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COMMUNICATION

Green synthesis of fluorescent hydrophobic carbon quantum dots and their use for 2,4,6-trinitrophenol detection

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Fluorescent hydrophobic carbon dots are synthesized through a green way and used for determination of 2,4,6-trinitrophenol in hydrophobic medium for the first time. The approach for TNP detection realized a wide linear response, excellent selectivity and high sensitivity, which provide a potential candidate for practical TNP sensing in hydrophobic condition.

Sensitive and selective detection of nitro aromatics is of great concern for homeland security, battlefield protection, and industrial and environmental safety control.¹ As a member of nitro aromatics family, 2,4,6-trinitrophenol (TNP) has been widely used in fields like explosives manufacture, leather, glass, pharmaceutical and dye industries.² However, it has been identified to be an important environmental contaminant and potential hazardous for animals and human. Once into human body, it will result in harm to human beings such as neurological disorders, convulsions, abdominal pain, headaches, light-headedness and even cancer.³ Thus, a wide variety of detection methods and materials are involved in this very challenging area. Various analysis methods are being developed including mass spectrometry,⁴ surface plasmon resonance,⁵ capillary electrophoresis,⁶ colorimetric⁷ and fluorescent methods,⁸ and so on. Among these competing methods, fluorescent quenching based sensing method has proven to be one of the most promising approaches owing to its advantages in sensitivity, selectivity and portability. The reported fluorescent method is mainly based on fluorescent material like fluorescent molecules,^{9, 10} metal-organic frameworks (MOFs),^{11, 12} covalent-organic frameworks (COFs),¹³ organic cage,¹⁴ metal-based nanoparticle¹⁵, carbon material,^{16, 17} and so on. Compared to other luminescent

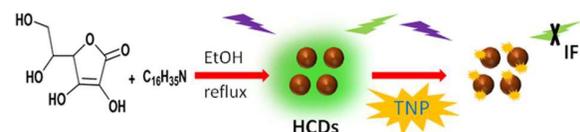
material, carbon quantum dots (CDs) are becoming a new potential candidate for TNP detection because they possess advantages of lower cost, easier synthesis and better optical property. As we know, the medium pollution of TNP is not only commonly found in aqueous media but also in hydrophobic media. Thus the quantitative and qualitative identification of TNP in hydrophobic media is very important for environmental safety control. Unlike other fluorescent material, CDs based chemical sensing system for TNP detection are focused merely on hydrophilic CDs in aqueous/alcohol media system, but there is no report of using CDs to detect TNP in hydrophobic media.^{17, 18}

In order to detect TNP in hydrophobic media, synthesis of hydrophobic carbon quantum dots (HCDs) with photoluminescence fluorescence character as fluorescent probe is required. To the best of our knowledge, there are seldom reports on the HCDs synthesis, which includes microwave irradiation¹⁹ and high temperature carbonization of glucose.²⁰⁻²³ However, these methods suffer from limitations like complicated carbon source, difficult synthesis process and harsh synthesis conditions. Therefore, designing an easier and green way for HCDs synthesis is still a worthwhile and challenging work.

Herein, a green method to synthesize HCDs was presented using ascorbic acid (AA) and hexadecylamine (HDA) as reactant sources at low temperature in absolute ethanol (Scheme 1). The morphology, size and optical properties of the HCDs were characterized. A novel method to detect TNP in hydrophobic medium using the HCDs as fluorescence probe was presented, and the quenching mechanism process was studied. The sensitivity and selectivity for detection of TNP were probed and were compared with other methods. A qualitative

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Electronic Supplementary Information (ESI) available: The details of XRD pattern of HCDs, FL under different add process, FL with different amounts of TNP, UV/Vis absorption spectra of the HCDs and TNP, Tables for different methods comparison, fluorescence quenching constant towards different nitro aromatics and fluorescence lifetime of HCDs with different TNP concentration. See DOI: 10.1039/x0xx00000x



Scheme 1 Synthesis of HCDs and their sensing of TNP.

identification for TNP in hydrophobic medium on a portable test strip with the naked eye was also presented.

In a special designed way, 0.1 g of AA and 0.18 g of HDA were dissolved in a round-bottom flask contained 100 ml of absolute ethanol and mixed uniformly through ultrasonic, then the mixture was heated to reflux in an oil bath. The mixture turned brown gradually with reaction time. After 8 h, the mixtures became dark red-brown and showed strong green fluorescence under UV lamp (365 nm), which indicated the formation of HCDs. The mixture was evaporated in a rotary evaporator to remove the solvent, and the reserved product was washed by acetone to remove other impurities. Then, the HCDs was dried in a vacuum oven, they showed good solubility in hydrophobic medium such as chloroform, toluene and tetrahydrofuran (THF), but it was nearly insoluble in water. Ethanol was reported to play a key role for the CDs' stability and uniformity by using AA as a carbon source.^{24, 25} Simultaneously, ethanol can promote the degradation of AA.²⁶ In the present work, absolute ethanol extremely promoted the degradation of AA and resulted in the formation of HCDs' precursor from AA and HDA. The long alkyl chain of HDA also improved the stability and hydrophobicity of the HCDs.

High resolution transmission electron microscopy (HRTEM) was used to obtain the morphology and size of HCDs. The HCDs with spherical shape are excellent dispersion in THF, and the average diameter is 2.1 ± 0.4 nm as shown in Fig. 1a. Atomic force microscopy (AFM) was further applied to confirm the homogeneous distribution and the average diameter is about 2.0 ± 0.4 nm, which is accorded with the result obtained by TEM in the experimental errors (Fig. 1b). The UV/Vis absorption and photoluminescence spectra of the HCDs display typical characteristics of CDs (Fig. 1c). In the UV/Vis spectra, the HCDs exhibit complicated shoulder absorption

peaks, which may be ascribed to the existence of different kinds of chemical bond such as C=O and C=C.²⁷ The photoluminescence spectra show the maximum emission peak of 492 nm at the excitation wavelength of about 410 nm. And the emission maxima shifts to a longer wavelength as increase of the excitation wavelength, which may be caused by the different emissive traps on the surface, and this has become one unifying character of CDs.²⁸

Characteristic functional groups on surface of these HCDs were probed by Fourier transform infrared spectroscopy (FTIR). The large broad peak at about 3500 cm^{-1} is attributed to the stretching vibration absorption bands of O-H and N-H as depicted in Fig. 1d. The peaks at 2925 cm^{-1} and 2850 cm^{-1} of C-H indicated the presence of abundant aliphatic hydrocarbon. These surface hydrophilic and hydrophobic groups significantly improve the stability of HCDs without any further surface passivation. The strong electrostatic interaction between O-H of HCDs and TNP may provide a much higher fluorescence quenching response. The HCDs have been characterized by elemental analysis, which were mainly composed of C (66.55 %), H (11.15 %) and N (3.77 %). A single broad peak at $2\theta = 21.48^\circ$ in the X-ray diffraction (XRD) pattern further confirmed the amorphous carbon composed structure of HCDs (Fig. S1).

The photoluminescence spectrum of these HCDs dispersed in THF exhibit strong emission at 495 nm at excitation of 410 nm, and the fluorescence intensity of HCDs was gradually quenched by the addition of TNP (Fig. 2a inset). The quenching efficiency was analyzed by using the Stern-Volmer (SV) equation: $(I_0/I) - 1 = K_{sv} [C]$, where I_0 is the initial fluorescence intensity before the addition of analyte, I is the fluorescence intensity in the presence of analyte, $[C]$ is the molar

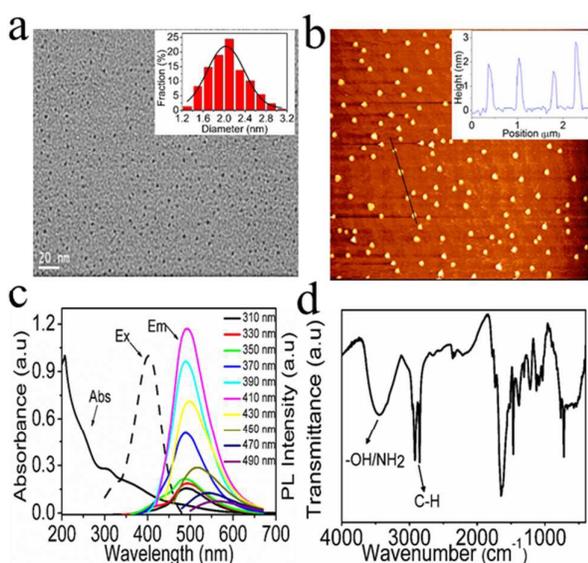


Fig. 1 (a) HRTEM image of HCDs. Inset: diameter distribution according to HRTEM analysis; (b) AFM image of HCDs. Inset: height profile analysis along the line; (c) Adsorption and photoluminescence emission spectra of HCDs; (d) FTIR spectra of HCDs.

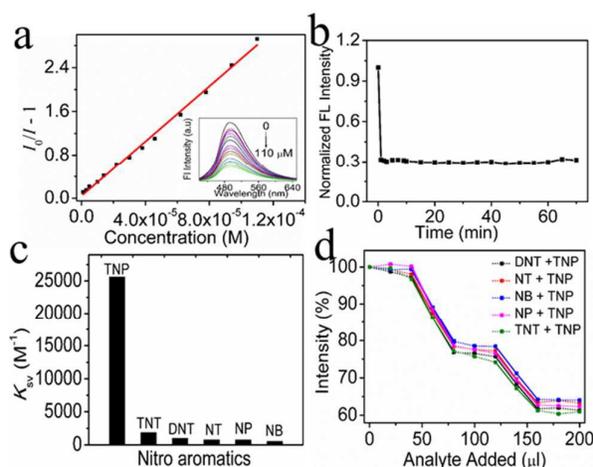


Fig. 2 (a) The linear relationship between $(I_0/I) - 1$ and TNP concentration in the range 1-110 μM . Inset: emission spectra of the HCDs excited at 410 nm in the presence of various concentrations of TNP; (b) Effect of incubation time on the fluorescence quenching of the HCDs based fluorescence sensor toward TNP analysis (72 μM); (c) Fluorescence quenching constant (K_{sv}) for different nitro compounds; (d) Effect of commutative titration of various nitro compounds on fluorescence quenching of the HCDs (solid line for TNP added process and dashed line for other analytes added process).

concentration of analyte, and K_{sv} is the quenching constant (M^{-1}) which represents the quenching efficiency. It is worth noting that fluorescence quenching was a distinct linear relationship with the TNP concentration in the range $1 \sim 110 \mu M$ ($R = 0.998$), with a limit of detection (LOD) of $1.8 \mu M$ at a signal-to-noise ratio of 3 (Fig. 2a). The detection range in this method was comparable with or superior to those obtained from other sensitive fluorimetric methods (Table S1). In order to probe the sensitivity and stability of HCDs for detection of TNP, the relationship of the HCDs fluorescence intensity in THF to time of adding TNP was shown in Fig. 2b, the results indicated the fast fluorescence quenching rate (within 1 min) and excellent stability of the detection system (over 70 min), which provided the possibility for stable and rapid detection of TNP in practice.

To explore the selectivity of HCDs towards TNP, the quenching efficiency for various nitro aromatics (2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT), 2-nitrotoluene (NT), 4-nitrophenol (NP) and nitrobenzene (NB)) in THF is estimated by using the Stern-Volmer (SV) equation, and the quenching constants are shown in Fig. 2c and Table S2. The quenching constant K_{sv} for TNP was found to be $2.6 \times 10^4 M^{-1}$, which is much larger than that for other nitro aromatics, suggesting the predominant selectivity of the HCDs toward TNP. Motivated by these results, selectivity of HCDs towards TNP in the presence of other nitro compounds was explored. The initial fluorescence intensity of HCDs (0.2 mg) dispersed in THF (2 ml) was recorded; then the other nitro compounds ($400 \mu M$) and TNP ($400 \mu M$) were added respectively by titration method (detail process in ESI). Effect of commutative titration of various nitro compounds on fluorescence quenching of the HCDs were demonstrated in Fig. 2d. The stepwise decrease in fluorescence intensity clearly demonstrates the high selectivity of HCDs for TNP, even in the presence of a higher concentration of other nitro compounds. The results provide the possibility for TNP detection in a complicated organic system contained several kinds of nitro aromatics.

In order to probe the origin of high selectivity of HCDs toward TNP, the quenching mechanism process was studied. In general, the fluorescence quenching can be mainly ascribed to two ways: (1) an inner filter effect (IFE) or fluorescence resonance energy transfer (FRET), (2) the formation of a

donor-acceptor charge-transfer complex.^{16, 17} Compared to other nitro compounds, there was larger spectral overlap between the absorption band of TNP and the emission spectrum of the HCDs which generally opened up the bigger possibility of IFE or FRET between TNP and HCDs as shown in Fig. 3a. Meanwhile, as displayed in Figure 3b, the lifetime of the HCDs in the absence and presence of different TNP concentration remained nearly constant, and the fluorescence decay was fitted using a two-exponential decay function to yield lifetimes of 9.45, 9.49, 9.45, 9.50 ns under the TNP concentrations of 0, 24, 48 and $96 \mu M$, respectively (Table S3). The unchanged lifetime excluded the possibility of the FRET process between the HCDs and TNP. On the other hand, TNP still possessed a non-ignorable K_{sv} value at largest emission excited at 500 nm, although no UV/Vis absorption spectral band of TNP appeared in the wavelength region above 500 nm (Fig. S3). The K_{sv} value was close to $4.2 \times 10^3 M^{-1}$, which was far less than that ($2.6 \times 10^4 M^{-1}$) excited at 410 nm, thus the IFE could be considered as one major but not the only process in the fluorescence quenching process. To exclude TNP's acid effect on HCDs' fluorescence intensity, TNP was replaced by two strong acidic organic compounds trifluoroacetic acid and p-Toluenesulfonic acid. The result shows that the HCDs fluorescence intensity is barely affected by acidic organic compounds even though at high concentration (Fig S4 and S5). In addition, the experimental UV/Vis absorption spectrum of the sum of HCDs and TNP was very similar to the theoretical (Fig. S6). No new absorption peak was found in the quenching process, but increased absorption intensity could be observed with the increasing concentration of TNP (Fig. S7). These results exclude the formation of Meisenheimer complex between the HCDs and TNP and exclude the possibility of a charge-transfer mechanism.^{16, 29}

To realize practical applications of this sensing system in industrial production field, portable test strips were prepared by immersing the silica gel TLC plate in the HCDs solution and followed dried in an oven. The fluorescent TLC plate with HCDs emits the bright blue-green light under 365 nm UV light. The fluorescence quench of TNP with various concentration were performed in portable fluorescent test strips at 365 nm UV light, and the results indicated that the fluorescence quench increased with concentration of TNP in range from 2 to 400

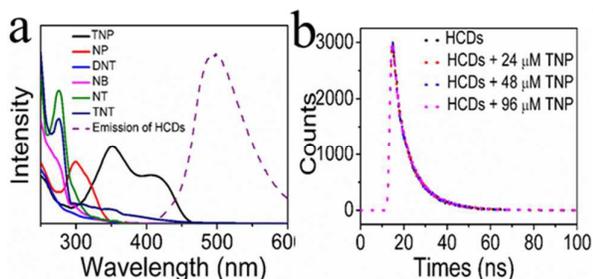


Fig. 3 (a) Spectral overlap between the absorption spectra of nitro compounds (solid line) and the emission spectrum of the HCDs (dashed line) in THF; (b) Time-resolved fluorescence decay of the HCDs with different concentrations of TNP in THF (excited at 410 nm and emission at 495 nm).

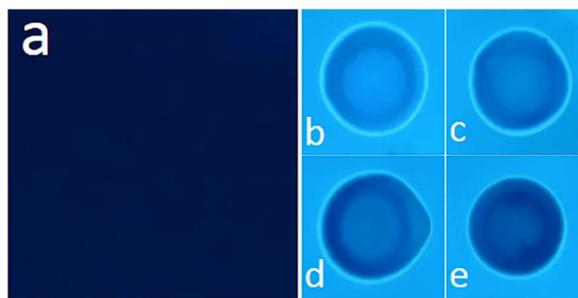


Fig. 4 Portable fluorescent test strips experiment under 365 nm UV light (a) Silica gel TLC plate without any treatment; (b), (c), (d) and (e) Silica gel TLC plate after treatment under the concentration of TNP 2, 20, 200, and $400 \mu M$, respectively.

μM (Fig. 4). These results validate the potential of the fluorescent HCDs as fluorescent sensor for local and instant detection of TNP, and further efforts are still needed for this method and field.

Conclusions

A green method to synthesize fluorescent HCDs was presented at low temperature in absolute ethanol for the first time. During the synthesis process neither harsh conditions nor surface passivation are needed. The morphology, size and optical properties of the HCDs were characterized. In particular, the HCDs based on the fluorescence quenching were firstly applied for TNP quantitative determination in hydrophobic medium. Quenching mechanism process was studied and the results showed that inner filter effect could be considered as one major reason in the fluorescence quenching. Owing to the inner filter effect, the fluorescent HCDs based TNP sensing system was constructed and displayed superiorities like broad linearity ranging, good sensitivity, selectivity and stability. By immobilization of the probes on silica gel TLC plate, a portable test strip has been presented to visualize trace TNP particulates in hydrophobic medium under a UV lamp. What's more, HCDs could be also high potential for the analytic determination and other use in the future.

Acknowledgements

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