Mucoadhesive In-Situ Gel for Intranasal Delivery of Resveratrol: In Vitro and In Vivo Studies

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Abstract
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment, neuronal degeneration, oxidative stress, and neuroinflammation. Although resveratrol (RES) exhibits potent antioxidants, anti-inflammatory, and neuroprotective properties, its therapeutic application is limited by poor aqueous solubility, extensive first-pass metabolism, and extremely low oral bioavailability. The present study aimed to develop and optimize a thermosensitive mucoadhesive in situ gel for intranasal delivery of resveratrol to enhance nose-to-brain transport and improve therapeutic efficacy against Alzheimer's disease. A Quality-by-Design (QbD) approach employing a 2^3 full factorial design was used to optimize the concentrations of Poloxamer 407, Poloxamer 188, and HPMC E15 LV based on gelation temperature, gelation time, and pH. The optimized formulation demonstrated desirable physicochemical characteristics, including rapid thermogelation, suitable nasal pH, excellent mucoadhesive strength, prolonged residence time, pseudoplastic rheological behavior, and sustained drug release over 12 h. Pharmacokinetic studies in Wistar rats revealed significantly enhanced brain delivery following intranasal administration, with approximately two-fold higher brain Cmax, faster Tmax, and improved brain bioavailability compared with oral administration of marketed resveratrol tablets. Pharmacodynamic evaluation in a trimethyltin-induced Alzheimer's disease model showed marked improvement in spatial learning, memory, and behavioral performance. Histopathological examination demonstrated neuroprotection with preservation of hippocampal architecture, while nasal ciliotoxicity studies confirmed the safety of repeated intranasal administration. The developed formulation also exhibited acceptable cytocompatibility in Neuro2a cells. Overall, the optimized thermosensitive mucoadhesive in situ gel represents a promising, safe, and non-invasive platform for efficient nose-to-brain delivery of resveratrol, offering significant potential for the management of Alzheimer's disease and other neurodegenerative disorders.

Keywords Resveratrol, Intranasal drug delivery, Thermosensitive in situ gel, Mucoadhesive gel, Alzheimer's disease.
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Introduction: Neurological disorders are becoming increasingly prevalent worldwide, [1] Alzheimer's disease (AD) is one of the most common neurodegenerative conditions, characterized by progressive neuronal degeneration, cognitive decline, memory impairment [2], and behavioral disturbances and is a leading cause of dementia in the elderly population [3]. The pathological condition includes the extracellular accumulation of amyloid-beta (Aβ) plaques which initiates a cascade of events that promote tau pathology synaptic dysfunction, neuronal loss [4], and ultimately neurodegeneration and the intracellular formation of neurofibrillary tangles composed of hyper phosphorylated tau protein [5]. In addition, impaired clearance of aggregated proteins, oxidative stress, neuroinflammation, and mitochondrial dysfunction further contribute to disease progression [6,7].

Resveratrol (RES), a naturally occurring non-flavonoid polyphenolic compound found in grapes, berries, peanuts, and red wine, has attracted considerable attention because of its potent antioxidant, anti-inflammatory, neuroprotective, and anti-aging properties [8,9]. Numerous studies have

demonstrated that resveratrol can modulate multiple pathways involved in Alzheimer's disease [8]. Despite its promising therapeutic potential, the clinical application of resveratrol remains limited because of its poor aqueous solubility, extensive first-pass metabolism, rapid systemic elimination, and extremely low oral bioavailability, which is reported to be less than 1% [10,11]. These limitations significantly reduce the amount of drug reaching the brain following conventional oral administration [12]. Intranasal drug delivery has emerged as a promising and non-invasive strategy for direct brain targeting [13,14]. This route provides a unique anatomical connection between the nasal cavity and the central nervous system through the olfactory and trigeminal neural pathways, enabling drugs to bypass the BBB, offers several advantages, including rapid onset of action, avoidance of hepatic first-pass metabolism, reduced systemic exposure, improved patient compliance, and enhanced brain bioavailability [15].

The highly vascularized and permeable nature of the nasal mucosa further facilitates efficient drug absorption and transport to the brain [16]. Various intranasal dosage forms have been investigated to improve nose-to-brain drug delivery [17]. Among them in situ gelling formulations have gained considerable interest because they combine the ease of administration of liquid formulations through sol-to-gel transition upon exposure to physiological conditions within the nasal cavity with the prolonged residence time of gels [18].

Poloxamers are widely used as thermo responsive polymers owing to their excellent biocompatibility, low toxicity, and reversible gel-forming properties [15]. At lower temperatures, poloxamers exist as individual polymer chains in solution; however, as the temperature increases, the hydrophobic PPO blocks aggregate to form micelles, which subsequently organize into a three-dimensional gel network [19,20].

In the present study, development of an alternative, non-invasive delivery system capable of enhancing brain targeting and bypassing the blood-brain barrier (BBB), the development of an alternative, non-invasive delivery system capable of enhancing brain targeting and bypassing the blood-brain barrier (BBB) a Quality-by-Design (QbD) and Design of Experiments (DoE) approach will be employed to systematically investigate the influence of formulation variables on critical quality attributes such as gelation temperature, gelation time, gelation time, viscosity, mucoadhesive strength, and drug release behavior. This statistical approach enables optimization of the formulation with a minimal number of experimental trials while ensuring robust product performance. Following optimization, the developed resveratrol-loaded mucoadhesive thermosensitive in situ gel will undergo comprehensive physicochemical characterization, in vitro release studies, ex vivo permeation studies, and in vivo evaluation to assess its potential as an effective nose-to-brain delivery system for the treatment of Alzheimer's disease [21,22].

Materials and methods

Materials

Resveratrol purchased from BLD Pharmatech (India) Pvt Ltd., India. Poloxamer 407 (P407), poloxamer 188 (P188), and HPMCE15 LV (Mw~40 000, 12–18 *cP*) were obtained from Sigma-Aldrich Chemicals Private Limited, India. All chemicals and solvents used in this study were of analytical grade from SD Fine Chemicals, Mumbai, India.

Preparation of Standard Stock Solution

A standard stock solution of Resveratrol (RES) was prepared by accurately weighing 10 mg of RES and dissolving it in methanol, followed by dilution to 100 mL in a volumetric flask to obtain a concentration of 100 µg/mL. Appropriate aliquots of this stock solution were subsequently diluted with methanol to prepare working standard solutions having concentrations of 1, 2, 4, 6, 8, and 10 µg/mL. The absorption spectrum of RES was recorded over the wavelength range of 200–400 nm using a UV–Visible spectrophotometer, with methanol serving as the blank. The wavelength corresponding to the maximum absorbance (λmax) was identified and selected for subsequent quantitative analysis [23].

Construction of Calibration Curve

The calibration curve for RES was constructed using standard solutions at concentrations of 1, 2, 4, 6, 8, and 10 µg/mL. The absorbance of each concentration was measured in triplicate at the determined λmax, and the mean absorbance values were calculated. A calibration graph was plotted by correlating absorbance against concentration, and the linear regression equation along with the correlation coefficient (R^2) was determined to evaluate the linearity of the method.

Method Validation

The developed UV spectrophotometric method was validated according to the International Council for Harmonisation (ICH) Q2(R1) guidelines. Validation parameters included specificity, linearity, range, accuracy, precision, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ).

Preparation of the Thermosensitive Gel

The thermosensitive gel formulations were prepared using the cold method. Initially, Poloxamer 407 (P407) was dispersed in 10 mL of deionized water at room temperature under continuous stirring. The resulting solution was then refrigerated at 4 °C overnight to ensure complete hydration and dissolution of the polymer. Afterward, Poloxamer 188

(P188) and HPMC E15 LV were gradually added to the chilled P407 solution while maintaining the temperature at 4 °C and stirring continuously until a clear and homogeneous solution was obtained. For the preparation of drug-loaded formulations, citric acid was first incorporated into the polymeric solution, followed by the addition of resveratrol. The mixture was stirred continuously until the drug was completely dissolved and uniformly distributed throughout the formulation. The prepared gels were stored at 4 °C until further characterization and evaluation Figure 1.

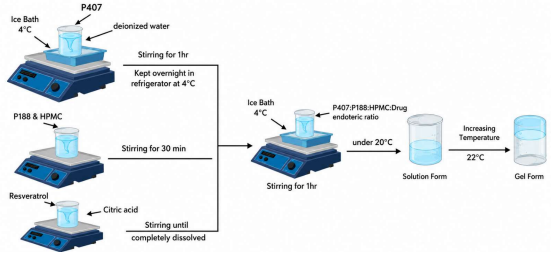

Figure 1. Illustration process of preparation of RES *in situ* gel formulation.

Optimization of the Thermosensitive Gel

A 2^3 full factorial design was employed to optimize the thermosensitive gel formulation for intranasal delivery of resveratrol using Design-Expert® software (Version 13, State-Ease Inc., USA). The study investigated the effects of three independent formulation variables: the concentrations of Poloxamer 407 (P407), Poloxamer 188 (P188), and HPMC E15 LV. The influence of these variables on critical formulation attributes, namely gelation temperature, gelation time, and pH, was evaluated.

The experimental design consisted of factorial points along with three center-point replicates to assess the curvature and reproducibility of the model within the selected design space. The coded and actual levels of the independent variables are presented in Table 1, while the experimental design matrix generated using Design-Expert software is shown in Table 2.

	Coded Level		
Factors	**High**	**Medium**	**Low**
Independent variables			
X1:P407 (%w/v)	23	21	19
X2:P188 (%w/v)	6	4	2
X3:HPMCE15 LV (%w/v)	1	0.8	0.6
Dependent variables	**Constraints for optimization**		
Y1: Gelation temperature (°C)	31 °C 30s		
Y2: Gelation time (Sec)	6		
Y3: pH			

Table 1. Variables used for the optimization of thermosensitive gel using 2^3-factorial.

Run	**Factor X1 P407 (%w/v)**	**Factor X2 P188 (%w/v)**	**Factor X3 HPMCE15 LV (%w/v)**
F1	19	2	0.6
F2	23	2	0.6
F3	19	6	0.6
F4	23	6	0.6
F5	19	2	1
F6	23	2	1
F7	19	6	1
F8	23	6	1
F9	21	4	0.8
F10	21	4	0.8
F11	21	4	0.8

Table 2. Compositions of the 2^3-factorial design for thermosensitive gel.

Based on the optimization results, a formulation containing 21% w/v P407, 4% w/v P188, and 0.8% w/v HPMC E15 LV was identified as the optimized composition. Drug-loaded thermosensitive gels were subsequently prepared by incorporating citric acid and resveratrol into the optimized polymeric system. The detailed composition of all developed formulations is provided in Table 3.

Batch Code	**P407 (%w/v)**	**P188 (%w/v)**	**HPMC E15 LV (%w/v)**	**Citric acid (%w/v)**	**Resveratrol (%w/v)**
F12	21	4	0.8	0.25	1
F13	21	4	0.8	0.25	-
F14	21	4	0.8	0.50	1.25
F15	21	4	0.8	0.50	-
F16	21	4	0.8	0.75	1.50
F17	21	4	0.8	0.75	-
F18	21	-	-	-	-
F19	21	4	-	-	-
F20	21	-	0.8	-	-
F21	21	4	0.8	-	-

Table 3. Composition of the gel formulations.

To evaluate the effect of resveratrol incorporation on the physicochemical characteristics of the gel, corresponding blank formulations (F13, F15, and F17) were prepared without the drug. In addition, reference

formulations (F18–F21) were developed for comparative evaluation. All blank, drug-loaded, and reference formulations were subjected to comprehensive characterization to assess their performance and suitability for intranasal administration.

Characterization of Thermosensitive Gel

Appearance

The prepared thermosensitive gel formulations were visually inspected under adequate lighting conditions to evaluate their physical appearance. Parameters such as color, homogeneity, and the presence of any visible particulate matter were observed. The clarity of each formulation was assessed by viewing the samples against contrasting black and white backgrounds [24].

Drug Content

The drug content of the formulations was determined by diluting 1 mL of the gel with phosphate buffer (pH 6.4). The resulting solution was filtered through a 0.22 µm membrane filter to remove any undissolved particles. After suitable dilution, the absorbance of the sample was measured at 269 nm using a UV–Visible spectrophotometer (Shimadzu UV-1900i, Japan). The concentration of Resveratrol was calculated from a previously established calibration curve, and the drug content was expressed as the percentage of theoretical drug loading [25].

Gelation Time

Gelation time was evaluated using a thermostatically controlled water bath maintained at 34 °C, simulating the physiological temperature of the nasal cavity. A 10 mL sample of the formulation was placed in a vial and stirred continuously at 200 rpm using a magnetic stirrer. The time required for the formulation to undergo sol-to-gel transition was recorded. Gelation was considered complete when the magnetic bar ceased to move due to the formation of a gel network. The reported values represent the mean of three independent measurements [26].

Gelation Temperature

The gelation temperature of the formulations was determined using the vial inversion method. Briefly, 10 mL of the sample was transferred into a glass vial and placed in a water bath maintained initially at 15 °C. The temperature gradually increased by increments of 2 °C every 5 minutes up to 25 °C, followed by increments of 1 °C every 5 minutes thereafter. At each temperature interval, the vial was inverted to monitor the sol-to-gel transition. The temperature at which the formulation no longer flowed upon inversion was recorded as the gelation temperature. All measurements were performed in triplicate, and the average value was reported [27].

Gel Strength

Gel strength was evaluated using a method adapted from a previously reported procedure [28]. Briefly, 30 mL of the formulation was transferred into a 100 mL measuring cylinder and maintained in a water bath at 34 °C until gelation occurred. After gel formation, a 15 g weight was carefully placed on the surface of the gel. The time required for the weight penetrate 1 cm into the gel was recorded and considered as an indicator of gel strength. The experiment was performed in triplicate, and the results were expressed as mean ± standard deviation.

In Vitro Mucoadhesive Strength

The mucoadhesive strength of the formulation was determined using a modified two-arm balance technique [29]. A mixture of porcine mucin (2%) and agar (1.5%) was prepared and placed in a Petri dish (2.5 cm diameter) to simulate the nasal mucosal surface. The experimental arrangement is illustrated in Figure 2. Mucoadhesive strength was quantified as the force required to detach the two surfaces and was expressed in g/cm². The amount of water needed to separate the dishes was recorded as a measure of adhesive strength. Each measurement was carried out three times, and the average value was reported.

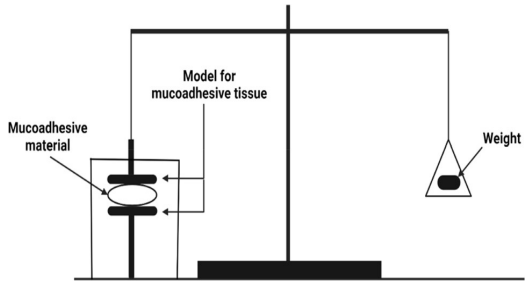

Figure 2. Modified balance for measuring *In vitro* mucoadhesive strength.

Mucoadhesive Time

The residence time of the gel on the mucosal surface was assessed using a modified in vitro method [30]. One gram of gel containing 0.1% carminic acid was applied onto the surface of an agar block composed of porcine mucin (2%) and agar (1.5%). The agar block was placed in a Petri dish and maintained at 34 °C. The dish was positioned at an angle of 45° inside a temperature-controlled chamber. Simulated nasal fluid (SNF, pH 6.5) was continuously passed over the gel surface at a flow rate of 1 mL/min using a peristaltic pump (EnerTech, Mumbai, India). The gel was monitored until it was completely removed from the surface. The time taken for the complete disappearance of the colored gel was recorded as the mucoadhesive residence time.

Rheological Properties

The rheological behaviour of the in-situ gel formulations was investigated using a Brookfield DV3T rheometer (Brookfield Engineering Laboratories, USA). An appropriate spindle was selected based on the viscosity range of the samples. Measurements were initially performed over a temperature range of 25–32 °C to evaluate the effect of temperature on viscosity and gel formation. Subsequently, viscosity measurements were carried out at 32 ± 2 °C, corresponding to nasal physiological conditions, over a rotational speed range of 10–50 rpm. Before analysis, each formulation was allowed to equilibrate at the test temperature for 10 minutes to ensure thermal stability. All measurements were performed in triplicate, and the relationship between shear rate and viscosity was used to characterize the flow behaviour of the formulations [31].

Texture Analysis

The mucoadhesive properties of the formulations were evaluated using a TA-XT Plus Texture Analyzer fitted with a 5 g load cell and a mucoadhesive test rig [32]. Fresh sheep nasal mucosa was used as the biological membrane model. Tissue samples measuring approximately 20 × 20 mm were equilibrated at 35.0 ± 0.5 °C for 10 minutes before testing and then secured onto the mucoadhesive holder. The probe was lowered towards the mucosal surface at a speed of 0.1 mm/s until contact was established. A contact force of 5 g was maintained for 20 seconds to allow interaction between the formulation and the mucosal tissue. Subsequently, the probe was withdrawn at a speed of 0.1 mm/s to 5 mm. The maximum detachment force (N), work of adhesion (mJ), and mucoadhesive strength (mJ/cm²) were recorded and used to assess the adhesive performance of the formulations.

Spreadability

The spreadability of the in-situ gel formulations was determined by measuring the area covered by the formulation over time and was expressed as cm²/min [33]. A 1 mL graduated pipette fitted with a rubber bulb was mounted vertically on a clamp stand. The pipette tip was positioned 2 cm above a horizontally placed Whatman filter paper (0.45 µm pore size). A volume of 0.1 mL of the formulation was carefully dispensed onto the center of the filter paper. The area covered by the spreading formulation was measured at fixed intervals of 20 seconds, and the spreadability was calculated accordingly.

In Vitro Drug Release Study

The in vitro release profile of Resveratrol (RES) from the in-situ gel formulations was evaluated using a Franz diffusion cell (Orchid Scientific and Innovative Ltd., Nashik, India) with an effective diffusion area of 9 mm diameter. A gel sample containing 10 mg of RES was placed on a pre-soaked cellophane membrane (MWCO 12–14 kDa), which was positioned between the donor and receptor compartments. The receptor chamber was filled with 5 mL of simulated nasal fluid (SNF, pH 6.4) and maintained at 37 ± 0.5 °C under continuous stirring at 600 rpm [34].

Samples were withdrawn at predetermined intervals (0, 0.5, 1, 2, 3, 4, 6, 8, and 12 h), and an equal volume of fresh SNF was added to maintain sink conditions. The collected samples were suitably diluted and analysed using a UV–Visible spectrophotometer at 269 nm. The cumulative percentage drug release was then calculated and plotted against time.

In Vivo Studies

Animals

A total of 98 male Wistar albino rats weighing between 200 and 250 g and aged 8–10 weeks were used for the in vivo experiments. The animals were procured from Vaarunya BioLabs Private Limited, Bengaluru, India. They were housed under standard laboratory conditions with a controlled temperature of 25 ± 1 °C and a 12 h light/dark cycle. Prior to the study, the animals were allowed to acclimatize for four weeks and were provided with free access to standard pellet diet and water throughout the experimental period.

Pharmacokinetic Studies

Pharmacokinetic evaluation was performed using 30 male Wistar rats, which were randomly divided into two groups of 15 animals each. Group I received the optimized Resveratrol (RES)-loaded thermosensitive in situ gel formulation via the intranasal route at a dose of 10 mg/kg. Group II received the marketed RES formulation, Resvae, orally at the same dose (10 mg/kg). For oral administration, Resvae tablets were dispersed in normal saline to obtain a uniform suspension [35].

Prior to dosing, the animals were fasted overnight for 12 h with free access to water. At predetermined time intervals (0, 5, 10, 20, 30, 60, 120, 180, 240, and 480 min), three rats from each group were selected for blood collection through the retro-orbital plexus. Following sample collection, the animals were euthanized by intraperitoneal administration of urethane (1.0 g/kg). Blood samples were centrifuged to separate plasma, which was stored at −20 °C until further analysis.

Brain tissues were carefully excised, homogenized, and extracted using acetonitrile. The concentrations of RES in both plasma and brain tissues following intranasal and oral administration were quantified using High-Performance Liquid Chromatography (HPLC). Chromatographic separation was achieved using a C18 column (4.6 × 250 mm, 5 µm particle size). The mobile phase consisted of acetonitrile and ammonium acetate (90:10, v/v), delivered at a flow

rate of 1.0 mL/min. The injection volume was 20 µL, and RES was detected at a wavelength of 269.6 nm with a total run time of 20 min. Pharmacokinetic parameters, including maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), and area under the concentration–time curve (AUC0–t), were calculated using non-compartmental analysis.

Pharmacodynamic Studies

To evaluate the therapeutic efficacy of the formulation, rats were randomly divided into four groups, each consisting of eight animals. The control group received intranasal phosphate-citrate buffer (pH 5.5). The disease-control group received the same intranasal buffer along with oral administration of Trimethyltin (8 mg/kg) for five consecutive weeks to induce Alzheimer's disease-like pathology [36].

The third group received oral Resveratrol solution in combination with Trimethyltin (8 mg/kg, orally), while the fourth group was treated with the optimized intranasal RES-loaded in situ gel formulation together with oral Trimethyltin administration. Resveratrol was administered at a dose of 4 mg/kg in both treatment groups [37]. The intranasal formulation was administered at a volume of 10 µL per nostril, 90 min before Trimethyltin administration.

The effects of treatment were assessed using a series of behavioral tests designed to evaluate learning, memory, and motor function. These included the Y-maze test for assessing spatial working memory, the conditioned avoidance response test for evaluating learning and memory associated with negative reinforcement, the Morris water maze test for measuring spatial learning and memory, and the swimming performance test for assessing motor coordination and physical endurance.

Y-Maze Test

The Y-maze test was performed to assess spatial working memory and cognitive function in rats during the 5-week treatment period [38]. The apparatus consisted of a black wooden maze with three identical arms arranged in a Y-shaped configuration around an equilateral triangular central area. Each arm measured 35 cm in length, 10 cm in width, and 25 cm in height.

For each trial, a rat was placed at the end of one arm and allowed to explore the maze freely for 8 min. The total number of arm entries and the sequence of entries into the three arms (designated as A, B, and C) were recorded. Spontaneous alternation behaviour was defined as consecutive entries into all three arms without repetition. The percentage of spontaneous alternation, which reflects spatial working memory performance, was calculated using the following equation:

$$\%\text{Alternation} = \frac{\text{Number of Actual Alternations}}{\text{Total Number of Arm Entries} - 2} * 100$$

Higher alternation percentages indicate improved cognitive performance and working memory.

Morris Water Maze Test

The Morris water maze test was employed to evaluate spatial learning and memory in rats [39]. The apparatus consisted of a circular pool measuring 150 cm in diameter and 60 cm in height, filled with water to a depth of 30 cm and maintained at 25 ± 2 °C. To prevent the platform from being visible, the water was rendered opaque using non-toxic white paint.

Training was conducted over three consecutive days, with four trials performed per day. During each trial, rats were released into the water from different quadrants while facing away from the pool wall and were allowed 60 s to locate the hidden platform. Animals that failed to find the platform within the allotted time were gently guided to it and allowed to remain there for 20 s. The time required to reach the platform (escape latency) was recorded for each trial.

On the fourth day, a probe trial was conducted by removing the platform from the pool. Rats were allowed to swim freely for 60 s, and the time spent in the target quadrant, where the platform had previously been located, was recorded as an indicator of memory retention. The swimming paths of the animals were monitored and recorded using a video camera mounted above the pool.

Conditioned Avoidance Test

The conditioned avoidance (CA) test was used to assess learning and memory following induction of Alzheimer's disease-like symptoms [40]. The apparatus consisted of five interconnected chambers. Four chambers had electrified stainless-steel grid floors capable of delivering a mild electric shock (50 V, 25 pulses/s), while the fifth chamber had a glass floor and served as a safe compartment.

During the training sessions, rats were exposed to an auditory stimulus (electric bell) for 5 s, followed by a mild foot shock for another 5 s. The ability of the animals to associate the auditory cue with the impending shock was evaluated by recording the number of times they successfully moved to the safe chamber within 5 s of hearing the bell. The avoidance responses were measured on both the first and second training days and used as an indicator of learning and memory performance.

Swimming Test

Motor coordination and physical performance were assessed using a swimming test adapted from a previously reported method [41]. The experiment was conducted in a transparent glass tank filled with water

maintained at 26 ± 2 °C. A ramp was positioned at one end of the tank to serve as an escape platform.
Each rat was placed at the opposite end of the tank and allowed 3 min to swim towards the ramp using its forelimbs. Performance was evaluated based on the swimming path taken to reach the ramp. Rats that reached the ramp directly received a score of 4. Minor deviations to either side were assigned a score of 3, multiple deviations in both directions received a score of 2 and pronounced erratic swimming patterns were assigned a score of 1.

Nasal Ciliotoxicity Study

The safety of the intranasal RES thermosensitive in situ gel was evaluated through histopathological examination of nasal mucosal tissues. Six rats were randomly divided into two groups, each containing three animals. The control group received 10 µL of normal saline intranasally, whereas the test group received 10 µL of the RES-loaded thermosensitive in situ gel formulation once daily for one week.
Twenty-four hours after the final administration, the animals were euthanized using an overdose of phenobarbital sodium. Nasal mucosal tissues were carefully exercised and fixed in 10% w/v formalin. The tissues were subsequently processed, sectioned, and stained with haematoxylin and eosin (H&E). Histopathological evaluation was performed under a light microscope to identify any signs of epithelial damage, inflammation, or structural abnormalities [42].

Histopathological Examination of Brain Tissue

At the completion of the behavioural studies, rats were euthanized, and their brains were carefully removed 24 h after the final behavioural assessment. The excised tissues were washed with isotonic saline to remove blood and immediately fixed in 10% neutral-buffered formalin.
Following fixation, the tissues were rinsed with water and dehydrated through a graded series of alcohol solutions. The samples were then embedded in paraffin wax, and sections of approximately 4 µm thickness were prepared using a microtome. The sections were mounted onto glass slides, deparaffinized, and stained with haematoxylin and eosin. Finally, the stained sections were examined under a light microscope to evaluate histopathological alterations and assess the neuroprotective effects of the treatment.

MTT Cytotoxicity Assay

The cytotoxicity of the Resveratrol (RES) solution prepared in dimethyl sulfoxide (DMSO) and the developed gel formulation was evaluated using the MTT assay on Neuro2a cells obtained from the National Centre for Cell Science (NCCS), Pune, India [43].
Neuro2a cells were cultured in Ham's F10 medium supplemented with 10% foetal bovine serum (FBS; Invitrogen, India) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were seeded into 96-well plates at a density of 5×10^4 cells per well in a total volume of 200 µL and allowed to attach overnight.
The cells were then exposed to different dilutions (1:10, 1:50, and 1:100) of the RES solution and gel formulations, both in the presence and absence of citric acid, and incubated for 12 h. Following treatment, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were further incubated for 4 h to allow the formation of formazan crystals by viable cells.
After incubation, 180 µL of the culture medium was carefully removed from each well and replaced with an equal volume of DMSO to dissolve the formed formazan crystals. The plates were gently shaken and incubated for 15 min to ensure complete dissolution. The absorbance of each well was measured at 490 nm using a microplate reader, and cell viability was calculated relative to untreated control cells.

Statistical Analysis

Optimization of the formulation variables was carried out using the 2^3 factorial design module of Design-Expert® software (Version 13, State-Ease Inc., USA). Statistical comparisons between experimental groups were performed using appropriate post hoc tests in GraphPad Prism® Version 8.0.
A p-value of less than 0.05 was considered statistically significant. All experimental data are presented as mean ± standard deviation (SD), and the error bars shown in graphical representations correspond to the standard deviation of the measurements.

Results

Determination of Maximum Absorption Wavelength of RES

The UV absorption spectrum of Resveratrol was recorded in methanol to determine its maximum absorption wavelength (λmax). Spectral scanning revealed a distinct absorption peak at 305 nm, which was selected for all subsequent UV spectrophotometric analyses (Figure 3).

Figure 3. Maximum absorbance wavelength of RES.

Calibration Curve of Resveratrol

A standard calibration curve for Resveratrol was constructed over a concentration range of 1–10 µg/mL. The calibration plot demonstrated excellent linearity within the studied range, with a correlation coefficient (R^2) of 0.9991. The regression equation obtained was:
$y = 0.1004x$ where y represents absorbance and x represents concentration (µg/mL) (Figure 4).

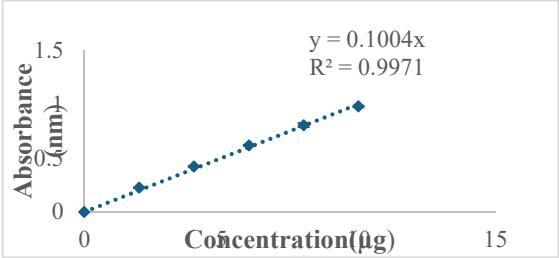

Figure 4. Standard calibration curve of RES.

Pre formulation Studies and Design of Experiments (DoE)

The formulation development of the Resveratrol (RES)-loaded thermosensitive in situ nasal gel was optimized using a 2^3 full factorial design. This experimental design was employed to systematically evaluate the influence of three formulation variables on the critical characteristics of the gel system. The independent variables selected for the study were the concentrations of Poloxamer 407 (P407, X_1), Poloxamer 188 (P188, X_2), and Hydroxypropyl Methylcellulose E15 (HPMC E15, X_3).

The effects of these formulation components were investigated on three key response variables: gelation temperature (Y_1), gelation time (Y_2), and pH (Y_3). These responses were chosen because they directly influence the performance, stability, and nasal applicability of the thermosensitive in situ gel. The experimental ranges selected for each independent variable are presented in Table 1.

Based on the factorial design, eleven different formulation batches were prepared with varying concentrations of the selected polymers. The formulations generated were subsequently evaluated to identify the optimal composition capable of providing suitable gelation characteristics under physiological nasal conditions.

For optimization, predefined target values were established for each response parameter. The desired gelation temperature was set within the range of 28–35 °C, with an optimum target of approximately 31 °C to ensure gel formation upon administration into the nasal cavity. The pH was maintained between 5.5 and 6.5, with an ideal value of 6.0 to ensure compatibility with the nasal mucosa while minimizing irritation. Gelation time was targeted at approximately 30 s, allowing a tolerance of ±10 s to achieve a balance between ease of administration and rapid gel formation, thereby reducing the possibility of formulation leakage from the nasal cavity.

The observed values of gelation temperature, gelation time, and pH for all developed RES in situ gel formulations are presented in Table 5.

Exp. no.	Gel temperature °C	Gel time sec	pH
1	34.5 ± 0.50	40.6 ± 1.15	5.3 ± 0.20
2	31.0 ± 1.00	26.0 ± 1.00	6.6 ± 0.05
3	34.1 ± 0.28	37.3 ± 1.52	5.2 ± 0.20
4	29.3 ± 0.28	24.0 ± 1.00	6.6 ± 0.15
5	32.3 ± 1.52	36.3 ± 0.57	5.9 ± 1.00
6	28.5 ± 0.50	21.6 ± 1.52	6.8 ± 0.05
7	33.5 ± 0.50	34.3 ± 0.57	5.4 ± 0.26
8	27.0 ± 1.00	19.0 ± 1.00	6.5 ± 0.00
9	30.1 ± 0.76	30.3 ± 0.57	5.8 ± 0.05
10	30.8 ± 0.76	30.6 ± 1.52	6.1 ± 1.00
11	32.0 ± 1.00	31.6 ± 1.52	6.0 ± 0.11

Table 5. Gelation temperature, gelation time, and pH of the prepared mucoadhesive *in situ* gel as per 2^3-factorial design. Data are mean ± SD ($n = 3$).

Equation 1 illustrates the effect of various independent variables on the gelation temperature (Y1).

Gelation temperature (Y1) =31.69 -2.56X1- 0.3125X2 - 0.5625X3. (1)

Effect of Formulation Variables on Gelation Temperature, Gelation Time, and pH

Statistical analysis of the factorial design revealed that the developed model was highly significant, as evidenced by an F-value of 382.93 ($p < 0.0001$). The coefficient of determination (R^2) was 0.9948, indicating excellent agreement between the experimentally observed and predicted values. Furthermore, the adjusted R^2 (0.9922) and predicted R^2 (0.9844) values were in close agreement, confirming the model's strong predictive capability. The adequate precision value, which measures the signal-to-noise ratio, was found to be 46.25, far exceeding the minimum desirable value of 4 and demonstrating an adequate signal for navigating the design space. In addition, the coefficient of variation (CV) was only 0.698%, indicating high precision and reproducibility of the experimental data.

Analysis of variance (ANOVA) demonstrated that the concentrations of Poloxamer 407 (X_1) and HPMC E15 (X_3) had a significant influence on the gelation temperature of the Resveratrol (RES) in situ gel formulations, whereas Poloxamer 188 (X_2) showed a comparatively smaller effect. The three-dimensional response surface plot (Figure 5) further

illustrated that increasing the concentrations of P407 and HPMC E15 within the studied range resulted in marked changes in the gelation behaviour of the formulations.

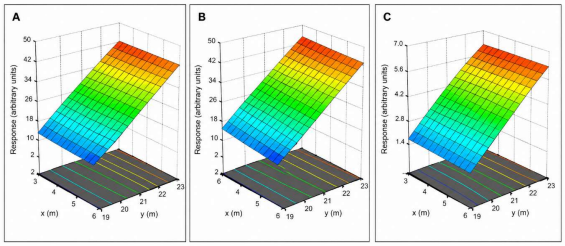

Figure 5. 3D Surface plots of the effect of P407 and P188 on gelation temperature, time, and pH.

The relationship between the independent variables and gelation time (Y_2) was represented by the following polynomial equation:

Gelation Time (Y2) = 30.12 - 6.62X1 - 1.12X2 - 1.87X3

The model developed for gelation time was also found to be statistically significant, with an F-value of 359.42 ($p < 0.0001$). The model exhibited excellent predictive performance, with an R^2 value of 0.9954. The adjusted R^2 and predicted R^2 values were 0.9917 and 0.9808, respectively, demonstrating strong agreement between predicted and experimental observations. The adequate precision value of 47.51 indicated a satisfactory signal-to-noise ratio, while the CV value of 1.99% confirmed the reliability and precision of the model.

Among the investigated variables, the concentration of Poloxamer 407 had the greatest influence on gelation time, exhibiting both linear and quadratic effects. An increase in P407 concentration resulted in a noticeable reduction in gelation time, leading to faster gel formation under simulated nasal conditions.

The effect of the formulation variables on pH (Y_3) was described by the following equation:

pH (Y3)= 6.30 + 0.5000X1

Optimization of the RES Thermosensitive In Situ Gel Formulation

The statistical analysis demonstrated that the developed model was highly significant, as indicated by an F-value of 103.23 ($p < 0.0001$). The coefficient of determination (R^2) was found to be 0.9281, indicating a strong correlation between the experimentally observed and model-predicted responses. Furthermore, the adjusted R^2 (0.9191) and predicted R^2 (0.8677) values were in reasonable agreement, confirming the model's ability to reliably predict formulation performance within the studied design space.

The adequacy of the model was further supported by an adequate precision value of 13.75, which is well above the minimum recommended value of 4, indicating an acceptable signal-to-noise ratio. In addition, the coefficient of variation (CV) was only 2.29%, demonstrating good precision, reproducibility, and reliability of the experimental data.

The polynomial model describing the pH response indicated a direct relationship between pH and the concentration of Poloxamer 407. As the concentration of Poloxamer 407 increased, a slight increase in formulation pH was observed. The robustness of the model was further confirmed through the predicted versus actual response plots shown in Figure 6. The close agreement between experimental and predicted values highlights the accuracy of the model and its suitability for optimization purposes. The high R^2 values obtained for the responses further demonstrate the excellent fit of the model to the experimental data.

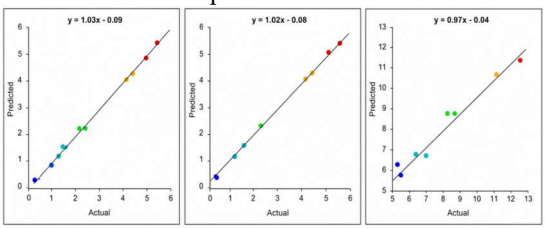

Figure 6. Predicted vs. actual plots of the gelation temperature, gelation time, and pH.

To better visualize the interaction between formulation variables and to identify the optimum formulation region, four-dimensional (4D) contour plots were generated (Figure 7). These plots provided a comprehensive representation of the effects of Poloxamer 407, Poloxamer 188, and HPMC E15 LV on the critical quality attributes of the Resveratrol (RES) thermosensitive in situ gel.

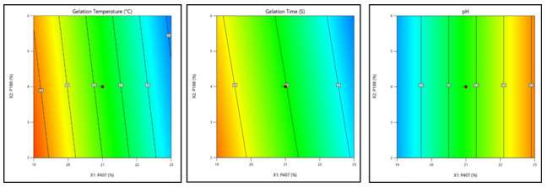

Figure 7. Responses 4D contour plots of gelation temperature, time, and pH.

The 4D contour plot for gelation temperature revealed that a Poloxamer 407 concentration of approximately 20.46% was required to achieve the desired gelation temperature of 32 ± 1 °C, which is considered ideal for intranasal administration. Variations in Poloxamer 188 concentration produced only minor changes in gelation temperature; however, P188 played an important role in fine-

tuning the response within the target range. Changes in HPMC E15 LV concentration showed a more pronounced interaction with the other formulation variables, particularly with Poloxamer 407. This interaction was consistent with the previously observed influence of HPMC concentration on gelation behaviour at different levels of P407.

Similarly, the contour plots generated for gelation time demonstrated that several combinations of the three formulation variables could achieve the target gelation time of approximately 30 s. Among these, formulations containing approximately 21% Poloxamer 407 and 0.8% HPMC E15 LV were found to provide gelation characteristics and pH values that closely matched the predefined optimization criteria. Overall, the Design of Experiments (DoE) approach successfully identified the optimal polymer concentrations required to produce a Resveratrol (RES)-loaded thermosensitive in situ nasal gel with desirable gelation temperature, gelation time, and pH suitable for intranasal drug delivery.

Optimization of the Mucoadhesive Gel

The interaction between formulation variables and their effects on the critical quality attributes of the gel was analyzed using Design-Expert® software. A quadratic polynomial model was generated to describe the relationship between the independent variables and the measured responses. The significance of the developed models was evaluated using analysis of variance (ANOVA), and the results are summarized in Table 6. The statistical significance of each independent variable is presented in Table 7.

Models	R^2	Adjusted R^2	Predicted R^2	SD	Adequate precision	% CV	Remarks
Gelation Temperature Y1 Quadratic	0.9948	0.9922	0.98844	0.2205	46.2505	0.698	Significant
Gelation time Y2 Quadratic	0.9945	0.9917	0.98808	0.60	47.514	1.99	Significant
pH Y3 Quadratic	0.9281	0.9191	0.86 77	0.139	13.756	2.29	Significant

Table 6. Regression analysis of different dependent variables.

Variables	P-value of gelation temperature	P-value of gelation time	P-value of pH
X1	<0.0001	<0.0001	<0.0001
X2	<0.0001	<0.0001	-
X3	0.0070	0.0018	-

Table 7. p-values of dependent factors.

Numerical optimization was performed using the point prediction function of Design-Expert® software to identify the optimal formulation composition. The target gelation temperature was set at approximately 31 °C to ensure gel formation under physiological nasal conditions. Similarly, the desired gelation time was fixed at 30 ± 10 s, while the target pH was maintained at 6.0 to ensure compatibility with the nasal mucosa.

Based on the optimization criteria, the software predicted that a formulation containing 21% w/v Poloxamer 407 (P407), 6% w/v Poloxamer 188 (P188), and 1% w/v HPMC E15 would provide the desired gel characteristics (Table 8). Experimental evaluation of the optimized formulation showed a gelation temperature of 30.8 ± 0.76 °C, gelation time of 30.6 ± 1.52 s, and pH of 6.1 ± 0.1. These values closely matched the predicted responses of 31.45 ± 0.22 °C, 28.78 ± 0.60 s, and 5.99 ± 0.13, respectively, confirming the reliability of the optimization model.

Formulation no.	Composition (X1:X2:X3)	Responses	Experimental value	Predicted value	% Prediction error
F10	21:4:0.8	Y_1 (°C)	30.8 ± 0.76	31.45 ± 0.22	0.21
		Y_2 (sec)	30.6 ± 1.52	28.78 ± 0.60	0.59
		Y_3	6.1 ± 1.00	5.99 ± 0.13	0.18

Table 8. Composition of the optimized formulation

The overall desirability value obtained for the optimized formulation was 0.860. Since desirability

values closer to 1 indicate a higher degree of agreement between predicted and experimental responses, the obtained value demonstrated excellent optimization performance and confirmed the suitability of the selected formulation.

Appearance and Drug Content

All developed intranasal in situ gel formulations (F12–F21) were clear, transparent, and free from visible particulate matter. The drug content of the formulations ranged from 80 ± 0.05% to 85 ± 0.11%, indicating uniform distribution of Resveratrol (RES) throughout the gel matrix.

Gelation Temperature

The gelation temperatures of the developed formulations ranged from 28.27 ± 0.25 °C to 35.0 ± 0.10 °C (Figure 8A). These values fall within the desirable range for thermosensitive nasal gels, ensuring rapid gel formation upon administration into the nasal cavity.

Gelation Time

The gelation times of all formulations were found to be within the acceptable range of 17.33 ± 0.57 s to 34.0 ± 1.0 s (Figure 8B). The observed gelation times suggest that the formulations can undergo rapid sol-to-gel transition, thereby minimize nasal drainage and enhance residence time.

Gel Strength

The developed formulations exhibited gel strengths ranging from 34 ± 1.0 s to 55 ± 1.0 s (Figure 8C), indicating adequate mechanical strength for intranasal applications. Furthermore, the gel strengths of RES-loaded formulations (F12, F14, and F16) were comparable to those of their corresponding drug-free formulations (F13, F15, and F17), suggesting that drug incorporation did not significantly affect gel integrity.

Mucoadhesive Strength

The mucoadhesive strength of the formulations varied between 4211 ± 4.04 and 6945 ± 3.51 dyne/cm² (Figure 8D). Among the tested formulations, F21 exhibited the highest mucoadhesive strength, significantly exceeding that of F18, which contained only Poloxamer 407. These findings demonstrate the beneficial role of combining Poloxamer 188 and HPMC E15 in enhancing the adhesive properties of the gel and promoting prolonged retention within the nasal cavity.

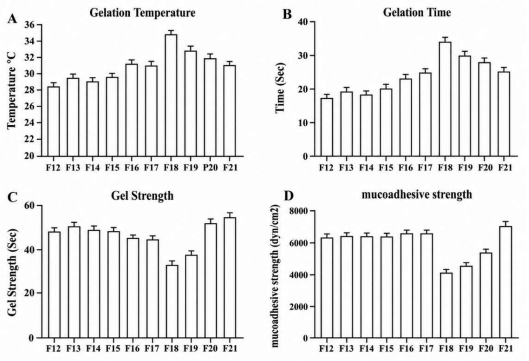

Figure 8. Gelation temperature, time, strength, and mucoadhesive strength of various thermosensitive mucoadhesive gel formulations. Data are mean ± SD ($n = 3$).

Mucoadhesive Time

The in vitro mucoadhesive residence time of the RES-loaded formulations ranged from 3.10 ± 1.15 h to 7.22 ± 0.57 h. Formulation F16, which contained the highest concentration of citric acid, exhibited the longest residence time of approximately 7 h and showed the greatest mucoadhesive strength. The prolonged residence time is expected to improve drug retention and absorption across the nasal mucosa.

Rheological Behaviour

The rheological characteristics of the formulations are illustrated in Figure 9A. All formulations exhibited pseudoplastic or shear-thinning behaviour, characterized by a decrease in viscosity with increasing shear rate. This property is advantageous for nasal administration, as it facilitates easy spraying or application while allowing the formulation to regain viscosity after administration.

The effect of temperature on viscosity is shown in Figure 9B. A marked increase in viscosity was observed with increasing temperature, confirming the thermosensitive nature of the formulations. Control formulations (F18–F20), containing a single polymer, showed comparatively smaller viscosity changes, highlighting the synergistic effect of polymer combinations on thermos gelation behaviour.

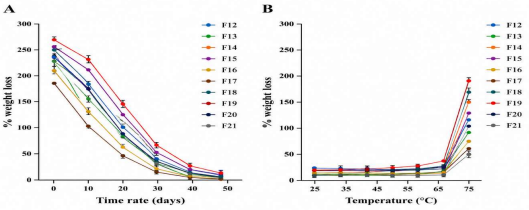

Figure 9. Rheological profile of thermosensitive *in situ* gel formulations. Data are mean ± SD ($n = 3$).

Texture Analysis

The mucoadhesive performance of the formulations was further evaluated using texture analysis by

measuring maximum detachment force, work of adhesion, and mucoadhesion energy (Table 9).

Formulations	(Mean ± SD) Mucoadhesion		
	Maximum detachment force (N)	Mucoadhesion (mJ)	Work of adhesion (mJ/cm²)
F12	0.32 ± 0.048	1.51 ± 0.076	1.91 ± 0.067
F13	0.33 ± 0.048	1.51 ± 0.078	1.93 ± 0.059
F14	0.33 ± 0.041	1.50 ± 0.069	1.92 ± 0.057
F15	0.34 ± 0.037	1.51 ± 0.070	1.93 ± 0.059
F16	0.33 ± 0.038	1.51 ± 0.075	1.93 ± 0.069
F17	0.34 ± 0.041	1.51 ± 0.062	1.94 ± 0.067
F18	0.03 ± 0.004	0.16 ± 0.042	0.19 ± 0.010
F19	0.04 ± 0.006	0.18 ± 0.050	0.21 ± 0.017
F20	0.27 ± 0.032	1.31 ± 0.073	1.52 ± 0.041
F21	0.35 ± 0.040	1.52 ± 0.073	1.97 ± 0.061

Table 9. Mucoadhesion characteristics of (F12-F21).

Formulations F12–F17 and F20–F21 exhibited strong adhesive properties, with maximum detachment forces ranging from 0.32 ± 0.048 N to 0.35 ± 0.040 N. Similarly, the mucoadhesion values ranged between 1.50 ± 0.069 and 1.52 ± 0.073 mJ, indicating strong interactions with the mucosal surface.

In contrast, formulations F18 and F19, which lacked HPMC E15, displayed significantly lower detachment forces (0.03 ± 0.004 N and 0.04 ± 0.006 N, respectively) and mucoadhesion values (0.16 ± 0.042 mJ and 0.18 ± 0.050 mJ, respectively), suggesting poor adhesive performance.

The work of adhesion followed a similar trend. Formulations F12–F17 and F20–F21 exhibited values ranging from 1.52 ± 0.041 to 1.97 ± 0.061 mJ/cm², whereas F18 and F19 showed markedly lower values of 0.19 ± 0.010 and 0.21 ± 0.017 mJ/cm², respectively. These findings confirm the crucial role of HPMC E15 in enhancing the mucoadhesive characteristics of gel formulations.

Spreadability

The spreadability of the developed RES in situ gels ranged from 11.12 ± 1.0 to 23.16 ± 0.05 cm²/min. The observed values indicate that the formulations possessed adequate spreading characteristics, facilitating uniform distribution across the nasal mucosal surface.

In Vitro Drug Release

The influence of citric acid on the release profile of Resveratrol (RES) from formulations F12, F14, and F16 was investigated and compared with an RES suspension. The presence of citric acid significantly enhanced drug release, increasing the cumulative release from 29.38 ± 1.2% to 39.59 ± 1.6% over a period of 12 h (Figure 10). This enhancement can be attributed to the solubilizing effect of citric acid, which improved the dissolution and diffusion of RES from the gel matrix.

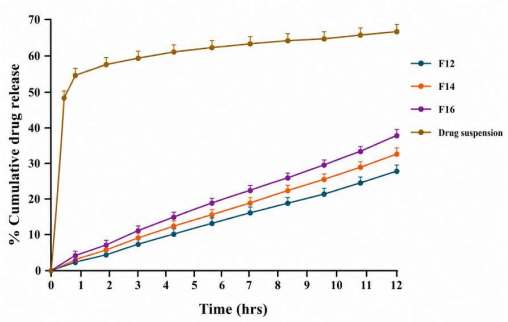

Figure 10. Drug release of RES from the gel (F12, F14, and F16) and RES sus-pension.
Data expressed as mean ± SD (n = 3).

All three formulations demonstrated a controlled and sustained release pattern over 12 h compared with the RES suspension, indicating their suitability for prolonged drug delivery. Based on the combined evaluation of gelation behaviour, mucoadhesive strength, residence time, rheological properties, and drug release performance, formulation F16 was identified as the most promising formulation and was selected for further in vivo studies.

Pharmacokinetic Studies

Pharmacokinetic evaluation demonstrated that intranasal administration of the optimized Resveratrol (RES) thermosensitive in situ gel at a dose of 10 mg/kg resulted in significantly enhanced brain delivery compared with oral administration. The intranasal formulation achieved a maximum brain concentration (Cmax) of 421.29 ng/mL, which was substantially higher than the 189.72 ng/mL observed following oral administration of the conventional RES formulation (Table 10 and Figure 11).

Parameters	Plasma		Brain	
	RES Oral	RES in-	RES Oral	RES in-

		suit gel		suit gel
C_{max}(ng/ml)	474.20 ± 18.37	284.44 ± 17.84	189.72 ± 17.23	421.29 ± 13.12
T_{max} (min)	60	60	120	30
$t_{1/2}$ (min)	174.93 ± 0.29	163.23 ± 0.10	203.87 ± 0.05	170.21 ± 0.04
Ke (min^{-1})	0.0039	0.0041	0.0034	0.0043
AUC_{0-t} (ng.min/ml)	65748.2 ± 4325.12	39520.9 ± 2432.04	32379 ± 1872.14	54821.3 ± 4643.21
DTI	—	—	—	1.31
F_{rel}	—	0.59	—	0.79

Table 10. Pharmacokinetic parameters of RES in rat plasma and brain. (values are expressed as mean ± SD, *n* = 3).

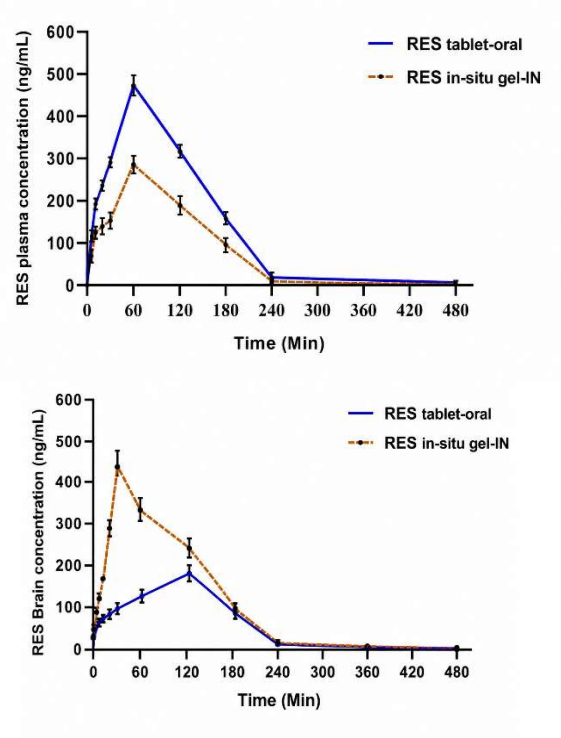

Figure 11. Compartmental distribution of marketed formulations and RES-based in-situ gel at different time intervals in plasma and brain concentration v/s time in Wistar rats. Results are expressed as mean ± SD.

The optimized intranasal gel exhibited approximately two-fold greater brain bioavailability than the orally administered formulation, highlighting the effectiveness of the nose-to-brain delivery pathway. Although the oral formulation produced a higher plasma concentration (Cmax = 474.20 ng/mL) than the intranasal gel (Cmax = 284.44 ng/mL), the intranasal formulation demonstrated more rapid brain uptake.

Furthermore, the time required to reach peak concentration in brain tissue (Tmax) was significantly shorter for the intranasal gel, occurring at 30 min compared with approximately 2 h following oral administration (Figure 11). These findings suggest that the developed RES thermosensitive in situ gel efficiently bypasses systemic circulation and facilitates direct transport of the drug to the brain, thereby improving both the rate and extent of brain targeting.

Pharmacodynamic Studies

Y-Maze Test

The Y-maze test was performed to assess spatial working memory and cognitive performance. The Alzheimer's disease (AD) group exhibited a significant decline in spontaneous alternation behaviour, with an approximately 25% reduction in the percentage of continuous alternations compared with the control group ($p < 0.001$) (Figure 12A). This reduction indicates impairment of working memory and cognitive function following AD induction.

Treatment with orally administered Resveratrol (RES) produced a noticeable improvement in cognitive performance, resulting in an approximate 5% increase in spontaneous alternation behaviour compared with the untreated AD group. A more pronounced improvement was observed in animals treated with the intranasal RES gel formulation, indicating superior restoration of working memory and enhanced neuroprotective activity.

Morris Water Maze Test

The Morris water maze test was conducted to evaluate spatial learning and memory retention. Rats in the AD group showed a two-fold increase in escape latency compared with the control group, reflecting substantial impairment in spatial memory (Figure 12B).

Administration of oral RES significantly reduced escape latency by approximately 40% ($p < 0.001$) compared with the untreated AD group, demonstrating partial recovery of cognitive function. However, treatment with the intranasal RES gel resulted in an even greater improvement, reducing escape latency by approximately 44% relative to the AD group and indicating enhanced restoration of learning and memory abilities.

Further analysis of memory retention revealed a marked reduction in the time spent within the target quadrant during the probe trial. The AD group exhibited an approximately 70% decrease in residence time compared with healthy controls ($p < 0.001$) (Figure 12C). In contrast, administration of

the intranasal RES gel significantly increased residence time, reaching nearly twice that observed in the untreated AD group. This improvement demonstrates enhanced memory retention and cognitive performance following treatment.

Conditioned Avoidance Test

The conditioned avoidance (CA) test was used to assess learning and short-term memory. On the first day of evaluation, the AD group displayed a substantial decline in memory performance, requiring nearly three times more trials to achieve successful avoidance behaviour compared with the control group (Figure 12D).

Treatment with oral RES improved learning ability, resulting in a significant reduction of approximately 15% ($p < 0.001$) in the number of trials required when compared with the untreated AD group. Notably, administration of the intranasal RES gel produced an even greater improvement, indicating enhanced learning capacity and more effective restoration of memory function. These findings suggest that intranasal delivery of RES provides superior cognitive benefits compared with conventional oral administration.

Swimming Test

The swimming test was performed to evaluate motor coordination and behavioural performance in the experimental animals. The Alzheimer's disease (AD) group exhibited a significant decline in swimming performance, showing approximately a 60% reduction in swimming score compared with the control group ($p < 0.001$) (Figure 12E). This reduction reflects impaired motor function and behavioural deficits associated with AD progression. Treatment with oral Resveratrol (RES) resulted in a marked improvement in swimming performance, with the swimming score increasing approximately two-fold compared with the untreated AD group. An even greater improvement was observed following administration of the RES-loaded intranasal in situ gel, which produced nearly a three-fold increase in swimming score ($p < 0.001$). These findings suggest that intranasal delivery of RES effectively improves motor coordination and behavioural deficits associated with Alzheimer's disease.

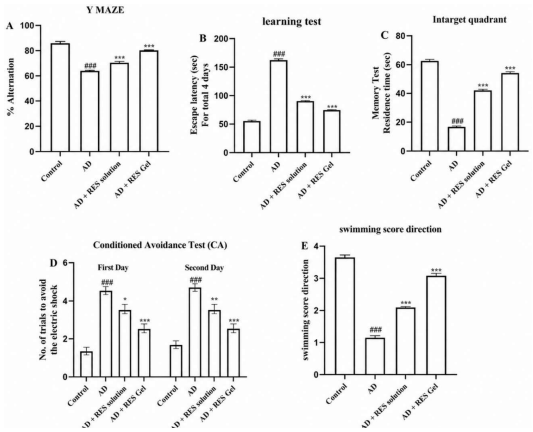

Figure 12. Impact of RES solution and RES gel on AD model. (A) Y-maze tests. (B) Morris water maze test. (C) Variations in residence time in the target quadrant of Morris water maze. (D) Conditioned avoidance test. (E) Swimming score direction. Results are presented as mean ± SEM ($n = 6$). Significance at $p < 0.05$. '###' denotes a significant difference from the control, and '***' denotes a significant difference from the AD group.

Nasal Ciliotoxicity Study

The safety of the optimized RES thermosensitive in situ gel was assessed through histopathological examination of rat nasal mucosal tissues following repeated intranasal administration. Representative histological images of the nasal mucosa from the saline-treated control group and the RES gel-treated group are presented in Figures 13A and 13B, respectively.

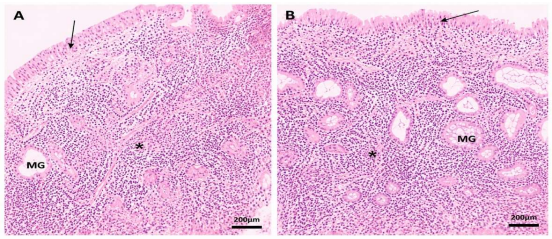

Figure 13. Histopathology of the nasal mucosa after treatment with normal saline solution (A), and RES *in situ* gel intranasal (B).

Microscopic examination revealed normal nasal mucosal architecture in both groups. The mucosal lining consisted of intact columnar epithelial cells with pink-stained cytoplasm and basally located blue-stained nuclei, along with numerous goblet cells. Importantly, no signs of ciliary damage, epithelial disruption, tissue atrophy, inflammatory infiltration, or degenerative changes were observed following the RES-loaded in situ gel. These findings demonstrate the biocompatibility and safety of the developed formulation for intranasal application.

Histopathological Changes in Brain Tissue

Histopathological evaluation revealed distinct differences between brain tissues obtained from healthy animals and those from the Alzheimer's disease group (Figure 14).

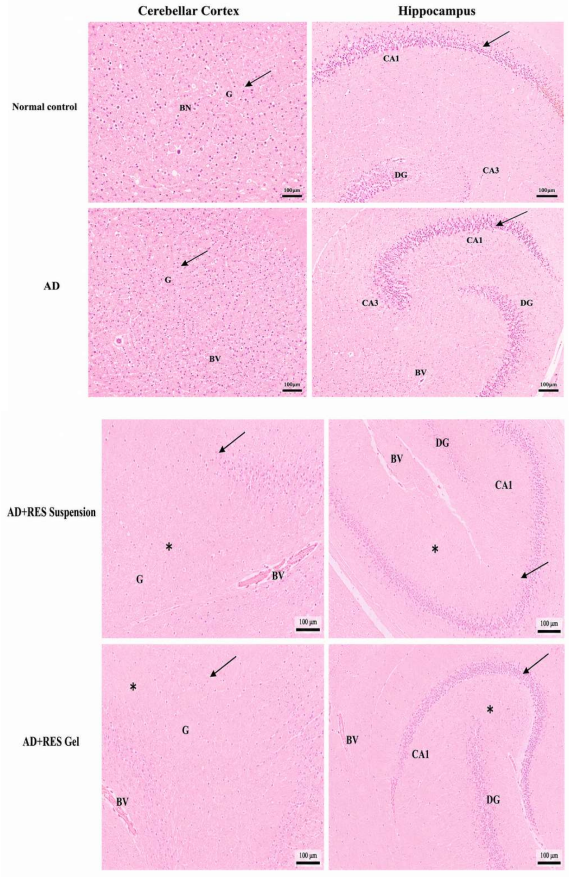

Figure 14. Histopathology of the frontal cerebellar cortex and hippocampus stained with haematoxylin and eosin. Blood vessels (BV), granular cells (G), cornu ammonis (CA), dentate gyrus (DG), and pyramidal (P).

In healthy rats, the frontal cerebellar cortex exhibited normal histological architecture, characterized by well-organized cortical neurons within the molecular layer, prominent basophilic nuclei, and normal vascular structures. In contrast, brain tissues from AD-induced animals showed severe pathological alterations, including neuronal degeneration, nuclear condensation, and disruption of normal tissue organization.

Treatment with oral RES solution and intranasal RES in situ gel resulted in substantial improvement in brain histology. Both treatment groups demonstrated restoration of normal cortical architecture, with cortical neurons exhibiting clearly defined basophilic nuclei and vascular structures resembling those observed in healthy animals. The granular layer contained abundant granular cells distributed within an eosinophilic neuropil matrix composed of neuronal and glial cell processes.

Examination of the hippocampal regions (CA1, CA2, and CA3) of healthy rats revealed the normal arrangement of pyramidal, polymorphic, and molecular layers. In contrast, AD-induced animals exhibited extensive pathological changes, including interstitial haemorrhage, inflammatory cell infiltration, neuronal degeneration, and vacuolation within the molecular layer and dentate gyrus.

Notably, treatment with both oral RES solution and intranasal RES gel markedly reduced these pathological changes. The hippocampal architecture in treated animals closely resembled that of healthy controls, suggesting significant neuroprotective effects. These findings indicate that RES treatment may help preserve neuronal integrity, reduce neuroinflammation, and protect against Alzheimer's disease-associated neurodegeneration.

Cytotoxicity Study

The cytotoxicity of the RES solution and the optimized formulation (F16) with and without citric acid was evaluated using the MTT assay on Neuro2a cells. The results are presented in Figure 15.

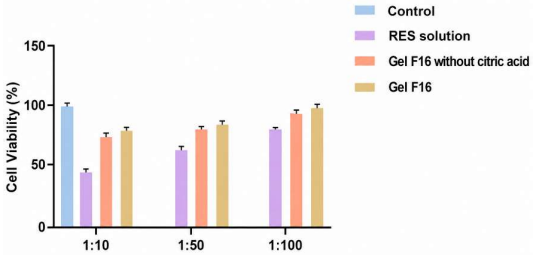

Figure 15. Neuro2a cell viability after 12 h exposure to RES solution and F16 with and without citric acid at different dilutions (1:10, 1:50, and 1:100).

Among the tested samples, the F16 formulation demonstrated superior cellular compatibility. Approximately 70% cell viability was maintained even at the highest tested concentration (1:100 dilution), indicating relatively low cytotoxicity. In contrast, the RES solution exhibited substantially lower cell viability, with values falling below 50% at comparable concentrations.

These findings suggest that incorporation of RES into the thermosensitive in situ gel matrix improves cellular compatibility and reduces cytotoxic effects, thereby supporting the safety of the optimized formulation for intranasal administration and potential nose-to-brain drug delivery applications.

Discussion

The 2^3 factorial design proved to be an effective and systematic approach for optimizing the formulation variables of the Resveratrol (RES)-loaded thermosensitive in situ nasal gel. This design enabled

the evaluation of both the individual and interactive effects of the selected formulation factors while minimizing the number of experimental runs. Such an approach is particularly valuable in identifying the optimal formulation conditions required to achieve the desired physicochemical and performance characteristics.

For successful intranasal administration, the formulation must exhibit an appropriate gelation temperature, gelation time, and physiological pH. The gelation temperature should be close to the temperature of the nasal cavity (approximately 32–34 °C) to ensure rapid gel formation following administration while maintaining patient comfort and minimizing mucosal irritation. Similarly, the gelation time should be sufficiently short to prevent drainage from the nasal cavity but long enough to allow convenient administration. Maintaining the pH within the physiological nasal range (5.5–6.5) is also essential to ensure compatibility with the nasal mucosa and avoid irritation.

Among the formulation variables studied, Poloxamer 407 (P407) was identified as the primary factor influencing gelation behaviour. Increasing the concentration of P407 resulted in a reduction in gelation temperature and gelation time, which can be attributed to enhanced micelle formation and stronger intermolecular interactions within the polymer network. These findings are consistent with previous reports describing the thermoresponsive properties of poloxamer-based systems. Poloxamer 188 (P188) acted as a secondary modifier, allowing fine adjustment of gelation characteristics, whereas HPMC E15 contributed significantly to viscosity enhancement and mucoadhesive performance.

Citric acid played an important role in improving the solubility and release behaviour of Resveratrol, a compound known for its poor aqueous solubility and limited bioavailability. The incorporation of citric acid enhanced drug dissolution within the gel matrix, thereby facilitating improved release of RES. A slight increase in gelation temperature and gelation time was observed following citric acid incorporation, likely due to interactions between citric acid and the poloxamer micellar system, which may delay the formation of the three-dimensional gel network.

All developed formulations were clear, transparent, and free from visible particulate matter, indicating successful formulation development. The gelation temperatures obtained were suitable for nasal administration, while the gel strengths remained within the desirable range required to maintain structural integrity without causing discomfort to the nasal mucosa. Importantly, incorporation of RES did not significantly alter the mechanical properties of the gel system.

Mucoadhesion is a critical characteristic for intranasal drug delivery because it prolongs residence time within the nasal cavity and improves drug absorption. The optimized formulations exhibited strong mucoadhesive properties, primarily due to the presence of HPMC E15. The observed mucoadhesive strengths were consistent with previously reported nasal gel systems and are expected to contribute to prolonged drug retention and enhanced nose-to-brain transport.

Rheological analysis demonstrated that all formulations exhibited pseudoplastic (shear-thinning) behaviour. This characteristic is highly desirable for intranasal administration because it facilitates easy spraying or administration under shear conditions while allowing the formulation to regain viscosity and form a stable gel after application. The thermosensitive increase in viscosity observed with increasing temperature further confirmed the suitability of the developed formulation for intranasal delivery.

Texture analysis further confirmed the strong mucoadhesive performance of the optimized formulations. Formulations containing HPMC E15 showed significantly greater detachment force, work of adhesion, and overall mucoadhesive strength compared with formulations lacking the polymer. These findings highlight the importance of polymer selection in achieving prolonged nasal residence and improved therapeutic efficacy.

The in vitro release studies demonstrated sustained release of Resveratrol over a period of 12 h. The inclusion of citric acid significantly enhanced RES release, likely through improved drug solubilization. The optimized formulation provided controlled drug release while maintaining desirable gelation and mucoadhesive properties, making it a promising platform for prolonged intranasal drug delivery.

Pharmacokinetic studies demonstrated that intranasal administration of the optimized RES gel achieved significantly greater brain delivery than oral administration. The higher brain concentration and shorter Tmax observed following intranasal administration suggest efficient nose-to-brain transport through olfactory and trigeminal pathways. Additionally, the increased brain bioavailability observed with the intranasal formulation may be attributed to avoidance of first-pass metabolism and prolonged residence time within the nasal cavity.

Behavioural assessments further supported the therapeutic potential of the developed formulation. Alzheimer's disease-induced animals exhibited significant impairments in learning, memory, and motor function, as demonstrated by the Y-maze,

Morris water maze, conditioned avoidance, and swimming tests. Treatment with the intranasal RSV gel significantly improved cognitive performance compared with both untreated and orally treated groups. These findings suggest enhanced brain targeting and neuroprotective effects of RES following intranasal administration.

The nasal ciliotoxicity study confirmed the safety of the optimized formulation. Histopathological examination of the nasal mucosa revealed no evidence of epithelial damage, inflammation, ciliary disruption, or tissue degeneration following repeated intranasal administration. Similarly, histopathological examination of brain tissue demonstrated substantial preservation of neuronal architecture and reduced pathological alterations in RES-treated groups compared with the Alzheimer's disease group.

The MTT cytotoxicity assay further demonstrated the safety profile of the optimized formulation. The RES-loaded gel maintained significantly higher cell viability compared with RSV solution alone, indicating that incorporation of the drug into the gel matrix reduced its cytotoxic effects and improved cellular compatibility.

Conclusion

The present study successfully developed and optimized a thermosensitive mucoadhesive in situ gel system for intranasal delivery of Resveratrol (RES). The optimized formulation, composed of Poloxamer 407, Poloxamer 188, HPMC E15, and citric acid, exhibited desirable gelation temperature, rapid gelation, strong mucoadhesive properties, suitable rheological behaviour, and sustained drug release characteristics.

The formulation demonstrated enhanced brain delivery following intranasal administration, resulting in improved pharmacokinetic performance compared with conventional oral administration. Furthermore, behavioural studies in an Alzheimer's disease model confirmed significant improvements in learning, memory, and motor performance following treatment with the optimized RES gel.

Safety evaluations, including nasal ciliotoxicity, histopathology, and cytotoxicity studies, confirmed the biocompatibility of the developed formulation. Overall, the optimized intranasal RES thermosensitive in situ gel represents a promising strategy for nose-to-brain delivery and may offer an effective therapeutic approach for the management of Alzheimer's disease and other neurodegenerative disorders.

Future investigations should focus on long-term safety studies, dose optimization, pharmacodynamic evaluation, and clinical translation to further establish the therapeutic potential of this intranasal delivery system.

References

1 Murphy MP, LeVine H. Alzheimer's disease and the amyloid-β peptide. JAD. 2010;19(1):311–323. doi: 10.3233/ JAD-2010-1221.

2 Long JM, Holtzman DM. Alzheimer disease: an update on pathobiology and treatment strategies. Cell. 2019;179(2):312–339. doi: 10.1016/j.cell.2019.09.001.

3 Aderibigbe B. In situ-based gels for nose to brain delivery for the treatment of neurological diseases. Pharmaceutics. 2018;10(2):40. doi: 10.3390/pharmaceutics10020040.

4 Agrawal M, Saraf S, Saraf S, et al. Nose-to-brain drug deliv-ery: an update on clinical challenges and progress towards approval of anti-Alzheimer drugs. J Control Release. 2018;281:139–177. doi: 10.1016/j.jconrel.2018.05.011.

5 de la Torre C, Ceña V. The delivery challenge in neurode-generative disorders: the nanoparticles role in Alzheimer's disease therapeutics and diagnostics. Pharmaceutics. 2018;10(4):190. doi: 10.3390/pharmaceutics10040190.

6 Hasa D, Voinovich D, Perissutti B, et al. Multidisciplinary approach on characterizing a mechanochemically activated composite of vinpocetine and crospovidone. J Pharm Sci. 2011;100(3):915–932. doi: 10.1002/jps.22331.

7 Bönöczk P, Panczel G, Nagy Z. Vinpocetine increases cere-bral blood flow and oxygenation in stroke patients: a near infrared spectroscopy and transcranial Doppler study. Eur J Ultrasound. 2002;15(1–2):85–91. doi: 10.1016/S0929-8266 (02)00006-X.

8 Deshmukh R, Sharma V, Mehan S, et al. Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine—a PDE1 inhibitor. Eur J Pharmacol. 2009;620(1–3):49–56. doi: 10.1016/j.ejphar.2009.08.027.

9 El-Laithy HM, Shoukry O, Mahran LG. Novel sugar esters proniosomes for transdermal delivery of vinpocetine: pre-clinical and clinical studies. Eur J Pharm Biopharm. 2011;77(1):43–55. doi: 10.1016/j.ejpb.2010.10.011.

10 Bereczki D, Fekete I. Vinpocetine for acute ischaemic stroke. Cochrane Database Syst Rev.

2008;2008(1):CD000480. doi: 10.1002/14651858.CD000480.pub2.

11 Hard SAAA, Shivakumar HN, Redhwan MAM. Development and optimization of in-situ gel containing chitosan nanoparticles for possible nose-to-brain delivery of vinpocetine. Int J Biol Macromol. 2023;253(Pt 6):127217. doi: 10.1016/j.ijbiomac.2023.127217.

12 Keller LA, Merkel O, Popp A. Intranasal drug delivery: opportunities and toxicologic challenges during drug development. Drug Deliv Transl Res. 2022;12(4):735–757. doi: 10.1007/s13346-020-00891-5.

13 Hanson LR, Frey WH. Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the cen-tral nervous system and treat neurodegenerative disease. BMC Neurosci. 2008;9(S3):1–4. doi: 10.1186/1471-2202-9-S3-S5.

14 Kaur P, Garg T, Rath G, et al. In situ nasal gel drug delivery: a novel approach for brain targeting through the mucosal membrane. Artif Cells Nanomed Biotechnol. 2016;44(4):1167–1176. doi: 10.3109/21691401.2015.1012260.

15 Brunner J, Ragupathy S, Borchard G. Target specific tight junction modulators. Adv Drug Deliv Rev. 2021;171:266–288. doi: 10.1016/j.addr.2021.02.008.

16 Rohrer J, Lupo N, Bernkop-Schnürch A. Advanced formu-lations for intranasal delivery of biologics. Int J Pharm. 2018;553(1–2):8–20. doi: 10.1016/j.ijpharm.2018.10.029.

17 Nguyen T-T-L, Maeng H-J. Pharmacokinetics and pharmacody-namics of intranasal solid lipid nanoparticles and nanostruc-tured lipid carriers for nose-to-brain delivery. Pharmaceutics. 2022;14(3):572. doi: 10.3390/pharmaceutics14030572.

18 Jacob S, Nair AB, Shah J, et al. Emerging role of hydrogels in drug delivery systems, tissue engineering and wound management. Pharmaceutics. 2021;13(3):357. doi: 10.3390/pharmaceutics13030357.

19 Ci L, Huang Z, Liu Y, et al. Amino-functionalized poloxamer 407 with both mucoadhesive and thermosensitive proper-ties: preparation, characterization and application in a vag-inal drug delivery system. Acta Pharm Sin B. 2017;7(5):593–602. doi: 10.1016/j.apsb.2017.03.002.

20 Schmolka IR. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. J Biomed Mater Res. 1972;6(6):571–582. doi: 10.1002/jbm.820060609.

21 Kabanov AV, Batrakova EV, Alakhov VY. Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. J Control Release. 2002;82(2–3):189–212. doi: 10.1016/S0168-3659(02)00009-3.

22 Dumortier G, Grossiord JL, Agnely F, et al. A review of poloxamer 407 pharmaceutical and pharmacological char-acteristics. Pharm Res. 2006;23(12):2709–2728. doi: 10.1007/s11095-006-9104-4.

23 Tavade S, Patil K, Kurangi B, et al. Development and validation of UV-spectrophotometric method for estimation of berberine hydrochloride in marketed formulation and poly lactic co-glycolic acid nanoparticles. IJPER. 2022;56(3):873–880. doi: 10.5530/ijper.56.3.140.

24 Nair AB, Shah J, Jacob S, et al. Experimental design, for-mulation and in vivo evaluation of a novel topical in situ gel system to treat ocular infections. PLoS One. 2021;16(3):e0248857. doi: 10.1371/journal.pone.0248857.

25 Makwana SB, Patel VA, Parmar SJ. Development and char-acterization of in-situ gel for ophthalmic formulation con-taining ciprofloxacin hydrochloride. Results Pharma Sci. 2016;6:1–6. doi: 10.1016/j.rinphs.2015.06.001.

26 Mura P, Mennini N, Nativi C, et al. In situ mucoadhesive-thermosensitive liposomal gel as a novel vehicle for nasal extended delivery of opiorphin. Eur J Pharm Biopharm. 2018;122:54–61. doi: 10.1016/j.ejpb.2017.10.008.

27 Altuntaş E, Yener G. Formulation and evaluation of ther-moreversible in situ nasal gels containing mometasone furoate for allergic rhinitis. AAPS PharmSciTech. 2017;18(7):2673–2682. doi: 10.1208/s12249-017-0747-8.

28 Yong CS, Choi JS, Quan Q-Z, et al. Effect of sodium chlo-ride on the gelation temperature, gel strength and bioad-hesive force of poloxamer gels containing diclofenac sodium. Int J Pharm. 2001;226(1–2):195–205. doi: 10.1016/ S0378-5173(01)00809-2.

29 Shelke S, Shahi S, Jadhav K, et al. Thermoreversible nanoethosomal gel for the intranasal delivery of Eletriptan hydrobromide. J Mater Sci Mater Med. 2016;27(6):103. doi: 10.1007/s10856-016-5713-6.

30 Khan S, Patil K, Bobade N, et al. Formulation of intranasal mucoadhesive temperature-mediated in situ gel containing ropinirole and evaluation of brain targeting efficiency in rats. J Drug Target. 2010;18(3):223–234. doi: 10.3109/ 10611860903386938.

31 Al-Kassas RS, El-Khatib MM. Ophthalmic controlled release in situ gelling systems for ciprofloxacin based on polymeric carriers. Drug Deliv. 2009;16(3):145–152. doi: 10.1080/ 10717540802689008.

32 Baloglu E, Karavana SY, Senyigit ZA, et al. In-situ gel formu-lations of econazole nitrate: preparation and in-vitro and in-vivo evaluation. J Pharm Pharmacol. 2011;63(10):1274–1282. doi: 10.1111/j.2042-7158.2011.01315.x.

33 Galgatte UC, Kumbhar AB, Chaudhari PD. Development of in situ gel for nasal delivery: design, optimization, in vitro and in vivo evaluation. Drug Deliv. 2014;21(1):62–73. doi: 10.3109/10717544.2013.849778.

34 Rukmangathen R, Yallamalli IM, Yalavarthi PR. Formulation and biopharmaceutical evaluation of risperidone-loaded chitosan nanoparticles for intranasal delivery. Drug Dev Ind Pharm. 2019;45(8):1342–1350. doi: 10.1080/03639045. 2019.1619759.

35 Aldawsari HM, Badr-Eldin SM, Assiri NY, et al. Surface-tailoring of emulsomes for boosting brain delivery of vinpocetine via intranasal route: *in vitro* optimization and *in vivo* pharmacokinetic assessment. Drug Deliv. 2022;29(1):2671–2684. doi: 10.1080/10717544.2022.2110996.

36 Abu-Elfotuh K, Tolba AMA, Hussein FH, et al. Anti-alzheimer activity of combinations of cocoa with vinpocetine or other nutraceuticals in rat model: modulation of Wnt3/β-catenin/ GSK-3β/Nrf2/HO-1 and PERK/CHOP/Bcl-2 pathways. Pharmaceutics. 2023;15(8):2063. doi: 10.3390/pharmaceu-tics15082063.

37 Wang M, Wang L, Sun J, et al. Simultaneous determination of vinpocetine and its major active metabolite apovin-caminic acid in rats by UPLC-MS/MS and its application to the brain tissue distribution study. J Chromatogr Sci. 2018;56(3):225–232. doi: 10.1093/chromsci/bmx104.

38 Hritcu L, Cioanca O, Hancianu M. Effects of lavender oil inhalation on improving scopolamine-induced spatial mem-ory impairment in laboratory rats. Phytomedicine. 2012;19(6):529–534. doi: 10.1016/j.phymed.2012.02.002.

39 Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods. 1984;11(1):47–60. doi: 10.1016/0165-0270(84)90007-4.

40 Gao J, Li M. Reinforcement attenuation as a behavioral tech-nique to suppress conditioned avoidance response in rats: a comparative study with olanzapine. J Psychopharmacol. 2019;33(1):86–100. doi: 10.1177/0269881118805497.

41 Ali AA, Khalil MG, Elariny HA, et al. Study on social isolation as a risk factor in development of Alzheimer's disease in rats. Brain Disord Ther. 2017;06(02):1–10. doi: 10.4172/2168-975X.1000230.

42 Youssef NAHA, Kassem AA, Farid RM, et al. A novel nasal almotriptan loaded solid lipid nanoparticles in mucoadhe-sive in situ gel formulation for brain targeting: preparation, characterization and in vivo evaluation. Int J Pharm. 2018;548(1):609–624. doi: 10.1016/j.ijpharm.2018.07.014.

43 Abdelmageed N, Twafik WAA, Seddek AL, et al. Vinpocetine-based therapy is an attractive strategy against oxidative stress-induced hepatotoxicity in vitro by target-ing nrf2/ho-1 pathway. EXCLI J. 2021;20:550–561. 10.17179/ excli2021-3463.

44 Sherafudeen SP, Vasantha PV. Development and evaluation of in situ nasal gel formulations of loratadine. Res Pharm Sci. 2015;10(6):466–476.

45 England RJA, Homer JJ, Knight LC, et al. Nasal pH measure-ment: a reliable and repeatable parameter. Clin Otolaryngol Allied Sci. 1999;24(1):67–68. doi: 10.1046/j.1365-2273. 1999.00223.x.

46 Giuliano E, Paolino D, Fresta M, et al. Mucosal applications of poloxamer 407-based hydrogels: an overview. Pharmaceutics. 2018;10(3):159. doi: 10.3390/pharmaceutics10030159.

47 Huang W, Zhang N, Hua H, et al. Preparation, pharmacokinet-ics and pharmacodynamics of ophthalmic thermosensitive in situ hydrogel of betaxolol hydrochloride. Biomed Pharmacother. 2016;83:107–113. doi: 10.1016/j.biopha.2016.06.024.

48 Li Q, Cao Y, Wang P. Recent advances in hydrogels for the diagnosis and treatment of dry eye disease. Gels. 2022;8(12):816. doi: 10.3390/gels8120816.

49 Kurniawansyah IS, Rusdiana T, Sopyan I, et al. In situ oph-thalmic gel forming systems of

poloxamer 407 and hydroxypropyl methyl cellulose mixtures for sustained ocu-lar delivery of chloramphenicole: optimization study by factorial design. Heliyon. 2020;6(11):e05365. doi: 10.1016/j.heliyon.2020.e05365.

50 Ning M, Zhou Y, Chen G, et al. Preparation and in vitro/in vivo evaluation of vinpocetine elementary osmotic pump system. Adv Pharmacol Sci. 2011;2011:385469. doi: 10.1155/2011/385469.

51 Ravi PR, Aditya N, Patil S, et al. Nasal in-situ gels for deliv-ery of rasagiline mesylate: improvement in bioavailability and brain localization. Drug Deliv. 2015;22(7):903–910. doi: 10.3109/10717544.2013.860501.

52 Wang R, Hartel RW. Citric acid and heating on gelatin hydro-lysis and gelation in confectionery gels. Food Hydrocoll. 2022;129:107642. doi: 10.1016/j.foodhyd.2022.107642.

53 Boraste SV, Patil SB. Formulation development and evaluation of nasal *in situ* gel of promethazine hydrochloride. Drug Dev Ind Pharm. 2023;50(1):11–22. doi: 10.1080/03639045.2023.2291463.

54 Liu W, Zou L, Liu J, et al. The effect of citric acid on the activity, thermodynamics and conformation of mushroom polyphenoloxidase. Food Chem. 2013;140(1–2):289–295. doi: 10.1016/j.foodchem.2013.02.028.

55 Karavasili C, Fatouros DG. Smart materials: in situ gel-forming systems for nasal delivery. Drug Discov Today. 2016;21(1):157–166. doi: 10.1016/j.drudis.2015.10.016.

56 Pathak R, Prasad Dash R, Misra M, et al. Role of mucoad-hesive polymers in enhancing delivery of nimodipine microemulsion to brain via intranasal route. Acta Pharm Sin B. 2014;4(2):151–160. doi: 10.1016/j.apsb.2014.02.002.

57 Rao M, Agrawal DK, Shirsath C. Thermoreversible mucoad-hesive *in situ* nasal gel for treatment of Parkinson's disease. Drug Dev Ind Pharm. 2017;43(1):142–150. doi: 10.1080/03639045.2016.1225754.

58 Zahir-Jouzdani F, Wolf JD, Atyabi F, et al. In situ gelling and mucoadhesive polymers: why do they need each other? Expert Opin Drug Deliv. 2018;15(10):1007–1019. doi: 10.1080/17425247.2018.1517741.

59 Newton AMJ, Lakshmanan P. Effect of HPMC - E15 LV premium polymer on release profile and compression characteristics of chitosan/pectin colon targeted mesalamine matrix tab-lets and in vitro study on effect of pH impact on the drug release profile. Recent Pat Drug Deliv Formul. 2014;8(1):46–62. doi: 10.2174/1872211308666140225143926.

60 Jose S, Ansa CR, Cinu TA, et al. Thermo-sensitive gels con-taining lorazepam microspheres for intranasal brain tar-geting. Int J Pharm. 2013;441(1–2):516–526. doi: 10.1016/j. ijpharm.2012.10.049.

61 Khattab A, Marzok S, Ibrahim M. Development of optimized mucoadhesive thermosensitive pluronic based in situ gel for controlled delivery of Latanoprost: antiglaucoma effi-cacy and stability approaches. J Drug Deliv Sci Technol. 2019;53:101134. doi: 10.1016/j.jddst.2019.101134.

62 Ahmed OAA, Badr-Eldin SM. Biodegradable self-assembled nanoparticles of PEG-PLGA amphiphilic diblock copolymer as a promising stealth system for augmented vinpocetine brain delivery. Int J Pharm. 2020;588:119778. doi: 10.1016/j.ijpharm.2020.119778.

63 Wei Y, Zhang J, Zheng Y, et al. Cubosomes with surface cross-linked chitosan exhibit sustained release and bio-availability enhancement for vinpocetine. RSC Adv. 2019;9(11):6287–6298. doi: 10.1039/C8RA10302J.

64 Dhuria SV, Hanson LR, Frey WH. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharm Sci. 2010;99(4):1654–1673. doi: 10.1002/jps.21924.

65 Amidon GL, Lennernäs H, Shah VP, et al. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 1995;12:413–420. doi: 10.1023/A:1016212804288.

66 Wong YC, Zuo Z. Intranasal delivery—modification of drug metabolism and brain disposition. Pharm Res. 2010;27(7):1208–1223. doi: 10.1007/s11095-010-0127-5.

67 Abu-Elfotuh K, Ragab GM, Salahuddin A, et al. Attenuative effects of fluoxetine and triticum aestivum against aluminum-induced Alzheimer's disease in rats: the possible consequences on hepatotoxicity and nephrotoxicity. Molecules. 2021;26(21):6752.

doi: 10.3390/molecules26216752.

68 Gadhave D, Khot S, Tupe S, et al. Nose-to-brain delivery of octreotide acetate in situ gel for pituitary adenoma: pharmacological and in vitro cytotoxicity studies. Int J Pharm. 2022;629:122372. doi: 10.1016/j.ijpharm.2022.122372.

69 Mohanty D, Alsaidan OA, Zafar A, et al. Development of atomoxetine-loaded NLC in situ gel for nose-to-brain deliv-ery: optimization, in vitro, and preclinical evaluation. Pharmaceutics. 2023;15(7):1985. doi: 10.3390/pharmaceu-tics15071985.