Development of a PDE4 Inhibitor-Loaded Ethosomal Nanogel: Physicochemical Characterization and In Vitro Release Studies

[1]Suraj Vishwas, [2]Dr Swarnali Das Paul, [3]Dr Anil Kumar Sahu

[1]Faculty of Pharmaceutical Sciences, Shri Shankaracharya Technical Campus, Bhilai (C.G.)

[2]Shri Shankaracharya College of Pharmaceutical Sciences, SSPU, Bhilai, 490020, C.G., India

[3]Royal College of Pharmacy, Raipur (C.G.)

*Corresponding author:

Suraj Vishwas

Faculty of Pharmaceutical Sciences, Shri Shankaracharya Technical Campus, Bhilai (C.G.)

Abstract

Transdermal drug delivery systems offer significant advantages over conventional routes, including improved patient compliance and sustained therapeutic effects. This study aimed to develop and characterize an ibudilast-loaded ethosomal nanogel formulation for enhanced transdermal delivery. Ethosomes were prepared and subsequently incorporated into a Carbopol 934 nanogel matrix. The ethosomal vesicles exhibited an average particle size of 193.20 ± 0.85 nm with a polydispersity index of 0.264 ± 0.002, indicating uniform size distribution. The zeta potential was -28.3 ± 0.32 mV, confirming excellent colloidal stability. SEM analysis revealed spherical vesicles with smooth surfaces and a discrete distribution. The entrapment efficiency was 79.83 ± 0.22%, demonstrating efficient drug loading. The nanogel formulation displayed transparency, physiological pH (6 ± 0.5), favorable spreadability (6.2 ± 1 cm), and pseudoplastic rheological behavior. SEM of the nanogel revealed a fibrous, interconnected porous network structure. The drug content was 97.95 ± 0.5%. In vitro release studies demonstrated that while free ethosomes released 78.4% of ibudilast within 8 hours, the ethosomal nanogel achieved

sustained release with 86.7% cumulative drug release over 24 hours. The PDE4 inhibitor-loaded ethosomal nanogel formulation exhibited optimal physicochemical properties and sustained drug release characteristics, demonstrating significant potential as an effective transdermal delivery system for ibudilast with improved patient compliance and therapeutic efficacy.

Keywords: Ibudilast, ethosomes, nanogel, transdermal delivery, sustained release, PDE4 inhibitor.

1. Introduction

Transdermal drug delivery has emerged as an attractive alternative to conventional oral and parenteral routes, offering numerous advantages including bypassing hepatic first-pass metabolism, maintaining steady-state plasma concentrations, reducing dosing frequency, minimizing systemic side effects, and improving patient compliance. However, the stratum corneum, the outermost layer of the skin, presents a formidable barrier to the permeation of drugs, limiting the transdermal delivery of many therapeutic agents. This challenge requires the development of advanced formulation strategies that can enhance drug penetration through the skin barrier while maintaining therapeutic efficacy [1].

Phosphodiesterase (PDE) inhibitors represent a significant therapeutic class that functions by preventing the enzymatic degradation of cyclic nucleotides, specifically cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), thereby elevating their intracellular concentrations and modulating critical cellular processes such as inflammation, immune responses, and smooth muscle relaxation [2]. Among the PDE enzyme family comprising multiple isoforms (PDE1-12), PDE4 inhibitors have garnered considerable attention due to their potent anti-inflammatory properties, which are mediated through the suppression of pro-inflammatory cytokine production [3].

Ibudilast, a pyrazolo-pyridine derivative, functions as a broad-spectrum PDE inhibitor with activity against PDEs 3, 4, 10, 11, and 12. By elevating intracellular cAMP levels, ibudilast effectively suppresses the synthesis of pro-inflammatory cytokines and attenuates inflammation. Despite these promising therapeutic attributes, the clinical utility of ibudilast is

compromised by limitations inherent to conventional oral administration, including poor bioavailability, first-pass metabolism, and systemic side effects. Transdermal delivery presents a viable strategy to overcome these limitations [4].

Ethosomes, novel lipid-based nano vesicular carriers containing high concentrations of ethanol (20-45%), have demonstrated superior skin penetration capabilities compared to conventional liposomes [5,6]. The incorporation of ethosomes into gel matrices, combining the penetration-enhancing properties of ethosomes with the controlled-release characteristics of hydrogels, represents a synergistic strategy. Carbopol 934 was selected for nanogel development due to its exceptional viscosity, mucoadhesive properties, and biocompatibility [7]. This investigation aimed to develop and comprehensively characterize an ibudilast-loaded ethosomal nanogel formulation, integrating penetration-enhancing capabilities with sustained-release properties to provide an effective, patient-friendly therapeutic alternative.

2. Experimental methods

2.1 Chemicals and Reagents

Ibudilast and soya lecithin were procured from Otto Chemie Pvt Ltd. All other chemicals used in the study were of analytical grade.

2.2 Preparation of ethosomal suspension

Ethosomes were formulated following the method described by Touitou et al. (2000) [8] with slight modifications. Briefly, lecithin (4% w/v) was dissolved in ethanol (30% v/v) containing ibudilast using a magnetic stirrer in a round-bottom flask, which was kept covered to minimize ethanol loss. Thereafter, distilled water was gradually added under continuous stirring, resulting in the formation of ethosomal colloidal dispersions. The resulting suspension was maintained at room temperature for 30 minutes with constant agitation, after which it was stored under refrigeration until further characterization. The prepared formulations were evaluated for vesicle size, zeta potential, polydispersibility index, morphology, entrapment efficiency, and in vitro drug release behavior.

2.2.1 Determination of Vesicle Size, Size Distribution, and Zeta Potential

The average vesicle size (VS), polydispersity index (PDI), and zeta potential (ZP) of ibudilast-loaded ethosomes were determined at room temperature using dynamic light scattering (DLS, Anton Paar Litesizer 500). For analysis, the samples were diluted to 0.5% (w/v) with deionized water and subjected to agitation for 3 minutes. This procedure was carried out in triplicate to ensure reproducibility [9].

2.2.2 Field emission scanning electron microscope (FESEM) analysis

The morphological features of ethosomes and nanogels were examined using a field-emission scanning electron microscope (FESEM, Carl Zeiss Ultra-High-Resolution Gemini SEM 500, KMAT, India). A drop of the sample was carefully placed on a clean glass stub, air-dried, and subsequently coated with a thin layer of gold to enhance conductivity. The specimens were then visualized under FESEM at a suitable magnification.

2.2.3 Entrapment efficiency

The entrapment efficiency (% EE) of the prepared ethosomes was determined by calculating the difference between the total drug added and the unentrapped drug. Unencapsulated ibudilast was quantified using a centrifugation method. Briefly, the formulations were centrifuged in an ultracentrifuge (Remi) with a TLA-45 rotor at 14,000 rpm and 4 °C for 30 minutes. The supernatant was carefully collected, and the residue was re-centrifuged for an additional 15 minutes under the same conditions. The total amount of unentrapped drug was then measured using a UV/Vis spectrophotometer at a wavelength of 227 nm. All measurements were performed in triplicate [10]. The percentage of encapsulated drug amount was calculated using the following formula:

%EE = (Amount of the drug in the ethosome /Total amount of drug loaded into the ethosome) ×100

2.2.4 In vitro permeation study

The in vitro drug release of ethosomes and ethosomal nanogel was evaluated using the dialysis bag diffusion method (molecular weight cut-off 12,000 Da). Briefly, 1 mL of each

formulation was placed in separate dialysis bags, which were then immersed in 20 mL of PBS buffer and maintained at 37 °C with continuous stirring at 100 rpm. Samples of the release medium were withdrawn at predetermined intervals (0.5, 1, 2, 4, 6, 8, 12, 16, 20, and 24 hours) and replaced with an equal volume of fresh buffer. The amount of ibudilast released was quantified using UV–visible spectroscopy [11].

2.3 Formulation of ethosomal nanogel

The ibudilast-loaded nanogel was prepared by incorporating Carbopol 934 into ibudilast-loaded ethosomes. Briefly, Carbopol 934 (0.75% w/v) was dispersed in distilled water and allowed to hydrate for 1 hour. The pH of the dispersion was then adjusted to 6-6.5 using triethanolamine. Ibudilast-loaded ethosomes were added to the hydrated Carbopol dispersion and gently stirred at 1200 rpm for 15 minutes (Remi Motors Ltd., India) to obtain the ethosomal nanogel. [12]

2.4 Characterization of Nanogel

2.4.1 Organoleptic properties

The organoleptic characteristics of the Ibudilast nanogel, including color, uniformity, and the presence of grittiness, were evaluated through visual inspection.

2.4.2 Clarity and Refractive Index Determination

The transparency of the formulated Carbopol-based nanogel was examined visually under natural light against both black and white backgrounds to detect any turbidity or suspended particles. For quantitative evaluation, the refractive index was measured using an Abbe-type digital refractometer (Anton Paar) maintained at 25 ± 0.5 °C. A small amount of the nanogel was applied to the prism surface, and readings were recorded once the sample reached equilibrium. Distilled water (n = 1.333 at 25 °C) served as the reference standard to validate the method. All measurements were performed in triplicate, and results were reported as mean ± SD [13].

2.4.3 Determination of pH

The pH of the Ibudilast nanogel was determined using a digital pH meter (Labcare pH Meter). The instrument was calibrated beforehand with standard buffer solutions of pH 4 and 7. For analysis, about 1 g of the nanogel was dispersed in 100 mL of distilled water, and the pH value was measured in triplicate (n = 3) [14].

2.4.4 Viscosity measurement

The rheological behavior of the Ibudilast-loaded nanogel was evaluated using a programmable Brookfield viscometer (Model DV2T Plus, AMETEK Brookfield, Middleboro, MA, USA) at 25 °C with Spindle LV-4. The spindle was carefully immersed vertically into the gel without touching the container walls. Measurements were taken at rotational speeds ranging from 2 to 50 rpm, with readings recorded after 1 minute once the gel level had stabilized. Prior to each test, the sample was equilibrated at 25 °C. The results were expressed as viscosity versus shear rate plots [15].

2.4.5 Percent (%) drug content

The content uniformity of the Ibudilast nanogel was evaluated by diluting 0.5 mL of the formulation with double-distilled water, followed by dissolution in 5 mL of ethanol using vortex mixing. The resulting solution was filtered through a 0.45 μm membrane filter, and the drug concentration in the filtrate was analyzed using UV–Visible spectrophotometry (UV-1700, Shimadzu, Japan) at 227 nm to ensure accuracy and reliability of the results.

$$(\%)\ \text{Content of Ibudilast} = \frac{amount\ of\ drug\ detected}{amount\ of\ drug\ employed} \times 100$$

2.4.6 Spreadability

The spreadability of the Ibudilast-nanogel was evaluated by placing 0.5 g of the formulation within a 1 cm diameter circle on a glass plate. A second glass plate weighing 250 g was then placed on top and left for 1 minute. The increase in diameter resulting from the gel's spreading provided a measure of its spreadability, an important parameter for its practical application in pharmaceutical and nanotechnological formulations.

3. Results and Discussion

3.1 Characterization of ethosomes

3.1.1 Vesicle Size Analysis

The average vesicle size (VS) of the ethosomal formulation was measured at 193.20 ± 0.85 nm (Figure 1A). The polydispersity index (PDI) was recorded as 0.264 ± 0.002, indicating a narrow and homogeneous particle size distribution, which is suitable for transdermal delivery.

3.1.2 Zeta Potential Measurement

The zeta potential (ZP) was determined to be -28.3 ± 0.32 mV (Figure 1B), indicating a highly negative surface charge. The negative surface charge was attributed to the presence of ethanol in the formulation, which enhances the electrostatic stability of ethosomes and minimizes the risk of particle aggregation and flocculation.

3.1.3 Morphological Characterization

Scanning electron microscopy (SEM) analysis revealed that the ethosomal vesicles were predominantly spherical in shape with smooth surfaces (Figure 1C). The vesicles exhibited a uniform morphology and a discrete distribution, without notable aggregation, indicating good dispersibility and structural integrity.

3.1.4 Entrapment Efficiency

The formulation demonstrated a high entrapment efficiency of 79.83 ± 0.22%, confirming efficient drug loading within the ethosomal carriers.

3.2 Characterization of nanogel

The evaluation of the PDE4 inhibitor-loaded nanogel formulation was carried out to assess its physical properties, drug content, and flow behavior, as shown in Table 1, which are essential for determining the suitability of the formulation for transdermal drug delivery. Below are the evaluation parameters and their corresponding values:

3.2.1 Appearance and Clarity

The formulation exhibited a transparent appearance with excellent clarity, demonstrating good aesthetic appeal for topical application.

3.2.2 Refractive Index

The refractive index was measured at 1.337 ± 0.002, indicating the absence of suspended particles in the formulation.

3.2.3 Homogeneity

The ibudilast-loaded Carbopol 934 nanogel demonstrated good homogeneity with no clogs or aggregates observed.

3.2.4 pH Measurement

The pH of the nanogel was measured at 6 ± 0.5, which falls within the physiological range and confirms its compatibility for skin application.

3.2.5 Spreadability

Spreadability assessment showed a favorable diameter of 6.2 ± 1 cm, indicating ease of application and potential for improved absorption across the skin surface.

3.2.6 Morphological Analysis

SEM analysis of the Carbopol nanogel (Figure 1D) revealed a fibrous, interconnected, and porous network structure characteristic of hydrated polymeric gels. The nanogel surface appeared smooth, free from cracks or phase separation, confirming the stability and structural uniformity of the formulation.

3.2.7 Rheological Properties

Rheological evaluation demonstrated pseudoplastic flow behavior, with viscosity decreasing as shear rate increased from 2 to 50 rpm (Figure 2A). This shear-thinning property enhanced the ease of spreading during application while maintaining structural integrity at rest.

3.2.8 Drug Content

The drug content was determined to be 97.95 ± 0.5%, indicating efficient incorporation and uniform distribution of ibudilast within the nanogel formulation.

3.2.9 In Vitro Drug Release Studies

Ibudilast-loaded ethosomes exhibited rapid drug release, with 78.4% of the drug released within 8 hours. The ethosomal nanogel formulation demonstrated a controlled and sustained release profile, achieving 86.7% cumulative drug release over 24 hours (Figure 2B). The sustained release pattern confirmed the gel matrix's role in prolonging drug diffusion and ensuring extended therapeutic action, which is suitable for transdermal delivery.

4. Discussion

The development of an ibudilast-loaded ethosomal nanogel represents a promising approach for transdermal drug delivery, combining the penetration-enhancing properties of ethosomes with the controlled release characteristics of a gel matrix. The characterization results demonstrate that the formulation possesses optimal physicochemical properties for topical application. The ethosomal vesicles exhibited a mean particle size of 193.20 ± 0.85 nm with a PDI of 0.264 ± 0.002, indicating a uniform size distribution ideal for skin penetration. The negative zeta potential of -28.3 ± 0.32 mV, attributed to ethanol content, provides excellent colloidal stability by preventing particle aggregation through electrostatic repulsion. SEM analysis confirmed the spherical morphology and smooth surface of the vesicles, further supporting their structural integrity. The high entrapment efficiency of 79.83 ± 0.22% validates the effectiveness of the preparation method in achieving optimal drug loading, which is crucial for therapeutic efficacy. The incorporation of ethosomes into a Carbopol 934 nanogel matrix enhanced the overall performance of the formulation. The nanogel exhibited excellent physical properties, including transparency, a suitable pH (6 ± 0.5) for skin compatibility, and favorable spreadability (6.2 ± 1 cm), thereby ensuring ease of application and patient compliance. The pseudoplastic rheological behavior is particularly advantageous, as it facilitates smooth application under shear stress while maintaining viscosity at rest, preventing premature drug leakage. SEM revealed a porous, interconnected network structure typical of hydrated polymeric gels, which plays a critical role in controlling drug release. The

in vitro release studies highlighted a significant advantage of the ethosomal nanogel over conventional ethosomes. While free ethosomes released 78.4% of ibudilast within 8 hours, the nanogel formulation achieved 86.7% release over 24 hours, demonstrating sustained and controlled drug delivery. This prolonged release profile is attributed to the gel matrix acting as a diffusion barrier, which ensures extended therapeutic action and reduces the frequency of dosing. Overall, the PDE4 inhibitor-loaded ethosomal nanogel exhibits promising potential for effective transdermal delivery with improved patient compliance.

5. Conclusions

The present study successfully developed and characterized an ibudilast-loaded ethosomal nanogel formulation for transdermal delivery of the drug. The ethosomal vesicles demonstrated optimal physicochemical properties, including a suitable particle size of 193.20 ± 0.85 nm, a narrow size distribution (PDI: 0.264 ± 0.002), and high colloidal stability, as indicated by a zeta potential of -28.3 ± 0.32 mV. The spherical morphology with smooth surfaces and high entrapment efficiency of 79.83 ± 0.22% confirmed the effectiveness of the preparation method and structural integrity of the vesicles. The incorporation of ethosomes into a Carbopol 934 nanogel matrix yielded a formulation with excellent physical characteristics, including transparency, a skin-compatible pH (6 ± 0.5), favorable spreadability (6.2 ± 1 cm), and pseudoplastic rheological behavior that facilitates ease of application. The fibrous, interconnected porous network structure observed through SEM analysis, combined with high drug content (97.95 ± 0.5%), confirmed the uniformity and stability of the nanogel formulation. In vitro release studies demonstrated a significant advantage of the ethosomal nanogel system over conventional ethosomes, achieving sustained and controlled drug release with 86.7% cumulative release over 24 hours compared to 78.4% release within 8 hours from free ethosomes. This prolonged release profile, attributed to the gel matrix's diffusion-controlling properties, ensures extended therapeutic action and reduces dosing frequency, thereby improving patient compliance. Overall, the PDE4 inhibitor-loaded ethosomal nanogel exhibited promising characteristics suitable for transdermal delivery, combining enhanced skin penetration potential with controlled drug release. This innovative formulation approach demonstrates significant potential as an

effective transdermal delivery system for ibudilast, warranting further in vivo evaluation to establish its clinical applicability.

CONFLICT OF INTEREST

The author(s) confirm that this article's content has no conflicts of interest.

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Figures

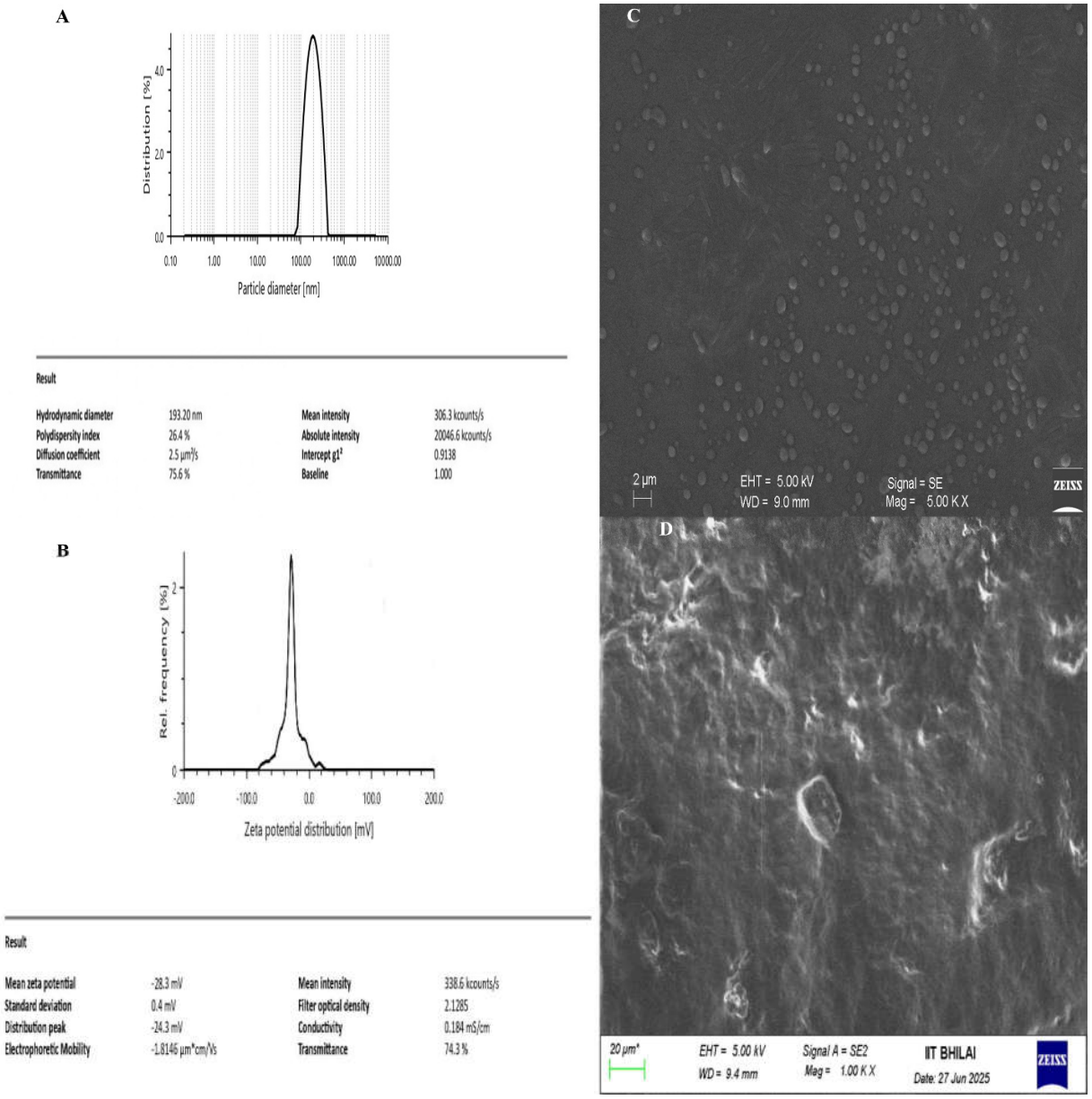

Figure 1. A. Particle size distribution report of Ibudilast-loaded Ethosomes B. Zeta potential graph of Ibudilast-loaded Ethosomes C. SEM image of the Ibudilast-loaded Ethosomes D. SEM image of Ibudilast-loaded Carbopol nanogel

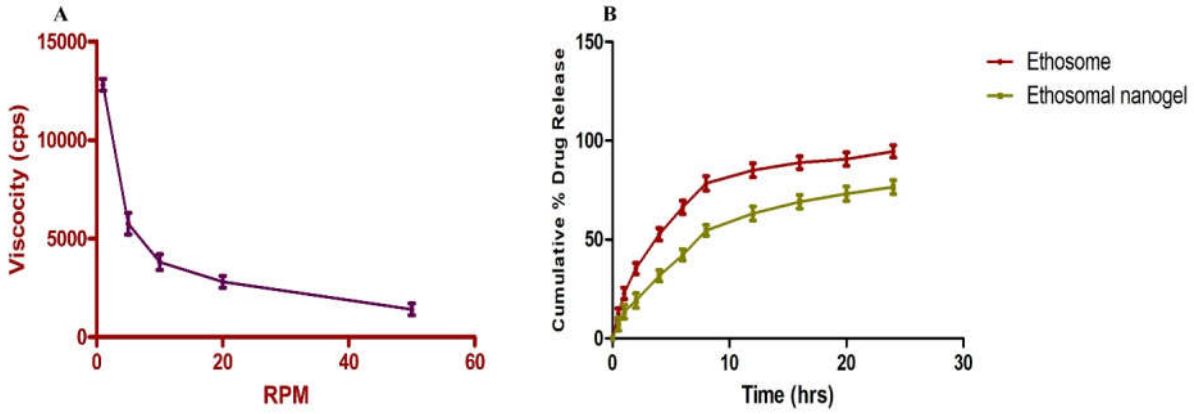

Figure 2. A. Effect of shear rate on the viscosity of ethosome-loaded Carbopol nanogel B. In vitro drug release study for Ibudilast-loaded Ethosomes and Ibudilast-loaded nanogel.

Table

Table 1. Physicochemical and rheological properties of prepared topical ibudilast nanogel formulations

Evaluation Parameters	Ibudilast-nanogel
Appearance	Transparent
Clarity	Yes
Homogeneity	Good
pH	6±0.5
Spreadability Diameter (cm)	6.2±1 cm
Drug content	97.95±0.5%
Flow behavior	Non-Newtonian (Pseudoplastic)