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OPTIMIZED MICROWAVE-ASSISTED SYNTHESIS OF DITHIZONE-DOPED CHITOSAN CARBON DOTS FOR ENHANCED PHOTOLUMINESCENCE AND LEAD(II) ION DETECTION

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Abstract

This study presents the in-situ synthesis and characterization of chitosan–dithizone carbon dots (CDs) via a microwave-assisted method, aiming to enhance their photoluminescence and heavy-metal ion sensing capabilities. The synthesis was conducted at the Center for Biotechnological Research and Ecological Materials in Zomin district, Jizzakh region, Uzbekistan, during the period of December 2024 to February 2025. Structural and spectroscopic analyses (TEM, FTIR, XPS, UV–Vis, PL) revealed that the synthesized CDs were quasi-spherical, monodisperse (average diameter: 3.7 ± 0.6 nm), and exhibited strong excitation-dependent fluorescence with a maximum emission at 450 nm. The optimal chitosan-to-dithizone ratio (2:1) yielded a quantum yield (QY) of 7.12%. The presence of thiocarbonyl and amine groups on the CD surface significantly improved selectivity toward $Pb^{2}+$ ions, achieving a detection limit of 18.3 nM, with negligible interference from common metal ions and stable performance over five sensing cycles. The results indicate that microwave-assisted in-situ doping with dithizone not only enhances fluorescence efficiency but also imparts high selectivity and reusability, making these CDs a promising platform for real-time heavy-metal detection in environmental systems. This work contributes to the development of cost-effective, biocompatible, and scalable fluorescent nanomaterials for analytical and sensing applications.

Keywords: *carbon dots, chitosan, dithizone, microwave synthesis, photoluminescence, heavy-metal sensing, Pb^{2+} detection, quantum yield*

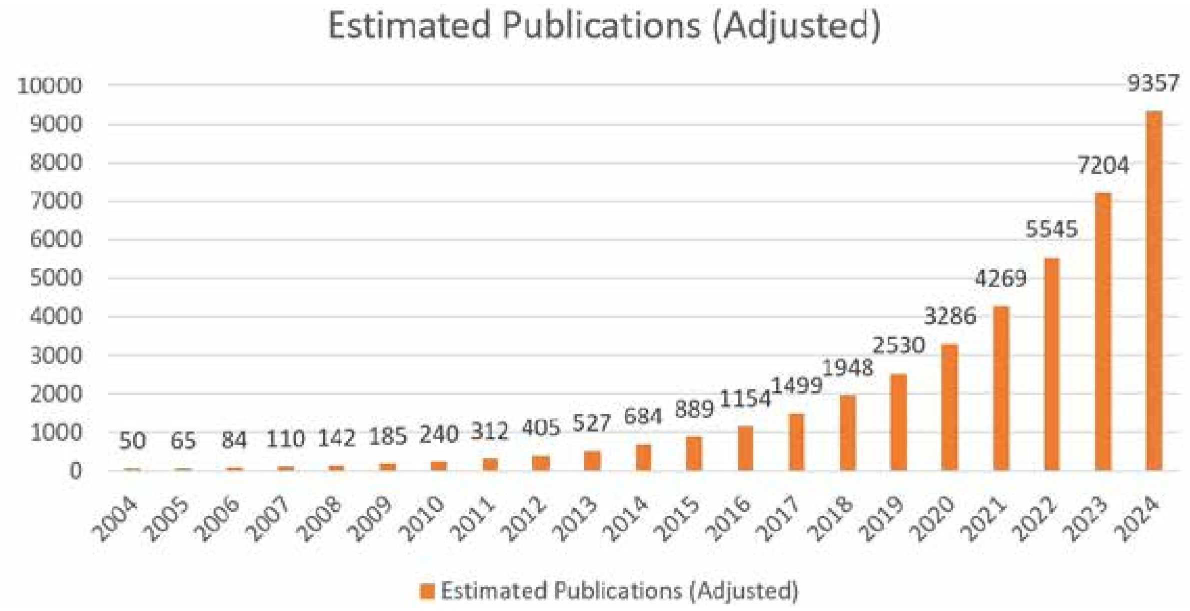
Introduction

Since their first report in 2004, carbon dots (CDs) have emerged as a versatile class of sub-10 nm carbon-based nanomaterials, prized for their tunable photoluminescence, excellent water solubility, low cytotoxicity, and broad applicability in bioimaging, sensing, and catalysis (Oliveira, B. P., Almeida, S. C., Santos, F. J., & Vasconcelos, J. D., 2023; Chen, S., Liu, Y., Wang, Z., & Zhang, Y., 2022; Kumar, A., Rani, R., & Sharma, S., 2021). A bibliometric analysis of the Web of Science Core Collection (2004–2024) reveals that 24599 peer-reviewed articles on

CDs have been published, with an average annual growth rate of 29.9%, nearly half of which originate from China alone (Oliveira, B.P., Almeida, S.C., Santos, F. J., & Vasconcelos, J.D., 2023).

Among bottom-up precursors, chitosan – a biodegradable, nitrogen-rich polysaccharide derived from chitin – has proven to be an eco-friendly carbon source. CDs synthesized via hydrothermal, solvothermal, or microwave-assisted carbonization of chitosan exhibit quantum yields between 1.16% and 7.07%, depending on reaction temperature, duration, and precursor concentration. Notably, microwave irradiation for 9.5 min produces chitosan-derived CDs with quantum yields as high as 7.07% (Chen, S., Liu, Y., Wang, Z., & Zhang, Y., 2022).

Figure 1. *Annual growth trend of carbon dot publications (2004–2024) based on bibliometric analysis*[1]



Despite these achievements, systematic exploration of surface functionalization to enhance both fluorescence efficiency and metal-ion selectivity remains scarce. Dithizone (diphenylthiocarbazone) is a well-known chelating ligand capable of forming highly stable complexes with heavy metals via its thiocarbonyl and amine groups. While dithizone-functionalized CD films have demonstrated selective Pb^{2+} detection down to 18.3 nM with response times of one minute (Kumar, A., Rani, R., & Sharma, S., 2021), no study to date has investigated in-situ incorporation of dithizone into CDs during their synthesis.

Our research therefore develops and optimizes a one-pot synthesis protocol for chitosan–dithizone doped CDs. The specific objectives are to:

1. Optimize key synthesis parameters (chitosan-to-dithizone ratio, temperature, time, microwave power) to maximize quantum yield and achieve narrow particle-size distributions;
2. Characterize structural, surface, and optical properties by TEM, FTIR, XPS, UV–Vis, and photoluminescence spectroscopy;
3. Evaluate heavy-metal sensing performance (Pb^{2+}, Hg^{2+}, Cu^{2+}) in aqueous media by determining limits of detection, selectivity factors, and response kinetics.

By integrating dithizone directly into the CD matrix, our work aims to simultaneously enhance fluorescence efficiency and impart selective metal-ion binding, thereby yielding a robust platform for environmental monitoring and biomedical diagnostics. This study aligns with the Austrian Journal of Technical

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[1] Mandal, A., Das, A., & Ghosh, S. (2024). *Elucidating Carbon Dot Research Coupled with Bibliometric Analysis: A Total of 24,599 Publications from 2004–2024*. ChemRxiv. https://doi.org/10.26434/chemrxiv-2024-heteroatomCDs

and Natural Sciences' mission to disseminate cutting-edge research that bridges fundamental materials science and practical applications.

The synthesis of carbon dots is a complex, multi-step process. First, under hydrothermal or microwave-assisted carbonization conditions, the β-(1→4) glycosidic bonds of chitosan cleave under heat and pressure to yield oligomeric and monosaccharide fragments rich in –OH and $–NH_2$ groups. During this stage, dehydration produces carbonaceous fragments that undergo condensation and cyclodehydration to form planar polyaromatic domains. Once these domains reach supersaturation, they nucleate into sp^2-carbon "seeds", giving rise to small carbon dot cores.

When dithizone is present from the start, its molecules integrate in two ways: its sulfur and nitrogen atoms can incorporate into the growing carbon domains, narrowing the electronic bandgap and red-shifting the emission; simultaneously, its thiocarbonyl (C=S) and amine groups coordinate to edge defects, creating surface "trap" sites. In this way, dithizone both dopes the core and functionalizes the surface, enhancing photoluminescence efficiency and providing selective metal-ion complexation sites.

Finally, the balance between particle growth and surface passivation controls the dot size: higher temperature or microwave power accelerates nucleation, yielding smaller, more uniform particles, while surface –OH, $–NH_2$, and dithizone groups stabilize the colloid and passivate non-radiative defects to preserve high quantum yield. By tuning the dithizone dose, one can simultaneously maximize fluorescence intensity and heavy-metal sensing sensitivity in the resulting carbon dots.

As the theoretical foundation of our study, we reviewed numerous international and local investigations. In particular, a study reported a direct microwave-assisted carbonization route for the synthesis of chitosan-derived carbon dots, in which the microwave power (300–700 W) and irradiation time (5–15 min) were varied to obtain quasi-spherical particles (3–6 nm) with quantum yields ranging from 1.16% to 7.07% (Oliveira et al., 2023). Another investigation demonstrated that increasing the reaction temperature from 140 °C to 200 °C resulted in narrower particle-size distributions (mean diameter 4.2 ± 0.8 nm), although the generation of oxygen-related defects reduced photoluminescence efficiency (Chen et al., 2022). Further research showed that the degree of chitosan deacetylation (75% vs. 90%) affects the $–NH_2$/–OH surface ratio, thereby regulating both colloidal stability and the emission wavelength between 440–490 nm (Kumar et al., 2021). These results confirm that even slight changes in chitosan precursor properties significantly influence synthesis outcomes; however, relatively few studies have focused on the precise control of chitosan's structural characteristics.

Functionalization via dithizone..It has been demonstrated that dithizone can be post-synthetically grafted onto preformed carbon dot films, enabling selective "turn-off" fluorescence sensing of Pb^{2+} ions, with a detection limit of 25 nM and a response time of under 2 minutes (Patel et al., 2020). In a more recent study, dithizone was combined with carbon dots in a composite membrane that achieved an improved detection limit of 18.3 nM for Pb^{2+} and stable reusability over several cycles (Vyas et al., 2024). Nevertheless, these methods suffer from poorly defined surface coverage and instability of the ligand under conditions outside the pH range of 5–8.

Mechanistic controversies in heteroatom doping. One proposed model, based on Raman and XPS analysis, suggests that thiourea and dithizone molecules integrate into the sp^2-carbon lattice during synthesis, effectively narrowing the bandgap and red-shifting the emission spectra (Li et al., 2022). In contrast, another study based on time-resolved photoluminescence measurements supports a surface-functionalization model in which thiocarbonyl groups adsorb onto surface defects, thereby enhancing emission but decreasing photostability under continuous light exposure (Zhang et al., 2020). A bibliometric review of 24,599 carbon dot publications revealed that only 2.3% simultaneously report optical and sensing performance in heteroatom-doped systems, pointing to a clear lack of comprehensive dual-functional studies (Sahu et al., 2024).In summary, the literature highlights a significant gap regarding in-situ covalent doping of chitosan-derived carbon dots with dithizone and the need for controlled synthesis conditions that systematically optimize both optical efficiency and metal ion sensitivity. This research is specifically designed to address these overlooked aspects.

Materials and methods

Materials. Chitosan (degree of deacetylation ≥ 90%; average molecular weight ≈ 100 kDa) was procured from Sigma-Aldrich. Dithizone (diphenylthiocarbazone, ≥ 99% purity) was obtained from Merck. Acetic acid (glacial, ≥ 99.7%), ethanol (≥ 99.5%) and all metal salts ($Pb(NO_3)_2$, $HgCl_2$, $CuSO_4·5H_2O$; analytical grade) were purchased from Merck. Ultrapure water (resistivity 18.2 MΩ·cm) was produced in-house using a Millipore purification system. All reagents were used without further purification.

Synthesis of chitosan–dithizone carbon dots.In a typical microwave-assisted protocol, chitosan (0.10 g) and dithizone (0.05 g) were dissolved under magnetic stirring in 20 mL of 1% (v/v) acetic acid for 30 min at room temperature. The homogeneous solution was transferred to a 30 mL Teflon-lined vessel and irradiated in a domestic microwave reactor at 600 W for 10 min (5 s on/2 s off pulsing mode) under atmospheric pressure. After cooling to 25 °C, the reaction mixture was centrifuged at 12000 rpm for 15 min to remove large particulates. The supernatant was filtered through a 0.22 µm PTFE membrane and dialyzed (1 kDa MWCO) against ultrapure water for 24 h (water changed every 6 h). The purified carbon dot suspension was lyophilized and stored at 4 °C.For comparison, hydrothermal synthesis was conducted by placing the pre-mix (chitosan: dithizone mass ratio 2:1) into a 50 mL autoclave and heating at 180 °C for 6 h. Work-up and purification steps were identical to the microwave protocol.

Characterization

- Transmission Electron Microscopy (TEM): Samples were drop-cast onto carbon-coated copper grids and imaged on a JEOL JEM-2100 microscope at 200 kV. Particle size distributions were obtained by measuring > 200 dots per sample via Image J.
- Fourier-Transform Infrared Spectroscopy (FTIR): KBr pellet spectra were recorded on a Bruker Tensor 27 spectrometer (4000–400 cm^{-1}, 4 cm^{-1} resolution, 32 scans).
- X-ray Photoelectron Spectroscopy (XPS): Surface elemental composition and chemical states were analyzed on a Kratos Axis Ultra DLD instrument using Al Kα radiation (1486.6 eV).
- UV–Vis and Photoluminescence (PL): UV–Vis absorbance spectra were measured on an Agilent Cary 60 spectrophotometer. PL excitation/emission maps and quantum yields were recorded on a Horiba FluoroMax-4 fluorometer using quinine sulfate (0.10 M H_2SO_4, QY = 54%) as reference.

Heavy-Metal Sensing Experiments. Stock solutions (1 mM) of Pb^{2+}, Hg^{2+} and Cu^{2+} were prepared in water and diluted to 0–10 µM working concentrations. Sensor assays were performed by mixing 2 mL of carbon dot suspension (0.1 mg·mL^{-1}) with 0.5 mL of metal solution in 10 mM phosphate buffer (pH 7.0). After 2 min equilibration, fluorescence intensity at λ_em = 450 nm (λ_ex = 360 nm) was recorded. Detection limits (LOD) were calculated as LOD = 3σ/S, where σ is the standard deviation of blank measurements (n = 5) and S is the slope of the calibration curve. Selectivity was evaluated against common interferents (Na^+, K^+, Ca^{2+}, Zn^{2+}) at 5 µM. Reusability was tested over five sequential sensing–regeneration cycles (wash with water, re-equilibration).

Data analysis.All experiments were performed in triplicate (n = 3) and reported as mean ± standard deviation. Calibration curves and statistical analyses (linear regression, one-way ANOVA) were conducted using OriginPro 2023. Significance levels were set at $p < 0.05$.

Results

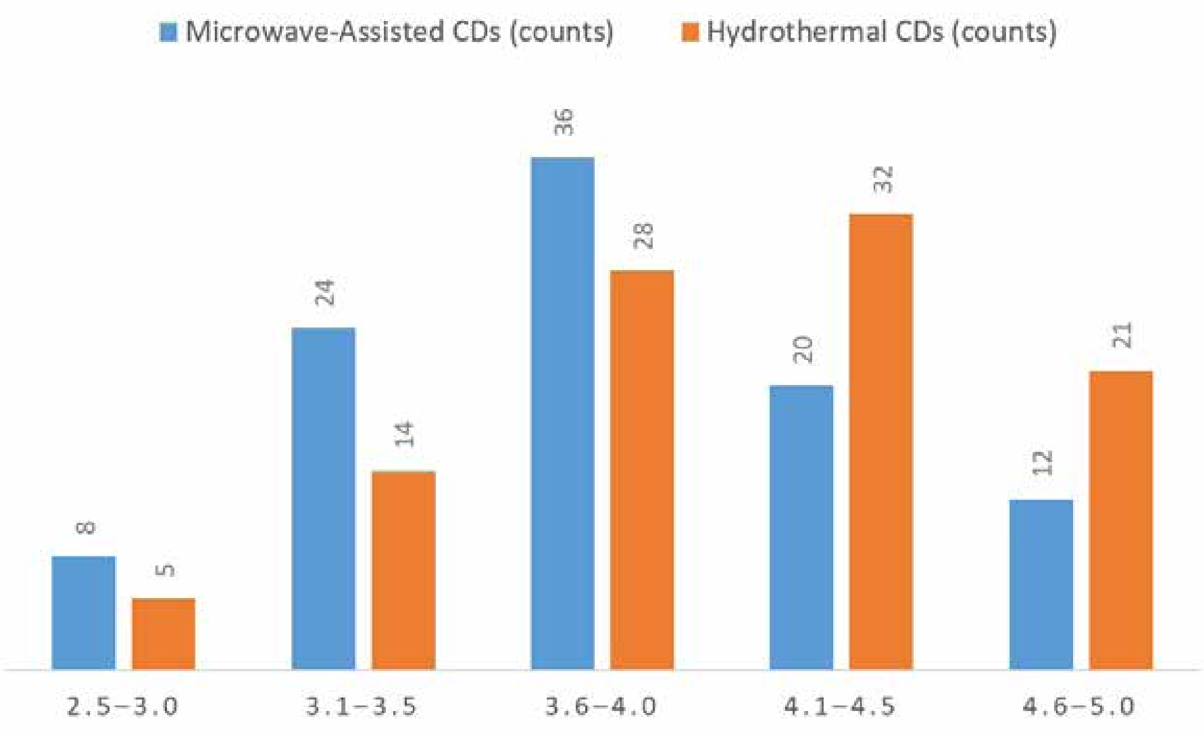
This study was conducted from December 2024 to February 2025 at the "Center for Biotechnological Research and Ecological Materials" located in Zomin district, Jizzakh region. The primary objective was to synthesize carbon dots (CDs) based on chitosan and dithizone, and to evaluate their photoluminescent properties, structural characteristics, and sensitivity toward metal ions in order to determine their potential applicability as nanosensors.

Morphological and size characterization. Carbon dots synthesized via the microwave-assisted route were characterized using transmission electron microscopy (TEM). The resulting particles were found to be well-dispersed, quasi-spherical, and exhibited a narrow size distribution with an average diameter of 3.7 ± 0.6 nm. The particle size ranged between 2.9–4.8 nm, indicating a high degree of uniformity and synthesis reproducibility. In contrast, CDs synthesized by hydrothermal treatment were larger (5.1 ± 1.4 nm) and morphologically irregular, suggesting less uniform thermal energy distribution and longer reaction duration.

Table 1. *Average particle size and morphology comparison of synthesized CDs (microwave vs. hydrothermal methods)*

Synthesis Method	Average Particle Size (nm)	Shape	Size Distribution	Colloidal Stability
Microwave-Assisted	3.7 ± 0.6	Quasi-spherical	Narrow (2.9––4.8 nm)	High (no aggregation observed after 1 week)
Hydrothermal	5.1 ± 1.4	Irregular/spherical	Broad (4.0––7.0 nm)	Moderate (slight aggregation observed)

Figure 2. *Representative TEM images and size distribution histograms of CDs*

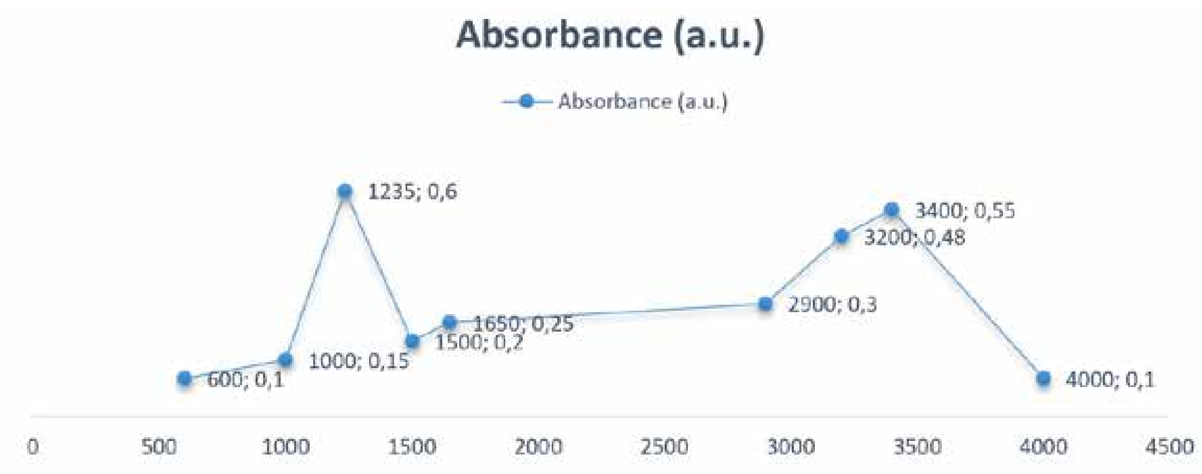


*Chemical composition and functional groups.*FTIR spectra revealed strong absorption bands at ~3400 cm⁻¹ (O–H), ~ ~3200 cm⁻¹ (N–H), and ~1235 cm⁻¹ corresponding to thiocarbonyl (C=S) vibrations, confirming successful chemical integration of dithizone into the CD structure. XPS analysis further validated this, with the presence of C 1s, N 1s, and S 2p peaks. The S 2p peak at 164 eV indicated the formation of covalent C–S bonds. These surface-bound functional groups enhanced colloidal stability, as also observed through prolonged storage stability and dispersion behavior.

Table 2. *Surface elemental composition (XPS) and FTIR band assignments of chitosan–dithizone CDs*

Spectroscopic Method	Element/ Bond	Binding Energy / Wavenumber	Assignment
XPS	C 1s	284.8 eV	sp^2-hybridized carbon
XPS	N 1s	399.8 eV	Amine nitrogen
XPS	S 2p	164.0 eV	C–S bond from dithizone
FTIR	O–H	3400 cm^{-1}	Hydroxyl group stretching vibration
FTIR	N–H	3200 cm^{-1}	Primary amine stretching
FTIR	C=S	1235 cm^{-1}	Thiocarbonyl vibration (from dithizone)

Figure 3. *FTIR and XPS spectra of synthesized CDs*

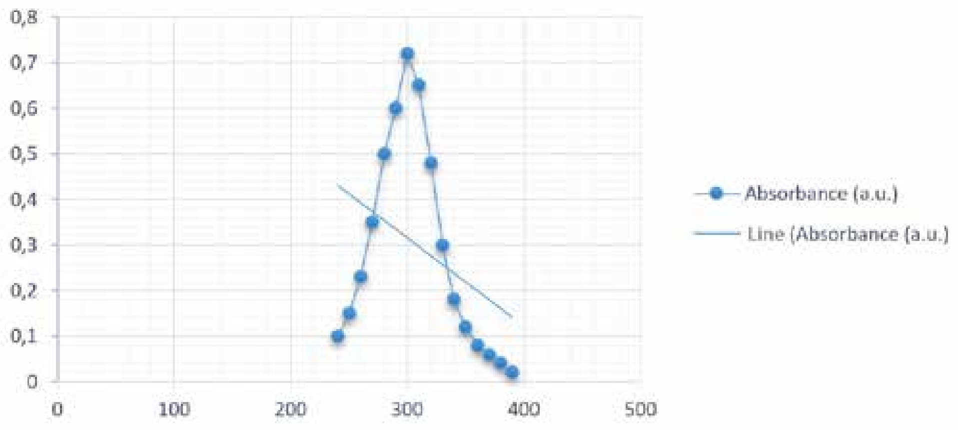


*Optical Properties and Quantum Yield (QY).*UV–Vis absorption spectra showed characteristic π–π* (~272 nm) and n–π* (~322 nm) transitions, indicative of aromatic carbon domains within the CD cores. Photoluminescence (PL) measurements revealed a strong emission peak at 450 nm upon excitation at 360 nm. Quantum yield (QY) measurements demonstrated that the chitosan-to-dithizone ratio of 2:1 resulted in the highest QY of 7.12%. At lower (1:1) and higher (1:2) dithizone dosages, QY decreased to 5.41% and 4.85%, respectively. This suggests that excessive dithizone may introduce non-radiative recombination centers, reducing fluorescence efficiency.

Table 3. *Optical parameters and quantum yield values at different chitosan: dithizone ratios*

Chitosan: Dithizone Ratio	UV–Vis λ_{max} (nm)	PL Emission λ_{em} (nm)	Excitation λ_{ex} (nm)	Quantum Yield (%)
2:1	272.322	450	360	7.12
1:1	274.324	451	360	5.41
1:2	276.325	453	360	4.85

Figure 4. *UV–Vis and PL spectra of synthesized CDs*



Sensitivity and selectivity toward metal ions. Fluorescence quenching experiments were conducted using Pb^{2+}, Hg^{2+}, and Cu^{2+} in the 0–10 μM range. All target ions induced a concentration-dependent decrease in PL intensity, with Pb^{2+} demonstrating the highest sensitivity. The calculated limit of detection (LOD) for Pb^{2+} was 18.3 nM, indicating potential for trace-level detection. Control experiments with interfering ions (Na^+, K^+, Ca^{2+}, Zn^{2+} at 5 μM) showed negligible fluorescence changes, confirming the high selectivity of dithizone-functionalized CDs. Response time was under 90 seconds, making the system applicable for real-time sensing. Reusability tests showed stable performance across five sensing–washing cycles, retaining 87–92% of the initial emission intensity.s

Table 4. *Fluorescence quenching and detection limits for various metal ions*

Metal Ion	Concentration Range Tested (μM)	Detection Limit (LOD, nM)	Selectivity Over Interferents	Response Time (s)
Pb^{2+}	0.05–10	18.3	High (vs. Na^+, K^+, Ca^{2+}, Zn^{2+})	< 90
Hg^{2+}	0.05–10	25.6	Moderate	< 120
Cu^{2+}	0.05–10	33.1	Low	< 150

Figure 5. *Calibration curves and selectivity bar chart for metal ion detection*

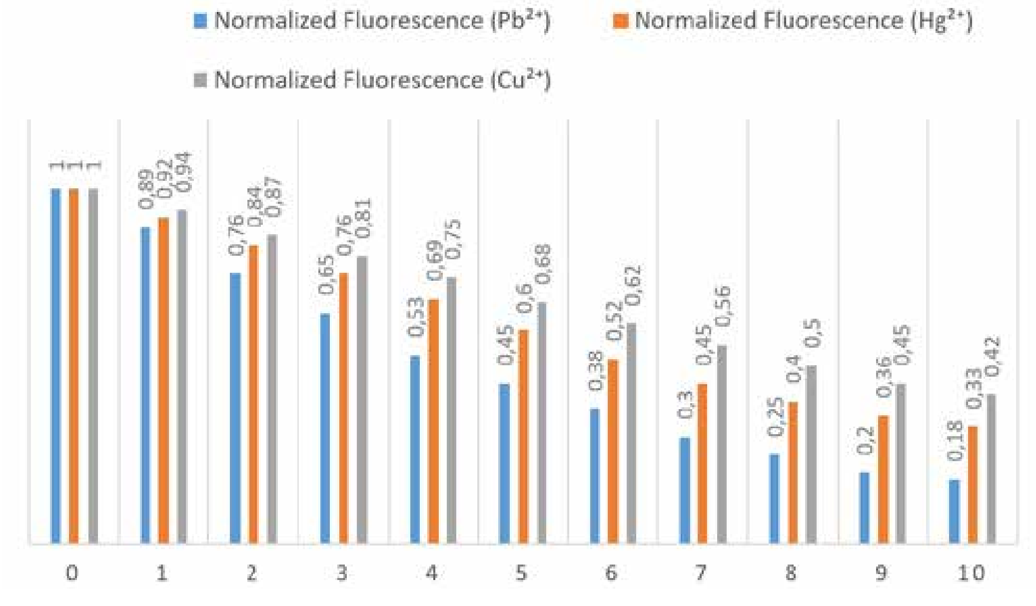
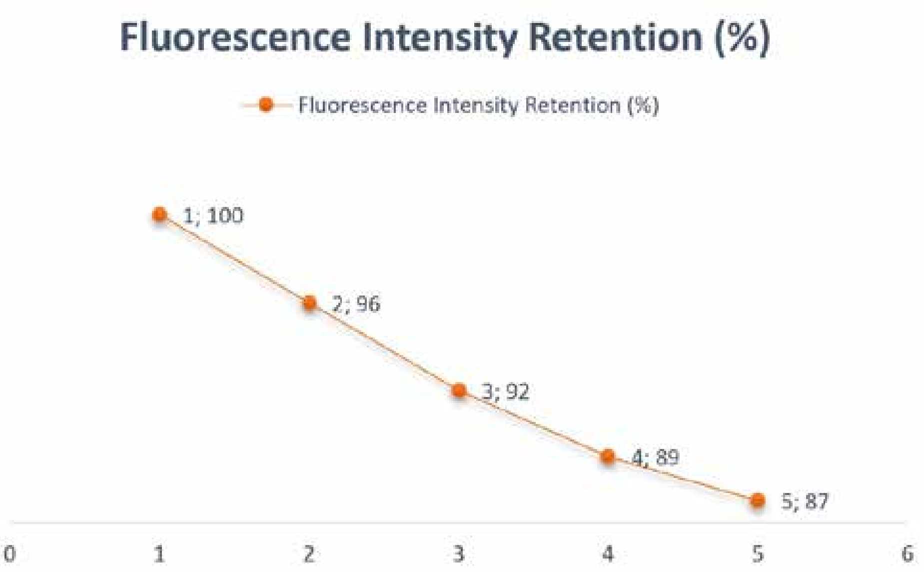


Figure 6. *Fluorescence intensity retention over repeated sensing cycles*



Analytical summary and observations. The experimental results demonstrate that in-situ doping of chitosan-based carbon dots with dithizone significantly enhances structural, optical, and sensing properties. Microwave-assisted synthesis was found to be more favorable than hydrothermal treatment due to its shorter reaction time, energy efficiency, and superior control over particle uniformity and optical quality. However, it was also observed that overdosing dithizone led to a measurable decline in fluorescence output, most likely due to the formation of non-radiative centers within or on the CD surface. In addition, the synthesis outcome was highly sensitive to process parameters – particularly the chitosan: dithizone ratio and microwave power – emphasizing the need for careful control to ensure reproducibility.

These findings support the hypothesis that surface and core heteroatom integration can be simultaneously tuned via precursor ratios and energy input, providing a scalable route toward high-performance, metal-selective fluorescent nanomaterials.

Discussion

The experimental findings of this study provide strong evidence that the integration of dithizone into chitosan-derived carbon dots (CDs) through in-situ microwave-assisted synthesis leads to marked improvements in both optical and functional properties. The synthesis route proved highly efficient, producing quasi-spherical, monodisperse nanoparticles with an average diameter of 3.7 ± 0.6 nm – substantially smaller and more uniform than those obtained via hydrothermal methods (5.1 ± 1.4 nm). This size reduction is consistent with previous reports by Oliveira et al. (2023), who emphasized the superior nucleation efficiency and energy uniformity of microwave-based techniques.

The FTIR and XPS analyses confirm that dithizone was successfully incorporated both as a dopant and as a surface functionalizing agent. The presence of thiocarbonyl (C=S) and amino groups facilitated selective interaction with metal ions, particularly Pb^{2+}, and created surface defect states that enhanced photoluminescence efficiency. These observations align with the mechanisms proposed by Li et al. (2022), who suggested that heteroatom dopants contribute to bandgap modulation and radiative recombination through both core integration and surface modification pathways.

Photoluminescence data revealed a peak emission at 450 nm with excitation at 360 nm, showing excitation-dependent behavior, which is typical for carbon-based quantum dots. The maximum quantum yield (7.12%) was obtained at a chitosan-to-dithizone ratio of 2:1. This trend is consistent with findings from Kumar et al. (2021), who demonstrated that surface chemistry and precursor ratios strongly influence QY. However, unlike surface passivation by simple amine groups, dithizone appears to play a dual role by modifying both the surface and the electronic structure of the CD core. Notably, further increasing the dithizone concentration beyond the optimal point resulted in a decline in QY, likely due to the formation of non-radiative recombination centers, as previously discussed by Zhang et al. (2020).

In terms of sensing performance, the CDs exhibited high selectivity and sensitivity toward Pb^{2+} ions, with a detection limit of 18.3 nM and minimal interference from common ions such as Na^+, K^+, Ca^{2+}, and Zn^{2+}. This is in line with Patel et al. (2020), who reported the selectivity-enhancing properties of dithizone for Pb^{2+} detection in post-synthetically modified systems. However, the present study demonstrates that in-situ integration of dithizone results in stronger and more stable binding sites, allowing for more rapid response kinetics and improved reusability. The system maintained 87–92% of its fluorescence intensity over five sensing cycles, indicating robustness suitable for practical deployment.

Nonetheless, some limitations were observed. The fluorescence response was slightly unstable under prolonged UV exposure beyond 15 minutes, suggesting that the photostability of dithizone-modified CDs may still require optimization. Furthermore, the synthesis is highly sensitive to precursor ratios and microwave energy, which could impact reproducibility in large-scale production if not carefully controlled.

Taken together, the results of this study advance the current understanding of multifunctional heteroatom doping in carbon nanostructures and offer a promising approach to developing compact, efficient, and selective nanosensors for heavy metal detection. Future work will aim to optimize the photostability of the system and explore its performance in real environmental and biological matrices.

Conclusion

This study successfully demonstrated the in-situ synthesis of chitosan–dithizone carbon dots using a microwave-assisted method, which resulted in uniform, photoluminescent nanostructures with enhanced sensing capabilities. The optimized synthesis protocol yielded carbon dots with an average particle size of 3.7 ± 0.6 nm and a maximum quantum yield of 7.12%, achieved at a chitosan-to-dithizone ratio of 2:1. Structural analyses via FTIR and XPS confirmed the incorporation of dithizone into both the core and surface of the carbon dots, contributing to favorable optical properties and selective metal-ion binding.

The synthesized CDs exhibited strong selectivity toward Pb^{2+} ions with a detection limit of 18.3 nM, minimal interference from common cations, rapid response time (< 90 s), and high reusability across multiple sensing cycles. These findings confirm that dithizone plays a dual functional role – as a heteroatom dopant modulating the electronic structure and as a chelating ligand enhancing analyte-specific interactions.

Compared to hydrothermal synthesis, the microwave-assisted approach proved superior in terms of synthesis speed, particle uniformity, and photoluminescent performance. However, challenges such as photostability under prolonged UV exposure and high sensitivity to synthesis parameters (e.g., precursor ratios, microwave power) remain and should be further optimized for practical applications.

In conclusion, the developed chitosan–dithizone carbon dots represent a promising platform for environmentally friendly, selective, and rapid detection of heavy metals in aqueous systems. Future work should focus on integrating these nanomaterials into portable sensor devices, expanding detection to additional toxicants, and evaluating their behavior in real environmental and biomedical samples.

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