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ARTICLE TYPE

A novel rapid and green synthesis of highly luminescence carbon dots with good biocompatibility for cell imaging

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A novel simple and green method was developed to synthesize highly luminescence carbon dots using ascorbic acid as carbon sources in the aqueous solution. The as-prepared C-dots showed downconversion and upconversion fluorescence properties. They were applied to imaging of human breast cancer cells and mammary normal cells, showing low cytotoxicity and good biocompatibility.

As a new class brightly photoluminescent nanomaterials, carbon dots are small carbon nanoparticles with sizes below 10 nm. Possessing superior properties such as high aqueous solubility, chemical inertness, easy functionalization, low photobleaching, low cytotoxicity and good biocompatibility, C-dots are a promising alternative for semiconductor quantum dots (QDs).[1, 2] As a result, much interests has been paid on their versatile applications in bioimaging,[3] photocatalysis,[4] sensing,[5] light emitting devices[6] and energy conversion/storage devices.[7] Several approaches have been developed for synthesizing C-dots with excellent characteristics, such as arc discharge,[8] laser ablation,[9] combustion,[10] thermal decomposition,[11] electrochemical synthesis,[12] hydrothermal,[13] microwave/ultrasonic[14] and so on. Among these approaches, the microwave approaches has become popular because it's rapid and facile.[3, 15] However, organic solvent with high boiling point[3] or alkali solution[15] was needed to avoid the evaporation of solvent or to catalyze the carbonization process in the microwave methods. And then complicated processes are needed to remove these organic solvent and inorganic salts. Attempting to overcome these shortcomings, a couple of literatures have presented so-called microwave-hydrothermal method without using organic solvent or inorganic salts to prepare carbon quantum dots.[16, 17] Nevertheless, the method is dangerous during the synthesis process because the synthesis temperature and pressure is difficult to control.

Herein, a novel method that combines the hydrothermal way and microwave digestion approach (M-H) was presented to synthesize C-dots. In this combination method, a microwave digestion apparatus was used to precisely control the reaction temperature and pressure and the method does not require any surface passivation agents or inorganic additives. The crystallinity and surface functional group information of C-dots were characterized. The normal and upconversion fluorescence properties were discussed. The morphology and optical properties of C-dots synthesized by different methods were compared. And the C-dots were used for image of human breast cancer cells and mammary normal cells.

The formation of C-dots can be confirmed by high resolution transmission electron microscopy (HRTEM). As shown in Fig. 1a, the C-dots fabricated by M-H method are spherical and well dispersed (Fig. 1b). The average diameter is 3.1 ± 0.3 nm. The result is supported by the atomic force microscopy (AFM) analysis with the average thickness of about 3.3 nm as shown in Fig. 1c-d. To comparison this approach with relative methods, traditional hydrothermal approach (H) and microwave method (M) were used to fabricate C-dots using the same carbon source. HRTEM images of C-dots made by M-H, H and M were shown in Fig. 1e, 1f and 1g, respectively. The size distribution of C-dots synthetized by different method were compared in Fig. 1h, and the average diameters of C-dots made by M, H and M-H method were 5.1±1.1 nm, 4.8±0.8nm and 3.1±0.3 nm, respectively. Obviously, the C-dots made by M-H method had small particle size and good monodispersity.

X-ray diffraction (XRD) was used to investigate the crystallinity of C-dots. There is a broad diffraction peak centred at 25° due to highly disordered carbon atoms,[18] which corresponds interlayer spacing in graphite structure of 0.34 nm as shown in Fig. 2. Fourier transform infrared spectroscopy (FTIR) was used to investigate the surface functional groups of C-dots. As depicted in Fig. 2b, the broad peak centred at 3384 cm^{-1} is attributed to the stretching vibration of large amount of residual

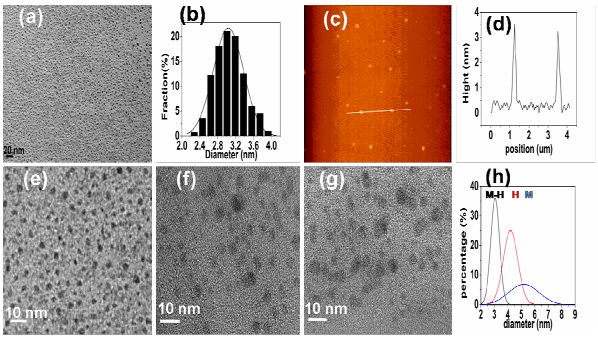

Fig. 1 (a) HRTEM image of C-dots made by M-H method. (b) Diameter distribution according to HRTEM analysis. (c) AFM image of C-dots. (d) The height profile analysis along the line (AFM). (e)-(g) HRTEM images of C-dots made by M-H, H and M methods. (h) The size distributions of C-dots synthesized by different methods.

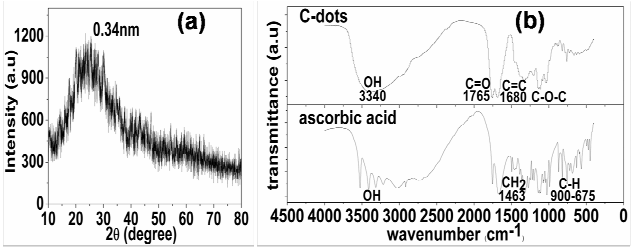

Fig. 2 (a) XRD pattern of C-dots. (b) FTIR spectra of C-dots and carbon source ascorbic acid.

hydroxyl groups. The peak at 1765 cm^{-1} and 1680 cm^{-1} correspond to C=O and C=C group respectively. The peaks between 1000 ~ 1400 cm^{-1} are attributed to the asymmetric and symmetric stretching vibration of C-O-C. Compared with the FTIR spectrum of ascorbic acid, the increases of relative intensity of 1765 cm^{-1} and the disappearing of characteristic peaks in the range of 900-675 cm^{-1} originated by C-H out-of-plane bending indicate the oxygenation process and formation of surface carboxylic acid groups of C-dots. These surface hydrophilic groups significantly improve solubility of C-dots in water, and facilitate applications in biochemistry and diagnostics. Moreover, the surface groups can dramatic improvements the stability of C-dots without any surface passivation agents or inorganic additives.

The UV-visible absorption, excitation and emission spectra of C-dots aqueous solution are presented in Fig. 3a. The UV absorption peaks of C-dots aqueous solution centred at 265nm. The absorption is ascribe to n-π^* transition of the C=O band or the π-π^* transition of the C=C band of conjugated system. A maximum emission peak (Fig 3a. blue line) at 405 nm is observed when the C-dots aqueous solution is excited by 330 nm (Fig 3a. green line). It's worth noting that the emission spectrum is characterized by good symmetry and narrow full width at half maximum (FWHM) of only 68 nm, which suggests a narrow size distribution of C-dots[13] or the limited emissive sites on the surface.[12]

Similarly to C-dots described in other literature,[19] the C-dots mentioned here showed excitation dependent PL spectra

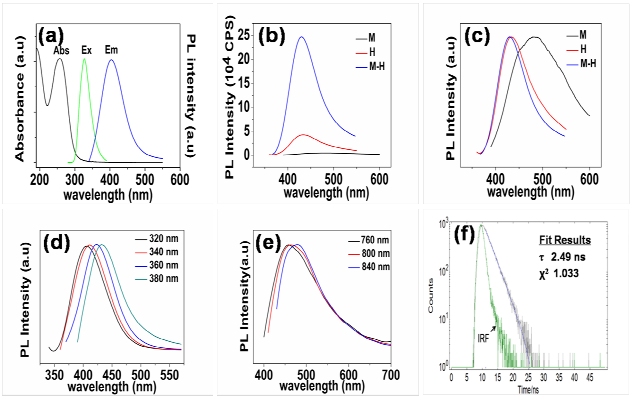

Fig. 3 (a) Typical UV/Vis absorption, excitation and emission spectra of C-dots. (b) PL emission spectra (original) of C-dots (0.6 mg/ml, λ_{ex}= 360 nm) made by different synthetic approach. (c) PL emission spectra (normalized) of C-dots (λ_{ex}= 360) made by different synthetic approach. (d) Normalized PL spectra of C-dots with various excitations. (e) Normalized upconversion emission spectra of C-dots with various excitations. (f) Fluorescence lifetime decay profiles of C-dots aqueous solution (0.6 mg/ml) at 330 nm excitation, emission monitored at 400 nm emission.

(Fig. S1a in ESI). And different maximum emission wavelength can be readily got by adjusting the heating time ascorbic acid or the concentration of C-dots (Fig. S1b and S1c). Additionally, the PL intensity of C-dots is pH sensitive as showed in Fig S1d.

To compare the effect of different synthetic method on C-dots PL properties, C-dots were prepared at various conditions using three different methods and the results were shown in Fig. S2. The PL spectra of C-dots fabricated by three methods were compared and shown in Fig. 3b-c. The fluorescence intensity of C-dots made by M-H method is much stronger than that made by H and M method with same measurement condition (Fig. 3b).

Besides, the FWHM of maximum emission peaks were 68 nm, 80 nm and 112 nm for C-dots made by M-H, H and M methods (Fig. 3c), respectively. These results demonstrate that the M-H method can obtain preferentially mono-disperse C-dots compared with relative methods. This FWHM results is consistent with the HRTEM analysis in Fig. 1h. The superior morphology and optical properties of C-dots made by M-H method may ascribe to homogeneous medium with better heat equilibrium and pressure balance to eliminate the effect of temperature gradient and differential pressure for whole synthesis. On the other hand, higher heat and pressure cause the reaction to be accelerated.

Remarkably, besides normal fluorescence (Fig. 3d), the as-prepared C-dots exhibit clear upconversion PL properties as shown in Fig 3e. The C-dots excited by long wavelength light (from 760 nm to 840 nm) emit the upconverted fluorescence with the PL peaks located in the range from 456 nm to 480 nm. And the maximum emission wavelength increased with the excitation wavelength. This upconverted PL property of C-dots should be attributed to the multiphoton active process, in which the absorption of two or more near-infrared photons leads to the observed visible luminescence of C-dots.[9] This upconverted PL of C-dots provide the intriguing potential in cell labelling with the advantage of low background signal, deep tissue penetration depth, and low phototoxicity associated with the use of near-IR excitation.

The fluorescence quantum yields (Φ_F) of C-dots is measured by using quinine sulphate and Rhodamine 6G as fluorescence standards. The Φ_F of C-dots prepared by M-H, H and M is 15%, 3% and 0.8%, respectively. (Fig S3 in ESI). The Φ_F of the C-dots obtained by M-H method is significantly higher than that obtained by H and M methods. And it also much higher than those C-dots produced using ascorbic acid as carbon source.[13]

The fluorescence decay profile of C-dots is shown in Fig. 3f, the calculated average life-time (τ_f) is 2.49 ns. It should be noted that the fluorescence decay curve could be fitted in mono-exponential function. As the nanoseconds τ_f refers to electron-hole recombination,[20] this mono-exponential function decay indicates that the recombination process undergoes within a homogenous microenvironment which correlates well with the narrow FWHM.[21]

To explore the possible application of the C-dots as bio-imaging material, human breast cancer Bcap-37 cells and human normal mammary Hs-578 cells were used to evaluate the cytotoxicity of the C-dots by the MTT assay. Fig 4a shows that C-dots exhibited low cytotoxicity with cells retaining viability of about 75% for BCap-37 cells and 88% for Hs-578 cells at 0.5 mg/ml after 24 h incubation. The retaining viability of BCap-37

is lower than that of human normal mammary, it may ascribe to the inhibition of residual ascorbic acid to cancer cells. As

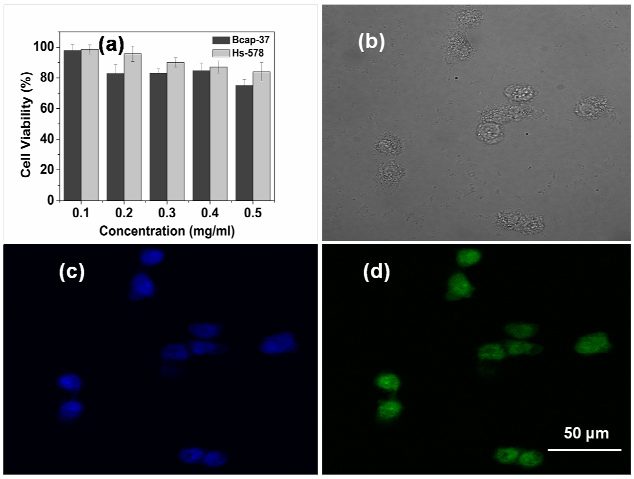

Fig. 4 (a) Cell viability of Bcap-37 cells and Hs-578 cells by MTT assay, (b), (c) and (d) are confocal fluorescence microphotograph of carbon-dots labeledBCap-37 cells under bright field, 405nm excitation and 488nm excitation respectively.

demonstrated to be safe for in vitro applications, Bcap-37 cells were incubated with C-dots in the medium, and the confocal fluorescence microphotograph of C-dots labelled BCap-37 cells under bright filed, 405nm and 488nm excitation were shown in Fig 4b-d. And these results suggest that C-dots are localized in the whole cell area. The similar cell images of Hs-578 cells are depicted in Fig. S3, which indicates that the C-dots can imaging both breast cancer cells and mammary normal cells. The C-dots were internalized into the cells possibly by endocytosis and localized in the cytoplasm and the nucleus of cells. In addition, there is little reduction in fluorescence intensity after excitation for 3h. All these studies indicated the C-dots are biocompatible and ideal cellular imaging agents.

In conclusion, a facile green method was developed to synthesize highly luminescence C-dots in aqueous solution. This method combines the merit of high efficiency of microwave treatment with the virtue of green solvent and controllable heating temperature of hydrothermal method. Besides safety, efficient and green, this method doesn't require complicated post-treatment such as surface passivation and separation of additives. Compare with microwave and hydrothermal methods both using the same carbon source, C-dots obtained by this M-H method exhibited narrowest size distribution and highest fluorescent intensity. The uniformed C-dots show high PL stability and low cytotoxicity. The C-dots has proven to be ideal cellular imaging agents for both human breast cancer BCap-37 cells and human normal mammary Hs-578 cells. Moreover, it can be believed that this M-H method is available to synthesize C-dots efficiently and greenly with various carbon sources.

Experimental

The C-dots were synthesized by rapid one step microwave-assisted hydrothermal method by employing a microwave digestion which can monitor and control the temperature and pressure via fiber optic and pressure transducer in a reference vessel, respectively (XH-800C, xianghu, China). In a typical procedure, 0.18g ascorbic acid was dissolved in 30ml water. Then place it in a microwave digestion container and heated at 180℃ for a period of time (1, 2, 3, 4, 5min) at 900w power. When cooled down to room temperature, the brown color solution was purified by centrifugation at 14000rpm for 10min to remove the large particles. Pure C-dots powder was obtained by rotary evaporating the C-dots colloid solution and subsequent drying the concentrated solution in a vacuum oven.

For comparison, hydrothermal and microwave approaches with ascorbic acid as carbon source were employed to synthesize C-dots. For hydrothermal process, 0.18g ascorbic acid was dissolved in 30ml water and then was transferred into an Teflon-lined stainless-steel autoclave and was heated at 180℃ for 4h. In the microwave procedure, 0.18g ascorbic acid was dissolved in 30ml NaOH aqueous solution (0.5M) and then the mixture was heated in a domestic microwave oven (700w) for 2min.

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Notes and references

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1. H. Li, Z. Kang, Y. Liu and S.-T. Lee, *Journal of Materials Chemistry*, 2012, **22**, 24230-24253.
2. S. N. Baker and G. A. Baker, *Angewandte Chemie International Edition*, 2010, **49**, 6726-6744.
3. A. Jaiswal, S. S. Ghosh and A. Chattopadhyay, *Chemical Communications*, 2012, **48**, 407-409.
4. H. Li, X. He, Z. Kang, H. Huang, Y. Liu, J. Liu, S. Lian, C. H. A. Tsang, X. Yang and S.-T. Lee, *Angewandte Chemie International Edition*, 2010, **49**, 4430-4434.
5. L. Zhou, Y. Lin, Z. Huang, J. Ren and X. Qu, *Chemical Communications*, 2012, **48**, 1147-1149.
6. F. Wang, Y.-h. Chen, C.-y. Liu and D.-g. Ma, *Chemical Communications*, 2011, **47**, 3502-3504.
7. C. Wang, X. Wu, X. Li, W. Wang, L. Wang, M. Gu and Q. Li, *Journal of Materials Chemistry*, 2012, **22**, 15522-15525.
8. X. Xu, R. Ray, Y. Gu, H. J. Ploehn, L. Gearheart, K. Raker and W. A. Scrivens, *Journal of the American Chemical Society*, 2004, **126**, 12736-12737.
9. L. Cao, X. Wang, M. J. Meziani, F. Lu, H. Wang, P. G. Luo, Y. Lin, B. A. Harruff, L. M. Veca, D. Murray, S.-Y. Xie and Y.-P. Sun, *Journal of the American Chemical Society*, 2007, **129**, 11318-11319.

10. H. Liu, T. Ye and C. Mao, *Angewandte Chemie International Edition*, 2007, **46**, 6473-6475.
11. A. B. Bourlinos, A. Stassinopoulos, D. Anglos, R. Zboril, M. Karakassides and E. P. Giannelis, *Small*, 2008, **4**, 455-458.
12. L. Bao, Z.-L. Zhang, Z.-Q. Tian, L. Zhang, C. Liu, Y. Lin, B. Qi and D.-W. Pang, *Advanced Materials*, 2011, **23**, 5801-5806.
13. B. Zhang, C.-y. Liu and Y. Liu, *European Journal of Inorganic Chemistry*, 2010, **2010**, 4411-4414.
14. H. Li, X. He, Y. Liu, H. Huang, S. Lian, S.-T. Lee and Z. Kang, *Carbon*, 2011, **49**, 605-609.
15. X. Wang, K. Qu, B. Xu, J. Ren and X. Qu, *Journal of Materials Chemistry*, 2011, **21**, 2445-2450.
16. L. Tang, R. Ji, X. Cao, J. Lin, H. Jiang, X. Li, K. S. Teng, C. M. Luk, S. Zeng, J. Hao and S. P. Lau, *ACS Nano*, 2012, **6**, 5102-5110.
17. Q. Wang, H. Zheng, Y. Long, L. Zhang, M. Gao and W. Bai, *Carbon*, 2011, **49**, 3134-3140.
18. S. Zhu, Q. Meng, L. Wang, J. Zhang, Y. Song, H. Jin, K. Zhang, H. Sun, H. Wang and B. Yang, *Angewandte Chemie International Edition*, 2013, **52**, 3953-3957.
19. X. Jia, J. Li and E. Wang, *Nanoscale*, 2012, **4**, 5572-5575.
20. X. Wen, P. Yu, Y.-R. Toh, X. Hao and J. Tang, *Advanced Optical Materials*, 2013, **1**, 173-178.
21. Y. Liu, C.-y. Liu and Z.-y. Zhang, *Journal of Materials Chemistry C*, 2013, **1**, 4902-4907.