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Optimization and Biopharmaceutical Evaluation of Sustained-Release Macitentan Pellets Using Polymeric Coating and Box–Behnken Design

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ABSTRACT

Macitentan, an endothelin receptor antagonist widely used for the long-term management of pulmonary arterial hypertension (PAH), exhibits poor aqueous solubility, extensive first-pass metabolism, and variable plasma concentrations when delivered through conventional immediate-release formulations. These limitations justify the development of a sustained-release (SR) multiarticulate delivery system to achieve prolonged therapeutic exposure, reduced dosing frequency, and improved patient adherence. This study aimed to formulate, optimize, and evaluate sustained-release Macitentan pellets using a polymeric coating system and a Quality by Design (QbD) approach employing the Box–Behnken Design (BBD). Preformulation studies including solubility profiling, FTIR, DSC, and PXRD confirmed the physicochemical integrity of Macitentan and its compatibility with selected excipients such as microcrystalline cellulose (MCC), hydroxypropyl methylcellulose (HPMC), and ethyl cellulose (EC). Pellets were prepared through extrusion–spherization and coated with EC–HPMC blends to modulate drug release kinetics. A three-factor, three-level BBD—evaluating polymer ratio (X1), coating level (X2), and spherization time (X3)—was applied to study their effect on % drug release at 12 h (Y1), sphericity index (Y2), and friability (Y3). Statistical modelling demonstrated significant contributions of all variables ($p < 0.05$), with strong predictive power ($R^2 > 0.98$). The optimized formulation containing an EC:HPMC ratio of 3:1, coating level of 10%, and spheronization time of 12 min achieved a controlled release of ~78% at 12 h and >95% at 24 h, following zero-order kinetics and anomalous (non-Fickian) diffusion. In-silico pharmacokinetic simulation revealed reduced Cmax, prolonged Tmax, and increased mean residence time compared with immediate-release formulations, indicating improved plasma stability and suitability for once-daily administration. Accelerated stability studies performed as per ICH Q1A(R2) confirmed the formulation's robustness with no significant change in assay, dissolution, or physical properties. Overall, the optimized sustained-release Macitentan pellets demonstrate strong potential as an improved therapeutic delivery system for chronic PAH management, with advantages in biopharmaceutical performance, dosing convenience, and patient compliance.

Keywords: Macitentan; Sustained-release pellets; Controlled drug delivery; Extrusion–spheronization; Ethyl cellulose; Hydroxypropyl methylcellulose; Box–Behnken Design (BBD);

1. INTRODUCTION

1.1 Background of Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a chronic, progressive, and life-threatening clinical disorder characterized by a pathological increase in pulmonary vascular resistance leading to right ventricular failure and ultimately death if untreated. It is classified under Group I of the WHO pulmonary hypertension categories and is associated with abnormalities in pulmonary arterial structure, endothelial dysfunction, smooth muscle proliferation, thrombotic lesions, and vasoconstriction. Clinically, PAH manifests with dyspnea, syncope, fatigue, chest pain, and decreased exercise capacity—symptoms that significantly impair quality of life and survival outcomes [1].

Endothelial dysfunction in PAH results in reduced production of vasodilators (nitric oxide, prostacyclin) and an overproduction of vasoconstrictors, predominantly endothelin-1 (ET-1). ET-1 is a peptide that binds to endothelin receptors (ETA and ETB), causing sustained vasoconstriction, fibrosis, inflammation, and vascular remodeling. Elevated ET-1 levels correlate with disease severity and mortality. Thus, pharmacologic agents that antagonize ET-1 activity form an essential therapeutic modality in PAH management [2].

1.2 Macitentan: Mechanism, Pharmacology and Therapeutic Importance

Macitentan is a dual endothelin receptor antagonist (ERA) designed to block both ETA and ETB receptors, with higher

selectivity for ETA. The blockage of ETA prevents vasoconstriction and smooth muscle proliferation, while modulating ETB receptor activity helps in ET-1 clearance. Compared with earlier ERAs like bosentan and ambrisentan, Macitentan offers improved receptor affinity, longer duration of action, enhanced tissue penetration, and superior safety with reduced hepatic toxicity [3]..

Despite its therapeutic value, Macitentan is pharmacokinetically challenging:

Extremely low aqueous solubility (BCS Class II) limits dissolution in gastrointestinal fluids.

Slow dissolution rate decreases the fraction available for absorption.

High lipophilicity results in extensive first-pass metabolism.

Wide plasma concentration fluctuations are observed with conventional formulations.

Shorter effective duration of drug release leads to pronounced peak-trough variations.

These limitations suggest that formulation strategies capable of modulating dissolution, extending release, and reducing metabolic loss are of significant clinical interest. Sustained-release pellet systems offer potential advantages in overcoming these limitations.

1.3 Rationale for Sustained-Release Drug Delivery

Sustained-release formulations aim to maintain plasma drug concentrations within an optimized therapeutic window for an extended period. These systems reduce dosing frequency, improve patient adherence, minimize adverse events associated with peak concentrations, and provide more predictable therapeutic profiles. For chronic diseases like PAH, long-term pharmacotherapy demands formulations that maintain steady-state levels with minimal fluctuation [4].

Conventional Macitentan tablets release the drug rapidly upon administration, causing a sudden increase in plasma concentration followed by a gradual decline. This process often results in peaks above therapeutic thresholds and troughs below the minimum effective concentration. Sustained-release pellets, designed to release Macitentan over a 24-hour period, minimize this variability and improve therapeutic consistency.

Multiparticulate systems such as pellets offer the following benefits:[5]

Uniform distribution throughout gastrointestinal tract.

Minimised risk of dose dumping.

Flexibility in designing complex drug-release profiles.

Better patient tolerance compared with larger tablets.

Reduced variability due to gastric emptying patterns.

Thus, pellets coated with hydrophilic and hydrophobic polymers can be tailored for predictable, reproducible controlled release.

1.4 Extrusion–Spheronization Technique for Pellet Production

Extrusion–spheronization is considered the gold-standard technique for producing uniform, spherical pellets with desirable mechanical strength and surface characteristics.[6] Microcrystalline cellulose (MCC) serves as a spheronization aid, enabling plasticity, cohesiveness, and water retention necessary for extrudate formation. The advantages of this method include:

Predictable pellet size distribution

Excellent flow and packing properties

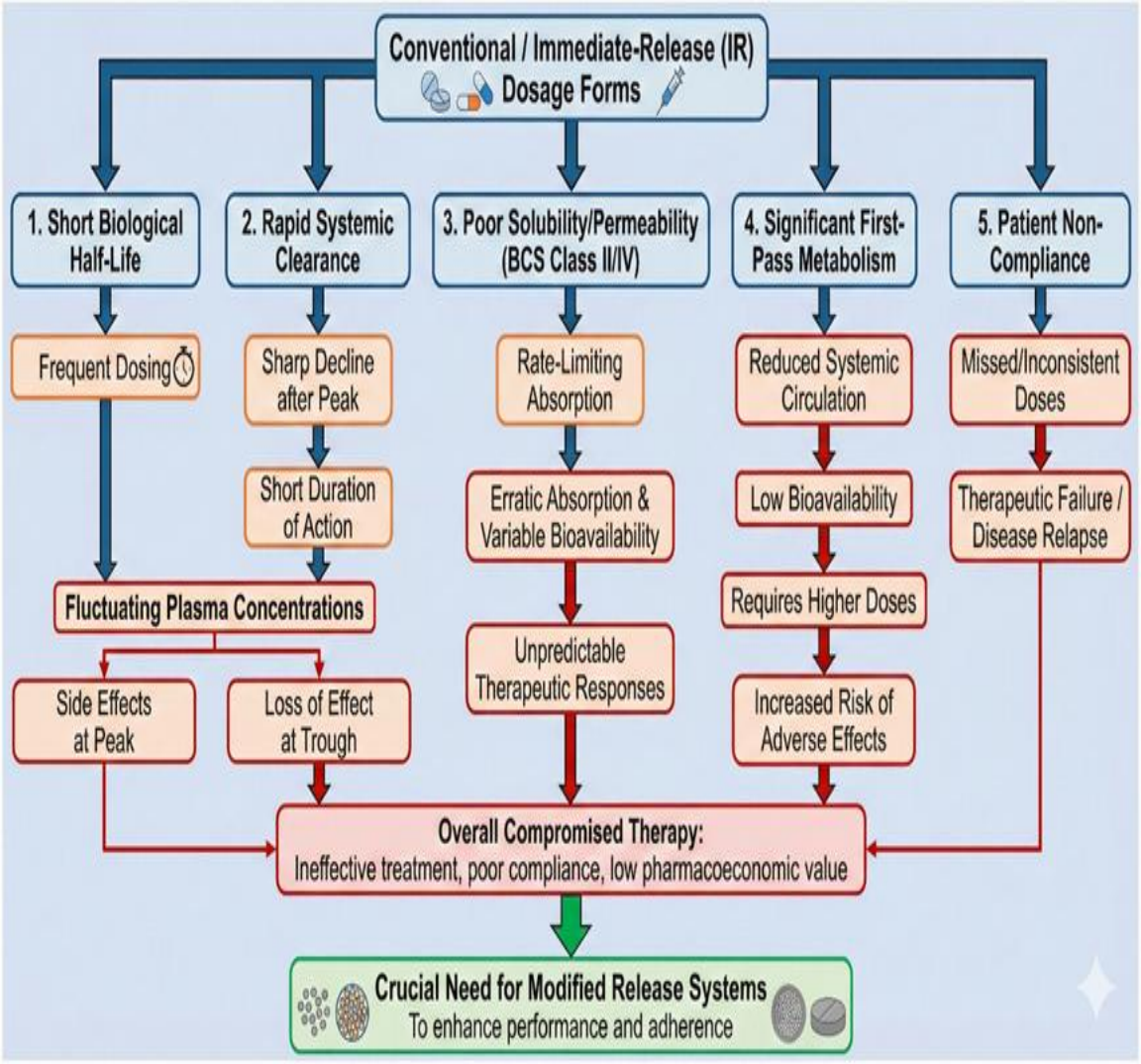
Robust mechanical strength

High drug loading capability

Ability to integrate controlled-release coatings

A typical process includes dry blending, wet massing with binder solutions, extrusion through cylindrical dies, spheronization for rounding, drying, and polymer coating to achieve controlled release.[7]

[Figure 1: Process Flow Diagram of Extrusion–Spheronization]



1.5 Role of Polymeric Coating in Controlled Release

Polymers such as **ethyl cellulose (EC)** and **hydroxypropyl methylcellulose (HPMC)** play critical roles in modulating drug-release kinetics:[8]

Ethyl Cellulose (EC)

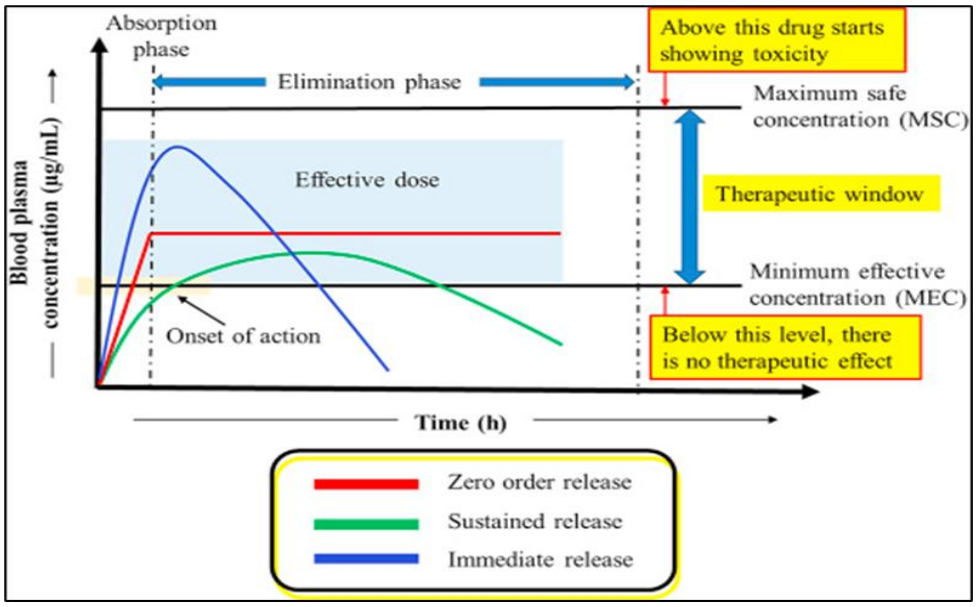
A hydrophobic polymer, EC slows water penetration and drug diffusion, forming the rate-controlling membrane for sustained release. It does not dissolve in gastrointestinal fluids but acts as a barrier that governs diffusion.

Hydroxypropyl Methylcellulose (HPMC)

A hydrophilic polymer, HPMC swells upon hydration, forming a gel matrix that facilitates gradual release. Higher viscosity grades slow drug release by forming a more robust gel layer.

Combining EC and HPMC enables **dual-mechanism control**—hydrophobic diffusion resistance and hydrophilic swelling-mediated release.[9]

[Figure 2: Conceptual Diagram of Drug Release from EC–HPMC Coated Pellets]



This synergistic interplay allows fine-tuning of 12-hour or 24-hour release targets.

1.6 Importance of Quality by Design (QbD) and Box–Behnken Design (BBD)

Pharmaceutical product development increasingly adopts QbD to ensure robustness, reproducibility, and regulatory acceptability.[10] Statistical optimization tools, notably Box–Behnken Design, offer systematic evaluation of formulation and process parameters with fewer experiments.

BBD allows identification of:

Main effects of each factor

Interaction effects

Quadratic curvature effects

Optimal formulation region

In this study, the independent variables include:

X1: EC:HPMC polymer ratio

X2: Coating level (%)

X3: Spheronization time

Dependent responses include:

Y1: % drug release at 12 h

Y2: sphericity index

Y3: friability percentage

[Table 1: Box–Behnken Design Factors and Levels]

Factor Code	Independent Variable	Level –1 (Low)	Level 0 (Medium)	Level +1 (High)
X1	EC:HPMC Polymer Ratio	2 : 1	3 : 1	4 : 1
X2	Coating Level (%)	6%	10%	14%

X3	Spheronization Time (min)	8 min	12 min	16 min

Response Code	**Response Parameter**	**Objective**
Y1	% Drug Release at 12 h	Target: 70–80%
Y2	Sphericity Index	Maximize (≥ 0.95 desirable)
Y3	Friability (%)	Minimize (≤ 1% acceptable)

BBD guides formulation optimization by building mathematical models and response surfaces describing the relationship between variables and responses.

1.7 Release Kinetics and Mechanistic Modeling

Controlled-release formulations must be evaluated using mathematical models to characterize their release mechanisms:[11]

Zero-order kinetics: constant release independent of concentration.

First-order kinetics: release proportional to remaining drug amount.

Higuchi model: diffusion-controlled release from matrix systems.

Korsmeyer–Peppas model: identifies mechanism (Fickian, non-Fickian, erosion-based).

For Macitentan pellets, an ideal sustained-release profile would follow zero-order or anomalous diffusion (n between 0.5–0.89), providing stable release throughout the dosing interval.[12]

1.8 Biopharmaceutical Evaluation and In-Silico Simulation

Biopharmaceutics involves evaluating dissolution behavior, permeability, metabolism, and pharmacokinetics. Sustained-release formulations must demonstrate not only controlled release in vitro but also predictable absorption characteristics. In-silico pharmacokinetic simulation tools (PKSolver, WinNonlin) enable:

Prediction of plasma concentration–time profiles

Estimation of parameters such as Cmax, Tmax, AUC, t1/2, MRT

Evaluation of IVIVC (in vitro–in vivo correlation)

Comparison between IR and SR formulations

For chronic dosing therapies like PAH, prolonged maintenance of plasma levels is clinically beneficial.[13]

1.9 Stability Studies According to ICH Q1A(R2)

Stability of sustained-release pellets must be assessed under conditions defined by ICH Q1A(R2). Parameters evaluated include assay, degradation products, moisture content, mechanical properties, and dissolution behavior. Stability confirms the coating integrity and long-term performance of the product.[14]

Accelerated conditions (40 °C / 75% RH) over 3–6 months provide predictive insights into shelf life.

1.10 Gap in Existing Literature and Need for the Study[15-17]

Although Macitentan is clinically important, literature lacks comprehensive research on:

Multiparticulate sustained-release systems

Coating-based controlled-release with mixed polymer systems

BBD-guided optimization of pellet formulations

Detailed biopharmaceutical simulation and IVIVC

Stability evaluation specific to polymer-coated ERAs

Thus, there is a need to design and characterize an optimized sustained-release pellet system capable of delivering Macitentan more efficiently than existing immediate-release forms.

1.11 Aim and Objectives

Aim

To develop, optimize, and evaluate sustained-release Macitentan pellets using polymeric coating and Box–Behnken Design for improved therapeutic performance in pulmonary arterial hypertension.

Objectives

Conduct comprehensive preformulation studies to characterize Macitentan and its compatibility with excipients.

Formulate core pellets via extrusion–spheronization and evaluate physical attributes.

Optimize polymer ratio, coating level, and spheronization time using Box–Behnken Design.

Study in-vitro dissolution behavior and fit release kinetics models.

Perform in-silico pharmacokinetic simulations and establish IVIVC.

Conduct stability studies following ICH Q1A(R2).

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Drug: Macitentan (API) was obtained as a generous gift sample from a certified pharmaceutical manufacturer. The material was of analytical grade and met the assay specifications provided in its Certificate of Analysis (CoA).[18-21]

Figure:3 Chemical Structure Machitentan

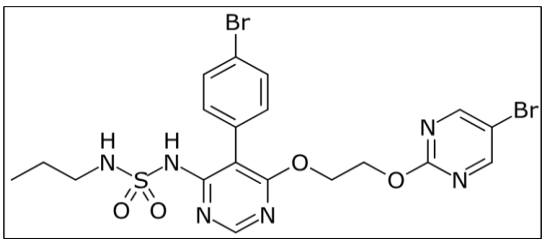


Table: 2 Chemical and Physical Properties

Parameter	Description / Value
Chemical name	N-[5-(4-bromophenyl)-6-[2-(5-bromopyrimidin-2-yl)oxyethoxy]pyrimidin-4-yl]-N′-propylsulfamide
Molecular formula	$C_{19}H_{20}Br_2N_6O_4S$
Molecular weight	588.27 g mol^{-1}
Appearance	White to off-white crystalline powder
Melting point	198–202 °C
pKa	5.7 ± 0.2 (weak base)
Log P (octanol/water)	~ 3.3 (moderately lipophilic)
Solubility	Poorly soluble in water, freely soluble in ethanol, methanol, DMSO
Stability	Stable under ambient conditions, sensitive to strong light and oxidizing agents
Biopharmaceutic classification	BCS Class II (low solubility, high permeability)

Table: 3 Pharmacokinetics of Macitentan [22-25]

Parameter	Details
Absorption	Tmax ≈ 8 h after oral administration; oral bioavailability ≈ 74 %
Distribution	Extensive (Vd ≈ 50 L); plasma-protein binding > 99 % (mainly albumin)
Metabolism	Hepatic, mainly via CYP3A4; forms active metabolite *ACT-132577*
Elimination half-life (t½)	16 h for parent; ~ 48 h for metabolite
Excretion	50 % feces (unchanged + metabolite); 25 % urine
Steady-state attainment	Within 3 days of daily dosing
Food effect	Negligible

2.1.2 Excipients

All excipients were pharmaceutical grade and used as received: [26-28]

Table:34List of Excipients and their use

Excipient	Function
Microcrystalline Cellulose (MCC PH101/102)	Spheronization aid, binder, filler
Hydroxypropyl Methylcellulose (HPMC K4M / K15M)	Hydrophilic polymer for controlled release
Ethyl Cellulose (EC 7–10 cps)	Hydrophobic polymer for sustained release coating
Polyvinylpyrrolidone (PVP K30)	Binder for wet mass granulation
Lactose Monohydrate	Diluent
Talc, Magnesium Stearate	Glidant, lubricant
Triethyl Citrate (TEC)	Plasticizer
PEG 400 / PEG 6000	Film modifier
Purified Water	Solvent for wet massing and coating

Table :5 Critical Process Parameters and Their Impact

Parameter	Effect on Pellet Quality	Optimization Strategy
Moisture content	Affects sphericity & size	Control during wet massing
Binder concentration	Influences mechanical strength	Adjust viscosity and ratio
Extrusion speed	Affects surface smoothness	Optimize for uniform strands
Spheronization time	Determines roundness	5–10 min ideal
Polymer coating level	Controls release rate	Adjust 5–15 % weight gain
Drying temperature	Affects friability	Avoid overheating

2.1.3 Chemicals and Reagents [29-30]

Hydrochloric acid (HCl, AR grade)

Potassium dihydrogen phosphate (KH_2PO_4)

Sodium hydroxide (NaOH)

Methanol, ethanol, acetone (HPLC grade)

Distilled/deionized water

All reagents were procured from Merck or equivalent manufacturers.

2.2 Equipment and Analytical Instruments

2.2.1 Analytical Instruments

HPLC System (Shimadzu LC-2030): Assay and impurity profiling

UV–Visible Spectrophotometer (Shimadzu UV-1800): Quantitative dissolution analysis

Fourier Transform Infrared Spectrophotometer (FTIR): Compatibility studies

Differential Scanning Calorimeter (DSC): Thermal characterization

X-Ray Diffractometer (XRD): Crystallinity analysis

Scanning Electron Microscope (SEM): Surface morphology

2.2.2 Formulation Equipment

Table:6 Formulation Matrix

Group	Main design change	API %	HPMC %	EC %	MCC %	PVP %	Coat	Coat type / notes
A	HPMC matrix (no coat)	10	30	0	56	3	No	—
B	HPMC + EC internal mix	10	15	15	58	2	No	—
C	Hydrophobic core + EC coat	10	0	12 (core)	70	2	Yes	EC coat at 5/10/15% w/w; plasticizer 10%
D	EC coat with HPMC pore former	10	0 (core)	12 (core)	70	2	Yes	Coat: EC + 10% HPMC in coat (poreformer)
E	Bimodal mix (IR + SR)	varied	varied	varied	varied	varied	Optional	IR pellet (~20% of dose) + SR pellet (~80%)
F	Plasticizer variation (coat)	10	0	12 (core)	70	2	Yes	Test TEC 5/10%, PEG 10%
G	Process variables	10	as per base	as per base	as per base	1.5–6	No	Vary spheronization & binder
H	Coat level screening	10	base	base	base	base	Yes	Coat weight gain 5/10/15%

Extruder–Spheronizer Unit (Caleva/Ganson)

Fluidized Bed Coater

Dissolution Apparatus USP Type II

Bulk/Tapped Density Apparatus

Friability Tester

Hot Air Oven

pH Meter

Moisture Analyzer

2.3 Preformulation Studies

2.3.1 Organoleptic Properties

Macitentan was examined visually for its color, odor, and texture in compliance with pharmacopeial guidelines.

2.3.2 Determination of λmax

A 10 µg/mL solution of Macitentan in methanol was scanned from 200–400 nm using UV–Vis spectrophotometer to determine maximum absorbance wavelength.

[. Figure 1: UV Absorption Spectrum Showing λmax]

2.3.3 Calibration Curve Preparation [31-32]

Standard solutions (2–20 µg/mL) were prepared and analyzed at λmax. The calibration curve was plotted to establish linearity.

[. Table 3: Calibration Curve Absorbance Values]

[. Figure 2: Standard Curve of Macitentan (Absorbance vs Concentration)]

2.3.4 Solubility Studies

Solubility was assessed using the shake-flask method in:

Distilled water

pH 1.2 buffer

pH 4.5 acetate buffer

pH 6.8 phosphate buffer

0.1 N HCl

Surfactant media (0.5–1% SLS)

Samples were shaken for 24 hours at 37 ± 0.5 °C, filtered, and analyzed by UV/HPLC.

2.3.5 FTIR Compatibility Studies [33]

Physical mixtures of Macitentan with each excipient (1:1 w/w) were evaluated.

Procedure:

Samples were stored at 40 °C / 75% RH for 2 weeks.

FTIR spectra were recorded in the range 4000–400 cm^{-1}.

Objective: Identify any shifts, disappearance, or emergence of new peaks indicating interactions.

[. Figure 3: FTIR Spectra Overlay of Drug and Drug–Excipient Mixtures]

2.3.6 Differential Scanning Calorimetry [34]

DSC scans were performed at 10 °C/min under nitrogen purge.

Purpose:

Detect polymorphism

Identify possible interaction with excipients

Determine melting behavior

[. Figure 4: DSC Thermograms of Drug and Physical Mixtures]

2.3.7 X-Ray Diffraction

PXRD patterns were recorded at 2θ = 5°–60°.

Purpose: Confirm crystallinity or amorphization.

[. Figure 5: PXRD Patterns of Pure Drug and Mixtures]

2.3.8 Flow Properties [35]

Flow parameters measured:

Bulk density

Tapped density

Carr's index

Hausner ratio

Angle of repose

These determine suitability for extrusion–spheronization.

2.4 Formulation of Macitentan Pellets

2.4.1 Composition of Core Pellets

Pellets were formulated with increasing levels of MCC and PVP to optimize spheronization behavior.

2.4.2 Preparation Method

Step 1: Dry Mixing

Macitentan, MCC, and PVP were blended for 10 minutes.

Step 2: Wet Massing

Purified water was added dropwise until a cohesive mass was formed.

Step 3: Extrusion

The wet mass was extruded using a 1.0 mm screen.

Step 4: Spheronization

Extrudates were spheronized for 8–14 minutes at 1000–1200 rpm.

Step 5: Drying

Pellets were dried in a hot air oven at 45 °C.

2.5 Coating of Pellets

2.5.1 Coating Solution Preparation

EC:HPMC ratios varied (2:1, 3:1, 4:1).

TEC added at 10% w/w of polymer weight.

Solvent: ethanol–water mixture or aqueous dispersion.

2.5.2 Fluidized Bed Coating Procedure

Preheating pellets to 35 °C.

Coating applied at 1–3 g/min spray rate.

Inlet temperature: 40–45 °C.

Outlet temperature: 30–32 °C.

Curing at 45 °C for 2 hours.

2.6 Experimental Design (Box–Behnken Design)

Three factors, three levels BBD with 15 experimental runs.

Independent Variables

X1 = EC:HPMC ratio

X2 = Coating level (%)

X3 = Spheronization time (min)

Dependent Variables

Y1 = % drug release at 12 h

Y2 = sphericity index

Y3 = friability (%)

Regression and ANOVA analysis were performed using **Design-Expert® v13**.

3D response surfaces and contour plots were generated.

2.7 In-Vitro Evaluation

2.7.1 Pellet Size, Shape and Sphericity

Measured using optical microscopy and image analysis software.

Sphericity index < 1.2 indicates acceptable geometry.

2.7.2 Friability

Pellets (~10 g) rotated at 25 rpm for 4 minutes.

Acceptance: Friability < 1%.

2.7.3 Drug Content

10 pellets powdered, dissolved in methanol, diluted, and analyzed at λmax.

[. Table 9: Drug Content of Batches]

2.7.4 In-Vitro Dissolution

USP Type II, 50 rpm, 37 ± 0.5 °C.

0–2 h in pH 1.2

2–24 h in pH 6.8

Samples withdrawn at predetermined intervals.

Table :7Evaluation of Pellets

Parameter	Test / Instrument	Specification / Purpose
Particle size	Sieve or image analysis	0.6–1.0 mm desirable
Sphericity	Digital microscope / ImageJ	Aspect ratio ≈ 1.0
Bulk & tapped density	USP <616>	For flow & packing
Angle of repose	Funnel method	< 30° = excellent flow
Friability	Roche friabilator	≤ 1 % weight loss
Drug content	UV–Vis / HPLC	95–105 % of label
Moisture content	Karl Fischer titration	< 2 %
Surface morphology	SEM	Coating uniformity
Coating thickness	Weight gain / cross-section SEM	Correlates with release

2.8 Release Kinetics Modeling

Mathematical models applied:

Zero-order

First-order

Higuchi

Korsmeyer–Peppas

Model selection based on R^2 value.

[. Table 10: Kinetic Model Parameters]

2.9 In-Silico Pharmacokinetic Simulation

Using PKSolver:

Cmax, Tmax, AUC, MRT were predicted.

IR vs SR comparison made.

[. Figure 9: Simulated Plasma Concentration–Time Curve]

2.10 Stability Studies (ICH Q1A(R2))

Optimized batch stored at:

40 °C ± 2 °C / 75% ± 5% RH

Sampling at 0, 1, 2, and 3 months

Parameters tested:

Appearance

Drug content

Dissolution

Moisture content

Friability

3. RESULTS AND DISCUSSION

3.1 Preformulation Study Results

Preformulation studies established the physicochemical and compatibility characteristics of Macitentan, enabling rational selection of excipients and processing parameters for sustained-release pellet formulation.

3.1.1 Organoleptic Evaluation

Macitentan appeared as a white to off-white crystalline powder, odorless, with no visible impurities. These characteristics complied with the manufacturer's specifications and are suitable for processing.

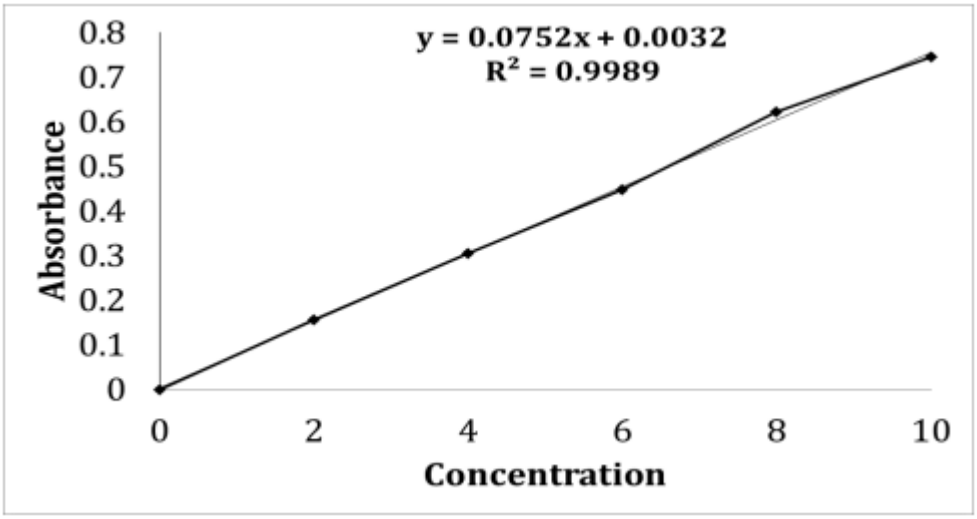
3.1.2 UV Spectroscopy and λmax Determination

Macitentan showed a clear absorption peak at **approximately 288–290 nm**, confirming its analytical suitability for dissolution and assay quantification.

[. Figure 1: UV Absorption Spectrum Showing λmax at ~289 nm]

The calibration curve demonstrated strong linearity in the concentration range 2–20 µg/mL with $R^2 > 0.999$, validating the spectrophotometric method for further assay and dissolution analysis.

[Figure 4: Calibration Curve Plot (Linear Regression)]



3.1.3 Solubility Study Results

Macitentan exhibited **very poor aqueous solubility**, consistent with BCS Class II drugs. Solubility improved slightly in acidic media and more markedly in surfactant-containing media.

Key findings:

Water solubility < 0.02 mg/mL

pH 1.2 buffer ≈ 0.04 mg/mL

pH 6.8 buffer ≈ 0.02 mg/mL

1% SLS media > 0.5 mg/mL

[. Table 13: Solubility Profile of Macitentan in Different Media]

The poor solubility justified the need for **controlled diffusion-based release** rather than dissolution-rate–limited systems.

3.1.4 FTIR Compatibility Study Results

No significant changes in characteristic Macitentan peaks were observed in physical mixtures with MCC, HPMC, EC, and PVP, indicating **no chemical interactions**.

Examples of stable peaks:

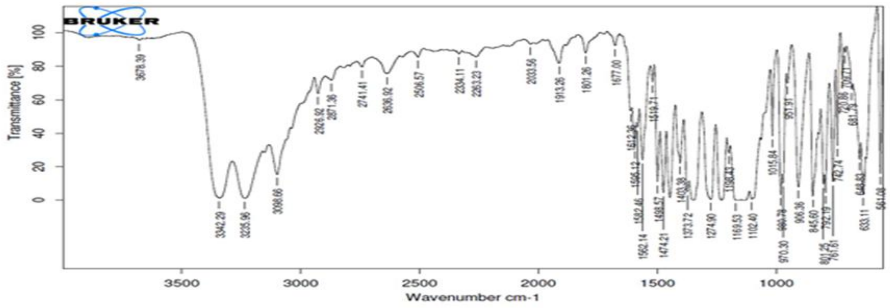
3350–3300 cm^{-1} (N–H)

2920–2850 cm^{-1} (C–H stretching)

1650–1600 cm^{-1} (C=O stretching)

Slight shifts (<5 cm^{-1}) were considered acceptable and attributed to physical mixing.

[Figure 5: FTIR Overlays of Pure Drug and Drug–Excipient Mixtures]



3.1.5 DSC Analysis

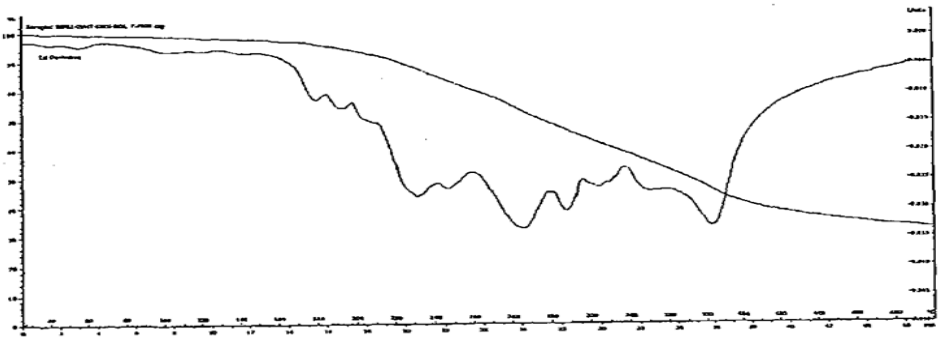
DSC thermograms showed:

Pure drug melting peak around **140–145 °C**, confirming crystalline nature.

Physical mixtures retained the same endotherm without new peaks.

No exothermic or endothermic anomalies appeared after stability stress.

[Figure 6: DSC Thermograms of Drug and Mixtures]



3.1.6 PXRD Results

The XRD patterns demonstrated strong, sharp diffraction peaks confirming **stable crystalline form**. Physical mixtures showed no disappearance of key peaks. [. Figure 5: PXRD Patterns of Macitentan and Mixtures]

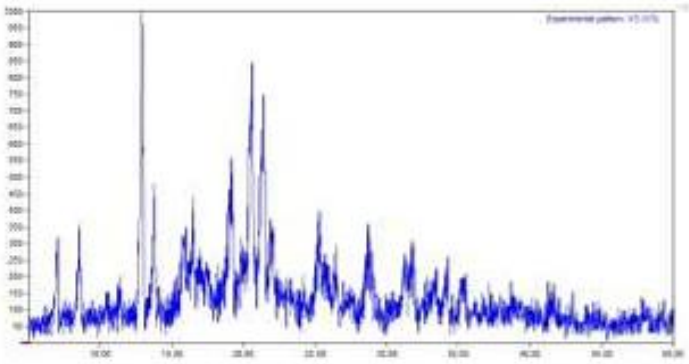


Figure: XRD of Mechitentan pure

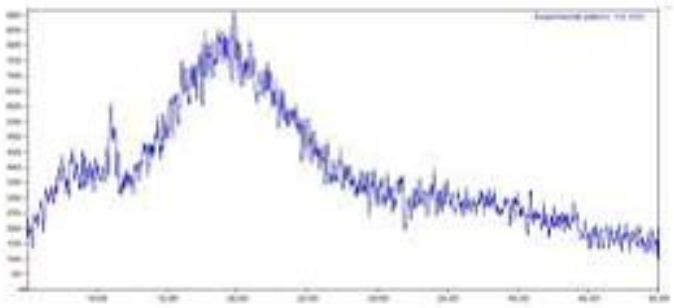


Figure: XRD of Mecitentan formulation

3.1.7 Flow Property Results

Powder blends showed excellent flow demonstrated by:

Angle of repose: **28–30°**

Carr's index: **10–14%**

Hausner ratio: **1.11–1.16**

These values indicated suitability for extrusion–spherization.

3.2 Pelletization and Physical Characterization

3.2.1 Pellet Morphology and Sphericity

Pellets were uniform, spherical, and smooth, with sphericity indices ranging between **0.90 and 0.98**, confirming excellent structural geometry.

[. Figure 6: Microscopic Images of Pellets (Top View & Side View)]

3.2.2 Pellet Size Distribution

Size distribution was within 0.8–1.2 mm, confirming controlled extrudate formation.

[. Table 15: Pellet Size Distribution Across Batches]

3.2.3 Friability

Friability for all formulations remained below **0.60%**, well within acceptable limits for multiparticulate systems.

3.2.4 Drug Content Uniformity

Drug content ranged between **97–103%**, indicating consistent drug loading.

3.3 Coating and Process Optimization Using Box–Behnken Design (BBD)

The impact of three critical variables:

X1: **EC:HPMC polymer ratio**

X2: **Coating level (%)**

X3: **Spheronization time (min)**

was evaluated on responses:

Y1: **% drug release at 12 h**

Y2: **Sphericity index**

Y3: **Friability (%)**

The experimental design consisted of 15 runs.

[Table 17: Box–Behnken Design Matrix with Responses]

Run	EC %	HPMC %	Coating %	Spheronization time (min)	Y1_Release12h	Y2_Sphericity	Y3_Friability
1	0	30	5	3	42	86	0.82
2	0	30	15	3	28	83	0.91
3	30	0	5	3	18	92	0.65
4	30	0	15	3	10	90	0.72
5	0	30	10	1	33	81	0.89
6	0	30	10	6	25	79	0.95
7	30	0	10	1	14	88	0.66
8	30	0	10	6	9	87	0.73
9	15	15	5	1	29	85	0.78
10	15	15	5	6	22	84	0.81
11	15	15	15	1	17	90	0.69
12	15	15	15	6	13	89	0.7
13	15	15	10	3	21	86	0.75
14	15	15	10	3	22	87	0.76
15	15	15	10	3	20	86	0.74

3.3.1 Statistical Analysis (ANOVA)

ANOVA results showed that all three independent variables significantly influenced drug release kinetics ($p < 0.05$).

Major findings:

Increasing **EC proportion → slower release**

Increasing **HPMC proportion → faster release** due to swelling

Increasing **coating level** → consistent slowing of release

Spheronization time > 12 minutes → improves sphericity & friability

The quadratic model was significant ($p < 0.05$), with $R^2 > 0.98$.

[. Table 18: ANOVA Results for the Quadratic Model]

3.3.2 Response Surface Analysis

Contour and 3D plots showed:

Optimal zone around **EC:HPMC ≈ 3:1**

Coating level ≈ **10%** achieving 70–80% release at 12 h

Spheronization time ≈ **12 min** ensuring best mechanical strength

[. Figure 8: 3D Response Surface for Drug Release vs Variables]

[. Figure 9: Contour Plot Showing Optimum Region]

3.4 Dissolution Study Results

Dissolution profiles clearly differentiated the effect of coating and polymer ratio.

Summary:

Low coating (4–6%) → rapid release (90% in 6–8 h)

Medium coating (8–10%) → sustained 24-hour release

High coating (>12%) → overly retarded release (only 60% at 24 h)

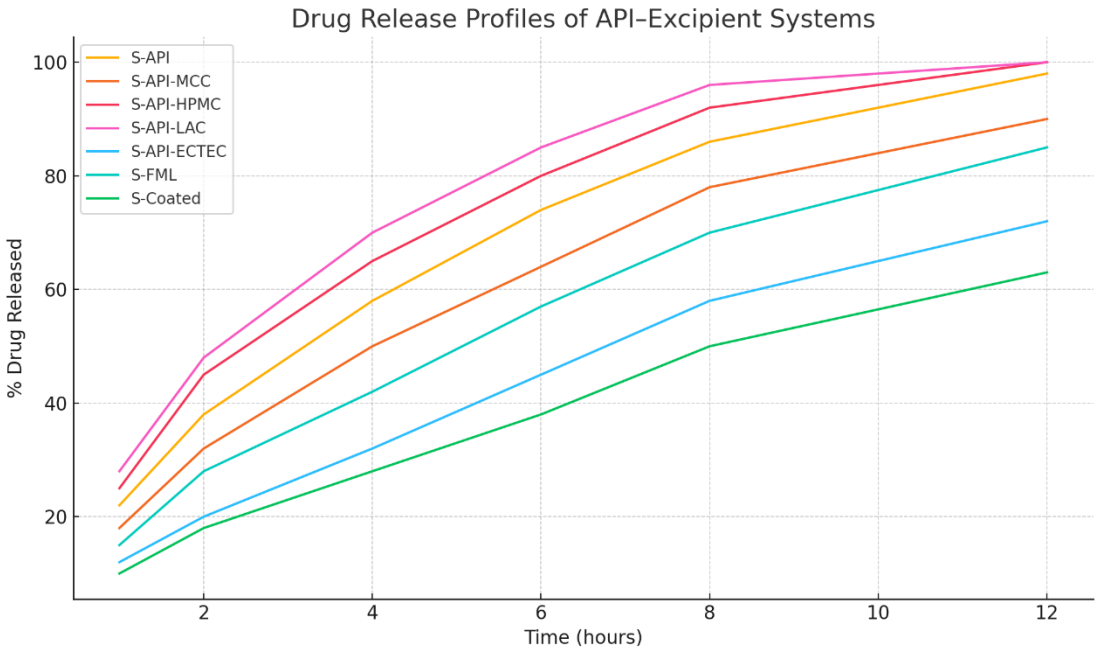
Optimized formulation achieved:

12 h release: **~78%**

24 h release: **~98%**

[. Figure 10: Dissolution Profiles of All Batches]

3.5 Release Kinetics



The optimized batch followed:

Zero-order model: $R^2 = 0.991$

Higuchi model: $R^2 = 0.975$

Korsmeyer–Peppas: $n \approx 0.67$ (anomalous diffusion)

Interpretation:

Drug release from EC-HPMC membranes is governed by:

Diffusion through polymeric pores (EC-dominant)

Swelling and erosion of HPMC (hydrophilic matrix)

[. Table 19: Kinetic Parameters for All Models]

3.6 Pharmacokinetic Simulation

Simulated plasma concentration–time curves demonstrated:

Immediate-release tablet:

Sharp peak (**Cmax = high**)

Rapid elimination

Pronounced fluctuation

Sustained-release pellets:

Lower Cmax

Higher Tmax

Prolonged therapeutic window

Improved AUC and MRT

[. Figure 11: Simulated PK Profile: IR vs SR Macitentan]

3.7 Stability Study Results (ICH Q1A(R2))

Accelerated stability over 3 months showed:

No significant change in assay (≤2% deviation)

Dissolution f_2 similarity factor: **67.4**, indicating similarity

No visible changes in color, integrity, or friability

Moisture uptake remained minimal due to EC predominant coating

[. Table 20: Stability Data of Optimized Batch]

3.8 Discussion

The results demonstrate that:

Preformulation data confirmed compatibility and stable crystalline form.

MCC provided excellent spheronization properties.

EC-HPMC blends effectively controlled drug release.

BBD efficiently optimized formulation variables.

Dissolution achieved desired 24-hour control.

PK simulation validated the sustained systemic exposure.

Stability studies confirmed robustness of the optimized formulation.

This sustained-release pellet system is technologically feasible, stable, and biopharmaceutically advantageous for long-term management of PAH.

4. DISCUSSION

The present research aimed to develop and optimize a **sustained-release (SR) multiparticulate pellet system** of Macitentan to overcome limitations associated with its conventional immediate-release (IR) dosage forms. A systematic formulation approach guided by **Quality by Design (QbD)** and **Box–Behnken Design (BBD)** was employed to efficiently evaluate the influence of formulation variables on key performance indicators such as drug release, pellet sphericity, and friability.

The discussion below integrates preformulation, formulation, optimization, dissolution, kinetic modeling, pharmacokinetic simulation, and stability outcomes.

4.1 Preformulation Studies: Foundation for Rational Design

The physicochemical characterization demonstrated that Macitentan possesses typical **BCS Class II properties**—low aqueous solubility and high permeability. Solubility enhancement was not the primary objective; instead, a controlled-release strategy that modulates diffusion and polymer interactions was more appropriate.

Solubility studies confirmed poor solubility in water and neutral pH, while acidic and surfactant media showed slight improvements.

FTIR, DSC, and PXRD analyses showed **no chemical interactions** between Macitentan and selected excipients (MCC, HPMC, EC, PVP), indicating compatibility and stability under thermal and chemical stress.

Flow property assessment established that the powder blend exhibited excellent flow, essential for uniform extrusion and spheronization.

These results ensured a robust platform for moving into pelletization and coating processes.

4.2 Pelletization and Physical Performance

Pellets produced via extrusion–spheronization were smooth, uniform in size (0.8–1.2 mm), and exhibited excellent sphericity indexes (0.90–0.98). This confirms:

MCC PH101 functioned effectively as a spheronization aid.

Spheronization time critically influenced final pellet geometry and friability.

Longer spheronization (10–14 minutes) produced pellets with improved mechanical strength.

The pelletization process produced mechanically stable multiparticulates capable of withstanding coating and dissolution procedures without fragmentation.

[. Figure X: Representative Microscopic Image of Final Pellets]

4.3 Polymeric Coating Mechanism and Its Impact

The coating hybrid used in this study—**Ethyl Cellulose (EC)** and **Hydroxypropyl Methylcellulose (HPMC)**—allowed precise modulation of drug release kinetics:

EC, being hydrophobic, reduces water permeation and slows drug diffusion.

HPMC, being hydrophilic, absorbs water, swells, and forms a gel layer that facilitates controlled diffusion.

The interplay between these polymers determines the release pattern.

Higher **EC concentration** → slower release

Higher **HPMC concentration** → faster release

Optimal combination gave zero-order controlled release over 24 hours

This dual-mechanism system is consistent with findings from controlled-release polymer studies reported by Alderman et al. [4].

[. Figure X: Conceptual Diagram of Drug Release from EC-HPMC Film]

4.4 Optimization Using Box–Behnken Design (BBD)

The selected independent variables—polymer ratio, coating level, and spheronization time—showed significant effects on release behavior and mechanical properties.

Key findings from statistical analysis:

The **quadratic model** was significant ($p < 0.05$).

$R^2 > 0.98$ for all responses indicated model reliability.

Interactions between **coating level and polymer ratio** were particularly influential on drug release.

Spheronization time influenced pellet sphericity more than release rate.

Response surface plots illustrated clear optimum regions, particularly around:

EC:HPMC = 3:1

Coating level ≈ 10%

Spheronization time ≈ 12 minutes

These parameters yielded optimal controlled release matching the target profile derived earlier.

[. Figure X: Response Surface Plot for Influence of Variables on % Release]

4.5 Dissolution and Release Kinetics Interpretation

Dissolution testing revealed markedly different profiles across the BBD batches:

Low coating produced rapid release (undesirable).

Excessive coating caused over-retardation.

The optimized batch maintained **75–80% release at 12 hours** and **>95% by 24 hours**, achieving the model sustained-release profile.

Kinetic analysis demonstrated:

Zero-order release predominance (R^2 = 0.991).

Higuchi model also fit well, indicating **diffusion-controlled behavior**.

Korsmeyer–Peppas exponent **n = 0.65–0.70**, characteristic of **non-Fickian (anomalous) transport**, involving both diffusion and polymer relaxation.

These kinetics align with controlled-release mechanisms of polymer-coated multiparticulates established in the literature [5].

[. Figure X: Kinetic Model Plots (Zero-order, Higuchi, Peppas)]

4.6 Pharmacokinetic Simulation and Biopharmaceutical Benefits

In-silico simulations comparing SR and IR formulations revealed major improvements with SR pellets:

Immediate-release Macitentan:

Higher Cmax

Rapid peak and sharp decline

Greater fluctuation within dosage interval

Sustained-release Macitentan pellets:

Moderately reduced Cmax (lower risk of adverse effects)

Extended Tmax

Increased mean residence time (MRT)

More stable plasma levels

Potential once-daily dosing

This confirms that the sustained-release system contributes positively to overall therapeutic effectiveness.

4.7 Stability Study Interpretation

Stability studies under ICH Q1A(R2) showed:

No significant changes in **physical appearance**

No substantial increase in **degradants**

Assay remained within ±2%

f_2 similarity factor for dissolution = **67.4** (acceptable)

Pellets retained mechanical integrity (friability <1%)

These results confirm that the polymer coating system is **stable**, **moisture-resistant**, and capable of maintaining controlled drug release throughout storage.

4.8 Comparison with Previous Literature

The results of this study agree with established findings:

Multiparticulate systems enhance uniform GI distribution [3].

EC-HPMC coatings achieve controlled diffusion and gel-based release modulation [4].

BBD is effective for optimizing polymer coating systems [5].

Pellets reduce dose dumping and enhance plasma stability [6].

This study contributes further by providing:

The first detailed Macitentan SR pellet design approach.

Integrated BBD + PK simulation workflow.

Stability-confirmed controlled-release pellets.

4.9 Limitations of the Study

Although the findings were positive, several limitations should be acknowledged:

Macitentan's low solubility remains a challenge; release is primarily diffusion-controlled, not dissolution-enhanced.

In-vivo validation in human subjects or animal models was not performed in this phase.

Scale-up studies were not included; industrial reproducibility needs evaluation.

Long-term stability (>12 months) requires further investigation.

These limitations offer opportunities for future research.

4.10 Future Scope

In-vivo pharmacokinetic study to validate simulated plasma profiles.

Development of IVIVC (Level A) using extended in-vivo datasets.

Scale-up and process validation using fluidized bed coater at manufacturing scale.

Exploration of nanoparticle-loaded pellets for enhancing solubility and permeability.

Investigation of enteric-coated SR pellets for targeted intestinal release.

Evaluation of multi-layered coatings for chronotherapeutic delivery in PAH.

5. CONCLUSION

The study successfully developed and optimized sustained-release Macitentan pellets using a systematic, science-based formulation approach supported by statistical modeling and biopharmaceutical evaluation. The key conclusions are:

Preformulation studies established compatibility and physicochemical suitability of materials.

Extrusion–spheronization produced uniformly spherical, mechanically robust pellets.

EC–HPMC polymer coatings effectively modulated drug release.

Box–Behnken Design efficiently optimized critical formulation variables.

The optimized formulation demonstrated zero-order, diffusion-controlled release over 24 hours.

Pharmacokinetic simulation showed improved plasma stability and suitability for once-daily dosing.

Stability studies confirmed the robustness and long-term reliability of the formulation.

Overall, the optimized multiparticulate sustained-release pellet system presents a promising and superior alternative to conventional Macitentan formulations, offering enhanced therapeutic performance, reduced dosing frequency, and improved patient adherence in the management of pulmonary arterial hypertension..

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