Research Article

Formulation, Mechanistic Evaluation, and Kinetic Release Profiling of Paclitaxel-Loaded Liposomes Synthesized via Thin-Film Hydration for Advanced Cancer Therapeutics

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ABSTRACT

Paclitaxel (PTX) has emerged as an essential element in the therapeutic regime for cancers originating from breast, ovaries, and non-small-cell lungs. Nonetheless, its use is drastically limited owing to low water solubility (~<0.01 mg/mL) and the high levels of systemic toxicity associated with toxic industrial surfactants such as Cremophor EL®. In the present study, six different combinations (F1-F6) were developed by optimizing the ratio of phosphatidylcholine and cholesterol molar concentration. Structural assessment indicated that F4 offered a perfect structure with a narrow vesicle diameter (162.8 ± 1.2 nm), extremely low polydisperse nature with 0.236 PDI and a substantial electrokinetic charge potential of −31.4 ± 0.5 mV. Furthermore, F4 offered a remarkable drug entrapment efficiency (88.2 ± 0.9%) along with high drug loading (98.1 ± 0.4%) in hydrophobic lipid bilayer. No adverse interactions were detected using FTIR analysis. The dialysis-based in vitro drug release test yielded a biphasic and sustained drug release pattern with a delivery of 89.4 ± 1.5% drug in 24 hours. Mathematical modeling of the release curve indicated that F4 follows Higuchi kinetic model of drug release (R^2 = 0.988) with predominantly non-Fickian transport characteristics (R^2 = 0.975 through Korsmeyer–Peppas model). Further, accelerated stability studies proved that these nanoparticles remained stable at 4 ± 2°C up to 30 days. Therefore, the results obtained in this study indicate that the proposed F4 nanovesicle provides a high-performance system that can avoid toxic surfactants and exploit EPR phenomenon to deliver maximum therapeutic dose of PTX.

Keywords: Paclitaxel; Liposomes; Thin Film; Nanocarriers; Higuchi Diffusion; Sustained Drug Release.

1. INTRODUCTION

Despite all the advancements made in modern medicine, cancer persists to be one of the major issues in global health care. It is characterized by uncontrollable cell growth, invasion into tissues, and metastasis[1] . Figure 1 presents the structure of paclitaxel (PTX), a taxane diterpenoid initially obtained from the bark of *Taxus brevifolia.* It represents one of the strongest cytotoxic drugs utilized in the medical practice today. Its mechanism of action involves selective binding to the β-subunit of tubulin that leads to microtubule polymerization and its stabilization, thus preventing microtubule disassembly.

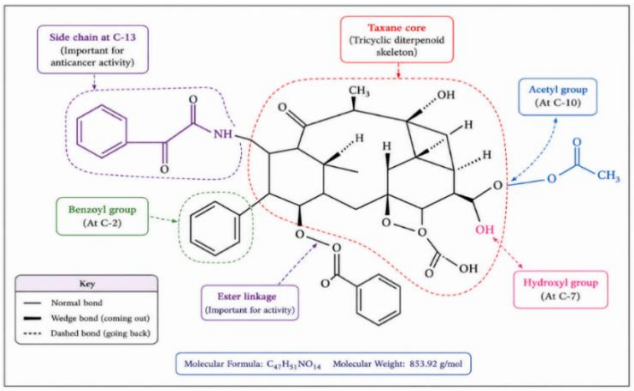

Figure 1: Chemical structure of paclitaxel

Image source: *https://upload.wikimedia.org/wikipedia/commons/5/59/Taxol.svg*

Research gap: Although PTX displays high clinical efficacy, it belongs to BCS Class IV because of its low intestinal permeability and poor aqueous solubility (less than 0.01 mg/mL). For overcoming such a physicochemical problem, the conventional marketed drug product (Taxol®) is prepared using a highly concentrated solvent system that consists of a 1:1 ratio (v/v) of polyethoxylated castor oil (Cremophor EL®) and anhydrous ethanol. Nevertheless, Cremophor EL® is not an inert carrier. It exhibits high toxicity, which was proven by many clinical adverse events that ranged from severe anaphylactoid hypersensitivity reactions and peripheral neuropathy to hyperlipidemia and non-linear pharmacokinetics[2,3].

Need for Novel Drug Delivery Systems

In order to overcome some of the limitations of classical treatment using paclitaxel, many carrier delivery systems such as polymeric nanoparticles, solid lipid nanoparticles, micelles, and liposomes have been evaluated[4,5,6]. The phospholipid bilayer is the best site for the incorporation of hydrophobic drugs, like paclitaxel, and no surfactants will be required during the process as in figure 2. In addition, liposomes having sizes smaller than 200 nm can accumulate in tumors via the EPR effect[7-15]. Only few methods could be identified after comprehensive literature search[16-23] in connection with this study.

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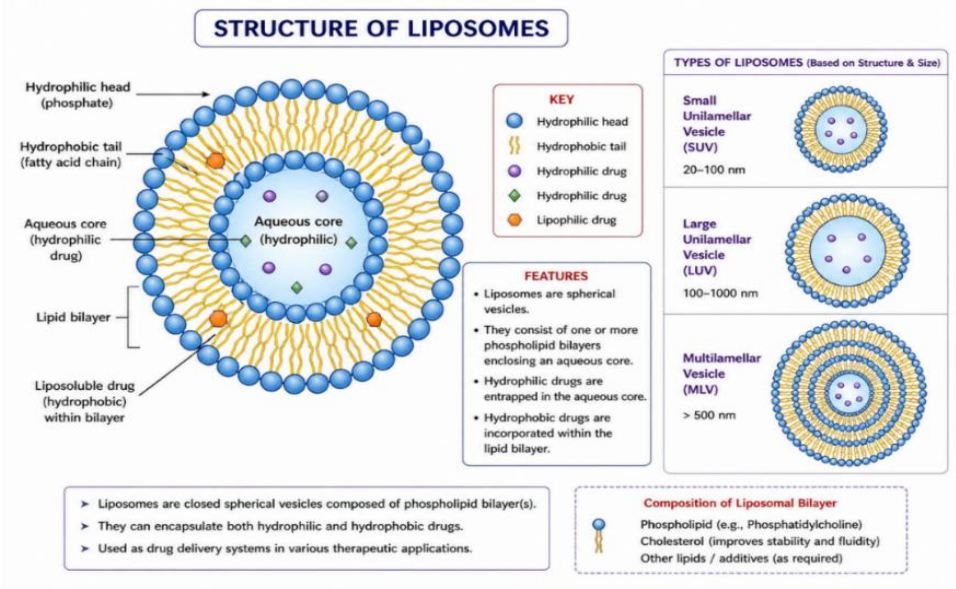

Figure 2: Diagram showing phospholipid bilayer enclosing an aqueous core with hydrophilic drugs inside and lipophilic drugs within the bilayer.

Source: *Adapted from Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: Classification, preparation, and applications. Nanoscale Research Letters. 2013;8:102. https://doi.org/10.1186/1556-276X-8-102.*

2. EXPERIMENTAL SECTION

The physicochemical and stability attributes of liposomes are influenced by the nature of the lipids used to prepare liposomes. This research involves the preparation of soya lecithin/cholesterol-based paclitaxel liposomes without any surfactants using the thin film hydration technique.

2.1 Materials and Reagents

Pure paclitaxel was supplied as a white crystalline solid. Soya lecithin (highly refined phosphatidylcholine component) and cholesterol (A.R. grade) were purchased to act as bases for the liposome matrices. High-grade chloroform and methanol were used as organic solvents. Distilled water was used throughout aqueous procedures to prepare phosphate-buffered saline solution (PBS, pH 7.4). Cellular-grade dialysis membranes with a molecular weight cut-off of 12,000–14,000 Da were utilized for mass transport analysis.

2.2 Optimization of Formulation Variables

Formulations were tested in the trial F1-F6 where different formulations with varied parameters such as lipid/drug ratio, phospholipid/cholesterol ratio, hydration process, sonication time, and solvent system as outlined in Table 1 were used. Complete hydration of the lipid thin film using PBS solution (pH 7.4) was done at 40-45°C in 30-60 minutes. An optimal sonication time for the probe was set at 5-10 minutes to get appropriate size distribution of vesicles and homogeneity; long sonication time leads to vesicle rupture and leakage of the drug. The chloroform-methanol solvent system (2:1) was chosen as an efficient system for dissolving phospholipids and paclitaxel and for producing lipid thin film completely.

2.3 Calibration Procedure for UV-Visible Spectroscopy

A solution of 10 mg paclitaxel was prepared by dissolving the weight of the drug in 10 ml methanol to obtain a concentration of 1 mg/ml (1000 µg/ml). Working solutions were diluted with methanol and prepared for calibration using concentrations of 2, 4, 6, 8, and 10 µg/ml.

2.4 Characterization of Solid-State Composition by Fourier Transform Infrared Spectroscopy

FTIR spectra were recorded for pure paclitaxel, raw soya lecithin, raw cholesterol, and lyophilized liposomal formulation.

Table 1: Formulation Trials of Liposomal Paclitaxel

Formulation Code	Paclitaxel (mg)	Soya Lecithin (mg)	Cholesterol (mg)	Lipid: Cholesterol ratio	Remarks
F1	10	80	20	4:1	Low cholesterol = less stability
F2	10	90	30	3:1	Moderate stability
F3	10	100	40	2.5:1	Balanced composition
F4	10	110	50	2.2:1	Improved rigidity
F5	10	120	60	2:1	High stability, reduced release
F6	10	130	70	1.8:1	Excess cholesterol = rigid membrane

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A small amount of each substance (1-2 mg) was dissolved with infrared grade potassium bromide in a dry atmosphere and placed in a high pressure press to obtain a clear KBr pellet. FTIR spectrum of each KBr pellet was measured over a wavenumber range of 4000 to 400 cm^{-1} with a spectral resolution of 4 cm^{-1}.

2.5 Fabrication of PTX Loaded Liposomes Using Thin Film Hydration Method

In this section, the creation of PTX liposomes was done by thin film hydration method. Six different lipid compositions (F1-F6) were systematically explored. The amount of PTX was kept constant (10 mg). The ratio between soya lecithin and cholesterol varied to pass from a cholesterol-poor matrix to highly loaded with structure of lipids.

2.6 Physicochemical Characterization of Nanovesicles

2.6.1 Dynamic light scattering

The hydrodynamic diameter of the vesicles, size distribution, and polydispersity index (PDI) for formulations F1-F6 was determined using dynamic light scattering analysis (DLS). In order to minimize the scattering effects of multiple scattering, the dispersion of the vesicles was diluted to 10 mL with ultra-pure water from a 0.1 mL liposome dispersion solution at 25°C.

2.6.2 Zeta Potential Surface Charge

Each sample of 1 mL diluted liposomal suspension was filled in a capillary zeta cell at 25°C and determined by the Helmholtz-Smoluchowski equation.

$$u = \frac{\varepsilon \zeta E}{\eta}$$

Where: u= electroosmotic velocity, ε= dielectric constant (permittivity) of the medium, ζ= zeta potential, E= applied electric field and η= viscosity of the medium.

2.6.3 Assessment of Total Drug Content

Formulation homogeneity was determined by measuring the total drug content. This entailed transferring 1.0 mL of the liposome suspension to a volumetric flask and lysing it using 9.0 mL of pure methanol, thereby solubilizing the lipid bilayers, resulting in the complete release of encapsulated paclitaxel into the solution. Following this, the filtrate was subjected to spectroscopic analysis at the validated λmax of 227 nm, utilizing the formula below:

$$\text{Drug Content } (\%) = \frac{\text{Amount of drug present}}{\text{Theoretical amount of drug}} \times 100$$

2.6.4 Determination of Entrapment Efficiency (EE%)

2.0 mL of the liposomal suspension was subjected to centrifugation in an ultra-centrifugation machine set at 4 °C and a speed of 10,000 rpm for 20 minutes. The supernatant solution was separated, diluted in methanol, and spectrophotometrically quantified at 227 nm using an indirect isolation technique. Percent entrapment efficiency can be expressed by the equation:

$$\text{Entrapment Efficiency } (\%) = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

Where total drug refers to the amount of drug initially added (10 mg), and free drug is the amount detected in the supernatant.

2.7 *In Vitro* Controlled Drug Release Profiling

Mass transport and the kinetics of drug release from the liposome formulations were carried out by means of an equilibrium dialysis bag method. This involved the sealing of a sample size of 2.0 mL of the liposome suspension within a cellulose dialysis bag (12–14 kDa). The sealed bag was then placed into a dissolution medium that was made up of 100 mL Phosphate Buffer Saline solution (pH 7.4) and supplemented with 0.5% tween 80. The system was kept at a physiological temperature of 37±0.5°C and stirred using a magnetic stirrer at a speed of 100 rpm. Samples were taken out at predetermined intervals of 1, 2, 4, 6, 8, 12 and 24 hours and replaced with a fresh amount of buffer solution and scanned at 227 nm.

2.8 Mathematical Modelling of Transport Kinetics

To determine the precise mechanism driving drug mass transport across the liposomal membrane, the cumulative in vitro release data were fitted into four established mathematical kinetic models such as Zero-Order Model, First-Order Model, Higuchi Model and Korsmeyer–Peppas Model.

2.9 Accelerated Thermal Stability testing

The optimized liposomal formulation (F4) was subjected to stability studies as per ICH guidelines (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)[24] to determine its shelf-life stability.

3. RESULTS AND DISCUSSION

3.1. Spectrophotometric analysis

Paclitaxel solution prepared in methanol was scanned in the wavelength range of 200–400 nm, and the maximum absorbance (λmax) was observed which corresponds to the reported value of paclitaxel as depicted in figure 3. The calibration curve for the same was shown in figure 4.

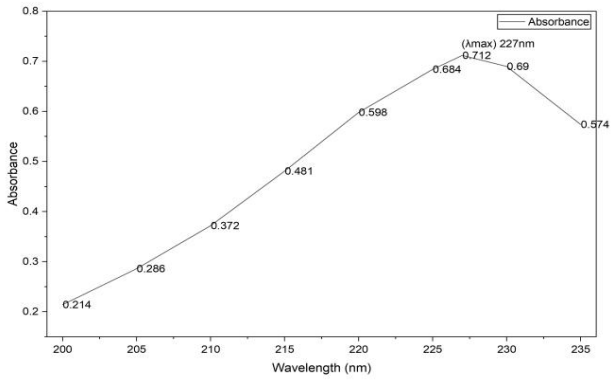

Figure 3: UV–Visible absorption spectrum of paclitaxel

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Table 2: Calibration Data of Paclitaxel

S. No.	Concentration (µg/mL)	Absorbance at 227 nm
1	2	0.112
2	4	0.221
3	6	0.337
4	8	0.448
5	10	0.561

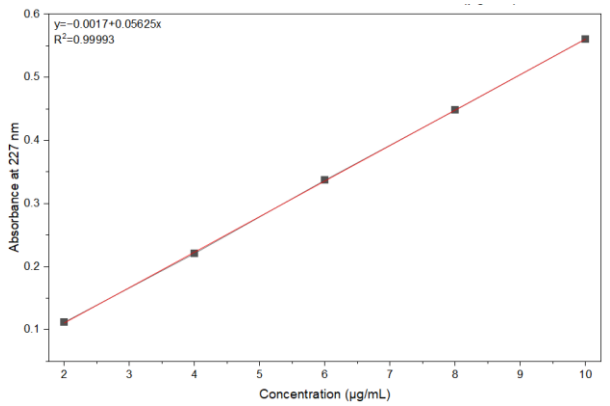

Figure 4: Calibration curve of paclitaxel in methanol at 227 nm

Discussion: The constructed calibration curve demonstrated excellent linearity with correlation coefficient (R^2) of 0.9999, confirming strict adherence to the Beer–Lambert law and ensuring high accuracy for all downstream drug quantitation steps.

3.2. Structural Integrity and Compatibility via FTIR

The collected FTIR spectra confirmed the chemical structure of the components and verified drug-excipient compatibility (figure 5).

Discussion: In the optimized lyophilized liposomal formulation of paclitaxel (F4), all characteristic peaks of paclitaxel were found, however, with slightly reduced intensity possibly due to hydrogen bonds or encapsulation in the bilayer lipid structure. Lack of new bands means that there were no interactions and degradation processes in the course of formulation.

3.3. Physicochemical Characterization of Formulations F1 to F6

Comprehensive analytical data for all six formulations (F1–F6) are detailed in table 3 and the respective figures were illustrated from 6-11.

Discussion: Formulation F4 exhibited the most favorable physicochemical properties, including the smallest vesicle size, low PDI, high zeta potential, maximum entrapment efficiency, and high drug content. The radar optimization analysis further confirmed F4 as the optimized formulation with superior overall performance among all formulations (F1–F6).

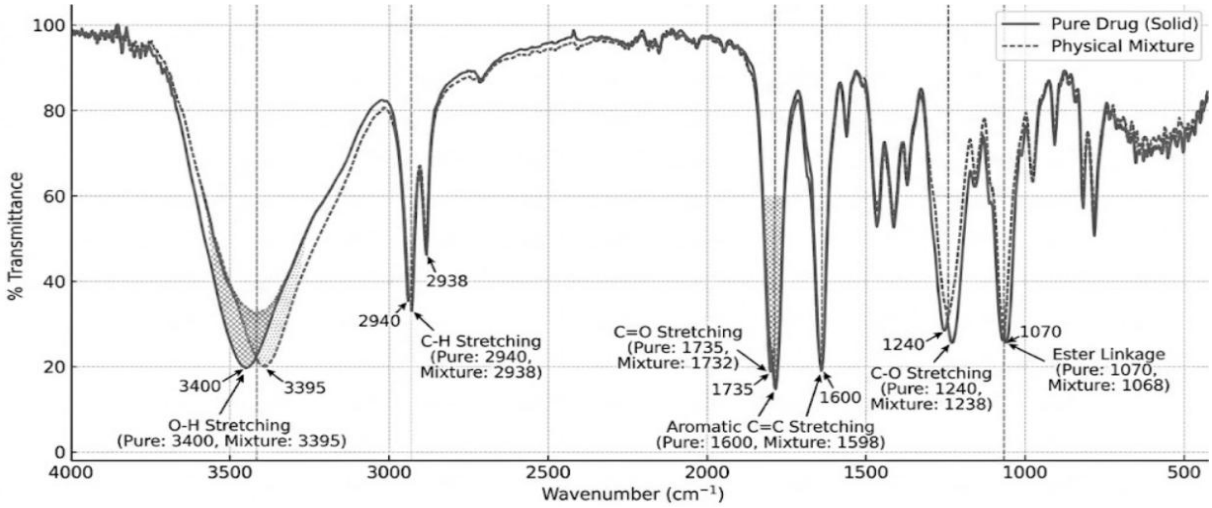

Figure 5: FTIR overlay spectrum of pure paclitaxel and physical mixture

Table 3: Physicochemical Performance Metrics of Liposomal Formulations (F1–F6)

Code	Vesicle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	Entrapment Efficiency (%)	Total Drug Content (%)
F1	245.3 ± 2.1	0.412	−18.2	68.5 ± 1.2	90.2 ± 0.8
F2	220.6 ± 1.8	0.365	−22.5	74.1 ± 1.5	92.4 ± 0.6
F3	185.4 ± 1.5	0.281	−28.3	81.6 ± 1.1	95.3 ± 0.5
F4	162.8 ± 1.2	0.236	−31.4	88.2 ± 0.9	98.1 ± 0.4
F5	175.2 ± 1.4	0.251	−30.2	85.5 ± 1.0	96.5 ± 0.7
F6	198.5 ± 1.7	0.298	−27.1	79.4 ± 1.3	94.2 ± 0.9

Note: All measurements executed in independent replicates and reported as mean ± standard deviation (n = 3).

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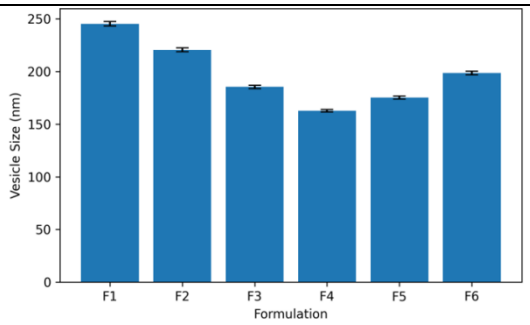

Figure 6: Vesicle size analysis of paclitaxel-loaded liposomal formulations (F1–F6)

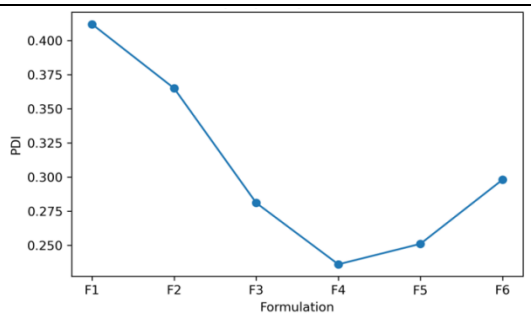

Figure 7: Polydispersity index (PDI) of paclitaxel-loaded liposomal formulations (F1–F6)

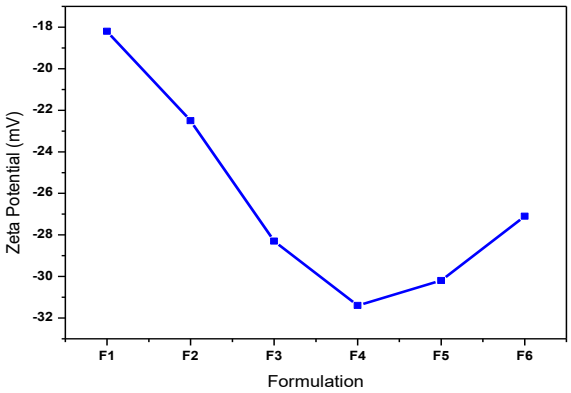

Figure 8: Zeta potential analysis of paclitaxel-loaded liposomal formulations (F1–F6)

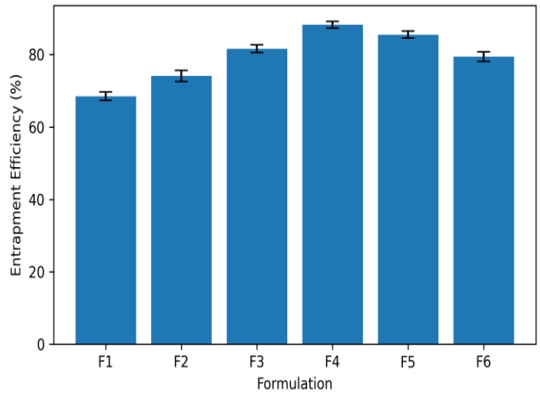

Figure 9: Entrapment efficiency (%) of paclitaxel-loaded liposomal formulations (F1–F6)

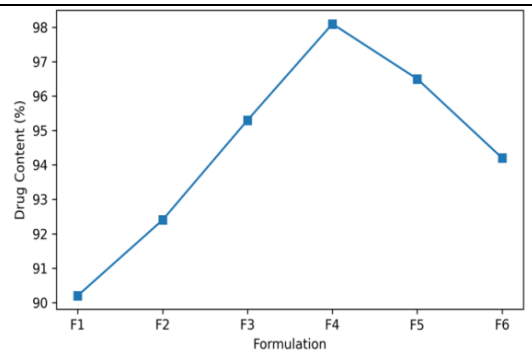

Figure 10: Drug content (%) of paclitaxel-loaded liposomal formulations (F1–F6)

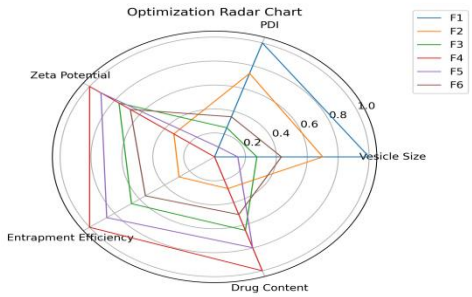

Figure 11: Optimization radar chart comparing vesicle size, PDI, zeta potential, entrapment efficiency, and drug content of paclitaxel-loaded liposomal formulations (F1–F6)

3.4. *In Vitro* Controlled Transport Kinetics

The in vitro mass transport behavior of the optimized formulation (F4) was monitored over 24 hours in PBS (pH 7.4) to simulate physiological conditions. The quantified release profile data are presented in table 4 and the graph was demonstrated in figure 12.

Discussion: The biphasic release kinetics exhibited by F4 followed the typical behavior of a controlled biphasic profile, beginning with a small but fast initial burst amounting to 12.4 ± 0.5% in the first hour. This was succeeded by a very sustained and smooth linear release for the next 24 hours, with an overall release reaching up to 89.4 ± 1.5%. This biphasic behavior proves that the liposomal carrier successfully prevents any instantaneous release of the drug.

Table 4: *In Vitro* dissolution release Profile for the Optimized F4 formulation

Chronological Sample Point (Hours)	Cumulative Paclitaxel Dissolution Release (%)
1	12.4 ± 0.5
2	20.8 ± 0.7
4	34.5 ± 0.9
6	46.3 ± 1.1
8	58.7 ± 1.2
12	72.6 ± 1.4
24	89.4 ± 1.5

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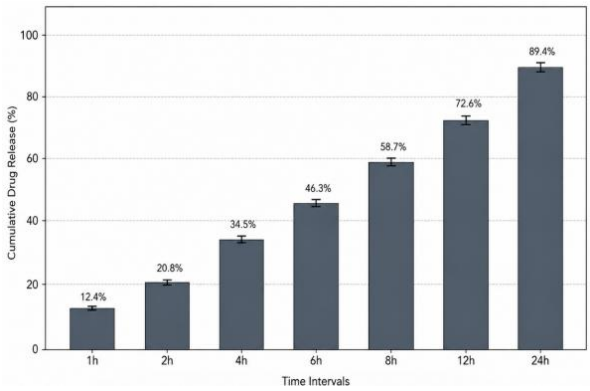

Figure 12: *In vitro* cumulative drug release profile of optimized formulation (F4)

3.5. Drug release kinetics

In order to elucidate the physical force behind the sustained release of paclitaxel, the release data obtained from experiments were fitted using kinetic theories (figure 13 and table 5).

Discussion: Linearization of the results indicated that Higuchi equation resulted in high linearity (R^2= 0.988). It means that drug release from the liposomal matrix is mostly determined by the classical Fickian diffusion. Korsmeyer-Peppas equation also exhibited good linearity (R^2 = 0.975), while the calculated value of diffusional exponent (n) was equal to 0.612. Anomalous or non-Fickian transport process occurs when diffusional exponent n takes values between 0.45 and 0.89 for vesicular systems.

3.6. Accelerated Stability testing

Accelerated physical and chemical stability tests conducted over 30 days highlighted a clear divergence in formulation shelf-life depending on storage temperature (figure 14 and table 6).

Table 5: Linear Regression Fitting Diagnostics for Mathematical Kinetic Modeling of F4

Kinetic Analytical Model	Linear Regression Formula (Fitted Equation)	Correlation Coefficient (R^2)	Elucidated Transport Parameters
Zero-Order	Q = 3.4682 × t + 12.351	0.884	Release rate independent of remaining concentration
First-Order	log Qrem = −0.0384 × t + 2.0012	0.912	Release rate dependent on remaining matrix core concentration
Higuchi Model	Q = 16.541 × t0.5 − 2.145	0.988	Controlled drug mass transfer via matrix diffusion
Korsmeyer–Peppas	log(Mt/M∞) = 0.612 × log t − 0.895	0.975	Anomalous transport exponent (n = 0.612)

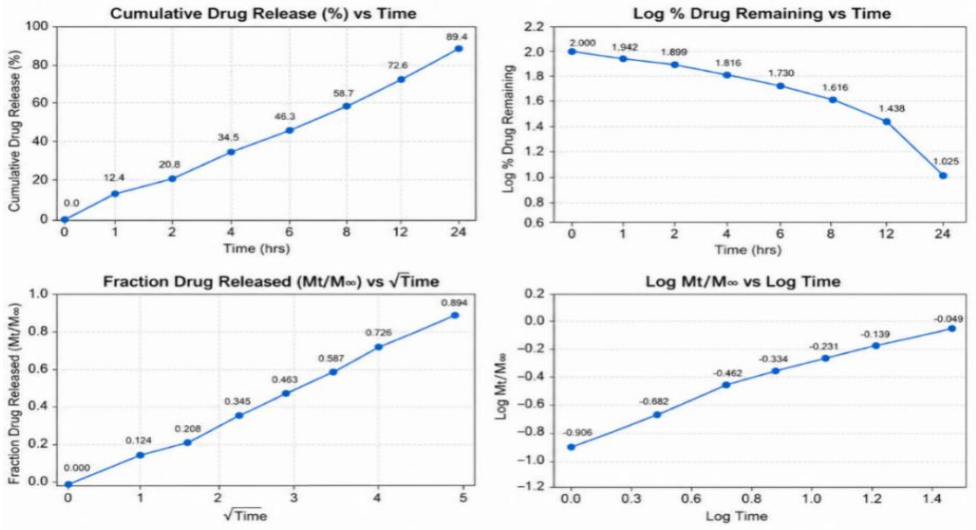

Figure 13: Drug release kinetic plots of optimized formulation (F4)

Table 6: Dynamic Structural Stability Profile of Optimized F4 Liposomes

Thermal Environment	Time Frame (Days)	Hydrodynamic Sizing (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	Entrapment Efficiency (%)	Total Drug Content (%)
Refrigerated Storage (4 ± 2°C)	Day 0	162.8 ± 1.2	0.236 ± 0.01	−31.4 ± 0.5	88.2 ± 0.9	98.1 ± 0.4
	Day 15	164.3 ± 1.4	0.241 ± 0.01	−31.0 ± 0.4	87.4 ± 0.8	97.5 ± 0.5
	Day 30	166.1 ± 1.6	0.246 ± 0.02	−30.6 ± 0.4	86.8 ± 0.7	96.9 ± 0.6
Ambient Storage (25 ± 2°C)	Day 0	162.8 ± 1.2	0.236 ± 0.01	−31.4 ± 0.5	88.2 ± 0.9	98.1 ± 0.4
	Day 15	169.7 ± 1.8	0.251 ± 0.02	−29.8 ± 0.5	84.9 ± 1.0	95.8 ± 0.7
	Day 30	175.6 ± 2.1	0.268 ± 0.02	−28.2 ± 0.6	82.5 ± 1.2	93.4 ± 0.8

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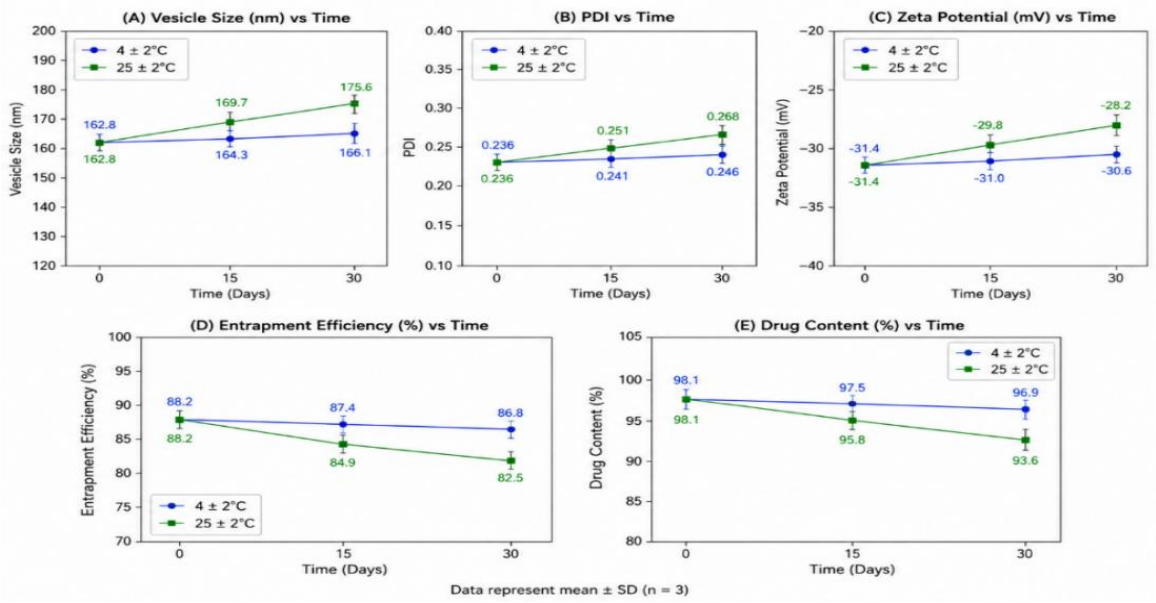

Figure 14: Stability study graphs of optimized paclitaxel-loaded liposomal formulation (F4) showing changes in subjected parameters.

Discussion: F4 formulation was observed to exhibit very good stability under refrigerated conditions (4 ± 2°C). After 30 days, only slight enlargement in the size of vesicles was observed (162.8 nm to 166.1 nm). The values of the PDI remained low, along with the high level of drug entrapment efficiency (86.8 ± 0.7%). On the contrary, the process of degradation was higher when the liposomes were kept at room temperature (25 ± 2°C). In a period of 30 days, an increase in vesicle sizes to 175.6 ± 2.1 nm was recorded; furthermore, the drug entrapment value fell to 82.5 ± 1.2%. This rapid degradation was caused by increased kinetic and thermal energy due to elevated room temperature.

4. CONCLUSION

The present work demonstrated the successful development and optimization of a surfactant-free paclitaxel-liposomal system based on thin-film hydration method. Optimization of the phospholipid to cholesterol molar ratio resulted in a stable nanosized system possessing favorable physicochemical parameters, a high drug entrapment rate and a sustained release pattern. FTIR results proved the compatibility of the drug with the components of the developed system; hence, the incorporation of paclitaxel into lipid bilayer without any chemical interaction was confirmed. The developed optimized liposomal system demonstrated a drug release from the liposomal system controlled by diffusion kinetics and stability during the whole course of the study under refrigerated storage conditions. The absence of Cremophor EL® from the drug composition is considered one of the advantages of the current formulation since the incidence of the hypersensitive reaction can be decreased by this way. Thus, the current findings have proved the effectiveness of the developed surfactant-free liposomal formulation as an alternative drug delivery system for enhancing the therapeutic activity of paclitaxel.

5. FUTURE SCOPE AND STRATEGIC DIRECTIONS

Future research on the subject must focus on increasing targeting efficiency and improving the clinical relevance of the created paclitaxel-containing liposome-based carrier. PEGylation and functionalization with tumor-targeting moieties such as antibodies, peptides, folic acid, and transferrin might help increase targeting efficiency and prolong the circulation time in the body. Development of the stimuli-responsive liposomes that are capable of responding to environmental changes such as pH, temperature, enzyme reaction, or other stimuli to release the loaded drug would contribute to improving targeting and therapeutic efficacy. The comprehensive in vivo studies should be conducted to determine biodistribution, pharmacokinetics, pharmacodynamics, safety, and anticancer activity of the drug using adequate animal models. Also, biocompatibility and long-term toxicity studies should be performed to assess the safety of the drug. With respect to pharmaceutical development, future research must have glance on manufacturing in a large scale using microfluidics, cryogenic transmission electron microscopy (Cryo-TEM), and atomic force microscopy (AFM) as well as lyophilization optimization.

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