

Long lifetime photoluminescence in N, S co-doped carbon quantum dots from an ionic liquid and their applications in ultrasensitive detection of pesticides

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ABSTRACT

Pesticides widely used in modern agriculture can impact on the environment and human health and hence it is important to develop highly sensitive detectors for pesticide residues. Optical methods based on photoluminescence (PL) properties provide an ideal approach to facile and accurate trace-level analysis of pesticides. Carbon Quantum Dots (CQDs) have been proposed as the photo-sensitizer for this purpose, however the optical properties of pure CQDs restrict the detection limit of such an approach. Doping is an effective strategy to introduce novel electronic structure into the CQDs to solve this problem. Here, using ionic liquids as a single source, novel N and S co-doped CQDs were obtained by a simple ultrasonic method. The doping in the structure introduces localized states which can trap photo-excited electrons and enhance their PL lifetime. These quantum dots are successfully used as the basis of a simple, efficient sensor for ultrasensitive pesticide detection (Limit of Detection = 5 ppb). Application of the analysis method to real fruit samples is demonstrated. The mechanism of the high sensitivity is also investigated and explained from experimental and calculation results.

1. Introduction

Carbon nanomaterials have attracted attention recently based on their variety of excellent chemical and physical properties.[1, 2] Doping is an efficient method to enhance their properties, by introducing localized states that change their electron transfer properties and

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potentially enhance their application in different areas, such as catalysis, energy science, sensors, etc.[1] So far, many successful studies on doping of carbon nanotubes, graphene, etc have been published.[1, 3, 4] Carbon quantum dots (CQDs) are a nano-spherical form of carbon that can be obtained by a variety of methods.[5-8] There are also reports of the property enhancing effects achieved by doping CQDs with a variety of dopants including B, Gd or N.[9-11] As benign, inexpensive, and nontoxic fluorescent carbon nanomaterials, CQDs could be an excellent candidate for environmental-friendly sensors, especially for pesticide detection as a fluorescent probe.[5, 8, 12-14]

Many analytical strategies have been explored to detect low levels of pesticides retained in food and the environment; these include bioprobes based on fluorescence,[15-18] field-effect transistors,[19] colorimetric assays,[20, 21] chromatographic detection,[22-24] electrochemical methods,[25-27] or enzyme-linked immunosorbent analysis.[28-30] These techniques can have high selectivity and sensitivity for pesticide detection; however, they suffer from a number of disadvantages, such as high cost, complex equipment, or complex pre-treatment of the sample. These often render the technique inappropriate for on-site detection, especially in some emergency situations. Sensing based on a fluorescent probe is a promising approach to overcome these issues.

Compared with other types of quantum dots, CQDs have special optical and electronic properties, good chemical stability, low toxicity and biocompatibility, [5, 8, 31, 32] which makes them ideal candidates in analytical detection. Multiple dopants (or “co-dopants”) in CQDs can further enhance their properties for sensor and catalytic applications.[33-35] However, their synthesis is complex, usually involving extreme conditions, or complex purification processes.[33, 36] In this work, we develop a simple and clean method to obtain N, S co-doped CQDs from an ionic liquid precursor, and show how this CQD-based material can be used as a highly sensitive sensor for a pesticide. We also investigate the mechanism experimentally and through computational modelling.

2. Experimental section

2.1 Materials

Chemicals in this work were from Sigma-Aldrich and Merck Chemical Reagents.

2.2 Synthesis of N,S-CQDs

An ionic liquid N-Methylethanolammonium thioglycolate (MEATG, Fig. S1) is used as a precursor. Firstly, the MEATG is pyrolyzed in a furnace with a heating rate of 0.5 °C/min, to reach the final temperature of 450 °C. This temperature is held for 1 hour, to produce the doped carbon material.[37] Then, 20mg of the carbon material is added into a mixture of 15ml H₂O and 15ml H₂O₂, and given an ultrasonic treatment for 3 hours. Finally the mixture is filtered to remove large particles, the filtrate is vacuum evaporated at 35 °C and then dispersed in pure water to obtain the N, S-CQDs aqueous suspension.

2.3 Synthesis of pure CQDs for comparison

These were prepared as a control sample by a literature method.[5] 9g glucose is added into 50 mL water to obtain a 1mol/L glucose solution. Similarly, a 50 ml NaOH (1 mol/L) solution is obtained and mixed with the glucose solution. The mixture is given ultrasonic treatment for 4 hours. Then the brown solution is dialyzed to obtain pure CQDs.

2.4 Characterization methods

The photoluminescence (PL) results were obtained on a Luminescence Spectrometer (Fluoro Max-4). The transmission electron microscopy (TEM) and HRTEM images were obtained with a FEI/Philips Tecnai G2 F20 S-TWIN TEM. The Raman spectra were obtained on a NT-MDT Integra AFM Raman System. The N, S-CQDs PL lifetimes were obtained using a Jobin Yvon Time-correlated Single Photon Counting System.

2.5 The calculation of quantum yield

The quantum yield (QY) of the N, S-CQDs was measured according to the method described in the literature.[38, 39] Briefly, quinine sulfate (0.1M H₂SO₄ as solvent) was chosen as a reference standard (QY = 54%); the N, S-CQDs were dissolved in distilled water. Then the absorbance of the standard and N, S-CQDs samples was measured at the excitation wavelength; the PL spectra of the solutions were then measured. The integrated PL intensity (that is, the total area of the fluorescence spectrum) was calculated based on the fully corrected PL spectrum. From a plot of integrated PL intensity vs. absorbance the gradient, M, was used to calculate the quantum yield based on the following equation:

$$\Phi_x = \Phi_s \frac{M_x \eta_x^2}{M_s \eta_y^2}$$

In this equation, the subscripts s and x are the standard (quinine sulfate) and test samples

respectively, Φ is QY, and η stands for the refractive index of the solvent. In this process, the excitonic absorption peak of the CQDs samples was set as the excitation wavelength for measurements of QY.

2.6 Experimental Method for Pesticide Detection:

In this work, we selected a commonly used pesticide (Fig. S2) in agriculture, carbaryl, as the target pesticide. The test was carried out in the presence of AChE and choline oxidase (ChOx) and is based on measuring the decrease in the N, S-CQDs quenching efficiency. Firstly, the AChE (2U/mL) is mixed with different amounts of carbaryl in phosphate-buffered saline (PBS) buffer (5 mM, pH 8.0, 100 μ L), and incubated for 10 min at 25°C. The obtained mixture was added to the test solution. The test solution is produced by mixing the N, S-CQDs and PBS (5 mM, pH 8.0, 550 μ L) containing ChOx (0.7 U/mL) & acetylcholine chloride (Ach, 1 mM). The final mixture is incubated in the dark at 37 °C for 15 minutes.

2.7 Pesticides Detection in real samples (apples):

Apples were selected as a real sample to further confirm the pesticide detecting ability of the N,S-CQDs. In the process, 0.1g/L carbaryl solution was sprayed onto the surface of the apples. The batch of apples was sampled (two apples) every two days thereafter during the testing period (8 days). The apples were cut into pieces and homogenised in a juice blender. The homogenate (40g) was mixed with acetonitrile (80ml), and filtered to remove insolubles. The obtained solution was dried on a water bath, then the obtained solid material was added into methanol to form an 8ml solution. A sample of this solution (60ul) was added into the detecting system (N,S-CQDs-AChE-ChOx-ACh, 2ml) and incubated at 37 °C for 15 min. The solution was then cooled to room temperature to test its PL response.

2.8 Simulation procedure:

Using density functional theory (DFT), the CQDs are modelled by a finite sized cluster, C₁₇₀H₃₂, based on our recent work.[32] N, S-co-doping is modelled with two nitrogens and one sulfur being introduced into the cluster to replace carbon. A single H₂O₂ molecule is then introduced to adsorb onto the CQD at different sites. All geometry optimizations and energies were calculated under the scheme of standard density functional theory.[40] Under the generalized gradient approximation, the Perdew-Burke-Ernzerhof functional has been employed for the exchange-correlation term,[41] as embedded in the Vienna ab-initio

Simulation Package (VASP).[42] To obtain the frontier molecular orbitals, a double-numeric basis set with polarization functions has been employed, as embedded in DMol3.[43]

3. Results and discussion

Our starting point was to synthesize novel N, S-doped CQDs. Uniquely, we choose certain ionic liquids as the precursor for the carbon and N/S to ensure that the dopants are already mixed with carbon framework atomically. The synthesis is performed through an ultrasonic method (Fig. 1a) using the IL precursor, MEATG (Fig. S1). As shown in Fig. 1b and 1c (inset is the High-resolution TEM (HRTEM) image), the synthesized CQDs are around 3~8 nm in diameter (also see Fig. S3 in supporting information), and the existence of N and S atoms as dopants is confirmed by Energy-dispersive X-ray (EDX) analysis. The overall composition of the N, S-CQDs from elemental analysis was C 61.56 wt%, O 19.85 wt%, N 9.23 wt%, and S 2.47 wt%. The FTIR spectrum (Fig. S4) indicates[5, 33, 44] that there are –OH, C=O, COO-, C-S and C-N groups present. The Raman spectrum shows that there is a substantial fraction of crystalline structure in the N, S-CQDs (Fig. S5a). The Fast Fourier Transforms (FFT) pattern from the TEM image (Fig. S5b) also confirms the crystalline structure in the CQDs. We also used glucose as an alternative precursor to synthesize the pure CQDs in a control experiment, and the EDX result shows that only C and O were detected (Fig. S6).

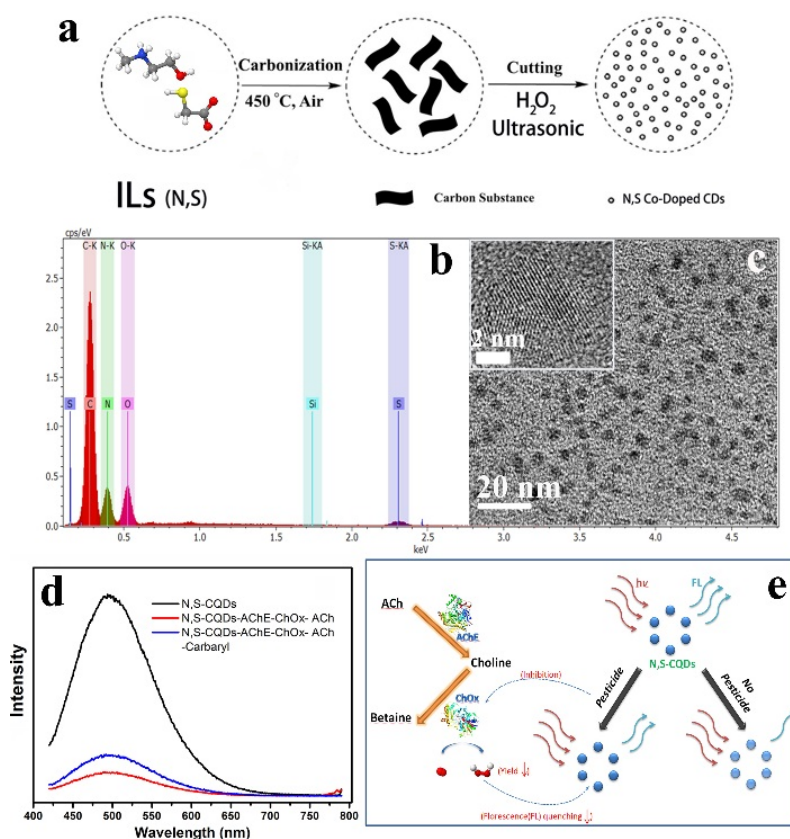


Fig. 1. (a) Synthesis of highly luminescent N,S-CQDs directly from Ionic Liquids; (b) EDX analysis of the N,S-CQDs (using a Si substrate); (c) TEM image of the N,S-CQDs (inset: the HRTEM image of a single N,S-CQD); (d) PL spectra of N,S-CQD mixtures, Black curve: only N,S-CQDs and buffer, Blue curve: N,S-CQDs + AChE (6 U/mL) + ChOx (2.1 U/mL) + ACh (3.0 mM) + carbaryl (8×10^{-4} g/L), Red Curve: N,S-CQDs + AChE (6 U/mL) + ChOx (2.1 U/mL) + Ach (3.0 mM). (e) The detection scheme of the ultra-sensitive biosensor based on the N, S-CQDs.

PL is commonly used to characterise the optical properties of CQDs. The PL spectrum (black curve, Fig. 1d) exhibits a typical feature of CQD dispersion, Fig. S7 also shows the excitation and emission (excited by 350nm light) spectra of the N, S-CQDs. Their PL properties are very stable even in solution with high ionic strength such as 2M NaCl (Fig. S8), and at different temperatures, and pH (Fig. S9). As expected, the PL lifetime of the N, S-CQDs is longer than the pure CQDs (2.8 ns) obtained from glucose, reaching as long as 3.9 ns under 370 nm excitation (Fig. S10). This longer lifetime is advantageous for application as a fluorescence probe. The quantum yield of the N, S-CQDs can reach 12.5%, compared with 7% for pure CQDs.[13] This also makes them excellent candidates for optical

applications.[45]

The N, S-CQDs were investigated in the detection of the pesticide carbaryl. As shown schematically in Fig. 1e, the hydrolysis of ACh can be catalyzed into choline under the action of AChE, and the ChOx can further oxidise choline into betaine with the concomitant generation of H₂O₂. [46] Most pesticides inhibit the activity of AChE strongly, [47] and thus such pesticides can be detected based on this detection strategy and the analysis of H₂O₂ produced. CQDs can be excellent detectors for H₂O₂ because their PL is known to be very effectively quenched (Fig. 2a) by electron transfer from the CQDs to H₂O₂. [48] To confirm this, Fig. 1d also shows the PL of the N, S-CQDs before (black curve) and after (red curve) the addition of the enzyme solution; as expected the generation of the H₂O₂ by the enzyme activity quenches the PL. After adding the pesticide (carbaryl), the PL intensity increases (blue curve) compared to the “blank” (the red curve). This occurs because the amount of H₂O₂ produced in the enzyme reaction is decreased by the inhibiting effect of the pesticide on the AChE activity. Furthermore, the PL results show that the quenching effect of H₂O₂ on the N, S-CQDs is stronger than that on the pure CQDs (Fig. S11&12) or on singly doped N-CQDs or S-CQDs (Fig. S13&S14); this indicates that the co-doped CQDs have superior optical properties in respect of this analytical application.

In the mixture of AChE and ChOx enzymes, their concentrations could affect the pesticide detection sensitivity in the proposed sensing system. In all tested cases, the PL intensity of the N, S-CQDs–Enzyme (AChE & ChOx) system decreases if the ACh concentration is increased (Fig. 2b). This shows that the optimal concentrations for detection are AChE = 2 U/mL and ChOx = 0.7 U/mL; hence, we have used these concentrations in further development of the fluorescence probe.

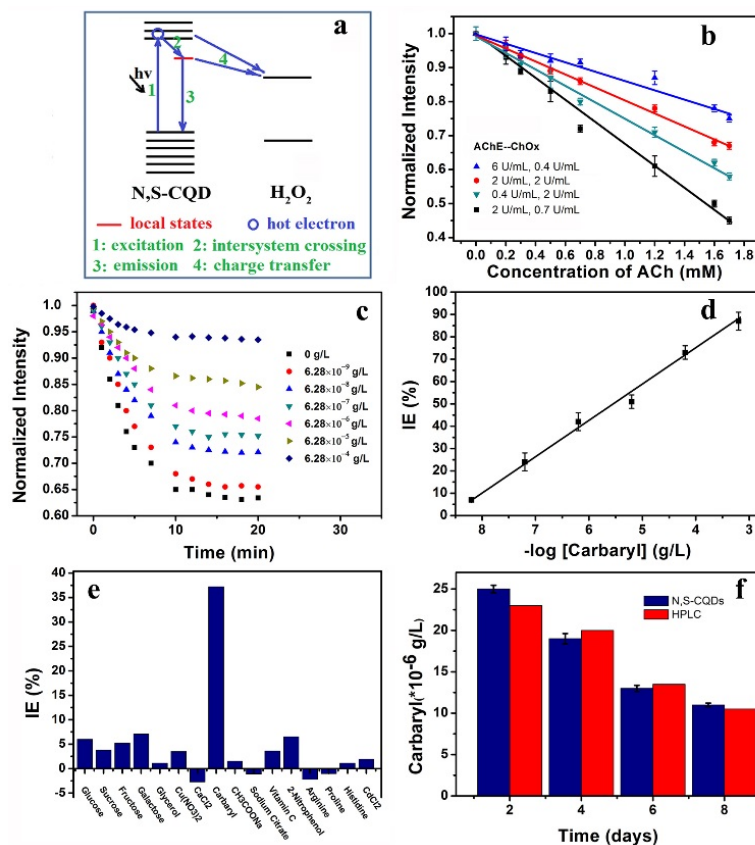


Fig. 2. (a) The electron transfer mechanism between the N, S-CQDs and H₂O₂. (b) PL intensity of N, S-CQDs as a function of ACh concentration. (c) Relationship between PL intensity and reaction time in the presence of N, S-CQDs, AChE, ChOx, Ach (1.0 mM), and carbaryl at different concentrations. (d) The relationship between the efficiency of inhibition and the log of carbaryl concentration (tested in PBS solution with pH = 8.0, and with the AChE (2 U/mL) and ChOx (0.7 U/mL)). (e) The inhibition efficiency as a function of various interference species for 7.0×10^{-7} g/L carbaryl. (f) The comparison of detection results for carbaryl in fruit (apple) samples; N, S-CQDs based optical method (black) and HPLC (red).

Fig. 2c shows the relationship between the time and PL intensity of N, S-CQDs in the N, S-CQDs–AChE–ChOx system by adding ACh (1.0 mM) at different concentrations of the carbaryl pesticide. As a function of time after dosing with the pesticide, the PL decreases, ultimately reaching a plateau, indicating that, as expected from Fig. 1e, the N, S-CQDs have been quenched by the H₂O₂ generated by the AChE - ChOx catalytic reactions. Stern-Volmer plots of the PL intensity vs reaction time data in Fig. 2c were constructed and K_{10} values were calculated from the slope of the plot over the first 10 min (via Eq S1). Values of inhibition

efficiency (IE) were obtained via Eq S2. Fig. 2d gives the relationship between IE and the concentration of carbaryl, indicating that the IE is linearly related to the logarithm of the concentration from 6.3×10^{-9} g/L to 6.3×10^{-4} g/L. Furthermore, the limit of detection (LOD), determined from the carbaryl concentration required to achieve 5% inhibition [49] is estimated to be 5.4×10^{-9} g/L. This result is substantially lower than the allowable limit for carbaryl in the European Union database of pesticide (0.05ppm) and also lower than the United States limit (0.02 ppm). It is also considerably more sensitive than other reported detection methods (Table S1, supporting information).

In the analytical process, lack of interference of the sensor from other species in the typical sample is necessary. Our work is targeted towards developing novel sensing systems for detecting the residues of pesticide in food, so some common chemicals in typical food samples, for example, glucosides, metal ions and acids, were tested. From Fig. 2e, we can see that glucose, sucrose, glycerol, etc., exhibited little effect on the detection of carbaryl, even under 10^3 times higher concentrations than the carbaryl. These results indicate that this N, S-CQDs based optical sensor has an excellent specificity for the detection of carbaryl and its anti-interference capability is strong. We also use the obtained N,S-CQDs to detect two other, different pesticides (Diazinon and Parathion); the results in Fig. S15 show that the inhibition efficiency (IE) are 29% and 27%, respectively, when their concentration is 7×10^{-7} g/L in the N,S-CQDs-AChE-ChOx -ACh system. This demonstrates that the detection system can be used for other pesticides as long as there is an inhibiting effect of the pesticide on AChE.

Detection in real fruit sample was also investigated. Here, apples were exposed to carbaryl spray and then sampled every two days for 8 days. The residue concentration of carbaryl in each sample was obtained and, for comparison, high-performance liquid chromatography (HPLC) was used to confirm the accuracy of the sensor approach (Fig. 2f). The results tested by the PL method are acceptably similar to the HPLC results.

We also further investigated the mechanism to explain why such doped CQDs can detect the pesticide at low levels. As described above, the detection of the pesticides is essentially determined by the fluorescence quenching of the CQDs. To understand the observed improvement due to doping, theoretical calculations by density functional theory have been performed to understand the role of the N and S dopants. We start from the analysis of H_2O_2

adsorption, which is the basis for effective detection in the solution.

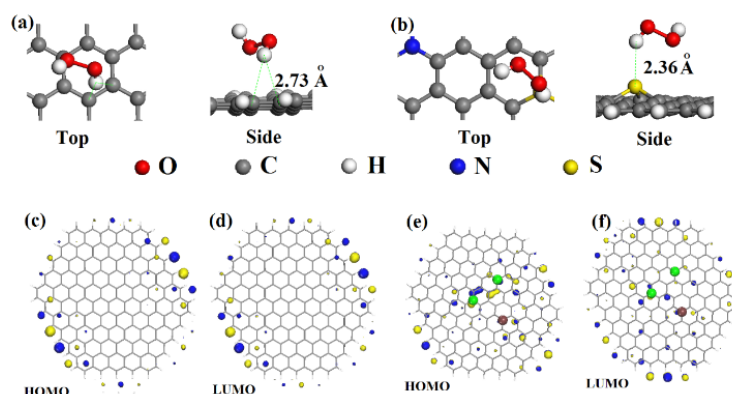


Fig. 3. DFT calculations: (a) & (b) Adsorption of H_2O_2 on CQD and N,S-CQD (partially shown), with the top and side view, and the distance between H_2O_2 and CQD is indicated by the blue line; (c) & (d) HOMO and LUMO for the CQDs; and (e) & (f) HOMO and LUMO for N,S-CQDs.

In the literature, N- or S-doped carbon nanotubes and graphene have been extensively studied and it is widely accepted that N/S dopants can partially oxidize carbon, leading to partial negative charges on the dopants.[3, 50] Thus stronger adsorption of H_2O_2 can be expected due to the $\text{H}^{\delta+} - \text{N}(\text{S})^{\delta-}$ interaction, which is confirmed by our calculations, Fig. 3. Fig. 3 a & b shows that the adsorption of H_2O_2 on N, S-CQD is slightly stronger (~ 0.1 eV) than that on an un-doped CQD, as reflected by the distance between H_2O_2 and the CQD. Such differences will lead to both more H_2O_2 adsorption and more facile charge transfer between the CQD and H_2O_2 .

Another key factor affecting the fluorescence quenching is the lifetime of the photo-excited electrons (PEEs). When the CQD is exposed to light, electrons are excited from the highest occupied molecular orbital (HOMO) to its empty states, generating active PEEs, followed by rapid decay to the lowest unoccupied molecular orbital (LUMO). For the un-doped CQD, calculated HOMO and LUMO levels are shown in Fig. 3c & d, which are dominated by the edges, in which case PEEs directly decay to the HOMO, producing band-gap determined fluorescence. However, with N/S doping, localized states below the inherent LUMO are introduced, as shown in Fig. 3e & f; consequently, a fraction of the PEEs

will be trapped by these states due to their localized nature. Such trapped PEEs can survive for longer before decaying, which increases the ratio of PEEs transferring to react with H_2O_2 . We also synthesized N-CQDs and S-CQDs as control experiments (Fig. S13 &14) to compare the quenching effect of singly doped CQDs. Comparing Figures S11-S14 it appears that the strongest quenching is seen with the co-doped N, S-CQDs. Thus, based on this reasoning, the novel N, S-doped CQDs can detect the pesticide more sensitively than could the undoped or singly doped equivalents.

4. Conclusions

In summary, novel, N, and S doped CQD materials have been prepared from an ionic liquid source. The doping remarkably enhances the PL lifetime. The doped CQDs have been used for pesticide detection based on a two-enzyme, coupled reaction system. The H_2O_2 generated by the enzyme can effectively decrease the PL intensity of the co-doped CQDs to create a highly responsive detector with LOD as low as 5 ppb. Through theoretical calculations, it is further clarified that the high sensitivity originates from the N and S dopants, which offer stronger capacity to adsorb H_2O_2 and generate local states to trap hot electrons, promoting the electron transfer to H_2O_2 and thus resulting in the quenching of the CQD fluorescence. We hope such conclusions may significantly stimulate further research in the development of doped CQDs for environmental sensing applications.

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Notes and references

- [1] Neto AC, Guinea F, Peres N, Novoselov KS, Geim AK. The electronic properties of graphene. *Reviews of modern physics*. 2009;81(1):109.
- [2] Georgakilas V, Perman JA, Tucek J, Zboril R. Broad Family of Carbon Nanoallotropes: Classification, Chemistry, and Applications of Fullerenes, Carbon Dots, Nanotubes, Graphene, Nanodiamonds, and Combined Superstructures. *Chem Rev*. 2015, 115(11), 4744-822.

- [3] Joucken F, Tison Y, Lagoute J, Dumont J, Cabosart D, Zheng B, et al. Localized state and charge transfer in nitrogen-doped graphene. *Physical Review B*. 2012;85(16):161408.
- [4] Choi YM, Lee DS, Czerw R, Chiu PW, Grobert N, Terrones M, et al. Nonlinear Behavior in the Thermopower of Doped Carbon Nanotubes Due to Strong, Localized States. *Nano Lett*. 2003;3(6):839-42.
- [5] Li H, Kang Z, Liu Y, Lee S-T. Carbon nanodots: synthesis, properties and applications. *J Mater Chem*. 2012;22(46):24230-53.
- [6] Dai L. Layered graphene/quantum dots: nanoassemblies for highly efficient solar cells. *ChemSusChem*. 2010;3(7):797-9.
- [7] Biswal M, Deshpande A, Kelkar S, Ogale S. Water Electrolysis with a Conducting Carbon Cloth: Subthreshold Hydrogen Generation and Superthreshold Carbon Quantum Dot Formation. *ChemSusChem*. 2014;7(3):883-9.
- [8] Lim SY, Shen W, Gao Z. Carbon quantum dots and their applications. *Chem Soc Rev*. 2015;44(1):362-81.
- [9] Bourlinos AB, Trivizas G, Karakassides MA, Baikousi M, Kouloumpis A, Gournis D, et al. Green and simple route toward boron doped carbon dots with significantly enhanced non-linear optical properties. *Carbon*. 2015;83:173-9.
- [10] Peng H, Li Y, Jiang C, Luo C, Qi R, Huang R, et al. Tuning the properties of luminescent nitrogen-doped carbon dots by reaction precursors. *Carbon*. 2016;100:386-94.
- [11] Shi Y, Pan Y, Zhong J, Yang J, Zheng J, Cheng J, et al. Facile synthesis of gadolinium (III) chelates functionalized carbon quantum dots for fluorescence and magnetic resonance dual-modal bioimaging. *Carbon*. 2015;93:742-50.
- [12] Li H, Ming H, Liu Y, Yu H, He X, Huang H, et al. Fluorescent carbon nanoparticles: electrochemical synthesis and their pH sensitive photoluminescence properties. *New J Chem*. 2011;35(11):2666-70.
- [13] Li H, He X, Liu Y, Huang H, Lian S, Lee S-T, et al. One-step ultrasonic synthesis of water-soluble carbon nanoparticles with excellent photoluminescent properties. *Carbon*. 2011;49(2):605-9.
- [14] Li H, He X, Kang Z, Huang H, Liu Y, Liu J, et al. Water-Soluble Fluorescent Carbon Quantum Dots and Photocatalyst Design. *Angew Chem Int Ed*. 2010;49(26):4430-4.

- [15]Zhang K, Mei Q, Guan G, Liu B, Wang S, Zhang Z. Ligand Replacement-Induced Fluorescence Switch of Quantum Dots for Ultrasensitive Detection of Organophosphorothioate Pesticides. *Anal Chem*. 2010;82(22):9579-86.
- [16]Zheng Z, Zhou Y, Li X, Liu S, Tang Z. Highly-sensitive organophosphorous pesticide biosensors based on nanostructured films of acetylcholinesterase and CdTe quantum dots. *Biosens Bioelectron*. 2011;26(6):3081-5.
- [17]Azab HA, Duerkop A, Anwar ZM, Hussein BHM, Rizk MA, Amin T. Luminescence recognition of different organophosphorus pesticides by the luminescent Eu(III)-pyridine-2,6-dicarboxylic acid probe. *Anal Chim Acta*. 2013;759:81-91.
- [18]Zou Z, Du D, Wang J, Smith JN, Timchalk C, Li Y, et al. Quantum Dot-Based Immunochromatographic Fluorescent Biosensor for Biomonitoring Trichloropyridinol, a Biomarker of Exposure to Chlorpyrifos. *Anal Chem*. 2010;82(12):5125-33.
- [19]Singh AK, Flounders AW, Volponi JV, Ashley CS, Wally K, Schoeniger JS. Development of sensors for direct detection of organophosphates. Part I: immobilization, characterization and stabilization of acetylcholinesterase and organophosphate hydrolase on silica supports. *Biosens Bioelectron*. 1999;14(8-9):703-13.
- [20]Fu G, Chen W, Yue X, Jiang X. Highly sensitive colorimetric detection of organophosphate pesticides using copper catalyzed click chemistry. *Talanta*. 2013;103:110-5.
- [21]Andreou VG, Clonis YD. A portable fiber-optic pesticide biosensor based on immobilized cholinesterase and sol-gel entrapped bromocresol purple for in-field use. *Biosens Bioelectron*. 2002;17(1-2):61-9.
- [22]Erney D, Gillespie A, Gilvydis D, Poole C. Explanation of the matrix-induced chromatographic response enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot on-column injection and flame photometric detection. *J Chromatogr A*. 1993;638(1):57-63.
- [23]Payá P, Anastassiades M, Mack D, Sigalova I, Tasdelen B, Oliva J, et al. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Analytical and bioanalytical chemistry*. 2007;389(6):1697-714.

- [24]Ma X, Wang J, Wu Q, Wang C, Wang Z. Extraction of carbamate pesticides in fruit samples by graphene reinforced hollow fibre liquid microextraction followed by high performance liquid chromatographic detection. *Food Chem.* 2014;157:119-24.
- [25]Viswanathan S, Radecka H, Radecki J. Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA. *Biosens Bioelectron.* 2009;24(9):2772-7.
- [26]Rao TN, Loo B, Sarada B, Terashima C, Fujishima A. Electrochemical detection of carbamate pesticides at conductive diamond electrodes. *Anal Chem.* 2002;74(7):1578-83.
- [27]Drechsel L, Schulz M, von Stetten F, Moldovan C, Zengerle R, Paust N. Electrochemical pesticide detection with AutoDip—a portable platform for automation of crude sample analyses. *Lab on a Chip.* 2015;15(3):704-10.
- [28]Liu Y, Guo Y, Zhu G, Tang F. Enzyme-Linked Immunosorbent Assay for the Determination of Five Organophosphorus Pesticides in Camellia Oil. *Journal of Food Protection®.* 2014;77(7):1178-83.
- [29]Qian G, Wang L, Wu Y, Zhang Q, Sun Q, Liu Y, et al. A monoclonal antibody-based sensitive enzyme-linked immunosorbent assay (ELISA) for the analysis of the organophosphorous pesticides chlorpyrifos-methyl in real samples. *Food Chem.* 2009;117(2):364-70.
- [30]Nunes GS, Toscano IA, Barceló D. Analysis of pesticides in food and environmental samples by enzyme-linked immunosorbent assays. *TrAC, Trends Anal Chem.* 1998;17(2):79-87.
- [31]Li H, Zhang X, MacFarlane DR. Carbon Quantum Dots/Cu₂O Heterostructures for Solar-Light-Driven Conversion of CO₂ to Methanol. *Advanced Energy Materials.* 2015;5(5):101477.
- [32]Li H, Sun C, Ali M, Zhou F, Zhang X, MacFarlane DR. Sulfated Carbon Quantum Dots as Efficient Visible-Light Switchable Acid Catalysts for Room-Temperature Ring-Opening Reactions. *Angew Chem Int Ed.* 2015;54(29):8420-4.
- [33]Dong Y, Pang H, Yang HB, Guo C, Shao J, Chi Y, et al. Carbon-Based Dots Co-doped with Nitrogen and Sulfur for High Quantum Yield and Excitation-Independent Emission. *Angew Chem Int Ed.* 2013;52(30):7800-4.

- [34] Xu Q, Pu P, Zhao J, Dong C, Gao C, Chen Y, et al. Preparation of highly photoluminescent sulfur-doped carbon dots for Fe(III) detection. *Journal of Materials Chemistry A*. 2015;3(2):542-6.
- [35] Xu Q, Liu Y, Gao C, Wei J, Zhou H, Chen Y, et al. Synthesis, mechanistic investigation, and application of photoluminescent sulfur and nitrogen co-doped carbon dots. *Journal of Materials Chemistry C*. 2015;3(38):9885-93.
- [36] Zhao A, Zhao C, Li M, Ren J, Qu X. Ionic liquids as precursors for highly luminescent, surface-different nitrogen-doped carbon dots used for label-free detection of Cu²⁺/Fe³⁺ and cell imaging. *Anal Chim Acta*. 2014;809:128-33.
- [37] Zhang S, Dokko K, Watanabe M. Carbon materialization of ionic liquids: from solvents to materials. *Materials Horizons*. 2015;2(2):168-97.
- [38] Briscoe J, Marinovic A, Sevilla M, Dunn S, Titirici M. Biomass-Derived Carbon Quantum Dot Sensitizers for Solid-State Nanostructured Solar Cells. *Angew Chem Int Ed*. 2015;54(15):4463-8.
- [39] Sahu S, Behera B, Maiti TK, Mohapatra S. Simple one-step synthesis of highly luminescent carbon dots from orange juice: application as excellent bio-imaging agents. *Chem Commun*. 2012;48(70):8835-7.
- [40] Kohn W, Sham LJ. Self-consistent equations including exchange and correlation effects. *Physical Review*. 1965;140(4A):A1133.
- [41] Perdew JP, Burke K, Ernzerhof M. Generalized gradient approximation made simple. *Phys Rev Lett*. 1996;77(18):3865.
- [42] Kresse G, Joubert D. From ultrasoft pseudopotentials to the projector augmented-wave method. *Physical Review B*. 1999;59(3):1758.
- [43] Delley B. An all-electron numerical method for solving the local density functional for polyatomic molecules. *The Journal of chemical physics*. 1990;92(1):508-17.
- [44] Lane MD. Mid-infrared emission spectroscopy of sulfate and sulfate-bearing minerals. *Am Mineral*. 2007;92(1):1-18.
- [45] Zhao A, Chen Z, Zhao C, Gao N, Ren J, Qu X. Recent advances in bioapplications of C-dots. *Carbon*. 2015;85:309-27.
- [46] Wang F, Liu X, Lu C-H, Willner I. Cysteine-mediated aggregation of Au nanoparticles:

the development of a H₂O₂ sensor and oxidase-based biosensors. ACS nano. 2013;7(8):7278-86.

[47]Guilhermino L, Barros P, Silva M, Soares AM. SHORT COMMUNICATION Should the use of inhibition of cholinesterases as a specific biomarker for organophosphate and carbamate pesticides be questioned. Biomarkers. 1998;3(2):157-63.

[48]Shan X, Chai L, Ma J, Qian Z, Chen J, Feng H. B-doped carbon quantum dots as a sensitive fluorescence probe for hydrogen peroxide and glucose detection. Analyst. 2014;139(10):2322-5.

[49]Abad J, Pariente F, Hernandez L, Abruna H, Lorenzo E. Determination of organophosphorus and carbamate pesticides using a piezoelectric biosensor. Anal Chem. 1998;70(14):2848-55.

[50]Liang J, Jiao Y, Jaroniec M, Qiao SZ. Sulfur and Nitrogen Dual-Doped Mesoporous Graphene Electrocatalyst for Oxygen Reduction with Synergistically Enhanced Performance. Angew Chem Int Ed. 2012;51(46):11496-500.