



Short Communication

## Development of a quercetin fluorescent sensor prepared from waste paper

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### Abstract

The present paper aims to develop a carbon quantum dots (CQDs)-based sensor through a fast method and using a green carbon source, for the detection of quercetin. To achieve the sensor goals, waste office paper, water, and a microwave oven were chosen. To reach the best condition synthesis, some parameters (mass of paper, microwave power and time of heating) were evaluated, leading to a remarkably 30 min heating time. The as-prepared CQDs showed 9% quantum yield and particle size of 11 nm. For quercetin detection, these nanoparticles displayed a fluorometric quenching response. In order to obtain higher sensibility, chemical parameters of analysis (pH, concentration of CQDs and reaction time) were assessed, enabling a practical analysis of only 3 min long, with wide linear range of two orders of magnitude, and detection limit of 0.8 mg L<sup>-1</sup>. As exemplification of the present sensor feasibility, the CQDs were applied to determine the quercetin concentration in a real sample of tea.

### Article Information

Received: 01 May 2023

Revised: 02 June 2023

Accepted: 10 June 2023

### Academic Editor

Radosław Kowalski

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### Keywords

Green carbon source, microwave heating, probe, quenching, fennel tea, carbon dots.

## 1. Introduction

Quercetin is a flavonoid that has biological activities including antioxidant, anticancer, anti-allergic, and also contributes to the prevention of cataracts and coronary diseases. It is present in natural products, such as fruit and vegetables, e.g., in onion, broccoli, apple and teas [1]. Thus, monitoring the amount of quercetin in these foods is important as a contribution to a healthier diet. Quercetin has been quantified by methods involving chromatography [2] and electrochemistry [3]. However, these methodologies can be expensive, time-consuming and require prior knowledge of equipment operation and data processing. In contrast, methods involving fluorescent nanoparticles are easy to apply, economical and quick. In this study, we synthesized

carbon quantum dots (CQDs) from waste office paper and water via microwave heating. This represents a faster, cheaper and simpler CQDs synthesis, yielding fluorescent nanoparticles solution which could be used as quercetin sensor. Several aspects of the CQDs synthesis were evaluated, some characterizations of the obtained CQDs were achieved and an attempt to apply it as quercetin sensor was performed.

## 2. Materials and methods

### 2.1 Materials

Solutions were prepared using ultrapure water (18.0 MΩ cm) from a Milli-Q system (Millipore Inc., Bedford, MA). Methanol (HPLC grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

A 1000 mg L<sup>-1</sup> quercetin stock solution was prepared from HPLC grade quercetin ( $\geq 95\%$ , Sigma-Aldrich, St. Louis, MO, USA) in methanol. Other reagents were of analytical grade. All buffer solutions were prepared according to Gomori [4], adapting the final volumes. Quinine sulfate (53%) in an aqueous H<sub>2</sub>SO<sub>4</sub> solution (0.05 M) was used as the reference for calculating quantum yield with excitation at 366 nm [5]. The paper used in the syntheses were A4 computer printout sheets which were no longer being used. These sheets were cut into strips by an office paper shredder and then cut with scissors, resulting in small rectangles of about 8 by 1 mm.

## 2.2 Apparatus

The Microwave oven was a Start D model (Milestone, Sorisole, Italy) with cavity of ten Teflon® bottles capacity. Centrifuge was a Hettich EBA 200 with speed of 6000 rpm. Spectrofluorometers Horiba-Jobin Yvon Fluorolog-3 with xenon lamp, Perkin Elmer model LS 55, and Shimadzu model RF-5301PC were used according to their availability. Absorption spectrophotometer was an Agilent model 8453. TGA was a Shimadzu model 50WS, and DSC was a Shimadzu model DSC-60, both operating in nitrogen flow (50 mL min<sup>-1</sup>; heating rate: 10 °C min<sup>-1</sup>). Rotator evaporator was a Büchi R-210 with B-491 bath and Vario PC 3001 vacuum pump. Nuclear magnetic resonance (NMR) device was a 400 MHz VNMRs from Varian (solvent: D<sub>2</sub>O). DLS device was a NanoBrook Omni. Zeta potentiometer was a Zetasizer. The X-ray diffractometer was a Bruker D8 Advance with K $\alpha$  radiation of Cu ( $\lambda = 1.5418 \text{ \AA}$ ). Microscopy images were acquired from a Delong LVEM5 with 5 kV acceleration using a grid covered with ultrafine carbon film as substrate.

## 2.3 CQDs synthesis

Aiming to evaluate the best conditions of CQDs synthesis the independent variables such as microwave power (P), microwave irradiation time (t) and mass of paper (m) were varied at two levels on a 2<sup>3</sup> factorial design. The upper (+) and lower (-) levels for P were respectively 200 and 100 W. The levels for t were 30 and 15 min, respectively. And these levels for m were 1.0 (+) and 0.5 g (-). For each combination of variable levels one synthesis using two microwave reactors was executed. The monitored responses were absorption and emission spectra. Statistica software

(StatSoft, Inc., version 12.0) was used to generate the matrix of experiments.

In a typical synthesis, for each reactor, 1 g of paper was weighed in a beaker, 10 mL of ultrapure water was added and the beaker was stirred until every piece of paper was wet; then the mixture was transferred to the reactor. The reactors were exposed to 200 W of power for 30 min. After reaching room temperature the reactors were opened. Both products were transferred to a single 50 mL Falcon tube, using water to help the transfer. Then, the tube was topped up to 45 mL. The liquid and solid remaining in the tube were equally divided among four 15 mL Falcon tubes and centrifuged for 10 min. The supernatant was equally divided into two other 15 mL Falcon tubes and centrifuged for further 5 min, then the supernatant was transferred to a glass and stored at room temperature.

## 2.4 Quercetin measurement

To a quartz cuvette, 0.5 mL of 5% (v/v) CQDs solution of the as-synthesized CQDs, 2 mL of TRIS-HCl buffer solution at pH 8 and 0.5 mL of 10 to 1000 mg L<sup>-1</sup> standard solution were added. At the time of adding the standard solution, a timer was triggered; the mixture was stirred and the fluorescence spectrum was obtained within 3 minutes.

## 2.5 Study of chemical parameters in quercetin analysis

To assess various conditions, emission intensities were collected at 485 nm (when excited at 400 nm) for the blank (methanol) and a quercetin standard (50 mg L<sup>-1</sup>). The volumes of buffer, CQDs and standard or blank solutions were kept the same and only the composition of these solutions was altered. For the pH study, buffer solutions of pH 5, 6, 7 and 8 were tested while the CQDs solution was maintained at a concentration of 3% (v/v) and the reaction time at 1 min. For the CQDs concentration study, the pH 8 was preserved, and the reaction time at 1 min while the CQDs solution was varied between concentrations of 1%, 3%, and 5% (v/v) (percentage relative to the solution obtained from synthesis). For the reaction time study, the pH was kept at 8 and CQDs solution at concentration of 5% (v/v) while the reaction time was measured every 30 s until reaching 5 min, after this, it was measured minute by minute up to 20 min of reaction time.

### 2.6 Tea sample

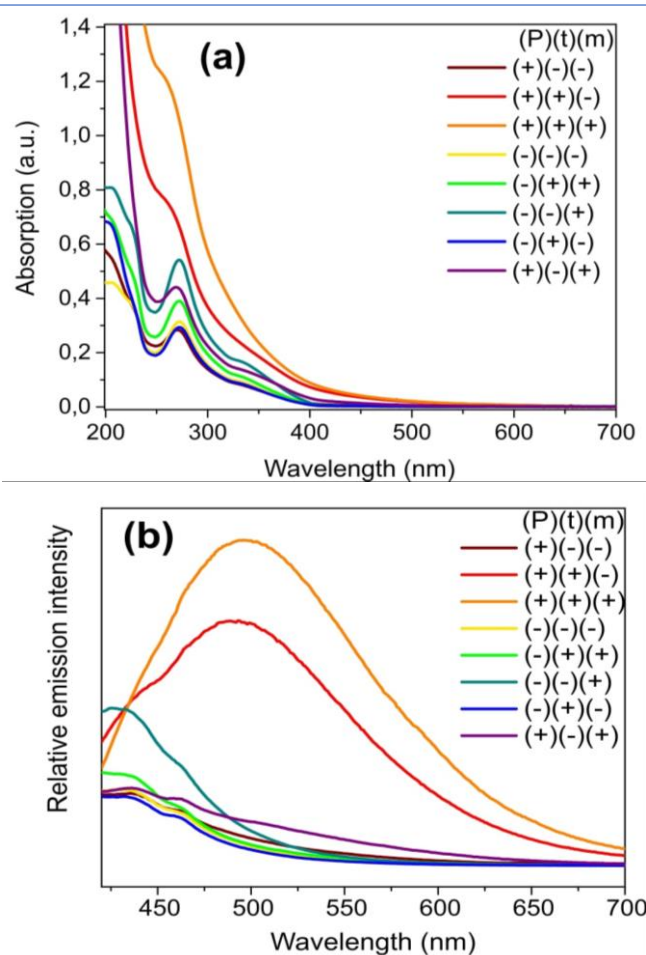
Fennel tea bags were acquired at a local supermarket and stocked at room temperature. The tea was prepared in infusion following the package instructions (1 bag of 2 g in 200 mL of hot water for 5 min) and left to cool down to room temperature. The brewed tea was then diluted to 10% (v/v), by taking 1 mL and completing the volume of 10 mL with methanol, subsequently, the diluted solution was stored in a refrigerator. Before analysis, the 10% solution of brewed tea was left to reach room temperature, when it was added to the cuvette containing 0.5 mL of 5% (v/v) CQDs solution and 2 mL of TRIS-HCl buffer solution at pH 8. The fluorescence spectrum was obtained after 3 minutes from the sample addition.

## 3. Results and discussion

Since 2004 [6] CQDs have been synthesized from different carbon sources, including expensive reagents like ionic liquids [7] or low-value materials like waste paper [8]. CQDs synthesis usually needs a heating source such as autoclave, microwave radiation, or plasma [9–13]. The methodologies so far developed for the synthesis of CQDs from office printing paper, paper filters, brown paper tissues, or newspaper are time consuming, multi-step or use chemical reagents [8, 14–21]. Then, we investigated the conditions of CQDs synthesis from waste office paper and water via microwave heating. First, a  $2^3$  factorial design was executed to assess the synthesis conditions. This factorial design varied the microwave irradiation power, P (200 W (+), 100 W (-)), the heating time, t (30 min (+), 15 min (-)), and the mass of paper, m (1.0 g (+), 0.5 g (-)). The best condition was expected to show higher intensities in absorption and emission spectra. As seen in absorption (Fig. 1(a)) and emission spectra (Fig. 1(b)) of the different CQDs suspensions obtained through the factorial design, the best synthesis condition used power of 200 W, 30 min of heating and 1.0 g of paper. Besides, a synthesis with one hour of heating was run; but no significant improvement in spectra intensities was observed.

At the end of the synthesis, a considerable amount of paper remained. This would be usually discarded, but it could be used again to produce more CQDs.

Using such residue in a new synthesis procedure,



**Fig. 1** Absorption (a) and emission ( $\lambda_{exc} = 400$  nm) (b) spectra of the CQDs synthesized with different conditions of power (P: 200 W (+), 100 W (-)), time (t: 30 min (+), 15 min (-)) and mass of paper (m: 1.0 g (+), 0.5 g (-)).

exhibits the advantage of producing a solution with higher fluorescence intensity (Fig. S1). This indicates that a greater number of nanoparticles were formed. Therefore, the usage of synthesis residue as starting material should be further investigated.

Apart from articles which make CQDs from other carbon sources [7, 9–13, 22, 23], the methodologies in literature that synthesize CQDs from paper may need several steps [15, 19, 21], take up to 15 hours of heating [16–19], or use reagents such as sodium hydroxide [16], sulfuric acid [14], urea [14, 19], or ionic liquids [15]. The synthesis proposed herein offers the advantage of using only waste paper and water, instead of reagents. Other advantages are the demand for only 30 min of heating, the use of a controllable heating system, and the presence of only one step for nanoparticle formation.

One feature that allowed the present synthesis methodology to be faster was the exclusion of a

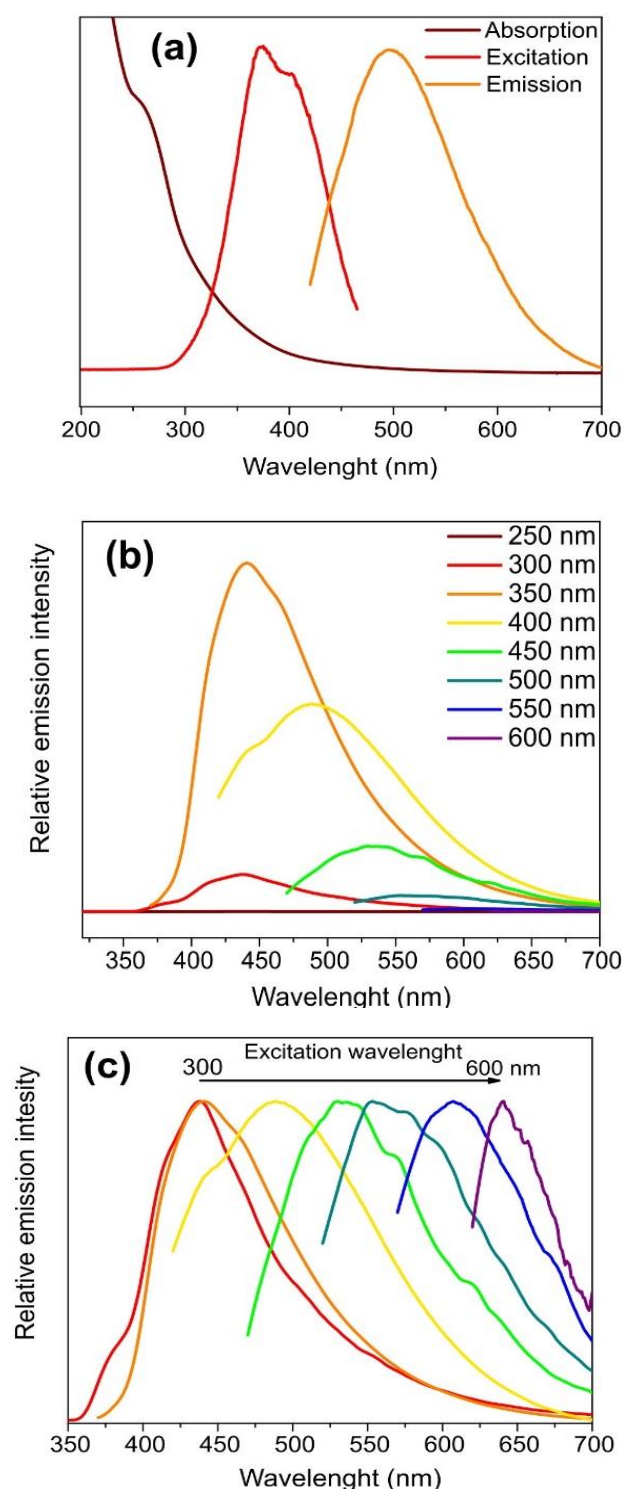


dialysis step usually found in many methodologies. Although dialysis is used as a purification step, for example to remove inorganic and molecular impurities, it takes a very long time. Thus, with a view to the target application, the obtained CQDs suspension after centrifugation required no further purification.

Observing the optical characteristics of the synthesized CQDs (Fig. 2) is possible to affirm the quality of CQDs and the feasibility of the method is very satisfactory. As shown in Fig. 2(a), there is a band in the excitation spectrum around 375 nm and a shoulder around 400 nm. The fluorescence emission spectrum (excited at 400 nm) has a band at 496 nm, showing a Stokes shift of 96 nm and a full width at half maximum (FWHM) of 137 nm. The fluorescence also presented a very common property to carbon dots reported in the literature: the excitation-dependent emission (Fig. 2(b) and 2(c)). The quantum yield was calculated to be 9%, relative to quinine, at 366 nm [5]. This 9% is acceptable when taking into account the range of quantum yields of other CQDs prepared from paper which varies from 0.27% to 20% [8, 15–18, 20]; although, it can appear to be very low when compared to other reported quantum yields [10]. The UV-Vis spectrum (Fig. 2(a)), showed an obvious shoulder around 263 nm associated to  $\pi$ - $\pi^*$  transition and some not so distinctive shoulders in the range of 270-300 nm and around 320 nm that are associated to  $n$ - $\pi^*$  and  $\pi$ - $\pi^*$  transitions of C=O, respectively [8, 15–17].

The obtained emission spectra were suspected to be composed of three components: the fluorescence from CQDs, the fluorescence from the paper fluorophore and the water autofluorescence. To verify that, the paper fluorophore was extracted from the paper by adding some pieces of paper into a beaker with water for a few minutes in a sonication bath and then blue fluorescent solution was obtained. The fluorescence spectra of the extracted fluorophore were acquired (Fig. S2 (a)), these spectra were also acquired for pure water (Fig. S2(b)). Paper fluorophore emission is fixed at 438 nm and varied its intensity with wavelength excitation. This fluorophore is added to paper during its fabrication and is composed mainly of TiO<sub>2</sub> [25].

On the other hand, water emission wavelength is variable with excitation. Both emissions are present



**Fig. 2** Absorption, excitation and emission spectra of CQDs produced after improvement of synthesis condition (a). Emission spectra of CQDs excited at different wavelengths (250 - 600 nm) (b). Normalized emission spectra of CQDs excited at different wavelengths (300 - 600 nm) (c).

together in the emission spectrum of the solution produced in the CQDs synthesis. In order to view the CQDs fluorescence and avoid water and fluorophore emissions, excitation at 400 nm was selected for further experiments.

Thermal analyses of TGA and DSC were performed with the as-prepared CQDs after evaporation under vacuum and drying under acetone (Fig. S3). TGA shows that some water is lost in the beginning, some other losses of weight happened and above 600 °C more than 40% of mass remained, demonstrating the presence of inorganic residue. This residue is attributed to the oxides added during fabrication for paper conditioning such as Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub> and TiO<sub>2</sub> [25]. In DSC, various endothermic signals around 117 and 160 °C can be seen, indicating CQDs undergo some reactions and these reactions might spoil the CQDs luminescent properties. Thus the thermal stability of CQDs was verified to be up to 115 °C.

The <sup>1</sup>H NMR of CQDs is shown in Fig. S4 where it is possible to see signals of H from: sp<sup>3</sup> carbons, hydroxyls, ethers, carboxyls or aldehydes [27]. No signals of H from aromatic or sp<sup>2</sup> carbon can be seen between 6 and 8 ppm. This indicates that the synthesized CQDs were not graphitic quantum dots (GQDs). The X-rays diffraction pattern of CQDs showed broad bands around 10° and 23° indicating a very amorphous carbon structure (Fig. S5) which corroborates to the deduction the CQDs were not GQDs.

The CQDs microscopy images were obtained from low voltage electron microscopy (LVEM). Fig. 3 presents a LVEM image where several well-dispersed nanoparticles are seen. In the inset, the histogram of these particles shows size dispersion around 11 nm. The zeta potential of CQDs was measured at -5.92 mV, due to its hydroxyl, carbonyl or carboxyl surface groups, and this negative charge promotes electrostatic repulsions that can indicate a stable CQDs dispersion [8, 11].

In order to assess the possibility of separating CQDs populations obtained together in the synthesis, a column was prepared with powder cellulose as stationary phase and water as mobile phase. During cellulose packaging luminescence was observed in the water that run-off before the CQDs were added, so the column was washed until the fluorescence was minimized. The CQDs solution was passed through the column and fractions of the output solution were collected according to visualized color or fluorescence changes. Fluorescence spectra of these fractions were obtained and shown in Fig. S6. Some intensities

variation is observed in 3 regions of the spectrum: 455, 500 and 560 nm, indicating that there are at least 3 majority populations of CQDs and that these populations are separable. It might be possible to obtain each population separately if a column with better separation or a preparative HPLC column were used. This is still ongoing. Perhaps, it would be advantageous to add this post-synthesis procedure in the future, in order to obtain different solutions of CQDs emitting in different regions of the spectrum with a narrow emission profile.

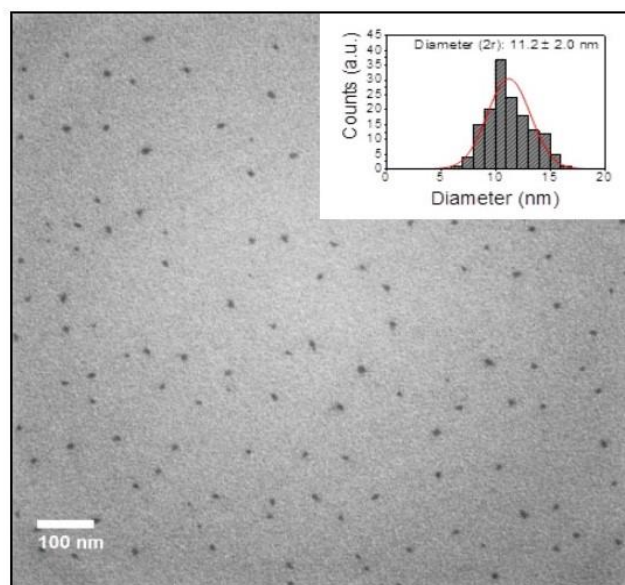
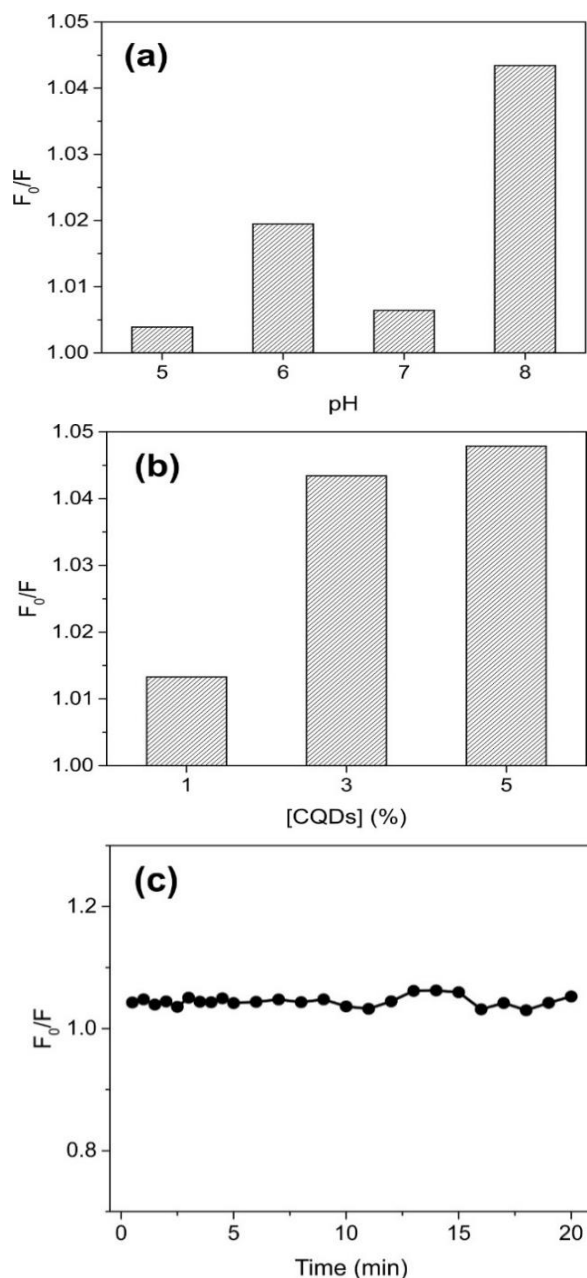


Fig. 3 LVEM image of the as-prepared CQDs. Inset: Histogram of size distribution of CQDs (n = 200).

With a view to its application, CQDs can be used to different purposes including analytical determinations of neutral, positively and negatively charged, organic or inorganic species [27]. These sensors may quantify species at the nanomolar level with great selectivity [22]. Benefic compounds in foods or drinks [7, 23, 28] and toxic species like mercury II [29] have been targets of their determinations. Herein, the as-prepared CQDs were used in an attempt to propose a fast and practical quercetin sensor, regarding the mentioned importance to determine quercetin.

Initially, the experimental conditions including pH, concentration of CQDs and reaction time were optimized in order to obtain the highest sensitivity, i.e., the greatest difference between the CQDs emission intensity in the presence (F) and in the absence (F<sub>0</sub>) of the analyte. The pH could influence the

reactivity and stability of the CQDs, affecting the fluorescence intensity or sensor sensitivity [7]. The study of the pH was performed using buffer solutions at different levels of pH (from 5 to 8) so its influence on quenching of CQDs fluorescence could be evaluated. As can be seen in Fig. 4(a), the pH which gives greater quenching is 8, therefore pH 8 was selected for further studies.



**Fig. 4** Effect of pH (a), CQDs concentration (b) and reaction time (c) to quenching of CQDs.

High concentrations of CQDs can result in self-quenching while low concentrations can lead to poor sensitivity, hence is necessary to establish the best concentration of CQDs to be used in the analysis [28].

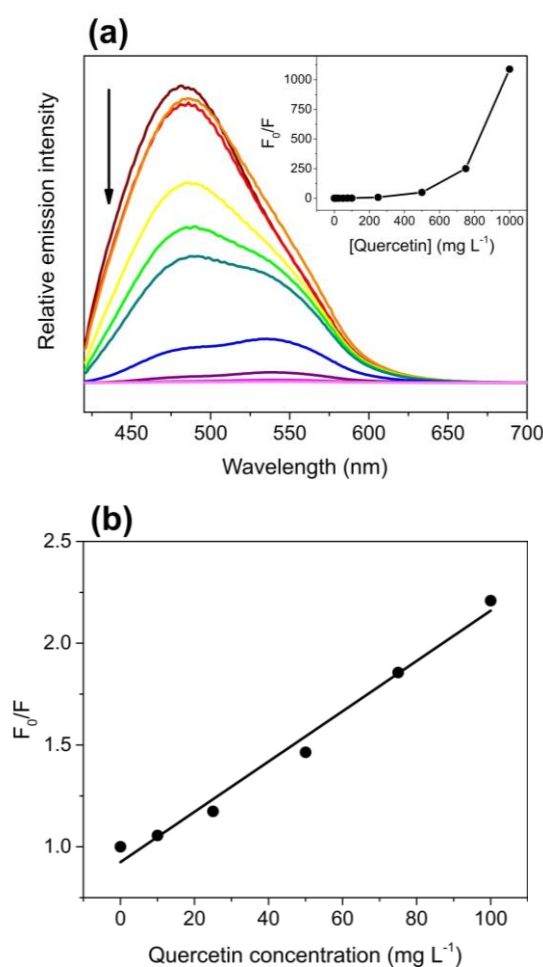
With the pH fixed at 8 as determined above, the CQDs concentration was varied between 1% and 5% (v/v) and the fluorescence signal quenching ( $F_0/F$ ) was obtained (Fig. 4(b)). The results showed a quenching slightly better for the concentration of 5% (v/v), so this concentration was chosen. The evaluation of the reaction time between CQDs and analyte is important to make sure the measured signal is stable. Furthermore, is convenient to evaluate the shortest time in which the quenching can be measured. The quenching (Fig. 4(c)) was measured up to 20 min. Less variation was observed between 1 and 9 min. Consequently, in order to have higher analytical frequency and a safety margin for variations in quenching signal, the selected measurement time was 3 min.

Given that, the following analyses were conducted using a buffer solution at pH 8 (TRIS-HCl), 5% (v/v) CQDs solution and 3 min of reaction time. Standard solutions with concentrations from 10 to 1000 mg L<sup>-1</sup> of quercetin were analysed through the sensor proposed (Fig. 5). A linear relationship was observed in the range of 10 to 100 mg L<sup>-1</sup> quercetin and a regression was determined to be  $F_0/F = 0.01236[Q] + 0.9245$  (where [Q] is the concentration of quercetin, in mg L<sup>-1</sup>) with a determination coefficient ( $R^2$ ) of 0.9811 and relative standard deviation (RSD) of 1.6% ( $n = 9$ ; 50 mg L<sup>-1</sup>). According to IUPAC criteria, the detection and quantification limits were 0.8 and 2.6 mg L<sup>-1</sup> quercetin, respectively. This detection limit is not as low as most of the limits reported for other quercetin analysis methodologies [3, 7, 28, 30–33], however, the sensor proposed here is more advantageous because of its low cost and simple preparation. Moreover, the present detection limit is slightly lower than the limit reported by de Paula et al. [23] (0.85 mg L<sup>-1</sup>) and quite lower than the reported by Jeevika et al. [34] (2.03 mg L<sup>-1</sup>). These authors also used carbon dots-based sensor for quercetin analysis, but their carbon dots were prepared with longer heating time and from sodium citrate or garlic peel as carbon source.

Considering the linear Stern-Volmer equation (Equation 1) and the linear regression found for the present sensor, a Stern-Volmer constant ( $K_{SV}$ ) of 0.01236 can be established [23, 34].

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1)$$





**Fig. 5** Fluorescence spectra of CQDs in the absence and presence of quercetin. The inset ( $F_0/F$  of these spectra at 485 nm) is an analytical curve with quercetin concentrations up to 1000 mg L<sup>-1</sup> (a). Analytical curve for quercetin in the linear range (b)

The CQDs-based sensor proposed here exhibited great potential to be used for quercetin monitoring in natural products and drinks. Although there are several features to be assessed about the present sensor, e.g., selectivity or interference tests, an exemplification of the sensor used for quercetin analysis in the real sample was performed. Once the amount of quercetin found in teas is usually high [1], this kind of sample does not require very low detection limits from the sensor neither large amounts from the sample. Therefore, brewed fennel tea was utilized in the exemplification. Because the tea has its fluorescence, it was necessary to be subtracted from the emission of the mixture of the CQDs with the tea. Then a mixture prepared without the CQDs was also recorded. Once the sample was properly analysed and submitted to the analytical curve, the result showed a concentration of 145.6 mg L<sup>-1</sup> quercetin in the brewed

tea. The same sample was spiked with 30 mg L<sup>-1</sup> quercetin and a recovery of 124% was found.

#### 4. Conclusions

Considering the acquired results, we assume the aims of the present work were mostly reached. The CQDs synthesis conditions were established at 200 W of microwave power, 30 min heating and 1 g of paper; signaling a greener, faster, and more feasible method than in the literature. The obtained CQDs showed good quality and a quantum yield of 9%, which appears to be low; however, is acceptable compared to other nanoparticles prepared from similar sources. And the application attempt as quercetin sensor was studied, determining the best analysis condition as pH 8, 5% (v/v) CQDs solution and reaction time of 3 min. The sensor showed a wide linear range from 10 to 100 mg L<sup>-1</sup>, limits of detection and quantification of 0.8 and 2.6 mg L<sup>-1</sup>, respectively, and RSD of 1.6% (n = 9), indicating its applicability. Finally, a real sample of brewed tea was successfully analysed and submitted to a recovery test, suggesting the potential application of this sensor. However, much study is still ongoing to improve and better explore the sensor performance.

#### Supplementary Data

[Supplementary Fig S1-S6](#)

DOI Link:

<https://doi.org/10.58985/jeopc.2023.v01i03.26>

#### Authors' contributions

Conceptualization, T.H.K.; Methodology, T.H.K., S.G.B.P. and A.P.S.P.; Validation, A.P.S.P.; Formal Analysis, S.G.B.P. and A.P.S.P.; Investigation, T.H.K., S.G.B.P. and A.P.S.P.; Resources, A.P.S.P.; Data Curation, T.H.K. and S.G.B.P.; Writing – Original Draft Preparation, T.H.K.; Writing – Review & Editing, A.P.S.P.; Visualization, T.H.K.; Supervision, S.G.B.P. and A.P.S.P.; Project Administration, A.P.S.P.; Funding Acquisition, A.P.S.P.

#### Acknowledgements

The English text of this paper has been revised by Sidney Pratt, Canadian, MAT (The Johns Hopkins University), RSAdip-TESL (Cambridge University), and by Roderick M. Mackenzie, teacher at Britanic® school in Pernambuco, Brazil.

## Funding

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship granted for T.H.K. (process no. 145834/2015-8); FACEPE/NUQAAPE (APQ-0346-1.06/14); FACEPE (APQ-0557-1.06/15); and the Instituto Nacional de Tecnologias Analíticas Avançadas - INCTAA (CNPq grant 465768/2014-8, and FAPESP grant 2014/50951-4) for supporting this work.

## Conflicts of interest

The authors declare no conflict of interest.

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