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Article

Use of Carbon Dots Synthesized from Citrate as a Fluorescent Probe for Quercetin Determination in Tea and Beer Samples

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In this work, carbon dots (CDs) prepared by a one-step hydrothermal method were employed for the determination of quercetin in teas and beers. The fluorescence quenching of CDs was proportional to the concentration of quercetin. Under optimum experimental conditions, linear quenching was observed for quercetin in the range of 1.0 to 10 mg L^{-1} (F/F_0 = (0.1511 ± 0.0075)[Q] + (0.8922 ± 0.0384), R^2 = 0.9926), at room temperature, using 80 μL CDs and 0.1 mol L^{-1} Na_2HPO_4/NaOH buffer solution (pH 11.0). A limit of detection (3σ criterion) of 0.85 mg L^{-1}, and a relative standard deviation of 0.21% (n = 3) for 4.0 mg L^{-1} quercetin solution was obtained. Addition and recovery tests with tea and beer samples were performed resulting in recoveries at the range between 80 and 118%. This procedure served successfully to determine quercetin in beer and tea samples presenting high sample throughput.

Keywords: flavonoids, quercetin, carbon dots, fluorescence

Introduction

There is a growing concern about the quality of life, leading to a greater number of people who care about having a healthier diet. Thus, there is an increase in the consumption of foods that, in addition to nutritional value, also provide health benefits. These are the so-called nutraceutical foods.[1] Nutraceuticals are foods from vegetable or animal sources which in addition to their nutritional value have bioactive compounds that are beneficial for human health.[2] One type of nutraceutical/micronutrient that has received increasing attention recently are the polyphenols, due to their antioxidant action in the prevention of a number of diseases that involve free radicals. They are the most abundant antioxidants present in the human diet, found in foods such as fruits, vegetables, nuts, chocolate, wine, beer, coffee and tea.[3,4] Among antioxidant polyphenols, flavonoids stand out because of their ability to fight free radicals.[5]

Quercetin (3,5,7,3'-4'-pentahydroxy-flavone) is one of the most abundant flavonoids, classified as a flavonol, with antioxidant and antitumoral activity due to the ability to act as a free radical scavenger.[6] The action of quercetin in the process of inhibiting the formation of free radicals can occur in three different stages, at initiation (by interaction with superoxide ions), in the formation of hydroxyl radicals (by chelating iron ions) and in lipid peroxidation (by reacting with peroxyl radicals of lipids).[7] In addition, quercetin also has antiallergic, antiviral, antimutagenic, cardiovascular protection, cataract prevention, and lipid peroxidation inhibitory properties.[8] It is reported that when compared to healthy people patients with diabetes have less ability to eliminate free radicals. One likely cause is that the concentration of natural antioxidants in people with diabetes is lower. Thus, increased consumption of foods high in quercetin may be useful in improving the side effects of chronic hyperglycemia.[9] This flavonol is found in various foods consumed daily in the human diet, especially in fruits and vegetables. It is also found in a higher concentration in drinks like beer and tea. Considering this fact, it is important to analyze foods that are sources of quercetin to know how much quercetin they contain.

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There are several methods for determination of quercetin in foods and beverages, such as high performance liquid chromatography (HPLC) with ultraviolet-visible (UV-Vis) and fluorescence detection,[3,10] spectrophotometric method,[11] capillary zone electrophoresis,[12] solid phase extraction,[13] and near-infrared spectroscopy and chemometrics.[14]

Although these methods may be reasonably accurate and sensitive, they have certain disadvantages, such as laborious analysis, high conductor consumption, expensive instruments and time-consuming analysis. The methods involving fluorescence as detection are fast, economical, simple and very sensitive.[15]

The carbon dots (CDs) are a new carbon fluorescent material with a diameter less than 10 nm. The CDs are becoming an alternative to the use of quantum dots (QDs), since the QDs are synthesized from metals and the CDs from a carbon source, thus being biocompatible, exhibiting good conductivity, high chemical stability, low toxicity, strong emission photoluminescence, easy and low cost synthesis, and a contribution to greener chemistry.[16,17] Owing to their properties, the carbon dots have been used as fluorescent sensors for detection of many species such as glucose,[18] microRNAs,[19] iron ions and pH,[20] dopamine,[21] ascorbic acid,[22] Cu^{2+},[23] Al^{3+},[24] and D-penicillamine.[25]

In the process of carbon dots synthesis, it is possible to perform doping or surface passivation which can significantly improve their properties.[26] CDs containing nitrogen present wide and strong emission of light when excited in the ultraviolet region, being promising in several applications like fluorophores.[27]

In this paper, an analytical procedure for quantification of quercetin based on quenching of the fluorescence of carbon dots is reported. The procedure was based on an interaction between synthesized carbon dots from citrate, using ammonium bicarbonate as resource of the nitrogen, and quercetin, providing a quenching on the fluorescence signal proportional to the concentration of analyte present in the tea and beer samples analyzed.

Experimental

Reagents, solutions and samples

All solutions were prepared with analytical grade chemicals. The solutions were prepared with water from a Milli-Q system (Millipore Inc., Bedford, USA) with resistivity of 18 MΩ cm, and all chemicals were of analytical reagent grade, and methanol (HPLC grade) from Merck (Darmstadt, Germany) and stored in amber glass bottles.

Stock solutions of 100 mg L^{-1} quercetin, kaempferol, myricetin, catechin, epicatechin and resveratrol (Sigma-Aldrich, Darmstadt, Germany) were prepared by dissolving an appropriate amount of the reagents in methanol (HPLC grade).

Stock solutions of 100 mg L^{-1} ascorbic (Vetec, Darmstadt, Germany), gallic (Sigma-Aldrich, Darmstadt, Germany), citric (Nuclear, Diadema, Brazil) and malic (Sigma-Aldrich, Darmstadt, Germany) acids were prepared by dissolving an appropriate amount of the reagents in water.

Buffer solutions were prepared with KCl/HCl 0.1 mol L^{-1} (pH 2.0); citric acid/K_2HPO_4 0.1 mol L^{-1} (pH 3.0; 4.0; 5.0; 6.0; 7.0 and 8.0); Na_2CO_3/$NaHCO_3$ 0.1 mol L^{-1} (pH 9.0); Na_2CO_3/HCl 0.1 mol L^{-1} (pH 10.0); Na_2HPO_4/NaOH 0.1 mol L^{-1}, $Na_2B_4O_7$/NaOH 0.1 mol L^{-1}, Na_2CO_3/HCl 0.1 mol L^{-1}, glycine in aqueous solutions of NaCl/NaOH 0.1 mol L^{-1} and $NaHCO_3$/NaOH 0.1 mol L^{-1} (pH 11.0); and Na_2HPO_4/NaOH 0.1 mol L^{-1} (pH 12.0).

Working solutions containing quercetin between 1.0 to 10.0 mg L^{-1} were prepared, by appropriate dilution of the stock solution to a volume of 5 mL for each solution.

Tea and beer samples were purchased from local stores. The tea samples were green and black tea infusions and a ready-to-drink green tea; the beer samples were Weissbier, Pilsen and Lager beers. All the samples were filtered and diluted adequately prior to analysis.

Apparatus

For the carbon dot synthesis, a temperature-controlled oven (MMM Ecocell Confort Line, Planegg, Germany) was used. Ultraviolet-visible (UV-Vis) absorption spectra of the carbon dot was obtained using a spectrophotometer (Agilent model 8453, Palo Alto, USA). Transmission electron microscopy (TEM) images were produced with a transmission electron microscope (JEOL model JEM 2100, Tokyo, Japan) operated at an acceleration voltage of 200 kV to characterize the synthesized carbon dot. For pH measurement, a bench pH meter (Q400AS, Quimis®, São Paulo, Brazil) was used. FTIR spectra were recorded on a Fourier transform infrared spectrophotometer-IRTracer-100 from Shimadzu, using the KBr pellet technique in the wavenumber region 4000-400 cm^{-1}. A spectrofluorometer (RF-5301PC, Shimadzu®, São Paulo, Brazil) was used for detection of quercetin. The measurement of the photoluminescence quantum yield of the synthesized carbon dot was carried out in a Quantaurus QY-C11347-11 Absolute QY Spectrometer equipped with an integration sphere (Hamamatsu, Japan). The photoluminescence properties were measured with a spectrofluorometer Horiba-Jobin

Yvon Fluorolog-3 with the continuous 450 W xenon lamp and UV xenon flash tube for excitation, double-grating monochromator in the excitation and UV-Vis emission position, single-grating monochromator in the NIR (near infrared) emission position, R928P and H10330A-75 Hamamatsu photomultipliers, respectively for the UV-Vis and NIR range emissions. All emission spectra were corrected by spectral response of the monochromators using a silicon photodiode reference detector to monitor and compensate for variation in the xenon lamp output, using typical correction spectra provided by the manufacturer. The nanosecond emission decay curves were acquired in this equipment operating in the time-correlated single photon counting mode (TCSPC) and using a pulsed NanoLED-340 (pulses at 339 nm) as the excitation source. Instrumental function response for the NanoLED-340 was obtained by use of a Ludox (colloidal silica) scattering water solution. Fluorescence decay analysis software, DAS6, was used to fit the model functions to the experimental data. All these instruments are installed either in the Laboratório de Análise Química, Inorgânica e Sensores (LAQIS) in the Department of Chemistry of Federal Rural University of Pernambuco (UFRPE, Recife, Brazil), in the Laboratório Associado para a Química Verde (LAQV) da Rede de Química e Tecnologia (REQUIMTE) in the Department of Applied Chemistry of Porto University (Porto, Portugal), in the Laboratório de Terras Raras (BSTR) in the Department of Fundamental Chemistry of Federal University of Pernambuco (UFPE, Recife, Brazil), in the Laboratório de Polímeros Não-Convencionais in the Department of Physics of Federal University of Pernambuco (UFPE, Recife, Brazil) and in the analytical central in the Chemistry Institute of USP (São Paulo, Brazil).

Carbon dot synthesis

A carbon dot from citrate was prepared using the hydrothermal method in a single step, according to a procedure described by Guo *et al*.[28] 0.2 g of sodium citrate and 1.5 g of ammonium bicarbonate were weighed separately, then dissolved in 10 mL of deionized water and transferred to a Teflon® reactor. Subsequently, the Teflon® reactor was coupled to a stainless-steel reactor and the reaction was carried out in a controlled oven at 180 °C over a period of 4 h, under high pressure with a heating ramp of 10 °C min^{-1}. After this, the vessel was cooled to room temperature and the solution containing 25 mg mL^{-1} of carbon dots was stored in a Falcon tube and kept under refrigeration.

Procedures for the detection of quercetin

Each Falcon tube had a total volume of 10.0 mL. To make this up, 0.1 mL of the carbon dot was added to 1 mL of buffer solution (pH 11), 3.9 mL of water (representing the blank solution) or different amounts of quercetin stock solution (100 mg L^{-1}) and the final volume (5.0 mL) completed with water. Then, the readouts were performed on the spectrofluorometer to verify the maximum fluorescence quenching. The excitation wavelength was 380 nm and the emission spectra were obtained in the 400 to 650 nm range with maximum emission at 450 nm and the slit widths of excitation and emission at 1.5 nm. The analytical curve was plotted for F_0/F (F_0 and F are the fluorescence intensity of the carbon dot solution in the absence and presence, respectively, of quercetin) *versus* quercetin concentrations.

Determination of quercetin in beer and tea samples

The proposed method was used to determine the amount of quercetin in beer (Weissbier, Pilsen and Lager beers) and tea (green and black tea infusions and a ready-to-drink green tea) samples that had been purchased from a local supermarket. The contents of three bags of each tea sample was weighed and 1.8 g of the leaves were placed in 100 mL of heated water (90 ºC). After 5 min, the tea was filtered and stored in amber glasses. The samples of tea and beer were filtered, diluted and the amount of quercetin concentration was detected.

Study of the chemical parameters

The pH study for the determination of quercetin employing the synthesized carbon dot was carried out using pH buffer solutions ranging from 1 to 12. For each pH, an analytical curve was obtained in the range of quercetin concentration from 1.0 to 10.0 mg L^{-1}. For this, fixed volumes of the carbon dot and the buffer solution were mixed in Falcon tubes, then different volumes of a quercetin stock solution (100.0 mg L^{-1}) were added, to obtain a quercetin concentration range of 1.0-10.0 mg L^{-1}. Finally, deionized water completed the solution to a total volume of 5 mL.

The study of the reaction time between the carbon dot and quercetin was evaluated. To do this, the following were mixed in a Falcon tube: 0.1 mL of the carbon dot, 1.0 mL of buffer solution (pH 11), 3.4 mL of deionized water and 0.5 mL of quercetin stock solution (100.0 mg L^{-1}) to obtain a final quercetin concentration of 10.0 mg L^{-1}. Immediately after the addition of quercetin, the Falcon tube content was transferred to the spectrofluorometer for readout to

obtain the transient signals from the fluorescence intensity variation as a time function.

In order to use the least amount of carbon dot in the determination of quercetin, a study was carried out by varying the amount of carbon dot from 0.05 to 0.10 mL and the analytical curves obtained for each were compared. In six Falcon tubes, fixed amounts of the buffer solution were added, varying the added amounts of quercetin to obtain different concentrations of quercetin from 1.0 to 10.0 mol L^{-1} and varying the amounts of deionized water and carbon dot (0.05, 0.06, 0.07, 0.08, 0.09 and 0.10 mL), maintaining the final volume in the tubes equal to 5 mL.

After evaluating the optimal pH in the determination of quercetin, a study was carried out to verify composition of the buffer solution to be used. A curve was obtained for each of the buffer solutions, pH 11: (1) $Na_2B_4O_7$ 0.05 mol L^{-1}/ NaOH 0.1 mol L^{-1}; (2) Na_2HPO_4/NaOH 0.1 mol L^{-1}; (3) $NaHCO_3$ 0.05 mol L^{-1}/NaOH 0.1 mol L^{-1}; (4) glycine in aqueous solutions of NaCl/NaOH 0.1 mol L^{-1}, and (5) Na_2CO_3/HCl 0.1 mol L^{-1}.

The influence of the concentration of the buffer solution was evaluated. The buffer solution concentration varied between 0.1 and 0.6 mol L^{-1} (0.1; 0.2; 0.3; 0.4; 0.5 and 0.6 mol L^{-1}) and a curve for each concentration of the buffer solution was obtained.

The fluorescence quenching as a function of temperature was monitored to verify the type of interaction between the carbon dot and quercetin. The carbon dot-quercetin interaction kinetics were recorded at different temperatures (varied from 25 to 40 ºC). Temperature control was maintained using a water bath and the variation of temperature was controlled to be within 0.1 ºC.

Interference test

The interference studies were performed using a quercetin standard solution of 5.0 mg L^{-1} containing from 0.02 to 20 mg L^{-1} of the possible interfering agents: ascorbic acid, gallic acid, citric acid, malic acid, catechin, epicatechin, kaempferol, resveratrol and myricetin, tested separately. Solutions with the quercetin mixed with each potential interfering compound in equal concentrations were prepared. When interference did not occur, the concentration of the interfering compound was increased or decreased until there was an effect.

Addition and recovery test

The accuracy of the method was determined using spiked beer and tea samples in two different concentration levels, 2.0 and 5.0 mg L^{-1} for six beer and six tea samples. These experiments were performed to evaluate matrix effects that may affect the measurements using the proposed method. Once the best operating conditions had been established, 12 samples were analyzed to test the usefulness of the proposed system.

Results and Discussion

Characterization of carbon dot

The carbon dot was synthesized according the method adapted from Guo *et al.*[28] with some modifications and showed a yellow color. The UV-Vis absorption and fluorescence emission and excitation spectra were obtained to study the optical properties of the CDs, as shown in Figure 1a. There are three bands in the excitation spectrum, around 260, 300 and 380 nm. Regardless of the chosen excitation wavelength, the fluorescence emission spectrum had a maximum of 450 nm, so the excitation wavelength at 380 nm was selected, as it presented the highest emission intensity. The synthesized material presented wide absorption in the ultraviolet and visible range of the electromagnetic spectrum with two bands characteristic of the CDs, as previously reported in the literature.[28] The UV-Vis absorption spectra, presented in Figure 1a, showed two bands around 230 and 340 nm. The band around 230 nm corresponds to the π-π* transition, corresponding to sp2-type carbon bonds of nanocarbon.[29,30] The band around 340 nm corresponds to the n-π* transition of the C=O bond and carboxylic groups C–O.[30,31] In both absorption and emission spectra, the CDs characteristic curves are similar to those in the literature.[28]

The synthesized carbon dot exhibits colloidal stability in an aqueous medium, being a translucent yellow when not under light irradiation, as shown in the photo insert in Figure 1a.

When the solution is exposed to an irradiation of ultraviolet light at 380 nm, the emission of a blue color occurs showing the formation of the carbon dot as displayed in the photo in Figure 1a. This indicates that even after practical changes were made to simplify the methodology, it was still possible to obtain the carbon dot with the same efficiency and quality as obtained by Guo *et al.*[28] The absolute quantum yield (QY) of CDs dispersed in water was 9.3% (excitation wavelength 380 nm), which is higher than most of the CDs previously reported.[32-34] We still did not reach a 68% QY as demonstrated in the work of Guo *et al.*[28] The decrease in the QY can be due to the non-realization of the dialysis step, which from the analytical point of view was not necessary in this work, because even without the dialysis step it was possible to perform the analyses in a simpler and faster way.

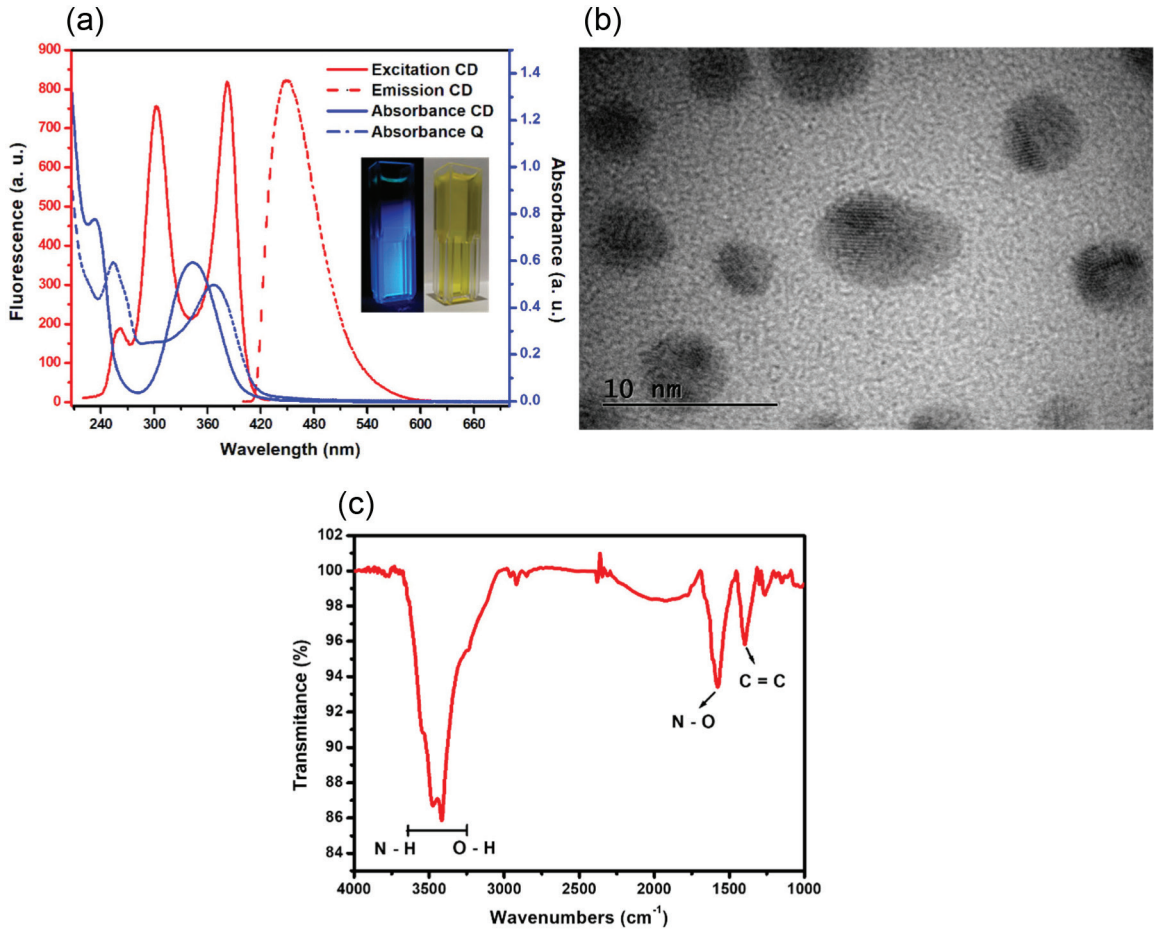

Figure 1. (a) An overlapping of absorption (–) excitation (–) and emission (···) spectra of the synthesized carbon dots and absorption spectrum of quercetin (···). The inset is the picture of carbon dots under light irradiation by a UV lamp at 380 nm (left) and without light irradiation (right); (b) A TEM image of the carbon dots synthesized; (c) FTIR (KBr) spectrum of CDs.

Through the analysis of the TEM images (Figure 1b), it is possible to observe that the nanoparticles have a spherical morphology, as already reported by other authors,[28] inferring that the CDs had been obtained as the product of the synthesis.

For a better characterization of the CDs, a FTIR analysis was performed to verify the functional groups present on the surface of the CDs. In Figure 1c, bands at 3200-3630 cm^{-1} are attributed to the stretching vibration of O–H and N–H.[35] Peaks at 1577 and 1396 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the carboxylate anions.[28] Thus, it is possible that the amino groups on the surface of the synthesized CDs interact with the phenolic hydroxyls present in quercetin. In this way the hydrogen bonding between the quercetin and the surface of the CDs occurs, which causes the quenching of the fluorescence.[35]

The synthesized carbon dot was applied to the analysis of quercetin. Consequently, studies were carried out to establish the best reaction conditions between the carbon dot and quercetin.

The experimental conditions used for the proposed procedure including media pH, reaction time, CDs amount, nature and concentration of the buffer solution, the interaction kinetics, and CDs selectivity were evaluated. Studies to evaluate the performance of the analytical assay were carried using univariate analysis with the purpose of attaining the highest sensitivity, i.e., maximum fluorescence quenching.

Study of the pH

The reactivity and stability of the carbon dot in a solvent are related to the charge on the surface of the nanoparticles, which is dependent on the nature of the binder and the pH of the medium. Due to the presence of carboxyl groups in acidic solution, these groups may be protonated, leading to colloidal instability, which may lead to an aggregation of the nanoparticles and consequent fluorescence inhibition.[28] In this study, the pH was varied from 1 to 12 using buffer solutions according to the buffering range required to evaluate the influence of pH on the quenching of carbon dot

fluorescence caused by quercetin. In Figure 2 it can be seen that there was an increase in sensitivity as a function of the pH increase. It was not possible to perform an analytical curve for pH 1, because at this pH the fluorescence intensity for all the points of the curve was close to zero, indicating that there was a degradation of the carbon dot. The highest sensitivity (Stern-Volmer constant, K_{sv}) and the best coefficient of determination (R^2) were obtained when the pH was equal to 11 (F_0/F = (0.1005 ± 0.0045)[Q] + (0.9980 ± 0.0234), R^2 = 0.9938), which was expected, since the synthesized carbon dot had pH 10.8. Therefore, pH 11 was selected for further studies.

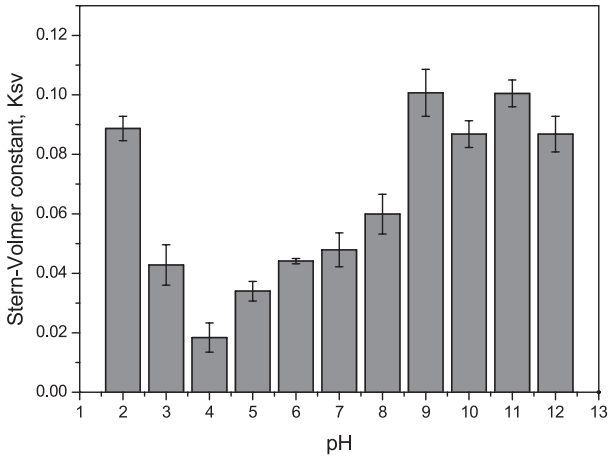

Figure 2. Stern-Volmer constant (K_{sv}) *versus* pH of the buffer solution used to determination of quercetin. Buffer solution varied from 2 to 12.

Reaction time

The stability of carbon dot solutions can affect the reproducibility and accuracy of successive fluorescence measurements during sample analyses. An unstable solution will affect the relationship between fluorescence intensity and analyte concentration because the loss of fluorescence may be due to the instability of the CDs solution and not to the presence of the analyte in different concentrations. This aspect becomes more important if the reaction requires a long time. Figure 3 shows the transient signal for the time of the reaction between CDs and quercetin (10 mg L^{-1}), pH 11, monitored for a period of 15 min. It can be seen that the reaction between the CDs and quercetin (10 mg L^{-1}) occurred rapidly and then the equilibrium was displaced in order to partially regenerate the CDs, visible by the partial recovery of the carbon dot fluorescence. Thus, the shortest possible time, 0.5 min, was selected for reading the reaction after the addition of quercetin to the vessel where the reaction occurred.

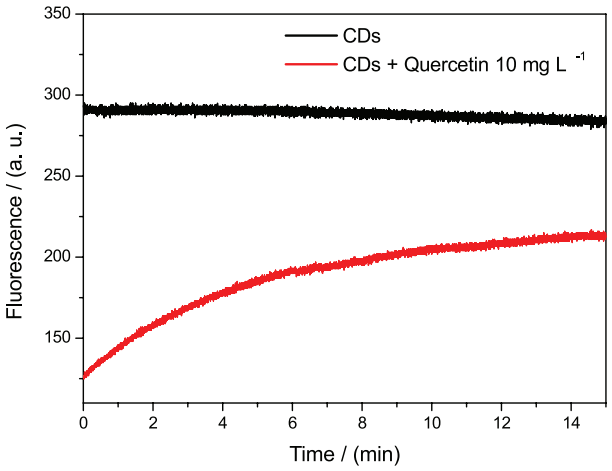

Figure 3. The transient signal for time of the reaction between carbon dot and quercetin (10 mg L^{-1}), pH 11. The signal was monitored at 450 nm.

Carbon dot volume

In each synthesis that was carried out, about 10 mL of the carbon dot solution was produced. Given this, the amount of carbon dot to be used in the quercetin analysis was evaluated to verify what would be the least amount of carbon dot possible that could be used in the analysis without loss of the sensitivity of the method.

The amount was varied between 0.05 and 0.10 mL and an analytical curve was obtained for each amount of carbon dot employed. In all volumes studied, a linear relationship between the fluorescence signal quenching (F_0/F) and the concentration of quercetin was obtained. The amount of 0.08 mL was selected because it presented a curve with

Table 1. Linear equation and coefficient of determination (R^2) obtained according to the carbon dot volume

Carbon dot volume / mL	Linear equation	R^2
0.05	F_0/F = (0.1513 ± 0.0049) × C + (0.8936 ± 0.0252)	0.9968
0.06	F_0/F = (0.1590 ± 0.0062) × C + (0.8639 ± 0.0319)	0.9954
0.07	F_0/F = (0.1654 ± 0.0067) × C + (0.8889 ± 0.0341)	0.9952
0.08	F_0/F = (0.1579 ± 0.0039) × C + (0.9316 ± 0.0202)	0.9982
0.09	F_0/F = (0.1749 ± 0.0085) × C + (0.8748 ± 0.0432)	0.9930
0.10	F_0/F = (0.1488 ± 0.0064) × C + (0.9814 ± 0.0329)	0.9944

C: quercetin concentration; F_0/F: F_0 and F are the fluorescence intensity of the carbon dot solution in the absence and presence, respectively, of quercetin.

Table 2. Linear equation and coefficient of determination (R^2) obtained according to the buffer solution nature

Buffer	Buffer solution	Linear equation	R^2
1	$Na_2B_4O_7$ 0.05 mol L^{-1}/NaOH 0.1mol L^{-1}	$F_0/F = (0.1338 \pm 0.0082) \times C + (0.8006 \pm 0.0416)$	0.9892
2	Na_2HPO_4/NaOH 0.1 mol L^{-1}	$F_0/F = (0.1511 \pm 0.0075) \times C + (0.8922 \pm 0.0384)$	0.9926
3	$NaHCO_3$ 0.05 mol L^{-1}/NaOH 0.1 mol L^{-1}	$F_0/F = (0.1761 \pm 0.0119) \times C + (0.8500 \pm 0.0608)$	0.9864
4	glycine/NaCl/NaOH 0.1 mol L^{-1}	$F_0/F = (0.1323 \pm 0.0043) \times C + (0.9250 \pm 0.0219)$	0.9968
5	Na_2CO_3/HCl 0.1 mol L^{-1}	$F_0/F = (0.1672 \pm 0.0091) \times C + (0.8606 \pm 0.0461)$	0.9912

C: quercetin concentration; F_0/F: F_0 and F are the fluorescence intensity of the carbon dot solution in the absence and presence, respectively, of quercetin.

greater sensitivity (a) and better coefficient of determination (R^2), as shown in Table 1.

Buffer solution nature

The study was carried out by varying the chemical composition of the buffer solution at pH 11: $Na_2B_4O_7$/NaOH, Na_2HPO_4/NaOH, $NaHCO_3$/NaOH, glycine/NaCl/NaOH and Na_2CO_3/HCl, as displayed in Table 2. As can be observed, there was a small loss of sensitivity (a) for buffer solutions 1 and 4. In the curves obtained with buffer solutions 3 and 5, although the sensitivity (K_{sv}) was higher, the quenching for the first concentration of the curve (1 mg L^{-1}) was lower than in the curve obtained for buffer solution 2. The results showed that the Na_2HPO_4/NaOH buffer solution promoted greater carbon dot stability and greater quenching effect for quercetin analysis. Thus, the Na_2HPO_4/NaOH buffer solution was chosen.

Buffer solution concentration

After establishing the pH (11.0) and nature of the buffer solution (Na_2HPO_4/NaOH), the concentration of the buffer was evaluated. The magnitude of the interaction of the analyte with the carbon dot surface can be influenced by the ionic strength of the medium (buffer solution concentration). The evaluation of this parameter was performed by varying the Na_2HPO_4/NaOH buffer solution concentrations between 0.1 and 0.6 mol L^{-1}. There was no significant variation in sensitivity (K_{sv}) and coefficient of determination (R^2) values in the quercetin analysis as a function of buffer concentration. Thus, the lowest concentration employed, 0.1 mol L^{-1} Na_2HPO_4/NaOH ($F_0/F = 0.1502 \times C + 0.9096$, $R^2 = 0.9978$) was maintained in subsequent studies.

Mechanism of interaction between carbon dot and quercetin

Figure 1 shows that the UV-Vis absorption spectra of the quercetin has bands around 255 and 370 nm. The synthesized carbon dot exhibits an absorption band around 340 nm and an emission band around 450 nm when excited at 380 nm. A partial overlap occurs between the absorption spectrum of quercetin and both the absorption and excitation spectra of the carbon dot. Fluorescence quenching of carbon dot occurs due to the absorbance of quercetin being similar to the excitation wavelength of the carbon dot. The quercetin shields part of the radiation for the excitation of the carbon dot, leading to the inner filter effect between quercetin and the carbon dot, causing the change in the intensity of fluorescence.[30,34]

In the spectra shown in Figure 4, a red-shift of the emission wavelength after the reaction between the quercetin and the carbon dot occurs. It is possible to observe that with the increase of the quercetin concentration, an increasing displacement for larger wavelengths (red-shift) occurs, suggesting that the quenching mechanism is not only related to the inner filter effect process, but also may be due to the fundamental state of the compounds formed.[36]

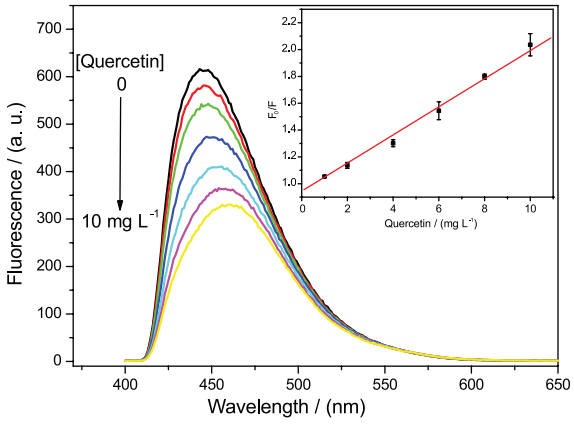

Figure 4. Fluorescence spectra for quercetin determination, reference solutions containing, 0; 1; 2; 4; 6; 8 and 10 mg L^{-1}, the insert is the analytical curve for quercetin.

Figure 5 shows the change in fluorescence intensities of CDs at 298, 303 and 305 K. It is possible to observe that there is a decrease in the Stern-Volmer constant (K_{sv}) with the increase in temperature of the reaction medium.

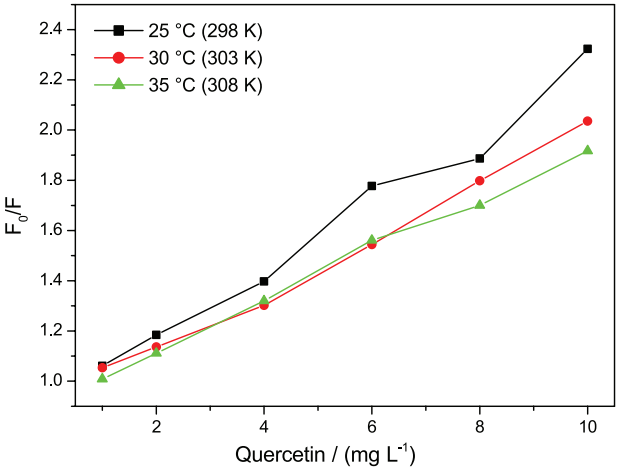

Figure 5. Analytical curves obtained from the reaction between the synthesized carbon dot and quercetin under different temperatures: 25, 30 and 35 ºC, respectively.

This phenomenon demonstrates that the mechanism involved in the fluorescence quenching of the carbon dot by quercetin is static. When the quenching mechanism is made more dynamic with the increase in temperature, an increase in the diffusion occurs as well as collisions between the carbon dot and the quencher, increasing the Stern-Volmer constant. When the mechanism is static, with the increase temperature occurs a dissociation of the complexes formed during the reaction between the carbon dot and the quencher, causing a decrease in the Stern-Volmer constant. Table 3 shows that this occurred between the reaction of the synthesized carbon dot and the quercetin.[37,38]

Table 3. Stern-Volmer quenching constants (K_{sv}) obtained from the reaction of the carbon dot and quercetin at different temperatures

Temperature / ºC	K_{sv} / (L mg^{-1})	R^2
25 (298 K)	0.1357	0.9819
30 (303 K)	0.1103	0.9934
35 (308 K)	0.1007	0.9964

R^2: coefficient of determination.

Another way to verify if the quenching is static is through the absorption spectrum of the CDs, shown in Figure 6. The formation of the ground state complex can result in the change of the absorption spectrum of the CDs.[39] It is possible to observe a change in the absorption spectrum of the CDs after the reaction with quercetin. There is a band at around 425 nm (Figure 6) indicating the occurrence of perturbation in the electron cloud, indicating that there was interaction between the CDs and quercetin.

The most efficient way to evaluate the mechanism involved in quenching, either static or dynamic, is through the measurement of the fluorescence lifetime. When

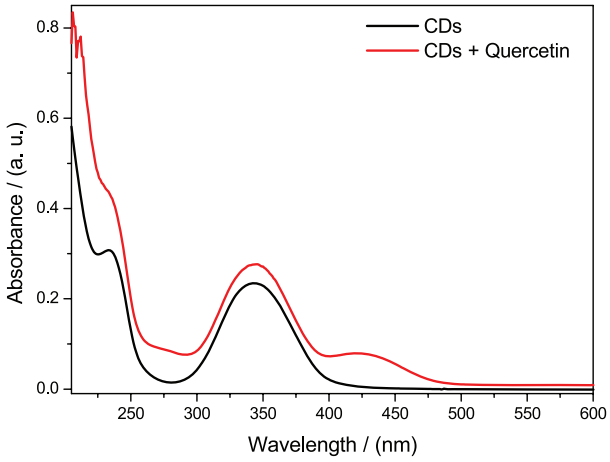

Figure 6. Absorbance spectrum of the CDs and of the product of the reaction between the CDs and quercetin.

quenching is static, the fluorescence lifetime does not change ($\tau_0/\tau = 1$), where τ_0 and τ, are the fluorescence lifetimes of CDs in the absence and presence of quercetin, respectively. The complexed CDs do not fluoresce, so the only observed fluorescence is from the uncomplexed CDs. In contrast, for dynamic quenching $F_0/F = \tau_0/\tau$. Therefore, it is possible to observe in Figure 7 that the fluorescence lifetime of CDs and CDs in the presence of 10 mg L^{-1} quercetin in buffer solution pH 11 was 6.43 and 6.54 ns, respectively, where $\tau_0/\tau = 1$, which proves that the mechanism of quenching of fluorescence involved in the reaction was static quenching.[35,37] Fluorescence emission decay profile of the other quercetin concentrations in buffer solution and in aqueous solution can be seen in Figures S1-S6 (Supplementary Information section).

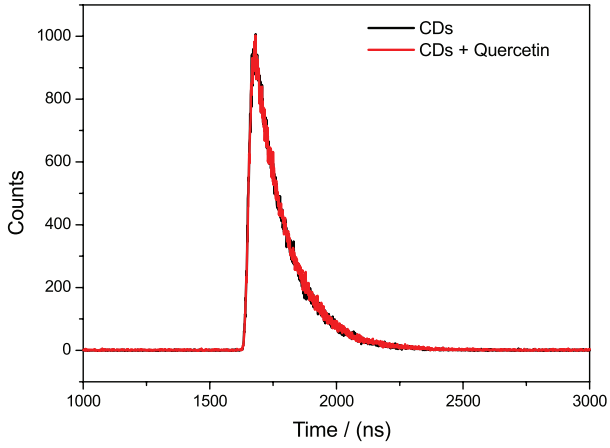

Figure 7. Fluorescence emission decay profile of the CDs and CDs in the presence of 10 mg L^{-1} quercetin in buffer solution pH 11 (λ_{ex} = 380 nm, λ_{em} = 450 nm).

Guo *et al.*[28] observed that CDs synthesized at a lower pH had low fluorescence and at a higher pH had high

fluorescence intensity. Given this, they concluded that there were carboxyl groups present in the nanoparticles synthesized. These groups can be protonated in acidic solutions, which will lead to the aggregation of CDs, resulting in quenching of the fluorescence. The interaction between quercetin and synthesized CDs may have occurred on the carbon dot surface.[40] It is believed to have occurred through the interaction between the CDs and the formed compounds due to the instability of the ring structure in quercetin under basic conditions, resulting in fragmentation of the ring structure[41] and this may have modified the surface of the CDs and caused the quenching of fluorescence.

Interference study

To evaluate the selectivity of the proposed method, we evaluated the effect of possible interferences that could affect the analysis in real samples. The effects on the analytical signal for reference solutions of 5.0 mg L^{-1} quercetin containing different concentrations of the potentially interfering species, ascorbic acid, gallic acid, citric acid, malic acid, catechin, epicatechin, kaempferol, resveratrol and myricetin were evaluated. The tolerance limit for interfering substances was defined as the amount of chemical species, which the signals obtained using the reference quercetin solution with the interfering substance causing a variation of ± 5% compared to the signal of the reference solution with 5.0 mg L^{-1} quercetin. As expected, the results presented in Table 4 show that the carbon dot employed gave a high selectivity to the method.

Table 4. Tolerance for potential interfering substances

Substance	Concentration / (mg L^{-1})	Variation signal / %
Ascorbic acid	5.00	−1.84
Gallic acid	5.00	−2.32
Citric acid	5.00	+1.08
Malic acid	10.00	−4.74
Catechin	2.00	+0.71
Epicatechin	15.00	+4.76
Kaempferol	0.04	−2.95
Resveratrol	10.00	−1.36
Myricetin	1.00	−4.85

Reference solution for quercetin 5.0 mg L^{-1}.

Analyzing the results shown in Table 4, we concluded that the species with the highest interference potentials are catechin, kaempferol and myricetin, as these substances showed a lower concentration limit than the interfering others.

In beer samples, the following contents have been identified: catechin ranging from 5.9 to 10.1 mg L^{-1};[42] kaempferol, 16.4 mg L^{-1};[43] and myricetin 0.08 mg L^{-1}.[44] When the beer samples were diluted 1:10 (v/v), the catechin, kaempferol and myricetin concentrations remained at maximums of 1.01, 1.64 and 0.008 mg L^{-1}, respectively. Since the limit of tolerance for catechin and myricetin is higher than the allowable concentration obtained after dilution, possible effects on the analysis would not be expected. If the analyzed beer does not have a maximum content of kaempferol, which is 16.4 mg L^{-1} as described in the literature,[43] there will also be no interference of this polyphenol after its dilution for quercetin analysis, as expected.

In tea samples, although the concentrations of catechin, kaempferol and myricetin are high as reported in the literature,[7] the recovery test confirmed the accuracy of the method, showing that there was no interfering effect on the analyzed tea samples. This way, through the recovery test (Table 5), the accuracy of the method was evaluated.

Analytical performance and recovery

Optimization of the proposed procedure for quercetin determination employed a reaction time of 0.5 min, a buffer solution Na_2HPO_4/NaOH and other parameters established, as shown in Table 6.

The fluorescent emission intensity of the carbon dot was sensitive to quercetin and decreased with the increase of the quercetin standard solution concentration. There was a red-shift of the emission wavelength. A linear response between 1.0 and 10.0 mg L^{-1} was obtained. The linear equation was estimated as $F_0/F = (0.1511 \pm 0.0075)[Q] + (0.8922 \pm 0.0384)$ ($R^2 = 0.9926$, n = 3), (the F_0 and F are the fluorescence intensities in the absence and presence of quercetin, respectively, and [Q] is the concentration of quercetin in mg L^{-1}), with a limit of detection of 0.85 mg L^{-1} and relative standard deviation (RSD) 0.21% (n = 3, 4 mg L^{-1}). This phenomenon can be described by the Stern-Volmer equation,[45] as shown in equation 1. The Stern-Volmer equation predicts a linear dependence of F_0/F on quencher concentration [Q]. Where F_0 and F are the fluorescence intensity of the carbon dot solution in the absence and presence, respectively, of quercetin and $K_{sv,}$ this is referred to as the quenching constant or the Stern-Volmer constant, established at 0.1511 L mg^{-1}.[45,46]

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

Given the above, it can be said that the proposed

Table 5. Recovery tests for quercetin

Sample	Added / (mg L^{-1})	Found / (mg L^{-1})	Recovery / %
Lager beer	0	5.14	–
	2.0	5.90	83
	5.0	8.65	85
Lager beer	0	1.73	–
	2.0	3.01	81
	5.0	6.09	91
Weissbier	0	4.37	–
	2.0	5.94	93
	5.0	8.12	87
Pilsen beer	0	1.88	–
	2.0	3.12	81
	5.0	7.01	101
Pilsen beer	0	2.96	–
	2.0	4.32	87
	5.0	7.70	97
Pilsen beer	0	1.51	–
	2.0	3.39	96
	5.0	5.22	80
Ready-to-drink green tea	0	3.87	–
	2.0	6.27	107
	5.0	8.30	94
Green tea infusion	0	8.45	–
	2.0	10.62	102
	5.0	13.17	98
Green tea infusion	0	6.54	–
	2.0	8.15	95
	5.0	13.64	104
Green tea infusion	0	8.72	–
	2.0	8.99	84
	5.0	11.24	82
Black tea infusion	0	7.48	–
	2.0	9.22	97
	5.0	11.32	91
Black tea infusion	0	6.37	–
	2.0	8.76	91
	5.0	10.07	89

procedure for quercetin analysis is feasible for analytical applications.

Spike recovery test is one of the ways to evaluate the accuracy of analytical results.[47] In a recovery experiment, a measured amount of the constituent to be analyzed (analyte) is added to the matrix and then the analysis is performed on this material. A comparison of the amount found in the matrix without addition *versus* the amount added provides the recovery of the method, which is an estimate of the accuracy of the method.[48] Six types of beer samples (Weissbier, Pilsen and Lager) and six types of tea samples (green and black teas infusion and ready-to-drink green tea) were tested. The addition was performed at two concentration levels of quercetin, 2.0 and 5.0 mg L^{-1}. Before analysis, the samples were filtered and diluted, 1:10 (v/v) for the beer samples and 1:30 (v/v) for the tea sample, maintaining the quercetin concentration within the linear working range. The results for the analysis of these beers and teas for the recovery test are shown in Table 5. The recovery values were between 80 and 107%, indicating the accuracy of the proposed method, demonstrating that there was no matrix effect. Thus, the method can be used to determine the content of quercetin in beer and tea samples.

Table 6. Parameters established after optimization of the proposed procedure

Parameter	Range	Selected value
Buffer solution pH	2-12	11
Carbon dot volume / mL	0.05-0.10	0.08
Buffer solution concentration / (mol L^{-1})	0.1-0.6	0.1

The proposed method was compared with other approaches previously reported in the literature for quercetin determination (Table 7), showing that our work has a linear range comparable to the methods reported in the literature.[49] In the analyses with high performance liquid chromatography mass spectrometry (HPLC-MS), heating and the use of solvents such as formic acid and acetonitrile were necessary, and the analyses were time consuming.[50] In some studies, the sample preparation made the method expensive and time consuming.[49,51,52] In the spectrophotometric method, although the analyses were performed directly due to the simplicity of the samples (drug), there were disadvantages such as the amount of solvent needed and reaction time of 4 min, being eight times slower than the reaction time reported in the present work.[53] There are fluorescent methods using carbon nanoparticles for determination of quercetin in an aqueous solution such as the method using luminescent organosilane-functionalized carbon dots[8] and using fluorescent carbon nanoparticles from ionic liquids,[40] although these methods are interesting, were not applied to real samples.

Our work has advantages over the others, such as high sample throughput, eco-friendly reagent economy, no extraction step using solvent, feasible analysis after simple filtration and dilution steps, without complicated pretreatment.

Table 7. Analytical features of methodologies employed on quercetin determination

Detection method	Linear range / (mg L^{-1})	RSD / %	Detection limit / (mg L^{-1})	Sample	Reference
Voltammetric	0.0002-7.55	3.5	0.0001	medicinal herbs	51
HPLC-MS	0.002-2.0	–	–	tea infusions	50
Electrochemical	0.12-9.07	5	0.012	tea	49
Fluorescence	0.02-0.8	4.1	0.005	urine and onion skin	52
Spectrophotometric	1.0-12.0	< 3	0.76	quercetin + C capsules	53
Fluorescence	0-12.0	–	0.02	aqueous solution	8
Fluorescence	0.87-9.54	2.9	0.03	–	40
Fluorescence	1.0-10.0	1.22	0.85	tea and beer	this work

RSD: relative standard deviation; HPLC-MS: high performance liquid chromatography mass spectrometry.

Conclusions

In this work, we demonstrated the applicability of a fluorescence procedure using carbon dots synthesized with citrate to determine the amount of quercetin in beer and tea samples. This was a simple and fast procedure, with a 0.5 min reaction time between the synthesized carbon dot and the quercetin, having a high sample throughput. The analysis was performed with a low consumption of carbon dots, using only 0.08 mL in each determination. As the carbon dots are obtained from carbon sources, they could provide eco-friendly sensors for the detection of quercetin. The interaction mechanism between carbon dots and quercetin is known to be not only associated with an inner filter effect but may also be due to the fundamental state of the compounds formed. Through the change of the temperature, it was possible to observe that the quenching was static. After the optimization of the analytical parameters, the method presented a linear range from 1 to 10 mg L^{-1}, limit of detection of 0.85 mg L^{-1} (pH 11), with recoveries of 80 and 107% for beer and tea samples, respectively, demonstrating that there was no matrix effect on the determination of quercetin in the samples analyzed. The proposed procedure was simple, fast, selective, and versatile for applications with different samples.

Supplementary Information

Supplementary information (Figure S1-S6) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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References

1. Abe-Matsumoto, L. T.; Sampaio, G. R.; Bastos, D. H. M.; *Cad. Saúde Pública* **2015**, *31*, 1371.
2. Santini, A.; Tenore, G. C.; Novellino, E.; *Eur. J. Pharm. Sci.* **2017**, *96*, 53.
3. Rodrígues-Delgado, M. Á.; González-Hernández, G.; Conde-González, J. E.; Pérez-Trujillo, J. P.; *Food Chem.* **2002**, *78*, 523.
4. Eleazu, C.; Obianuju, N.; Eleazu, K.; Kalu, W.; *Biomed. Pharmacother.* **2017**, *88*, 644.
5. Wang, Y.; Gao, Y.; Ding, H.; Liu, S.; Han, X.; Gui, J.; Liu, D.; *Food Chem.* **2017**, *218*, 152.
6. Bhuiya, S.; Haque, L.; Pradhan, A. B.; Das, S.; *Int. J. Biol. Macromol.* **2017**, *95*, 177.
7. Behling, E. B.; Sendão, M. C.; Francescato, H. D. C.; Antunes, L. M. G.; Bianchi, M. L. P.; *Aliment. Nutr.* **2004**, *15*, 285.
8. Zou, Y.; Yan, F.; Zheng, T.; Shi, D.; Sun, F.; Yang, N.; Chen, L.; *Talanta* **2015**, *135*, 145.
9. Mazloom, Z.; Abdollahzadeh, S. M.; Dabbaghmanesh, M. H.; Rezaianzadeh, A.; *J. Health Sci Surveill. Sys.* **2014**, *2*, 8.
10. Belmiro, T. M. C.; Pereira, C. F.; Paim, A. P. S.; *Microchem. J.* **2017**, *133*, 114.
11. Pejic, N.; Kuntic, V.; Vujic, Z.; Micic, S.; *Farmaco* **2004**, *59*, 21.

12. Memon, A. F.; Solangi, A. R.; Memon, S. Q.; Mallah, A.; Memon, N.; Memon, A. A.; *Food Anal. Methods* **2017**, *10*, 83.
13. Molinelli, A.; Weiss, R.; Mizaikoff, B.; *J. Agric. Food Chem.* **2002**, *50*, 1804.
14. Meenu, M.; Kamboj, U.; Sharma, A.; Guha, P.; Mishra, S.; *Int. J. Food Sci. Technol.* **2016**, *51*, 2520.
15. Liu, L.; Feng, F.; Paau, M. C.; Hu, Q.; Liu, Y.; Chen, Z.; Bai, Y.; Guo, F.; Choi, M. M. F.; *Talanta* **2015**, *144*, 390.
16. Namdari, P.; Negahdari, B.; Eatemadi, A.; *Biomed. Pharmacother.* **2017**, *87*, 209.
17. Tuerhong, M.; Yang, X.; Xue-Bo, Y.; *Chinese J. Anal. Chem.* **2017**, *45*, 139.
18. Shi, W.; Wang, Q.; Long, Y.; Cheng, Z.; Chen, S.; Zheng, H.; Huang, Y.; *Chem. Commun.* **2011**, *47*, 6695.
19. Liu, Q.; Ma, C.; Liu, X.-P.; Wei, Y.-P.; Mao, C.-J.; Zhu, J.-J.; *Biosens. Bioelectron.* **2017**, *92*, 273.
20. Wang, R.; Wang, X.; Sun, Y.; *Sens. Actuators, B* **2017**, *241*, 73.
21. Zhu, L.; Xu, G.; Song, Q.; Tang, T.; Wang, X.; Wei, F.; Hu, Q.; *Sens. Actuators, B* **2016**, *231*, 506.
22. Fong, J. F. Y.; Chin, S. F.; Ng, S. M.; *Biosens. Bioelectron.* **2016**, *85*, 844.
23. Kumari, A.; Kumar, A.; Sahu, S. K.; Kumar, S.; *Sens. Actuators, B* **2018**, *254*, 197.
24. Zou, Y.; Yan, F.; Dai, L.; Luo, Y.; Fu, Y.; Yang, N.; Wun, J.; Chen, L.; *Carbon* **2014**, *77*, 1148.
25. Gunjal, D. B.; Gore, A. H.; Naik, V. M.; Pawar, S. P.; Anbhule, P. V.; Shejwal, R. V.; Kolekar, G. B.; *Opt. Mater.* **2019**, *88*, 134.
26. Sun, S.; Guan, Q.; Liu, Y.; Wei, B.; Yang, Y.; Yu, Z.; *Chinese Chem. Lett.* **2019**, *30*, 1051.
27. Wang, Y.; Hu, A.; *J. Mater. Chem. C* **2014**, *2*, 6921.
28. Guo, Y.; Wang, Z.; Shao, H.; Jiang, X.; *Carbon* **2013**, *52*, 583.
29. Jaiswal, A.; Ghosh, S. S.; Chattopadhyay, A.; *Chem. Commun.* **2012**, *48*, 407.
30. Vaz, R.; Bettini, J.; Júnior, J. G. F.; Lima, E. D. S.; Botero, W. G.; Santos, J. C. C.; Schiavon, M. A.; *J. Photochem. Photobiol., A.* **2017**, *346*, 502.
31. Zhao, Y.; Liu, X.; Yang, Y.; Kang, L.; Yang, Z.; Liu, W.; Chen, L.; *Fullerenes, Nanotubes, Carbon Nanostruct.* **2015**, *23*, 922.
32. Bourlinos, A. B.; Stassinopoulos, A.; Anglos, D.; Zboril, R.; Karakassides, M.; Giannelis, E. P.; *Small* **2008**, *4*, 455.
33. Yang, Y.; Cui, J.; Zheng, M.; Hu, C.; Tan, S.; Xiao, Y.; Yang, Q.; Liu, Y.; *Chem. Commun.* **2012**, *48*, 380.
34. Zheng, M.; Xie, Z.; Qu, D.; Li, D.; Du, P.; Jing, X.; Sun, Z.; *ACS Appl. Mater. Interfaces* **2013**, *24*, 13242.
35. Yang, H.; Long, Y.; Li, H.; Pan, S.; Liu, H.; Yang, J.; Hu, X.; *J. Colloid Interface Sci.* **2018**, *516*, 192.
36. Huang, S.; Qiu, H.; Zhu, F.; Lu, S.; Xiao, Q.; *Microchim. Acta* **2015**, *182*, 1723.
37. Lakowicz, J. R.; *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: Baltimore, 2006.
38. Zhao, C.; Jiao, Y.; Hu, F.; Yang, Y.; *Spectrochim. Acta, Part A* **2018**, *190*, 360.
39. Zu, F.; Yan, F.; Bai, Z.; Xu, J.; Wang, Y.; Huang, Y.; Zhou, X.; *Microchim Acta* **2017**, *184*, 1899.
40. Xiao, D.; Yuan, D.; He, H.; Gao, M.; *J. Lumin.* **2013**, *140*, 120.
41. Chen, C.; Zhou, J.; Ji, C.; *Life Sci.* **2010**, *87*, 333.
42. Cortacero-Ramírez, S.; Segura-Carretero, A.; Cruces-Blanco, C.; Romero-Romero, M. L.; Fernández-Gutiérrez, A.; *Anal. Bioanal. Chem.* **2004**, *380*, 831.
43. Siqueira, P. B.; Bolini, H. M. A.; Macedo, G. A.; *Aliment. Nutr.* **2008**, *19*, 491.
44. Jandera, P.; Škeříková, V.; Řehová, L.; Hájek, T.; Baldriánová, L.; Škopová, G.; Kellner, V.; Horna, A.; *J. Sep. Sci.* **2005**, *28*, 1005.
45. de Souza, G. C. S.; de Santana, E. E. A.; da Silva, P. A. B.; Freitas, D. V.; Navarro, M.; Paim, A. P. S.; Lavorante, A. F.; *Talanta* **2015**, *144*, 986.
46. Keizer, J.; *J. Am. Chem. Soc.* **1983**, *105*, 1494.
47. Xu, J.; Zhu, L.; Shen, H.; Zhang, H.; Jia, X.; Yan, R.; Li, S.-L.; Xu, H.-X.; *J. Pharm. Biomed. Anal.* **2012**, *62*, 210.
48. Betz, J. M.; Brown, P. N.; Roman, M. C.; *Fitoterapia* **2011**, *82*, 44.
49. Rezazadeh, F.; Mohamadi, M.; Afzali, D.; Shamspur, T.; Mostafavi, A.; *Food Compos. Addit.* **2015**, *98*, 1375.
50. Jeszka-skowron, M.; Krawczyk, M.; Zgoła-Grzeskowiak, A.; *J. Food Compos. Anal.* **2015**, *40*, 70.
51. Ziyatdinova, G.; Kozlova, E.; Budnikov, H.; *J. Electroanal. Chem.* **2018**, *821*, 73.
52. Hu, Y.; Feng, T.; Li, G.; *Spectrochim. Acta, Part A* **2014**, *118*, 921.
53. Pejic, N.; Kuntic, V.; Vujic, Z.; Micic, S.; *II Farmaco* **2004**, *59*, 21.

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