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A dual-mode sensor for colorimetric and fluorescent detection of nitrite in hams based on carbon dots-neutral red system

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Abstract

Nitrite residue in hams was detected by a fluorescent and colorimetric sensor based on carbon dots (C-dots) and neutral red (NR). C-dots with green fluorescence was synthesized by a microwave-assisted method. This novel sensor was fabricated by C-dots as donors and NR as acceptors. The presence of nitrite led to decrease of absorbance and increase of fluorescence. Colorimetric and fluorescent methods for nitrite detection were developed with excellent correlation coefficients (R^2= 0.995 and 0.991) and low limits of detection (196 nM and 0.518 nM). Moreover, nitrite residue in seven types of ham was detected by the colorimetric and fluorescent methods which were verified by a standard method. The results obtained by the proposed method were comparable and agree with that of the Griess-based method (relative errors< 5%). C-dots-NR system as a sensor has a potential application for nitrite detection in hams to monitor its quality and safety.

Keywords: Carbon dots, Neutral red, Fluorescence resonance energy transfer, Nitrite, Ham

1 Introduction

Ham is a meat product highly consumed in the daily diet. Nitrite is a permitted additive in hams to inhibit the growth of spoilage bacteria, and it can improve recognizable colors and flavors of hams (Addiscott & Benjamin, 2004). High intake of nitrite has an adverse impact on human health for nitrite reacting with amines and amides can form a series of carcinogenic N-nitroso compounds (Honikel, 2008; James, Jayashree, & Alice, 2009). In contrast, modest dietary intake of nitrite can mediate physiological effects including vasodilation, modulation of mitochondrial function, and protection from ischemia-reperfusion injury (Lundberg, Weitzberg, Shiva, & Gladwin, 2011). Control of nitrite quantities is necessary to reduce the potential for N-nitroso compound formation and make nitrite have functions of mediating physiological effects. Therefore, accurate measurement of residual unreacted nitrite is required to guarantee quality and safety of hams.

Recently, various methods for nitrite detection have been developed including electrochemistry (Gayathri & Balasubramanian, 1999), chromatography (Li, Yu, Jiang, Zhou, & Liu, 2003;) and optical method (Gayathri & Balasubramanian, 1999; Li, Yu, Jiang, Zhou, & Liu, 2003; Zhen, Xue, Chen, & Lin, 2011). Chromatography and Griess-based method were standard methods for nitrite detection set by International Standardization Organization(ISO, 1975), Association of Official Analytical Chemists (AOAC, 1984), Chinese Ministry of Health(CHM, 2016) and the European Committee for Standardization(CEN, 1999). Chromatographic method is sensitive and reliable in the determination of nitrite. However, it is time-consuming with sophisticated operations and high-cost with expensive instrument. The Griess-based colorimetric method has a widespread application with advantages of simplicity and low cost. It is easily affected by colored pigments that may exist in hams due to relying on the color of azo-compounds. Moreover, it is not sensitive to trace-level nitrite. Development of a new method with high sensitivity, simplicity and low cost for nitrite detection is still a research focus.

Fluorescence-based methods have attracted increasing interest because of their

high sensitivity, simplicity, and ease of operation. In addition, it possesses high selectivity without interference from color components. Carbon dots (C-dots) as inorganic fluorescent nanomaterials has gained considerable attention due to their excellent fluorescence properties, low toxicity, and low cost (Amjadi & Jalili, 2018; Y. Wang & Hu, 2015; Yang. et al., 2011). Fluorescence resonance energy transform (FRET) systems based on C-dots have been successfully developed for bioimaging (Chatzimarkou et al., 2018), biological sensing (Kudr et al., 2017), and chemical sensing (Wu et al., 2017). They have many advantages, such as fast response, low toxicity and high sensitivity(Liu, Zeng, Liu, & Wu, 2012). Therefore, FRET systems based on C-dots have potentials for nitrite detection.

A novel FRET system based on C-dots and NR was developed for nitrite detection in hams. C-dots is synthesized by a microwave-assisted method. C-dots-NR system displaying faint fluorescence is fabricated by C-dots as donors and NR as acceptors for the presence of NR can quench fluorescence emission. Colorimetric and fluorescent methods are developed for nitrite detection based on absorbance and fluorescence signals of C-dots-NR system. Nitrite in hams is detected by this colorimetric and fluorescent methods.

2 Materials and methods

2.1 Materials and instruments

All chemicals were of analytical grade. Polyethylene glycol 200 (PEG 200) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The remaining reagents (sucrose, sodium nitrite ($NaNO_2$), sodium chloride, sodium bromide, and so on) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All aqueous solutions were prepared by using ultrapure water obtained from Milli-Q filter system (Millipore Co., USA) with a resistivity of 18.2 MΩ cm^{-1}. Seven types of ham at different levels were purchased from the local supermarket.

The size and morphology of C-dots were characterized by JEM-2100 transmission electron microscopy (TEM) (JEOL Ltd.) at a maximum accelerating

voltage of 200 kV. A 5 μL of C-dots solution was drop-cast on carbon-coated copper grids and subsequently air-dried before TEM analysis. A portable apparatus composed of several commercially devices was used as ultraviolet-visible (UV-Vis) spectroscopy and fluorescence spectroscopy. It includes two light sources (Ocean optics, Halma Company), a USB 2000 spectrometer (Ocean optics, Halma Company), three fiber optics (Ocean optics, Halma Company), a 1-cm pathlength quartz cuvette (Ocean optics, Halma Company), and a tablet PC equipped with Spectrasuite software. Tungsten light or 365 nm laser light source is successively guided into a cuvette carriage through fiber optics for generation of absorbance or fluorescence signals. These signals are gathered by the micro spectrometer and transferred to the tablet PC for analysis (Fig. S1).

2.2 Detection of nitrite residue in hams using Griess-based colorimetric method

A Griess-based colorimetric method was used to detect nitrite residue (NO_2^-) in seven types of ham according to GB 5009.33-2016 in China(CHM, 2016) and ISO 2918-1975(ISO, 1975). The preprocessing procedures for extraction and isolation of nitrite in hams included: (1) 5 g of homogenized ham was macerated with 14 mL borax solution and 100 mL of distilled water (70℃); (2) the mixture was heated in boiling water for 15 min; (3) 5 mL ferrous potassium cyanide and 5 mL zinc acetate solution were subsequently added; (4) the solution was made up to a final volume of 200 mL with water followed by filtration through 0.45 μm micro-filter. The filtrates were stored at 4℃ for analysis.

Sodium nitrite standard solution or ham extraction was added into the Griess reagent containing sulfanilic acid solution (0.4 mL, 2 g/L) and N-(1-naphthyl) ethylenediamine dihydrochloride solution (0.2 mL, 1 g/L) incubating for 15 min. The concentration of $NaNO_2$ was 0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25 μg/mL, respectively. Then absorbance spectra of those mixtures were obtained. A linear calibration curve was established by plotting absorbance values at 538 nm to the corresponding $NaNO_2$ concentrations (0- 0.25 μg/mL). The absorbance values of ham

extraction at 538 nm were recorded and the nitrite concentration in ham was detected according to equation (1). All measurements were performed in triplicate on different days.

$$Q = \frac{(A_{538} - 0.0098) \times 5 \times 200}{6.076 \times 1.371 \times 5} (\text{mg/kg}) \quad (1)$$

Where Q is the nitrite residue in hams, A_{538} represents the absorbance value at 538 nm.

2.3 Development of colorimetric and fluorescent methods for nitrite detection

2.3.1 Synthesis of C-dots

Synthesis of C-dots was according to a microwave-assisted method with minor modifications (Gong et al., 2014). Sucrose solution (1 mL, 30% (w/v)) and concentrated H_2SO_4 (200 μL) were sequentially added to 6 mL of PEG in a 10 mL glass tube. The mixture was heated in a 900W domestic microwave oven (Midea, China) for 15 seconds. It turned golden yellow indicating the formation of C-dots. The C-dots solution was centrifuged to remove the insoluble substance, and small molecules were removed using a dialysis membrane (molecular weight cut-off =1000) for 24 h. The resultant C-dots was diluted into 100 mL and stored at 4°C for further characterization and utilization.

2.3.2 Optimization of experimental conditions

The concentration of C-dots, concentration of NR, pH and reaction time were optimized to achieve the maximum sensitivity for nitrite detection. Different volumes of C-dots (1, 2, 3 and 4 mL) were analyzed to select the optimal volume of C-dots. C-dots (1 mL) mixing with different amounts of NR (0, 40, 80, 120, 160, 200 and 300 μL) was used for selecting the optimal amount of NR. Different pH values (0.9, 1.0, 1.2, 1.7, 2.0, 3.0, 4.0, 5.0, and 6.0) were investigated in presence of 0.10 μg/mL nitrite, 1mL C-dots and 160 μL NR. The mixture (1 mL C-dots, 160 μL NR, 100 μL HCl and 0.10 μg/mL nitrite) was incubated for 5, 10, 15 and 20 min to select the optimal reaction time. All solutions were diluted into 5 mL with water before spectroscopy measurements.

2.3.3 Colorimetric and fluorescent methods for nitrite detection

Colorimetric and fluorescent methods were developed for nitrite detection. NR and of C-dots were mixed to form C-dots-NR system. Then $NaNO_2$ solution and HCl were added into the C-dots-NR system followed by diluted to 5.0 mL. The final concentration of $NaNO_2$ were 0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.1, 0.12, 0.015, 0.2, 0.25, and 0.3 μg/mL, respectively. Absorbance and fluorescence spectra were acquired as an average of three independent measurements. Absorbance values and fluorescence intensities were recorded to establish linear calibration curves. The response of C-dots-NR system to other ions was also studied to validate the specificity for NO_2^- detection. All experiments were performed in triplicate on different days.

2.4 Colorimetric and fluorescent detection of nitrite residue in ham

Nitrite residue in seven types of ham was detected by the colorimetric and fluorescent methods based on C-dots-NR system according to the Section 2.3.3. The unknown concentration was calculated by putting fluorescent intensity or absorbance value into the corresponding calibration curve. All experiments were performed in triplicate on different days (sample size is 12 for each ham).

3 Results and discussion

3.1 Nitrite in hams detected by the Griess-based method

Nitrite residue (NO_2^-) levels in hams detected by Griess-based colorimetric method were between 0.102 and 22.276 mg/kg (Fig. 1). Absorbance values enhanced with increasing concentration of NO_2^- (Fig. 1a). Excellent linearity between nitrite concentrations (0-0.25 μg/L) and absorbance values at 538 nm was observed with correlation coefficient of 0.995 (inset in Fig. 1a). The linear equation was y= (6.076±0.015) x+ (0.010±0.023) (x and y represented the $NaNO_2$ concentration (μg/mL) and absorbance value at 538 nm, respectively). The limit of detection (LOD, 3s) was calculated to be 15.8 μg/L (229 nM) according to the International Union of Pure and Applied Chemistry (IUPAC). The average absorbance spectra of Griess

reagent in presence of each ham extraction were shown in Fig. 1b. The NO_2^- levels in seven types of ham were 0.102, 0.700, 6.769, 11.612, 15.872, 16.397, and 22.276 mg/kg by plugging absorbance value at 538 nm into the linear equation (1).

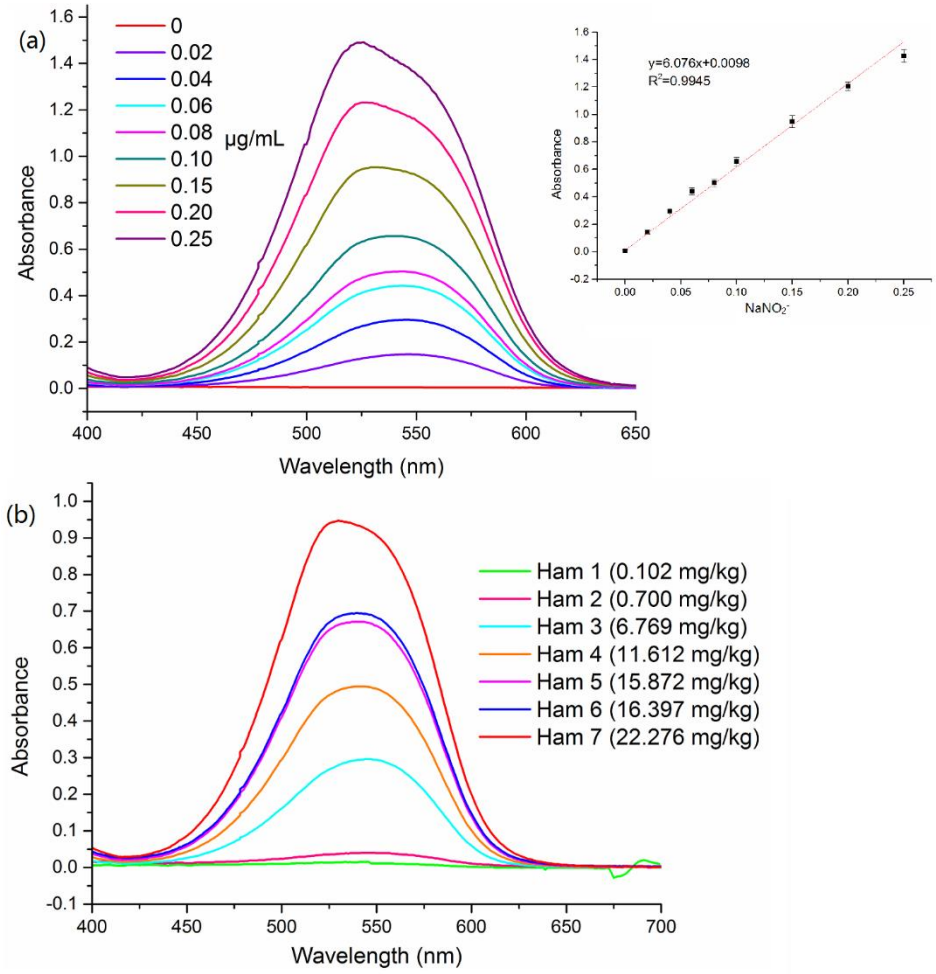

Fig.1 (a) absorbance spectra of Griess reagent in presence of $NaNO_2$ with various concentrations (inset: A calibration curve between $NaNO_2$ concentrations and absorbance values at 538 nm); (b) absorbance spectra of Griess reagent in presence of ham extractions.

3.2 Development of colorimetric and fluorescent methods for nitrite detection

3.2.1 Characterization of C-dots

C-dots prepared in this paper displayed excellent fluorescence properties and favorable dispersibility (Fig. 2). It was synthesized using a one-step method (microwave-assisted method) with sucrose as a carbon source and PEG 200 as a passivating agent(Yang et al., 2013). C-dots emitted bright green fluorescence after

being irradiated by UV light at 365 nm (inset in Fig. 2). The fluorescent intensity at 520 nm achieved the highest when the C-dots was irradiated at 365 nm (Fig. 2). TEM image of C-dots displayed that C-dots were well-dispersed with spherical shape (inset in Fig. 2). The average diameter of C-dots was 5.3 nm. Those results are consistent with observation reported previously (Gong et al., 2014).

C-dots is selected as a fluorescent material to analyze NO_2^- for its unique characteristics (e.g., low cost, simple preparation, excellent dispersibility, favorable biocompatibility, and easy modification). It is synthesized from common carbon sources, such as carbohydrate, natural gas soot and candle soot (Egorova, Tomskaya, An, & Sa, 2017). These raw materials are easily acquired and have no toxicity. The synthesis of C-dots in this paper is fast and simple including: (1) preparation of fluorescent C-dots by heating mixture of sucrose and PEG for 15 seconds in microwave and (2) purification of C-dots by centrifugation and dialysis. Hydroxyl, carbonyl, and carboxylic acid groups on surfaces endow C-dots with excellent water-solubility, favorable biocompatibility and easy modifications (Y. Wang & Hu, 2015). All characteristics are conducive to analysis of targets.

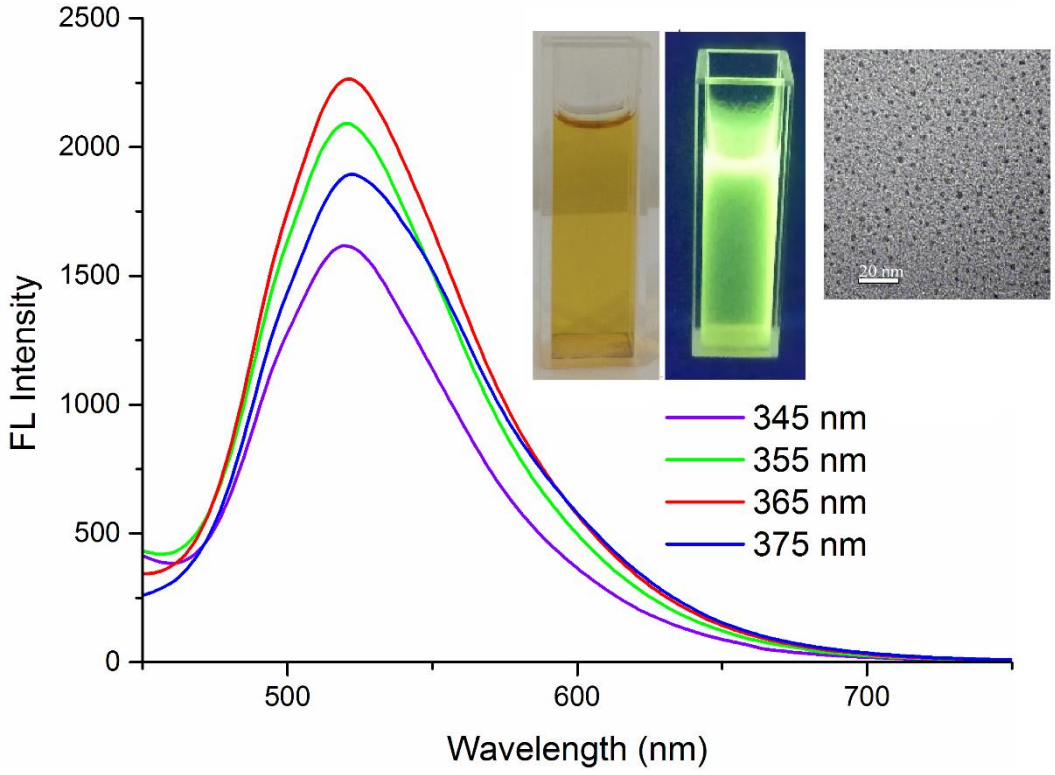

Fig. 2 fluorescent spectra of C-dots excited at 345, 355, 365, and 375 nm (inset (from left to right): image of C-dots under natural light (left); fluorescent image of C-dots under UV light at 365 nm (middle); and TEM image of C-dots (right)).

3.2.2 Mechanism of colorimetric and fluorescent detection of nitrite

C-dots-NR system has colorimetric and fluorescent responses to nitrite (Fig. 3). C-dots-NR system showed red color under natural light and faint green fluorescence under UV light of 365 nm. It turned blue in the presence of NO_2^- and the absorbance was decreased. Therefore, C-dots-NR system could be developed as a colorimetric sensor for nitrite detection, where NR acted as the receptor unit and chromogenic agent. Simultaneously, the fluorescent emission of this system recovered by blocking off FRET process with addition of NO_2^-. C-dots-NR also could be designed as a fluorescent sensor for nitrite detection, where NR acted as the receptor unit and C-dots acted as the fluorophore. The nitrite is residual unreacted and free(AbbasAfkhami & AbbasAliMogharnesband, 1994; Li et al., 2003). Some reaction products (nitroso compounds and diazonium salts) were synthesized from free nitrite and amines (or amides). It cannot interact with neutral red and interfere the detection (AbbasAfkhami & AbbasAliMogharnesband, 1994; Gayathri & Balasubramanian, 1999; Han et al., 2014). Therefore, colorimetric and fluorescent detection of nitrite is realized by the C-dots-NR system.

Fluorescence and absorbance spectra of NR and diazonium salts displayed that NR had a sensitive response to nitrite (Fig. S2a and b). NR solution with red color (Fig. S2a) has maximal absorbance band at 517 nm (a red line in Fig. S2b). NR can be oxidized in presence of trace amounts of nitrite, forming diazonium salts which exhibit blue color with a maximal absorbance band at 631 nm (a blue line in Fig. S2b). The absorbance of NR at 517 nm was decreased greatly with addition of nitrite. NR has been used to determine nitrite in previous researches (Gayathri & Balasubramanian, 1999).

NR quenched fluorescence of C-dots effectively via FRET process based on NR

as acceptors and C-dots as donors (Fig. S2c). Fluorescent intensity of C-dots at 520 nm was decreased with addition of NR. This observation was in agreement with these fluorescent images (inset in Fig. S2c). Nitrite, diazonium salts and nitrosative species had no impact on C-dots for the fluorescence spectra remained unchanged in the presence of nitrite, diazonium salts (reaction products of nitrite and neutral red) and N-Nitrosodimethylamine (Fig. S2c). As shown in Fig. S2b, the UV-vis absorbance peak of NR (red line) and fluorescence emission peak of C-dots (green line) were at 517 and 520 nm, respectively. The absorbance spectrum of NR almost exactly overlapped with the emission spectrum of C-dots. The principle of fluorescence resonance energy transform (FRET) is that absorption spectrum of acceptor should overlap with emission spectrum of donor and the distance between them must be linked in close proximity(Tang, Du, & Su, 2013). Therefore, an efficient FRET system could be established with NR as acceptors and C-dots as donors because of their overlaps and close distance. The FRET efficiency was estimated by $E=1-I/I_0$, where I and I_0 were fluorescent intensity of C-dots in presence of NR and C-dots alone (no NR), respectively. The quenching efficiency exceeded 77% by adding 160 μL of NR.

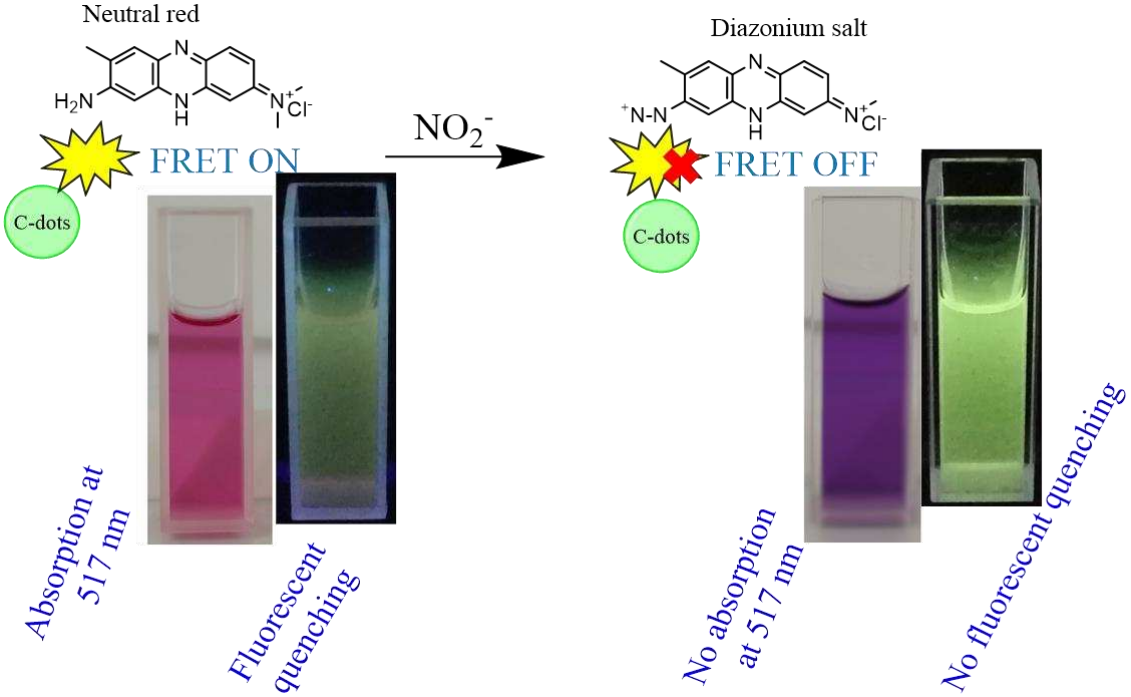

Fig. 3 illustration of the mechanism for nitrite detection using C-dots-NR system

3.2.3 Colorimetric and fluorescent detection of nitrite

C-dots(1 mL), NR (160 μL), HCl (100 μL) and reaction time (10 minutes) were selected as the optimal reaction parameters (Fig. S3). One milliliter of C-dots had strong fluorescent emission (Fig. S3a) and their fluorescent emission was almost quenched by adding 160 μL of NR (Fig. S3b). The ratio of fluorescent intensity (F/F_0) reached the maximum at pH value of 1.2 indicating that the reaction was the fastest (Fig. S3c). The fluorescence ratio (F/F_0) remained stable after 10 min, indicating that the reaction was almost completed (Fig. S3d).

Under these optimal assay conditions, C-dots-NR system was applied successfully for colorimetric detection of NO_2^- (Fig. 4a). The absorbance spectrum of C-dots-NR system was centered at 517 nm, which displayed in red color. The color changed from red to blue with addition of NO_2^-, which can be recognized by the naked eye (inset in Fig. 4a). Absorbance values at 517 nm decreased gradually and a new absorbance peak at 575 nm raised with wavelength shift of 58 nm. There was a good linear correlation (R^2= 0.995) between absorbance intensity and $NaNO_2$ concentration in the range of 0- 0.2 μg/mL (0- 2.9 μM) (inset in Fig. 4a). The associated linear regression equation was y= (-6.367±0.092) x+ (2.141±0.039) (x and y represented the $NaNO_2$ concentration (μg/mL) and absorbance value at 517 nm, respectively). The limit of detection for $NaNO_2$ was approximately 13.5 μg/L (196 nM) at a 3:1 signal-to-noise (S/N) ratio, which was lower than the maximum concentration limit of nitrite in drinking water(about 1 μM)(Keeton, 2017).

Simultaneously, fluorescent detection of NO_2^- was performed successfully using C-dots-NR system (Fig. 4b). Fluorescence intensities of C-dots-NR system at 520 nm enhanced gradually with increasing NO_2^- concentrations (Fig. 4b). The fluorescence recovered with addition of nitrite which was attributed to decrease of NR and reduction of FRET efficiency (inset in Fig. 4b). There was an excellent linear correlation (0.991) between fluorescent intensities at 520 nm and $NaNO_2$ concentrations in the range of 0- 0.3 μg/mL (0- 4.34 μM) (inset in Fig. 4b). The

277 associated linear regression equation was y= (5034.570±2.597) x+ (380.690±1.743) (x
278 and y represented the $NaNO_2$ concentration (μg/mL) and absorbance value at 520 nm,
279 respectively). Limit of detection for $NaNO_2$ detection was calculated to be 35.75 ng/L
280 (0.518 nM), which was lower than most methods reported to nitrite detection (Table 1)
281 (Abbas & Mostafa, 2000; Büldt & Karst, 1999; Jedličková, Paluch, & Alušík, 2002;
282 Li et al., 2003; Losada, Armada, García, Casado, & Alonso, 2017; Zheng, Liang, Li,
283 Zhang, & Qiu, 2016).

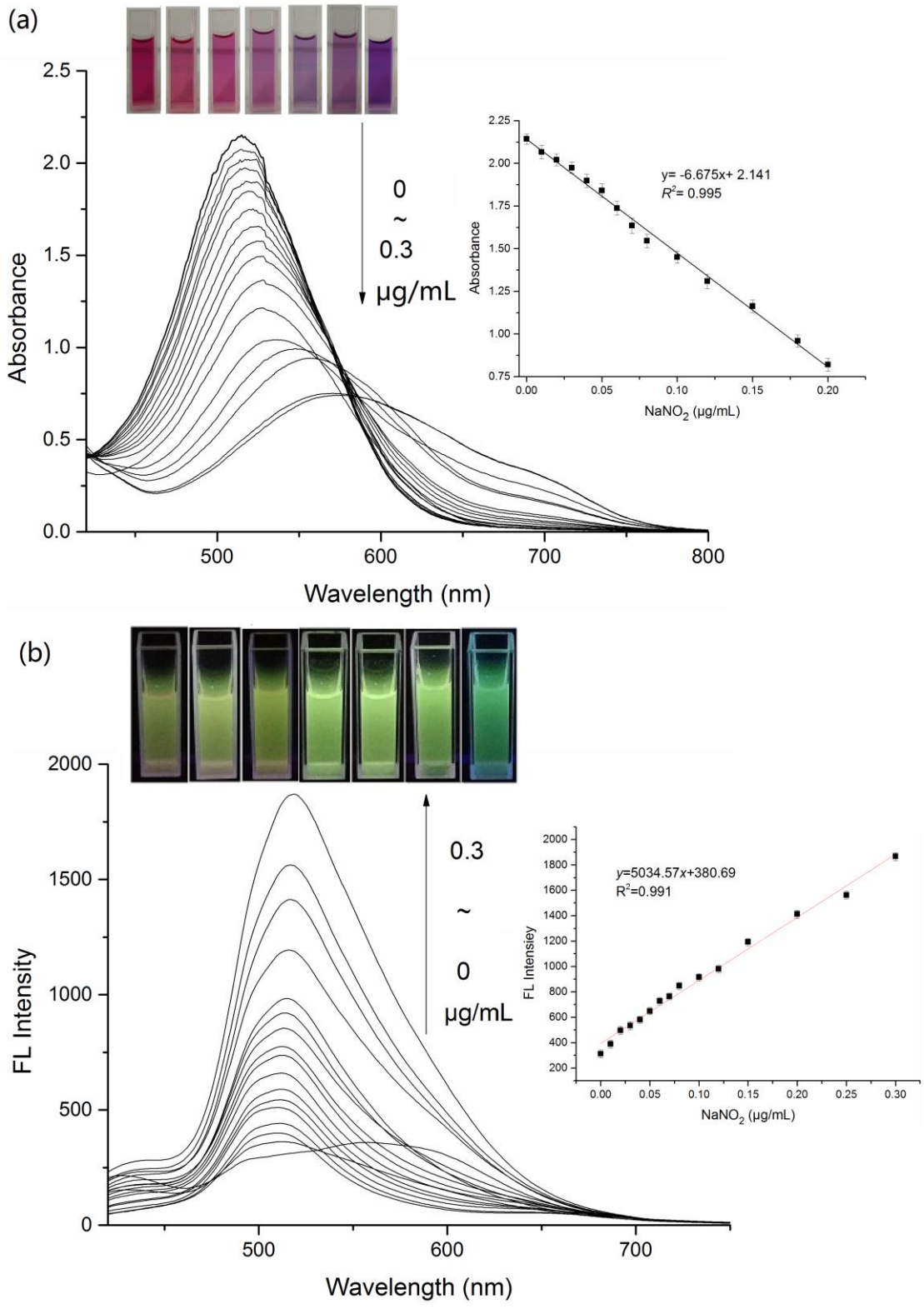

Fig. 4 (a) absorbance spectra of C-dots-NR system after addition of $NaNO_2$ with different concentrations (0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.1, 0.12, 0.15, 0.18, 0.2, 0.25, 0.3 μg/mL) (inset: photographs of C-dots-NR system with different concentrations of $NaNO_2$ and a calibration curve established by absorbance

289 values at 517 nm and $NaNO_2$ concentrations (0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07,
290 0.08, 0.1, 0.12, 0.15, 0.18, 0.2 μg/mL)); (b) fluorescence spectra of C-dots-NR system
291 with addition of $NaNO_2$ with various concentrations (0, 0.005, 0.01, 0.015, 0.02, 0.03,
292 0.04, 0.05, 0.06, 0.07, 0.08, 0.1, 0.12, 0.015, 0.2, 0.25, 0.3 μg/mL) (inset: photographs
293 of C-dots-NR system with addition of $NaNO_2$ under UV light at 365 nm and a
294 calibration curve established by fluorescence intensities at 520 nm and $NaNO_2$
295 concentrations(0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.1, 0.12,
296 0.015, 0.2, 0.25, 0.3 μg/mL)).

297 The detectable concentration range based on fluorescence signal (0.072- 4.34 μM)
298 was wider than that of absorbance signal (0.145- 2.9 μM); the LOD of fluorescence
299 signal (0.518 nM) was much lower than that of absorbance signal (196 nM); the
300 relationship coefficient of absorbance signal (R^2= 0.995) was higher than that of
301 fluorescence signal (R^2= 0.991). Both absorbance and fluorescent signals were
302 successfully used for nitrite detection and they had their own unique characteristics.
303 Therefore, signal fusion of fluorescence and absorbance was performed in this study.
304 Binary regression equation y (μg/mL) =(0.188 ± 0.024)- (0.098 ± 0.006) x_1+
305 (6.180±0.037)E-5 x_2 (R^2= 0.995, x_1 and x_2 were the absorbance value at 517 nm and
306 the fluorescence intensity at 520 nm, respectively, y was the $NaNO_2$ concentration
307 (μg/mL)) was obtained by combining absorbance and fluorescence signal. The
308 correlation coefficient based on dual signals was higher than that based on
309 colorimetric signals and was equal to that based on fluorescent signals.

310 Cu nanoclusters(Zheng et al., 2016), Au nanoclusters(H. Liu, Yang, Abdel-Halim,
311 & Zhu, 2013), CdSe quantum dots(X. Liu, Guo, Cheng, & Ju, 2009; B. Wang, Huang,
312 Ma, Shi, & Cai, 2014) and upconversion nanoparticles (UCNPs) (Han et al., 2014)
313 have been successfully used for determination of NO_2^-. It is simple and sensitive to
314 detect nitrite based on these nanosensors. However, CdSe quantum dots are
315 synthesized from semiconductors containing heavy metals (Cd) and their applications
316 are subject to their toxicity and potential hazard to the environment (Guo et al., 2013;

Zhen et al., 2011). Cu nanoclusters and Au nanoclusters have low water-solubility and are harmful to the environment as their preparations are based on heavy metals (Cu, Au). UCNPs doped with certain rare-earth ions need complex procedures to convert hydrophobic form to hydrophilic variants for making them water-soluble. In addition, the rare earth elements are scarce (Han et al., 2014). Compared with other methods, the proposed method exhibits superiority in nitrite detection.

Table 1 the results of NO_2^- detection reported by other methods

Technique	Linear range (μM)	LOD(nM)	Reference
Neutral red (colorimetry)	0.87- 4.35	176.1	(Li, Yu, Jiang, Zhou, & Liu, 2003)
Griess reagent (colorimetry)	0.22- 48	20	(Abbas & Mostafa, 2000)
HPLC-UV-vis[a] (chromatography)	0- 20	100	(Jedličková, Paluch, & Alušík, 2002)
HPLC-FL[b] (chromatography)	0.215- 7.17	60	(Büldt & Karst, 1999)
Amperometry (electrochemistry)	0.005- 10	0.02	(Losada, Armada, García, Casado, & Alonso, 2017)
Cu nanoclusters (fluorometry)	0.0125- 125	3.6	(Zheng, Liang, Li, Zhang, & Qiu, 2016)
C-dots-NR system (colorimetry and fluorometry)	0.145- 4.34 (colorimetry) 0.072- 4.34 (fluorometry)	196 0.518	This work

[a] High performance liquid chromatography with UV-vis absorbance detection

[b] High performance liquid chromatography with fluorescence detection

3.2.4 Selectivity of C-dots-NR system for nitrite detection

The C-dots-NR system as a colorimetric and fluorescent sensor displayed superb selectivity in NO_2^- detection (Fig. 5). The presence of NO_2^- led to significant color change (from red to blue), absorbance reduction at 517 nm (Fig. 5a and b). The fluorescence emission of 520 nm recovered by adding NO_2^- (Fig. 5c). Potentially competing compounds (F^-, Cl^-, Br^-, HSO_4^-, SO_4^{2-}, SO_3^{2-}, NO_3^- , dianoium salts (reaction products of nitrite and neutral red), and N-Nitrosodimethylamine) were

added into the C-dots-NR system under identical conditions. Absorbance at 517 nm and fluorescent intensity at 520 nm showed no change indicating that those compounds did not interfere with nitrite detection (Fig. 5b and c). The high selectivity of NO_2^- detection using this sensor may be attributed to the unique reaction between NO_2^- and NR.

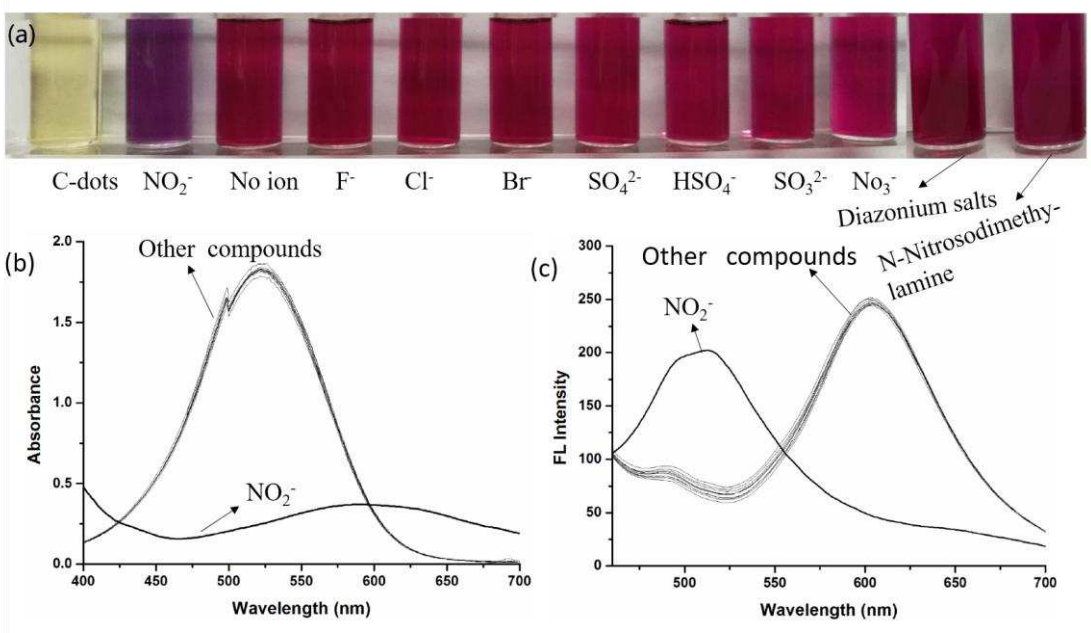

Fig. 5 images (a), absorbance spectra (b), and fluorescent spectra (c) of C-dots-NR system in presence of NO_2^-, F^-, Cl^-, Br^-, HSO_4^-, SO_4^{2-}, SO_3^{2-}, NO_3^-, diazonium salts and N-Nitrosodimethylamine.

3.3 Colorimetric and fluorescent detection of nitrite in hams

Nitrite in the seven types of ham was detected by the proposed method (Fig. 6) and the performance was evaluated by the Griess-based colorimetric method (Table 2). Absorbance spectra of C-dots-NR system in presence of ham extractions were shown in Fig. 6a. Based on the absorbance values at 517 nm (A) and linear correlation (Q= (2.141- A)/6.675), the concentrations of NO_2^- in hams were 0.098, 0.677, 7.059, 12.020, 16.205, 17.189 and 22.969 mg/kg, respectively. Relative errors were between 2.098% and 4.830% compared with results detected by the Griess-based method. The results demonstrate that the NO_2^- levels predicted by absorbance signal are in accordance with that detected by the standard method. Fluorescent spectra of

C-dots-NR system in presence of these ham extractions were presented in Fig. 6b. Based on fluorescent intensities at 520 nm (F) and the linear correlation (Q= (F-380.69)/ 5034.57), the concentrations of NO_2^- were 0.099, 0.676, 6.714, 11.628, 15.899, 16.429 and 22.190 mg/kg, respectively. Relative errors (0.138-3.429%) indicate that the NO_2^- concentration levels predicted by fluorescent signal are in agreement with that detected by Griess-based method perfectly. The nitrite content in hams were 0.087, 0.850, 6.921, 11.709, 15.788, 16.599 and 22.276 mg/kg based on absorbance values and fluorescence intensities combined with binary regression equation (Q=0.188- 0.098 A+ 6.180E-5 F), respectively. Relative errors (0.000-21.429%) are not as good as those obtained by colorimetric or fluorescent method.

Compared with these results of nitrite detection in seven types of ham, the fluorescent detection results were more excellent than colorimetric detection results and dual-signal detection results. It may be ascribed to that pigments in hams have a negative impact on colorimetric detection results. All results, especially the fluorescent detection results indicate that C-dots-NR system can be utilized to realize simple and sensitive detection of nitrite in hams.

The difference between this proposed method and Griess-based method was investigated by using Student's two-tailed t test. The developed colorimetric, fluorescent and dual-signal methods are comparable to the standard method since the differences in nitrite concentration between them are fairly negligible (P value (0.939, 0.980 and 0.987)> 0.05). In addition, the LOD of this method (196 nM for colorimetric detection and 0.518 nM for fluorescent detection) is much lower than that of Griess-based method (229 nM). This method yields excellent quantification results (R^2=0.995 and 0.991) as accurate as Griess-based method (R^2=0.994). Those results demonstrate that most of common compounds (e.g., ions and reaction products of nitrite) do not interfere with the measurement of nitrite with the use of the proposed method(Li et al., 2003).

The other standard methodology for nitrite detection is ion chromatography (CHM, 2016; ISO, 1975). The detection time (about 30 min) by using this method to measure a set of 7 samples is less than ion chromatography (about 3 hours). This method has an advantage of requiring simpler sample preparation because less centrifugation and filtration, as well as no cleanup steps (Siu & Henshall, 1998). Generally, only 3 steps are needed to measure nitrite after sample preparation for this method: (1) mixing nitrite extraction and C-dots-NR system; (2) acquiring spectra; (3) calculating nitrite concentration. This method is much faster and simpler than ion chromatography for nitrite detection in hams(Q.-H. Wang et al., 2017).

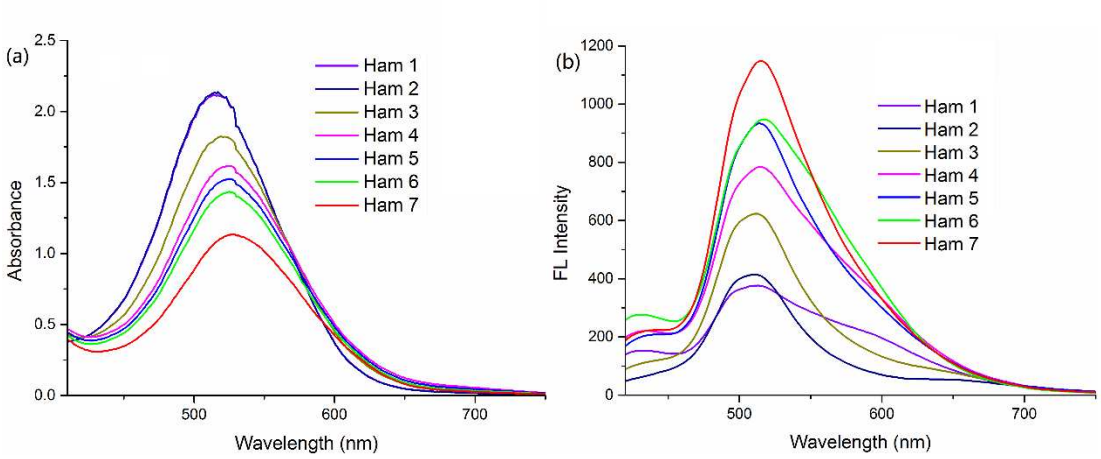

Fig. 6 absorbance (a) and fluorescent (b) spectra of C-dots-NR system with addition of ham extractions.

Table 2 detection results of nitrite in hams based on Griess reagent and C-dots-NR system

Sample	Griess reagent	C-dots-NR system					
		absorbance		fluorescence		signal fusion	
	$C(NO_2^-$ mg/kg)	$C(NO_2^-$ mg/kg)	Error (%)	$C(NO_2^-$ mg/kg)	Ratio (%)	$C(NO_2^-$ mg/kg)	Error (%)
Ham 1	0.102	0.098	3.922	0.099	2.914	0.087	14.706
Ham 2	0.700	0.677	3.286	0.676	3.429	0.850	21.429
Ham 3	6.769	7.059	4.284	6.714	0.813	6.921	2.245
Ham 4	11.612	12.020	3.514	11.628	0.138	11.709	0.835
Ham 5	15.872	16.205	2.098	15.899	0.170	15.788	0.529
Ham 6	16.397	17.189	4.830	16.429	0.195	16.599	1.232
Ham 7	22.276	22.969	3.110	22.190	0.386	22.276	0.000

4 Conclusion

A novel FRET system based on C-dots and NR has been applied successfully for colorimetric and fluorescent detection of nitrite residue in hams. Nitrite concentrations had excellent linear relation with absorbance values at 517 nm (R^2= 0.995) and fluorescent intensities at 520 nm (R^2=0.991). Limit of detection for nitrite using colorimetric and fluorescent method was 169 nM and 0.517 nM, respectively. Furthermore, the performance based on this proposed method was accordance with that of the Griess-based method for detection of nitrite in hams (relative errors< 5%). The dual-mode optical sensor has several advantages in sensitive detection, convenient instrumentation and simple operation over conventional methods. In summary, the developed C-dots-NR system is a potential colorimetric and fluorescent sensor for simple, selective and sensitive detection of nitrite in hams.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent is not applicable for the nature of this study.

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