

**PHYTOCHEMICAL SCREENING AND PRE-FORMULATION STUDIES OF
NASAL *IN-SITU* GEL OF ETHANOLIC EXTRACT OF *MIMOSA PUDICA*
FOR ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITY**

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ABSTRACT

This study investigates the potential of *Mimosa pudica*, known as chuimui or lajwanti in Hindi, as a herbal remedy for anxiety and depression. The plant is recognized for its diverse pharmacological activities, encompassing analgesic, antidiarrheal, anti-inflammatory, hepatoprotective, antiasthmatic, anti-ulcer, and antioxidant properties. The emphasis on its antidepressant and anxiolytic activity positions it as a promising candidate for mental health treatment, with anticipated minimal side effects compared to synthetic agents. The study also explores pre-formulation considerations, with a specific emphasis on determining the ideal concentration of noacetylated gellan gum for in-situ gelation. Preliminary investigations involve qualitative phytochemical screening of *Mimosa Pudica* extract by cold maceration process, revealing the presence of bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, and saponins. These components are known for their potential in modulating neurotransmission and exhibiting anxiolytic effects. The research fine-tunes the concentration of deacetylated gellan gum for gelation using simulated nasal fluid, with the objective of achieving gelation with minimal viscosity. Compatibility studies using Fourier-transform infrared spectroscopy (FTIR) confirm the absence of significant interactions between the drug and polymer. Additionally, differential scanning calorimetry (DSC) provides

insights into the thermal behaviour of the pure drug and the drug-polymer complex. Overall, this comprehensive investigation anticipates inspiring advanced research into the manifold benefits of *Mimosa pudica*, particularly in the realm of mental health treatment, and lays the foundation for potential herbal formulations with reduced side effects.

Keywords: Anxiolytic, Antidepressant, Intranasal, Mucoadhesive, Herbal formulations, Treatment

1. INTRODUCTION

For millennia, nature has served as a reservoir of medicinal compounds, with diverse cultures worldwide harnessing the therapeutic properties of various plants in daily life to combat diseases. Herbal medicine operates on the belief that plants inherently harbor natural substances capable of enhancing well-being and mitigating illnesses [1]. Presently, a significant portion of the global population grapples with depression and anxiety, as evidenced by widespread prevalence. Global anxiety statistics, as reported by the World Health Organization, reveal that approximately 264 million people, constituting 3.6 percent of the world's population, are afflicted by anxiety disorders. Notably, anxiety affects 4.6 percent of females and 2.6 percent of males on a global scale [2]. While synthetic drugs such as Clomipramine, Imipramine, Desipramine, and Nortriptyline are commonly employed as conventional treatments for clinically depressed and anxious patients, their efficacy is often accompanied by adverse effects that pose challenges to the overall therapeutic process [3]. In light of potential drug-drug

interactions and adverse effects associated with synthetic medications, there arises an opportunity to explore alternative treatments for anxiety and depression, particularly through the utilization of medicinal plants or plant-based formulations with antianxiety and antidepressant properties. In the Ayurvedic system of Indian Medicine, intranasal administration has long been recognized as an accepted and effective form of therapy [15]. These intranasal administered products are self-administered by the patient, with the proper efficiency of drug delivery [16]. The plant *Mimosa pudica*, known for possessing both anxiolytic and antidepressant attributes, emerges as a viable herbal treatment option, emphasizing the necessity for comprehensive documentation of research conducted on traditional herbal medicines [4].

1.1 Plant Profile:

Mimosa Pudica referred to as "chumui" or "lajwanti" in Hindi and known for its distinctive characteristic of drooping or collapsing upon touch, *Mimosa Pudica* is also recognized as "Lajjuki lota" in Assamese [4].

Scientific Classification:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Subfamily	Mimosoideae
Genus	Mimosa
Species	M. pudica



1.2 Polymer Used in *in situ* Gel Drug Delivery System

To enhance the efficacy of drug products, the careful selection of an appropriate polymer for formulation is crucial. Polymers exhibiting a sol-to-gel transition in aqueous solutions, known for in-situ gelation, play a key role. Examples of such polymers include poloxamer, pluronic, and various copolymers like PEO-PLLA and PEG-PLGA-PEG. Additionally, materials like pectin, gelrite, cellulose acetophthalate latex, gellan gum, alginate, matrigel, carbopol, and chitin are capable of in-situ gelation. The gel formation can be triggered by factors such as temperature change in the case of poloxamer, cellulose acetophthalate latex, and carbopol, while pH change induces gelation in Carbopol [5]. Gellan gum, an anionic deacetylated exocellular polysaccharide produced by *Pseudomonas elodea*, features a tetra saccharide repeating unit composed of 1b-l-rhamnose, 1b D-glucuronic acid, and 2b D-

glucose. By considering the existence of roughly 0.1 ml of mucus containing sodium, potassium, and calcium ions on the human nasal mucosa, an expected transition from solution to gel phase can be foreseen [6]. This study seeks to create a nasal in-situ gel using a gel-forming solution with temperature-sensitive and mucoadhesive polymers, addressing oral administration limitations such as fatigue, diarrhea, nausea, and vomiting [7].

2. MATERIAL AND METHODS:

The *Mimosa Pudica* plant has collected from the local nurseries of Guwahati (Name of the Nursery: Dream Flower Nursery, Khanapara) Assam and it authenticated by Dr. Sourav Bora from Botany department of Guwahati University, Authentication No. Acc. No. GUBH20430 dt.11.08.2023 and has been preserved.

2.1 Chemical Requirement

Table 1: Chemicals required

S. No.	Chemical Name	Manufacture
1	Ethanol	Assam petrochemical ltd.
2	Ferric chloride	Fisher scientific
3	Lead acetate	ACS
4	Sodium Hydroxide	Fisher scientific
5	Conc. Sulphuric acid	Fisher scientific
6	Fehling's reagents A & B	Organo biotech labs
7	Benedict's reagent	Bullux laboratories
8	Alpha-naphthol	Alpha chemika
9	Mayer's reagent	Bio rapid

Fig 02. Drug (Ethanolic extract of *Mimosa pudica*)

METHODS:

2.2 Preparation of plant extract:

The preparation involved washing raw plant material, shade drying, and crushing to obtain a dry coarse powder of *Mimosa pudica*. Approximately 500 gm of the powder underwent extraction with ethanol using a percolator apparatus. After standing for 16 hours, the percolate was collected, and the extraction process was repeated four times. The combined extract was filtered, concentrated under vacuum at 40°C using a rotavapor, resulting in a 1.5% yield [8].

2.3 PHYTOCHEMICAL ANALYSIS:

The crude fractions have been subjected to different qualitative phytochemical screening to identify the presence of various phytoconstituents as described by Harborne [9].

Preliminary phytochemical screening was conducted on all extracts in accordance with established standard procedures.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

- **Mayer's Test:** The application of Mayer's reagent (Potassium Mercuric Iodide) to the filtrates resulted in the formation of a yellow-coloured precipitate, indicating the presence of alkaloids in the tested sample [17].

- **Dragendorff's Test:** The filtrates were subjected to treatment with Dragendorff's reagent, a solution of Potassium Bismuth Iodide and the appearance of a red precipitate served as an indicative signal for the presence of alkaloids in the tested sample [18].

- **Fehling's Test:** The filtrates were subjected to hydrolysis with dilute HCl, neutralized with alkali, and subsequently heated with Fehling's A and B solutions. The emergence of no red precipitate following this process indicated the absence of reducing sugars in the tested sample.

Detection of glycosides: The extracts were treated with diluted hydrochloric acid (dil. HCl) to hydrolyse them, after which they

underwent testing to detect the presence of glycosides.

• **Modified Borntrager's Test:** Treatment of extracts with Ferric Chloride, boiling, benzene extraction, and subsequent ammonia treatment resulted in a rose-pink colour, confirming anthranol glycosides [19].

Legal's Test: The sodium nitroprusside and sodium hydroxide treatment reveal cardiac glycosides in plant extracts, evident by a distinctive pink to blood-red colour change [20].

Detection of saponins:

• **Froth Test:** After diluting the extracts with distilled water to 20ml and vigorously shaking the mixture in a graduated cylinder for 15 minutes, the emergence of a 1 cm layer of foam served as an indication of the existence of saponins in the analysed sample.

• **Foam Test:** Agitating 0.5 gm of the extract with 2 ml of water led to the generation of foam, and the sustained presence of this foam over ten minutes served as a reliable indicator for the presence of saponins in the tested sample [21].

Detection of phenols

• **Ferric Chloride Test:** Upon exposure to 3-4 drops of ferric chloride solution, the extracts displayed the development of a bluish-black hue, providing a clear

indication of the presence of phenols in the tested sample.

Detection of tannins

• **Gelatin Test:** Incorporating the extract with a 1% gelatin solution containing sodium chloride led to the emergence of a white precipitate, affirming the existence of tannins in the analysed sample.

Detection of flavonoids

• **Alkaline Reagent Test:** Upon treatment with a few drops of sodium hydroxide solution, the extracts exhibited the formation of an intense yellow colour, which turned colourless upon the addition of dilute acid, indicating the presence of flavonoids. Additionally, in the lead acetate test, the extracts, when treated with a few drops of lead acetate solution, resulted in the formation of a yellow-coloured precipitate, further confirming the presence of flavonoids in the tested sample [22].

Detection of steroids

Liebermann Burchard test

Following the treatment of extracts with chloroform and filtration, the filtrates were subjected to acetic anhydride, boiling, and subsequent cooling. The addition of concentrated sulfuric acid revealed the absence of a brown ring at the junction, indicating the non-existence of phytosterols in the analysed sample.

Detection of diterpenes

Copper acetate Test: After dissolving the extracts in water, introduction of 3-4 drops of copper acetate solution led to the development of a vivid emerald green hue, serving as distinct signal confirming the existence of diterpenes was evident in the analysed sample [10].

2.4 Preliminary investigations were undertaken to ascertain the most effective concentration of nonacetylated gellan gum for in-situ gel formation:

In the preliminary investigations, different concentrations of gellan gum, as detailed in Table 3, were utilized to fine-tune the gelling concentration of nonacetylated gellan gum. Gelation studies were conducted using simulated nasal fluid, prepared by dissolving sodium chloride (2.1925 g), calcium chloride (0.145 g), and potassium chloride (0.745 g) in 250 ml of double distilled water. The optimization process focused on achieving gelation with the minimal viscosity, and the experiments were conducted in simulated nasal fluid with a pH of 6.4 ± 0.1 at a temperature of $34 \pm 1^\circ\text{C}$ [11].

2.5 Formulation of in situ gel systems:

The formulation process involved dissolving non-acetylated gellan gum in varying concentrations in double distilled water, heating to 90°C , and cooling. The drug (*Mimosa Pudica* extract) was combined with PEG 400 and added to the

polymer solution. Mannitol and methyl paraben were sequentially added, serving as an isotonic agent and preservative, respectively. The final formulation underwent evaluation [12].

PREFORMULATION STUDIES:

2.6 FTIR spectral studies

FTIR analysis assessed interactions in 1:1 w/w physical mixtures, including the drug with gellan gum, drug with formulation, and gellan gum with nasal fluid components. These mixtures underwent a one-month incubation at room temperature ($25^\circ\text{C} \pm 2^\circ\text{C}$, $60\% \pm 5\%$ relative humidity) to ensure complete interaction between the drug and polymer. Subsequently, drug and drug-polymer samples were dried in a hot air oven at 60°C for 30 minutes to remove moisture. The samples were then subjected to FTIR scanning from 4000 to 400 cm^{-1} , and the spectra obtained were compared with the drug sample spectra to identify any changes in peaks [13].

2.7 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis was employed to assess the thermal behaviour of the pure drug, utilizing a DSC-60 instrument from Shimadzu Corporation, Japan. Samples weighing 10 mg were meticulously measured and sealed in standard aluminium pans. Subsequently, the samples underwent

scanning across a temperature spectrum lies from 50°C to 300°C, the process involved a gradual heating rate of 10°C per minute [13].

3. RESULTS AND OBSERVATIONS

3.1 Drying: The plant material was air-dried in shade under controlled conditions

at ambient temperature, following which it was pulverized into a dry coarse powder.

3.2 Extraction: The composite extract underwent filtration and was subsequently concentrated under vacuum utilizing a rotary evaporator at a temperature of 40°C, and the mass of extract has been noted and resulting in a 1.5% yield.

Drying and Extraction:



Fig 03: *Mimosa pudica* after shade Dry



Fig 04: Dry coarse powder of *Mimosa pudica*

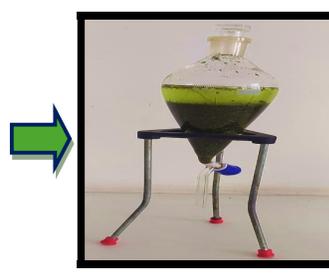


Fig 05: Cold maceration process with ethanol & dry Coarse powder of *Mimosa pudica*

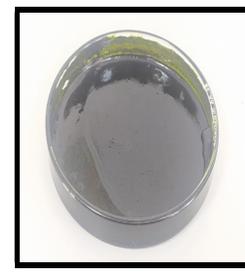


Fig 06: Ethanolic extract of *Mimosa pudica*

3.3 Phytochemical analysis:

Table 2: Preliminary identification test of phytoconstituents of Ethyl alcohol extract of *Mimosa pudica*

Tests	Extract of <i>Mimosa pudica</i>
Terpenoids	Present
Flavonoids	Present
Steroids	Absent
Alkaloids	Present
Glycosides	Present
Sugars	Absent
Phenols	Present
Tannins	Present
Saponins	Present

Discussion: The preliminary Phytochemical screening of *Mimosa Pudica* extract showed the presence of bioactive components like Terpenoids, *Flavonoids, Glycosides, *Alkaloids, Quinines, *Phenols, Tannins, Saponins as

mentioned in **Table 2** and these phytoconstituents are responsible for modulating neurotransmission to display anxiolytic effects [14].

3.4 Initial investigations to determine the optimal concentration of deacetylated gellan gum for in-situ gelation.

During initial investigations, diverse concentrations of gellan gum (polymer) were examined, utilizing simulated nasal fluid containing sodium chloride (2.195 g), calcium chloride (0.165 g), and potassium chloride (0.755 g) dissolved in 250 ml of

double-distilled water. The optimization of deacetylated gellan gum's gelling concentration aimed at achieving gelation with minimal viscosity. Gelation studies were carried out using simulated nasal fluid with a pH of 6.4 ± 0.1 at a temperature of $34 \pm 1^{\circ}\text{C}$, yielding the most favorable outcomes with a concentration of 0.2% w/v gellan gum in double-distilled water [11].

Table 3: Tests were conducted to explore the gelation characteristics of deacetylated gellan gum

Formulations	Gellan gum (GG) deacetylated concentration (%w/v)	Observation
Gellan gum 1	0.1	Absence of gel development.
Gellan gum 2	0.2	Sturdy gel development.
Gellan gum 3	0.3	Intensely viscous gel
Gellan gum 4	0.4	Intensely viscous gel

3.5. Formulation of in situ gel systems: The formulation process involved dispersing nonacetylated gellan gum at optimized concentration (0.2% w/v) in distilled water, followed by heating to 90°C with stirring persistently until all solids are fully dissolved, and subsequent cooling to room temperature ($25^{\circ}\text{C} \pm 20^{\circ}\text{C}$). The ethanolic extract of *Mimosa Pudica* (drug) was combined with distilled water

containing PEG 400. Subsequently, this pharmaceutical solution was integrated into the polymer solution, and the sequential addition of mannitol and methyl paraben ensured continuous agitation. The final formulation was stored in adequately sealed glass containers at room temperature and subjected to evaluation based on various parameters [12].

3.6 FTIR Studies:

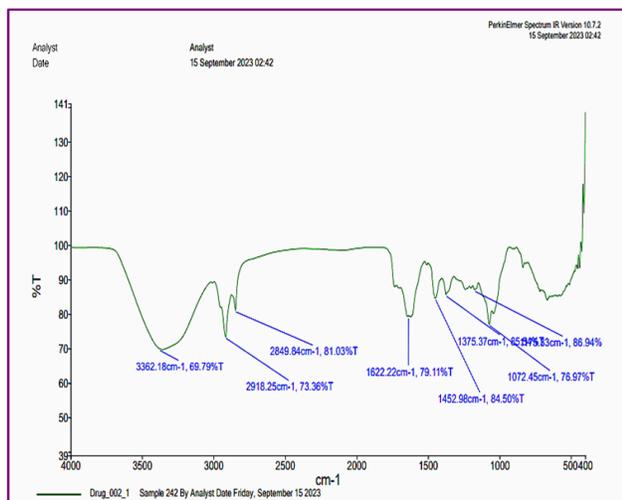


Fig: 07(Drug)

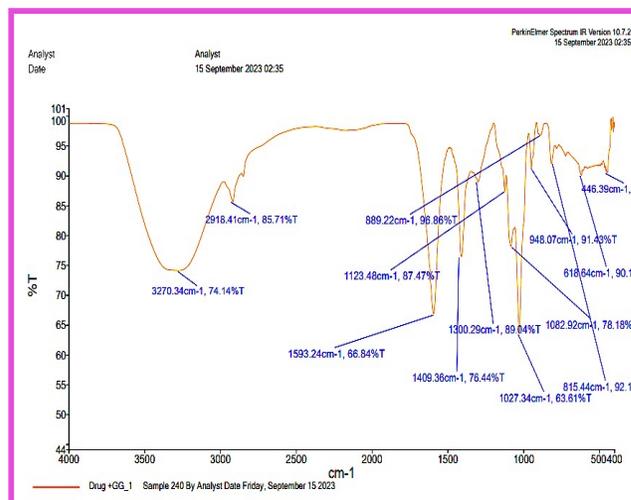


Fig: 08(Drug+ GG)

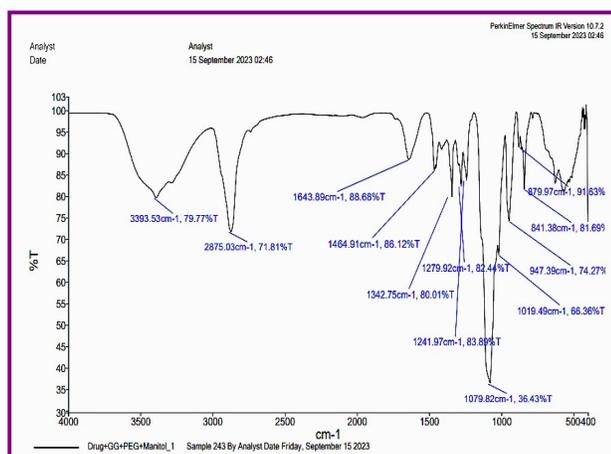


Fig: 09 (Drug+ GG+ PEG+ Mannitol)

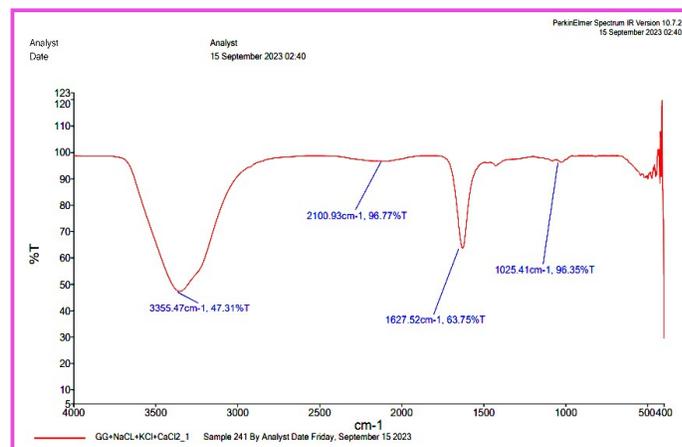
Fig: 10(GG+NaCl+KCl+CaCl₂)

Table 4: FT-IR Data interpretation

S. No.	Component	Peak Position (cm ⁻¹)	Functional Group
1	Ethanolic extract of <i>Mimosa Pudica</i> (Drug)	3362.18	N-H stretching of secondary amine
		2918.25	C-H bending of aldehyde
		2849.84	C-H bending of aldehyde
		1622.22	Aromatic C=C stretching
		824.73	Aromatic C=C stretching
2	Drug + GG	3270.34	N-H stretching of secondary amine
		2918.41	C-H bending
		1593.24	C=C aromatic stretching
3	Drug+ PEG+ GG+ Mannitol	3393.53	N-H stretching of secondary amine
		2875.03	C-H bending
		1643.89	C=C stretching
4	GG+NaCl+KCl+CaCl ₂	3355.47	N-H stretching of secondary amine
		2100.93	C-C Alkyne stretching
		1627.52	C=C aromatic stretching

Discussion: The drug and polymer exhibit no significant interaction, as indicated by the absence of appreciable shifts in characteristic peaks in their respective spectra. Similarly, minimal interaction is

observed between the formulation components and gellan gum in simulated nasal fluid [13].

3.7 DSC Studies:

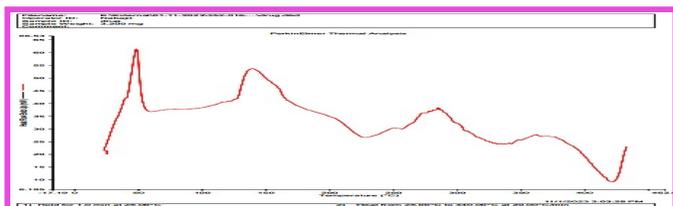


Fig:11(Drug)

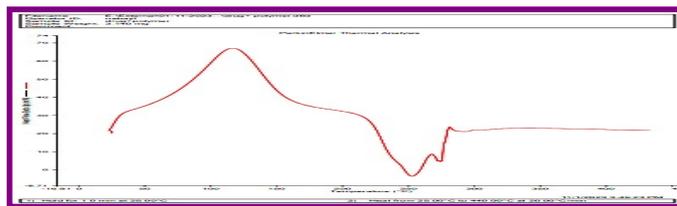


Fig:12(Drug + Polymer)

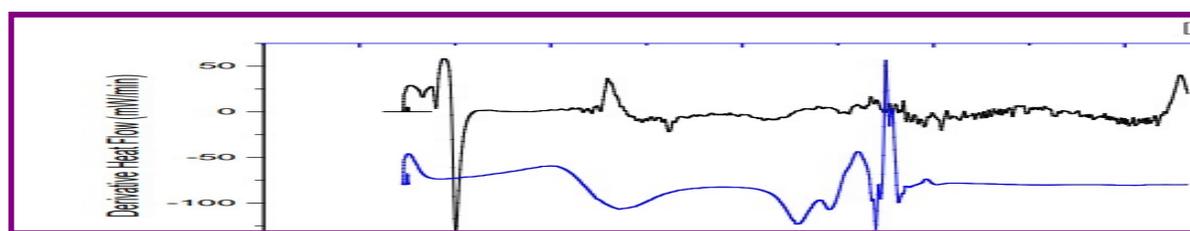


Fig:13(Drug +Drug: Polymer)

Table: 5 DSC Data interpretation

Data	Sample Temperature (°C)	Derivative Heat Flow (mW/min)	Data	Sample Temperature (°C)	Derivative Heat Flow (mW/min)
Drug + Polymer	101.2322	14.33333	Drug	104.026	2.265331
	101.5571	14.3009		104.3551	2.354953
	101.882	14.2284		104.6943	2.555714
	102.2169	14.20993		105.0235	2.893922
	102.5418	14.30317		105.3523	3.129408
	102.8668	14.38377		105.6911	3.138856
	103.1916	14.3073		106.0199	2.966056
	103.5263	14.07762		106.3587	2.685586
	103.8513	13.8142		106.6778	2.460447
	104.1864	13.61306		107.017	2.327645
104.5116	13.41954	107.3463	2.275309		

Discussion: DSC analysis revealed no significant peak shifts, indicating no interaction between amorphous drug and polymer, with melting points at 105.69°C and 102.86°C [6].

3. CONCLUSION:

Plant material was collected and after drying and extraction various

phytochemical constituents were found like flavonoids, tannins etc., which provides evidence for anxiolytic and anti-depressant activity. Preliminary investigations aimed at determining the optimal concentration of deacetylated gellan gum for in-situ gelation have been completed, unveiling optimal outcomes with a concentration of 0.2% w/v

gellan gum in double-distilled water. Pre formulation (FTIR, DSC) studies were performed for the drug along with other polymers and excipients which confirms that the characteristic peaks did not shift significantly, indicating the absence of any interaction between the drug and polymer. Furthermore, the formulation optimization can be accomplished in terms of different physico-chemical parameters.

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