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QBD ENABLED OPTIMIZATION STUDY OF THE VARIABLE CONCENTRATION OF PHOSPHOLIPID AND STABILIZER IN THE DEVELOPMENT OF LIPOSOMAL PASTILLES OF SOLID DISPERSION POLYMERIC COMPOSITE OF ANTIHYPERTENSIVE DRUG

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ABSTRACT
Background: The study aimed to develop and optimize liposomes of the antihypertensive drug Felodipine (FH) using the Quality by Design (QbD) approach with a 3^2 Central Composite Design (CCD) in Design Expert software, followed by the development of pastilles. **Methodology:** Liposomes were prepared using the solvent injection method, with soya lecithin and cholesterol as key excipients, and a solid dispersion of FH. The impact of their concentrations on particle size (PS), drug content (DC), entrapment efficiency (EE), and in vitro and ex vivo drug release was analyzed using response surface methodology. The optimized formulation was validated using four batches (optimized batch, VC1, VC2, and VC3), ensuring a minimal percentage error. The liposomal formulation was incorporated into pastilles to enhance patient compliance, and these were evaluated for drug content, dissolution, bioadhesion, and stability. **Results and Discussion:** The optimized liposomes exhibited desirable properties, including a positive surface charge (PS, 1.41±0.12), a high DC (94.323±1.03), a high EE (69.61±1.13), in vitro drug release (70.73±1.08), and ex vivo drug release (66.88±0.23). The validation batches showed minimal percentage error, confirming the optimization process. The pastilles demonstrated excellent physical stability and bioadhesion, indicating their potential for improved patient compliance. **Conclusion:** The study showed the effectiveness of the QbD approach in optimizing a liposomal drug delivery system for FH, thereby minimizing the need for extensive trials. The incorporation of liposomes into pastilles provided a patient-friendly dosage form with enhanced bioadhesion and stability, making it a promising alternative for antihypertensive drug delivery.

INTRODUCTION

Limited aqueous solubility presents a significant challenge in transforming new compounds into oral dosage forms, as their absorption is mainly dependent on dissolution. Poorly water-soluble compounds often exhibit several critical drawbacks, including low bioavailability, variability in absorption

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depending on whether the individual is fed or fasted, inconsistent dose-response relationships, and challenges in achieving optimal dosing. Additionally, their formulation may require the use of harsh excipients, such as co-solvents, and extreme pH conditions to enhance solubility, which may lead to uncontrolled precipitation post-administration and ultimately affect patient adherence [1]. Liposomes are tiny, spherical-shaped vesicles made up of a lipid bilayer membrane. They were first identified by Bangham and Thorne in 1964 when they found phospholipid dispersions in an aqueous medium under an electron microscope [2]. Liposomes gained popularity in vesicular research due to their biocompatibility and structural features similar to those of biological cells [3]. Liposomal pastilles enhance bioavailability by enabling sublingual absorption and protecting drugs from degradation through liposomal encapsulation. They offer sustained release, improved taste, and better patient compliance compared to traditional forms, such as tablets or syrups. This makes them particularly suitable for sensitive or poorly absorbed compounds. Felodipine is a calcium channel antagonist belonging to the dihydropyridine class, primarily used for managing hypertension and angina pectoris. Due to its poor water solubility and significant hepatic first-pass metabolism, the oral bioavailability of this compound is low, ranging from 15% to 20% [2]. The growing global focus on nanotechnology has significantly advanced various scientific fields, particularly in medical and pharmaceutical research, leading to innovative drug delivery approaches [4]. Nanotechnology enables precise drug targeting, enhanced solubility, and protection of actives, making it a transformative tool in drug delivery. Its integration in this research highlights a novel approach for improving therapeutic efficacy and patient outcomes.

MATERIALS

Felodipine was procured from Geneka Healthcare, India, while soya lecithin and cholesterol were obtained from Finar Chemicals Limited, Ahmedabad. Chloroform was sourced from Molychem, Mumbai, and gelatin from Sisco Research Lab, Mumbai. All other chemicals were of analytical grade.

METHODS

Preparation of PVP-PEG Polymeric composite

Polymeric composites of felodipine (FH)-loaded PVP-PEG polymeric composite were developed using the process of solvent-evaporation. The FH, along with excipients, was entirely solubilized in ethanol (99.5%) to get a transparent solution, ensuring a homogeneous mixture at the molecular level. The prepared solution was then subjected to drying, yielding a solid product with evenly dispersed drug molecules within the polymeric matrix. For each batch, precise amounts of FH and polymers (PVP-K30 & PEG 6000) were added to ethyl alcohol to form a clear solution. The prepared solution was evenly distributed on a tray and dried until a constant weight was achieved using a tray dryer maintained at 40°C. Once completely dried, the entire mass was subjected to trituration using a pestle and mortar for 15 minutes, then passed through the sieve (Sieve No. 60) and stored. The experimental design for the formation of drug-loaded PVP-PEG solid dispersion polymeric complexes is detailed in Table 1 [5].

Formulation of liposomes using PVP-PEG polymeric composite

Preparing batches utilized a 3^2 CCD with $\alpha = 1$, in which analysis of the variables was involved. This was one of the reasons why DoE was considered optimal, as it tends to reduce the number of experiments needed to study the individual impact of each variable (main effects) as well as how such variables combine together (interaction effects). Additional information was attained from the average of the five center point replicates. The dependent variables for the study were PS, DC, EE, ex vivo, and in vitro drug release rate. Table 1 provides details of the various batches prepared. The solvent injection method was employed, where the mixture was injected at a controlled rate of 1 mL/min and then evaporated using a rotary evaporator at 60°C for 1 hour. This process ensured the complete removal of residual chloroform, with constant stirring to ensure the product was evenly distributed. The liposomal dispersion volume was diluted to 10 mL with phosphate buffer (pH 7.4) and stored at 2°C to 8°C in sealed, light-tight containers, ready for use. For each formulation, three validation batches of Felodipine formulations were developed using the same procedure to calculate the average. All the developed batches are shown in Table 1.

Surface Topography

The surface topographic study of the formulated liposomes was analysed using a scanning electron microscope. A drop of liposome dispersion was placed on a sterile, clean slide, air-dried, and then coated with a thin layer using a Sputter Coating Unit-E 5100 (Polaron Engineering Ltd., Watford, UK). The coated sample was subsequently analyzed using a scanning electron microscope (Leo-435VP, Leo, Cambridge, UK) [6].

Particle Size Distribution

The zeta potential was measured to assess the electrostatic stability of the liposomes, ensuring they remain well-dispersed without aggregation. Higher absolute zeta potential values indicate better stability due to stronger repulsive forces between particles. The particle size distribution of the developed liposomes was determined using a Zeta Sizer 3000HSA (Malvern Instruments, Malvern, UK) via dynamic light scattering. The analysis was performed for 60 seconds at a 90° angle of scattering, with the samples placed in a cuvette for measurement. The uniformity of the liposome particle size was determined using the zeta potential of the optimized liposomal formulation. Dynamic and static light scattering techniques are widely used in nanotechnology research for characterization [7].

Table 1: Experimental Design for Preparation of Felodipine Liposomes

Formulation code	Soya Lecithin X_1		Cholesterol X_2		Chloroform(mL)	Buffer (mL)
	Coded Value	Actual Value	Coded Value	Actual Value		
F1	0	15	0	7.5	10	10
F2	+1	20	-1	5	10	10
F3	0	15	0	7.5	10	10
F4	0	15	-1	5	10	10
F5	-1	10	0	7.5	10	10
F6	0	15	0	7.5	10	10
F7	0	15	0	7.5	10	10
F8	0	15	0	7.5	10	10
F9	+1	20	+1	10	10	10
F10	-1	10	+1	10	10	10
F11	0	15	+1	10	10	10
F12	-1	10	-1	5	10	10
F13	+1	20	0	7.5	10	10

Drug Entrapment Efficiency

The drug entrapment efficiency was determined using the ultracentrifugation technique, which involved measuring the drug concentration in three distinct regions of the vesicular system. This includes the amount integrated into the internal core phase, the amount adsorbed on the vesicular membrane, and the amount incorporated into the vesicle membrane bilayer. The dispersed liposomes were subjected to centrifugation using a Remi CPR-30 centrifuge (India) at 5°C and 18,000 rpm for 1 hour to estimate the entrapment efficiency. The supernatant containing unentrapped drug was further subjected to dilution and analyzed using a UV-visible spectrophotometer, Shimadzu, UV1800. The following formula was used to estimate the entrapment efficiency for each formulation [8].

$$EE = \left[1 - \frac{Unentrapped\ drug\ content}{Unentrapped\ drug\ content}\right] \times 100$$

Ex vivo drug permeation study

The drug permeation studies were carried out ex vivo using goat gut, which had a thickness of 0.18 mm. The fresh intestine was stored in a refrigerator at -20°C after purchase from a local abattoir. The intestine was thawed to room temperature, dissected, and placed into a Franz diffusion cell with the mucosal side of the inner lining of the intestine facing the donor compartment, whose orifice diameter was 9 mm [Figure 4]. The process of drug permeation was evaluated using a Franz diffusion cell, where 5 mL of the solution was introduced into the donor compartment. Samples of 2 millilitres each were removed from the sampling port every hour for 5 hours, and at each removal, the receiver compartment medium (0.01 N HCl) was changed. A thermostatic system was used to maintain the temperature for each diffusion cell at 37±2°C [9].

Statistical analysis and optimization

Response plots help predict how dependent variables will respond to variations in independent variables, facilitating the study of the influence of various factors at a particular point in time. The best conditions will be determined using the RSM, which encompasses optimization by graphs. An overlay curve can be generated with the aid of the plots to produce new formulations that meet the required responses. The practical values were compared with the anticipated values to validate the

selected experimental design. The relative error (%) will then be calculated using the formula. The application of RSM has been used for prediction and optimization in various fields [10].

$$\textbf{Relative Error} = \frac{(\textbf{Predicted Value} - \textbf{Experimental Value})}{\textbf{Predicted Value}} \times 100$$

Knowledge of the key and interaction effects of these factors is further facilitated by RSM plots of the response surface, which depict the interaction between independent variables at a time while keeping the rest of the factors fixed. Optimization of all responses with different objectives was done using a multi-criteria decision strategy. This method integrates the graphical optimization technique employing an overlay plot. By limiting the responses of the dependent and independent variables, the optimal formulation was found. The suggested concentrations for the independent factors were determined using the Statease software [11].

Stability Studies of Felodipine Liposomes

The stability of Felodipine liposomes in various formulations was assessed under two storage conditions. For three months, one sample was kept at room temperature, while the other was stored at a temperature of 2–8 °C under refrigerated conditions. Samples were observed to assess the physical stability of the liposomal dispersion over this period. Stability characterization is a crucial aspect of pharmaceutical liposome product development, with regulatory considerations playing a significant role in ensuring product viability [12].

Formulation of Liposomal Pastilles

Liposomal pastilles were developed using the previously optimised liposomal formulation, with each pastille containing 10 mg of Felodipine. A modified technique based on the British Pharmaceutical Codex (1907) was used for their preparation. Initially, a glass beaker (25mL) was filled with an accurately weighed quantity of gelatin (1.5g). Phosphate buffer (5 mL, pH 7.4) was added to the gelatin, and the mixture was heated in a water bath at 60°C until the gelatin had fully dissolved. Subsequently, sugar (1 g) and tamarind gum (445 mg) were added to the mixture and stirred while heating until both components dissolved in the gelatin base. Glycerol (1g) and sodium saccharin (5 mg) were then weighed and added to the pre-formed mixture. Following this, 50mg of the liposomal dispersion of FH was stirred into the mixture. The completed liquid was then poured into five molds, each with a diameter of 15 mm, and stored in a deep freezer for solidification. As a control, pastilles containing pure FH were prepared using the same process. The pastilles were stored in a refrigerator at 2-8°C in firmly sealed containers until further use. Studies have demonstrated the formulation and evaluation of pastilles for pharmaceutical applications, including their potential in dental care [13].

EVALUATION TESTS FOR FELODIPINE LIPOSOMAL PASTILLES AND PURE FELODIPINE PASTILLES

Physical Characterization

The diameter and thickness of the formulated liposomal pastilles and the pure drug-containing pastilles were measured using a Vernier calliper.

Weight Variation Test

A total of 20 pastilles were selected, and their weights were noted both individually and collectively using a digital weighing balance. The weight variation was then determined [14].

$$\textbf{Weight variation} = \frac{(\textbf{W1} - \textbf{W2})}{\textbf{W2}} \times 100$$

Where, W_1- Individual weight of tablet, W_2- Average weight of tablet

Drug Content

The drug content was determined by dissolving a single pastille in an appropriate volume of phosphate buffer (pH 7.4) and diluting the solution to 100 mL in a volumetric flask. The sample obtained was then subjected to filtration and suitably diluted before measuring its absorbance at 362 nm using a UV-visible spectrophotometer, with phosphate buffer (pH 7.4) serving as a blank. Based on the recorded absorbance, the drug content in the pastilles was calculated. UV spectrophotometry is a well-established technique for determining the drug content in various dosage forms. [15].

Swelling and Erosion Studies

The swelling and erosion behaviour of pastilles was evaluated in phosphate buffer (pH 6.8). Each pastille was affixed to a pre-weighed glass Petri dish using a bonding sealant. Three pastilles were individually weighed (W_1) and then immersed separately in phosphate buffer (pH 6.8). After 1 hour, they were removed, and any excess surface moisture was gently blotted using filter paper. The swollen pastilles were reweighed (W_2), and the swelling index (SI), along with the erosion rate, were calculated using the formula provided below. [16].

$$\textbf{Swelling Index} = \frac{(W2 - W1)}{W1} \times 100$$

$$\textbf{Erosion} = \frac{(W1 - W2)}{W1} \times 100$$

Where, W1 = pastille initial weight, W2 = pastille final weight

Moisture Absorption Study

The moisture absorption was performed to determine the extent of water retention capacity of the polymers used in the preparation and characterization of the structural stability of the pastilles after absorbing moisture. To conduct this study, a 5% w/v agar solution was prepared in distilled water, poured into Petri dishes while still hot, and left to solidify. The pastilles were first weighed and then kept in desiccators overnight to remove residual moisture. Afterwards, they were positioned on the surface of the agar plate for 2 hours, following which they were reweighed. The percentage of absorbed moisture was then calculated using the appropriate formula. Moisture uptake and diffusion mechanisms in composite materials have been widely studied to understand their impact on formulation stability [17].

$$\%\ \textbf{Moisture Absorbed} = \frac{(W2 - W1)}{W1} \times 100$$

W1 & W2 are the initial and final weights of pastilles, respectively

Mucoadhesion Time

The mucoadhesion time was assessed by applying the dosage form onto freshly excised porcine buccal mucosa. The mucosal tissue was affixed to a microscopic glass plate, and a drop of phosphate buffer (pH 6.8) was applied to one side of the pastille to moisten it. The pastille was then gently pressed against the mucosa using a fingertip for 30 seconds. The glass slide was placed in a beaker containing 200 mL of phosphate buffer pH 6.8, which was continuously stirred at 300 rpm using a magnetic stirrer to maintain homogeneity. The duration required for the pastille to detach from the mucosa was recorded as the mucoadhesion time. Mucoadhesive systems are widely explored for enhancing drug retention in the buccal cavity [18].

Bioadhesive Strength

The adhesive strength of the pastille was evaluated using a modified physical balance method, utilizing porcine buccal mucosa as the model membrane. The freshly excised mucosa was trimmed, rinsed with phosphate buffer (pH 6.8), and affixed to a microscopic glass plate, which was lightly moistened with the same buffer. The pastille was attached to the underside of another glass plate using an adhesive. To balance the setup, an appropriate weight was placed on the left-hand pan. The glass plate holding the mucosa was positioned with adequate support to establish contact between the pastille and the mucosal surface. Additional weights were gradually added to the right-hand pan until the pastille separated from the mucosal tissue. The weight required for detachment was recorded as the bioadhesive strength. The experiment was conducted in triplicate, and the average value was calculated [19].

pH Study

A single pastille was dissolved in distilled water (100mL), and maintained at 37°C. The pH was then measured using a pH meter, allowing the solution to stabilize for one minute before recording the reading.

***In vitro* Drug Release Studies**

The drug release study was conducted using a Paddle-type dissolution test apparatus. The dissolution study was conducted in phosphate buffer (500 mL) of pH 7.4 as the dissolution medium. The paddle speed was maintained at 75 rpm, and the temperature was controlled at 37 ± 0.5 °C. A single pastille was placed for drug release, and a sample of 5 mL was withdrawn at a regular interval (10, 20, 30, 45, and 60 minutes) and replaced with the same volume of fresh dissolution medium. The collected samples were appropriately diluted and examined at 362 nm using a UV-visible spectrophotometer. The % of drug release at different time intervals was determined, and the experiment was performed in triplicate to ensure reliability [20].

Fourier Transform Infrared Spectroscopy

The FTIR spectroscopic studies were carried out using an FTIR spectrophotometer (M/s. BRUKER, Model Alpha). In the KBr pellet technique, the sample was finely ground with potassium bromide to minimize scattering effects caused by large crystals. The resulting powder mixture was then compressed using a mechanical die press to form a translucent pellet, enabling the spectrometer beam to pass through for analysis. The pressed sample was carefully removed from the die and placed in the FTIR sample holder. The IR spectrum was recorded from 4000 cm^{-1} to 400 cm^{-1} [21].

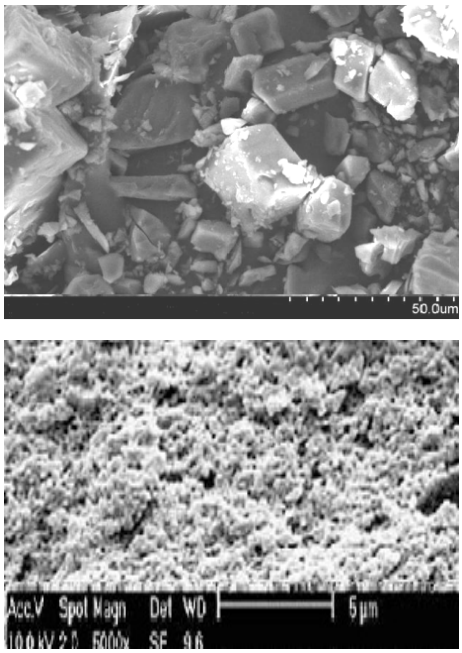
Stability Studies of Liposomal Pastilles on Storage

The stability of the optimized liposomal pastilles was assessed by examining and contrasting the best formulation under storage conditions of 2–8 °C and 25 °C/60% RH for six months. During the stability study, the physicochemical properties of the pastilles were evaluated at regular intervals.

RESULT AND DISCUSSION

Scanning Electron Microscopy

The morphology of the optimized liposomal formulation was studied using Scanning Electron Microscopy, and the images are shown in Figure 1 (A and B). The electron microscopy image of the pure drug and the optimized liposomal formulation showed a spherical, smooth, and regular surface.



A B

Figure 1: SEM Images for (A) Pure drug and **(B) Optimized Formulation**

Particle Size Determination

The particle size distribution of felodipine liposomes is shown in Table 2. The mean particle size of all the liposome formulations was found to be in the range of 110±0.09 nm to 205±0.04 nm. The results demonstrated that as the lecithin-to-cholesterol weight ratio increased, the vesicle size decreased. The polydispersity index (PDI) of the liposomal vesicles in the optimized formulation was determined to be 0.439. Small values of P.I. (< 0.3) indicate a homogenous population, whereas high P.I. (> 0.3) indicates heterogeneity. The findings revealed that the vesicles in the dispersion were of consistent size, possibly attributed to the membrane-stabilizing properties of cholesterol. The zeta potential for the optimized formulation was found to be 38.1 mV. The results indicate good dispersion stability as the value is less than 50 mV [23].

Drug Entrapment

The entrapment efficiency (EE) of all liposomal formulations (F1 to F13) ranged from 55.7±0.17% to 69.2±0.28%, as shown in Table 2. Formulation F11 showed the highest entrapment efficiency of 69.2±0.28%, whereas formulation F2 showed the lowest percentage entrapment efficiency of 55.7±0.17%. An increase in phospholipid and cholesterol content resulted in a significant improvement in entrapment efficiency. The results align with previous studies, which demonstrated that an increase in cholesterol enhances the stability and entrapment efficiency of the drug in the liposomes [24].

In Vitro Release Profile of Liposomal Formulations

The in vitro drug release profile of liposomes was evaluated using a semipermeable membrane over 24 hours, with the percentage of Felodipine release presented in Table 2. The findings demonstrated a sustained and gradual release of Felodipine throughout the study duration. Among the various liposomal formulations, F9 exhibited the highest drug release, reaching 69.69% w/w within 24 hours. The observed biphasic release pattern, characterized by a slow and continuous drug release, can be attributed to the gradual diffusion of both free and encapsulated Felodipine from the liposomal membrane through the dialysis membrane [25].

Ex Vivo Diffusion Studies on Prepared Liposomes

The findings from the ex vivo diffusion studies, presented in Table 2, demonstrated a gradual and sustained release of Felodipine from the liposomes over 24 hours. The drug release from various liposomal formulations ranged from 53.4% w/w (F5) to 71.7% w/w (F9), with the highest release observed in formulation F9. These results indicate that all liposomal formulations, prepared with varying ratios of soya lecithin and cholesterol, exhibited a controlled drug release profile compared to the pure drug. The data further suggest that an increase in lipid and cholesterol content contributed to an enhanced percentage of drug release in the liposomal formulations [26,27].

Statistical Analysis for Physicochemical Parameters of Felodipine Liposomes

Felodipine-loaded liposomes were developed using various ratios of soya lecithin and cholesterol, based on the design of the experiment generated by Design-Expert trial version software. The formulations were assessed for PS, DC, and EE, in vitro and ex vivo drug release. All responses were analyzed using linear and quadratic models within the software, with the quadratic model being selected for PS, DC, EE, in vitro, and ex vivo drug release. The obtained p-values for these four responses

were 0.0012, 0.0036, 0.0090, and 0.0015, respectively, all of which were below 0.05, indicating statistical significance. The calculated R^2 value in the present models for all four responses was close to 1, indicating a good model fit. In all cases, the adjusted R^2 values are in reasonable agreement with the predicted R^2 values (0.8435 and -6.1539 for particle size, 0.2002 and -37.6980 for drug content, 0.5944 and -18.5535 for entrapment efficiency, and 0.6711 and -14.9230 for percentage drug release). "Adeq Precision" measures the signal-to-noise ratio, and a ratio greater than 4 was desirable. In all cases, precision values for the four responses were found to be 10.366, 4.144, 5.539, and 6.728, indicating an adequate signal and that the model can be used to navigate within the design space. The application of response surface methodology yielded the following regression equations (A: Soya Lecithin; B: Cholesterol).

Table 2: Physicochemical Parameters of Different Felodipine Liposomal Formulations

Batch Code	Particle Size (nm±SD)*(n=3)*	Drug Content (%±SD)*(n=3)*	Entrapment Efficiency (%±SD)*(n=3)*	In vitro drug release (%)*(n=3)*	*Ex vivo* drug release (% w/w±SD)*(n=3)*
F1	123±0.03	96.21±0.14	68.8±0.15	69.69±0.41	64.1±0.11
F2	155±0.01	95.34±0.19	55.7±0.17	58.93±0.31	64.7±0101
F3	128±0.03	96.29±0.05	69.2±0.29	69.54±0.18	64.5±0.33
F4	156±0.05	93.13±0.06	66.1±0.18	68.51±0.35	60.9±0.53
F5	196±0.07	91.89±0.08	61.5±0.31	65.80±0.30	53.4±0.83
F6	121±0.11	96.28±0.03	69.1±0.11	69.66±0.16	64.2±1.39
F7	127±0.06	96.20±0.17	68.7±0.27	69.19±0.28	64.8±0.49
F8	122±0.02	96.19±0.23	68.6±0.22	69.40±0.22	64.5±0.76
F9	134±0.04	94.20±0.13	55.8±0.13	60.0±0.22	71.7±0.88
F10	167±0.07	93.82±0.24	59.2±0.18	63.42±0.27	57.8±0.78
F11	110±0.09	97.27±0.28	69.2±0.16	70.86±0.34	67.4±1.04
F12	205±0.04	92.22±0.30	60.6±0.17	64.21±0.17	58.2±0.82
F13	142±0.15	94.21±0.07	57.9±0.33	60.20±0.30	71.2±0.41

***Particle Size** = + 1.28414 - 0.27000A - 0.11000B + 0.26750AB + 0.30052A2 + 0.060517B2 - 0.097500A2B + 0.28750AB2*

***Drug Content** = + 95.65724 + 1.16000 A + 0.53500 B - 1.64250 AB - 1.16534 A2 -0.55034 B2 + 0.34750 A2B - 1.24250 AB2*

***Entrapment Efficiency** = + 67.57586 - 1.80000A - 5.20000B - 1.27500 AB - 4.61552A2 -3.41552B2 + 8.2250 A2B - 1.92500 AB2*

***In vitro Drug release** = + 68.62414-2.80000A - 4.25500B - 0.54000AB - 3.33448 A2 -2.07948B2 + 7.04000A2B - 0.38000AB2*

***Ex vivo drug release**= +64.72+8.90A+5.40B-4.03AB-3.16A2+0.84 B2-4.83A2B-9.67AB2*

Response surface Plot

The response surface curve (Figure 2A) indicates that soya lecithin has a positive influence on particle size, meaning that higher values of soya lecithin lead to larger particle sizes. The particle size decreases, approaching the minimum region (yellow area). This suggests that lower values of soya lecithin contribute to finer particles. A similar increase in particle size is observed in some regions, but the effect is not uniform across all cholesterol values. The surface curvature suggests that cholesterol has a non-linear effect, meaning moderate values of cholesterol might yield smaller particle sizes, but extreme values increase. The contour plot illustrates the impact of factors, such as soya Lecithin and Cholesterol, on particle size. Increasing the amount of soya Lecithin decreases the particle size, as seen in Figure 2. Cholesterol has a weaker but interactive effect, where low soya Lecithin and low Cholesterol result in the largest particle size, while high soya Lecithin and high cholesterol minimize it. The steep gradient in certain areas indicates a strong interaction between soya Lecithin and Cholesterol. To achieve a minimum particle size, soya Lecithin should be high, and soya Lecithin should be moderate to high. Conversely, low soya Lecithin and low Cholesterol lead to a maximum particle size. The response surface plot (Figure 2B) illustrates the effect of soya lecithin and cholesterol on drug content, with higher values of both resulting in increased drug content. The blue region (lowest drug content, ~91.89%) is observed at lower levels of soya lecithin and cholesterol. In contrast, the red region (highest drug content, ~97.27%) appears at higher levels of soya lecithin and cholesterol, indicating a positive correlation. The gradual transition from blue to red suggests a synergistic effect, meaning

both components contribute significantly. Maximizing drug content requires keeping soya lecithin and cholesterol at higher levels. The optimal formulation should focus on higher levels of soya lecithin and cholesterol to achieve the best results.

The response surface plot (Figure 2C) illustrates the effect of soya lecithin and cholesterol on entrapment efficiency, with higher levels of both resulting in increased efficiency. The blue region (~55.7%) represents the lowest entrapment efficiency, observed at lower levels of soya lecithin and cholesterol, while the red region (~69.2%) indicates the highest efficiency, found at higher levels. Increasing soya lecithin significantly improves entrapment efficiency, while cholesterol also contributes, but an optimal balance is required. The combined effect of both factors enhances entrapment efficiency, showing a synergistic interaction. For maximum entrapment, both soya lecithin and cholesterol should be kept at higher levels. The response surface plot (Figure 2D) shows the effect of soya lecithin (A) and cholesterol (B) on in vitro drug release. The blue region (~58.93%) represents the lowest drug release, observed at higher cholesterol levels. The red region (~70.86%) indicates the highest drug release, found at lower cholesterol and higher soya lecithin levels. Increasing soya lecithin enhances drug release, while improving cholesterol reduces it, likely due to the formation of a denser lipid matrix. The combined effect reveals an inverse relationship, indicating that an optimal balance is necessary. For maximum in vitro drug release, soya lecithin should be increased, and cholesterol should be kept at lower levels. The response surface plot (Figure 2E) illustrates the effect of soya lecithin and cholesterol on ex vivo drug release. The blue region (~53.4%) represents the lowest drug release, occurring at higher cholesterol levels, while the red region (~71.7%) indicates the highest drug release, found at lower cholesterol levels. Increasing soya lecithin enhances drug release, whereas higher cholesterol reduces it, likely due to increased membrane rigidity. The combined effect suggests an inverse relationship, where optimizing soya lecithin while balancing cholesterol is crucial. For higher drug release, the amount of soya lecithin should be increased, and cholesterol should be maintained at an optimal level.

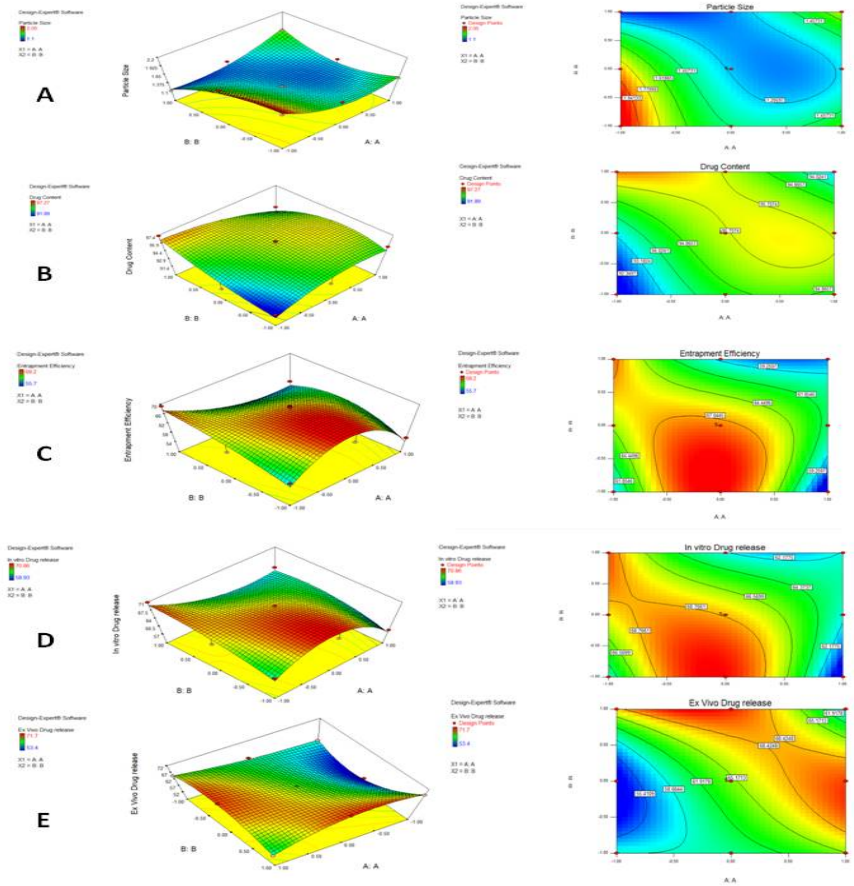


Figure 2: Response Surface and Contour plot for (A) particle Size, (B) Drug content, (C) Entrapment efficiency, (D) in-vtro Drug Release and (E) ex-vivo drug release

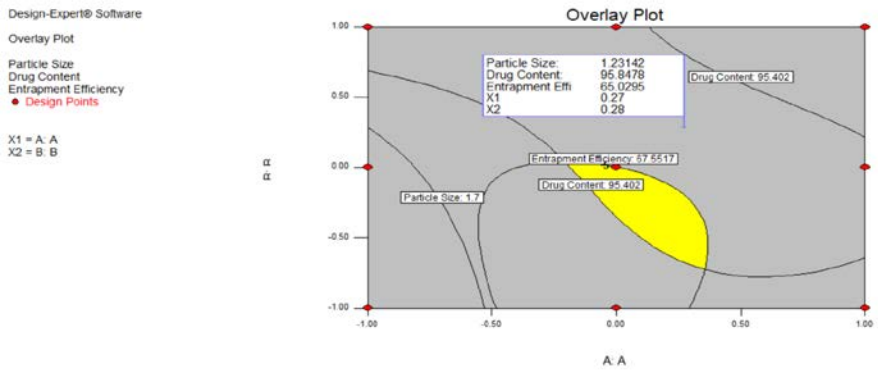


Figure 3: Overlay plot showing optimized region

Overlay Plot

Optimized formulation was for particle size (1.1-1.7nm), drug content (95.402-100%), (67-70%), and in vitro drug release (58.93-70.86). The overlay plot in Figure 3 shows the optimized region (yellow area) where particle size, drug content, and entrapment efficiency meet the desired criteria.

The black contour lines indicate constraints on these responses. The optimal formulation occurs with soya lecithin (A) at 16.35 mg and cholesterol (B) at 8.715 mg, resulting in a particle size of 1.231 μm, a drug content of 95.84%, and an entrapment efficiency of 65.02%. The gray regions represent non-optimal areas, where one or more responses fall outside the desired range. This plot aids in selecting the optimal formulation parameters for achieving high drug content and entrapment efficiency, as well as an optimal particle size.

Data validation

The predicted responses for all the formulated batches and their corresponding experimentally observed values were found to be in close agreement, as shown in Table 3. This indicates that the models developed to predict the responses were not only statistically significant but also valid, as they yielded values that closely matched the experimentally observed results.

Table 3: Data validation of optimized data*(n=3)*

Formulation code	X1	X2	Response	Predicted Value	Experimental Value	% Error
Optimized Batch	-0.04	-0.75	Particle Size (nm)	1.413±0.02	1.41±0.12	0.2128
			Drug Content (%)	94.876±	94.323±	0.5863
			Entrapment Efficiency (%)	69.61±1.13	69.59±0.87	0.0244
			Iv-vitro drug release (%)	70.73±1.08	70.82±1.22	-0.1271
			Ex-vivo drug release (%)	67.09±0.59	66.88±0.23	0.3140
VC1	-0.11	0.01	Particle Size (nm)	1.315±0.02	1.32±0.0	-0.3788
			Drug Content (%)	95.52±1.13	95.61±0.12	-0.0805
			Entrapment Efficiency (%)	67.644±0.23	67.594±1.37	0.0740
			Iv-vitro drug release (%)	68.826±0.53	68.791±0.72	0.0509
			Ex-vivo drug release (%)	67.617±1.11	67.593±0.89	0.0355
VC2	0.07	-0.22	Particle Size (nm)	1.29±0.02	1.28±0.19	0.7813
			Drug Content (%)	95.609±1.27	95.586±0.74	0.0241
			Entrapment Efficiency (%)	68.248±0.57	68.201±0.38	0.0689
			Iv-vitro drug release (%)	69.413±1.37	69.397±1.19	0.0231
			Ex-vivo drug release (%)	70.12±0.47	69.98±0.65	0.2001
VC3	0.35	-0.36	Particle Size (nm)	1.2572±0.09	1.25±0.16	0.5760
			Drug Content (%)	95.793±0.87	95.836±0.69	-0.0449
			Entrapment Efficiency (%)	67.518±1.89	67.535±0.62	-0.0252
			Iv-vitro drug release (%)	68.236±0.55	68.231±	0.0073
			Ex-vivo drug release (%)	71.555±0.78	71.471±1.03	0.1175

FTIR spectroscopy

The FTIR curve (Figures 4 & 5) of the formulated liposomal pastilles shows all characteristic peaks corresponding to FH, indicating that there is no interaction between FH and polymers.

Dimensions of Pastilles

The results of dimensions for optimized liposomes pastilles and pure drug pastilles are shown in Table 4. The diameter and thickness of the FH liposomal pastilles formulation (optimized liposome pastilles) were found to be 15 ± 0.2 mm and 5 ± 0.2mm, respectively. The results indicated uniformity in the size and shape of the pastilles.

Average Weight and Weight Variation

The results of the average weight and weight variation for optimized liposome pastilles and pure drug pastilles are shown in Table 4. The average weight of optimized liposomes pastilles and pure drug pastilles was found to be 1.5 ± 0.05 g and 1.3 ± 0.10 g, respectively.

Drug content estimation

The results of the drug content for optimized liposomes pastilles and pure drug pastilles are shown in Table 4 and were found to be 99.0 ± 1.15% w/w and 98.6 ± 1.00% w/w, respectively.

Swelling and Erosion Studies

The results of the swelling and erosion studies for optimized liposome pastilles and pure drug pastilles are presented in Table 4. The swelling index for optimized liposome pastilles and pure drug pastilles was found to be 4.5±0.29% w/w and 5±0.59% w/w, respectively. The % mass loss for optimized liposomes pastilles and pure drug pastilles after swelling was found to be 28.0±0.89% and 26.5±0.95% in 2 h.

Study of pH

The pH of optimized liposome pastilles and pure drug pastilles in distilled water was found to be 6.2 and 6.5, respectively.

Moisture Absorption, Bio-adhesive Strength, and Bio-adhesion Time

The results for moisture absorption, bioadhesion time, and bioadhesive strength are presented in Table 4. Moisture absorption for optimized liposome pastilles and pure drug pastilles was found to be 2.95 ± 0.15% and 2.65 ± 0.27%, respectively, within 2 hours.

The bio-adhesion time to porcine buccal membrane for optimized liposomes pastilles and pure drug pastilles was found to be 1.5 h. The bio-adhesive strength observed to detach optimized liposomes pastilles and pure drug pastilles from the glass slide was found to be 20 g and 15 g, respectively.

Table 4: Physicochemical Parameters of Optimized Liposomal Pastilles Formulation *(n=3)*

Characteristics	Optimized liposomal Pastilles	Pure Drug Pastilles
Diameter (mm)	15.5±0.1	15.6±0.21
Thickness (mm)	5.0±0.2	4.9±0.33
Weight (g)	1.5±0.05	1.5±0.14
Drug (%)	98.9±1.15	96.98±1
SwellingIndex (%)	5.5±0.29	5.0±0.69
Erosion (%)	30.0±0.89	26.8±1.1
Moisture absorption(%)	3.15±0.15	2.85±1.01
pH	6.0±0.06	6.5±0.04
Bioadhesion time (h)	2.1±0.29	1.8±0.13
Bioadhesive strength(g)	24±1.50	20±1.4

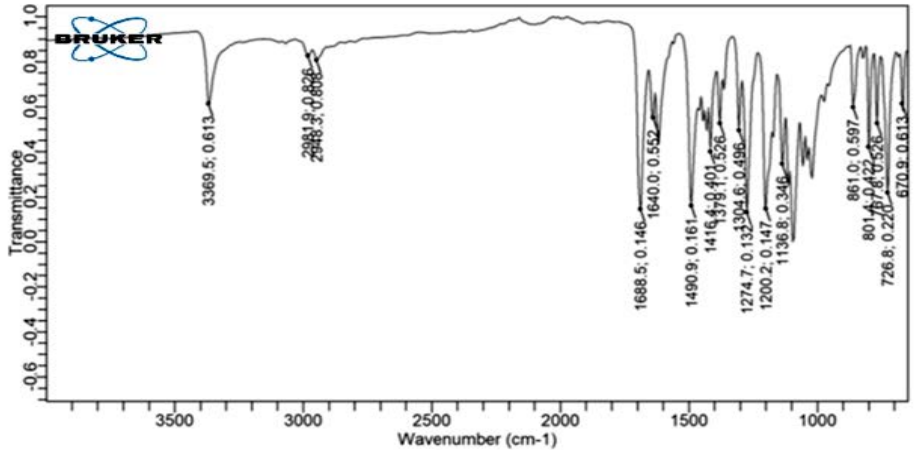


Figure 4: FTIR spectra of felodipine

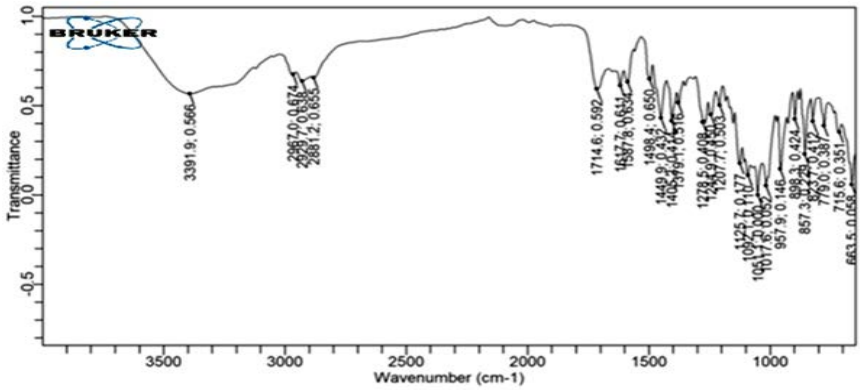


Figure 5: FTIR of optimized felodipine liposomes

Drug Release profile

The drug release study was conducted using the Paddle type dissolution test apparatus for one hour. The cumulative drug release of Felodipine from the optimized liposomal pastilles and pure drug pastilles is presented in Table 5 and Figure 6. The findings demonstrated that Felodipine was completely released from the liposomal pastilles within one hour. The in vitro drug release from the optimized liposomal pastilles reached 99.88±1.03 % in one hour, whereas the pure drug pastilles exhibited a release of 81.43±1.18% w/w over the same period.

Table 5: *In Vitro* Drug Release Data of optimized formulation and pure drug pastilles*(n=3)*

Time (Min)	Cumulative Drug Release (%w/w ± SD, n=3)	
	Optimized Liposomes pastilles	PureDrug pastilles
10	58.8±1.24	26.59±0.84
20	72.6±1.35	45.32±1.04
30	83.5±1.66	59.35±0.98
45	91.5±1.51	74.23±1.41
60	99.88±1.03	81.43±1.18

Evaluation of Stability for Liposomal Pastilles

The optimized liposomal pastilles exhibited excellent physical stability after being stored for six months at 2–8 °C as well as room temperature. As presented in Table 6, there were no significant changes were observed in physical appearance, diameter, thickness, average weight, or drug content throughout the stability study. The findings suggest that maintaining the liposomal pastilles at 2–8 °C is an appropriate condition for ensuring long-term stability.

CONCLUSION

The study effectively employed the QbD approach to optimize a liposomal drug delivery system for Felodipine, achieving an ideal particle size, drug content, entrapment efficiency, and controlled drug release. Validation batches demonstrated the robustness of the optimization process with minimal percentage error.

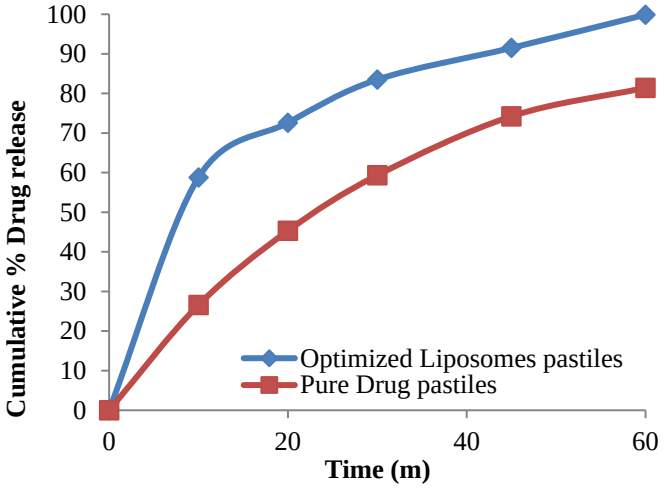


Figure 6: *In Vitro* Drug Release Data of optimized formulation and pure drug pastilles

Table 6: Stability Data of optimized FH*(n=3)*

S. No.	Property	Storage at 2-8 °C (6 Months)	Storage at 25 °C, 60% RH(6 Months)
1	Visual Appearance	No Change	No Change
2	Diameter(mm)	15.000.12	15.100±0.15
3	Thickness(mm)	5.00±0.11	4.9±0.19
4	AverageWeight(g)	1.50±0.02	1.50±0.12
5	Drug content (%)	99.10±1.09	98.60±0.95

Additionally, the incorporation of liposomes into pastilles improved patient compliance by offering a stable and user-friendly dosage form reduce in chronic disease management. This formulation holds significant potential as an efficient and convenient alternative for antihypertensive drug delivery.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS
Deepti Aggarwal designed, conceptualized and performed the study. She also performed the writing work of the manuscript while Vijay Sharma drafted and edited it. R D Gupta read and approved the conent of the manuscript.

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