RESEARCH ARTICLE

Formulation, Optimization, and in Vitro Release Study of Felodipine-Loaded Liposomes for Enhanced Solubility and Therapeutic Efficacy

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ABSTRACT

Liposomal drug delivery systems have revolutionized pharmaceutical formulations by enhancing bioavailability and therapeutic efficacy. This study focused on developing a liposomal formulation of felodipine (FLD), a Biopharmaceutical Classification System (BCS) Class II antihypertensive drug with poor oral bioavailability. Liposomes were prepared using the thin-film hydration method, followed by characterization for particle size, zeta potential, entrapment efficiency, and in vitro drug release. The optimized formulation (L12) exhibited a particle size of 155.3 nm, a zeta potential of -22.9 mV, and an entrapment efficiency of 93%, ensuring stability and high drug encapsulation. In vitro drug release studies demonstrated a sustained release profile, with a significant percentage of drug released over 24 hours, fitting the Korsmeyer-Peppas kinetic model. The findings suggested that liposomal encapsulation significantly improved the solubility and controlled release of felodipine, providing a promising approach for enhancing its bioavailability and therapeutic potential.

Keywords: Liposomes, Felodipine, Formulation, Zeta Potential, Particle size Entrapment efficiency, Drug release Etc

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INTRODCUTION

Liposomal drug delivery systems have significantly advanced pharmaceutical research and therapy. Initially described by Alec Bangham in 1961 **(Bangham et al., 1965),** liposomes are spherical vesicles composed of a lipid bilayer enclosing an aqueous core. Their structural components primarily consist of phospholipids or synthetic amphiphiles, with sterols like cholesterol incorporated to regulate membrane permeability. Among various fabrication techniques, thin-film hydration is the most widely employed. This method involves dissolving lipid components, with or without the drug, in an organic solvent, which is then evaporated using rotary evaporation. The resulting thin film is subsequently rehydrated with an aqueous solution. Alternative techniques include reverse-phase evaporation, freeze-drying, and ethanol injection **(Torchilin and Weissig, 2003).** To achieve precise size and uniform distribution, processes such as membrane extrusion, sonication, homogenization, and freeze-thawing are commonly utilized. Liposomes can be engineered to vary in size, composition, surface charge, and lamellarity, allowing for tailored drug delivery applications **(Izadiyan et al., 2025)**

The encapsulation of drugs within liposomes enhances their therapeutic potential by modifying pharmacokinetic and pharmacodynamic properties. Liposomes can accommodate drugs with diverse solubility characteristics—hydrophobic drugs integrate into the lipid bilayer, whereas hydrophilic drugs are retained within the aqueous compartment **(Bulbake et al., 2017).** This versatile drug delivery system improves bioavailability, prolongs circulation time, and optimizes targeted therapy.

Felodipine (FLD) is a Biopharmaceutical Classification System (BCS) Class II drug that belongs to the therapeutic category of antihypertensives **(Saltiel et al., 1988).** Despite its effectiveness, FLD exhibits poor oral bioavailability due to its high lipophilicity and extensive hepatic metabolism. The oral route remains the preferred method of administration due to its physiological benefits and enhanced patient compliance. However, improving the bioavailability of FLD remains a challenge. Various formulation strategies, such as microparticles, solid dispersions, and nanoparticles, have been explored to overcome these limitations **(Bazzo et al., 2012; Sarode et al., 2014).** In this study, we developed a liposomal formulation of felodipine to enhance its bioavailability and improve its therapeutic efficacy.

MATERIAL AND METHODS

- **Formulation of liposomes via thin film hydration**

A solution of phosphatidylcholine and cholesterol in specified weight ratios was dissolved in 5 mL of chloroform. The chloroform solution was placed in a 250 mL round-bottom flask and rotated in a single direction. The flask was maintained in a thermostatic water bath at a temperature of 30–35 °C while continuously rotating. Rotation continued until all the chloroform had evaporated, resulting in the deposition of a thin lipid film on the inner wall of the flask. Subsequently, an aqueous phosphate-buffered saline (PBS) solution (pH 7.4) containing the drug (15 mL) was added to the flask and rotated at the same speed for 30 minutes or until complete removal of the lipid film from the flask wall. The resulting suspension was

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allowed to stand in the water bath at 30 °C for 15 minutes to ensure complete hydration. The hydrated suspension was then subjected to sonication using a bath-type sonicator for 30 minutes. Separation of the non-entrapped drug was performed by centrifugation at 3700 rpm for 40 minutes. Prior to centrifugation, the liposomal suspension was cooled by placing the test tubes in ice-cold water. Finally, the liposomal pellet was collected and resuspended in distilled water for further analysis **(Lombardo et al., 2022).**

1. Determination of particle Size (PS) and Zeta Potential

The vesicle size was determined at 25 °C using dynamic light scattering (DLS) with a Zetasizer Nano ZS. Prior to analysis, the samples were diluted 100-fold in Milli-Q water. The polydispersity index (PDI) was used to assess the homogeneity of the vesicle size distribution, with the hydrodynamic diameter expressed as the Z-average, obtained from the autocorrelation fit of the data.

The stability of the formulation was further evaluated by analyzing the zeta potential, as surface charge plays a crucial role in preventing vesicle aggregation. For zeta potential measurements, the samples were diluted in Milli-Q water, and their zeta potential was assessed using the Malvern Zetasizer **(Blanken et al., 2020).**

2. **Optimisation**

The influence of independent variables on each response variable was analyzed using 3D response surface plots. To determine the optimal composition, the optimization goals were set as follows: phospholipid concentration (X_1) within a specified range, cholesterol content (X_2) within a defined range, and stirring time (X_3). The dependent variables considered were particle size (Y_1) and zeta potential (Y_2).

The predefined optimization criteria for both independent and response variables, as outlined in the table below, were input into the software. Based on these parameters, the software predicted an optimized composition with the highest desirability value among multiple alternative formulations **(Pattni et al., 2015).**

Table 1: DOE run for the optimized formulation

std	run	Phospholipid	cholesterol	Stirring time	PS	ZP
4	1	60	30	27.5	198	23.4
17	2	35	20	27.5	248.2	38.9
10	3	35	30	20	191.1	24.1
6	4	60	20	20	265.3	33.9
5	5	10	20	20	260.1	39.5
13	6	35	20	27.5	227.3	37.3
3	7	10	30	27.5	193.8	28.4
9	8	35	10	20	341.5	46.8
11	9	35	10	35	319.3	46.3
1	10	10	10	27.5	331.5	41.3
14	11	35	20	27.5	271.4	31.8
8	12	60	20	35	291.9	32.3
2	13	60	10	27.5	389.1	48.5
15	14	35	20	27.5	256.2	31.4
7	15	10	20	35	294.7	36.3
12	16	35	30	35	198.1	27.2
16	17	35	20	27.5	295.4	35.1

Table 2: Factors

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Phospholipid	mg	Numeric	10.00	60.00	-1 ↔ 10.00	+1 ↔ 60.00	35.00	17.68
B	cholestrol	mg	Numeric	10.00	30.00	-1 ↔ 10.00	+1 ↔ 30.00	20.00	7.07
C	stirring time	min	Numeric	20.00	35.00	-1 ↔ 20.00	+1 ↔ 35.00	27.50	5.30

- **Optimized formulation preparation**

The optimized formulation containing Felodipine (100 mg) was prepared using a combination of phosphatidylcholine and cholesterol (10 mg:20 mg) in a specified weight ratio. These components were dissolved in 5 mL of chloroform to form a homogeneous solution. The chloroform solution was transferred to a 250 mL round-bottom flask and rotated in a thermostatic water bath while maintaining a temperature of 30–35°C. The rotation was continued until complete evaporation of chloroform, resulting in the formation of a thin lipid film on the inner wall of the flask. To hydrate the lipid film, 15 mL of phosphate-buffered saline (PBS, pH 7.4) containing the drug was added to the flask. The mixture was rotated at the same speed for 30 minutes or until complete detachment of the lipid film. The resulting suspension was allowed to stand in a water bath at 30°C for 15 minutes to ensure complete hydration. Subsequently, the suspension underwent sonication in a bath-type sonicator for 30 minutes to reduce vesicle size. The separation of non-entrapped drug was carried out by centrifugation at 3700 rpm for 40 minutes. To maintain stability, the liposomal suspension was cooled by immersing the test tubes in ice-cold water before centrifugation. Finally, the liposome pellet was collected and resuspended in distilled water for further characterization **(Ba et al., 2021).**

Table 3: Optimized liposomes formulation

S.No	Formulation code	Drug (felodipine)	Phosphatidylcholine (mg)	Cholesterol (mg)	Pbs buffer 7.4 (ml)	Stirring time (min)
1	L12	100 mg	10	20	15	30

- **Characterisation of the liposomes**

1. Particle size and zeta potential

The particle size and zeta potential of the liposome were measured at room temperature using a Malvern Particle Sizer and Zeta Potential Analyzer (Malvern Instruments Ltd., UK). A 1 mL aliquot of the liposomal suspension was diluted with deionized water before analysis. The zeta potential was determined using the Henry equation, which accounts for electrophoretic mobility, solvent dielectric constant, viscosity, and other relevant factors. Colloidal dispersions in fluids with a high dielectric constant exhibited zeta potential, with most measurements conducted in aqueous systems **(Bulbake et al., 2017).**

2. Entrapment efficiency

The liposomal formulation was subjected to centrifugation at 4000 rpm for 18 minutes at 4 °C using a Remi cooling centrifuge to separate the free drug. The supernatant contained the suspended liposomes, while the free drug was deposited on the walls of the centrifuge tube. The supernatant was then centrifuged again at 12,000 rpm for 38 minutes at 4 °C. This process resulted in a transparent supernatant and a liposome pellet. The pellet, consisting of liposomes, was redispersed in distilled water before further analysis **(Seleci et al., 2017).**

The liposomes, free from unentrapped drug, were mixed with 10 mL of a methanol-water mixture (7:3 v/v) and sonicated for 5 minutes. The sonication process disrupted the liposomes, releasing the encapsulated drug. The amount of released drug was then analyzed to determine the entrapment efficiency.

Percentage Entrapment Efficiency = Wc / Wt × 100

Where amount of drug content (entrapped) in the liposomes is denoted as Wc and total amount of drug in the dispersion is denoted as Wt **(Seleci et al., 2017).**

3. SEM

Scanning Electron Microscopy (SEM) was utilized to examine the morphology of the liposomal vesicles. The vesicles were dried on a copper grid and excess moisture was removed using filter paper. After complete drying, the sample was analyzed under the SEM at a magnification range of 10–100k and an accelerating voltage of 100 kV **(Seleci et al., 2017).**

4. In vitro drug release

The in vitro drug release of felodipine-loaded liposomes was evaluated using the dialysis method. A dialysis membrane (Himedia, India) with a molecular weight cut-off of 12,000–14,000 Da was activated following a previously reported protocol. A precisely measured 1 mL of liposomal dispersion was placed into a dialysis membrane bag, which was sealed at both ends with closure clips. The bag was then immersed in 250 mL of 0.1 N HCl, maintained at 37°C ± 0.5°C, and stirred at 75 rpm. At predetermined intervals (0.5, 1, 3, 6, 9, 12, and 24 hours), aliquots of the release medium were collected, and the amount of drug released was quantified using a UV spectrophotometer (Shimadzu 1700) at 362 nm.

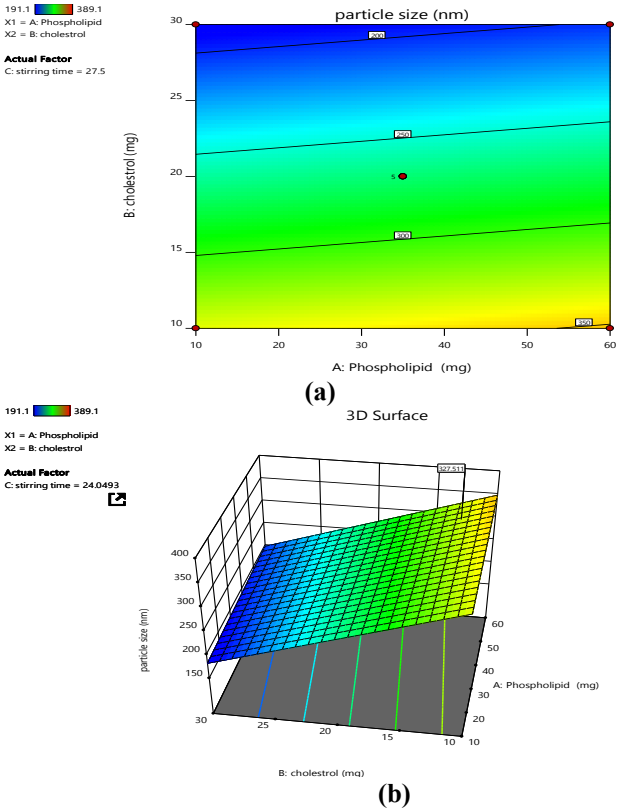
The in vitro drug release of the optimized liposomal formulation was evaluated using the previously described dialysis method. The amount of drug released was quantified using a UV spectrophotometer (Shimadzu 1700) at 362 nm, the isosbestic point for simultaneous estimation. The drug release kinetics of the optimized liposomal formulation was analyzed by statistically fitting the release data into various kinetic models, including Zero-order, First-order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell models **(Bulbake et al., 2017)**

- **Stability study of liposome**

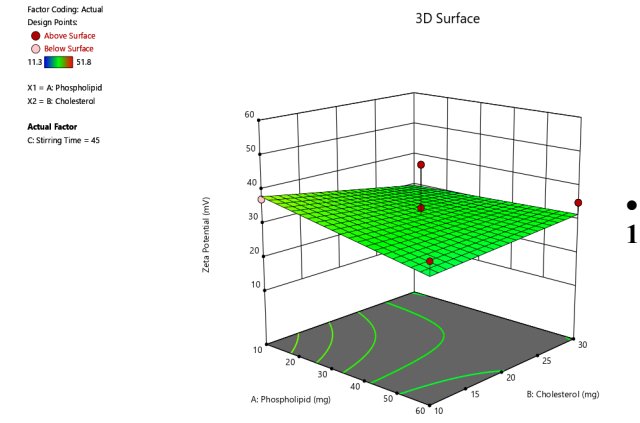
The particle size, size distribution, changes in mean particle size over time, and physical appearance of the liposomal suspension served as key indicators of its kinetic stability. The particle size and size distribution of both conventional and liposomal formulations were measured. Additionally, 5 mL of each formulation was stored at 4°C, room temperature (24°C), and physiological temperature (37°C). At predetermined time intervals of 1, 2, and 3 months, samples were collected, and their physical appearance was evaluated **(Bulbake et al., 2017).**

RESULT AND DISCUSSION

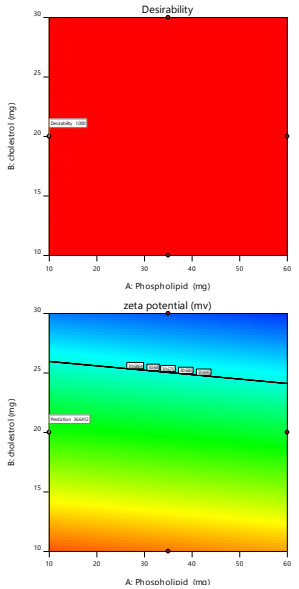
- **Optimisation of formulation**



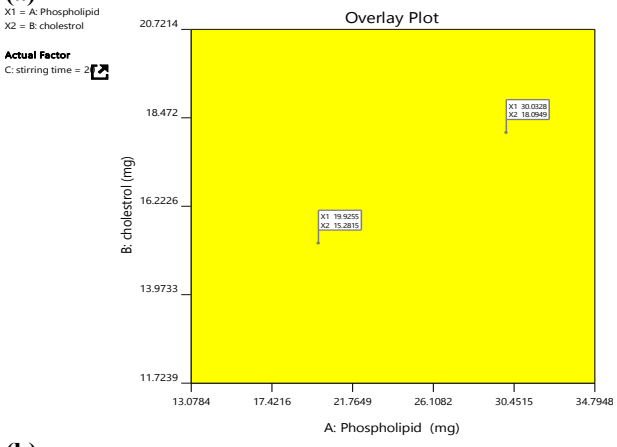
Graph 1: (a) Counter plot showing the actual factor dependent variables against particle size and (b) Response surface study of independent variables



Graph 2: Response surface study of independent variables



(a)



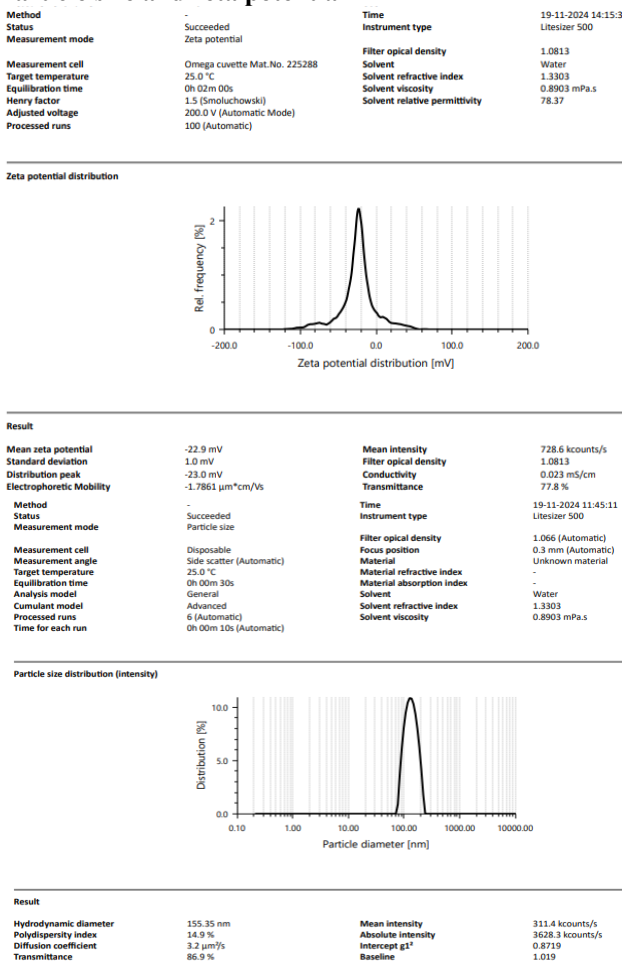
(b)

Graph 3: Response surface and overlay plot for the optimized dependent variables

The liposomes were prepared using the thin-film hydration method, followed by optimization using a Box-Behnken design. A Box-Behnken design (Statease software; Version 1.0.7.2 ID – DS-1, ReliaSoft Corporation, USA) of response surface methodology was used to evaluate two or more factors simultaneously. The treatments were combinations of different factor levels. The advantages of this optimization technique via Stat-Ease software over one-factor-at-a-time experiments were its higher efficiency and ability to detect interactions. In this optimization approach, independent and dependent variables were utilized to optimize the formulation.

- **Characterization of optimised formulation**

1. Particle size and zeta potential



Graph 4: Zeta potential and Particle size of optimized formulation L12

Table 4: Showing the Particle Size Distribution, Polydispersity Index and Zeta potential of optimized formulation

S. No.	Parameters	Formulation code L12
1	Particle sizer	155.35nm
2	PDI	14.9%
3	Zeta Potential	-22.9mv

The particle size and zeta potential of the liposomes in the optimized formulation (L12) were 155.3 nm and -22.9 mV, respectively. The zeta potential results indicated that the formulation was stable, as an increase in charge enhances formulation stability.

2. Entrapment efficiency

Table 5: Entrapment efficiency of the optimised formulation L12

S.N	Parameters	Formulation code L12
1	Entrapment Efficiency	93±0.023

The entrapment efficiency of the liposomes in the optimized formulation (L12) was 93%, indicating that 93% of the drug was successfully encapsulated within the vesicles, while the remaining portion constituted the free drug content.

3. SEM

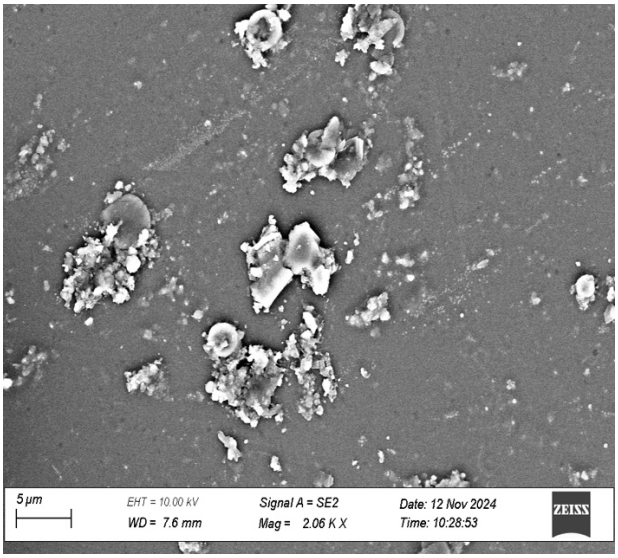
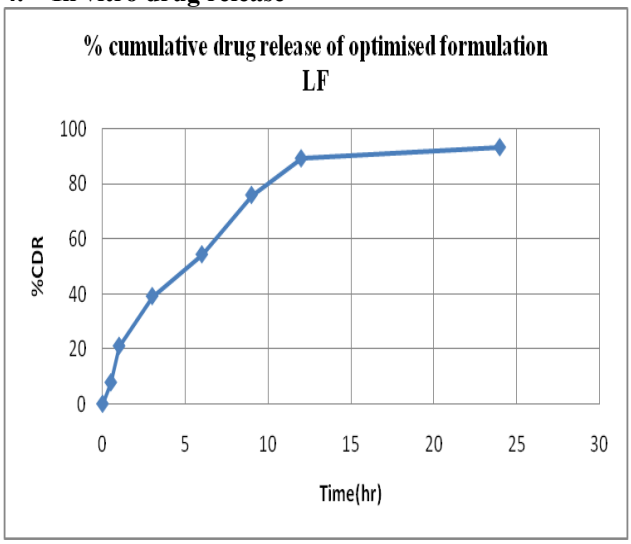


Figure 5: SEM of the optimized formulation liposomes L12

4. In vitro drug release

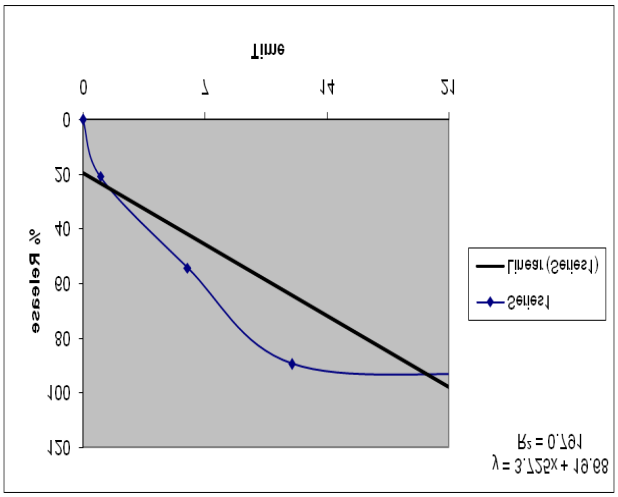


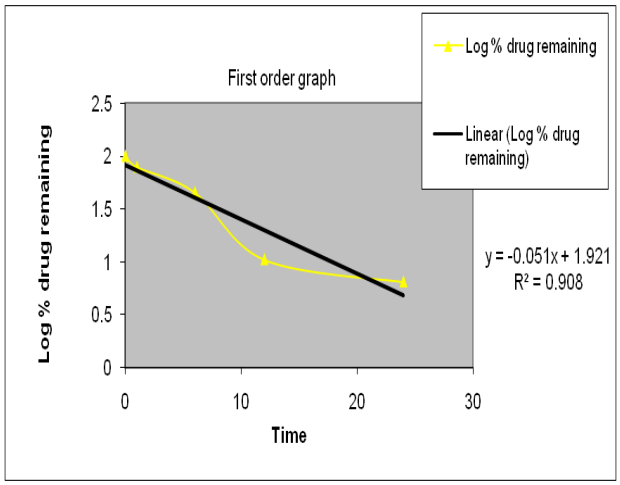
Graph 6: % cumulative drug release of liposomes (L12) loaded with combination drug

- **Kinetic release model for the optimized formulation L12**

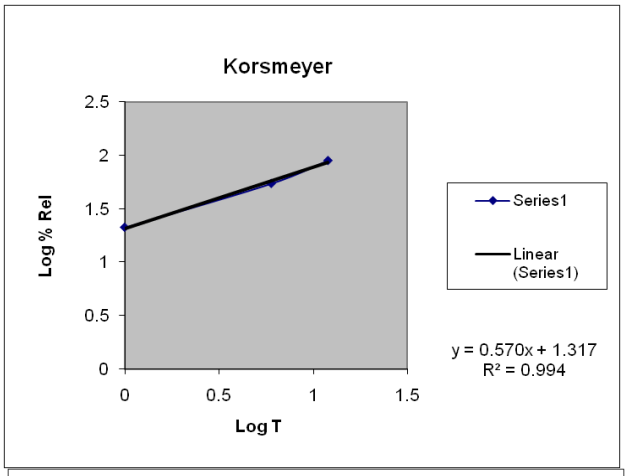
Table 6: Represent the % cumulative drug release of the liposomes (L12) loaded drug

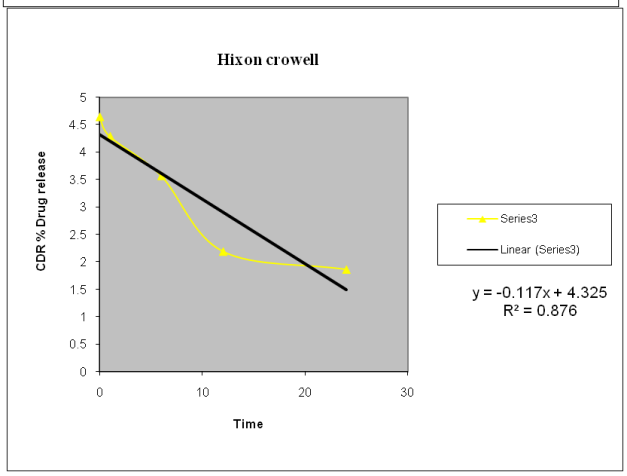
S.N.	Time (hrs)	L12	% drug remaining	Log % drug remaining	Sqr root of time	log T	Log % Rel	CDR % drug remaining
1	**0**	**0**	100	2	0	0	0	4.641589
2	0.5	7.8±0.21	92.2	1.964731	0.707107	0.30103	0.892095	4.517626
3	1	21.11±0.25	78.89	1.897022	1	0	1.324488	4.288848
4	3	39.26±0.11	60.74	1.783475	1.732051	0.477121	1.59395	3.930896
5	6	54.48±0.14	45.52	1.658202	2.44949	0.778151	1.736237	3.570541
6	9	76.12±0.26	23.88	1.378034	3	0.954243	1.881499	2.879684
7	12	89.47±0.28	10.53	1.022428	3.464102	1.079181	1.951677	2.191843
8	24	93.52±0.29	1.72	0.235528	4.898979	1.380211	1.992465	1.198145



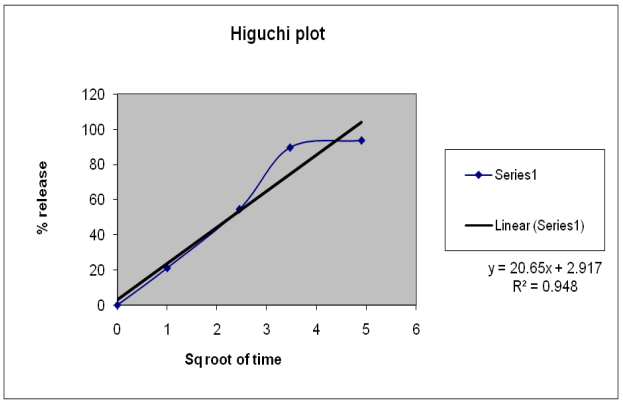


Graph 7: Zero and first order kinetic release model for L12





Graph 81: Korsmeyer and Hixoncrowell kinetic release model for L12



Graph 9: Higuchi kinetic release model for L12

The drug release profile of the L12 formulation was illustrated through tables and graphs depicting cumulative release. The results indicated a high cumulative percentage of drug release, with the optimized L12 formulation demonstrating sustained release for up to 24 hours. Various kinetic models were applied to the in vitro release data to determine the regression coefficients (R^2), as shown in the table. The drug release from all investigated L12 formulations followed the Korsmeyer–Peppas kinetic model, with a correlation coefficient greater than zero, and exhibited first-order kinetics. The Korsmeyer–Peppas release exponent data indicated a non-Fickian anomalous release, with a regression value of $R^2 = 0.994$ for the optimized L12 formulation.

- **Stability study**

Table 7: Represent stability of the liposome (L12) loaded drug

Formulation	Test condition	Temp	% EE	Particle size (nm)	Physical appearance
Liposomes	0 Month	-	96.23	107.23	Milky suspension
	1 Month	4°C	95.23	105	Milky suspension
		24°C	95.09	110	Milky suspension; slight coagulation
		37°C	95	112	Slightly dark color and thicker suspension, with growth of fungus on top, dispersed upon shaking.
	2 Month	4°C	95.23	109	Milky suspension
		24°C	95.11	121	Slight dark color suspension with formation of big clumps which were dispersible
	3 Month	4°C	95.23	110	Milky suspension
		24°C	95.01	134	Slight dark color suspension with formation of big clumps of, which were not dispersible upon shaking

The physical appearance of both conventional and liposomal formulations was evaluated after storage at 4°C, 24°C, and 37°C for 1, 2, and 3 months. After 1 month, all liposomal formulations stored at 4°C and 24°C remained stable, whereas those stored at 37°C exhibited changes in color and viscosity. By the end of the 2nd and 3rd months, fungal growth was observed in formulations stored at 24°C, while those stored at 4°C remained stable. Additionally, the particle size distribution and mean particle size of both formulations were assessed as a function of temperature over the same period. Except for liposomes stored at 37°C, no significant changes were observed in particle size or size distribution in formulations stored at 4°C and 24°C.

Conclusion

The study successfully formulated and optimized a liposomal delivery system for felodipine using the thin-film hydration technique. The optimized liposomal formulation demonstrated favorable physicochemical properties, including appropriate particle size, high entrapment efficiency, and sustained drug release. The stability and controlled drug release profile of the formulation suggested that liposomal felodipine could be a viable strategy for improving the bioavailability of this antihypertensive drug.

Future studies, including in vivo pharmacokinetic and pharmacodynamic evaluations, were warranted to further validate its therapeutic benefits and clinical applicability.

Reference

1. Bangham, A. D., Standish, M. M., & Watkins, J. C. (1965). Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of molecular biology*, *13*(1), 238-IN27.
2. Torchilin, V., & Weissig, V. (Eds.). (2003). *Liposomes: a practical approach* (No. 264). Oxford University Press.
3. Izadiyan, Z., Misran, M., Kalantari, K., Webster, T. J., Kia, P., Basrowi, N. A., ... & Shameli, K. (2025). Advancements in Liposomal Nanomedicines: Innovative Formulations, Therapeutic Applications, and Future Directions in Precision Medicine. *International Journal of Nanomedicine*, 1213-1262.
4. Bulbake, U., Doppalapudi, S., Kommineni, N., & Khan, W. (2017). Liposomal formulations in clinical use: an updated review. *Pharmaceutics*, *9*(2), 12.
5. Blanken, D., Foschepoth, D., Serrão, A. C., & Danelon, C. (2020). Genetically controlled membrane synthesis in liposomes. *Nature communications*, *11*(1), 4317.
6. Saltiel, E., Gray Ellrodt, A., Monk, J. P., & Langley, M. S. (1988). Felodipine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension. *Drugs*, *36*, 387-428.
7. Bazzo, G. C., Caetano, D. B., Boch, M. L., Mosca, M., Branco, L. C., Zétola, M., ... & Pezzini, B. R. (2012). Enhancement of felodipine dissolution rate through its incorporation into eudragit® E–PHB polymeric microparticles: In vitro characterization and investigation of absorption in rats. *Journal of pharmaceutical sciences*, *101*(4), 1518-1523.
8. Lombardo, D., & Kiselev, M. A. (2022). Methods of liposomes preparation: formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. *Pharmaceutics*, *14*(3), 543.
9. Pattni, B. S., Chupin, V. V., & Torchilin, V. P. (2015). New developments in liposomal drug delivery. *Chemical reviews*, *115*(19), 10938-10966.-8
10. Seleci, M., Ag Seleci, D., Scheper, T., & Stahl, F. (2017). Theranostic liposome–nanoparticle hybrids for drug delivery and bioimaging. *International Journal of Molecular Sciences*, *18*(7), 1415.
11. Ba, S., Qiao, M., Jia, L., Zhang, J., Zhao, X., Hu, H., & Chen, D. (2021). Construction of hierarchical-targeting pH-sensitive liposomes to reverse chemotherapeutic resistance of cancer stem-like cells. *Pharmaceutics*, *13*(8), 1205.
12. Sarode, A. L., Malekar, S. A., Cote, C., & Worthen, D. R. (2014). Hydroxypropyl cellulose stabilizes amorphous solid dispersions of the poorly water soluble drug felodipine. *Carbohydrate polymers*, *112*, 512-519.