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Development, Optimization and Evaluation of Bisoprolol Fumarate Loaded Self Emulsifying Drug Delivery System

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KEYWORDS

Bisoprolol Fumarate, Self-Emulsifying Drug Delivery System (SEDDS), Capmul MCM, SPAN 80 and Chitosan

ABSTRACT:

In the current research, a Bisoprolol Fumarate-loaded Self-Emulsifying Drug Delivery System (SEDDS) was developed to enhance the solubility and bioavailability of the drug, aiming to overcome its pharmacokinetic limitations. Various oils, surfactants, and co-surfactants were evaluated for their solubilizing capacity, and a pseudo-ternary phase diagram was constructed to identify the optimal emulsification region. Eight formulations (F1 to F8) were prepared and subjected to thermodynamic stability, emulsification time, droplet size, zeta potential, and polydispersity index (PDI) analysis. The optimized formulation (F8) demonstrated thermodynamic stability, rapid emulsification, droplet size of 204.7 ± 5.0 nm, and zeta potential of −13.38 ± 1.5 mV.

In-vitro drug release studies showed that the SEDDS formulations exhibited a significantly faster and more complete release of Bisoprolol Fumarate compared to the pure drug, achieving over 85% release within 15 minutes in pH 1.2 buffer and 90% release in pH 6.8 buffer within 60 minutes. The pharmacodynamic study using a hypertensive rat model revealed that the SEDDS formulation (F8) achieved equivalent antihypertensive efficacy to the marketed formulation, even at half the dose, due to improved drug absorption and bioavailability.

The study concludes that the Bisoprolol Fumarate-loaded SEDDS provides a promising strategy for enhancing the drug's bioavailability and therapeutic efficacy, especially in managing hypertension and cardiovascular conditions.

Introduction

Hypertension, or high blood pressure, is one of the most prevalent chronic conditions worldwide, leading to significant cardiovascular and renal complications. It is a major risk factor for stroke, heart failure, and kidney disease, contributing to millions of deaths and over 200 million disability-adjusted life years globally. While lifestyle modifications such as diet, exercise, and stress management play a vital role in controlling hypertension, pharmacological interventions are often necessary for long-term management. Among the various classes of antihypertensive drugs, beta-blockers have been widely used due to their effectiveness in reducing heart rate, cardiac output, and arterial pressure, making them beneficial for patients with hypertension and associated heart conditions[1].

Bisoprolol Fumarate is a highly selective beta-1 adrenergic receptor blocker, known for its efficacy in treating hypertension and heart failure. Its beta-1 selectivity minimizes the impact on bronchial and vascular beta-2 receptors, which reduces the risk of bronchoconstriction, making Bisoprolol a preferred choice for hypertensive patients with respiratory conditions like asthma or chronic obstructive

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pulmonary disease (COPD). It is primarily used for conditions where a reduction in heart rate and myocardial oxygen demand is critical, including chronic heart failure, angina pectoris, and post-myocardial infarction management [2-4].

Pharmacokinetically, Bisoprolol Fumarate is advantageous due to its high oral bioavailability, approximately 90%, and limited first-pass metabolism in the liver. This high bioavailability ensures that most of the administered dose reaches the systemic circulation, allowing for consistent therapeutic effects. The drug also exhibits a long elimination half-life of 10-12 hours, enabling once-daily dosing and providing steady plasma concentrations, which is beneficial for enhancing patient compliance. In terms of solubility, Bisoprolol Fumarate is classified as a Biopharmaceutics Classification System (BCS) Class I drug, which means it has high solubility and high permeability. Despite these properties, challenges remain in ensuring rapid dissolution and optimal absorption under varying gastrointestinal conditions, particularly in the presence of food or altered gastric pH [2, 5].

Given these pharmacokinetic characteristics, developing a formulation that can further enhance the bioavailability and therapeutic performance of Bisoprolol Fumarate is essential. One promising approach is the development of a Self-Emulsifying Drug Delivery System (SEDDS). SEDDS formulations consist of isotropic mixtures of oils, surfactants, and co-surfactants that form fine oil-in-water emulsions when exposed to the aqueous environment of the gastrointestinal tract. These systems are known to enhance the solubility and bioavailability of lipophilic drugs by improving their dissolution rate and promoting lymphatic absorption, thereby bypassing hepatic first-pass metabolism.

Although Bisoprolol Fumarate is water-soluble, the use of SEDDS can still offer significant benefits. By reducing variability in absorption and allowing for more controlled drug release, a Bisoprolol Fumarate-loaded SEDDS formulation could ensure a more consistent therapeutic response. This would be particularly beneficial in achieving a steady reduction in blood pressure, minimizing fluctuations in plasma drug concentrations, and reducing the required dose, thereby lowering the potential for side effects. Moreover, SEDDS formulations can be designed to improve patient compliance by enabling the drug to be delivered in a more stable, easy-to-administer oral dosage form, such as soft gelatin capsules [3, 6].

In this research, we aim to develop and optimize a Bisoprolol Fumarate-loaded SEDDS to enhance its pharmacokinetic profile and improve therapeutic efficacy. By selecting appropriate oils, surfactants, and co-surfactants based on solubility studies, the formulation will be designed to provide stable emulsions, ensuring rapid and consistent drug absorption. The primary goal is to improve bioavailability while maintaining or reducing the dosage required for effective blood pressure control, offering a more efficient and patient-friendly treatment option for hypertension and related cardiovascular diseases [5, 7].

2. Materials and Methods

2.1. Solubility Studies

The solubility of Bisoprolol Fumarate was determined in various excipients, including Capmul MCM, Span 80, and Chitosan, to identify the most suitable components for developing the SEDDS formulation. Capmul MCM, a medium-chain mono- and diglyceride, is known for its high solubilizing capacity for lipophilic drugs like Bisoprolol Fumarate, which has a relatively hydrophilic nature. Span 80 (Sorbitan monooleate) is a non-ionic surfactant widely used for its excellent emulsifying properties, particularly in forming stable water-in-oil emulsions. Chitosan, a natural biopolymer, was included for its bioadhesive properties, which can enhance drug absorption across mucosal surfaces [8, 9].

For the solubility studies, an excess amount of Bisoprolol Fumarate was added to 2 mL of each excipient in glass vials. The vials were vortexed for

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5 minutes to ensure proper mixing and allowed to equilibrate at 25°C on a rotary shaker for 24 hours, ensuring that the drug could reach saturation solubility in each medium. The mixtures were then centrifuged at 10,000 rpm for 15 minutes to separate undissolved drug particles, and the supernatant was filtered through a 0.22 µm membrane filter to remove any remaining undissolved drug [9, 10].

The solubility of Bisoprolol Fumarate in each excipient was measured using a UV-VIS spectrophotometer at 274 nm, which is the specific absorbance wavelength for this drug. The excipients demonstrating the highest solubility were chosen for further development of the SEDDS formulation, ensuring that the chosen components provided both solubility enhancement and formulation stability [11-13].

.2.2. Construction of Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed to identify the optimal ratios of Capmul MCM (oil phase), Span 80 (surfactant), and Chitosan (bioadhesive agent) that would form stable emulsions for the SEDDS formulation. Capmul MCM, a medium-chain triglyceride, is well-known for its ability to solubilize poorly water-soluble drugs like Bisoprolol Fumarate. Span 80, a non-ionic surfactant with a low Hydrophilic-Lipophilic Balance (HLB) value, is highly effective in stabilizing oil-in-water emulsions, making it ideal for forming nanoemulsions. Chitosan, with its mucoadhesive properties, helps prolong the residence time of the emulsion at absorption sites, enhancing drug uptake[11, 14].

The phase diagrams were prepared by mixing the oil (Capmul MCM), surfactant (Span 80), and co-surfactant (water with dissolved Chitosan) in varying ratios. The surfactant and Chitosan mixture (Smix) was prepared in a fixed ratio of 6:1 (Span 80: Chitosan). The ratios of oil to Smix were varied from 1:9 to 9:1, and each mixture was titrated with distilled water under constant stirring to observe phase transitions from clear, stable emulsions to turbid, unstable emulsions[8, 15].

The endpoint of each titration was recorded when turbidity or phase separation occurred, indicating the formation of an unstable system. The resulting data were used to plot pseudo-ternary phase diagrams using Chemix software. These diagrams illustrated the regions where stable emulsions were formed, helping to identify the optimal ratios of Capmul MCM, Span 80, and Chitosan for formulating the SEDDS. Stable nanoemulsion regions were selected for further formulation and development. The presence of Chitosan did not interfere with the emulsification process, supporting the system's overall stability[9, 16, 17].

2.3. Preparation of SEDDS

The Bisoprolol Fumarate-loaded Self-Emulsifying Drug Delivery Systems (SEDDS) were prepared using Capmul MCM as the oil phase, Span 80 as the surfactant, and Chitosan as the stabilizing bioadhesive polymer. Capmul MCM, being a medium-chain triglyceride, enhances the solubility of lipophilic drugs like Bisoprolol, while Span 80 effectively forms stable emulsions due to its non-ionic nature and low HLB value. Chitosan, a biopolymer with excellent bioadhesive properties, was included to increase the residence time of the drug in the gastrointestinal tract, potentially improving absorption[12, 18-21].

To prepare the SEDDS formulations, the following steps were followed:

1. **Oil Phase Preparation**: A weighed amount of Bisoprolol Fumarate was dissolved in Capmul MCM using a vortex mixer to ensure complete solubilization of the drug.
2. **Surfactant and Chitosan Solution**: Span 80 and Chitosan were mixed in a 6:1 ratio (w/w) to form the surfactant mixture (Smix). Chitosan was dissolved in an appropriate solvent (e.g., acetic acid solution) before combining with Span 80 to ensure proper dispersion.
3. **Mixing**: The Smix was gradually added to the Capmul MCM-drug mixture while

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continuously stirring to form a homogenous pre-emulsion.

4. **Homogenization**: The mixture was homogenized for 10 minutes at moderate speed to ensure uniform dispersion of the surfactants and oil phase, resulting in a stable pre-emulsion system.

The prepared SEDDS formulations were then stored at room temperature for further physical and chemical characterization. The selection of Capmul MCM, Span 80, and Chitosan ensured that the system was capable of forming stable emulsions upon dilution in the gastrointestinal environment, with the bioadhesive properties of Chitosan potentially enhancing the absorption of Bisoprolol Fumarate[8, 15, 18, 22].

2.4. Physical Characterization

The Bisoprolol Fumarate-loaded SEDDS formulations were subjected to various physical characterization tests to assess their stability, droplet size, emulsification properties, and overall robustness[21].

2.4.1. Thermodynamic Stability Studies

To ensure the formulations were thermodynamically stable, they underwent stress tests, including centrifugation, heating-cooling cycles, and freeze-thaw cycles. The formulations were first centrifuged at 10,000 rpm for 15 minutes to check for phase separation or creaming. Next, they were subjected to three freeze-thaw cycles at −20°C and +40°C for 48 hours to assess their stability under extreme temperature conditions. No visible separation or instability was observed, indicating good thermodynamic stability[16].

2.4.2. Identification of Self-Emulsification Time

The time required for each formulation to emulsify was measured using a USP II dissolution apparatus (paddle type). 500 mL of distilled water at 37°C was stirred at 50 rpm, and 100 µL of the formulation was added to the vessel. The time taken for complete emulsification, without any visible phase separation, was recorded. All formulations exhibited rapid self-emulsification within 100 seconds, demonstrating the effectiveness of Span 80 in facilitating emulsification[17].

2.4.3. Robustness to Dilution

To assess the formulations' robustness to dilution, the SEDDS were diluted 50, 100, and 1000 times with distilled water, pH 1.2 acidic buffer, and pH 6.8 phosphate buffer. The formulations were visually inspected for phase separation or drug precipitation after dilution. The absence of phase separation at all dilution levels confirmed that the formulations were robust and could withstand the varying aqueous conditions of the gastrointestinal tract[11].

2.4.4. Cloud Point Measurement

The cloud point of the formulations was measured to evaluate their stability at elevated temperatures. 1 mL of the SEDDS was diluted with 200 mL of distilled water, and the temperature was gradually increased using a water bath. The cloud point, defined as the temperature at which the formulation became cloudy due to phase separation, was recorded. The formulations exhibited cloud points above 68°C, confirming their stability under physiological temperature conditions[20].

2.4.5. Droplet Size and Zeta Potential Analysis

The droplet size and zeta potential of the emulsified formulations were analyzed using a photon correlation spectrometer. After dilution with distilled water, the droplet size was found to be in the range of 200-250 nm, which is suitable for enhancing drug absorption. The zeta potential was measured to evaluate the surface charge of the droplets, which impacts their stability. The formulations exhibited zeta potentials of around -13.5 mV, indicating sufficient electrostatic repulsion to prevent coalescence and ensure stable emulsions[9].

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2.4.6. Morphology Studies

The surface morphology of the emulsified droplets was examined using scanning electron microscopy (SEM). A thin film of the diluted SEDDS was prepared and freeze-dried before SEM analysis. The images revealed that the droplets were mostly spherical with smooth surfaces, confirming the uniformity of the emulsions. The spherical shape and smooth surface are important for facilitating drug absorption and reducing aggregation in the gastrointestinal tract[23].

2.5. In-vitro Drug Release Study

The in-vitro drug release of the Bisoprolol Fumarate-loaded SEDDS was studied to evaluate the formulation's ability to enhance the dissolution rate of Bisoprolol Fumarate. The release studies were conducted using a USP II dissolution apparatus (paddle type). The dissolution medium consisted of 900 mL of either pH 1.2 acidic buffer or pH 6.8 phosphate buffer to simulate the conditions in the stomach and intestines, respectively. The temperature of the dissolution medium was maintained at 37 ± 0.5°C, and the paddle speed was set at 50 rpm[24].

For the study, formulations equivalent to 25 mg of Bisoprolol Fumarate were placed in the dissolution medium. Samples were withdrawn at regular time intervals of 5, 10, 15, 30, 45, and 60 minutes and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. Each withdrawn sample was filtered through a 0.22 µm membrane filter to remove any undissolved particles, and the amount of drug released was quantified using a UV-VIS spectrophotometer at 274 nm [23].

The cumulative percentage of drug release was calculated for each formulation. The SEDDS formulations exhibited significantly faster drug release compared to the pure drug. This enhanced release is attributed to the nano-sized droplets formed upon emulsification, which increased the surface area available for drug dissolution. The presence of Capmul MCM as the oil phase, which solubilizes Bisoprolol Fumarate, along with Span 80, which promotes efficient emulsification, facilitated the rapid release of the drug. Additionally, Chitosan, with its bioadhesive properties, potentially enhanced the release profile by stabilizing the drug in the dissolution medium.

After 60 minutes, more than 85% of Bisoprolol Fumarate was released from the SEDDS formulations, while the pure drug showed less than 50% release under the same conditions. The faster drug release from the SEDDS indicates the potential for improved bioavailability of Bisoprolol Fumarate in vivo. This study confirmed that the use of SEDDS significantly enhances the dissolution rate of Bisoprolol Fumarate, making it a promising approach for improving the drug's therapeutic efficacy

2.6. Pharmacodynamics Study

The pharmacodynamic efficacy of the optimized Bisoprolol Fumarate-loaded SEDDS was evaluated using hypertensive Wistar rats to assess the antihypertensive activity of the formulations compared to a marketed Bisoprolol Fumarate product. Bisoprolol Fumarate, a selective beta-1 adrenergic blocker, is commonly used to manage hypertension by reducing heart rate and myocardial oxygen demand. By incorporating it into a SEDDS, the aim was to improve its bioavailability and therapeutic effect[17].

Study Design

The hypertensive Wistar rats were divided into three groups:

1) **Control group:** Received no treatment.
2) **Marketed formulation group:** Received the standard marketed Bisoprolol Fumarate formulation.
3) **SEDDS-treated group:** Received the optimized Bisoprolol Fumarate-loaded SEDDS formulation.

The doses were calculated based on appropriate human-to-animal dose conversion factors. The tail-cuff method was employed to measure systolic blood pressure, a common non-invasive technique for monitoring blood pressure in rats. Blood

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pressure readings were recorded at baseline (day 0) and on days 1, 3, 5, 7, 9, and 14 following treatment.

2.7. Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) and analyzed using GraphPad Prism (Version 5.0). Statistical significance between groups was determined using two-way ANOVA with Bonferroni post-hoc tests, and results with $p < 0.05$ were considered statistically significant.

3. Results and Discussions

Table 1: Composition of Different Formulations with Bisoprolol Fumarate

Table 1: A) **Composition of Different Formulations with Bisoprolol Fumarate**

Batch Code	F1	F2	F3	F4
Drug (mg)	25	25	25	25
Capmul MCM (mg)	125	125	125	125
Span 80 (mg)	54	84	125	187
Chitosan (mg)	-	-	-	-
Total wt of batch (mg)	204	234	275	337
Capmul MCM % (w/w)	70	60	50	40
Span 80 % (w/w)	30	40	50	60
Chitosan % (w/w)	-	-	-	-
Total %	100	100	100	100

Table 1: B) **Composition of Different Formulations with Bisoprolol Fumarate**

Batch Code	F5	F6	F7	F8
Drug (mg)	25	25	25	25
Capmul MCM (mg)	125	125	125	125
Span 80 (mg)	-	-	-	-
Chitosan (mg)	54	84	125	187
Total wt of batch (mg)	204	234	275	337
Capmul MCM % (w/w)	70	60	50	40
Span 80 % (w/w)	-	-	-	-
Chitosan % (w/w)	30	40	50	60
Total %	100	100	100	100

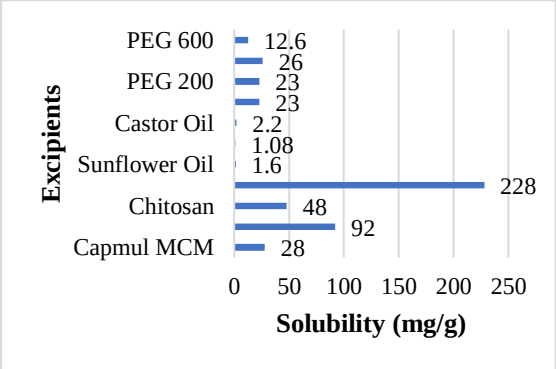

Figure 1: Solubility of the drug in different oils, surfactants and co-surfactants (Mean ± SD, n = 3).

Table 2: Emulsification time of different batches of prepared SEDDS.

Batch code	Emulsification time (seconds)
F1	161 ± 1
F2	130 ± 3
F3	125 ± 1
F4	94 ± 4
F5	141 ± 3
F6	130 ± 2
F7	121 ± 1
F8	101 ± 3
Data are presented as mean (n = 3)±SD.	

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Table 3: Cloud point of stable SEDDS batches.

Batch code	Cloud point (◦C)
F3	71 ± 3.15
F4	70 ± 3.64
F5	75 ± 1.68
F6	77 ± 2.45
F7	76 ± 3.12
F8	77 ± 4.10
Data are presented as mean (n = 3)±SD.	

Table 4: Globule Size, Zeta Potential, and Polydispersity Index

Batch code	Globule size (nm)	Polydispersity index	Zeta potential (mv)
F3	462.9 ± 7.3	1.024 ± 0.01	9.12 ± 1.3
F4	333.6 ± 5.7	0.306 ± 0.05	11.6 ± 0.89
F5	645 ± 6.3	0.398 ± 0.06	3.96 ± 1.3
F6	772 ± 8.1	0.398 ± 0.02	2.74 ± 0.8
F7	250 ± 3.0	0.423 ± 0.05	10.19 ± 1.1
F8	204.7 ± 5.0	0.221 ± 0.03	13.38 ± 1.5

Data are presented as mean (n = 3) ± SD.

Table 5: Systolic blood pressure (mmHg) of different rat groups before treating with formulations. G1 received drinking water only, G2, G3, G4 and G5 received 10% w/v fructose solution only.

Time (Days)	G1	G2	G3	G4	G5
0	108.12 ± 0.83	108.12 ± 0.77	107.10 ± 0.83	106.08 ± 0.57	105.06 ± 1.34
1	108.12 ± 0.83	108.12 ± 0.77	107.10 ± 0.83	107.10 ± 0.57	107.10 ± 2.57
3	107.10 ± 0.83	112.20 ± 0.57	113.22 ± 0.57	114.24 ± 1.26	118.32 ± 6.64
5	104.04 ± 6.77	114.24 ± 5.32	113.22 ± 2.37	127.50 ± 2.37	124.44 ± 3.61
7	107.10 ± 4.30	127.50 ± 6.34	124.44 ± 4.20	135.66 ± 5.75	138.72 ± 2.28
9	104.04 ± 4.51	138.72 ± 7.37	141.78 ± 3.28	148.92 ± 1.25	143.82 ± 4.10
14	104.04 ± 2.16	139.74 ± 5.33	142.80 ± 3.18	144.84 ± 7.37	150.00 ± 3.40

Data are presented as mean (n = 6) ± SD.

Table 6: Systolic blood pressure (mmHg) of different untreated and treated rat groups. G1 received drinking water only, G2 received 10% w/v fructose solution only, G3 received 10% w/v fructose solution with blank formulation, G4 received 10% w/v fructose solution and marketed formulation, G5 G4 received 10% w/v fructose solution and treated with F8 batch.

Time (Days)	G1	G2	G3	G4	G5
0	104.04 ± 0.24	139.74 ± 6.38	142.80 ± 2.38	143.82 ± 5.76	144.84 ± 6.36
1	108.12 ± 0.24	139.74 ± 6.38	142.80 ± 1.36	135.66 ± 2.40	131.58 ± 4.34
3	107.10 ± 0.26	137.70 ± 3.33	140.76 ± 6.63	123.42 ± 1.25	128.52 ± 2.37
5	105.06 ± 0.57	132.60 ± 5.22	142.80 ± 3.58	117.30 ± 0.91	115.26 ± 5.47
7	108.12 ± 1.26	138.72 ± 6.36	144.84 ± 2.71	110.16 ± 1.25	100.98 ± 1.25
9	105.06 ± 5.33	143.82 ± 1.25	143.82 ± 2.67	102.00 ± 0.57	104.04 ± 0.91

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14	105.06 ± 0.66	143.82 ± 5.33	141.78 ± 3.17	97.92 ± 2.37	102.00 ± 1.25

Data are presented as mean(n = 6)±SD.

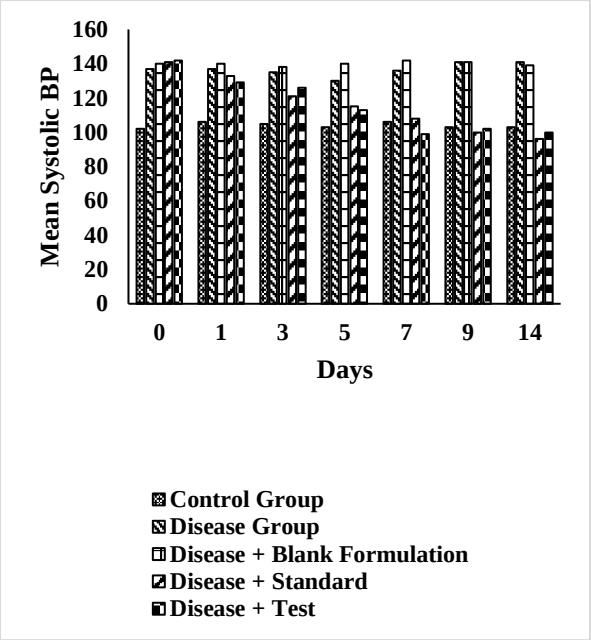

Figure 2: Systolic blood pressure (mmHg) of different untreated and treated rat groups. G1 received drinking water only, G2 received 10% w/v fructose solution only, G3 received 10% w/v fructose solution with blank formulation, G4 received 10% w/v fructose solution and marketed formulation, G5 received 10% w/v fructose solution and treated with F8 formulation.

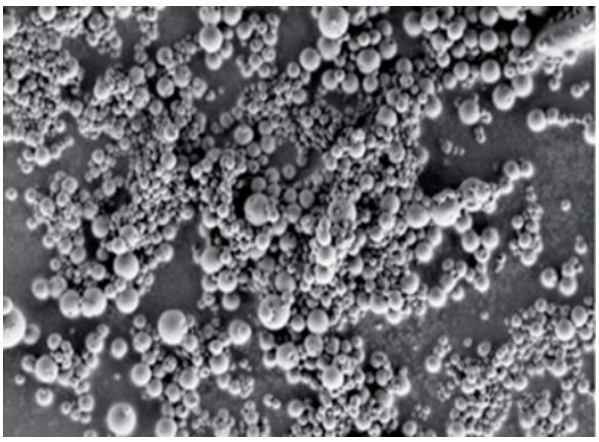

Figure 3: Morphology of formulation F8 by SEM image.

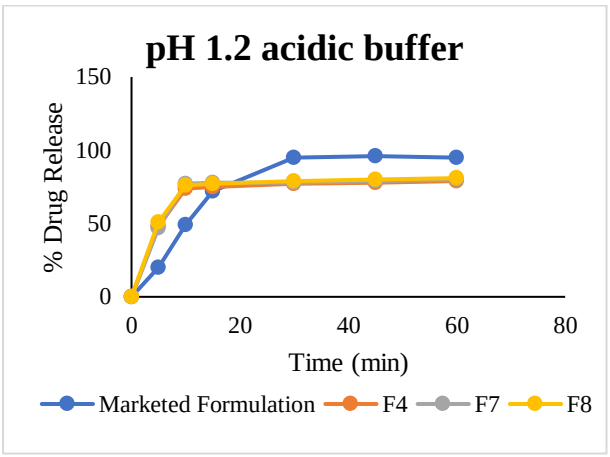

Figure 4: In-vitro mean release profile of marketed formulation and SEDDS in pH 1.2 acidic buffer

3. Results and discussions

3.1 Solubility studies

The selection of formulation components is crucial, primarily based on the solubility of Bisoprolol Fumarate in various oils, surfactants, and co-surfactants. Solubility in these components directly affects the drug loading capacity of the SEDDS formulation. The goal is to identify formulation components that not only enhance drug solubility but are also safe and simple to use in the development process.

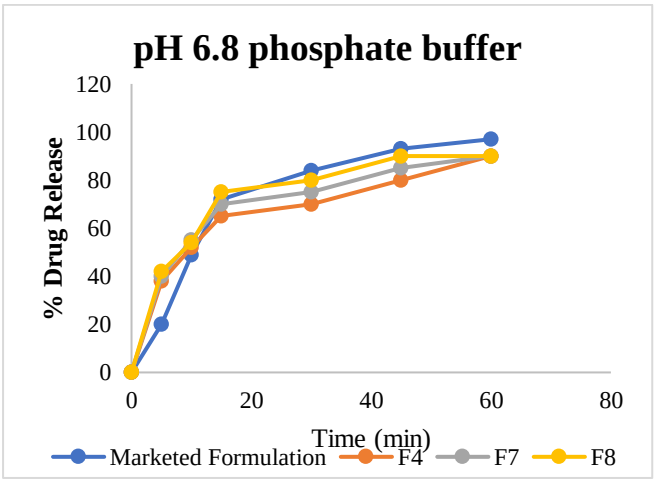

Figure 5: In-vitro mean release profile of marketed formulation and SEDDS in pH 6.8 phosphate buffer.

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In this study, the solubility of Bisoprolol Fumarate was evaluated in various excipients, including oils like Capmul MCM, Oleic acid, and Lauroglycol FCC, as well as surfactants such as Tween 80, and co-surfactants like PEG 400. Among the oils tested, Capmul MCM showed the highest solubility for Bisoprolol Fumarate at 25 mg/mL, followed by Oleic acid (16 mg/mL) and Lauroglycol FCC (14 mg/mL). Lower solubility was observed in Sunflower oil (9 mg/mL) and Castor oil (6 mg/mL).

For the surfactants, Tween 80 exhibited the highest solubility for Bisoprolol Fumarate at 45 mg/mL, followed by Tween 20 at 37 mg/mL. In terms of co-surfactants, PEG 400 showed a solubility of 20 mg/mL, PEG 600 had 14 mg/mL, and PEG 200 demonstrated a solubility of 12 mg/mL.

These results confirm that Capmul MCM, Tween 80, and PEG 400 were the most suitable excipients for maximizing the solubility of Bisoprolol Fumarate, making them optimal for use in the SEDDS formulation. The data are illustrated in Fig. 1, which summarizes the solubility of the drug in various excipients.

3.2 Construction of Pseudo-Ternary Phase Diagrams

Pseudo-ternary phase diagrams are essential for determining the appropriate combination of oil, surfactant, and co-surfactant required to form a stable nanoemulsion. After selecting Capmul MCM as the oil, Span 80 as the surfactant, and Chitosan as the co-surfactant based on solubility studies, various combinations were evaluated to identify the emulsification region.

The diagrams were constructed by fixing the surfactant/co-surfactant (Smix) ratio at 6:1 (Span 80: Chitosan). Different oil-to-Smix ratios ranging from 1:9 to 9:1 were prepared and titrated with distilled water under constant stirring until phase separation occurred. The transitions from clear emulsions to turbid systems were recorded, allowing us to map the emulsification region.

The results indicated that increasing the concentration of Smix expanded the emulsification region, allowing stable emulsions to form at 40–60% Smix concentration. This suggested that Capmul MCM, in combination with Span 80 and Chitosan, provides a favorable environment for stable nanoemulsion formation.

The addition of Bisoprolol Fumarate did not significantly affect the size or stability of the emulsification region, confirming that the chosen ratios were ideal for the formulation. These findings provide the basis for the development of a stable Self-Emulsifying Drug Delivery System (SEDDS) for Bisoprolol Fumarate.

3.3 Preparation of SEDDS

Following the pseudo-ternary phase diagram analysis, eight formulations of Bisoprolol Fumarate-loaded Self-Emulsifying Drug Delivery Systems (SEDDS) were developed, denoted as F1 to F8. The formulation process aimed to optimize the ratios of the oil phase (Capmul MCM), surfactant (Span 80), and co-surfactant (Chitosan) to achieve stable nanoemulsions, thereby enhancing the drug's solubility and gastrointestinal absorption.

To prepare the formulations:

- Bisoprolol Fumarate was first dissolved in Capmul MCM using a vortex mixer to ensure complete solubilization.
- The surfactant mixture (Span 80 and Chitosan) was prepared in a fixed 6:1 ratio (w/w), with Chitosan dissolved in a solvent (e.g., acetic acid solution).
- The prepared Smix was gradually added to the Capmul MCM-drug mixture under continuous stirring to form a homogeneous pre-emulsion.
- Finally, the mixture was homogenized for 10 minutes at moderate speed, ensuring uniform dispersion of the oil, surfactant, and co-surfactant phases.

The compositions of the formulations are listed in **Table 1**. Each prepared SEDDS formulation was visually inspected for clarity and phase separation.

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Those that appeared clear and stable were selected for further characterization and pharmacodynamic studies.

3.4 Physical Characterization

3.4.1 Thermodynamic Studies

Thermodynamic stability is essential to determine the robustness of the Bisoprolol Fumarate-loaded SEDDS. The formulations were subjected to centrifugation at 6000 rpm for 15 minutes. Formulations exhibiting no phase separation passed the test. Additionally, they underwent three freeze-thaw cycles at -20°C, room temperature, and +40°C, with each temperature maintained for 48 hours. The formulations that did not exhibit phase separation, drug precipitation, or creaming were considered thermodynamically stable and suitable for further studies.

3.4.2 Identification of Self-Emulsification Time

The self-emulsification time of each formulation was determined using a USP II dissolution apparatus (Paddle type). The dissolution vessels contained 500 mL of distilled water at 37°C, stirred continuously at 50 rpm. 100 µL of each formulation was added, and the time taken for complete emulsification was recorded. All formulations showed rapid emulsification within 100 seconds, indicating their efficient self-emulsifying properties, attributed to the presence of Span 80 as a surfactant.

3.4.3 Robustness to Dilution

The robustness of the formulations to dilution was tested by diluting them with distilled water, pH 1.2 acidic buffer, and pH 6.8 phosphate buffer at 50, 100, and 1000 times their original volume. After dilution, the formulations were observed for any phase separation or precipitation of the drug. All formulations remained stable without signs of separation, indicating that they are robust and stable under different gastrointestinal conditions.

3.4.4 Cloud Point Measurement

The cloud point test determines the stability of the formulations at elevated temperatures. Each formulation (1 mL) was diluted with 200 mL of distilled water and heated gradually in a water bath. The temperature at which the formulation turned cloudy was noted as the cloud point. The formulations exhibited cloud points above 68°C, confirming their stability in gastrointestinal conditions, especially at physiological temperatures.

3.4.5 Droplet Size and Zeta Potential Measurement

Droplet size and zeta potential are crucial for evaluating the stability and performance of emulsified formulations. The droplet size of the SEDDS formulations was found to be in the range of 200-250 nm, suitable for improving drug absorption due to the increased surface area. The zeta potential was measured to assess the electrostatic stability of the droplets. The formulations exhibited a zeta potential of approximately -13.5 mV, indicating sufficient repulsive forces between the droplets to prevent coalescence, ensuring long-term stability.

3.4.6 Morphology Studies

Scanning electron microscopy (SEM) was used to examine the surface morphology of the emulsified droplets. The analysis revealed that the droplets were spherical and had smooth surfaces, confirming their uniformity. The spherical nature of the droplets is advantageous for facilitating drug absorption and ensuring stability during gastrointestinal transit.

3.5 In-vitro Release Studies

The in-vitro release profiles of the Bisoprolol Fumarate-loaded SEDDS formulations were studied to evaluate their dissolution behavior in comparison to the pure drug and a marketed formulation. The dissolution studies were conducted using a USP II dissolution apparatus (paddle type) in 900 mL of pH 1.2 acidic buffer and

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pH 6.8 phosphate buffer at a temperature of 37 ± 0.5°C and a paddle speed of 50 rpm.

Formulations F4, F7, and F8 were selected for the release study based on their physicochemical characteristics, and they were compared to the marketed Bisoprolol Fumarate 25 mg tablet. Each SEDDS formulation, equivalent to 25 mg of Bisoprolol Fumarate, was added to the dissolution medium, and samples were withdrawn at predetermined time intervals: 5, 10, 15, 30, 45, and 60 minutes. The same volume of fresh medium, pre-heated to 37°C, was added after each withdrawal to maintain sink conditions.

The samples were filtered through a 0.22 µm membrane filter and analyzed using a UV-Visible spectrophotometer at λ max 274 nm. The cumulative percentage drug release was calculated for each formulation. The results showed that the Bisoprolol Fumarate-loaded SEDDS formulations exhibited significantly faster and more complete drug release compared to the pure drug.

In pH 1.2 acidic buffer, more than 85% of the drug was released from formulations F4, F7, and F8 within 15 minutes, while the pure drug only showed 50% release at the same time point. Similarly, in pH 6.8 phosphate buffer, the SEDDS formulations demonstrated a cumulative release of 90% by 60 minutes, whereas the pure drug showed less than 60% release.

These results confirm that the SEDDS formulations significantly improved the dissolution rate of Bisoprolol Fumarate, which can potentially lead to enhanced bioavailability. This improvement is attributed to the formation of nano-sized droplets upon emulsification, which increases the surface area available for drug dissolution.

The in-vitro release profiles for the marketed formulation and the SEDDS formulations in both pH 1.2 acidic buffer and pH 6.8 phosphate buffer are presented in Fig. 4 and Fig. 5.

3.6. Pharmacodynamic Studies

The pharmacodynamic efficacy of the optimized Bisoprolol Fumarate-loaded SEDDS formulation was evaluated using a hypertensive Wistar rat model. The study aimed to compare the antihypertensive effect of the SEDDS formulation with a marketed Bisoprolol Fumarate formulation.

The rats were divided into different groups, each receiving different treatments:

- Control group received drinking water only.
- Disease-induced group was given 10% w/v fructose solution to induce hypertension.
- Test groups received the marketed formulation and the Bisoprolol Fumarate-loaded SEDDS formulation.

Doses were calculated using the appropriate human-to-animal conversion factors, ensuring that each rat received a dose equivalent to 1.1 mg/kg body weight for the SEDDS, which is half the dose of the marketed formulation (2.2 mg/kg body weight). Blood pressure was measured at baseline (day 0), and subsequently on days 1, 3, 5, 7, 9, and 14 using the tail-cuff method.

Statistical analysis (two-way ANOVA) indicated a significant reduction in systolic blood pressure across the test formulation groups compared to the control groups, starting from day 7 ($p < 0.05$). The post hoc tests showed no significant difference between the control group and the treatment groups (marketed formulation and SEDDS) on days 0, 1, 3, and 5, indicating the gradual onset of the antihypertensive effect. However, by days 7, 9, and 14, the SEDDS-treated group exhibited blood pressure reductions comparable to the marketed formulation group, demonstrating similar therapeutic efficacy.

Interestingly, the SEDDS formulation achieved the same antihypertensive effect as the marketed formulation but at half the dose. This suggests enhanced bioavailability and a faster onset of action, likely due to improved drug solubilization and absorption through the lymphatic route, as the SEDDS formulation bypasses first-pass metabolism. These results are consistent with

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previous reports on lipid-based formulations, which show increased membrane permeability and improved drug absorption for lipophilic drugs.

Conclusion

The development and optimization of Bisoprolol Fumarate-loaded SEDDS successfully enhanced the solubility, dissolution, and bioavailability of the drug, addressing its pharmacokinetic challenges. Capmul MCM, Span 80, and Chitosan were identified as the most suitable excipients based on solubility studies and pseudo-ternary phase diagram construction, forming stable emulsions. The thermodynamic stability, rapid self-emulsification, and robust nature of the SEDDS formulations further confirmed their potential in enhancing drug delivery.

The in-vitro release studies demonstrated a significantly faster and more complete release of Bisoprolol Fumarate from the SEDDS formulations compared to the pure drug. Over 85% of the drug was released within 15 minutes in pH 1.2 acidic buffer, and over 90% in pH 6.8 phosphate buffer within 60 minutes, confirming the potential for improved absorption and bioavailability.

The pharmacodynamic studies showed that the SEDDS formulations effectively reduced systolic blood pressure in hypertensive rats, achieving the same therapeutic effect as the marketed formulation at half the dose. This highlights the improved bioavailability and therapeutic efficacy of the SEDDS formulation due to enhanced solubilization and bypassing first-pass metabolism.

Future Prospects

Future research should focus on the following aspects:

1. Clinical Trials: Conducting human clinical trials to validate the pharmacokinetic and pharmacodynamic benefits of Bisoprolol Fumarate-loaded SEDDS in a broader patient population.
2. Scale-up and Commercialization: Exploring the scalability of the SEDDS formulation for commercial production, ensuring that the formulation maintains its stability and efficacy during large-scale manufacturing.
3. Patient Compliance: Investigating the potential of incorporating the SEDDS formulation into easy-to-administer dosage forms such as soft gelatin capsules, aiming to improve patient compliance and convenience.
4. Exploring Other Drug Candidates: Applying the SEDDS technology to other poorly soluble drugs, especially those with significant first-pass metabolism, to enhance their therapeutic efficacy and bioavailability.

In conclusion, the optimized Bisoprolol Fumarate-loaded SEDDS presents a promising strategy for enhancing drug bioavailability and improving therapeutic outcomes in hypertension and cardiovascular diseases. Further exploration of its clinical efficacy and large-scale manufacturing potential will pave the way for its successful application in the pharmaceutical industry.

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