

Synthesis of Maltitol-derived fluorescent carbon dots for selective detection of thyroxine in biological and pharmaceutical samples

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ABSTRACT

In this work, blue-emissive maltitol-derived carbon dots (ML-CDs) with an average particle size of ~ 2.99 nm were successfully synthesized via a hydrothermal method. These ML-CDs were exhibited a strong and stable fluorescent signal owing to their rich surface functionalities and hydrophilic character. The prepared ML-CDs were driven as a very sensitive and selective fluorescent sensor for thyroxine (T4) detection, with an outstanding detection limit of as 0.564 nM. Significantly, this sensor protocol exhibited low minimal interference with similar drugs to T4, while demonstrating high selectivity in biologically relevant environments. The ML-CDs were successfully shown for practical use in real biological samples and pharmaceutical samples. The recovery ranges of 95.3 to 100.5% confirm the high accuracy and good precision (0.35 to 3.51% RSD) of the proposed method for T4 measurements. This cost-effective and eco-friendly detection technique opens up new opportunities for rapid T4 monitoring in complex biological and environmental matrices. It also highlights the massive potential of CDs through from ML as next-generation fluorescent probes for detection of T4 in biological and pharmaceutical samples.

1. Introduction

Thyroid hormones primarily thyroxine (T4) and triiodothyronine (T3) are fundamental regulators of metabolism, growth, and especially brain development. T4 circulates in the bloodstream predominantly bound to T4-binding globulin. Although ingestion of small doses of L-thyroxine (3 mg / day) is usually asymptomatic, excessive doses can lead to serious outcomes including multi organ failure and coma. Thyroid related disorders have become increase common worldwide, particularly among women and they include structural abnormalities (goiter, thyroid nodules, and thyroid cancer) and functional (hypothyroidism and hyperthyroidism). Adequate iodine intake remains essential for hormone synthesis, and congenital hypothyroidism affects around 1 in 3000 to 4000 new-borns continues to poses clinical challenges [1–3]. Because of this growing global burden, developing a rapid and sensitive platform for detecting T4 is of significant importance. To achieve such sensitive detection of T4 and T3, fluorescence-based techniques offer a powerful analytical foundation due to their exceptional sensitivity, cost-

effectiveness, and rapid signal generation.

The fluorescence, first described by George G. Stokes in 1852, refers to the emission of light from a material after it absorbs electromagnetic energy, generally in the ultraviolet (UV) region. This phenomenon, known as the Stokes shift, made fluorescence a cornerstone in biological imaging and biochemical sensing due to its high sensitivity and rapid signal response. Traditional fluorescence systems relied on water-soluble organic dyes that delivered specificity and industrial expanded significantly after the 1994 Inter-Society Color Council (ISCC) Williamsburg Conference on Colorimetry of Fluorescent Materials [4–6]. The success of fluorescence sensing has encouraged the development of new fluorescent nanomaterials (NMs) with enhances stability and biocompatibility. Carbon dots (CDs), nanometer scale carbon-based materials typically smaller than 2–20 nm were found by accident in 2004 during nanotubes purification. Since, they have gained tremendous attention due to their multi color fluorescent, water solubility, biocompatibility, and simple synthesis routes [7,8]. CDs exhibit excitation-dependent emission and tunable photoluminescence arising

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from π -conjugated domains and surface defects states. Their properties can be further enhanced by heteroatoms, making them attractive for sensing, catalysis, bioimaging, and nanomedicine [9–12].

CDs have been synthesized using a range of variety of organic precursors and ligands, including small organic acids (citric acid, ascorbic acid), amino acids, polymers, sugars, plant waste, and biomass-derived molecules. The chemical nature of these precursors plays a crucial role in determining the surface states, functional groups, hydrophilicity, and photoluminescence behavior of the resulting CDs. For example amino acids-derived CDs provide nitrogen-containing surface functionalisation that quantum yield and photo bleaching, while polymer-, plant waste- and biomass-based precursors introduce complex surface defect structure and excitation depended emission. Recently, sugar- and polyol-based precursors have attracted increasing attention owing to their low toxicity, renewable origin, and ability to generate oxygen rich surface functionalization favourable for aqueous sensing applications. However, many reported synthesis strategies require additional passivating and heteroatom doping agents to achieve desirable fluorescence properties, increasing synthetic complexity and limiting scalability. Moreover, several previous reported CDs-based sensing platform demonstrate sensing mechanism complex biological matrices. In the context, maltitol (ML, $C_{12}H_{24}O_{11}$), a non-reducing disaccharide polyol, represent an attractive and underexplored green precursor for CDs synthesis. Due to its abundant hydroxyl groups, chemical stability, high aqueous compatibility, non-cariogenic nature, and sweetness level comparable to 75–90% of sucrose carbon source capable of generating highly hydrophilic and surface functionalized CDs without doping agents. It is safe at low doses, resistant to the fermentation of oral bacteria, and suitable for diabetic-friendly foods due to its low glycemic response [13,14].

In this study, CDs were synthesized from ML via a simple hydrothermal process, yielding stable and highly fluorescent CDs. The ML provides a uniform carbon frameworks enriched hydroxyls and carboxyls groups, resulting in strong photoluminescence and excellent water solubility. These ML-derived carbon dots (ML-CDs) exhibit low cytotoxicity, strong binding affinity toward T4 molecules, enabling their use as an effective fluorescent probe. Their structural and optical characteristics uniquely supports sensitive fluorescent quenching-based detection of T4. Compared with previous reported colorimetric and fluorescent platforms, ML-CDs demonstrate superior sensitivity and selectivity for T4 due to exchanged fluorescence quenching. Their selective interaction enables rapid and label-free T4 detection using simple and cost-effective setup. Therefore, ML-CDs represent a promising sensing material for biological and environmental applications requiring accurate T4 determination.

2. Experimental details

2.1. Solvents and chemicals

Maltitol, thyroxine, sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), monosodium phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), L-cysteine (L-Cys), L-histidine (L-His), citric acid (CA), uric acid (UA), creatinine (Creat), gallic acid (GA), sucrose (Suc), tannic acid (TA), guanine (Gua), adenine (Ade), dopamine (DA), thiamine (B1), uracil (Ura), L-proline (L-Pro), L-ascorbic acid (AA), L-threonine (L-Thr), potassium iodide, acetic acid, potassium chloride were purchased from sigma-Aldrich and used without further purification. NaH_2PO_4 and Na_2HPO_4 are used to prepare pH 1 to pH 12 (0.2 M) phosphate buffer solutions, and the pH was adjusted using NaOH and H_2SO_4 . All the chemicals are analytical grade and solutions were prepared with Millipore water.

2.2. Instruments and characterization

A Perkin Elmer (lambda-25) UV–visible (UV–Vis) spectrophotometer

was used for the absorption spectral measurements. The identification of ML-CDs and ML functional groups were studied using an Fourier transform infrared spectroscopy (FT-IR) JASCO-6300 spectrometer in the spectrum region 400 to 4000 cm^{-1} . Transmission electron microscopy (TEM) images were obtained using a JEOL-2100 (Peabody, Massachusetts, USA) at an acceleration voltage at 200 kV. The X-ray diffraction (XRD)-Rigaku MiniFlex 6th generation unit ($\lambda = 1.5406$) CuK, Ni-filtered radiation was used for measuring the XRD pattern. Dry powder samples had been produced were employed in X-ray powder diffraction patterns. The emission spectral studies and quantum yield were performed by spectrofluorometer FluoroMax Plus (HORIBA's). The measurement of the fluorescence spectrum was done at room temperature, using a scan rate of 200 nm / min, a slit width of 2 nm, and wavelengths between 350 and 650 nm. The Surface functional groups of ML-CDs were characterized by X-ray photoelectron spectroscopy (XPS) using ULVAC-PHI instrument (Japan). High-performance liquid chromatography (HPLC) with photodiode array (PDA) and fluorescence detector was performed using LC-2010CHT HPLC system (Shimadzu, Japan, 2013).

2.3. Synthesis procedure of ML-CDs

The ML-CDs were synthesized hydrothermally by dissolving 0.5 g of ML in 15 mL of Millipore water. The completely dispersed precursor was transferred to a Teflon autoclave with a stainless steel cover. The hydrothermal autoclave was then annealed for 10 h at 180 °C (4 °C / min) in a muffle furnace, after which it was allowed to cooled to room temperature. The obtained ML-CDs solution showed brownish-yellow color. Further, it was centrifuged to eliminate unreacted residues and further purified using syringe filters (diameter: 0.25 μm). The final filtered ML-CDs solution were stored at 4 °C for further use. The hydrothermal technique is a reaction process occurs in water under high pressures and temperatures inside of a sealed Teflon-lined autoclave. Under these condition, the elevated pressures and temperature act as drawing forces that convert small molecules of organic molecules into nano-sized carbon structures. During the forming ML-CDs from organic precursor as ML, the material typically undergoes four stages such as (Scheme 1).

2.4. Spectrofluorometric detection of T4 in an aqueous medium

For spectrofluorometric analysis, 0.3 mL of ML-C dots and 2.3 mL of phosphate buffer solution (pH 7) were transferred to the quartz cuvette, and fluorescence spectra were recorded from 360 to 650 nm at an excitation wavelength 350 nm. Different concentration of T4 were prepared in a 10 mL centrifuge tube, and various amount of T4 were subsequently added to the ML-CD solution at room temperature.

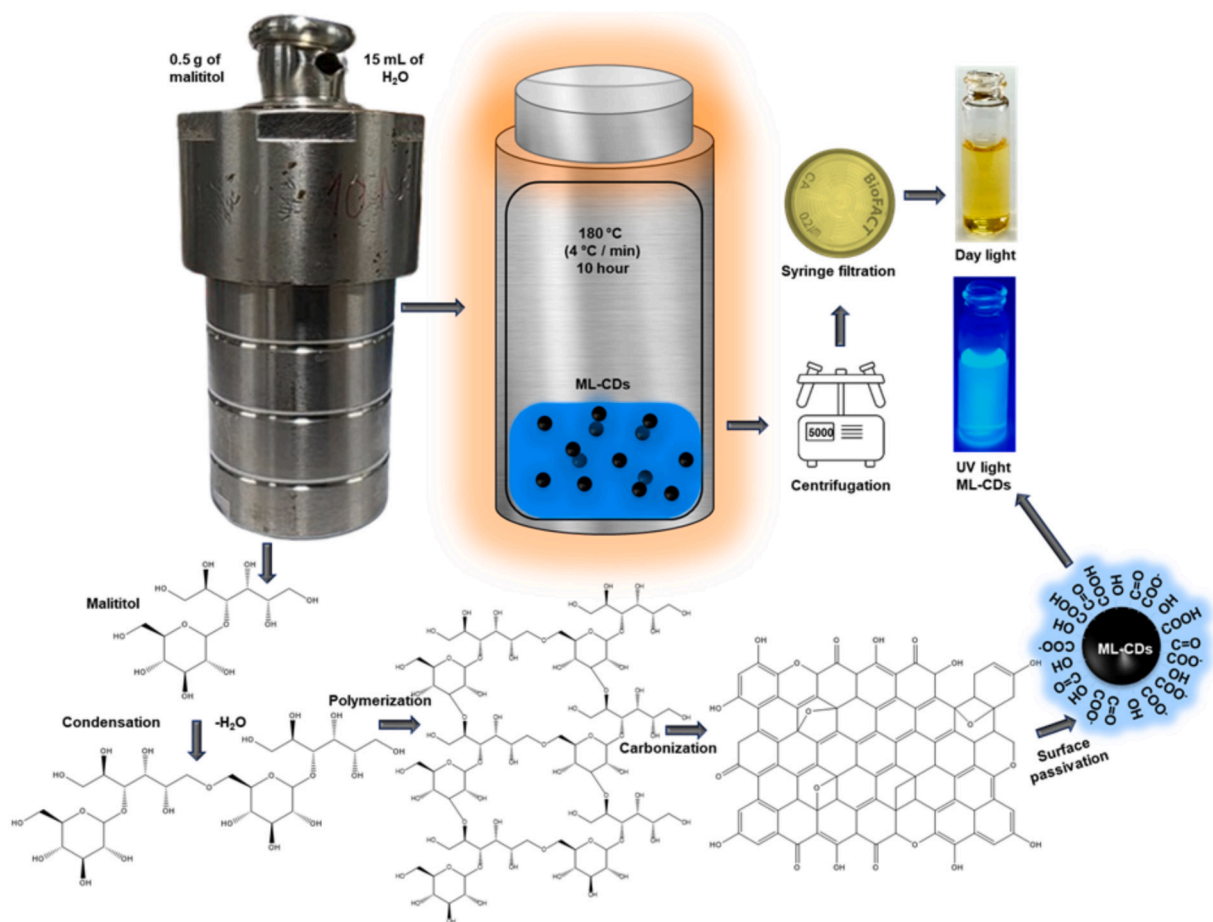
2.5. Detection of T4 in real samples

Blood serum sample and T4 sodium tablets were obtained from a nearby health center and used without further purification. Urine and saliva samples were collected from the author and filtered using Whatman filter paper No. 1. Known volumes of T4 were then spiked into the ML-CDs solution, and the fluorescence response was recorded using a spectrofluorometric technique.

3. Results and discussion

3.1. Absorbance and emission behavior of ML-CDs

Fig. 1 A shows the absorbance and emission spectra of ML-CDs. The synthesized ML-CDs display absorption in the optimal wavelengths were 221, 259 nm and 356 nm, for the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition may be correlated to the peaks centered at 221, 259 nm and 356 nm (curve a). The emission intensity of ML-CDs observed at 438 nm (curve b). Fig. 1 A inset i and ii shows the photographs of day and UV-light of ML-CDs. The



Scheme 1. Schematic representation for hydrothermal synthesis and proposed formation mechanism of ML-CDs.

synthesized ML-CDs fluorescent color determined through CIE chromaticity coordinate. The fluorescence was classified as to the blue fluorescence region based on $(x, y) = (0.17, 0.18)$ (Fig. 1B) [7,8]. Fig. 1C presents the 3D fluorescence spectra of ML-CDs obtained at different excitation wavelengths. The fluorescence intensity increases as the excitation wavelength ranges from 310 nm to 350 nm. However, beyond 360 nm of excitation, the fluorescence intensity begins to decrease. The ML-CDs exhibit higher emission intensity at 438 nm when excited at 350 nm. Fig. S1 shows normalized emission spectra of ML-CDs obtained with different excitation wavelengths from 310 to 450 nm. Fig. S1 revealed that the emission intensity undergoes a red shifted, when the excitation of 310 to 450 nm.

The fluorescence behavior of CDs is primarily attributed to both intrinsic state emission (arising from quantum size effect / zigzag sites, and electron-hole recombination) and defect state emission (resulting from surface energy traps). The fluorescence color of the CDs indicates that the intrinsic state emission produce blue light (longer wavelength) and defect state emission results in green (shorter wavelength). The variable emission behavior can also be attributed to the abundance of carboxyl functional groups and the presence of various emissive sites layer. Despite extensive, the precise mechanism of photoluminescence in CDs remain uncertain [15–17].

3.2. Vibrational spectral analysis of ML and ML-CDs

Fig. 1D shows the XRD pattern, including the actual and smoothed spectra of ML-CDs. The broad diffraction peaks observed at 20.18° and 35.84° correspond to the (002) and (100) planes, respectively. Notably, no sharp peaks were detected in the XRD data. Based on these observed peaks, it can be concluded that the ML-CDs exhibit amorphous structure,

which is in good agreement with previously published report. The d-spacing value (the spacing between the layer of atom) were calculated using the Bragg Equation, based on the position of the (0 0 2) and (1 0 0) reflection peaks. The calculated interlayer distance were approximately 0.44 nm for the (0 0 2) and 0.25 nm for the (1 0 0) plane [18,19].

The FT-IR spectra of the ML and ML-CDs are shown in Fig. 2A (curve a and b). The FT-IR spectrum of ML reveals the presence of primary and secondary alcoholic -OH groups, with peaks observed at 3383 to 3262 cm^{-1} , and the stretching vibration of -CH groups at 2964 cm^{-1} [20–22]. Additionally, the -CH₂- deformation and -CH rocking frequencies are found at 1428 and 1346 cm^{-1} , respectively [20,22]. The stretching vibration of C-O-C ether group in the glucose unit) and the -C-OH mode were detected at 1129 and 1001 cm^{-1} (Fig. 2A; curve a) [23,24]. The FT-IR spectra of ML-CDs exhibits all the characteristic peaks of ML. However, two new peaks are present at 1703 and 1662 cm^{-1} corresponding to the -C=O double bonds of the carboxylic group [23,24]. A broad band at 3301 cm^{-1} represented the alcoholic -OH groups in aqueous solution (Fig. 2A; curve b) [21]. The XRD and FT-IR analyses reveal that ML-CDs exhibit an amorphous structure, with XRD showing broad diffraction peaks corresponding to the (0 0 2) and (1 0 0) planes, and FT-IR spectra indicating the presence of functional groups such as carboxylic (-C=O), alcohol (-C-OH), and hydroxyl (-OH), with new peaks observed at 1703 and 1662 cm^{-1} confirming the successful formation of ML-CDs from ML.

3.3. XPS spectrum, TEM images, and Raman spectrum of ML-CDs

The elemental composition of the ML-CDs was further characterized by XPS. The survey spectrum displayed two dominant peaks at 530 eV (O_{1s}) and 283 eV (C_{1s}), corresponding to atomic concentration of 30% and 70%, respectively (Fig. 2B). These results indicate that the ML-CDs

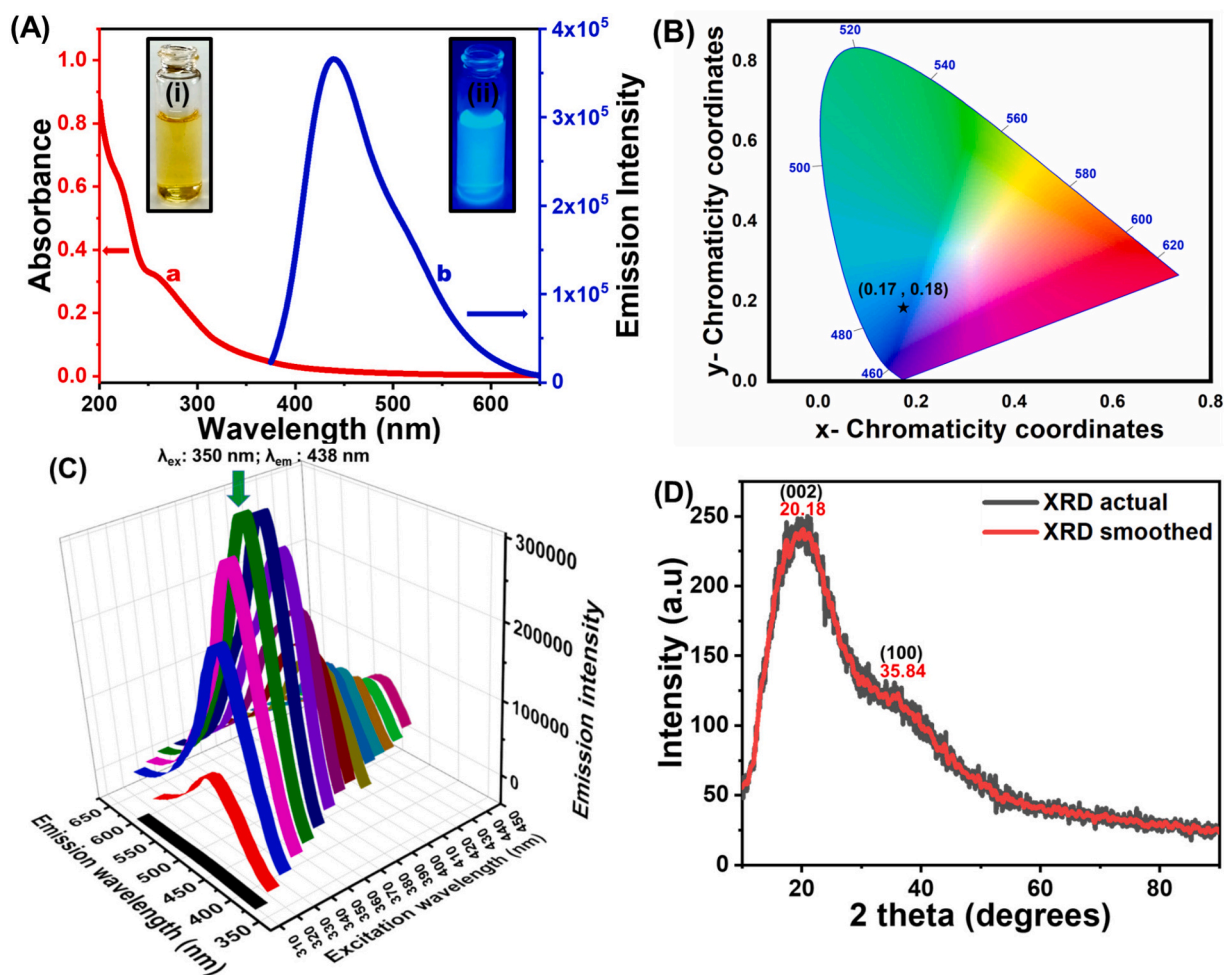


Fig. 1. (A) (a) Absorption and (b) emission spectrum of ML-CDs; inset (A): photographs of ML-CDs under (i) day light and (ii) UV light. (B) Chromaticity diagram of ML-CDs. (C) Fluorescence 3D spectra of ML-CDs obtained with different excitation wavelengths from 310 to 450 nm. (D) XRD pattern of ML-CDs.

are composed primarily of carbon and oxygen. The high-resolution C_{1s} spectrum was deconvoluted into four components assigned to O-C=O, C=O, C-O, and C-C at 289.1, 288.7, 286.5, and 284.8 eV respectively (Fig. 2C). The high-resolution O_{1s} spectrum showed four peaks attributed to O-C=O, C-O-C, C-O-H, and C=O at 535, 533.7, 532.6, and 531.7 eV, respectively (Fig. 2D). Overall, the FT-IR and XPS results are consistent, confirming that the ML-CDs are dominated by carbon and oxygen abundant surface functionalities, including C-OH, C=O, C-O, C-C, and C-O-C groups [25].

Fig. 3A and B display TEM images of ML-CDs at magnification of 10 nm and 5 nm, respectively. The particle size histogram for the ML-CDs shown in Fig. 3C. The synthesized ML-CDs were uniformly dispersed on the carbon grid, exhibiting an almost spherical morphology. The average particle size of the ML-CDs, calculated from the particle size histogram in Fig. 3C, was found to be ~ 2.99 nm. Image J software was used for particle size analysis. Clear lattice fringes were clearly observed in the HR-TEM image of single ML-CD, and the lattice spacing was determined to be 0.211 nm (Fig. 3D) [19,23].

Fig. S2 presents the Raman spectrum of ML-CDs, showing both the actual and smoothed spectra. The spectrum exhibits a broad peak at 1542 cm^{-1} and a shoulder peak at lower wavenumber about 1340 cm^{-1} , corresponding to the G and D bands, respectively. The G band is attributed to the vibrational mode (E_{2g}) of carbon (sp^2), while the D band (sp^3) corresponds to the (A_{1g}) symmetry and arises from phonons near the k-zone boundary. The calculated intensity ratio (I_D / I_G) of the D and G bands for ML-CDs is 1.18, confirming the presence of sp^3 carbon lattice site and surface defects in the ML-CDs. The TEM and Raman

analyses indicate that the synthesized ML-CDs have a uniform spherical morphology with an average particle size of 2.99 nm, and exhibit surface defects, as evidenced by an I_D / I_G ratio of 1.11 [26,27].

3.4. Effect of pH and time

A 0.2 M phosphate buffer system covering pH 1–12 in was prepared to evaluate the pH-dependent fluorescence behavior of ML-CDs (Fig. S3 and S4). The ML-CDs exhibited strong emission in the acidic to neutral range (pH 1–7), followed by a marked decrease in fluorescence intensity under alkaline conditions (pH 8–12). The enhanced fluorescence at lower pH values is attributed to the higher net surface charge and improved hydrophilicity of the ML-CDs, which promote colloidal stability and suppress non-radiative decay pathways. In contrast, above pH 7, deprotonation of functional groups and the consequent reduction in surface charge likely induce particle aggregation and fluorescence quenching, resulting in decreased emission intensity.

The incubation study of the ML-CDs in the presence 0.16 μM of T4 was carried out by monitoring the fluorescence intensity over a time interval of 1–90 min (Fig. S5). The ML-CDs-T4 mixture exhibited an increase in fluorescence intensity within the initial min, with noticeable enhancement from 1 to 3 min. The fluorescence continued to rise gradually until 4 min, after which it remained essentially constant throughout the remaining incubation period (4–90 min). These results indicate that the interaction between ML-CDs and T4 reaches equilibrium within the 4 min of incubation (Fig. S5). The ML-CDs exhibited maximum fluorescence under acidic-neutral pH (1–7) and showed rapid

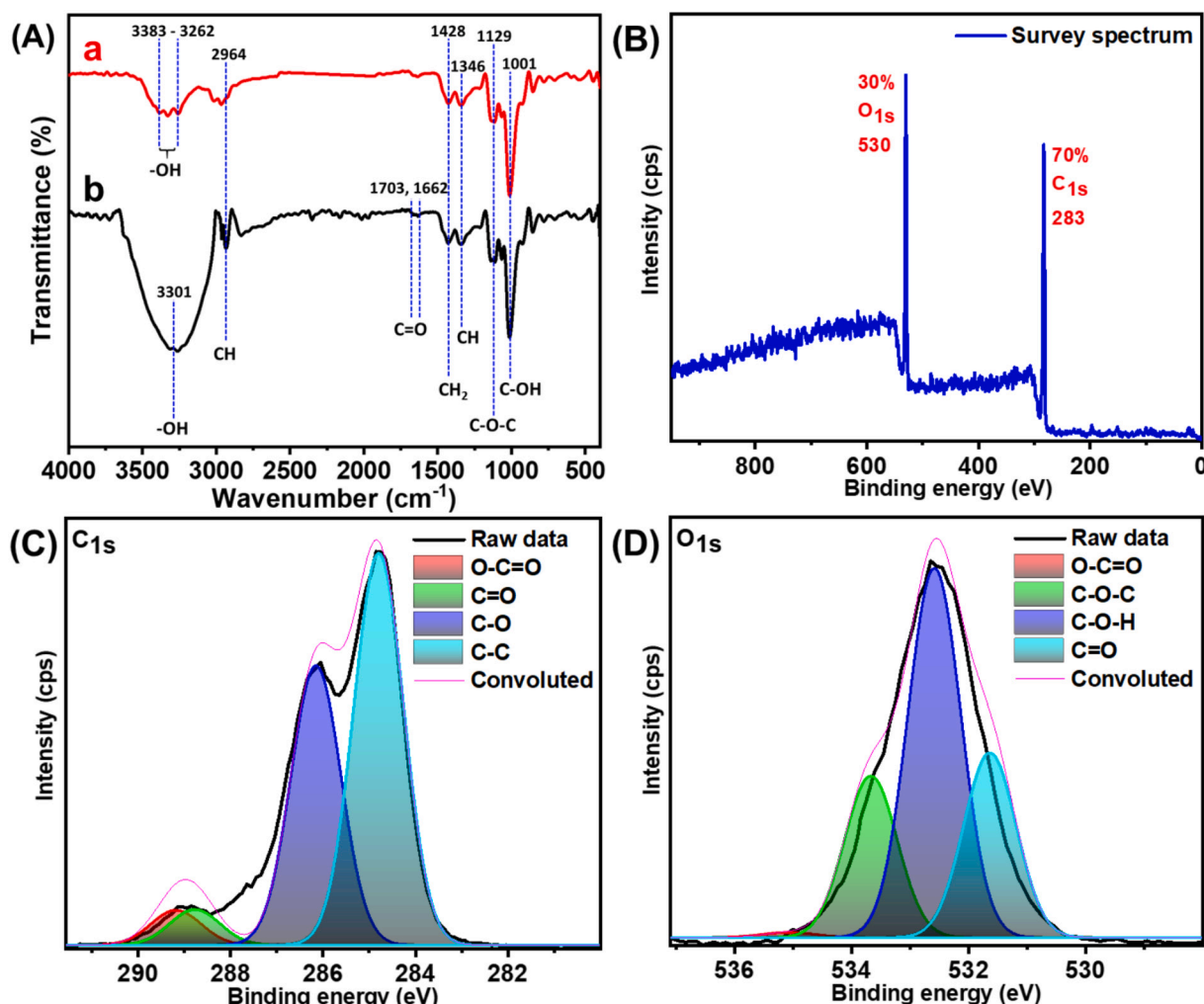


Fig. 2. (A) FT-IR spectra of (a) ML and (b) ML-CDs. (B) XPS survey spectrum of ML-CDs. High resolution spectra of (C) C_{1s} and (D) O_{1s}.

binding kinetic with T4, reaching fluorescence equilibrium within 4 min. Therefore, all further studies were carried out at pH 7 with an incubation time of 4 min.

3.5. Spectrofluorometric determination of T4 using ML-CDs

Fig. 4A shows the emission spectra of ML-CDs, which was measured using spectrofluorometric determination of T4. The emission spectrum of ML-CDs in the absence of T4 is shown in Fig. 4A (curve a), exhibiting a maximum fluorescence intensity at 438 nm. Upon the addition of 0.384 μ M T4, a pronounced decrease in fluorescence intensity at 438 nm was observed (curve b). With increasing T4 concentration from 1.15 to 11.3 μ M, a progressive and concentration-dependent quenching of the ML-CDs fluorescence occurred (curves c-n). Further increases in T4 concentration to 14.9 and 18.5 μ M did not cause additional quenching, indicating saturation of the interaction between T4 and ML-CDs (curves o and p). The inset photographs in Fig. 4A (i and ii) show ML-CDs in the absence and presence of 18.5 μ M T4 under 365 nm UV illumination, confirming the fluorescence quenching behavior. Quantitative determination of T4 was achieved by correlating the change in fluorescence intensity with T4 concentration. A linear calibration curve was obtained in the concentration range of 0.384–11.3 μ M (Fig. 4B). Overall, the limit of detection (LOD) of 0.564 nM was calculated using $3S / m$, accompanied by excellent calibration linearity ($R^2 = 0.9974$) [28–30].

3.6. Absorption spectral study of ML and ML-CDs in the presence of T4

Fig. S6 presents the UV–Vis absorbance spectra of ML alone and ML in the presence of increasing concentrations of T4, ranging from 1.92 to 18.9 μ M. The absorption spectrum of ML alone exhibits characteristic absorption bands at 217, 274, and 313 nm (Fig. S6; curve a). Upon gradual addition of T4 (1.92–18.9 μ M), a slight and consistent enhancement in absorbance intensity was observed without any noticeable shift in peak position, suggesting a weak association between ML and T4, along with partial spectral overlap from T4 absorption (Fig. S6; curve b-k). Fig. S7 shows the UV–Vis spectrum of T4 (1mM), exhibiting a prominent absorption maximum at 277 nm. Fig. S8 shows the UV–Vis absorbance spectra of ML-CDs in the absence and presence of T4 (1.92–18.9 μ M). ML-CDs alone exhibits characteristic absorption bands at 221, 259, and 356 nm (Fig. S8; curve a). Upon gradual addition of T4, a pronounced hyperchromic effect was observed without any noticeable shift in wavelength (Fig. S8; curve b-k). This behavior indicates stronger interaction of T4 on the ML-CDs surface through hydrogen bonding and π - π interaction, leading to enhanced absorbance while maintaining the same electronic transition energies.

3.7. TEM images of ML-CDs in the presence of T4

Fig. 5 presents TEM images of ML-CDs in the presence of 18.5 μ M of T4 (ML-CDs / T4). Compared with the TEM images of pristine ML-CDs (Fig. 3), the TEM images of the ML-CDs / T4 system exhibits a

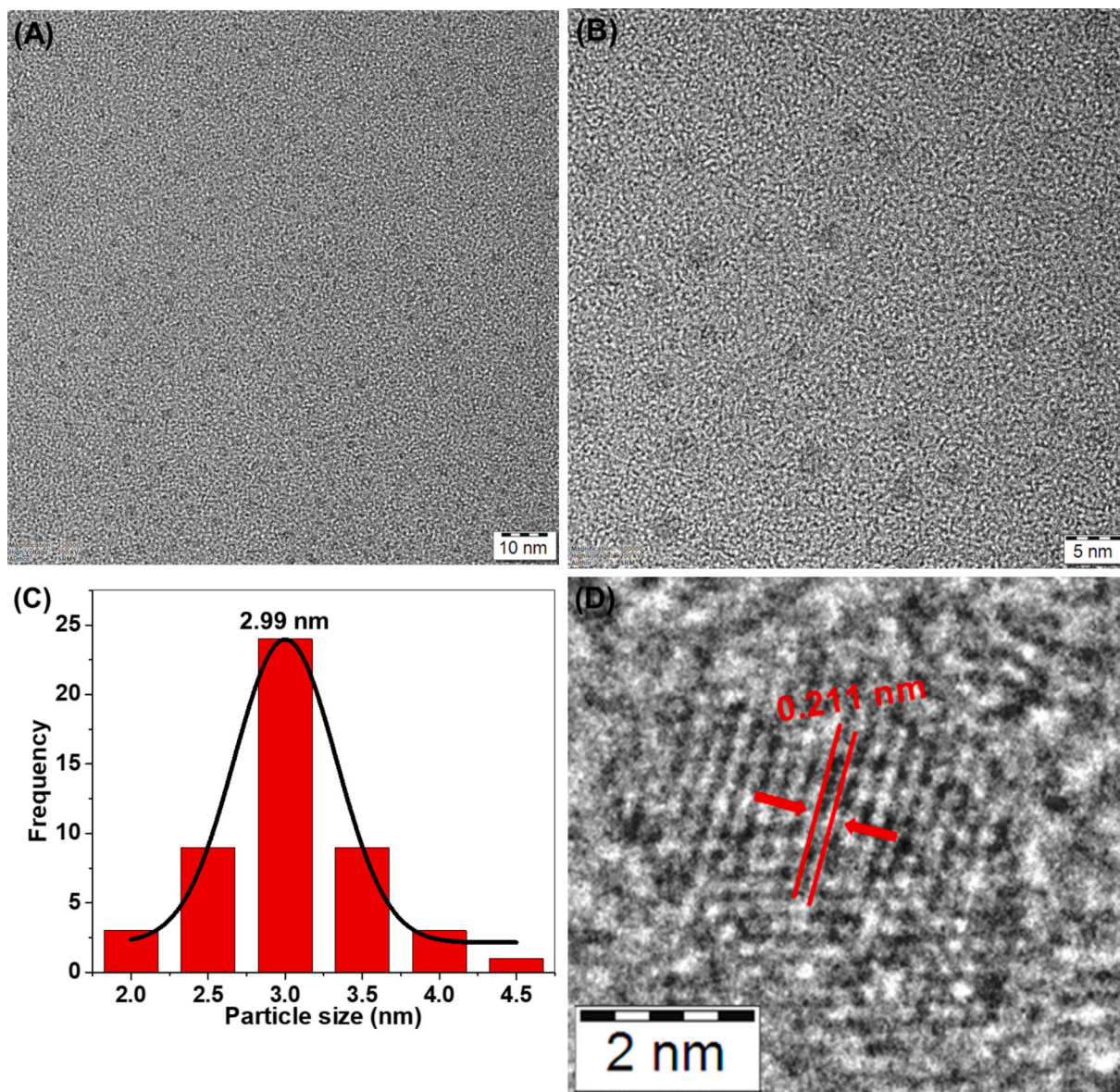


Fig. 3. TEM images ML-CDs at (A) 10 nm, (B) 5 nm magnification. (C) Particle size histogram. (D) HR-TEM image of ML-CD at 2 nm magnification.

noticeable increase in particle size, approximately 2.39 fold. The aggregated ML-CDs / T4 particles show an average size 7.14 nm (Fig. 5 A and B). These TEM observations indicated that the binding of T4 onto the ML-CDs surface induces aggregation of the CDs, which is consistent with the fluorescence quenching behavior observed in the sensing system.

3.8. Sensing mechanism of T4 using ML-CDs

The fluorescence sensing of T4 using by ML-CDs is governed by a non-covalent surface binding-induced aggregation mechanism by static fluorescence quenching. ML-CDs possess abundant oxygen and contain functional groups such as $-\text{COOH}$, $-\text{OH}$, and $-\text{C}=\text{O}$ on their surface, which impart a negative surface charge and ensure good dispersion stability in aqueous solution at neutral pH due to electrostatic repulsion between CDs. Upon addition of T4, strong intermolecular interactions occur between T4 molecules and the negatively charged ML-CD surface via hydrogen bonding between negative functional groups of ML-CDs surface. The interaction primarily involves electrostatic interaction between protonated amine groups ($-\text{NH}_3^+$) of T4 and negative charge

functional groups of ML-CDs, along with hydrogen bonding between hydroxyl/carbonyl groups of ML-CDs and phenolic/amine groups of T4. In addition, possible π - π interactions between the aromatic ring of T4 and graphitic domains of ML-CDs further enhance formation of ML-CDs/T4 complex. The presence of four iodine atoms in T4 exerts a strong electro-withdrawing effect, making the aromatic ring electron-deficient and enhancing surface binding affinity toward ML-CDs. The combined non-covalent interactions reduce electrostatic repulsion between ML-CDs, leading to aggregation of ML-CDs after T4 binding, as supported by TEM images observation. The aggregation promotes formation of non-emissive ground state ML-CDs/T4 complexes, resulting static fluorescence quenching. UV-Vis spectra show a hyperchromic effect without a peak shift, which confirms that the interactions are non-covalent. The fluorescence decrease results from aggregation-induced formation of non-emissive complexes, which enhance non-radiative pathways and suppresses radiative emission promotes to significant quenching of ML-CDs. The concentration of T4 was quantify by correlating the fluorescence quenching intensity of ML-CDs with T4 concentration (Scheme 2) [28–30].

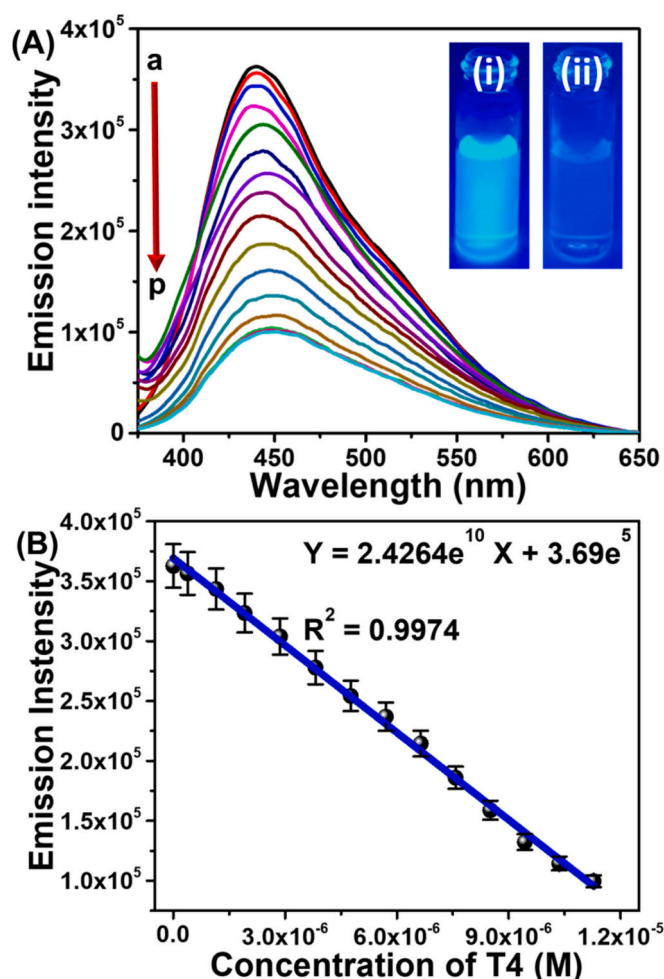


Fig. 4. (A) Emission spectra of ML-CDs in the presence of different concentrations of T4: (a) 0, (b) 0.384, (c) 1.15, (d) 1.92, (e) 2.87, (f) 3.82, (g) 4.76, (h) 5.70, (i) 6.64, (j) 7.58, (k) 8.51, (l) 9.43, (m) 10.4, (n) 11.3, (o) 14.9, and (p) 18.5 μM ; **inset (A):** photographs of (i) only ML-CDs (ii) ML-CDs in the presence of 18.5 μM T4 under UV light. (B) Calibration curve showing the linear response in the T4 concentration range of 0.384–11.3 μM .

3.9. Selectivity studies

Fig. 6 illustrates the selectivity performance of the ML-CD-based sensor, demonstrating a pronounced fluorescence quenching response toward T4 compared with other potential interfering species. To evaluate selectivity, various amino acids, including L-Cys, L-His, L-Pro, and L-Thr, as well as biomolecules such as UA, GA, TA, CA, Creat, Suc, Gua, Ade, DA, B1, Ura, AA, and iodide anion, were employed as common interferences. For the selectivity assessment, 9.43 mM (836-fold) of L-Cys, L-His, UA, GA, TA, and iodide as well as 18.5 mM (1641-fold) of CA, Creat, Suc, Gua, Ade, DA, B1, Ura, L-Pro, AA, and L-Thr were individually introduced into the ML-CDs solution. As shown in **Fig. 6**, the addition of these interfering species caused negligible changes in the relative fluorescence emission intensity of ML-CDs and did not interfere with detection of 11.3 μM T4 alone and mixed analyte systems. In contrast, the presence of T4 alone and mixed analyte systems resulted in a clear fluorescence “turn-off” response, as visually confirmed by the photographic images (**Fig. 6**; inset), whereas the fluorescence of ML-CDs remained essentially unchanged in the presence of other common interferences. Quantitatively, the variation in emission intensity of ML-CDs caused by interfering species was limited to 0.05–4.4%, confirming minimal interference effects (Table S1). Moreover, since iodothyronine analogues such as triiodothyronine (T3), diiodothyronine (T2), and moniodothyronine (T1) share a highly similar backbone with T4 and have been reported to exhibit comparable fluorescence-quenching behavior in optical systems, they may also affect the ML-CDs fluorescence response with varying quenching efficiencies [31,32].

3.10. Real samples analysis in biological, drug, and water samples

The ML-CDs probe was to find in samples of tap water, urine, saliva, serum, and the T4 drug to see their real life applications (Table 1). No detectable T4 was initially observed in drug, tap water, urine, saliva, and serum samples, whereas a T4 concentration of 2.73 μM was successfully quantified in the T4 drug sample. Subsequently, real-sample analysis was performed using T4-spiked tap water, urine, saliva, serum, and T4 drugs samples. Three different concentration of T4 1.15, 1.92, and 2.87 μM were spiked into each sample. As summarized in Table 1, satisfactory recoveries ranging from 95.3 to 100.5% were obtained, demonstrating the high accuracy and good precision (0.35 to 3.51% RSD), as well as the applicability of the ML-CDs-based sensing platform for T4 detection in complex real matrices.

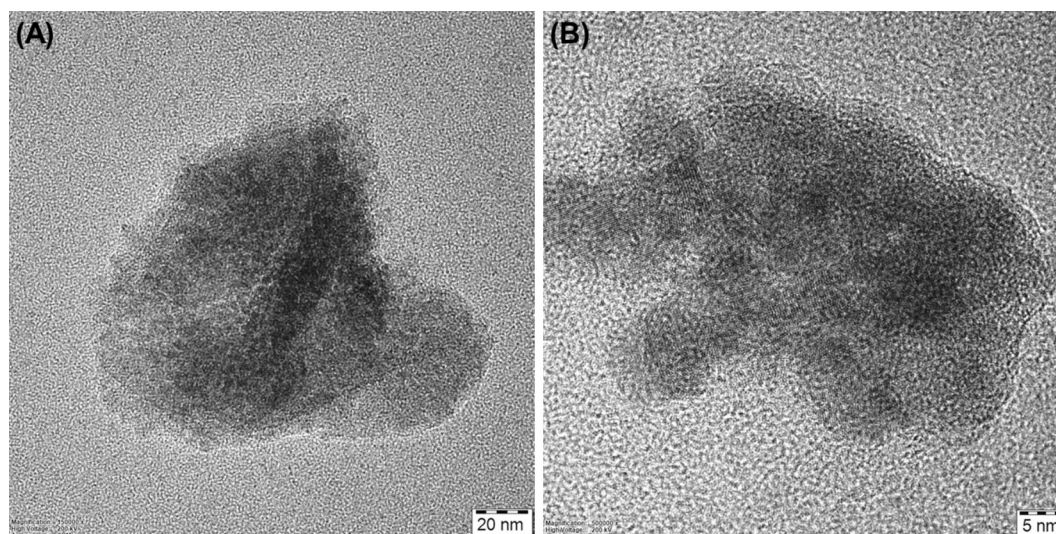
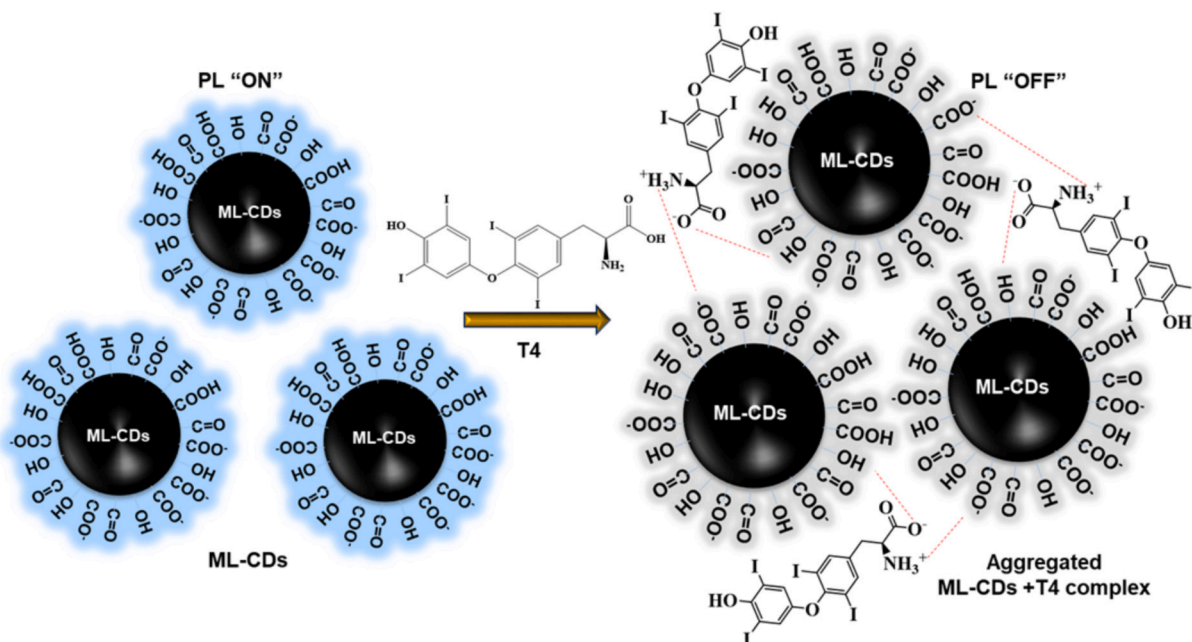


Fig. 5. TEM images of ML-CDs in the presence of 18.5 μM T4 at (A) 20 nm and (B) 5 nm magnifications.



Scheme 2. Schematic representation of the sensing mechanism of the proposed ML-CDs probe for detection T4.

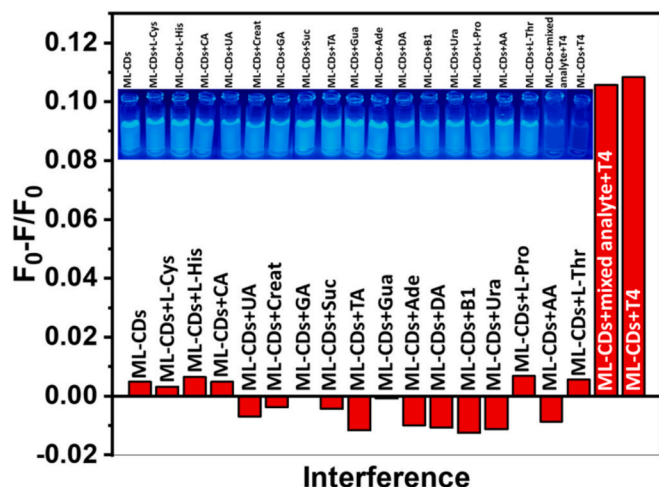


Fig. 6. Selectivity study for T4 detection. Relative fluorescence emission intensity of ML-CDs in the presence of common interfering species, including L-Cys, L-His, UA, GA, TA, and iodide (9.43 mM; 836-fold) and CA, Creat, Suc, Gua, Ade, DA, B1, Ura, L-Pro, AA, and L-Thr (18.5 mM; 1641-fold), compared with 11.3 μ M of T4 alone and mixed analyte systems; **inset:** photographs of ML-CDs alone and ML-CDs in the presence of the corresponding interfering species and T4.

3.11. Validation by HPLC

The ML-CDs probe on HPLC method to validate the accuracy of our T4 sensing system. Tap water samples were spiked with known concentration of T4 (0.502 to 7.91 μ M), and the HPLC responses were recorded. Fig. S9 shows HPLC of tap water spiked with T4 at the concentration of (A) 0.502, (B) 2.0, (C) 3.98, (D) 5.95, and (E) 7.91 μ M. As the T4 concentration increased, the corresponding HPLC peak response area also increased (Fig. S9). A calibration plot of peak area versus T4 concentration showed a linear response in the range of 0.502 to 7.91 μ M (Fig. S10). Based on the slope of the calibration curve, T4 concentration in the spiked samples was calculated and compared with the concentration obtained from our sensing method. The results were in good

Table 1

Real sample analysis of T4 in biological, drug, and water samples.

Real sample	T4 spiked (μ M)	T4 found (μ M)	Recovery (%)
Tap water	-	0	-
	1.15	1.14 $\pm$ 0.04	99.1 $\pm$ 0.3
	1.92	1.93 $\pm$ 0.03	100.5 $\pm$ 0.2
	2.87	2.85 $\pm$ 0.01	99.3 $\pm$ 0.2
Urine	-	0	-
	1.15	1.14 $\pm$ 0.04	99.1 $\pm$ 0.2
	1.92	1.89 $\pm$ 0.01	98.4 $\pm$ 0.2
	2.87	2.75 $\pm$ 0.03	95.8 $\pm$ 0.4
Saliva	-	0	-
	1.15	1.12 $\pm$ 0.01	97.3 $\pm$ 0.3
	1.92	1.85 $\pm$ 0.02	96.3 $\pm$ 0.2
	2.87	2.78 $\pm$ 0.01	96.8 $\pm$ 0.3
Serum	-	0	-
	1.15	1.14 $\pm$ 0.04	99.1 $\pm$ 0.4
	1.92	1.83 $\pm$ 0.03	95.3 $\pm$ 0.1
	2.87	2.75 $\pm$ 0.02	95.8 $\pm$ 0.2
T4 drug	-	0	-
	1.15	1.14 $\pm$ 0.03	99.1 $\pm$ 0.2
	1.92	1.84 $\pm$ 0.02	95.8 $\pm$ 0.2
	2.87	2.78 $\pm$ 0.01	96.8 $\pm$ 0.3

agreement with the reported sensing method, indicating acceptable accuracy and recovery thereby confirming the reliability of the proposed sensor.

Table 2 compares the performance of the present ML-CDs-based probe with previously reported optical sensor for the T4 detection. Compared with earlier reports, the proposed ML-CDs sensor demonstrates several notable advantages, including reliable operation under biological pH conditions, rapid assay response time, a lower limit of detection, a wide linear detection range, and low analytical cost. In addition, the present method was successfully validated across six different real matrices, including water, saliva, urine, serum, and T4 drug samples, highlighting its broad practical applicability. These advantages suggest that the developed ML-CDs-based sensor platform holds strong potential for clinical and pharmacological diagnostics of T4 in medical and healthcare applications.

Table 2

Comparison of the reported nanomaterial-based optical sensor and the present work for T4 detection.*

Sl. No.	Mode of detection	Nanomaterial	Linear range	LOD	Assay response time (min)	Real sample	Ref.
01	FS	L-cysteine-ZnS QDs	0.11–4.0 μ M	62 nM	5	Saliva	[28]
02	CS	AgNPs with epigallocatechin gallate and CTAB	0.1–0.4 μ M	330 nM	10	Drug	[33]
03	CS	Au triangular nanoplate	0–50 μ M	200 nM	10	Drug	[34]
04	CI	Anti-T4 labelled AuNPs	0.8–100 pM	14 pM	15	–	[35]
05	FS	<i>J.adhatoda</i> -CuNCs	0.1–2.5 μ M	7.4 nM	–	Human serum	[36]
06	PS	Mercaptopropionic acid-Mn:ZnS QDs	7.9 nM–1.6 μ M	3.43 nM	20	Human serum	[37]
07	FS	T4-imprinted mesoporous organosilica	0.5 nM–5 μ M	0.47 nM	15	–	[38]
08	FS	ML-CDs	0.384–11.3 μ M	0.564 nM	4	Water, urine, saliva, serum, T4 drug	Present Work

* CM- colorimetric sensor; CI-colorimetric immunosensor; FS- fluorometric sensor; PS-phosphorescent sensor.

4. Conclusion

In summary, the simple and eco-friendly hydrothermal strategy was successfully developed for the synthesis of ML-CDs and their applications as a highly sensitive and selective fluorescent probe for T4 detection. The synthesized ML-CDs exhibited uniform nanoscale dimensions (~2.99 nm), superior water solubility, consistent blue fluorescence, and an abundance of surface functional groups, all contributed to their effective interaction with T4 molecules. The proposed sensing platform outperformed numerous previously published optical sensing systems with an ultra-low detection limit of 0.564 nM, a broad linear response range, and a quick assay time of 4 min. Importantly, the ML-CDs-based sensor demonstrated excellent selectivity toward T4 even in the presence of structurally similar amino acids, biomolecules, and anions, confirming its robustness in complex matrices. The practical applicability of the sensing system was validated through successful detection of T4 in water, urine, saliva, serum, and drug samples, yielding satisfactory recoveries ranging from 95.3 to 100.5% confirming high accuracy and good precision (0.35 to 3.51% RSD). These findings demonstrate the developed method's accuracy and dependability for real sample analysis. Overall, this work establishes ML as a promising green precursor for the production of biocompatible fluorescent CDs and demonstrates the strong potential of ML-CDs as low-cost, rapid, and efficient nanoprobe for T4 monitoring. The proposed sensing platform shows considerable promise for clinical diagnostics, pharmacological analysis, and may extended to other hormone-related and biomolecules sensing applications in future studies.

CRediT authorship contribution statement

Joseph Sartho Immaculate Mercy: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Veera Sangili Veera Muthammal:** Validation, Methodology, Formal analysis, Data curation. **Mani Chandhru:** Validation, Formal analysis, Data curation, Conceptualization. **Srinivasalu Kutti Rani:** Validation, Methodology, Formal analysis, Data curation. **Muniyandi Maruthupandi:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Nagamalai Vasimalai:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jphotochem.2026.117225>.

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Data availability

Data will be made available on request.

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