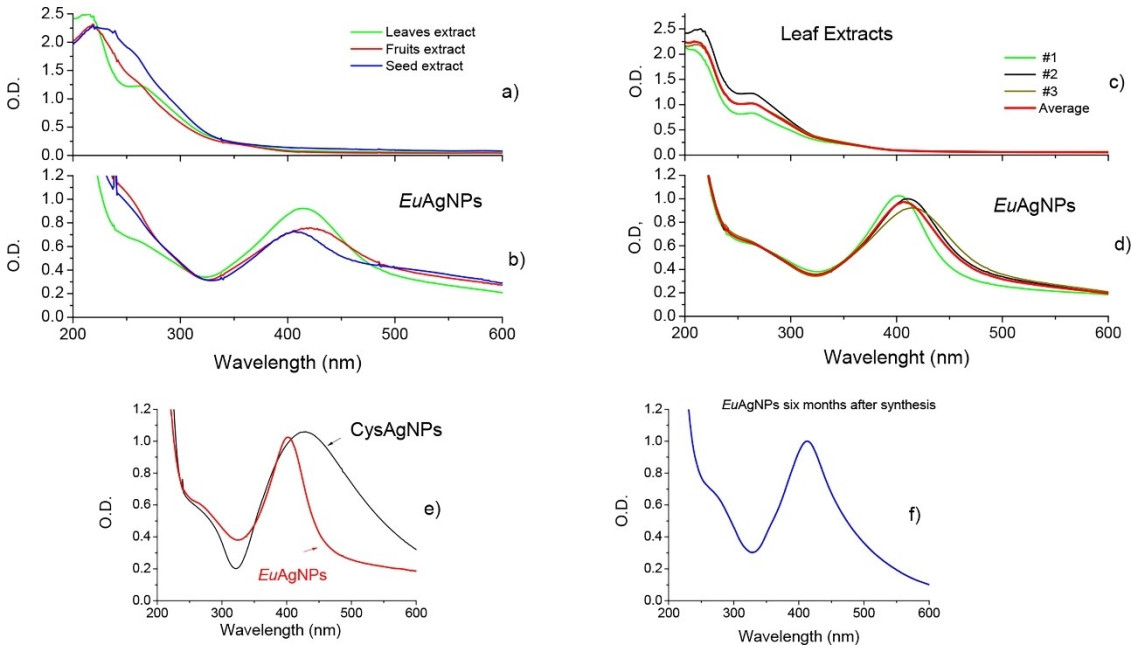

Figure 3. a) UV/VIS spectra of extracts prepared with *E. uniflora L.* leaves, fruits, and seeds; b) UV/VIS spectra of *Eu*AgNPs prepared with *E. uniflora L.* leaves, fruits, and seeds; c) Leaves extracts of tree threes (#1, #2, and #3); d) *Eu*AgNPs prepared with leaves extracts by photoreduction of leaves obtained from three trees (#1, #2, and #3); e) Comparison between *Eu*AgNPs and CysAgNPs UV/VIS spectra. f) *Eu*AgNPs Uv-vis spectra six months after synthesis.

Figure 3d shows the UV/VIS spectrum of *Eu*AgNPs synthesized using aqueous extracts of leaves of three trees. The averaged spectra show an SPR peak around 406.47 ± 0.30 nm (x_c), with width (w) of 36.12 ± 0.36 nm (GaussAmp curve fit $y = y_0 + Ae^{-\frac{(x-x_c)^2}{2w^2}}$). Although some differences were observed, the synthesis procedure was very reproductive and reliable.

Figure 3e shows the absorption spectra for cysteine silver nanoparticles CysAgNPs compared with *Eu*AgNPs. The oxidation of cystine, cysteine dimer, generated by forming a disulfide bond, promoted the formation of nanoparticles. The position of the SPR band peak was ~427 nm.[43] *Figure 3f* shows UV/VIS spectra of *Eu*AgNPs six months after synthesis.

The TEM images and size distribution obtained for *Eu*AgNPs (plant #3) and CysAgNPs are presented in *Figures 4a* and *4c*, respectively. The *Eu*AgNPs prepared by the photoreduction process were spherical with diameters of ~21 nm (*Figure 4b*). CyAgNPs are more polydisperse, and their diameter ranges around 24 nm (*Figure 4d*).

The results obtained for Zeta potential measurements for *Eu*AgNPs (#3) are shown in *Figure 5a.* The zeta potential was −15.1 mV, and the polydispersity index (PI) was 0.256. CysAgNPs Zeta potential was > -30 mV.[43]

Figure 5b presents the Fourier Transform Infrared Spectroscopy (FTIR) obtained for *E. uniflora L.* leaves extract and *Eu*AgNPs prepared with leaves extract. In the spectra are observed bands around 3400 cm^{-1} related hydroxy groups,[44,45] 2980–2800 cm^{-1} due to stretching vibration of C–H, 1600 and 1700 cm^{-1} originated from the stretching band of carbonyl groups C=O and C=C,[46] 1635 cm^{-1} related to flavonoids[24] and 1317 cm^{-1} in the *Eu* extract that shifts to 1387 cm^{-1} in the *Eu*AgNPs attributed to the symmetric deformation in the plane of CH_2.[46]

3.2. Cysteine Quantification

With the addition of an increasing concentration of cysteine (0.002–1 mM) in *Eu*AgNPs prepared with leaf extract (#2) was observed a gradual change from yellow color (diluted solution) to orange (as shown in *Figure 6a*) and finally, red. Variations of SPR band peak wavelength (x_c), width (w), amplitude (A), and FWHM can be observed in *Figure 6a* with the increase in cysteine concentration in the solution. A good linear relationship with a correlation coefficient of 0.99 was obtained over the range from 2 to 140 μM, plotting w

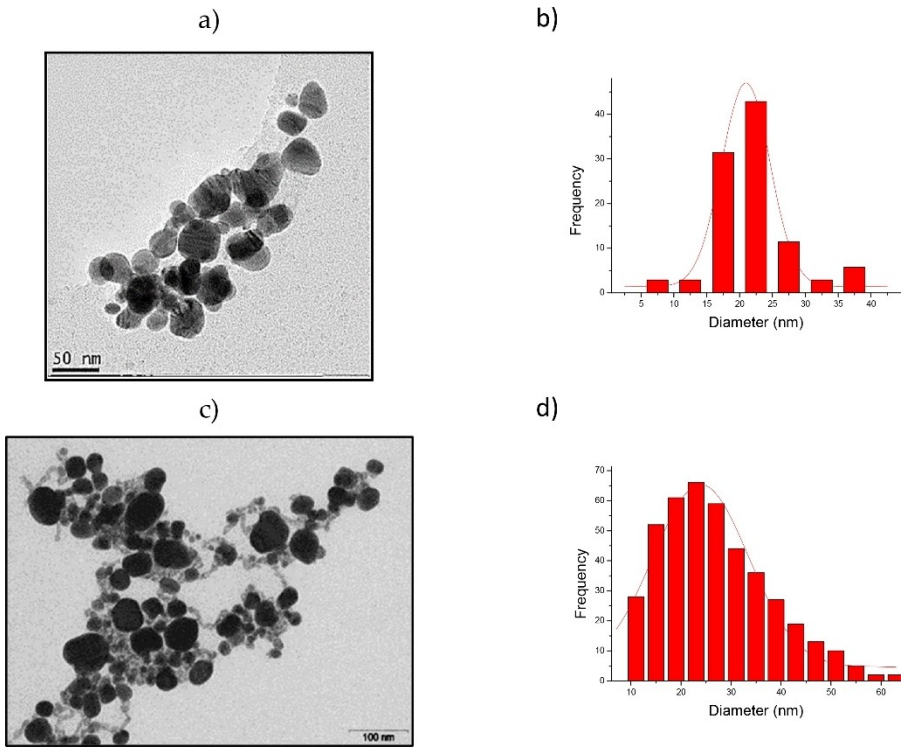

Figure 4. TEM image for a) *Eu*AgNPs (#3) prepared with leaves extract, c) TEM image CysAgNps, and b, d) size distributions.

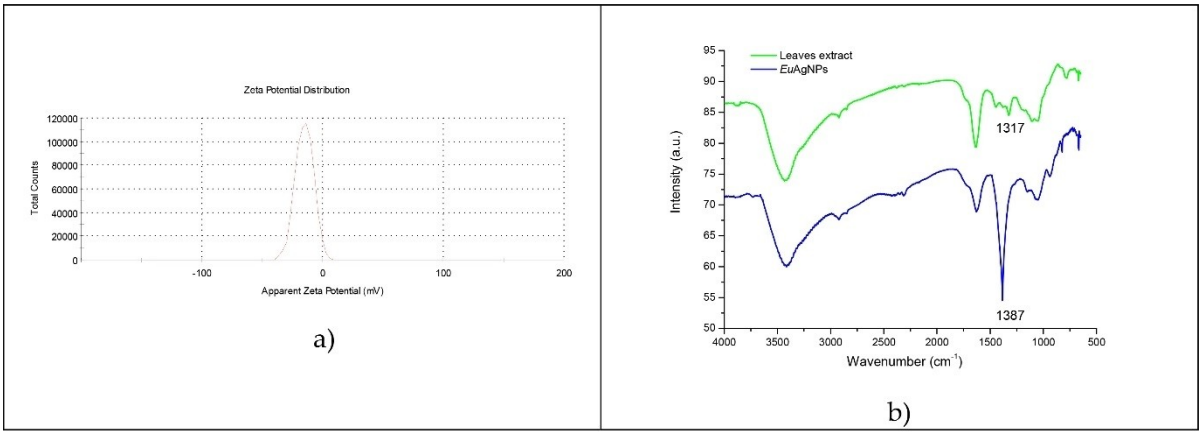

Figure 5. a) Zeta potential analysis of synthesized *Eu*AgNPs (#3). b) FTIR spectra leaves extract and *Eu*AgNPs (#3).

in the function of cysteine concentration. The limit of detection (LOD) was calculated considering $LOD = 3.3\frac{\sigma_B}{b}$ where σ_B is the standard deviation of response and b is the slope obtained by ordinary least squares regression. The obtained LOD was 3.8 nM.

The selectivity was determined by comparing results for other amino acids: L-arginine, L-glutamine, glycine, L-histidine, L-methionine, L-phenylalanine, L-serine, and L-tryptophan. The absorption spectra of *Eu*AgNPs in the presence of different amino acids are shown in *Figure 7a*. In the presence of all investigated amino acids, no change in the absorption spectra of referent *Eu*AgNPs colloids was observed. The amino acid methionine, with has a sulfur bridge in its molecular structure, did not induce optical changes in *Eu*AgNPs since L-methionine silver nanoparticles are stable only in an acidic medium.[43] Under identical experimental conditions, the *Eu*AgNPs colloidal solution turned red in the presence of Cys due to a shift and enlargement of the SPR band.

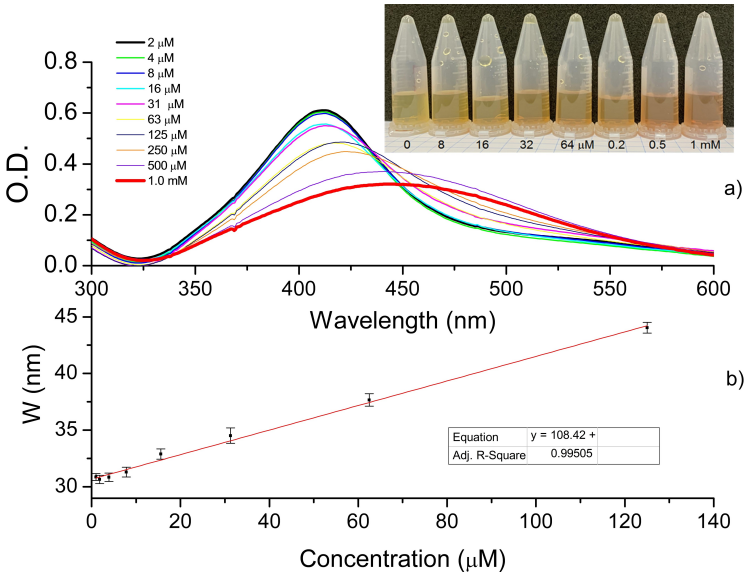

Figure 6. a) Changes in *Eu*AgNPs SPR band in the function of cysteine concentration. b) Variations of SPR width (w) in the function of cysteine concentration and linear fit.

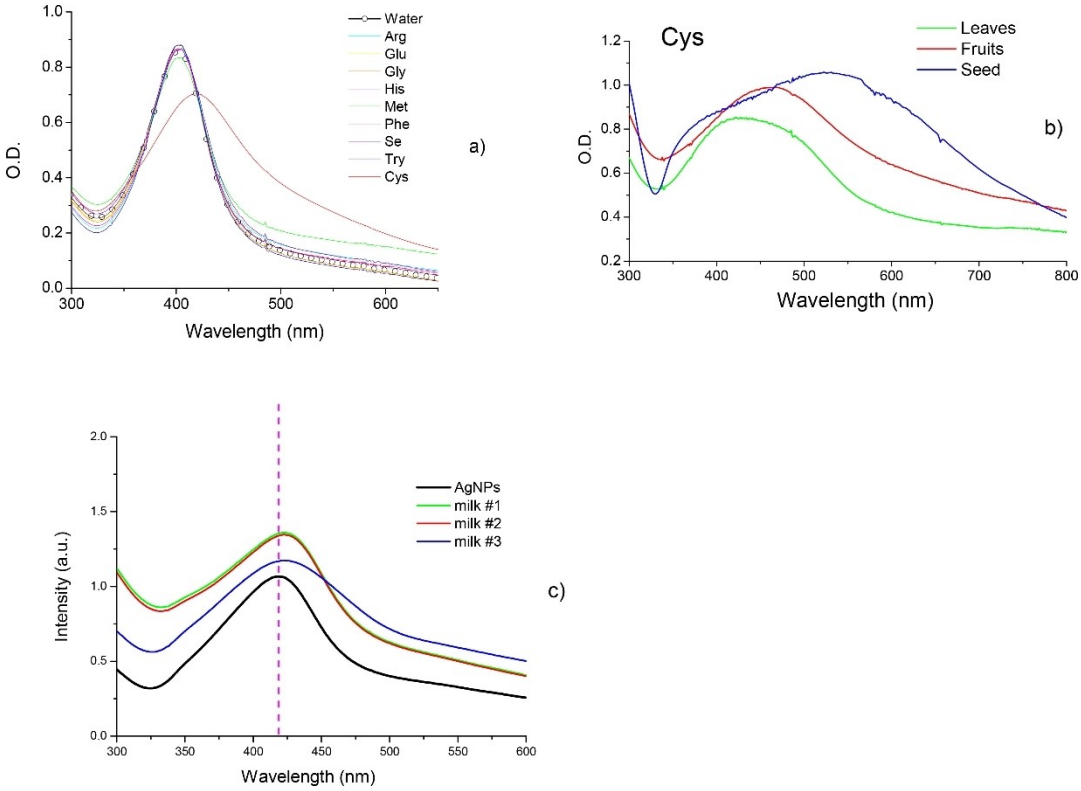

Figure 7. a) The selectivity of the colorimetric sensing method toward cysteine. Variations in UV/VIS spectra of the *Eu*AgNP system in the presence of 100 mM of L-arginine, L-glutamine, glycine, L-histidine, L-methionine, L-phenylalanine, L-serine, L-tryptophan and L-cysteine, b) Comparison in UV/VIS spectra of *Eu*AgNPs prepared with leaves, fruits, and seed extracts in the presence of Cys (2 mM). c) Effect of the presence of three samples of milk in *Eu*AgNPs. Each spectrum represents an average of three spectra for each sample.

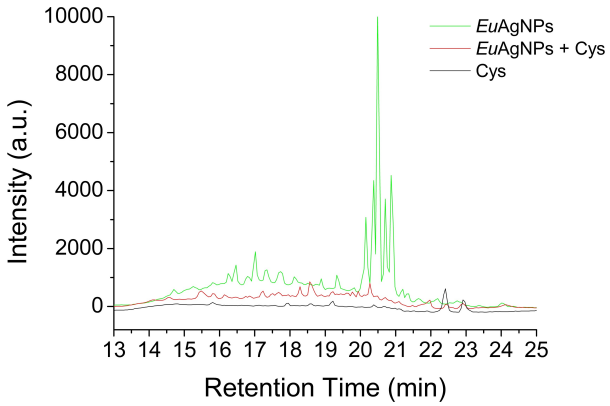

Figure 8. Chromatograms for *Eu*AgNPs from leaves extract (green line) and *Eu*AgNPs with cysteine (red line) and cysteine solution (black line). Detection at 254 nm.

Figure 7b compares the cysteine effect on *Eu*AgNPs prepared with leaves, fruits, and seeds extracts of *E. uniflora*. Although AgNPs prepared with leaf extract present a more intense SPR band, changes in the SPR band in the presence of Cys were also observed for *Eu*AgNPs prepared with fruit and seed extracts.

The selectivity of *Eu*AgNPs to detect Cys in milk was tested. Commercial milk samples were diluted 60 times using deionized water, added to *Eu*AgNPs, and the UV/VIS spectra were measured and shown in *Figure 7c*. *Eu*AgNPs ($x_c = 415.44$ nm and $w = 33.31$ nm) presented a shift and enlargement of SPR bands in the presence of milk samples. Milk #1 and #2 showed similar band profiles ($x_c = 419.88$ nm and 420.87 nm, and $w = 33.77$ nm and 33.48 nm, respectively), and milk #3 presented a shift and an enlargement in bandwidth ($x_c = 428.17$ nm and $w = 42.46$ nm). These results indicate that the Cys concentration in milk samples should follow the order #1 < #2 < #3. So, *Eu*AgNPs can be used to control the presence of Cys concentration in milk samples easily and practically.

The analysis by HPLC from the leaf extract, *Eu*AgNps in the presence of cysteine, and a cysteine solution (1 mM) are shown in *Figure 8* in intervals between 13 to 25 min. It was noted that *Eu*AgNPs, in the presence of cysteine, present the retentions peaks attributed to cysteine (22.4, 22.9 min), the probable quercetin peak (~20.2 min), and the absence of the intense peaks in the interval 20–21 min.

4. Discussion

Using plant extracts for nanoparticle synthesis is a straightforward green and economically viable.[47] Several works in the literature evidence satisfactory results of synthesis and antimicrobial activities of nanoparticles prepared with plant extracts.[48–51] This article discusses biogenic silver nanoparticles prepared with *E. uniflora* extracts associated with the photoreduction method and pH control. The aqueous extracts present differences in flavonoid composition (*Figures 2a* and *3a*) but leaves, fruits, and seed extracts promoted silver reduction, as observed in *Figure 3b*. The presence of the SPR band confirmed the presence of nanoparticles. The *Eu*AgNPs synthesized with leaves extract presented good optical properties (high intensity and low FWHM indicating monodispersity), and the fact of leaves are easy to collect and over-distributed all the year, more studies were performed with nanoparticles prepared with leaves extract.

The leaves of *E. uniflora* have a brownish-green color when new, whereas the adult leaves are dark green. Although the variability, biotype, and environmental factors, the UV/VIS spectra of aqueous *E. uniflora* extracts prepared with leaves of three different trees without any purification method, generated similar spectra profiles. The photoreduction promoted the oxidation of flavonoids of *E. uniflora L.*, which reduced Ag^+ ions to elemental silver. The photoreduction method offered steric repulsion within nanoparticles, preventing agglomeration, and giving rise to a mutual stabilization system.[52] Silver nanoparticles prepared by photoreduction presented sizes around 21 nm (*Figure 4b*). The nanoparticles prepared by this method are stable for at least six months.

The peak around 1387 cm^{-1}, observed in the FTIR spectrum for *Eu*AgNPs prepared with leaves extract, *Figure 5b*, indicates the presence of C–H bending and the involvement of flavonoids in silver reduction and stabilization.

The interaction between Cys and *Eu*AgNPs induced the color change of the colloidal solution from yellow to red. Due to the specific and strong interaction of cysteine with AgNPs, there was a drastic decrease in SPR band intensity (*Figure 6a*) and an increase in width (w) (*Figure 6b*), resulting in color change. Considering the linear range in the curve w in the function of Cys concentration was possible to obtain the limit of detection (LOD) of 3.8 nM. The LOD was comparable to cysteine sensors reported in *Table 1*.

The selectivity of *Eu*AgNPs colloid toward Cys is demonstrated in *Figure 7a*. The SPR band of *Eu*AgNPs

Table 1. Other methods for cysteine detection compared with *Eu*AgNPs.

Probe	Linear range (μM)	LOD (μM)	Ref.
Pear juice	0.5 – 10	0.68	[12]
Dextran	100 – 1000	12.0	[53]
C. procera	0.1 – 1	0.1	[54]
In the presence of Ca^{2+}	0.1 – 1000	0.10	[55]
Bagasse	0.1 – 1000	0.035	[13]
Castor oil		25.37	[56]
Quercetin	0.25 – 15	0.021	[10]
Eugenia Uniflora	0.02 – 130	0.004	Present work

colloid in the presence of amino acids L-arginine, L-glutamine, glycine, L-histidine, L-methionine, L-phenylalanine, L-serine, and L-tryptophan (100 μM) do not result in changes in the SPR band.

The proposed interaction between Cys and *Eu*AgNPs that caused the color change of the colloidal solution from yellow to red is presented in *Figure 9*. The addition of Cys involved the replacement of flavonoids (quercetin, for example) by Cys on the surface of *Eu*AgNPs. The terminal thiol part of L-cysteine bound on the surface of NPs through Ag–S bond creating a new nanoparticle CysAgNPs (*Figure 3e*). With increasing Cys concentration, *Eu*AgNPs were replaced by CysAgNPs in the colloidal solution, and the SPR band was enlarged. Increasing the Cys concentration, hydrogen bound between hydroxyl/ketone groups of flavonoids and the amino groups of Cys were formed, enhancing the red color of solutions.

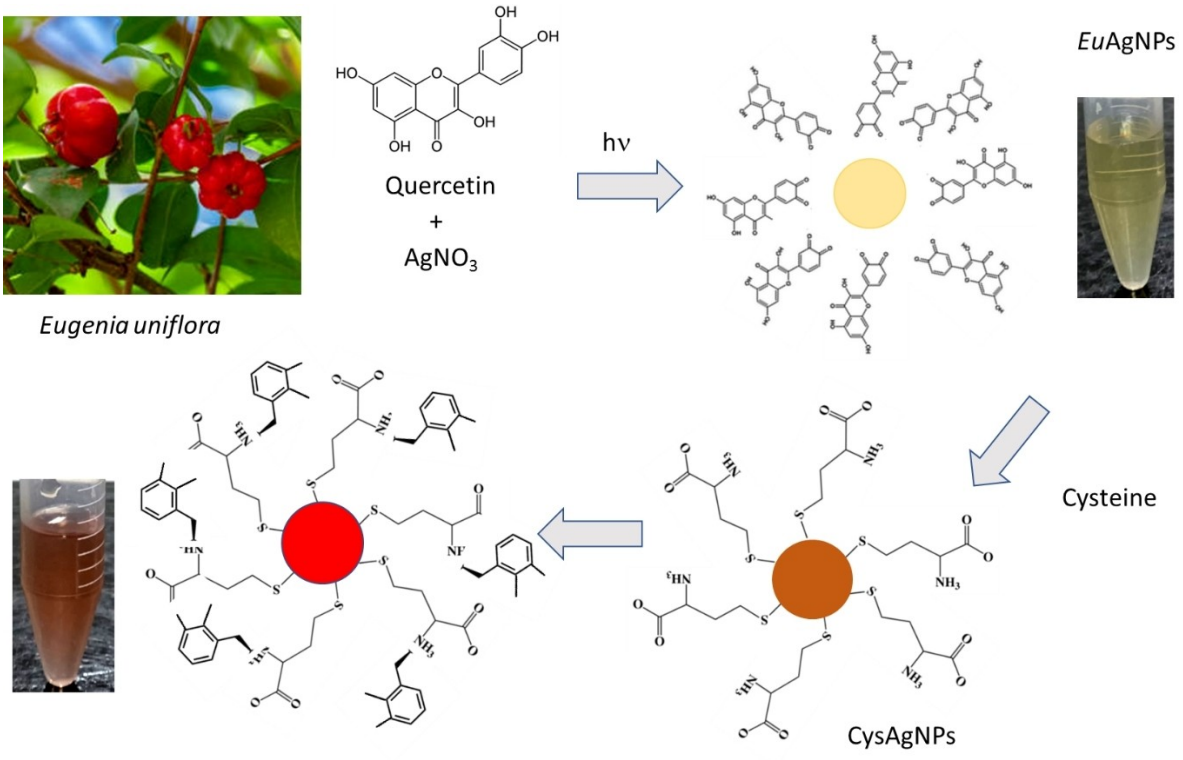

Figure 9. Diagram showing proposed interaction between Cys and *Eu*AgNPs. The Photoreduction process promotes flavonoid oxidation (quercetin, for example) and the formation of *Eu*AgNPs. With the addition of Cys in the solution, the terminal thiol part of L-cysteine binds on the surface of NPs through Ag–S bond. The *Eu*AgNPs and CysAgNPs coexist until flavonoids bound the amino group of Cys, enhancing the red color of solutions.

Conclusions

*Eu*AgNPs prepared with leaves extract and photoreduction are spherical with a size range of ~21 nm. The Zeta potential results indicate that nanoparticles are negatively charged (−15.1 mV). When *Eu*AgNPs are exposed to cysteine, strong interaction of the –SH group of cysteine with silver nanoparticle surface causes color change noticeably even by the naked eye. With increasing Cys concentration, *Eu*AgNPs are replaced by CysAgNPs in the colloidal solution, and the SPR band is increased. The limit of detection for cysteine using *Eu*AgNPs was 3.8 nM. This system can be used to detect cysteine in different biological fluids and food products with the advantages of low-cost synthesis, fast response, high selectivity, and sensitivity.

Ethics Declarations

Not applicable.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author Contribution Statement

Lilia C. Courrol: Formal analysis, Investigation, Validation, Resources, Writing – original draft. Isabela Santos Lopes, Fernando Cassas, and Flavia Rodrigues de Oliveira Silva: Data obtaining. Thiago André Moura Veiga: HPLC resources. All authors drafted the manuscript authors to read and approve the final manuscript.

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