Full Paper

Synthesis of highly fluorescent carbon dots from fermented soya bean crisp for determination of mercuric ions

Suphawuth Siriket[1, 3], Nisachon Praoboon[1], Surasak Kuimalee[2], Sirirat Phaisansuthichol[1], Pusit Pookmanee[1] and Sakchai Satienperakul[1, *]

[1] Department of Chemistry, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand

[2] Department of Industrial Chemistry and Textile Technology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand

[3] Department of Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket 83000, Thailand

* Corresponding author, sakchais@mju.ac.th

Abstract: A simple and rapid synthesis of highly fluorescent and water-soluble carbon dots (CDs) is reported. *Thua-nao khap* (fermented soya bean crisp or Thai natto sheet) was used as carbon source through low-temperature carbonisation at 220˚C for 12 hr with no other reagent required. The CDs with bright and stable blue photoluminescence was afforded under UV irradiation at a maximum fluorescent emission wavelength of 450 nm. The 7-nm spherical-shape i.d. of CDs can serve as fluorescent sensors for mercuric ions (Hg^{2+}) detection without nitrogen or sulfur doping. The quenching effect of Hg^{2+} on the fluorescence intensity of CDs was found to be concentration dependent over the linear range of 0.05 - 100 μg L^{-1}, while the lowest detectable concentration of Hg^{2+} ions was observed at 0.05 μg L^{-1}. The results demonstrate that the CDs from *Thua-nao khap*, which can be prepared easily and inexpensively, have an excellent fluorescence property for Hg^{2+} determination.

Keywords: carbon dots, fermented soya bean crisp, carbonisation, fluorescent sensor, carbon dots, Hg^{2+} detection

INTRODUCTION

Fluorescence spectroscopy has attracted great interest in environmental monitoring since it inherently provides a lower limit of detection than any other molecular spectroscopy. Sophisticated instruments such as cold-vapour atomic absorption spectrometer or inductively coupled plasma mass spectrometer may be used for detecting a very low concentration of mercury. Thus, the design and

development of highly sensitive and selective fluorescent sensors with ease of use, rapid implementation and inexpensive instrumentations, is needed in the fluorometric analysis of mercury.

Intensive research on carbonaceous nanomaterials has recently focused on the exploration of photoluminescent fluorophore because of its higher photoactivity, lower toxicity and less-expensive cost, compared with quantum dots containing heavy metals. Fluorescent carbon dots (CDs), spherical carbon nanoparticles with normally less than 10-nm i.d., have aroused much interest and intensive research due to their intriguing properties such as low toxicity, high aqueous solubility, robust chemical stability, high resistance to photo-bleaching and excellent biocompatibility [1, 2]. CDs can be prepared from various raw materials by both 'top-down' [3-5] and 'bottom-up' [6-8] methods. Numerous top-down technique approaches for carbon quantum dots have been developed, including carving graphite crystallites using high-resolution electron-beam lithography [9], cutting graphene oxide sheet via hydrothermal route [10], reoxidation [11], electrochemical technique [12] and chemical oxidation by treating carbon black and carbon fibres [13, 14]. However, most top-down methods usually suffer from some major disadvantages such as the requirement of special equipment, expensive chemicals with and frequent generation of harmful or highly toxic waste. The carbonisation of some natural materials such as fruits [15], flowers [16], leaves [17] and animal products [18, 19] by thermal treatment is called the 'bottom-up' method. The advantages of using organic precursors for the synthesis of CDs are cost-effectiveness and eco-friendliness.

Fermented soya bean is well known as a protein-rich dietary supplement with a high protein component (54.6%) [20] and consists of nitrogen- and sulfur-containing amino acids [21, 22]. A high amino acid content makes fermented soya bean suitable as an ideal carbon source for CDs synthesised through low-temperature carbonisation.

Recently graphene CDs from microwave-assisted pyrolysis of aspartic acid have been reported as a fluorescent probe sensor for Fe^{3+}, which exhibited a linear-response concentration range of 0–50 μM [23]. Gonçalves et. al. [24] also described the application of an optical fibre sensor with a sol-gel immobilised CDs nanoparticles functionalised with PEG_{200} and N-acetyl-l-cysteine for Hg^{2+} in aqueous solution based on fluorometric measurements. Moreover, nitrogen-doped CDs from lotus root were recently used to detect Hg^{2+} in environmental water samples and applied to multicolour fluorescence bioimaging [25]. However, the precursors used in those methods involved tedious synthetic procedures which are difficult to achieve.

Herein, a simple method of synthesising CDs has been developed using fermented soya bean crisp (*Thua-nao khap* or Thai natto sheet) as a CDs precursor. A pyrolysis process was used without any recourse to reducing or oxidising agents. The properties and morphology of *Thua-nao khap* CDs (TNK-CDs) were characterised and studied. The fluorescence system is proposed as a sensitive and environmental friendly method for the determination of Hg^{2+}, which is a highly toxic heavy metal ion occasionally found in natural water and industrial wastewater samples.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used were analytical-grade reagents and were used without any further purification. Ultrapure deionised water was used in all preparations and prepared by a compact ultrapure water system (18.2 M□, Elga, UK). A mercuric chloride standard solution (1000 mg L^{-1}) for atomic absorption measurement and quinine sulphite were acquired from Fluka (USA). Stannous chloride, nitric acid and hydrochloric acid were obtained from Merck (Germany). Sulphuric acid

solution (0.05M) was prepared by appropriate dilution of concentrated sulphuric acid (Merck, Germany).

Synthesis and Characterisation of CDs

TNK-CDs were prepared using a pyrolysis method without any reagent doping. The preparation and purification methods were modified from Feng et al. [16]. Briefly, 50 g of fermented soya bean crisp commonly available from Maejo municipal market in Chiang Mai was ground to a fine powder and placed in a hot-air oven (Memmert, Germany) at 220°C for 12 hr. Then the black carbon residue after grinding with a porcelain mortar was weighed to 1.0000 g and placed into a cellophane membrane bag (CelluSep, USA) and dialysed with 100 mL of ultrapure distilled water. After 6 hr, TNK-CDs were filtered and dispersed completely in 100 mL of ultrapure deionised water. Appropriate dilution of the TNK-CDs solution was applied prior to use. The preparation process of the CDs can be briefly illustrated in Figure 1. The bright blue, stable fluorescence and excellent water dispersion of TNK-CDs can be observed under UV irradiation using a mercury-vapour lamp.
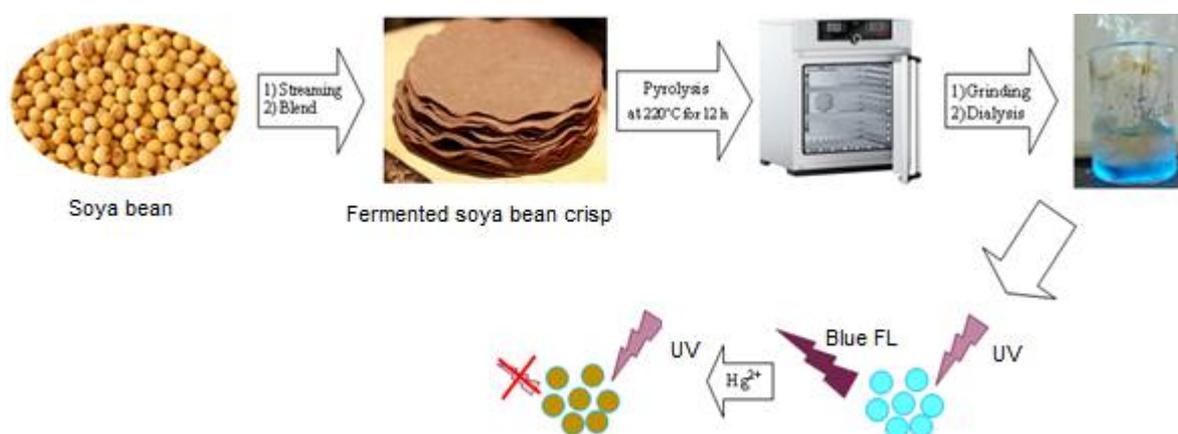
Figure 1. Schematic illustration of the synthesis process of fluorescent TNK-CDs and its fluorescence property under UV irradiation

Absorption spectra of TNK-CDs were studied by a UV-visible spectrometer (U-2900, Hitachi, Japan). The fluorescence spectrophotometric measurements were carried out on a luminescence spectrometer (LS50B, Perkin Elmer Corporation, USA). The TNK-CDs solution at appropriate dilution was put in a quartz fluorescence cuvette with 10-mm optical path length, while the excitation and emission slits were set at 5 nm. The emission spectra were observed when the excited wavelength was set at 337 nm and recorded over the wavelength range of 400-600 nm.

Determination of the fluorescence quantum yield of TNK-CDs was carried out by comparison with a standard solution of quinine sulphite (quinine sulphite dissolved in 0.05M H_2SO_4, quantum yield 54%) as reference compound. The quantum yield was calculated using the slope of the regression line generated by plotting the integrated fluorescence intensity at the emission wavelength of 450 nm under the excitation wavelength of 337 nm for multiple concentrations of TNK-CDs solutions [26].

Images from bright field transmission electron microscopy (TEM) of the TNK-CDs were obtained from a transmission electron microscope (JOEL-2100, JEOL, Japan) operated at 200 keV. Before the TEM measurements, the TNK-CDs were dispersed in ethanol using ultrasonic vibration

for approximately 5 min. The suspended mixture was then dropped on a 200-mesh copper grid coated with continuous carbon film and allowed to dry at room temperature. The TEM image obtained was then used for size and morphology investigation.

Fluorescence Quenching Detection of Hg^{2+} and Hg^{2+} Analysis in Real Samples

A 1000 μL suspension of TNK-CDs solution (about 400 mg L^{-1}, based on an appropriate dilution of the solution of 1.0000 g of black carbon residue) was added and mixed well with Hg^{2+} standard solution at different concentrations in a 25-mL volumetric flask. The quenching fluorescence spectra were recorded at $\lambda_{ex}/\lambda_{em}$ 337/450 nm 5 min. after the addition of TNK-CDs to the Hg^{2+} solution. The fluorescence intensities were used to plot the quenching external calibration curve for Hg^{2+}.

Three wastewater samples were collected from university laboratories and a hospital wastewater treatment plant. To remove the solid, the samples were filtered through Whatman filter paper (No.1). The filtrate was collected into 4 polypropylene bottles and added with Hg^{2+} solution (0, 20, 50, 100 μg L^{-1}). All of the sample solutions were stored at 4°C and kept away from light before fluorescent measurement. Finally, a 1000-μL suspension of TNK-CDs was applied to each 25 mL of standard Hg^{2+} and wastewater sample solutions, and fluorescence observation was carried out 5 min. after the addition of TNK-CDs to the Hg^{2+} solution.

Comparative atomic absorption method was performed by a cold-vapour atomic absorption spectrometer (model FIAS-AAnalyst 100, Perkin Elmer, USA). Standard mercury solutions containing 0-5 mg L^{-1} were prepared by appropriate dilution of the stock solution with deionised water containing 0.1% (v/v) nitric acid. The carrier stream solution was 3% (v/v) hydrochloric acid. The reducing stream solution was an aqueous solution of 10% (w/v) stannous chloride in a 3% (v/v) hydrochloric acid solution, which was freshly prepared prior to use [27].

RESULTS AND DISCUSSION

**Characterisation and Optical Properties of TNK-CDs**

TEM was used to characterise the morphology and size distribution of the TNK-CDs. The TEM image shows that TNK-CDs were uniform in size (5-10 nm) with an average diameter of about 7 nm and a narrow size distribution (Figure 2) and were well dispersed with spherical particles in aqueous solution.

The UV spectrum of purified TNK-CDs (Figure 3) shows two distinct peaks at 230 and 235 nm, which arise from $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions associated with C=C and carbonyl groups exposed on the CDs surface [28]. To investigate the fluorescence property of the TNK-CDs, its dispersed solution was placed in a 10-mm quartz fluorescence cuvette under visible and UV lights. It can be seen from the inset of Figure 3 that TNK-CDs are easily dispersed in water and transparent under visible day light while emitting bright blue fluorescent light under UV light excitation (365 nm).

The TNK-CDs have a high quantum yield of 40% (compared with quinine sulphite). The high quantum yield is probably due to the existence of nitrogen- and sulfur-containing functional groups, which come from the amino acid content in fermented soya bean. The fluorescence intensity of TNK-CDs was stable for more than 3 months under ambient condition.
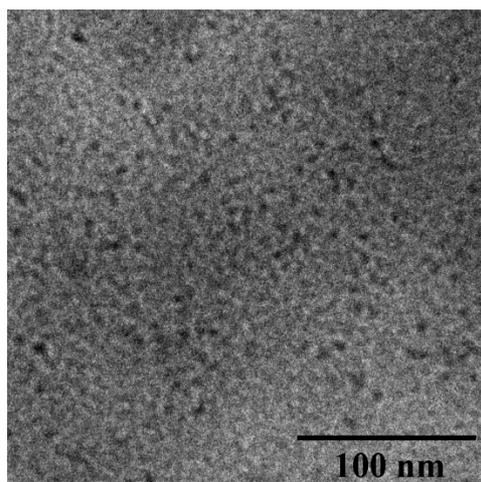
Figure 2. Typical TEM image of the TNK-CDs
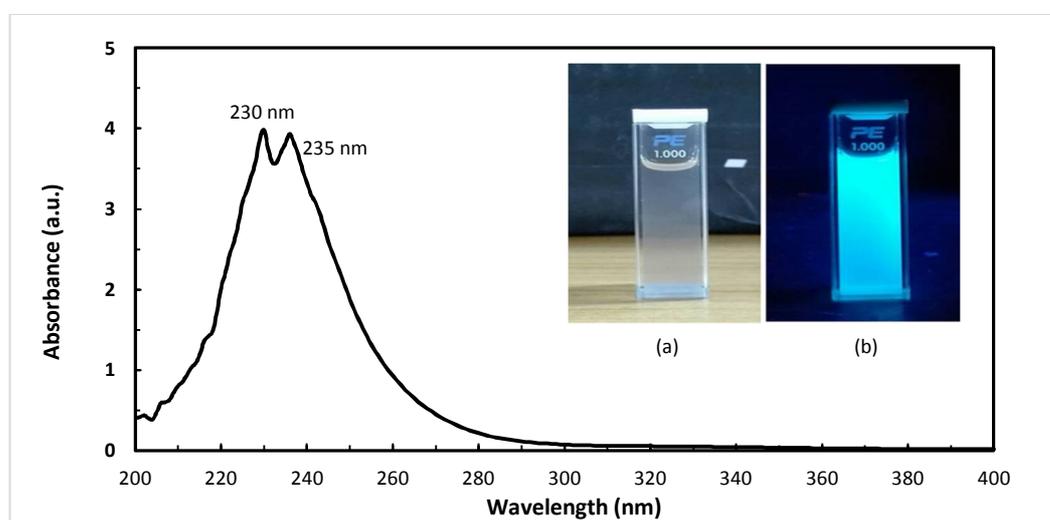
Figure 3. UV spectrum of purified TNK-CDs; inset shows photographs of TNK-CDs aqueous solution under (a) daylight and (b) 365-nm UV light

## Spectral Characteristics of TNK-CDs and Hg^{2+}

The fluorescence spectra of TNK-CDs dispersed in deionised water are shown in Figure 4. The maximum photoluminescent emission wavelength was observed at 450 nm under 337-nm irradiation. It is illustrated in Figure 5 that Hg^{2+} at different concentrations can quench the fluorescence intensity of the 400-mg L^{-1} TNK-CDs solution, and the fluorescence quenching is concentration dependent, which makes it possible for TNK-CDs to be used as an Hg^{2+} sensor.

Application of TNK-CDs to Hg^{2+} Detection in Real Samples

The practical application of the prepared TNK-CDs sensor for Hg^{2+} was investigated using wastewater from two scientific laboratories and a local hospital. Under the optimum condition, a calibration curve was constructed which showed a linear response at the Hg^{2+} concentration range of 0.05-100 μg L^{-1}. The regression equation of y = 3.682x + 0.371 (R^2=0.9986) was observed with the limits of quantitation and detection of 10 μg L^{-1} and 0.05 μg L^{-1} respectively.
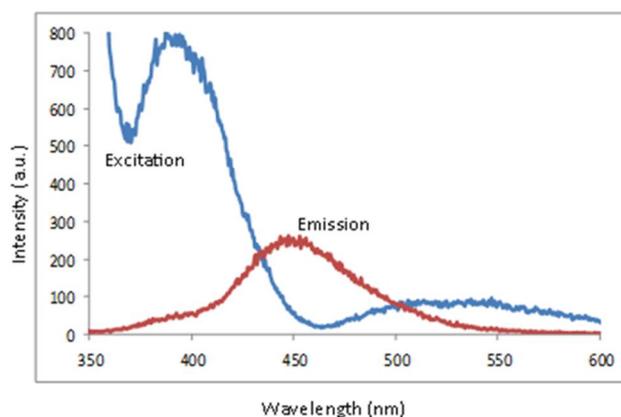
Figure 4. Fluorescence excitation (blue line) and emission (red line) spectra of TNK-CDs

(a)	(b)
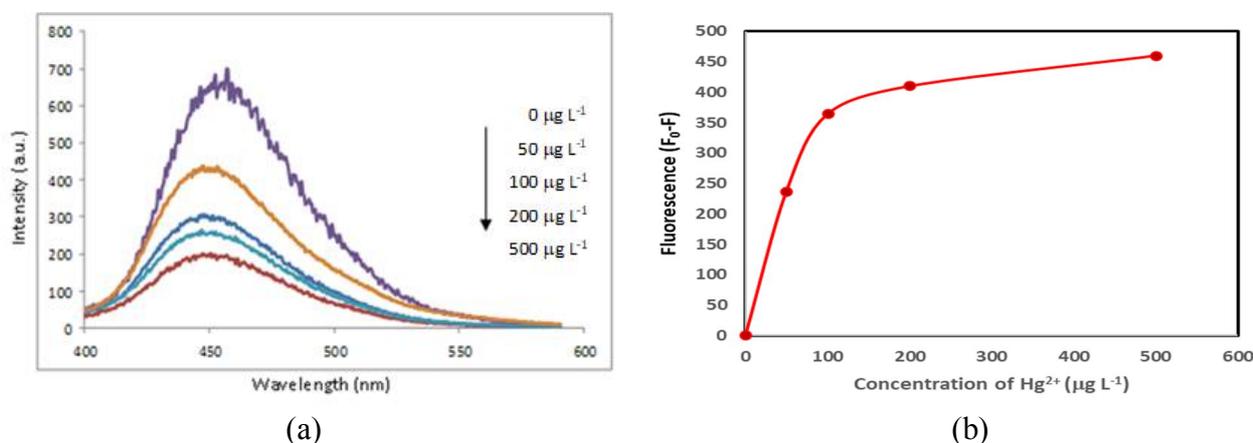
Figure 5. (a) Fluorescence spectra of 400 mg L^{-1} TNK-CDs solution after addition of Hg^{2+} at different concentrations; (b) Fluorescence/concentration graph for quenched Hg^{2+}, showing linear portion of the curve between 0-100 mg L^{-1}

Because of the relatively low values of the Hg^{2+} concentration in all wastewater samples, none of the samples quenched the TNK-CDs fluorescence intensity, indicating that the Hg^{2+} content of these wastewater samples was below detection limit. Likewise, the cold-vapour atomic absorption spectrometer did not detect any Hg^{2+} from the tested samples. Good recovery from three spiked samples with different Hg^{2+} concentrations are shown in Table 1.

These findings demonstrate the potential of TNK-CDs for routine analysis of Hg^{2+} level in water samples. The US Environmental Protection Agency limits mercury in drinking water to 0.002 mg L^{-1}, while the pollutant control department in Thailand sets the maximum mercury level permitted by the industrial effluent standards at 0.005 mg L^{-1} [29, 30]. A detection limit of 0.05 mg L^{-1} is equal to the typical detection limit achieved with a cold-vapour atomic absorption spectrometer [31, 32]. The TNK-CDs can achieve a much lower detection limit than those previously reported in other fluorescent probes, as shown in Table 2.

Table 1. Results of Hg^{2+} analysis in wastewater samples using TNK-CDs sensor

Source of sample	Amount Hg^{2+}added ($\mu g\ L^{-1}$)	Amount found ± SD * ($\mu g\ L^{-1}$)	Recovery (%)
Lab A	20	18.09±0.02	90.45
	50	46.76±0.02	93.52
	100	98.88±0.05	98.88
Lab B	20	18.49±0.01	92.45
	50	48.13±0.01	96.26
	100	103.73±0.04	103.73
Hospital	20	17.97±0.01	89.85
	50	45.71±0.02	91.42
	100	93.84±0.04	93.84

* Standard deviation from triplicate measurements

Table 2. Comparison of sensing performance of different fluorescent sensors for Hg^{2+}

Fluorescent sensor	Detection Limit ($\mu g\ L^{-1}$)	Linear range ($\mu g\ L^{-1}$)	Ref.
Thymine-functionalised CDs	7.0	0 - 200	[33]
N-doped CDs (from lotus root)	3.74	20 - 1200	[25]
Hydrothermal synthesis CDs	2.0	0 - 100	[34]
TNK-CDs	0.05	0.05 - 100	This work

CONCLUSIONS

CDs synthesised from fermented soya bean crisp without using any further reagent modification, were applied as a metal sensor for the quantitation of Hg^{2+} by fluorescence quenching. The fluorometric method developed herein was successfully used to determine Hg^{2+} ions in waste-water at μg level.

REFERENCES

1. V. Sharma, P. Tiwari and S. M. Mobin, "Sustainable carbon-dots: Recent advances in green carbon dots for sensing and bioimaging", *J. Mater. Chem. B*, 2017, *5*, 8904-8924.
2. M. Tuerhong, Y. Xu and X.-B. Yin, "Review on carbon dots and their applications", *Chin. J. Anal. Chem.*, 2017, *45*, 139-150.
3. I. Singh, R. Arora, H. Dhiman and R. Pahwa, "Carbon quantum dots: Synthesis, characterization and biomedical applications", *Turk. J. Pharm. Sci.*, 2018, *15*, 219-230.
4. P. Zuo, X. Lu, Z. Sun, Y. Guo and H. He, "A review on syntheses, properties, characterization and bioanalytical applications of fluorescent carbon dots", *Microchim. Acta*, 2016, *183*, 519-542.

5. S. Bak, D. Kim and H. Lee, "Graphene quantum dots and their possible energy applications: A review", *Current Appl. Phys.*, 2016, *16*, 1192-1201.

6. X. Yang, Y. Luo, S. Zhu, Y. Feng, Y. Zhuo and Y. Dou, "One-pot synthesis of high fluorescent carbon nanoparticles and their applications as probes for detection of tetracyclines", *Biosens. Bioelectron.*, 2014, *56*, 6-11.

7. R. Das, R. Bandyopadhyay and P. Pramanik, "Carbon quantum dots from natural resource: A review", *Mater. Today Chem.*, 2018, *8*, 96-109.

8. M. R. Pacquiao, M. D. G. de Luna, N. Thongsri, S. Kladsomboon and P. Paoprasert, "Highly fluorescent carbon dots from enokitake mushroom as multi-faceted optical nanomaterials for Cr^{6+} and VOC detection and imaging applications", *Appl. Surf. Sci.*, 2018, *453*, 192-203.

9. L. A. Ponomarenko, F. Schedin, M. I. Katsnelson, R. Yang, E. W. Hill, K. S. Novoselov and A. K. Geim, "Chaotic dirac billiard in graphene quantum dots", *Science*, 2008, *320*, 356-358.

10. D. Pan, J. Zhang, Z. Li and M. Wu, "Hydrothermal route for cutting graphene sheets into blue-luminescent graphene quantum dots", *Adv. Mater.*, 2010, *22*, 734-738.

11. J. Shen, Y. Zhu, C. Chen, X. Yang and C. Li, "Facile preparation and upconversion luminescence of graphene quantum dots", *Chem. Commun.*, 2011, *47*, 2580-2582.

12. Y. Li, Y. Hu, Y. Zhao, G. Shi, L. Deng, Y. Hou and L. Qu, "An electrochemical avenue to green-luminescent graphene quantum dots as potential electron-acceptors for photovoltaics", *Adv. Mater.*, 2011, *23*, 776-780.

13. Y. Dong, C. Chen, X. Zheng, L. Gao, Z. Cui, H. Yang, C. Guo, Y. Chi and C. M. Li, "One-step and high yield simultaneous preparation of single- and multi-layer graphene quantum dots from CX-72 carbon black", *J. Mater. Chem.*, 2012, *22*, 8764-8766.

14. J. Peng, W. Gao, B. K. Gupta, Z. Liu, R. Romero-Aburto, L. Ge, L. Song, L. B. Alemany, X. Zhan, G. Gao, S. A. Vithayathil, B. A. Kaipparettu, A. A. Marti, T. Hayashi, J.-J. Zhu and P. M. Ajayan, "Graphene quantum dots derived from carbon fibers", *Nano Lett.*, 2012, *12*, 844-849.

15. R. Atchudan, T. N. J. I. Edison, D. Chakradhar, S. Perumal, J.-J. Shim and Y. R. Lee, "Facile green synthesis of nitrogen-doped carbon dots using *Chinanthus retusus* fruit extract and investigation of their suitability for metal ion sensing and biological applications", *Sens. Actuators B Chem.*, 2017, *246*, 497-509.

16. Y. Feng, D. Zhong, H. Miao and X. Yang, "Carbon dots derived from rose flowers for tetracycline sensing", *Talanta*, 2015, *140*, 128-133.

17. S. Thambiraj and D. R. Shankaran, "Green synthesis of highly fluorescent carbon quantum dots from sugarcane bagasse pulp", *Appl. Surf. Sci.*, 2016, *390*, 435-443.

18. X. Yang, Y. Zhuo, S. Zhu, Y. Luo, Y. Feng and Y. Dou, "Novel and green synthesis of high-fluorescent carbon dots originated from honey for sensing and imaging", *Biosens. Bioelectron.*, 2014, *60*, 292-298.

19. L. Wang and H. S. Zhou, "Green synthesis of luminescent nitrogen-doped carbon dots from milk and its imaging application", *Anal. Chem.*, 2014, *86*, 8902-8905.

20. E. Chukeatirote and P. Thakang, "Chemical composition of thua nao--a fermented soybean food of northern Thailand", *Chiang Mai J. Sci.*, 2006, *33*, 243-245.

21. B. Z. Han, F. M. Rombouts and M. J. R. Nout, "Amino acid profiles of sufu, a Chinese fermented soybean food", *J. Food Compos. Anal.*, 2004, *17*, 689-698.

22. P. K. Sarkar, L. J. Jones, G. S. Craven, S. M. Somerset and C. Palmer, "Amino acid profiles of kinema, a soybean-fermented food", *Food Chem.*, 1997, *59*, 69-75.

23. C. Zhang, Y. Cui, L. Song, X. Liu and Z. Hu, "Microwave assisted one-pot synthesis of graphene quantum dots as highly sensitive fluorescent probes for detection of iron ions and pH value", *Talanta*, 2016, *150*, 54-60.

24. H. M. R. Gonçalves, A. J. Duarte and J. C. G. E. da Silva, "Optical fiber sensor for Hg(II) based on carbon dots", *Biosens. Bioelectron.*, 2010, *26*, 1302-1306.

25. D. Gu, S. Shang, Q. Yu and J. Shen, "Green synthesis of nitrogen-doped carbon dots from lotus root for Hg(II) ions detection and cell imaging", *Appl. Surf. Sci.*, 2016, *390*, 38-42.

26. J. N. Demas and G. A. Crosby, "The measurement of photoluminescence quantum yields: A review", *J. Phys. Chem.*, 1971, *75*, 991-1024.

27. A. Hineman, "Determination of As, Se and Hg in waters by hydride generation/cold vapor atomic absorption spectroscopy", 2011, https://www.perkinelmer.com/lab-solutions/resources/docs/ APP_PinAAcle-ToxicMetalsinWaterbyHG-CVAA.pdf (Accessed: August 2020).

28. S. Pandey, A. Mewada, G. OZa, M. Thakur, N. Mishra, M. Sharon and M. Sharon, "Synthesis and centrifugal separation of fluorescent carbon dots at room temperature", *Nanosci. Nanotechnol. Lett.*, 2013, *5*, 775-779.

29. US Environmental Protection Agency, "National primary drinking water regulations", 2020, https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations (Accessed: August 2020).

30. Pollution Control Department, "Effluent water quality standards", 2016, http://www.pcd.go.th/ info_serv/reg_std_water04.html (Accessed: August 2020).

31. E. Bulska, W. Kandler, P. Pasławski and A. Hulanicki, "Atomic absorption spectrometric determination of mercury in soil standard reference material following microwave sample pretreatment", *Microchim. Acta*, 1995, *119*, 137-146.

32. M. F. M. Noh, T. A. Hamid and Z. Ismail, "Determination of mercury in urine by on-line digestion with a flow injection mercury system", *At. Spectrosc.*, 1998, *19*, 95-99.

33. Y. Li, Z.-Y. Zhang, H.-F. Yang, G. Shao and F. Gan, "Highly selective fluorescent carbon dots probe for mercury(II) based on thymine-mercury(II)-thymine structure", *RSC Adv.*, 2018, *8*, 3982-3988.

34. Y. Guo, Z. Wang, H. Shao and X. Jiang, "Hydrothermal synthesis of highly fluorescent carbon nanoparticles from sodium citrate and their use for the detection of mercury ions", *Carbon*, 2013, *52*, 583-589.