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One pot selective synthesis of water and organic soluble carbon dots with green fluorescent emission†

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In this work, two types of carbon dots (CDs), water soluble carbon dots (WCDs) and organic soluble carbon dots (OCDs), have been selectively synthesised via a simple, energy-saving process at room temperature. By using cetylpyridinium chloride monohydrate (CPC) as the carbon source, CDs with strong green fluorescent emission could be obtained only in the presence of NaOH, without the need for external heating or additional energy input such as microwave, ultrasound. By controlling the reaction conditions of concentration of NaOH and reaction time, WCDs and OCDs were synthesised and this is the first time of controllable synthesise of WCDs and OCDs at ambient conditions. The as-prepared WCDs and OCDs were characterised by UV-vis absorption, photoluminescence (PL) spectroscopy, transmission electron microscopy, X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, and PL lifetime. The results show that both WCDs and OCDs have uniform particle distribution, better photostability and strong luminescence with a quantum yield of 7.2 and 16.7% for WCDs and OCDs, respectively. The formation mechanisms of WCDs and OCDs were postulated by detailed examining the reaction process and characterisation of the WCDs and OCDs. Owing to the favourable properties of strong emission, good photostability and low toxicity, the WCDs have been successfully applied for bioimaging. This new bottom-up synthetic strategy results in harvesting high-quality CDs, exhibiting the potential of easy and inexpensive large-scale production of WCDs and OCDs.

Introduction

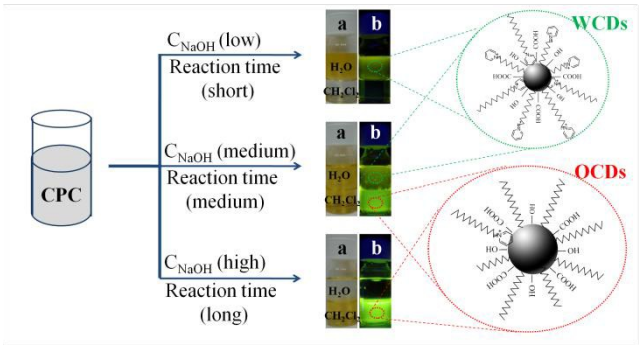
As a new comer of carbon nanomaterials, fluorescent carbon dots (CDs) have recently attracted a tremendous attention.[1,2] CDs have promising photoluminescent (PL) properties such as tuneable excitation and emission spectra, non-blinking emission, excellent photostability and upconversion PL.[3,4] In addition, unlike semiconductor quantum dots, which contain toxic elements (*e.g.*, Cd, In, Mn, Pb, Se, Te, Zn) and expensive synthesis routes, CDs are mainly composed of C, O, H and N, and are considered to be more biocompatible.[5] These advantages of nontoxicity, low-cost, higher quantum yield (Φ_S) and biocompatibility have led to the recognition of the outstanding potential of CDs for a range of applications such as bioimaging and biolabelling,[6,7] biomedicine, optoelectronic devices,[8,9] chemical and biochemical sensors,[10,11] solar cells,[12] and photocatalysis.[13]

Since the first reported amorphous carbon nanoparticles with visible light emission,[14] lots of methods such as microwave,[15] ultrasonication,[16,17] electrochemical method,[18,19] thermal oxidation of suitable molecular precursors,[15,20] exfoliation in organic solvent by modified Hummer's method have been proposed to synthesise CDs.[13] However, the high power consumption, high pressure and temperature (usually about 200°C or even higher) turn to be the main drawbacks of these syntheses, limiting their widespread and large-scale preparation.[21-23] In addition, some other synthesis methods of CDs such as dehydration of carbohydrates by concentrated sulfuric acid,[24] oxidation of wastes like barbeque char, paper and capsicum[25-27] have been described. However, the requirement for a large amount of strong acid create problems for their application.[18,24,28-29] An alternative way to overcome these drawbacks would be to carry out in situ and energy saving synthesis of CDs without any other assistance except the precursor of CDs.

So far most reported CDs to date emit blue light under UV excitation. Due to the so called "water window" effect,[30] it is not suitable for bioimaging because cells and tissues are mostly composed of carbohydrate and emit blue light too, which would interfere with the fluorescent signals form CDs. Therefore, significant efforts have been invested to shift the maximum emission light from blue to green, yellow, red and even near-infrared regions. It has been reported that the emission wavelength of the CDs could shift with the excitation wavelength, that is to say CDs may emit longer wavelength light under longer wavelength excitation. This is the so called "excitation-dependent CDs". In fact, their longer wavelength emissions are achieved with a longer wavelength excitation, not at a maximum excitation; thus, the Φ_S of the red-shifted fluorescence would decrease significantly. It is very challenging to fabricate CDs with strong long-wavelength emission through a facile strategy, and the difficulties in preparing large-scale and high-quality (narrow size distribution) CDs are also the problems.[31]

To overcome the disadvantages associated with higher temperature and pressure controlled reactions, herein we present a novel strategy to selectively synthesise strong green emission water soluble carbon dots (WCDs) and organic soluble carbon dots (OCDs) with cetylpyridinium chloride monohydrate (CPC) as the carbon source and sodium hydroxide (NaOH) as the condensation reagent. This is a simple and energy saving method because the only step in CDs synthesis is to mix CPC with NaOH

solutions, leaving them alone for a relatively period of time and followed with purification of the as-prepared CDs. This is also a green method because no other reagents are used except CPC and NaOH throughout the whole processes. The reaction simply occurs at room temperature without supplying any other external energy, *e.g.*, heating, microwave, ultrasound irradiation and pressurisation. More importantly, WCDs and OCDs could be selectively synthesised by simply controlling the reaction conditions as depicted in Scheme 1. When the reaction time is short and the initial concentration of NaOH is low, WCDs are generated. By contrast, OCDs are harvested at longer reaction time and higher NaOH concentration. The as-synthesised CDs have higher Φ_S at room temperature. This method exhibits the potential of easy and inexpensive large-scale production of both OCDs and WCDs. The OCDs can be applied in the fields of optoelectronic devices, sensors, and solar cells. Finally, since our as-synthesised WCDs possess good biocompatibility and low toxicity, they have been successfully applied for cell imaging.



Scheme 1. The synthesis of water soluble (WCDs) and organic soluble carbon dots (OCDs) by varying the experimental conditions. Photographic images of the as-prepared CDs under (a) daylight and (b) UV light at 365 nm. The upper layer is water and the bottom layer is CH_2Cl_2. CPC: Cetylpyridinium chloride. C_{NaOH}: Concentration of NaOH.

Materials and methods

Materials

Cetylpyridinium chloride monohydrate (CPC) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (HCl), methanol (MeOH) and dichloromethane (CH_2Cl_2) were purchased from Labscan (Bangkok, Thailand). Sodium di-hydrogen phosphate, glycerol, *m*-xylene and NaOH were from Guangdong Chemical Reagent Engineering-Technological Research and Development Center (Guangzhou, Guangdong, China). Quinine sulfate was purchased from J & K Chemical (Beijing, China). Ethanol, petroleum ether, acetone, carbon tetrachloride (CCl_4) were obtained from Tianjin Fuyu Fine Chemical Co., Ltd. Dialysis membrane was obtained from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Solarbio (Beijing, China). Distilled deionised (DDI) water was obtained from a Millipore Milli-Q-RO4 water purification system with a resistivity higher than 18 MΩ·cm (Bedford, MA, USA). All reagents of analytical reagent grade or above were used as received without further purification.

Synthesis of WCDs and OCDs

The syntheses of WCDs and OCDs were based on CPC and various concentrations of NaOH and reaction times. Typically, 2.0 M NaOH was added into a CPC aqueous solution (15 mM) and left to stand at room temperature without applying any external energy. The colour of the mixture solution turned from colourless to light yellow and then to wine red as the reaction time proceeded, indicating the carbonisation of CPC. The reaction could be terminated anytime by adjusting the pH of the solution to neutral (pH 7) with HCl. Therefore, aliquots of reaction mixture could be taken out at different time intervals for optical measurements by quenching with HCl. WCDs and OCDs could be conveniently synthesised by controlling the experimental conditions of reaction time and initial concentration of NaOH. After reaction, WCDs and OCDs could be separated by adding CH_2Cl_2 to extract the OCDs into the bottom organic layer. Dialysis was applied to remove the excess reagents and other water soluble ions of the WCDs sample. Both WCDs and OCDs samples were vacuum-dried at 45°C prior to further characterisation and spectroscopic studies.

Characterisation of CDs

The UV-vis absorption spectra were recorded on a Varian Cary 300 Scan UV-vis absorption spectrophotometer (Palo Alto, CA, USA) and PL measurements were performed on a Perkin Elmer LS55 spectrofluorometer (Waltham, MA, USA). The Fourier transform infrared (FTIR) spectra were acquired on a Perkin-Elmer Paragon 1000 FTIR spectrometer. The X-ray photoelectron spectra (XPS) were recorded on a Leybold Heraeus SKL-12 X-ray photoelectron spectrometer (Shenyang, China). The XPS were processed by the Casa XPS software. The elemental analysis was carried out on an Elementar Analysensysteme vario EL cube organic elemental analyser (Hanau, Germany). The transmission electron microscopic (TEM) images were acquired on a JEOL JEM-2010 transmission electron microscope (Tokyo, Japan) with an accelerating voltage of 300 kV using carbon-coated copper grids. Thermogravimetric analysis (TGA) was conducted on a PE TGA thermogravimetric analyser at a heating rate of 1.0 ℃/min from room temperature to 750 ℃ under a stream of nitrogen. The PL lifetime measurements were performed on a HORIBA TemPro 01 fluorescence lifetime system (Glasgow, UK). A 390 nm NanoLED pulsed diode light source was used to excite the samples. The zeta potentials of samples were measured on a Malvern Nano ZS90 Zetasizer Nano instrument (Worcestershire, UK).

Fluorescence imaging

The cellular uptake of WCDs was determined by an Olympus IX71 fluorescence microscope (Olympus, Tokyo, Japan). Hela cells were cultured in 2.0 mL Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic (Gibco) at 37 ℃ in a 5.0% CO_2/95% air incubator. After 4h incubation with 20 μg/mL WCDs, the medium was removed and the cells were washed three times with phosphate buffer saline (PBS containing 1.8 mM KH_2PO_4, 10.1 mM Na_2HPO_4, 2.7 mM KCl, and 140 mM NaCl at pH 7.4).

For the cell viability study, the Hela cells cultured in the presence of WCDs were determined by the MTT assay. The Hela

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cells were first grown by using a 96 well plates (5000 cells/well) and then cultured in culture media with various concentrations of WCDs (0.0, 1.0, 5.0, 10, and 20 μg/mL) at 37°C in a 5.0% CO_2/95% air incubator. All samples had five parallel wells. After culturing for 24h, the culture medium was replaced by 90 μL serum-free appropriate medium and 10 μL MTT solution (5.0 mg/mL) and then incubated at 37°C for 4h. The reaction solution was carefully aspirated, and 150 μL DMSO was added to dissolve the formazan crystals. The optical density (absorbance) was read by a Bio-Tek Instrument Power Wave XS2 multi-detection microplate reader (Winooski, VT, USA).

Results and discussion

Synthesis of WCDs and OCDs

The synthesis of CDs in this work is rather simple by using only CPC as the carbon source. Green emissive CDs could be obtained quickly after adding NaOH into the CPC aqueous solution. Two types of CDs, *i.e.*, WCDs and OCDs could be synthesised by controlling the reaction time and concentration of NaOH without applying external energy.

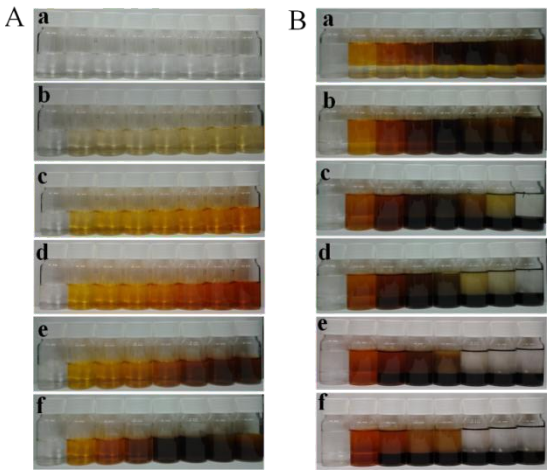

Figure 1. (A) The colour changes of 15 mM CPC aqueous solutions (a) without and (b)-(f) with NaOH at different reaction times (b) 0.0, (c) 1.0, (d) 4.0, (e) 8.0, and (f) 28h. Various concentrations of NaOH were used (from left to right bottles): 0.0, 15, 30, 45, 90, 180, 270, and 360 mM NaOH. (B) The photographic images of the reaction mixtures after addition of CH_2Cl_2 at (a) 28, (b) 48, (c) 96, (d) 108, (e) 132, and (f) 180h.

Figure 1A shows the CDs synthesised with different NaOH concentrations (from left to right: 0.0, 15, 30, 45, 90, 180, 270, and 360 mM) and 15 mM CPC. It can be seen that the solutions turn from colourless to yellow after adding NaOH and the colour is darkened with the increase in NaOH concentration, indicating that the reaction rate increases with the increase in NaOH concentration since NaOH could facilitate the carbonisation of CPC.[32] In addition, the mixed solutions display green fluorescence under UV irradiation (Figure 2B), attributing to the presence of CDs in the solution. When the reactions proceed, the solutions gradually turn from colourless to yellow, to dark yellow and then to wine red at last. The reaction mixtures are darker in colour at higher concentrations of NaOH. Figure 2C displays the UV-vis absorption spectrum of one of the reaction mixtures against reaction time. The UV-vis absorption band grows with the increase in the reaction time, indicating the formation of CDs. More interesting is that the solution begins to be turbid at the highest NaOH concentration (360 mM) when the reaction time is over 24h (Figure S1†). If the reaction time is too long, the reaction mixtures also turn to be turbid even with lower concentrations of NaOH such as 180 and 270 mM (Figure 1A, f). This could be explained by the formation of OCDs which have lower solubility in aqueous solution.

In order to testify this conclusion, CH_2Cl_2 was added to the reaction solution at a reaction time of 28h. Two phases were formed with the upper aqueous and the bottom organic phases (Figure 1B). The colour of the bottom phase was darkened gradually, probably attributing to the conversion of WCDs in the aqueous phase to OCDs in the organic phase. This process is promoted with longer reaction time and higher concentration of NaOH. For instances, more OCDs were obtained at higher NaOH concentration (360 mM) after 96h (Figure 1Bc). But the same phenomenon was occurred at lower NaOH concentration (180 and 270 mM) only at 132h (Figure 1Be). By contrast, more WCDs were found lowest NaOH concentration (15 mM) even after 180h (Figure 1Bf). In summary, WCDs are initially formed but they are converted to OCDs if higher concentration of NaOH and longer reaction time are allowed. In other words, WCDs and OCDs can be selectively synthesised by just adjusting the concentration of NaOH and reaction time: At low NaOH concentration or short reaction time, WCDs will be obtained. OCDs can be produced at high NaOH concentration or long reaction time.

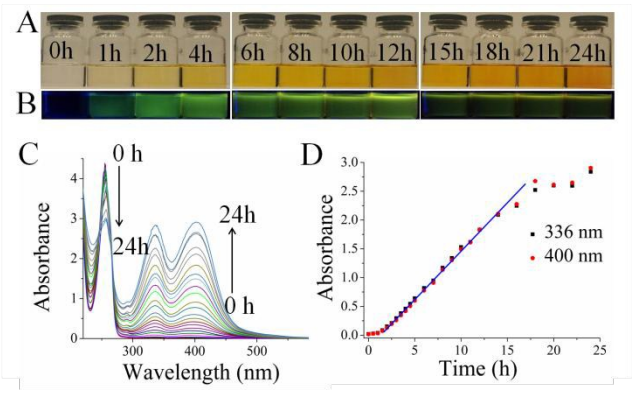

Figure 2. Photographic images of water solutions of WCDs under (A) daylight and (B) UV 365 nm irradiation at reaction times from 0.0 to 24h. (C) UV-vis absorption spectra of water solutions of WCDs at different reaction times. (D) The absorption changes of the WCDs solution at 336 and 400 nm with time.

To better understand the temporal evolution during the reaction time, the absorption and fluorescent spectra of the WCDs and OCDs solutions prepared with 45 mM NaOH were recorded at different reaction times. Figure 2A and B displays the photographic images of the solutions under daylight and UV, respectively at various reaction times. It can be seen that the colour of the solution changes from colourless to light yellow and then dark yellow as the reaction time increases (Figure 2A). The green emission grows stronger with the increase in reaction time (Figure 2B). Figure 2C displays the absorption spectra of the solution at different reaction times. An absorption peak at 256 nm corresponding to CPC and two absorption peaks at 336 and 400 nm corresponding to WCDs are observed. The absorption peak at 256 nm decreases as the time increases, indicating the consumption of CPC during the reaction. Figure S2† shows the absorption spectrum of pure CPC for comparison. By contrast, the absorption peaks at 336 and 400 nm increase with the reaction time, attributing to the formation of WCDs and corresponding to the π–π* and n–π* transitions of WCDs, respectively.[33,34] Figure 2D displays the absorbances at 336 and 400 nm against reaction time. At the first 1.5h, the absorbances increase slowly and then linearly increase with the time at 1.5–14h. After 14h, the reaction slows down. Similarly observations were obtained for OCDs. The absorption spectra of OCDs in CH_2Cl_2 against time are depicted in Figure S3†. The absorption bands (353 and 415 nm) of OCDs are bathochromically shifted as compared to that of WCDs.

The generation of CDs should be attributed to the decomposition of CPC in the presence of NaOH, which could be monitored by the IR spectra of the reaction mixture at different reaction times (Figure S4†). It is obvious that peak III and IV corresponding to the C-H and C=N stretching vibration[32] respectively of CPC decrease with the increase in the reaction time from to 24h. By contrast, Peak II and V corresponding to the O-H/N-H stretching and C-N bending respectively on the surface of CDs increase with time. These results clearly indicate that CPC was consumed in the reaction and CDs were produced with the increase in reaction time.

Characterisation of carbon dots

TEM, XPS, IR, elemental analysis and TGA were used to characterise the CDs synthesised with NaOH and CPC. Figure 3 display the TEM images of the WCDs and OCDs. Both types of CDs are spherical shape and are well dispersed from each other. The absence of discernible lattice structures of CDs on the higher magnification TEM image (Figure S5†) indicates that the resultant CDs are amorphous. The average size of OCDs is 6.2 nm which is larger than that of WCDs (2.8 nm). The much larger size of OCDs should attribute to the growth of CDs with more carbonisation of CPC under the stronger alkaline medium and longer reaction time.

The surface functionality of CDs could determine their solubility in water or organic solvent. It is well known that IR is a common technique to assess the surface functionality of CDs. Figure 4 depicts the IR spectra of WCDs and OCDs. Both of them exhibit some common absorption characteristic of these CDs.[35,36] Nitrogen-containing bonds interpreted as C=N are indicated by the spectral bands at around 1639 cm^{-1}.[37] Similar peaks are observed in CPC (Figure S6†).

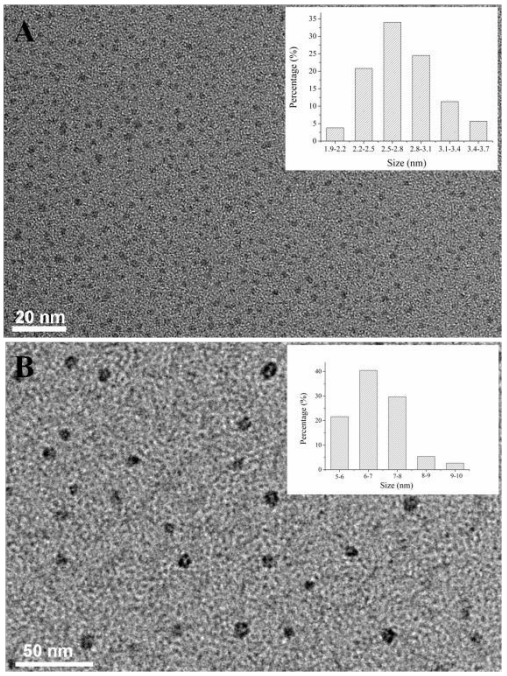

Figure 3. TEM images of (A) WCDs and (B) OCDs prepared with CPC. The insets are the corresponding particle size distribution histograms.

WCDs and OCDs indicate the presence of pyridine group or unmodified surfactant molecules on the surface of the carbon cores. The peaks at 2920 and 2850 cm^{-1} should be attributed to the stretching vibration of C-H, corresponding to CH_3 and CH_2 moieties in CPC, respectively. Several bands from 1374 to 1467 cm^{-1} are assigned to a CH_2 stretching vibration deformation. These results confirm not only the presence of pyridinium rings but also the aliphatic carbon on the surface of the CDs. In addition, the broad absorption peak (both for WCDs and OCDs) at about 3441 cm^{-1} should be attributed to the O-H/N-H stretching[32,38] which is stronger for WCDs than that of OCDs, suggesting that WCDs have better dispensability in water than OCDs.

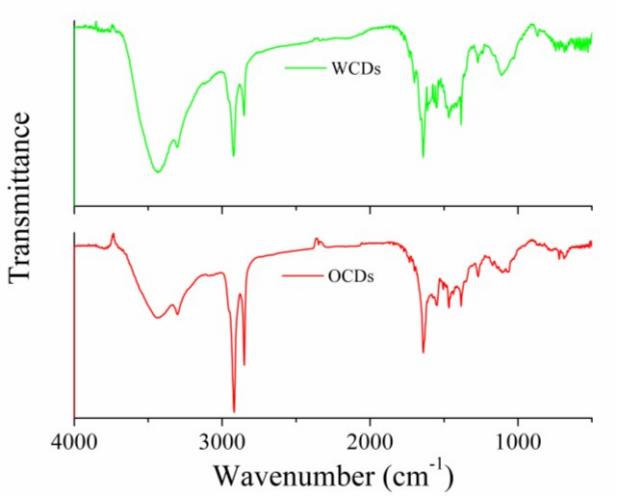

Figure 4. FTIR spectra of WCDs (green line) and OCDs (red line).

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In addition, the stretching C-H vibration bands at 2920 and 2850 cm^{-1} are stronger for OCDs, indicating more aliphatic carbon chains $–(CH_2)_n-CH_3$ ($n \leq 15$) on the surface of OCDs. These results explain why OCDs are soluble in organic solvent whereas WCDs are soluble in water.

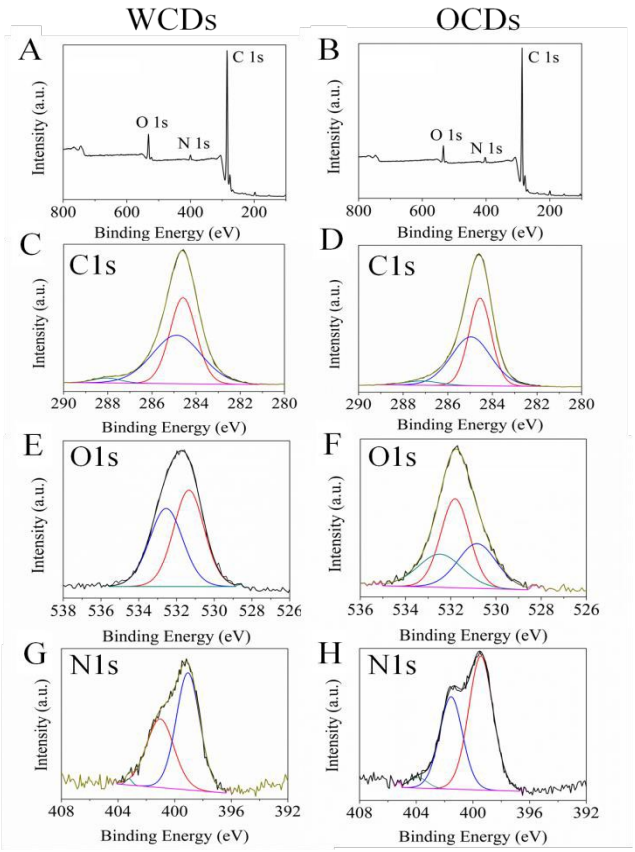

Figure 5. XPS survey scan of (A) WCDs and (B) OCDs. XPS C1s, O1s and N1s of WCDs (C, E and G) and OCDs (D, F and H), respectively.

To gain further insight into the surface functional groups and element states of CDs, XPS measurements were performed to determine the surface composition of the WCDs and OCDs. Figure 5A and B depicts the XPS survey scan of the WCDs and OCDs, respectively. For both types of CDs, three peaks at about 285, 400 and 532 eV associated with C1s, N1s and O1s are identified. It can be seen that the peak intensity of O1s for WCDs is higher than that of OCDs, inferring a higher O content for WCDs and attributing to the hydroxyl functionality on WCDs. This is consistent with the IR analysis. Figure 5C and D show the high-resolution XPS spectra of C1s XPS of WCDs and OCDs, respectively. The C1s spectra are deconvoluted into three peaks. For the WCDs, the peaks at 284.6, 285.0 and 288.0 eV are corresponding to C-C, C-O/C-N and C=O/O-C=O, respectively.[39,40] For the OCDs, the peaks at 284.6, 284.9 and 287.2 eV are corresponding to C-C, C-O and C=O, respectively.[41] They represent the existence of carboxylic and hydroxyl groups on the surface of two kinds of CDs.[42] On the other hand, the O1s region possesses two peaks at 531.3 and 523.5 eV for WCDs (Figure 5E) whereas three major deconvoluted peaks at 530.5, 531.5, and 533.2 eV) for OCDs (Figure 5F), corresponding to the C-O, C=O, and C-OH/C-O-C groups, respectively.[43] These results support the existence of plenty of oxygen-containing groups on the surface of CDs. Figure 5G and H depicts the N1s spectra of WCDs and OCDs, deconvoluting to the C-N-C peaks at 399.4 eV for OCDs and 399.1 eV for WCDs, $N-(C)_3$ peaks at 401.5 eV for OCDs and 401.1 eV for WCDs, and N-H peaks at 403.2 eV for OCDs and 403.0 eV for WCDs.[44] Table S1† summarises the elemental analyses of WCDs and OCDs. Again, higher O content is found for WCDs which is in good agreement with the IR and XPS results.

Figure S7† displays the TGA of CPC, OCDs and WCDs. For CPC, two weight loss steps at *ca.* 100 and 170–250°C are observed, corresponding to the water loss and decomposition of CPC, respectively. For WCDs and OCDs, three major steps are found. The first 35.4% weight loss at 170–380°C is due to the decomposition of the surface-attached CPC ligand on the CDs. The second step at 380–480°C is attributed to the combustion of the carbon-based core containing alkyl groups, which are firmly attached on the surface of CDs.[45] The weight loss for WCDs and OCDs in this step is 16.2 and 20.0%, respectively, indicating that more alkyl groups are on the surface of OCDs and is consistent with the IR and elemental analysis. The third step at 520–650°C is corresponding to other functional groups such as C=O, COOH, OH on the surface of CDs.

Furthermore, the zeta potentials of CPC, WCDs and OCDs were determined as +21.45, +8.05, and -5.35 mV, respectively. It is obvious that CPC has more positive zeta potential as it comprises a cationic quaternary ammonium moiety. For WCDs, it is capped with higher contents of CPC, resulting in positive zeta potential. For OCDs, it is capped with lesser amounts of CPC because of the further decomposition of CPC under higher NaOH concentration and longer reaction time; thus, the surface-attached anionic carboxylate moiety on OCDs renders the negative zeta potential of the overall OCDs.

In essence, it is clear that WCDs and OCDs have been successfully synthesised. The composition and functional groups on the surface of the WCDs and OCDs are different according to their TEM, FT-IR, XPS, TGA, zeta potential and elemental analyses. WCDs contain higher O content than that of OCDs, leading to its better water solubility.

The most attractive feature of CDs relates to their emissive qualities, therefore, the fluorescence of the prepared CDs was also studied in this work. Generally, cationic surfactants such as CPC are referred as fluorescence quenchers.[46,47] However, the CDs (WCDs and OCDs) prepared from CPC in this work exhibit good PL. The excitation and emission spectra of the prepared WCDs (in H_2O) and OCDs (in CH_2Cl_2) are shown in Figure S8†. Two excitation bands are observed for WCDs at 336 and 400 nm

and for OCDs at 343 and 403 nm. For WCDs, an emission peak at 540 nm (Figure S8A†) is found whereas OCDs emit at 510 nm (Figure S8B†). Figure 6 depicts the emission spectra of (A) WCDs and (B) OCDs at various excitation wavelengths. For WCDs, the emission peaks remain almost the same at *ca.* 540 nm when excited in the range 300–400 nm. At excitation wavelengths > 400 nm, the emission spectrum are red-shifted with the increasing in excitation wavelength, *i.e.*, it turns to be excitation-dependent which is similar to most luminescent CDs.[2] The excitation dependent emission may be associated with the aromatic C=C bonds and surface defects resulted from C-OH and C=O groups in the CDs.[48,49] Similar results were obtained for OCDs (Figure 6B).

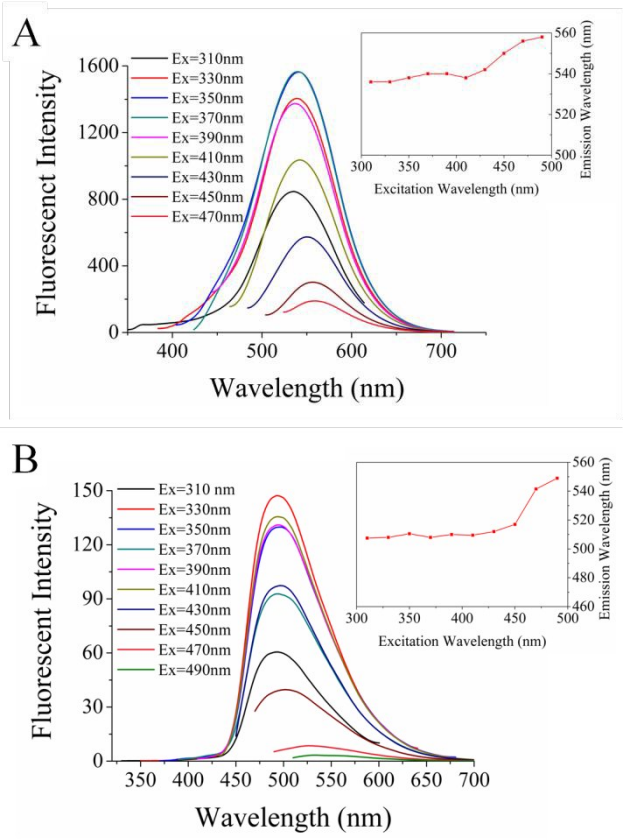

Figure 6. The photoluminescence emission spectra of (A) WCDs and (B) OCDs at different excitation wavelengths. The insets display the changes in the emission wavelength against the excitation wavelength.

The PL properties of OCDs and WCDs in different organic solvents such as ethanol, CCl_4, acetone, glycerol, petroleum ether, *m*-xylene are investigated (Figure S9†). In general, the emission bands of WCDs in these solvents are bathochromically shifted as compared with that of OCDs. Both CDs display strongest emission in ethanol, probably they have good dispensability in ethanol.

Φ_S is one of the most important features to be determined. It is noted that most of the reported green CDs have low Φ_S. Herein, quinine sulfate in 0.10 M H_2SO_4 (Φ_R = 54%) was utilised as the reference. The detail of the measurement is depicted in ESI†. The Φ_S of the WCDs and OCDs in aqueous solution and CH_2Cl_2 solution are determined as 7.2% (in H_2O) and 16.7% (in CH_2Cl_2), respectively. This result indicates that both of the WCDs and OCDs prepared with this method have stronger fluorescence and that of OCDs is stronger than WCDs. The time-resolved fluorescence decay curves and data of WCDs and OCDs were measured and shown in Figure S10† and Table S2†. The decay curve of WCDs in H_2O can be fitted by a three exponential function with lifetimes of 0.337 ns (20.81%), 1.6 ns (71.76%) and 3.89 ns (7.43%) at excitation/emission 390/520 nm with an average lifetime of 1.52 ns. For OCDs in CH_2Cl_2, the time-resolved fluorescence curve exhibits a double-exponential decay with the lifetimes of 2.3 ns (16.3%) and 7.0 ns (83.7%) excitation/emission 390/520 nm with an average lifetime of 6.23 ns. It is well-known that diverse fluorophores or energy levels present in the samples are responsible for their multiple lifetimes.[37] Therefore, it can be deduced that new energy levels are generated in WCDs compared with OCDs which may be due to the effect of solvent. In order to testify this, the lifetimes of WCDs and OCDs in MeOH were also measured. Both time-resolved fluorescence curves of WCDs and OCDs in MeOH exhibit double-exponential decay behaviour and the average lifetimes for WCDs and OCDs are 2.34 and 2.81 ns, respectively which are almost the same (Table S2†). It seems that the lifetime of WCDs or OCDs is determined by the solvent, following with the order: CH_2Cl_2 > MeOH > H_2O. It is possibly attributed to the formation of hydrogen bonds between the OH/COO^- moieties and H_2O with a concomitant effect on stabilising the excited state of WCDs and leading to longer emission wavelengths and shorter fluorescence lifetime.

The photostability of WCDs and OCDS were examined by time-based intensity measurements for 2.5h with continuous UV irradiation as shown in Figure S11†. There was no obvious photo-bleaching observed after long-term illumination for the CDs. In addition, WCDs in H_2O was used as an ink to write on a weighting paper (Figure S12A†). It is hard to see the words under daylight but they are visible and clear under UV light (Figure S12B†). This result and the excellent photostability suggest that the WCDs have potential applications for biological labelling and bioimaging. Table S3† summarises the comparison of the preparation CDs using hydrothermal method with our proposed method. This work possesses the advantages of cost-effectiveness and ease of large-scale production of WCDs and OCDs

Mechanisms of WCDs and OCDs formation

The studies of the formation mechanisms of CDs from the bottom-up method are very important and challenging and usually not employed owing to the difficulties in tracking and characterising the complicated reactions. Herein we commit to understand the formation of WCDs and OCDs from CPC based on the possible routes of reactions and the available characterisation data of the obtained products. In the synthesis of CDs, only CPC as the precursor and NaOH were used and no other external energy such as heating, microwave, ultrasonic or electric potential was applied. Therefore, the energy required for formation of CDs should come from mixing the aqueous solutions of CPC with NaOH.[50] Upon addition of water to NaOH, a large amount of heat will be generated which can then be utilised to carbonise CPC. The higher the concentration of NaOH, the more thermal energy will be provided for carbonisation of

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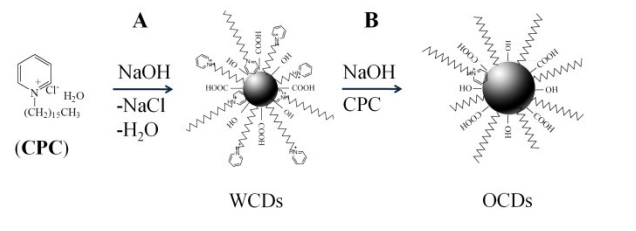
CPC. This proposition has been confirmed by the results that higher NaOH concentration could increase the formation rate of CDs as depicted in Figure 1. The deprotonation and carbonisation of CPC will take place preferentially on the alkyl chain of CPC in the presence of NaOH with a concomitant formation of carbon core.[32] The carbon core is partially capped with the residual unreacted CPC and is also simultaneously surface-functionalised with -OH or -COOH moieties by the OH^- ions to form the WCDs (Scheme 2). As a result, the obtained WCDs could be dispersed well in aqueous solution. By contrast, when higher NaOH concentrations were used, more CPC molecules could be carbonised to produce OCDs of larger core size (Scheme 2), confirming by the TEM image in Figure 3. The carbonisation process is mainly taken place on the pyridine group of CPC by NaOH and the resultant OCDs are capped with the alkyl chains from CPC, thus, making it soluble in organic solvents. This can explain why OCDs are synthesised at higher NaOH concentration whereas WCDs are produced at lower NaOH concentration. Reaction time is another critical factor for CDs preparation. WCDs could be synthesised at a specific NaOH concentration and a shorter reaction time. When the reaction time increases, more CPC molecules are carbonised and CDs are grown and covered with the alkyl chains, resulting in the formation of OCDs. As such, WCDs and OCDs could also be selectively synthesised by just controlling the reaction time. In summary, the synthesis of WCDs or OCDs could be simply tuned by controlling the NaOH concentration and/or reaction time.



Scheme 2. The mechanism of CDs formation with CPC and NaOH, and the transformation of WCDs to OCDs.

Cytotoxicity assay and fluorescence bioimaging

The most important potential applications of CDs are biological applications because of their remarkable biocompatibility. Since the hydrophilic CDs are more appropriate for cell imaging than the hydrophobic CDs, WCDs were used for cell imaging and toxicity measurement. WCDs were applied as a bioimaging agent to label the cervical cancer cell line Hela. A WCDs solution (20 μg/mL) was mixed with the cell culture media along with cells, incubated for 4h, and the washed cells were then imaged under bright field and UV excitation as depicted in Figure 7A and B, respectively. The cells turn to bright green under UV excitation, suggesting that WCDs are successfully internalised by Hela cells and localise in the cell membrane and cytoplasm region surrounding the nuclei of the Hela cells.

The cytotoxicity of the WCDs was studied by the MTT assay using the cervical cancer cell line Hela as the model. Figure 7C shows the cell viability after incubation with WCDs at different concentrations (0–20 μg/mL WCDs) for 24h. The viability of the cells exceeds 92% in all the investigated concentrations, demonstrating that WCDs almost show no toxicity to the cells.

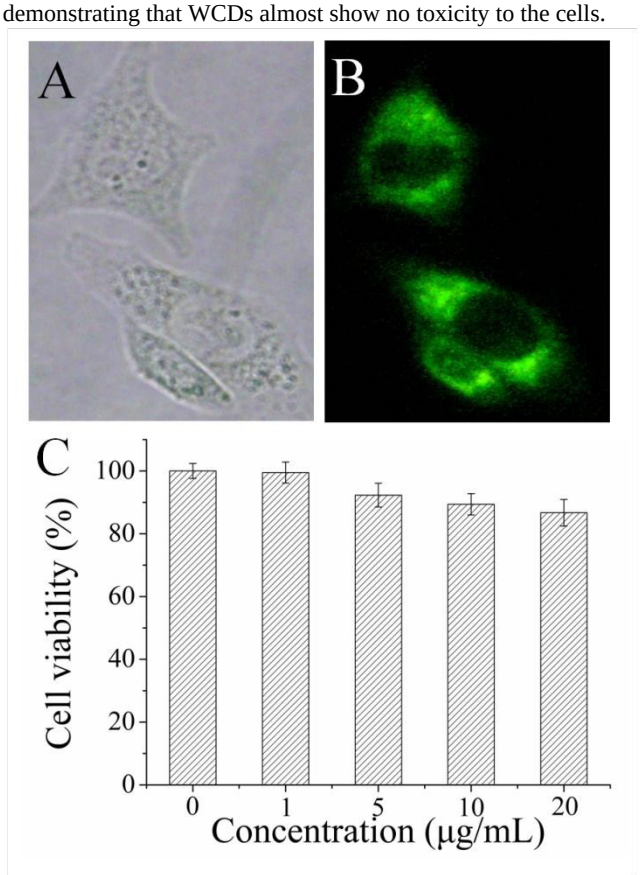


Figure 7. Laser scanning confocal microscopy images of Hela cells incubated with WCDs (20 μg/mL) for 4h: (A) Bright field and (B) fluorescent images at 405 nm excitation. (C) Cell viability at various concentrations of WCDs.

Conclusion

In summary, we have presented a simple and energy-saving strategy to selectively synthesise WCDs and OCDs at ambient conditions by controlling the reaction conditions such as NaOH concentration and reaction time. Two types of CDs could be obtained by only mixing CPC with NaOH without supplying any external energy. This process is likely to be of interest to a range of researchers requiring cheap, simple, and safe synthesis of green-luminescent water soluble and organic soluble CDs. The as-prepared WCDs and OCDs have spherical sizes, good photostability and higher Φ_S (7.2% for WCDs and 16.7% for OCDs). The formation mechanisms of WCDs and OCDs were also investigated in detail, indicating that WCDs could be

obtained at lower NaOH concentration and shorter reaction time whereas OCDs are formed at higher NaOH concentration and longer reaction time. The as-synthesised WCDs display low toxicity to cells and can be employed as a new class of fluorescent imaging agent for bioimaging whereas OCDs can be potentially useful in the fields of optoelectronic devices, sensors, and solar cells. Moreover, our proposed synthesis method of CDs requires little infrastructure and is promising for industrial scalability.

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†Electronic supplementary information (ESI) available: Colour changes of CPC aqueous solutions with NaOH at different reaction times 0–28h, UV-vis absorption spectrum of CPC, absorption spectra and photographic images of OCDs at different reaction times under daylight and UV irradiation, absorption changes of the reaction solution in CH_2Cl_2 at 353 and 415 nm, HRTEM images of WCDs and OCDs, FTIR spectra of CPC and reaction mixture at various times, TGA curves of CPC, OCDs and WCDs, PL of WCDs and OCDs in different solvents, time-resolved spectra of WCDs and OCDs in different solvents, photostability of WCDs and OCDs, WCDs fluorescent characters written on a weight paper, elemental analyses of WCDs and OCDs, fluorescence lifetimes of WCDs and OCDs, comparison with hydrothermal method, and measurement of quantum yield. See DOI: 10.1039/c0××00000×.

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