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Green tea synthesized silver nanoparticles as sensing platform for determination of tetracycline in honey samples

Running title: Silver nanoparticles for determination of tetracycline in honey

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Abstract

Background: Silver nanoparticles (AgNPs) can be easily obtained in aqueous solution by chemical reduction using appropriate reducing agents and stabilizers. The development of environmentally-friendly methods using non-toxic solvents and reagents has become an alternative for the synthesis of these particles and their future application as sensor probe for agricultural products. In this work a straightforward method based on green tea extracts as reducing and capping agent is proposed for the synthesis of AgNPs, followed by their evaluation as sensing platform for determination of tetracycline in honey samples.

Results: Highly-stable nanoparticles were easily obtained by combining green tea aqueous extracts and ultrasound irradiation during 2 min. The as-synthesized AgNPs, spherical in shape and with average size of 8.5 nm, were evaluated for determination of tetracycline by following the changes on the localized surface plasmon resonance band (LSPR) at 450 nm induced by the presence of this antibiotic at pH= 5.8. The method was successfully applied in the concentration range between 200 and 800 μg L^{-1} with R^2 > 0.996 and limit of detection (LOD) of 52.7 μg L^{-1}. Multiple honey samples were analyzed and obtained recovery values were ranged between 82.8 – 116 %, with relative standard deviation values lower than 6.69%.

Conclusion: Obtained results demonstrate the synthesized AgNPs, only using green tea extracts, represents a promising and sustainable alternative tool for the cost-effective determination of tetracycline antibiotics in honey.

Keywords: Green nanotechnology, Honey, Silver nanoparticles, Tetracyclines

1. INTRODUCTION

Tetracyclines (TCs) are a class of broad-spectrum antibiotics that have been widely applied in veterinary medicine due to their effective antimicrobial properties and low cost[1]. These compounds are often administered through food or applied directly to the animal, not only to treat bacterial diseases, but also for preventive and prophylactic purposes. However, the abuse of these antibiotics can lead to the proliferation of resistant bacteria and potential accumulation in the human body through the intake of food from animal origin, including milk, meat and honey, among others[2]. Regarding their occurrence in apiculture products, several countries have established maximum residue levels (MRLs) for TCs in honey, while others banned their use[3, 4]. Particularly, Argentina is the third largest producer and exporter of honey worldwide, so the quality of this product must fulfil the international standards and commercial requirements[5]. Most of the analytical methods applied for determination of TCs are based on the high performance liquid chromatography (HPLC) with UV-Vis detection, molecular fluorescence or mass spectrometry (MS) [2, 6]. However, chromatography-based methods require expensive instrumentation, as well as high-purity reagents that are often not entirely environmentally friendly and cheap. For this reason, the development of simple and low-cost methods, with minimal consumption of samples and reagents, for determination of TCs residues in honey is highly required.

In the last years, the application of nanosensors for optical detection of TCs has emerged as alternative to the conventional methodologies[7, 8]. Particularly, optical sensors based on the localized surface plasmon resonance band (LSPR) of metallic nanoparticles, such as gold and silver, have attracted attention for determination of TCs because their good reproducibility and overall performance[7]. LSPR is induced by the absorption and/or scattering of radiation due to the oscillation of the conduction electrons at the surface of the particle. This phenomenon is very sensitive to the refractive index and is strongly affected by the particle size, shape, inter-particle distance of nanoparticles, and also by the interaction with target molecules, including TCs [3, 9].

Silver nanoparticles (AgNPs) can be easily obtained in aqueous solution by chemical reduction of Ag^+ ions to Ag^0 using appropriate reducing agents and stabilizers to prevent their aggregation[10, 11]. However, in most of the cases, highly reactive and toxic chemicals, such as sodium borohydride, are employed along with organic agents, including surfactants, polymers, and solvents, which can generate harmful effects to the environment and living organisms. Hence, the development of environmentally-friendly methods using non-toxic solvents and reagents has become a challenge for the synthesis of nanoparticles. Mainly, the use of natural, renewable and widely distributed sources, such as plants, roots and fungi, that have compounds with reducing and

stabilizing properties, including polyphenols, flavonoids, carbohydrates and vitamins, is receiving great attention[12, 13]. The use of green tea for generation of AgNPs was recently proposed because of the synergistic effect between both, the antioxidant and anti-inflammatory properties of tea components and antimicrobial effects of nanoparticles, leading for biomedical and industrial applications[12, 14]. Nevertheless, the use of AgNPs, obtained by green tea extracts as unique source of reducing and stabilizing compounds, has not been yet explored as sensor for TC determination.

In this work, an environmentally-friendly method based on green tea extracts and ultrasound energy is presented for the generation of AgNPs in aqueous solution. The as-synthesized particles are then proposed as chemical sensor to evaluate the TCs concentration in multiple honey samples.

2. MATERIAL AND METHODS

2.1 Reagents and solutions

The reagents used were of analytical grade and all solutions were prepared with ultrapure water. Tetracycline hydrochloride (TC) was obtained from Parafarm (Argentina) and used to prepare a 1000 mg L^{-1} stock solution in methanol (Anedra, Argentina). This solution was kept at 4ºC under light protection. A working solution of 100 mg L^{-1} was prepared by taking 1.00 mL of the TC stock solution and diluting to 10.00 mL with ultrapure water. For the synthesis of AgNPs in aqueous solution, 8 mg mL^{-1} 0.8% (w/v) green tea extract, 0.1 M NaOH (Cicarelli, Argentina) and 0.1000 M $AgNO_3$ (Biopack, Argentina) were selected. Folin Ciocalteu reagent (Biopack), gallic acid (Biopack) and Na_2CO_3 (Anedra) were used to determine the total polyphenolic content in the tea extracts. Regarding the flavonoid content, $AlCl_3$ (Merck, Germany) and quercetin (Cicarelli) were used. Disodium phosphate (Timper, Argentina) and sodium dihydrogen phosphate (Cicarelli) were used to prepare a 0.020 mol L^{-1} phosphate buffer solution (pH= 5.8 ± 0.1) while disodium phosphate and citric acid (Timper) were used to prepare a McIvaine Buffer (pH= 4.1 ± 0.1). A mixture of methanol and ammonia (90:10 v/v) was used as the elution solvent in the solid phase extraction (SPE) procedure.

2.2 Tea selection and preparation of aqueous extracts

Black tea, two of local brands and one international, and three kinds of green tea with the same origin as the previous ones were selected to assure the natural variability of tea composition. Subsequently, a quartering process was carried out for all types of

tea, thus ensuring the representativeness of the laboratory subsamples. Then, 0.8 g of dried tea leaves were added to 100 mL of ultrapure water to obtain the aqueous extracts. The mixture was boiled for 20 min, cooled to room temperature (22°C), filtered with Whatman® paper (8µm pore size) and stored at 4°C under light protection for further analysis.

2.2.1 Determination of total phenolic content and flavonoids

The total polyphenols content in the tea extract was analyzed by the reference method (Folin Ciocalteu) [15]. A calibration curve was constructed in the concentration range between 2.0 - 10 mg L^{-1} of gallic acid. Subsequently, 55 µL of the tea extract (8 mg mL^{-1}) were taken, mixed with 500 µL of the Folin-Ciocalteau reagent, 1.5 mL of the 100 mg mL^{-1} Na_2CO_3 solution and made up to a final volume of 10.00 mL with ultrapure water. After reacting for 2 h absorbance was measured at 765 nm using an UV-Vis spectrophotometer (Agilent Cary 60, Agilent Technologies, USA). The quantitative determination of flavonoids was carried out by the Zhishen's method based on the use of $AlCl_3$ [16]. A calibration curve (2.5 - 30 mg L^{-1}) was prepared by directly mixing 2.50 mL of 20 mg mL^{-1} solution with different volumes of a 277 mg L^{-1} quercetin working solution, to a final volume of 5.00 mL. In the case of the tea extract, 364 µL were taken and treated in the same way as the standard solutions. Standard solutions and tea extracts were left for reaction during 10 min and then quantification of flavonoids was performed at 415 nm.

2.3 Synthesis and characterization of AgNPs

AgNPs were easily obtained by adding 3.75 mL of the tea extract (8 mg mL^{-1}), 40 µL of 0.1000 M $AgNO_3$, 240 µL of 0.1 M NaOH, and 970 µL of ultrapure water in a glass tube. The mixture was sonicated with an ultrasound bath (BK-9050, 40 KHz, Baku, China) for 2 min (50W, 22°C) until a change in the color of the solution from colorless to brownish-yellow indicated the formation of AgNPs. Suspension of nanoparticles were kept at 4°C under light protection for further analysis.

The LSPR of AgNPs were analyzed by UV-Vis spectroscopy and spectra were collected in the range between 300 and 800 nm. Morphology and size of AgNPs were determined by transmission electron microscopy-TEM (JEOL 100 CX II microscope, Tokyo, Japan). Samples were prepared by placing a drop of fresh suspension on copper grids and dried at room temperature before analysis. The electrophoretic mobility and hydrodynamic size of nanoparticles were determined by dynamic light scattering (DLS) with a Zetasizer Nano ZS90 instrument (Malvern Instruments Ltd., Worcestershire, UK).

Because no additional steps were performed to isolate AgNPs from the reaction medium and considering highly colored suspensions were obtained, dilution of AgNPs with ultrapure water (1:3) and filtration with syringe membranes (Acrodisc, Gelma®, 0.2 µm) were required prior to analysis. Infrared spectroscopy (IR) measurements were carried out in the ranges between 4000 and 400 cm^{-1} and 4000 – 10000 cm^{-1} (4 cm^{-1} resolution), aiming to identify the functional groups which are distinctively bound on the surface of AgNPs. All spectra were recorded with a FTIR-NIR Nexus 470 Spectrophotometer (Thermo Scientific Nicolet iS50) and samples were prepared in KBr discs. To this end, AgNPs were decanted from the solution by adding 0.3 M K_2CO_3 and centrifuged during 60 min (7000 rpm), by following the reported procedure [17]. After drying the precipitate in a vacuum desiccator for 24 h, 2 mg of AgNPs were mixed with 100 mg of KBr, grounded into a fine powder and pressed in a mechanical press to prepare the mentioned discs.

2.4 Sample preparation

In order to evaluate the applicability of AgNPs for determination of TC in real samples, four commercially available honey samples were collected from different geographical locations (Fig. S1). Samples were subjected to a solid-liquid extraction procedure for isolation of TC [18]. For this purpose, 1.0 g of honey was accurately weighed and mixed with 6.0 mL of acetone. Then, it was immersed in an ultrasonic bath (50 W, 25°C) for 30 min to promote the extraction. This step was performed twice and supernatants were collected and evaporated to dryness (35°C, N_2). Dry extracts were suspended with 10.00 mL of McIvaine buffer solution (pH = 4.1) as recommended in the literature [19].

2.4.1 Solid phase extraction (SPE) procedure

Due to TC residues can be found in honey at concentrations lower than 1.0 mg L^{-1} and different co-existing substances are also present in the matrix, including fructose, glucose and sucrose, inorganic ions, among others, a solid phase extraction step (SPE) was included [20]. To this end, a C18 column (100 mg, Strata®, Phenomenex, USA) was used for the isolation and preconcentration of the analyte from sample extracts. The procedure adopted for SPE was implemented as follow: methanol (3.0 mL) and water (3.0 mL) were used for activation of the sorbent. In a second step 10.00 mL of TC solution or samples (pH 4.1) were pumped through the column (2 mL min^{-1}). The column was then dried with an air stream for 5 min and the retained analyte was finally eluted with

2.0 mL of a methanol: ammonia (90:10 v/v) mixture. The eluate was evaporated to dryness (35°C, N_2) and reconstituted in 2.00 mL of phosphate buffer (pH 5.8) for analysis.

2.5 Determination of TC by synthesized AgNPs

The synthesized AgNPs were evaluated for determination of TC by following the changes in the LSPR in presence of different concentrations of the analyte. To this purpose, reconstituted eluates were mixed with 200 µL of AgNPs and left for equilibration during 30 minutes. Absorbance measurements were recorded at 450 nm by using a 1.5 mL-quartz cuvette. Calibration curve was constructed in the range between 200 – 800 µg L^{-1} of TC.

3. RESULTS AND DISCUSSION

3.1 Synthesis of AgNPs by green tea extracts

3.1.1 Selection of tea aqueous extracts

Selection of the most suitable tea extract for synthesis of AgNPs was based on the polyphenols content. In fact, it has been previously demonstrated that spherical AgNPs with controllable size distribution can be obtained when varying the polyphenolic content [21]. The possible mechanism for reduction of Ag^+ to Ag^0 involves the ionization of polyphenols in alkaline conditions, followed by the transfer of one electron to Ag^+, resulting in the oxidation of tea polyphenols to quinone and generation of AgNPs [22]. However, there are several factors that affect the polyphenolic content and availability, including location, climate, variety, harvest and manufacturing conditions and process, among others. Hence, tea extracts were analyzed to choose that one with the highest total polyphenolic content (Table S1). In particular, extract n°5, obtained from green tea, showed the highest content of polyphenols. Flavonoids content was also assessed in this extract (22.0 ± 4.9 mg g^{-1}), which agreed with the mean values reported in the literature for good qualified green tea [23]. Hence, extract n°5 was selected as unique source of the reducing and capping agents involved in the synthesis of AgNPs.

3.1.2 Study of experimental conditions

Preliminary studies were performed to evaluate the effect of the green tea concentration in the reaction mixture on the synthesis of AgNPs under alkaline conditions. Three concentrations were tested (0.7, 3.4 and 6.0 mg mL^{-1}) following the

common conditions for biogenic synthesis of nanoparticles by green tea extracts [14, 24]. According to the obtained results, high intensity of LSPR was observed when the concentration of green tea was above 0.7 mg mL^{-1}, showing the favorable effect when the reducing compounds concentration increases (Fig. S2). Although the highest intensity was observed when working with tea at 3.4 mg mL^{-1}, associated with a high concentration of nanoparticles in the reaction solution, an increase in the concentration to 6.0 mg mL^{-1} allowed to obtain a narrow band, suggesting the presence of AgNPs with a narrow size distribution. As a compromise on these factors, we decided to set the concentration of green tea in the mixture to 6.0 mg mL^{-1} for the subsequent studies aiming to assure the reduction of silver ions while maintaining the satisfactory size distribution and stabilization of AgNPs in aqueous solution. The pH of solutions (6.0 and 10.0), contact time (1-10 min) and agitation mode (manual, vortex and ultrasounds) were also evaluated. It was observed that broad LSPRs, with a maximum at λ= 429 nm, were obtained when the synthesis was performed at pH 6.0, while the LSPRs showed a narrow distribution at alkaline conditions, with a maximum around λ= 415 nm, mainly attributed to monodisperse particles, being independent of the agitation mode (Fig. 1). These findings can be explained considering tea polyphenols are mostly in their ionized form under alkaline conditions, transferring one electron to Ag^{+} and being fast oxidized to quinone, thus leading to the generation of AgNPs with monodisperse distributions [24, 25].

Regarding the time dependence, AgNPs were obtained in the first 2 min by the assistance of vortex and ultrasound energy, but remaining stable when using ultrasounds (Fig. 1B). On the other hand, no significant changes on the LSPR that evidence the generation of AgNPs were noticed when the contact time was lower than 2 min (Fig. S3). Hence, the synthesis of AgNPs was performed with 6.0 mg mL^{-1} of green tea, under alkaline conditions and the assistance of ultrasound energy (50 W, 22°C) for 2 min.

3.2 Characterization of AgNPs

Synthesized AgNPs were characterized by TEM microscopy, FTIR-NIR spectroscopy and DLS. As showed in the TEM micrographs in Fig. 2, AgNPs obtained under optimal conditions (pH 10.0) resulted mostly spherical in shape with an average size of 8.5 nm ± 2.6 nm (n= 200). On the contrary, polyhedral nanoparticles with sizes around 50 nm were observed at pH 6.0. In fact, aggregation of particles can also be observed in the TEM image (Fig. 2A). Particularly, the statistical size distribution data

presented in Fig. 2B show a narrow size distribution that support the tendency observed in the LSPR of AgNPs under optimal conditions.

FTIR analysis was performed to evaluate the presence of bioactive compounds from the green tea extract on the surface of AgNPs synthesized under alkaline conditions (Fig. 3A). As observed in the figure, the broad band at 3292 cm^{-1} is associated with the O-H stretching vibration assigned to the hydroxyl group of polyphenols and the bands at 2930 and 2733 cm^{-1} are related to the C-H stretching of aromatic rings. The band at 1630 cm^{-1} could be associated with the C=O vibration of conjugated ketones, while the band at 1455 cm^{-1} is related to C-C stretch in aromatic compounds. Moreover, the band at 1040 cm^{-1} is connected to the stretching vibration of C-O-C [24]. The bands located between 900–600 cm^{-1} might be linked to the $–NH_2$ wagging of amine and amide groups, as recently stated by Hussein et.al. [26]. As complementary study, the analysis in the range between 4000 – 10000 cm^{-1} (2500 – 1000 nm) was also assessed by NIR spectroscopy (Fig. S4). Distinctive bands associated with the functional groups of tea polyphenols were evidenced at 4520 cm^{-1} (=CH stretching), 5189 cm^{-1} (O-H stretching first overtone) and 7243 cm^{-1} (C-H stretching) [27].

As showed in Fig. 3B and 3C, average hydrodynamic diameter of AgNPs was estimated by DLS and resulted about 23.7 nm (measured by triplicate), with a size distribution range between 10.1 and 37.8 nm. The zeta potential value was -43.4 mV at pH 5.8, being AgNPs negatively charged in the pH range between 3.4 – 9.0. Therefore, the high stability of AgNPs in aqueous solution may be attributed to the surface modification by the bioactive groups from the green tea extract [25]. In order to test the effective capping of particles by the phytocompounds, stability of AgNPs towards aggregation was evaluated for a period of time and under temperature effect. A slight band broadening was observed in the following 7 days after synthesis but remained for the next weeks, without significant changes on the position of the maximum LSPR, demonstrating the long-term stability of nanoparticles (Fig. S5). The effect of temperature was also evaluated in the range between 15 – 55°C immediately after synthesis of AgNPs and after 7 days (inset in Fig. S5). In both cases, no significant changes were noticed in the position of the maximum LSPR indicating the rapid reduction rate of Ag^+ and the high stabilization of particles by the bioactive compounds.

Table 1 shows a brief comparison between the AgNPs synthesized in the present work and those previously reported [24, 28 - 31]. As can be seen, the proposed method allows obtaining AgNPs with small particle size, being fully comparable with the published methodologies, even when conventional or green procedures are applied. Distinctively, in this work, the combined use of green tea as reducing and capping agent, and ultrasound energy results suitable for the synthesis of AgNPs in only 2 min. Special

attention can be paid on the differences between hydrodynamic size and zeta potential values for AgNPs synthesized by both, the borohydride-based and the proposed method, in the latter also suggesting an active role of the capping agent in controlling stability and size in aqueous solution.

Table 1. Comparison between AgNPs synthesized in the proposed work and those obtained from the literature

	Ref. [24]	Ref. [28]	Ref. [29]	Ref. [30]	Ref. [31]	**This work**
Synthesis parameters						
Reducing agent/capping	Green tea	*Persea americana* seed	*Berberis asiática* root	*Parkia Speciosa* leaves	$NaBH_4$	Green tea
Assistance	Mechanical stirring	Magnetic stirring	n.r.†	n.r.	Mechanical stirring	Ultrasound energy
Temperature (ºC)	RT ‡	RT	RT	25	n.r.	22
Time	15 min	5 h	4 h	24 h	30 min	2 min
TEM analysis						
Average diameter (nm)	3.9	50	9.8	35	12	8.5
Shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
DLS measurements						
Hydrodynamic size (nm)	34.7	n.r.	n.r.	155	2.87	23.7
Zeta potential (mV)	-35.5	n.r.	n.r.	-14.9	-10.1	-43.4
UV-Vis spectroscopy						
LSPR (nm)	410	430	427	410.5	390	415

† n.r.: not reported
‡ RT: room temperature

3.3 Evaluation of AgNPs for determination of TC

AgNPs were evaluated for determination of TC through changes in the LSPR under various pH conditions considering the species distribution of TC in aqueous solution and the surface charge of AgNPs. Under acidic conditions (pH = 2.5), AgNPs showed an initial decrease in the LSPR intensity (Fig. S6). This behavior can be associated with the partial loss of the capping agent and a release of Ag^+ ions by

dissolution processes, leading to the possible AgNPs aggregation and/or Ag^+ adsorption on the particle surface, particularly evidenced during long-term exposition [32]. Therefore, the interaction between AgNPs-TC was found unfavorable at acidic conditions, also considering TC exists as cation. In the same way, incomplete interaction between AgNPs and TC was evidenced when the pH was adjusted to 4.1 or set above 7.0, possible attributed to the co-existence of various TC species. On the contrary, intensity of LSPR was enhanced when the concentration of TC varied between 0 and 20 mg L^{-1} at pH 5.8 (Fig. S7). A possible explanation is the interaction between the oxidized polyphenols on the surface of AgNPs with the protonated dimethylamine group of the TC molecules, which can enhance the stability in aqueous solution [33]. However, a minor shift in the maximum LSPR to higher wavelengths was observed after interaction with the antibiotic, suggesting a particle size increase because of the change in the surrounding media without significant aggregation (Fig. 4). Similar behavior has been observed for the interaction between citrate-capped AgNPs with TC at concentrations lower than 22 mg L^{-1} under analogous experimental conditions [34].

Aiming to evaluate the interaction of AgNPs with other substances, cations and anions were also mixed with the nanoparticles suspension and the changes on the LSPR were registered. Zinc, aluminum and fluoride were selected considering their occurrence in honey may arise from environmental factors or can result from different anthropogenic activities and diclofenac was chosen as organic model containing amino and carboxylic moieties [35]. The change on the LSPR of nanoparticles in presence of these substances showed that the response of AgNPs toward TC was five-fold higher than that of the inorganic ions (Fig. S8). As a mention, and without considering the differences in concentration, the change on the LSPR in presence of TC was about eight time higher if compared with diclofenac. In this case, diclofenac presents a pKa value around 4.2 and exists as anion at the working pH value, which can affect the interaction with the negatively stabilized AgNPs, as similarly noticed with fluoride.

3.3.1 SPE procedure

Considering the complexity of honey matrix, a SPE was included for the pretreatment of samples. SPE procedures based on C18 sorbents have been previously used for extraction and preconcentration of TC from honey samples [36]. In this work, a C18 cartridge was employed and the extraction conditions, including pH of sample extracts, composition and volume of the elution solvent, were evaluated as shown in Table 2.

Table 2. Optimization of the variables for SPE procedure

Parameter	Evaluated	Optimal condition
Sample pH	4.0 - 6.0	4.1
Elution solvent	Methanol Methanol / ammonium hydroxide Methanol / acetic acid	Methanol / ammonium hydroxide
Acid or base content (% v/v)	10 – 50	10
Elution volume (mL)	1.0 – 6.0	2.0

As previously mentioned TC is an amphoteric molecule, which ionization forms are strongly affected by the pH of the medium. Here, the pH of samples was studied and satisfactory retention of TC was obtained at pH 4.1. Considering the characteristics of the adsorbent and the physicochemical properties of this analyte, methanol was selected as elution solvent and its performance was evaluated at different pH conditions and volumes. It was observed that a mixture containing 10% (v/v) of ammonium hydroxide provided a higher extraction efficiency. At alkaline conditions, TC remains negatively charged, thus decreasing its affinity to the adsorbent. Finally, the effect of the elution volume was studied in the range between 1.0 and 6.0 mL. The extraction of TC was not complete when using 1.0 mL, while the recovery was maximum with 2 mL of elution solvent and then remained almost constant for higher volumes. Therefore, satisfactory results were obtained when the pH of samples or standards were set at 4.1 (± 0.1) with McIlvaine buffer, and then using 2.0 mL of methanol with 10% (v/v) ammonium hydroxide as the elution solvent.

3.3.2 Analytical performance

The performance of AgNPs for determination of TC was evaluated by following the changes in the LSPR at 450 nm when increasing the TC concentration in phosphate buffer (pH= 5.8). The calibration curve was constructed in the concentration range between 200 and 800 µg L^{-1}. The equation of the calibration curve was Y = (0.1537 ± 0.0053) X + (0.0803 ± 0.0029), with R^2 = 0.9964. The limit of detection (LOD), calculated at 3 $S_{y/x}$/slope, was 52.7 µg L^{-1} [37]. Intermediate precision, expressed as relative standard deviation (RSD%), was 6.22% by analyzing a 300 µg L^{-1} TC solution (n = 5). Compared with the existing analytical data, the obtained LOD value meet those from the literature, resulting even lower than that from electrochemical and spectrophotometric analyses

(Table 3)[8,18,38-41]. Moreover, recovery rates and RSD values are in agreement with those reported when more sophisticated methodologies were applied, mainly liquid chromatography coupled to UV detection or mass spectrometry, thus supporting the reliability and validity of the AgNPs as chemical sensor.

Table 3. Comparison of methods for determination of TC in honey samples

Method	Probe	Linear range	LOD	RSD (%)	Recovery (%)	Ref.
Electrochemical	Antimony film electrode	178 –1.33x10^3 µg L^{-1}	67.0 µg L^{-1}	0.41 - 8.23	91.81 – 109.7	18
Colorimetric	Aptamer-AuNPs	17.8 – 311 µg L^{-1}	12.4 µg L^{-1}	-	-	38
Fluorescent	BCDs-Eu/CMP-cit †	22.2 – 1.33x10^4 µg L^{-1}	3.50 µg L^{-1}	2.4	94.2	8
Spectrophotometric	Yttrium (III) - complex	4.44x10^3 – 1.78x10^5 µg L^{-1}	2.18x10^3 µg L^{-1}	1.5 - 3.3	99.8 – 100.6	39
HPLC-UV	-	50.0 – 1.00x10^4 µg L^{-1}	16.1 µg L^{-1}	4.5 – 6.7	71.2 – 82.3	40
LC-MS/MS	-	0.100 – 100 µg L^{-1}	0.66 µg L^{-1}	7.9 – 14.8	87.4	41
LSPR	AgNPs	200 – 800 µg L^{-1}	52.7 µg L^{-1}	6.22	82.8 - 116	**This work**

† BCDs-Eu/CMP-cit: carbon quantum dots (BCDs) and cytidine monophosphate (CMP)/europium coordination polymer nanoparticles

3.4 Analysis of honey samples

To evaluate the applicability of the proposed method for determination of TC, four commercially available honey samples, representative of different geographical locations in Argentina, with climate and flora diversity, were selected and prepared according to the protocols described in Section 2.4 [42]. Under optimal experimental conditions, TC was not detected in the samples about the detection limit. The accuracy of the method was further investigated by determining the recovery rates of the target analyte in the spiked samples at two levels, according to the reported amounts of this antibiotic in honey from treated hives [43]. For this purpose, known amounts of TC were added to the samples before any pretreatment, and then subjected to the whole procedure. As shown in Table

4, satisfactory recovery values were obtained in the range between 82.8 and 116 % with RSD values less than 6.69% for all samples. It is noteworthy that the recovery rates are comparable to those obtained when using an imprinted monolithic sorbent for selective isolation of TCs coupled to HPLC-DAD [40]. Moreover, the results obtained in the present study meet the acceptability criteria for analysis of contaminants in food from animal origin [44].

Table 4. Recovery study of TC in honey samples

Samples	Added ($\mu g\ L^{-1}$)	Found † ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%)
S1	0	-	-	-
	300	313	104	5.39
	600	630	105	2.38
S2	0	-	-	-
	300	249	82.8	6.69
	600	690	115	0.93
S3	0	-	-	-
	300	347	116	6.09
	600	580	96.7	6.38
S4	0	-	-	-
	300	287	95.8	3.52
	600	550	91.6	4.61

† mean of 3 replicates

4. CONCLUSION

A straightforward and cheap method was proposed for the synthesis of AgNPs only based on green tea extracts as reducing and stabilization agent and low-frequency ultrasound irradiation, at 22°C during 2 minutes. Polyphenols from green tea are marked as responsible for abatement of aggregation of nanoparticles in aqueous solution. TEM analysis revealed spherical in shape AgNPs with an average size lower than 10 nm. In addition, DLS measurements showed a hydrodynamic size around 23.7 nm and zeta potential values about -43.4 mV, confirming the high stability of AgNPs. The developed strategy provides environmental and economic benefits, being two important issues to be addressed for the synthesis of nanomaterials. Based on the LSPR changes at 450 nm, the AgNPs were directly applied for determination of tetracycline residues in honey samples. The analysis was carried out after an SPE procedure to assure the clean-up of samples and preconcentration of the analyte. The obtained results demonstrate the satisfactory application of the AgNPs as chemical sensor, with acceptable LOD value

and recovery rates between 82.8 and 116%, being comparable with those values reported when more sophisticated methodologies were applied. Hence, this approach represents a low-cost way to address the need of TC determination in honey with minimal instrumentation required.

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Figure Legends

Figure 1. Synthesis of AgNPs at (A) pH= 6.0, and (B) pH= 10.0. Dashed line, manual agitation; Dotted line, ultrasound energy; Continuous line, vortex agitation. (Inset: Effect of contact time during synthesis)

Figure 2. TEM images of synthesized AgNPs at (A) pH= 6.0 and (B) pH= 10.0. Insets: size distribution

Figure 3. Characterization of AgNPs by (A) FTIR spectroscopy, (B) hydrodynamic size (three single measurements) and (C) zeta potential vs pH

Figure 4. LSPR of AgNPs in presence of various concentrations of TC, mg L^{-1}: a) 0; b) 3.0; c) 10; d) 15 and e) 20. AgNPs, 200 µL; pH 5.8; volume, 2.00 mL

Table 1. Comparison between AgNPs synthesized in the proposed work and those obtained from the literature

	Ref. [24]	Ref. [28]	Ref. [29]	Ref. [30]	Ref. [31]	**This work**
Synthesis parameters						
Reducing agent/capping	Green tea	*Persea americana* seed	*Berberis asiática* root	*Parkia Speciosa* leaves	$NaBH_4$	Green tea
Assistance	Mechanical stirring	Magnetic stirring	n.r.†	n.r.	Mechanical stirring	Ultrasound energy
Temperature (ºC)	RT ‡	RT	RT	25	n.r.	22
Time	15 min	5 h	4 h	24 h	30 min	2 min
TEM analysis						
Average diameter (nm)	3.9	50	9.8	35	12	8.5
Shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
DLS measurements						
Hydrodynamic size (nm)	34.7	n.r.	n.r.	155	2.87	23.7
Zeta potential (mV)	-35.5	n.r.	n.r.	-14.9	-10.1	-43.4
UV-Vis spectroscopy						
LSPR (nm)	410	430	427	410.5	390	415

† n.r.: not reported
‡ RT: room temperature

Table 2. Optimization of the variables for SPE procedure

Parameter	Evaluated	Optimal condition
Sample pH	4.0 - 6.0	4.1
Elution solvent	Methanol Methanol / ammonium hydroxide Methanol / acetic acid	Methanol / ammonium hydroxide
Acid or base content	10 – 50 % (v/v)	10 % (v/v)
Elution volume (mL)	1.0 – 6.0	2.0

Table 3. Comparison of methods for determination of TC in honey samples

Method	Probe	Linear range	LOD	RSD (%)	Recovery (%)	Ref.
Electrochemical	Antimony film electrode	178 –1.33x10^3 µg L^{-1}	67.0 µg L^{-1}	0.41 - 8.23	91.81 – 109.7	18
Colorimetric	Aptamer-AuNPs	17.8 – 311 µg L^{-1}	12.4 µg L^{-1}	-	-	38
Fluorescent	BCDs-Eu/CMP-cit †	22.2 – 1.33x10^4 µg L^{-1}	3.50 µg L^{-1}	2.4	94.2	8
Spectrophotometric	Yttrium (III) - complex	4.44x10^3 – 1.78x10^5 µg L^{-1}	2.18x10^3 µg L^{-1}	1.5 - 3.3	99.8 – 100.6	39
HPLC-UV	-	50.0 – 1.00x10^4 µg L^{-1}	16.1 µg L^{-1}	4.5 – 6.7	71.2 – 82.3	40
LC-MS/MS	-	0.100 – 100 µg L^{-1}	0.66 µg L^{-1}	7.9 – 14.8	87.4	41
LSPR	AgNPs	200 – 800 µg L^{-1}	52.7 µg L^{-1}	6.22	82.8 - 116	**This work**

† BCDs-Eu/CMP-cit: carbon quantum dots (BCDs) and cytidine monophosphate (CMP)/europium coordination polymer nanoparticles

Table 4. Recovery study of TC in honey samples

Samples	Added ($\mu g\ L^{-1}$)	Found † ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%)
S1	0	-	-	-
	300	313	104	5.39
	600	630	105	2.38
S2	0	-	-	-
	300	248	82.8	6.69
	600	690	115	0.93
S3	0	-	-	-
	300	347	116	6.09
	600	580	96.7	6.38
S4	0	-	-	-
	300	287	95.8	3.52
	600	550	91.6	4.61

† mean of 3 replicates