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Optimization and Evaluation of in Situ Nasal Gel of Memantine Hydrochloride for Alzheimer's Disease

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Nasal gel, gelling agent, cold method, Memantine Hcl, HPMC K4M etc.

Abstract
The objective of this study was to optimize, design and evaluate the in situ nasal gel of Memantine Hydrochloride in Alzheimer's disease. In situ nasal gel was prepared by cold method and was optimized by the Box-Behnken design using Design-Expert Software (version 13). Concentration of polymer (%) (Poloxamer 407), concentration of gelling agent (%) HPMC and stirring speed (rpm) were selected as a independent variables, whereas viscosity(mpa), drug release (%) and gelation time (sec) were selected as a dependent variables. All the batches were assessed for various parameters such as viscosity (mpa), drug release (%), gelation time (sec), pH, drug content and gel strength. The F1 was found as an optimized formulation of nasal insitu gel. The optimized formulation of the insitu nasal gel for Alzheimer's disease was found to have a viscosity of 3867 ± 5.5 mpa, drug release of 77.02 %, and a gelation time of 48 sec respectively. These results indicate that the nasal insitu gel was able to maintain its viscosity over time and was able to release the drug at a sustained rate over the course of the study. The gelation time was also found to be within an acceptable range, indicating that the gel was able to form in the nasal cavity. These results suggest that the developed Memantine Hydrochloride insitu nasal gel may be the promising drug delivery for Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that worsens over time. It is distinguished by progressive cognitive deterioration, decreased activities of daily living, and behavioural changes. It is the most common form of pre- and senile dementia. According to the World Health Organization (WHO), Alzheimer's type dementia affects 5% of men and 6% of women over the age of 60 worldwide. The clinical manifestation of Alzheimer's disease (AD) is dementia, which typically begins with subtle and poorly recognized memory loss and gradually worsens until it is incapacitating. Currently available treatments, such as acetylcholinesterase inhibitors (rivastigmine, galantamine, donepezil) and N-methyl d-aspartate receptor antagonists (memantine), have a minor impact on the disease and focus on late-stage symptoms. This lack of understanding of the pathogenic process is likely to be the cause of the lack of effective treatment that can prevent disease onset and progression. Because of significant progress in pathophysiology over the last few years, new therapeutic targets are now available that should allow the underlying disease process to be addressed directly. [1] Memantine Hydrochloride (MH) is a medication used in the treatment of Alzheimer's disease, which acts as an NMDA receptor antagonist and helps to improve cognitive function in affected individuals. However, conventional drug delivery systems have limitations such as poor bioavailability and rapid elimination, which can lead to suboptimal therapeutic outcomes.

In situ nasal gel is a novel drug delivery system that provides sustained drug release and improved bioavailability compared to conventional drug delivery systems. The mucoadhesive properties of the gel allow it to adhere to the nasal mucosa, resulting in a longer residence time and improved drug absorption. The gel can also protect the drug from degradation and eliminate the need for frequent dosing. [2] Therefore, the development of a in situ nasal gel of Memantine Hydrochloride can provide a promising approach for the treatment of Alzheimer's disease. This delivery system has the potential to improve patient compliance, reduce dosing frequency, and provide better therapeutic outcomes.[3]

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2. Materials and Method

Memantine Hydrochloride was obtained as a gift sample from Emcure Pharmaceuticals Limited (Pune, Maharashtra). Poloxamer 407 was purchased from Evonik Catalysts India Private Ltd (Mumbai, Maharashtra), HPMC were purchased from Colorcon Asia Private Limited (Verna, Goa) and benzalkonium chloride were purchased from Research Fine Lab, (Mumbai, Maharashtra).

Optimization of the in-situ nasal gels using Box–Behnken design

On the basis of the results obtained from preliminary trials and use of Design–Expert software (version-13), Box–Behnken designs was constructed based on three independent variables, namely, concentration of polymer (poloxamer 407) (X_1) and concentration of gelling agent (HPMCK4M) (X_2) and stirring speed(X_3). The dependent variables measured were viscosity (Y_1) and percent drug release (Y_2) and gelation time (Y_3) as shown in Table No s no [1-3].

3. Methodology

The In-situ nasal gel was prepared by cold method. The Poloxamer 407 was slowly added to cold water (5^0C) maintaining at constant stirring. All other excipients were added with continuous stirring. The dispersions were then stored in a refrigerator until clear solution was obtained. The HPMC K4M with different concentrations were dissolved in distilled water and stirred for 1 hr. From the each prepared HPMC K4M solution, required amount of drug was added. Then the Poloxamer 407 solution was slowly added to the above HPMC K4M solution containing drug & stirred for 1 hr.

Table No 1: Independent Variables and their corresponding Levels for Optimization Studies

Sr.No.	Independent Variables	Factor	-1	+1
1.	Concentration of Polymer **Poloxamer 407** (%)	X_1	8	12
2.	Concentration of Gelling agent **HPMC K4M** (%)	X_2	0.1	0.5
3.	Stirring speed (rpm)	X_3	1000	1500

Table No 2: Dependent Variables for Optimization Studies

Sr.No.	Dependent Variables	Response
1.	Viscosity(mpa)	Y_1
2.	Drug release (%)	Y_2
3.	Gelation time (sec)	Y_3

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Table No 3: Formulation and composition of insitu nasal gel

Formulation	Memantine Hydrochloride (mg)	Concentration of Polymer (Poloxamer 407) (%)	Concentration of gelling agent (%)	Benzalkonium Chloride (%)	Water (q.s)
F1	0.25	10	0.3	0.01	50
F2	0.25	8	0.3	0.01	50
F3	0.25	12	0.1	0.01	50
F4	0.25	10	0.5	0.01	50
F5	0.25	12	0.3	0.01	50
F6	0.25	12	0.5	0.01	50
F7	0.25	10	0.3	0.01	50
F8	0.25	10	0.1	0.01	50
F9	0.25	10	0.3	0.01	50
F10	0.25	10	0.3	0.01	50
F11	0.25	10	0.3	0.01	50
F12	0.25	10	0.1	0.01	50
F13	0.25	12	0.3	0.01	50
F14	0.25	10	0.5	0.01	50
F15	0.25	8	0.1	0.01	50
F16	0.25	8	0.5	0.01	50
F17	0.25	8	0.3	0.01	50

4. Evaluation for In-Situ Nasal Gel:

Appearance

The appearance of insitu nasal gel was monitored for clarity. The clarity of different formulations was assessed through visual observation against a black and white background.[4]

Determination of pH

A calibrated pH meter was used to determine the pH of the insitu nasal gel. The measurements were made in triplicate, and the average of these measurements was used to calculate the pH of the gel.[5]

Drug content determination

1 gm of gel was placed in a 10 ml volumetric flask and diluted with distilled water to a volume of 10 ml. 1 ml

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of the above solution was taken and diluted to 10 ml with distilled water. The absorbance of the prepared solution was then measured at a 254 nm wavelength using an ultraviolet (UV)-visible spectrophotometer. The tests were performed in triplicate.[6]

Gelation Time

The formulation's sol-gel temperature dependence (Tsol-gel) was determined using the test tube inversion method, which involved transferring 2 ml of prepared formulation to a test tube (10 ml) and sealing it with paraffin. This test tube was immersed in a 37±0.5°C constant temperature water bath.[7]

Gelation Temperature

The formulation's Tsol-gel was determined using the test tube inversion method, which involved transferring 2 ml of the formulation into a test tube and sealing it with paraffin. This test tube was immersed in a bath of constant temperature water. The temperature of the water bath was raised in 2±0.5°C increments and allowed to equilibrate at each new temperature. However, in the Tsol-gel region, temperature was gradually increased in 0.5°C increments. The formulation was tested for gelation, which was said to occur when the gel meniscus would no longer move when tilted at 90°.[8]

Gel Strength

The gel strength, which represents the viscosity of the nasal gel at specific temperature, was determined by measuring the force required to settle the gel at 35-37°C. A sample of 20 g nasal gel was placed in a 100 ml graduated cylinder and gelled in a 35-37°C water bath. A 35 g weight was placed on top of the gelled solution. Thus, the time in seconds required by the weight to penetrate 5 cm into the nasal gel was measured.[9]

Viscosity

The viscosity of prepared gel formulations was determined using a Brookfield DV-II pro-plus viscometer. The formulations' viscosities were measured at two different temperatures, such as room temperature and 37±0.5°C with varying shear rate. Measurements were taken in triplicate. [10,11]

In vitro Diffusion Study

This study was carried out using a Franz diffusion cell with a diameter of 2.0 cm and a capacity of 25 ml that was water jacketed and made of glass. As a diffusion membrane, dialysis membrane was used. Pieces of dialysis membrane were soaked in phosphate buffer with a pH of 6.4 for 24 hours prior to the experiment. The diffusion cell's receptor compartment was filled with phosphate buffer pH 6.4. The dialysis membrane was placed in the diffusion cell's donor and receptor compartments. The donor compartment's position was adjusted so that the membrane just touched the diffusion medium. The temperature was kept at 35-37°C. A magnetic stirrer was used to stir the content of the receptor membrane. The donor compartment was then filled with 2 ml of formulation. For an 8-hour period, 1 ml samples were withdrawn from the receptor compartment at 1 hr intervals and replaced with the same volume of fresh phosphate buffer pH 6.4 after each sampling. The withdrawn samples were filtered and spectrophotometrically analysed at 254 nm with a UV-visible spectrophotometer. [12,13]

Stability Study

Memantine Hydrochloride nasal gel stability studies were carried out in real-world storage conditions (refrigeration condition). Gels are kept away from light in clean, dry, airtight, moisture-proof bottles. Appearance, pH, drug content, and drug release of the gel samples of Memantine Hydrochloride were evaluated at 15, 30, 45-day intervals. [14]

5. Results:

Appearance

The appearnce of all the formulations were clear in solution form.

pH

The pH of all formulations was found to be between 5.2 ± 0.5 and 5.7± 0.5, which is within the nasal (4.5-6.4) pH range.

Drug Content

The percent drug content of all the formulations was found in the range of 80.11±0.5-84.77±0.5%.

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Gelation Time

Gelation time refers to the time taken for the liquid mixture to transform into a solid gel under specific conditions. The effect of concentration of polymer and gelling agent on the Gelation time, a series of experiments were conducted at different concentrations of both the polymer and the gelling agent. The gelation time was measured for each experiment, and the results were analysed using statistical methods. The results showed that the gelation time was influenced by the concentration of both the polymer and gelling agent. Generally, increasing the concentration of the polymer and gelling agent resulted in a shorter gelation time. However, there was a threshold concentration beyond which increasing the concentration had no significant effect on the gelation time.[15]

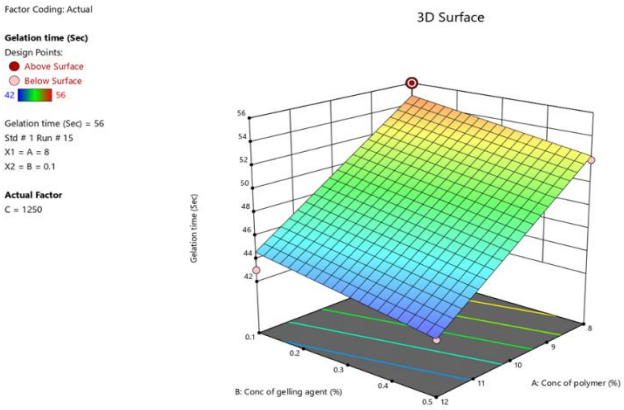

Figure No 1: 3D Response surface plot for effect of concentration of polymer (%) and gelling agent (%) on the Gelation time (sec).

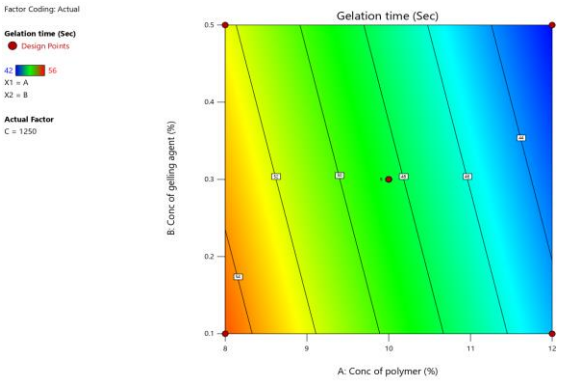

Figure No 2: Counter plot for the effect of concentration of polymer (%) and gelling agent (%) on the Gelation time (sec).

Gelation Temperature

The gelation temperature is an important parameter for in-situ nasal gel formulations because it determines the time required for the liquid to solidify and form the gel, and also affects the properties of the resulting gel. The gelation temperature for in-situ nasal gel must be carefully chosen to ensure that the gelation process occurs at a temperature close to the physiological temperature of the nasal cavity. This is because the gelation process should occur quickly to prevent the liquid from flowing out of the nasal cavity, but not too quickly as it can cause irritation to the nasal mucosa. Poloxamer and HPMC K4M have the ability to form gels when in solution under certain conditions. In general, adding HPMC K4M to a Poloxamer solution can increase the gelation temperature. This is because HPMC K4M is more rigid and less soluble than poloxamer, and therefore requires more energy to overcome the entanglement of its polymer chains in order to form a gel. However, the effect of HPMC K4M and the poloxamer on the gelation temperature can depend on several factors, such as the molecular weight, concentration of the polymers and the ratio of Poloxamer 407 to HPMC K4M. Studies show that the adding HPMCK4M to poloxamer may actually decrease the gelation temperature, especially if the concentration of HPMC K4M is low or if the HPMCK4M has a lower molecular weight.[16]

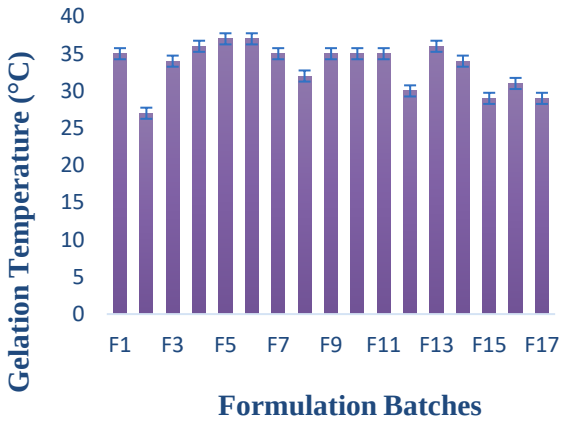

Figure No 3: Gelation temperature for formulation batches

Gel Strength

Gel strength refers to the ability of a gel to resist deformation or flow under stress. In the context of

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insitu nasal gel, gel strength is an important property that can affect the performance and effectiveness of the gel.Insitu nasal gels are designed to be applied in a liquid state and then transform into a gel once they come into contact with the nasal mucosa. Gel strength can determine how well the gel adheres to the nasal mucosa, which can affect drug delivery and absorption.Gel strength was found to be affected by gelling and thermosensitive polymer concentrations. In situ gel must have a suitable gel strength to be administered easily and to be retained at the nasal mucosa after administration without leakage. HPMC and Poloxamer 407 were found to increase gel strength with increasing concentrations in the gels. There are more molecules available to crosslink and form a stronger, denser network. This increases the physical strength of the gel, making it more resistant to deformation and easier to handle. Additionally, the increased concentration of gelling agents and thermoreversible polymers can lead to a more viscous gel, which can further contribute to its strength by preventing the movement of the gel matrix. [17]

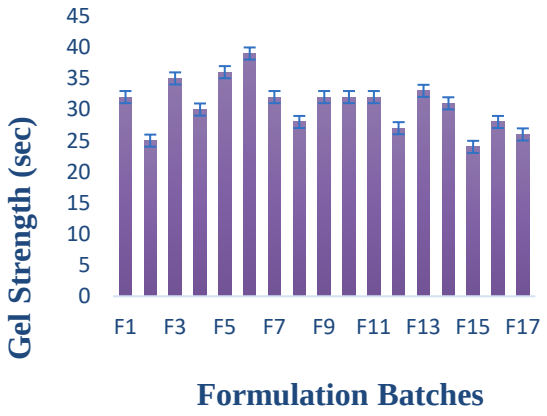

Figure No 4: Gel strength for formultion batches

Viscosity

Viscosity is an essential property of liquids and semisolids that refers to their resistance to flow. In the context of pharmaceuticals, viscosity plays a critical role in determining the product's stability, efficacy, and patient acceptance. The viscosity of prepared formulations was determined using a Brookfield DV-II pro-plus viscometer. The results of the formulations in both solution and gel form are shows in [Table No 4].[17]

Table No 4: Viscosity of in situ nasal gel in solution and after gel formation

Batches	Solution Form (mpa)	Gel Form(mpa)
1	123± 0.5	3867± 0.5
2	102± 0.5	2820± 0.5
3	162± 0.5	4571± 0.5
4	149± 0.5	3991± 0.5
5	152± 0.5	4567± 0.5
6	208± 0.5	4734± 0.5
7	109± 0.5	3865± 0.5
8	119± 0.5	3521± 0.5
9	123± 0.5	3867± 0.5
10	123± 0.5	3867± 0.5
11	123± 0.5	3867± 0.5
12	135± 0.5	3710± 0.5
13	171± 0.5	4636± 0.5
14	167± 0.5	4219± 0.5
15	99± 0.5	2542± 0.5
16	100± 0.5	2568± 0.5
17	119± 0.5	3020± 0.5

3D response surface plot that visually displays the relationship between the concentration of polymer and gelling agent and the resulting viscosity in units of mPa.The analysis revealed that increasing the concentration of both the polymer and gelling agent led to a significant increase in viscosity. Additionally, we observed higher polymer concentration and gelling agent can increase the viscosity of a solution due to their ability to increase the entanglement and intermolecular interactions between polymer chains by creating a three-dimensional network within the

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solution that traps the solvent and polymer chains. This network also increases the resistance to flow and results in a higher viscosity. Overall, results suggest that the concentration of both the polymer and gelling agent play critical roles in determining the viscosity of the final product. [18]

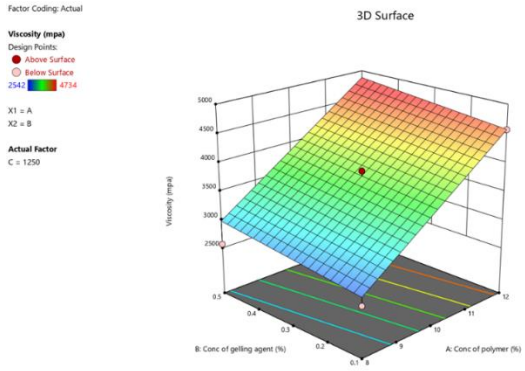

Figure No 5: 3D Response surface plot for effect of concentration of polymer (%) and gelling agent (%) on the viscosity (mpa)

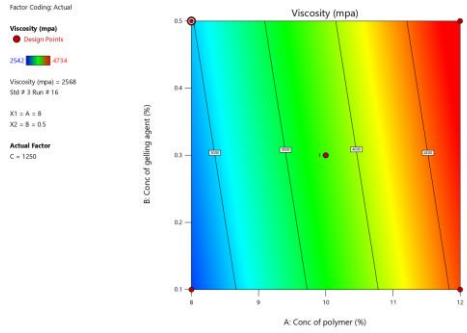

Figure No 6: Counter plot for effect of concentration of polymer (%) and gelling agent (%) on the viscosity (mpa)

In vitro Diffusion Study

Figure No 7: Drug diffusion study

The 3D response surface plot reveals the relationship between the concentration of polymer and gelling agent and their effect on the percent drug release. The plot shows that increasing the concentration of the polymer and gelling agent leads to a decrease in the percent drug release. This is likely due to the formation of a thicker gel layer that slows down the diffusion of the drug molecules through the matrix. Additionally, the interaction between the two factors suggests that there is an optimal balance between the concentration of polymer and gelling agent that results in maximum drug retention. These findings provide important insights for the development of drug delivery systems and can be used to optimize the formulation parameters to achieve the desired drug release profile.

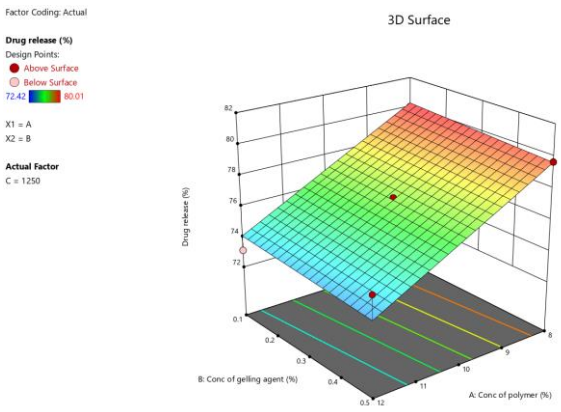

Figure No 8: 3D Response surface plot for effect of concentration of polymer (%) and gelling agent (%) on the percent drug release (%)

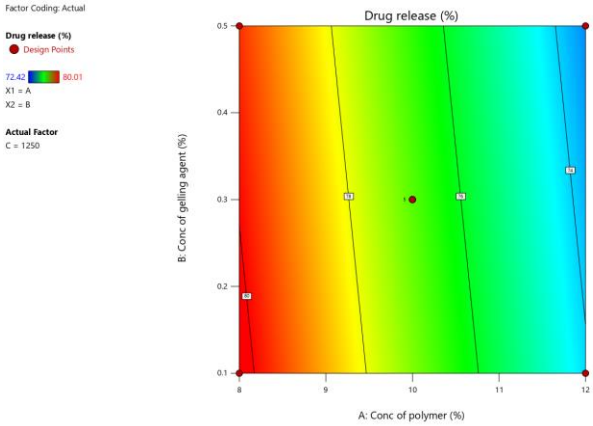

Figure No 9: Counter plot for effect of concentration of polymer (%) and gelling agent (%) on the percent drug release (%)

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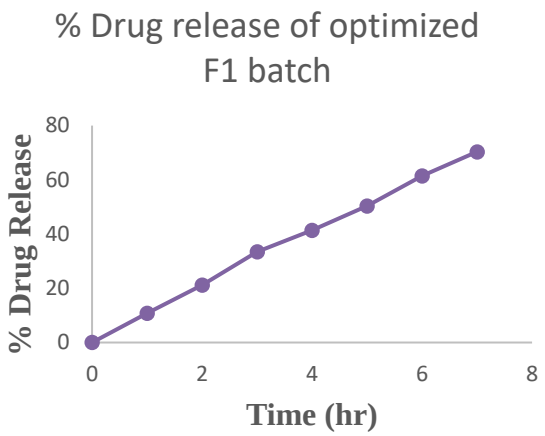

Figure No 10: Percent drug release of optimized formulation (F1)

Stability Study

After storing the optimised formulation (F1) for 15, 30, and 45 days, studies were conducted. The data for appearance, pH, dug content, and drug release are shown in [Table No 5]. After 15, 30, and 45 days, the formulation F1 showed no change in appearance. After 15, 30, and 45-day intervals, the drug content decreased from 84.38± 0.5% to 84.19± 0.5%. After 15, 30, 45-day intervals, the drug release decreased from 77± 0.5% to 69.87± 0.5%. Furthermore, the formulation's pH was altered. The results showed a slight change in the evaluation parameters, indicating that the formulation was suitable according to the guidelines

Table No 5: Stability study for optimized formulation (F1)

Time period	Appearance	pH	Drug content	%Drug Release
15 days	Clear	5.42 ± 0.59	84.38± 0.5	77.00± 1.4
30 days	Clear	5.42± 0.53	84.25± 0.5	71.99± 1.2
45 days	Clear	5.42± 0.42	84.19± 0.5	69.87± 2.1

6. Conclusion

The optimization and evaluation of an in situ nasal gel of Memantine Hydrochloride for the treatment of Alzheimer's disease represents a promising avenue for improving drug delivery and patient outcomes. The use of nasal delivery offers several advantages over traditional oral administration, including rapid onset of action, avoidance of first-pass metabolism, and improved bioavailability. Through the use of the cold method, a pH 5.42 ± 0.5 was achieved, which is compatible with the nasal cavity. The optimization of the formulation was carried out using response surface methodology and Box-Behnken design, resulting in the selection of an optimized formulation (F1) that exhibited desirable properties, including good viscosity (3867 ± 5.5 mPa), sustained drug release (77.02%), and a reasonable gelation time (48 seconds). These results suggest that the in situ nasal gel of Memantine Hydrochloride has the potential to overcome the limitations of current treatment options for Alzheimer's disease. However, further studies are needed to assess its safety and efficacy in clinical trials. In addition, the long-term effects of the optimized formulation should be investigated.

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