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Easy synthesis of photoluminescent N-doped carbon dots from winter melon for bio-imaging

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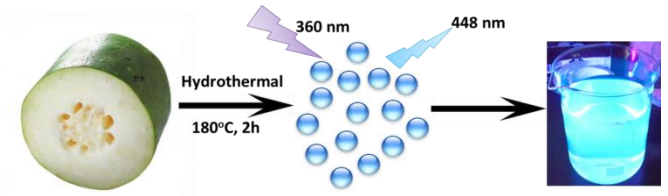
N-doped carbon dots were successfully synthesized via one-step hydrothermal method by using readily edible winter melon as source materials. Mono-dispersed CDs with 4.5-5.2 nm in diameter and quantum yield (QY) of 7.51 % were achieved. The photoluminescent CDs were demonstrated as effective bio-imaging agents for hepG2 (liver hepatocellular carcinoma) cells.

Hepatocellular carcinoma is one of the severest malignancies worldwide threatening the health of human being. Therefore, it is particularly significant to elucidate comprehensively the pathogenesis of hepatocellular carcinoma for diagnosis and treatment. As the intrinsic fluorescence of biological molecules is weak and non-specific, fluorescent labelling and imaging techniques have become a sensible visual analysis to clarify the biological process on the cellular and molecular level.[1] Traditionally, owing to the prominent advantages of well-defined structures, feasibility of synthesis, high quantum yield, and remarkable photostability.[2,3] semiconductor quantum dots (QDs), such as CdS and CdSe, were the preferred choice for cell imaging.[4] Unfortunately, semiconductor QDs are toxicity and poor dispersity in water, thus bringing in serious safety and health concerns, especially for long-term imaging of cells. In addition, Pluronic F127 or hydrophilic chemicals were introduced to minimize the toxicity of QDs, but not totally eliminate.[5,6] The greatest challenge, therefore, is to confidently report the bio-imaging of the observed living cells by using non-toxic fluorescent alternatives while maintaining advantageous optical performances.

As an emerging class of carbon nanomaterials, carbon dots (CDs) exhibit strong fluorescence, outstanding photostability, biocompatibility, aqueous dispersibility and non-toxicity with favorable size advantage (below 10 nm),[7-10] These features make CDs a promising candidate to replace the traditional fluorescent materials in many applications such as sensors, optoelectronic devices, bio-imaging, drug delivery and catalysts.[11-18] Many complex synthetic methods, such as laser ablation, hydrothermal oxidation, electrochemical oxidation, high-temperature calcination, solvothermal and microwave assisted pyrolysis, were devoted to prepare CDs till now.[19-25] However, two issues that need to addressed for the effective synthesis of cost-effective CDs still remain: the size non-uniformity and the time-consuming fabrication process.[26,27] CDs were commonly prepared by using graphite powder and rods,[19,20,28] carbon nanotubes,[29] carbon fibers,[30] carbohydrates,[21,31] ammonium citrate,[32] petroleum coke[33] as carbon sources. Recently, the utilization of eco-friendly materials has drawn much attention.[34] A diverse set of cheap, natural and non-toxic available materials like food waste, hair waste, potato, chitosan, cow milk, coffee grounds have been described for the preparation of photoluminescent CDs. [24,35-42]



Scheme 1 The formation process of N-doped carbon dots by one-step hydrothermal method.

Conventionally, the fluorescence properties and bio-imaging efficiencies of CDs are influenced by using surface passivation, which requires both passivating agents and multiple time consuming steps.[10,11] By doping CDs with other non-metallic components is beneficial for adjusting the structures and composition of CDs till now.[43] N-doped CDs have been found efficiently to induce charge delocalization and enhance performance in bio-imaging and catalysis with N atoms as dopants.[44] Winter melon is an edible and the widely grown

vegetables over the world that is rich in vitamins, proteins, carbohydrates. In this study, we utilized winter melon as both carbon source and nitrogen source to direct prepare photoluminescent N-doped CDs with 4.5-5.2 nm in size and quantum yield (QY) of 7.51 % through an efficient one-step hydrothermal treatment without rigid reaction conditions, surface passivation, and tedious post-processing. And then the N-doped CDs were used as fluorescent agents for liver hepatocellular carcinoma (hepG2) cell imaging.

The synthetic procedure is illustrated in Scheme 1 and more details can be seen from the Electronic Supplementary Information (ESI). The WMJ was extracted from the crushed winter melon and filtered with a 0.22 μm microporous membrane. Then the homogeneous and water-soluble CDs can be directly archived by the hydrothermal process at 180 ℃ for 2 h after the subsequent centrifugation and dialysis. The dispersibility of CDs is ascribed to the abundant functional groups derived from the carbonization of WMJ. The morphology and particle size of CDs were examined by TEM and DLS analysis, as shown in Fig.1. Fig. 1A reveals that the N-doped CDs are uniform and regularly spherical shape with an average diameter of about 5 nm without aggregation. The corresponding particle size measured by DLS (Fig. 1B) indicates that N-doped CDs have a relatively narrow size distribution between 4.5 and 5.2 nm. The inset in Fig. 1A is the typical HRTEM image of N-doped CDs, indicating certain crystallinity with a lattice space of approximately 0.31 nm, which is in agreement with values in the literature.[17]

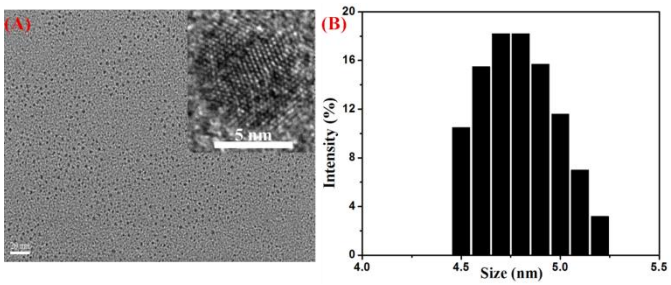


Fig. 1 (A) HRTEM image of N-doped CDs, the inset describes the magnification of a single CD; (B) particle size distribution of CDs.

XPS measurements and FTIR spectrum were performed to identify the effective incorporation of nitrogen and surface functional groups of CDs. In the survey scan of XPS spectrum (Fig. 2A), it is obvious that three distinct peaks centred at 284.5 eV, 399.1 eV, and 531.1 eV corresponding to C1s, N1s, and O1s, respectively. In detail, the C1s spectrum (Fig. 2B) displays three distinct peaks at 284.6 eV, 286.0 eV, and 287.5 eV, which are attributed to C−C, C−N, C=O, respectively.[45,46] The N1s spectrum has two typical peaks at 398.3 (pyridinic Ns) and 400.2 eV (pyrrolic Ns) in Fig. 2C,[46,47] indicating that nitrogen is present in a π-conjugated system where two p-electrons are present in the system of as-prepared N-doped CDs.[44] FTIR spectrum in Fig. 2D shows the characteristic absorption bands at 3416 cm^{-1} corresponding to the stretching vibrations O−H and N-H.[48] The peak at 2926 cm^{-1} is ascribed to the C−H bonds. The absorption bands at 1624 cm^{-1} and 1404 cm^{-1} are due to the C−O stretching vibrations and C−N stretching vibrations, respectively. The existence of carboxylic groups can be clearly proven by the peak at 1732 cm^{-1} corresponding to C=O and the peak at 1071 cm^{-1} attributed to C−O, suggesting the partial oxidation of CDs surfaces.[49] Furthermore, the band centered at 1245 cm^{-1} is assigned to asymmetric stretching vibrations of C−O−C bond.[50] It can be concluded from the results of XPS and FTIR analysis that the hydrothermal degradation of WMJ offered the as-synthesized N-doped CDs with hydrophilic groups such as –COOH and –OH, which are beneficial for the improvement of aqueous solubility of CDs for potential application in biochemistry, drug delivery and diagnostics.

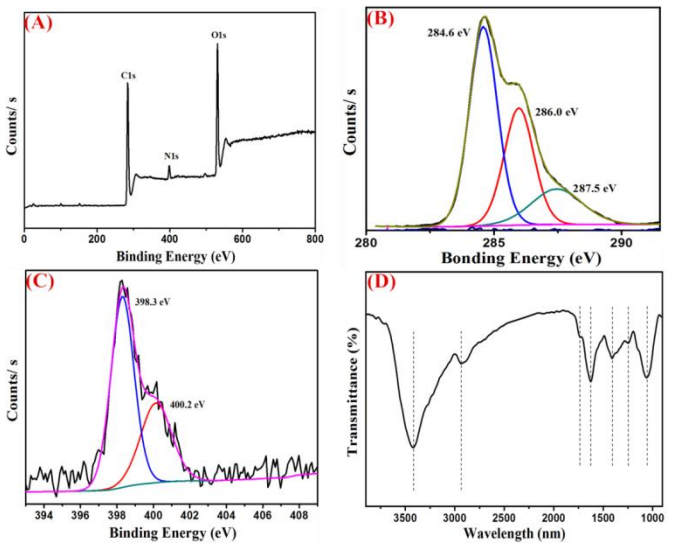


Fig. 2 XPS and FTIR spectra of N-doped CDs. (A) XPS full scan spectrum; (B) C1s spectrum; (C) N1s spectrum; (D) FTIR spectrum.

Photoluminescence (PL) is the more attractive property of N-doped CDs than other carbon-materials. The UV−vis absorption spectra of the WMJ and N-doped CDs are given in Fig. 3, respectively. No obvious absorption band can be detected in the UV-vis spectrum of WMJ. In comparison with WMJ, the optical properties of the N-doped CDs depicts a strong absorption peak at around 280 nm, which can be attributed to the presence of π-π* of N-doped CDs.[51,52] In addition, a broad shoulder at around 325 nm has also been found, perhaps attributing to the excited defect surface states induced by N atoms.[41]

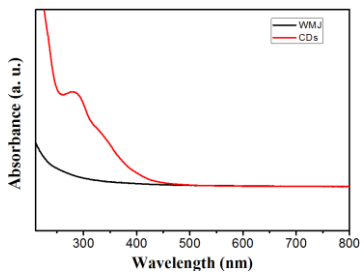


Fig. 3 UV-Vis absorption spectra of winter melon juice (WMJ) and N-doped CDs aqueous solutions.

Fig. 4A shows that the excitation and emission spectra peaks of the N-doped CDs aqueous solution. When irradiated under the short-wavelength laser, N-doped CDs generate strong photoluminescence between 450 and 550 nm. The N-doped CDs aqueous solution is transparent and yellow in bright field and turns into strong blue-fluorescence under UV excitation (inset, Fig. 4A). As shown in the Fig. 4B, the N-doped CDs solution has obvious excitation-dependent PL behaviour. The solution presents strongest luminescence intensity when excited at 360 nm, while the maximum emission peaks located at 448 nm. Besides, with the increase of excitation wavelength from 360 nm to 450 nm, the intensity of emission peaks remarkably decreased and the spectrum are red-shifted from 448 nm to 480 nm. The full width at a half maximum (FWHM) under 360 nm excitation was calculated and the result was 80 nm further verifying narrow size distribution of the as-synthesized N-doped CDs. The PL stability of N-doped CDs to the effects of pH of solutions and UV exposure temperature were investigated. The PL intensity is largely controlled by pH values and the maximum intensity is at neutral pH. The PL intensity increases significantly as the pH increases from 4 to 6, and decreases slightly in alkaline solution with the pH increasing from 8 to 13 (Fig. S1). This stimulus-response property is advantageous for the exploitation of CDs-based pH sensing systems. Furthermore, the temperature-independent PL behaviour was also observed, it can be shown that the PL intensity of the N-doped CDs is nearly unchanged at different exposure temperatures (Fig. S2). Additionally, N-doped CDs showed excellent photostability, as the PL intensity exhibited no meaningful reduction (~6.7%) even after continuous excitation with a Xe lamp from 0.5 days to 12 days (Fig. S3). The QY of N-doped CDs without surface passivation in aqueous solution at pH 7 as measured at an excitation wavelength of 360 nm is estimated to be 7.51 % by using quinine sulphate as standard. Thus, the excellent PL of N-doped CDs will doubtless enhance their applicability used in cell imaging fields.

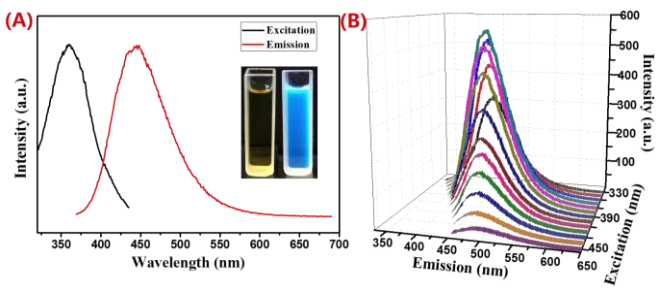


Fig.4 (A) Fluorescence excitation and emission spectra of the N-doped CDs aqueous solutions, inset: photographs of the N-doped CDs aqueous solutions when exposed to daylight and 360 nm UV irradiation, respectively; (B) Emission spectra of the N-doped CDs at different excitation ranged from 340 to 450 nm with a 10 nm increase in each step.

In order to investigate the biocompatibility of the as-synthesized N-doped CDs, CKK-8 assay was used to assess the cytotoxicity of N-doped CDs by using hepG2 cells, and the results are shown in Fig. 5. The cells viability was barely affected when N-doped CDs concentration varied from 0 to 0.8 mg/mL for 24 h. The cells survival rates are still exceeding 90% at all the experimental concentrations, indicating that the N-doped CDs are no apparent toxicity and cyto-compatible with hepG2 cells, which can be a potential candidate for in biosensors and *in vivo* imaging. Based on the excellent photoluminescence property, aqueous dispersibility, narrow size distribution, and low cytotoxicity, N-doped CDs can serve as promising probes for cell imaging applications. Here the hepG2 cells were incubated with N-doped CDs concentration of 0.8 mg/mL and the imaging performance was examined to testify the cells viability by confocal fluorescence microscope. As shown in Fig. 6, all the hepG2 cells showing bright blue fluorescence at UV light excitation can be clearly imaged, and no damage was observed in the microscope after being labelled with N-doped CDs. The N-doped CDs are mainly internalized into the cytoplasm region surrounding the nucleus. All above demonstrate that the N-doped CDs prepared from winter melon can be uptaken by hepG2 cells and served as promising bio-imaging agent.

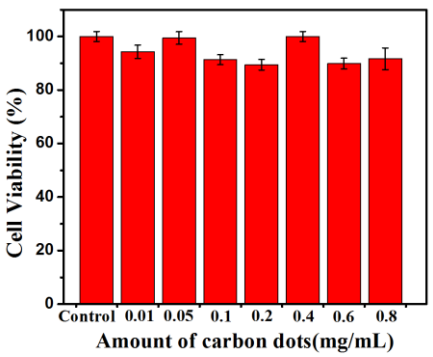


Fig. 5 CKK-8 cytotoxicity assay of hepG2 cells incubated with N-doped CDs at different concentrations for 24 h.

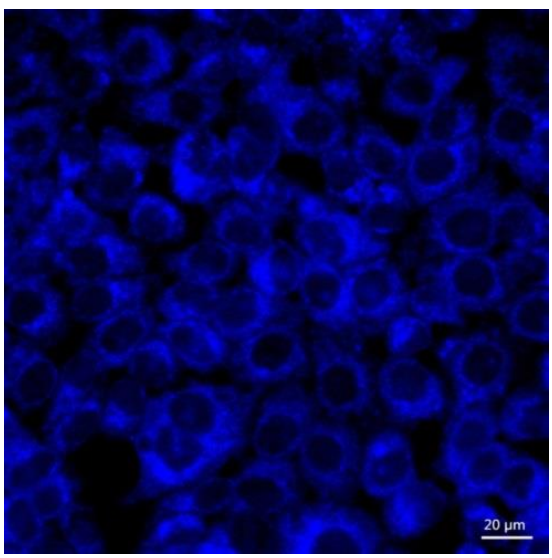


Fig. 6 Confocal laser microscopic image of hepG2 cells after the cellular uptake of N-doped CDs (0.8 mg/mL) for 24 h. The image was obtained by 405 nm excitation and the emission is recorded at 420–520 nm.

Conclusions

In summary, an efficient one-step hydrothermal route was successfully utilized to synthesize photoluminescent N-doped CDs from winter melon. As far as we know, this is the first time to direct prepare N-doped CDs using winter melon juice as both carbon source and nitrogen source without any additional surface passivation. The obtained N-doped CDs with narrow size distribution from 4.5 nm to 5.2 nm exhibit outstanding aqueous dispersibility, strong photoluminescence, and excellent photostability. Based on the favorable biocompatibility and low cytotoxicity as verified by CKK-8 assay, the N-doped CDs were internalized into hepG2 cells as cell-imaging agent showing bright blue fluorescence at UV light excitation. Moreover, the N-doped CDs produced from an edible and the widely grown vegetables would strengthen the applications prospect for green type fluorescent label, drug delivery, and bio-sensors.

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