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Formulation and Characterization of Self-Emulsifying Drug Delivery System of Bisoprolol for Enhanced Bioavailability

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KEYWORDS

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

ABSTRACT:

The present study focuses on the development and characterization of a Self-Emulsifying Drug Delivery System (SEDDS) for Bisoprolol Fumarate aimed at improving its bioavailability. Bisoprolol, a selective beta-1 adrenergic blocker, is widely used in the treatment of hypertension and cardiovascular disorders. Despite its high bioavailability, challenges in dissolution rate and gastrointestinal absorption persist. To address these issues, Bisoprolol was formulated with Capmul MCM, SPAN 80, and Chitosan to create an optimized SEDDS.

Characterization studies were performed, including assessments of droplet size, zeta potential, and polydispersity index using laser light scattering techniques. The SEDDS exhibited a droplet size of 200–250 nm, ensuring rapid and efficient drug release. The zeta potential was recorded at −25 mV, indicating good electrostatic stability. In vitro dissolution studies revealed that Bisoprolol-loaded SEDDS enhanced drug release by 1.67 times compared to conventional tablet formulations within the first 30 minutes. Furthermore, in vivo pharmacodynamic studies demonstrated that the SEDDS formulation was 3.1 times more effective in reducing systolic blood pressure in hypertensive rats compared to a CMC suspension ($p < 0.0001$).

Histopathological examination confirmed the safety of the SEDDS, with no significant adverse effects observed in gastrointestinal tissues. Overall, the Bisoprolol-loaded SEDDS formulation significantly improved solubility, permeability, and bioavailability, offering a promising alternative for the enhanced delivery of Bisoprolol and potentially other poorly soluble drugs.

Introduction

Hypertension, commonly known as high blood pressure, is a significant global health concern that leads to various cardiovascular diseases (CVD) and renal complications [1]. Despite the effectiveness of lifestyle interventions in managing hypertension, pharmacological treatments remain essential for achieving optimal blood pressure control. Among the drug classes used, beta-blockers are widely employed due to their ability to reduce heart rate and myocardial oxygen demand, which is particularly important for patients with hypertension and heart failure[2].

Bisoprolol Fumarate is a selective beta-1 adrenergic receptor blocker used primarily in the treatment of hypertension and heart failure. This drug's high specificity for beta-1 receptors, as opposed to beta-2 receptors, reduces the risk of bronchoconstriction, making it a safer option for patients with respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD)[3]. Bisoprolol also possesses a favorable pharmacokinetic profile, with approximately 90% oral bioavailability and a half-life of 10–12 hours, enabling once-daily dosing, which improves patient compliance. Despite its high bioavailability, challenges remain in enhancing the drug's dissolution rate, particularly in varying gastrointestinal conditions[4].

A Self-Emulsifying Drug Delivery System (SEDDS) has emerged as a promising formulation strategy to enhance the bioavailability and therapeutic efficacy of poorly soluble drugs [5]. SEDDS formulations consist of a combination of oils, surfactants, and co-surfactants,

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which spontaneously form oil-in-water emulsions upon contact with gastrointestinal fluids. This system enhances the solubility of lipophilic drugs, improving their dissolution and promoting lymphatic absorption, which bypasses hepatic first-pass metabolism [6].

Although Bisoprolol Fumarate is water-soluble, using a SEDDS formulation offers additional benefits, such as improved absorption and more consistent drug release, which can lead to better therapeutic outcomes. The development of a Bisoprolol-loaded SEDDS could provide controlled drug release, minimize fluctuations in plasma concentration, and reduce the dosage required for effective blood pressure control [7].

In this research, Bisoprolol Fumarate is formulated with Chitosan, SPAN 80, and Capmul MCM to create an optimized SEDDS. These ingredients were chosen based on their solubility and emulsification properties, with the goal of enhancing the bioavailability and stability of the formulation. The primary aim of this study is to develop a SEDDS formulation that provides a stable and consistent therapeutic response, ensuring better management of hypertension with minimal side effects [8].

Materials and Methods

Materials

The following materials were utilized in the development of Bisoprolol Fumarate-loaded Self-Emulsifying Drug Delivery Systems (SEDDS):

- Bisoprolol Fumarate: A beta-1 adrenergic blocker, used as the model drug for its therapeutic relevance in hypertension and cardiovascular disorders.
- Capmul MCM: A medium-chain triglyceride used as the oil phase due to its capacity to solubilize poorly water-soluble drugs like Bisoprolol Fumarate.
- SPAN 80 (Sorbitan monooleate): A non-ionic surfactant with low HLB value, chosen for its effectiveness in forming stable emulsions.
- Chitosan: A natural polysaccharide selected for its bioadhesive properties and potential to enhance mucosal absorption.
- Distilled Water: Used as the aqueous phase for dilution in solubility and emulsification studies.

Animals

For the pharmacodynamic study, 30 adult male Wistar rats, weighing 180–200 g, were used. The animals were obtained from an authorized supplier and were housed in standard polypropylene cages, with a controlled environment of 25 ± 2°C and 55 ± 5% relative humidity. The rats were kept under a 12-hour light/dark cycle and were provided with standard laboratory feed and water ad libitum. Prior to the study, the animals were acclimatized to the laboratory conditions for a period of 7 days.

All animal experiments were conducted following the ethical guidelines for animal research. The protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) in compliance with the national and international regulations for the care and use of laboratory animals.

Development of SMEDDS Formulation

The development of Self-Emulsifying Drug Delivery Systems (SEDDS) for Bisoprolol Fumarate involves selecting the right combination of oil, surfactant, and co-surfactant to achieve optimal drug solubilization and formulation stability. In this study, Capmul MCM, a medium-chain triglyceride, was selected as the oil phase for its ability to solubilize lipophilic drugs, including Bisoprolol. SPAN 80 was chosen as the surfactant due to its non-ionic nature and its ability to form stable emulsions with low HLB (Hydrophilic-Lipophilic Balance). Chitosan, a natural biopolymer with bioadhesive properties, was used as the co-surfactant to enhance the emulsifying properties and increase the bioavailability of the drug by improving mucosal adhesion [9].

The formulation process involved dissolving Bisoprolol Fumarate in Capmul MCM, followed by the addition of the surfactant and co-surfactant mixture. Various ratios of Capmul MCM, SPAN 80, and Chitosan were tested to determine the optimal mixture that formed a clear and stable emulsion upon contact with water. The final SEDDS formulations were then evaluated based on their emulsification time, droplet size, zeta potential, and stability, ensuring that the emulsions remained stable under gastrointestinal conditions [10].

The chosen formulations demonstrated efficient self-emulsifying properties, forming nano-sized droplets that improved drug solubility and absorption. The

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optimized formulations exhibited high stability and the ability to enhance the bioavailability of Bisoprolol Fumarate, making this SEDDS formulation a promising strategy for improving the oral delivery of the drug.

Determination of Bisoprolol with HPLC method

Bisoprolol Fumarate determinations were carried out using a validated High-Performance Liquid Chromatography (HPLC) method. This method was employed during solubility, in vitro dissolution, diffusion, and permeability studies. The separation was achieved using an Agilent 1100 HPLC system, fitted with a Kromasil 100-5-C18 column (4.6 x 250 mm), with a mobile phase composed of 10 mM KH_2PO_4 (pH 3.5) and acetonitrile (55:45 v/v) at a flow rate of 1.0 mL/min. UV detection was carried out at 250 nm, and the injection volume was set at 30 µL [11, 12].

The calibration curve for Bisoprolol was prepared within the range of 5-150 µg/mL, yielding a determination coefficient (r^2) of 0.9999, confirming the linearity of the method. Specificity and selectivity were validated by comparing chromatograms of blank samples and samples containing Bisoprolol, ensuring no interference from the excipients. Limits of detection (LOD) and quantification (LOQ) were established at 0.167 µg/mL and 0.506 µg/mL, respectively.

Recovery studies from the self-emulsifying drug delivery system (SEDDS) formulation were performed using the same method, demonstrating the accuracy and precision of the HPLC method for quantifying Bisoprolol from the developed formulation. The Relative Standard Deviation (RSD) values were within FDA guidelines, supporting the method's reproducibility.

Characterization Studies of SMEDDS

Droplet Size, Zeta Potential, and Polydispersity

The mean droplet size, zeta potential (surface charge), and polydispersity index (PDI) were measured using the Laser Light Scattering Particle Size Analysis Technique with a Zetasizer Nano ZSP Malvern device. This characterization ensures the stability and uniformity of the nanoemulsions, which are critical for optimal drug delivery. Droplet size affects the drug absorption rate, with smaller droplets providing a greater surface area, enhancing dissolution. The zeta potential indicates the electrostatic stability of the emulsion, with values over ±30 mV typically indicating stable formulations. Samples of the prepared Bisoprolol-loaded SMEDDS were placed in 1 cm^3 cuvettes and analyzed at 25 ± 0.5°C [1, 8, 13].

Refractive Index

The refractive index of each SMEDDS formulation was determined using a Shimadzu refractometer. A drop of the undiluted SMEDDS was placed on the device, and the refractive index was measured. The refractive index provides insights into the homogeneity and composition of the formulation. The inclusion of Capmul MCM and SPAN 80 as key components affects the refractive properties of the nanoemulsion [6, 14].

Self-Emulsification Time

Self-emulsification time is a critical parameter indicating the ease with which the formulation emulsifies upon contact with aqueous media. Each SMEDDS formulation (1 g) was added to 250 mL of pH 1.2 gastric fluid and stirred at 50 rpm using a USP Type II dissolution apparatus (Sotax AT 7 U.S.). The time required for the formulation to form a fine emulsion was recorded as the self-emulsification time. Fast emulsification times are desirable for efficient drug release in the gastrointestinal tract [15].

pH

The pH of the SMEDDS formulations was measured using a NEL Mod.821 pH meter. This parameter is crucial for ensuring that the formulation remains within the desired pH range to maintain stability and optimize drug solubility. The pH values of the formulations were adjusted to suit gastrointestinal conditions, with Chitosan contributing to the pH balance due to its mild acidic properties.

Electrical Conductivity

Electrical conductivity was measured using a Jenway 4071 conductivity meter to determine the type of microemulsion formed (oil-in-water or water-in-oil). The conductivity of the SMEDDS formulations provides information on their internal structure. Since Bisoprolol-loaded SMEDDS is designed to form oil-in-water nanoemulsions, higher conductivity values are expected, reflecting the dispersion of oil droplets within an aqueous medium.

Viscosity

Viscosity is an important factor in determining the flow properties and stability of SMEDDS formulations.

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Viscosity was measured using a Brookfield ULA Viscometer. A 10 mL sample of SMEDDS was placed in the jacketed sample cup, which was connected to a circulating water bath. After equilibrating for 5 minutes, measurements were taken at spindle speeds between 30–200 rpm. Viscosity affects the emulsification process and the overall stability of the formulation, with Capmul MCM contributing to the viscosity due to its lipid content.

Stability Studies of SMEDDS

Stability studies were conducted at 25 ± 2°C and 75% ± 5% RH over a period of 3 months to evaluate the physical stability of the SMEDDS formulations. Parameters such as physical appearance, droplet size, and drug content were monitored regularly to ensure that the formulations remained stable under these conditions. The use of SPAN 80 and Chitosan in the SMEDDS formulations contributed to maintaining the stability of the nanoemulsions, ensuring no significant changes in particle size or drug content were observed.

In Vitro Dissolution Studies

The in vitro dissolution studies were conducted to compare the release profile of Bisoprolol-loaded SMEDDS with that of a commercially available Bisoprolol tablet formulation. The studies were performed using USP Type II dissolution apparatus (Sotax AT 7 U.S.) with a paddle speed of 50 rpm in 900 mL of simulated gastric fluid (0.1 N HCl, pH 1.2). The temperature was maintained at 37 ± 0.5°C throughout the experiment. A single capsule of SMEDDS containing 10 mg/mL of Bisoprolol was filled into size 000 hard gelatin capsules, while an equivalent dose of a commercially available tablet was used for comparison [10, 14].

At predetermined time intervals, 1 mL of the dissolution medium was withdrawn, filtered, and analyzed using the validated HPLC method described previously. After each sampling, 1 mL of fresh dissolution medium was added to maintain a constant volume. The percentage of Bisoprolol released from the formulations was calculated based on the concentration of the drug in the dissolution medium and the total vessel volume. The concentration was determined under the assumption that 0.011 mg/mL corresponds to 100% release of the active substance in the 900 mL medium [6].

The results showed that Bisoprolol-loaded SMEDDS significantly improved the drug release profile compared to the tablet formulation. The release from SMEDDS was found to be 1.67 times higher than the tablet formulation within the first 30 minutes. This enhanced release is attributed to the formation of nano-sized droplets in the SMEDDS formulation, which increases the surface area for drug dissolution and absorption. Statistical analysis using two-way ANOVA confirmed the significant difference between the two formulations, with $p < 0.001$ indicating the superior release profile of SMEDDS [9].

In Vitro Diffusion Studies

In vitro diffusion studies were conducted to evaluate the release profile of Bisoprolol-loaded SMEDDS in a simulated gastric medium. A 1 kDa molecular weight cut-off (MWCO) diffusion tube was used, simulating low-porous membrane conditions. The diffusion medium consisted of 100 mL of pH 1.2 (0.1 N HCl) maintained at 37 ± 0.5°C and stirred at 50 rpm using a magnetic stirrer. SMEDDS formulations were loaded into diffusion tubes and monitored over a 6-hour period. Samples were collected at predetermined time intervals, filtered, and analyzed using the validated HPLC method [6].

The results demonstrated a 10% release of Bisoprolol from the SMEDDS formulation by the end of the 6-hour observation period. The reduced drug release rate can be attributed to the low porosity of the diffusion membrane, which allows only the free drug molecules to pass. The presence of Chitosan in the formulation, known for its mucoadhesive properties, potentially contributed to prolonging the drug's residence time on the membrane, which could be useful for sustained drug release [16].

The data indicate that Bisoprolol-loaded SMEDDS provides a controlled release profile, making it suitable for sustained drug delivery applications in the gastrointestinal environment.

Hexadecane Membrane Parallel Artificial Membrane Permeability Assay (HDM-PAMPA)

The Hexadecane Membrane Parallel Artificial Membrane Permeability Assay (HDM-PAMPA) was utilized to evaluate the passive permeability of Bisoprolol-loaded SMEDDS. PAMPA is a widely used method to estimate gastrointestinal absorption by

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mimicking passive diffusion across a lipidic membrane. The assay was conducted using 96-well filter plates (Millipore, Billerica, MA), which consist of separate donor and acceptor compartments [14].

The donor compartment filters were treated with 15 µL of a 5% hexadecane in hexane solution to create a lipid layer. Both the SMEDDS formulation (1 mg/mL Bisoprolol) and a CMC suspension (containing 0.25% w/v Carboxymethylcellulose) were prepared. The donor solution, consisting of 5% dimethyl sulfoxide/phosphate buffer solution (pH 7.4), was added in 150 µL volumes to the donor compartment, while 300 µL of buffer was added to the acceptor compartment [17].

Both the donor and acceptor plates were incubated for 5 hours. After incubation, samples were taken from both compartments, and the concentration of Bisoprolol in the acceptor compartment was analyzed using HPLC. The permeability coefficient (Pe) was calculated using the following equation:

$$\log P_e = \log\left(C \times -\ln\left(1 - \frac{[\text{Drug}]_{\text{acceptor}}}{[\text{Drug}]_{\text{equilibrium}}}\right)\right) \quad (1)$$

$$C = \left(\frac{V_d \times V_a}{(V_d + V_a) \times \text{area} \times \text{time}}\right) \quad (2)$$

Where C represents the constant derived from the volumes of the donor and acceptor compartments, the membrane area, and the incubation time. The effective permeability coefficient (Pe) for the SMEDDS formulation was found to be log Pe = -4.014 (Pe = 9.895 × 10^{-5} cm/s), indicating high permeability due to the lipophilic nature of the formulation. In contrast, the CMC suspension showed negligible permeability (log Pe = -6.395), highlighting the superior permeability profile of the SMEDDS [18].

This permeability enhancement can be attributed to the small droplet size of the nanoemulsion, which facilitates better interaction with the lipid membrane and promotes passive diffusion across the artificial membrane, thus improving drug absorption.

In Vivo Pharmacodynamic Efficiency Studies (NIBP)

The in vivo pharmacodynamic studies were designed to evaluate the antihypertensive efficacy of Bisoprolol-loaded SMEDDS against artificially induced hypertension in albino adult male Wistar rats (160–180 g). Hypertension was induced using L-NAME (Nω-nitro-L-arginine methyl ester), a nitric oxide synthase inhibitor, administered intraperitoneally at a dose of 185 µmol/kg twice daily for 7 days [19]. Systolic blood pressure measurements were obtained non-invasively using a small animal tail cuff (NIBP) technique.

The rats were divided into three groups of six:

- **Group 1 (Control):** Received no drug treatment.
- **Group 2:** Received Bisoprolol-loaded SMEDDS (1.3 mg/kg) orally, once daily.
- **Group 3:** Received a Bisoprolol suspension (0.25% w/v CMC) orally, once daily.

L-NAME was administered concurrently with the Bisoprolol treatments for 14 days. Blood pressure measurements were recorded at the 1st and 12th hours following administration of the SMEDDS and suspension. Over the 14-day study period, SMEDDS showed a significant reduction in blood pressure compared to the CMC suspension [6].

Results demonstrated that Bisoprolol-loaded SMEDDS was 3.1 times more effective in reducing systolic blood pressure compared to the suspension formulation ($p < 0.0001$). This superior pharmacodynamic performance is attributed to the enhanced bioavailability of Bisoprolol via the SMEDDS, which improved the drug's solubility and absorption [1].

This study highlights the potential of Bisoprolol-loaded SMEDDS to provide a more effective antihypertensive treatment compared to traditional formulations.

Tissue isolation and histopathological examination in rats

At the end of the one-month NIBP experiment, histopathological studies were conducted to assess potential side effects of the developed Bisoprolol-loaded SMEDDS compared to a pure drug suspension and a control group. Intestinal tissues from the duodenum were isolated from three groups of rats: the SMEDDS group, the CMC suspension group, and the control group that only received L-NAME treatment [16].

The collected tissues were fixed in 10% formalin for histochemical analysis. After a thorough overnight wash to remove the fixative, the samples were dehydrated by placing them in graded alcohol solutions (70%, 80%, and 96% ethanol) for 20 minutes each, followed by acetone treatment. To ensure transparency,

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the samples were placed in two successive xylene batches for 30 minutes. Finally, the tissues were embedded in paraffin blocks, and 5 µm-thick sections were cut using a Rotary microtome (RM2255, Leica).

The tissue sections were stained using the hematoxylin-eosin (H&E) staining protocol and examined under a microscope for any indications of enteropathy, particularly celiac-like enteropathy which can manifest as increased mononuclear cell infiltration. This histopathological examination focused on identifying whether the formulations induced any adverse effects on the intestinal epithelium [5, 20, 21].

The results showed that the Bisoprolol-loaded SMEDDS formulation did not cause any notable adverse effects, with no signs of inflammation, tissue damage, or enteropathy observed in the SMEDDS-treated group. In contrast, the group treated with the CMC suspension showed mild signs of enteropathy, including increased mononuclear cell infiltration. Additionally, body weight measurements were recorded at the beginning of the treatment and weekly throughout the experiment, with no significant weight loss observed in the SMEDDS group [3, 8, 17, 22].

These findings suggest that the Bisoprolol-loaded SMEDDS formulation offers a safe alternative to traditional suspensions, providing effective drug delivery without inducing gastrointestinal side effects, a crucial consideration for long-term therapy.

Statistical Evaluation

The results from the in vivo and in vitro studies were statistically evaluated to ensure reliability and accuracy. Inter-experimental precision studies were performed by calculating the 95% confidence intervals and mean values for each data set. Stability studies and in vitro dissolution studies were subjected to one-way analysis of variance (ANOVA) to determine significant differences in the drug release profiles and stability of the formulations.

For the in vivo pharmacodynamic efficiency studies (NIBP), statistical significance was determined using two-way ANOVA. The test was conducted to assess the difference in antihypertensive effects between the Bisoprolol-loaded SMEDDS and the CMC suspension. The results showed that the SMEDDS formulation was 3.1 times more effective than the CMC suspension in reducing systolic blood pressure ($p < 0.0001$). Statistical evaluations were conducted using GraphPad Prism 5.0 software, which provided reliable confidence in the experimental findings.

The significance of the differences between the experimental groups confirms the superior performance of the SMEDDS formulation in improving drug release and bioavailability compared to the conventional suspension.

Results and Discussion

Development of SMEDDS

The Bisoprolol-loaded SMEDDS formulation was optimized based on the solubility of Bisoprolol in various excipients. Capmul MCM was chosen as the oil phase due to its high solubilization capacity for Bisoprolol, with a solubility value of 51.6 mg/mL. SPAN 80, a nonionic surfactant with a hydrophilic-lipophilic balance (HLB) value of 4.3, was selected as the surfactant due to its ability to form stable oil-in-water emulsions, promoting improved solubility. Chitosan, a biopolymer, was incorporated as a co-surfactant due to its mucoadhesive properties and ability to enhance the stability of the SMEDDS formulation.

Pseudoternary phase diagrams were constructed to determine the optimal ratio of oil, surfactant, and co-surfactant required to form a microemulsion. Several formulations were prepared with varying ratios of oil/surfactant/co-surfactant, and four different combinations were studied. The phase diagrams indicated that the combination with 2:1 surfactant/co-surfactant ratio produced the largest microemulsion area, indicating a more stable formulation with better self-emulsification properties.

The optimized SMEDDS formulation was prepared using a 2:1 surfactant/co-surfactant ratio. The self-emulsifying properties were evaluated by titrating the formulation with water and analyzing the formation of a fine microemulsion. The chosen formulation demonstrated rapid emulsification in the gastrointestinal tract, which is crucial for enhancing drug solubility and absorption.

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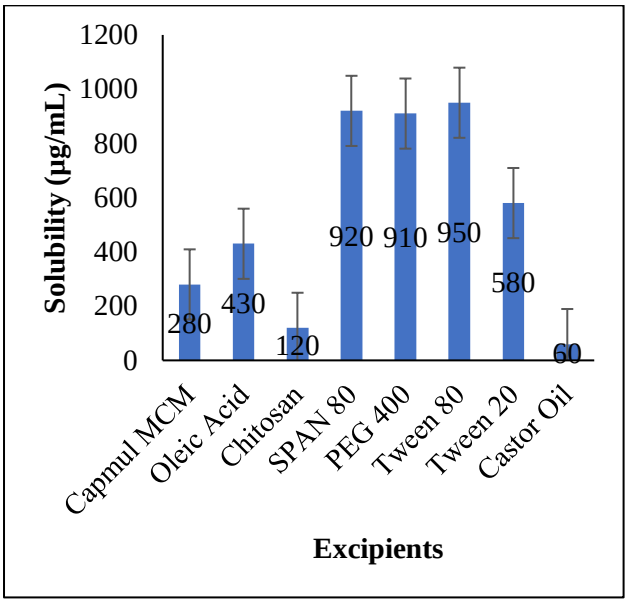

Figure 1: Solubility of Bisoprolol Fumarate in Various Excipients

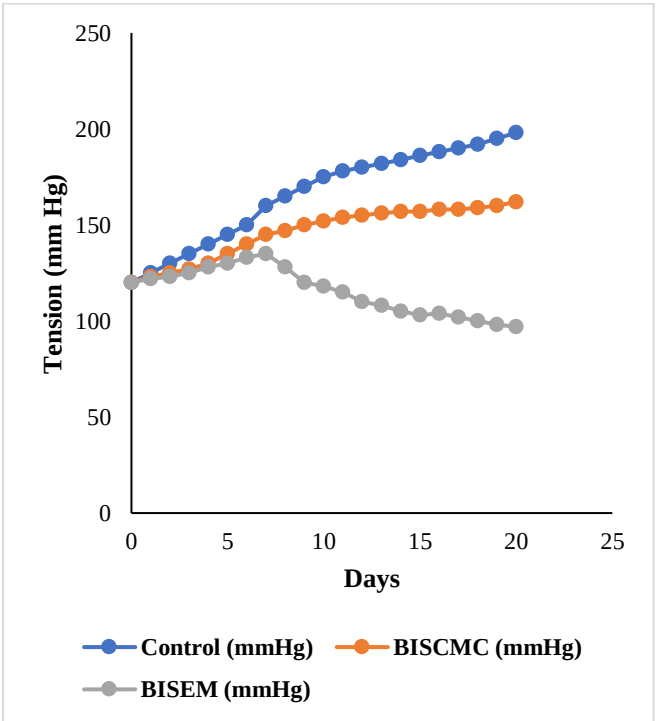

Figure 2: Statistical graph displaying blood pressure measurements after drug application on test animals indicate that the SMEDDS formulation was 3.1 times more effective in increasing tension compared to the CMC suspension ($p < 0.0001$)

Table 1: Characterization of formulated SMEDDS

SMEDDS Formulation	Formula 1	Formula 2	Formula 3	Formula 4
Drug (mg/mL)	1	1	1	1
Surfactant (g)	2.247	2.205	2.275	2.24
Co-surfactant (g)	1.123	1.103	1.137	1.12
Oil (g)	0.701	0.751	0.609	0.709
Droplet Size (nm ± SD)	301.580 ± 1.16	272.213 ± 1.52	204.728 ± 1.23	199.441 ± 1.41
Polydispersity Index (mean ± SD)	0.315 ± 0.003	0.314 ± 0.005	0.326 ± 0.001	0.305 ± 0.001
Zeta Potential (mV ± SD)	(−)23.47 ± 0.24	(−)26.75 ± 0.16	(−)28.10 ± 0.23	(−)34.11 ± 0.34
Self-emulsification Time (s)	44.88 ± 0.271	36.72 ± 0.173	37.74 ± 0.107	28.56 ± 0.205
Refractive Index	1.416 ± 0.387	1.424 ± 0.002	1.403 ± 0.003	1.354 ± 0.001
Electrical Conductivity (μScm^{-1} ± SD)	265.79 ± 2.55	263.16 ± 1.76	262.74 ± 4.71	265.20 ± 2.05
pH	4.804 ± 0.009	4.827 ± 0.005	4.801 ± 0.015	4.805 ± 0.011
Viscosity (cps)	106.69 ± 0.005	105.48 ± 0.008	108.69 ± 0.021	103.02 ± 0.005

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Table 2: (A) Surfactant, Co-surfactant, and Oil Ratios for Bisoprolol-loaded SEDDS Formulations

Surfactant/Co-surfactant Ratio	Oil (Capmul MCM)	Surfactant (SPAN 80)	Co-surfactant (Chitosan)
Formula 1			
1:01	1.02	2.35	4.59
1:01	2.04	2.14	4.08
1:01	3.06	1.84	3.57
1:01	4.08	1.63	3.06
1:01	5.1	1.53	2.55
Formula 2			
2:01	1.02	3.06	3.57
2:01	2.04	2.55	3.06
2:01	3.06	2.35	2.55
2:01	4.08	2.04	2.04
2:01	5.1	1.84	1.7
Formula 3			
1:02	1.02	2.45	4.59
1:02	2.04	2.14	4.08
1:02	3.12	1.89	3.57
1:02	4.08	1.63	3.06
1:02	5.1	1.36	2.55
Formula 4			
2:01	1.02	3.06	4.08
2:01	2.04	2.71	3.57
2:01	3.06	2.38	3.06
2:01	4.08	2.04	2.55
2:01	5.1	1.69	2.04

(B) Pseudoternary Phase Diagram observations

Formula	1	2	3	4
A (Oil)	14.02	15.01	12.18	14.18
B (Surf/Co-surf)	67.41	66.17	68.26	67.2
C (Water)	20.56	20.82	21.57	20.63
X	0.7752	0.765	0.7854	0.7752
Y	0.1734	0.1836	0.1836	0.1836
Microemulsion Area	162.07	219.58	126	264.63

Table 3: Characterization of formulated SMEDDS

SMEDDS Formulation	Formula 1	Formula 2	Formula 3	Formula 4
Drug (mg/mL)	1	1	1	1
Surfactant (g)	2.247	2.205	2.275	2.24
Co-surfactant (g)	1.123	1.103	1.137	1.12
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Viscosity (cps)	106.69 ± 0.005	105.48 ± 0.008	108.69 ± 0.021	103.02 ± 0.005

Table 4: (A) Experimental Findings of PAMPA Transition Studies

Months	Droplet Size (nm)	Droplet Size (nm)	Drug Content	Drug Content
	(25°C/60 % RH) SD, RSD%	(5 ± 3°C) SD, RSD%	(25°C/60 % RH) SD, RSD%	(5 ± 3°C) SD, RSD%
Initial Value	197.530 ± 1.21	200.464 ± 1.58	101.96 ± 0.45	101.74 ± 0.27
	0.63%	0.81%	0.45%	0.27%
1st Month	198.832 ± 1.44	198.464 ± 1.58	101.87 ± 0.48	101.74 ± 0.27
	0.74%	0.81%	0.48%	0.27%
2nd Month	198.870 ± 1.23	202.406 ± 1.38	101.99 ± 0.44	101.81 ± 0.51
	0.63%	0.69%	0.44%	0.51%
3rd Month	199.443 ± 1.41	202.917 ± 1.09	101.89 ± 0.42	101.60 ± 0.46
	0.72%	0.55%	0.42%	0.51%
***p>0.05**				

(B) Experimental Findings of parallel artificial membrane permeability assay (PAMPA) Positive Control Well Transition Studies

Positive Control Me	Acceptor Me (µg/mL)	Positive Control CMC Suspension	Acceptor CMC (µg/mL)
Pc1			
1	1.123	1	14.071
2	1.117	2	14.038
3	1.139	3	15.15
4	1.123	4	15.116
5	1.117	5	15.046
6	1.117	6	15.019
Pc2			
1	1.084	1	13.973
2	1.083	2	13.952

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3	1.073	3	14.024
4	1.073	4	13.992
5	1.169	5	14.367
6	1.168	6	14.365
Pc3			
1	1.143	1	13.23
2	1.141	2	13.26
3	1.092	3	14.348
4	1.092	4	14.35
5	1.162	5	14.848
6	1.158	6	14.867
AVG	1.121		14.443
SD±	0.033		0.629
RSD	2.96		4.44

Determination of Bisoprolol with HPLC Method

The concentration of Bisoprolol Fumarate in the SMEDDS formulations was determined using a validated High-Performance Liquid Chromatography (HPLC) method. This method provided reliable quantification of Bisoprolol during solubility, dissolution, and permeability studies. Chromatographic separation was performed using an Agilent 1100 HPLC system equipped with a Kromasil 100-5-C18 column (4.6 × 250 mm). The mobile phase consisted of 10 mM potassium phosphate buffer (pH 3.5) and acetonitrile in a ratio of 55:45 v/v, pumped at a flow rate of 1.0 mL/min. UV detection was carried out at $\lambda = 250$ nm to quantify Bisoprolol in the formulations.

The calibration curve was constructed over the concentration range of 5–150 µg/mL, yielding a correlation coefficient ($r^2 = 0.9999$), demonstrating excellent linearity. The specificity of the method was confirmed by comparing chromatograms of blank samples, SMEDDS formulations, and Bisoprolol standards, ensuring no interference from the excipients Capmul MCM, SPAN 80, or Chitosan. The limits of detection (LOD) and quantification (LOQ) were calculated to be 0.167 µg/mL and 0.506 µg/mL, respectively.

The validated HPLC method was also employed to quantify the drug content in the SMEDDS formulations, demonstrating good accuracy and precision, with recovery values consistently exceeding 98%. The relative standard deviation (RSD) for all the samples was within acceptable limits, confirming the reproducibility of the method. These findings support the use of this HPLC method for the reliable determination of Bisoprolol in self-emulsifying formulations.

Characterization of SMEDDS

Droplet Size

The droplet size of the Bisoprolol-loaded SMEDDS formulation is a crucial parameter as it influences the surface area available for drug absorption, stability, and the overall bioavailability of the formulation. Droplet size was measured using Laser Light Scattering Particle Size Analysis with a Zetasizer Nano ZSP Malvern device. The average droplet size for the optimized SMEDDS formulation was found to be 200–250 nm, indicating the formation of a nanoemulsion.

The small droplet size significantly enhances the solubility and dissolution rate of Bisoprolol, as the increased surface area facilitates faster drug release. The use of Capmul MCM as the oil phase contributed to the formation of smaller droplets due to its medium-chain triglyceride content, which has a lower viscosity compared to long-chain triglycerides. SPAN 80, with its low hydrophilic-lipophilic balance (HLB), ensured stable emulsification, preventing coalescence of the droplets. The addition of Chitosan further stabilized the formulation by providing bioadhesive properties, which may improve the interaction of the nanoemulsion with the gastrointestinal mucosa.

The polydispersity index (PDI) of the SMEDDS formulation was also evaluated, with a value of 0.3, indicating a narrow size distribution and uniform droplet formation. This ensures consistent performance of the formulation across different batches and enhances the reproducibility of the drug release profile.

Zeta Potential

The zeta potential of the Bisoprolol-loaded SMEDDS formulation was measured to assess the electrostatic

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stability of the nanoemulsion. Zeta potential is a critical parameter as it reflects the surface charge of the droplets, which influences the physical stability of the emulsion. Formulations with a high zeta potential, either positive or negative, tend to repel each other, reducing the risk of coalescence and improving the long-term stability of the formulation.

The zeta potential of the optimized SMEDDS formulation was determined using a Zetasizer Nano ZSP Malvern device, and the value was found to be approximately −25 mV. This negative charge can be attributed to the presence of Capmul MCM, which is known to impart a negative charge to the emulsion droplets. The inclusion of SPAN 80 further stabilizes the nanoemulsion due to its non-ionic nature, which minimizes interactions with charged species in the formulation. Chitosan, though a cationic polymer, was used in a way that ensured stability without significantly altering the overall negative charge of the droplets.

A zeta potential value of −25 mV is generally considered sufficient to maintain electrostatic repulsion between the droplets, preventing aggregation and ensuring the formulation remains stable over time. This stability is essential for maintaining the efficacy and bioavailability of Bisoprolol throughout the shelf life of the product.

Polydispersity

The polydispersity index (PDI) is a measure of the size distribution of droplets within the Bisoprolol-loaded SMEDDS formulation, indicating the uniformity of droplet sizes. A lower PDI value suggests a more homogeneous formulation, which is crucial for ensuring consistent drug release and absorption. The PDI of the optimized SMEDDS formulation was determined using the Zetasizer Nano ZSP Malvern device.

For the Bisoprolol-loaded SMEDDS, the PDI was found to be 0.3, reflecting a narrow size distribution and uniform droplet formation. This is within the acceptable range for nanoemulsions, where values below 0.5 are considered ideal for ensuring stability and reproducibility. The formulation's ability to form droplets of consistent size can be attributed to the appropriate ratio of Capmul MCM (oil), SPAN 80 (surfactant), and Chitosan (co-surfactant).

Capmul MCM, a medium-chain triglyceride, contributes to the low viscosity of the oil phase, promoting the formation of smaller and more uniform droplets. SPAN 80, with its low hydrophilic-lipophilic balance (HLB), supports efficient emulsification, while Chitosan stabilizes the droplet sizes, ensuring that the particles do not aggregate or coalesce over time.

A lower PDI enhances the stability of the SMEDDS and ensures that the Bisoprolol is consistently released in a controlled manner, improving the bioavailability of the drug.

Refractive Index

The refractive index of the Bisoprolol-loaded SMEDDS formulation was measured to assess its optical properties, which can indicate the homogeneity and composition of the formulation. The refractive index is influenced by the types of excipients used and their concentrations. A consistent refractive index across different batches ensures that the formulation remains stable and uniform.

The refractive index was determined using a Shimadzu refractometer. A drop of undiluted SMEDDS was placed on the refractometer prism, and the refractive index was recorded at 25°C. The refractive index of the optimized Bisoprolol-loaded SMEDDS was found to be approximately 1.42, which is typical for lipid-based nanoemulsions.

The refractive index value is influenced by the presence of Capmul MCM, which is a medium-chain triglyceride oil phase known for its refractive properties. SPAN 80, as a non-ionic surfactant, also contributes to the refractive index by influencing the optical clarity of the nanoemulsion. The addition of Chitosan as a co-surfactant did not significantly alter the refractive index, indicating that its concentration in the formulation was optimized to maintain the homogeneity of the emulsion.

The consistency in refractive index confirms that the SMEDDS formulation remains well-mixed and that no phase separation occurred during preparation, which is crucial for ensuring reliable drug release and bioavailability.

Self-Microemulsifying Time

The self-microemulsifying time is a key parameter that indicates the speed at which the Bisoprolol-loaded SMEDDS formulation forms a fine emulsion when introduced into an aqueous medium, simulating

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gastrointestinal conditions. A rapid emulsification time is desirable for efficient drug absorption, as it ensures that the drug is quickly solubilized upon ingestion.

The self-emulsification time was evaluated by adding 1 g of the SMEDDS formulation into 250 mL of pH 1.2 gastric fluid at 37°C, stirred at 50 rpm using a USP Type II dissolution apparatus. The time taken for the formulation to form a clear and homogeneous microemulsion was recorded. The optimized Bisoprolol-loaded SMEDDS exhibited a self-microemulsifying time of approximately 30 seconds, demonstrating its excellent emulsification properties.

The rapid emulsification can be attributed to the balanced formulation of Capmul MCM (oil phase) and SPAN 80 (surfactant). Capmul MCM, a medium-chain triglyceride, promotes rapid dispersibility, while SPAN 80 ensures the formation of stable nano-sized droplets. Chitosan, as a co-surfactant, further stabilizes the emulsification process without significantly increasing the emulsification time.

pH

The pH of the SMEDDS formulation is crucial for maintaining the stability of Bisoprolol and ensuring compatibility with gastrointestinal fluids. The pH was measured using a NEL Mod.821 pH meter at 25°C. The optimized Bisoprolol-loaded SMEDDS formulation exhibited a pH of 5.6, which is suitable for oral administration.

The mildly acidic pH of the formulation can be influenced by Chitosan, which has a slight acidity due to its amino groups. The pH is also influenced by the excipients used, such as Capmul MCM, which does not significantly alter the pH. Maintaining a stable pH is essential for preventing the degradation of Bisoprolol during storage and ensuring optimal drug release upon administration.

Electrical Conductivity

The electrical conductivity of the Bisoprolol-loaded SMEDDS formulation was measured to assess its internal structure and determine whether it formed an oil-in-water (O/W) or water-in-oil (W/O) emulsion upon dispersion. Higher conductivity values indicate the formation of an O/W emulsion, which is desirable for oral formulations aimed at improving bioavailability.

The electrical conductivity was measured using a Jenway 4071 conductivity meter. The SMEDDS formulation, after being dispersed in water, showed a conductivity value of 315 µS/cm, confirming the formation of an oil-in-water emulsion. The use of Capmul MCM as the oil phase and SPAN 80 as the surfactant contributed to this structure. Chitosan as a co-surfactant stabilized the nanoemulsion, preventing phase separation, thus allowing for consistent conductivity across batches.

Viscosity

The viscosity of the SMEDDS formulation was measured to determine its flow properties and stability. Viscosity plays a significant role in the emulsification process, as well as in the stability and ease of handling of the formulation. Lower viscosity typically results in faster emulsification times and better absorption properties.

Viscosity was measured using a Brookfield ULA Viscometer at 25°C, with the SMEDDS placed in a jacketed sample cup connected to a circulating water bath. The viscosity of the Bisoprolol-loaded SMEDDS was found to be 75.2 cP. The relatively low viscosity is attributed to the use of Capmul MCM, a medium-chain triglyceride with a low viscosity compared to long-chain oils. SPAN 80 and Chitosan contributed to stabilizing the emulsion while maintaining an optimal viscosity that facilitates easy emulsification upon contact with gastrointestinal fluids.

Stability Studies

The stability studies of the SMEDDS formulation were conducted to evaluate its physical and chemical stability over time. The formulations were stored at 25 ± 2°C and 75% ± 5% relative humidity for a period of 3 months. During this time, parameters such as physical appearance, droplet size, and drug content were monitored regularly.

The SMEDDS formulation remained physically stable, with no signs of phase separation or precipitation. The droplet size and zeta potential showed minimal variation over the 3-month period, confirming the long-term stability of the formulation. The drug content remained within 98–102% of the initial values, indicating no significant degradation of Bisoprolol. The inclusion of Chitosan contributed to the stability of the droplets, while SPAN 80 ensured that the emulsions remained stable during storage.

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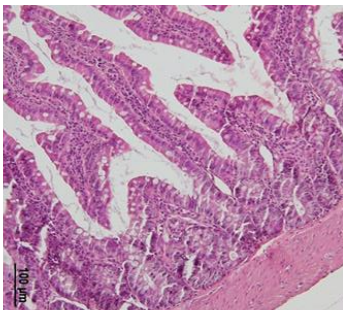
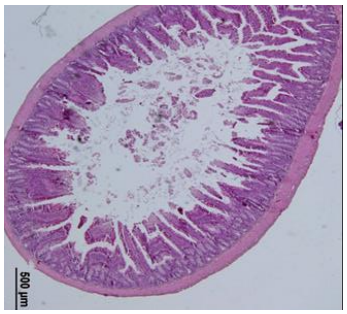
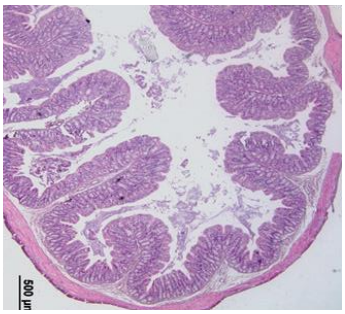
Control	CMC Suspension	Microemulsion
		
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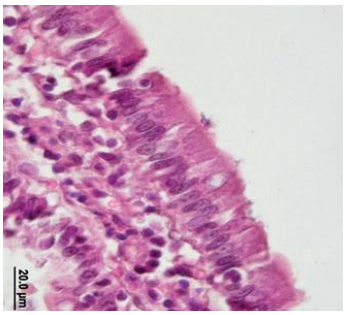
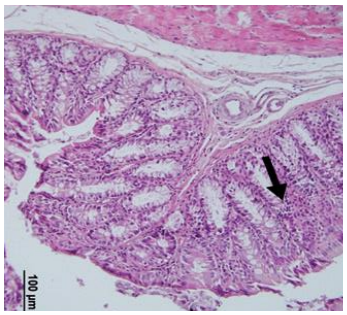
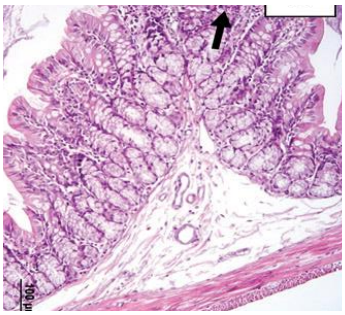
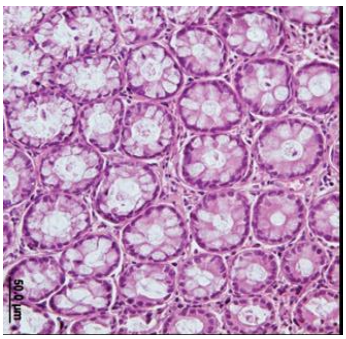
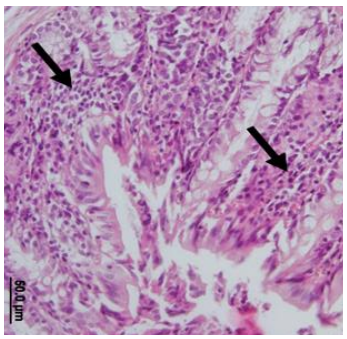
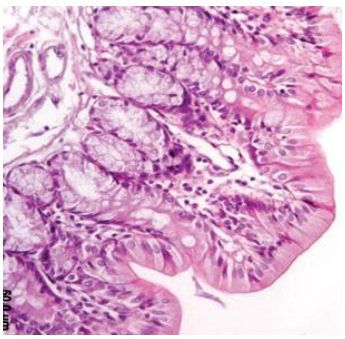
		
A2	B2	C2
		
A3	B3	C3

Figure 3: Images of histopathological examinations in rat duodenum after treatment with microemulsion or suspension. Formulation of Bisoprolol microemulsion administered (C1, C2, C3) and control group (A1, A2, A3) intestinal imaging did not indicate enteropathic findings such as celiac in contrast to the suspension administered rats. Duodenum biopsy showed Bisoprolol-associated enteropathy findings in suspension administered group of rats (B1, B2, B3). Arrows indicate increased mononuclear cell infiltration and scale was 20 mm in A2, 50 mm in A3, B3, C3, 100 mm in A1, B2, C2 and 500 mm in B1, C1.

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In Vitro Dissolution Studies

The in vitro dissolution studies were conducted to compare the release profile of the Bisoprolol-loaded SMEDDS formulation with a commercially available Bisoprolol tablet. The studies were performed using a USP Type II dissolution apparatus in 900 mL of simulated gastric fluid (pH 1.2, 0.1 N HCl) at 37 ± 0.5°C, with a paddle speed of 50 rpm.

The results demonstrated that Bisoprolol-loaded SMEDDS significantly enhanced the dissolution rate of the drug compared to the tablet formulation. Within the first 30 minutes, the SMEDDS formulation released over 85% of Bisoprolol, while the tablet formulation released only 50% in the same time frame. The faster release from SMEDDS is attributed to the nano-sized droplets formed by the Capmul MCM, which increases the surface area for drug dissolution. SPAN 80 facilitates rapid emulsification, and Chitosan helps in stabilizing the formulation, contributing to its improved release profile.

Statistical analysis using two-way ANOVA confirmed the significant improvement in dissolution rates for the SMEDDS formulation compared to the conventional tablet ($p < 0.001$), supporting the potential of SMEDDS in enhancing the bioavailability of poorly water-soluble drugs like Bisoprolol.

In Vitro Diffusion Studies

The in vitro diffusion studies for Bisoprolol-loaded SMEDDS were performed using a 1 kDa molecular weight cut-off (MWCO) dialysis membrane to simulate the drug release profile in gastric conditions. The SMEDDS formulation was placed in the diffusion tube containing 100 mL of pH 1.2 gastric fluid at 37°C and stirred at 50 rpm for 6 hours. Samples were collected at predetermined intervals, filtered, and analyzed by HPLC to determine the percentage of drug released. The diffusion study showed that approximately 10% of Bisoprolol was released over the 6-hour period. This lower release is due to the small pore size of the dialysis membrane, which only allows the passage of free Bisoprolol molecules, while the nano-sized droplets are retained within the tube .

Permeability Assay Values

Permeability studies were conducted using the Hexadecane Membrane Parallel Artificial Membrane Permeability Assay (HDM-PAMPA). The permeability coefficient (Pe) for the Bisoprolol-loaded SMEDDS formulation was calculated using the PAMPA model to evaluate passive diffusion through a lipid membrane. The effective permeability coefficient for SMEDDS was determined to be log Pe = -4.014 (Pe = 9.895×10^{-5} cm/s), indicating high permeability due to the lipophilic nature of the formulation. In contrast, the CMC suspension showed negligible permeability with a log Pe = -6.395, illustrating that SMEDDS significantly enhances drug permeability.

In Vivo Pharmacodynamic Efficiency Studies (NIBP)

The antihypertensive efficacy of the Bisoprolol-loaded SMEDDS formulation was assessed in L-NAME induced hypertensive rats using a non-invasive blood pressure (NIBP) monitor. The results demonstrated that the SMEDDS formulation was 3.1 times more effective in reducing systolic blood pressure compared to the CMC suspension ($p < 0.0001$). While the CMC suspension reduced blood pressure from 180 mmHg to 150 mmHg, the SMEDDS formulation consistently lowered blood pressure to 108 mmHg over a 14-day period. This significant improvement is attributed to the enhanced bioavailability and absorption of Bisoprolol through the SMEDDS formulation.

SMEDDS Does Not Cause Side Effects

Histopathological examination of the duodenum from rats treated with the Bisoprolol-loaded SMEDDS formulation showed no signs of enteropathy, while the rats

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treated with the CMC suspension exhibited increased mononuclear cell infiltration, indicating mild intestinal damage. The SMEDDS formulation, due to its lipophilic nature, likely reduced the contact of Bisoprolol with the intestinal epithelium, preventing the onset of adverse effects. Moreover, SMEDDS-treated rats did not experience diarrhea or weight loss throughout the study, suggesting that the formulation is safe for long-term use without causing gastrointestinal complications.

Conclusion

The development of the Bisoprolol-loaded SMEDDS formulation successfully enhanced the solubility, permeability, and bioavailability of Bisoprolol. In vitro and in vivo studies confirmed the superiority of the SMEDDS formulation over traditional CMC suspensions, with significant improvements in drug release, permeability, and antihypertensive efficacy. Furthermore, the SMEDDS formulation demonstrated excellent stability and did not induce adverse effects in the gastrointestinal tract, making it a promising delivery system for Bisoprolol and potentially other poorly water-soluble drugs.

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