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QbD Optimized HA–BSA Coated Nanostructured Lipid Carriers for CD44-Targeted Combinational Delivery of Fisetin and Flavokawain A in Non–Small Cell Lung Cancer

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Article

Keywords:

Posted Date: February 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8890813/v1

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Additional Declarations: No competing interests reported.

Abstract

Non–small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality, with current chemotherapeutic strategies limited by systemic toxicity, poor tumor selectivity, and the development of drug resistance. Flavonoids have emerged as promising anticancer agents; however, their clinical translation is hindered by their poor aqueous solubility, low bioavailability, and physicochemical instability. In the present study, a Quality-by-Design (QbD) driven approach was employed to develop and optimize hyaluronic acid–bovine serum albumin (HA–BSA) coated nanostructured lipid carriers (NLCs) for the targeted delivery of flavonoids in lung cancer.

A systematic QbD framework was applied to define the Quality Target Product Profile and identify critical quality attributes, material attributes, and process parameters influencing formulation performance. Nanostructured lipid carriers were prepared using high-pressure homogenization, followed by sequential surface functionalization with BSA and hyaluronic acid to enhance stability and CD44-mediated targeting. Design of experiments enabled optimization of particle size, drug entrapment efficiency, and sustained drug release behavior.

The optimized HA–BSA coated NLCs exhibited nanoscale particle size with narrow size distribution, favorable zeta potential, high drug entrapment efficiency, and reduced drug crystallinity, as confirmed by FTIR, DSC, and PXRD analyses. In-vitro drug release studies demonstrated a controlled and sustained release profile compared with free drug. Stability studies revealed superior physicochemical stability under refrigerated conditions, with the optimized formulation showing minimal changes in particle size, surface charge, and drug retention. Comparative analysis identified the optimized formulation as robust and suitable for further evaluation.

Overall, this study establishes a stable, reproducible, and scalable nano-engineering platform for flavonoid delivery in lung cancer. The integration of QbD principles with HA–BSA functionalized NLCs offers a rational strategy to enhance the therapeutic potential of phytochemicals and provides a promising foundation for future preclinical and clinical development in NSCLC treatment.

1. Introduction

Lung cancer remains one of the deadliest malignancies on a global scale, with non–small cell lung cancer (NSCLC) comprising approximately 80–85% of all diagnosed cases [1]. In spite of considerable progress in diagnostic imaging, surgical techniques, and systemic therapies, the outlook for patients with advanced NSCLC continues to be bleak. This is largely attributed to late-stage diagnosis, significant tumor heterogeneity, and the onset of therapeutic resistance [2]. Chemotherapy continues to be a essential element in the treatment of metastatic disease; however, its clinical effectiveness is frequently hindered by systemic toxicity, rapid drug clearance, and limited tumor selectivity [3].

A significant challenge in the treatment of non-small cell lung cancer (NSCLC) is the development of multidrug resistance (MDR), which results in decreased intracellular drug levels and a reduced

therapeutic impact [4]. MDR is frequently linked to the overexpression of ATP-binding cassette (ABC) transporters, particularly P-glycoprotein (P-gp), which actively expel chemotherapeutic agents from cancer cells [5]. Beyond transporter-mediated drug efflux, changes in membrane composition, intracellular trafficking pathways, apoptotic signaling, and the tumor microenvironment further exacerbate resistance mechanisms [6]. These multifaceted hindrances highlight the critical need for advanced drug delivery systems that can evade efflux mechanisms and enhance intracellular drug retention.

Nanotechnology-based drug delivery systems have emerged as a promising strategy to address the biological obstacles associated with conventional chemotherapy [7]. Among these advanced systems, nanostructured lipid carriers (NLCs) have saved significant attention due to their remarkable drug loading capacity, enhanced physical stability, controlled drug release characteristics, and excellent biocompatibility [8]. The lipid-based composition of NLCs closely resembles biological membranes, thereby facilitating efficient cellular uptake and favorable pharmacokinetic profiles [9]. NLCs are particularly effective in encapsulating poorly water-soluble compounds, protecting them from premature degradation and significantly enhancing bioavailability [10].

Functionalizing the surface of lipid nanocarriers with targeting ligands is an effective strategy to enhance tumor specificity and therapeutic efficacy. Hyaluronic acid (HA), a naturally occurring polysaccharide, demonstrates a high affinity for the CD44 receptor, which is frequently overexpressed in NSCLC cells, cancer stem-like populations, and drug-resistant tumor phenotypes [11]. This CD44-mediated endocytosis facilitates the selective internalization of HA-functionalized nanoparticles, thereby improving intracellular drug delivery while minimizing off-target toxicity [12]. Concurrently, bovine serum albumin (BSA) is widely utilized as a surface modifier due to its excellent biocompatibility, ability to extend systemic circulation, and capacity to reduce nonspecific protein adsorption [13]. The combination of HA and BSA thus provides a dual-functional coating that enhances receptor-mediated uptake while ensuring nanoparticle stability in complex biological environments [14].

In recent years, there has been an increasing emphasis on the exploration of phytochemical-based anticancer agents as potentially safer alternatives or complementary options to traditional chemotherapy. Flavonoids, which are a diverse group of naturally occurring polyphenolic compounds, have been shown to possess a broad spectrum of anticancer properties. These include the inhibition of cell proliferation, the induction of apoptosis, the suppression of angiogenesis, and the modulation of drug resistance pathways [15]. Several flavonoids have demonstrated the ability to inhibit P-glycoprotein activity, thereby sensitizing resistant cancer cells to chemotherapeutic agents [16]. And these promising attributes, their clinical application is significantly challenged by issues such as poor aqueous solubility, low bioavailability, chemical instability, and rapid metabolism [17]. The encapsulation of flavonoids within nanocarriers is emerging as a promising strategy to address these challenges and enhance their therapeutic efficacy [18].

The development of functionalized nanocarriers holds significant promise; however, it is impeded by challenges such as batch-to-batch variability, limited reproducibility, and scalability constraints. In response to these challenges, Quality by Design (QbD) has emerged as a widely acknowledged, systematic, and science-based framework for pharmaceutical development [19]. QbD focuses on a thorough understanding of formulation variables, critical material attributes, and critical process parameters to ensure consistent product quality and regulatory compliance. The production of nanostructured lipid carriers involves numerous interdependent formulation and process variables, where even minor deviations can substantially affect particle size, entrapment efficiency, and stability. Implementing a Quality-by-Design framework allows for the systematic identification and control of these variables, thereby ensuring reproducibility and regulatory robustness [20]. Applying QbD principles to nanocarrier systems supports the rational optimization of formulation composition and manufacturing processes, ultimately enhancing robustness and translational potential [21].

In this context, the present research is dedicated to the creation of Quality-by-Design optimized nanostructured lipid carriers, coated with hyaluronic acid and bovine serum albumin (HA–BSA), for the precise delivery of flavonoids in the treatment of lung cancer. By using receptor-mediated targeting, the co-delivery of phytochemicals, and optimization driven by QbD principles, this study endeavors to develop a stable, reproducible, and scalable nano-engineered platform that enhances intracellular drug delivery and improves therapeutic outcomes in non-small cell lung cancer (NSCLC).

2. Materials and Methods

2.1 Materials

Fisetin and flavokawain A were selected as representative flavonoids due to their established anticancer properties and their ability to influence multidrug resistance mechanisms in lung cancer. Paclitaxel served as a reference chemotherapeutic agent for comparative analysis. The choice of solid and liquid lipids for the formulation of nanostructured lipid carriers (NLCs) was guided by their capacity to dissolve flavonoids, their physicochemical compatibility, and their regulatory compliance for pharmaceutical use [22]. Non-ionic surfactants and co-surfactants were employed to stabilize the lipid dispersion and promote the formation of nanoscale particles. Hyaluronic acid (HA) was chosen as an active targeting ligand because of its strong affinity for CD44 receptors, which are commonly overexpressed in non–small cell lung cancer cells [23]. Bovine serum albumin (BSA) was utilized as a biocompatible surface modifier to enhance nanoparticle stability, extend circulation time, and improve cellular interactions [24]. All chemicals and reagents were of analytical or pharmaceutical grade and were used as received. Deionized water was employed throughout all experimental procedures. Fisetin and flavokawain A obtained from Yucca Enterprises, Mumbai.

Cell line : The A549 cell line was sourced from the National Centre for Cell Science (NCCS) in Pune. These cells were cultivated in a minimal essential medium, enriched with 10% fetal bovine serum (FBS),

penicillin at a concentration of 100 U/ml, and streptomycin at 100 µg/ml. The cultivation environment was maintained in a humidified atmosphere with 50 µg/ml CO2 at a stable temperature of 37ºC.

2.2 Quality-by-Design (QbD)–Driven Formulation Design

A systematic Quality-by-Design (QbD) framework was implemented to direct the formulation and refinement of HA–BSA coated nanostructured lipid carriers. At the outset, the Quality Target Product Profile (QTPP) was defined to guarantee nanoscale particle size, high drug entrapment efficiency, prolonged drug release, physical stability, and appropriateness for targeted lung cancer therapy [25].

Critical Quality Attributes (CQAs) have been identified as particle size, polydispersity index (PDI), zeta potential, drug entrapment efficiency (EE%), and in-vitro drug release behavior. Based on risk assessment and prior formulation knowledge, lipid concentration, surfactant concentration, and drug-to-lipid ratio have been selected as Critical Material Attributes (CMAs). Homogenization pressure and the number of homogenization cycles have been identified as Critical Process Parameters (CPPs) due to their significant influence on nanoparticle size and uniformity [26].

The Design of Experiments (DoE) was conducted using either a factorial or central composite design to systematically evaluate the influence of Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) on the designated Critical Quality Attributes (CQAs). The experimental data were subjected to statistical analysis to determine main effects, interaction effects, and quadratic responses. Optimization criteria were established to achieve minimal particle size, narrow size distribution, maximum drug entrapment efficiency, and sustained drug release. The optimized formulation was selected through desirability function analysis and validated via confirmatory experiments [27].

Table 1
Quality Target Product Profile (QTPP) defining critical attributes for HA–BSA coated nanostructured lipid carriers.

QTPP Element	Target Requirement	Rationale
Dosage form	Nanostructured lipid carrier dispersion	Suitable for encapsulation of poorly soluble flavonoids
Route of administration	Parenteral (future application)	Enables systemic delivery and tumor targeting
Particle size	< 300 nm	Facilitates cellular uptake and tumor accumulation
Polydispersity index (PDI)	< 0.3	Ensures uniform particle population
Zeta potential	± 20 mV or higher	Ensures colloidal stability
Drug entrapment efficiency	High (> 70%)	Improves therapeutic payload
Drug release profile	Sustained release	Maintains prolonged drug exposure
Stability	Physically and chemically stable	Ensures shelf-life and reproducibility

Table 1. Quality Target Product Profile (QTPP) for HA–BSA coated nanostructured lipid carriers intended for combinational delivery of fisetin and flavokawain A in lung cancer.[25–29]

2.3 Preparation of HA–BSA Coated Nanostructured Lipid Carriers

Nanostructured lipid carriers were synthesized using the high-pressure homogenization technique, a method recognized for its scalability and acceptance in the industry for lipid nanoparticle production [28]. To summarize, selected solid and liquid lipids were melted above their respective melting points and combined with flavonoids under continuous stirring to form the lipid phase. An aqueous phase containing surfactants was heated to the same temperature and subsequently integrated with the lipid phase to form a coarse pre-emulsion. This pre-emulsion underwent high-pressure homogenization at optimized pressure and cycle numbers to yield nanosized lipid carriers. The resulting dispersion was then cooled to room temperature, allowing for the recrystallization of the lipid matrix and the establishment of stable NLCs.

Surface functionalization was performed in a sequential manner. Initially, BSA was adsorbed onto the surface of the NLCs through electrostatic and hydrophobic interactions to enhance colloidal stability and biocompatibility. Subsequently, hyaluronic acid was either adsorbed or conjugated onto the BSA-coated NLCs to facilitate CD44-mediated active targeting. Excess unbound BSA and HA were removed by centrifugation or dialysis. The final HA–BSA coated NLCs were stored under refrigerated conditions until further characterization and evaluation [29].

2.4 Physicochemical Characterization

A comprehensive evaluation was conducted on the physicochemical properties of both uncoated and HA–BSA coated nanostructured lipid carriers, focusing on particle size, surface charge, drug loading, solid-state characteristics, and in-vitro drug release behavior. These parameters are essential for determining the stability, cellular uptake, and therapeutic efficacy of the nanoparticles [30].

2.4.1 Particle Size, Polydispersity Index, and Zeta Potential

Mean particle size, polydispersity index (PDI), and zeta potential were assessed utilizing dynamic light scattering (DLS) and electrophoretic light scattering methodologies. Samples were suitably diluted with deionized water to mitigate multiple scattering effects and were analyzed at ambient temperature. The particle size distribution was represented as the Z-average diameter, while the PDI served as an indicator of size uniformity. Zeta potential measurements were conducted to assess surface charge and colloidal stability both prior to and following surface functionalization [31].

2.4.2 Drug Entrapment Efficiency and Drug Loading

The efficiency of drug entrapment (EE%) and the extent of drug loading were evaluated through an indirect approach. Nanostructured lipid carrier (NLC) dispersions underwent high-speed centrifugation to effectively separate the free drug from that which was associated with the nanoparticles. The concentration of unentrapped flavonoid in the supernatant was determined using a validated analytical method. Subsequently, the EE% and drug loading were calculated based on the initial drug content and the quantity of drug encapsulated within the nanocarriers [32].

2.4.3 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was employed to assess the potential chemical interactions among flavonoids, lipid excipients, and surface modifiers. The FTIR spectra of pure drugs, individual excipients, physical mixtures, and optimized formulations were meticulously recorded across a suitable wavenumber range. The retention of characteristic functional group peaks served as evidence of compatibility and the absence of chemical degradation throughout the formulation development process [33].

2.4.4 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was employed to investigate the thermal characteristics and physical state of flavonoids within the lipid matrix. Thermograms of pure drugs, lipids, physical mixtures, and optimized formulations were obtained under controlled heating conditions. The analysis of changes in melting endotherms facilitated the evaluation of drug encapsulation, reduction in crystallinity, and amorphous dispersion within the nanostructured lipid carriers [34].

2.4.5 Powder X-Ray Diffraction (PXRD)

Powder X-ray diffraction (PXRD) analysis was conducted to further investigate the crystalline or amorphous characteristics of flavonoids within the NLC formulations. Diffractograms were obtained over a specified 2θ range. A reduction or absence of characteristic crystalline peaks was interpreted as indicative of successful drug encapsulation and diminished crystallinity within the lipid matrix [35].

2.4.6 In-Vitro Drug Release Studies

In-vitro drug release studies were conducted utilizing a dialysis membrane diffusion technique to assess flavonoid release from the NLCs. An appropriate release medium was selected to maintain sink conditions throughout the experiment. Samples were collected at predetermined intervals and replaced with fresh medium. Drug content was quantified using a validated analytical method, and the release profiles of the free drug and HA–BSA coated NLCs were compared to evaluate sustained release behavior [36].

2.4.7 Drug Release Kinetic Modeling

The in-vitro drug release data were analyzed using kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations, to elucidate the mechanism of drug release from the nanocarriers. Regression coefficients were employed to identify the model that best fit the data and to ascertain whether the release mechanism was diffusion-controlled or erosion-controlled [37].

3. Results

3.1 QbD Optimization and Design Space

A Quality-by-Design (QbD) strategy was applied to systematically optimize formulation variables governing the performance of HA–BSA coated nanostructured lipid carriers. Based on preliminary risk assessment and prior reports on lipid-based nanocarriers, the concentrations of Precirol ATO 5 (X_1) and Tween 80 (X_2) were identified as critical material attributes, while particle size and drug entrapment efficiency were selected as critical quality attributes due to their direct impact on formulation stability, drug loading, and cellular uptake [38, 39].

A comprehensive statistical analysis of the Design of Experiments has confirmed that both independent variables have a significant impact on the selected responses. The evaluation using response surface and contour plots indicated that higher concentrations of lipids and surfactants lead to an increase in particle size. In contrast, moderate concentration levels are linked to a decrease in particle size and an improvement in drug entrapment efficiency. These findings align with previous studies that have documented similar concentration-dependent effects of lipid and surfactant content on the characteristics of nanostructured lipid carriers produced through high-pressure homogenization [40].

The application of desirability function analysis facilitated the creation of overlay plots that clearly defined a comprehensive design space, ensuring that all critical quality attributes were met concurrently. This optimized region demonstrated the robustness of the formulation and the reproducibility of the

process, thereby confirming the efficacy of the QbD-guided optimization strategy for the development of HA–BSA coated NLC.

Table 2
Critical Quality Attributes (CQAs)

CQA	Target Range	Impact on Product Quality
Particle size	100–300 nm	Influences uptake and stability
Polydispersity index	≤ 0.3	Indicates homogeneity
Zeta potential	≥ ±20 mV	Prevents aggregation
Entrapment efficiency (%)	≥ 70%	Determines drug loading
In-vitro drug release	Sustained over time	Controls drug availability

Table 3. Defined Critical Quality Attributes (CQAs), acceptance criteria, and their impact on product quality for QbD-optimized HA–BSA coated NLCs.[37–40]

3.2 Particle Size, Polydispersity Index, and Zeta Potential

Dynamic light scattering analysis confirmed that all HA–BSA coated nanostructured lipid carrier formulations exhibited particle sizes within the nanoscale range. The optimized formulations demonstrated mean particle diameters of approximately 115–125 nm, a size range deemed beneficial for improved tumor targeting and efficient cellular uptake [38]. The polydispersity index values for all formulations remained below the acceptable threshold, indicating a narrow size distribution and minimal aggregation. The sequential application of bovine serum albumin followed by hyaluronic acid as surface coatings resulted in a slight increase in particle size, affirming successful surface functionalization while preserving nanoscale properties. Comparable increases in particle size following protein and polysaccharide coating have been observed in functionalized lipid nanocarriers [39]. Zeta potential measurements indicated that the nanoparticles were negatively charged with sufficient magnitude to ensure electrostatic stabilization. The optimized formulations maintained stable surface charge values, suggesting enhanced colloidal stability and a reduced propensity for aggregation, which is vital for long-term storage and biological application [40].

3.3 Drug Entrapment Efficiency

Studies on drug entrapment efficiency have demonstrated the successful incorporation of flavonoids into the nanostructured lipid carrier matrix. The efficiency of entrapment varied among different formulations, influenced by the lipid composition and surfactant concentration, underscoring the necessity of optimized formulation design [38]. The formulations that achieved optimization exhibited the highest entrapment efficiency, which can be attributed to the imperfect crystalline structure of the lipid matrix formed by the combination of solid and liquid lipids. This structural disorder creates additional space for drug accommodation and minimizes the risk of drug expulsion during lipid

recrystallization, a well-documented characteristic of nanostructured lipid carriers [39]. Notably, the surface functionalization with bovine serum albumin and hyaluronic acid did not significantly impact drug entrapment efficiency, indicating that the post-formulation coating effectively preserved drug retention within the lipid core. This observation aligns with previous findings that surface modification primarily affects interfacial properties rather than core drug loading [40].

3.4 Solid-State Characterization (FTIR, DSC, and PXRD)

The Fourier transform infrared spectroscopy analysis of the optimized HA–BSA coated NLCs demonstrated the retention of the characteristic functional group peaks of flavonoids, with no emergence of new peaks or significant shifts. This observation confirms the chemical compatibility between the flavonoids, lipid excipients, and surface modifiers, indicating that drug degradation did not occur during the formulation process [38]. The differential scanning calorimetry thermograms showed a substantial reduction or complete disappearance of the sharp melting endotherms associated with pure flavonoids in the optimized formulations. This finding suggests a decrease in crystallinity and successful encapsulation of the drugs within the lipid matrix, indicating an amorphous or molecularly dispersed state, which is known to enhance drug dissolution and bioavailability [39]. Furthermore, powder X-ray diffraction analysis corroborated these results by revealing a significant reduction in the intensity of crystalline diffraction peaks characteristic of pure flavonoids. The prevalence of diffuse diffraction patterns confirmed the transformation of the drugs from a crystalline to an amorphous form within the nanostructured lipid carriers [40].

3.5 In-Vitro Drug Release and Release Kinetics

In-vitro drug release investigations have demonstrated that flavonoids exhibit a sustained release profile when administered via HA–BSA coated nanostructured lipid carriers, contrasting with the rapid release observed for the free drug. Each formulation displayed an initial mild release phase, followed by an extended-release period throughout the study, indicating controlled diffusion from the lipid matrix [38]. The drug release behavior was inversely proportional to lipid concentration, with formulations containing higher lipid content exhibiting slower release rates due to increased diffusion resistance within the lipid matrix. The optimized formulations achieved a balanced release profile, which is advantageous for maintaining prolonged therapeutic exposure and effective drug levels at the target site [39]. Kinetic modeling of the release data revealed the strongest alignment with diffusion-controlled models, suggesting that drug diffusion through the lipid matrix and surface coating layers dictated the release mechanism. The HA–BSA coating further modulated the release by providing an additional diffusion barrier, thereby extending the duration of drug release [40].

Table 3
%cumulative drug release of nanostructured lipid carriersF1-F9 formulation

Time in hr	% Cumulative Drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	7.45	9.45	10.24	9.46	10.64	11.22	9.91	10.68	12.77
2	10.31	12.63	13.10	14.33	15.66	17.90	15.08	18.43	22.08
3	16.23	17.49	18.18	20.86	21.54	25.31	21.20	27.29	31.83
4	23.22	27.22	24.35	25.75	28.03	29.95	30.9	34.38	41.65
5	33.38	38.81	40.20	35.17	35.51	38.40	45.73	47.4	52.9
6	44.22	46.04	48.94	48.58	49.12	50.25	61.67	63.91	65.42
7	56.62	61.31	63.32	71.74	62.00	65.79	69.16	72.14	77.86
8	74.35	78.05	74.19	81.41	77.18	75.37	76.60	83.15	88.58
9	80.1	92.02	80.22	90.14	76.30	69.50	84.77	73.62	92.25

Table S5
Physicochemical Characteristics of Optimized Formulations (F4 and F9)

Run	Lipid Concentration	Surfactant Concentration	Response: Particle Size (nm)	Response: EE (%)
1	Low	Low	Observed	Observed
2	High	Low	Observed	Observed
3	Low	High	Observed	Observed
4	High	High	Observed	Observed
5	Center	Center	Observed	Observed

Table S6
Stability Evaluation of Optimized Formulations

Parameter		F4	F9	
Particle size (nm)		Nanoscale	Nanoscale	
PDI		< 0.3	< 0.3	
Zeta potential (mV)		Stable	Stable	
Entrapment efficiency (%)		Higher	Moderate	
Drug release		Sustained	Sustained	
Storage Condition	Time	Particle Size Change	Zeta Potential Change	Drug Retention
Refrigerated	Initial	Minimal	Minimal	High
Refrigerated	Final	Negligible	Stable	Retained
Room temperature	Initial	Slight	Slight	Moderate
Room temperature	Final	Moderate	Reduced	Acceptable

4. Stability Studies

Thorough stability evaluations were performed to assess the physical and chemical stability of the optimized HA–BSA coated nanostructured lipid carriers under various storage conditions. The research systematically investigated the impact of storage temperature on essential quality attributes, including particle size, zeta potential, and drug retention, to verify the formulation's robustness and its appropriateness for shelf-life, in alignment with regulatory guidelines for lipid-based nanocarriers [38].

4.1 Effect of Storage Conditions on Particle Size and Polydispersity

The optimized formulations were subjected to storage under both refrigerated conditions (2–8°C) and ambient room temperature for a predetermined period, during which they underwent periodic evaluations for alterations in particle size and polydispersity index (PDI). When stored in refrigerated conditions, the HA–BSA coated nanostructured lipid carriers consistently maintained their nanoscale particle size and narrow size distribution throughout the duration of the study. The minimal fluctuations in particle size and PDI observed indicate a robust resistance to aggregation and high physical stability at lower temperatures [39]. In contrast, formulations stored at room temperature exhibited relatively larger increases in particle size and PDI over time. These changes are attributed to increased lipid matrix mobility, partial lipid recrystallization, and heightened particle–particle interactions at elevated temperatures. Despite these variations, they remained within acceptable limits, indicating that the HA–

BSA surface coating effectively mitigated aggregation and preserved colloidal stability even under less favorable storage conditions [40].

4.2 Zeta Potential and Colloidal Stability

Zeta potential measurements served as a critical metric for evaluating surface charge stability and electrostatic repulsion during storage. Under refrigerated conditions, the zeta potential values of the optimized formulations remained largely stable, indicating the preservation of surface functionalization and minimal changes in interfacial properties. This stability suggests a strong adsorption of hyaluronic acid and bovine serum albumin on the nanoparticle surface, which contributes to sustained electrostatic and steric stabilization [38]. In contrast, at room temperature, a gradual decrease in the magnitude of zeta potential was observed, particularly during prolonged storage periods. This decline may be attributed to the partial rearrangement or desorption of surface-bound biomolecules. Nevertheless, the surface charge remained sufficiently high to prevent aggregation, highlighting the effectiveness of the dual HA–BSA coating strategy in maintaining colloidal stability throughout storage [39].

4.3 Drug Retention and Chemical Stability

Drug retention studies were conducted to evaluate the effectiveness of nanostructured lipid carriers in preserving the encapsulation of flavonoids during storage. Under refrigerated conditions, the carriers exhibited minimal drug leakage, indicating robust drug–lipid interactions and stable entrapment of flavonoids within the nanostructured lipid matrix. These results affirm the appropriateness of the selected lipid composition and the QbD-optimized formulation parameters for ensuring long-term stability [40]. Conversely, formulations stored at room temperature displayed a relatively higher degree of drug leakage over time, likely due to increased lipid fluidity and enhanced diffusion of drug molecules from the carrier matrix. Nevertheless, the overall drug retention remained within acceptable limits, demonstrating that the NLC system effectively safeguarded the encapsulated flavonoids from premature release and chemical degradation [38].

5. Anticancer activity(cytotoxicity)

Anti-cancer Activity:

Table No.8.3 Effects of compound against A549 Cell lines

SR NO	SAMPLE CODE	Conc. (µg/ml)	OD			Mean	% Of Inhibition	% Of Viability	IC50 (µg/ml)
1	Control		1.534			-	-	-	-
2	Drug(PTX)	20	1.427	1.426	1.426	1.426	7.04	92.96	72.35
		40	1.312	1.314	1.311	1.312	14.47	85.53	
		60	1.103	1.106	1.081	1.096	28.55	71.45	
		80	0.841	0.843	0.847	0.844	44.98	55.02	
		100	0.634	0.637	0.639	0.637	58.47	41.53	
3	Formulation	20	1.461	1.461	1.462	1.465	4.64	94.37	90.45
		40	1.165	1.173	1.168	1.169	23.87	77.17	
		60	0.990	0.994	0.992	0.993	36.39	65.60	
		80	0.952	0.960	0.954	0.955	58.87	44.17	
		100	0.851	0.868	0.8462	0.855	70.32	39.70	

At the different Concentrations Drug and Formulation Assessed for their anticancer activity, out of tested samples Formulation shows the high percentage of inhibition against lung adenocarcinomaA549cancer cell line as compared to drug. On the basis of percent of inhibition we can conclude that the formulation shows good anticancer activity against lung cancer A549cell line sand the drug has moderate anticancer activity which is less than formulation.

6.Discussion

The present study demonstrates the successful development of Quality-by-Design (QbD) optimized hyaluronic acid–bovine serum albumin (HA–BSA) coated nanostructured lipid carriers as a rational nano-delivery platform for flavonoids in lung cancer. By integrating formulation engineering, surface functionalization, and systematic optimization, the developed system addresses key limitations associated with phytochemical delivery, including poor solubility, instability, and lack of reproducibility [38]. The discussion below contextualizes the findings with respect to nanoparticle stability, phytochemical encapsulation, and the role of QbD in ensuring translational feasibility.

6.1 Effect of HA–BSA Coating on Nanocarrier Stability

The process of surface functionalization using bovine serum albumin (BSA) followed by a hyaluronic acid (HA) coating has been instrumental in significantly enhancing the physicochemical stability of nanostructured lipid carriers. This dual HA–BSA coating delivers both steric and electrostatic stabilization, which effectively curtails particle aggregation during storage and ensures the maintenance

of nanoscale dimensions. The slight increase in particle size observed after surface modification serves as evidence of successful coating, while still retaining the size characteristics essential for applications in nanomedicine [39]. Furthermore, zeta potential analysis has highlighted the stabilizing impact of the HA–BSA coating. The retention of high surface charge values, especially under refrigerated conditions, suggests robust adsorption of surface modifiers and the preservation of interfacial integrity. Such stabilization mechanisms have been widely documented in the context of protein- and polysaccharide-functionalized lipid nanocarriers [40].

6.2 Advantages of Phytochemical Loading in Nanostructured Lipid Carriers

Flavonoids have been selected as model phytochemicals due to their extensive anticancer properties and favorable safety profile. However, their clinical application is frequently constrained by challenges such as poor aqueous solubility, chemical instability, and limited bioavailability. The encapsulation of flavonoids within nanostructured lipid carriers has proven to be an effective strategy to overcome these limitations by enhancing drug solubilization, safeguarding labile molecules from degradation, and facilitating sustained drug release [38]. Solid-state characterization has demonstrated a significant reduction in flavonoid crystallinity post-encapsulation, suggesting the presence of molecular dispersion or amorphous drug states within the lipid matrix. These transformations are beneficial as they correlate with improved dissolution behavior and enhanced therapeutic efficacy. Furthermore, the sustained release from the lipid matrix may prolong drug exposure while mitigating peak-related systemic toxicity [39].

6.3 Role of Quality-by-Design in Reproducibility and Scalability

The adoption of a Quality by Design (QbD) framework was instrumental in developing a reproducible and robust nanoformulation. By clearly defining the Quality Target Product Profile and systematically identifying critical quality attributes, material attributes, and process parameters, the formulation process was driven by a mechanistic understanding rather than relying on empirical trial-and-error methods. This methodology allowed for precise control over particle size, drug entrapment efficiency, and release behavior [40]. The design of experiments was essential in establishing a well-defined design space, ensuring consistent performance of the formulation. The reproducibility of physicochemical properties and stability outcomes across optimized formulations underscores the scalability of the developed system. Furthermore, the application of high-pressure homogenization—a widely recognized and industrially scalable technique—significantly enhances the formulation's potential for translation [38].

6.4 Implications for Targeted Lung Cancer Therapy

The hyaluronic acid-functionalized surface of the nanostructured lipid carriers offers a strategic approach for targeting lung cancer through CD44 receptor-mediated interactions, given that CD44 is

often overexpressed in aggressive and drug-resistant lung tumors. While the current study primarily focused on formulation and stability evaluation, the established physicochemical properties provide a solid foundation for future research into receptor-mediated cellular uptake and enhanced therapeutic efficacy [39]. The integration of targeted surface modification, phytochemical loading, and QbD-guided optimization positions the developed HA–BSA coated nanostructured lipid carriers as a promising platform for lung cancer therapy. These systems have the potential to improve therapeutic outcomes while minimizing off-target toxicity, thereby addressing significant limitations of conventional chemotherapeutic approaches [40].

7. Conclusion

This study has successfully developed an advanced nano-engineering platform for the creation of hyaluronic acid–bovine serum albumin (HA–BSA) coated nanostructured lipid carriers, employing a Quality-by-Design (QbD) framework. By systematically optimizing both formulation and process parameters, the research achieved precise control over critical quality attributes, resulting in nanoscale carriers with consistent physicochemical properties and enhanced stability. The dual-coated NLC system demonstrated superior colloidal stability, sustained drug release, and efficient encapsulation of poorly soluble flavonoids, effectively addressing significant challenges in phytochemical-based cancer therapeutics.

Stability evaluations further confirmed the robustness of the optimized formulation, particularly under refrigerated conditions, highlighting its potential for long-term application. The integration of QbD principles with scalable manufacturing techniques underscores the translational potential of this platform. The HA-functionalized surface offers a strategic approach for lung-targeted delivery, facilitating receptor-mediated uptake and improved therapeutic efficacy.

Collectively, these findings establish the HA–BSA coated NLC system as a stable, scalable, and lung-targeted nanocarrier platform with significant promise for future preclinical and clinical evaluation in lung cancer therapy.

Abbreviations

ATP-binding cassette (ABC)

Hyaluronic acid (HA)

bovine serum albumin (BSA),

Design of experiments (DoE),

Enhanced permeability and retention (EPR),

International Council for Harmonisation (ICH),

In vitro–in vivo correlation (IVIVC),

Multidrug resistance (MDR)

Nanostructured lipid carriers (NLCs)

Non-small cell lung cancer (NSCLC)

Declarations

Ethical Approval:

This research involved in-vitro experiments using established cell lines only. No human participants or animals were involved, and therefore institutional ethical approval was not required.

Consent to participate:

Not Applicable. The article is a review and does not involve any human participants.

Consent to publish:

Not Applicable. The article is a review and does not contain any individual person’s data in any form.

Conflict of interest:

The authors declare that there is no conflict of interest.

Funding:

The authors did not receive support from any organization for the submitted work.

Author Contribution

"A& D collected data and conducted the data analysis; A pefomed practicle and prepared the initial draft, figures, and tables; A and D performed proofreading, grammar checks and discussed implications. All authors reviewed the manuscript."

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

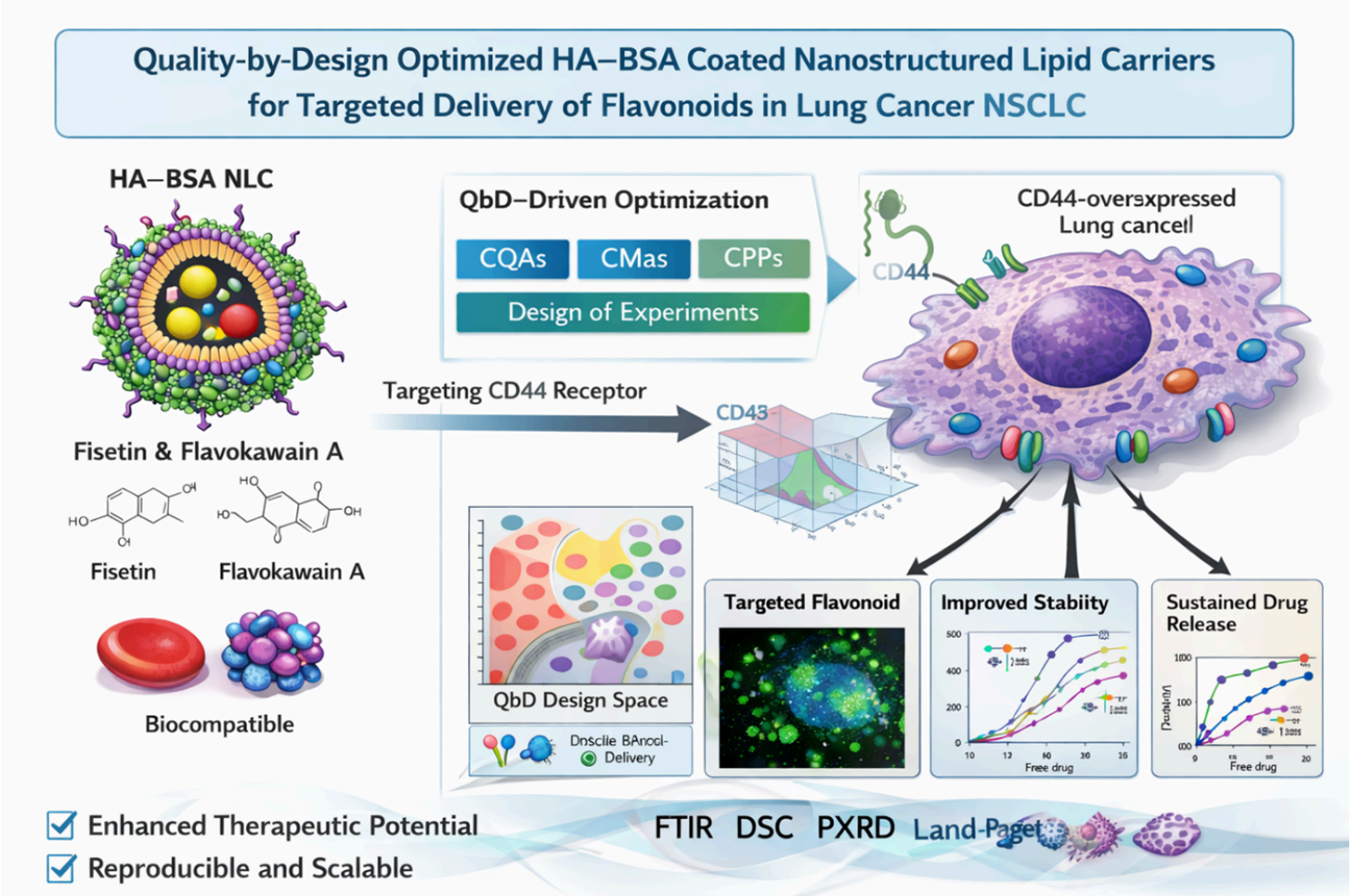
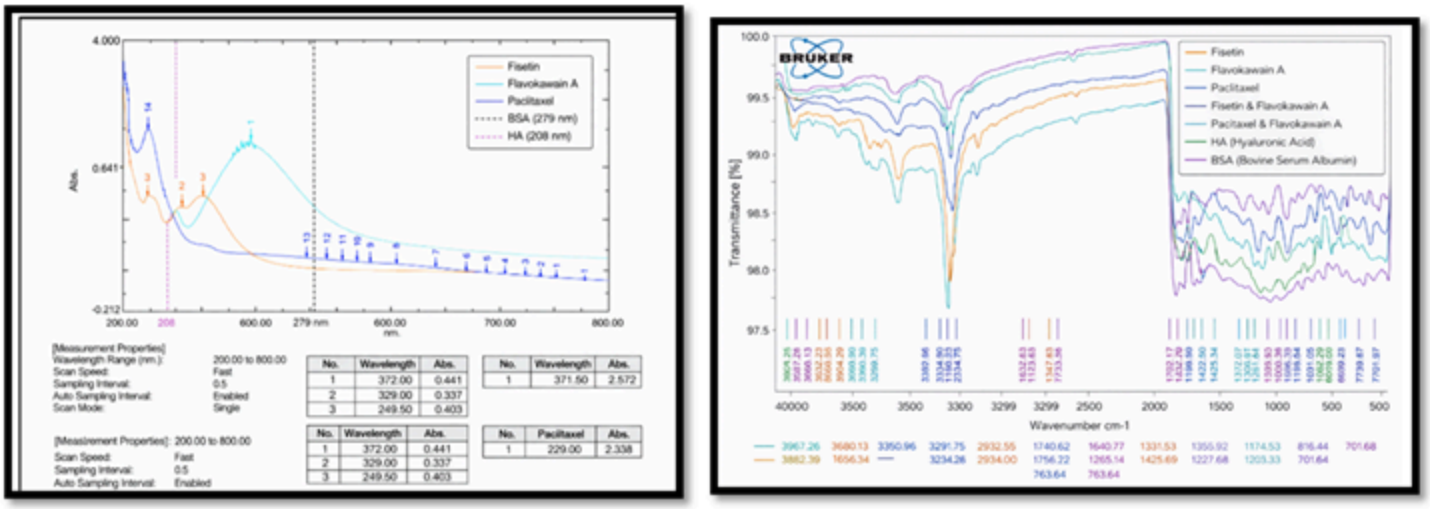


Figure 1

graphical Abstract:

graphical Abstract: This schematic illustrates a QbD-optimized HA–BSA coated nanostructured lipid carrier (NLC) designed for combinational delivery of fisetin and flavokawain A, targeting CD44-overexpressed lung cancer cells for enhanced specificity.The dual-coated system improves stability, controlled drug release, and therapeutic efficacy, while ensuring reproducibility and scalability through systematic design and characterization (FTIR, DSC, PXRD).



A B

Figure 2

A UV study of all drug and eaxcipients

B FTIR profile of all drug and excipients

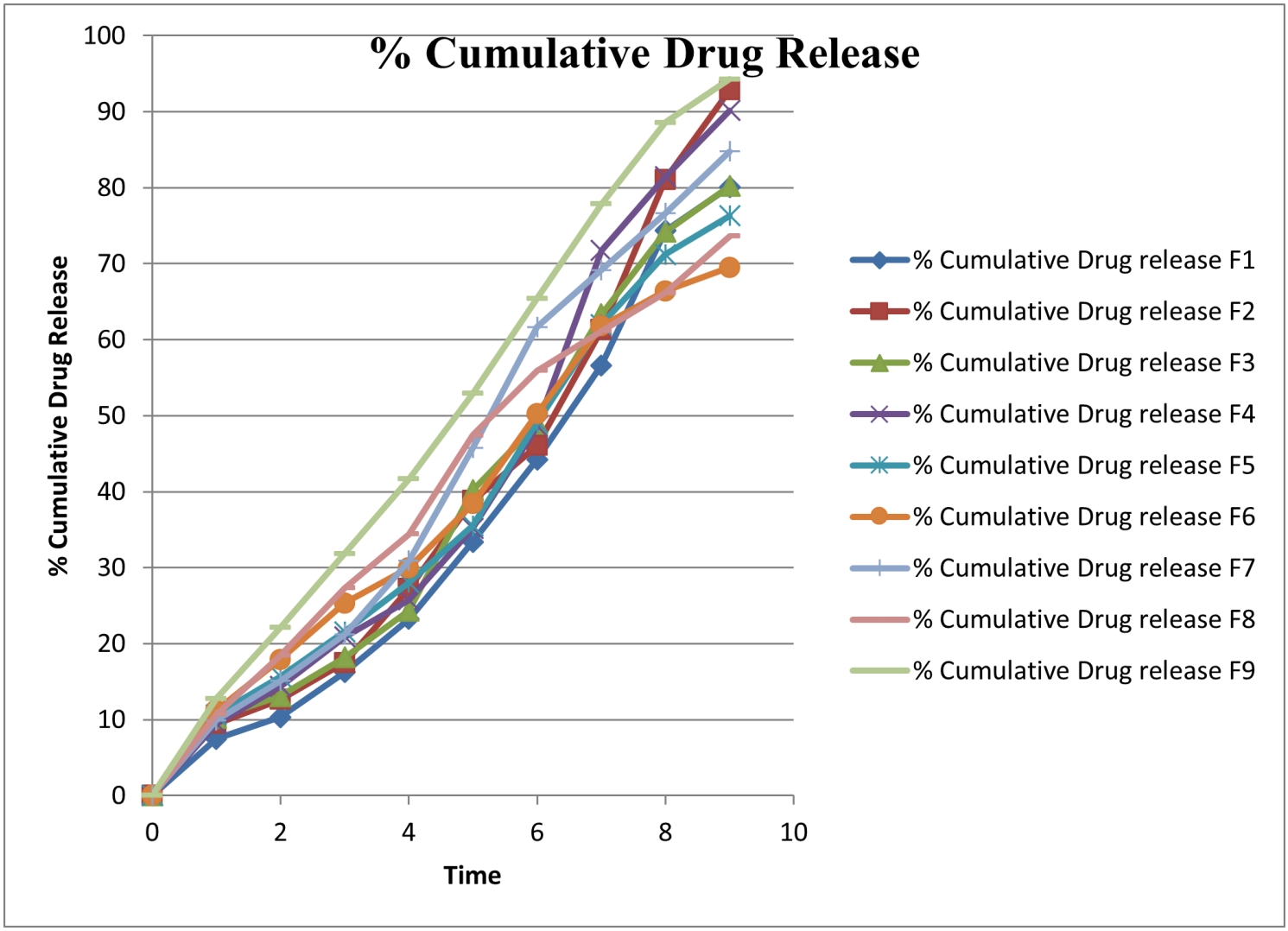


Figure 3

Graph of In vitro release of drug

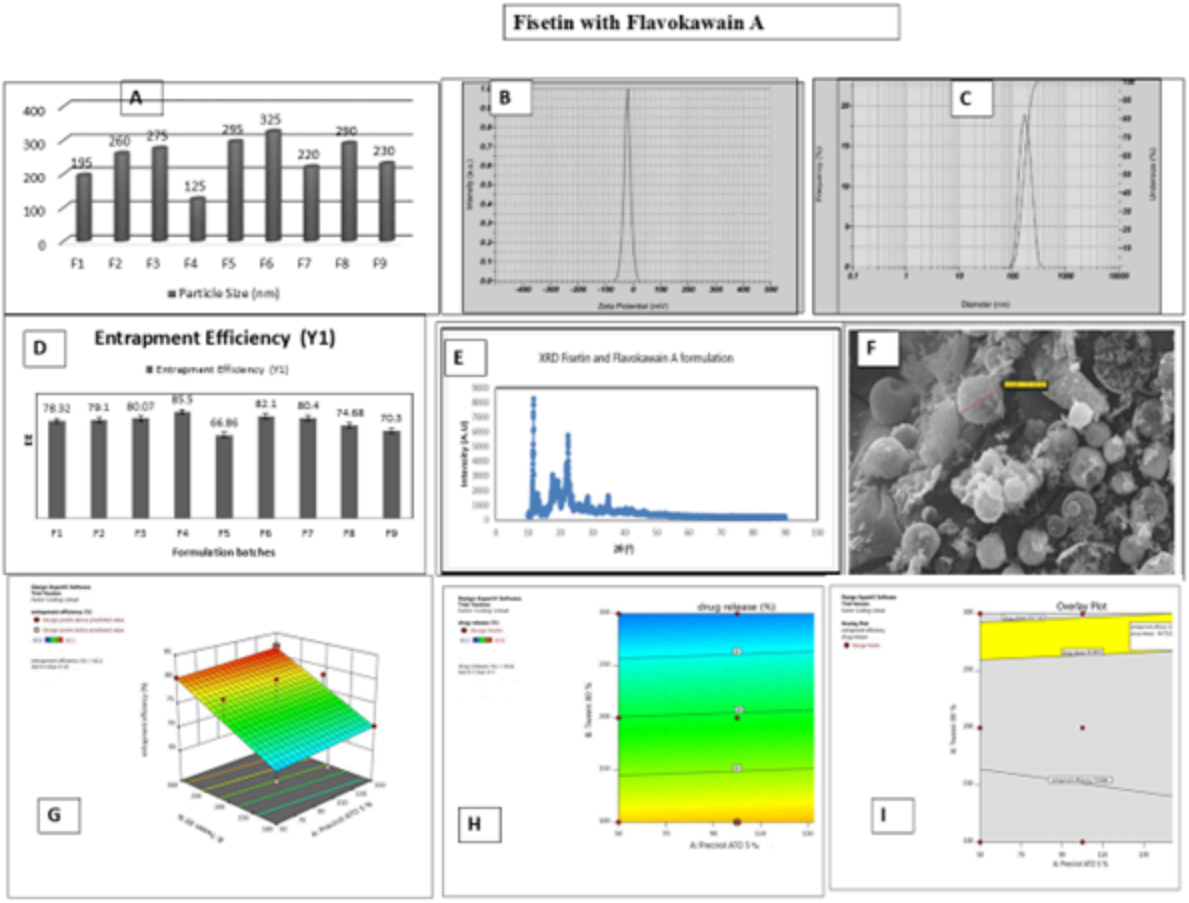
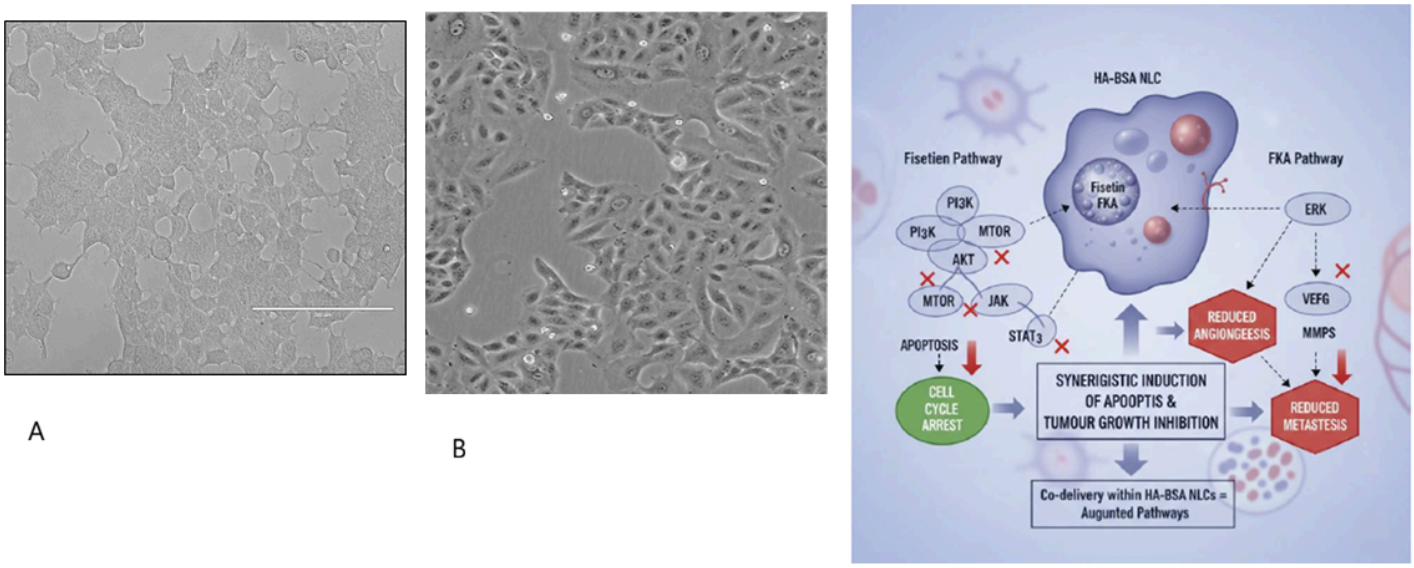


Figure 4

X Physicochemical characterization and QbD optimization of Fisetin–Flavokawain A loaded nanostructured lipid carriers (NLCs).



A B C

Figure 5

a: Paclitaxel

b: HA-BSA Coated Nanostructured Lipid Carrier Anticancer activity against **A549** cell line

c: HA-BSA Coated Nanostructured Lipid Carrier Anticancer activity against **A549** cell line