



Nitrogen Doped Carbon Quantum Dots Modified by *Lens culinaris* β -Galactosidase as a Fluorescent Probe for Detection of Lactose

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Abstract

Nitrogen doped carbon quantum dots (NCQDs) were synthesized via hydrothermal route. The NCQDs are thermally and optically stable with high fluorescence yield. For the synthesis of NCQDs, citric acid and urea was taken as carbon and nitrogen sources, respectively. The Transmission Electron Microscopy (TEM) of these quantum dots revealed nearly spherical shape and average size of 1.5 nm, which was calculated using Image J software. The quantum dots were also well-characterized using spectroscopic techniques such as FTIR, UV-Visible absorption and fluorescence. These synthesized and characterized dots were utilized for selective detection of lactose in Milli Q water. The bioprobe provide a wide linear range varying from (10.00–77.41) μM with limit of detection 11.36 μM and sensitivity equal to $(0.0065 \pm 0.0002) \mu\text{M}^{-1}$.

Keywords Lactose · Fluorescence · β -Galactosidase · *Lens culinaris* · Biosensor

Introduction

Lactose (disaccharide) is an important ingredient of milk and milk products. A large number of people are suffering from lactose intolerance cannot digest lactose easily [1, 2]. The lactose intolerant people suffer from β -galactosidase (Lactase) deficiency are prime cause of lactose indigestion [3]. The undigested lactose causes several problems such as abdominal pain, bloating, loose stool and flatulence [4–6]. This widespread problem can be controlled to a large extent

by precise measurement of the quantity of lactose and subsequently reducing the lactose amount.

Earlier, enzymatic spectrometry [7], polarimetry, cryoscopic techniques [8], ion exchange chromatography [9, 10] are used to evaluate the quantity of lactose present in milk and other dairy products. However, these methods have inherent limitations such as time consuming, low sensitivity and lack of stability as well as expensive.

Recently, electrochemical biosensors based on conductimetric [11, 12], amperometric [13], potentiometric techniques

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Highlights

- Sensitive and selective detection of lactose using fluorescent bioprobe.
- Amide bond formation between *Lsbgal* and nitrogen doped carbon quantum dots (NCQDs)
- Static quenching of the fluorescent bioprobe after addition of different amounts of lactose prepared in the Milli Q water.

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have been reported for selective detection of lactose. However, preparation of electrodes in electrochemical method is very tedious and involves multistep process such as extensive pretreatment (polishing, surface contamination like oxidation) of the electrode. Fluorescent sensors are highly sensitive, simple, reproducible and selective [14–16]. Nitrogen doped carbon quantum dots (NCQDs) have large fluorescence quantum yield and they can be used in bioimaging [17–19] and detection of various heavy metal ions and toxins [17, 18, 20–27].

In the present work a thermally and optically stable NCQDs were prepared through hydrothermal method and they were characterized by transmission electron microscopy (TEM), Fourier-transform infrared (FTIR), UV-Visible absorption and fluorescence (both steady state and time-resolved) fluorescence spectroscopic techniques. The NCQDs were then immobilized with *Lens culinaris* β -galactosidase (*Lsbgal*) for the selective detection of lactose into Milli Q water.

Experimental Section

Materials

N-hydrooxysuccinimide (NHS), *N*-ethyl-*N*-(3-dimethylaminopropyl) carbodiimide (EDC), bovine serum albumin (BSA), citric acid, urea, and other chemicals of analytical grade with 99% purity used in this work were procured from Sigma-Aldrich, India. *Lens culinaris* seeds were purchased from agriculture seed store. The chemicals and buffers required for extraction of enzyme were procured from Merck Eurolab GmbH Darmstadt, Germany. All reagents and materials used in chromatography, Size-Exclusion Chromatography FPLC column (Superdex-200) (GE Healthcare) and molecular marker were purchased from Sigma (St. Louis, MO, USA). Milli Q (Millipore, Bedford, MA, USA) water with resistance of more than 18 M Ω .cm was used throughout the experiment.

Enzyme Preparation

β -Galactosidase was isolated and purified from *Lens culinaris* seeds by the following published procedure [28].

Synthesis of Nitrogen Doped Carbon Quantum Dots (NCQDs) Via Hydrothermal Route

The NCQDs were synthesized by hydrothermal route using citric acid and urea as carbon and nitrogen source, respectively. In the previous work, microwave assisted green method was used with same precursors [15]. First of all transparent, clear and colorless solution of citric acid and

urea with concentration 1 mM and 3 mM, respectively, were prepared in the Milli Q water and subsequently these solutions were transferred into a teflon tube. The tube containing this solution was autoclaved for 12 h at 200 °C and at atmospheric pressure. After this time duration, the colorless solution gets converted into brown color and cooled down to room temperature. The solution obtained finally was centrifuged at 12,557 g for 30 min to separate the uniform sized quantum dots.

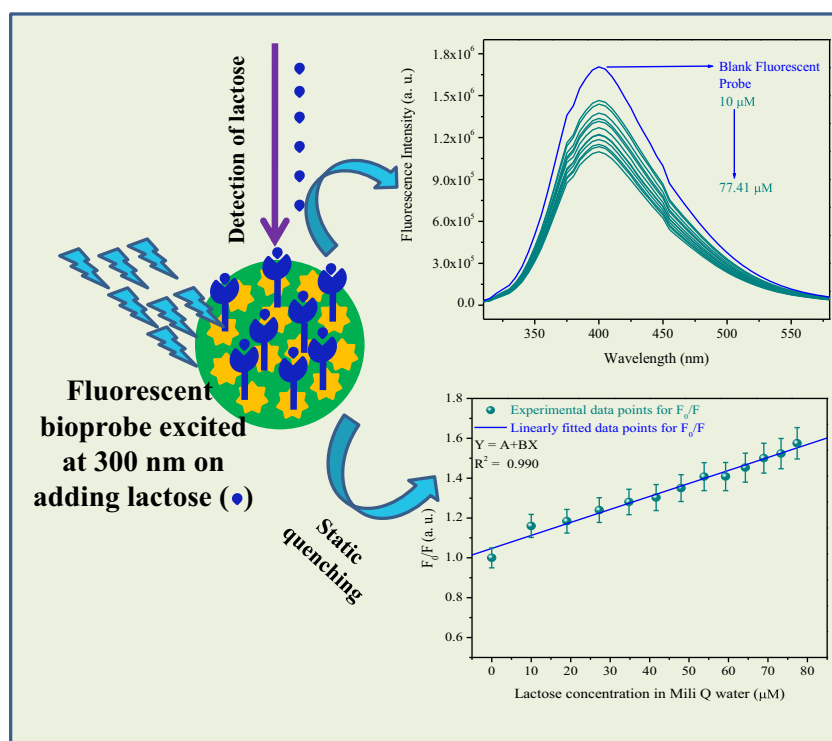
Immobilization of *Lens culinaris* β -Galactosidase (*Lsbgal*) onto NCQDs

The conjugation of *Lsbgal* and NCQDs was performed via amide condensation between the carboxylic group of NCQDs and primary amine of enzyme as per *N*-hydrooxysuccinimide/*N*-ethyl-*N*-(3-dimethylaminopropyl) carbodiimide (EDC/NHS) chemistry [15, 29]. Briefly, NCQDs, EDC (10 μ l, 1 mg/ml) and NHS (10 μ l, 1 mg/ml) were mixed to activate COOH groups of NCQDs. Thereafter, BSA (1 μ l; 1 mg/ml) has been added to block the non-specific sites of the fluorescent bioprobe [29]. Then *Lsbgal* (100 μ g), freshly prepared (25 mM, Sodium phosphate buffer, pH 6.7) was added into EDC/NHS/BSA activated NCQDs and incubated at room temperature for $\sim$ 12 h. Formation of covalent bond between NH₂ functional groups of *Lsbgal* and COOH groups of NCQDs [17] has been observed. It was experimentally confirmed through FTIR spectrum of NCQDs/*Lsbgal*. Schematics of immobilization of *Lsbgal* on highly fluorescing and thermally stable NCQDs treated with EDC/NHS and BSA along with sensing of lactose *via* steady state fluorescence quenching mechanism (static quenching) in Milli Q water has been depicted in Scheme 1.

Material Characterizations

The size and morphologies of the NCQDs were determined using Transmission Electron Microscopy (TEM). FTIR studies was performed on Spectrum 65 FTIR spectrometer, Perkin Elmer using KBr pellet techniques. The optical properties of NCQDs were recorded using UV-Visible (Lambda 25 UV-Visible spectrometer, Perkin Elmer) and fluorescence spectroscopic techniques. Emission, excitation spectra and fluorescence decay profiles were recorded using fluorescence spectrophotometer (Edinburg FL 900) with spectral slit widths 2 nm for both the excitation and the emission monochromators. The excitation source was xenon lamp for emission and excitation profile and light emitting diode (LED) with a low full width at half-maximum at fixed excitation wavelength $\sim$ 369 nm for time decay measurements.

Scheme 1 EDC/NHS/BSA treated and subsequently *Lsbgal* immobilized NCQDs fluorescent bioprobe and detection of lactose in Milli Q water using steady state fluorescence spectroscopic techniques with mechanism of reduction in the intensity of the fluorescent bioprobe.



Results and Discussion

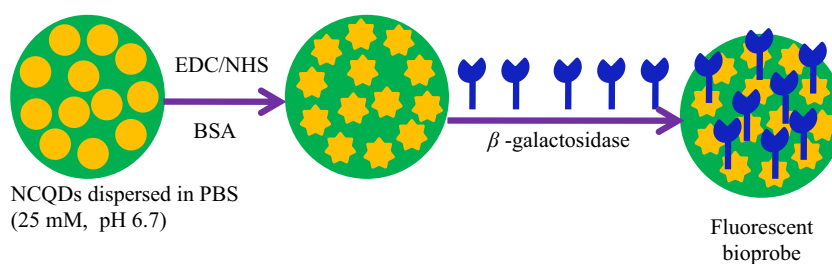
As shown in Scheme 2, NCQDs were selected as a fluorophores. The obtained NCQDs exhibited abundant carboxylic groups, which was activated by EDC/NHS chemistry and subsequently modified with enzyme.

Characterization of NCQDs

NCQDs were synthesized via typical hydrothermal method using urea and citric acid. The size, morphology and photophysical properties were characterized, respectively. As shown in TEM images [Fig. S1(a), supplementary material] showed almost spherical shape of NCQDs (highlighted with yellow color) with an average size ~ 1.5 nm [Fig. S2(a), supplementary material]. The selected area electron diffraction (SAED) pattern showed the amorphous nature of NCQDs, shown in left corner inset Fig. S1(a), [supplementary material]. The UV-Visible absorption spectrum of NCQDs dispersed in the phosphate buffer solution (PBS, 25 mM, pH ~ 6.7) has

been shown by blue color in Fig. S1(b), [supplementary material]. The UV-visible absorption bands at 275 nm and 300 nm corresponds to π - π^* of the C=C, n- π^* transition of the C=O bond present in NCQDs, respectively [15, 30]. The NCQDs exhibited maximum fluorescence emission centered at 400 nm shown in brown color Fig. S1(b), [supplementary material]. It was recorded at 300 nm excitation wavelengths. Corresponding excitation spectrum has been represented with green color [Fig. S1(b)]. It was obtained at fixed emission maximum at 400 nm wavelength. Effects of different excitation wavelengths [300–330 nm] on the emission spectra of NCQDs have been also studied which showed that an increasing excitation wavelength emission maxima slightly shifted towards higher wavelength Fig. S2(b)]. This excitation-dependent fluorescence properties associated with NCQDs may be attributed due to different sizes and distribution of the different surface states [23, 31]. The two-dimensional (2D) fluorescence intensity map of NCQDs is depicted in Fig. S1(c), [supplementary material] as a function of fluorescence emission wavelength (λ_{emc}) under different excitation

Scheme 2 Schematic to prepare fluorescent bioprobe modifying NCQDs treated with EDC/NHS/BSA and subsequently immobilized with β -galactosidase



wavelengths (λ_{exc}) varying from 300 nm to 330 nm. The position of maximum intensity of emission spectra of NCQDs is represented with white circle shown in Fig. S1(c) [supplementary material]. The international commission on illumination (CIE) map software was used to determine the emission-colors of NCQDs. It gives emission blue color (0.18, 0.40) of the fluorescence spectra at the excitation wavelength 300 nm Fig. S1(d) [supplementary material]. The optical images of NCQDs dispersed in PBS, under white light (left) and UV lamp of wavelength 365 nm (right) have been shown in the inset of Fig. S1(d) [supplementary material]. Under UV-lamp illumination the NCQDs also provides the blue color which strongly supports the color observed with CIE map.

Characterization of Bioprobe (NCQDs-*Lsbgal*)

To confirm the successful conjugation of NCQDs-*Lsbgal* UV-Visible absorption and FTIR studies was carried out as shown in [supplementary material, Figs. S3(a) and (b)].

The UV-Visible absorption spectra of NCQDs, NCQDs/EDC/NHS, NCQDs/EDC/NHS/BSA and NCQDs/EDC/NHS/BSA/*Lsbgal* have been shown in Fig. S3(a) [supplementary material] which showed typical peak at 280 nm in case of NCQDs/EDC/NHS/BSA/*Lsbgal*. FTIR spectra for NCQDs, *Lsbgal* and NCQDs/*Lsbgal* shown in Fig. S3(b) [supplementary material] by blue, green and violet colors, respectively. Both result implies the successful conjugation of NCQDs and enzyme.

Steady State and Time Domain Fluorescence Studies of NCQDs and Modified NCQDs

Fluorescence spectra of NCQDs (violet color) (5 μ l of NCQDs dispersed in 2 ml PBS) and successively modified NCQDs with EDC/NHS (green color), EDC/NHS/BSA (brown color) and EDC/NHS/BSA/*Lsbgal* (blue color) were recorded at similar excitation wavelength 300 nm. These

spectra have been shown in Fig. 1(a). Gradual decrease in the intensity was monitored at successive steps of fluorescent biosensor preparation. The reduction in the intensity is also represented on bar graph in the inset of Fig. 1(a). An important parameter fluorescence quenching efficiency defined as F_0/F [here F_0 = fluorescence intensity of NCQDs and F = fluorescence intensity of the modified NCQDs at successive steps] is depicted in Fig. 1(b). The parameter F_0/F gives a linear response at different stages of biosensors preparation which might be due to static or dynamic quenching. The mechanism of reduction in intensity, time domain fluorescence measurements were carried out using light emitting diode (LED) of 369 nm excitation wavelength [Fig. S4]. NCQDs contain three life times which were also observed with modified NCQDs. The first life-time changes at each step while other two remain almost unchanged after second step [Table S1] [supplementary material]. An important parameter τ_0/τ [where τ_0 = life-times associated with NCQDs and τ = life-times with modified NCQDs] was calculated for these three life-times. For first life-time this parameter [black color] changes at each step whereas for other two life-times after second step remain constant [τ_0/τ for second life-time represented by red color and for third with green color]. These parameters are also provided in the form of Table S1 [supplementary material]. Reduction in the intensity could be attributed due to dynamic and static quenching in the excited and ground states, respectively.

Detection of Lactose

These optically characterized fluorescent biosensors were employed to detect various concentration of lactose prepared in Milli Q water. From 0.2 mM stock solution of lactose 10 μ M was added to the fluorescent probe incubated at 58 $^{\circ}$ C for 10 min as temperature is an important parameter for catalytic activity of enzyme. Thereafter fluorescence

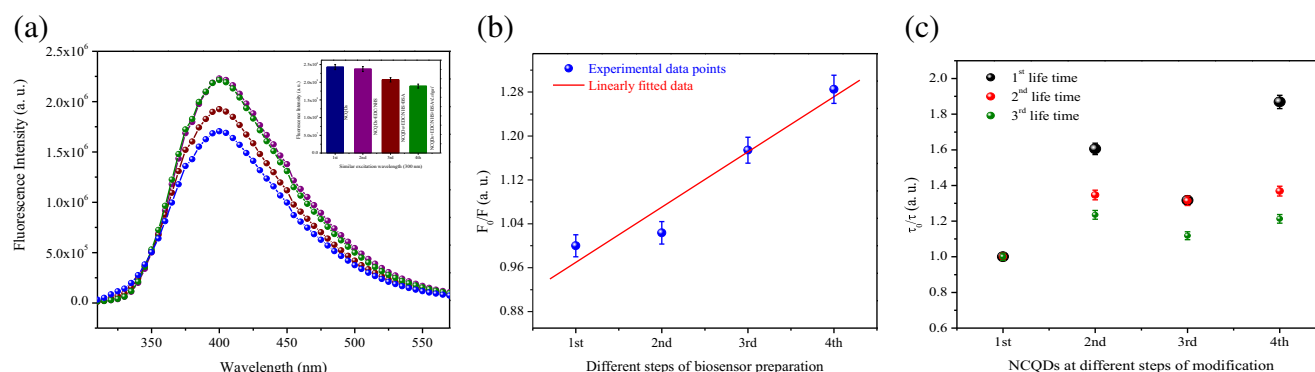


Fig. 1 (a) Fluorescence spectra of NCQDs (violet color), NCQDs/EDC/NHS (green color), NCQDs/EDC/NHS/BSA (red color) and NCQDs/EDC/NHS/BSA/*Lsbgal* (blue color) excited at 300 nm (b) Fluorescence quenching efficiency of the modified NCQDs excited at similar

wavelength 300 nm by blue color and (c) τ_0/τ for three life times at similar excitation wavelength 369 nm shown by black, red and green color

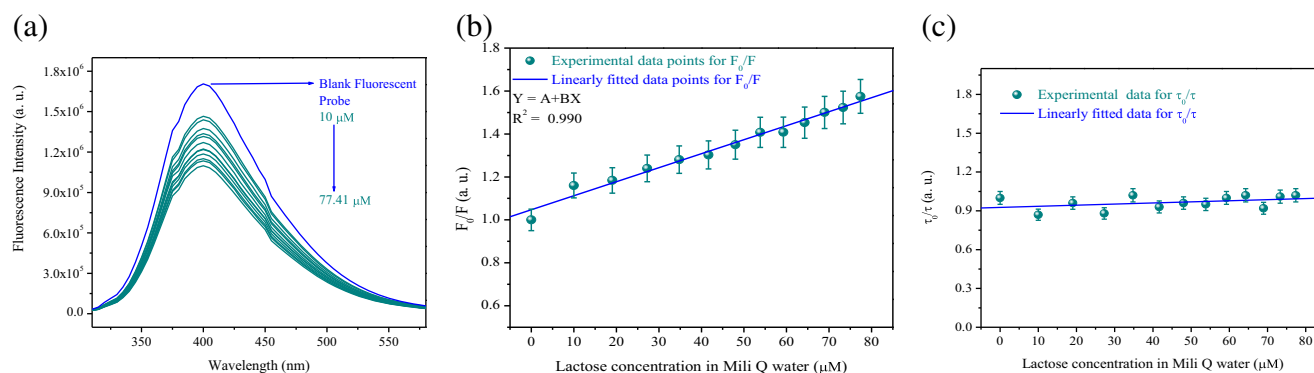


Fig. 2 (a) Fluorescence emission spectra of fluorescent bioprobe [blue color] and after the addition of various concentration of lactose (10, 19.04, 27.27, 34.78, 41.66, 48.00, 53.84, 59.25, 64.28, 68.96, 73.33, 77.41 μM) prepared in phosphate buffer 25 mM at pH 6.7 [blue color]

spectrum was recorded at 300 nm excitation wavelength and continued at similar excitation wavelength 300 nm by adding diverse amounts of lactose [10–77.41 μM] prepared in Milli Q water. The fluorescence spectrum of bioprobe with different concentration of lactose is depicted in Fig. 2(a). The UV-visible absorption spectrum of bioprobe with different concentration of lactose (10–77.41 μM) is depicted in Fig. S5 (supplementary material) which showed that fluorescence intensity of the probe declines on adding diverse amounts of lactose [from Fig. 2(a)]. The linear response of quenching efficiency (F_0/F) versus concentrations of lactose has been shown in Fig. 2(b). The linear nature of the graph indicates towards static quenching. In order to verify this mechanism of reduction in the fluorescence intensity, its time domain fluorescence measurements were done with excitation at 369 nm [Table S1 & Fig. S6]. The lifetimes of probe and probe with lactose at different concentrations were observed to be almost unaffected which confirmed that the nature of quenching is static. At each step, decay lifetime of probe and probe with different concentrations of lactose [10–77.41 μM] have been recorded and a graph is shown by red color for τ_0/τ [where τ_0 is decay lifetime for probe and τ is decay lifetime for each step of addition different amount of lactose, excited at 369 nm, Fig. 2(c)]. This parameter is found to be more or less constant which suggest the presence of static quenching on adding different amount of lactose.

The linear part graph shown in (Fig. 2b) upto 77.41 μM was fitted and presented in Fig. 2(c). The linear equation

(b) The dependence of F_0/F on the concentration of lactose prepared in phosphate buffer (25 mM, pH 6.7) within the range of [10–77.41 μM] (c) Dependency of τ_0/τ on the concentration of lactose prepared in Milli Q water in the range of [10–77.41 μM]

giving best fit has intercept (1.0471 ± 0.01451) and slope (0.0065 ± 0.0002) μM^{-1} . The values of linear correlation coefficient [R^2] and standard deviation [SD] for the close-fitting linear curve are 0.990 and 0.024 Fig. 2(c). The limit of detection [LOD] was estimated to be 11.36 μM [32]. It has been calculated based on three times the standard deviation [SD] rule [$\text{LOD} = 3 \times \text{SD}/m$], where m is the slope of the linearly fitted graph [15, 32]. The sensitivity was observed to be equal to (0.0065 ± 0.0002) μM^{-1} which has been determined by the slope of the linearly fitted graph Fig. 2(c). The parameters observed with fluorescent bioprobe were compared with other detection methods and represented in the form of Table 1.

Reproducibility, Selectivity and Stability of the Fluorescent Biosensor

In order to verify the reproducibility, four fluorescent biosensors were prepared under similar conditions and treated at 58 °C for 10 min. Fluorescence spectra were recorded at same excitation wavelength at 300 nm. The fluorescence intensity of four sensors is shown by blue bar in Fig. 3(a) and that of the fluorescent biosensor treated at 58 °C by green bar in Fig. 3(a). Thermal stability was monitored at each step of probe preparation and data for first three steps are included in the supplementary material in Fig. S7. The fluorescence intensity of these two sets of the four probes was same with average error $\sim 2.5\%$ Fig. 3(a).

Table 1 Sensing characteristic of proposed fluorescent bioprobe NCQD/EDC/NHS/BSA/*Lsbgal* are compared with some reports in literature

Detection techniques for lactose detection	Detection Range	Detection sensitivity limit	Stability	References
Conductometric biosensor	60–800 μM		5 days (operational stability)	[11]
Amperometric biosensor	44–339 μM	–	3 days without stabilizers	[33]
Amperometric biosensor	200–20,000 μM			[34]
Biosensor based on AC Electrophoresis	Linear range upto 14 mM	–	–	[35]
Fluorescent bioprobe biosensor	10.00–77.41 μM	11.36 μM	4 weeks	Present work

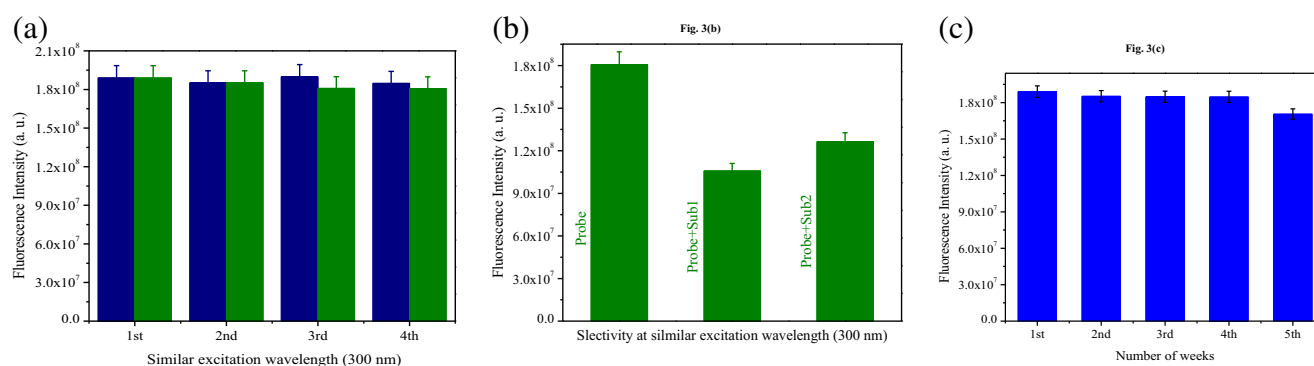


Fig. 3 (a) Fluorescence Intensity of the four fluorescent bioprobes (navy blue color bar)/fluorescent bio-probes (treated at 58 °C, green color bar) prepared under similar conditions and excited at similar wavelength 300 nm (b) Fluorescence Intensity of the fluorescent bioprobe and its complex with sub 1(lactose) and sub 2 (4-methyl-umbelliferyl- α -D-

galactopyranoside) each having concentration 85.71 μ M at $\lambda_{\text{exc}} = 300$ nm (c) Variation of fluorescence response of a fluorescent bioprobe with respect to time for stability determination of the probe at regular interval of one week excited at 300 nm wavelength

The assay was based on enzyme specificity for lactose. Therefore, high catalysis is expected. Fluorescence intensity of the probe (control) and probe with two different substrates were measured at same excitation wavelength 300 nm for lactose (85.71 μ M named as sub1) and 4-methylumbelliferyl- α -D-galactopyranoside (85.71 μ M named as sub2). The fluorescence response of the probes on adding same concentration of lactose and 4-methylumbelliferyl- α -D-galactopyranoside shown in Fig. 3(b). On adding same concentration of both substrates resulted in decrease of fluorescence intensity of the probes as shown in Fig. 3(b). It is clearly evident from graph that on adding 4-methylumbelliferyl- α -D-galactopyranoside (85.71 μ M) there is only 30.06% reduction in fluorescence intensity while addition of lactose (85.71 μ M) results in 41.44% decrease in PL intensity of the probe Fig. 3(b). Stability of the enzyme modified bioprobe was recorded at an equal interval once in a week upto five weeks by monitoring fluorescence spectra. Intensity decrease by $\sim$ 2.22 and 9.05% at the end of 4th and 5th weeks, respectively. The value of average error was below $\sim$ 2.5%, which has been shown by error bar in Fig. 3(c). This result showed good stability fluorescent bioprobes upto 4th week.

Conclusions

Thermally and optically stable biosensor to detect the trace amount of lactose was fabricated using fluorescence properties of NCQDs. The NCQDs were synthesized by hydrothermal process, COOH groups were activated by adding EDC/NHS and the non-specific sites were blocked by BSA. The *Lsbgal* that binds with lactose was covalently attached to the probe via EDC/NHS cross-linking chemistry. At every stage of probe preparation a heat treatment at 58 °C for 10 min was given to achieve maximum yield. The probe prepared in this way was mixed with lactose (10–77.41 μ M) and the reduction

in fluorescence intensity was noted. The quenching efficiency is linear with concentration that gives a detection range 10–77.41 μ M with detection limit 11.36 μ M. The minimum detection limit achieved in this biosensor is far more improved than the earlier proposed bioprobe. This bioprobe has additional advantage of being thermally as well as optically stable.

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Compliance with Ethical Standards

Conflict of Interests The authors declare no competing financial interest.

References

- Heyman MB (2006) Lactose intolerance in infants, children, and adolescents. *Pediatrics* 118(3):1279–1286
- Vesa TH, Marteau P, Korpela R (2000) Lactose intolerance. *J Am Coll Nutr* 19(sup2):165S–175S
- Dwevedi A, Singh AK, Singh DP, Srivastava ON, Kayastha AM (2009) Lactose nano-probe optimized using response surface methodology. *Biosens Bioelectron* 25(4):784–790
- Deng Y, Misselwitz B, Dai N, Fox M (2015) Lactose intolerance in adults: biological mechanism and dietary management. *Nutrients* 7(9):8020–8035
- Saha M, Parveen I, Shil BC, Saha SK, Banik RK, Majumder M, Salam MU, Islam AN (2016) Lactose intolerance and symptom pattern of lactose intolerance among healthy volunteers. *Euroasian J Hepatogastroenterol* 6(1):5–7

6. Kishore D, Talat M, Srivastava ON, Kayastha AM (2012) Immobilization of β -galactosidase onto functionalized graphene nano-sheets using response surface methodology and its analytical applications. *PLoS One* 7(7):e40708
7. Lynch JM, Barbano DM, Fleming JR (2007) Determination of the lactose content of fluid milk by spectrophotometric enzymatic analysis using weight additions and path length adjustment: collaborative study. *J AOAC Int* 90(1):196–216
8. Colinas C, Barrera I, Blanco CA (2006) A novel correlation for rapid lactose determination in milk by a cryoscopic technique. *J AOAC Int* 89(6):1581–1584
9. Geisser A, Hendrich T, Boehm G, Stahl B (2005) Separation of lactose from human milk oligosaccharides with simulated moving bed chromatography. *J Chromatogr A* 1092(1):17–23
10. Kucerova P, Komenska P, Tomkova H, Skopalova J, Bartak P (2017) Determination of lactose in milk products: a comparison of three-enzyme amperometric biosensor and gas chromatography/tandem mass spectrometry. *Monatsh Chem* 148(3):517–524
11. Marrakchi M, Dzyadevych SV, Lagarde F, Martelet C, Jaffrezic-Renault N (2008) Conductometric biosensor based on glucose oxidase and beta-galactosidase for specific lactose determination in milk. *Mat Sci Eng C-Bio S* 28(5–6):872–875
12. Soldatkin OO, Peshkova VM, Saiapina OY, Kucherenko IS, Dudchenko OY, Melnyk VG, Vasylenko OD, Semenycheva LM, Soldatkin AP, Dzyadevych SV (2013) Development of conductometric biosensor array for simultaneous determination of maltose, lactose, sucrose and glucose. *Talanta* 115:200–207
13. Pfeiffer D, Ralis EV, Makower A, Scheller FW (1990) Amperometric Bienenzyme based biosensor for the detection of lactose - characterization and application. *J Chem Technol Biotechnol* 49(3):255–265
14. Singh AK, Prakash P, Singh R, Nandy N, Firdaus Z, Bansal M, Singh RK, Srivastava A, Roy JK, Mishra B, Singh RK (2017) Curcumin quantum dots mediated degradation of bacterial biofilms. *Front Microbiol* 8:1517
15. Singh R, Kashayap S, Singh V, Kayastha AM, Mishra H, Saxena PS, Srivastava A, Singh RK (2018) QPRTase modified N-doped carbon quantum dots: a fluorescent bioprobe for selective detection of neurotoxin quinolinic acid in human serum. *Biosens Bioelectron* 101:103–109
16. Miao P, Tang Y, Han K, Wang B (2015) Facile synthesis of carbon nanodots from ethanol and their application in ferric(III) ion assay. *J Mater Chem A* 3(29):15068–15073
17. Dai H, Shi Y, Wang Y, Sun Y, Hu J, Ni P, Li Z (2014) A carbon dot based biosensor for melamine detection by fluorescence resonance energy transfer. *Sensors Actuators B Chem* 202:201–208
18. Jiang G, Jiang T, Zhou H, Yao J, Kong X (2015) Preparation of N-doped carbon quantum dots for highly sensitive detection of dopamine by an electrochemical method. *RSC Adv* 5(12):9064–9068
19. Xu Q, Pu P, Zhao J, Dong C, Gao C, Chen Y, Chen J, Liu Y, Zhou H (2015) Preparation of highly photoluminescent sulfur-doped carbon dots for Fe(III) detection. *J Mater Chem A* 3(2):542–546
20. Baker SN, Baker GA (2010) Luminescent carbon nanodots: emergent nanolights. *Angew Chem Int Ed Engl* 49(38):6726–6744
21. Dong Y, Li G, Zhou N, Wang R, Chi Y, Chen G (2012) Graphene quantum dot as a green and facile sensor for free chlorine in drinking water. *Anal Chem* 84(19):8378–8382
22. Karfa P, Roy E, Patra S, Kumar S, Tarafdar A, Madhuri R, Sharma PK (2015) Amino acid derived highly luminescent, heteroatom-doped carbon dots for label-free detection of Cd²⁺/Fe³⁺, cell imaging and enhanced antibacterial activity. *RSC Adv* 5(72):58141–58153
23. Liao J, Cheng Z, Zhou L (2016) Nitrogen-doping enhanced fluorescent carbon dots: green synthesis and their applications for bioimaging and label-free detection of Au³⁺ ions. *ACS Sustain Chem Eng* 4(6):3053–3061
24. Yu J, Xu C, Tian Z, Lin Y, Shi Z (2016) Facilely synthesized N-doped carbon quantum dots with high fluorescent yield for sensing Fe³⁺. *New J Chem* 40(3):2083–2088
25. Li L, Li L, Wang C, Liu K, Zhu R, Qiang H, Lin Y (2015) Synthesis of nitrogen-doped and amino acid-functionalized graphene quantum dots from glycine, and their application to the fluorometric determination of ferric ion. *Microchim Acta* 182(3):763–770
26. Li Q, Ohulchanskyy TY, Liu R, Koynov K, Wu D, Best A, Kumar R, Bonoiu A, Prasad PN (2010) Photoluminescent carbon dots as biocompatible Nanoprobes for targeting Cancer cells in vitro. *J Phys Chem C* 114(28):12062–12068
27. Zhang R, Chen W (2014) Nitrogen-doped carbon quantum dots: facile synthesis and application as a “turn-off” fluorescent probe for detection of Hg²⁺ ions. *Biosens Bioelectron* 55:83–90
28. Yadav A, Pandey SK, Agrawal DC, Mishra H, Srivastava A, Kayastha AM (2019) Carbon nanotubes molybdenum disulfide 3D nanocomposite as novel nanoscaffolds to immobilize Lens culinaris β -galactosidase (Lsbgal): robust stability, reusability, and effective bioconversion of lactose in whey. *Food Chem* 297:125005
29. Sarkar P, Nicholson PS (1996) Electrophoretic deposition (EPD): mechanisms, kinetics, and application to ceramics. *J Am Ceram Soc* 79(8):1987–2002
30. Li S, Li Y, Cao J, Zhu J, Fan L, Li X (2014) Sulfur-doped graphene quantum dots as a novel fluorescent probe for highly selective and sensitive detection of Fe³⁺. *Anal Chem* 86(20):10201–10207
31. Dong Y, Chen C, Lin J, Zhou N, Chi Y, Chen G (2013) Electrochemiluminescence emission from carbon quantum dot-sulfite coreactant system. *Carbon* 56:12–17
32. Singh R, Rai SK, Tiwari MK, Mishra A, Tewari AK, Mishra PC, Singh RK (2017) An excellent stable fluorescent probe: selective and sensitive detection of trace amounts of Hg²⁺ ions in natural source of water. *Chem Phys Lett* 676:39–45
33. Tkac J, Sturdik E, Gemeiner P (2000) Novel glucose non-interference biosensor for lactose detection based on galactose oxidase-peroxidase with and without co-immobilised [small beta]-galactosidase. *Analyst* 125(7):1285–1289
34. Rajendran V, Irudayaraj J (2002) Detection of glucose, galactose, and lactose in Milk with a microdialysis-coupled flow injection Amperometric sensor. *J Dairy Sci* 85(6):1357–1361
35. Ammam M, Franssaer J (2010) Two-enzyme lactose biosensor based on beta-galactosidase and glucose oxidase deposited by AC-electrophoresis: characteristics and performance for lactose determination in milk. *Sensor Actuat B-Chem* 148(2):583–589