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Turn–on fluorescent sensor for the detection of periodate anion following photochemical synthesis of nitrogen and sulphur co–doped carbon dots from vegetables

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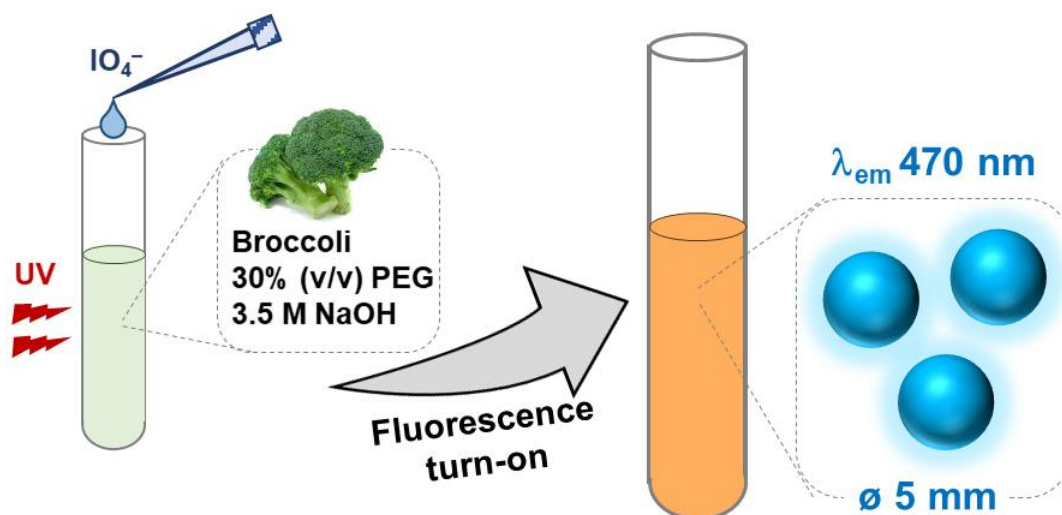**Corresponding author: Carlos Bendicho. E–mail address: bendicho@uvigo.es*

Turn–on fluorescent sensor for the detection of periodate anion following photochemical synthesis of nitrogen and sulphur co–doped carbon dots from vegetables

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Abstract

In this work, a novel one–step 'bottom–up' synthetic approach is described for obtaining highly fluorescent nitrogen (N) and sulphur (S) co–doped carbon dots (CDs) following photochemical oxidation of carbohydrates naturally occurring in vegetables. N and S co–doping allows more active sites in the CDs surface resulting in an enhancement of their luminescent properties. Among the 18 vegetables studied as green precursors of CDs, those rich in proteins and glucosinolates (natural S–linked glucosides), such as cruciferous vegetables (*Brassicaceae* family), *i.e.* broccoli, cauliflower and Romanesco, facilitate an efficient N and S co–doping of the CDs during the photochemical reaction without the need for further post–synthetic treatments. A comprehensive characterization of CDs obtained from broccoli was performed by transmission electron microscopy, elemental analysis, X–ray fluorescence (total reflection), UV–Vis absorption and Fourier transform–infrared spectroscopy. Quantum yields and fluorescence up–conversion properties were also investigated.

Monodisperse CDs (~ 8 nm average size) with up–conversion fluorescence properties and a quantum yield (QY) of 22% are obtained. More importantly, a dramatic increase in the CDs fluorescence (turn–on) is observed when the highly oxidant periodate anion (IO_4^-) is added to the reaction medium, which allowed us to construct a sensitive and selective fluorescent assay for the detection of periodate anion in wastewater samples. The detection limit was 19 µM IO_4^- and the repeatability expressed as the relative standard deviation was 3.2% (N=5).

Keywords

Carbon dots; Vegetables; Photochemical oxidation; Nitrogen sulphur co–doping; Turn–on fluorescent sensing; Periodate anion

1. **Introduction**

In the last years, carbon dots (CDs) have emerged as one of the most promising fluorescent nanoprobes because of their great advantages including low toxicity, good biocompatibility, low cost synthesis, high aqueous stability and easy functionalization [1–3]. CDs with typical sizes in the range of 2–10 nm can be synthesized from natural and non–toxic precursors such as carbohydrates. A wide variety of approaches have been developed for obtaining highly fluorescent CDs, including 'top–down' strategies, *e.g.* arc–discharge [4], laser ablation [5,6] and electrochemical exfoliation [7, 8], as well as 'bottom–up' strategies, *e.g.* microwave treatment [9,10], sonochemical treatment [11], thermal decomposition [12,13] and thermal treatment [14–17]. Main drawbacks of these methods are the long operation times, high costs related to the instrumentation needed and the harsh synthesis conditions typically involved. So, more efforts should be focused

on the development of simpler, greener and less expensive synthetic pathways for obtaining fluorescent CDs.

Different precursors have been proposed as green carbon sources, mainly fruits peels (*e.g.* grape, pomelo, orange or lemon peels) [18–21] and fruit juices (*e.g.* pear, orange, lime, apple, papaya, banana or lemon juices) [22–29]. One of the most common approaches for CDs production from fruit is the hydrothermal synthesis based on the treatment of aqueous reaction mixtures at high temperature and pressure in an autoclave system. The use of greener carbon sources brings about low environmental impact and cost–effectiveness, but the reaction time and temperature involved in hydrothermal processes remain a drawback. Besides, assays based on fluorescent CDs typically consist of two stages, *i.e.* firstly, synthesis and purification of fluorescent CDs and secondly, detection of the target analyte.

Recently, *in situ* detection strategies for methylmercury and hydrogen peroxide have been developed in our group. These strategies are based on the integration of synthesis of CDs (using both sonochemical and photochemical approaches along with pure commercial carbohydrates) and sensing within a single step [11, 30]. Although these approaches eliminate the need for large and tedious procedures and shorten the time required to accomplish the assay, quantum yields (QYs) are typically below 10%. In order to obtain higher QYs, chemical doping of CDs with N or S elements is a very effective strategy to intrinsically improve the photoluminescence QYs of CDs [31]. Higher QY and fluorescent intensity could be attributed to the heteroatom passivation, which stabilizes the effective radiative recombination of electrons and holes from CDs surface [32].

Herein, we report on a simple synthetic route of N and S co–doped highly blue–fluorescent CDs following photochemical treatment of broccoli aqueous extracts. The fluorescence enhancement caused by the presence of periodate anion (IO_4^-) in the reaction

mixture can be the basis for a fast, green, low cost and sensitive fluorescent assay for detecting this oxidizing agent in wastewater. The described approach does not require large and tedious procedures for purification of CDs and the synthesis time is remarkably shortened as compared to conventional hydrothermal methods.

2. Experimental

2.1. Materials and methods

Ultrapure water obtained from an Ultra Clear TWF EDI UV TM system from Siemens (Barsbuettel, Germany) was used for preparing vegetables extracts and working solutions. Broccoli, carrot, cauliflower, celery, chard, collard greens, courgette, endive, fennel, green beans, green Italian pepper, pumpkin, red bell pepper, romanesco, tomato, turnip greens, white cabbage and yellow bell pepper were purchased in local markets and used as carbon source for CDs photochemical synthesis.

Poly(ethylene glycol) Bio Ultra 200, polyethylenimine (PEI), Tween 80 and bovine serum albumin (BSA) from Sigma–Aldrich were tried as stabilizing agents. NaOH from Prolabo (Paris, France) was used for adjusting pH in the reaction medium.

Potassium periodate (KIO_4) AnalR Normapur® from VWR Chemicals (Fontenay–sous–Bois, France) was used as analyte for the fluorescent sensing assay. Potassium perchlorate ($KClO_4$) from Sigma Aldrich (St Lois, MO, USA), hydrogen peroxide 30% (w/w) solution (H_2O_2) from Merck (Darmstadt, Germany), and sodium hypochlorite (NaClO) from VWR (Fontenay–sous–Bois, France) were used for evaluating the response of the synthesized CDs in the presence of other highly oxidant species.

The following chemicals were tested as potential interferents: potassium iodate, magnesium chloride (Merck, Darmstadt, Germany), sodium chloride, sodium sulphate (Sigma–Aldrich, St Lois, USA), potassium nitrate (Probus SA, Badalona, Spain), sodium

carbonate (Panreac, Barcelona, Spain), humic acid (Fluka Sigma Aldrich, St Lois, USA) and potassium phosphate monobasic (Panreac, Barcelona, Spain).

For preparing the synthetic wastewater samples, humic acid, NaCl, $CaCO_3$ and $FeSO_4 \cdot 7H_2O$ from Sigma–Aldrich (St Louis, USA), $Mg(NO_3)_2 \cdot 6H_2O$, $CuSO_4 \cdot 5H_2O$ and KNO_3 from Probus (Barcelona, Spain), $CuCl_2$ and $MgCl_2$ from Merck (Darmstadt, Germany), Na_2CO_3 (Panreac, Barcelona, Spain), $NaHCO_3$ (Carlo Erba, Rodano, Italy), EDTA (Prolabo, Leuven, Belgium) and potassium hydrogen phthalate (Scharlab, Barcelona, Spain) were used. Experimental details for synthetic wastewater preparation are included in the Supplementary Material.

2.2. Instrumentation

Photochemical oxidation was carried out using a 705 UV digester (Metrohm, Herisau, Switzerland) equipped with a high–pressure mercury lamp (500 W), cooling system and a holder for twelve digestion quartz tubes (15 mm i.d.; 125 mm length).

A Thermo Scientific NanoDrop 3300 Fluorospectrometer equipped with three solid–state light emitting diodes (LEDs) as excitation source, which are oriented 90° in respect to the 2048–element CCD array detector, was used for performing the fluorescence measurements. The fluorescence intensity was measured at 480 nm after excitation at 365 nm using the UV LED.

For studying the effect of the excitation wavelength over the emission wavelength of the synthesized broccoli–CDs, a FluoroMax®–4 spectrofluorometer (Horiba, Bensheim, Germany) equipped with a xenon arc lamp (150 W) was used. Besides, for monitoring the degradation of carbohydrates during photochemical treatment of broccoli extracts, UV–Vis absorption measurements were performed using a Cary–100 Bio UV–visible spectrophotometer (Agilent, Mulgrave, Australia).

A JEOL–1010 microscope operating at an acceleration voltage of 100 kV was used for performing the transmission electron microscopy (TEM) characterization. Fourier transform infrared (FT–IR) spectra were recorded using a Nicolet 6700 spectrometer (Thermo Scientific, Waltham, USA) in the range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1}.

Total reflection X–ray fluorescence (TXRF) was applied to the determination of trace elements (S, K, Ca, Mn, Fe, Cu, Zn) in the different green precursors using a Bruker S2–Picofox® spectrometer equipped with a molybdenum tube as X–ray source (1000 µA, 50 kV, 50 W) and high resolution XFlash® detector (silicon–drift detector, SDD) with an active surface of 10 mm^2.

Elemental analysis measurements (N, S, C and H) were carried out with a Fisions–Carlo Erba microanalyzer model EA1108.

An IKA ultraturrax model T–25 digital homogenizer was employed for sample pretreatment in the extraction of carbohydrates from vegetables.

2.3. Synthetic procedures

2.3.1. Procedure for carbohydrate extraction from vegetables

18 different vegetables were tried as green precursors for CDs synthesis, *i.e.* broccoli, carrot, cauliflower, celery, chard, collard greens, courgette, endive, fennel, green beans, green Italian pepper, pumpkin, red bell pepper, romanesco, tomato, turnip greens, white cabbage and yellow bell pepper. For the preparation of aqueous extracts, 40 g of each fresh vegetable were weighed, rinsed thoroughly with ultrapure water, cut into small pieces with a knife and placed in a polystyrene vessel. Then, 100 mL of ultrapure water were added, and the sample was firstly ground with a beater. Next, the sample was further homogenized using an Ultra–Turrax® system for 2 min at a speed of 10,000 rpm. Afterwards, the vegetable sample was transferred to a polypropylene tube and placed

inside an ultrasound bath for 60 min in order to achieve the extraction of carbohydrates. Finally, the mixture was filtered under vacuum and the liquid phase was stored in the freezer at –5 °C (being stable for at least 6 months). The frozen extract was defrosted at ambient temperature for 2 h before use. The excess of defrosted extract was stored at 4 °C in the dark.

2.3.2. Photochemical synthesis of CDs

For synthesis of CDs from the different vegetables, a 1.5 mL aliquot of each vegetable aqueous extract, 0.2 mL of 3.5 M NaOH and 0.8 mL of 20% (v/v) PEG were placed in a quartz tube. The reaction mixture was homogenized using a vortex mixer for 30 s. After that, the quartz tubes were placed in the sample holder of an UV digester and irradiated for 15 min. Then, the obtained mixtures were transferred to Eppendorf™ tubes for separation the aggregates formed by centrifugation at 14,000 rpm (at 5 °C) for 10 min. Finally, a 2 µL aliquot of the synthesized CDs was placed onto the pedestal of a microfluorospectrometer to measure the intrinsic fluorescence at 480 nm of each sample after excitation at 365 nm. Three replicates for each vegetable were performed.

2.3.3. Integration of broccoli–based CDs–PEG synthesis and IO_4^- sensing

To construct the fluorescent probe for sensing of IO_4^-, an aliquot of 1.5 mL of broccoli aqueous extract prepared following extraction of carbohydrates from 80 g of fresh broccoli in 100 mL of ultrapure water was placed in a quartz tube. Then, 1.5 mL of wastewater sample containing IO_4^- was added. After that, 0.2 mL of 3.5 M NaOH and 0.8 mL of 30% (v/v) PEG were introduced in the quartz tube. Next, the mixture was shaken in a vortex mixer for 30 s. Afterwards, the quartz tube was placed in a sample holder of an UV digester and irradiated for 20 min. The color of the bulk solution changed from light green to brown. After that, the mixture was introduced in an Eppendorf™ tube

and centrifuged at 14,000 rpm at 5 °C for 10 min to separate the aggregates formed in the medium from the synthesized CDs–PEG. Finally, a 2 µL aliquot of CDs–PEG was placed onto the pedestal of a microfluorospectrometer for measuring the analytical response. The analytical response is expressed as I/I_0, where I_0 and I represent the fluorescence intensity of CDs synthesized in the absence of IO_4^- (analytical blank) and in the presence of IO_4^-, respectively. For blank measurements, the same procedure was followed, but an aliquot of 1.5 mL of ultrapure water (without IO_4^-) instead of wastewater sample was used. The synthesis procedure integrated with IO_4^- sensing is illustrated in **Fig. 1**.

3. Results and discussion

3.1. Selection of the carbon source

Intrinsic fluorescence of CDs varies as a function of the precursor used for synthesis (**Fig. 2**). Fluorescent CDs are observed from all vegetables tested as precursors, but higher fluorescence signals occurred for cruciferous vegetables (*Brassicaceae* family), *i.e.* broccoli, cauliflower and romanesco. All vegetables contain vitamins (at different concentration levels) which could be a nitrogen source for natural doping of the obtained CDs. In addition, cruciferous vegetables are characterized for containing high amount of glucosinolates, natural S–linked glucosides, which would promote the surface doping with sulphur resulting in higher intrinsic fluorescence compared to others. Heteroatom doping was reported to improve the optical properties of CDs, specially their QY. In order to probe the presence of sulphur in the obtained CDs using broccoli, cauliflower and romanesco, CDs synthesized using the different precursors were characterized by total reflection X–ray fluorescence (TXRF). Results are shown in **Table S1**. As can be observed, those CDs synthesized using cruciferous vegetables contain higher concentration of sulphur in their composition, *e.g.* 25 mg g^{-1}, 17 mg g^{-1} and 22 mg g^{-1} for the CDs synthesized using broccoli, cauliflower and romanesco as precursors,

respectively. Romanesco is usually available in supermarkets and groceries only in winter season and it is more expensive than others. On the other hand, broccoli and cauliflower are available in supermarkets and groceries all year. Aqueous extracts from broccoli (stored at 4 °C in the darkness) were stable at least for 2 weeks. On the contrary, cauliflower aqueous extracts (stored at 4 °C in the darkness) undergo degradation after 2 days being unsuitable for CDs synthesis. Thus, among these natural precursors, broccoli was selected for further experiments.

The effect of precursor concentration used for preparing the aqueous extracts was studied in the range of 0.2–1 g mL^{-1}. Results are shown in **Fig. S1**. As can be observed, intrinsic fluorescence increases up to a concentration of 0.8 g mL^{-1} of broccoli subjected to extraction. For higher concentrations, a decrease in the fluorescence was observed. When 1 mg mL^{-1} of broccoli was used to perform the extraction, homogenization of the mixture was troublesome. Therefore, a concentration of 0.8 g mL^{-1} of broccoli was used to obtain aqueous extracts for further optimization studies.

3.2. Optimization of experimental parameters

The effect of the different parameters involved in the procedure procedure, *i.e.* pH of the medium, type of stabilizing agent, stabilizing agent concentration and UV irradiation time, over the analytical response of IO_4^- anion was investigated. All experiments were performed using broccoli as carbon source and a 0.5 mM IO_4^- solution.

3.2.1. Effect of pH

The mechanism for the formation of CDs involves the photooxidation of the major carbohydrate constituents of broccoli, *i.e.* glucose, fructose and sucrose. In broccoli, fructose represents around the 60% of the total sugars content [33], being the primary CDs precursor. In addition, studies concerning photooxidation of sugars indicate that alkaline medium is needed for obtaining both good photodegradation yields and highly

fluorescent CDs [30, 34]. The effect of pH was tested in the range of 2 – 14. For adjusting the pH to 2, a 0.2 mL volume of 1 M HCl was added to the quartz tube together with 1.5 mL of broccoli extract and 0.8 mL of 20% (v/v) PEG. For testing neutral pH, 0.2 mL of ultrapure water were added to the quartz tube. Finally, pH values 9, 10 and 14 were adjusted by adding different concentrations of NaOH (0.2 mL), *i.e.* 0.5 M, 1.0 M and 3.5 M respectively, to the quartz tube. All mixtures were UV irradiated for 15 min after homogenization by vortex mixing. After that, the reaction mixtures were transferred to Eppendorf™ tubes and centrifuged at 14,000 rpm (at 5 °C) for 10 min to separate the aggregates formed. Finally, fluorescence measurement of each sample was carried out. In view of the obtained results (**Fig. 3A**), a 3.5 M NaOH concentration (reaction medium pH~14) was selected to obtain the best fluorescent response.

Although sugars are non–UV absorbing molecules, carbonyl compounds are produced after photooxidation, which can be characterized by a maximum absorbance peak at *ca.* 268 nm [34], so photodegradation of sugars can be monitored by UV absorption measurements. For monitoring the photodegradation of the sugars present in broccoli, UV spectra of the reaction medium after photooxidation at different times in the range of *ca.* 200–400 nm were recorded (**Fig. S2**). An increased absorbance at *ca.* 268 nm is observed on increasing the photooxidation time, thereby confirming the structural changes of sugars occurring in the broccoli extract after UV irradiation.

3.2.2. Effect of stabilizing agent and its concentration

Both functionalization and passivation of CDs using stabilizing agents in the reaction medium plays a critical role in determining the optical properties of the obtained CDs [35, 36]. Fluorescent emission from naked CDs synthesized from broccoli without any deliberate surface functionalization in aqueous media is observed, but it is quite low (**Fig. 3B**). Highly fluorescent CDs can be obtained using an effective surface functionalization.

This would also stabilize the surface defects thus enabling more efficient radiative electron/hole recombination after photoexcitation, and in turn, providing higher QY [37]. In the present work, several stabilizing agents including polyethyleneglycol (PEG), polyethyleneimine (PEI), 20% (v/v) Tween–80 and 0.1 g mL^{-1} bovine serum albumin (BSA) were tested as stabilizing agents.

A 0.8 mL aliquot of each stabilizing agent solution was added to different quartz tubes containing 1.5 mL of broccoli extract and 0.2 mL of 3.5 M NaOH. The highest fluorescence intensity was provided by PEG as stabilizing agent. To evaluate the influence of the concentration of PEG, different aqueous solutions of PEG, *i.e.* 10% (v/v), 20% (v/v), 30% (v/v), 40% (v/v), and 50% (v/v) were prepared. 0.8 mL of each PEG solution together with 1.5 mL of broccoli extract and 0.2 mL of 3.5 M NaOH were added to different quartz tubes. The mixtures were vortex mixed followed by UV irradiation for 15 min. Next, aggregates were separated by centrifugation (14,000 rpm; 10 min; 5 °C) and the intrinsic fluorescence of the CDs in the supernatant was measured (λ_{em} 365 nm; sample volume 2 μL). An increase in the PEG concentration up to 30% (v/v) led to an increased analytical response for IO$_4^-$ (**Fig. 3C**), the latter concentration being chosen for further experiments.

3.2.3. Effect of irradiation time

Another relevant factor influencing the optical properties of the synthesized CDs is the UV irradiation time (*i.e.* synthesis time) since it would affect the size of the obtained nanoparticles. Different synthesis times in the range of 1–25 min were tried. As shown in **Fig. 3D**, both the fluorescence intensity of CDs and the analytical response (I/I$_0$) for IO$_4^-$ increased on increasing UV irradiation time. For times longer than 20 min, only a slight increase in the fluorescence response was observed. In order to assess the effect of the UV irradiation on CDs size, TEM images at two different synthesis times, *i.e.* 10 and 20

min, were recorded. As shown in **Fig. 4 (A and B)**, application of longer irradiation times led to smaller CDs. Average sizes of 10 nm and 8 nm were obtained after a synthesis time of 10 and 20 min, respectively. The decrease in CDs size would promote a higher quantum confinement of the emissive energy traps resulting in enhanced QY and, in turn, in a higher fluorescence emission. This finding is in agreement with the mechanism firstly reported for photochemical synthesis of CDs from sucrose [30].

3.3. Study of potential interferences

In order to assess the effect of potential matrix effects in the sample, the detection of IO_4^- anion (0.5 mM) in the presence of several foreign substances was tried. Several compounds commonly present in wastewater samples were tested (a matrix effect was considered to be significant when the analytical response varied beyond $\pm10\%$). As shown in **Table 1**, concentrations of 100 mg L^{-1} humic acid and 5000 mg L^{-1} potassium hydrogen phthalate cause a small depressive effect since IO_4^- is consumed during oxidation of those compounds. It is remarkable to observe that those concentrations are above their typical levels in wastewaters (see Supplementary Material). KIO_3 did not cause any interferent effect up to at least a 500 mg L^{-1} (~2.3 mM) concentration. For the rest of studied concomitants, no significant effects were observed in the studied concentration ranges.

In addition, some metal and organometal species of interest, *i.e.* Se(IV), Se(VI), Sb(III), Sb(V), As(III), As(V), Hg, Cd(II), Te(VI), Cr(VI), Cu(II), Me_3Pb, $MeHg^+$ y $SnBu_4$, have been also evaluated as potential interferences. No significant interferent effect was observed for those species at least up to a concentration of 10 mg L^{-1}.

Finally, the response of CDs in the presence of other highly oxidant species, *i.e.* perchlorate (ClO_4^-), hydrogen peroxide (H_2O_2), and hypochlorite (ClO^-) at a concentration of 5 mM, was also studied (**Fig. S3**). No effect was observed showing the high selectivity of the fluorescent assay toward IO_4^-.

3.4. Sensing mechanism and analytical figures of merit

When CDs are synthesized in the presence of IO_4^-, a reduction in their size (~5 nm) is observed (**Fig. 4C**), resulting in fluorescence enhancement without spectral shift of the emission band. Periodate is a highly oxidizing agent that can promote the scission of simple polysaccharides (glycol cleavage) such as glucose, fructose or sucrose, thereby acting as a catalyst during carbohydrates oxidation [38]. Furthermore, irradiation of IO_4^- anion under alkaline conditions leads to the generation of highly reactive radicals such as $IO_3^{\bullet}$, $\bullet OH$, and $IO_4^{\bullet}$ [39], which facilitates the oxidation of the carbohydrates yielding CDs with smaller size. As stated above, a decrease in CDs size would promote the confinement of emissive energy traps leading to a fluorescent enhancement.

Analytical characteristics of the fluorescent assay were established under optimal conditions. As is shown in the inset of **Fig. 5,** a linear relationship between I/I_0 and the concentration of IO_4^- in the range of 0.04 – 4 mM IO_4^- was obtained. The detection limit (LOD) calculated following the 3σ IUPAC criterion was 19 μM IO_4^-. The repeatability expressed as relative standard deviation was 3.2% (N=5) whereas the reproducibility studied as between–day precision was 5.2% (N=3).

It should be noted that broccoli–based CDs are selective for IO_4^- in the presence of other highly oxidant species. Even IO_3^- at a concentration 10–fold higher did not cause any interference over the detection of IO_4^-. In addition, the novel CDs–based fluorescent assay required neither use of organic dyes, typically involved in conventional fluorometric/photometric approaches for IO_4^- determination in water [40–43], nor expensive reagents, which considerably diminishes the analysis cost. The UV–digestion equipment employed in this work allows parallel processing of up to 12 samples per run thus yielding a sample throughput of 36 h^{-1}, being advantageous for routine analysis.

3.5. Detection of periodate anion in wastewater samples

The fluorescent assay was applied to the detection of IO_4^- in two different synthetic wastewater samples following the procedure described in *Section 2.3.3*. Synthetic wastewater samples were prepared following the procedure described in the Supplementary Material and stored at 4 °C in the dark before use. To demonstrate the accuracy of the fluorescent assay, recovery studies at three different levels were performed. The analytical results show recoveries in the range of 90–97% (**Table 2**) indicating that the assay is reliable for IO_4^- sensing in wastewater.

3.6. Characterization of the CDs

Experimental details for characterization of CDs are described in the Supplementary Material. The composition of the CDs synthesized under optimal conditions (2.5 mL of broccoli aqueous extract, 0.8 mL of 30% (v/v) PEG and 0.2 mL of 3.5 M NaOH) was studied by elemental analysis and FT–IR measurements. **Table S2** shows the results for elemental analysis. CDs synthesized from broccoli in the presence of PEG contains around 26% of carbon and 0.5% of nitrogen thus confirming N–doping. In addition, **Fig. 6** shows IR spectra obtained for dried broccoli, PEG and CDs. As can be observed, for PEG and CDs–PEG, a broad band appears at around 3400 cm^{-1} corresponding to OH group stretching vibrations. In the case of broccoli, the broad band in the range of 3400–3500 cm^{-1} can be ascribed to OH and NH groups stretching vibrations. Furthermore, a band at 1640 cm^{-1} appears in the CDs–PEG IR spectrum, which can be attributed to carbonyl groups formed after photodegradation of the sugars present in broccoli. Moreover, the sharp band around 1100 cm^{-1} can be assigned to C–O stretching vibrations of primary alcohols groups (from PEG) thereby confirming that photogenerated CDs–PEG is surrounded by hydrophilic groups. This finding accounts for the high aqueous stability of CDs–PEG.

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Photogenerated CDs showed a blue color when they were irradiated at 365 nm (UV), easily visible to the naked eye (**Fig. S4**). In addition, fluorescent spectra of CDs recorded under UV–Vis and NIR excitations showed that the fluorescent emission was dependent on the excitation wavelength. As shown in **Fig. S5A**, the emission band of CDs is shifted from 405 nm to 510 nm when excitation is performed at a wavelength in the range of 300–500 nm (UV–Vis). Moreover, CDs irradiated with low wavelength light, *i.e.* 700–900 nm showed up–conversion fluorescence emissions located between 415 and 500 nm (**Fig. S5B**). Low–energy light (NIR or IR) is converted into higher–energy light (UV–Vis), which may be attributed to a multiphoton active process leading to anti Stokes type emission.[44, 45] In addition, the QY of the synthesized CDs using a photooxidation time of 20 min was calculated using fluorescein as the reference fluorophore. The relative method gave a QY of 22% for the broccoli–based CDs–PEG (**Fig. S6**), which is comparable to that of CDs synthesized from many fruit juices and peels (**Table 3**). Furthermore, one of the main advantages of the photochemical treatment compared to conventional hydrothermal methods is the shorter synthesis time.

4. Conclusions

In sum, blue fluorescent N,S co–doped CDs have been successfully synthesized by photochemical treatment of broccoli aqueous extracts. Monodisperse CDs (~8 nm) with stable photoluminescence and up–conversion behavior are obtained. IO_4^- can promote the scission of carbohydrates present in broccoli thereby acting as a catalyst during the photochemical reaction. Since the sensing event is integrated with the preparation of the fluorescent probe, slow and tedious purification steps typically needed for the subsequent application of CDs as sensors are avoided. The involvement of other oxidizing species and inhibitors of the photochemical reaction could expand the scope of this assay.

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Figure captions

Figure 1. Scheme for in situ photochemical synthesis of broccoli–based CDs–PEG and sensing of periodate (IO_4^-) in wastewater procedure.

Figure 2. Intrinsic fluorescence (excitation at 365 nm) of CDs using different natural precursors *(in alphabetic order)*.

Figure 3. (A) Effect of pH medium (B) Effect of type of stabilizing agent (C) Effect of PEG concentration (C) Effect of irradiation time. FI: fluorescence intensity (relative fluorescence units, RFU); PEI: polyethyleneimine; PEG: polyethylene glycol; BSA: bovine serum albumin. The solid and dotted lines represent the analytical response (I/I_0) for IO_4^- and the intrinsic fluorescence (as relative fluorescence units, RFU) of CDs, respectively. Errors bars are expressed as the standard deviation from three replicates.

Figure 4. (A) TEM images of broccoli–based CDs–PEG after 10 min of UV irradiation (scale bar: 50 nm) (B) TEM images of broccoli–based CDs–PEG after 20 min of UV irradiation (scale bar: 50 nm) (C) TEM images of broccoli–based CDs synthesized in the presence of IO_4^- (scale bar: 20 nm).

Figure 5. Fluorescence spectra of photogenerated CDs in the presence of different concentrations of periodate (IO_4^-) and representation of the calibration curve. Experimental conditions: 0.8 mg mL^{-1} broccoli extract volume, 1.5 mL; [IO_4^-], 0.04–4 mM; 30% (v/v) PEG volume, 0.8 mL; 3.5 M NaOH volume, 0.2 mL; irradiation time, 20 min.

Figure 6. FT–IR spectra for the different studied samples.

Table 1. Study of potential interferent species on the detection of IO_4^-.

Interferent	Concentration (mg L^{-1})	IO_4^- recovery (%)	Effect (%)
KIO$_3$	50	98 ± 5	−2
	500	105 ± 8	+6
Na$_2$CO$_3$	500	102 ± 3	+3
	5000	92 ± 10	−9
NaHCO$_3$	500	100 ± 3	+1
	5000	96 ± 5	−5
NaCl	100	99 ± 3	−2
	1000	96 ± 4	−4
MgCl$_2$	100	103 ± 4	+4
	500	108 ± 10	+9
NaSO$_4$	100	101 ± 2	+1
	500	100 ± 1	+1
KNO$_3$	100	105 ± 6	+5
	2000	110 ± 11	+8
KH$_2$PO$_4$	100	99 ± 3	−2
	500	95 ± 6	−6
Humic acid	10	95 ± 7	−6
	100	91 ± 11	−12
Potassium hydrogen phthalate	500	93 ± 8	−8
	5000	80 ± 15	−16

Table 2. Analytical results and recovery studies for synthetic wastewater analysis

Sample	IO_4^- added (mM)	IO_4^- found (mM ± sd, N=3)	Recovery (% ± sd, N=3)
WW–1	–	–	–
	0.1	0.09 ± 0.02	90 ± 7
	0.5	0.48 ± 0.05	97 ± 4
WW–2	–	–	–
	0.1	0.09 ± 0.03	95 ± 6
	0.5	0.45 ± 0.08	92 ± 9

sd: standard deviation

Table 3. Comparison of photosynthesized broccoli–based CDs with others prepared from fruits as carbon source.

Carbon source	Synthesis strategy	Synthesis time	QY (%)	Application	Ref
Onion and grape juice	Hydrothermal treatment	4 h	n.a.	Fe(II)–Fe(III) speciation in wastewater water	[18]
Pomelo peel	Hydrothermal treatment	3 h	6.9	Hg(II) sensing in river water	[19]
Orange peel	Hydrothermal treatment	12 h	36	Photocatalyst	[20]
Lemon peel	Hydrothermal treatment	12 h	14	Cr(VI) sensing in wastewater	[21]
Orange juice	Hydrothermal treatment	2.5 h	26	Cell imaging	[23]
Lime juice	Hydrothermal treatment	7 h	40	Hg(II) sensing	[24]
Apple juice	Hydrothermal treatment	12 h	4.27	Cell imaging	[25]
Papaya juice	Hydrothermal treatment	12 h	7	Cell imaging	[26]
Banana juice	Hydrothermal treatment	4 h	8.95	n.a.	[27]
Lemon juice	Hydrothermal treatment	10 h	28	Cell imaging	[29]
Broccoli	Photochemical oxidation	20 min	22	IO_4^- sensing in wastewater	This work