INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study	:	PhD

Thesis Title:
Studies on the synthesis, characterization, mechanism of peroxidase-like activity, and the application potential of carbon dots prepared from L-glutamic acid and D-(+)-glucose.

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SHORT ABSTRACT

The advent of nanomaterials as enzyme mimics has drawn significant attention, owing to their high stability, robustness, low cost, and easy synthesis protocol. Carbon dots (CDs) with attractive intrinsic characteristics are a propitious candidate for peroxidase mimic. The present investigation is designed to focus and systematically realize the peroxidase-like activity of CDs synthesized from a widely available organic compound in nature, L-glutamic acid. The compound was selected based on a systematic investigation following a theoretical-cum-experimental approach, which led to identify the -COOH group as a highly active moiety for strongly binding and degrading H_2O_2 in the presence of a reducing equivalent, i.e., ABTS. We explored the influence of different functional groups and identified the -NH_2 group present near the -COOH group exerting the highest peroxidase-like activity as validated by the compound, L-glutamic acid. Following a pyrolysis method, the L-glutamic acid was transformed into CDs having a diameter of 3.18 ± 0.53 nm with its core made of pyroglutamic acid. The CD exhibited thermostability (~90 °C) and higher peroxidase-like activity than the L-glutamic acid. The higher activity of the CD was attributed to its binding affinity with ABTS (2, 2'-azino-bis(3 ethylbenzothiazoline-6-sulfonic acid) and H_2O_2. The K_m and K_{cat} of the CD for H_2O_2 were 5.85 mM and 0.011 s^{-1}, respectively. Based on spectroscopic investigations, we confirmed that the peroxidase-like activity is a surface phenomenon that did not have a significant link to the photophysical property of the CD. Based on the above facts and spectroscopic analysis, one of the critical issues concerning the obscure mechanism of the peroxidase-like activity of the CDs has been addressed. We also highlighted the application potential of the synthesized CDs for the colorimetric detection of peroxide. The colorimetric reaction could be reproduced on a paper platform, confirming the application potential of the CD for developing a low-cost peroxide sensor.

As the application potential of CDs for clinical diagnosis has not been adequately explored, we examined the suitability of pyroglutamate (PGA) CDs for detecting glucose, cholesterol, and alcohol in blood serum through their peroxidative activity in the respective enzyme-catalysed reactions following fluorimetric and colorimetric approaches. In buffer, the CD's fluorescence

intensity ($\lambda_{ex\ 354\ nm}$) enhanced over 115 % after interaction with the enzyme proteins due to different lifetime components on its surface. The enhancement was also linked to FRET with the proteins ($\lambda_{ex\ 274\ nm}$ for TRP/TYR). As revealed from zeta potential study, the electrostatic interactions generated binding energy (ΔG, kcal/mol) in the range of -5.8 to -6.3 and significantly shifted the protein's secondary structure to *β*-strands contents. The CD's fluorescence in the blood serum medium was also enhanced where serum's particulate components contributed to the emission. All these subvert fluorescence emissions could be substantially cleaned for detection of peroxide generated in the enzymatic reaction by filtering the serum particulates and redox proteins prior addition of CDs to the reaction systems. The CD, however, could complement well in ABTS-based (absorbance at λ_{max} 414 nm) colorimetric reaction in blood serum without introducing protein or particle separation steps for sensitive detection of peroxide. The limit of detection, dynamic range, and sensitivity discerned for peroxide in glucose oxidase catalysed reaction system were 183 μM, 0.02 mM-0.10 mM (R^2 = 0.98), and 0.2482 AU mM^{-1}, respectively. Overall, these findings will stand as guidance for clinical application of the peroxidative CDs to detect various analytes in blood serum following fluorescence and colorimetric-based principles.

To further increase the efficiency of CDs as peroxidase-mimic, it is also desirable to understand the modification of CD's geometry during the catalytic reaction. We concentrated on the change in material property of the CDs upon their reaction with H_2O_2 during the peroxidase reaction. For this, we utilized D-(+)-glucose to synthesize of chiroptical CDs bearing peroxidase-like activity, which could detect H_2O_2 with a limit of detection of 630 μM. Further investigation revealed that the addition of H_2O_2 to the CDs results in its increased molecular orderliness leading to the introduction of polycrystallinity without affecting its peroxidase-like activity.