RESEARCH PAPER

Development and Characterization of Ranolazine Nanoliposomes and Nanocochleates Using Quality by Design Approach

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ABSTRACT

Introduction

Ranolazine is an anti-anginal drug used for the treatment of chronic angina, but its therapeutic effectiveness is limited due to poor aqueous solubility and low bioavailability. The present study aimed to develop Ranolazine-loaded nanoliposomes and nanocochleates to improve drug stability, entrapment efficiency, and sustained drug release. Preformulation studies such as melting point determination, λmax analysis, FTIR, and DSC were carried out to confirm the purity, identity, and compatibility of the drug.

Materials and Methods

Ranolazine-loaded nanoliposomes were prepared by thin film hydration method and further converted into nano cochleates using calcium chloride by trapping method. Optimization of formulations was carried out using Design of Experiments (DOE) and Central Composite Design (CCD). The prepared formulations were evaluated for particle size, polydispersity index (PDI), zeta potential, drug content, entrapment efficiency, in-vitro drug release, FTIR, DSC, PXRD, TEM, and FESEM studies.

Results and Conclusion

The observed melting point of Ranolazine was 123°C and λmax was found at 274 nm, confirming drug purity and identity. Among all formulations, optimized batch NF6 showed particle size of 150.3 nm, PDI of 0.153, zeta potential of −29.5 mV, drug content of 96.45%, and entrapment efficiency of 90.56%. The optimized formulation exhibited sustained drug release of 92.85% up to 24 hours and followed Higuchi diffusion kinetics ($R^2 = 0.9828$). FTIR, DSC, PXRD, TEM, and FESEM studies confirmed the successful formation and stability of nanocochleates. The study concluded that Ranolazine-loaded nanocochleates are a promising nano-delivery system for sustained and improved drug delivery.

Keywords: Ranolazine, Nanoliposomes, Nanocochleates, Quality by Design, Central Composite Design, Sustained Release.

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Introduction

Cardiovascular diseases are among the leading causes of mortality worldwide, and chronic angina pectoris is one of the most common cardiovascular disorders affecting quality of life. Ranolazine is a well-known anti-anginal drug that acts by inhibiting the late sodium current in cardiac cells, thereby improving myocardial relaxation and reducing oxygen demand. However, Ranolazine exhibits poor aqueous solubility and limited oral bioavailability, which may reduce its therapeutic efficiency. Therefore, development of an advanced drug delivery system is necessary to improve its pharmacokinetic and therapeutic performance.

Nanotechnology-based drug delivery systems have emerged as promising approaches for improving solubility, bioavailability, stability, and controlled release of poorly soluble drugs. Among various lipid-based carriers, nanoliposomes have gained considerable attention because of their biocompatibility, ability to encapsulate hydrophilic and lipophilic drugs, and enhanced drug targeting capability. Nanoliposomes also protect the encapsulated drug from degradation and improve systemic circulation time.[1]

Nanocochleates are cigar-shaped lipid-based carriers formed by the interaction of negatively charged phospholipids with divalent cations such as calcium ions. These systems possess a stable multilayered structure with minimal internal aqueous space, which provides enhanced stability and sustained drug release characteristics. Nanocochleates are also capable of protecting drugs from enzymatic degradation and improving oral bioavailability.[2]

Several researchers have reported successful applications of nanocochleates for improving therapeutic efficacy of poorly soluble drugs. Fisetin-loaded nanocochleates showed enhanced bioavailability and anticancer activity due to improved drug entrapment and sustained release behavior.[3] Similarly, chitosan-functionalized nanocochleates demonstrated enhanced oral absorption and stability of Cyclosporine A.[4]

The Quality by Design (QbD) approach and Design of Experiments (DOE) are widely used for optimization of pharmaceutical formulations. Response Surface Methodology (RSM) using Central Composite Design (CCD) helps in studying the influence of formulation variables on critical

quality attributes such as particle size and entrapment efficiency with minimum experimental runs.[5]

Preformulation studies including melting point determination, λmax determination, Fourier Transform Infrared Spectroscopy (FTIR), and Differential Scanning Calorimetry (DSC) are important for identification, purity assessment, and compatibility analysis of drug and excipients prior to formulation development. FTIR analysis helps identify functional groups and possible drug–excipient interactions, whereas DSC provides information regarding thermal behavior and crystallinity of the drug.[6]

In the present study, Ranolazine-loaded nanoliposomes were prepared and optimized, followed by conversion into nanocochleates using calcium chloride by trapping method. The developed formulations were characterized for particle size, polydispersity index (PDI), zeta potential, drug content, entrapment efficiency, and in-vitro drug release behavior. Advanced characterization studies such as TEM, FESEM, FTIR, DSC, and PXRD were also carried out to evaluate morphology, compatibility, crystallinity, and stability of optimized formulations.[7]

The main objective of this work was to develop a stable and effective Ranolazine-loaded nanocochleate system with improved drug entrapment and sustained release characteristics, which may ultimately enhance therapeutic efficacy and patient compliance in the management of chronic angina.[8]

Materials and Methodology

Materials

Ranolazine was used as the model drug. Phospholipids, cholesterol, calcium chloride, methanol, phosphate buffer pH 6.8, distilled water, and all other analytical grade reagents were used for the preparation of nanoliposomes and nanocochleates. Mannitol was used as a cryoprotectant during lyophilization. Double distilled water was used throughout the study.

Methodology

1. Identification of Drug

Appearance

The organoleptic properties of Ranolazine were evaluated visually for appearance, odor, taste, and physical nature.

2. Melting Point Determination

The melting point of Ranolazine was determined by capillary tube method. A small quantity of pure drug was filled into a capillary tube sealed at one end. The capillary tube was attached to a thermometer and immersed in Thiele's tube containing liquid paraffin. The assembly was heated gradually until the drug melted completely. The temperature at which the drug started melting was recorded

3. λmax Determination

The λmax of Ranolazine was determined using UV-Visible spectrophotometer (Jasco V-630). A stock solution was prepared by dissolving 10 mg of Ranolazine in 10 ml methanol to obtain a concentration of 1000 µg/ml. From this solution, 1 ml was diluted to 10 ml with methanol to obtain 100 µg/ml solution, and further diluted to obtain 10 µg/ml solution. The prepared solution was scanned between 200–400 nm and the wavelength showing maximum absorbance was recorded.

4. Linearity Study

Linearity studies were carried out in different media including methanol, distilled water, phosphate buffer pH 6.8, phosphate buffer pH 7.4, and phosphate buffer pH 1.2 using UV spectrophotometric method at 274 nm.

I. Calibration Curve of Ranolazine in Methanol

Preparation of Stock Solution

Standard stock solution was prepared by dissolving 10 mg of Ranolazine in 10 ml methanol to obtain a concentration of 1000 µg/ml.

Preparation of Standard Dilutions

Aliquots corresponding to concentrations of 10–60 µg/ml were prepared by suitable dilution with methanol. Absorbance was measured at 274 nm using methanol as blank.

II. Calibration Curve of Ranolazine in Distilled Water

Preparation of Stock Solution

Standard stock solution was prepared by dissolving 10 mg of Ranolazine in 10 ml distilled water to obtain 1000 µg/ml solution.

Preparation of Standard Dilutions

Different concentrations ranging from 10–60 µg/ml were prepared and analyzed at 274 nm.

III. Calibration Curve of Ranolazine in Phosphate Buffer pH 6.8

Preparation of Stock Solution

Stock solution containing 1000 µg/ml was prepared in phosphate buffer pH 6.8.

Preparation of Standard Dilutions

Different concentrations ranging from 10–60 µg/ml were prepared and absorbance was measured at 274 nm.

IV. Calibration Curve of Ranolazine in Phosphate Buffer pH 7.4

Preparation of Stock Solution

A standard stock solution of Ranolazine (1000 µg/ml) was prepared in phosphate buffer pH 7.4.

Preparation of Standard Dilutions

Dilutions of 10–60 µg/ml were prepared and analyzed spectrophotometrically at 274 nm.

V. Calibration Curve of Ranolazine in Phosphate Buffer pH 1.2

Preparation of Stock Solution

Standard stock solution was prepared in phosphate buffer pH 1.2 to obtain 1000 µg/ml concentration.

Preparation of Standard Dilutions

Serial dilutions were prepared in the concentration range of 10–60 µg/ml and analyzed at 274 nm.

5. FTIR Spectroscopy

Drug-excipient compatibility study was performed using Fourier Transform Infrared (FTIR) spectroscopy. The Ranolazine sample was scanned over the range of 500–4000 cm^{-1} using diffuse reflectance scanning technique.

6. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was performed to study the thermal behavior of Ranolazine. Approximately 4.6 mg of sample was placed in an aluminum pan and scanned under controlled heating conditions.

The DSC thermogram exhibited a sharp endothermic peak corresponding to the melting point of Ranolazine, indicating its crystalline nature. The onset temperature was observed at approximately 130.02°C. The narrow peak confirmed the purity of the sample. The endothermic transition indicated absorption of heat during melting. The observed thermal behavior was found to be consistent with reported polymorphic forms of Ranolazine.

7.Preparation of Ranolazine -Loaded Nanocochleates

1. Nanocochleates were prepared by 'trapping method'.
2. 200-250 litre of calcium chloride solution (0.1 M) was added drop-wise into the prepared Ranolazine -loaded unilamellar vesicles under vortex.
3. The vesicle phase immediately turned turbid because of nanocochleate formation. Precipitated nanocochleates were refrigerated at 2-8C.[1]

7.1.Experimental design

The response surface methodology (RSM) was employed to perform Quality by Design approach for constructing and investigating the polynomial models, using fewer experimental runs. Central composite Design comprising of 2-factors and 3-levels was employed to examine the quadratic response surfaces by assessing the effect of pre-defined independent variables on different response dependent variables Particle size (nm) and Entrapment efficiency (%) was coded as Y1 and Y2. Two independent variables namely Calcium Chloride Concentration (µl) (A) and Vortex Time (min) (B) were chosen. Each of the variables was varied at three different levels, known as high, medium and low levels. All the finalized independent variables and the response variables are described in Table 1.

Table 1: List of Independent and Dependent variables in Central Composite design

Independent variables	Low value(-1)	Medium (0)	High value(+)
Calcium Chloride Concentration (µl)	200	225	250
Vortex Time (min)	10	15	20
Dependent variables	Constraints		
Particle size (nm)	Minimize		
Entrapment efficiency (%)	Maximize		

Table 2: DOE suggested and Experimental batches

Formulation Code	Optimized Liposomal Suspension (ml)	Calcium Chloride Concentration(µl)	Vortex Time(min)
NF1	10.40	200	15
NF2	10.40	225	10
NF3	10.40	250	15
NF4	10.40	250	10
NF5	10.40	200	20
NF6	10.40	250	20
NF7	10.40	225	20
NF8	10.40	225	15
NF9	10.40	200	10

7.2. Application of DOE for Nanoliposomes

Quality by Design (QbD) approach using Design of Experiments (DOE) was employed for optimization of nanoliposomes. Response Surface Methodology (RSM) with Central Composite Design (CCD) was used to evaluate the effect of formulation variables on particle size and entrapment efficiency.

Table 3: Two independent variables were selected:

Independent Variables	Low (-1)	Medium (0)	High (+1)
Lipid concentration	Low	Medium	High
Sonication time	Low	Medium	High

Table 4: Dependent variables:

Response Variables	Goal
Particle size	Minimize
Entrapment efficiency	Maximize

Different experimental batches were prepared according to DOE software suggestions and evaluated for optimization.

7.3. Characterization of Nanoliposome Batches

The prepared nanoliposomal batches were evaluated for the following parameters:

a) Particle Size and Polydispersity Index (PDI)

Particle size and PDI were measured using dynamic light scattering method by particle size analyzer. Samples were diluted appropriately with distilled water before analysis.

b) Zeta Potential

Zeta potential was determined using electrophoretic light scattering technique to evaluate stability of nanoliposomes.

c) Drug Content

Drug content was determined by dissolving formulation in methanol followed by UV spectrophotometric analysis at 274 nm.

d) Entrapment Efficiency

Entrapment efficiency was determined indirectly by centrifugation method. The amount of free drug present in supernatant was analyzed using UV spectroscopy at 274 nm.

e) In-vitro Drug Release

In-vitro drug release study was carried out using dialysis bag diffusion technique in phosphate buffer pH 6.8 at 37 ± 0.5°C under continuous stirring. Samples were withdrawn at predetermined time intervals and analyzed spectrophotometrically at 274 nm.

7.4. Selection of Optimized Batch of Nanoliposomes

The optimized nanoliposomal batch was selected on the basis of:

- Minimum particle size
- Low PDI
- High zeta potential
- Maximum drug content
- High entrapment efficiency
- Controlled drug release profile

The optimized liposomal suspension was further utilized for preparation of nanocochleates.

7.5. Preparation of Nanocochleates

Ranolazine-loaded nanocochleates were prepared by trapping method. Nanocochleates were formed by the addition of calcium chloride solution into the optimized liposomal suspension under vortex mixing.

Procedure:

1. Optimized liposomal suspension was taken in a suitable container.
2. Calcium chloride solution (0.1 M) was added drop-wise into the liposomal suspension under vortex conditions.
3. Immediate turbidity formation indicated conversion of liposomes into nanocochleates.
4. The formed nanocochleates were refrigerated at 2–8°C for further studies.

7.6. Application of DOE for Nanocochleates

Central Composite Design (CCD) was employed for optimization of nanocochleates using Response Surface Methodology.

Table 5: Two formulation variables were selected:

Independent Variables	Low (-1)	Medium (0)	High (+1)
Calcium chloride concentration (µL)	200	225	250
Vortex time (min)	10	15	20

Table 6: Dependent responses:

Response Variables	Goal
Particle size	Minimize
Entrapment efficiency	Maximize

Nine experimental batches (NF1–NF9) were prepared according to DOE suggested runs.

7.7. Characterization of Nanocochleate Batches

Prepared nanocochleate formulations were evaluated using the following parameters:

a) Particle Size, PDI and Zeta Potential

Particle size, PDI and zeta potential were determined using HORIBA SZ-100 particle size analyzer at 25°C after suitable dilution with distilled water.

b) Drug Content

Drug content was determined by dissolving nanocochleates in methanol followed by UV spectrophotometric analysis at 274 nm.

c) Entrapment Efficiency

Entrapment efficiency was determined by centrifugation method using UV spectrophotometric estimation of free drug in supernatant at 274 nm.

d) In-vitro Drug Release Study

Drug release study was performed using dialysis membrane diffusion technique in phosphate buffer pH 6.8 at 37 ± 0.5°C under stirring conditions. Samples were analyzed at 274 nm.

e) Drug Release Kinetics

The release data of optimized formulation were fitted to:

- Zero order model
- First order model
- Higuchi model

to determine release mechanism.

f) Transmission Electron Microscopy (TEM)

TEM analysis was performed to study morphology and surface characteristics of optimized nanocochleates. Samples were stained with phosphotungstic acid and analyzed under TEM.

g) Fourier Transform Infrared Spectroscopy (FTIR)

FTIR study was carried out in the range of 650–4000 cm^{-1} to evaluate drug–excipient compatibility.

h) Differential Scanning Calorimetry (DSC)

DSC analysis was performed to investigate thermal behavior and compatibility of formulation components. Samples were scanned between 20–300°C under nitrogen atmosphere.

j) FESEM Analysis

Field Emission Scanning Electron Microscopy (FESEM) was performed to study surface morphology and particle characteristics of optimized nanocochleates.

k) Lyophilization

Optimized nanocochleates were freeze dried using mannitol as cryoprotectant. Samples were pre-frozen at −80°C and lyophilized under vacuum at −55°C.

7.8. Selection of Optimized Batch of Nanocochleates

The optimized nanocochleate batch was selected based on:

- Minimum particle size
- Low PDI
- High zeta potential
- Maximum drug content
- High entrapment efficiency
- Sustained drug release behavior
- Good surface morphology and stability

Among all formulations, batch NF6 was identified as the optimized nanocochleate formulation due to superior particle size, drug content, entrapment efficiency, and drug release characteristics.

Result

1. Appearance

The observed physical characteristics confirmed the identity and purity of Ranolazine and were found to comply with reported standards.

Table 7: Appearance

Parameter	Observation
Appearance	White to off-white crystalline powder

Parameter	Observation
Odor	Odorless or faint smell
Taste	Bitter
Solubility	Slightly soluble in water and soluble in organic solvents

2. Melting Point of Ranolazine

Table 8: Melting Point of Ranolazine

Drug	Observed	Reported
Ranolazine	123°C	120–124°C

The observed melting point of Ranolazine (123°C) was found to be within the reported range (120–124°C), confirming the purity and crystalline nature of the drug.

3. λmax Determination

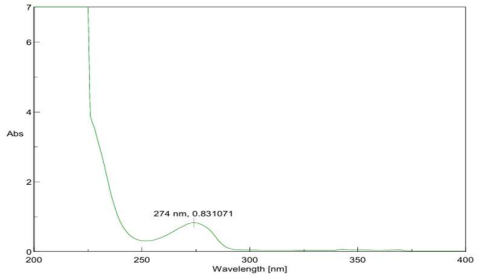

Figure: UV spectrum of Ranolazine in methanol (10µg/ml)

Table.9: λmax of Ranolazine

Drug	λmax	Reported λmax
Ranolazine	274	274

The λmax of Ranolazine was experimentally determined to be 274 nm, which is in exact agreement with the reported λmax value of 274 nm. This confirms the accuracy of the UV spectrophotometric method used and validates its suitability for the identification and further quantitative analysis of Ranolazine.

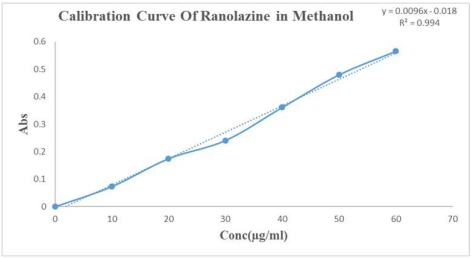

Figure.1: Calibration Curve of Ranolazine in Methanol

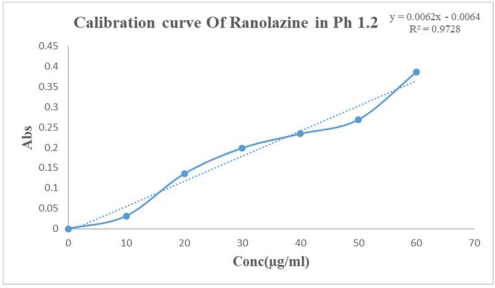

Figure.2: Calibration Curve of Ranolazine in pH 1.2

3. FTIR spectroscopy :

The drug excipients compatibility study was performed by FTIR technique. The Ranolazine samples were scanned over wave number range of 500-4000 cm-1 with diffraction reflectance scanning technique.

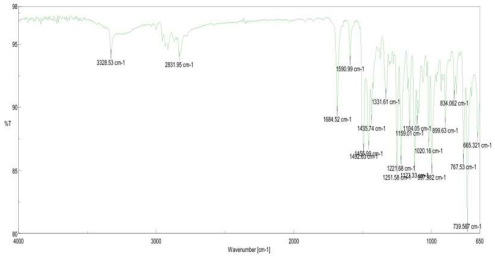

Figure.3: FTIR Spectrum of Ranolazine

Table.10: FTIR interpretation of Ranolazine

Functional group	Observed range (cm^{-1})	Reported range (cm^{-1})
N–H stretching (secondary amine)	~3328	3300–3400
C–H stretching (aliphatic)	~2832	2850–2960
C=O stretching (amide)	~1684	1650–1700
C=C stretching (aromatic ring)	~1591	1500–1600
C–N stretching (amine)	~1331	1200–1350
C–O stretching	~1104–1020	1000–1200
Aromatic C–H bending (out of plane)	~899–834	800–900
C–Cl stretching	~766–665	600–800

4. DSC:

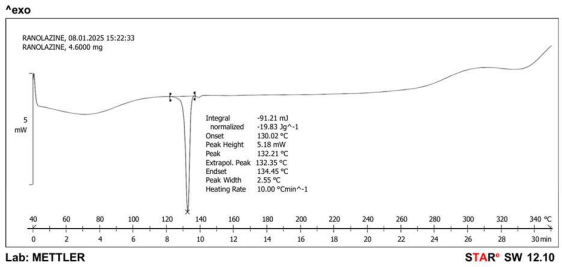

Figure.4: DSC graph of Ranolazine

The provided differential scanning calorimetry (DSC) thermograph shows the thermal properties of a ranolazine sample with a mass of 4.6000 mg (p. 1). The prominent downward peak indicates an endothermic event, which is likely the drug's melting point.

Pure crystalline substances typically exhibit a sharp, symmetrical melting peak. The relatively narrow peak width suggests a high degree of purity for this specific ranolazine sample.

The negative value for the normalized integral indicates that heat was absorbed during the process (an endothermic reaction), confirming a phase transition like melting.

This specific sample melts with an onset temperature of approximately 130.02 °C. This value is consistent with some reported melting points for specific polymorphic forms of ranolazine, which have been observed in the range of 118-137 °C, depending on the crystal form and preparation method. The value of $-19.83\ Jg-1$is different from

the enthalpy values of pure polymorph I (−54 Jg−1) or polymorph II (−27 Jg−1) reported in literature, suggesting this may be a different form or a mixture.

5. Evulation and Characterization of nanocochleate

1. Particle Size, PDI and Zeta Potential

The mean z-average diameter and polydispersity index (PDI) were determined by photon correlation spectroscopy using a HORIBA SZ-100 particle size analyzer (HORIBA Ltd.) at 25 °C. Samples were appropriately diluted with double-distilled water prior to measurement. Zeta potential was measured using the same instrument based on electrophoretic light scattering.[2]

Table.11: Particle Size, PDI and Zeta Potential of NF1- NF9

Formulation Code	Particle Size (nm)	PDI	Zeta Potential (mV)
NF1	199.3	0.345	-22.3
NF2	222.4	0.385	-18.6
NF3	173.6	0.265	-20.7
NF4	220	0.321	-19.5
NF5	248.2	0.432	-15.3
NF6	150.3	0.153	-29.5
NF7	214.9	0.251	-24.7
NF8	194.6	0.312	-23
NF9	208.5	0.264	-17.1

Particle Size and PDI

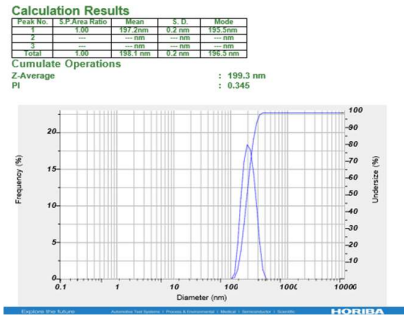

Figure.5: Particle size and PDI of NF1

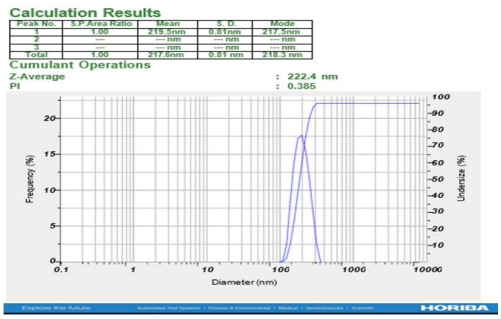

Figure.6: Particle size and PDI of NF2

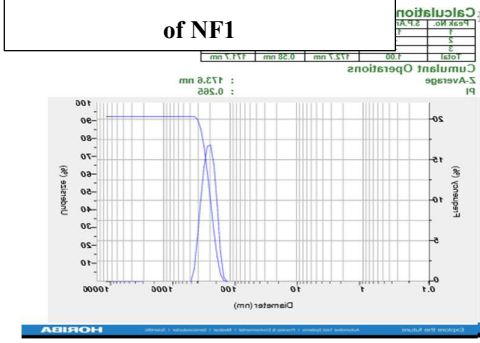

Figure.7: Particle size and PDI of NF3

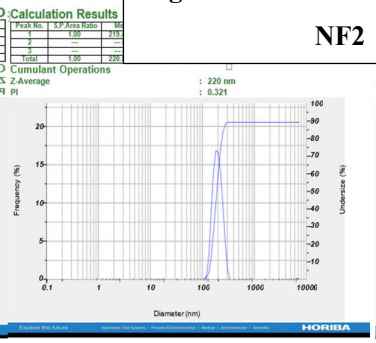

Figure.8: Particle size and PDI of NF4

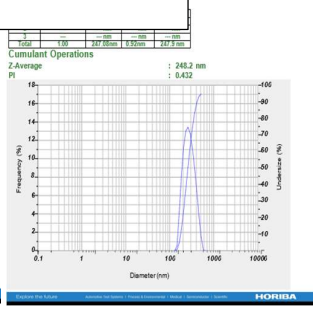

Figure.9: Particle size and PDI of NF5

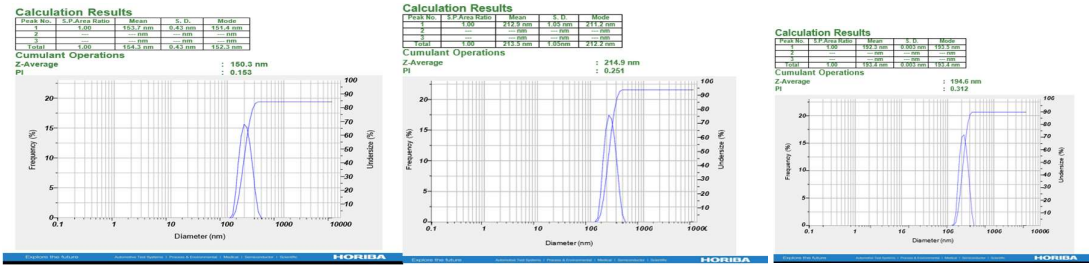

Figure.10: Particle size and PDI of NF6 Figure.11:

2.Zeta Potential

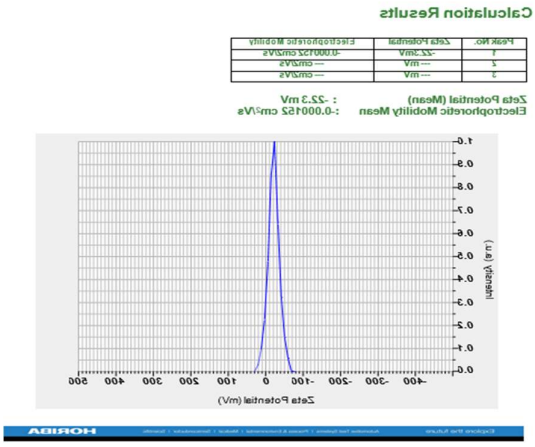

Figure 13: Zeta Potential of NF1

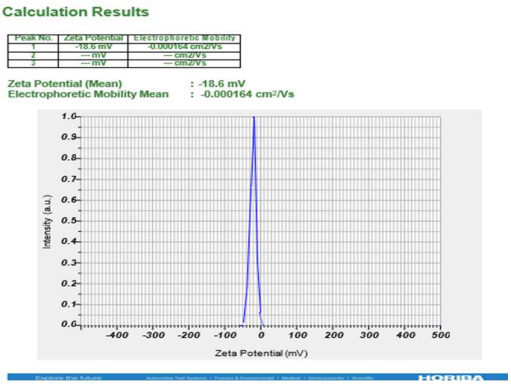

Figure 14: Zeta Potential of NF2

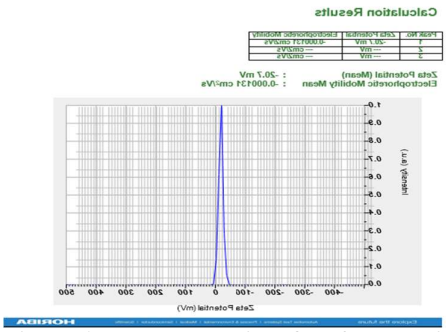

Figure 15: Zeta Potential of NF3

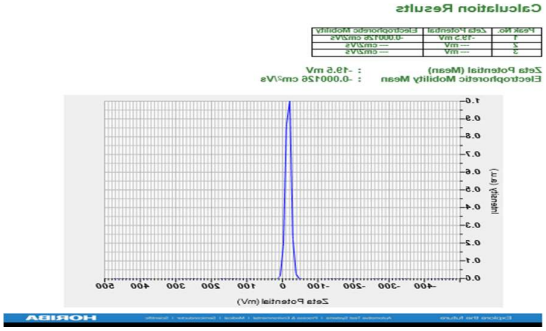

Figure 16: Zeta Potential of NF4

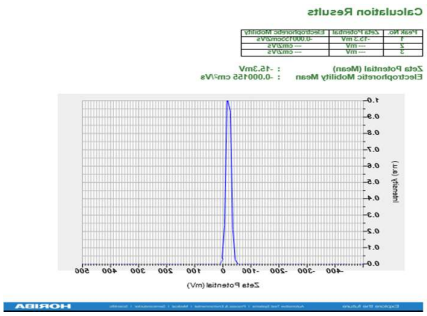

Figure 17: Zeta Potential of NF5

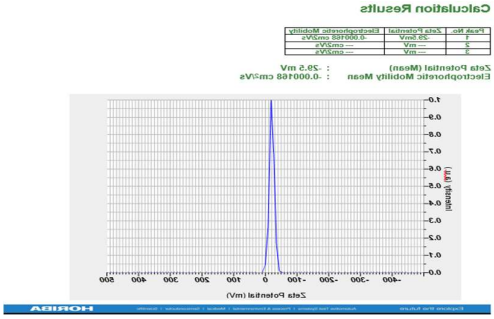

Figure 18: Zeta Potential of NF6

ANOVA for Quadratic model

Table.12: Response 1: Particle Size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6476.52	5	1295.30	30.58	0.0089	significant
A-Calcium Chloride Concentration	2094.40	1	2094.40	49.44	0.0059	
B-Vortex Time	234.37	1	234.37	5.53	0.1001	
AB	2992.09	1	2992.09	70.63	0.0035	
A^2	226.85	1	226.85	5.36	0.1036	
B^2	928.80	1	928.80	21.93	0.0184	
Residual	127.08	3	42.36			
Cor Total	6603.60	8				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**
The **Model F-value** of 30.58 implies the model is significant. There is only a 0.89% chance that an F-value this large could occur due to noise.
P-values less than 0.0500 indicate model terms are significant. In this case A, AB, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
Fit Statistics

Table 13: Fit Statistics

Std. Dev.	6.51	**R^2**	0.9808
Mean	203.53	**Adjusted R^2**	0.9487
C.V. %	3.20	**Predicted R^2**	0.7699
		Adeq Precision	17.3246

Final Equation in Terms of Coded Factors

Table 14.: Final Equation in Terms of Coded Factors

Particle Size	=
+196.27	
-18.68	A
-6.25	B
-27.35	AB
-10.65	A^2
+21.55	B^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.
Final Equation in Terms of Actual Factors

Table.15:Final Equation in Terms of Coded Factors

Particle Size	=
-1023.98333	
+10.20267	Calcium Chloride Concentration
+22.12000	Vortex Time
-0.218800	Calcium Chloride Concentration * Vortex Time
-0.017040	Calcium Chloride Concentration ²
+0.862000	Vortex Time ²

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

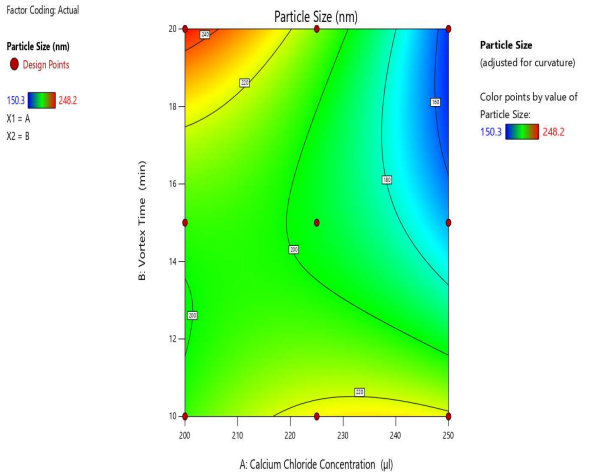

Figure.19:Counter plot

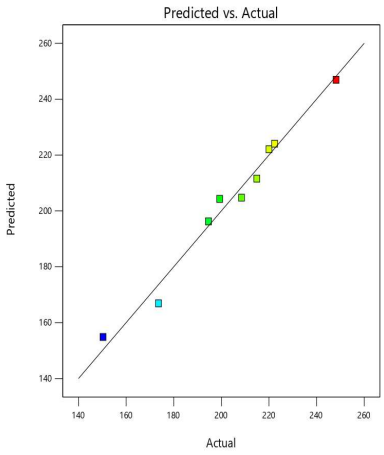

Figure.20 :Predicted Vs Actula plot

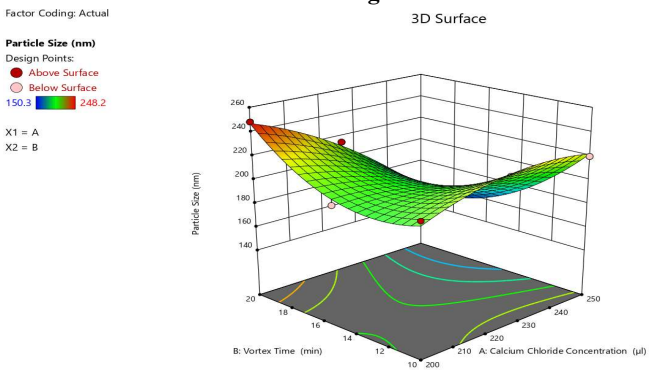

Figure.21:3D Surface plot

2. Drug content

For the determination of drug content, p nl) were dis solved in 10 ml Methanol, sonicated, filtered through nylon mem brane filter (0.45 µ). The absorbance of the filtrate was detected using a UV spectrophotometer at 274 nm (Jasco v-630)[3]

Table 16: Drug Content of NF1-NF9

Formulation Code	Drug Content (%)
NF1	88.17±0.071
NF2	91.14±0.95
NF3	86.20±0.33
NF4	94.32±0.54
NF5	85.57±0.41
NF6	96.45±0.89
NF7	93.58±0.47
NF8	83.40±0.53
NF9	89.85±0.27

3. Entrapment efficiency (%)

The entrapment efficiency (EE %) of nano-cochleate was determined indirectly by calculating the difference between the total amount of ranolazine added to the formulation and remaining is the aqueous medium after separation the nano-cochleates. This separated dispersion was centrifuged at 10,000 rpm for 1 hr using Micro centrifuge. Then the supernatant was removed and amount of corporated drug was measured by taking the absorbance of appropriate dilutes supernatant at 274 nm using UV spectrophotometer.[4]

Table.17: Entrapment efficiency (%) of NF1-NF9

Formulation Code	Entrapment efficiency (%)
NF1	78.01± 0.17
NF2	84.27± 0.43
NF3	81.17± 0.21
NF4	89.48± 0.57
NF5	82.8± 0.18
NF6	90.56± 0.68
NF7	83.34± 0.72
NF8	79.06± 0.96
NF9	86.4± 0.052

ANOVA for Quadratic model

Table.18:Response 2: Entrapment efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	143.30	5	28.66	15.16	0.0244	significant
A-Calcium Chloride Concentration	32.67	1	32.67	17.28	0.0253	
B-Vortex Time	1.98	1	1.98	1.05	0.3811	
AB	5.48	1	5.48	2.90	0.1874	
A^2	12.63	1	12.63	6.68	0.0814	
B^2	90.54	1	90.54	47.88	0.0062	
Residual	5.67	3	1.89			
Cor Total	148.97	8				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**
Final Equation in Terms of Coded Factors

Table.18: Final Equation in Terms of Coded Factors

Entrapment efficiency	=
+77.74	
+2.33	A
-0.5750	B
+1.17	AB
+2.51	A^2
+6.73	B^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table.19:Final Equation in Terms of Actual Factors

Entrapment efficiency	=
+354.18778	
-1.85667	Calcium Chloride Concentration
-10.29500	Vortex Time
+0.009360	Calcium Chloride Concentration * Vortex Time
+0.004021	Calcium Chloride Concentration ²
+0.269133	Vortex Time ²

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

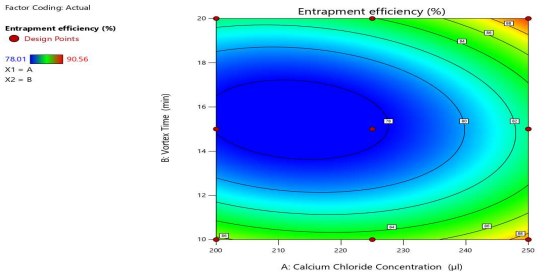

Figure 22:Predicted vs Actual plot

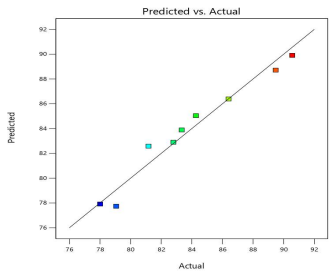

Figure 23 :Counter plot

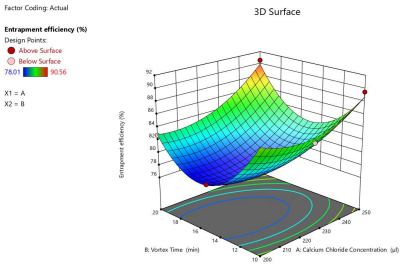

Figure 24 :3D Surface plot

6. In-vitro drug release

- In vitro release studies of Ranolazine from nanocochleates were carried out in phosphate buffered saline (pH 6.8) using dialysis bag diffusion method
- Drug equivalent of prepared formulation were placed in dialysis bags (cellulose membrane, molecular weight cut off 120,000 Da), completely sealed and immersed in 200 mL of dissolution medium.
- The assembly was maintained at 37 ± 0.5 ◦C with constant stirring by a magnetic stirrer at 100 rpm.
- At predetermined intervals, (1, 2, 3, 4,5,6,7 and 8 hr) samples were withdrawn and an equal volume of fresh medium was added to achieve sink conditions.
- The absorbance of Ranolazine in solution was determined using a UV-Vis spectrophotometer 274 nm (Jasco v-630).[5]

Table.20: Drug release of NF1-NF5

Time (hr)	NF1	NF2	NF3	NF4
0	0	0	0	0
1	22.84 ± 0.52	14.44 ± 0.38	20.35 ± 0.47	16.60 ± 0.41
2	26.02 ± 0.58	19.89 ± 0.45	23.15 ± 0.52	25.34 ± 0.56
4	35.55 ± 0.66	26.75 ± 0.60	30.67 ± 0.63	36.85 ± 0.68
6	49.39 ± 0.74	36.92 ± 0.69	41.20 ± 0.72	48.16 ± 0.76
8	55.71 ± 0.81	44.23 ± 0.75	46.41 ± 0.78	55.68 ± 0.83
12	64.16 ± 0.89	52.80 ± 0.82	55.70 ± 0.84	62.07 ± 0.88
20	77.15 ± 0.97	60.11 ± 0.90	68.15 ± 0.93	71.31 ± 0.95
24	82.67 ± 1.05	77.97 ± 1.02	81.12 ± 1.04	80.27 ± 1.03

Table.21: Drug release of NF6-NF9

Time (hr)	NF5	NF6	NF7	NF8	NF9
0	0	0	0	0	0
1	12.70 ± 0.35	23.07 ± 0.55	18.79 ± 0.46	20.09 ± 0.48	20.67 ± 0.49
2	25.23 ± 0.54	30.67 ± 0.62	25.51 ± 0.55	27.75 ± 0.57	30.87 ± 0.64
4	37.96 ± 0.71	42.55 ± 0.75	34.96 ± 0.65	31.21 ± 0.62	44.08 ± 0.78
6	47.68 ± 0.75	54.07 ± 0.82	49.64 ± 0.77	43.09 ± 0.73	53.55 ± 0.80
8	55.49 ± 0.82	62.71 ± 0.90	55.08 ± 0.81	48.45 ± 0.77	60.62 ± 0.88

12	65.02 ± 0.91	75.82 ± 1.02	62.90 ± 0.89	57.80 ± 0.85	71.65 ± 0.97
20	73.01 ± 0.98	84.30 ± 1.08	73.74 ± 0.96	68.49 ± 0.92	76.80 ± 1.00
24	82.53 ± 1.06	92.85 ± 1.15	87.29 ± 1.10	77.33 ± 1.01	80.78 ± 1.04

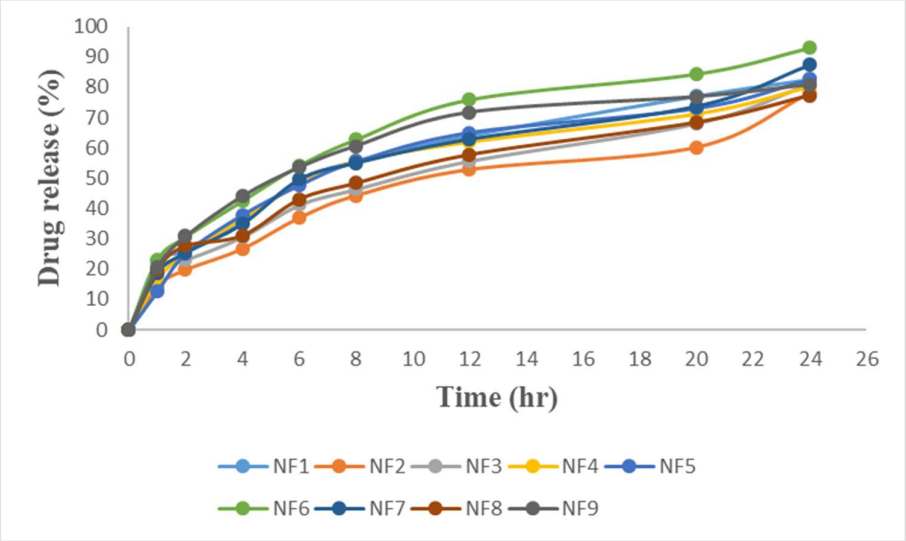

Figure 25: Drug release of NF1-NF9

Kinetic analysis of drug release-

In order to define the release mechanism that gives the best description of the release pattern; the in vitro release data for optimized batch (NF6) were fitted to kinetic equations models.

The kinetic equations were used i.e., zero, first-order and Higuchi model. Both the kinetic rate constant (k) and the determination coefficient (R2) were calculated and presented in below graphs.

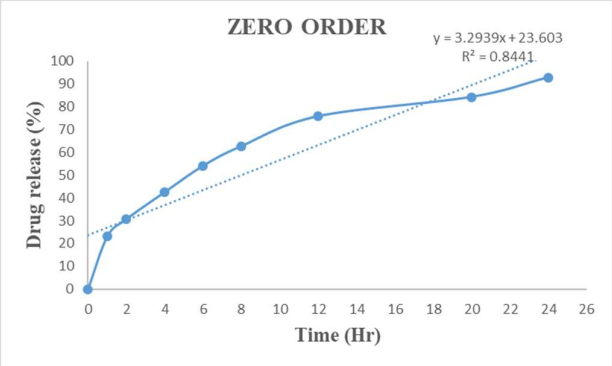

Figure 26: Zero order model of NF6

Table.22: ZERO ORDER MODEL

ZERO ORDER MODEL	
Formulation Code	**R2 Value**
NF6	0.8441

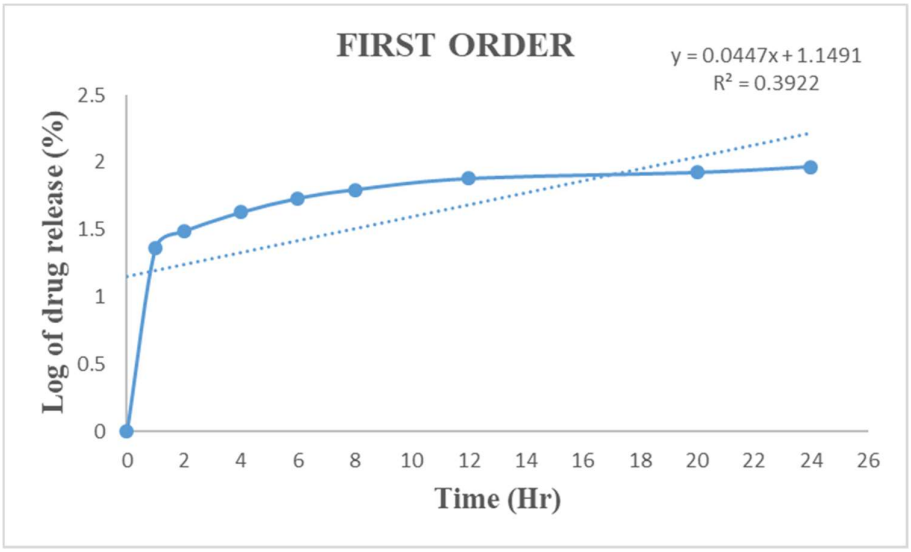

Figure.27: First order model of NF6

Table.23: FIRST ORDER MODEL

FIRST ORDER MODEL	
Formulation Code	**R2 Value**
NF6	0.3922

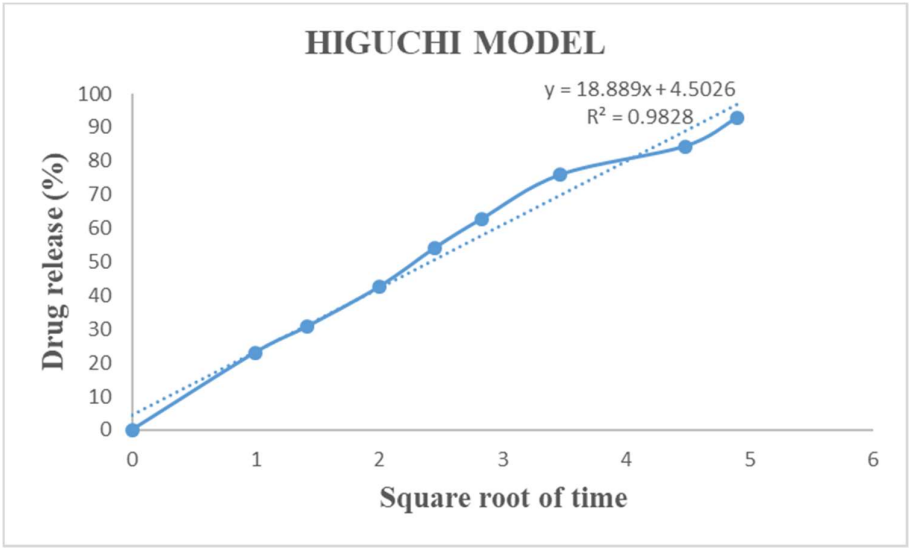

Figure.28: Higuchi model of NF6

Table 24: HIGUCHI MODEL

HIGUCHI MODEL	
Formulation Code	**R2 Value**
NF6	0.9828

5. TEM

TEM is done for analysis of surface morphology. Transmission Electron Microscopy (TEM) The surface morphology of the optimized batch NF6 was established by using Transmission Electron Microscopy (TEM).

Few microliters of diluted nanocochleates solution of optimized batch NF6 was put on 300 mesh copper grid film coated with copper and were air-dried at room temperature. Once it was dried completely, the sample was stained using a 2 %w/v phosphotungstic acid solution removing the excess with a filter paper. Further, the analysis and images of samples were captured by using digital micrograph and Soft Imaging Viewer Software.[6]

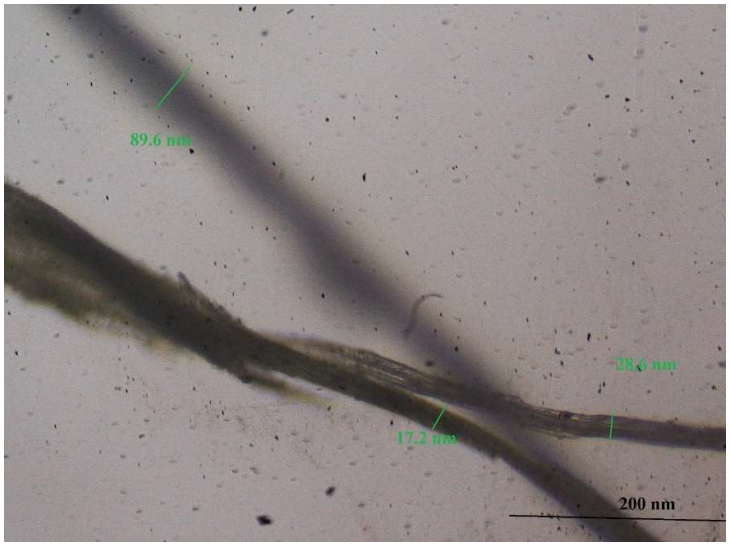

Figure.29: TEM of NF6

6. FTIR spectroscopy

The drug excipients compatibility study was performed by FTIR technique. The Optimized batches NF6 samples were scanned over wave number range of 650-4000 cm-1 with diffraction reflectance scanning technique.[7]

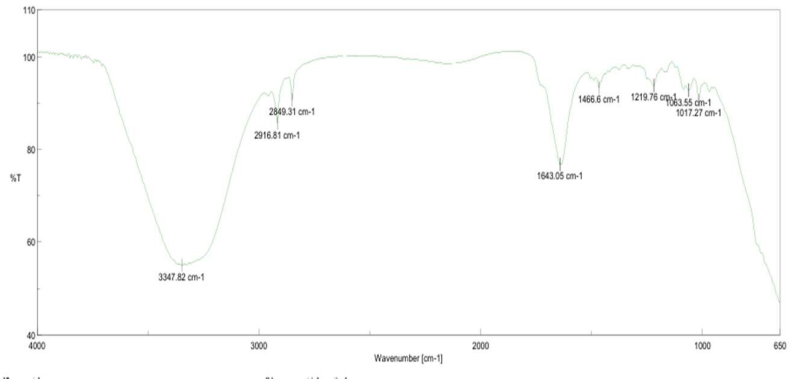

Figure.30: FTIR Spectrum of NF6

Table 25: FTIR interpretation of NF6

Functional Group	Observed Wavenumber (cm^{-1})	Reported Range (cm^{-1})
O–H stretching (alcohol / hydroxyl group)	3347.82	3200–3500
C–H stretching (aliphatic $–CH_2/–CH_3$)	2916.81	2850–2950
C–H stretching (aliphatic)	2849.31	2850–2920
C=O stretching / Amide band	1643.05	1600–1700
C–H bending	1466.6	1450–1470
C–O stretching (alcohol/ether)	1219.76	1200–1300
C–O stretching	1035.55	1000–1100
C–O–C stretching	1017.27	1000–1100

Lyophilization

- The freeze-drying technology was used to prepare freeze-dried Ranolazine -Cochs to increase its storage stability. 10.65 mL samples were placed in a 25 mL vial respectively, and then different freeze-drying protective agents were added to ensure that the physical and chemical properties of samples remained unchanged before and after freeze-drying.

- Mannitol (5% (*w*/*v*) respectively) were selected as freeze-dried protective agents.
- Firstly, prepared samples were put in a −80 °C refrigerator to pre-freeze for 24 h.
- After that, samples were put into a vacuum freeze dryer (Labfreez Instruments Group Co., Ltd) for 24 hat −55 °C and a vacuum pressure under 100 Pa to obtain Ranolazine -Cochs freeze-dried powder.[8]

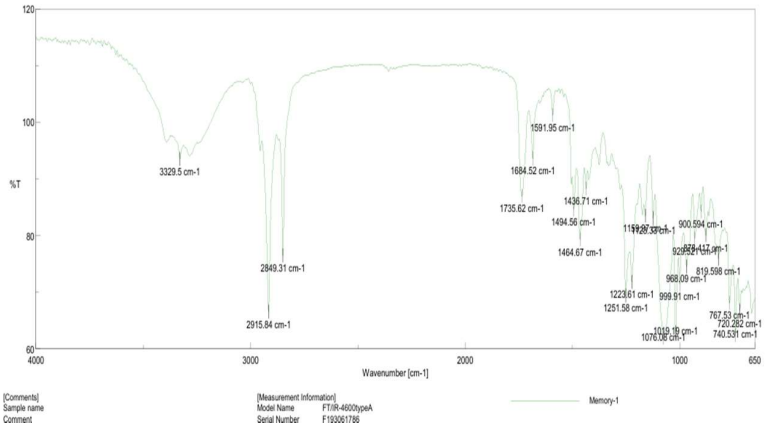

Figure.31: FTIR Spectrum of NF6

Table 26: FTIR interpretation of NF6

Functional Group	Observed Wavenumber (cm^{-1})	Reported Range (cm^{-1})
O–H stretching (hydroxyl)	3329.5	3200–3600
C–H stretching (alkanes)	2915.84, 2849.321	2850–2960
C=O stretching (carbonyl)	1735.62	1700–1750
C=C stretching (aromatic/alkene)	1684.52, 1591.95	1500–1680
CH_2 bending	1494.56, 1464.67,1436.71	1400–1470
C–O stretching (alcohol/ether)	1236.32, 1223.61, 1251.58	1000–1300
C–O–C / C–O vibrations	1159.97,1076.08,1019.1	1000–1150
C–H bending (fingerprint region)	999.91,968.09,928.521,900.594	900–1100
Aromatic C–H out-of-plane bend	878.417,819.598,767.53,740.531	650–900

7. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric (DSC) measurements were carried out on a modulated DSC (Mettler Toledo, SW STARe, and USA). The Optimized batch NF6 were weighed (5.1000 mg), the aluminium pans were used and hermetically covered with lead. The heating rage was 20-300 °C for sample with constant increasing rate of temperature at 10°C /min under nitrogen atmosphere (50-60ml/min). The resultant thermograms of formulation was obtained.[9]

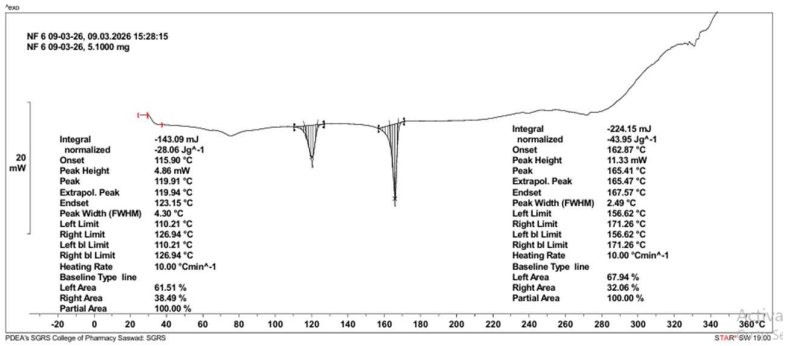

Figure.32: DSC thermogram of NF6

8.FESEM

Field Emission Scanning Electron Microscopy (FESEM) (Nova NanoSEM NPEP303) at the central

instrumental facility (Sppu Pune). Photographs of samples were taken by a different magnification power (5000x).electron microscopy is used to determine the morphology of fractured, surface topography, and texture. The surface morphology of optimized batches was determined.[11]

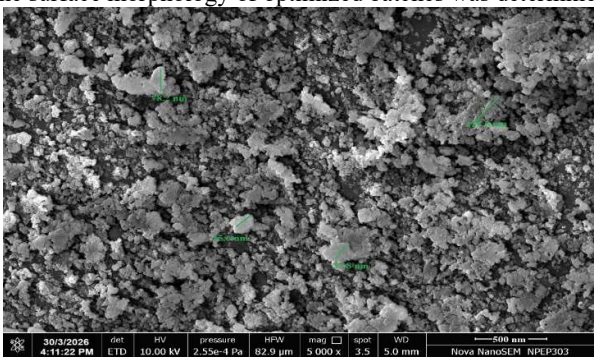

Figure 33: FESEM of NF6

Discussion

The present study successfully developed and optimized Ranolazine-loaded nanoliposomes and nanocochleates for enhanced drug delivery and sustained release. Preformulation studies confirmed the identity, purity, and compatibility of Ranolazine. The observed melting point of 123°C was found within the reported range of 120–124°C, confirming the crystalline nature and purity of the drug. Similarly, the λmax was observed at 274 nm, which matched the reported value and validated the suitability of the UV spectrophotometric analytical method. FTIR studies confirmed the presence of characteristic functional groups without significant peak shifting, indicating compatibility between drug and excipients. DSC thermogram exhibited a sharp endothermic peak around 130.02°C, confirming the crystalline nature of Ranolazine.

The nanocochleates were successfully prepared using calcium chloride-induced trapping method. Optimization using Central Composite Design (CCD) demonstrated that calcium chloride concentration and vortex time significantly influenced particle size and entrapment efficiency. The ANOVA results indicated that the developed quadratic model was significant for both responses.

Among all formulations, batch NF6 showed the most desirable characteristics with particle size of 150.3 nm, PDI of 0.153, and zeta potential of −29.5 mV, indicating good stability and uniformity of the formulation. The optimized formulation also exhibited maximum drug content (96.45%) and high entrapment efficiency (90.56%), suggesting effective incorporation of Ranolazine into the lipidic structure of nanocochleates.

In-vitro drug release studies demonstrated sustained release behavior of the optimized batch NF6 with cumulative drug release of 92.85% up to 24 hours. Drug release kinetic analysis showed that the formulation followed Higuchi diffusion model with R^2 value of 0.9828, indicating diffusion-controlled drug release from the nanocochleate matrix.

TEM and FESEM studies confirmed the formation of nanocochleates with suitable surface morphology and structural integrity. PXRD and DSC studies further indicated partial conversion of crystalline drug into amorphous or less crystalline form, which may contribute to enhanced drug release and bioavailability. Lyophilization using mannitol as cryoprotectant successfully improved storage stability of the optimized formulation.

Overall, the study demonstrated that nanocochleate-based lipidic delivery systems could serve as an effective strategy for improving stability, entrapment efficiency, and sustained release characteristics of poorly soluble drugs like Ranolazine.

Conclusion

The present research work successfully developed and optimized Ranolazine-loaded nanoliposomes and nanocochleates using lipid-based nanocarrier technology. Preformulation studies confirmed the purity, identity, and compatibility of Ranolazine for formulation development. The prepared nanocochleates exhibited desirable physicochemical properties including nanosized particle distribution, low PDI, high zeta potential, excellent drug content, and high entrapment efficiency.

Among all formulations, batch NF6 was identified as the optimized formulation with particle size of 150.3 nm, PDI of 0.153, zeta potential of −29.5 mV, drug content of 96.45%, and entrapment efficiency of 90.56%. The optimized formulation showed sustained drug release up to 24 hours and followed Higuchi diffusion kinetics.

Characterization studies such as FTIR, DSC, PXRD, TEM, and FESEM confirmed successful formation, stability, compatibility, and appropriate morphology of the nanocochleates. The freeze-dried formulation also exhibited improved physical stability.

Therefore, Ranolazine-loaded nanocochleates may serve as a promising lipid-based nano-drug delivery system for enhancing drug stability, controlled release, and therapeutic performance. The developed formulation has potential for further in-

vivo and pharmacokinetic studies for improved management of chronic angina.

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