Article

Carbon Dots from *Pilosocereus gounellei* for Fluorimetric Determination of Tannin in Tea Using a Flow-Batch System

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In this study, xiquexique (*Pilosocereus gounellei*) native endemic cactus from the Brazilian semiarid was used for the first time as a natural carbon source ("green precursor") for the synthesis of highly fluorescent carbon dots (CDs). These CDs were successfully used to develop a fast, low-cost, eco-friendly fluorescence method for the determination of tannins in teas. This method was automatized employing a flow-batch system coupled to an inexpensive ultraviolet light-emitting diode (UV-LED) used as an excitation source and a cheap handheld spectrometer used as a detector. CDs were characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), UV-Vis, and fluorescence spectroscopy. The proposed method presented a linear range from 2.0-30.0 mg L^{-1}, relative standard deviation (RSD) < 3.0%, limit of detection (LOD) = 0.102 mg L^{-1}, and recovery rates from 90.5-109.1%. A paired *t*-test at a 95% confidence level indicated no statistically significant difference between the proposed and reference methods, revealing that it is a useful alternative for the determination of tannins.

Keywords: carbon dots, *Pilosocereus gounellei*, flow-batch analyzer, UV-LED, tannins

Introduction

Tannins (TA) are natural phenolic compounds commonly found in fruits, coffees, wines, and teas. They present antioxidant, antiangiogenic, antimutagenic, and anticarcinogenic properties, and health benefits when taken in doses at safe levels.[1-4] Adversely, high consumption of TA may cause incidences of cancers, immune response, hepatotoxicity, lipid metabolism, and antinutritional activities, such as the reduction in digestive enzymatic activities.[4-6] TA has been used as a food additive; a safe dosage should be maintained in the range of 10 to 400 mg L^{-1} in food products.[6]

TA content has been determined using non-automatic spectrophotometric,[7] chromatographic,[8] electrochemical,[9] diffuse reflectance,[10] automatic spectrophotometric,[11] and spectrofluorimetric methods.[12,13] The ferrous tartrate and Folin-Ciocalteu spectrophotometric methods have been respectively adopted by Japan[14] and the Association of Official Analytical Chemists (AOAC)[15] as the official methods for quantification of TA in teas. The ferrous tartrate method has been successfully automatized using the flow-batch analysis system.[16,17]

Carbon dots (CDs) are a class of promising fluorescent nanomaterials used in analytical applications due to their distinct properties, including high stability, excellent water solubility, good biocompatibility, ease of synthesis, and functionalization.[18] CDs have been generally synthesized by a hydrothermal carbonization process using various natural resources, including fruits, seeds, leaves, and grains.[19] This use of natural, readily available carbon sources ("green precursor") has attracted the attention of researchers due to its low cost, nontoxic nature, and eco-friendly process.[20] Besides, CDs when doped with heteroatoms, especially N, exhibit enhancement of their fluorescent properties, including high quantum yield, and adjustment of their electronic and chemical properties.[1,3,21] Various hypotheses have been reported to explain the fluorescence mechanism behavior of CDs, including

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the effects of quantum confinement, quantum size and crosslink-enhanced emission, and surface and molecule states.[22]

CDs and fluorescent detection have been also combined to develop high sensitivity methods for the TA determination in beer,[1] wine,[2,23] and water.[6,23,24] However, to the best of our knowledge, only one work[6] has been described for TA determination using CDs synthesized from green resources and with spectrofluorimetric detection; this method is indirect, however, needs the previous addition of a Fe[III] solution, and uses a high-priced benchtop spectrofluorometer which employs an expensive xenon lamp as the source of excitation. In addition, this non-portable instrument is also unsuited for field applications. To overcome these inconveniences, a high-intensity ultraviolet light-emitting diode (UV-LED)[25,26] and a cheap handheld spectrometer,[27] both coupled to a notebook, can be used as a stable monochromatic excitation source and fluorimetric detector.

In this study, CDs, an inexpensive UV-LED, and a cheap handheld spectrometer were used for the first time to develop an analytical method based on fluorescence quenching for TA determination in tea. High fluorescent CDs were synthesized via a hydrothermal carbonization process using xiquexique (*Pilosocereus gounellei*), a native endemic cactus from the Brazilian semiarid,[28] as a promising carbon source from nature ("green precursor"). To increase the quantum yield, ethanolamine was chosen as the functionalizing agent to dope CDs with N. To decrease the consumption of reagents, time, and cost analysis, as well as to improve precision and accuracy, the proposed method was automatized using a flow-batch analysis (FBA) system.[27]

The advantage of this cactus plant (*Pilosocereus gounellei*) is that it is strongly resistant and easily found in semiarid regions. Moreover, xiquexique cladodes are composed of carbohydrates, proteins, amino acids, ascorbic acid, flavonoids, glucose, sucrose, and fructose,[29] which acts as excellent precursors for the synthesis of carbon dots.[30] In addition, the production of CDs from xiquexique can convert low-value biomass into valuable and useful materials for the semiarid region, encouraging the establishment of sustainable applications.

Experimental

Reagents, solutions, and samples

The reagents used in this work were of analytical grade, and all solutions were prepared using fresh distilled-deionized water (> 18 MΩ cm).

For the synthesis of the fluorescent CDs, the xiquexique (*Pilosocereus gounellei*) cladodes were collected from the semiarid region located near the Ipanguaçu City, Rio Grande do Norte, Brazil. Before use, xiquexique cladodes had their spikes removed, and after were washed twice with distilled water, and stored at 5 °C. Ethanolamine was purchased from Sigma-Aldrich (Saint Louis, USA). The quinine sulfate used as fluorescence standard was acquired from Acros Organic (Geel, Belgium).

To build the analytical curve, the tannic acid was chosen as appropriate water-soluble and stable standard for analysis of tannins. Most of the methods available use this standard for determining tannins.[1-3,6-16,23,24,31,32] A stock solution of tannic acid (1000 mg L^{-1}) was prepared daily by the dissolution of solid standard (Synth, Diadema, Brazil) in deionized water. The working solution of tannic acid (50 mg L^{-1}) was freshly prepared by diluting the stock solution with deionized water. The standard solutions of tannic acid ranging from 2.0 to 30.0 mg L^{-1} were automatically prepared in the FBA system from adequate dilutions of the working solution.

A 0.1 mol L^{-1} phosphate buffer solution (pH 8.0) was prepared by adding 50.0 mL of 0.1 mol L^{-1} potassium dihydrogen phosphate (Synth, Diadema, Brazil) and 46.1 mL of 0.1 mol L^{-1} sodium hydroxide (Synth, Diadema, Brazil) in a 250.0 mL volumetric flask, and completed with deionized water.

For reference method, a ferrous tartrate reagent solution was prepared by dissolving 1.0 g of heptahydrate ferrous sulfate (Vetec, São Paulo, Brazil), 5.0 g of potassium sodium tartrate (Vetec, São Paulo, Brazil), and 0.1000 g of sodium bisulfite (Vetec, São Paulo, Brazil) in water to 1.0 L. A 0.1 mol L^{-1} phosphate buffer solution (pH 6.9) was prepared by adding 50.0 mL of 0.1 mol L^{-1} potassium dihydrogen phosphate (Synth, Diadema, Brazil) and 22.4 mL of 0.1 mol L^{-1} sodium hydroxide (Synth, Diadema, Brazil) in a 250.0 mL volumetric flask, and completed with deionized water.

Six tea samples (green and black tea) from different manufacturers were purchased from local retail suppliers in João Pessoa, Paraíba, Brazil. These samples were prepared according to a procedure developed elsewhere:[16] about 2.0 g of tea was added to 50.0 mL of deionized water and heated at 90 °C for 10 min. After cooling, the mixture was filtered and completed to 100.0 mL with deionized water.

Apparatus

The fluorescence measurements were performed using a cheap handheld spectrometer (Ocean Optics, model USB4000, Orlando, USA) as the detector, and a UV-LED

(Lite-on, model LTPL-C034UVH370, Shanghai, China) as the excitation source at the 370 nm wavelength.

The optical characterization of CDs was performed employing a Cary Eclipse fluorescence spectrophotometer (Agilent, model G9800A, California, USA) equipped with a 1.0 cm × 0.5 cm quartz cell with slit widths set at 10/10 nm, and a UV-Vis spectrophotometer (Hewlett-Packard/Agilent, model 8453, California, USA) equipped with a 1.0 cm × 1.0 cm quartz cuvette. The structural characterization was carried out using a Fourier transform infrared (FTIR), spectrophotometer (Shimadzu, model IR Prestige-21, Kyoto, Japan), X-ray diffraction (XRD, Shimadzu, model XRD 6000, Kyoto, Japan), and transmission electron microscopes-TEM (Jeol, JEM-2100, Tokyo, Japan). For the TEM measurements, the sample was prepared by dropping diluted CDs solution onto a carbon-coated copper grid. The synthesis of the CDs was carried out in a muffle furnace (Edg, model 7000, São Carlos, Brazil) equipped with a 100.0 mL Teflon-lined stainless-steel autoclave. Then, the unwanted particulate material was separated by centrifugation (Hermle, model Z206A, Wehingen, Germany).

Synthesis of CDs

The fluorescent CDs were synthesized using the xiquexique cladode juice as a renewable inexpensive and natural carbon source and ethanolamine as the nitrogen source. For this purpose, the following one-step hydrothermal method was employed: 100 g of xiquexique cladode was cut into small pieces, mixed with 100.0 mL of deionized water, and blended using a kitchen food multiprocessor (Black & Decker, model HC31x, Shanghai, China). After filtering, 30.0 mL of pulp-free xiquexique juice was mixed with 3.0 mL of ethanolamine and 7.0 mL of deionized water. This mixture was transferred into a 100.0 mL Teflon-lined stainless-steel autoclave, heated at a constant temperature of 200 ºC for 8 h, and cooled to room temperature. The resulting brownish dark mixture was filtered through a quantitative filter paper, centrifuged at 6000 rpm for 20 min, and dialyzed against deionized water through a dialysis tubing cellulose membrane (Sigma-Aldrich, MWCO 1 kDa, Saint Louis, USA) for 72 h to remove small molecules of unreacted raw materials and by-products. Finally, a CDs powder was obtained by evaporation, re-dispersed in deionized water, and kept at 4 °C for further analysis.

Heating at 200 ºC for a long time is not energy friendly. However, the CDs synthesis involves dehydration and carbonization and the temperature range between 100 to 350 ºC is commonly used.[21] On the other hand, xiquexique cladode is a very cheap and abundant natural precursor in the semiarid region.

## Fluorescence quantum yield of CDs

The fluorescence quantum yield (FQY) of the CDs was determined by a well-established comparative method[1,21] using as a fluorophore reference a quinine sulfate (QS) solution in 0.1 mol L^{-1} H$_2$SO$_4$ (FQY$_{QS}$ = 54%). The FQY$_{CDs}$ value was calculated according to the following equation: FQY$_{CDs}$ = FQY$_{QS}$ × (Grad$_{CDs}$/Grad$_{QS}$) × (η_{CDs}/η_{QS})^2, where Grad is the slope of the emission intensity (excitation at 370 nm) *versus* absorbance curves and η is the refractive indexes of CDs and QS. To prevent the re-absorption effect, absorbances of CDs and QS solutions were adjusted to less than 0.05.[1,21]

Flow-batch analysis (FBA) system

A schematic diagram of the FBA system used for the fluorimetric determination of tannin in teas is presented in Figure 1. This system consists of a 2 mL mixing chamber (MC) in Teflon® with three quartz windows (W1-W3) mounted at 180 and 90° from each other; a cheap handheld spectrometer coupled to the window W1 and a UV-LED with its heat sink coupled to the window W2; an eight-channel peristaltic pump (Ismatec, model MCP-Z, Wertheim, Germany) equipped with pumping Tygon® tubes; five three-way solenoid valves (Cole Parmer, model EW-01540-13, Vernon Hills, USA); a magnetic stirrer (IKA, model Lab Disc White, Staufen, Germany); and a stirring bar (diameter: 2.0 mm; length: 5.0 mm) inside the MC. Teflon® tubes with 1.0 mm internal diameter were used for transporting fluids.

The spectrometer, UV-LED, magnetic stirrer, and MC were mounted inside a black box (10.0 cm × 12.0 cm × 15.0 cm) for minimizing the effects of spurious environmental radiation during analysis. The flow rates of 87.8 ± 0.6, 87.7 ± 1.5, 78.8 ± 1.1, 84.2 ± 1.2, and 257.7 ± 3.6 μL s^{-1} (n = 20) were respectively employed for CDs (1.2 mg mL^{-1}), pH 8.0 phosphate buffer, working standard solutions (50 mg L^{-1} of tannic acid) or samples, water, and waste.

The FBA system was computer-controlled using software developed in LabVIEW® 2013 graphical programming language (National Instruments, Austin, USA), a USB interface (National Instruments, USB6009, Austin, USA), and a lab-made electronic actuator to switch ON/OFF three-way solenoid valves. The circuit of the lab-made electronic actuator was based on an integrated circuit ULN2803 as previously described elsewhere.[33]
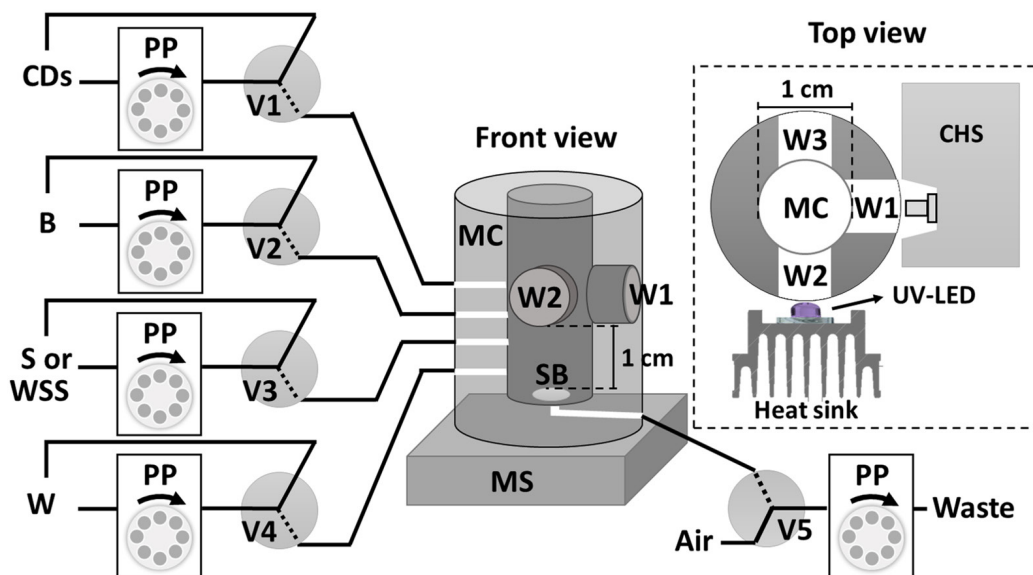
Figure 1. Schematic diagram of the FB system. CDs: carbon dots solution (1.2 mg mL^{-1}); B: 0.10 mol L^{-1} KH$_2$PO$_4$/0.10 mol L^{-1} NaOH phosphate buffer (pH 8) solution; S or WSS: sample or working standard solution (50 mg L^{-1} of tannic acid); W: deionized water; V1-V5: three-way solenoid valves; PP: peristaltic pump; MC: flow-batch mixing chamber; W1-W3: quartz windows; MS: magnetic stirrer; SB: stirring bar; UV-LED: high-intensity ultraviolet light-emitting diode (λ_{max} = 370 nm) and CHS: cheap handheld spectrometer.

Automatic analytical procedure

Before any analysis, all working solutions are pumped and re-circulated to their respective reservoirs. After the (V1-V4) solenoid valves are switched ON for 5 s, pumping the working solutions and filling the channels located between valves of and the MC. Solutions inside the MC are then removed when the V5 solenoid valve is switched ON for 15 s. Subsequently, the MC cleaning step is performed by switching the V4 valve ON for 21.4 s. Immediately after, the V5 valve is switched ON for 22.8 s to empty the MC. The channel filling and cleaning steps should be always performed whenever CDs, buffer, working standard solutions, or sample is changed.

The steps of the automatic analytical procedure for the determination of tannin in teas are shown in Table 1. Initially, valves V1, V2, V3, and V4 are simultaneously switched ON to add CDs (1.2 mg mL^{-1}), buffer (0.10 mol L^{-1} KH$_2$PO$_4$/0.10 mol L^{-1} NaOH phosphate buffer pH 8.0), sample, or WSS (50 mg L^{-1} of tannic acid), and water into the MC (step 1). The times for valves V3 and V4 to be switched ON and, consequently, the volumes of WSS and water to be added may vary according to the standard solution of the analytical curve being prepared. After the mixing time (step 2) for homogenization of the mixture into MC, the analytical signal is measured (step 3). Finally, valve V5 is switched on to empty the MC (step 4). Before starting to analyze the next sample, a cleaning procedure is carried out in triplicate by switching ON valve V4 to add water to the MC (step 5) and then valve V5, to empty the MC (step 6). The blank measurement procedure is performed in a similar way (steps 1 to 6), except that valve V3 is not switched ON.

Table 1. Steps of the automatic analytical procedure

Step	Description	V1	V2	V3	V4	V5	Volume / µL	time / s
	additions of:							
	CDs						250	2.8
1	B	on	on	on	on	off	300	3.4
	S and W						48 and 1202	0.6 and 14.3
	or						or	or
	WSS and W						72-1080 and 1178-170	0.9-13.7 and 14.0-2.0
2	mixing	off	off	off	off	off	–	5.0
3	measurement	off	off	off	off	off	–	5.0
4	emptying MC	off	off	off	off	on	1800	22.8
5[a]	addition of water	off	off	off	on	off	1800	21.4
6[a]	emptying MC	off	off	off	off	on	1800	22.8

[a]Steps 5 and 6 are carried out in triplicate. CDs: carbon dots; MC: mixing chamber; W: quartz windows; S or WSS: sample or working standard solution.

Reference method

For comparison with the proposed method, the teas were also analyzed by the official Japanese ferrous tartrate method.[14,34] For this purpose, standard solutions of tannic acid ranging from 2.0 to 30.0 mg L^{-1} were prepared by adding appropriate volumes of stock solution of the tannic acid (1000 mg L^{-1}) in water. Samples or standard, phosphate buffer (pH 6.9), and the ferrous tartrate reagent solutions were mixed and, after 10 min of reaction, the absorbance of these solutions was measured at 550 nm. The concentrations of tannic acid in tea samples were determined using the estimated equation of the analytical curve.

Results and Discussion

Morphological and structural characterization and optical properties of CDs

Morphology, structure, size distribution, and surface functional groups of the CDs were investigated using TEM, XRD and FTIR. The surface morphology of the CDs was investigated by TEM. For the TEM measurements, the sample was prepared by dripping 0.3 mg mL^{-1} CDs solution onto a carbon-coated copper grid. The TEM image (Figure 2a) shows that the CDs exhibit a dispersion without aggregation and a structure nearly spherical with an average diameter (Figure 2b) of around 3.5 nm. The XRD pattern of the CDs (Figure 2c) had a wide diffraction peak centered at 22°, which corresponds to the amorphous structure of CDs.

FTIR was used to evaluate the functional groups of the CDs. As shown in Figure 2d, the absorption bands in the region of 2900-3400 cm^{-1} were attributed to the stretching vibrations of N–H, O–H, and C–H bonds.[32] The peaks centered in 1600 and 1400 cm^{-1} were caused by C=C stretching vibration and the vibration C–N groups.[3] Besides, the peak at 1050 cm^{-1} was assigned to the stretching of C–N groups.[35]

To investigate the optical properties of CDs, the UV-Vis absorption, excitation, and emission spectra of 0.3 mg mL^{-1} CDs solution were recorded. The UV-Vis absorption spectrum (black line) exhibits absorption peaks at 280 and 340 nm (Figure 2e), which were attributed to the typical absorption for the π–π* and n–π* transitions of groups such as C=O or C=C.[21] The bright blue color under UV light (Figure 2b) of CDs occurs because the fluorescence spectra (Figure 2e) exhibit the maximum excitation at 370 nm (red line) and emission at 460 nm (blue line).

The excitation-dependent photoluminescence behavior of CDs can be observed in the fluorescence spectra of the CDs recorded under different excitation wavelengths from 320 to 410 nm with 10 nm increments (Figure 2f). The fluorescence emission peak of CDs gradually shifted to higher wavelengths. This phenomenon is frequently attributed to surface defects and particle size distributions of CDs.[2,21] The calculated quantum yield of CDs was 13.6% against quinine sulfate as the fluorophore reference, which is quite satisfactory when compared to other studies that used biomass for the synthesis of CDs.[19]

### Choice of pH and CDs amount for TA determination

To choose the optimal pH for TA determination in teas, the TA concentration and volume of the 1.2 mg mL^{-1} CDs solution were fixed at 10.0 mg L^{-1} TA and 250 μL. pH was changed from 4.5-9.6 and fluorescence response (FR) was calculated according to the following equation: $FR = (F_0 - F)/F_0$, where F_0 and F are the fluorescence intensities of CDs at 460 nm in the absence and presence of TA, respectively. As can be seen in Figure 2g, the maximum fluorescence response was obtained at pH = 8 and this was then taken as the optimum value.

The amount of CDs usually influences the fluorescence response. To select the optimal volume of the 1.2 mg mL^{-1} CDs solution, the pH was fixed at 8, TA concentration at 10.0 mg L^{-1}, and volume was changed from 150 to 450 μL. The fluorescence response was maximum (Figure 2h) using a volume equal to 250 μL as the optimum value for the proposed FBA method.

Effects of potentially interfering species on the CDs-tannin reaction have been studied in previous papers.[1,2,6,23,24] It was found that cations, anions, organic acids, phenols (gallic acid and others), amino acid, substances containing aromatic moieties and other species that might co-exist in the analyzed samples do not appreciably affect the analytical signal under the chemical analysis conditions of those works.

Analytical curve, sample analysis, and recovery study

The fluorescence intensity of CDs decreases gradually with the increase in the concentration of TA in the range from 2.0 to 30.0 mg L^{-1}. The analytical curve exhibited a satisfactory linear relationship between fluorescence response (FR) and TA concentrations. The estimated regression equation was $FR = (0.20 \pm 0.02) \times 10^{-2} + (0.30 \pm 0.02) \times 10^{-2} C$ (in mg L^{-1}), yielding a linear correlation coefficient, $(r^2) = 0.996$. Limits of detection (LOD) and quantification (LOQ) calculated at $3s/\beta$ and $10s/\beta$ (s is the standard deviation of the blank and β is the slope of the analytical curve)[36] were respectively equal to 0.102 and 0.304 mg L^{-1}. To evaluate the fit to a linear model
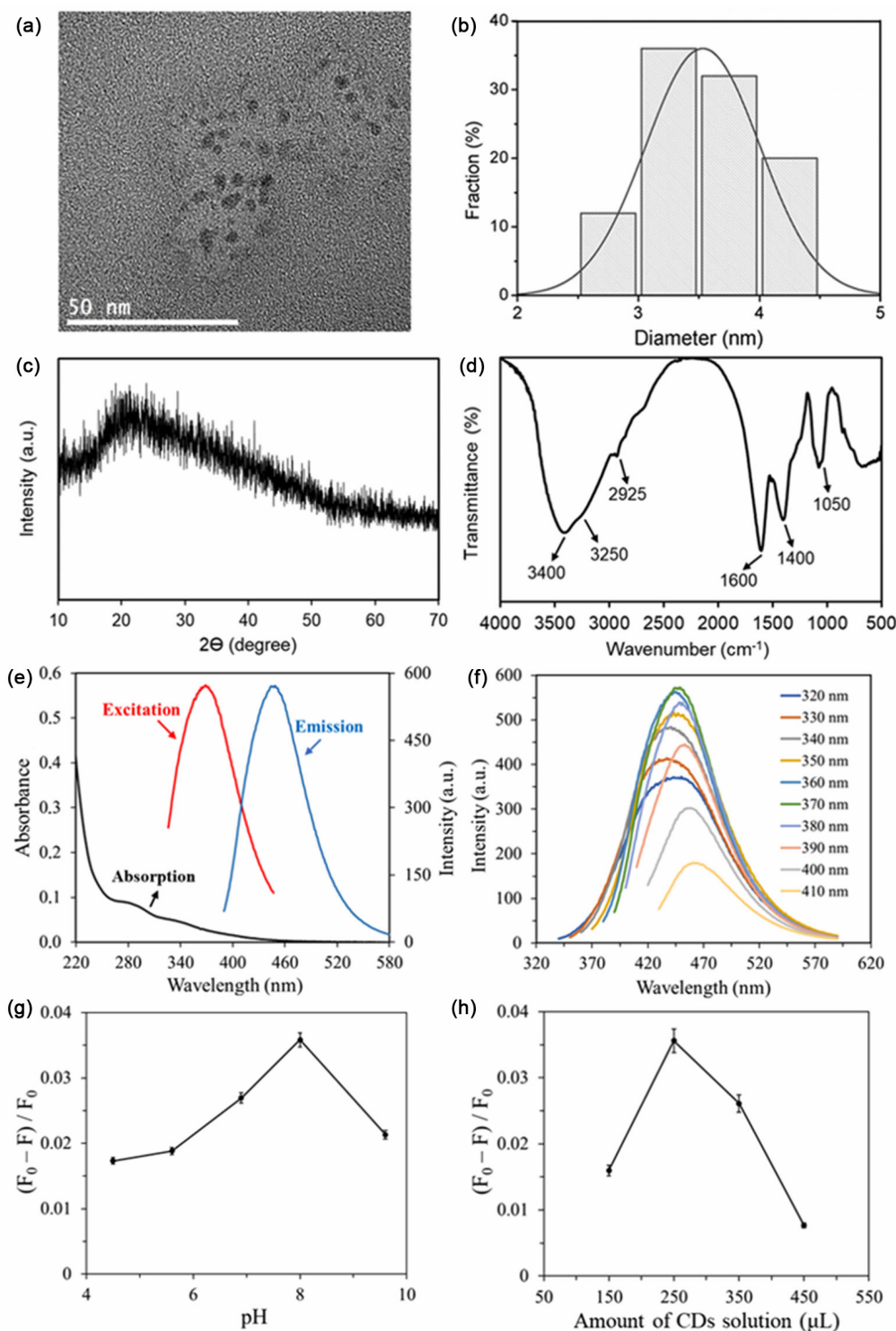
Figure 2. (a) TEM image, (b) the particle size distribution, (c) XRD pattern, (d) FTIR spectrum, (e) UV-Vis absorption, excitation, and emission (fluorescence) spectra of 0.3 mg mL^{-1} CDs solution. (f) Fluorescence emission spectra of 0.3 mg mL^{-1} CDs solution under different excitation wavelengths from 320 to 410 nm with 10 nm increments. (g) Fluorescence response *versus* pH and (h) volume of the 1.2 mg mL^{-1} CDs solution.

of the analytical curve, an analysis of variance (ANOVA) was carried out according to recommendations described elsewhere.[37] The linear regression was considered unbiased and valid for the estimation of TA concentrations in tea samples since the linear regression was significant. The residual plot presented a random distribution approaching zero and there was no evidence of lack of fit for the linear model at a 95% confidence level.

The concentration of TA in green and black tea samples from different brands was quantified using the proposed

FBA and the official Japanese ferrous tartrate methods.[14] The results are presented in Table 2. No statistically significant differences were observed among the results when applying the paired *t*-test at a confidence level of 95%. The relative standard deviation (RSD%) was less than 2.9% (n = 3), revealing that the developed method using CDs of *Pilosocereus gounellei* is a useful alternative for the determination of TA in tea.

The accuracy of the proposed method was evaluated using the recovery studies. For this purpose, standard solutions of TA (5.0, 10.0, and 15.0 mg L^{-1}) were added

employing authentic triplicates in green and black teas. As can be seen in Table 2, the recovery values were found to be in the range from 90.5-109.1% with a standard deviation of less than 2.7%, which indicated the reliability of the proposed method.

Analytical features of the proposed FBA and other methods

A comparative study among the proposed with other methods which use CDs for the determination of TA[1-3,6,23,24,32] was carried out. As can be seen in Table 3, the proposed

Table 2. Results and recovery values for tannin (TA) determination in tea samples

Tea sample	Proposed method		Reference method		Recovery ± SD / %		
	TA ± SD / (mg L^{-1})	RSD / %	TA ± SD / (mg L^{-1})	RSD / %	5.0 mg L^{-1}	10.0 mg L^{-1}	15.0 mg L^{-1}
Green 1	25.3 ± 0.3	1.1	25.3 ± 0.3	1.0	109.1 ± 1.0	90.5 ± 2.3	103.4 ± 0.8
Black 2	5.8 ± 0.1	0.9	5.8 ± 0.1	1.6	–	–	–
Green 3	22.1 ± 0.1	0.2	22.1 ± 0.0	0.1	107.3 ± 0.5	97.6 ± 1.0	105.7 ± 2.7
Black 4	8.9 ± 0.3	2.9	8.8 ± 0.1	0.3	98.1 ± 2.2	90.9 ± 2.4	102.3 ± 0.7
Green 5	4.0 ± 0.1	1.8	3.9 ± 0.1	2.2	–	–	–
Black 6	13.4 ± 0.3	2.3	13.8 ± 0.2	1.7	99.3 ± 1.4	105.5 ± 0.7	102.4 ± 1.1

SD: standard deviation (n = 3); RSD: relative standard deviation.

Table 3. Analytical features of the proposed FBA method with other methods which use CDs for the determination of TA

Parameter	Reference							
	1	2	3	6	23	24	32	This work
Precursor	sodium citrate and aminopyrazine	6-bromohexyl-boronic acid and polyethyleneglycol bis(3-amino-propyl)	ascorbic acid and ammonia	lemon	xylan and branched polyethyleneimine	diethylenetriamine-pentaacetic acid	citric acid, melamine, and formaldehyde	*Pilosocereus gounellei*, and ethanolamine
Synthesis method	hydrothermal	hydrothermal	ultrasonic	hydrothermal	microwave-assisted	solid-state	hydrothermal	hydrothermal
Synthesis conditions	200 °C for 4 h	180 °C until obtaining a reddish-brown solution	50 °C for 12 h	160 °C for 3 h	180 °C for 15 min	180 °C for 5 min	180 °C for 12 h	200 °C for 8 h
Quantum yield / %	11.8	0.3	10.2	1.4	8.0	–	63.7	13.6
Measurement	fluorescence	fluorescence	chemiluminescence	fluorescence	fluorescence	fluorescence	chemiluminescence	fluorescence
Excitation source	xenon lamp	xenon lamp	–	xenon lamp	xenon lamp	xenon lamp	–	UV-LED λ_{max} 370 nm
Spectrophotometer	benchtop	benchtop	benchtop	benchtop	benchtop	benchtop	benchtop	handheld
Automatic procedure	no	no	FIA	no	no	no	FIA	FBA
Sampling rate / h^{-1}	–	–	120	–	–	–	140	22
CDs consumption *per* determination / μL	200	100	60	–	20	600	–	250
Sample consumption *per* determination / μL	–	100	60	–	5000	2000	–	48
Waste generation *per* determination / mL	10.0	5.0	18.9	–	10.0	4.0	–	3.6
Working range / (μmol L^{-1})	0.40-9.0	0.06-6.0	0.07-7.0	1-47.5	0.10-5.0	0.2-10.0	0.2-10.0	1.87-28.0
LOD / (nmol L^{-1})	120	10.6	26.0	310	36.8	9.0	39.3	95.2
RSD (n = 3) / %	< 5.0	< 0.4	< 4.2	< 2.95	< 5.4	< 5.9	< 2.4	< 2.9
Sample	beer	white and red wine	Chinese gall	river water	lake water and white wine	tap and river water	red wine	green and black tea

CDs: carbon dots; LOD: limit of detection; RSD: relative standard deviation; FIA: flow injection analysis; UV-LED: ultraviolet light-emitting diode; TA: tannin.

method presents satisfactory features such as quantum yield, working range, LOD, RSD, sample consumption, and low waste generation. The proposed method also uses a green precursor (*Pilosocereus gounellei*) similar to the Xavier *et al.*[6] method, which used lemon. On the other hand, the TA determination is performed in the proposed method without the previous addition of Fe^{3+} or any other ion and presents a higher quantum yield (13.6% against 1.4%).

As with the automated FIA systems used by Liu and Han[3] and Li *et al.*,[32] the proposed method is also carried out automatically, but using an automated FBA system. Previous automated FIA methods,[3,32] however, employed chemiluminescent detection which has some limitations: the lack of selectivity in many chemiluminescent reactions, the shape of the tubular spiral flow cell restricts the area of contact with the detector window, and the reaction begins before reaching the flow cell, thus impairing sensitivity of the method. Moreover, the proposed method uses as a stable excitation source a simple and inexpensive UV-LED instead of a high-priced xenon lamp; as a fluorimetric detector, the method used a cheap handheld spectrophotometer instead of a non-portable and expensive benchtop spectrophotometer.

Conclusions

This paper presents for the first time the use of xiquexique (*Pilosocereus gounellei*), a native endemic cactus from the Brazilian semiarid region, as a promising natural carbon source ("green precursor") for the synthesis of highly fluorescent carbon dots (CDs). These CDs, a cheap UV-LED and handheld spectrometer were successfully employed to develop a fast, accurate, precise, low-cost, eco-friendly fluorescence method for determining tannins in tea samples. To improve the precision and accuracy as well as to decrease the consumption of reagents, time, and cost analysis, the proposed method was automated using a FBA system. The CDs were successfully characterized by TEM, XRD, UV-Vis, FTIR, and fluorescence spectroscopy and exhibited excellent water solubility, with a high quantum yield of 13.6%. The proposed method exhibited a satisfactory positive linear relationship between fluorescence response and tannin concentrations in the range from 2.0 to 30.0 mg L^{-1}. Therefore, the proposed methods presented satisfactory parameters when compared with other reported methods using CDs for tannins quantification.[1-3,6,23,24,32]

Acknowledgments

Author Contributions

Kelly D. Silva was responsible for conceptualization, methodology, validation, formal analysis, investigation, writing - original draft, writing-review and editing; Stéfani Iury E. Andrade for conceptualization, software, validation, formal analysis, investigation, supervision, writing - original draft, writing-review and editing; Marcelo B. Lima for conceptualization, validation, resources; Severino S. Monte-Filho for investigation, validation; Mário César Ugulino Araújo for conceptualization, investigation, validation, formal analysis, writing - original draft. resources writing-review and editing; Ricardo A. C. Lima for conceptualization, investigation, validation, formal analysis, supervision, writing - original draft, writing-review and editing, resources.

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