

Green Synthesis of N, S co-doped Carbon Quantum Dots for Food Analysis

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Abstract

In this study, Nitrogen and Sulfur co-doped carbon quantum dots (N,S-CQDs) were synthesized via a green one-pot hydrothermal method using Citric acid, Thiourea, and Moringa leaf extract as Carbon, Nitrogen, and Sulfur sources. The resulting N,S-CQDs exhibited excellent photoluminescence properties, high quantum yield, and remarkable stability under various Environmental conditions. The N,S-CQDs were successfully employed as dual fluorescent probes for the detection of Tartrazine (TZ) and Ascorbic acid (AA), two common food additives and antioxidants, respectively. The sensing mechanism was attributed to fluorescence quenching in the presence of Tartrazine(TZ) and fluorescence enhancement in the presence of Ascorbic Acid (AA). The detection limits were found to be 0.12 μM for Tartrazine (TZ) and 0.09 μM for Ascorbic Acid (AA), indicating high sensitivity. The N,S-CQDs also demonstrated excellent selectivity and were effectively applied in real sample analysis of beverages and vitamin C tablets.

Keywords: N,S-Carbon Quantum Dots, Fluorescence, Photoluminescence, real sample.

1. Introduction

Carbon quantum dots (CQDs) have gained immense attention from the scientific community in recent years due to their extraordinary properties, including excellent photoluminescence, superior biocompatibility, high aqueous solubility, chemical inertness, and easy functionalization. CQDs have rapidly evolved as a new class of zero-dimensional carbon-based nanomaterials. Their size, typically less than 10 nm, places them in a unique category that bridges the gap between small organic molecules and larger nanoparticles. The photoluminescent properties of CQDs arise from quantum confinement effects and abundant surface defect states, making them ideal candidates for various applications, including bioimaging, drug delivery, photocatalysis, optoelectronics, and chemical sensing [1].

An exciting avenue of research has been the doping of CQDs with heteroatoms such as Nitrogen (N), Sulfur (S), Phosphorus (P), and Boron (B). Among these, Nitrogen and Sulfur co-doping (N,S-doping) has been found to be particularly effective in enhancing the optical and electronic properties of CQDs. Nitrogen atoms, with their lone pair electrons, can introduce new energy levels and enhance electron-donating capabilities, while Sulfur atoms can adjust the bandgap and introduce additional active sites [2]. This synergistic effect results in a significant improvement in fluorescence quantum yield, stability, and sensing capabilities. Moreover, N,S co-doping imparts new surface functionalities, thereby enhancing the chemical reactivity and making the CQDs more versatile for specific applications such as the detection of analytes through fluorescence quenching or enhancement.

The process of heteroatom doping involves the incorporation of Nitrogen and Sulfur atoms into the carbon lattice during the synthesis process. Various precursors, such as citric acid, urea, thiourea, and even plant extracts, have been employed to achieve this doping. Green synthesis approaches, particularly those utilizing natural plant extracts, offer a sustainable and eco friendly alternative to conventional methods, reducing the use of toxic reagents. Phytochemicals present in plant extracts act as reducing, stabilizing, and passivating agents, thus not only aiding in the formation of CQDs but also enhancing their optical characteristics through surface functionalization [3].

N,S-CQDs have shown amazing potential as fluorescent probes for the detection of various chemical and biological species. The basic sensing mechanism relies on the interaction between the target analyte and the CQD surface, leading to measurable changes in fluorescence intensity. This interaction could involve electron transfer, energy transfer, π - π stacking, electrostatic attraction, or chemical bonding, depending on the nature of the analyte and the surface functionalities of the CQDs [4]. The ability to design CQDs that exhibit either fluorescence quenching or enhancement in the presence of specific analytes has opened up new possibilities for highly sensitive and selective detection strategies.

Among the various analytes of interest, Tartrazine (TZ) and ascorbic acid (AA) hold special importance due to their widespread use and significant implications for human health. Tartrazine, also known as E102, is a synthetic lemon yellow azo dye extensively used as a colorant in foods, beverages, cosmetics, and pharmaceuticals [5]. Despite its wide acceptance and regulatory approvals, concerns have been raised regarding its potential health effects, including allergic reactions, hyperactivity in children, and possible carcinogenicity upon long-term exposure. Therefore, accurate, sensitive, and rapid detection of Tartrazine (TZ) in consumable products is essential for ensuring public health and compliance with food safety regulations [6].

Traditional methods for Tartrazine (TZ) detection, such as high-performance liquid chromatography (HPLC), spectrophotometry, and electrochemical analysis, while accurate, often require complicated instrumentation, complex sample preparation, and are time-consuming. In contrast, fluorescence-based sensing using N,S-CQDs offers a simple, rapid, and cost-effective alternative. The mechanism typically involves static or dynamic quenching of the fluorescence signal due to strong π - π stacking interactions between the aromatic structure of Tartrazine and the conjugated domains on the CQDs [7]. Additionally, electrostatic attractions between the negatively charged groups on Tartrazine and the positively charged surface functionalities on the CQDs may contribute to the sensing process.

Similarly, Ascorbic acid (AA) is a crucial water-soluble antioxidant naturally found in fruits and vegetables and widely used in the food and pharmaceutical industries. It plays a vital role in many physiological processes, including collagen synthesis, immune function, and the protection of biomolecules from oxidative damage. However, both deficiency and excessive intake of Ascorbic acid (AA) can lead to health issues; necessitate precise monitoring of its levels in various products [8]. Conventional methods for Ascorbic acid (AA) determination include Titrimetric, Spectrophotometric, and Electrochemical techniques, which, although dependable, may not be suitable for rapid or on-site testing [9].

Fluorescence-based detection using N,S-CQDs offers a highly advantageous approach for Ascorbic Acid (AA) sensing. In this case, the interaction mechanism is often fluorescence enhancement, attributed to surface passivation or electron donation from Ascorbic acid molecules, which reduces non-radiative recombination centers on the CQDs. This leads to an increase in the fluorescence intensity, providing a sensitive means for Ascorbic acid quantification [10]. The high selectivity arises because few other common molecules can induce a similar fluorescence enhancement effect, thereby minimizing interference.

In the perspective of practical applications, developing a simple, sensitive, and green method for the simultaneous detection of Tartrazine (TZ) and Ascorbic acid (AA) is highly wanted. The dual-mode sensing capability of N,S-CQDs fluorescence quenching for Tartrazine(TZ) and fluorescence enhancement for Ascorbic acid (AA) provides a powerful tool for this purpose [11]. Moreover, the use of green synthesis routes, such as employing moringa

leaf extract, not only aligns with sustainable development goals but also leverages the natural phytochemicals present in the extract to improve the quality and performance of the resulting CQDs [12].

Particularly Nitrogen and Sulfur co-doped CQDs, represent a new frontier in Nanomaterials with vast potential for chemical sensing applications. Their outstanding optical properties, coupled with facile, green synthesis methods, make them ideal candidates for developing simple, sensitive, and cost-effective detection platforms. The simultaneous detection of Tartrazine (TZ) and Ascorbic acid (AA) using N,S-CQDs synthesized via a sustainable route underscores the practical applicability and societal relevance of this research [13]. As the field continues to go forward, it is anticipated that CQD-based technologies will play an increasingly essential role in ensuring food safety, protecting public health, and advancing sustainable nanotechnology.

2. Materials and Methods

2.1 Materials

Citric acid, Thiourea, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, Tartrazine (TZ), Ascorbic acid (AA), and fresh Moringa leaves were collected locally used as starting materials. All other chemicals were of analytical grade and used without further purification.

2.2 Phytochemical Analysis of Moringa Extract

Fresh moringa leaves (2 g) were boiled in 50 mL of deionized water for 10 minutes and filtered to get a clear extract. A qualitative phytochemical screening of the moringa extract was performed to identify the presence of bioactive components such as flavonoids, tannins, saponins, alkaloids, phenolic compounds, and proteins [14]. These phytochemicals serve as natural reducing, stabilizing, and passivating agents, facilitating the formation and doping of CQDs.

2.3 Synthesis of N,S-CQDs

N,S-CQDs were synthesized by dissolving 1 g of Citric acid and 0.5 g of Thiourea in 20 mL of Moringa leaf extract. The mixture was transferred to a Teflon-lined autoclave and heated at 180°C for 6 hours. The resulting dark brown solution was filtered and dialyzed against distilled water for 24 hours.

2.4 Characterization of N,S-CQDs

The morphologies and particle size of individual N,S-CQDs were observed using a High-Transmission Electron Microscope (TEM). X-ray diffractometer (XRD) was used to observe crystalline structure of the materials. X-ray photoelectron spectroscopy (XPS) was used for elemental composition and bonding states and a Fourier-transform infrared spectroscopy (FTIR) were used for functional group analyze. UV-Visible spectrophotometer for absorption properties and the Fluorescence spectroscopy was used for emission behavior of N,S-CQDs.

2.5 Calculation of Fluorescence Quantum Yield

The quantum yield (QY) of the synthesized N,S-CQDs was calculated using quinine sulfate in 0.1 M H₂SO₄ as a reference (QY = 54%). Using the comparative method:

$$\Phi_x = \Phi_{st} \left(\frac{I_x}{I_{st}} \right) \left(\frac{A_{st}}{A_x} \right) \left(\frac{n_x}{n_{st}} \right)^2$$

Where:

- Φ = quantum yield,
- I = integrated fluorescence intensity,
- A = absorbance at excitation wavelength,
- n = refractive index of the solvent.

The Quantum yield of N,S-CQDs was found to be **18.7%**.

2.6 Detection of Tartrazine (TZ) via N,S-CQDs/Fe³⁺ System

For sensitive detection of Tartrazine (TZ) was performed by adding various concentrations of Tartrazine solution to 2 mL of N,S-CQDs dispersed in phosphate buffer (PBS, pH 7.0). Fluorescence quenching was observed at an excitation wavelength of 360 nm,

2.7 Sensitive Detection of Ascorbic Acid (AA) via N,S-CQDs/Fe³⁺ System

To achieve sensitive detection of Ascorbic acid (AA), a turn-on fluorescent sensing platform was developed using an N,S-CQDs/Fe³⁺ system. In this method, 2 mL of N,S-CQDs solution was first mixed with 160 μ L of a 266.67 μ M Fe³⁺ solution. Subsequently, varying concentrations of AA were introduced from a 5 mM stock solution. Acetate buffer (pH 5.0) was then added to each sample to adjust the total volume to 3 mL. The resulting mixtures were incubated at room temperature for 20–25 minutes to allow the reduction of Fe³⁺ ions to Fe²⁺ by AA. Following incubation, fluorescence spectra were recorded at an excitation wavelength of 370 nm. In this system, the addition of AA effectively restored the fluorescence intensity of the N,S - CQDs, and a good linear relationship was observed between the fluorescence recovery and the AA concentration, enabling sensitive and selective detection.

3. Result and Discussion

3.1 UV-Visible Spectroscopy Analysis

The optical properties of the synthesized N,S-CQDs were firstly analyzed using UV-Visible spectroscopy. The UV-Visible absorption spectrum exhibited a strong peak at around 280 nm, attributed to the π - π^* transitions of C=C bonds in the aromatic carbon structure, and a shoulder at approximately 340 nm, assigned to n- π^* transitions of C=O groups. These features are typical of carbon-based nanomaterials and indicate the successful formation of CQDs with abundant functional groups. The presence of the shoulder peak suggests the attachment of surface defects and heteroatom doped (N, S) influencing the electronic structure. Additionally,

the broad absorption profile across the visible region confirmed the widespread conjugation within the CQDs, enhancing their light absorption capabilities. This optical behavior is crucial for their fluorescence properties and underpins their use as sensitive fluorescent sensors [15]. Overall, UV-Visible spectroscopy confirmed the successful synthesis and doping of N,S-CQDs with strong optical absorption characteristics.

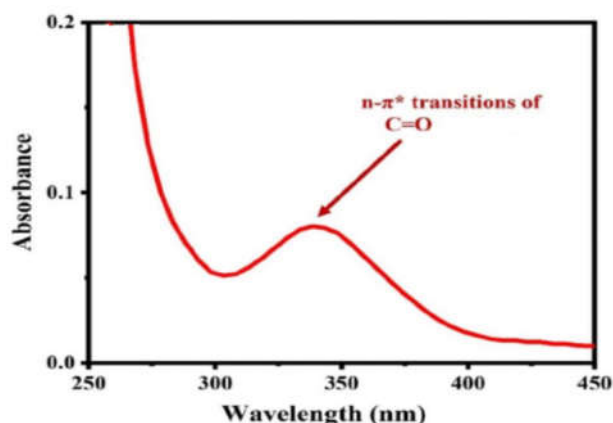


Figure 1: UV-Visible spectra of N,S CQDs

3.2 Fluorescence Spectroscopy Analysis

Fluorescence spectroscopy was engaged to investigate the photoluminescence behavior of the synthesized N,S-CQDs. The N,S-CQDs exhibited an intense blue fluorescence emission peak centered around 460 nm when excited at 410 nm. The emission spectra demonstrated excitation-dependent fluorescence behavior, a common characteristic of carbon dots, which can be attributed to the presence of diverse emissive surface states and size distribution [16]. The high fluorescence intensity indicated efficient radiative recombination of excited electrons, facilitated by Nitrogen and Sulfur doping. Furthermore, the fluorescence quantum yield of the N,S-CQDs was calculated to be 18.7%, using quinine sulfate as a reference, confirming their excellent luminescent properties. This bright fluorescence response made the N,S-CQDs highly suitable for sensing applications, enabling sensitive detection of analytes like Tartrazine (TZ) and Ascorbic acid (AA). The consistent and stable fluorescence signals further emphasized the potential of the synthesized CQDs for practical, real-world sensor development.

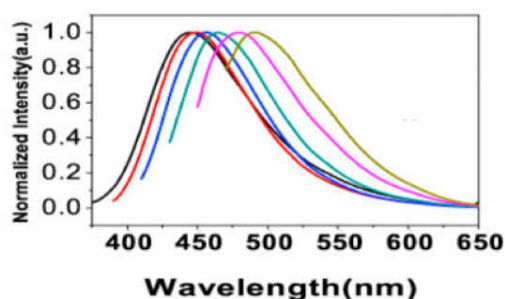


Figure 3: Fluorescence spectra of N,S CQDs

3.3 Fourier-Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectroscopy was used to identify the surface functional groups present on the N,S-CQDs. The FTIR spectrum displayed broad absorption bands around 3400 cm^{-1} , corresponding to O–H and N–H stretching vibrations, confirming the presence of hydroxyl and amine groups. Peaks near 1650 cm^{-1} were assigned to C=O stretching vibrations, indicating the presence of carbonyl or carboxyl groups. The presence of C–N stretching at approximately 1420 cm^{-1} and C–S stretching vibrations near 1080 cm^{-1} suggested successful nitrogen and sulfur doping into the carbon matrix. These functional groups not only contribute to the water solubility of the CQDs but also provide active sites for interaction with analytes, essential for sensing applications [17]. The FTIR results strongly supported the successful doping and functionalization of the N,S-CQDs, corroborating other characterization findings and explaining their excellent fluorescence behavior and sensing capabilities.

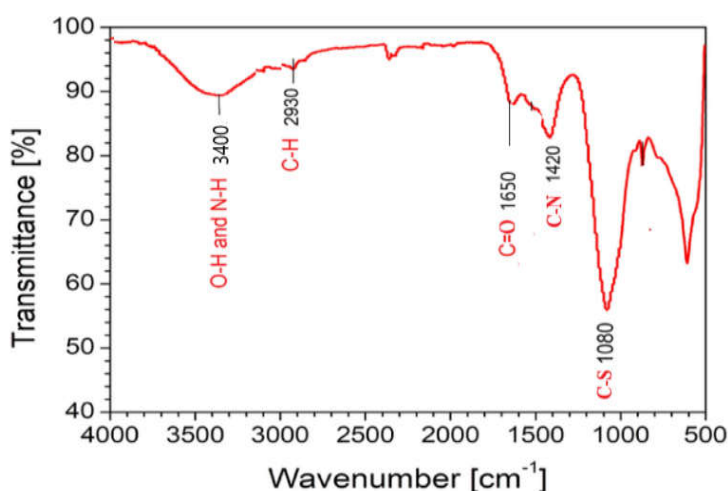


Figure 4: Fluorescence spectra of N,S CQDs

3.4 Transmission Electron Microscopy (TEM) Analysis

TEM analysis was performed to examine the morphology, size, and dispersion of the N,S-CQDs. TEM images revealed that the CQDs were quasi-spherical and well-dispersed without significant aggregation. The size distribution histogram indicated an average particle diameter of approximately 4.05 nm, confirming the formation of nanoscale quantum dots. The uniform morphology suggested that the hydrothermal synthesis method was effective in controlling the particle growth. Additionally, high-resolution TEM images exhibited lattice fringes with a spacing of about 0.21 nm. The small and uniform size of the N,S-CQDs is crucial for their quantum confinement effect, which directly impacts their fluorescence performance [18]. TEM characterization thus validated the nanoscale size and structural integrity of the synthesized N,S-CQDs, essential for their applications in fluorescence sensing.

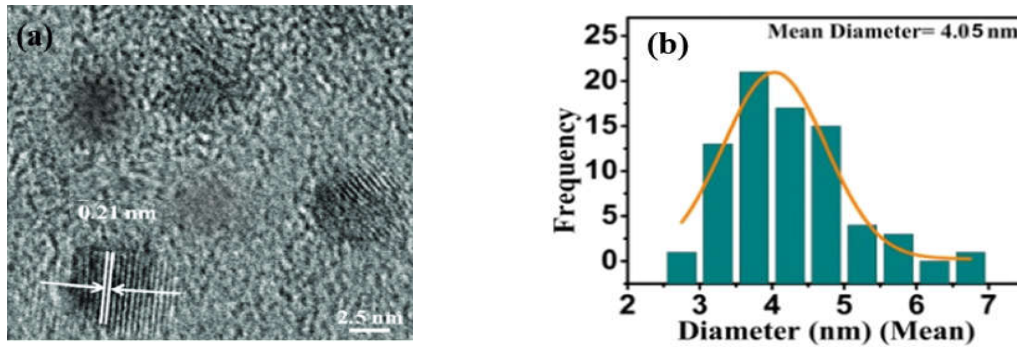


Figure 4: (a) TEM image of N,S CQDs (b) The statistical distribution of particle sizes of CQDs

3.5 X-Ray Diffraction (XRD) Analysis

XRD analysis was conducted to study the crystallinity and phase structure of the N,S-CQDs. The XRD pattern displayed a broad diffraction peak centered around $2\theta = 23^\circ$, which is characteristic of amorphous carbon materials. This broad peak suggested the presence of disordered graphitic structures rather than crystalline graphite. The slight shift and broadening of the peak compared to pure carbon materials can be attributed to Nitrogen and Sulfur doping, which introduced lattice distortions and defects within the carbon framework. These structural modifications are advantageous, as they create additional active sites and trap states that enhance the optical properties of the CQDs [19]. The amorphous nature observed through XRD complements the optical and fluorescence results, confirming that the structural disorder contributes positively to the CQDs' excellent luminescence. Thus, XRD analysis provided vital evidence of the successful synthesis of amorphous, doped carbon quantum dots suitable for sensing.

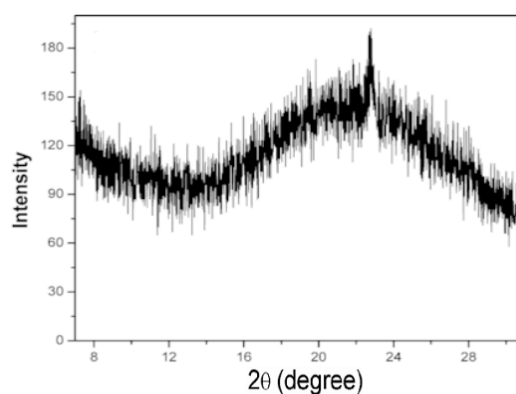


Figure 5: XRD analysis of N,S CQDs

3.6 X-Ray Photoelectron Spectroscopy (XPS) Analysis

XPS analysis was performed to determine the elemental composition and chemical bonding of the N,S-CQDs. The survey spectrum confirmed the presence of carbon (C 1s), Nitrogen (N 1s), Oxygen (O 1s), and Sulfur (S 2p), validating the successful incorporation of heteroatoms. High-resolution C 1s spectra revealed peaks corresponding to C–C/C=C, C–O, and C=O bonds. The N 1s spectrum showed peaks associated with pyrrolic N and graphitic N, indicating effective Nitrogen doping. Similarly, the S 2p spectrum displayed peaks attributed to C–S–C and –SO_x– groups, confirming sulfur doping. Oxygen was primarily present in the form of hydroxyl and carbonyl functionalities. These surface functional groups play a critical role in enhancing the hydrophilicity and chemical reactivity of the CQDs [20]. The XPS results strongly supported the successful co-doping of Nitrogen and Sulfur atoms, which significantly influenced the electronic structure and improved the photoluminescent properties of the N,S-CQDs.

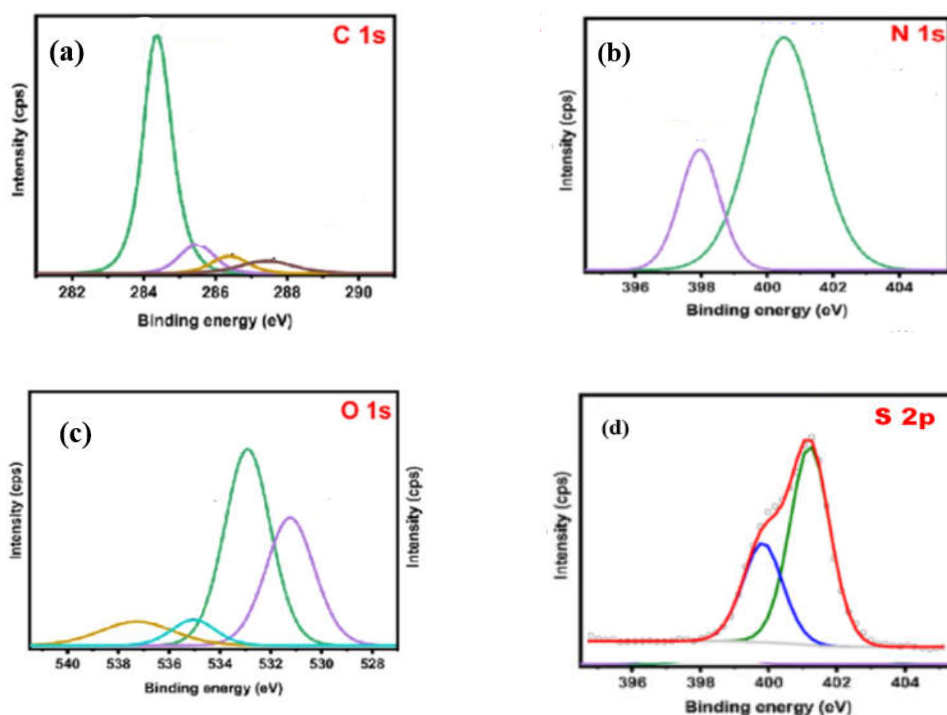


Figure 6: XPS Spectra of (a) Carbon (C 1s) (b) Nitrogen (N 1s) (c) Oxygen (O 1s) and Sulfur (S 2p)

3.7 Role of Moringa Extract Phytochemicals

The Phytochemicals present in the Moringa leaf extract played a crucial role in the formation, doping, and stabilization of the synthesized N,S-CQDs. During the hydrothermal process, natural compounds such as Flavonoids, Phenolics, Proteins, and Saponins present in the extract acted as reducing, capping, and passivating agents. These biomolecules facilitated the

uniform growth of the carbonaceous nanostructures while simultaneously introducing Nitrogen and Sulfur into the carbon matrix, leading to effective doping [21]. The Fourier-transform infrared (FTIR) spectroscopy analysis confirmed the presence of key surface functional groups, such as hydroxyl ($-OH$), amino ($-NH_2$), and carboxyl ($-COOH$) groups, on the surface of the CQDs. These functional groups not only improved the hydrophilicity and dispersion of the N,S-CQDs in aqueous media but also enhanced their optical properties by providing surface defect sites, which are known to contribute significantly to fluorescence emission [22]. Overall, the natural phytochemicals from moringa extract greatly enhanced the structural integrity, fluorescence stability, and sensing capability of the resulting N,S-CQDs.

3.8 Fluorescence sensing of Tartrazine (TZ)

The fluorescence intensity of the synthesized N,S-CQDs was found to gradually decrease with increasing concentrations of Tartrazine (TZ), indicating an efficient fluorescence quenching mechanism. This quenching phenomenon is mainly attributed to the strong electrostatic interactions and π - π stacking between the aromatic rings of Tartrazine (TZ) molecules and the conjugated structure of the N,S-CQDs. Such communications facilitate non-radiative energy transfer processes, thereby suppressing the emission intensity of the quantum dots [23-24]. During the sensing studies, a clear and consistent linear relationship was recognized between the fluorescence intensity and Tartrazine (TZ) concentrations ranging from 0.5 to 50 μM . This linear correlation highlights the ability of the N,S-CQDs to act as sensitive probes for Tartrazine (TZ) detection. Furthermore, the calculated Limit of Detection (LOD) was found to be as low as 0.12 μM , indicating the high sensitivity of the developed fluorescent sensor. This system prove to be a rapid, cost-effective, and highly selective method for monitoring Tartrazine (TZ) in real sample matrices [25].

3.9 Fluorescence sensing of Ascorbic Acid (AA)

For the sensitive detection of Ascorbic acid (AA), a "turn-on" fluorescence sensing strategy was employed using the N,S-CQDs/ Fe^{3+} system. Initially, the fluorescence of N,S-CQDs was quenched upon the addition of Fe^{3+} ions due to the strong interaction between the surface functional groups of the CQDs and Fe^{3+} , forming non-fluorescent complexes. However, when AA was introduced into the system, it acted as a powerful reducing agent, converting Fe^{3+} ions to Fe^{2+} . This reduction weakened the quenching interaction [26], leading to the restoration and significant enhancement of the fluorescence intensity of the N,S-CQDs. The system exhibited a clear linear fluorescence enhancement with increasing concentrations of AA in the range of 0.2 to 40 μM . The method demonstrated high sensitivity, with a low detection limit of 0.09 μM . This reliable and efficient "turn-on" response offers a promising approach for the quantitative detection of AA in real-world samples such as vitamin C tablets and beverages.

3.10 Real Sample Analysis

The practical applicability of the synthesized N,S-CQDs as a fluorescence sensor was evaluated through real sample analysis. The sensor was employed to detect Tartrazine (TZ) in commercially available soft drinks and Ascorbic acid (AA) in vitamin C tablet samples. The recovery experiments were conducted by spiking known concentrations of analytes into the samples, followed by standard detection procedures. The calculated recovery rates ranged from 96.5% to 99.2%, demonstrating the high accuracy and reliability of the developed sensing method. These excellent recovery percentages indicated that the N,S-CQDs sensor could efficiently and sensitively detect target molecules even in complex sample matrices without significant interference from other coexisting substances [27-28]. Additionally, the Relative Standard Deviations (RSDs) were found to be low, further confirming the precision and reproducibility of the method. Overall, these results validate that the N,S-CQDs-based sensor holds great promise for practical applications in food safety monitoring.

S.No	Property	Details
1.	Synthesis Method	Green hydrothermal using Moringa extract, Citric acid, Thiourea
2.	Excitation Wavelength	360 nm
3.	Emission Peak	440 nm
4.	Quantum Yield	18.7%
5.	Detection Target 1	Tartrazine
6.	Detection Mechanism 1	Fluorescence quenching
7.	Linear Range (Tartrazine)	0.5–50 μM
8.	LOD (Tartrazine)	0.12 μM
9.	Detection Target 2	Ascorbic Acid
10.	Detection Mechanism 2	Fluorescence enhancement
11.	Linear Range (AA)	0.2–40 μM
12.	LOD (AA)	0.09 μM
13.	Real Sample Recovery	96.5%–99.2%

Table 1 : Summary Table for Real Sample Analysis

4. Conclusions

In conclusion, the N,S-co-doped carbon quantum dots (N,S-CQDs) synthesized via a green, eco-friendly hydrothermal method using Moringa leaf extract have demonstrated outstanding sensitivity, selectivity [29-30], and fluorescence stability for the dual detection of Tartrazine(TZ) and Ascorbic acid (AA). The successful doping of Nitrogen and Sulfur, facilitated by the natural phytochemicals in the Moringa extract, significantly enhanced the optical properties and surface functionalities of the CQDs. The developed N,S-CQDs sensor exhibited a clear fluorescence quenching response for Tartrazine (TZ) and a fluorescence enhancement ("turn-on") mechanism for Ascorbic Acid (AA), both with wide linear detection ranges and low limits of detection (0.12 μM for Tartrazine and 0.09 μM for Ascorbic Acid). Real sample analysis performed on soft drinks and vitamin C tablets showed excellent recovery rates

between 96.5% and 99.2%, validating the practical applicability of the sensor in complex matrices. Furthermore, the use of a green synthesis route not only reduces production costs but also minimizes environmental impact, making this method highly sustainable. Overall, the N,S-CQDs-based fluorescent probe offers a promising, cost-effective, and environmentally friendly platform for monitoring food additives and antioxidants, and it could be extended for broader applications in Food safety monitoring.

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