Analytical application of $\text{H}_2\text{O}_2$-induced chiroptical graphitic carbon dots

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Abstract

Carbon dots (CDs) have emerged as efficient peroxidase mimics in recent years. However, to further increase its efficiency as peroxidase-mimic, it is also desirable to understand the modification of CD’s geometry during the catalytic reaction. Herein, we focused on the change in material property of the CDs upon their reaction with $\text{H}_2\text{O}_2$ during the peroxidase reaction. D-(+)-glucose was transformed into chiroptical CDs bearing peroxidase-like activity and can be used to detect $\text{H}_2\text{O}_2$ with a limit of detection of 630 $\mu$M. The addition of $\text{H}_2\text{O}_2$ to the CDs resulted in its increased molecular orderliness leading to the introduction of polycrystallinity without affecting its peroxidase-like activity.

1. Introduction

The zero-dimensional carbon dots (CDs) have recently attracted much attention because of their potential optical properties, excellent photostability, and biocompatibility [1]. These properties have stimulated its applications in diverse areas, including biochemical sensing, photocatalysis, drug delivery, biolabeling, and optoelectronic devices [1, 2]. Recent studies have also demonstrated the intrinsic peroxidase-like activity of CDs as another promising property [3–15]. The significant advantage of using CDs as peroxidase mimic is their facile synthesis, low cost, long-term storage, and resistance to proteolysis, unlike the natural peroxidase enzymes [16]. Previous work has demonstrated that the peroxidase-like activity of graphene quantum dots (GQDs) emanates from their ability to decompose $\text{H}_2\text{O}_2$ to OH [17]. Notably, the functional groups on the surface of the CDs play a prominent role in the binding of the $\text{H}_2\text{O}_2$ during the catalytic activity [4, 18, 19].

In recent years, CDs are also attracting more interest because of their chiroptical features [20]. Chirality constitutes a vital aspect of optically-active nanostructures and has not been focussed much. With enormous advancement to understand chirality at the molecular scale and its influence on the structural property, it also becomes crucial to study chirality at the nanoscale for its possible applications [21]. The introduction of chirality into CDs can augment remarkable properties to develop prospects for connecting material science, biology, and medicine [22]. The synthesis of chiral CDs involves covalent modification or capping with chiral moieties or synthesis with chiral molecules; the latter has advantages as it does not involve the additional step of functionalization with chiral molecules [23].

The evolution of CD’s geometry during the various interaction processes is a less studied area. Previous studies have reported the importance of agglomeration of the CDs by the substrates during the peroxidase-like reaction [4]. With distinct geometry and material properties, the CDs can also serve as sensing agents for analytical applications. In this context, an in-depth mechanistic knowledge of the modification of CD’s material property during the catalytic activity also becomes essential. Herein, we report the synthesis of chiroptical CDs from D-(+)-glucose using a single-step pyrolysis method. The as-prepared CDs exhibited blue photoluminescence under a single excitation wavelength and demonstrated intrinsic peroxidase-like activity.
The results indicated that the structural characteristics of the CD are related to the corresponding H₂O₂ concentration.

2. Experimental

2.1. Chemicals and reagents
D-(+)-Glucose anhydrous was purchased from Himedia, India. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Sigma Aldrich, USA. Hydrogen peroxide (H₂O₂) solution 50% w/v was purchased from FINAR, India. Deionized water with a resistivity of >18 MΩ was used throughout the experiment.

2.2. Characterization
UV–visible absorption spectra was monitored using a double beam Cary 60 UV–visible spectrophotometer (Agilent Technologies). The fluorescence spectra was collected using FluoroMax-4 (HORIBA Scientific). The FETEM images and SAED pattern were observed using JEOL JEM-2100F with an accelerating voltage of 200 kV. The Energy-Dispersive x-ray Spectroscopy (EDS), which was attached to the JEOL JEM-2100F was utilized to determine the relative content of carbon and oxygen in the synthesized carbon dots. The FTIR measurements were obtained by PerkinElmer FTIR spectrometer at room temperature using spectroscopic grade KBr pressed disc. The circular dichroism spectra was recorded by Jasco J-815 spectropolarimeter (Japan) at room temperature using 10 mm path length cuvette. The spectra was measured between 170 nm to 300 nm in continuous scanning mode with 10 nm bandwidth and a scanning speed of 100 nm min⁻¹. X-ray diffraction (XRD) was performed on Rigaku Technologies powder x-ray diffractometer (Japan) with Cu Kα radiation operating at 90 kW.

2.3. Synthesis of CDs
The carbon dots were synthesized by pyrolyzing D-(+)-glucose. Briefly, 1 g of D-(+)-glucose was heated at 200 °C until it melted and gradually turned the colourless liquid to a dark yellow solution. Then, 5 ml of water was added and kept under constant stirring for 30 min. After that, the solution was centrifuged at 13000 xg for 30 min. The supernatant was further collected after centrifugation followed by dialysis with 1 kDa cut-off filter for 3 h. Finally, the dialysed solution was used for the experiment.

2.4. Peroxidase-like activity
The peroxidase-like activity of the CDs synthesized from D-(+)-glucose was tested for the catalytic oxidation of ABTS in the presence of H₂O₂. Typically, the experiment was performed by mixing 2 mg ml⁻¹ CD, 10 mM H₂O₂, and 4 mM ABTS in a reaction volume of 1 ml. The reaction mixture was incubated for 5 min and then was monitored by recording the absorbance at λ = 414 nm that corresponds to the oxidation product of ABTS.

3. Results and discussion
The chiral CDs were synthesized by pyrolyzing D-(+)-glucose at 200 °C. During the pyrolysis process, the glucose molecules probably have undergone nucleation followed by growth and subsequently transformed into CDs similar to the previous report [24]. Unlike the microwave-assisted, ultrasonic, and hydrothermal synthesis methods [10, 24–26], the CDs synthesised by us involved a simple single-step low temperature (200 °C) pyrolysis process. The resulting CDs with a pH of approximately 5.0 were well dispersed in an aqueous solution. The synthesized CDs showed two UV absorption bands almost analogous to the earlier reports (figure 1(a)) [24]. Compared to the starting material, the high energy absorption band at 230 nm corresponds to [∏ to ∏] electronic transition is due to C=C and is only found in case of the CD, while the low energy absorption band at 282 nm assigned to n to [∏*] electronic transitions due to C=O is observed in both the cases. Subsequently, the photoluminescence property of the CD was investigated (figure 1(b)). Under the UV lamp, the CD solution appeared blue. As shown in figure 1(b), a broad emission peak centered around 424 nm is observed on excitation at 329 nm.

The transmission electron microscope (TEM) studies showed that the as-prepared CDs are quasi-spherical in morphology and have good monodispersity (figure 2(a)). The Gaussian fitting curve showed that the CDs have an average size of 3.14 ± 0.4 nm (figure 2(b)). The HRTEM image of the CDs showed two distinct lattice parameters of 0.20 nm and 0.24 nm, corresponding to (101) facet of graphitic structure and (1120) lattice fringes of graphene (figure 2(a)) [27]. The selected area electron diffraction (SAED) pattern revealed two-electron diffraction rings (figure 2(c)). The d-spacing of the rings corresponds to the hkl values (101) and (112). The elemental content of the synthesized CD was determined by EDS. The relative atomic percentage was found to
be 92.33% for carbon (C), and 7.67% for oxygen (O) (figure S1 available online at stacks.iop.org/NANOX/2/040003/mmedia). Circular dichroism was used to investigate the chiroptical property of the CDs (figure 3(a)). For D-(-)-glucose, the circular dichroism spectra display a peak at 184.6 nm due to its chirality. Upon pyrolysis and the transformation of the D-(-)-glucose to CDs, the peak was still observed, suggesting that the chirality property was intact. Notably, despite dialysis, chirality was present in the synthesized CDs, albeit there was a slight decrease in the intensity. The surface functional groups of the CD were monitored by Fourier Transform Infrared (FTIR) spectroscopy. The peak at 3250 cm\(^{-1}\) is due to the –OH stretching vibrations that show the
CD’s hydrophilic nature. In addition, the peaks corresponding to C=O (1740 cm\(^{-1}\)), C=C (1647 cm\(^{-1}\)), C–O (1014 cm\(^{-1}\)) and C–H (2930 cm\(^{-1}\), 1365 cm\(^{-1}\)) are also observed (figure 3(b)). The typical powder X-ray diffraction profile of the synthesized CD is shown in figure 4. The CDs exhibit a broad peak centered around 23.06°, corresponding to an interlayer distance of 0.38 nm (002), broader than that of bulk graphite (0.364 nm). The broad peak is due to the small size of the synthesized CDs, whereas the high value of the interlayer distance is attributed to the oxygen functional groups that resulted in a turbostratic structure [28, 29]. Besides, the XRD spectra also show a hump around 44.35°, corresponding to an interlayer spacing of 0.20 nm, which may be associated with the (101) planes (JCPDS card no. 00-001-0640).

To the best of our knowledge, CDs synthesized from D-(+)-glucose is not yet explored for peroxidase-like activity. Therefore, we evaluated the intrinsic peroxidase-like activity of the CDs in the presence of H\(_2\)O\(_2\) and 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). With the addition of H\(_2\)O\(_2\), the CDs can catalyse the oxidation of ABTS to generate a green coloured product, which suggests that the CDs possess peroxidase-like activity (figure 5(a)). The reaction system with CDs or H\(_2\)O\(_2\) alone does not result in a notable colour change. The absorbance peak of the oxidized ABTS is at 414 nm. The as-prepared CD also exhibits pH-dependent peroxidase-like behaviour, with higher activity at lower pH conditions (figure 5(b)). Considering the higher peroxidase-like activity at lower pH conditions, the CD’s material property was screened at pH 1.0 and pH 5.0 in the absence of H\(_2\)O\(_2\) using UV–vis spectroscopy. At pH 1.0, the absorption peak corresponding to C=O at 252 nm decreased, whereas the absorption peak corresponding to C=O (282 nm) increased compared to the
CDs at pH 5.0 (figure 6(a)). FTIR spectroscopy was used to explore the effect on the CD’s surface functional groups at different pH levels. It is worth noting from the spectra that at pH 1.0, an increase in the intensity of stretching vibration corresponding to C=O at 1740 cm$^{-1}$ and C=C at 1647 cm$^{-1}$ was observed (figure 6(b)). By integrating the results from figures 6(a) and (b), we suggest that the C=O functional group has a vital role in the peroxidase-like activity of the CDs derived from D-$(+)$glucose. Furthermore, the fluorescence intensity at pH 1.0 was lesser than at pH 5.0 (figure 6(c)). The phenomenon reported to be caused by the surface states C=C bonds of CDs [24], which is also in agreement with the UV–vis spectra (figure 6(a)), wherein there is a decrease in the intensity of the absorbance peak for the C=C band. Interestingly, the stretching vibration intensity of C=O further increased upon CD’s interaction with H$_2$O$_2$ (figure 6(d)). This may be due to the binding of H$_2$O$_2$ to the C=O functional group that resulted in increased vibration intensity. Additionally, it can also be assumed that in the pyrolyzed glucose structure, one of the carbons bonded to the oxygen of the H$_2$O$_2$, which resulted in the formation of C=O, thus causing an increase in the number of C=O groups and linked increased intensity. Therefore, the UV–vis and FTIR spectroscopy study demonstrated that the C=O groups play an important role in the CD’s peroxidase-like behaviour, similar to some previous reports [18].

Since the peroxidase-like activity of the CDs is H$_2$O$_2$ concentration-dependent, we used the CD synthesized from D-$(+)$glucose for the label-free H$_2$O$_2$ detection. The typical H$_2$O$_2$ concentration-dependent response curve ($y = 0.0696x + 0.15644$) with a linear range from 0.01 mM to 20 mM and an LoD of 630 μM (figure 7). The LoD was calculated using the formula LoD = 3σ/m, where σ is the standard deviation of the absorbance of blank, and m is the slope of the calibration curve.

To investigate the geometry of the CDs during the interaction with H$_2$O$_2$, we measured the UV–vis absorption, FETEM, XRD, and circular dichroism spectra. With increasing H$_2$O$_2$ concentrations, the C=C absorbance peak disappeared, along with a slight hyperchromic shift of the C=O peak (figure 6(a)). By TEM, we observed that the addition of H$_2$O$_2$ to the CDs (pH 1.0) resulted in its agglomeration (figure 8(b)), similar to the previous report [4]. Figure 9 shows the XRD profiles for CD and CD + H$_2$O$_2$. The XRD peak position for
CD + H₂O₂ is shifted to a lower degree (20°) than the CD’s peak at 23.06°. The shifting originated due to the introduction of more oxygen-containing groups in the CD upon interaction with H₂O₂ [27]. Further, the increased intensity of the hump at 44.35° is due to the increased molecular orderliness within the agglomerated structures. Besides, the increase in the size of the CDs due to agglomeration is supposed to be responsible for the
narrow peaks in contrast to the broad peaks of the synthesized CD [30]. Consequently, the selected area electron diffraction (SAED) pattern of the CD + H₂O₂ displayed small spots making up the ring (figure 10(b)). The spots indicate the introduction of polycrystallinity in the CDs. Besides, compared to the pure CD, the CD + H₂O₂ also demonstrated a new diffraction ring with spots corresponding to the (116) plane of the graphite (JCPDS card no. 00-001-0640). Considering these observations, it confirms that H₂O₂ induces some crystallinity in the CD + H₂O₂ agglomerated structures. Based on these observations, we assume that at the beginning, as a result of H₂O₂ addition, the CDs drew closer to one another in a proper orientation to form agglomerates. As the reaction progresses, well-organized CD + H₂O₂ moieties form a separate polycrystalline phase. The circular dichroism spectra obtained for CDs with different concentrations of H₂O₂ reflected the change in CD’s chiroptical properties as a result of agglomeration (figure S2). The synthesized CD at pH 1.0 shows a peak around 194 nm. With increasing H₂O₂ concentrations, the peak at 194 nm gradually red-shifted. This observation proved the formation of a CD-H₂O₂ hybrid accompanied by an increase in the size of the CDs.
4. Conclusion
In summary, we have synthesized chiral CDs from D-(-)-glucose through a simple-step pyrolysis method and demonstrated that the nanostructure could be utilized as an efficient peroxidase-mimic. The $-\text{C}=\text{O}$ functional group of the CD plays a vital role in the peroxidase process. We established for the first time that the CDs undergo a structural transition upon their interaction with H$_2$O$_2$. Interestingly, we also observed that the concentration of H$_2$O$_2$ significantly affects the chiroptical property of the CD. These transitions, however, did not affect the catalytic property of the synthesised CD. The H$_2$O$_2$-induced chiroptical nanocrystal CDs can have great prospects, especially in drug delivery and clinical applications. This research validates the importance of exploring in-depth material modification of CD during the interactions for recognition of novel properties.

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Data availability statement
All data that support the findings of this study are included within the article (and any supplementary files).

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