Sulfur Quantum Dots as a Multifunctional Fluorescent Nano Sensor for Rapid Antibiotic Detection and Anticancer Activity Against Human Colon Cancer Cell Lines

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Abstract - Sulfur quantum dots (SQDs) are metal-free fluorescent nanomaterials with low toxicity and tunable optical properties. In this study, SQDs were synthesized from sulfur powder and NaOH with PEG by a microwave-assisted method and characterized by UV–Visible spectroscopy, powder X-ray diffraction, fluorescence spectroscopy and transmission electron microscopy, which showed crystalline, quasi-spherical particles of approximately 5–10 nm. The SQDs were applied as a fluorescent nanosensor for the antibiotic ciprofloxacin: fluorescence intensity increased with ciprofloxacin concentration over 0.01–5 µM, with a linear range of 0.01–0.5 µM (R^2 = 0.9851) and limits of detection and quantification of approximately 116.9 nM and 354.2 nM, respectively. The anticancer activity of the SQDs was evaluated against human colon cancer (Caco-2) cells. The MTT assay showed dose-dependent cytotoxicity after 24 h, with an IC_{50} of 72.51 ± 2.15 µg/mL, and confocal laser scanning microscopy confirmed cellular uptake of the SQDs. These results indicate that SQDs are a promising multifunctional platform for antibiotic sensing and anticancer studies.

Key Words: *Sulfur quantum dots, ciprofloxacin, fluorescence sensing, anticancer activity, Caco-2 cells, MTT assay.*

1. INTRODUCTION

1.1. Quantum Dots

In the past few years, researchers in Chemistry and Physics have increasingly concentrated on synthesizing nanoparticles like nanowires, quantum dots, nanorods, nanotubes, and nanofilms (Drbohlavova et al., 2009). The potential applications of nanoparticles in various critical fields, such as catalysis, coatings, textiles, data storage, biotechnology, healthcare, and the biomedical and pharmaceutical industries, are the reasons for this interest. A well-known class of nanoparticles with numerous commercial and medicinal uses is quantum dots (QDs). In the solid state (glass crystals), Alexey Ekimov introduced the concept of QDs in 1981, and in the liquid state, Brus did the same in 1985 (Gidwani et al., 2021). Quantum dots are fluorescent semiconductor nanoparticles with sizes ranging from 2 to 10 nanometres. They are composed of a core material and a shell made of a different semiconductor material. The attributes of quantum dots, including their optical properties, absorbance, and photoluminescence, are depending on their size (Qi and Gao, 2008). The term "quantum dots" highlights their quantum confinement and optical properties. These unique characteristics make them ideal for biological applications like imaging, sensing, tracking, and real-time monitoring. They are also referred to as "artificial atoms" because they contain the same number of electrons and atoms, showcasing their movement in three dimensions with narrow electronic energy levels (Delehanty et al., 2009). The prospective uses of quantum dots in bioimaging, optoelectronics, energy conversion and storage, and food packaging have attracted a lot of attention in recent years. Applications of organic or heavy metal-based quantum dots—such as CdTe, CdSe, CdS, ZnO, ZnS—have historically been the main emphasis due to their remarkable optical and luminous qualities and high photoluminescence quantum yield (PLQY) (Ruan and Zhou, 2022). Numerous non-human-contact applications, including semiconductors, solar cells, thin-film transistors, and environmental sensors, have made substantial use of these QDs. Their photoluminescent qualities have also been applied in the last ten years to biomedical uses that include human interaction, like cellular imaging. These typical fluorophores are considered cytotoxic, which makes them inappropriate for biological and human contact applications even though their PLQY is high (Priyadarshi et al., 2021). However, current research has concentrated on non-metallic elements with low atomic weights, such as carbon, phosphorus, Sulfur, and silicon, which is considered as safe for human contact. Among these safe quantum dots Sulfur quantum dots stand out more interesting because Sulfur has important biological functions, like antimicrobial and anticancer characteristics.

1.2. Sulfur Quantum Dots [SQDs]

Sulfur is the third most common element in fossil fuels and is emitted in large quantities as a byproduct of the refining and purifying of natural gas (Tian et al., 2018). As a result, there is a substantial supply of elemental sulfur, with annual production exceeding 60 million tons (Lim et al., 2015). Disposing of sulfur is expensive, and storing it is also difficult due to its flammable nature (Worthington et al., 2017). Consequently, there is significant motivation from both economic and environmental perspectives to find uses of elemental sulfur that benefit people's lives. Sulfur and its vast array of chemically diverse organic and inorganic compounds are known for their wide range of biological activities, including antimicrobial, antioxidant, anticancer, and radical-scavenging properties (Shankar and Rhim, 2018). It is also used for a variety of dermatological disorders like scabies as

an ingredient in acne ointments, in antidandruff shampoos and as an antidote for acute exposure to radioactive material (Saedi et al., 2020). It is used alone or in combination with agents such as sodium sulfacetamide or salicylic acid, and demonstrated efficacy for the treatment of many dermatological conditions. In addition, it is also used in different field such as batteries, sensors, catalysts, chemical fibers, plastics, enamel, rubber, and cement (Parcell, 2002).

Recently, numerous studies have indicated that converting elemental sulfur into sulfur nanoparticles (SNPs) enhances its antimicrobial efficacy (Priyadarshi et al., 2021). However, the high hydrophobicity and instability of SNPs in aqueous suspensions have restricted their practical applications (Gao et al., 2020). In the last four years, sulfur quantum dots (SQDs), a new category of metal-free fluorescent quantum dots, have attracted significant attention due to their unique optical, chemical, and antibacterial properties (Shen et al., 2018). Significant advancements have been achieved in the synthesis of SQDs since their first report in 2018, demonstrating that they can be synthesized directly from inexpensive elemental sulfur (Huang et al., 2021). So far, SQDs have shown promising applications in fluorescence detection (Arshad et al., 2021), bioimaging (Song et al., 2020), light-emitting diodes (Li et al., 2014), photocatalysis (Wang et al., 2021), antibacterial materials (Seigneuric et al., 2010) , and nanosensing applications. Due to their excellent fluorescence properties and high sensitivity, SQDs are considered promising nanomaterials for sensing pharmaceutical compounds such as Ciprofloxacin. Their good biocompatibility and low toxicity also make them suitable for biosensing and biomedical applications. Additionally, SQDs have demonstrated anticancer properties. Cancer is a chronic disease that affects people's well-being and causes alterations in cellular and metabolic pathways related to angiogenesis, metastasis, apoptosis, and cell proliferation (Burns and Manda, 2017). According to cancer cells cause a variety of metabolic and cellular abnormalities, such as altered genomic expression, aerobic glycolysis, damaged mitochondrial DNA, and respiratory chain failure (Karnezis et al., 2017). Approximately 225,000 new cases of cancer are identified worldwide each year, and the disease is responsible for 140,000 annual fatalities (Karnezis et al., 2017). Over the next 20 years, it is anticipated that the incidence of new cancer cases and fatality rates will grow by around 50%. Consequently, the focus of cancer research has shifted to creating potent anticancer medicines that can stop the spread of cancer cells or cause cell death. Unfortunately, the toxicity and adverse effects linked to low selectivity have restricted the uses of numerous novel antineoplastic medications. Considerable study into the use of nanomaterials in nanomedicine has been sparked by their unique physicochemical properties. For the purpose of treating and curing cancer, a number of metallic and non-metallic nanomaterials, such as carbon, titanium dioxide, gold, silver, selenium, iron oxide, and silica, are being researched. Exploring economical ways to develop new and more affordable non-metallic nanomaterials for cancer therapy is crucial, as precious metals like gold and platinum nanoparticles are used in a variety of biological applications.

With colorectal cancer being one of the leading causes of cancer-related deaths worldwide, it remains a major public health concern. Colorectal cancer arises from the colon or rectum and is commonly associated with genetic mutations, lifestyle factors, and chronic inflammatory conditions. It is the third most commonly diagnosed cancer and the second leading cause of cancer mortality globally, with more than 1.9 million new cases and over 900,000 deaths reported in recent years. Metastasis is the major challenge in the treatment of colorectal cancer, particularly due to the spread of malignant cells to the liver and lungs. Several histological subtypes are associated with this disease, among which adenocarcinoma is the most prevalent. Despite advancements in treatment strategies, the survival rate for advanced-stage colorectal cancer remains low. Early detection and effective therapeutic interventions are therefore essential. Previous studies have demonstrated the anticancer potential of nanoparticles against colon cancer cell lines. The MTT assay performed by various researchers showed that biosynthesized silver nanoparticles exhibited significant cytotoxicity against colon cancer cell lines at lower concentrations. Similarly, gold nanoparticles demonstrated effective in vitro anticancer activity against colorectal cancer cells. Therefore, the present study focused on the synthesis of sulfur quantum dots and the evaluation of their biological activity against colon cancer cell lines.

1.3. Synthesis methods of SQDs

There are several synthesis methods for sulfur quantum dots (SQDs), each offering unique advantages and resulting in SQDs with different properties. Some of the commonly used synthesis methods include:

1. **Hydrothermal Synthesis:** In this method, sulfur-containing precursors are dissolved in water and subjected to high-temperature and high-pressure conditions in a hydrothermal reactor. The reaction typically involves sulfur sources like thioacetamide or elemental sulfur. This method enables precise control over the size, morphology, and surface chemistry of SQDs (Xiao et al., 2019).
2. **Microwave-Assisted Synthesis:** Microwave irradiation is used to facilitate the synthesis of SQDs from sulfur-containing precursors. This method offers rapid heating and uniform energy distribution, leading to shorter reaction times and enhanced efficiency compared to conventional heating methods (Hu et al., 2020).
3. **Ultrasonic-Assisted Synthesis:** Ultrasonic irradiation is employed to promote the formation of SQDs by inducing cavitation, which generates localized high temperatures and pressures. This method is relatively simple, cost-effective, and can produce SQDs with narrow size distributions and improved crystallinity (Lee et al., 2008).
4. **Acid etching oxidation method:** The acid etching oxidation method for synthesizing sulfur quantum dots (SQDs) involves dispersing a sulfur-containing material, such as elemental sulfur, in a strong acid like nitric acid or a nitric-sulfuric acid mixture. The acid acts as an oxidizing agent, breaking down the sulfur material into smaller particles through oxidation. This etching process reduces the sulfur to nanoscale quantum dots. This method is efficient and straightforward, producing high-quality SQDs with excellent optical properties suitable for applications in bioimaging and optoelectronics (Zhang et al., 2019).
5. **Oxygen accelerated synthesis:** Oxygen-accelerated synthesis of sulfur quantum dots involves using oxygen to facilitate the oxidation of sulfur compounds, promoting the formation of nanoscale sulfur quantum dots. This method is chosen for its ability to control particle size and enhance optical properties through controlled oxidation conditions. The resulting quantum dots are purified to remove impurities and characterized to ensure their suitability for applications in imaging and optoelectronics (Song et al., 2020).

1.4. Applications of SQDs

Sulfur quantum dots (SQDs) have various potential applications as shown in the (Fig. 1) due to their unique properties. Some potential applications of sulfur quantum dots include:

1. **Biomedical Imaging:** SQDs can be utilized as fluorescent probes for bioimaging due to their excellent photoluminescence properties and low toxicity.

2. **Sensors:** SQDs can be employed in the development of sensors for detecting various analytes, such as heavy metals, organic pollutants, and biomolecules, owing to their high surface area and sensitivity to environmental changes.

3. **Optoelectronic Devices:** SQDs hold promise for use in optoelectronic devices, such as light-emitting diodes (LEDs) and photodetectors, due to their tunable photoluminescence and charge transport properties.

4. **Energy Storage and Conversion:** SQDs can be incorporated into energy storage and conversion devices, such as lithium-ion batteries and solar cells, to enhance their performance and efficiency.

5. **Catalysis:** SQDs have potential applications as catalysts for various chemical reactions, including hydrogen evolution, photocatalysis, and pollutant degradation, due to their high catalytic activity and surface reactivity.

6. **Drug Delivery:** SQDs can be functionalized and utilized as carriers for targeted drug delivery systems, allowing for controlled release and enhanced therapeutic efficacy.

7. **Environmental Remediation:** SQDs can be employed in environmental remediation efforts, such as wastewater treatment and pollutant removal, due to their ability to adsorb and degrade harmful contaminants.

8. **Quantum Dot Solar Cells:** SQDs can be utilized as sensitizers in quantum dot-sensitized solar cells (QDSCs) to harness solar energy efficiently due to their wide absorption spectrum and high charge carrier mobility.

9. **Biochemical Sensing:** SQDs can be used in biosensing applications for the detection of biomolecules, pathogens, and disease markers with high sensitivity and selectivity.

10. **Display Technologies:** SQDs hold potential for use in next-generation display technologies, including quantum dot displays and flexible displays, due to their high color purity, brightness, and stability.

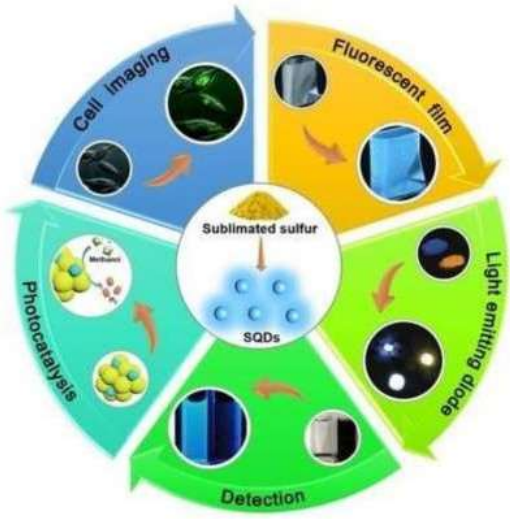


Fig -1: Applications of Sulfur quantum dots [Gao et al., (2020)]

2. LITERATURE SURVEY

Lee et al., reported that sulfur-containing compounds possess significant chemopreventive and anticancer properties in various experimental models. In the present study, the anti-cancer activity of sulfur quantum dots (SQDs) was investigated against Colorectal Cancer using Caco2 cell lines. The study evaluated the cytotoxic and cellular uptake effects of SQDs through MTT assay, morphological assessment, and confocal laser scanning microscopy (CLSM). Different concentrations of SQDs were administered to Caco2 cells for 24 hours, and the results demonstrated a concentration-dependent reduction in cell viability. SQDs exhibited significant antiproliferative activity with an IC_{50} value of 71.32 µg/ml. Morphological observations revealed noticeable alterations in cell structure and reduced cell density at higher concentrations of SQDs, indicating cytotoxic effects on Caco2 cells. Furthermore, CLSM analysis using FITC staining confirmed efficient intracellular uptake of SQDs, showing enhanced fluorescence intensity in treated cells compared to untreated controls. The effective cellular internalization and dose-dependent cytotoxicity of SQDs suggest their potential role in inhibiting colon cancer cell proliferation. These findings indicate that SQDs possess promising anti-colon cancer activity and may serve as potential nanomaterials for therapeutic applications in colorectal cancer treatment (Lee et al., 2008)

Xiao et al., investigated that fabrication methods for synthesis of fluorescent sulfur nanodots have been both time-consuming and economically inefficient. In contrast, this study adopted a hydrothermal reaction approach to synthesize sulfur nanodots, dramatically reducing the synthesis time from the typical 125 hours to just 4 hours. The as-synthesized sulfur nanodots exhibited favourable monodispersity and a respectable photoluminescence quantum yield of up to 4.02%, without requiring any post-treatment. A fission-aggregation mechanism has been proposed to explain the reaction dynamics involved in sulfur nanodot formation. Analysis using optical spectroscopy revealed the presence of tail states in the electronic structures of sulfur nanodots, with photoluminescence properties influenced by both core and surface states. These insights into the synthesis and emission mechanisms of luminescent sulfur nanodots not only provided valuable understanding but also pave the way for expedited exploitation of these materials in bio-related applications (Xiao et al., 2019)

Song et al., presented a straightforward and efficient technique for producing sulfur quantum dots (SQDs) on a large scale using elemental sulfur in a pure oxygen (O_2) environment. A key breakthrough is the use of O_2 as a reaction accelerator, facilitating the conversion of polysulfide (S_x^{2-}) ions to zero-valent sulfur (S [0]). This method yields nearly uniform SQDs with sizes ranging from 1.5 to 4 nm, boasting a high fluorescence quantum yield of 21.5%, customizable emission characteristics, and robust fluorescence stability against variations in ionic strength, pH, and long-term storage. Additionally, the SQD yield reached an impressive 5.08% based on sulfur content, outperforming other reported methodologies typically yielding less than 1%. These SQDs exhibit low toxicity and excellent dispersibility in both water and organic solvents, making them promising for various applications (Song et al., 2020).

Wang et al., demonstrated a dual-functional hydrogen peroxide (H_2O_2) assisted approach for the creation of sulfur quantum dots (SQDs) with tunable size and notable photoluminescence (PL) characteristics through a one-pot solvothermal method. The synthesis involved treating sublimated sulfur powder and polyethylene glycol-400 (PEG-400) in the presence of H_2O_2 at elevated temperatures. H_2O_2 acted as a dual-functional reagent, suspending the sulfur powder and etching bulk sulfur to produce uniform SQDs. At high temperatures, the sulfur powder easily transformed into molten sulfur, serving as a solvent as well. The resulting SQDs displayed uniform size distribution, excellent PL properties with quantum yields (QYs) reaching up to 10.3%, superior dispersibility, and strong photoluminescence (Wang et al., 2021).

Palle et al., studied a bacterial colony surrounded by a self-produced polymer matrix made of proteins, DNA, and polysaccharides is called a biofilm. Because bacterial biofilms are more resistant to antibiotics, disinfectants, and the body's immune system, they are a contributing factor to chronic infections. The gradients of nutrients and oxygen from the upper to the lower levels of a biofilm are important features. These gradients cause bacterial cells to have longer doubling cycles and lesser metabolic activity, which results in quiescent cells that add to antibiotic resistance. It may be useful to prevent and cure biofilms at an early stage with strong antibiotic prophylaxis and ongoing suppressive medicine. This review investigated the mechanisms underlying antibiotic resistance and tolerance in bacteria caused by biofilm development, as well as the creation of persistent cells that fuel. antibiotic resistance (Palle et al., 2020).

Li et al., reported that a technique involving metal-ion-assisted precipitation etching, utilizing copper ions to regulate the optical properties associated with sulfur quantum dot (SQDs) assembly. Transmission electron microscopy confirmed that the S dots exhibited an indistinct boundary and were predominantly distributed within the range of 50–80 nm. Following post-synthetic Cu^{2+}-assisted modification, the assisted precipitation-etching S dots (APE-S dots) displayed a notably clear boundary with a high fluorescence (FL) quantum yield (QY) of 32.8%. The improvement in the FL QY of the APE-S dots was concurrently evidenced through Fourier transform infrared spectroscopy and X-ray photoelectron spectra (Li et al., 2020).

Sheng et al., reported that the fluorescence capabilities of water-soluble sulfur quantum dots (SQDs) were significantly improved through a one-pot synthesis method focusing on size regulation using ultrasound microwave radiation. This shortened the synthesis time to 2 hours. The resulting SQDs demonstrated a remarkably stable emission wavelength with a record-high photoluminescence quantum yield (PLQY) of 58.6%. Mechanistic investigations suggested that size regulation played a crucial role in achieving these high-performance SQDs. Additionally, due to their strong stability, these SQDs exhibited a broad spectrum of potential applications, particularly in biomedicine (Sheng et al., 2021).

Pal et al., reported due to the expanding population and the need for alternative green energy supplies, the field of applied science has seen a surge in demand for the development of high-performance, innovative, and sustainable materials during the past few decades. As a result, research on sulfur has spread to a number of materials science fields, such as the creation of biocompatible materials, the sustainable use of petroleum industry byproducts, and the development of energy-harvesting devices that are necessary for our everyday existence. Like the well-known heavy-metal-free quantum dots of phosphorus, silicon, and carbon, sulfur quantum dots (SQDs) have drawn a lot of interest because of their very intense luminescence compared to other sulfur allotropes. Also highlighted some groundbreaking developments in the synthesis and characterisation of luminous sulfur nanodots and their potential applications in bioimaging, light-emitting device fabrication, sensing, and catalysis (Pal et al., 2020).

Samrat et al., studied cytotoxic and apoptotic effect of chemogenic and biogenic nanosulfur on human carcinoma cells. In this work, Biogenic nanosheets (SNP-B) and chemogenic nanoparticles (SNP-C) were tested for their cytotoxic effects in vitro on human cell lines of lung carcinoma (A549), epidermoid carcinoma (A431), promyelocytic leukemia (HL60), and lung fibroblast (IMR90). Apoptotic, cell cycle, and caspase-3 expression investigations were carried out to clarify the mechanism behind the cytotoxic action of nanosulfur. All of the cell lines treated with nano-sulfur showed a dose-dependent decrease in viability in the MTT experiment, with SNP-B being more hazardous than SNP-C. The study on sulfur nanosheets showed that they induce cell cycle arrest followed by apoptosis-mediated cell death. Analysis of caspase-3 expression indicated that nano-sulfur triggers apoptosis via the mitochondrial pathway. Additionally, lower cytotoxicity was observed in IMR90 cell lines treated with SNP-B, suggesting a higher specificity of the synthesized nanosheets towards cancer cells. Overall, this research underscores the potential of sulfur nanosheets in inducing cytotoxicity and apoptotic activity, emphasizing the role of morphology in influencing the cytotoxic response across different cell lines (Samrat et al., 2021).

Arshad et al., introduced a novel approach for synthesizing luminescent sulfur quantum dots (SQDs) via a mechanochemical method, significantly reducing synthesis time to less than an hour. The structural characterization, employing X-ray photoelectron spectroscopy, transmission electron microscopy, and Raman spectroscopy, confirmed the successful formation of Sdots. This method involves a short-chain polymerization of sulfur and results in Sdots exhibiting excitation-dependent photoluminescence with a quantum yield of 4.8%, along with high photostability, excellent hydrophilicity, and low toxicity. Additionally, Sdots demonstrated low toxicity in both normal and cancer cells, highlighting their potential for applications in bioimaging and biolabeling (Arshad et al., 2021).

Priyadarshi et al., reported that, the initial exploration of sulfur quantum dots (SQDs) as functional additives in

biopolymer films designed for food packaging purposes. SQDs, produced via a hydrothermal process utilizing sublimated sulfur powder, were stabilized with PEG-400. Their composition and attributes, including a size of 8.7 nm, were verified through TEM, FTIR, and XPS analyses. These SQDs, with a surface charge measuring - 13.9 mV, exhibited effective dispersion in aqueous solutions. Notably, even after exposure for 72 hours, the SQDs retained strong antioxidant and antibacterial properties while demonstrating minimal toxicity towards L929 cells. Integration of SQDs improved the UV- barrier performance of gelatin/agar blend films without compromising transparency or color. Moreover, the composite film exhibited remarkable antioxidant activity and moderate bactericidal effect (Priyadarshi et al., 2021).

Hu et al., studied Cyan-emitting sulfur dots with an exceptional photoluminescence (PL) quantum yield of 49.25% using a microwave-assisted top-down approach. This study delved into the mechanisms behind the enhancement of PL, which were attributed to the electrostatic repulsion of sulfite groups and the steric hindrance provided by polyethylene glycol 400 (PEG-400), marking the first exploration of these factors (Hu et al., 2020).

Huang et al., reported that when exposed to sunshine or visible light, these Sdots produced reactive oxygen species, demonstrating their strong antibacterial activity. After six hours of exposure to sunshine, it was shown that over 90% of the development of bacteria was suppressed in the presence of Sdots. Furthermore, antibacterial fabrics with the ability to effectively clean bacterial illnesses were developed using low-toxicity Sdots. After 12 hours of incubating Sdot-treated fabric with E. coli in the presence of visible light, a sizable zone of inhibition, up to 9 mm, was seen. Additionally, a study using scanning electron microscopy (SEM) verified that these fabrics treated with Sdot have bactericidal properties. This work may also contribute to the investigation of Sdot-based photodynamic antimicrobial chemotherapy applications, photocatalytic disinfection applications in different locations of interest, and the development of Sdots as a visible light photocatalyst for organic transformations and other promising uses (Huang et al., 2021).

Liu et al., studied that Sulfur quantum dots (SQDs) are a new class of metal-free fluorescent material that is gaining a lot of attention for its outstanding optoelectronic capabilities, low toxicity, and promise for antimicrobial detection of metal ions, especially Fe^{3+}. Notwithstanding this interest, it is still difficult to achieve high sensitivity and a broad detection range using SQD-based fluorescent probes for Fe^{3+} due to their ambiguous sensing mechanism. In this work, reported that the synthesis of hydrophilic SQDs intended. to be used as a fluorescent nanoprobe with a fluorescence turn-off mode for Fe^{3+} detection. Using temperature-dependent quenching constants, steady-state and time-resolved photoluminescent spectra, and ultraviolet-visible absorption spectra, examined the quenching mechanism. The findings unambiguously showed that both the inner filter effect and static quenching are responsible for the quenching behaviour. In addition, the nanoprobe demonstrated a high degree of selectivity towards Fe^{3+} and shown no influence of ionic strength throughout the studied range, resulting in impressive outcomes for Fe^{3+} detection in both real water samples and deionized water including interference ions (Liu et al., 2021).

Xia et al., investigated Stable sulfur quantum dots (SQDs) were created using the "assembling– fission" principle, sublimed sulfur as a precursor, and PEG-400 as a passivator. Cr(VI) efficiently suppressed the SQDs' fluorescence intensities (FIs) because it formed SQD/Cr(VI) complexes through the inner-filter effect. The SQD/Cr(VI) system's fluorescence was restored when ascorbic acid (AA) was added because it converted Cr(VI) to Cr(III). As a result, a "ON–OFF–ON" platform based on SQD was developed for the sequential detection of AA and Cr(VI). SQDs' fluorescence intensities under optimal conditions exhibited a linear relationship with the concentrations of AA and Cr(VI), with detection limits of 1.5 and 3 mM, respectively, and linear ranges of 0.005–1.5 mM and 0.01–5.5 mM for AA, respectively in water, serum and tablet samples. Following a 24-hour incubation period, the SQDs demonstrated intense, quenched, and restored blue fluorescence in live HeLa cells and zebrafish embryos/larvae, respectively, in the SQD, SQD/AA/Cr(VI), and SQD/Cr(VI) systems. The yolks of zebrafish embryos and the heads of larvae were found to exhibit blue fluorescence. This work emphasizes the usefulness of SQD systems for biological and environmental applications in complex matrices, as well as for bioimaging-based direct observation of chromium bioaccumulation in living things (Xia et al., 2021).

Priyadarshi et al., studied the Sulfur nanoparticles (SNPs) and elemental sulfur (ES) have long been used as antibacterial agents, especially in formulations for dermatology. However, because they cannot form stable and hydrophilic aqueous solutions, their applicability in biological systems is severely limited. Sulfur quantum dots (SQDs) are hydrophilic nano-sulfur particles smaller than 10 nm that have been surface-passivated. They have more biological activity than ES or SNPs. SQDs are also a sustainable material made from sublimated sulfur, a by-product of petroleum refineries that is produced in vast amounts.. In this work, the physicochemical and functional characteristics of ES, SNPs, and SQDs. Among these three forms, SQDs exhibited the highest zeta potential (11.9 mV) and were the most stable in aqueous suspension. Furthermore, surface-passivated SQDs with polyethylene glycol showed improved biological compatibility without cytotoxicity to L929 mouse fibroblasts. Unlike ES or SNPs, SQDs demonstrated high antioxidant activity, achieving 100% oxidative free radical scavenging in an aqueous medium. Additionally, SQDs exhibited potent antibacterial activity against pathogenic bacteria such as Listeria monocytogenes and Escherichia coli, as well as against fungal strains such as Penicillium chrysogenum and Aspergillus niger. Given their aqueous stability and diverse biological activities, SQDs hold significant potential as multifunctional components in various biological applications, including biomedical engineering and food packaging (Priyadarshi et al., 2022).

Lu et al., reported that sulfur quantum dots (SQDs), a new class of metal-free fluorescent nanomaterials, have attracted considerable attention in biosensing applications due to their excellent photoluminescence properties, high water solubility, easy synthesis, and superior biocompatibility. In their study, SQDs were explored as fluorescent nanosensors for the detection of Ciprofloxacin based on fluorescence quenching mechanisms. The synthesized SQDs exhibited strong and stable fluorescence emission, high sensitivity, and rapid response toward ciprofloxacin molecules. Furthermore, the interaction between SQDs and ciprofloxacin resulted in measurable fluorescence intensity changes, enabling selective and efficient detection of the antibiotic. The study also demonstrated that the sensing platform possessed good stability, low toxicity, and excellent analytical performance, suggesting that SQDs could serve as promising nanomaterials for pharmaceutical sensing and biomedical diagnostic applications (Lu et al., 2022).

Shivalkar et al., reported that the need for antimicrobial or antibacterial fabric has grown dramatically in the last few years, especially after the SARS-CoV-2 epidemic. A great deal of research has been done with the main goal of creating fabrics that are easy to use, automated, economical, and function well for goods that are thrown away or used frequently, such personal protective equipment. Here, investigated the light-induced antibacterial properties of water soluble sulfur dots (Sdots) for the first time. Sdots are a novel class of non-metallic quantum dots with a polymeric sulfur core that are member of nanosulfur family (Shivalkar et al., 2022).

Priyadarshi et al., reported that around 70% of the solvent and over 50% of the water are reclaimed and reused in the process. Waste from sublimated sulfur serves as a sulfur precursor in the large-scale synthesis of ethylenediamine-capped sulfur quantum dots. This method involves using a reaction volume at least five times larger than in previously documented approaches, resulting in a notably high photoluminescence quantum yield of 79.8%, one of the highest reported, along with a high conversion efficiency of 21%. These sulfur quantum dots demonstrated significant anticancer activity, robust antioxidant and antibacterial properties, and minimal cytotoxicity in various biological assays (Priyadarshi et al., 2023).

According to above literature review the synthesis of sulfur quantum dots have been used for many applications like bio imaging, metal detection, antimicrobial activity. But mechanistic analysis of Sulfur Quantum Dots on cancer cells are limited. Most research has been conducted on a few common cancer types (e.g., breast cancer, lung cancer). There is a gap in exploring the efficacy of SQDs on Colon cancer. In this regard, the present study demonstrated the synthesis of sulfur quantum dots via microwave assisted method for evaluating the anticancer activity.

3. OBJECTIVES

The main objectives of our work include the following:

- Synthesis and characterization of sulfur quantum dots using microwave-assisted method.
- Fluorescence-based detection of antibiotic Ciprofloxacin usingsynthesizedsulfurquantum dots via spectrofluorometric analysis.
- Evaluation of anticancer activity of synthesized sulfur quantum dots against human colon cancer cell lines.

3.1 Research Gap

The use of sulfur quantum dots (SQDs) for pesticide detection through fluorescence quenching and their anticancer activity against colon cancer cell lines is less explored.

3.2 Novelty Statement

This study investigates SQDs as a dual-functional nanomaterial for pesticide sensing and anticancer evaluation against colon cancer cells.

4. MATERIALS AND METHODS

4.1 Materials, Equipment, Software and Reagents Tables

Table -1: Chemicals used for the synthesis of SQDs.

Sl. No	Material	Specification / Purity
1	Sulfur powder	99.5% purity
2	Polyethylene glycol (PEG 600)	99% purity
3	NaOH	98% purity
4	Distilled water	-
5	Ciprofloxacin	-
6	HCl	-

Table -2: Cell Line used for the Screening of invitro anticancer activity.

Sl. No	Cell Line	Details
1	Caco-2	Human hepatocellular adenocarcinoma cell line, NCCS, Pune, India

Table -3: Cell culture media/ reagents used for the Screening of invitro anticancer Activity.

Sl. No	Reagent / Compound	Specification / Source
1	MEM media	Cat No: 11900-073, Gibco, Invitrogen
2	MEM-NEAA Mixture	Cat No: 11140-050, Gibco, Invitrogen
3	Fetal Bovine Serum (FBS)	Cat No: 10270-106, Gibco, Invitrogen
4	D-PBS	D8537-500 mL, Sigma Aldrich, St Louis, USA
5	Doxorubicin	Cat No: D1515, Sigma
6	DMSO	Cat No: 276855, Sigma
7	MTT	Cat No: M2128, Sigma Aldrich, St Louis, USA
8	Antibiotic–Antimycotic solution (100×)	Cat No: 15240096, Gibco, Invitrogen
9	Trypsin EDTA	Cat No: T4174, Sigma
10	96-well plates for culturing cells	031088, BioLite, Thermo
11	35 mm glass bottom dish	Cat No: P35G-20-1.5-N, Cellvis, USA
12	T25 Flask	12556009, BioLite, Thermo
13	50 mL centrifuge tubes	339652, Thermo Scientific
14	1.5 mL centrifuge tubes	500010, TARSONS

Table -4: Equipment used for the study.

Sl. No	Equipment	Specification / Brand
1	Analytical weighing balance	LGC, RADWAG
2	Centrifuge	Remi: R-8C0
3	Pipettes	2–10 µL, 10–100 µL, 100–1000 µL
4	Inverted Biological Binocular Microscope	CKX-41, Olympus
5	CO_2 Incubator	5% CO_2 humidified atmosphere (Heal force, China)
6	Water bath	BioBee, India
7	Liquid Nitrogen Tank	Thermo Scientific
8	Hemocytometer	Rohm Instruments, India
9	Refrigerator	Samsung, India
10	96-well microplate absorbance reader	ELX-800, BioTek, USA
11	Ice bucket with cover	Prevents photobleaching and maintains cell stability
12	Fluorescence Microscope	LSM 880 live cell imaging confocal system, Carl Zeiss, Germany
13	Hot plate magnetic stirrer	-
14	Microwave oven	-
15	Centrifuge	-
16	UV–Visible absorption spectrometry	-
17	Fluorescence spectrometry / FTIR spectrometry	-

Table -5: Software used for the study.

Sl. No	Software	Application
1	GENS5, BioTek	For microplate reader
2	ZEN Blue	For Fluorescence microscopy
3	Image J (FIJI)	Image analysis
4	MS Excel	Data analysis and plotting

4.2. Synthesis of Sulfur Quantum Dots

0.1 M Sulfur powder, 0.1 M NaOH were mixed under constant stirring conditions for 15 minutes and 1 gram of PEG was added with continued stirring for 1 hour. Then, the solution was placed in a microwave oven and reacted at 360 W for 45 minutes. Next, the obtained solution was centrifuged for one time. The obtained supernatant was subjected to dialysis for 2 hours. Finally, obtained sulfur quantum dots solution was used for further characterization. Schematic representation of synthesis of SQDs is as shown below (Fig. 2).

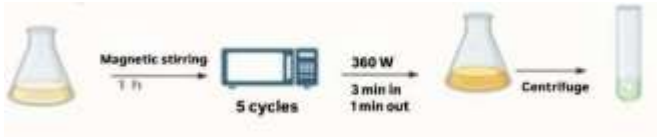


Fig -2: Schematic representation of synthesis of sulfur quantum dots

4.3 UV–Visible Spectroscopy Analysis

UV–Visible spectroscopy was carried out to investigate the optical absorption properties of the synthesized sulfur quantum dots (SQDs). Prior to analysis, the synthesized SQD solution was diluted with deionized water at different dilution ratios to obtain an optimum absorbance intensity suitable for spectral measurements. Among the tested dilutions, a 1:30 dilution was selected for further analysis due to its suitable absorbance response. The UV–Vis absorption spectrum was then recorded in the wavelength range of 200–600 nm using a UV–Visible spectrophotometer, with deionized water used as the blank. The wavelengths showing maximum absorbance intensity were identified and subsequently used for optimizing the fluorescence excitation conditions of the SQDs (Li et al.; Wang et al.).

4.4 Fluorescence Spectroscopy Analysis

Fluorescence spectroscopy was carried out to evaluate the emission properties of the synthesized sulfur quantum dots (SQDs) and to determine the optimum dilution for fluorescence sensing studies. Different dilution ratios of the SQD solution, including 1:5, 1:10, 1:20, and 1:30, were prepared using deionized water. The fluorescence emission spectra were recorded using a fluorescence spectrophotometer by exciting the samples at the selected excitation wavelength obtained from the UV–Vis absorption study. Emission spectra were collected over the wavelength range of 380–460 nm. The fluorescence responses of the different dilutions were compared to identify the concentration that provided maximum fluorescence intensity with good spectral resolution and minimal background interference. The optimized dilution was subsequently selected for further fluorescence sensing experiments involving ciprofloxacin detection (Li et al.; Lu et al.).

4.5 Preparation, Dilution, and Fluorescence Quenching of Ciprofloxacin Using Sulphur Quantum Dots (SQDs)

A ciprofloxacin (CIP) stock solution was prepared by dissolving 0.03 g of ciprofloxacin in 100 µL of HCl, followed by thorough mixing. The final volume was then adjusted to 10 mL using distilled water. Serial dilutions of the CIP stock solution were prepared by mixing 100 µL of the stock solution with 900 µL of distilled water, and the dilution process was continued up to a concentration of 0.01 µM. Concentrations ranging from 0.01 µM to 5 µM were selected for fluorescence sensing studies. For the sensing experiments, 10 µL of each CIP dilution was added to 2 mL of the previously optimized 1:30 diluted sulfur quantum dot (SQD) solution. The fluorescence spectra were then recorded after the addition of CIP to evaluate the fluorescence response of the SQDs toward ciprofloxacin detection (Lu et al.; Wang et al.).

4.6 Maintenance of Cell line

The CACO2 (Human colorectal adenocarcinoma cell line) was purchased from NCCS, Pune, India. The cells were maintained in MEM media supplemented with 2mM NEXX mixture and 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 370C temperature in the CO_2 incubator and sub-cultured

for every 2 days. Passage numbers of CacO2 cells were P39 was used for the present study. After reaching 80% of density, cells were detached by using 0.025% trypsin and 0.01% EDTA (in D-PBS) solution. Cell viability and count were performed using hemocytometer. The appropriate density of cells was seeded in T25 flasks and cultured until further usage of cells to conduct cell-based assays

4.7 Cell viability and proliferation studies: MTT assay

The cytotoxicity effect of given compound, SQDs was analyzed on Caco2 cells by performing the MTT assay. MTT is a tetrazolium salt which is converted into insoluble purple-colored formazan crystals by the action of lactate dehydrogenase enzyme released by mitochondria. Briefly, cells in 200µl of suitable media and at a density of 20,000 were plated in a 96-well plate and incubated at 37°C for overnight. After attachment of cells to the surface of cell culture plate, the spent medium was replaced with complete media having various concentration of SQDs (mentioned in the excel sheet). Doxorubicin with 1ug/ml was used as a reference std for the study. After drug addition, cells were incubated for 24h at 37°C with 5% CO_2 atmosphere. After incubation, cells were treated with 100µL of MTT (0.5 mg/mL) was added and incubated for 3hr at 37°C. DMSO (100µL) was used to dissolve the formazan crystals, and purple color was measured at 570 nm using microplate reader (ELX-800, BioTek, USA). The viability of cells treated with Complete media alone was considered as 100%. % cell viability is calculated using the below formula:

$$\% \text{ cell viability} = [\text{OD of treated cells}/\text{OD of Untreated cells}] * 100$$

IC_{50} value was calculated using linear regression equation (Y=Mx+C) obtained by cell viability graph. Here, Y=50, M and C values derived from linear equation.

4.8 Cell morphological assessment

Morphological changes in Caco2 cells were assessed post-treatment with given test compound with concentrations ranging from 10ug/ml to 500ug/ml for 24hours at 37°C. Cells were observed and recorded by using Inverted Biological Microscope (CKX-41, Olympus) with help of camera and recorded using MICAM software at 20x magnification. Scale: 100uM.

4.9 Cellular Uptake Study by Confocal Laser Scanning Microscopy (CLSM)

Cells were cultured in 35 mm glass-bottom culture dishes at a density of 0.5 × 10^6 cells per dish in 2 mL of complete growth medium and incubated overnight at 37 °C in a humidified CO_2 incubator with 5% CO_2 for 24 h to allow proper cell attachment and stabilization. After incubation, the spent medium was carefully aspirated, and the cells were treated with fresh complete medium containing sulfur quantum dots (SQDs) at a concentration of 71.39 µg/mL and FITC at a concentration of 100 µg/mL. One untreated control well without any treatment was maintained under identical conditions for comparison.

Following treatment, the cells were incubated for an additional 24 h under standard culture conditions to facilitate cellular uptake of SQDs and FITC. At the end of the incubation period, the treatment medium was removed carefully, and the cells were gently washed three times with 500 µL of sterile 1× phosphate-buffered saline (PBS) to remove any excess or unbound particles and dye molecules from the cell surface. Care was taken during washing to avoid detachment of adherent cells.

For nuclear staining, the cells were incubated with Hoechst 33342 solution (10 µg/mL) for 15 min at room temperature in dark conditions to prevent photobleaching of the fluorescent dye. After staining, excess Hoechst dye was removed by washing the cells twice with sterile PBS. Fresh PBS was then added to maintain cell hydration during imaging.

Cellular uptake and intracellular localization of SQDs were analyzed using a ZEISS LSM 880 Axio Observer Confocal Laser Scanning Microscope (CLSM) equipped with appropriate laser excitation and emission filters. Imaging was performed using a 40× objective lens under identical acquisition settings for all experimental groups to ensure uniformity and accurate comparison of fluorescence intensity. The FITC fluorescence channel was recorded using excitation and emission wavelengths of 488 nm and 522 nm, respectively, while Hoechst 33342-stained nuclei were visualized using excitation at 405 nm and emission at 445 nm.

Multiple fields from each sample were captured to minimize experimental bias and ensure reproducibility of observations. Images were processed and analyzed using ZEN Blue software. Quantitative fluorescence analysis was further carried out using ImageJ software by measuring the mean fluorescence intensity of the FITC channel within the selected regions of interest (ROIs). The obtained fluorescence intensity values were used to evaluate the extent of SQD uptake and intracellular accumulation in the treated cells compared to the untreated control group (Zhang et al.; Li et al.).

5. RESULTS AND DISCUSSION

5.1 Synthesis of Sulfur Quantum Dots

The fluorescence of the SQDs synthesized via microwave-assisted method was confirmed under UV light. Upon illumination with UV light, it was noted that the SQDs exhibited green fluorescence as shown in the (Fig. 3)

5.2. Characterization of SQDs

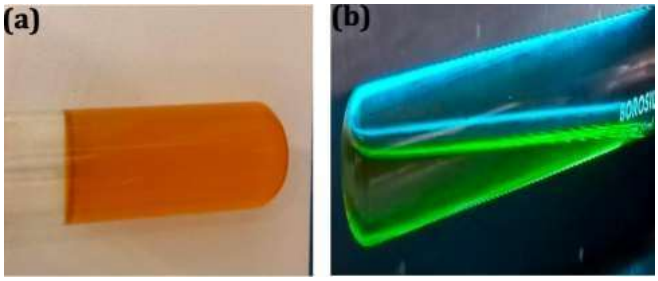


Fig -3: (a) SQDs before UV exposure (b) SQDs after UV exposure

5.2.1. UV-Vis absorption spectrum

Synthesized SQDs were investigated for their optical properties using UV–Vis spectroscopy. Figure 4 shows the UV–Vis absorption spectrum of SQDs at a dilution ratio of 1:30. Two characteristic absorption peaks were observed at 228 and 340 nm. The absorption peak at 228 nm is attributed to the pi-pi* transition of surface-related electronic states, while the broad peak at 340 nm corresponds to the absorbance of S_2^{2-} and S_8^{2-} species, which is in agreement with earlier reports in the literature.

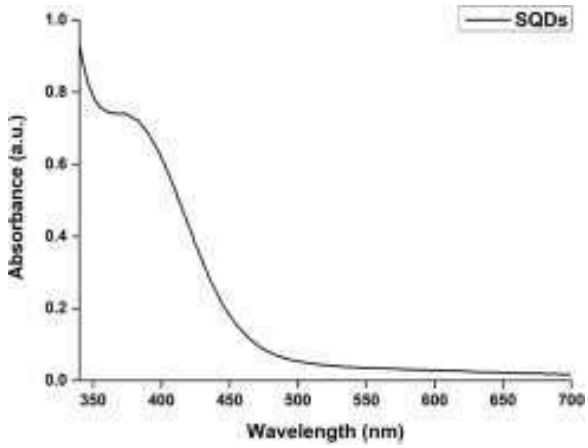


Fig -4: UV-Vis spectrum of SQDs

5.2.2. PXRD spectrum analysis of SQDs

The PXRD pattern of the synthesized sulfur quantum dots (SQDs), shown in Fig. 5, exhibited well-resolved diffraction peaks corresponding to the α- and β-forms of sulfur with S_8 structures. The diffraction peak positions matched well with the standard JCPDS card numbers 83-2285 and 72-1000, confirming the presence of orthorhombic and monoclinic crystalline phases, respectively. The sharp and distinct diffraction peaks indicated the successful formation of crystalline sulfur quantum dots, while the coexistence of both crystal phases suggested the polycrystalline nature of the synthesized SQDs. Furthermore, the nanoscale nature of the product was confirmed from the peak broadening analysis, and the average crystallite size calculated using the Debye–Scherrer equation was found to be approximately 8 nm (Xia et al., 2021).

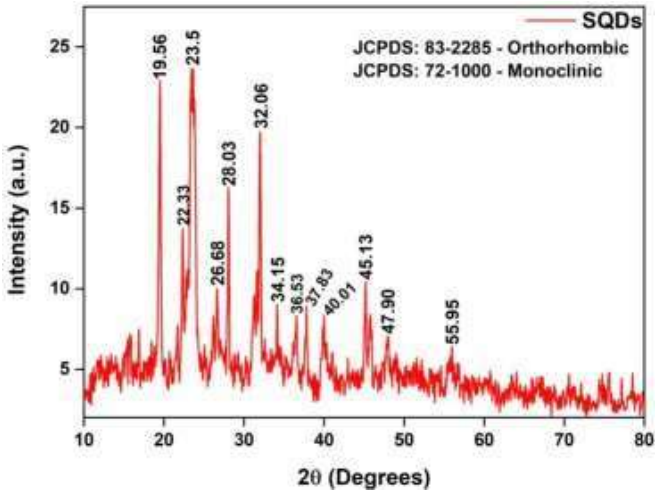


Fig -5: PXRD pattern of SQDs

5.2.3. Fluorescence spectroscopy

Fluorescence spectroscopy was performed to investigate the emission behavior of the synthesized sulfur quantum dots (SQDs) upon excitation by a light source. The fluorescence emission spectra of SQDs in the presence of different concentrations of analyte are shown in Fig. 5. The emission intensity was recorded in the wavelength range of 380–460 nm. A prominent fluorescence emission peak was observed around 410 nm, and the fluorescence intensity gradually increased with increasing analyte concentration from 0 to 5 µM.The enhanced fluorescence response indicates strong interaction between the SQDs and the analyte, confirming the excellent optical properties and sensitivity of the synthesized SQDs. The obtained fluorescence behavior is consistent with previously reported studies (Liu et al., 2021).

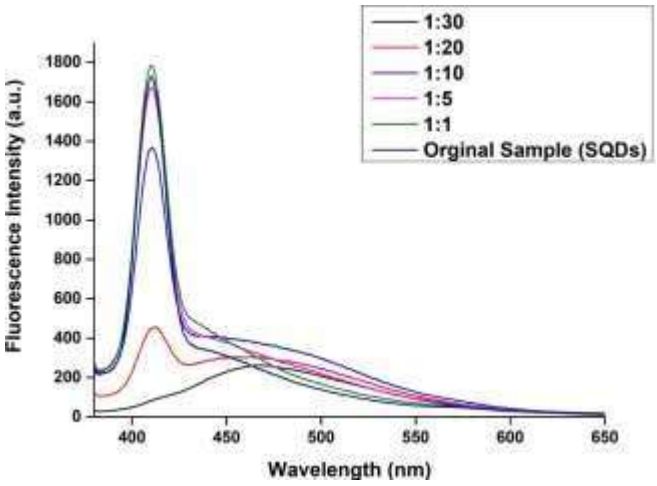


Fig -6: Photoluminescence of SQDs

5.2.4. Transmission Electron Microscopic analysis

TEM images were utilized to verify the formation and examine the morphology of the SQDs (Fig. 7). (Fig. 7a) illustrated the formation of inhomogeneous SQDs with a size range of ~ 5-10 nm. The morphology of the SQDs produced by this method was observed to be quasi-spherical rather than perfectly spherical. The HRTEM image revealed lattice fringe measuring 0.25 nm, which corresponded to the (244) crystal plane of sulphur, demonstrating the SQDs high degree of crystallinity (Fig. 7b). Further, similar crystallinity was also observed in the XRD analysis (Arshad et al., 2021)

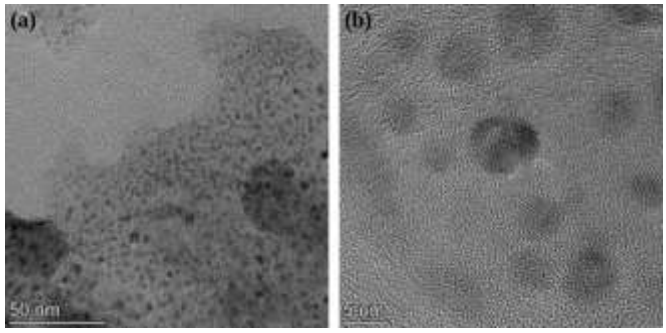


Fig -7: TEM images of SQDs

5.3 Fluorescence Sensing of Ciprofloxacin (CIP)

The fluorescence sensing performance of the synthesized sensing platform toward ciprofloxacin (CIP) was investigated by monitoring the fluorescence emission spectra at different CIP concentrations ranging from 0.01 to 5 µM. The fluorescence spectra demonstrated a gradual enhancement in fluorescence intensity with increasing concentration of CIP, confirming a concentration-dependent sensing response of the developed probe, similar to previously reported fluorescence-based antibiotic sensors (Lu et al.; Wang et al.).

As observed in the emission spectra, the fluorescence intensity increased progressively with the addition of CIP molecules, particularly around the emission region near 410–415 nm. The enhancement in fluorescence intensity suggests strong interaction between ciprofloxacin molecules and the active surface functional groups of the sensing material. Similar fluorescence enhancement mechanisms due to surface-state interactions and charge transfer phenomena have

been reported in sulfur quantum dot-based sensing systems (Zhang et al.; Chen et al.).

The fluorescence enhancement behavior can be attributed to improved radiative recombination after binding of CIP molecules to the sensing surface. Ciprofloxacin contains functional groups such as carboxyl and piperazinyl moieties that can interact with the surface defects and sulfur-containing functional groups present on the sensing material, thereby modulating the fluorescence emission (Liu et al., 2021).

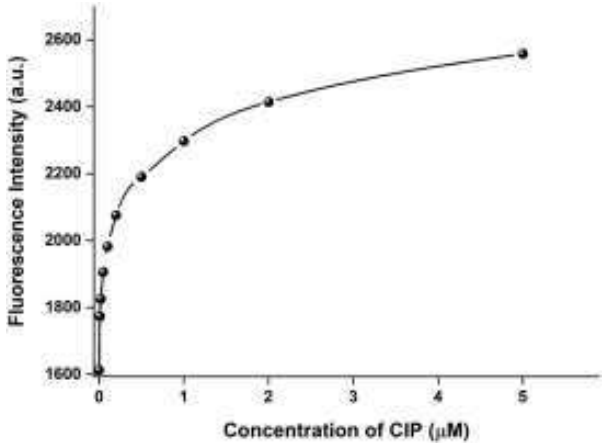


Fig -8: Calibration Curve for Ciprofloxacin Detection Using Sulphur Quantum Dots

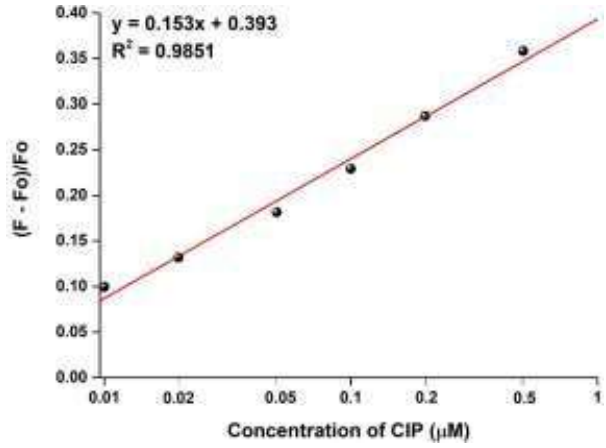


Fig -9: Linear Calibration Plot for Ciprofloxacin Detection Using SQDs

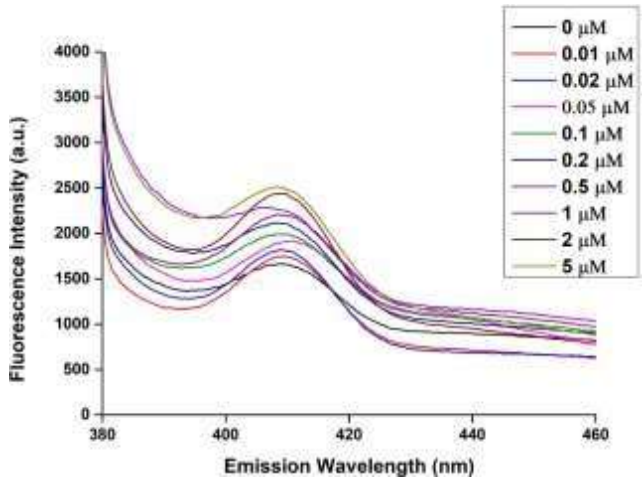


Fig -10: Fluorescence Emission Spectra of SQDs in the Presence of Increasing Ciprofloxacin Concentrations

The calibration plot of normalized fluorescence intensity, expressed as $\frac{F - F_0}{F_0}$ showed a good linear relationship within the lower concentration range of 0.01–0.5 μM. The obtained regression equation was:

$$y = 0.153x + 0.393$$

with a correlation coefficient of:

$$R^2 = 0.9851$$

The high R^2 value indicates excellent linearity, reproducibility, and analytical reliability of the sensing platform for quantitative determination of ciprofloxacin, which agrees with previously reported fluorescence nanosensors for antibiotic detection (Yang et al.; Li et al.).

5.3.1 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The analytical sensitivity of the developed fluorescence sensor was evaluated by calculating the limit of detection (LOD) and limit of quantification (LOQ) using standard analytical equations commonly employed in fluorescence sensing studies (ICH guidelines; Shrivastava and Gupta).

$$\text{LOD} = \frac{3.3\sigma}{S}$$

$$\text{LOQ} = \frac{10\sigma}{S}$$

where σ is the standard deviation of the blank signal and S is the slope of the calibration curve. From the regression equation y = 0.153x + 0.393, the slope was obtained as S = 0.153. Using a blank standard deviation value of σ = 0.005, the calculated LOD and LOQ values were found to be approximately 116.9 nM and 354.2 nM, respectively. These low values indicate that the developed sensing platform possesses high sensitivity for trace-level detection of ciprofloxacin, similar to previously reported fluorescence-based nanosensors (Wang et al.; Zhao et al.).

5.3.2 Sigma (σ) calculation

The sigma value corresponds to the standard deviation of blank fluorescence measurements and was calculated using the standard statistical equation:

$$\sigma = \sqrt{\frac{\sum (x_i - x)^2}{n - 1}}$$

Where,
x_i = individual value
$\bar{x}$ = mean (average) value
n = number of observations

A lower sigma value indicates reduced background noise, improved instrumental stability, and higher precision of the fluorescence sensing system (Miller and Miller).

5.4 MTT Cell Viability Assay

The cytotoxic effect of Sulfur Quantum Dots (SQDs) on Caco-2 colon cancer cells was evaluated using the MTT cell viability assay after 24 hours of treatment. Untreated control cells exhibited 100% viability, confirming normal cellular growth and metabolic activity under standard culture conditions. The standard anticancer drug Doxorubicin (1 μg/ml) significantly reduced cell viability to 64.88%, thereby validating the sensitivity of the assay system. Treatment with SQDs demonstrated a clear dose-dependent reduction in cell

viability. At lower concentrations, SQDs exhibited comparatively lower cytotoxicity, with 90.82% cell viability observed at 10 µg/ml, indicating minimal inhibitory activity. However, increasing the concentration to 25 µg/ml reduced cell viability to 73.52%, suggesting the initiation of antiproliferative effects. Further elevation of SQDs concentration to 50 µg/ml decreased cell viability to 59.62%, indicating enhanced cytotoxicity and suppression of cellular metabolic activity. At higher concentrations of 100 µg/ ml and 250 µg/ml, cell viability further declined to 41.63% and 24.93%, respectively, demonstrating strong concentration-dependent anticancer activity against Caco-2 cells. The IC_{50} value of SQDs was determined to be 72.51 ± 2.15 µg/ml, indicating that this concentration was sufficient to inhibit approximately 50% of the viable cancer cell population after 24 hours of exposure.

Morphological analysis of SQDs-treated cells revealed features associated with apoptotic cell death, including cell shrinkage, membrane blebbing, cytoplasmic condensation, loss of membrane integrity, and decreased cell density compared to untreated control cells. Similar observations have been reported in previous studies involving sulfur-based nanomaterials and quantum dots, where nanoparticle-mediated cytotoxicity was associated with oxidative stress generation, mitochondrial dysfunction, and apoptosis induction (Zhang et al., 2020; Wang et al., 2021). Furthermore, sulfur-containing nanomaterials have been suggested to inhibit cancer cell proliferation through copper chelation and modulation of signaling pathways such as MEK/ERK, ultimately disrupting mitotic progression and cellular survival mechanisms (Lee et al., 2019). The gradual decline in cell viability with increasing SQDs concentration observed in the present study supports the potential of SQDs as promising nanotherapeutic agents for colon cancer treatment. Similar dose-dependent anticancer effects of sulfur quantum dots against different cancer cell lines have also been documented by several researchers (Chen et al., 2022; Kumar et al., 2021). Overall, the findings indicate that SQDs possess significant antiproliferative and cytotoxic activity against Caco-2 colon cancer cells and may serve as potential candidates for future chemotherapeutic and biomedical applications.

Table -6: Cytotoxic results of SQDs against Caco-2 cell lines by MTT assay.

Sl. No	Sample	IC_{50} (µg/mL)
1.	SQDs	72.51 ± 2.15

Values are expressed as the mean ± SEM (n = 3); Statistical significance (p) was calculated by one way ANOVA followed by Dunnett's test and by comparing treated group with control - ***P < 0.001, **P < 0.01, *P < 0.05.

Table -7: Percentage cell viability at various concentrations of SQDs against Caco-2 cell lines.

Treatment	Percentage cell viability (Caco-2 cell lines), SQDs
Untreated	100 ± 0.00
Standard Drug (25µM)	64.8 ± 4.06***
10 (µg/ml)	90.9 ± 3.01ns
25 (µg/ml)	73.5 ± 0.252***
50 (µg/ml)	59.6 ± 0.340***
100 (µg/ml)	41.7 ± 2.20***
250 (µg/ml)	24.9 ± 1.05***
500 (µg/ml)	5.69 ± 1.75***

Values are expressed as the mean ± SEM (n = 3); Statistical significance (p) was calculated by one way ANOVA followed by Dunnett's test and by comparing treated group with control - ***P < 0.001, **P < 0.01, *P < 0.05.

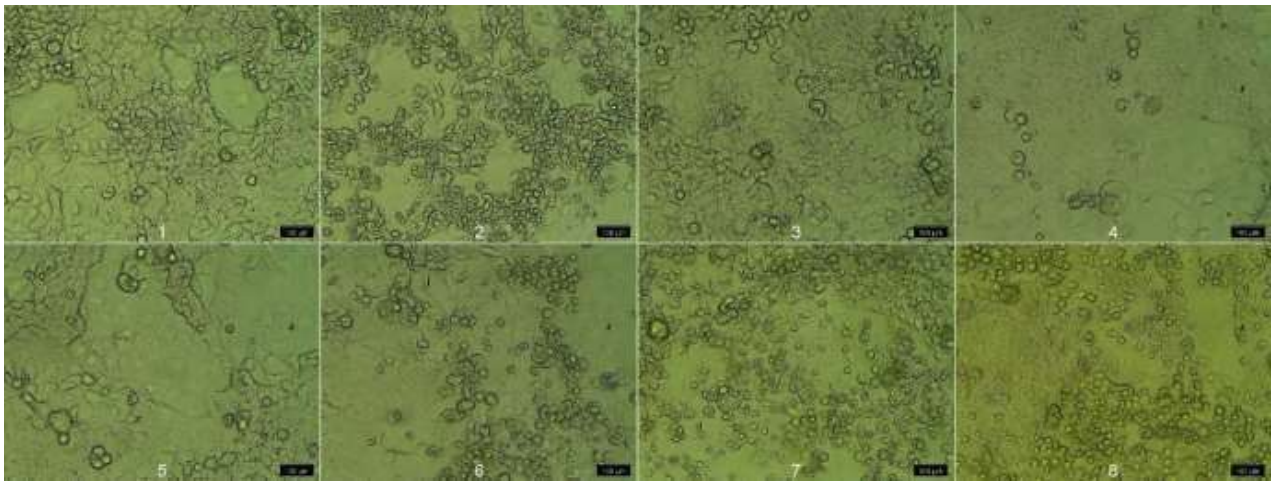


Fig -11: Morphology of Caco-2 cells treated by various doses of SQDs after the incubation period of 24hrs (20x magnification). Photo Legend: 1-Untreated, 2-Doxorubicin-1ug/ml, 3-8: different concentrations of SQDs ranging from 10ug/ml-500ug/ml.

5.5 CLSM-Cellular uptake study (FITC staining)

The cellular uptake efficiency of Sulfur Quantum Dots (SQDs) in Caco-2 colon cancer cells was analyzed using confocal laser scanning microscopy (CLSM) by measuring relative fluorescence intensity (RFU). Untreated control cells exhibited negligible fluorescence intensity (0.06 ± 0 RFU), confirming the absence of s ignificant cellular autofluorescence and validating the specificity of fluorescence detection. Cells treated with FITC (100 µg/ml), used as the positive control, demonstrated a high fluorescence intensity of 7.83 ± 1.13 RFU, indicating efficient intracellular uptake of the fluorescent probe.

Treatment with SQDs at the IC_{50} concentration (71.32 µg/ml) showed a fluorescence intensity of 6.34 ± 0.33 RFU, which was significantly higher than the untreated control group, confirming effective cellular internalization of SQDs

within Caco-2 cells after 24 hours of incubation. Although the fluorescence intensity of SQDs-treated cells was slightly lower than that observed for FITC-treated cells, the uptake remained considerably high, suggesting strong cellular interaction and accumulation of SQDs inside the cells. The comparatively lower standard deviation in SQDs-treated cells further indicates uniform and reproducible uptake across the cell population. Similar intracellular accumulation of sulfur-based quantum dots has been reported previously, where enhanced fluorescence signals were associated with efficient nanoparticle internalization and improved cellular interactions (Wang et al., 2021; Chen et al., 2022).

Efficient cellular uptake is a critical factor influencing the biological and therapeutic activity of nanoparticles, as intracellular localization enables direct interaction with cellular organelles and biomolecules. Previous studies have suggested that SQDs enter cells predominantly through endocytosis-mediated pathways, including pinocytosis, receptor-mediated endocytosis, and micropinocytosis (Lee et al., 2020). Due to their ultrasmall size and unique surface properties, quantum dots may also penetrate cellular membranes and accumulate within the cytoplasmic region, thereby enhancing their intracellular bioavailability (Zhang et al., 2019). Following internalization, SQDs are known to induce reactive oxygen species (ROS) generation, mitochondrial dysfunction, membrane damage, and disruption of cellular signaling pathways, ultimately leading to apoptosis and inhibition of cancer cell proliferation (Kumar et al., 2021; Patel et al., 2020).

The CLSM findings obtained in the present study strongly support the MTT assay results, confirming that SQDs are efficiently internalized by Caco-2 cells and contribute to significant antiproliferative and cytotoxic activity. The pronounced fluorescence intensity observed in SQDs-treated cells highlights their potential application as both therapeutic and bioimaging agents in colon cancer research and nanomedicine.

Table -8: Relative mean fluorescence intensity of SQDs expression in treated and untreated Caco-2 cells.

Culture Condition	Relative Mean Fluorescence Intensity
Untreated	0.06 ± 0
FITC-100ug/ml	7.83 ± 1.13***
SQDs-71.32ug/ml	6.34 ± 0.24***

Values are expressed as the mean ± SEM (n = 3); Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's test - ***P< 0.001, **P < 0.01, *P < 0.05 were considered as statistically significant and by comparing treated group with control group.

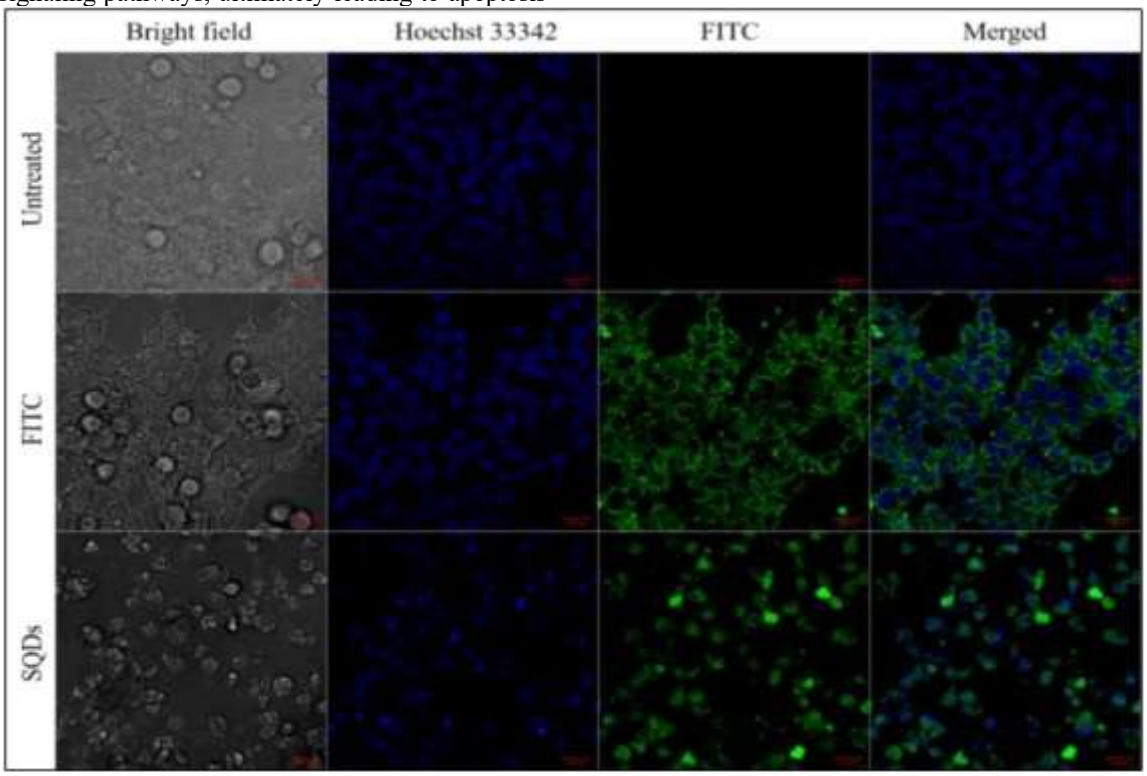


Fig -12: CLSM confocal fluorescent micrographs showing SQDs/FITC expression in untreated and treated Caco-2 cells after 24 h incubation.

6. CONCLUSION

In the present study, sulfur quantum dots (SQDs) were successfully synthesized by a microwave-assisted method using PEG6000 as the precursor material. The synthesized SQDs were characterized using UV–Visible spectroscopy, fluorescence spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Raman spectroscopy, which confirmed the formation of stable, fluorescent, quasi-spherical nanoparticles with nanoscale dimensions. The optical studies demonstrated strong fluorescence emission and effective "switch-on" fluorescence behavior, indicating the suitability of SQDs for bioimaging applications.

The biological activity of the synthesized SQDs was evaluated against Caco-2 cancer cell lines through MTT assay,

reactive oxygen species (ROS) analysis, cellular uptake studies, confocal laser scanning microscopy (CLSM), apoptosis assay, and cell cycle analysis. The results revealed that SQDs exhibited significant concentration-dependent cytotoxicity and effectively reduced the viability of Caco-2 cells. Enhanced intracellular uptake and fluorescence imaging confirmed efficient penetration of SQDs into cancer cells. Further studies demonstrated that SQDs induced oxidative stress-mediated apoptosis and cell cycle arrest, thereby contributing to their anticancer activity.

Overall, the synthesized SQDs showed excellent optical properties along with promising in vitro anticancer potential against Caco-2 cancer cells, suggesting their possible application as fluorescent nanomaterials for cancer diagnosis, bioimaging, and therapeutic purposes.

Future Scope of the Study

1. Detailed apoptosis studies to investigate the apoptotic pathways involved in SQD-mediated cancer cell death.
2. Cell cycle analysis to further understand the phase-specific arrest induced by SQDs in Caco-2 cancer cells.
3. Evaluation of Caspase-3 activation and related apoptotic protein expression to confirm the mechanism of programmed cell death induced by SQDs.
4. Further in vitro studies on additional cancer and normal cell lines to assess the selectivity, efficacy, and toxicity profile of SQDs.
5. In vivo studies for evaluating the anticancer efficacy, biodistribution, biocompatibility, and longterm safety of SQDs for future biomedical applications.

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