Facile synthesis of N-rich carbon quantum dots by spontaneous polymerization and incision of solvents as efficient bioimaging probes and advanced electrocatalysts for oxygen reduction reaction†

Zhouyue Lei,‡ Shengjie Xu,‡ Jiaxun Wan and Peiyi Wu*

In this study, uniform nitrogen-doped carbon quantum dots (N-CDs) were synthesized through a one-step solvothermal process of cyclic and nitrogen-rich solvents, such as N-methyl-2-pyrrolidone (NMP) and dimethyl-imidazolidinone (DMEU), under mild conditions. The products exhibited strong light blue fluorescence, good cell permeability and low cytotoxicity. Moreover, after a facile post-thermal treatment, it developed a lotus seedpod surface-like structure of seed-like N-CDs decorating on the surface of carbon layers with a high proportion of quaternary nitrogen moieties that exhibited excellent electrocatalytic activity and long-term durability towards the oxygen reduction reaction (ORR). The peak potential was −160 mV, which was comparable to or even lower than commercial Pt/C catalysts. Therefore, this study provides an alternative facile approach to the synthesis of versatile carbon quantum dots (CDs) with widespread commercial application prospects, not only as bioimaging probes but also as promising electrocatalysts for the metal-free ORR.

Introduction

Xu et al. accidentally discovered carbon quantum dots (CDs) during the purification of single-walled carbon nanotubes, which triggered intense research on novel properties of this viable fluorescent nanomaterials.1 Compared to conventional semiconductor quantum dots (QDs), CDs can be obtained from a variety of sources, and possess remarkable photostability, chemical inertness, tunable fluorescence and excellent biocompatibility.2–9 Thus, they are regarded as promising and advanced alternatives for organic dyes or semiconductor QDs in biomedical and optical imaging areas.10,11

Although there have been abundant raw materials and methods proposed to prepare CDs over the past few years and CDs have been applied in a wide variety of fields, the reported methods suffer various drawbacks and there are few reports on the usability of CDs as electrocatalysts. In general, the synthesis routes proposed thus far have always required a specific equipment and some strict conditions, which limit the practical applications of CDs. For example, top-down methods to prepare CDs refer to breaking down larger carbon structures, including chemical oxidation of graphite,10 activated carbon,12 graphite oxide and lampblack,13 laser ablation of graphite,14 electrochemical oxidation of graphite or multi-walled carbon nanotubes (MWCNTs).15 These methods are accompanied by a strong oxidization procedure and still remain a challenge for the scale-up production of CDs under mild conditions. Regarding bottom-up methods, they can synthesize CDs from various precursors, such as polyethylenimine (PEI),16 ethylenediaminetetraacetic acid (EDTA),17 phenylenediamine,18 diethylene glycol19 and even orange juice20 or natural proteins,21,22 through hydrothermal treatments or microwave synthetic routes. However, traditional bottom-up methods are limited by poor control over the sizes and the products usually tend to aggregate into carbon nanoparticles instead of uniform CDs.23 Therefore, it is of interest to develop more efficient and facile routes to synthesize water-soluble CDs on a large scale, in
which the complex procedures and strict reaction conditions can be bypassed, and the size, fluorescent properties and the solubility of CDs can be well controlled.

On the other hand, the introduction of nitrogen atoms into sp²-hybridized carbon frameworks can effectively modulate their chemical activity and electrical property, which results in excellent electrocatalytic performance towards the oxygen reduction reaction (ORR). For example, there are abundant reports on nitrogen-doped carbon frameworks, such as nitrogen-doped carbon nanotube arrays, nitrogen-doped graphene, 3D N-doped graphene foam and nitrogen-doped carbon nanocages, utilized as metal-free and high-performance ORR electrocatalysts. In particular, Qu et al. reported nitrogen doped GQDs (N-GQDs) decorated on graphene sheets manifesting impressive electrocatalytic activity toward ORR. However, there are few reports on sole CDs as high-performance ORR electrocatalysts, and further optimizing the performance of CD-based ORR electrocatalysts through heteroatom doping and desired assembly is worthwhile.

In the current study, relatively uniform nitrogen-doped carbon quantum dots (N-CDs) were prepared through a facile and one-step solvothermal treatment by employing only common solvents, such as N-methyl-2-pyrrolidone (NMP) and dimethyl-imidazolidinone (DMEU), with cyclic structures and rich amino groups as raw materials. In contrast to previous study requiring an additional carbon source, our study provides a facile method in which the solvents themselves can serve as the carbon source. The previous methods produce CDs in a heterogeneous system; thus the size distribution is affected by ambiguous kinetic factors, whereas the N-CDs in this study are synthesized in homogeneous system, which is more beneficial for their size control. More importantly, these N-CDs can be efficiently prepared in mild experimental condition, even at a low temperature down to 90 °C in air, and easily performed in a flask instead of commonly used high-pressure stainless steel autoclaves. The as-prepared N-CDs exhibit excellent water solubility, strong fluorescence and low cytotoxicity, which enable them to be promising and efficient probes in bioimaging. Moreover, these N-rich CDs are also interesting precursors for N-doped carbon-based electrocatalyst towards ORR. After a simple post-thermal treatment, the original separated N-CDs assemble into a lotus seedpod surface-like structure accompanied with the quaternary and pyridine-like nitrogen atoms increasing, and thus exhibit excellent electrocatalytic activity towards ORR. Therefore, this study might provide an alternative facile way to prepare versatile N-CDs with widespread commercial application prospect on a large scale.

Results and discussion

Preparation and characterizations of N-CDs

Typical water-soluble uniform N-CDs were synthesized directly from common organic solvents with the participation of O₂ through a one-step cost-effective solvothermal reaction, in which the solvents themselves, such as NMP and DMEU, were able to produce N-CDs with the incorporation of O₂ under mild conditions, even at low temperatures down to 90 °C. Therefore, the target product N-CDs can be prepared simply even in a flask instead of traditional high-pressure stainless steel autoclaves. It is deduced that during the bottom-up procedure, NMP-derived N-CDs might go through two steps, i.e., spontaneous oxidative polymerization of NMP (or DMEU), which probably share a similar mechanism as phenylenediamine, dopamine or some other organic compounds with both amino and carboxyl groups (i.e. 2-aminomethyl-propane-1,3-diol, ethylenediaminetetraacetic acid, glycine, and cadaverine), and followed with carbonization and incision of the polymers in the solvothermal process. The detailed synthesis mechanism is showed in Scheme 1.

Fig. 1a and 1b show the transmission electron microscopy (TEM) images of N-CDs prepared at 140 °C for 24 h. Uniform N-CDs with an average size of ~3 nm were prepared after solvothermal treatment, and the size distribution of the N-CDs is provided in Fig. S1.† The high-resolved TEM image shows that the interplanar spacing of N-CDs is 0.23 nm, corresponding to the (002) face of amorphous carbon, which is composed of aromatic carbon sheets oriented in a considerably random fashion. The dim aureole in the selected area electron diffraction (SAED) patterns reveal these N-CDs are polycrystalline. To further investigate the morphology and thickness of the N-CDs, AFM measurements were carried out. As shown in Fig. 1c and 1d, the N-CDs are dispersed uniformly and the height of these N-CDs is about 1 nm, which is very close to the height of monolayer graphene oxide (0.6–0.8 nm), suggesting that most of these N-CDs are monolayer.

Scheme 1. Schematic of the synthesis process to prepare NMP-derived N-CDs via a facile one-step solvothermal treatment approach.
To trace the formation and evolution process of N-CDs from NMP, time-resolved experiments were performed. Fig. 2 shows TEM images of the products prepared after different solvothermal times. After 0.5 h, some large, irregular and amorphous aggregates are formed, which probably resulted from the oxidative polymerization of NMP (inset in Fig. 2). Moreover, some nanoparticles with a size of 10–20 nm were also obtained, which might be derived from the incision of polymers or the oligomers of NMP during the solvothermal process. When the solvothermal time was increased to 1.5 h, the large aggregates disappear, instead, some small particles were observed. The nanoparticles become smaller, confirming the incision and carbonization of the polymers during the solvothermal process (Fig. 2b). Prolonging solvothermal time to 6 h resulted in a further decrease in the size of nanoparticles (3–8 nm). Interestingly, these nanoparticles gradually crystallized, which are probably subjected to the further dehydration and carbonization process at high temperatures (Fig. 2c). Until 24 h, there are many uniform N-CDs with high ordered lattice fringes as prepared, indicating that the dehydration and carbonization process are almost completed. After prolonging the solvothermal time to 24 h, the morphology of the N-CDs was almost unchanged, only the average size was slightly smaller, and their photophysical properties are also similar to that of the N-CDs prepared in 24 h (Fig. S2†). This suggests that the size and photophysical properties of the N-CDs are mainly controlled by the solvothermal time in the initial 24 h. Subsequently, their properties are stable. Compared to the initial morphology, we deduce that the N-CDs are derived from the incision, dehydration and carbonization of polymers, which are prepared by the oxidative polymerization of NMP. Moreover, as shown in Fig. S3,† other common solvents with high-boiling points, such as DMF (N,N-dimethylformamide) and DMSO (dimethyl sulfoxide), cannot produce N-CDs under the same conditions. We deduce that this is probably related to the chemical structures of the NMP and DMEU. NMP and DMEU possess a ring structure and ketone groups, which are oxidized easily in the presence of oxygen, which initiates the self-polymerization of the solvents. This process is the key factor in the formation of N-CDs; subsequently, the polymers can be carbonized further and incised at high temperatures.

In addition to TEM results, time-resolved AFM is also performed to certify the formation mechanism according to the height profiles. After 0.5 h of solvothermal treatment, the height of the large particles, which are probably derived from the initial incision of polymers, was about 15 nm (Fig. S4a†). Increasing the solvothermal time to 1.5 h, resulted in a gradual increase in the height and size, along with many more nanoparticles appearing. This suggests the further incision and carbonation in the solvothermal process (Fig. S4b†). As the solvothermal time is prolonged to 6 h, the height of such nanoparticles further decreases to 3 nm (Fig. S4c†). After 24 h, the nanoparticles finally turn to uniform N-CDs with an average height of 1 nm close to that of monolayer graphene oxide (Fig. S4d†).

The chemical and structural evolution was confirmed by the FTIR spectra, XPS and NMR spectra. As shown in Fig. S5,† the broad band at 1680 cm⁻¹ was assigned to the C=C stretching mode of the polycyclic aromatic hydrocarbons and C=O stretching mode of the amide linkage. The broad and increasing band at 1400 cm⁻¹ was assigned to the overlap of the bending vibration of the O–H and C–N deformation. In addition, the peaks at around 3480 and 2970 cm⁻¹ probably correspond to the O–H and C–H stretching modes.22 The time-resolved FTIR spectra further reveal the chemical and structural evolution of N-CDs. Carbon-rich, N-doped nanodots with a crystal structure are derived from NMP, whereas the polycyclic aromatic feature and aromatic CN species arise by prolong-
ing the reaction time. Moreover, the XPS results in Fig. S6† suggest that the doped N atoms are mostly pyrrole-like N, and the NMR result in Fig. S7† also confirms an N-doped, oxygen-rich and aromatic ring structure of the as-prepared CDs, indicating the spontaneous polymerization of NMP.

Optical properties of N-CDs

The N-CDs aqueous solution is light orange with a typical bright blue color under UV irradiation, which is similar to other common CDs. The UV-Vis spectrum (Fig. 3a) shows a broad peak at ∼400 nm, which was attributed to the aromatic structures of N-CDs.32 The photoluminescence (PL) spectra suggest that the N-CDs prepared after 24 h solvothermal treatment have a maximum emission at 516 nm under the excitation wavelength of 450 nm. The overall quantum yield is approximately 11.6%, which was measured by the absolute method according to a previous report.33 Furthermore, these N-CDs exhibit excitation dependent of PL behaviors, which are consistent with other CDs9,21,22, i.e. as the excitation wavelength ranges from 360 to 520 nm, the PL peak shifts to a longer wavelength from 462 to 556 nm (Fig. 3b). This may be due to the synergistic effects of the carbogenic core and the external oxygen-rich functional groups, even though the exact mechanism is still unclear.9,21 Moreover, the time-resolved PL spectra (Fig. S8†) were also studied. With increasing solvothermal time, the maximum emission peak shifted from 415 nm to 516 nm. The fluorescence of nanoparticles prepared after 0.5 and 1.5 h is very weak and there are extra emission peaks located at 300–350 nm, which are probably due to the chromophore in the initial polymers. As for the PL spectra of N-CDs prepared after more than 6 h solvothermal treatment, the peaks located at 300 to 350 nm almost disappear, along with the drastic improvement in the fluorescence intensity, suggesting that the polymers have been incised efficiently and carbonized to N-CDs with strong fluorescence.

Bioimaging with N-CDs

Taking advantage of the excellent water-solubility and strong fluorescence, CDs have been extensively investigated as ideal cell-imaging probes with minimal cytotoxicity. The N-CDs incubated with the Hela cells exhibited remarkable intracellular fluorescence, as shown in the confocal laser scanning microscopy (CLSM) images (Fig. 4). Clearly, the fluorescence of LysoTracker and N-CDs probe can overlap very well in the cells, suggesting that the N-CDs are supposed to enter into cells by endocytosis. Moreover, the bright areas inside the cells are located at the regions of the perinuclear cytosol and the cell membranes, instead of the central region corresponding to the nucleus. This indicates that the N-CDs can permeate easily into the cell but are unable to be integrated into the nuclei. Therefore, these N-CDs will not disrupt the genetic sequence, confirming the low cytotoxicity of this probe. The inherent cytotoxicity of N-CDs was further evaluated using HEK 293T cell lines through a MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, as shown in Fig. S9†. The cells were mixed with N-CDs at different concentrations for 24 and 48 h. After 24 h, the cell viabilities were decreased only by ∼15% upon the addition of the N-CDs probes at 250 μg mL−1, which is comparable to or even better than many fluorescent carbon nanoparticles (FCNs).17,21,34 Furthermore, upon the addition of the fluorescent probes up to 1000 μg mL−1, the cell viability still maintains ∼73%, indicating the good biocompatibility of N-CDs. After cultivation for 48 h, the cell viability is almost unchanged, which further shows that these N-CDs are harmless to cells. Overall, the as-prepared N-CDs have great potential to be applied as low toxic, biocompatible, and good cell-permeability probes for in vitro imaging.

Electrocatalysis application of N-CDs

Although there have been considerable research focusing on the synthesis of nitrogen-doped carbon nanomaterials and
their catalytic activity toward the ORR, the reports on sole CDs being applied as ORR electrocatalysts are still rare. This is probably because the N-CDs along with a considerable amount of H and O are not beneficial to the catalytic activity for the ORR. Nevertheless, according to previous reports, high temperature treatment in an inert atmosphere can efficiently eliminate H and O, and further carbonize nitrogen-doped carbon nanomaterials with the effective active sites, which is probably a key factor to enhance the N-CDs catalytic activity.\textsuperscript{35,36} Therefore, in this study, the N-CDs are also subjected to a simple post-thermal treatment at 800 °C. Interestingly, the thermal treatment significantly alters the morphology of the N-CDs. As shown in Fig. 5a, after the post-thermal treatment, the products are shiny and metalic in physical appearance. With the further examination of their micromorphology, it appears that the N-CDs assemble together at high temperatures, whereas the original oxygen-rich functional groups around the QDs are carbonized to form some carbon atom layers onto which abundant N-CDs are decorated (Fig. 5a and b). In addition, a typical AFM image and its height profile also confirm that there are many smaller QDs decorated on the ultra-thin carbon layers (Fig. 5c and d). This unique morphology is supposed to provide abundant active sites for the catalysis of ORR, and also increases the electron transfer efficiency between the N-CDs with the support of the underlying ultra-thin and highly graphitic carbon atom layers.

The thermal treatment not only alters the morphology of the N-CDs, but also tunes the layered structure and chemical states. The distinct layered structures of N-CDs and post-thermal treated samples were characterized by XRD (Fig. S10†), in which the (002) peak centred at 19° in N-CDs is shifted to 25° after the post-thermal treatment. This indicates the decreasing interlayer spacing in post-treated N-CDs. Moreover, a new peak located at 43° appears, which suggests a higher crystallinity due to the further reduction of oxygen containing groups.\textsuperscript{20} The chemical state and surface composition of these post-treated products were detected by XPS. As shown in Fig. 6, compared to the initial N-CDs (Fig. S6†), the number of oxygen and nitrogen atoms was significantly decreased and the half-peak width of the C 1s spectra becomes narrower, demonstrating the reduction of N-CDs after thermal treatment at 800 °C. In particular, the distribution of N species varies from the pristine N-CDs, i.e., the number of pyrrole-like N species are clearly decreased accompanied by an increasing amount of quaternary and pyridine-like nitrogen atoms. According to previous reports, quaternary nitrogen atoms are supposed to offer abundant active sites and play a crucial role in the ORR performance.\textsuperscript{36,37}

The electrochemical activity of the as-prepared N-CDs was characterized by cyclic voltammetry (CV) measurements in O\textsubscript{2} or N\textsubscript{2} saturated 0.1 M KOH at a scan rate of 50 mV s\textsuperscript{−}1, as described in our previous study.\textsuperscript{38,39} Although original N-CDs (Fig. S11a†) already manifested impressive ORR activity, the post-treated sample had an ORR peak potential at −160 mV (Fig. 7a), which is even lower than that of the commercial Pt/C catalysts (Fig. S11b†). Remarkably, it exhibits outstanding performance among the previously reported nitrogen-doped carbon materials, as summarized in Fig. S12 and Table S1.† This probably originated from the increasing active sites and high electron transfer efficiency after the thermal treatment, as discussed above. Linear-sweep voltammetry (LSV) curves of oxygen reduction for the N-CDs in an O\textsubscript{2}-saturated 0.1 M KOH solution were also recorded on a rotating disk electrode (RDE), as shown in Fig. 7b. The measured current density evidently

---

**Fig. 5** (a, b) TEM images of the N-CDs after thermal treatment at 800 °C; inset of (a): typical digital image of the N-CDs after thermal treatment at 800 °C; inset of (b): typical HRTEM image of the N-CDs decorating on ultra-thin carbon layers; (c) typical AFM image of the N-CDs after thermal treatment at 800 °C; and (d) the corresponding height profile.

**Fig. 6** XPS spectra of the N-CDs after thermal treatment at 800 °C: (a) survey spectrum; (b) high-resolution C 1s; (c) high-resolution N 1s; (d) high-resolution O 1s.
increased with increasing rotation rate due to the enhanced diffusion of electrolytes, suggesting that the diffusion rate is a key factor for the catalytic process of this catalyst. In addition, the corresponding ORR current density of post-treated N-CDs reaches a flat region at around −0.35 V with a current density of about 1–1.5 mA cm⁻², and thus the Koutecky–Levich plots are utilized at various electrode potentials from −0.25 to −0.60 V to calculate the transferred electron number \( n \) per oxygen molecule. The almost straight and parallel lines reveal a first-order reduction reaction of dissolved \( \text{O}_2 \) catalyzed by post-treated N-CDs.\(^{29,40,41}\) The transferred electron number per oxygen molecule was calculated to be 3.04–3.71 according to the K–L equation, which suggests a direct four-electron transfer pathway for the ORR, i.e., \( \text{O}_2 \) is reduced directly to \( \text{OH}^- \) in the presence of this catalyst.

To further investigate the electrochemical stability of post-treated N-CDs, continuous CV scans were performed in an \( \text{O}_2 \)-saturated 0.1 M KOH solution. As shown in Fig. S13a,\(^{†}\) the peak potential is almost unchanged after 1000 cycles, suggesting the stable ORR activity of these thermal-treated N-CDs. On the other hand, when the catalysts were applied to the fuel cells, the fuel molecules such as methanol in the anode might permeate through the barrier membrane to the cathode. Thus the methanol-tolerant ability is also crucial and should be considered to evaluate the properties of the electrocatalyst.\(^{42}\) The electrocatalytic selectivity against the electrooxidation of methanol was measured in \( \text{O}_2 \)-saturated 0.1 M KOH with 3 M \( \text{CH}_3\text{OH} \) (Fig. S13b).\(^{†}\) No evident changes in the oxygen-reduction current were observed on the as-prepared catalyst, suggesting the impressive methanol-tolerant ORR activity of this electrocatalyst.\(^{43}\)

**Experimental section**

**Materials**

DMEU and NMP were purchased from Sinopharm Chemical Reagent Co. Ltd. Nafion solution (5 wt%) was purchased from Sigma-Aldrich. All reagents were of analytical grade and used as received. \( \text{N}_2 \) with a purity of 99.9% was provided from Shanghai Jifu Gas Co. Ltd.

**Preparation of N-CDs**

The N-CDs were synthesized using a one-pot facile method. Typically, 200 mL of NMP (or DMEU) was heated at 140 °C with magnetic stirring in atmosphere for 24 h (as shown in Fig. S1,\(^†\) NMP was stirred and heated at 90 °C in air for 24 h can also form N-CDs). The as-prepared solution containing plenty of N-CDs showed transparent orange color. The residual solvent was removed after rotary evaporation, dialysis and freeze drying. The collected solid samples were soluble in water and prepared for further use. To further enhance the electrochemical activity, a post-thermal treatment of the solid N-CDs was carried out in a tube furnace in a \( \text{N}_2 \) atmosphere at 800 °C for 2 h.

**In vitro cell experiments**

Hela cells were incubated in \( \varphi \sim 15 \) mm thin bottom culture chambers with 50 \( \mu \text{g mL}^{-1} \) of N-CDs. After 7 h incubation, the cells were washed with DMEM and phosphate buffer solution (PBS, \( \text{pH} = 7.4 \)) three times to remove the remaining extracellular N-CDs, followed by 0.001 mg mL⁻¹ of LysoTracker stained for 20 min. After the LysoTracker was removed by PBS washing, the samples were observed by CLSM with excitation wavelengths of 450 nm.

**Cell culture and MTT assay**

The cellular cytotoxicity of N-CDs was tested on Hela and HEK 293T cells according to a previous study.\(^{22,44}\) Briefly, Hela cells were cultured routinely in flasks, which contained 10 mL of Dulbecco’s Modified Eagle’s medium (DMEM, High Glucose) supplemented with 10% fetal bovine serum, streptomycin (100 U mL⁻¹) and penicillin (100 U mL⁻¹). In addition, all the cells were incubated in a humidified hood filled with 5% CO₂ at 37 °C. When the cells reached 80–90% confluence, they were lifted with trypsin-EDTA. The trypsinized cells were then dispersed and diluted in DMEM (High Glucose) medium, followed by centrifugation at 1000 rpm for 5 min. After removing the supernatant, the cells were resuspended in DMEM (High Glucose) medium and the cell number was counted using a hemocytometer. The cells were then plated at a density of approximately \( 2 \times 10^4 \) cells per well with 100 \( \mu \text{L} \) of medium in a 96-well plate, and subsequently incubated in a 5% CO₂ humid incubator at 37 °C for 24 h and 48 h. The number of viable cells was determined using a MTT assay with 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide. The N-CDs were added to the wells with increasing concentrations at 250, 500, 750, and 1000 \( \mu \text{g mL}^{-1} \). The absorbance was measured at 570 nm in a Multiskan MK3 microplate photo-
meter (Thermo Scientific, USA). The cells cultured with the pure culture medium were set as controls. The absorbance was measured on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain the sample signal.

Electrochemical measurements

CV measurements were carried out in a standard three-electrode cell using a platinum wire as the counter electrode, an Ag/AgCl electrode as the reference electrode and a glassy carbon electrode as the working electrode (CHI660D). The preparation of the working electrode was carried out as described elsewhere. Briefly, 10 mg of post-treated N-CDs were first dispersed ultrasonically in 10 mL deionized water to obtain a homogeneous suspension. Then, 5 μL of the suspension was attached to a glass carbon (GC) electrode with 3 mm diameter. After the suspension was dried, 5 μL of a Nafion solution (5 wt%) was dropped to cover the catalyst. For comparison, the original N-CDs and commercial Pt (20 wt% Pt/C) catalysts were also measured. Three electrodes were placed into a 0.1 M KOH solution with a flow of O2 or N2 maintained for 40 min to achieve an O2-saturated or an O2-free state. The current densities, respectively, and ω were related to the angular velocity ω according to

\[
\frac{1}{J} = \frac{1}{J_L} + \frac{1}{J_K} = \frac{1}{B \omega^{3/2}} + \frac{1}{J_K}
\]

in which \(J_L\) and \(J_K\) are the kinetic and diffusion-limiting current densities, respectively, and \(\omega\) is the angular velocity. \(B\) is the Levich slope calculated as

\[
B = 0.2nF \alpha C_D D_0 v^{-1/6}
\]

in which \(n\) is the number of electrons transferred in one O2 molecule reduction, \(F\) is the Faraday constant (96,485 C mol⁻¹), \(F\) is the Faraday constant (96,485 C mol⁻¹), \(\nu\) is the kinematic viscosity for KOH (0.01 cm² s⁻¹), \(C_D\) is the concentration of O2 in solution (1.2 × 10⁻⁶ mol cm⁻³), and \(D_0\) is the diffusion coefficient of O2 in solution (1.9 × 10⁻⁵ cm² s⁻¹).

Characterization

AFM images were obtained using a Multimode V8 in tapping mode after the samples were deposited on a freshly cleaved mica surface by spin coating. The TEM images were taken with a JEOL JEM2011 at 200 kV equipped with SAED. The fluorescence spectra and photoluminescence excitation spectra were obtained using a Shimadzu RF-5301PC spectrophotometer at room temperature. The FTIR spectra were obtained on a Nicolet 6700 spectrometer. The UV-Vis spectra were obtained on a Hitachi U-2910 spectrophotometer. The UV-Vis spectra were obtained on a Nicolet 6700 spectrometer. The XPS spectra were obtained using Cu Kα radiation. The XPS spectra were obtained on a RBD upgraded PHI-5000 ESCA system (Perkin Elmer) with Mg Kα radiation (hv = 1253.6 eV). The ¹H NMR spectrum was obtained on a Varian Mercury plus (400 MHz) spectrometer using CDCl₃ as the solvent.

Conclusions

Relatively uniform N-CDs were synthesized using a one-step solvothermal process of NMP and DMEU at a relatively low temperature without expensive experimental set-ups. By time-resolved experiments, the formation process of the N-CDs was traced, and a probable bottom-up mechanism was proposed. The as-prepared N-CDs showed strong fluorescence, good cell permeability and low cytotoxicity in vitro, which make them a promising candidate for bioimaging. Furthermore, after a facile post-thermal treatment, the N-CDs assemble into a lotus seedpod surface-like structure and exhibited excellent ORR activity, impressive methanol-tolerant ability and long-term durability. The peak potential was ~160 mV, which is comparable to or even lower than commercial Pt/C catalysts. Therefore, such low-cost metal-free electrocatalysts have great potential to replace traditional Pt-based ORR catalysts in future practical applications.

Acknowledgements

We gratefully acknowledge the financial support from the National Science Foundation of China (NSFC) (No. 21274030, 51473038). We thank Professor Jiachun Feng and Mr Bin Chen for their help with quantum yield measurement. We also appreciate the help from the group of Professor Changchun Wang for in vitro cell experiments.

Notes and references
