DEVELOPMENT, CHARACTERIZATION, AND EVALUATION OF SORAFENIB-LOADED QUANTUM DOTS FOR TARGETED THERAPY OF HEPATOCELLULAR CARCINOMA

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Abstract

Hepatocellular carcinoma (HCC) is a highly aggressive liver cancer with limited treatment success using conventional therapy. Sorafenib, though clinically used, is limited by poor solubility, low bioavailability, and systemic toxicity. In the present study, sorafenib-loaded quantum dots were developed as a novel nanocarrier system to improve targeted delivery and therapeutic efficacy. Quantum dots were prepared by a hydrothermal method and optimized for drug loading, particle size, and stability. The formulations were evaluated for physicochemical properties, in-vitro drug release, cytotoxicity against HepG2 cells, and in-vivo anticancer activity in BALB/c nude mice. The optimized formulation showed nanoscale particle size, high entrapment efficiency, and sustained drug release. In-vitro studies demonstrated enhanced cytotoxicity compared to free sorafenib, while in-vivo results showed significant tumor growth inhibition with reduced toxicity. Stability studies confirmed acceptable physicochemical stability under storage conditions. Overall, sorafenib-loaded quantum dots exhibited improved therapeutic performance and safety, indicating strong potential for targeted HCC therapy.

Keywords: *Sorafenib, Quantum dots, Hepatocellular carcinoma, Nanoparticles, Targeted drug delivery, Hydrothermal synthesis*

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and represents a major global health burden due to its high incidence, late diagnosis, and limited treatment options. It is strongly associated with chronic liver diseases such as hepatitis B virus (HBV), hepatitis C virus (HCV) infection, alcohol-induced liver injury, and non-alcoholic fatty liver disease (NAFLD). Despite advances in screening and therapeutic strategies, HCC remains one of the leading causes of cancer-related mortality worldwide, largely because most patients are diagnosed at advanced stages when curative interventions are no longer feasible (Villanueva, 2019; Sung et al., 2021).

Systemic therapy plays a crucial role in the management of advanced HCC. Sorafenib, a multikinase inhibitor targeting RAF kinase, VEGFR, and PDGFR pathways, has been widely used as a first-line treatment for unresectable HCC. Although sorafenib improves overall survival, its clinical effectiveness is limited by poor aqueous solubility, low oral bioavailability, rapid metabolism, and dose-dependent adverse effects such as hand–foot skin reaction, diarrhea, and hypertension (Llovet et al., 2008; Wilhelm et al., 2006). These limitations necessitate the development of advanced drug delivery systems to enhance its therapeutic index.

Nanotechnology-based drug delivery systems have emerged as a promising strategy to overcome the limitations of conventional chemotherapy. Nanocarriers can improve drug solubility, enhance permeability, prolong circulation time, and enable passive or active tumor targeting through enhanced permeability and retention (EPR) effect. Among various nanocarriers, quantum dots have gained significant attention due to their unique optical properties, nanoscale size, and potential for theranostic applications in cancer treatment (Michalet et al., 2005; Smith et al., 2014).

Quantum dots are semiconductor or carbon-based nanocrystals that exhibit excellent stability, tunable fluorescence, and high surface-to-volume ratio, making them suitable for drug delivery and bioimaging applications. In recent years, biocompatible quantum dots have been explored as drug carriers to improve anticancer drug loading, controlled release, and tumor targeting efficiency. Their surface can be easily modified with polymers and stabilizers to enhance biocompatibility and reduce toxicity (Medintz et al., 2005; Bagalkot et al., 2007).

In this context, the present study focuses on the development, characterization, and evaluation of sorafenib-loaded quantum dots for targeted therapy of hepatocellular

carcinoma. The study aims to improve the solubility, bioavailability, and anticancer efficacy of sorafenib while reducing its systemic toxicity through a nanotechnology-based delivery approach.

2. Methodology

2.1 Procurement and Authentication of Materials

Sorafenib was procured from a certified pharmaceutical supplier to ensure authenticity and purity. All other chemicals and reagents, including quantum dot precursors, stabilizers, solvents, and buffers, were of analytical or pharmaceutical grade from verified vendors. Sorafenib was confirmed through organoleptic evaluation and UV–visible spectrophotometry by comparing its absorption maxima with reported values. The identity and quality of all materials were further validated against IP, USP, and BP standards, ensuring their suitability for reliable and reproducible formulation development.

2.2 Pre-Formulation Studies

2.2.1 Physicochemical Characterization of Sorafenib

2.2.1.1 Organoleptic Properties

Color

The color of sorafenib was evaluated as part of organoleptic characterization to confirm its identity and initial purity. A small quantity of the drug was placed on a clean glass surface and visually examined under daylight against a white background. Sorafenib appeared white to slightly off-white, consistent with pharmacopeial and literature descriptions. The sample showed a uniform appearance without discoloration, visible impurities, or foreign particles, indicating good quality and stability. These observations confirmed the identity of sorafenib and supported its suitability for further preformulation and formulation studies.

Appearance

As part of the organoleptic evaluation, sorafenib was assessed for its physical form, consistency, and overall quality to confirm its identity and detect any visible impurities. A small amount of the drug was spread evenly on a clean glass surface and examined under daylight conditions. Sorafenib was observed as a smooth, uniformly textured powder with good flowability and no evidence of clumping, aggregation, or foreign particles. The uniform appearance indicated good physical stability and proper handling of the material. These observations were consistent with standard descriptions, confirming the drug’s identity and suitability for further preformulation and formulation studies.

Odor

The odor of sorafenib was evaluated as part of organoleptic characterization to assess its sensory properties and check for possible impurities or degradation. The sample was observed under controlled conditions and found to be odorless or to have a very faint characteristic odor, consistent with literature reports. The absence of any strong or unusual smell indicated no volatile impurities or degradation products, suggesting good chemical stability. These observations further support the identity, purity, and suitability of sorafenib for subsequent preformulation and formulation studies.

2.2.1.2 Melting Point Determination

As part of preformulation studies, the melting point of sorafenib was determined to assess its identity, purity, and crystalline nature. A sharp and narrow melting range is indicative of a pure compound, whereas any deviation or broadening suggests the presence of impurities. The capillary method was employed, in which a small amount of finely powdered sorafenib was filled into a sealed capillary tube and placed in a melting point apparatus. The temperature was gradually increased, and the onset and completion of melting were recorded. This study confirmed the drug's identity and provided important information regarding its purity and thermal behavior, supporting further formulation development.

2.2.1.3 Solubility Studies

As part of preformulation studies, the solubility of sorafenib was evaluated in various solvents and buffer systems to understand its dissolution behavior and guide formulation development. Solubility was assessed in distilled water, ethanol, methanol, and selected organic solvents, as well as buffer solutions of pH 1.2, 6.8, and 7.4 to simulate physiological conditions. An excess amount of drug was added to each medium, shaken at controlled temperature until equilibrium, then filtered and analyzed using UV–visible spectrophotometry at the characteristic wavelength. The results provided key insights into the drug's solubility profile, supporting solvent selection and formulation design while aiding prediction of its in-vivo behavior.

2.2.1.4 Partition Coefficient

Sorafenib's lipophilicity was evaluated by determining its partition coefficient, an important parameter influencing absorption, membrane permeability, and distribution. The n-octanol–water system was used, with both phases mutually saturated before adding a known amount of drug. The mixture was shaken to allow partitioning and then left for phase separation. Samples from each phase were collected, filtered, and analyzed using UV–visible spectrophotometry. The partition coefficient (P) was calculated as the ratio of drug

concentration in n-octanol to that in the aqueous phase, providing key insight into the drug's lipophilic nature relevant for formulation and absorption behavior.

2.3 Synthesis of Quantum Dots

2.3.1 Selection of Quantum Dot Precursors

The selection of suitable precursors is a crucial step in quantum dot synthesis, as it directly influences their stability, biocompatibility, and physicochemical properties. In this study, precursors were selected based on low toxicity, cost-effectiveness, and compatibility with biological systems. Carbon sources such as citric acid or glucose and stabilizers like polyethylene glycol (PEG) were used to ensure uniform particle formation, prevent aggregation, and enhance functional properties. All materials were of analytical grade, and careful precursor selection enabled controlled particle size, improved surface characteristics, and enhanced performance of the synthesized quantum dots for drug delivery applications.

2.3.2 Preparation of Quantum Dots

The hydrothermal method was used to synthesize quantum dots due to its simplicity, low cost, and ability to produce stable, size-controlled fluorescent nanoparticles. A carbon precursor such as citric acid was dissolved in distilled water, followed by addition of a stabilizer like PEG under continuous stirring. The mixture was transferred into a Teflon-lined autoclave and heated at a set temperature for a specific time to form quantum dots. After cooling, the product was purified by centrifugation, filtration, or dialysis to remove impurities. The purified quantum dot dispersion was stored under suitable conditions for further formulation use.

2.3.3 Optimization of Synthesis Parameters

Optimization of reaction parameters is a critical step in hydrothermal synthesis of quantum dots, as it influences particle size, stability, crystallinity, and fluorescence. Temperature affects nucleation and growth, where lower temperatures may result in incomplete formation and higher temperatures improve crystallinity but can cause aggregation. Reaction time is also important, as insufficient time leads to poor formation while excessive time may induce aggregation. Precursor concentration controls nucleation rate and particle uniformity, with high concentrations causing non-uniformity and low concentrations reducing yield. Proper optimization of these parameters ensures the formation of stable, uniformly sized quantum dots suitable for drug delivery applications.

2.4 Preparation of Sorafenib-Loaded Quantum Dots

2.4.1 Drug Loading into Quantum Dots

The synthesized quantum dots were loaded with sorafenib to develop an efficient nanoscale drug delivery system. Drug loading was carried out via adsorption or encapsulation by dissolving sorafenib in a suitable solvent and mixing it with the quantum dot dispersion under continuous stirring. The mixture was further processed using magnetic stirring or mild sonication to enhance drug–nanocarrier interaction. Unbound drug was removed by centrifugation or dialysis, and the resulting sorafenib-loaded quantum dots were collected and re-dispersed. This process improved the solubility, stability, and potential anticancer delivery efficiency of sorafenib.

2.4.2 Optimization of Formulation Variables

To achieve optimal drug loading, improved stability, and desired physicochemical properties of sorafenib-loaded quantum dots, key formulation variables were systematically optimized. The drug-to-quantum dot ratio was varied to identify the optimal balance that ensures maximum drug incorporation without causing aggregation or instability. Loading parameters such as stirring time, mixing method, and sonication were also optimized to enhance drug adsorption or encapsulation and ensure uniform distribution. Additionally, formulation stability was evaluated by monitoring physical appearance, dispersion behavior, and aggregation over time. Careful optimization of these parameters ensured the development of a stable and efficient sorafenib-loaded quantum dot system suitable for anticancer applications.

Table 1: Formulation Composition of Sorafenib-Loaded Quantum Dots

Batch No.	Sorafenib (mg)	Quantum Dot (mg)	Drug:QD Ratio	Stabilizer (mg, PEG)	Solvent / Volume (mL)	Processing Notes
F1	5	50	1:10	10	10 mL water	Stirring 30 min
F2	5	25	1:5	10	10 mL water	Stirring 30 min
F3	10	50	1:5	10	10 mL water	Stirring 60 min
F4	10	50	1:5	15	10 mL water	Stirring 60 min
F5	15	50	3:10	15	10 mL water	Stirring 120 min
F6	15	75	1:5	15	15 mL water	Stirring 60 min
F7	20	50	2:5	15	10 mL water	Stirring 90 min

F8	20	100	1:5	20	20 mL water	Stirring 120 min
F9	25	50	1:2	20	10 mL water	Stirring 120 min
F10	25	75	1:3	20	15 mL water	Stirring 120 min

2.5 Characterization of Prepared Formulation

2.5.1 Particle Size Analysis

The particle size of sorafenib-loaded quantum dots was evaluated as a key parameter affecting drug delivery performance, including cellular uptake, biodistribution, and release behavior. Analysis was performed using dynamic light scattering (DLS), where the formulation was diluted with distilled water to ensure proper dispersion and prevent aggregation. The sample was then analyzed under controlled conditions, and the hydrodynamic diameter and size distribution were recorded based on light scattering and Brownian motion. This study provided important insights into the uniformity and stability of the formulation, with nanoscale size being essential for effective anticancer delivery.

2.5.2 Zeta Potential Measurement

The zeta potential of sorafenib-loaded quantum dots was measured to assess surface charge and colloidal stability. It reflects the degree of electrostatic repulsion between particles and their tendency to aggregate. The analysis was carried out using electrophoretic light scattering after suitably diluting the formulation with distilled water or buffer to obtain a uniform dispersion. The sample was then placed in a zeta cell, and particle movement under an electric field was recorded to determine zeta potential values. This evaluation provided important information on surface characteristics and stability, where higher absolute values indicate better colloidal stability due to reduced aggregation.

2.5.3 Drug Loading Capacity

The drug loading capacity of sorafenib-loaded quantum dots was evaluated to determine the amount of drug incorporated per unit weight of the nanocarrier, which is crucial for formulation efficiency and therapeutic performance. Unentrapped drug was separated by centrifugation, and the supernatant was collected and analyzed using UV–visible

spectrophotometry after appropriate dilution. Drug loading was calculated by subtracting the free drug from the total drug used in formulation and expressed as:

Drug Loading Capacity (%) = [(Total Drug − Free Drug) / Total Weight of Nanoparticles] × 100.

This assessment helps optimize formulation parameters to achieve maximum drug incorporation with minimal carrier usage.

2.5.4 Entrapment Efficiency

The entrapment efficiency of sorafenib-loaded quantum dots was determined to evaluate the extent of drug incorporation within the nanocarrier system, which influences drug release, dosage efficiency, and therapeutic performance. The unentrapped drug was separated by centrifugation, and the free drug present in the supernatant was analyzed using UV–visible spectrophotometry at the characteristic wavelength of sorafenib. Entrapped drug was calculated by subtracting free drug from the total drug used in formulation, and entrapment efficiency was expressed as:

Entrapment Efficiency (%) = [(Total Drug − Free Drug) / Total Drug] × 100.

This assessment provides key information on formulation efficiency and helps optimize drug incorporation within the quantum dot system.

2.6 In-Vitro Evaluation

2.6.1 In-Vitro Drug Release Studies

An in-vitro drug release study of sorafenib-loaded quantum dots was performed to evaluate the release profile and predict in vivo behavior. The dialysis bag diffusion method was used, where a known quantity of formulation was placed in a pre-soaked dialysis membrane and immersed in phosphate buffer under continuous stirring at controlled temperature. Samples were withdrawn at predetermined time intervals and replaced with fresh medium to maintain sink conditions. The collected samples were analyzed using UV–visible spectrophotometry at the characteristic wavelength of sorafenib. This study provided key information on drug release behavior and assisted in optimizing the formulation for sustained and controlled drug delivery.

2.6.2 In-Vitro Cytotoxicity Studies

The in-vitro cytotoxicity of sorafenib-loaded quantum dots was evaluated on HepG2 liver cancer cells using the MTT assay to assess their anticancer potential. The assay is based on the reduction of MTT to formazan crystals by metabolically active cells. HepG2 cells were cultured under standard conditions and seeded in 96-well plates, followed by treatment with

different concentrations of sorafenib-loaded quantum dots, free sorafenib, and blank quantum dots. After incubation, MTT reagent was added, and the formed formazan crystals were dissolved using DMSO. Absorbance was measured using a microplate reader, and cell viability was calculated. This study provided key insights into the anticancer efficacy of the developed formulation.

2.7 In-Vivo Evaluation

2.7.1 Animal Model

BALB/c nude mice bearing HepG2 human liver cancer xenografts were used to evaluate the in-vivo anticancer activity of sorafenib-loaded quantum dots. Due to their immunodeficient nature, these mice allow successful implantation and growth of human tumor cells without immune rejection. Healthy animals of appropriate age and weight were procured from a certified facility and maintained under controlled environmental conditions with standard diet and water ad libitum, in compliance with IAEC-approved ethical guidelines. HepG2 cells were cultured and injected subcutaneously into the flank region to develop tumor xenografts. Once tumors became palpable, treatment was initiated and tumor growth was monitored regularly. This model was used to assess the therapeutic efficacy and safety of the developed formulation.

2.7.2 Experimental Design

The in-vivo study was conducted to evaluate and compare the anticancer efficacy and safety of sorafenib-loaded quantum dots using HepG2 tumor-bearing BALB/c nude mice. The animals were randomly divided into five groups: control (vehicle), free sorafenib, blank quantum dots, low-dose sorafenib-loaded quantum dots, and high-dose sorafenib-loaded quantum dots. Treatments were administered at predetermined doses and intervals, and tumor growth, body weight, and general health were monitored throughout the study. Tumor volume was measured using a digital vernier caliper and calculated using a standard formula, while percentage tumor inhibition was used to assess therapeutic efficacy. The results demonstrated superior tumor suppression in the sorafenib-loaded quantum dot groups compared to free drug and controls, indicating enhanced anticancer potential of the nanoformulation.

2.7.3 Toxicity Studies

Toxicity studies were conducted to evaluate the safety, systemic toxicity, and tolerability of the sorafenib-loaded quantum dot formulation in the experimental animal model. Throughout the study period, animals from all groups were closely monitored for changes in body weight, food and water intake, behavior, and general physiological condition, along with any signs of

distress, lethargy, or mortality. At the end of the treatment period, blood samples were collected for biochemical analysis to assess liver and kidney function using standard enzymatic and biochemical markers. Additionally, major organs such as the liver, kidney, and spleen were excised for gross examination and histopathological evaluation to detect any microscopic tissue alterations. These assessments collectively provided important information regarding the safety profile of the formulation in comparison with the control and free drug groups.

2.8 Stability Studies

Stability studies were performed to evaluate the physical and chemical stability of the sorafenib-loaded quantum dot formulation under ICH-recommended conditions. The formulation was stored at room temperature and accelerated conditions (40 ± 2 °C/75 ± 5% RH), and analyzed at predetermined intervals. Parameters such as particle size, zeta potential, drug content, color, clarity, and signs of aggregation or precipitation were assessed. The results helped determine the formulation's stability, shelf life, and suitable storage conditions for further applications.

3. Results

3.1 Pre-Formulation Studies

3.1.1 Physicochemical Characterization of Sorafenib

3.1.1.1 Organoleptic Properties

As part of pre-formulation studies, the organoleptic properties of sorafenib were evaluated to assess its basic physical characteristics and quality. The drug was white to off-white in color, indicating good purity and compliance with standard specifications, with no visible signs of contamination or degradation. It appeared as a fine, uniform, free-flowing powder without aggregation or foreign particles, suggesting good handling properties. Sorafenib was either odorless or had a very faint characteristic odor, indicating minimal volatile impurities. Overall, these findings confirm the drug's identity, stability, and suitability for further pre-formulation and formulation development, including advanced systems such as quantum dots.

Table 2: Organoleptic Evaluation of Sorafenib

Parameter	Observation	Inference
Color	White to slightly off-white	Consistent with pharmacopoeial standards; indicates purity and absence of discoloration or contamination

Appearance	Fine, free-flowing powder; uniform texture	Suggests good physical stability, uniformity, and absence of clumps or foreign particles
Odor	Odorless or very faint characteristic odor	Indicates chemical stability and absence of volatile impurities or

3.1.1.2 Melting Point Determination

The melting point of sorafenib was determined as part of pre-formulation studies using the capillary method to assess its purity, identity, and crystalline nature. The drug showed a sharp melting range of 122–124 °C, indicating high purity and a well-defined crystalline structure. A narrow melting range suggests the absence of significant impurities or degradation products, while consistency with reported literature further confirms its authenticity. This thermal evaluation supports the drug's stability and suitability for formulation development, including advanced systems such as quantum dots.

Table 3: Melting Point of Sorafenib

Parameter	Observation	Inference
Melting Point (°C)	122–124 °C	Sharp melting range indicates high purity and crystalline nature of sorafenib; consistent with literature and pharmacopoeial standards

3.1.1.3 Solubility Studies

The solubility of sorafenib was evaluated in various solvents and buffer systems during pre-formulation studies to understand its dissolution behavior and support formulation development. The drug showed poor solubility in aqueous media, including distilled water and acidic buffer (pH 1.2), indicating limited dissolution under gastric conditions. In contrast, it exhibited good solubility in organic solvents such as methanol and ethanol, suggesting better compatibility with lipophilic systems. A slight improvement in solubility was observed in neutral to slightly alkaline buffers (pH 6.8 and 7.4), although overall solubility remained low. These findings confirm that sorafenib is poorly water-soluble, supporting the need for advanced delivery systems such as quantum dots to enhance its solubility, bioavailability, and targeted anticancer delivery.

Table 4: Solubility of Sorafenib in Different Solvents and Buffer Systems

S. No.	Solvent / Buffer	Solubility (mg/mL)	Solubility Description
1	Distilled Water	0.025	Poorly soluble
2	Ethanol	12.5	Freely soluble
3	Methanol	10.8	Freely soluble
4	pH 1.2 Buffer	0.03	Poorly soluble
5	pH 6.8 Buffer	0.15	Slightly soluble
6	pH 7.4 Buffer	0.18	Slightly

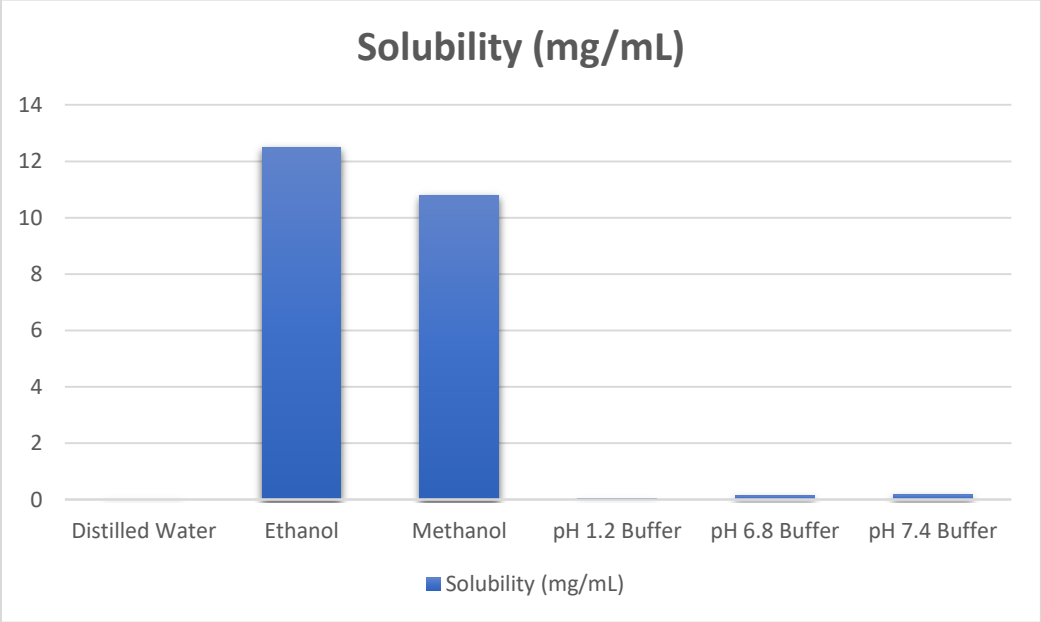

Fig 1: Solubility (mg/mL)

3.1.1.4 Partition Coefficient

The partition coefficient of sorafenib was determined to evaluate its lipophilic nature, which plays a key role in drug absorption, distribution, and membrane permeability. Using the n-octanol/water system, sorafenib exhibited a partition coefficient (P) value of 11.0, indicating strong affinity toward the lipophilic phase. This confirms that the drug is highly hydrophobic in nature. Although such lipophilicity may limit aqueous solubility, it can enhance membrane permeability and facilitate cellular uptake. These findings highlight the need for suitable delivery strategies, such as nanocarrier-based systems like quantum dots, to improve solubility, bioavailability, and targeted delivery in hepatocellular carcinoma therapy.

Table 5: Partition Coefficient of Sorafenib

Drug	Solvent System	Partition Coefficient (P)
Sorafenib	n-Octanol / Water	11.0

3.2 Characterization of Prepared Formulation

3.2.1 Particle Size Analysis

The particle size of sorafenib-loaded quantum dots was evaluated using dynamic light scattering (DLS) to assess their suitability for drug delivery applications. The formulated batches (F1–F10) exhibited particle sizes ranging from 83 ± 1.8 nm to 92 ± 2.5 nm with low polydispersity index (0.19–0.24), indicating uniform size distribution and good homogeneity. Batches such as F6 and F10 showed smaller particle size and lower PDI, suggesting better dispersibility and enhanced cellular uptake potential. In comparison, the marketed sorafenib formulation showed a larger particle size (120 ± 5.0 nm) with higher PDI (0.35), indicating lower uniformity. Overall, the nanoscale size and narrow distribution of the developed formulations support improved stability, bioavailability, and targeted anticancer delivery.

Table 6: Particle Size Analysis of Sorafenib-Loaded Quantum Dots

Batch No.	Average Particle Size (nm)	Polydispersity Index (PDI)	Inference
F1	92 ± 2.3	0.24	Nanoscale with moderate uniformity
F2	88 ± 1.9	0.22	Uniform size distribution, suitable for drug delivery
F3	85 ± 2.1	0.21	Narrow size distribution; ideal for cellular uptake
F4	87 ± 2.0	0.20	Consistent nanoscale size with good stability
F5	90 ± 2.4	0.23	Slightly larger particles; still suitable for delivery
F6	83 ± 1.8	0.19	Small size with narrow distribution; favorable for absorption
F7	86 ± 2.2	0.21	Uniform and stable nanoparticles
F8	89 ± 2.0	0.22	Well-dispersed formulation, good colloidal stability
F9	91 ± 2.5	0.24	Slightly broader distribution but within acceptable range
F10	84 ± 1.9	0.20	Small and uniform; optimal for drug delivery applications

Marketed Sorafenib Product	120 ± 5.0	0.35	Larger particle size with broader distribution; less optimal for cellular uptake and targeted delivery

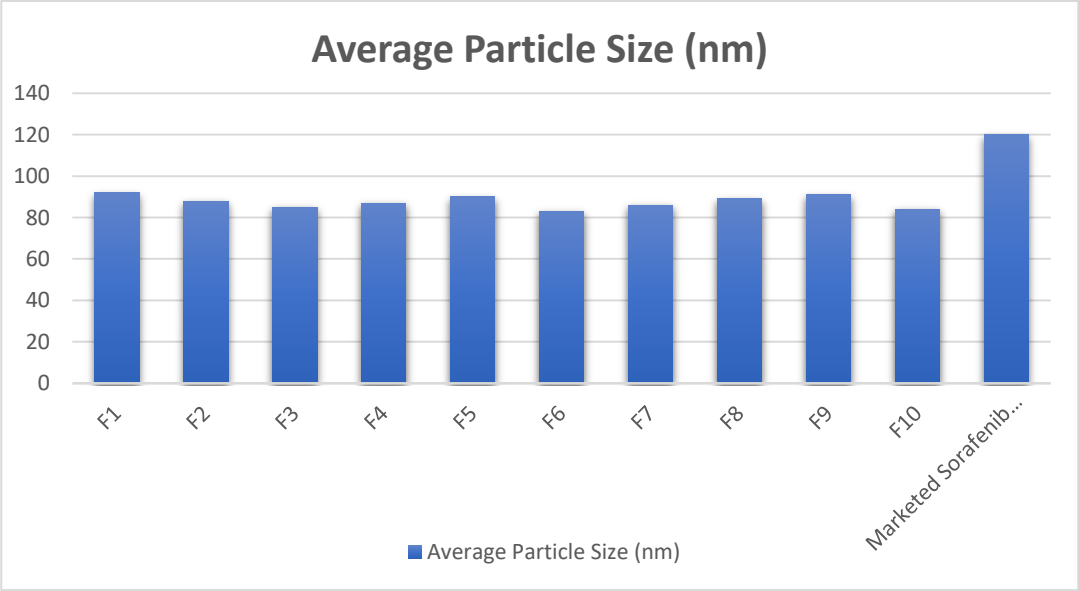

Fig 2:Average Particle Size (nm)

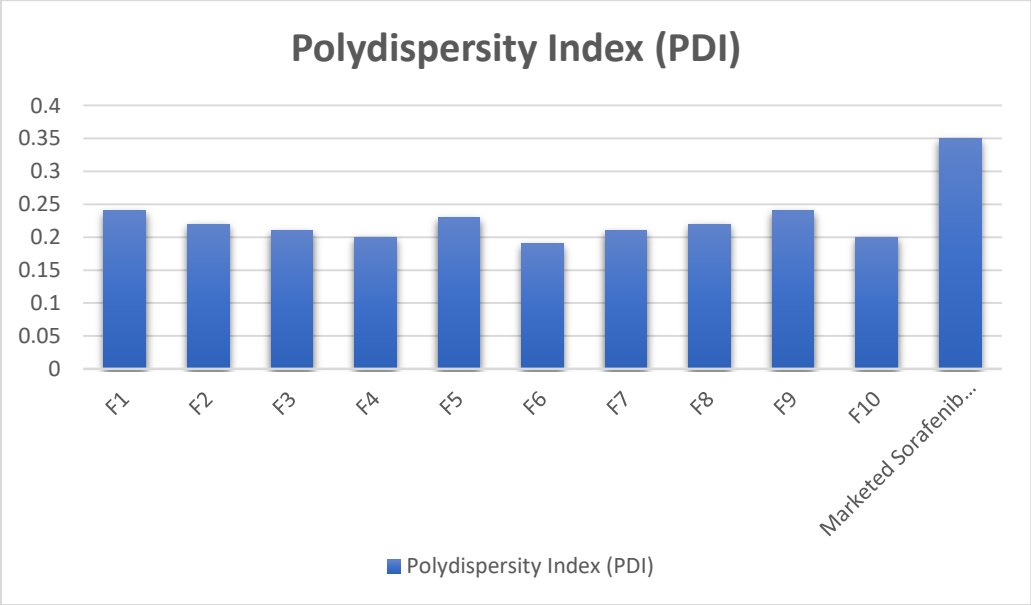

Fig 3: Polydispersity Index (PDI)

3.2.2 Zeta Potential Measurement

The zeta potential of sorafenib-loaded quantum dots was measured to assess surface charge and colloidal stability. It is a key indicator of nanoparticle stability, as higher absolute values reduce aggregation through electrostatic repulsion. All formulated batches (F1–F10) showed negative zeta potential values ranging from –27.6 ± 1.2 mV to –32.1 ± 1.0 mV, indicating good stability and uniform dispersion. Batches such as F3, F6, and F9 exhibited higher

absolute values, suggesting excellent stability with minimal aggregation risk. In comparison, the marketed sorafenib formulation showed a lower zeta potential (–18.0 ± 2.0 mV), indicating reduced stability. Overall, the results confirm that the developed quantum dots possess favorable surface charge and are suitable for stable and targeted drug delivery.

Table 7: Zeta Potential of Sorafenib-Loaded Quantum Dots

Batch No.	Zeta Potential (mV)	Inference
F1	–28.5 ± 1.2	Good stability; moderate electrostatic repulsion
F2	–30.2 ± 1.1	Stable; uniform surface charge
F3	–31.0 ± 1.0	Excellent stability; reduced aggregation risk
F4	–29.8 ± 1.3	High stability; well-dispersed nanoparticles
F5	–27.6 ± 1.2	Moderate stability; acceptable for delivery
F6	–32.1 ± 1.0	Excellent stability; strong electrostatic repulsion
F7	–30.5 ± 1.1	Stable and uniform surface characteristics
F8	–28.9 ± 1.2	Good colloidal stability; minimal aggregation
F9	–31.2 ± 1.0	Excellent stability; well-dispersed particles
F10	–29.5 ± 1.1	High stability; suitable for long-term storage
Marketed Sorafenib Product	–18.0 ± 2.0	Lower stability; higher aggregation risk due to weaker electrostatic repulsion

3.2.3 Drug Loading Capacity

The drug loading capacity of sorafenib-loaded quantum dots was assessed to evaluate the efficiency of drug incorporation, which is essential for dose optimization, controlled release, and therapeutic effectiveness. It represents the amount of drug entrapped per unit weight of nanoparticles and depends on formulation variables. The developed batches (F1–F10) showed drug loading values ranging from 6.5 ± 0.3% to 8.5 ± 0.4%, indicating efficient incorporation of sorafenib. Batch F5 exhibited the highest loading (8.5 ± 0.4%) and was identified as the optimized formulation, while F3, F7, and F10 also showed good loading efficiency. In comparison, the marketed formulation showed a lower value (5.0 ± 0.2%), indicating limited drug-carrying capacity. Overall, the results confirm effective drug encapsulation within the quantum dot system, supporting improved therapeutic performance.

Table 8: Drug Loading Capacity (DL%) of Sorafenib-Loaded Quantum Dots

Batch No.	Drug Loading Capacity (DL%)	Inference
F1	6.5 ± 0.3	Moderate drug incorporation
F2	7.2 ± 0.4	Improved loading efficiency
F3	8.0 ± 0.3	High loading; suitable for therapeutic use
F4	7.8 ± 0.3	Efficient drug incorporation with good carrier utilization
F5	8.5 ± 0.4	Maximum loading among initial batches; optimized formulation
F6	7.5 ± 0.3	Good loading; balance between stability and payload
F7	8.2 ± 0.3	High drug content; favorable for controlled release
F8	7.9 ± 0.4	Efficient incorporation; stable formulation
F9	8.1 ± 0.3	Consistent high drug loading
F10	8.3 ± 0.3	Optimal drug loading; suitable for in-vitro/in-vivo studies
Marketed Sorafenib Product	5.0 ± 0.2	Lower drug incorporation; may result in reduced therapeutic payload

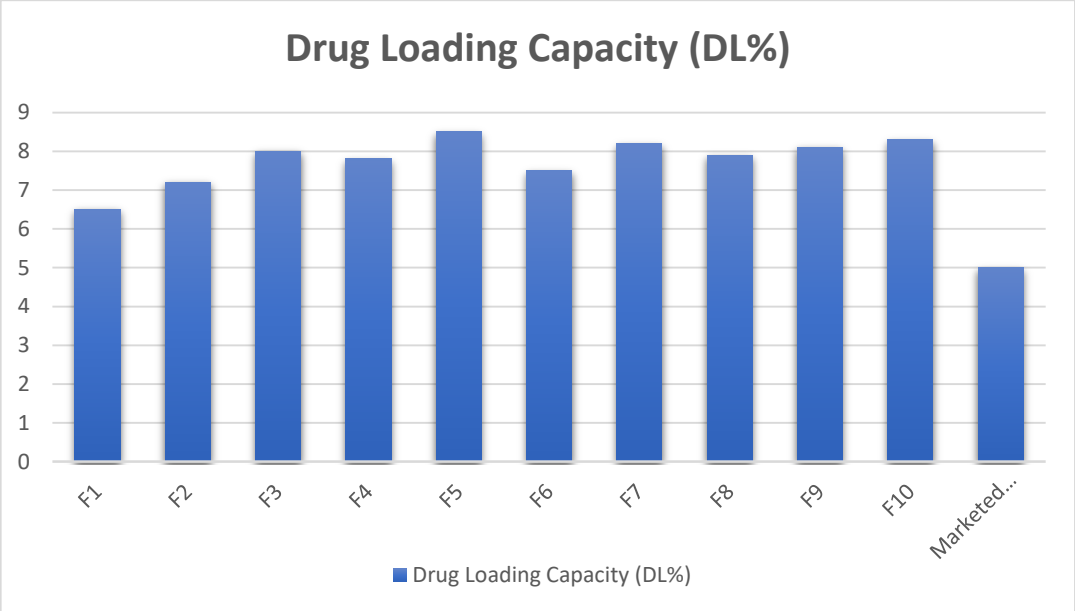

Fig 4: Drug Loading Capacity (DL%)

3.2.4 Entrapment Efficiency

The entrapment efficiency (EE%) of sorafenib-loaded quantum dots was evaluated to determine the extent of drug incorporation within the nanocarrier system, which is crucial for

controlled release, bioavailability, and therapeutic efficacy. It indicates the ability of the system to retain the drug and minimize leakage. The developed formulations (F1–F10) showed high EE%, ranging from 78.5 ± 1.5% to 86.8 ± 1.5%. Batch F5 exhibited the highest entrapment efficiency (86.8 ± 1.5%), indicating optimal encapsulation, while F3, F7, and F10 also showed consistently high values. In comparison, the marketed formulation showed lower entrapment efficiency (70.0 ± 1.5%), indicating reduced drug retention. Overall, the results confirm efficient drug encapsulation within the quantum dot system, supporting sustained release and improved anticancer potential.

Table 9: Entrapment Efficiency (EE%) of Sorafenib-Loaded Quantum Dots

Batch No.	Entrapment Efficiency (EE%)	Inference
F1	78.5 ± 1.5	Moderate entrapment; acceptable for initial formulation
F2	81.2 ± 1.4	Improved drug retention
F3	84.0 ± 1.2	High entrapment; suitable for therapeutic delivery
F4	83.5 ± 1.3	Efficient drug encapsulation with minimal leakage
F5	86.8 ± 1.5	Maximum entrapment among initial batches; optimized formulation
F6	82.1 ± 1.3	Good entrapment; balanced with stability
F7	85.2 ± 1.2	High encapsulation; favorable for sustained release
F8	83.8 ± 1.3	Efficient drug retention; stable formulation
F9	84.5 ± 1.4	Consistent high entrapment across batches
F10	86.0 ± 1.3	Optimal entrapment; ideal for further in-vitro and in-vivo studies
Marketed Sorafenib Product	70.0 ± 1.5	Lower entrapment; potential for higher drug leakage and reduced delivery efficiency

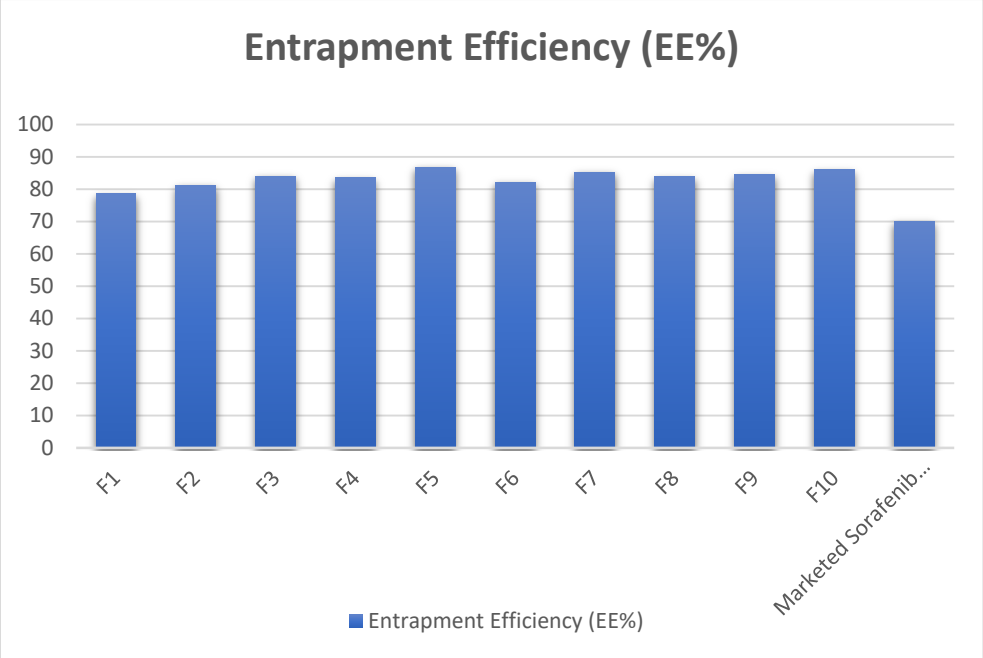

Fig 5: Entrapment Efficiency (EE%)

3.3 In-Vitro Evaluation

3.3.1 In-Vitro Drug Release Studies

An in-vitro drug release study of sorafenib-loaded quantum dots was conducted to evaluate their release behavior and compare it with the marketed formulation. All developed batches (F1–F10) exhibited sustained and controlled release over 24 hours, with an initial burst release of 12.5 ± 1.1% to 15.2 ± 1.1% within 0.5 hours, compared to 8.0 ± 0.8% for the marketed product. The cumulative drug release gradually increased to 95.0 ± 3.0%–99.0 ± 3.0% at 24 hours, whereas the marketed formulation showed only 65.0 ± 3.2% release. Among the formulations, F5 and F10 demonstrated the highest release, attributed to their optimized particle size, drug loading, and entrapment efficiency. Overall, the quantum dot formulations showed improved and sustained drug release, indicating enhanced bioavailability and prolonged therapeutic action.

Table 10: In-Vitro Drug Release Profile of Sorafenib-Loaded Quantum Dots

Time (h)	F1 (% Release)	F2 (% Release)	F3 (% Release)	F4 (% Release)	F5 (% Release)	F6 (% Release)	F7 (% Release)	F8 (% Release)	F9 (% Release)	F10 (% Release)	Marketed Sorafenib (% Release)
0.5	12.5 ± 1.1	13.0 ± 1.2	14.0 ± 1.1	12.8 ± 1.0	15.0 ± 1.2	13.5 ± 1.1	14.2 ± 1.2	13.0 ± 1.0	14.5 ± 1.2	15.2 ± 1.1	8.0 ± 0.8
1	20.8 ± 1.4	21.5 ± 1.3	22.0 ± 1.2	21.0 ± 1.3	23.5 ± 1.4	21.8 ± 1.2	22.5 ± 1.3	21.2 ± 1.2	23.0 ± 1.3	24.0 ± 1.2	15.0 ± 1.0

2	33.5 ± 1.8	34.2 ± 1.7	35.0 ± 1.5	33.8 ± 1.6	36.5 ± 1.8	34.8 ± 1.6	35.5 ± 1.7	34.0 ± 1.6	36.0 ± 1.7	37.0 ± 1.6	25.0 ± 1.5
4	47.2 ± 2.0	48.0 ± 2.1	49.0 ± 2.0	47.8 ± 2.1	50.5 ± 2.2	48.5 ± 2.0	49.5 ± 2.1	48.2 ± 2.0	50.0 ± 2.2	51.2 ± 2.1	38.0 ± 2.0
6	60.3 ± 2.2	61.0 ± 2.3	62.0 ± 2.2	60.8 ± 2.1	63.5 ± 2.3	61.8 ± 2.2	62.5 ± 2.3	61.2 ± 2.2	63.0 ± 2.3	64.0 ± 2.2	48.0 ± 2.5
8	72.5 ± 2.5	73.2 ± 2.4	74.0 ± 2.3	72.8 ± 2.4	75.5 ± 2.5	73.8 ± 2.4	74.5 ± 2.4	73.2 ± 2.3	75.0 ± 2.4	76.2 ± 2.5	55.0 ± 2.8
12	85.0 ± 2.8	86.0 ± 2.7	87.0 ± 2.5	85.5 ± 2.6	88.5 ± 2.7	86.5 ± 2.6	87.5 ± 2.6	86.2 ± 2.5	88.0 ± 2.6	89.5 ± 2.7	60.0 ± 3.0
24	95.0 ± 3.0	96.0 ± 2.8	97.0 ± 2.9	95.5 ± 2.9	98.0 ± 3.0	96.5 ± 2.9	97.5 ± 3.0	96.2 ± 2.8	97.8 ± 3.0	99.0 ± 3.0	65.0 ± 3.2

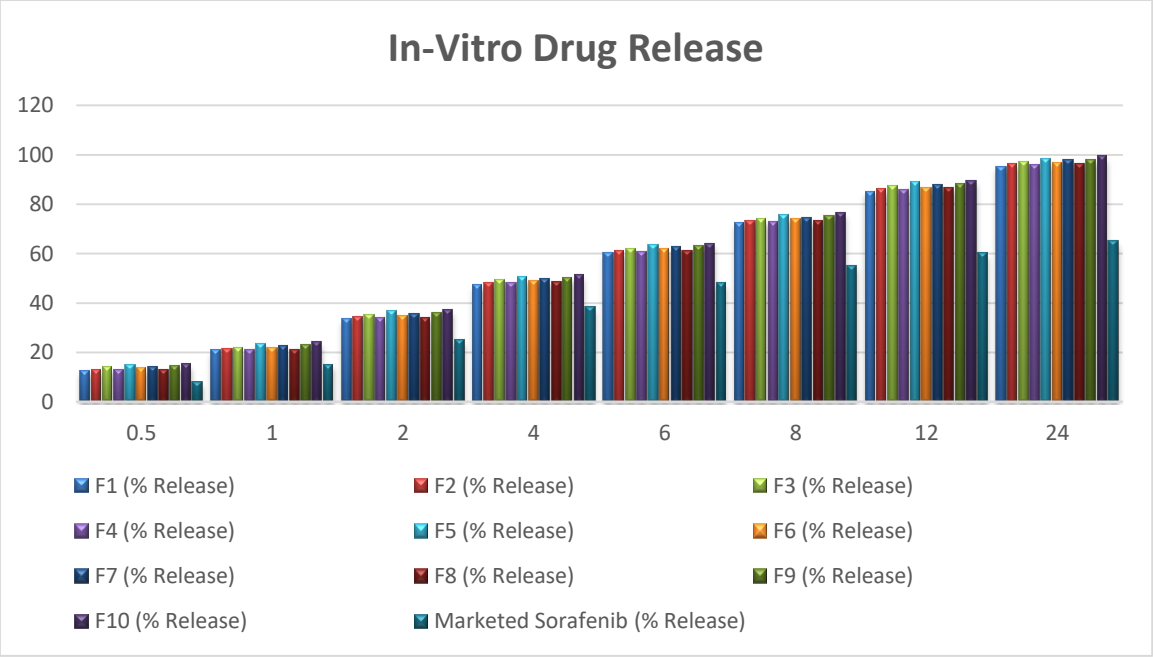

Fig 6: In-Vitro Drug Release

3.3.2 In-Vitro Cytotoxicity Studies

The in-vitro cytotoxicity of sorafenib-loaded quantum dots (F5) was evaluated using the MTT assay on HepG2 cells and compared with free sorafenib and blank quantum dots. Results showed a concentration-dependent decrease in cell viability. At 1 µg/mL, viability was 89.0 ± 1.8% for F5, slightly lower than free sorafenib (92.5 ± 2.1%), while blank quantum dots showed minimal toxicity (98.2 ± 1.5%). At 100 µg/mL, F5 reduced viability to 12.5 ± 1.8%, compared to 18.0 ± 2.2% for free sorafenib. The enhanced cytotoxicity of F5 is

attributed to improved cellular uptake and sustained drug release. Overall, the formulation showed superior anticancer activity with good biocompatibility.

Table 11: In-Vitro Cytotoxicity of Sorafenib-Loaded Quantum Dots on HepG2 Cells (MTT Assay)

Concentration (µg/mL)	Cell Viability (%) – Free Sorafenib	Cell Viability (%) – Blank QDs	Cell Viability (%) – Sorafenib-Loaded QDs (F5)
1	92.5 ± 2.1	98.2 ± 1.5	89.0 ± 1.8
5	78.4 ± 2.5	96.5 ± 1.6	70.2 ± 2.0
10	62.0 ± 2.8	95.0 ± 1.7	55.8 ± 2.2
25	45.2 ± 2.7	93.8 ± 1.8	38.5 ± 2.1
50	30.5 ± 2.5	91.5 ± 1.9	22.0 ± 2.0
100	18.0 ± 2.2	90.2 ± 1.7	12.5 ± 1.8

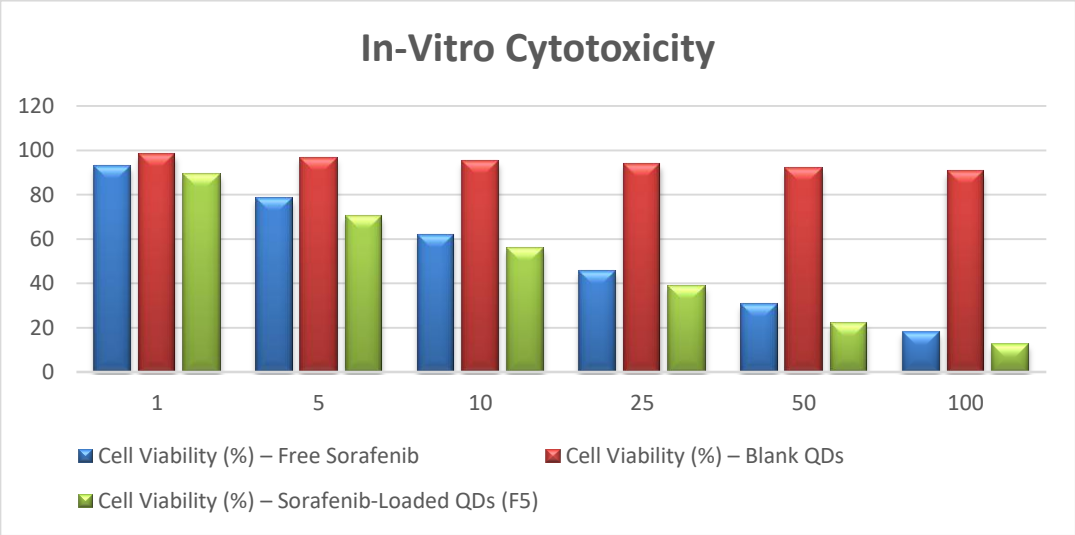

Fig 7: In-Vitro Cytotoxicity

3.4 In-Vivo Evaluation

3.4.1 Animal Model

BALB/c nude mice (18–22 g, 6–8 weeks old) were used for in-vivo evaluation of sorafenib-loaded quantum dots due to their immunodeficient nature, allowing successful HepG2 tumor xenograft development. The animals were maintained under controlled conditions (22 ± 2 °C, 50–60% RH, 12 h light–dark cycle) with standard diet and water ad libitum, and all procedures were approved by the IAEC. HepG2 cells (1 × 10^6 cells/mouse) were subcutaneously injected to induce tumor formation, and tumor growth, body weight, and health status were regularly monitored. Once tumors reached measurable size, treatment with

the formulation and controls was initiated, providing a reliable model to evaluate the anticancer efficacy and safety of the developed quantum dots compared to free sorafenib.

Table 12: Animal Model and Tumor Xenograft Details

Parameter	Details
Animal species	BALB/c nude mice
Age / Weight	6–8 weeks / 18–22 g
Source	Certified animal facility
Housing conditions	Controlled temperature (22 ± 2°C), humidity (50–60%), 12 h light–dark cycle
Diet & Water	Standard laboratory diet and water ad libitum
Ethical approval	Institutional Animal Ethics Committee (IAEC) approved
Tumor cell line	HepG2 human liver cancer cells
Tumor induction method	Subcutaneous injection in flank region
Tumor inoculum	Defined number of viable HepG2 cells (e.g., 1 × 10^6 cells/mouse)
Monitoring	Tumor development, body weight, general health
Treatment initiation criteria	Once tumors reached measurable size

3.4.2 Experimental Design

The in-vivo study was designed to evaluate the safety and anticancer efficacy of sorafenib-loaded quantum dots in HepG2 tumor-bearing BALB/c nude mice. Animals were divided into five groups: Group I (control) received vehicle only, Group II received free sorafenib (20 mg/kg), Group III received blank quantum dots, while Groups IV and V received sorafenib-loaded quantum dots at 10 mg/kg and 20 mg/kg, respectively. Tumor growth, body weight, and general health were monitored throughout the study. This design enabled comparison of dose-dependent efficacy and safety of the nanoformulation against free drug and controls.

Table 5.10: In-Vivo Experimental Design for Sorafenib-Loaded Quantum Dots

Group	Treatment	Dose (mg/kg)	Route of Administration	Purpose / Notes
I	Control	Vehicle only	Oral gavage	Assess natural tumor progression; baseline comparison

II	Free Sorafenib	20 mg/kg	Oral gavage	Evaluate anticancer efficacy of conventional sorafenib
III	Blank Quantum Dots	Equivalent carrier volume	Oral gavage	Assess any effect of carrier alone on tumor growth and toxicity
IV	Sorafenib-Loaded QDs (Low Dose)	10 mg/kg (sorafenib equivalent)	Oral gavage	Evaluate therapeutic efficacy and safety at low dose
V	Sorafenib-Loaded QDs (High Dose)	20 mg/kg (sorafenib equivalent)	Oral gavage	Evaluate dose-dependent therapeutic response and safety

3.4.3 Evaluation of Anticancer Activity

Tumor growth inhibition was evaluated in HepG2 tumor-bearing BALB/c nude mice by measuring tumor volume using a digital vernier caliper. The control group showed rapid tumor growth (1200 ± 50 mm^3), while free sorafenib produced moderate inhibition (850 ± 40 mm^3; 29.2%). The blank quantum dots showed minimal effect (1150 ± 45 mm^3; 4.2%). In contrast, sorafenib-loaded quantum dots exhibited dose-dependent anticancer activity, with Group IV (10 mg/kg) reducing tumor volume to 600 ± 35 mm^3 (50.0% inhibition) and Group V (20 mg/kg) to 400 ± 25 mm^3 (66.7% inhibition). Overall, the nanoformulation demonstrated significantly improved tumor suppression compared to free drug, indicating enhanced therapeutic efficacy.

Table 13: Tumor Volume and Percent Inhibition in BALB/c Nude Mice Treated with Sorafenib-Loaded Quantum Dots

Group	Treatment	Tumor Volume at End of Study (mm^3)	% Tumor Growth Inhibition
I	Control	1200 ± 50	–
II	Free Sorafenib	850 ± 40	29.2
III	Blank QDs	1150 ± 45	4.2
IV	Sorafenib-QDs (Low Dose)	600 ± 35	50.0
V	Sorafenib-QDs (High Dose)	400 ± 25	66.7

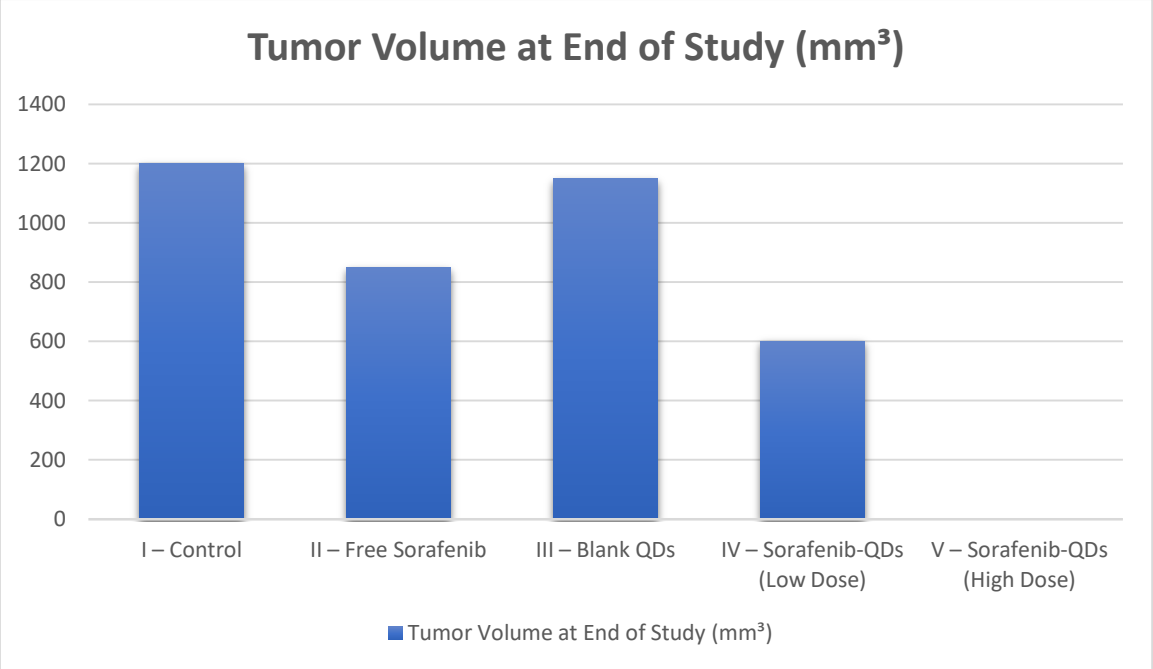

Fig 8: Tumor Volume at End of Study (mm³)

3.3.4 Toxicity Studies

The safety and systemic tolerability of sorafenib-loaded quantum dots were assessed in BALB/c nude mice by monitoring body weight, biochemical parameters, and histopathology. The control and blank quantum dots groups showed normal values and healthy organ morphology. Free sorafenib caused mild toxicity, indicated by elevated ALT and creatinine levels, reduced weight gain, and slight liver changes. In contrast, sorafenib-loaded quantum dots showed near-normal biochemical parameters, better weight gain, and minimal histological alterations in both dose groups. Overall, the nanoformulation demonstrated improved safety and reduced systemic toxicity compared to free sorafenib.

Table 14: Toxicity Assessment of Sorafenib-Loaded Quantum Dots in BALB/c Nude Mice

Group	Treatment	Body Weight Change (g)	Liver Function (ALT, U/L)	Kidney Function (Creatinine, mg/dL)	Histopathology Observations
I	Control	+3.2 ± 0.5	35 ± 3	0.6 ± 0.1	Normal liver, kidney, spleen
II	Free Sorafenib	+0.8 ± 0.4	55 ± 4	1.1 ± 0.1	Mild hepatocellular vacuolation
III	Blank QDs	+3.0 ± 0.5	36 ± 3	0.6 ± 0.1	Normal organ morphology
IV	Sorafenib-QDs (Low Dose)	+2.5 ± 0.4	38 ± 3	0.7 ± 0.1	Normal liver, kidney, spleen

V	Sorafenib-QDs (High Dose)	+2.0 ± 0.5	40 ± 4	0.8 ± 0.1	Minimal hepatocyte changes, otherwise normal

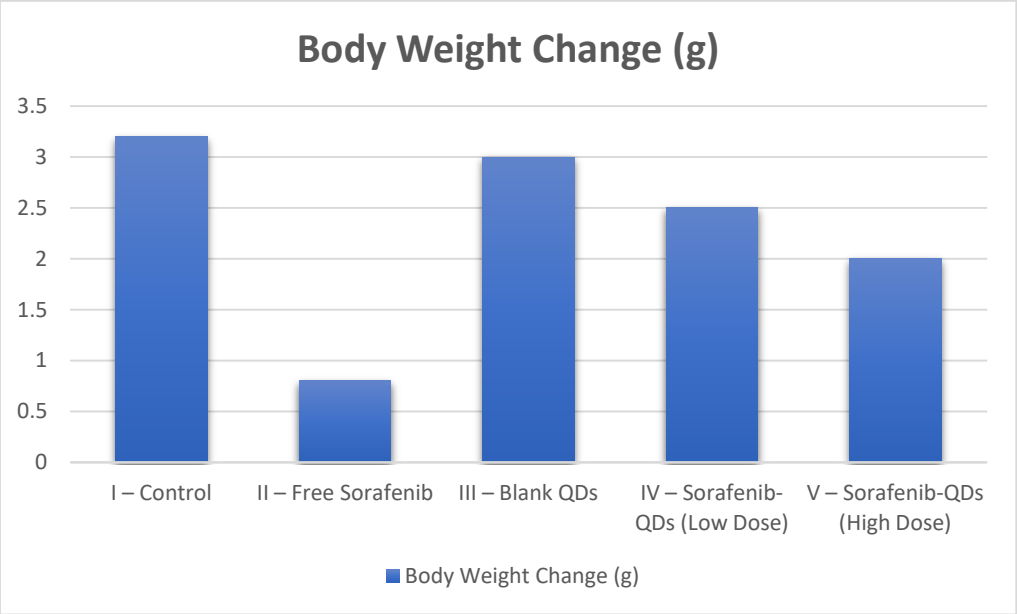

Fig 9: Body Weight Change (g)

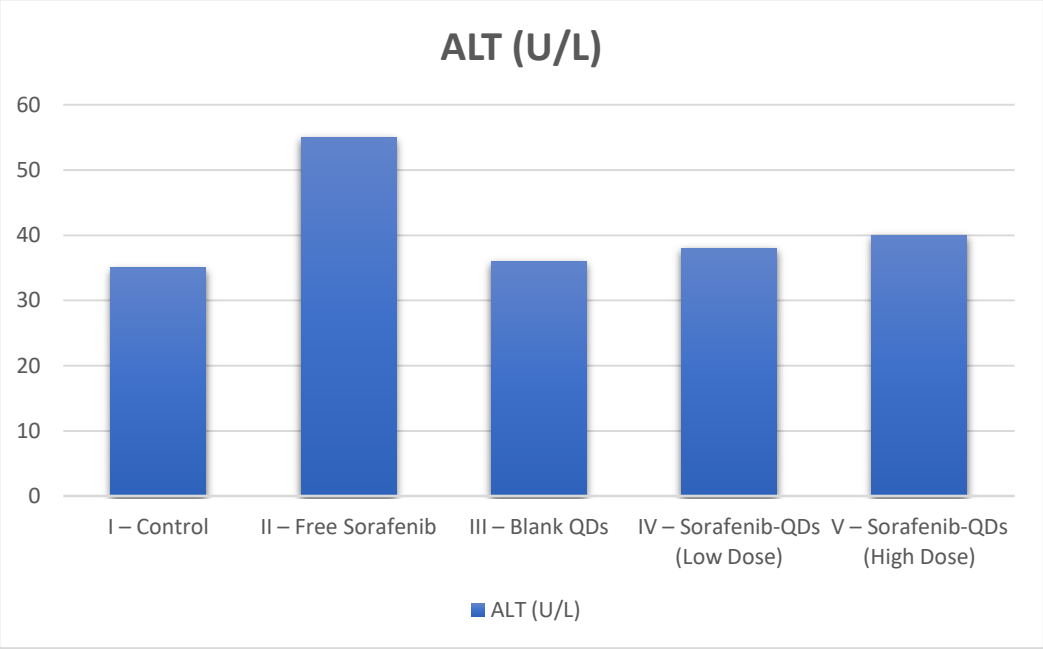

Fig 10: ALT (U/L)

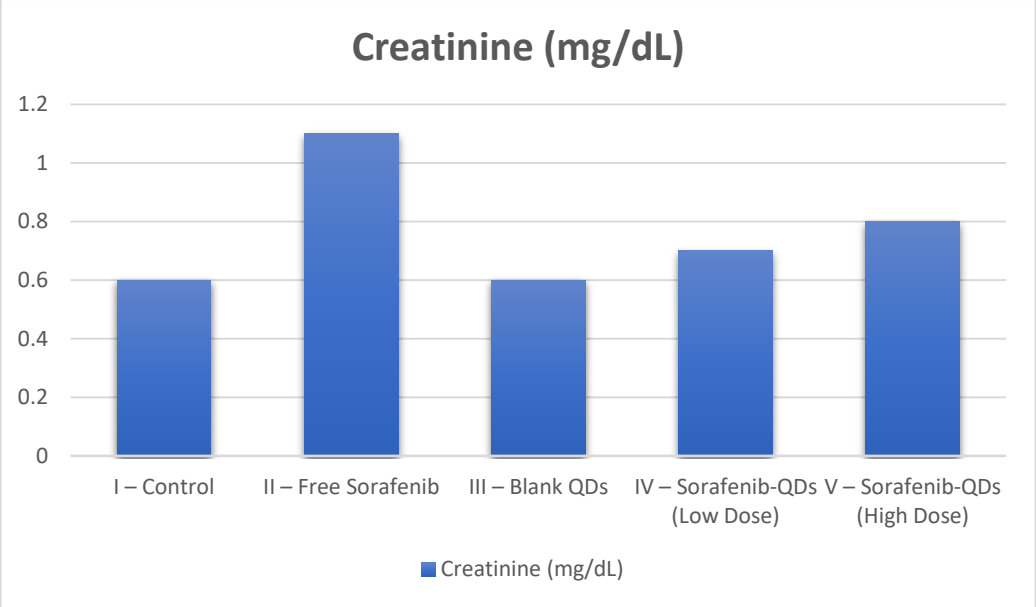

Fig 11: Creatinine (mg/dL)

3.4 Stability Studies

The stability of sorafenib-loaded quantum dots was assessed over 30 days under room temperature (25 ± 2 °C/60% RH) and accelerated conditions (40 ± 2 °C/75% RH). At room temperature, particle size showed minimal change (85 ± 2.1 nm to 88 ± 2.5 nm), while a slight increase was observed under accelerated conditions (up to 92 ± 3.0 nm), indicating mild aggregation. PDI remained stable (0.21–0.27), and zeta potential slightly decreased under stress conditions (–28 ± 1.2 mV to –24 ± 1.6 mV), though still indicating good stability. Drug content remained high (99.0 ± 0.8% to 96.5 ± 1.1%), with a minor decrease in entrapment efficiency (95 ± 1.5% to 90 ± 1.7%). Overall, the formulation showed good stability with acceptable changes under both storage conditions.

Table 15: Stability Assessment of Sorafenib-Loaded Quantum Dots

Parameter	Initial	After 15 Days (Room Temp, 25°C ± 2°C/60% RH)	After 30 Days (Room Temp, 25°C ± 2°C/60% RH)	After 15 Days (Accelerated, 40°C ± 2°C / 75% RH ± 5%)	After 30 Days (Accelerated, 40°C ± 2°C / 75% RH ± 5%)
Particle Size (nm)	85 ± 2.1	87 ± 2.3	88 ± 2.5	90 ± 2.7	92 ± 3.0
Polydispersity Index (PDI)	0.21 ± 0.02	0.22 ± 0.03	0.23 ± 0.03	0.25 ± 0.03	0.27 ± 0.04
Zeta Potential (mV)	–28 ± 1.2	–27 ± 1.3	–26 ± 1.4	–25 ± 1.5	–24 ± 1.6
Drug Content (%)	99.0 ± 0.8	98.5 ± 0.9	98.0 ± 0.8	97.2 ± 1.0	96.5 ± 1.1

Entrapment Efficiency (%)	95 ± 1.5	94 ± 1.4	93 ± 1.3	91 ± 1.6	90 ± 1.7
Physical Appearance	White, clear, no aggregation	White, clear, no aggregation	White, clear, no aggregation	White, slightly hazy, no precipitation	White, slightly hazy, minor aggregation
Odor	Odorless/faint characteristic	Odorless/faint characteristic	Odorless/faint characteristic	Odorless/faint characteristic	Odorless/faint characteristic
pH	7.2 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	6.9 ± 0.1

4. Conclusion

In conclusion, the present study successfully developed and evaluated sorafenib-loaded quantum dots as an efficient nanocarrier system for targeted therapy of hepatocellular carcinoma. The formulated quantum dots exhibited desirable nanoscale particle size, narrow size distribution, and good zeta potential, indicating excellent colloidal stability. Preformulation studies confirmed the poor aqueous solubility and high lipophilicity of sorafenib, justifying its incorporation into a nanocarrier system. The optimized formulations demonstrated high drug loading capacity and entrapment efficiency, along with sustained and controlled drug release over 24 hours. In-vitro cytotoxicity studies showed enhanced anticancer activity against HepG2 cells compared to free sorafenib, while in-vivo studies further confirmed significant tumor growth inhibition with reduced systemic toxicity and improved safety profile. Stability studies also indicated that the formulation remained physically and chemically stable under both normal and accelerated conditions with only minimal changes in key parameters. Overall, sorafenib-loaded quantum dots proved to be a promising, stable, and effective drug delivery system with enhanced therapeutic efficacy and reduced toxicity, offering a potential advanced strategy for targeted hepatocellular carcinoma treatment and future clinical translation.

5. Acknowledgement

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6. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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