QUALITY BY DESIGN-DRIVEN FORMULATION DEVELOPMENTAND OPTIMIZATION OF LERCANIDIPINE HYDROCHLORIDE LOADED MICROSPHERES: *IN VITRO* AND *IN VIVO* CHARACTERIZATION

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Abstract

The purpose of this research was to design, formulate and optimize the floating microspheres of lercanidipine hydrochloride so as to prolong its gastric residence time and increase its bioavailability as it has less solubility. The solvent evaporation technique was used for preparing the floating microspheres and optimized using Box-Behnken design. The independent variables were the ethyl cellulose (X1), HPMC (X2), and stirring speed (X3) while Vesicle size (Y1), drug entrapment efficiency (Y2), and yield (%Y3) were considered as dependent variables. The prepared microspheres underwent the following in vitro evaluation tests, including those for micrometric properties, tapped density, particle size measurement, percentage yield, entrapment efficiency, in vitro buoyancy, and drug content. The formulation F8 was found to be the optimum formulation predicted by the point prediction of the design expert software. Microsphere flow was found to be fair to good according to research on micrometric characteristics. Studies using FT-IR and DSC showed that there was no chemical interaction between the Drug and the employed polymers. Using SEM, the shape of microspheres was investigated. A hollow, spherical structure with a rough surface morphology was visible in the view of the microspheres. Microspheres have a particle size between 135.6±2.6 to 384.2±3.8µm. The formulation F8 demonstrated complete release in less than 12 hours and higher entrapment efficiency was chosen as the optimized formulation and used for additional in vivo investigations and bioavailability experiments in rats. According to the regression values derived from the in vitro release kinetics, the drug release from the improved formulation F8 follows the Korsmeyer-Peppas model. The stability studies on the improved formulation F8 showed no discernible change, and they were discovered to be stable under storage circumstances for six months. Due to controlled floating technology, an in vivo bioavailability research done on rabbits revealed enhanced bioavailability of the optimized formulation F8 when compared to the reference IR tablets. According to the commercial formulation, relative bioavailability was found to be 124.9. The longer-lasting floating mechanism

of the dose form in the stomach and the extended drug release may be responsibility for the improved bioavailability.

Keywords: Lercanidipine hydrochloride, Microspheres, Box-Behnken design, Drug release.

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Introduction

The oral route is the common way for the consumption of drugs among various routes of drug administration due to the patient compliance and cost involved in therapy. Absorption of drugs from the GIT is a very complicated process as it is difficult to confine and locate the system within anticipated regions of the GIT and also absorption varies with the conditions of GIT [1]. Relatively brief gastric emptying time leads to inadequate drug release causing reduced efficacy. Thus, the optimum site of absorption and the mechanism of action of the drug usually recommend site-specific delivery of drugs which demonstrate a window of absorption [2]. Accordingly, the increase in intimate contact of the dosage form with the GIT membrane has the prospect to enhance rate and extent of drug absorption [3]. Considering the gastric drug delivery, it is used not only in the treatment of gastric diseases but also for certain drugs getting absorbed in the stomach. Many approaches are used to enhance gastric residence time such as swellable systems, mucoadhesive formulations, and altering the density of dosage forms [4].

Floating drug delivery systems (FDDS) based on low density for gastric retention have been studied widely and are designed to float on stomach fluid while releasing the drug slowly at a predetermined rate from the delivery system [5]. Effervescent delivery systems are composed of swellable polymers such as alginate, chitosan, polyethylene oxide, and hydroxypropyl methylcellulose and effervescent components (sodium bicarbonate and citric or tartaric acid), as when these dosage forms enter the stomach, carbon dioxide is released from the dosage form by the reaction and is entrapped in the jellified polymer allowing tablet to move upwards and maintains its buoyancy [6]. For treating some diseases showing circadian rhythms in symptoms such as cardiovascular diseases, arthritis, bronchial asthma, cancer, duodenal ulcers, diabetes, and neurological disorders, it is essential to deliver the maximum drug at the time when symptoms are observed wherein release of drug can be controlled by lag time [7]. For example, in cardiovascular diseases, as frequencies of occurrence of myocardial infarction and cardiac arrest are more common from morning to noon, optimal antihypertensive and/or antianginal drug is to be delivered in morning time [8].

Lercanidipine is a dihydropyridine calcium channel antagonist which shows antihypertensive activation by peripheral and coronary vasodilation. It has poor water solubility and high lipophilic nature and is thus classified as a BCS class II drug [9]. Owing to these properties, it exhibits poor oral bioavailability (~ 20–30%) and a high first-pass effect [10]. Although solid dispersions, cyclodextrin complexes, micronization, etc. have been prepared in the literature to enhance the solubility of lercanidipine [11], limited success has been achieved so far. These oral formulations, however, are highly ineffective in avoiding hepatic first-pass metabolism and improving the permeability of the drug through oral route [12]. This has led us to design an

alternative therapeutic system for delivering the selected drug candidate with enhanced bioavailability characteristics and antihypertensive activity.

Systematic development of drug delivery systems using Design of Experiments (DoE) has been considered as an indispensable tool in drug delivery across the globe [13]. These techniques save a great deal of time, effort, and resources, as they choose "the best" formulation using lesser experimentation [14]. The use of experimental designs also helps in improving product and process understanding of the prepared drug delivery system and yields the best formulation with enhanced therapeutic performance [15].

Materials & Methods

Materials

Lercanidipine Hydrochloride was purchased from Apotex Research Private Limited, Bangalore, India. Ethyl cellulose was purchased from Degussa Chemical Co. Ltd., China. Hydroxypropyl methylcellulose was purchased from Shin-Etsu Chemical Ind. Co. Ltd., Japan. All other chemicals and reagents used are of analytical grade and are purchased from standard chemical manufacturers.

Methods

Preparation of Lercanidipine HCl loaded floating Microspheres Preliminary trial batches

Solvent evaporation was used to create a microsphere of Lercanidipine HCl. Solvent mixture (DCM: Ethanol = 1:1) was used to dissolve 20 ml of Ethyl cellulose, HPMC, and various mixtures thereof. After the necessary amount of the drug was added, it was dissolved using a combination of vortexing and sonication to produce a clear solution [16]. In a 500-milliliter beaker, 50 mL of heavy liquid paraffin were emulsified with 1 mL of Span 80 using a stirrer to create the exterior phase. At room temperature, the mechanical stirrer with a three-blade propeller was used to slowly pour the internal phase into the external phase while stirring. For three hours, the entire system was shaken up. After being separated by filtration, the microspheres were washed three times with n-hexane (50 ml) to remove any remaining paraffin oil and dried overnight at room temperature to produce a spherical product that flows freely.

Optimization of Independent Variables by Box Behnken Design

Box-Behnken design used to construct seventeen trial runs. The variables were optimized using a full factorial design. Vesicle size (Y1), drug entrapment efficiency (Y2), and floating efficiency (%Y3) were chosen as dependent responses, with ethyl cellulose (X1), HPMC (X2), and stirring speed (X3) serving as independent variables [17]. To describe the impact of the independent variables on the drug loading, particle size, and drug release, a polynomial equation was generated, the complexity of which was decreased by eliminating the non-significant interacting factors from the ANOVA. Each trial's answers (Y) are measured.

$$Y = b0 + b1X1 + b2X2 + b12X1X2 + b11X12 + b22X22$$

Where Y is the observed response, b0 represents the average of the eight trials, and b1 represents the calculated coefficient for the independent variable of interest (X1).

When one variable is adjusted from its minimum to maximum value, the average effect of those steps is what we call the main effect (X1& X2). The X1, X2, and X3 interaction terms reveal how a response shifts in response to simultaneous changes in two independent variables. In order to find statistically relevant variables, a multiple regression analysis and an F-statistics test were used to refine the model. Next, a contour plot and a three-dimensional plot were generated, and last, the suspension was optimized. At least three repetitions of each experiment were carried out [18].

Characterization of Microspheres

Drug encapsulation

The microspheres, at 100 mg, were precisely weighed. The drug was evaporated from dichloromethane into a buffer at pH 7.4 after dissolution [19]. After this, the solution was diluted appropriately before being tested by spectrophotometry at 239 nm against a pH 7.4 buffer to determine drug concentration.

$$\%EE = 1 - \frac{Concentration\ of\ free\ drug\ in\ the\ supernatent}{Concentration\ of\ total\ intial\ drug}$$

Particle size determination

Using a Zetasizer and photon correlation spectroscopy, the dispersions' average particle size and polydispersity index were calculated (DTS Ver. 4.10, Malvern Instruments, UK).

Buoyancy

Microsphere formulation (100 mg) was sprinkled over 100 ml of 0.1 N HCL containing 0.02% v/v Tween 80 in 200 ml glass beaker [20]. The ingredients were mixed together and left alone for a full day and night. Microspheres in suspension were decanted to separate them. Filtration was used once more to distinguish floating from sinking particles. The weight of the particles of both types was kept constant by drying them in a desiccator.

$$\%\ Buoyancy = \frac{wf}{wf+ws} X100$$

Where ws = weight of sinking microspheres and wf =weight of floating microspheres.

Preparation of Lercanidipine-loaded microspheres based oral suspension

The solvent evaporation process was used to create Lercanidipine-loaded microspheres, which were then precipitated. The emulsifier and floating agents were HPMC and tween 80. The ethyl cellulose polymer was dissolved in the solvent chloroform. In organic solvents, ethyl cellulose dissolves primarily [21]. Ethyl cellulose was first made by dissolving it in chloroform and incorporating the resulting liquid into the water-based solution (PVA, HPMC dissolved in water). When the magnetic stirrer's heating element is activated, chloroform quickly evaporates. Therefore, because ethyl cellulose is insoluble in water, microspheres that contain drug-entrapped molecules

precipitate. Above the organic phase (dielectric constant: 78.30 at 25°C), water was used as a suitable continuous aqueous phase for dispersal.

Flow Properties of Microspheres

Angle of repose

The horizontally-oriented pile represents the sample's weight, which was transported from the scale to the funnel. The ruler is used to calculate the pile's height (h) and the base's radius (r) [22].

$\tan\theta = h/r$

$\theta = \tan^{-1}(h/r)$

Where, h = Height of the pile, r = Radius of the pile

Carr's Compressibility Index

This method for determining the flowability of powders is straightforward, quick, and generally accepted [23].

$$\text{Consolidation Index} = \frac{Tapped\ Density - fluff\ density}{Tapped\ density}$$

Density Determination Bulk Density

Microsphere bulk density was calculated by sizing up a sample with a known mass and calculating the resulting volume then transfer of microspheres to a 10 ml measurement cylinder [24].

$$\text{Bulk density} = \frac{mass\ of\ a\ sample}{volume\ of\ a\ sample}\ g/cc$$

True Density

The microspheres were loaded with drug, and their true density was calculated using the liquid displacement method using Benzene [25].

Solid State Characterization

Fourier Transform Infrared Spectroscopy (FTIR)

By applying pressure to a triturated sample, we were able to create a thin, clear pellet of pure drug, ethyl cellulose, HPMC, a physical mixing of the drug with polymer (1: 1), and an optimal formulation. Preparation and analysis of KBr discs of the compositions were performed in the 4000-400 cm^{-1} wavelength range [26].

Differential scanning calorimetry (DSC)

DSC Q600 was used for a differential scanning calorimetry examination of Lercanidipine-microspheres, ethyl cellulose, and HPMC drug and polymer (1: 1). (TA Instruments, New Castle, DE). Each sample was weighed, and then placed in a standard aluminium pan with a tight seal; an empty pan was used as a benchmark. Both pans were heated at a rate of 10 degrees per minute from

40 to 200 degrees Celsius. The environment of the sample cells was kept inert using nitrogen purge gas [27].

X-ray diffraction (XRD)

Pure drug, ethyl cellulose, a drug: HPMC (1:1) physical combination and Lercanidipine-microspheres were all used in the P-XRD analysis [28]. An X-ray diffractometer was used in the research (Malvern PANalytical BV, Netherlands) with a Cu anode material, samples were exposed to nickel-filtered CuK radiation at 40 kV and 40 mA, with steps of 0.026° as the angle range from 2° to 70°.

Scanning electron microscopy (SEM)

The Drug and its optimal microspheres formulation were analyzed SEM, or scanning electron microscopy (SEM; JSM-6390LV; JEOL; Tokyo, Japan). Double-sided carbon tape was used and a metal-coated samples were analyzed using a 30 kV accelerating voltage and a scanning electron microscope equipped with 30 kV acceleration voltage was used to analyze all samples [29].

Determination of Particle Size

The Zetasizer was utilized for the measurement of microparticle size and polydispersity index (PDI) 300HS (Malvern Instruments, UK) after Lercanidipine-loaded Microspheres were produced. Additionally, laser Doppler anemometry was used to determine their proper zeta potentials. Fresh double-distilled water was used to dilute each sample by a factor of 10 [30].

In-vitro Drug release studies

The US Pharmacopoeia XXIII Dissolution apparatus I was used for the in-vitro dissolution tests (Basket type). Lercanidipine HCl loaded microspheres weighing 10 mg were suspended hydrochloric acid buffer (pH 1.2) volume (900 ml) heated to 37.0 ±0.5 ºC and swirled at 50 rpm. Five milliliters (ml) of aliquots of the material were taken out the volume was supplied at regular intervals using a straightforward dissolving liquid maintained at 37 ºC. The collected samples were sterilized, filtered, and the drug concentration was determined with a UV-Visible spectrophotometer set at 239 max with a hydrochloric acid buffer at pH 1.2 serving as a blank [31]. The in vitro release data of microspheres were analyzed with PCP Disso software using five kinetic models to determine the equation with the best fit, including Higuchi matrix, Peppas Korsmeyer, Hixson-Crowell, zero order, and first-order release equations [31].

In vivo floating behavior

The toxicity study of Lercanidipine Microspheres in rabbits before injecting the floated microspheres was conducted as an acute toxicity study as per OECD guideline 425. Rabbits (2–2.5 kg) were treated with floated microspheres (optimized formulation) and observed through X-ray images with modification. Animals were housed separately, fasted overnight maintaining all standard conditions, and deprived of food for the experimental duration to standardize the conditions of GI motility. Animals were kept for 1 week in the animal house to acclimatize them and fed a fixed standard diet. A total of six, healthy, rabbits were used to monitor the in vivo transit behavior of the floating microspheres. Photographs were taken to ensure the absence of opaquant in the stomach (t = 0 h). Barium sulfate (500 mg) was added to the optimized Lercanidipine Microspheres and orally administered to the rabbits with a sufficient amount of water, and for

radiographic imaging, their legs were properly tied over a piece of plywood (10x10 inch), and the source of the X-ray machine and the rat were kept uniform throughout the procedure, and finally, images of the gastric region were captured at 0, 5, and 10 h to observe the floatability of microspheres [32]. After the imaging, animals were free to carry out normal activities, but not allowed to take food for 6 h, including the experimental time. This experimental design was conducted at the Osmania University, School of Pharmacy, Hyderabad, and approved by the Institutional Animal Ethics Committee (Protocol No: SIP/IAEC/2019/06)

Pharmacokinetics studies

Twelve male SD rats (220 10 g) were separated into two groups of six after fasting for 24 hours with free access to water. These rats were given an oral dose of 25 mg/kg of raw Lercanidipine suspension in the form of microspheres that floated in the liquid. At 0, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours, 0.5 ml of blood was drawn from the fossa orbitalis vein. Blood samples were collected in microcentrifuge tubes pretreated with heparin (Sigma 3K30 centrifuge; Sigma Laborzentrifugen GmbH, Postfach, Germany). As a result, 200 l of plasma was collected and frozen at 20 C for later study. N-hexane: dichloromethane (2:1, v/v) was used twice to extract the drug, which was then dried in a water bath at 37 degrees Celsius with nitrogen gas before being dissolved in methanol. Lercanidipine levels using HPLC to analyze the results using a UV detector set at 239 nm. The mobile phase contained methanol and water (50:50, v/v) at a flow rate of 1 ml/min [33].

The experimental data were used to directly obtain the maximum plasma concentration (Cmax), and the amount of time that would be required to Cmax (Tmax). Using linear regression, the terminal semi-log plasma concentration vs time plot was used to determine the elimination rate constant (Ke). A value of 0.693/Ke was found to be the value of the half-life (T1/2). The latest detectable plasma concentration (Ct) was used to derive the AUC0- as AUC0-t + Ct/Ke. The t-test was used to see if statistically significant variations existed between the parameters, and differences were judged to be significant when the resulting P value was less than 0.05[34].

In-vivo **pharmacodynamic study**

A pharmacodynamic study was done by employing a non-invasive blood pressure arrangement (NIBP 200 A; Biopac System, Inc., Goleta,) depending on the cuff tail method. Twenty-four male Wistar rats were distributed equally into four groups (1–4). Group 1 served as normal control. Hypertension was made in the other groups (2, 3, and 4) by administration of methylprednisolone acetate for 2 weeks subcutaneously. Group 2 acted as hypertensive control. Groups 3 &4 received oral capsules (10 mg NCP) (Cardene SR® 20 mg) that were mashed and administered with the aid of oral gavage syringe [40]. Group 4 received optimal NCP microspheres suspension (10 mg NCP). The blood pressure (BP) from the tail was then taken at planned time points. Statistical analysis was done by one-way ANOVA using SPSS® software 22.0. Post-hoc test was performed using Tukey's HSD test. Percent of decrease in BP from hypertensive control was determined.

Stability studies

Lercanidipine-optimized formulations were used for storing the microspheres at 5±2°C, and 25± 2°C for three months, as required by ICH's study duration and storage condition guidelines. The drug concentration in the samples was then characterized [35].

Results & Discussion

Evaluation of Preliminary trial batches

The yield of microspheres was significantly impacted by temperature. The yield dropped from 75.64 to 46.21% w/w when the temperature rose from 30^0C to 45^0C, as indicated in Table 1. Due to the solvent system's fast evaporation at 45^0C, the emulsion was unable to stabilize, yielding non-spherical microspheres. There is a decline in yield at 60 ^{0}C. At 30^0C, 82.34% w/w encapsulation efficiency was reported; at 45^0C and 60^0C, it fell to 67.34 and 52.51%, respectively. This is due to the fact that when the temperature rose, the solvent evaporated more quickly, allowing for insufficient mixing time and the formation of big placebo microspheres. Sphericity, stirring duration, and encapsulation effectiveness were taken into account when selecting 30^0C as the temperature for more research.

Increase in HPMC from 50 to 150 mg, drug release increased from 68.92% to 88.37%. Decreases in microsphere size and floating capacity are also caused by an increase in HPMC concentration. For additional research, a fixed HPMC concentration of 50–150mg was chosen.
Agglomeration of the polymer caused by higher EC concentration led to lower % encapsulation effectiveness. Agglomeration caused the yield to be lower at high concentrations, only 61.25%. Microsphere diameter rose from 135.46 m to 325.67 m as polymer concentration increased from 750 to 1250. Sphericity was seen to decrease when EC concentration increased as a result of the polymer's poor coating. For additional research, EC concentration was selected as an independent variable (X1) for optimization at three levels: 750, 1000, and 1250.
With an increase in stirring speed, more microspheres were produced. As a result, the percentage yield increased from 78.92 to 87.36%w/w after increasing the stirring speed from 800 to 1600 rpm, as indicated in Table. Low stirring speeds caused the polymer to clump around the propeller shaft, lowering the microsphere yield. This was shown when the stirring speed was increased to 1600, the microsphere size was discovered to have fallen from 357.64 m to 140.38 m, and the size distribution was discovered to be uneven. This is due to the fact that increased turbulence and stronger shear pressures resulted in smaller emulsion droplets. With an increase in stirring speed, encapsulation efficiency went from 75.64 to 88.16% w/w. Speed was chosen to be the factor of study (X2) for optimization at three levels, i.e., 900, 1200, and 1500 rpm.

Table 1: Trial batches of Lercanidipine

F	EC	HPMC	Temp	Stirring	Sphericity	Size (µm)	EE (%)	Yield (%)
1	750	50	30	800	Spherical	135.64	82.35	76.34
2	850	100	40	1200	Spherical	150.34	76.39	68.34
3	950	150	60	1600	Spherical	160.59	70.35	73.34
4	1050	50	30	800	Less Spherical	250.37	76.34	59.37

5	1150	100	40	1200	Less Spherical	351.05	55.39	63.82
6	1250	150	60	1600	Less Spherical	364.52	52.19	49.35

*All values are expressed as Mean ±SD, n = 3.

Experimental design

The three independent variables were total ethyl cellulose, HPMC, and stirring speed. The second-order polynomial equation for Lercanidipine-loaded microspheres' vesicle size, percent EE, and floating efficiency. Model summary statistics, fit summary, and ANOVA were used to evaluate the significance and degree of interactions between independent and dependent variables. Counter plots and 3D response surfaces plots were generated using the regression model to examine the interactions between the independent variables.

Pre-optimization studies

The concentration of HPMC and the solvent ratio (ethanol: dichloromethane ratio) were tuned in the pre-optimization investigations. For the creation of floating microspheres, EC was chosen as the polymer due to its capacity for adjusting the release rate, the ability to create a film, and maintaining stability at gastrointestinal (GI) pH without causing any harm.

By changing the solvent ratio, various floating microsphere formulations were created. The concentration of the polymer was set at 750–1250 and 50–150 mg. In contrast to formulation F8, which produced unlike the expected results of the formulations, which would have resulted in spherical hollow microspheres with extremely strong floating ability and entrapment efficiency, the microspheres generated were irregular solid microspheres with no appreciable buoyancy.

By varying the amounts of EC and HPMC, 17 distinct formulations were created for the optimization of HPMC concentration tests. The Drug was added in a constant quantity of 10 mg. The amount of EC utilized was found to significantly affect the proportion of drug released at the 10-hour mark and the effectiveness of drug entrapment. For formulations F9 to F11, it was discovered that entrapment efficiency increased from 69.42±2.8 to 78.35±2.1% in proportion to the rise in EC concentration. This is because the higher the polymer content, the more likely it is that the drug will be polymer-bound, which prevents drug leakage to the surrounding environment and boosts entrapment efficiency. Drug release was observed to be noticeably slowed at higher concentrations of EC because ethyl cellulose is a release rate retardant when used in large doses. The percentages of drug release fluctuated between 56.06 and 41.12 percent. Therefore, HPMC was chosen to be administered in conjunction with EC in order to improve the Drug release rate. Combining EC and HPMC resulted in formulations F11 to F17. Drug release rose (from 62.14 to 77.73%) when HPMC concentration increased, but at the same time, the effectiveness of drug entrapment decreased, falling from 71.54 percent to 60.71 percent. A higher concentration of EC was associated with a higher percentage of floating ability, while a higher concentration of HPMC was associated with a lower percentage of floating capacity (for preparations with the same EC concentration). Formulation F8 showed adequate drug release and drug entrapment efficiency due to the amounts of EC and HPMC. Consequently, the formulation is essential for optimization studies F8 with entrapment efficiency (89.16±6.4%), floating ability (94.58±0.3%), and vesicle size (135.6±2.6 nm) was chosen.

Effect of Independent variables on Vesicle size

According to the table, the particle sizes of lercanidipine formulations ranged from 135.6±2.6 to 384.2±3.8 nm. When the acquired data were subjected to polynomial analysis and fitted with a quadratic model, the significance of the model was determined by the value of $p<0.0001$. The following polynomial equation illustrates the relationship between independent formulation factors and particle size:

Vesicle Size = 213.72 + 38.16A -11.21B – 16.43C – 17.80AB +27.48AC – 19.53 BC + 6.81A2 + 85.92B2 – 0.6100C2

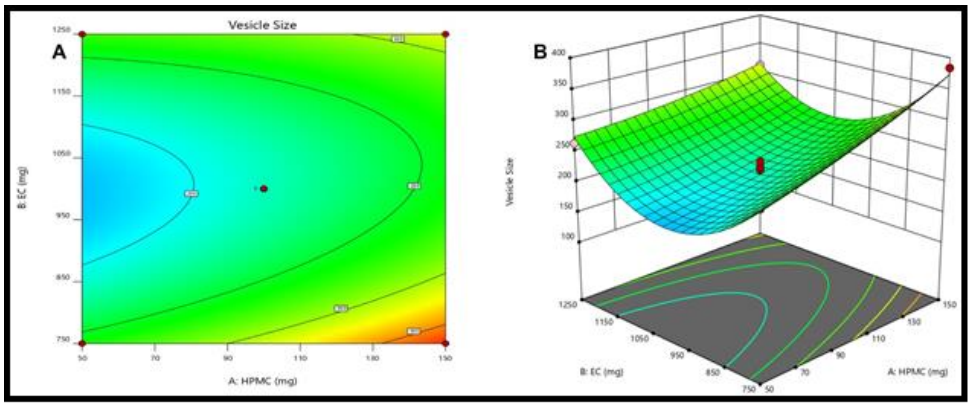


Figure1: Effect of independent variables X1, X2, on particle size (Y1).

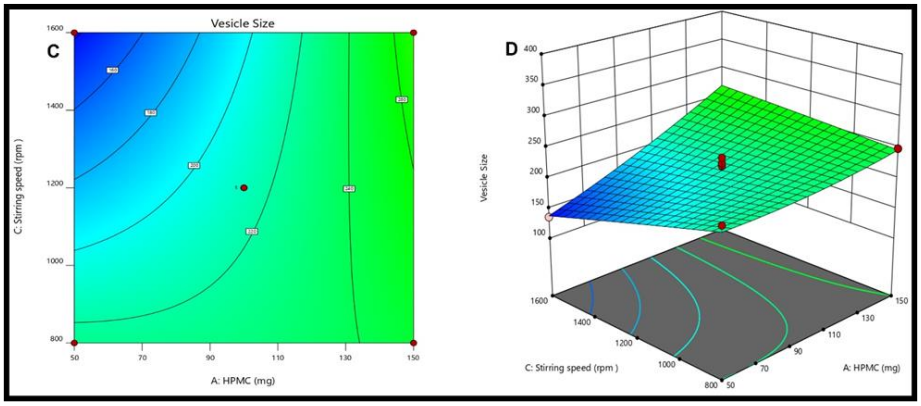


Figure 2: Effect of independent variables X1, X3, on particle size (Y1).

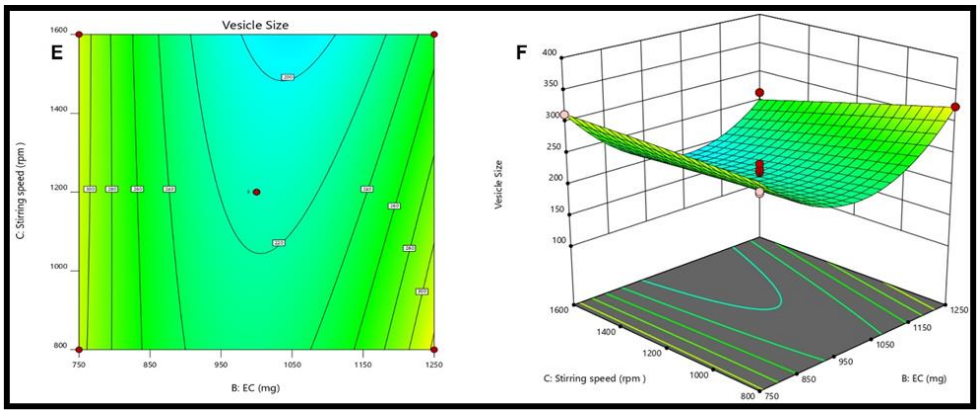


Figure 3: Effect of independent variables X2, X3, on particle size (Y1)

Effect of independent variables on Entrapment Efficiency

Drug encapsulation differed because the formulations were different. For example, we found the encapsulation effectiveness of formulation (F2) to be lowest with the formulation composition X1 concentration (150mg), X2 concentration (750mg), and X3 (1200 rpm). Using the formulation (F8), we achieved the smallest size with the highest encapsulation efficiency, the lowest EC concentration (50mg), and the longest stirring speed (1600 rpm). The encapsulation efficiency improved with increasing EC (A). Because the organic phase's increased viscosity reduces the two processes' partitioning, increasing the X1 concentration decreases entrapment efficiency. Reduces the amount of medicine released into the water by at least 80%. The encapsulation efficiency of the second liquid lipid (B) variable showed similar dual behavior. The entrapment efficiency improves when the X2 concentration is raised from 750 to 1000 mg. As the X2 concentration is more than 1000 mg, the encapsulation efficiency decreases. The hydrophobicity of the drug molecules may partly cause the reduced encapsulation efficiency in the particles. When the concentration of X1 is raised externally, lercanidipine may diffuse from the particles and form micelles in the aqueous phase. Entrapment efficiency improves with increasing stirring speed (C) since drug leaks are possible due to a high-energy supply.

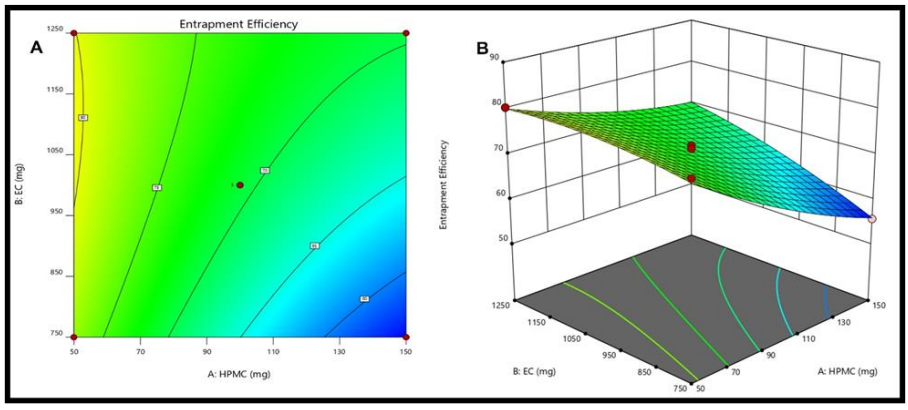


Figure 4: Effect of independent variables X1, X2, on Entrapment Efficiency (Y2).

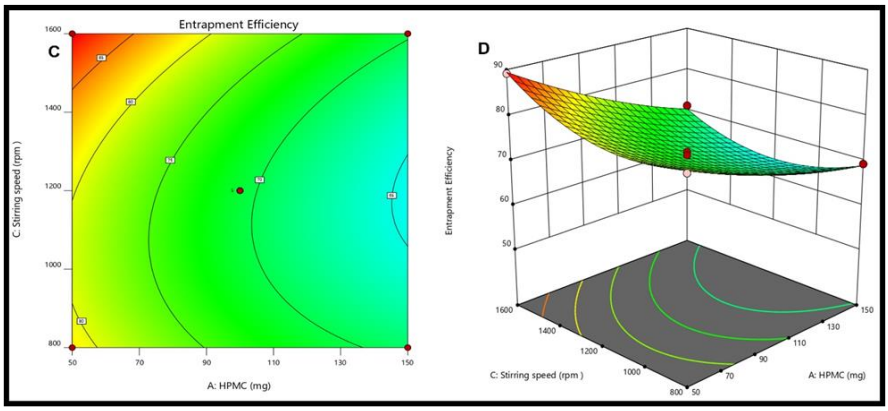


Figure 5: Effect of independent variables X1, X3, on Entrapment Efficiency (Y2)

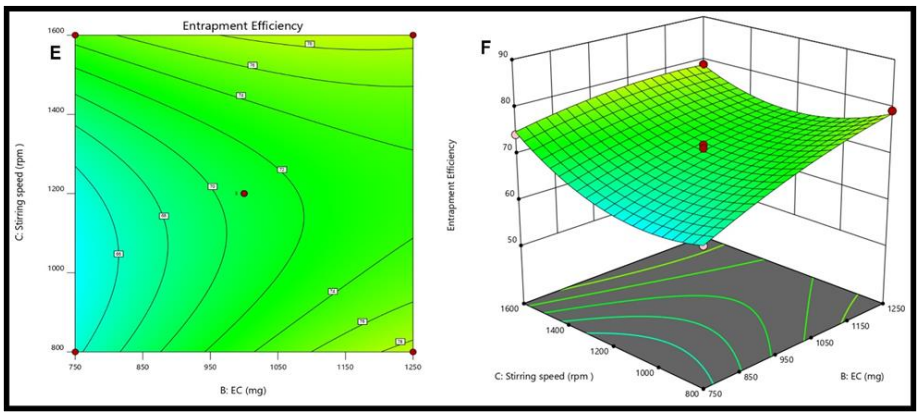


Figure 6: Effect of independent variables X2, X3, on Entrapment Efficiency (Y2)

Effect of independent variables on Floating Capacity

When compared to other formulations, formulation F8 had a higher percentage floating ability. Despite having the best floating ability, the formulation F7 particle size was excessive, making it inappropriate.

Floating Capacity = +72.72 -3.34A +2.18B+0.5300C -2.09AB -3.26AC -0.2875BC -2.92A2 -4.56B2 +18.91C2.

The yield of the formulations F8, F12, and F13 (stirring speed 1600 rpm) was low even though they produced smaller microspheres than the other formulations because of the production of microsphere coagulant masses from the rapid stirring. And so, the microspheres' ability to float likewise declined.

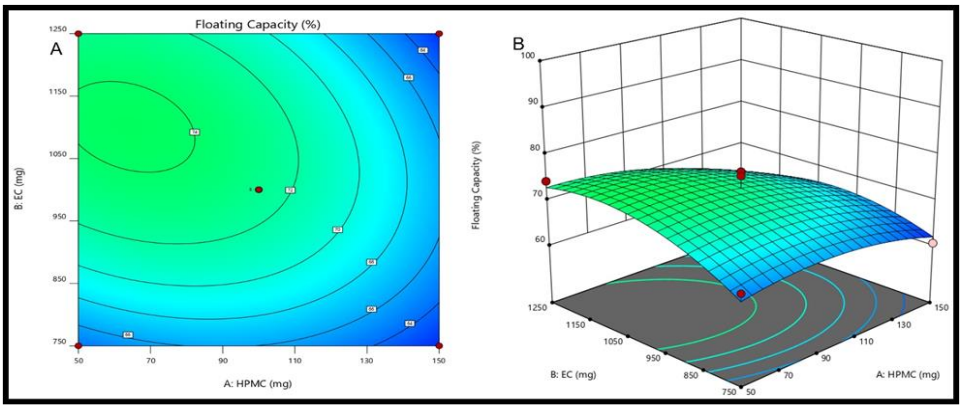


Figure 7: Effect of independent variables X1, X2, on Floating Capacity (Y3)

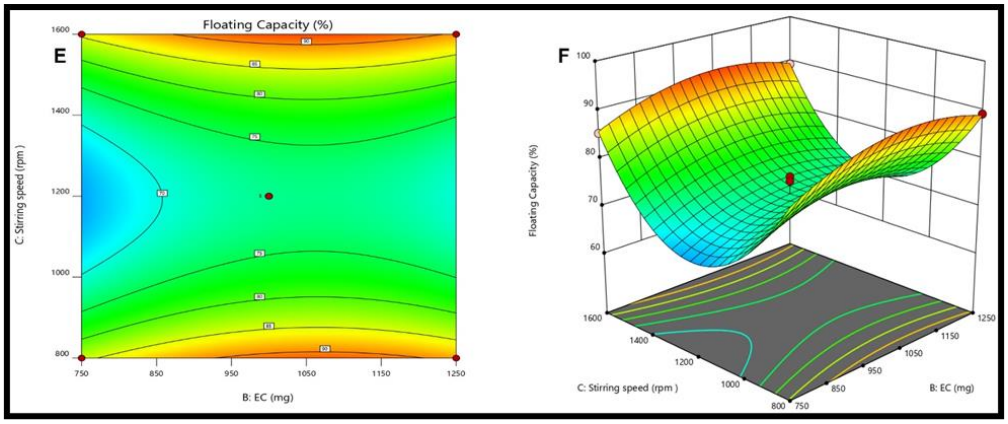


Figure 8: Effect of independent variables X1, X3, on Floating Capacity (Y3)

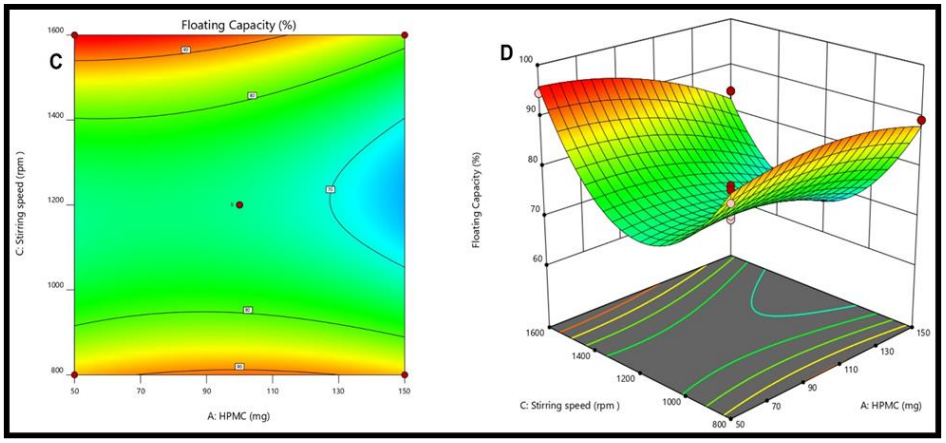


Figure 9: Effect of independent variables X2, X3, on Floating Capacity (Y3)

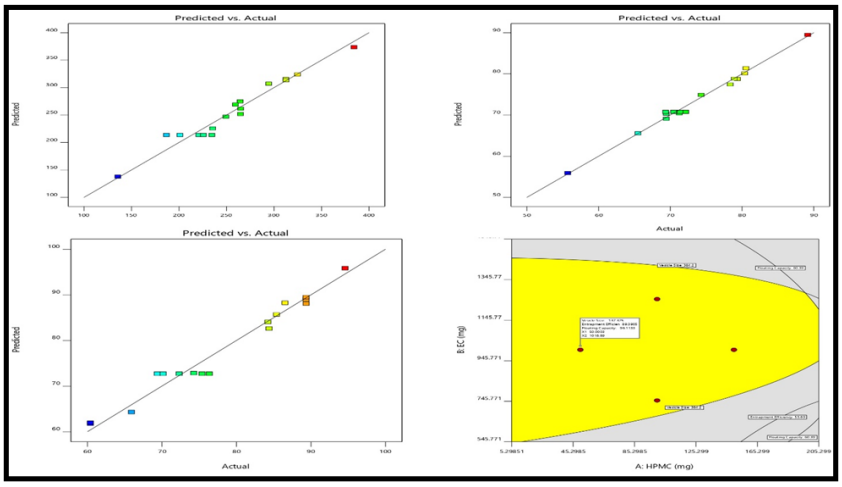


Figure 10: Actual vs. predicted values of Y1, Y2, Y3 and overly plot of independent variable effected on dependent variables

By using the Design Expert software's® numerical point prediction optimization method, the optimal formulation of the Lercanidipine-loaded microspheres system was selected based on the criteria of optimizing for the highest value of entrapment efficiency, the highest floating capacity, and the smallest particle size. The optimal formulation for lercanidipine-loaded microspheres was determined to be composed of EC (50 mg), HPMC (1000 mg), and stirring speed (1600 rpm).

Micromeritic Properties

With the exception of formulation F13, all formulations demonstrated outstanding flowability as measured in terms of angle of repose (400), most likely because of the formulation's larger amount of floating material. The improved flow characteristic shows that the created floating microspheres are not aggregated (Table 2).

Table 2: Physico-chemical evaluation of microspheres

F. Code	Bulk density (g/cm3)	Tapped density (g/cm3)	compressibility index (%)	angle of repose	Hausners ratio	Porosity (%)
F1	2.35±0.21	0.87±0.01	35.4±1.5	26.5±2	1.26±0.1	86.5±6
F2	1.79±0.26	0.61±0.05	29.8±0.6	23.4±3	1.35±0.5	88.2±1
F3	1.64±0.16	0.89±0.09	24.6±2.5	28.7±1	1.16±0.3	73.5±2
F4	1.92±0.14	0.91±0.06	31.5±2.4	31.4±5	1.43±0.2	68.4±3
F5	1.69±0.09	0.95±0.04	29.8±1.4	35.2±2	1.38±0.4	66.2±8
F6	2.15±0.24	0.83±0.03	26.4±2.1	29.7±1	1.29±0.6	69.5±5
F7	2.34±0.13	0.68±0.05	28.3±1.6	22.5±3	1.37±0.2	75.3±4
F8	1.62±0.27	0.53±0.01	29.5±0.9	31.6±2	1.52±0.1	79.1±6
F9	1.68±0.12	0.72±0.09	35.4±2.4	29.4±4	1.64±0.2	81.4±1
F10	1.79±0.05	0.69±0.07	36.1±1.9	26.5±2	1.35±0.5	88.3±2

F11	1.83±0.64	0.54±0.01	38.7±1.3	33.8±3	1.39±0.4	76.3±3
F12	2.05±0.93	0.69±0.05	26.4±2.1	35.4±2	1.27±0.6	69.4±5
F13	2.26±0.05	0.82±0.04	28.1±1.7	26.9±4	1.05±0.5	71.5±6
F14	2.19±0.17	0.64±0.06	29.5±0.5	31.7±5	1.64±0.1	78.3±2
F15	1.83±0.32	0.86±0.02	30.6±2.5	33.5±2	1.35±0.6	92.5±5
F16	1.94±0.28	0.75±0.09	33.2±1.6	36.4±3	1.07±0.3	81.4±8
F17	2.06±0.09	0.94±0.04	38.5±2.9	39.1±5	1.09±0.4	79.2±4

The findings of the compressibility index for all microsphere formulations range from 20.5±0.4 to 36.4±2.3, demonstrating the effective microspheres for fluid transport. All formulations' Hausner's ratios were smaller than 1.53±0.4, which indicates that they have free-flowing properties. The angle of repose's effects further supports the microspheres' free-flowing characteristic. The angle of repose's effects of the gelatin-based microsphere preparations is above 20, showing that the microspheres have good flow characteristics. Tween 80's high HLB value may be the cause of the microspheres' exceptional flow characteristics. Adding to their flow characteristics, the microspheres made with tween 80 were smoother and more spherical, which reduced the cohesion and adhesion between the microspheres. Because of the big microspheres that were created, microspheres generated with a lower drug-to-polymer ratio displayed a higher angle of repose.

Drug Excipient Compatibility Study by FTIR

FTIR scan of Lercanidipine and optimized formulation of Microspheres (F8) was taken. The peaks corresponding to the characteristic bands of the drug were found to be preserved in the spectra of the optimized drug matrix tablet (F8), there were no such peaks present in the scan of polymers, HPMC, and sodium alginate. This shows that the Drug and polymer are not interacting chemically has been taken place during the preparation of the formulations.

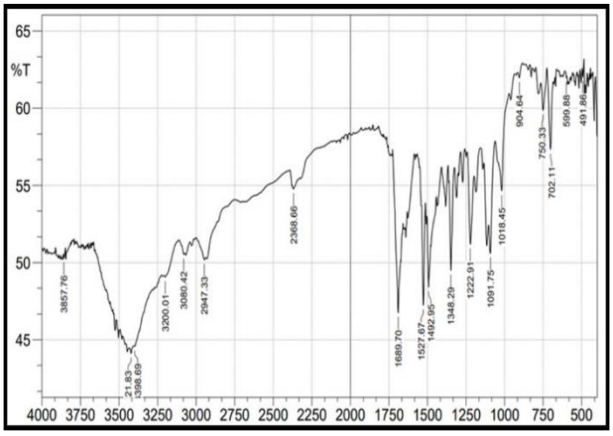


Figure 11: FTIR of Pure Lercanidipine

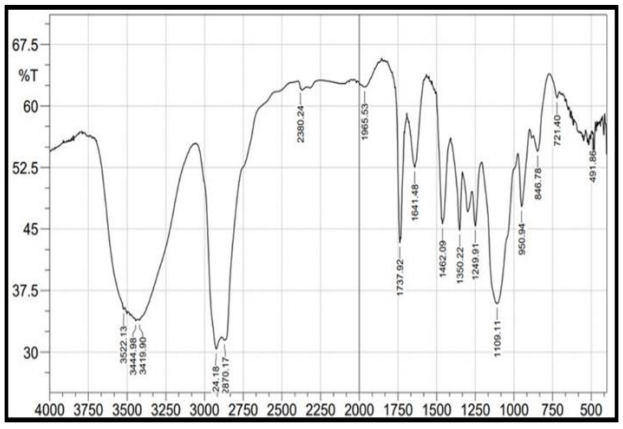


Figure 12: FTIR spectral studies of F8

The IR peaks observed in the scan of pure drug were: 3537.48 (O-H stretch); 3416.55 (N-H stretch, amide); 3082.21, 3060.87, 3026.62 (C-H stretch); 2924.24, 2869.21, 2850.93 (C-H stretch); 1649.83 (C=O stretch, amide merged); 1622.60 (N-H bend); 1544.75 (C=C stretch, aromatic). The same peaks, with slight changes in FTIR intensity, were found to be present in F8. As Identifiable peaks did not move or change the shape of Lercanidipine in F8, it indicated no significant drug-polymer interaction. Hence, Lercanidipine is compatible with the polymer used in the formulation of its floating matrix microspheres.

Thermal analysis

With a T beginning at 175.07 °C and a T peak at 179.80 °C and an associated fusion enthalpy of 26.51 J g1, the pure lercanidipine's thermal profile was initially flat, but then a sharp endothermic spike was seen, indicating that it is in the anhydrous crystalline state. However, no distinctive drug peak was seen in the DSC curves of the drug-loaded microspheres, indicating that the drug has been molecularly disseminated in the matrix of the polymer. At 46.28-79.76°C, the ethyl cellulose peak is attained. As a result, there was no discernible difference in where the drug's peak was located in the microspheres. However, because there are fewer drugs in the microspheres, there is a change in the relative intensities of the drug peak. This suggests that the drug is physically entrapped in the polymer matrix without any interaction with the polymers. It proved that even after the solvent evaporated during the microencapsulation procedure, the drug remained suspended in the ethyl cellulose matrix.

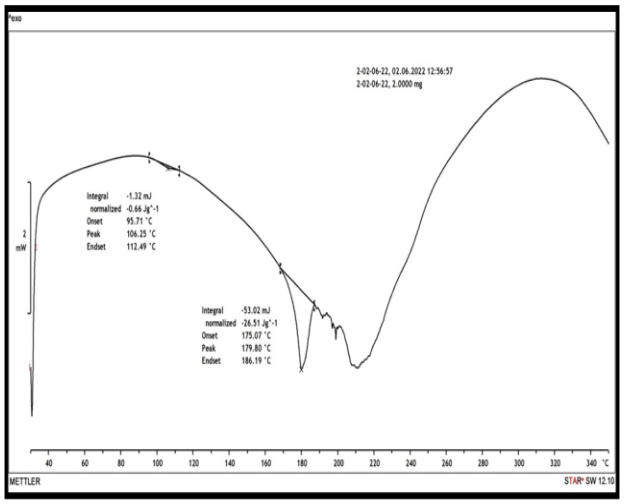


Figure 13: DSC thermogram of Pure Drug

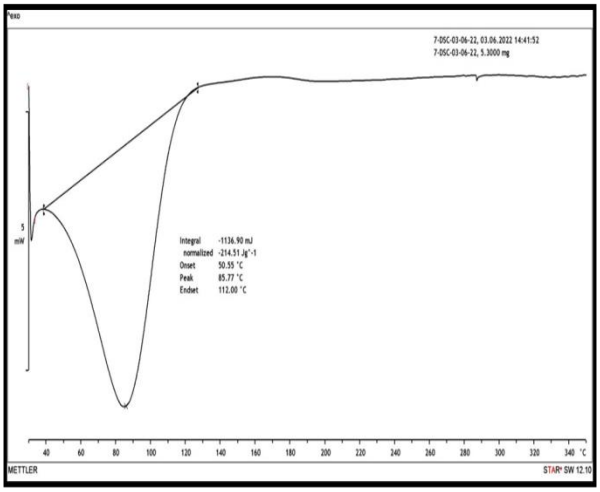
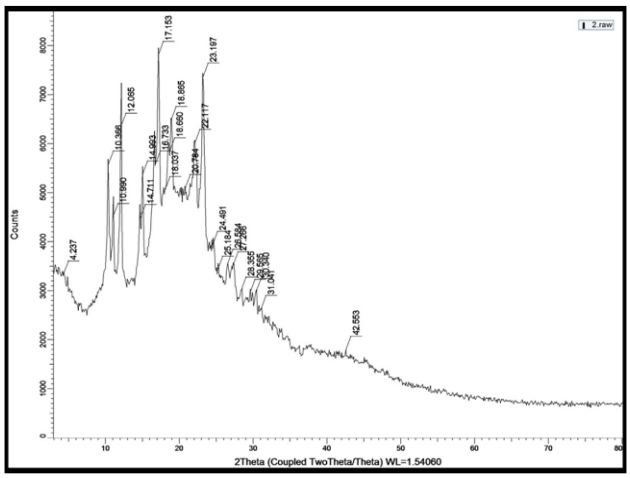


Figure 14: DSC thermogram of F8

X-Ray Diffraction Study

X-ray diffraction pattern of lercanidipine and microspheres did not contain any peaks associated with crystalline nature of drug, suggesting that drug might have changed into amorphous state during microencapsulation process due to solubilization in internal solvent and early re precipitation.



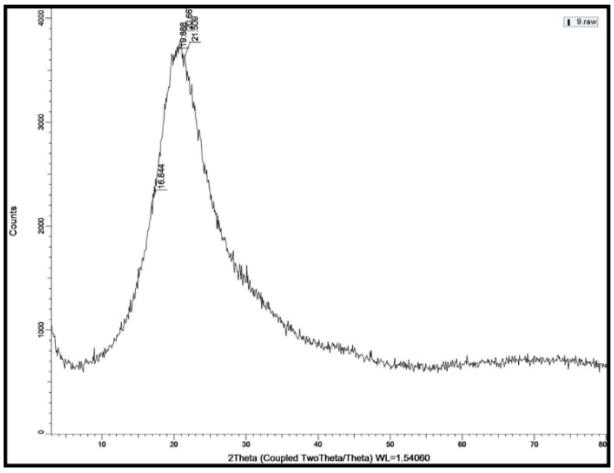


Figure15: Xray diffractogram of pure drug **Figure16: Xray diffractogram of F8**

Studies using X-ray diffraction are helpful in determining the drug's crystallinity. The spectra for the pure drug, the F8 formulation, and the physical mixture were taken (Figure 16). The presence of the drug's peaks at 2 of 12°, 17°, and 24° suggests that it is crystalline. However, formulation F8 does not contain these peaks, indicating that the Drug has been molecularly disseminated in the matrix of the polymer.

SEM studies

Different magnifications of optimized lercanidipine-loaded microspheres from the SEM. The pure substance had an erratic form. The formulation F8 had a smooth surface, was spherical with a range of sizes and was uniformly distributed throughout with no obvious porosity. Additionally, no drug crystals were seen on the formulation's surface. SEM photomicrographs showed a compact structure without interior porosity-containing cavities.

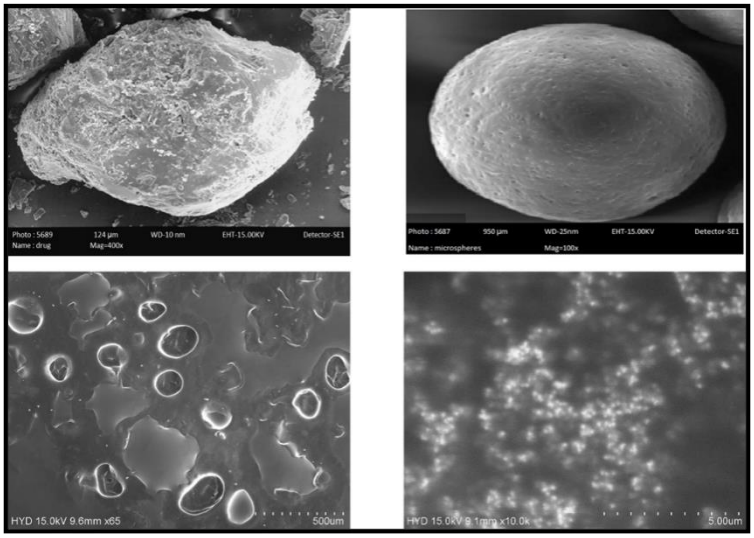


Figure 17: SEM images of pure drug and optimized microspheres

In-vitro Drug Release Study

The box Behnken design-based release investigation of all batches of lercanidipine floating microspheres is under usual conditions indicated by the Indian Pharmacopoeia, the release research was carried out in 500ml of 0.1N HCl (IP). High-performance liquid chromatography was used to evaluate samples of the dissolving fluid that were taken at regular intervals (HPLC).

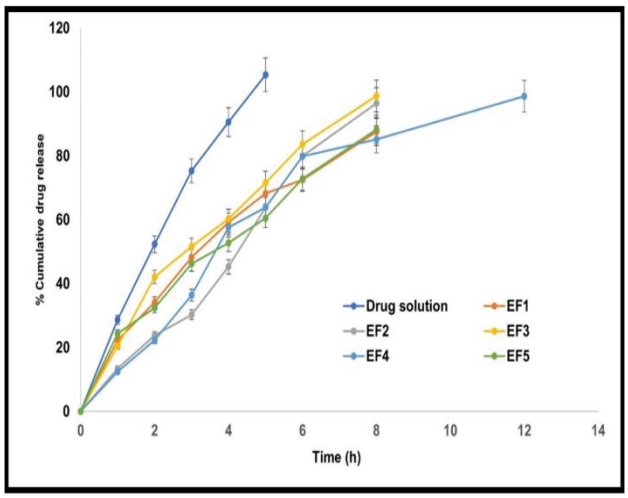


Figure 18: In vitro release studies of F1-F5

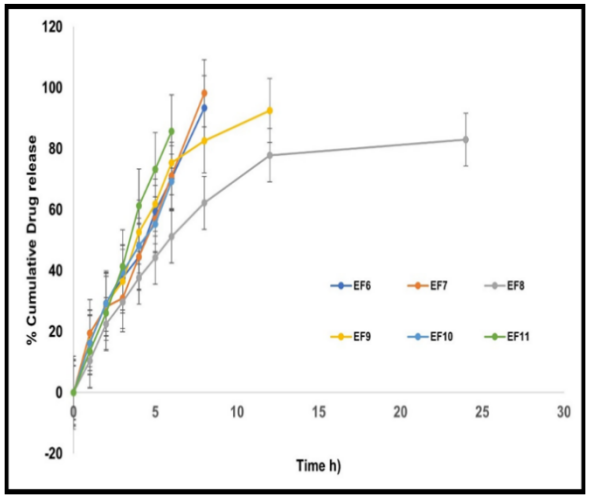


Figure 19: In vitro release studies of F6-F11

The sink conditions were maintained in the dissolution apparatus by replacing the withdrawn dissolution fluid with fresh 0.1N HCl.

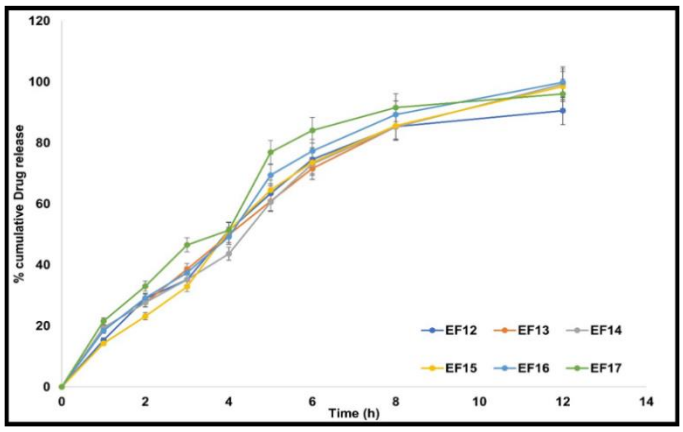


Figure 20: In vitro release studies of F12 to F17 formulation

In-vitro Drug Release Kinetics

The mechanisms of the total release of the Drug from the dose forms are described by model-dependent release kinetics. For a period of 24 hours, it was discovered that the release from optimized floating microspheres followed the Korsmeyer-Peppas model, with an R^2 value near 1. It followed a non-Fickian transport mechanism based on the value of "n." A non-Fickian transport

mechanism combines Drug release that is controlled by erosion and diffusion. This denotes that the dose form is appropriate for sustained release.

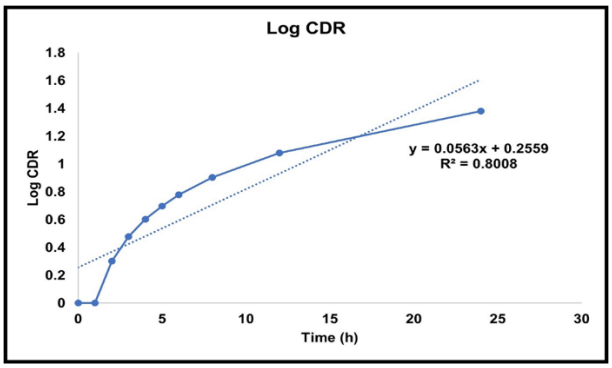


Figure 21: First order release kinetics

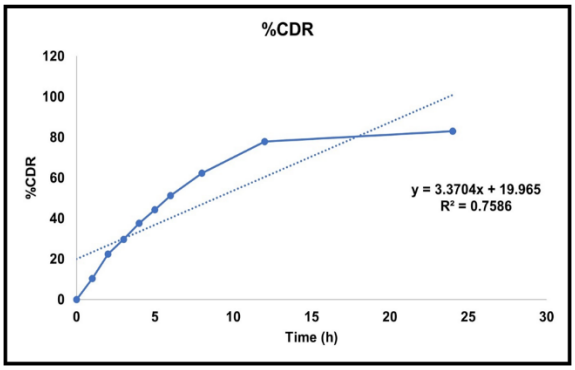


Figure 22: Zero release kinetics

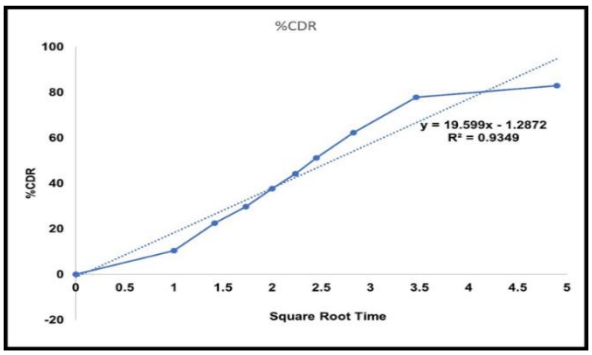


Figure 23: Higuchi model

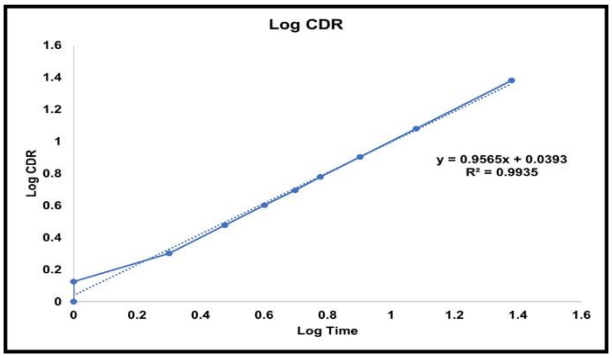


Figure 24: Korsmeyer model

In-vivo floating behavior

For increased residency in the stomach, the floating capacity of dried lercanidipine microspheres was evaluated in simulated gastric fluid. F8 was prepared with HPMC: EC (8:1); F8 exhibiting good in vitro buoyancy was further selected for study by the radiological technique. X-ray images taken at 2, 8, and 12 h for the buoyancy study indicate the gastro-retentive property of microspheres, emphasizing a significant gastric residence time for optimum release and absorption of the drug due to the porous nature of lercanidipine floating microspheres.

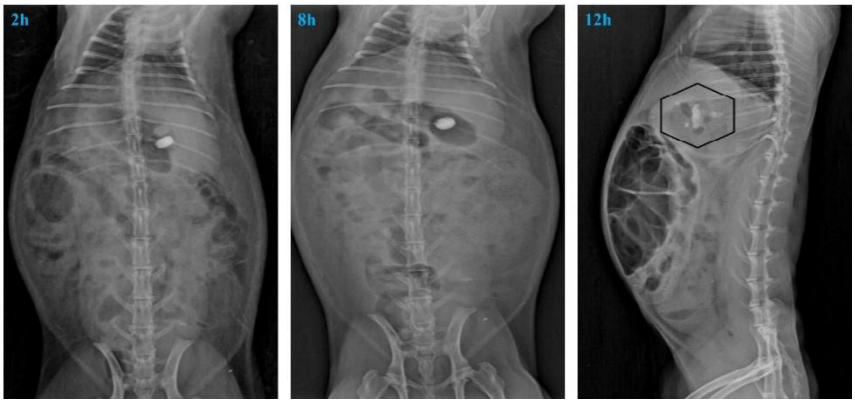


Figure 25: X-ray images of formulation F8 in the gastric region of a rabbits at 2, 8 & 12 hr

In-vivo **pharmacodynamic study**

When the ideal formula was applied to the GIT of hypertensive rats, blood pressure (BP) was measured to determine the antihypertensive effect of orally administered F8. Following treatment, at 1, 2, 4, or 6 hours, neither the oral tablet-treated group nor the microspheres-treated group significantly differed from the normal control group in terms of antihypertensive activity. At 8, 24, and 48 hours, oral Drugs failed to lower blood pressure to normal ranges or 36 hours after therapy, although they were able to keep it there for the first six hours after treatment. On the other hand, when taken orally, microspheres F8 decreased blood pressure to normal ranges, with the biggest percentage reduction of 35.64±5.00% occurring at the 24-hour mark. With better percent reductions in BP values of 15.64±6.00% and 7.38±4.00% compared to 1.00±0.00% and 3.00±1.00% for F8 and oral tablet treated group at 36 and 48 hours, respectively, there was a significant difference ($p > 0.05$) between the F8 treated group and the oral tablet treated group. This was true even though the BP values of the microspheres F8 treated group at 36 and 48 hrs were not at normal values, just like oral tablets. Additionally, a post-hoc analysis revealed that at 8, 24, and 36 hours after treatment, here, we see a statistically significant difference ($p<0.05$) between the oral tablet-treated group and the normal control group, but no such difference ($p>0.05$) between the normal control group and the F8-treated group. The results could be explained by the presence of floating components in the microspheres that successfully prevent mononuclear phagocytes from clearing lercanidipine from the body in vivo. According to these findings, the microspheres were more effective than oral tablets at maintaining the rats' blood pressure over time.

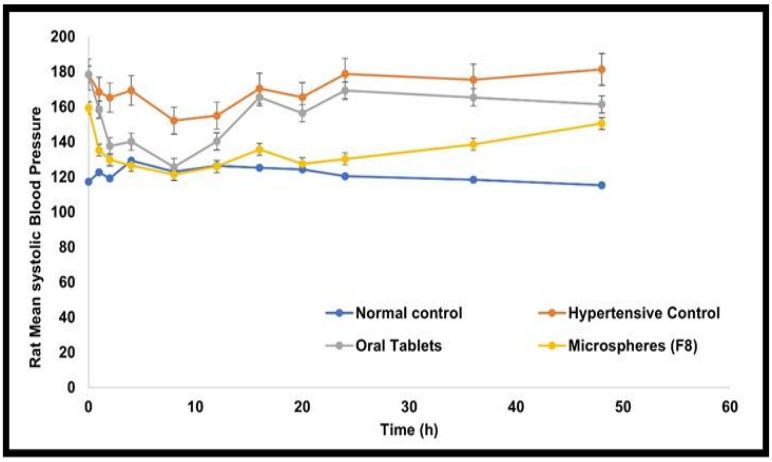


Figure 26: F8 and oral tablets' effects on the mean blood pressure of rats with hypertension brought on by methylprednisolone acetate.

In-vivo pharmacokinetic study

In order to evaluate the transdermal bioavailability of lercanidipine from microspheres F8, it is hypothesized that esterase enzymes exist, which cause NCP to be converted into the bloodstream. As a result, lercanidipine was employed as an indication to determine lercanidipine in-vivo pharmacokinetic behavior. Both Cmax and tmax were similar between microspheres F8 and the oral tablet ($p = 0.96$ and $p = 0.10$). There was a significant difference in AUC0-48 and AUC0- between microspheres F8 and oral tablet ($p<0.05$). The AUC0-48 and AUC0- values of the oral pill treatment group against the microspheres F8 treatment group were about 2.35 times higher, respectively. Additionally, compared to oral pill, the Tmax value of microspheres F8 was numerically higher although not noticeably so. The high relative bioavailability of F8 (235.04%) confirmed the initial hypothesis and demonstrated the superiority of microspheres F8 over oral tablets, which may be related to transdermal microspheres' ability to avoid the liver's first-pass metabolism, which in turn increases lercanidipine bioavailability. Additionally, based on the adjusted formula composition, the presence of floating materials may have increased bloodstream stability by limiting interactions with plasma opsonin’s, which may have lengthened blood circulation and increased systemic efficacy. Surfactant may also have made it easier for microspheres to reach the lymph nodes, which would have enhanced the vesicles' capacity to avoid the first-pass impact, as was already discussed. In a nutshell, the results showed that lercanidipine may be effectively delivered transdermally using microspheres F8 by avoiding oral side effects.

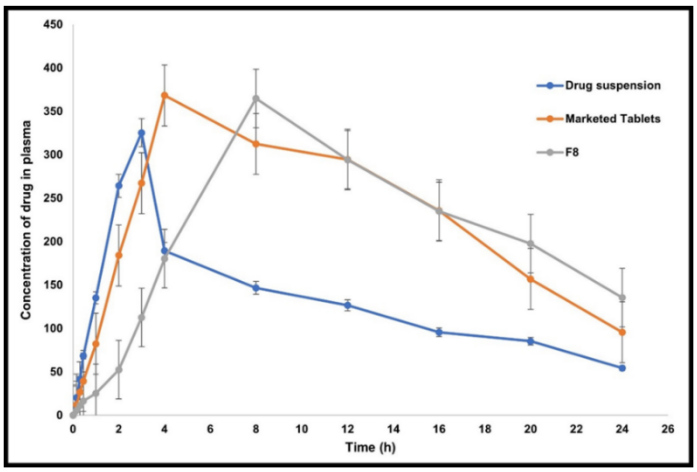


Figure 27: Comparison of Pharmacokinetic profiles of lercanidipine F8, marketed tablets, drug suspension. Information displayed as mean±SD (n=6)

Stability data of optimized formulation

According to ICH recommendations, stability tests on the improved microspheres (F8) were conducted at three distinct temperatures: 5 ±2°C and 25± 2°C The formulation was evaluated for size, Floating Capacity, entrapment effectiveness at 1, 3, and 6 months. Precipitation was visually inspected in the formulations. For three months, each drug's potency was measured once every 30 days. It was noted that the formulation's outward appearance had not changed. Analyses of the drug content revealed slight variations among the formulations stored at various temperatures. Throughout the investigation, the microsphere formulations exhibited good stability.

Table 3: Stability studies of optimized formulation of microspheres (F8)

Time	%EE		Size		Floating Capacity	
Months	5 ± 2°C	25°C ± 2°C	5 ± 2°C	25°C ± 2°C	5 ± 2°C	25°C ± 2°C
0	89.16±6.4	89.16±6.4	135.6±2.6	135.6±2.6	94.58±0.3	94.58±0.3
1	83.3±1.05	86.9±2.41	146.3±5.64	142.3±6.23	80.64±0.12	90.41±0.05
3	74.9±0.09	81.6±0.94	185.4±2.91	158.2±8.15	76.51±0.31	84.36±0.09
6	68.5±1.35	78.1±1.65	200.6±3.46	180.6±4.29	69.53±0.26	75.92±0.01

Conclusion

The purpose of this research was to design, formulate and optimize the floating microspheres of lercanidipine hydrochloride so as to prolong its gastric residence time and increase its bioavailability as it has less solubility. The solvent evaporation technique was used for preparing the floating microspheres and optimized using the Box-Behnken design. The prepared microspheres underwent the following in vitro evaluation tests, including those for micrometric properties, tapped density, particle size measurement, percentage yield, entrapment efficiency, in vitro buoyancy, and drug content. The formulation F8 was found to be the optimum formulation predicted by the point prediction of the design expert software.

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