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REVIEW Article

Development and Evaluation of Irinotecan-Loaded Liposomes for Targeted Cancer Drug Delivery

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Abstract

Background: Irinotecan, a topoisomerase I inhibitor, is widely employed in the management of colorectal, pancreatic, and lung malignancies. Its clinical utility is, however, constrained by rapid plasma clearance, conversion to an inactive carboxylate species at physiological pH, and dose-limiting gastrointestinal toxicity. Liposomal encapsulation offers a rational strategy to address these limitations by prolonging systemic circulation, protecting the drug from hydrolytic degradation, and exploiting passive tumor targeting via the enhanced permeability and retention (EPR) effect.

Objective: The present study aimed to fabricate irinotecan-loaded liposomes using the thin-film hydration method, optimize lipid composition with respect to the phosphatidylcholine-to-cholesterol ratio, and comprehensively characterize the resulting nanocarriers for physicochemical attributes, in vitro drug release behavior, release kinetics, and short-term storage stability.

Methods: Eight formulations (F1–F8) were prepared by systematically varying the cholesterol content from 100 to 450 mg while keeping the phosphatidylcholine concentration constant at 200 mg. Vesicles were characterized for particle size, polydispersity index (PDI), zeta potential, and percentage entrapment efficiency (%EE). In vitro drug release was assessed over eight hours using Franz diffusion cells with a cellulose acetate membrane. Release kinetics were modeled using zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations. Formulation morphology was examined by scanning electron microscopy, and drug–excipient compatibility was confirmed by Fourier-transform infrared spectroscopy. Accelerated stability was evaluated at 25°C/60% RH, 30°C/75% RH, and 40°C/75% RH over three months.

Results: The optimized formulation F5 (PC: cholesterol = 200:300 mg) achieved the smallest mean particle size (118 ± 4 nm), a narrow size distribution (PDI = 0.12), a highly negative zeta potential (−48.6 ± 1.3 mV), and the highest encapsulation efficiency (92.1 ± 1.5%). Cumulative drug release over eight hours reached 98.42% with a sustained profile devoid of an initial burst. Release followed first-order kinetics with a Korsmeyer–Peppas exponent of 0.60, indicative of non-Fickian diffusion. SEM confirmed a spherical vesicle morphology. FTIR analysis revealed no new or missing peaks, confirming physicochemical compatibility. The formulation remained stable across all storage conditions, retaining more than 95% release capacity after three months.

Conclusion: The thin-film hydration method yielded stable irinotecan-loaded liposomes with favorable physicochemical properties and a controlled release profile. These nanocarriers hold significant promise for targeted oncological therapy, with the potential to reduce dosing frequency and systemic adverse effects. Further in vivo pharmacokinetic and efficacy studies are warranted to advance the formulation toward clinical evaluation.

Keywords: *Irinotecan; liposomal nanocarriers; thin-film hydration; phosphatidylcholine; cholesterol; entrapment efficiency; controlled drug release; topoisomerase I inhibitor; cancer nanomedicine.*

1. Introduction

Cancer remains the second leading cause of mortality worldwide, accounting for approximately 9.7 million deaths in 2022 [1]. The majority of approved cytotoxic agents suffer from inherent pharmacokinetic limitations including short plasma half-life, nonselective tissue distribution, and significant systemic toxicity, all of which compromise the therapeutic window and patient quality of life [2]. Nanotechnology-based drug delivery platforms, and liposomal systems in particular, have attracted substantial scientific and regulatory attention over the past two decades as a means of addressing these challenges [3].

Liposomes are phospholipid bilayer vesicles that can encapsulate both hydrophilic and hydrophobic drug molecules in their aqueous core and lipid membrane, respectively [4]. Structurally analogous to biological membranes, liposomes are fully biocompatible and biodegradable, rendering them intrinsically safe for parenteral administration [5]. Their ability to accumulate preferentially in tumor tissue through the EPR effect—arising from hyperpermeable tumor vasculature and impaired lymphatic drainage—endows them with a passive targeting capability that markedly reduces off-target drug exposure [6]. Several liposomal products have received regulatory approval, including liposomal doxorubicin (Doxil®), liposomal daunorubicin (DaunoXome®), and, most pertinently, liposomal irinotecan (Onivyde®) for the treatment of metastatic pancreatic adenocarcinoma [7].

Irinotecan (CPT-11), a semi-synthetic water-soluble camptothecin derivative, functions as a prodrug that undergoes carboxylesterase-mediated hepatic conversion to SN-38, a metabolite 100 to 1,000 times more potent in inhibiting topoisomerase I [8]. Inhibition of topoisomerase I stabilizes the cleavable complex between the enzyme and DNA, preventing the relegation of single-strand breaks and ultimately triggering cell death predominantly in the S phase of the cell cycle [9]. Despite its broad-spectrum antitumor efficacy—validated in colorectal, gastric, pancreatic, and small-cell lung cancers—free irinotecan is burdened by delayed-onset diarrhea and myelosuppression as dose-limiting toxicities, and by lactone ring opening to an inactive carboxylate form at physiological pH [10]. Liposomal encapsulation shields the lactone moiety from hydrolysis and allows sustained SN-38 generation within the tumor microenvironment, offering a mechanistic rationale for formulation development [11].

Although Onivyde® has validated the clinical concept of liposomal irinotecan, its cost and the complexity of its PEGylated formulation create barriers to broader access [12]. There is therefore a compelling scientific and public-health rationale for developing generic, non-PEGylated irinotecan liposomes that can be produced through scalable, reproducible processes. The thin-film hydration method, one of the most well-characterized laboratory-scale preparation techniques, is amenable to optimization through systematic variation of lipid composition and provides a reliable starting point for such development [13]. The phosphatidylcholine-to-cholesterol ratio is a particularly influential variable: cholesterol intercalates between phospholipid acyl chains, reducing membrane fluidity at moderate concentrations and thereby improving encapsulation efficiency and colloidal stability, while excessive incorporation rigidifies the bilayer to the point where structural defects and drug leakage can paradoxically increase [14].

Against this background, the present study reports the preparation, optimization, and characterization of irinotecan-loaded liposomes formulated across a broad range of cholesterol concentrations. The primary endpoints encompass particle size, PDI, zeta potential, encapsulation efficiency, in vitro release profile, release kinetic modeling, SEM morphology, FTIR compatibility, and three-month stability under ICH-aligned storage conditions.

2. Materials and Methods

2.1 Materials

Irinotecan hydrochloride was procured from Aurobindo Laboratories Ltd. (Hyderabad, India). Soy phosphatidylcholine (Lipoid S-75, 70% PC content) and cholesterol (>99% purity) were sourced from Synpharma Research Labs (Hyderabad, India). Chloroform (AR grade), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and sodium chloride were also obtained from Synpharma Research Labs. All reagents were used as received without further purification. Ultrapure water generated by a Milli-Q system (Millipore, USA) was used throughout.

2.2 Preformulation Studies

2.2.1 Organoleptic and Physicochemical Characterization of Irinotecan

Approximately 100 mg of the active pharmaceutical ingredient (API) was spread on a clean Petri dish and examined visually for color, odor, and physical form. Melting point was determined in triplicate using a capillary melting-point apparatus (Thiele's tube method). Solubility was assessed qualitatively by adding 100 mg of drug to 20 mL of each solvent—water, absolute ethanol, methanol, chloroform, acetone, and hexane—followed by vortex agitation for five minutes and visual inspection for complete dissolution.

2.2.2 Spectrophotometric Analysis and Calibration Curve

Irinotecan exhibits a characteristic ultraviolet absorption maximum (λmax) at 276 nm in phosphate buffer saline (PBS, pH 7.4), consistent with published pharmacopoeial data [15]. A standard stock solution of 1 mg/mL was prepared in PBS pH 7.4, and serial dilutions yielding concentrations of 10, 20, 30, 40, and 50 µg/mL were prepared in triplicate. Absorbance was recorded at 276 nm on an ELICO SL164 double-beam UV–Vis spectrophotometer. A calibration curve was constructed by linear regression of absorbance against concentration ($R^2 = 0.9994$, Beer–Lambert range confirmed across 10–50 µg/mL).

2.2.3 FTIR Drug–Excipient Compatibility

Fourier-transform infrared (FTIR) spectra of pure irinotecan, blank liposomes (phosphatidylcholine and cholesterol only), and the optimized irinotecan-loaded formulation were recorded using an ATR-FTIR spectrophotometer (Jasco ATR-4000, Japan) in the 400–4000 cm^{-1} range. Potassium bromide (KBr) pellets dried at 60°C for one hour were used for transmission measurements. Spectra were compared for the emergence of new absorption bands or disappearance of characteristic peaks, both of which would indicate potential chemical interaction between the drug and excipients.

2.3 Preparation of Irinotecan-Loaded Liposomes

Liposomes were prepared by the thin-film hydration method according to a protocol adapted from Bangham and colleagues [16]. Briefly, the required quantities of phosphatidylcholine and cholesterol (Table 1) were co-dissolved in 10 mL of anhydrous chloroform in a 100 mL round-bottom flask. The organic solvent was removed at 40°C under reduced pressure using a rotary vacuum evaporator (Aditya Scientific, India) rotating at 60 rpm, yielding a uniform translucent lipid film. Residual solvent traces were eliminated by overnight desiccation under high vacuum at room temperature.

The dried lipid film was hydrated with 10 mL of PBS (pH 7.4) containing 10 mg of irinotecan hydrochloride. Hydration was performed at 37°C for two hours with continuous gentle agitation by reattaching the flask to the rotary evaporator at 30 rpm under atmospheric pressure. The resulting milky-white multilamellar vesicle (MLV) suspension was allowed to equilibrate at room temperature for an additional 30 minutes before characterization. A total of eight formulation batches (F1–F8) were prepared as detailed in Table 1, with cholesterol concentration varying from 100 to 450 mg.

Table 1: Composition of irinotecan-loaded liposomal formulations (F1–F8).

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Drug (mg)	10	10	10	10	10	10	10	10
PC (mg)	200	200	200	200	200	200	200	200
Cholesterol (mg)	100	150	200	250	300	350	400	450
Chloroform (mL)	10	10	10	10	10	10	10	10
PBS pH 7.4 (mL)	10	10	10	10	10	10	10	10

*PC = Phosphatidylcholine; PBS = Phosphate Buffer Saline; *F5 = Optimized formulation.*

2.4 Physicochemical Characterization

2.4.1 Particle Size, PDI, and Zeta Potential

Mean hydrodynamic diameter and PDI of all formulations were assessed by dynamic light scattering (DLS) using an optical microscope (Motic, Germany) supported by particle size analysis software. Samples were diluted 10-fold with Milli-Q water prior to measurement to avoid multiple scattering. All measurements were

conducted at 25°C in triplicate, and results are reported as mean ± standard deviation. Zeta potential was measured electrophoretically under the same conditions; a zeta potential magnitude exceeding ±30 mV was taken as indicative of acceptable colloidal stability [17].

2.4.2 Encapsulation Efficiency

Entrapment efficiency was determined by ultracentrifugation. Aliquots of 500 µL from each batch were placed in Amicon Ultra centrifugal filter units (0.5 mL, 10 kDa MWCO, Merck, USA) and centrifuged at 10,000 rpm for 20 minutes in a Tarson MC-1 Spinwin microcentrifuge. The filtrate representing non-encapsulated (free) drug was collected and assayed spectrophotometrically at 276 nm. Entrapment efficiency was calculated as:

$$EE\ (\%) = [(\text{Total drug} - \text{Free drug}) / \text{Total drug}] \times 100$$

Where total drug was defined as the theoretical amount of irinotecan added to each batch, and free drug was quantified from the ultrafiltrate using the validated calibration curve.

2.4.3 Scanning Electron Microscopy (SEM)

Morphological examination of the optimized formulation was performed by scanning electron microscopy (SEM) using a Hitachi S-3700 instrument operating at an accelerating voltage of 15 kV. Liposome dispersions were lyophilized overnight in a freeze-drier, and the resultant powder was mounted on aluminum stubs, sputter-coated with a 10 nm gold layer under an argon atmosphere, and imaged at a beam current of 750 mA.

2.5 In Vitro Drug Release Study

Cumulative drug release was evaluated using a Franz diffusion cell system with an 8 mL receptor compartment. A regenerated cellulose acetate membrane (pore size 0.45 µm, pre-soaked for 12 hours in PBS pH 7.4) was interposed between the donor and receptor compartments. The donor compartment was loaded with the liposomal formulation corresponding to approximately 1 mg of irinotecan, covered with aluminum foil to minimize evaporation, and maintained at 32 ± 0.5°C on a magnetic hot-plate stirrer with continuous agitation using magnetic beads. The receptor compartment contained PBS pH 7.4, replenished with equal-volume aliquots of fresh buffer following each sampling event to maintain sink conditions. Samples of 1 mL were withdrawn at 0, 1, 2, 3, 4, 5, 6, 7, and 8 hours, filtered through a 0.22 µm membrane filter, and analyzed spectrophotometrically at 276 nm. All experiments were performed in triplicate.

2.6 Release Kinetic Modeling

The cumulative drug release data from all eight formulations were fitted to four pharmacokinetic models to determine the governing release mechanism: (i) zero-order model (cumulative drug release versus time); (ii) first-order model (log residual drug fraction versus time); (iii) Higuchi model (cumulative drug release versus the square root of time); and (iv) Korsmeyer–Peppas power law model (log cumulative drug release versus log time). Goodness of fit was evaluated by the coefficient of determination (R^2), and the release exponent n from the Korsmeyer–Peppas model was interpreted according to established criteria: $n \leq 0.45$ (Fickian diffusion), $0.45 < n < 0.89$ (non-Fickian or anomalous transport), and $n \geq 0.89$ (case II transport or zero-order release) [18].

2.7 Stability Studies

Accelerated and long-term stability of the optimized formulation (F5) was assessed in accordance with ICH Q1A (R2) guidelines over a 90-day period. Samples were stored in nitrogen-flushed laminated aluminum containers (10 mL capacity) under three temperature–humidity conditions: 25°C/60% RH (long-term), 30°C/75% RH (intermediate), and 40°C/75% RH (accelerated). Aliquots were withdrawn at 0, 30, 60, and 90 days and evaluated for percentage in vitro drug release using the Franz diffusion cell method described in Section 2.5. Stability was considered acceptable if cumulative drug release remained not less than 85% of the initial value throughout the study period [19].

3. Results and Discussion

3.1 Preformulation Characterization

Irinotecan was obtained as a pale yellow to yellow crystalline powder with no discernible odor and tasteless character, consistent with published physicochemical descriptions [8]. The melting point ranged from 250 to 260°C, confirming the identity and purity of the API. Solubility screening indicated high solubility in

water and methanol, limited solubility in absolute ethanol, and negligible dissolution in non-polar solvents such as hexane. This predominantly hydrophilic solubility profile supports aqueous-core encapsulation within liposomal systems.

The UV spectrum of irinotecan in PBS pH 7.4 displayed a well-defined absorption maximum at 276 nm, corresponding to the π→π* electronic transition of the fused quinoline ring system [15]. The calibration curve constructed over the 10–50 µg/mL range was linear, with a regression equation of A = 0.01125C + 0.0042 (R^2 = 0.9994), confirming compliance with the Beer–Lambert law across the working concentration range.

FTIR analysis of pure irinotecan revealed characteristic absorptions attributable to O–H stretching (broad band at ~3912 cm^{-1}), C–H stretching (~2939 cm^{-1}), and C–H bending (~1346 cm^{-1}). In the spectrum of the optimized liposomal formulation, these signature peaks were retained with modest bathochromic shifts (O–H: 3333 cm^{-1}; C–H: 2818 and 1263 cm^{-1}), attributable to hydrophilic interactions between the drug and the polar phospholipid head groups. The absence of novel absorptions or complete peak suppression confirmed physicochemical compatibility between irinotecan and the lipid excipients [20].

3.2 Physicochemical Properties of Liposomal Formulations

The physicochemical characteristics of all eight formulations are summarized in Table 2. Particle size, PDI, zeta potential, and encapsulation efficiency each displayed a non-monotonic dependence on cholesterol concentration, reflecting the dual role of cholesterol in modulating membrane biophysics.

Table 2: Physicochemical characterization of irinotecan-loaded liposomes (n=3, mean ± SD).

Batch	Particle Size (nm)	PDI	Zeta Pot. (mV)	%EE	Remarks
F1	235 ± 8	0.32	−12.4 ± 1.1	58.2 ± 2.4	High PDI, low EE
F2	198 ± 6	0.28	−18.7 ± 1.4	66.5 ± 3.0	Moderate stability
F3	175 ± 5	0.25	−22.1 ± 1.2	71.0 ± 2.8	Acceptable
F4	160 ± 6	0.20	−28.9 ± 1.0	78.3 ± 2.1	Good stability
F5*	118 ± 4	0.12	−48.6 ± 1.3	92.1 ± 1.5	Optimized batch
F6	140 ± 5	0.18	−30.5 ± 1.2	81.0 ± 2.0	Near-optimal
F7	210 ± 7	0.30	−15.6 ± 1.0	63.8 ± 2.6	Excess cholesterol
F8	260 ± 9	0.36	−8.9 ± 0.9	52.7 ± 3.1	Aggregation noted

**F5 = Optimized formulation. PC = Phosphatidylcholine.*

3.2.1 Particle Size and Polydispersity

In formulations F1 through F5, progressive addition of cholesterol reduced mean particle size from 235 ± 8 nm (F1, 100 mg cholesterol) to a minimum of 118 ± 4 nm (F5, 300 mg cholesterol). This size reduction is consistent with the condensing effect of cholesterol on the phosphatidylcholine bilayer: sterol intercalation fills interchain voids, reduces acyl chain mobility, and promotes the formation of compact gel-phase domains that resist bilayer expansion [14]. A parallel decline in PDI—from 0.32 in F1 to 0.12 in F5—indicates a progressive narrowing of the vesicle size distribution, yielding a nearly monodisperse population in the optimized batch. PDI values below 0.2 are generally considered indicative of a homogeneous dispersion suitable for parenteral applications [17].

Beyond the optimal cholesterol concentration (F6–F8, 350–450 mg), particle size rebounded to as high as 260 ± 9 nm and PDI rose to 0.36. Excess cholesterol is known to induce membrane crystallization or phase separation, disrupting orderly bilayer curvature and favoring the formation of multilamellar or aggregated vesicles [21]. These observations align with findings reported by Wei et al. [22], who demonstrated that lipid phase behavior profoundly influences the structural integrity and drug-loading capacity of irinotecan liposomes.

3.2.2 Zeta Potential

Zeta potential values became increasingly negative with rising cholesterol content up to formulation F5 (−48.6 ± 1.3 mV), then returned toward neutral values in F6–F8. Phosphatidylcholine is a zwitterionic lipid that presents a net neutral charge under physiological conditions; the observed negative zeta potentials likely arise from residual phosphate groups, trace fatty acid oxidation products, or chloride adsorption during hydration [23]. Cholesterol-mediated bilayer compaction may restrict the rotational freedom of phospholipid head groups, effectively exposing negatively charged moieties to the bulk aqueous phase. The zeta potential of −48.6 mV recorded for F5 substantially exceeds the −30 mV threshold commonly cited as the lower boundary for adequate electrostatic stabilization [17], indicating that inter-vesicular electrostatic repulsion will effectively prevent aggregation during storage.

3.2.3 Encapsulation Efficiency

Encapsulation efficiency followed the same trend as colloidal stability, reaching a maximum of 92.1 ± 1.5% for F5 and declining symmetrically on both sides of this optimum (Table 2). At sub-optimal cholesterol levels, the membrane is too fluid to retain hydrophilic drug molecules efficiently; conversely, at supra-optimal levels, the rigidity-induced structural defects and phase boundaries provide pathways for drug leakage [24]. An EE of 92.1% is comparable to the 94.6% reported by Mohammadi et al. [25] for supercritical fluid-prepared PEGylated irinotecan liposomes and superior to the 90% achieved with simple soybean phospholipid formulations by Wei et al. [22], underscoring the importance of cholesterol optimization.

3.3 In Vitro Drug Release

Table 3 and the corresponding release profiles demonstrate that all formulations exhibited a sustained, burst-free drug release pattern over the eight-hour study period, with cumulative release values ranging from 93.67% (F1) to 98.42% (F5). The absence of an initial burst release is noteworthy and indicates that drug molecules were genuinely encapsulated within the vesicular interior rather than adsorbed on the outer membrane surface [26].

Table 3: Cumulative percentage drug release (%) from formulations F1–F8 over 8 hours (n=3).

Time	F1	F2	F3	F4	F5*	F6	F7	F8
0h	0	0	0	0	0	0	0	0
1h	15.96	16.93	16.80	17.20	18.15	17.10	16.70	16.50
2h	28.47	27.58	27.70	27.53	29.64	26.80	27.10	27.40
3h	36.50	37.95	37.50	36.12	38.46	37.60	37.10	37.40
4h	46.98	47.90	48.10	48.46	49.51	47.90	48.20	48.70
5h	57.45	58.12	56.10	55.02	58.20	55.30	56.20	55.90
6h	68.10	69.35	68.15	67.43	70.15	68.40	67.60	67.20
7h	81.23	82.26	84.43	85.12	86.43	85.60	83.40	84.50
8h	93.67	95.16	95.42	96.15	98.42	95.80	96.10	96.43

**F5 = Optimized formulation.*

Formulation F5 achieved the highest cumulative release at each time point, releasing 49.51% by hour 4 and 98.42% by hour 8. At intermediate cholesterol concentrations (F2–F6), drug release was facilitated by an optimal bilayer fluidity that allows aqueous pore formation and diffusion of the hydrophilic drug across the membrane. At higher cholesterol loading (F7, F8), membrane rigidity attenuated transmembrane water flux and drug permeation, yielding marginally lower but still clinically acceptable cumulative release values (96.10–96.43% at 8 h). These observations are consistent with the thermosensitive liposome release studies reported by Lu et al. [27], who identified membrane rigidity as a primary determinant of drug egress kinetics from lipid vesicles, and with the in vitro data of Messerer et al. [28], who demonstrated controlled irinotecan release from DSPC/cholesterol liposomes over comparable time periods. The sustained release achieved with F5 is particularly relevant given irinotecan's short plasma half-life of 6–12 hours [8]; a formulation capable of maintaining therapeutic concentrations over eight or more hours could significantly reduce dosing frequency in clinical practice.

3.4 Release Kinetic Analysis

The results of kinetic modeling are presented in Table 4. For formulations F1–F4 and F6, the Higuchi diffusion model provided the best fit (R^2 = 0.989–0.994), with Korsmeyer–Peppas exponents (n = 0.46–0.52) consistent with Fickian diffusion, wherein drug transport is governed by a concentration gradient across the membrane [18]. The optimized formulation F5 best fitted first-order kinetics (R^2 = 0.987) with n = 0.60, indicative of non-Fickian or anomalous transport—a combined mechanism in which both diffusion and membrane relaxation contribute to release. This mechanistic transition likely reflects the increased membrane order in F5: the more compact bilayer slows simple aqueous diffusion, making membrane swelling and erosion processes comparably significant. Formulations F7 and F8, with the most rigid bilayers, also exhibited first-order kinetics with higher n values (0.62–0.64), reinforcing the role of membrane dynamics in dictating release behavior. These findings parallel the Korsmeyer–Peppas modeling results reported by Lu et al. [27] for thermosensitive liposomes under modified Laplace pressure conditions.

Table 4: Release kinetic modeling parameters for formulations F1–F8.

Batch	Best-Fit Model	R^2	KP Exponent (n)	Release Mechanism
F1	Higuchi	0.992	0.48	Fickian diffusion
F2	Higuchi	0.991	0.46	Fickian diffusion
F3	Higuchi	0.994	0.50	Fickian diffusion
F4	Higuchi	0.992	0.52	Fickian diffusion
F5*	First-Order	0.987	0.60	Non-Fickian (anomalous)
F6	Higuchi	0.989	0.50	Fickian diffusion
F7	First-Order	0.985	0.62	Anomalous transport
F8	First-Order	0.989	0.64	Anomalous transport

**F5 = Optimized formulation; n = Korsmeyer–Peppas release exponent.*

3.5 SEM Morphology

SEM examination of the lyophilized F5 formulation at 15 kV revealed discrete, spherical vesicles without surface irregularities or particle fusion. The observed morphology is characteristic of well-formed unilamellar or low-lamellarity liposomes, consistent with the narrow PDI of 0.12 determined by DLS. Spherical geometry minimizes surface energy and is associated with superior structural stability and uniform drug loading [29]. No significant aggregation was visible in the micrographs, confirming the colloidal stabilization inferred from the zeta potential data.

3.6 Stability Studies

The stability data for the F5 formulation under three storage conditions are presented in Table 5. After 90 days, cumulative drug release remained above 95% under all conditions tested: 95.52% at 25°C/60% RH, 95.36% at 30°C/75% RH, and 95.30% at 40°C/75% RH. In each case, values remained well above the pre-specified acceptance criterion of not less than 85%, confirming satisfactory formulation robustness. The modest progressive decline in drug release over three months—approximately 3% under each condition—most likely reflects minor cholesterol oxidation or phospholipid hydrolysis during storage, processes known to subtly modulate bilayer permeability [30]. Nitrogen flushing of the storage containers mitigated but did not entirely eliminate these degradation pathways. These stability outcomes are consistent with those reported by Mohammadi et al. [25] for PEGylated irinotecan liposomes stored over eight weeks, lending confidence to the long-term physical and chemical integrity of the present formulation.

Table 5: Accelerated and long-term stability data for optimized formulation F5 (% cumulative drug release; n=3).

Batch	Condition	Initial (%)	1 Month	2 Months	3 Months
F5	25°C / 60% RH	98.42	97.56	96.38	95.52
F5	30°C / 75% RH	98.42	97.28	96.23	95.36
F5	40°C / 75% RH	98.42	97.25	96.12	95.30

All values remain above the ≥85% acceptance criterion over 90 days.

4. Conclusion

The present study demonstrates that irinotecan-loaded liposomes prepared by the thin-film hydration method can be systematically optimized through rational variation of the phosphatidylcholine-to-cholesterol ratio. The optimized formulation F5, incorporating 200 mg of phosphatidylcholine and 300 mg of cholesterol, yielded nanosized vesicles (118 ± 4 nm) with a narrow size distribution (PDI = 0.12), high colloidal stability (ζ = −48.6 mV), and excellent encapsulation efficiency (92.1%). A sustained, burst-free drug release profile over eight hours—governed by first-order, non-Fickian kinetics—was observed, suggesting simultaneous diffusion and membrane relaxation contributions to drug transport. FTIR analysis confirmed the absence of chemical interactions between irinotecan and the lipid matrix, and three-month accelerated stability testing validated formulation robustness across ICH-recommended storage conditions.

These findings collectively support the potential of the described liposomal system to overcome key pharmacokinetic limitations of free irinotecan, including rapid plasma clearance and pH-dependent lactone ring hydrolysis. By providing sustained drug levels over clinically relevant time periods, this formulation strategy could reduce dosing intervals, improve patient adherence, and attenuate systemic adverse effects. Future investigations should encompass in vivo pharmacokinetic and tissue-distribution studies in tumor-bearing animal models, assessment of cellular uptake and cytotoxicity in irinotecan-sensitive cell lines, scale-up feasibility evaluation, and comparison with the reference product Onivyde® to establish bioequivalence criteria for generic development purposes.

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