RESEARCH ARTICLE

Design, Formulation, and Optimization of Nicardipine-Loaded Gastroretentive Floating Microspheres: *In-vitro, Ex-vivo* and *In-vivo* Characterization

Sepuri Vijayalaxmi[1*], Abbaraju Krishna Sailaja[2]

[1]*University College of Technology, Department of Pharmaceutics, Osmania University, Hyderabad, Telangana, India.*
[2]*Department of Pharmaceutics, RBVRR Women's College of Pharmacy, Osmania University, Hyderabad, India.*

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ABSTRACT

Nicardipine hydrochloride is highly soluble at low pH values. The objective of this investigation was to design, manufacture, and refine the drug's floating microspheres to increase its bioavailability and prolong its period of residence in the gastrointestinal tract. To optimize the Box-Behken and prepare the floating microspheres, solvent evaporation was employed. The formulation F5 was discovered to be the best as anticipated by the design expert software's point prediction. Evaluations of pre- and post-compressional parameters were conducted. Utilizing several kinetic models, the drug release pathways were examined. The drugs and excipients did not interact, according to the FTIR/DSC investigations. According to the radiological approach, F5 showed good *in-vitro* buoyancy. The gastroretentive property of microspheres is revealed by X-ray pictures obtained at 2, 8, and 12 hours for the buoyancy investigation. F5 (235.04%) demonstrated a high relative bioavailability over oral tablets, according to the *in-vivo* pharmacokinetic investigation. In comparison to the marketed formulation group, the F5-treated group experienced a considerably larger percent decline in BP values (20.00 ± 6.00 and 13.00 ± 4.00%, respectively) at 36 and 48 hours of the *in-vivo* pharmacodynamic testing. For 90 days, the F5 formulation was maintained at 5 ± 2 and 25 ± 2°C, indicating the stability of nicardipine microspheres.

Keywords: Nicardipine, Optimization, Floating Microspheres, Ethylcellulose, Experiment design.

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INTRODUCTION

To provide more effective therapeutic benefits and lower the frequency of adverse effects, Systems for the continuous delivery of drugs have been developed for multiple administration routes. By preventing the entry of extracellular calcium across cell membranes, the dihydropyridine calcium channel-blocking drug nicardipine HCl promotes peripheral and coronary vasodilation.[1,2] Cardiovascular disorders, angina pectoris, and hypertension can all be successfully treated with it. When the pH is low, it dissolves well; when the pH is high, it dissolves poorly. As a result, only the stomach and upper intestinal tract will likely absorb nicardipine hydrochloride. Much work has been done to convert NIC or its hydrochloride to a form that has improved solubility in intestinal juices because of its poor solubility in biological fluids with a pH of 5 to 8.[3,4]

Nicardipine is metabolized extensively in the liver during the first pass after oral administration, and its systemic bioavailability ranges from 20 to 33%. The drug must be administered frequently (30 mg, three times a day) due to its short half-life (2-4 hours). Additionally, nicardipine has a few side effects that may be brought on by its rapid absorption, including nausea, vomiting, flushing, headaches, dyspepsia, anorexia, and diarrhea [5]. Because floating dosage forms stay at the absorption site longer, their bioavailability is increased. Various methods have been documented to regulate the residence time, including superporous, biodegradable hydrogel systems, mucoadhesive, extensible, or expandable systems, high-density systems, and intragastric floating systems (FDDS).[6,7]

One strategy to attain the maximum degree of therapeutic efficacy is to use microspheres as drug carriers. Usually composed of protein and semi- or synthetic polymers, they are free-flowing, biodegradable particles. The stomach can hold onto floating microspheres in the gastric region for extended periods when low-density systems have adequate floating.[8,9] Substances such as polymers and polysaccharides start to

**Author for Correspondence: vijji.9965@gmail.com*

gel when buoyant microspheres are introduced into stomach contents. It can control the flow of liquid penetration in the dosage form and, therefore, the release of active ingredients from the device by hydrating to assume the shape of a gel-based barrier.[10] Using Ethylcellulose (EC) as a release modifier and floating aid, along with HPMC as a hydrophilic polymer, formulated and designed an SR formulation of nicardipine and designed, and the *in-vivo* and *in-vitro* performance (bioavailability) of the proposed formulations.

MATERIALS AND METHODS

Materials

Nicardipine HCl was acquired from Apotex Research Private Limited, located in Bangalore, India. Degussa Chemical Co. Ltd. supplied the EC from Shin-Etsu Chemical Ind. Co. Ltd. in Japan, hydroxypropyl methylcellulose was acquired. Purchased from reputable chemical manufacturers, all additional chemicals and reagents utilized are of analytical quality.

Methods

Preparing initial trial batches of nicardipine hcl-loaded floating microspheres

Nicardipine HCL microspheres were produced by solvent evaporation. Dissolve 20 mL of EC, HPMC, and their different combinations using a solvent mixture (DCM: Ethanol = 1:1). Once the required quantity of the drug was included, it was dissolved into a transparent solution by combining vortexing and sonication.[11,12] Using a stirrer, 50 mL of heavy liquid paraffin and 1-mL of Span 80 were emulsified to generate the external phase using a 500 mL beaker. The internal phase was gradually poured into the exterior phase while stirred by a mechanical stirrer at room temperature. For 3 hours, the system was agitated. The microspheres were filtered to separate them, and any remaining paraffin oil was removed by cleaning them three times with 50 mL of n-hexane. They were finally allowed to dry overnight at ambient temperature to yield a freely flowing, spherical product.

Box-behnken design for the optimisation of independent variables

17 trial runs were built using the Box-Behnken design. With a full factorial design, the variables were optimized. The present study included vesicle size (Y1), drug entrapment efficiency (Y2), yield (%Y3), EC (X1), HPMC (X2), stirring speed (X3), and vesicle size (Y1) as independent variables. To generate a polynomial equation that would better represent the effects of the independent variables on drug loading, particle size, and drug release, the non-significant interaction factors from the ANOVA were removed. The outcomes (Y) of each trial are quantified.

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

In this case, Y is the observed response, b0 is the overall trial average, and b1 is the computed coefficient for the relevant independent variable (X1).

The model was refined using an F-test and multiple regression analysis to identify statistically significant variables. Subsequently, a three-dimensional and contour plot was produced, and ultimately, the suspension underwent optimization. Every experiment was conducted at least three times.[13]

Evaluation

Drug encapsulation

The 100 mg of microspheres were weighed exactly. After the drug was dissolved in dichloromethane, it was evaporated into a pH 7.4 buffer. Following this, the solution was diluted before the drug concentration was measured using spectrophotometry at 236 nm.

Particle size determination

The polydispersity index and average particle size of the dispersions were determined using a Zetasizer and photon correlation spectroscopy (DTS Ver. 4.10, Malvern Instruments, UK).

Buoyancy

In a 200 mL glass beaker, 100 mL of 0.1 N HCl with 0.02% v/v Tween 80 was used, and 100 mg of the microsphere formulation. After the materials were combined, they were left alone for the entire day and night. To separate the suspended microspheres, a centrifuge was used. To separate floating from sinking particles, filtration was utilized once more. By drying them in a desiccator, the mass of the particles of both kinds was maintained constant.

Preparation of nicardipine-loaded microspheres-based oral suspension

Nicardipine-loaded microspheres were produced by solvent evaporation and precipitated thereafter. HPMC and Tween 80 served as emulsifiers and stabilizing agents. The solvent chloroform was used to dissolve the EC polymer. Typically, EC dissolves in organic solvents.[19,20] When EC was first produced, it was dissolved in chloroform and then added to a water-based solution (PVA, HPMC dissolved in water). When the heating element of the magnetic stirrer is turned on, chloroform evaporates quickly. Consequently, microspheres containing drug-entrapped molecules precipitate because EC is insoluble in water. A suitable continuous aqueous phase for dispersion was found to be water above the organic phase (dielectric constant: 78.30 at 25°C).

Flow Properties

Angle of repose

The sample's weight is represented by the horizontally oriented pile, which was moved from the scale to the funnel. h & r are determined with the ruler.[21]

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

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

Carr's compressibility index

This approach to determining a powder's flowability is simple, fast, and widely recognized.

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

Bulk density

By measuring the volume of a sample with a known mass, the bulk density of microspheres was computed. Next, a 10 mL measuring cylinder received the microspheres.

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

True density

Benzene was used in the liquid displacement method to determine the true density of the drug-loaded microspheres.

Solid State Characterization

Fourier transform infrared spectroscopy (FTIR)

A thin, transparent pellet of pure drug, EC, and HPMC was produced by applying pressure to a triturated sample. The drug and polymer were physically mixed (1:1), and an optimal formulation was produced. The 4000 to 400 cm^{-1} wavelength range was used to prepare and analyze the KBr discs for the compositions

Differential scanning calorimetry (DSC)

Using the DSC Q600 (TA Instruments, Delaware, New Castle), analysis was carried out on EC, HPMC, drug and polymer (1: 1), and Nicardipine microspheres. After each sample was weighed, it was put into a standard aluminum pan and sealed tightly; an empty pan served as a reference. About 10°C were added to each pan per minute, bringing them up to 200°C. Nitrogen purge gas was used to maintain the sample cells' surroundings inert.

X-ray diffraction (XRD)

For the P-XRD study, Nicardipine microspheres, EC, a drug:HPMC (1:1) physical blend, and a pure drug were all utilized. Samples were exposed to CuK radiation that had been nickel-filtered at 40 kV and 40 mA, with increments of 0.026° covering the angle range of 2 to 70° in the investigation. Cu anode material was used with an X-ray diffractometer (Malvern PANalytical BV, Netherlands).

Morphology of the particles

Scanning electron microscopy (SEM)

By SEM (JSM-6390LV; JEOL; Tokyo, Japan), the drug and its ideal microsphere formulation were examined with a 30 kV acceleration voltage, double-sided carbon tape, and a metal-coated sample analysis tool.

Determination of particle size

After the production of microspheres loaded with nicardipine was completed, the Zetasizer (Malvern Instruments, UK) was used to measure the size of microparticles and the polydispersity index (PDI) 300HS. They also employed laser Doppler anemometry to ascertain their accurate zeta potentials. Every sample was diluted by a factor of 10 using brand-new double-distilled water.

In-vitro drug release studies

The apparatus used for dissolving US Pharmacopoeia XXIII was employed in the Basket-type *in-vitro* dissolution tests. About 10 mg of nicardipine microspheres were suspended in nine hundred milliliters of pH 1.2 hydrochloric acid buffer, heated to 37.0 ± 0.5ºC, and stirred at fifty rotations per minute. The material was extracted into five ml aliquots, with the volume provided at regular intervals. After the samples were filtered, the drug content was determined using a UV-visible spectrophotometer with a 236 λ_{max}

In-vivo floating behavior

As per OECD guideline 425, an acute toxicity assessment was carried out on rabbits before injecting floating microspheres containing nicardipine microspheres. Modified X-ray pictures were used to monitor the floating microspheres (optimized formulation) treatment administered to 2- to 2.5-kg rabbits. To standardize the conditions of GI motility, the animals were housed separately, fasted for the entire night while adhering to all standard procedures, and were not fed during the experiment. To acclimatize the animals, they were housed in the animal home for one week and fed a set, conventional diet. 6 healthy rabbits were used to observe the floating microspheres' *in-vivo* transit performance. Images were captured to confirm that there was no opacifier in the stomach (t = 0 hours). The rabbits' legs were securely tied over a 10-by-10-inch piece of plywood for radiographic imaging, and the rat and the X-ray machine's source were kept constant during the process. Lastly, to monitor the microspheres' flowability, pictures of the stomach area were captured at 0, 5, and 10 hours. The optimized Nicardipine Microspheres were given to the rabbits orally along with adequate water, and 500 mg of barium sulfate was added. Following the imaging, the animals were released to resume their regular activities; however, they were not permitted to eat for six hours, during which time the experiment was conducted. The Institutional Animal Ethics Committee permitted this experimental design.

Pharmacokinetics studies

After a 24-hour fast with unrestricted access to water, 12 male SD rats (220–10 g) were divided into 2 groups of six. Nicardipine suspension in the form of floating microspheres was administered orally to these rats at a dose of 25 mg/kg. The fossa orbitalis vein was used to extract 0.5 ml of blood at 0, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours. Microcentrifuge tubes (Sigma 3K30 centrifuge; Postfach, Germany) were used to collect blood samples and were prepared with heparin. Thus, 200 µL of plasma was gathered and stored for subsequent analysis at 20 degrees Celsius. The drug was twice extracted using a 2:1 v/v N-hexane:dichloromethane mixture, dried in a water bath with nitrogen gas at 37°C, and then dissolved in

Table 1: Trail Batches Physicochemical evaluations

F. code	*% Yield*	*%EE*	*Particle size*	*Buoyancy*
F1	65.38 ± 1.05	45.26 ± 0.51	394.23 ± 11.35	69.35 ± 0.43
F2	69.34 ± 2.01	51.29 ± 1.24	382.15 ± 12.51	74.51 ± 0.36
F3	70.26 ± 1.38	59.68 ± 1.08	298.64 ± 10.26	69.42 ± 0.51
F4	73.29 ± 2.04	46.28 ± 1.06	276.45 ± 8.95	80.24 ± 0.06
F5	80.35 ± 1.39	64.52 ± 0.04	259.73 ± 12.37	79.35 ± 1.08
F6	86.51 ± 0.97	59.38 ± 1.08	243.51 ± 6.59	84.53 ± 1.02
F7	84.23 ± 2.31	78.35 ± 0.26	264.51 ± 8.23	79.28 ± 0.26
F8	94.36 ± 2.61	85.67 ± 1.05	136.45 ± 12.04	91.56 ± 0.35

methanol. Nicardipine levels are measured with a UV detector set to 236 nm and HPLC. In the mobile phase, methanol and water were combined 50:50, v/v, with a flow rate of 1 mL/min. Using the experimental data, the maximum plasma concentration (Cmax) and the time needed to attain Cmax (Tmax) were directly computed. Using the terminal semi-log plasma concentration vs. time plot and linear regression, the elimination rate constant (Ke) was determined. It was determined that the half-life ($t_{1/2}$) was 0.693/Ke. AUC0-t + Ct/Ke was the formula used to derive the latest detectable plasma concentration (Ct). A P value of less than 0.05 indicated that the differences were statistically significant. The t-test was used to ascertain whether there were any differences between the parameters.

RESULTS AND DISCUSSION

%Yield and %EE

Table 1 illustrates the production yield range for various microsphere trial batches, which were determined to be 65.38 ± 1.05 (F 1 batch) to 94.36 ± 2.61% (F 8 batch). However, no significant difference was seen between the F1 and F6 samples ($F < 0.05$). The microspheres containing nicardipine had a high entrapment efficacy (ranging from 85.67 ± 1.05 for batch F8 to 45.26 ± 0.51% for batch F1).

Particle size analysis

Table 1 shows that the particle sizes of the various experimental batches of microspheres loaded with nicardipine varied from 136.45 ± 12.04 m (batch F8) to 394.23 ± 11.35 m (batch F1). Particle size is positively correlated with the drug-to-polymer ratio.

Experimental design

The vesicle size, yield, and percent EE of microspheres loaded with nicardipine are expressed as a second-order polynomial equation. The format and significance of interactions between independent and dependent variables were assessed using ANOVA, model summary statistics, and fit summary. The regression model was used to create contour plots and 3D response surface plots to explore the associations between the independent variables (Table 2).

Effect of Formulation Factors on Vesicle Size

Vesicles containing Nicardipine microspheres varied in size, ranging from 125.4 ± 5.37 (F5) to 328.1 ± 9.25µm (F13). There was a substantial difference in vesicle size between the two groups. The formulation (F5), for instance, contained 400 EC, 150 HPMC, and a stirring speed of 600 rpm. For this formulation, the smallest size was noted. The formulation (F13), for instance, that has the highest concentrations of X1 (200), X2 (200), and X3 (450) has the largest size. It was demonstrated that the viscosity of the organic phase decreases as the EC (A) increases. There were two effects of the second variable, which was the increase in HPMC concentration (B). A 25% increase in B content initially causes a size decrease.

Table 2: Independent and dependent factors of microspheres

Std	*Run*	*X1*	*X2*	*X3*	*Y1*	*Y2*	*Y3*
2	1	400	100	450	326.5 ± 3.52	59.37 ± 0.69	65.39 ± 0.05
4	2	400	200	450	269.1 ± 1.39	64.23 ± 2.13	71.26 ± 0.31
13	3	300	150	450	237.8 ± 2.54	71.18 ± 1.03	74.26 ± 0.05
12	4	300	200	600	267.5 ± 8.62	76.35 ± 1.46	84.28 ± 0.42
8	5	400	150	600	125.4 ± 5.37	82.51 ± 2.05	94.68 ± 0.06
6	6	400	150	300	238.2 ± 4.23	73.28 ± 1.23	79.35 ± 0.27
9	7	300	100	300	254.8 ± 9.15	71.29 ± 2.06	82.37 ± 0.31
7	8	200	150	600	253.7 ± 3.28	73.08 ± 1.04	86.49 ± 0.52
10	9	300	200	300	167.9 ± 8.67	79.34 ± 3.28	90.53 ± 0.61
1	10	200	100	450	324.5 ± 3.51	53.94 ± 2.26	70.84 ± 0.07
15	11	300	150	450	237.8 ± 4.29	71.62 ± 1.04	71.29 ± 0.06
14	12	300	150	450	239.6 ± 6.18	72.59 ± 1.35	70.59 ± 0.34
3	13	200	200	450	328.1 ± 9.25	62.83 ± 2.08	71.48 ± 0.08
16	14	300	150	450	240.7 ± 1.27	73.28 ± 2.22	81.39 ± 0.01
11	15	300	100	600	159.8 ± 3.24	78.61 ± 3.24	89.37 ± 0.02
17	16	300	150	450	259.3 ± 2.59	72.38 ± 1.36	75.38 ± 0.03
5	17	200	150	300	152.4 ± 6.34	79.21 ± 3.21	89.37 ± .05

Lowering the interfacial tension among the organic and aqueous phases should be accomplished by using the optimal concentration. Particle size is also increased by stirring speed (C) due to the high energy supply. The results of the investigation might be further explained by the researchers using the polynomial equation as shown in Figure 1.

Vesicle Size = +243.04 -12.44A -4.12B -0.8625C -15.25AB -53.53AC +48.65BC +24.47A2 +44.54B2 -75.08C2

Effect of Formulation Factors on Entrapment Efficiency (EE)

The percentage of Nicardipine-microspheres that were found to have an EE (Y2) ranged from 53.94 ± 2.26 (F10) to 82.51 ± 2.05% (F5). As the amount of polymer increased, the encapsulation efficiency increased (A). More X1 concentration results in better entrapment efficiency because the higher viscosity of the organic phase reduces the partitioning between the two phases and minimizes drug release into the water by at least 80%. The second HPMC (B) variable's encapsulation efficiency exhibited a dual behavior that was comparable. An increase in X2 concentration from 100 to 150 results in an improvement in entrapment efficiency. The effectiveness of encapsulation declines as the concentration of X2 rises above 200. The lower particle encapsulation efficiency could be partially attributed to the drug molecules' hydrophobicity. As drug outflows are possible due to a high energy source, entrapment efficiency increases with increasing stirring speed (C). The influence of independent factors is also explained mathematically by the polynomial equation, which connects independent variables and encapsulation efficiency, as shown in Figure 2.

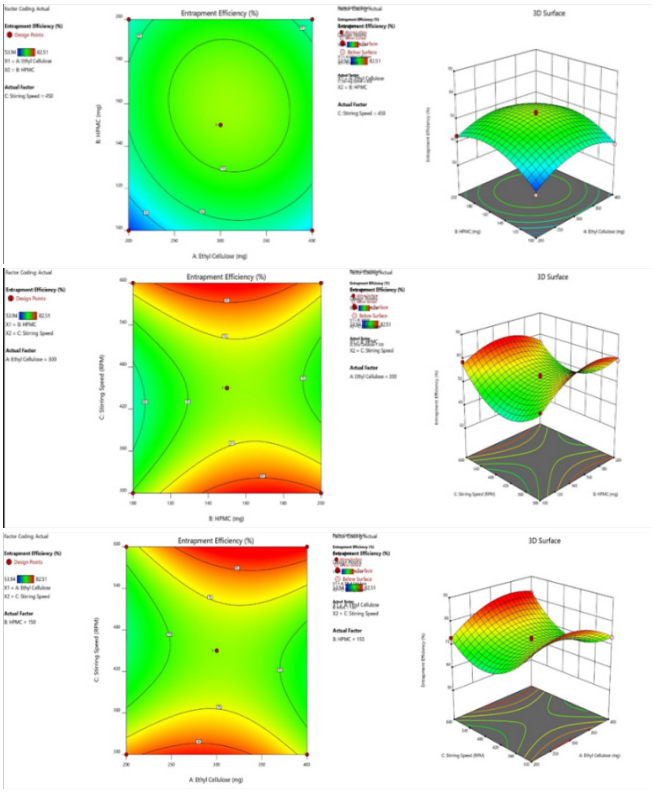

Figure 2: Contour and response surface plots interaction of independent variables against EE

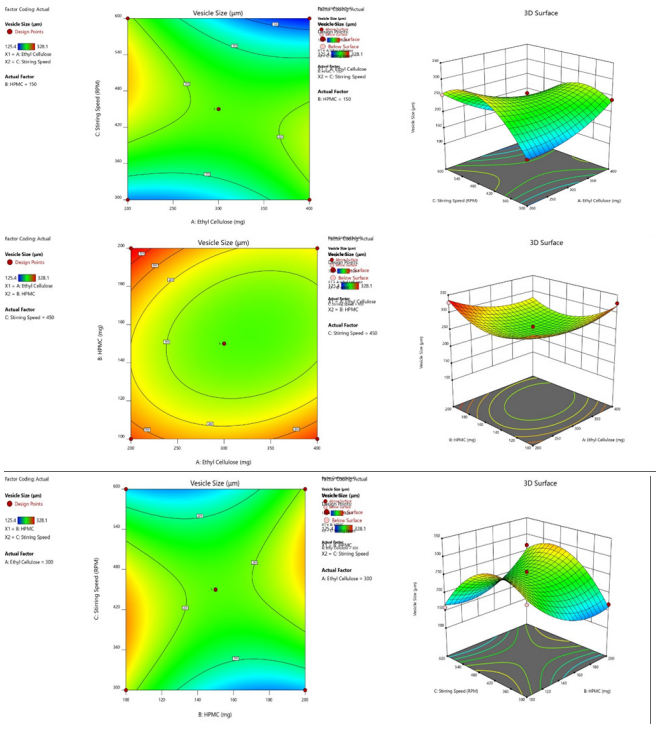

Figure 1: Contour and response surface plots interaction of independent variables against Vesicle size

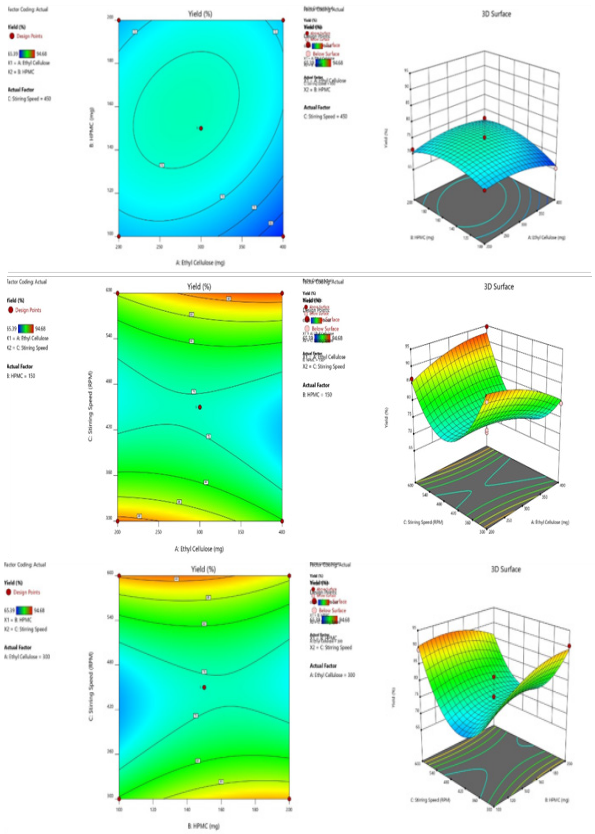

Figure 3: Contour and response surface plots interaction of independent variables against %Yield

Entrapment Efficiency = +72.21 +1.29A +2.44B +0.9287C -1.01AB +3.84AC -2.58BC -5.75A2 -6.37B2 +10.56C2

Effect of formulation factors on % yield

The drug percentage yield of oral suspensions based on nicotine-loaded microspheres ranged from 65.39 ± 0.05 to 68 ± 0.06% for 17 runs and the results were displayed in Figure 3. Yield = +74.58 -0.9375A +1.20B +1.65C +1.31AB +4.55AC -3.31BC -2.00A2 -2.84B2 +14.89C2

Point Prediction (Table 3)

From among the several compositions evaluated, the optimal nicardipine-loaded microspheres were selected using the program's point prediction approach. The results showed a strong correlation between the expected and actual values, as illustrated graphically in Figure 3. This observation might indicate that the process employed is the most effective way to produce microspheres. The desirability value of each piece was calculated using software, and the results ranged from 0.9786 to 0.9786, indicating the procedure's reliability. Small particle size and high entrapment efficacy were important selection factors. Microsphere optimisation employing X1-X3 concentrations of 400 (mg), 150 (mg), and 600 rpm of stirring speed (F5) for microspheres loaded with nicardipine.

Micromeritic Properties

The ideal diameters of the microparticles loaded with nicardipine were 125.4 ± 5.37 µm. All of the formulations had bulk densities between 1.43 and 1.84 g/cm3, while their tapped densities varied from 0.48 to 0.93 g/cm3. A low density of small nicardipine particles could be the reason for this discrepancy. Porosities ranging from 60 to 80% were observed in all of the formulations. The compressibility index varied between 21.8 and 34.6%. The compressibility index results for every formulation of microspheres varied from 20.5 ± 0.4 to 36.4 ± 2.3, indicating the microspheres' exceptional permeability and flow properties. All formulations exhibited free-flowing qualities, as evidenced by Hausner's ratios less than 1.53 ± 0.4. The angle of repose results further supports the microspheres' free-flowing property.

Table 3: Actual values vs. predicted values

Parameters	*Observed values*	*Predicted values*	*Difference*
X1	400	384.406	15.594
X2	150	141.008	8.992
X3	600	600.00	0.00
Y1	125.4 ± 5.37	124.598	0.802
Y2	82.51 ± 2.05	83.904	-1.394
Y3	94.68 ± 0.06	92.840	1.84
Desirability	0.979		

Drug and Excipient Compatibility

FTIR was used to test the optimal formulation and determine whether the microspheres were compatible with HPMC polymers and EC. The spectra of the enhanced formulation (F5) showed no significant change, and the characteristic peak of the pure drug did not change, implying that the drug and the polymer did not interact in any way. This is shown in Figure 4.

The DSC data that are displayed in Figures 5a to 5c reveal that Nicardipine HCl has a distinct endothermic peak between 135 and 150ºC. The endothermic peaks for EC, HPMC, and Tween 80 are at 164.16, 79.76, 80.95, and 206.84ºC, respectively, and are likewise the points of melting for these materials. With a reported melting point of 136.79ºC, nicardipine HCl is a crystalline drug. The DSC thermogram is displayed in Figure 5.

Conversely, the melting peak at 136.79ºC was absent from the microsphere thermograms. The finding of a drug melting peak in the physical mixture provided more evidence for this. At 71.94 & 206.84°C, the raw material EC displays a melting peak. Following the preparation of EC as microparticles, the melting peak progressively shifted to a lower temperature as the amount of polymer in the matrix grew.

Powder X-ray Diffraction studies

On nicardipine's diffractogram, clear peaks can be seen at 2 = 14.4, 19.5, and 24.4°. Rather, the drug's amorphism within the microparticles was revealed as the prominent peaks of the microspheres disappeared. Significant peaks can be seen at 2 equals 5.5°, 18.8°, and 23.2° for EC and HPMC, respectively, on their diffraction curves as shown in Figure 6.

Peak intensities of the optimized microspheres and physical mixture are less than those of the physical mixture, suggesting that the microparticles have less crystallinity than the raw materials. The microparticle diffractograms also demonstrate gradual alterations with a rise in the floating content of the polymer matrix. Optimal microspheres (10 weight) show a smaller peak when 2 = 5.5° than the other formulations, although EC shows a noticeable peak in the

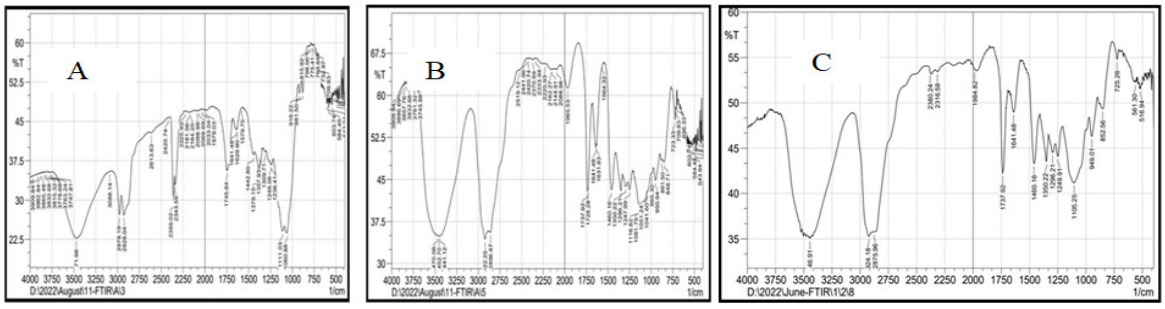

Figure 4: FTIR of A) Nicardipine, B) Physical mixture and C) optimized microspheres (F5)

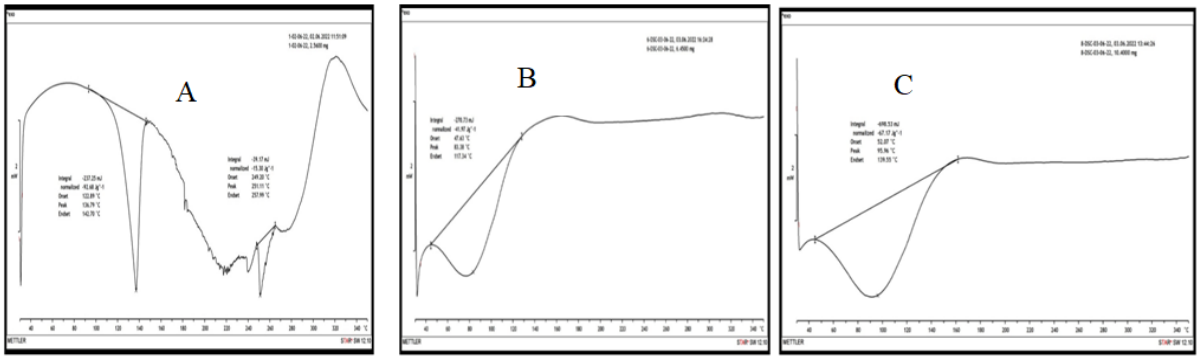

Figure 5: DSC thermogram of A) Nicardipine,B)Physical mixture and C)optimized microspheres (F5)

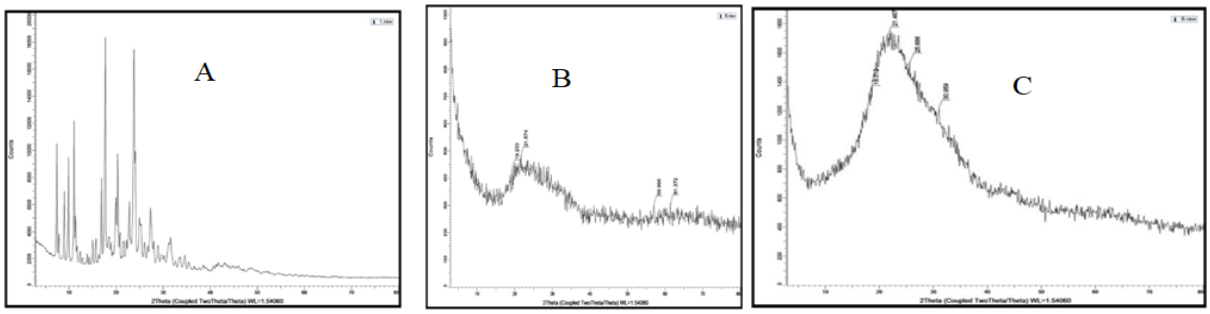

Figure 6: X-RD of A) Nicardipine,B)Physical mixture, and C)optimized microspheres (F5)

physical combination. When the ideal formulation's floating content reaches 20%, the peak at 5.5° broadens and eventually disappears in the microspheres' 30% weight percentage diffraction curve. These results demonstrate that the addition of floating to the polymeric matrix leads to less ordered crystals in microspheres, confirming the inference drawn from the DSC examination.

Morphology

Nicardipine-based HPMC microspheres were elongated in shape despite their mainly spherical appearance. Numerous holes and cracks may be seen in the microspheres, as shown by the photomicrograph. It's possible that uneven droplet coalescence formed the non-spherical, deformed particle shape. Multiple "craters of drug crystals on the surface" were discovered by the SEM study, further damaging the integrity and shape of the microspheres, and the same was depicted in Figure 7.

In-vitro Drug Release Study

Nicardipine release from EC microspheres was assessed at pH 1.2 and 7.4. No appreciable amount of drug is released from the microspheres in SGF because the acrylic polymer utilized in the procedure is soluble at acidic pH and begins to break down above pH 7. Both erosion and diffusion of the drug from HPMC occurred in the simulated intestinal fluid (SIF) (pH 7.4), which was shown in Figure 8.

In-vivo Floating behavior

The ability of dried Nicardipine microspheres to float in the gastric fluid was assessed to enhance their residence in the stomach. F5 was made using EC: HPMC (1:2.5); it was chosen for further investigation by the radiological approach because it showed good *in-vitro* buoyancy. The gastroretentive feature of microspheres is indicated by X-ray pictures collected at 2, 8, and 12 hours for the buoyancy study. This highlights the importance of a substantial gastric residence time for optimal drug release and absorption because floating microspheres are porous, which was depicted in Figure 9.

In-vitro Pharmacokinetic study

The $AUC_{0\text{-}48}$ and $AUC_{0\text{-}96}$ showed a significant change between the two (p <0.05), while the Cmax and tmax for the oral formulation and microspheres F5 did not differ significantly (p = 0.10 and 0.96, respectively). The microsphere group's AUC0-48 and AUC0-values were nearly 2.35 times higher than the oral formulation groups. Additionally, microspheres F5 displayed a quantitatively greater tmax value than a commercial formulation, albeit not considerably. This was demonstrated by the high relative bioavailability of F5 (235.04%), which validated the initial assumption, as shown in Figure 10.

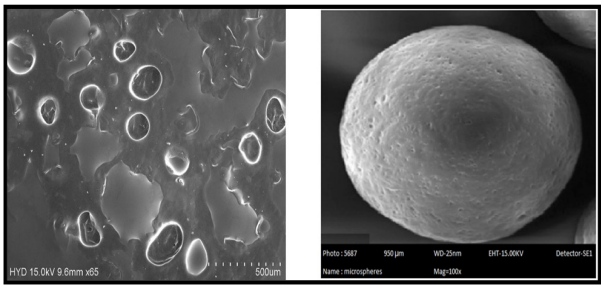

Figure 7: SEM images of optimized Nicardipine-loaded Microspheres (F5)

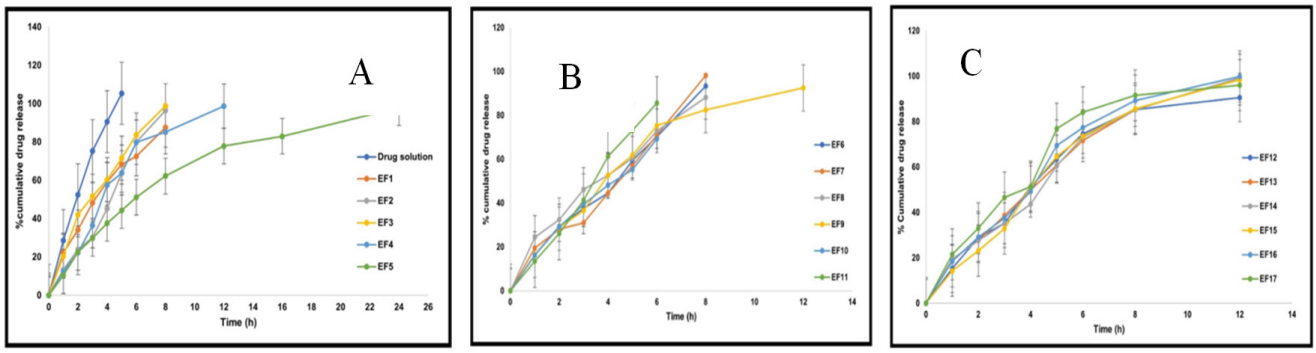

Figure 8: *In-vitro* drug release of A) F1 to F5 B) F6 to F11 C) F12 to F17

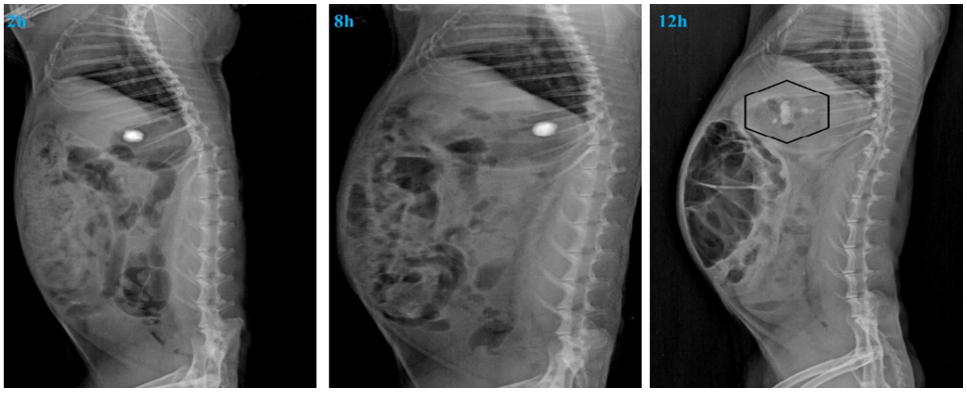

Figure 9: X-ray pictures of formulation F5 were taken at 2, 8, and 12 hours in the rabbits' stomach

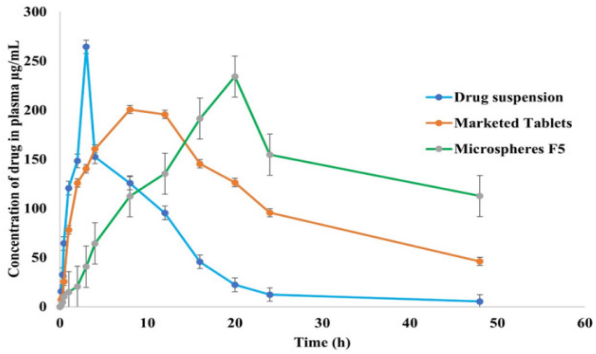

Figure 10: Pharmacokinetic profiles of NCP concentration over time from a tablet taken by mouth and microspheres F5. The data are shown as mean SD (n = 6)

CONCLUSION

To increase solubility, bioavailability, and patient compliance, floating microspheres containing Nicardipine hydrochloride were successfully created and optimized in the current study. The Box-Behnken design was utilized to optimize the solvent evaporation procedure, which was utilized to prepare the floating microspheres. The formulation F5 was discovered to be the best as anticipated by the design expert software's point prediction. Many physicochemical characteristics, surface morphology, drug-excipient interaction, *in-vitro* release, buoyancy tests, *in-vitro* floating behavior, and *in-vivo* pharmacokinetic study were used to characterize the optimized formulation of floating microspheres.

REFERENCES

1. Goswami A, Jain NK, Goyal M. An Updated Review on Gastro Retentive Drug Delivery System. International Journal of Pharmaceutical Sciences Review and Research 2020; 65.
2. C. K, Seth N, Gill NS. Gastro retentive drug delivery system: a significant tool to increase the gastric residence time of drugs. International Journal of Current Pharmaceutical Research 2021.
3. Kajal D. Chaudhari, Minakshee G. Nimbalwar, Nikita S. Singhal, Wrushali A. Panchale, Jagdish V. Manwar, Ravindra L. Bakal. A comprehensive review on characterizations and application of gastro-retentive floating drug delivery system. GSC Advanced Research and Reviews 2021; 7.
4. Nitave SA, Patil VA, Kagalkar AA. Review on gastroretentive drug delivery system (GRDDS). International Journal of Pharmaceutical Sciences Review and Research. 2014; 27.
5. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. Floating drug delivery systems: A review. AAPS PharmSciTech. 2005; 6.
6. Airemwen OC, Uhumwangho MU. Gastro-floating drug delivery systems - A review. Tropical Journal of Natural Product Research. 2017; 1.
7. Lodh H, FR S, Chourasia PK, Pardhe HA. Floating Drug Delivery System: A Brief Review. American Journal of PharmTech Research 2020; 10.
8. Kurniawansyah IS, Sopyan I, Wathoni N, Fillah DL, Praditya RU. Application and characterization of in situ gel. International Journal of Applied Pharmaceutics. 2018; 10.
9. Padmasri B, Nagaraju R, Prasanth D. A comprehensive review on in situ gels. International Journal of Applied Pharmaceutics. 2020; 12.
10. Liu L, Gao Q, Lu X, Zhou H. In situ forming hydrogels based on

chitosan for drug delivery and tissue regeneration. Asian Journal of Pharmaceutical Sciences. 2016; 11.
11. Mohanty D, Bakshi V, Simharaju N, Haque MA, Sahoo CK. A Review on in situ Gel: A Novel Drug Delivery System Dibyalochan. International Journal of Pharmaceutical Sciences Review and Research 2018; 50.
12. P.Vamsikrishna, T.Malyadri, Ch.Saibabu. Formulation development and *in-vitro* evaluation of bilayer tablets nicardipine. World Journal of Current Medical and Pharmaceutical Research 2021.
13. Singh C, Yashwant, Gupta AK, Garg V. Formulation Development and Evaluation of Divalproex Sodium Extended-release Tablets. International Journal of Drug Delivery Technology. 2022;12(4):1769-1773.DOI: 10.25258/ijddt.12.4.46