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DESIGN, OPTIMIZATION AND EVALUATION OF AN ION-TRIGGERED IN SITU NASAL GEL OF SELEGILINE FOR ANTIDEPRESSANT THERAPY

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ABSTRACT

Background: Oral delivery of selegiline is limited by extensive first-pass metabolism, low and variable bioavailability, and restricted brain access due to the blood–brain barrier. Intranasal drug delivery offers a non-invasive strategy for direct nose-to-brain transport. The present study aimed to design, optimize, and evaluate an ion-triggered in situ nasal gel of selegiline using sodium alginate and HPMC to potentiate brain delivery and antidepressant efficacy. **Methodology:** Preliminary screening was performed using the Plackett–Burman design to identify critical formulation variables. Further optimization was carried out using the Box–Behnken design with sodium alginate (0.5–2% w/v), HPMC (0.5–1% w/v), and $CaCl_2$ (10–50 mM) as independent variables. The optimized formulation was evaluated for physicochemical properties, rheology, gelation time, gel strength, mucoadhesive strength, in vitro and ex vivo drug release, histopathology, in vivo antidepressant activity, and stability. **Results and Discussion:** The optimized formulation (1.25% sodium alginate, 0.75% HPMC, 30 mM $CaCl_2$) exhibited suitable sol viscosity (~150 mPa·s), rapid gelation (~34 s), adequate gel strength (~350 g), and good mucoadhesive strength (~3140 dyne/cm²). Sustained drug release (~98% in 8 h in vitro; ~94% in 6 h ex vivo) was achieved. The formulation was non-irritant to nasal mucosa, showed enhanced antidepressant activity in vivo, and remained stable for 3 months under stability studies. **Conclusion:** The optimized sodium alginate–HPMC ion-triggered in situ nasal gel of selegiline offers a safe, stable, and effective nose-to-brain delivery system with sustained release and improved antidepressant activity, making it a promising alternative to oral therapy.

INTRODUCTION

Depression is a leading global cause of disability, affecting about 280 million people (~5% of adults), with particularly high prevalence among adolescents, young adults, and students, nearly one-third of whom show depressive symptoms. Driven by social determinants such as poverty, education, and unemployment, depression causes major health and economic losses, substantial disability-adjusted life years, and large treatment gaps, especially in low- and middle-income countries [1,2]. Conventional antidepressant therapy predominantly relies on oral administration; however, this approach has several drawbacks, including extensive first-pass metabolism, poor and

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variable bioavailability, delayed onset of therapeutic action, and systemic side effects, all of which contribute to poor patient adherence [3]. Additionally, drug delivery to the brain is hindered by the blood–brain barrier (BBB), which restricts the entry of more than 98% of small-molecule drugs and nearly all large molecules. Efflux transporters such as P-glycoprotein further limit drug penetration, resulting in subtherapeutic brain concentrations even after systemic administration [4]. Most oral drugs for brain delivery exhibit poor oral bioavailability due to solubility issues, enzymatic degradation, first-pass metabolism, and efflux, resulting in inadequate systemic levels to cross the BBB. Even lipophilic drugs that cross membranes often undergo rapid clearance or cause off-target toxicity [5,6].

These limitations highlight the need for alternative drug-delivery routes that bypass hepatic metabolism and enable direct brain targeting. The intranasal route has gained attention for its direct access to the brain via the olfactory and trigeminal pathways, enabling rapid, efficient delivery of therapeutic agents while minimizing systemic exposure [7]. This method can provide faster onset of action, reduced dosing frequency, and improved compliance, which are particularly beneficial in depression management [7].In situ gels, especially those based on biopolymers, are emerging as promising carriers for intranasal brain delivery. These systems undergo a sol-to-gel transition triggered by physiological factors such as temperature, pH, or ionic strength, forming a gel in situ that prolongs nasal residence and provides sustained drug release [8].

Natural polysaccharides, including alginate and hydroxypropyl methylcellulose (HPMC), are widely used for their biodegradability, safety, low toxicity, and excellent mucoadhesive properties, which enhance drug retention and absorption in the nasal cavity [9]. Selegiline, a selective monoamine oxidase B (MAO-B) inhibitor with proven antidepressant activity, increases central dopamine availability and improves dopaminergic neurotransmission. At higher doses, it also inhibits MAO-A, enhancing serotonin and norepinephrine levels. Despite its therapeutic potential, oral selegiline suffers from extensive first-pass metabolism, low bioavailability, and dose-related side effects [10]. Alternative approaches, such as transdermal delivery, address some of these issues but are often associated with skin irritation or inconsistent absorption [11]. Developing a natural polysaccharide-based in situ nasal gel of selegiline represents a novel strategy to overcome these challenges. Selegiline, a selective MAO-B inhibitor, shows antidepressant potential but suffers from poor oral bioavailability. Therefore, the present study aimed to develop and optimize a sodium alginate–HPMC ion-triggered in situ nasal gel of selegiline for improved brain delivery and antidepressant efficacy.

MATERIAL AND METHOD

The drug selegiline was purchased from Sigma-Aldrich India. Sodium alginate was purchased from Loba Chemie Pvt Ltd., Maharashtra, and HPMC K15 M, calcium chloride, Sodium chloride, & Potassium chloride from Alpha Chemika, Maharashtra. All other chemicals and solvents were analytical grade. Distilled water was used throughout the preparation.

Method

Sodium alginate & HPMC were dispersed in distilled water under continuous stirring until a homogeneous mixture was obtained and allowed to hydrate overnight at 4°C. The drug was dissolved separately and added slowly with stirring. Calcium chloride solution (0.1-0.3% w/v) was then gradually incorporated as the ion source to enable ionic crosslinking. This resulted in a free-flowing sol at room temperature that gels rapidly upon contact with divalent cations (Ca^{2+}) in simulated nasal fluids (NaCl 7.45 g/L, KCl 1.29 g/L, $CaCl_2$ 0.32 g/L). [12].

Preliminary screening

Preliminary screening was performed to identify the critical formulation concentration for sodium alginate–HPMC ion-triggered nasal in situ gel. A Plackett–Burman design was employed to evaluate the effect of sodium alginate (0.5–2% w/v), HPMC (0.5–1% w/v), and calcium chloride (10–50 mM) on gelation, viscosity, and solution-to-gel transition. This design was chosen because it enables rapid identification of significant factors with minimal experiments, which is useful in early formulation development [13,14]. The results of this screening study were used to define the variable concentration ranges for further optimization using response surface methodology.

Design of experiment

A Box–Behnken design (BBD) was applied to optimize a sodium alginate–HPMC ion-triggered in situ nasal gel. Three independent variables (Table 1) sodium alginate (0.5–2% w/v), HPMC (0.5–1% w/v), and $CaCl_2$ (10–50 mM)—were studied at three levels (−1, 0, +1) in 15 runs, including three centre points. Viscosity, gelation time, and gel strength were selected as responses. A quadratic polynomial model was fitted to the data, and the significance of terms was assessed using ANOVA.

The general form of the model is:

$$Y=\beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{12}AB+\beta_{13}AC+\beta_{23}BC+\beta_{11}A^2+\beta_{22}B^2+\beta_{33}C^2+\epsilon$$

Where Y is the response (viscosity, gelation time, gel strength, and mucoadhesive strength), A = sodium alginate, B = HPMC, C = $CaCl_2$, and ϵ is the residual error.

The experimental design minimized variability and identified factor interactions.

Table 1: Independent variables and their level

Factor (code)	Symbol	Low (−1)	Centre (0)	High (+1)
Sodium alginate (% w/v)	A	0.50	1.25	2.00
HPMC (% w/v)	B	0.5	0.75	1.00
$CaCl_2$ (mM)	C	10	30	50

Gelation time

Gelation time was determined by the vial inversion method as described by *Ranch et al. (2019)* [16]. Two milliliters of formulation were placed in a vial at 37 ± 0.5 °C, and 1 mL of simulated nasal fluid (pH 6.4) was added to initiate gelation. The time required for the solution to transition to a nonflowing gel was recorded. Tests were performed in triplicate, and results are expressed as mean ± SD.

In vitro drug release

In vitro drug release from sodium alginate–HPMC–calcium chloride in situ nasal gels was studied using a Franz diffusion cell. 1 mL of gel was placed in the donor compartment, separated by a cellophane membrane from the receptor chamber containing 20 mL of phosphate buffer (pH 6.4) maintained at 34 ± 0.5 °C and stirred at 50 rpm. At predetermined intervals, 1 mL samples were withdrawn and replaced with fresh medium. The drug concentration was determined spectrophotometrically at 288 nm, and the cumulative release was calculated [17]. The analytical method was validated over a concentration range of 2–20 µg/mL, and the calibration curve exhibited good linearity with a regression coefficient (R^2) of 0.999. The LOD and LOQ were 0.06 µg/mL and 0.18 µg/mL, respectively. Drug release kinetics were analyzed by fitting the in vitro release data to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, and the best-fit model was selected based on the highest correlation coefficient (R^2).

Rheological study & spreadability

Viscosity was measured using a Brookfield viscometer (LV, spindle 63, 50 rpm, 25 ± 1 °C) before and after adding simulated nasal fluid to assess sol–gel transition, following *Ranch et al.* (2019) [18]. Measurements were done in triplicate and expressed as mean ± SD. Spreadability was evaluated using the slip-and-drag method on two glass plates, as described by *Rathore & Desai* (2018) [19]. 1 g of gel was placed between plates under a 100 g load for 1 min, and spreadability was calculated using:

$$S = \frac{w \times L}{T}$$

where w is weight (g), L is distance moved (cm) & T is time (s).

pH: The pH of in situ nasal gels was determined using a calibrated digital pH meter (Century) at 25 ± 1 °C. Approximately 1 mL of the formulation was dispersed in 10 mL of distilled water, allowed to equilibrate for 5 min, and measured in triplicate [20].

Gel strength: Gel strength was determined by the standard weight-penetration method. The gel (0.5 mL) was filled into a 10 mL cylindrical device with a plunger containing simulated nasal fluid at 34 ± 0.5 °C, and the weight required for 5 mm plunger penetration in 5 seconds was recorded in triplicate [21].

Mucoadhesive strength

Mucoadhesive strength was evaluated using a modified two-arm balance method. Fresh sheep nasal mucosa was collected from a local slaughterhouse and rinsed with phosphate-buffered saline (pH 7.4). Collected mucosa was fixed on glass slides & a 0.5 g gel sample was placed between tissues for 2 min. The weight required to detach mucosal surfaces was noted in triplicate [22].

$$Mucoadhesive\ strength\ (dynes/cm2) = \frac{wg}{A}$$

where w is the weight required for separation in grams, g is the acceleration brought on by gravity (980 cm/s2), and A is the exposed mucosa area.

Drug content: Drug content was determined by dissolving 1 mL of the in situ nasal gel in 100 mL of phosphate buffer (pH 6.4), followed by filtration and UV spectrophotometric analysis at the λ_{max} 288 nm in triplicate [23].

Ex-vivo

Ex-vivo release was studied using Franz diffusion cells with sheep nasal mucosa as the membrane. The donor compartment contained 1 mL of gel, and the receptor phase had phosphate buffer (pH 6.4) at 34 ± 0.5 °C with constant stirring. Samples were withdrawn at set intervals, filtered, and analyzed spectrophotometrically (n = 3) [24].

Histopathological study

A histopathological study using freshly excised sheep nasal mucosa was conducted to evaluate the biocompatibility of the

optimized in situ nasal gel. The gel was applied to the mucosa used in the ex vivo study. Samples were fixed in 10% formalin and stained with eosin. Untreated mucosa and tissue treated with ethanol and in situ gel served as the negative, positive, and test groups, respectively [25].

In vivo study

An in vivo behavioral study was conducted to assess the antidepressant activity of the optimized in situ nasal gel using the forced swim test (FST) and locomotor activity in mice. Healthy mice (20-30 g) were divided into control, standard, and test groups (n = 6) and received a pure selegiline solution, a selegiline tablet (Selgin), and an in situ gel formulation, respectively. Animal activity was performed under institutional animal ethics approval KNIMT/PHAR/IAEC/18/02. For the FST, mice were individually placed in a cylindrical tank (height 40 cm, diameter 20 cm) filled with water (25 ± 1 °C) to a depth of 15 cm. After an initial 15-minute pre-swim session, immobility time during the final 6-minute test session was recorded [26]. Locomotor activity was evaluated using an actophotometer. Mice were placed in the activity chamber, and total movement counts were recorded over 5 min to assess CNS depression or stimulation [27]. All in vivo data were expressed as mean ± SD (n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, to determine significant differences between groups. A p-value of less than 0.05 was considered statistically significant.

Stability study

A stability study of the optimized in situ nasal gel of sodium alginate was conducted in accordance with ICH guidelines. Formulations were stored in tightly closed containers at 4 °C ± 2/55 ± 5% RH, 25±2 °C/60 ± 5% RH (room temperature), and 40 ± 2 °C/75 ± 5% RH (accelerated conditions) for 3 months. Samples were withdrawn at 0, 30, 60, and 90 days and evaluated for appearance, pH, viscosity, gelation time, gel strength, and drug content. Any change in physical characteristics or performance parameters was recorded [28].

RESULT AND DISCUSSION

Preliminary screening

Preliminary screening using the Plackett–Burman design revealed that sodium alginate and calcium chloride were the most influential factors on gelation, viscosity, and gel strength. In contrast, HPMC primarily contributed to viscosity modulation (Table 2). Formulations with low alginate (0.5% w/v) and calcium chloride (10 mM) showed delayed gelation (<90 s), low viscosity (44–113 cp), and weak gels (179–312 g). Increasing calcium chloride to 50 mM reduced gelation time (~24 s) and improved gel strength (~320 g). At high alginate levels (2% w/v), gels formed rapidly (16–18 s) with strong gel strength (420–440 g) but were excessively viscous, limiting practical nasal use. Intermediate conc. (1.25% alginate, 0.75% HPMC, 30 mM $CaCl_2$) provided balanced properties, with viscosity (~150 cp), gelation time (~34 s), and gel strength (~350 g). These findings defined the working ranges for further optimization.

Design of experiment

The Box–Behnken design efficiently evaluated the effect of formulation variables on viscosity, gelation time, gel strength, and mucoadhesive strength of the in situ nasal gel. The quadratic models for all responses were statistically significant ($p < 0.05$) with high coefficients of determination, indicating good agreement between predicted and experimental values. Sodium alginate showed a significant positive effect on viscosity, gel strength, and mucoadhesive strength, which may be attributed to increased polymer chain entanglement and formation of a stronger three-dimensional gel network at higher concentrations. The enhancement in mucoadhesion can be explained by the greater availability of hydroxyl and carboxyl groups in alginate, which interact with mucin through hydrogen bonding.

Similar findings have been reported for alginate-based in situ gels, in which increasing polymer concentration improved viscosity and mucoadhesive properties. $CaCl_2$ significantly decreased gelation time due to rapid ionic cross-linking between calcium ions and guluronic acid residues of sodium alginate, forming the characteristic "egg-box" structure responsible for instant gel formation. Increased calcium ion concentration also contributed to higher gel strength and improved mucoadhesion due to formation of a compact cross-linked network. HPMC predominantly influenced viscosity and contributed moderately to mucoadhesive strength, likely due to its hydrophilic swelling and chain interpenetration with mucus glycoproteins. The interaction between sodium alginate and $CaCl_2$ was also found to be significant, indicating that gel characteristics depend on both polymer concentration and availability of cross-linking ions. The three-dimensional response surface plots further illustrated the combined influence of formulation variables on the measured responses (Figure 1). Increasing concentrations of

sodium alginate & $CaCl_2$ increased viscosity, gel strength & mucoadhesive strength while reducing gelation time. The optimized formulation showed close agreement between predicted and experimental values with less than 5% deviation, confirming the adequacy and predictive capability of the developed model.

Gelation time

Gelation time ranged from 18.8 to 80.3 seconds (Table 3) and was influenced mainly by the concentrations of sodium alginate and calcium chloride. Higher polymer and ion levels accelerated gelation through stronger Ca^{2+}–alginate crosslinking, while lower levels delayed gelation. The optimized formulation F13 (1.25% alginate, 0.75% HPMC, 30 mM $CaCl_2$) exhibited a gelation time of 34.5 ± 0.2 seconds, ensuring rapid gel formation. These results agree with previous reports on ion-activated alginate gels.

In vitro drug release

The formulations (F1–F15) showed sustained drug release over 8 h, ranging from 67.5% to 98.9% (Figure 2). Higher concentrations of sodium alginate and HPMC slowed diffusion due to stronger gel networks, whereas lower concentrations promoted faster release [17]. F13 achieved nearly complete release, confirming controlled diffusion behavior consistent with earlier alginate–HPMC nasal gel studies. In vitro drug-release data for the optimized formulation were analyzed using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models.

Table 2: Preliminary screening and their evaluations

Run	Sod. Alginate (% w/v)	HPMC (% w/v)	$CaCl_2$ (mM)	Viscosity (cp)	Gelation Time (s)	Gel Strength (g)
1	0.5	0.5	10	44.33±1.5	94.7±1.2	179.6±0.7
2	2.0	0.5	50	312.67±1.2	12.4±0.5	450.7±0.8
3	0.5	1.0	50	123.60±0.5	24.5±0.5	321.5±0.5
4	2.0	1.0	10	282.87±0.8	17.7±0.6	420.8±0.4
5	0.5	0.5	50	52.87±0.8	90.1±1.1	187.4±0.8
6	2.0	0.5	10	307.53±0.5	16.9±0.8	440.4±0.5
7	0.5	1.0	10	113.33±0.6	38.1±0.9	312.5±0.6
8	2.0	1.0	50	291.77±0.3	14.3±0.6	431.3±0.5
9	1.25	0.75	30	152.17±0.8	35.6±0.5	351.4±0.8
10	1.25	0.75	30	152.60±0.5	33.9±0.8	354.6±0.6
11	1.25	0.75	30	151.33±0.6	32.1±1.0	352.2±1.1
12	1.25	0.75	30	151.93±0.9	33.6±0.5	352.0±0.9

Table 3: Formulation of in situ gel and their evaluations

Run	Selegiline (% w/v)	Sodium Alginate (% w/v)	HPMC (% w/v)	$CaCl_2$ (mM)	Viscosity (cp)	Viscosity of Gel (cp)	Gelation Time (s)	Gel Strength (g)	Mucoadhesive Strength (dyne/cm²)
F1	0.5	1.25 (0)	0.5 (−1)	10 (−1)	120.30±0.5	6420.5 ± 185.6	55.1±0.2	279.8±0.7	2965.3±28.4
F2	0.5	1.25 (0)	1.0 (+1)	10 (−1)	171.50±1.2	8360.7 ± 242.3	45.4±0.2	320.8±0.3	3042.6±31.2
F3	0.5	1.25 (0)	0.5 (−1)	50 (+1)	140.57±0.7	9580.4 ± 276.9	28.8±0.6	341.1±0.5	3098.4±25.7
F4	0.5	1.25 (0)	1.0 (+1)	50 (+1)	201.30±0.6	12740.6 ± 365.8	22.3±0.5	380.4±0.4	3176.2±29.4
F5	0.5	0.5 (−1)	0.75 (0)	10 (−1)	92.87±0.8	3980.5 ± 118.4	80.3±0.4	200.2±0.9	2911.2±24.6
F6	0.5	2.0 (+1)	0.75 (0)	10 (−1)	251.23±0.5	14860.8 ± 421.7	20.6±0.4	421.1±1.0	3289.4±35.1
F7	0.5	0.5 (−1)	0.75 (0)	50 (+1)	112.03±0.7	6950.3 ± 204.6	41.1±0.4	250.4±0.8	2998.7±26.8
F8	0.5	2.0 (+1)	0.75 (0)	50 (+1)	280.57±0.3	20540.9 ± 598.2	18.8±0.3	450.4±0.3	3374.8±39.6
F9	0.5	0.5 (−1)	0.5 (−1)	30 (0)	101.17±0.4	4520.6 ± 132.5	75.3±0.4	210.4±0.7	2945.6±23.1
F10	0.5	2.0 (+1)	0.5 (−1)	30 (0)	260.90±0.6	16780.2 ± 486.3	25.1±0.7	430.5±0.8	3325.1±36.4
F11	0.5	0.5 (−1)	1.0 (+1)	30 (0)	130.77±0.3	7460.8 ± 214.7	59.4±0.8	240.1±0.4	3021.8±27.3
F12	0.5	2.0 (+1)	1.0 (+1)	30 (0)	291.30±1.4	24566.6 ± 725.4	21.3±0.4	440.5±0.6	3356.9±38.1
F13	0.5	1.25 (0)	0.75 (0)	30 (0)	150.47±0.3	10920.4 ± 318.6	34.5±0.2	349.9±0.2	3142.5±30.6
F14	0.5	1.25 (0)	0.75 (0)	30 (0)	153.73±0.4	11080.6 ± 325.4	34.4±0.5	352.0±0.3	3151.2±29.8
F15	0.5	1.25 (0)	0.75 (0)	30 (0)	150.47±0.6	10840.3 ± 309.2	32.0±0.4	348.1±0.4	3138.9±28.9

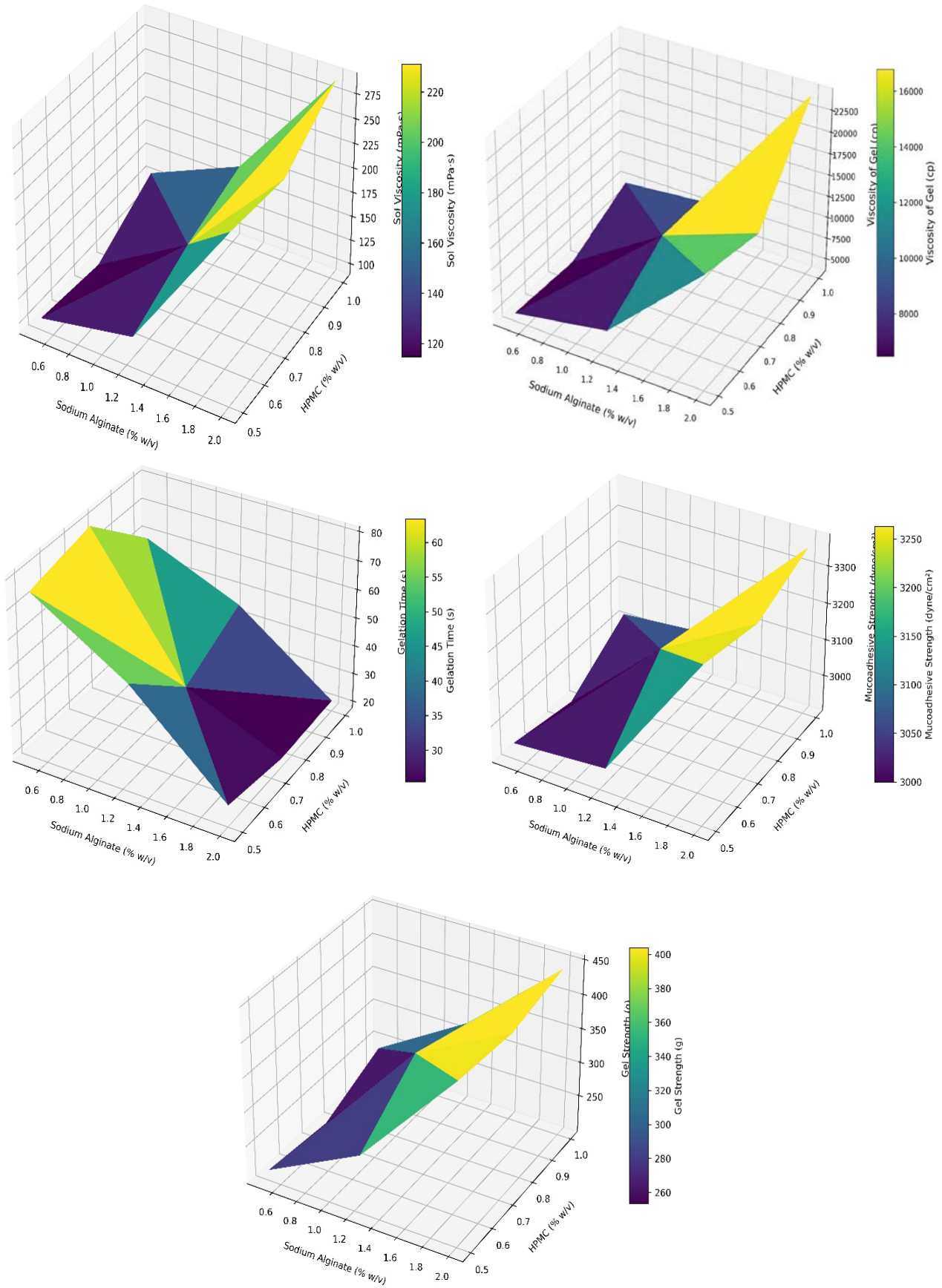

Figure 1: 3D surface plot showing the concentration effect of Sodium alginate on HPMC (a) Sol viscosity, (b) Viscosity of gel, (c) Gelation time, (d) Mucoadhesive strength and (e) Gel strength.

The highest correlation coefficient (R^2 = 0.99) was observed with the Higuchi model, indicating that drug release is predominantly diffusion-controlled from the polymeric matrix. Further analysis using the Korsmeyer–Peppas model indicated a release exponent (n) between 0.5 and 0.89, suggesting a non-Fickian (anomalous) transport mechanism in which both drug diffusion and polymer relaxation contribute to the release process. These results confirm the sustained release behavior of the sodium alginate–HPMC in situ gel system.

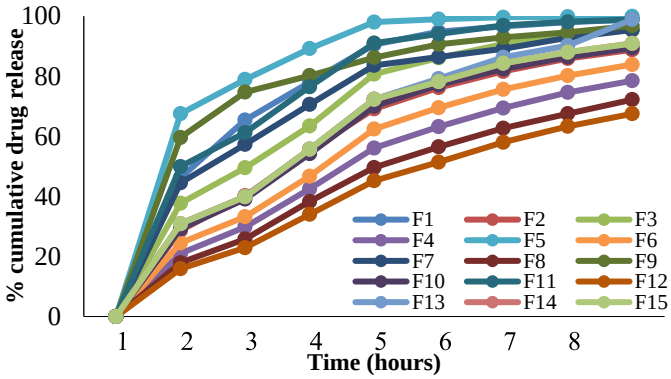

Figure 2: In vitro drug release of sodium alginate in situ gel

Viscosity & spreadability

Viscosity of sol and gel ranged from 92.87–291.30 cp and 3980.5 – 24566.6 cp, respectively (Table 3), increasing with higher sodium alginate and HPMC levels. The optimized formulation showed 150.47 cp and 10920.4 cp for the sol and gel states, respectively. The observed pseudoplastic flow supports smooth application and suitability for nasal delivery. Spreadability ranged from 19.5 to 28.8 g·cm/s (Figure 3), indicating good mechanical behavior. The optimized gel exhibited a flow rate of 24.6 ± 0.5 g·cm/s, ensuring easy application. Higher HPMC levels slightly decreased spreadability, aligning with previous reports [18].

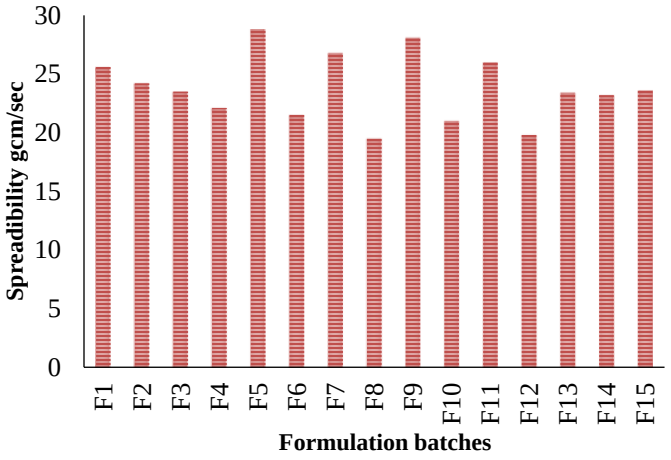

Figure 3: Spreadability of sodium alginate in situ gel

pH: The pH of all formulations ranged from 5.6 ± 0.1 to 6.1 ± 0.2 (Table 3), falling within the physiological range of nasal mucosa (pH 5.0–6.5). This indicates that the formulations are non-irritant and compatible with nasal tissues. A near-neutral pH also supports drug stability and prevents premature ion-triggered gelation during storage.

Gel strength

Gel strength of all formulation batches ranged from 210.4 to 450.4 g (Table 3), indicating adequate firmness for nasal retention. Higher alginate and $CaCl_2$ levels increased strength via cross-linking, while higher HPMC produced softer gels, consistent with previous findings [29].

Mucoadhesive strength

Mucoadhesive strength ranged from 2911.2 ± 24.6 to 3374.8± 39.6 dyne/cm^2 (Figure 4), confirming sufficient adhesion for nasal residence. Increased concentrations of HPMC and sodium alginate enhanced adhesion through stronger hydrogen bonding, consistent with earlier reports on mucoadhesive nasal gels [30].

Drug content

Drug content of all formulation batches ranged from 97.2 ± 0.4% to 98.6 ± 0.3%, indicating uniform distribution and minimal drug loss during formulation. The high recovery reflects good solubility & compatibility of the drug within the polymer matrix.

Ex-vivo

Ex vivo drug release from the optimized formulation F13 was 94.6 ± 0.9% after 6 hours (Figure 4), indicating sustained diffusion. Higher alginate and HPMC concentrations reduced release due to increased matrix viscosity.

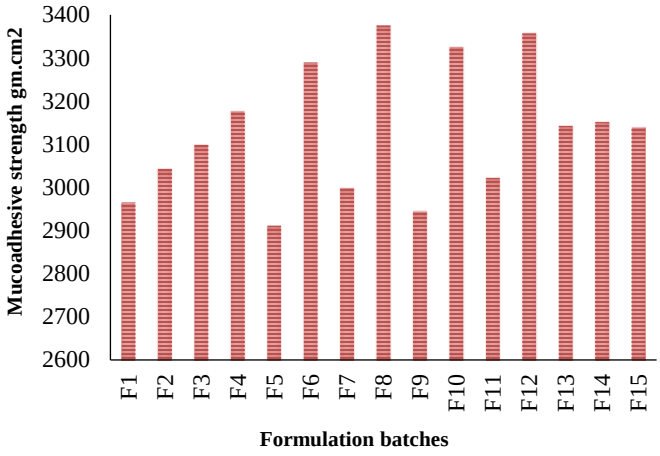

Figure 4: Mucoadhesive strength of sodium alginate in situ gel

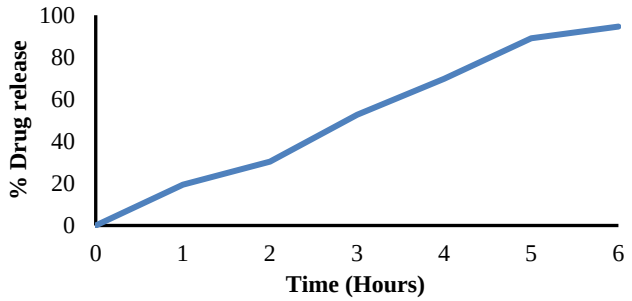

Figure 4: Ex vivo drug release of sodium alginate in situ gel

Histopathological study

A histopathological study of the optimized formulation batch F13 was carried out on sheep nasal mucosa, which was divided into three groups: control, in situ gel-treated, and positive (Figure 5). Histopathological evaluation of the optimized nasal mucosa revealed normal architecture in the control group with intact epithelium and organized lamina propria. The in situ gel-treated group showed preserved epithelial integrity without inflammation or tissue damage. In contrast, the positive control exhibited epithelial disruption, inflammatory cell infiltration, and evident mucosal irritation. These findings confirm the formulation's safety and non-irritant nature for nasal application. Histopathological evaluation showed no epithelial damage after acute exposure, indicating good short-term safety. However, long-term effects on mucociliary function and nasal tissue were not assessed, and no recovery studies were conducted. Further studies are needed to evaluate long-term safety and reversibility.

In vivo test

In vivo testing of the optimized formulation batch was carried out using the two-parameter forced swim test (FST) and Locomotor activity. For both tests, animals were divided into three groups: control, orally marketed-treated, and optimized in situ gel-treated. The reported animal dose of selegiline is 1 mg/kg; thus, a 25±5 µg/mg dose is required for each mouse weighing 25±5 gm [31]. A marketed Selgin 5 mg tablet was taken; 5 mg tablets were diluted in 100 ml of water, and 5 ml of this dilution was taken. In the FST, the test formulation significantly reduced immobility, while climbing and swimming time were higher than in the control and standard groups, indicating potent antidepressant activity (Figure 6). Locomotor activity counts showed greater locomotion in the test group than in the standard and control groups (Figure 7). These results support the nasal gel's potential for effective brain delivery and antidepressant action. In the forced swim test, the optimized intranasal gel significantly reduced immobility time compared to the control ($p < 0.001$) and oral formulation ($p < 0.05$), with increased climbing and swimming behavior indicating enhanced antidepressant activity. Locomotor activity was also significantly higher than control ($p < 0.001$) and oral groups ($p < 0.05$), suggesting improved CNS stimulation without sedation. Overall, the intranasal gel showed a superior pharmacodynamic response compared to oral therapy.

Stability study

The stability study was conducted on the formulation in its sol (pre-administration) state, as the in situ gel is designed to undergo gelation only upon contact with nasal ions under physiological conditions. Therefore, the stability of the preformed gel state was not evaluated, as it is not relevant for storage conditions. A stability study of the optimized formulation batch F13 was carried out at 4 ± 2 °C/55 ± 5% RH, 25 ± 2 °C/60 ± 5% RH, and 40 ± 2 °C/75 ± 5% RH for three months. The formulation was studied at the initial time and after 1, 2, and 3 months for pH, viscosity, and drug content. The formulation retained its clear appearance, pH, viscosity, and gelation behavior throughout the study. No significant changes in gel strength or drug content were observed, and all values remained within acceptable limits (Table 4). These results indicate good physical & chemical stability of the sod. alginate in situ nasal gel under storage conditions.

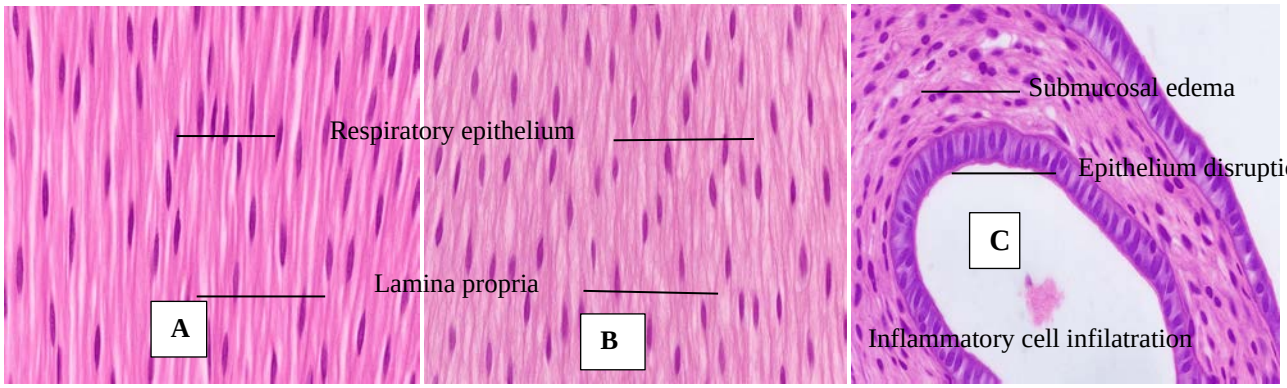

Figure 5: Histopathological study of (A) Control group, (B) In situ gel treated & (c) Positive control

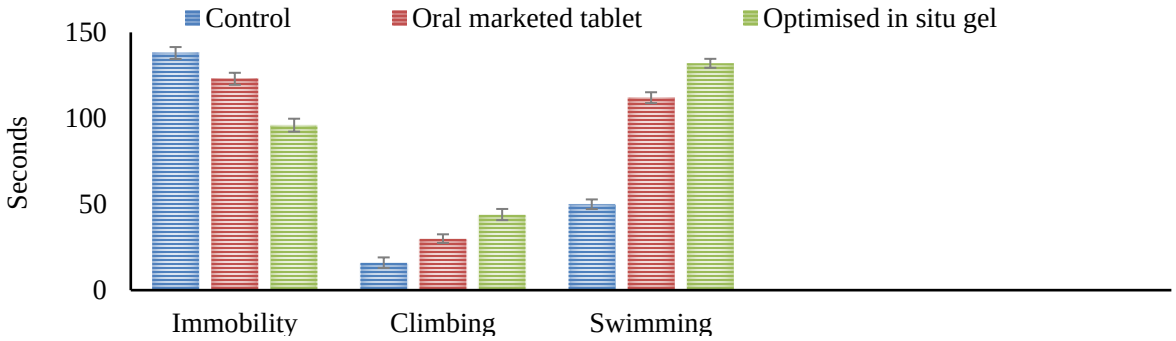

Figure6: Forced swim test

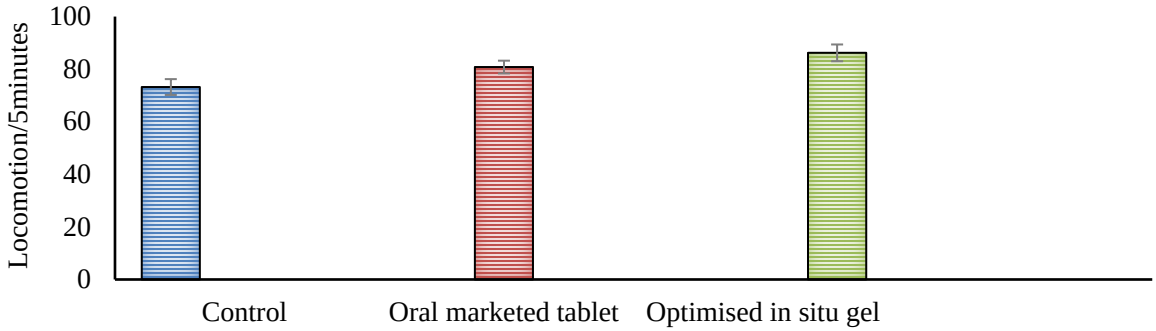

Figure 7: Locomotor activity

Table 4. Stability Evaluation of Sodium Alginate in Situ Nasal Gel

Parameter	Storage Condition	Initial	After 1 Month	After 2 Months	After 3 Months
Appearance	**—**	**Clear, transparent**	**No change**	**No change**	**No change**
pH	4±1°C	5.9 ± 0.3	5.6 ± 0.1	5.8 ± 0.2	6.2 ± 0.2
	25±2°C	5.8 ± 0.1	5.8 ± 0.1	6.0 ± 0.2	6.4 ± 0.4
	40±2°C	5.7 ± 0.2	5.8 ± 0.1	5.8 ± 0.2	5.8 ± 0.2
Viscosity (cp)	4±1°C	152 ± 2.5	150 ± 3.1	149 ± 2.9	149 ± 2.8
	25±2°C	151 ± 2.2	148 ± 2.9	147 ± 3.1	147 ± 3.0
	40±2°C	150 ± 2.8	146 ± 3.2	145 ± 3.3	144 ± 3.5
Drug Content (%)	4±1°C	98.5 ± 0.4	98.3 ± 0.5	98.2 ± 0.6	98.1 ± 0.6
	25±2°C	98.5 ± 0.4	97.9 ± 0.6	97.7 ± 0.7	97.5 ± 0.8
	40±2°C	98.5 ± 0.4	97.2 ± 0.7	97.0 ± 0.8	96.8 ± 0.9

CONCLUSION

The present study successfully developed and optimized a sodium alginate–HPMC-based ion-activated in situ nasal gel of selegiline with potential for improved brain targeting. The optimized formulation demonstrated desirable physicochemical properties, including suitable viscosity, rapid gelation, adequate gel strength, and good mucoadhesive characteristics, as well as sustained in vitro and ex vivo drug release. It exhibited pseudoplastic behavior, an acceptable pH, and a uniform drug content, and was found to be non-irritant to the nasal mucosa. In vivo studies indicated significant antidepressant activity, suggesting effective nose-to-brain delivery and improved therapeutic performance. Stability studies confirmed that the formulation remained stable under accelerated conditions. The formulation did not include additional chemical permeation enhancers. Instead, sodium alginate and HPMC were used to improve nasal residence time through mucoadhesion. Due to the lipophilic, low-molecular-weight nature of selegiline, passive diffusion across the nasal membrane may occur without additional enhancers, thereby minimizing the risk of mucosal irritation. These findings highlight the potential of the developed in situ nasal gel as an alternative to conventional oral therapy, particularly in improving drug bioavailability and targeting efficiency for central nervous system disorders such as depression, which remains a major global health concern. However, the study is limited by the lack of detailed pharmacokinetic and long-term clinical evaluations, which are necessary to confirm translational applicability in humans. A limitation of the present study is the absence of a control group receiving an intranasal selegiline solution. The inclusion of such

a group would allow differentiation between the effects of the intranasal route itself and the contribution of the in situ gel formulation to enhancing drug performance. Future studies will focus on comparative evaluation of intranasal drug solution, in situ gel formulation, and oral administration to better elucidate the relative roles of route and formulation. Overall, the formulation represents a promising, safe, and effective drug delivery approach with potential clinical relevance, warranting further investigation for its application in the management of depressive disorders.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Mahesh Prasad introduced the concept. Shiv Kumar Srivastava and Antesh Kumar Jha analyzed and interpreted the experimental design data related to the work. Shiv Kumar Srivastava, Shashi Shankar, and Abhishek Kumar Singh performed the studies in the laboratory, recorded observations, and contributed to drafting the manuscript. All authors read and approved the final manuscript.

DECLARATION OF USE OF AI TOOLS
The authors used ChatGPT (OpenAI) to assist with grammar correction, language editing, and readability improvement of the manuscript. All content, analysis, and conclusions were reviewed and verified by the authors. The authors take full responsibility for the originality, accuracy, and integrity of the work.

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