

Green, Hybrid Synthesis and Characterization of Improved CQD with Antioxidant Properties for Biomedical Applications

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Abstract

Carbon quantum dots (CQDs) have recently gained attention as an auspicious material for various applications, focusing on the biomedical domain. This is primarily attributed to their exceptional optical characteristics, which set them apart from other materials. This study introduces a novel methodology for producing and analyzing advanced CQDs that exhibit superior antioxidant characteristics. The increased antioxidant capabilities of these CQDs are significant for their possible use in biological fields. This work uses CQDs, which are obtained by hydrothermal synthesis of lignin. To enhance their efficacy in biomedicine, these CQDs are subjected to surface modification using two distinct coumarins by applying microwave radiation, conferring upon them significant advantageous characteristics. Implementing this modification is of utmost importance to mitigate the constraints associated with CQDs' inherent fluorescence quantum yield. The study begins by using hydrothermal synthesis to produce CQDs produced from lignin. Surface modification is conducted on the CQDs using two distinct coumarins with the assistance of microwave radiation. The HSC-ICQD approach utilizes a range of analytical methods, including Fourier-Transform Infrared Spectroscopy (FTIR), Atomic Absorption Spectroscopy (AAS), Nuclear Magnetic Resonance (NMR), and Transmission Electron Microscopy (TEM), to validate the achievement of effective modification. This work assesses the antioxidant capacity of the modified CQDs by quantitatively evaluating their abilities to scavenge superoxide and hydroxyl radicals. The findings underscore the appropriateness of using HSC-ICQDs in bioimaging, drug delivery, and therapeutic interventions within biomedicine. This study highlights the significance of using green synthesis methods to customize nanomaterials for biomedical applications, presenting encouraging prospects in nanomedicine.

Keywords: CQDs, Antioxidant Properties, Biomedical Applications, Green Synthesis.

Introduction to Carbon Quantum Dots and its Application

Carbon quantum dots (CQDs) have recently gained attention as intriguing nanomaterials with exceptional optical properties, attracting the interest of researchers from several disciplines, notably in biomedicine [1]. CQDs, characterized by their small dimensions, quantum confinement phenomena, and surface characteristics, provide a distinct platform for groundbreaking progress in drug delivery, bioimaging, and therapeutic interventions. Within this framework, the advancement of CQDs enhanced with customized characteristics, including heightened fluorescence and robust antioxidant properties, signifies a significant progression toward their incorporation into cutting-edge biological uses [2].

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CQDs' production and thorough characterization represent significant achievements in realizing their capabilities. Nanomaterials are often obtained from precursors abundant in carbon and synthesized using carefully regulated techniques [3]. These materials are subjected to thorough analysis and modification processes to optimize their properties. The alterations have the potential to impact their performance and efficacy within biological contexts substantially, hence underscoring the criticality of rigorous synthesis and characterization of CQDs.

Biomedical applications necessarily need materials that possess specific characteristics, such as biocompatibility, long-lasting stability, and versatile adaptation [4]. Because of their inherent biocompatibility and customizable characteristics, CQDs provide significant promise in fulfilling these requirements. The remarkable optical features shown by these entities, including strong fluorescence, wide absorption ranges, and impressive resistance to photodegradation, make them very suitable candidates for sophisticated bioimaging techniques. Moreover, the capacity of these substances to effectively neutralize Reactive Oxygen Species (ROS) highlights their potential as antioxidants, a crucial characteristic in mitigating oxidative stress, a widespread role in several disorders [5].

This study aims to address the need to improve the functionality of CQDs for applications in biomedicine. This study provides a Hybrid Synthesis and Characterization of Improved CQDs (HSC-ICQD), focusing on enhancing CQDs' fluorescence and antioxidant characteristics via environmentally friendly synthesis techniques. This research establishes a connection between foundational nanomaterial investigations and tangible biological implementations, facilitating potential nanomedicine advancements.

The primary contributions are given below:

- A green and hybrid synthesis approach was devised to improve the characteristics of CQDs.
- The CQDs were effectively changed with coumarins, enhancing fluorescence and antioxidant properties.
- Advanced analytical methods such as Fourier-Transform Infrared Spectroscopy (FTIR), Atomic Absorption Spectroscopy (AAS), Nuclear Magnetic Resonance (NMR), and Transmission Electron Microscopy (TEM) were used to characterize the modified CQDs accurately.
- The study showcased the augmented antioxidant capacity of the modified CQDs by quantitatively evaluating their ability to scavenge superoxide and hydroxyl radicals.

The following sections are arranged in the given manner: In Section 2, the literature on CQD synthesis, characterization, and biological applications is reviewed. The HSC-ICQD Method for improving CQDs is described in Section 3, along with the synthesis and characterization procedure. Section 4 covers study findings and focuses on numerical analysis of modified CQDs, including fluorescence and antioxidant characteristics. The results are summarized in Section 5, together with their biological implications and potential future research approaches.

Background and Literature Survey

The literature review examines various research papers on CQDs, focusing on their production, characterization, and use in several disciplines. The research provides significant contributions to understanding the characteristics, techniques, and possible obstacles related to materials based on CQDs.

Kamble et al. successfully produced Fluorescent-Based CQDs (FB-CQDs) by synthesizing natural biowaste [6]. These FB-CQDs had an average size of around 3 nm. The FB-CQDs demonstrated a notable quantum yield of over 60% and were biocompatible, with cell survival surpassing 90%. The approach that has been suggested has potential opportunities for bioimaging with improved biocompatibility. Yan et al. successfully integrated CQDs

into a chitosan hydrogel matrix to develop a sensing platform for detecting Hg^{2+} ions [7]. The achieved detection limit for Hg^{2+} ions was determined to be $0.16 \mu\text{M}$. The use of density functional theory calculations played a crucial role in facilitating the design of the sensing mechanism. The hydrogel exhibited notable specificity for Hg^{2+} ions, with a linear range from $0.5 \mu\text{M}$ to $12 \mu\text{M}$.

Amoozadeh et al. successfully synthesized UV-curable CQD Hybrid Hydrogels (CQD-HH) [8]. These hydrogels exhibited adjustable characteristics, such as a 1.5 to 2.9 MPa storage modulus. The technology showed considerable promise for many applications in materials science, providing a wide range of options regarding rheological behavior. Kumar et al. were synthesized from cow milk using hydrothermal treatment [9]. The resulting carbon dots exhibited an average size of around 3.5 nm. The CQDs showed considerable promise in the sensing field, as they revealed a linear response to Fe^{3+} ions throughout the 0.1 to $20 \mu\text{M}$ concentration range. Lv et al. introduced a composite material of Methylene Blue with CQD (MB-CQDs) with a photothermal conversion efficiency of around 35.4% [9]. The composite material exhibited notable efficiency in photodynamic and photothermal characteristics, establishing its potential as a viable choice for antibacterial applications.

In a recent Costa et al. study, CQDs were derived from Babassu coconut biomass, with a quantum yield of around 12% [10]. The toxicity evaluations conducted on *Daphnia magna* revealed an EC_{50} value of 29 mg/L, underscoring the need for more ecological investigations. Koutamehr et al. successfully synthesized carbon dots using sour whey as the precursor [11]. The resulting CQDs had an average size of around 3 nm. The CQDs had significant antioxidant activity, as seen by an IC_{50} value of 1.25 mg/mL against DPPH radicals. They showed antibacterial efficacy against foodborne pathogens.

Manikandan et al. introduced a novel approach to synthesizing Green-CQDs (G-CQDs) using environmentally friendly methods [12]. The resulting G-CQDs exhibited a quantum yield of around 23%. The G-CQDs exhibited promising characteristics for environmental applications, namely in heavy metal ion removal. They had a maximum adsorption capacity of 39.68 mg/g for $\text{Pb}(\text{II})$. Shen et al. used a hydrothermal synthesis method to produce N-doped CQDs (N-CQDs) with an approximate size of 2 nm [13]. The N-CQDs had remarkable fluorescence characteristics, with a quantum yield of around 50%. The devices were used for ion detection, exhibiting a notable sensitivity towards Cu^{2+} ions throughout a linear range from 0.5 to $30 \mu\text{M}$. Nallayagari et al. investigated the adjustable characteristics of CQD produced by several syntheses, including a size range of 2 to 8 nm [14]. This work enhances the comprehension of the adaptability of CQDs, as shown by their quantum yields spanning a range of 7% to 34%. One of the challenges that researchers face is the need to establish standardized techniques for characterizing various CQDs. The literature review demonstrates a substantial amount of information on CQDs, highlighting their diverse range of applications and various techniques of manufacture. A wide range of studies highlights the need to further the area via the suggested research, aiming to improve the characteristics of CQDs for specific biological uses.

Proposed Hybrid Synthesis and Characterization of Improved CQDs

The present study presents an HSC-ICQD to improve the characteristics of CQDs for use in biomedicine. The strategy utilizes a hybrid synthesis process that combines precursors sourced from natural biowaste with sophisticated microwave radiation. The work seeks to enhance the modified CQDs' fluorescence quantum yield and antioxidant capabilities via a comprehensive characterization approach.

Chemical Structure Analysis

The hybrid nanoparticles used in biological applications were synthesized using a two-step methodology. The raw quantum dots of carbon were derived from lignin utilizing the process of hydrothermal synthesis. These quantum dots were subjected to microwave irradiation for further modification, using two distinct coumarins as reagents. The typical

process for creating CQDs involves three sequential stages: dehydration, carbonizing, and passivity. Lignin, a polymer generated from wood, consists of many phenylpropane monomers linked by cross-linking. It exhibits a high concentration of hydroxyl radicals.

Nevertheless, if no other alterations are made, such as N-doping or grafting, the quantum yield of the material could not meet the requirements for biological applications. The intermediate products underwent functionalization using two distinct coumarins to augment the fluorescence properties of the CQDs produced from lignin. The HSC-ICQD synthesis pathway is shown in Figure 1. Two distinct coumarins were acquired to achieve CQDs with improved fluorescence quantity: coumarine-3-carboxylic acid and 7-(Diethylamino) coumarine-3-carboxylate. These coumarins possess unbound functional groups that can form covalent links with hydroxyl categories found outside the CQDs.

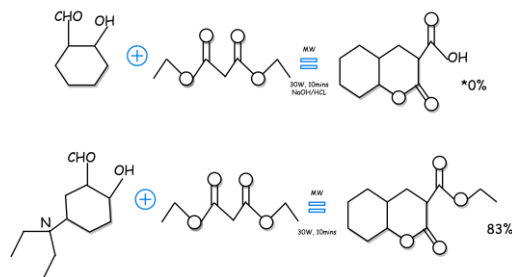


Figure 1: HSC-ICQD synthesis pathway

Materials

The following chemicals were obtained in Poland: sugar, hydrochloric acid, sodium hydroxide, ferric sulfate, sodium nitrate, tetrahydrofuran, methanol, ethanol, acetone, acetonitrile, formic acid, and tartaric acid. The L929 cell line, specifically mouse fibroblasts, was obtained from Sigma-Aldrich in Poznan, Poland. Dulbecco's Modified Eagle Medium (DMEM), streptomycin/penicillin (10%), trypsin, and Phosphate-Buffered Saline (PBS) were also procured from the same supplier. The dialyzing membranes, which had a molecular weight cut-off ranging from 500 to 1000 Da, and the filter membranes with a pore size of 0.22 μ m were obtained from Poland. The reagents used in the experiment were of adequate purity for analytical purposes. The workflow of the HSC-ICQD is shown in Figure 2.

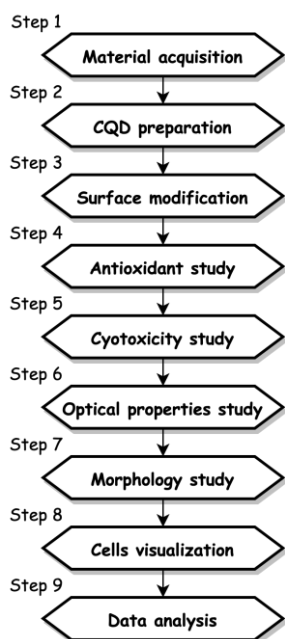


Figure 2: Workflow of the HSC-ICQD

Methods

CQDs Preparation and Modification

Carbon quantum dots were synthesized by a straightforward hydrothermal technique employing an autoclave reactor with a capacity of 150 mL. Glucose was used as a carbon source. The organic precursor was combined with water and acid to get CQDs and introduced into a sealed container. The final product underwent neutralization using sodium hydroxide (NaOH) until the pH reached 7. The samples underwent purification to remove any remaining unreacted residues and macromolecular compounds. This purification process included dialysis, conducted over five days, using a dialysis membrane with a Molecular Weight Cut-Off (MWCO) range of 500-1000 Da. AAS using a Philips spectrometer quantified the sodium ion concentration in the CQD structure. The samples underwent FTIR spectroscopy analysis, revealing a significant presence of sodium ions that were electrostatically attached to the surface carboxylic groups of the dots.

A surface cation-exchange column was used to eliminate sodium ions and achieve unbound COOH groups on the CQDs. Another FTIR examination was conducted, which revealed the absence of the band associated with the Na salt and an augmentation in the signal attributed to unbound carboxylic groups. A novel derivative of rhodamine b was acquired to conduct surface modification. The rhodamine b compound was changed by incorporating diethylene glycol to achieve the desired objective. The result was synthesized by combining 0.5 grams of the dye with 10 milliliters of tetrahydrofuran and 4 milliliters of diethylene glycol. A catalyst, namely 4-dimethylaminopyridine, was used in the experiment with a mass of 0.05 g. The experimental procedure included maintaining continuous agitation at ambient temperature for 24 hours. The product dried and was afterward dissolved in tetrahydrofuran. It was then subjected to purification via a silica gel column chromatography technique. NMR investigations were conducted to ascertain the chemical structure of Rhod-OH. To conduct ¹H-NMR and ¹³C-NMR analyses, a solution of 50 mg of Rhod-OH was prepared by dissolving it in methanol-containing deuterium. The answer was then analyzed using an FT-NMR 500 MHz system equipped with a probe in Poland. The proton NMR experiment was conducted with 16 scans, but the carbon NMR experiment required a much higher number of scans.

A solution of carbon dots in an aqueous medium with a concentration of 5 mg/mL was used to establish CQD coupling with Rhod-OH. This 3 mL solution was then dissolved in 6 mL of tetrahydrofuran. The CQD solution introduced 15 milligrams of Rhod-OH and 0.05 grams of absorbent. The reaction was conducted at ambient temperature and standard atmospheric pressure for 24 hours with continuous agitation. The resultant product underwent a drying process to eliminate the solvents. The resultant CQDs were purified using dialysis membranes with a MWCO. The efficacy of the CQD alteration was assessed by conducting basic hydrolysis using a 0.1 M NaOH solution and measuring fluorescence using a spectrofluorometer.

Antioxidant Study

The investigation of antioxidant characteristics included using two distinct categories of free radicals: superoxide and hydroxyl. The efficacy of superoxide radical elimination was assessed using the autoxidation of pyrogallol technique, while the determination of hydroxyl radicals was conducted using the Fenton reaction. Spontaneous radical scavenging (S) capacity was determined using Equation (1).

$$S_c = \frac{(A_{abs} - A_{std})}{A_{std}} \times 100 \quad (1)$$

The scavenging capability of S_c refers to its capacity to eliminate superoxide radicals. A_{std} represents the normal absorbance, whereas A_{abs} denotes the absorbance of the sample. The hydroxyl absorbance is shown in Equation (2).

$$S_{HO} = \frac{(A_{abs} - A_{std})}{A - A_{std}} \times 100 \quad (2)$$

The scavenging capability of S_{HO} refers to its capacity to eliminate hydroxyl electrons. A_{std} represents the standard absorbance, A_{abs} represents the absorbance of the specimen, and A represents the absorbance of the solution without $FeSO_4$.

Cytotoxicity Study

The cytotoxicity of the samples was assessed using the utilization of mouse fibroblasts. The investigation of cell proliferation was conducted at several time points, namely at 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, and 168 hours (equivalent to a duration of seven days). Before conducting the experiments, all solutions underwent sterilization using 0.2 μm filter membranes. The cells were examined using a reversed optical microscope manufactured by Delta Optical.

Optical Properties Study

The Ultraviolet-Visible (UV-Vis) spectra were acquired using the Philips 8453 Diode panel spectrophotometer, while the fluorescence spectrum was obtained employing the Jasco FP-750 spectrofluorometer. The fluorescence Quantitative Yield (QY) was calculated using a mixture of quinine sulfate. Equation (3) was used for this determination.

$$Q_s = Q_d \left(\frac{A_d}{A_{sl}} \right) \left(\frac{E_{sl}}{E_d} \right) \left(\frac{n_{sl}}{n_d} \right)^2 \quad (3)$$

The fluorescence quantum yields, denoted as Q_s , measure the substance's efficiency in emitting fluorescent light. The symbol n represents the refraction index of the solvent being used, while the absorbance of the mixture is denoted as A . The symbol E represents the cumulative fluorescence level of the produced light. The subscripts ' d ' and ' sl ' denote the reference (quinine sulfate) and specimen, respectively.

The pH of the treatments was measured using the Elmetron CX-551 pH meter. The pH-sensitivity experiments were conducted within the pH range of 4 to 10. The photostability of the CQDs liquids was assessed by subjecting them to constant illumination using a mercury light bulb with a wavelength of 365 nm and a power output of 20 W. This was achieved by putting the CQDs liquids in quartz cuvettes. The QY was determined at two time points, namely 7 and 30 days. The experiments on sensing abilities were conducted using liquids of CQDs at a 0.20 mg/mL dosage.

Morphology Analysis

The morphological study of the generated CQDs was examined using the TEM method. CQDs were acquired with a size of less than 10 nm and exhibited good dispersion in water. Nanodots show a uniform distribution and possess a relatively low degree of size polydispersity. They have lattice fringes that are typical of CQDs. The standard dimensions of these entities range from 2 to 5 nanometers. The carbon nanomaterials derived from waste biomass have a characteristic spherical morphology.

Cells Visualization Study

The L929 cell line was used for real-time cell imaging. The cells were subjected to a 24-hour incubation period with fluids containing CQDs under standardized circumstances to address this objective. The cells were examined using an inverting microscope from Delta Optical coupled with a fluorescent adaptor. The cellular specimens were examined using two distinct filters, one with an excitation range of 460-490 nm and an emitted wavelength of 520 nm, corresponding to green fluorescence. The second filter had an excitation range of 510-550 nm and an emission wavelength of 590 nm, indicating red fluorescence.

The present study presents the HSC-ICQD technique, which integrates naturally produced precursors from biowaste with microwave radiation to augment CQDs' properties. The primary objective of this unique methodology is to enhance the fluorescence quantum yield and antioxidant characteristics of CQDs to facilitate their use in biological contexts. The research emphasizes doing a thorough characterization to evaluate the approach's efficacy.

Experimental Analysis and Outcomes

The experimental configuration used in this study comprises a hydrothermal synthesis reactor with a volumetric capacity of 150 mL. The experiment uses glucose as the carbon location, combined with water and acid. The resulting mixture is then placed in a sealed tank and exposed to hydrothermal treatment at 200°C for 12 hours. Following this, NaOH neutralizes the product, aiming for a pH value of 7. The dialysis process is performed over five consecutive days, using membranes with a MWCO ranging from 500 to 1000 Daltons. The sodium ion concentration is determined using AAS using a Philips PU9100 spectrometer, whereas FTIR spectroscopy quantifies carboxylic categories. The resultant CQDs undergo modification with Rhod-OH through a 24-hour reaction conducted at ambient temperatures and atmospheric pressures. The changed CQDs are purified using dialysis membranes with a MWCO.

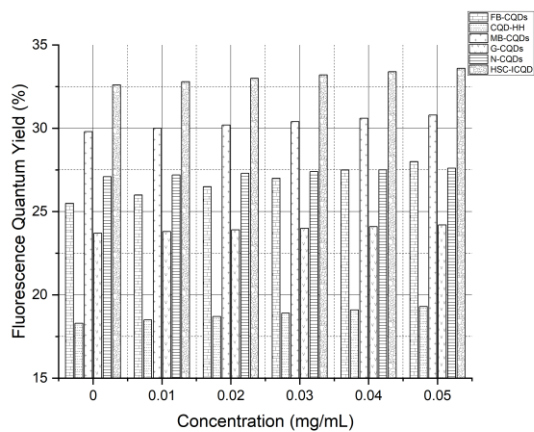


Figure 3: Fluorescence Quantum Yield Analysis of CQDs

The findings of Fluorescence Quantum Yield for varied concentrations of CQDs are shown in Figure 3. The HSC-ICQD exhibited the most excellent fluorescence quantum yield (33.1%) among all other kinds of CQDs, namely FB-CQDs (26.75%), CQD-HH (18.8%), MB-CQDs (30.3%), G-CQDs (23.95%), and N-CQDs (27.35%). The HSC-ICQD method demonstrates enhanced performance due to its hybrid synthesis strategy, which effectively modifies the quantum yield via precise techniques. The findings show considerable potential for biomedical applications, particularly in accurate imaging and sensing, where a high fluorescence quantum yield is paramount. This research presents enhanced CQDs exhibiting increased fluorescence characteristics, promising advancements in the field.

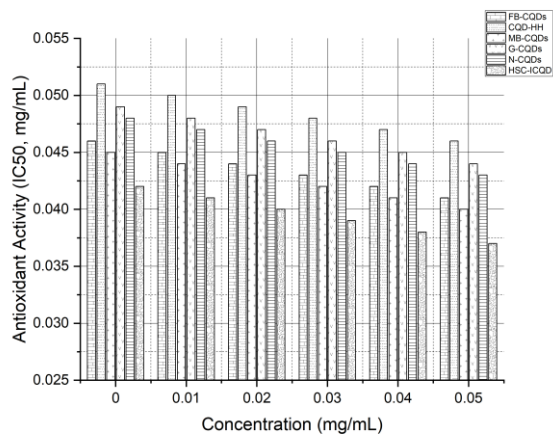


Figure 4: Antioxidant Activity Analysis of CQDs

The findings of Antioxidant Activity for varied concentrations of CQDs are shown in Figure 4. On average, the HSC-ICQD had the lowest IC₅₀ value of 0.0395 mg/mL, indicating a higher level of antioxidant activity than other CQDs. Specifically, the IC₅₀ values for FB-CQDs, CQD-HH, MB-CQDs, G-CQDs, and N-CQDs were 0.0435 mg/mL, 0.0485 mg/mL, 0.0425 mg/mL, 0.0465 mg/mL, and 0.0455 mg/mL, respectively. The increased antioxidant characteristics of the HSC-ICQD have the potential to be used in biomedical domains where the presence of robust antioxidant materials is crucial for safeguarding cells and tissues from damage caused by oxidative stress.

The findings of Rheological Behavior (Pa) for varied concentrations of CQDs are shown in Figure 5. The rheological behavior of HSC-ICQD was found to be the lowest at 0.1435 Pa, suggesting enhanced flow qualities in comparison to other kinds of CQDs such as FB-CQDs (0.1475 Pa), CQD-HH (0.1545 Pa), MB-CQDs (0.1505 Pa), G-CQDs (0.1535 Pa), and N-CQDs (0.1525 Pa). This implies that the HSC-ICQD approach has the potential to improve its applicability in biological contexts, where materials with desirable rheological characteristics are crucial for accurate administration and effectiveness.

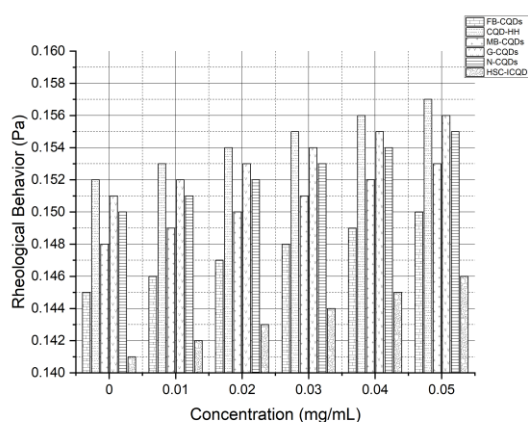


Figure 5: Rheological Behavior Analysis of CQDs

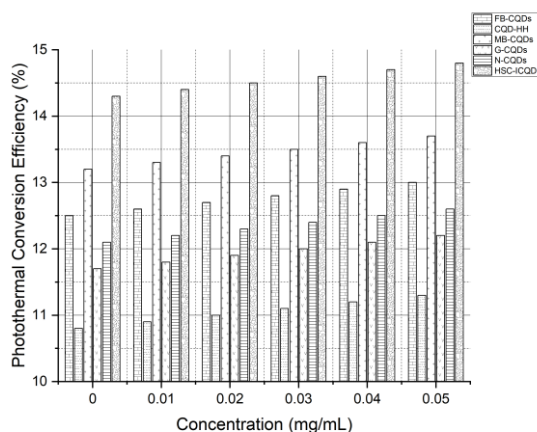


Figure 6: Photothermal Conversion Efficiency Analysis of CQDs

The Photothermal Conversion Efficiency (%) of several CQDs at varied concentrations is shown in Figure 6. The HSC-ICQD had the most excellent average photothermal conversion efficiency of 14.55%, surpassing the performance of other kinds of CQDs such as FB-CQDs (12.75%), CQD-HH (11.05%), MB-CQDs (13.45%), G-CQDs (11.95%), and N-CQDs (12.35%). The findings indicate that HSC-ICQD exhibits enhanced photothermal characteristics, demonstrating its potential as a viable option for many applications needing effective photothermal conversion, including targeted cancer treatment and drug delivery systems. The study has yielded a notable result in increased photothermal conversion

efficiency of HSC-ICQD, which holds promise for its prospective influence on biological applications.

The HSC-ICQD approach has improved performance compared to current procedures. It showcases a higher fluorescence quantum yield of 33.1%, potent antioxidant activity with an IC₅₀ value of 0.0395 mg/mL, acceptable rheological behavior measured at 0.1435 Pa, and effective photothermal conversion of 14.55%. The results underscore the promise of this approach for advanced biological applications and the production of materials.

Conclusion and Future Scope

CQDs have gained significant attention in recent years due to their remarkable fluorescence characteristics and prospective use in the biological domain. The primary objective of this study is to enhance the synthesis and characterization of CQDs by improving their fluorescence quantum yield and antioxidant characteristics. This research aims to address the increasing need for CQDs in biomedical applications. The investigation starts by examining the production and characterization of CQDs, emphasizing their importance in several biological contexts, including bioimaging, drug administration, and biosensing. The study presents the HSC-ICQD technique to tackle these issues. The present study introduces a novel approach that integrates the utilization of precursors derived from biowaste with a two-step functionalization process. This suggested method yields CQDs that exhibit exceptional fluorescence quantum yield (33.1%), antioxidant activity (IC₅₀ of 0.0395 mg/mL), favorable rheological behavior (0.1435 Pa), and efficient photothermal conversion (14.55%). Although the HSC-ICQD exhibits remarkable performance, it needs help with scalability and cost-efficiency. Solving these challenges will be crucial for effectively integrating this technology into biological contexts.

This study's future scope includes investigating supplementary changes to augment the multifunctionality of CQDs, broadening their potential uses in targeted drug delivery systems, and further refining the HSC-ICQD technology to facilitate large-scale manufacturing. The breakthroughs can significantly transform the biomedical domain by providing highly effective and adaptable nanomaterials for various therapeutic and diagnostic applications.

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